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BASES MOLECULARES E ELECTROFISIOLÓGICAS DA INFLUÊNCIA DA ACTIVIDADE AUTONÓMICA NA GÉNESE E MANUTENÇÃO DA FIBRILHAÇÃO AURICULAR PAROXÍSTICA

Mário João Martins Oliveira

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Orientadores: Prof. Doutor Luis Silva Carvalho

Prof.ª Doutora Isabel Rocha

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À Alexandra
À Marta, à Mariana e ao Pedro
Aos meus pais
À memória de Luís Silva Carvalho

“The most beautiful thing we can experience is the mysterious. It is the source of all true art and science.”

Albert Einstein

"It is not what the man of science believes that distinguishes him, but how and why he believes it"

Bertrand Russell

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Abreviaturas

AAD - apêndice auricular direito

AAE - apêndice auricular esquerdo

AD - aurícula direita

AE - aurícula esquerda

ATP - adenosina trifosfato

FA - fibrilhação auricular

FAP - fibrilhação auricular paroxística

FC - frequência cardíaca

Cx - conexina

HF - high frequency

LF - low frequency

LF/HF - relação low frequency/high frequency

PA - pressão arterial

PRE - período refractário efectivo

SC - seio coronário

SNA - sistema nervoso autónomo

VP - veias pulmonares

Índice

Capítulo I.

Introdução

1. Fibrilhação Auricular

1.1 Epidemiologia e Impacto Clínico

A FA é a arritmia cardíaca mais frequente na prática clínica e a que envolve um maior número de hospitalizações (Benjamin et al, 1998; Lip, 2001; Peters et al, 2002; Rich, 2009). Caracteriza-se pela presença de actividade eléctrica auricular extremamente rápida e irregular, com variações na duração do ciclo, polaridade, configuração e amplitude dos electrogramas, condicionando deterioração da função contráctil das aurículas, resposta ventricular irregular, com redução do desempenho cardíaco e da capacidade funcional, que se associam a maior ocorrência de insuficiência cardíaca congestiva (Olgin & Zipes, 2008).

A sua prevalência varia de 0,4% a 1% na população global, sendo ligeiramente superior nos homens (Camm, 1997; Go et al, 2001; Rich, 2009). Apresenta um aumento significativo com a idade, afectando 4% dos individuos com mais de 60 anos, cerca de 10% depois dos 80 anos e mais de 17% numa população com idade $\geq$ 85 anos (Feinberg et al, 1995; Fuster et al, 2006; Heeringa et al, 2006). De acordo com dados do estudo de Framingham, o risco de aparecimento de FA depois dos 40 anos de idade é de cerca de um em cada quatro indivíduos, com valores ligeiramente superiores para o sexo masculino (Lloyd-Jones et al, 2004), enquanto no estudo de Roterdão são referidos valores de um em cada seis mesmo na ausência de enfarte do miocárdio ou insuficiência cardíaca (Heringa et al, 2006). No estudo de Framingham, ao longo dum período de 38 anos a incidência de FA na população com idades entre os 55 e os 64 anos foi de 3/1000 pessoas-ano nos homens e 2/1000 pessoas-ano nas mulheres (Kannel & Benjamin, 2008). Também no Reino Unido, o estudo Renfrew/Paisley mostrou, durante um período de 20 anos, uma incidência de FA semelhante numa população da mesma faixa etária (Stewart et al, 2001). Diferentes registos têm revelado um aumento significativo e consistente da incidência de FA e do número de episódios tratados nos serviços de urgência médica ao longo das últimas décadas, estimando-se que, actualmente, o número de doentes com FA ultrapasse os 6 milhões na

Europa e os 2,3 milhões nos Estados Unidos da América (Kannel & Benjamin, 2008; McDonald et al, 2008), e que, com o envelhecimento esperado da população a prevalência de FA possa duplicar nos próximos 25 anos (Go et al, 2001). No entanto, estas projecções podem estar subvalorizadas, se considerarmos que a FA assintomática ("silenciosa") pode afectar 25-30% dos indivíduos com mais de 65 anos e que muitos episódios não estão documentados devido a serem de auto-limitados e de curta duração (Savelieva & Camm, 2000; Ellinor et al, 2005). Além disso, na comparação com uma população sintomática, a ausência de sintomas não se associa a prognóstico mais favorável (Flaker et al, 2005). A FA é habitualmente associada à presença de cardiopatia estrutural, mas pode ocorrer sem evidência de patologia cardíaca num terço de todos os casos e em cerca de metade dos doentes jovens (Flaker et al, 1995; Peters et al, 2002; Lip et al, 2004). No entanto, para alguns autores o acompanhamento a longo-prazo de doentes com FA mostrou que apenas numa proporção bastante inferior a arritmia pode ser considerada verdadeiramente idiopática (Jahangir et al, 2007). Esta discrepância pode resultar de diferentes definições dado que, recentemente, no âmbito da nomenclatura e classificação da FA, se estabeleceu como forma *isolada* a que ocorre na ausência de doença cardíaca e como *idiopática* aquela que surge na ausência de qualquer patologia concomitante (Levy et al, 2003).

O perfil clínico e a evolução da FA são heterogéneos, variando de acordo com a doença cardíaca subjacente, as co-morbilidades, o tipo de FA e a faixa etária. A classificação dos tipos de FA inclui as formas *paroxística* e *persistente*, quando se apresenta com episódios recorrentes (respectivamente com duração inferior a 7 dias e habitualmente de terminação espontânea, ou mantida, com duração superior a 7 dias ou necessidade de conversão a ritmo sinusal), e a forma *permanente*, quando a duração é superior a 1 ano sem recurso a cardioversão ou quando a tentativa de cardioversão foi ineficaz (Fuster et al, 2006). Recentemente, foi introduzida a designação de FA *persistente de longa duração*, para os casos que apresentam FA contínua com mais de 1 ano de evolução, mas que são referenciados para ablação de FA por cateter (Calkins et al, 2007). No Euro Heart Survey, um estudo efectuado no âmbito da Sociedade

Europeia de Cardiologia, 10% dos doentes apresentavam FA idiopática, a FA *de novo* foi diagnosticada em 18%, a FAP em 28%, a FA *persistente* em 22% e a FA *permanente* em 29% dos doentes (Nieuwlatt et al, 2005). A FAP pode constituir cerca de 25-62% dos casos observados na clínica, sendo esta variação resultante de diferentes definições de FAP e da diversidade de populações estudadas (Lip & Hee, 2001). A FA é considerada *idiopática* ou "isolada" em 30-50% dos casos com FAP e em 15-25% nos doentes com FA *persistente*, sendo o diagnóstico mais comum em jovens (Peters et al, 2002, Fuster et al, 2006).

A evolução para FA *persistente* é relativamente frequente em doentes com FAP, estimando-se que possa variar entre 14% e 24% (Schoonderwoerd et al, 2005; Fuster et al, 2006; Saksena et al, 2007). De acordo com dados do Canadian Registry of Atrial Fibrillation, 25% dos doentes com FAP *de novo* podem evoluir para FA *permanente* decorridos 5 anos (Kerr et al, 2005). Além disso, 40% dos casos com FA *persistente* desenvolvem FA *permanente* após 1 ano (Lehto et al, 2003). Numa população com FA isolada, um *follow-up* médio de 25 anos mostrou progressão para a forma *permanente* em 30% dos doentes (Jahangir et al, 2007).

A FA tem um impacto desfavorável na qualidade de vida, morbilidade e mortalidade, mais acentuado nos idosos e na presença de patologia cardíaca subjacente (Feinberg et al, 1995; Benjamin et al, 1998; Van de Berg et al, 2001; Bilato et al, 2009). As manifestações clínicas resultam dos efeitos hemodinâmicos adversos relacionados com a perda da função contráctil auricular e a resposta ventricular irregular, que podem causar taquicardiomiopatia com disfunção ventricular, insuficiência cardíaca congestiva e aumento significativo do risco de tromboembolismo (Lip & Hee, 2001). Neste contexto, para além de reduzir o desempenho cardíaco e a capacidade funcional, contribuindo significativamente para a maior prevalência de insuficiência cardíaca congestiva, a FA é um potente marcador independente do risco de acidente vascular cerebral, sendo responsável por 17%-27% de todos os acidentes vasculares cerebrais isquémicos, cujo risco aumenta 2 a 7 vezes na sua presença (Gottdiener et al, 2000;

Wolff et al, 2001; Feigin et al, 2003; Kannel & Benjamin, 2008). A taxa de mortalidade em doentes com FA é cerca do dobro para ambos os sexos quando comparada com a de doentes em ritmo sinusal, e está relacionada com a gravidade da doença cardíaca subjacente (Kannel & Benjamin, 2008). A presença de co-morbilidades e a idade podem influenciar significativamente a evolução clínica e complicações da FA (Jahangir et al, 2007). Por outro lado, a FA é responsável por aproximadamente um terço das hospitalizações por distúrbios do ritmo cardíaco (Fuster et al, 2006), que representam 50-70% dos gastos de saúde nesta área (Reynolds et al, 2007; Ringborg et al, 2008, Stewart et al, 2001).

O impacto epidemiológico e os riscos clínicos inerentes à FA constituem um problema *major* de saúde pública e um desafio no que se refere à investigação dos mecanismos fisiopatológicos subjacentes à génese e manutenção desta arritmia, aos factores predisponentes e às estratégias de prevenção e terapêutica mais eficazes. Neste âmbito, o objectivo da manutenção do ritmo sinusal tem sido difícil de obter com o recurso a fármacos antiarrítmicos, que além do mais se associam a efeitos adversos potencialmente graves (Savelieva & Camm, 2008). Por outro lado, a terapêutica ablativa da FA é atractiva mas ainda não atingiu níveis de eficácia e segurança semelhantes aos verificados no tratamento doutras arritmias. Neste contexto, a compreensão dos diferentes fenómenos ligados ao substrato electrofisiológico da arritmia pode ser importante para melhorar aspectos relacionados com a técnica de intervenção e definir a abordagem mais adequada no tratamento da FA.

1.2 Factores de Risco e Condições Cardiovasculares Associadas

A idade tem sido considerada como a variável com maior valor predizente para a ocorrência de FA, prevendo-se uma duplicação da sua prevalência e sua incidência por cada década de vida (Fuster et al 2006; Kannel & Benjamin, 2008). Cerca de 75% dos doentes com FA tem mais de 65 anos sendo a mediana da idade da população afectada aproximadamente de 75 anos (Go et

al, 2001). De facto, o envelhecimento acompanha-se de alterações cardíacas que incluem a perda gradual de fibras nodais, o aumento de zonas de fibrose e de tecido adiposo por vezes com infiltração amilóide, alterações do relaxamento ventricular e dilatação auricular (Everett, 2007; Spach, 2007). Além disso, Anyukhovsky e colaboradores, sugeriram que a idade se associa, também, a alterações das propriedades electrofisiológicas nomeadamente da dispersão da refractariedade que podem facilitar o início de FA (Anyukhovsky, 2005).

A hipertensão arterial, a insuficiência cardíaca congestiva, a cardiopatia valvular (em particular a patologia mitral reumatismal) e o enfarte do miocárdio são situações clínicas comuns que se associam de forma independente à predisposição para a ocorrência de FA (Kannel & Benjamin, 2008), sendo a hipertensão arterial, a cardiopatia isquémica e a insuficiência cardíaca as co-morbilidades mais prevalentes na população com FA (Levy et al, 1999; Go et al, 2001; Nieuwlaat et al, 2005) (tabela I). Recentemente, foi sugerido que alguns doentes com FA podem representar casos "ocultos" de hipertensão arterial, que só se torna aparente depois de diagnosticada a arritmia (Katritsis et al, 2005).

Tabela I – Caracterização populacional e clínica dos doentes com FA incluídos nos estudos ALFA, ATRIA e EHS-AF

	ALFA	ATRIA	EHS-AF
idade média (anos)	68,6	71,2	66,6
sexo masculino (%)	57,7	56,6	57,8
AVC prévio (%)	8,4	8,9	10,1
ICC (%)	29,8	29,2	31,1
cardiopatia valvular (%)	3,3	4,9	10,2
cardiopatia isquémica (%)	16,6	34,6	29,6
HTA (%)	60,8	49,3	63,1
FA idiopática (%)	29,3	-	14,3

AVC=acidente vascular cerebral; ICC=insuficiência cardíaca congestiva; HTA=hipertensão arterial; ALFA=the ALFA study (Levy et al, 1999); ATRIA=The Anticoagulation and Risk Factors in Atrial Fibrillation Study (Go et al, 2001); EHS-AF=The European Heart Survey on Atrial Fibrillation (Nieuwlaat et al, 2005)

O aumento das dimensões da AE, a hipertrofia ventricular esquerda e a disfunção diastólica, que se observam também na hipertensão arterial, têm sido considerados como marcadores cardíacos que aumentam o risco de FA (Varisi et al, 1994; Tsang et al, 2002; Kizer et al, 2006). No contexto de cirurgia cardíaca 20% a 40% dos doentes têm episódios de FA durante a primeira semana após a intervenção (Fuster et al, 2006). Outras causas menos comuns de FA incluem as cardiopatias congénitas, em particular a comunicação interauricular, todas as formas de miocardiopatia, a miocardite, pericardite e *cor pulmonale*. A FA pode ainda estar associada a outras arritmias como o *flutter* e taquicardia auriculares, a síndrome de Wolf-Parkinson-White ou a taquicardia de reentrada nodal (Fuster et al, 2006). O consumo excessivo de álcool (com efeitos agudos na refractariedade e condução auriculares e repercussão crónica na função ventricular), características da personalidade como a hostilidade e fúria, a obesidade, a síndrome metabólica e a *diabetes mellitus* têm sido apontados como condições clínicas que aumentam significativamente o risco de FA (Djousse et al, 2004; Eaker et al, 2004; Dublin et al, 2006; Kannel & Benjamin, 2008). Outras situações descritas como tendo valor independente para risco de FA incluem as patologias do foro pulmonar, o hipertiroidismo, a apneia do sono, o *stress* psicológico e situações de inflamação sistémica com valores elevados de proteina C reactiva (Fuster et al, 2006; Auer et al, 2001; Tsang et al, 2005; Boos & Lip, 2008). Noutro âmbito, o exercício físico vigoroso, sobretudo em jovens, parece aumentar o risco de FA por mecanismos que permanecem por esclarecer, havendo uma relação entre o número de horas de prática desportiva, a presença de bradicardia e a probabilidade de desenvolver a arritmia (Aizer et al, 2009; Mont et al, 2009; Grimsmo et al, 2010).

Tem sido estabelecida uma relação entre as correntes iónicas transmembranares e os genes responsáveis pela configuração e distribuição dos diferentes canais iónicos e, assim, com as características electrofisiológicas celulares e FA. Estudos recentes evidenciaram, num pequeno grupo de doentes, uma predisposição genética para FA associada a alterações nos genes

SCN5A (que codifica um canal de sódio voltagem-dependente), KCNE, KCNE2, KCNE3, KCNJ2, KCNQ1 e KCNH2 (que codificam canais do potássio) e a variantes genéticas da emerina, uma proteína associada às lâminas nucleares, encontrada nas fibras musculares cardíacas e cuja função ainda é desconhecida (Darbar et al, 2008; Tsai et al, 2008; Karst et al, 2008). Em indivíduos sem história familar de FA foram descritos polimorfismos no gene do angiotensinogénio associados a cardiopatia estrutural (Tsai et al, 2008) e mutações nos genes das conexinas (Cx40) em casos com FA *idiopática* e perturbação da condução eléctrica auricular (Gollob et al, 2006). De facto, têm sido identificadas mutações de diferentes genes com repercussão nas propriedades electrofisiológicas cardíacas em doentes com história familiar de FA (Otway et al, 2007). Brugada e colaboradores (1997) descreveram uma família com um padrão autossómico dominante para transmissão de FA associado a um gene no cromossoma 10q (região 10q22-q24), cuja função ainda não foi identificada (Brugada et al, 1997). Na população com Síndroma de Brugada, uma doença eléctrica de transmissão autossómica dominante, sem alterações cardíacas estruturais e com risco de morte súbita por taquiarritmias ventriculares a prevalência de FA tem sido estimada em 20% (Letsas et al, 2007).

Num estudo envolvendo populações da Europa e Hong-Kong foi identificada uma associação entre variantes de duas sequências no cromossoma 4q25 e FA (Gudbjartsson et al, 2004). Estas variantes encontram-se adjacentes ao gene PITX, uma sequência de ADN envolvida na regulação do desenvolvimento, que intervem na diferenciação de fibras músculares nas VP (Mommersteeg et al, 2007). Dados do estudo de Framinghan apontam um risco relativo de 1.85 para o aparecimento de FA quando existe história daquela arritmia em pelo menos um membro da família (Fox et al, 2004). Por outro lado, a análise duma população com história familiar de FA, envolvendo predominantemente caucasianos, não permitiu identificar mutações em genes de subunidades de canais do potássio (KCNJ2 e KCNE1-5) potencialmente associadas à FA (Ellinor et al, 2006).

Assim, os dados disponíveis apontam para a associação de diferentes polimorfismos genéticos com a FA, sugerindo um substrato genético heterogéneo, que, numa proporção indeterminada de casos, pode desempenhar um papel na complexa fisiopatologia da FA. Apesar da evidência crescente de que a FA familiar possa ser mais comum do que o previsto, nomeadamente no âmbito da FA *idiopática*, permanece por definir o verdadeiro impacto dos factores genéticos na prevalência desta arrítmia (Wiesfeld et al, 2005; Lubitz SA et al, 2009).

Estudos recentes têm sugerido que alterações na actividade neurohormonal e na regulação do SNA com influência na electrofisiologia do tecido auricular possam desempenhar um papel importante na susceptibilidade para FA (Van Wagoner, 2007; Aldhoon et al, 2009). Também factores como os distúrbios electrolíticos, os digitálicos, a isquemia aguda, a hipóxia, o *stress* oxidativo, o estiramento (*stretch)* das fibras auriculares e a inflamação, por facilitarem o aparecimento de actividade focal e influenciarem a condução e refractariedade auriculares são considerados como potenciais responsáveis pelo início de FA (Alessie et al 2001; Kasper et al, 2006; Marchlinski, 2008; Kourliouros & Camm, 2009).

As modificações neurohormonais que ocorrem na insuficiência cardíaca, frequentemente como consequência de hipertensão arterial ou após enfarte do miocárdio, relacionam-se com a activação do sistema renina-angiotensina-aldosterona, com o aumento dos péptidos natriuréticos e do tónus simpático, promovendo alterações do automatismo, na actividade *trigger,* na velocidade de condução e na refractariedade auriculares que, quando combinados com hipertrofia, fibrose e aumento da pressão nas cavidades cardíacas aumentam a vulnerabilidade para a ocorrência de FA (Padeletti et al, 2004; Byrne et al, 2008). Em particular, os péptidos natriuréticos, aceites como marcadores de sobrecarga cardíaca de volume e pressão, com utilidade demonstrada no diagnóstico, monitorização terapêutica e prognóstico na insuficiência cardíaca congestiva, alteram as propriedades electrofisiológicas cardíacas e podem estar aumentados mesmo em doentes com FA *isolada* (Yamada et al, 2005). A comparação dos

valores do péptido natriurético B entre doentes com FAP isolada e um grupo controlo da mesma idade e sexo, mostrou que, apesar de não haver diferenças entre o diâmetro da AE e a fracção de ejecção ventricular esquerda, o grupo com FAP apresentava níveis séricos mais elevados, permanecendo por explicar o significado clínico deste achado no contexto da FA (Li et al, 2006). No entanto, num estudo em cães anestesiados com inervação autonómica preservada ou bloqueada com atropina e propranolol, demonstrou-se que os efeitos do factor natriurético auricular no potencial de acção e refractariedade auriculares são dose-dependentes e mediados pelo SNA (Stambler & Guo, 2005).

A angiotensina II parece influenciar a excitabilidade auricular ao intervir na regulação das correntes do cálcio (I_{CaL} e I_{CaT}) e do potássio (I_{Kr}, I_{Ks}, I_{Kur}) (Ferron et al, 2003; Caballero et al, 2000), influenciando deste modo a duração do potencial de acção e propagação do impulso e facilitando a ocorrência de circuitos de reentrada (Ehrlich et al, 2006). Além disso, há evidência de que a estimulação dos receptores AT1 e aumento da síntese de TGF-β1 (*Transforming growth factor beta 1*) promove o aparecimento de fibrose e dilatação auriculares, condicionando a heterogeneidade na condução auricular e a predisposição para FA (Ehrlich et al, 2006; Verheule et al, 2004).

A influência da actividade simpática e da actividade vagal na arritmogénese cardíaca tem sido avaliada em investigação básica e clínica, admitindo-se que o SNA possa desempenhar um papel potencial na modulação dos fenómenos electrofisiológicos associados à FA (Chen & Chen 2006; Darge et al, 2009; Lombardi et al, 2004). De facto, num trabalho recente, mostrou-se que a estimulação beta-adrenérgica promove a ocorrência de pós-potenciais tardios (na fase 4 do potencial de acção), encurta os PRE e aumenta a velocidade de condução no tecido auricular, enquanto a estimulação vagal tem sido considerada mais arritmogénica que a simpática com um efeito potente na redução heterogénea dos PRE auriculares (Zhang & Mazgalev, 2008). Os trabalhos de Zipes, no cão, mostraram que a estimulação vagal cervical direita diminui o PRE de forma mais acentuada na AD que na AE, documentando pela primeira vez a presença dum

gradiente nos PRE auriculares em resultado da actividade vagal (Zipes et al, 1974). Outros estudos mostraram que a estimulação simpática, por seu lado, facilita também os pós-potenciais precoces (nas fase 2 e 3 da repolarização, através do aumento da concentração de cálcio intracelular) e a actividade *trigger,* o automatismo e circuitos de microreentrada (Coumel, 1997; Chen & Tan, 2007).

Parece haver maior influência da actividade vagal na FA isolada, enquanto os efeitos do tónus simpático têm sido considerados como tendo mais importância na presença de doença cardíaca (Chen & Tan, 2007). No entanto, para alguns autores a designação de FA "adrenérgica" aplica-se, à semelhança da FA "vagal", também numa população jovem, habitualmente sem patologia cardíaca associada, sendo desencadeada, especificamente, por situações de exercício ou *stress* emocional (Fuster et al, 2006). Além disso, tem sido sugerido que pode haver alguma forma de sinergismo envolvendo simultaneamente actividade simpática e parassimpática na génese da FA (Tan et al, 2007; Ogawa et al, 2007). A designação de FA *neurogénica* tem sido utilizada especificamente nos casos em que sejam identificados mecanismos autonómicos com influência na génese da arritmia (Siotia et al, 2004), uma vez que o miocárdio e tecido de condução cardíaco são inervados por fibras do SNA que podem ser determinantes nas propriedades electrofisiológicas do tecido auricular. Permanece, no entanto, por esclarecer se a FA *neurogénica* é uma entidade pura e qual a sua verdadeira importância na etiologia da FA, nomeadamente no que se refere à dependência da dinâmica do balanço simpático-parassimpático no início, manutenção e interrupção dos episódios recorrentes de FA (Efimov & Fedorov, 2005; Krummen & Narayan, 2009).

1.3 Mecanismos Fisiopatológicos

Apesar da investigação desenvolvida nas últimas décadas no sentido da compreensão da fisiopatologia e definição da base científica para o tratamento da FA, os mecanismos ligados à génese e manutenção de FA não se encontram totalmente esclarecidos. As hipóteses da origem

focal, fundamentada no aumento do automatismo e da excitabilidade com formação de impulsos de frequência elevada, e a da existência de múltiplos circuitos de reentrada em simultâneo ou circuitos de reentrada únicos com despolarização auricular rápida, têm sido consideradas como responsáveis pela FA (figura 1), havendo ao longo da investigação que tem vindo a ser realizada diferentes resultados relativamente à contribuição de cada uma destas teorias para o substrato da FA (Cosio et al, 1997; Nattel, 2006; Chou & Chen, 2008). Além disso, tem sido atribuído ao SNA um efeito modulador das propriedades que contribuem para o substrato electrofisiológico da FA. No entanto, apesar da diversidade de estudos efectuados nesse sentido permanece controverso o impacto das variações da actividade autonómica na fisiopatologia da FAP (Chen et al, 2006; Arora & Kadish, 2008; Krummen & Narayan, 2009). É provável que, de acordo com as diferentes fases da história natural da FA ou com a situação clínica subjacente, possa haver envolvimento de múltiplos mecanismos com influência no início (factores *trigger*) e manutenção da arritmia (dependendo de alterações auriculares eléctricas e/ou estruturais) (Wit & Boyden, 2007; Aldhoon et al, 2009).

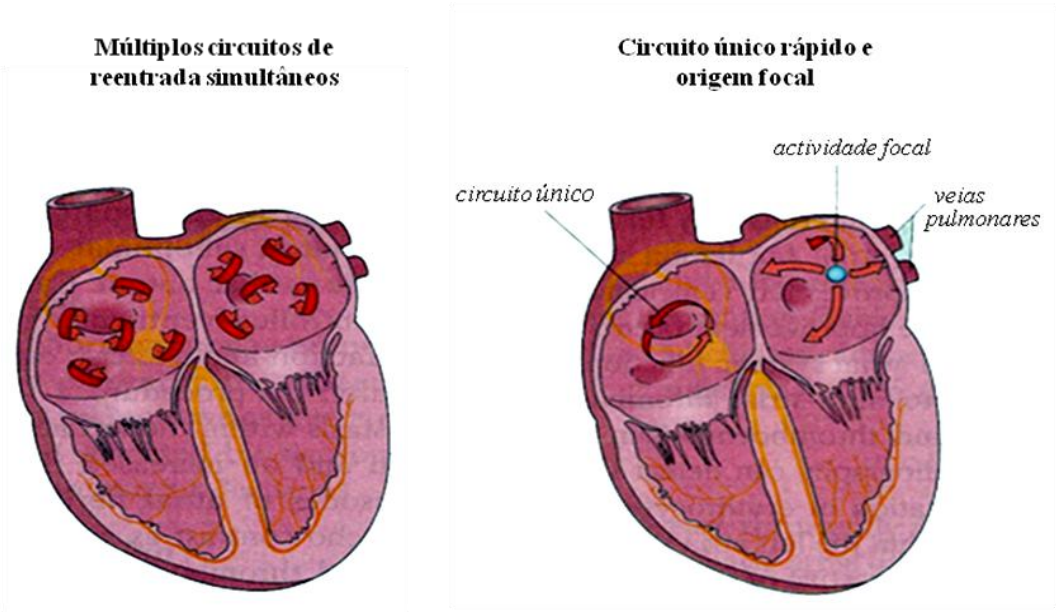

Figura 1 – Representação esquemática dos principais mecanismos electrofisiológicos da fibrilhação auricular

A indução repetida de FA por Scherf numa experiência com aplicação focal de aconitina a nível auricular, estabeleceu a possibilidade da FA depender de um ou mais focos com capacidade para descargas rápidas de impulsos (Scherf, 1947). A interrupção imediata da FA mediante arrefecimento do local de aplicação da aconitina, permitiu mostrar que o início e manutenção da FA eram atribuíveis à actividade focal de alta-frequência. No entanto, Moe e colaboradores, ao repetirem a experiência de Scherf, demonstraram que durante estimulação vagal, a FA se mantinha apesar do isolamento da zona de aplicação da aconitina, traduzindo a capacidade de perpetuação da arritmia resultante do efeito vagal na refractariedade, sem depender da actividade focal (Moe & Abildskov, 1959). Estes autores, viriam a formular a hipótese de que a FA dependeria da coexistência de múltiplos circuitos de reentrada independentes (*Multiple Wavelet hypothesis*) (Moe et al, 1964). Esta teoria, baseia-se na observação de ondas de activação auricular que se fraccionam (condicionadas por barreiras anatómicas ou pela refractariedade do tecido auricular), encontrando outras ondas de propagação, que se podem extinguir ou reiniciar a activação de modo sinérgico, levando à perpetuação de vários circuitos com orientação espacial e velocidades de condução diferentes (*daughter wavelets*). A manutenção da FA seria possível com um número mínimo de três a seis circuitos de reentrada, que, por sua vez, dependeriam da superfície auricular (envolvendo uma massa auricular mínima), da velocidade de condução e da refractariedade (Rensma et al, 1988; Konings et al, 1994).

A presença de múltiplos circuitos necessita dum substrato apropriado em que actividade ectópica funciona como o factor iniciador do mecanismo de reentrada e em que a arritmia termina quando todos os circuitos alcançam, em simultâneo, uma área de tecido não excitável (Nattel & Opie, 2006). Outros estudos, com recurso a múltiplos eléctrodos e mapeamento de alta-densidade, vieram confirmar esta hipótese em modelos animais e no Homem, demonstrando a coexistência de circuitos de reentrada em segmentos auriculares de diferentes dimensões, com ondas de propagação múltiplas fundindo-se com outras frentes de activação ou

interrompendo-se por colisão com estruturas anatómicas das aurículas, outras ondas de despolarização ou por alcançar tecido refractário (Alessie et al, 1985; Konings et al, 1994). Assim, foram descritos três padrões de activação da AD durante FA: o tipo I, em que uma frente de activação se propaga de modo uniforme, com zonas de condução lenta muito localizadas; o tipo II, caracterizado por uma ou duas ondas de activação com condução não uniforme; e o tipo III, em que a activação é fragmentada mostrando três ou mais ondas de propagação, que mudam de direcção em resultado de múltiplos bloqueios funcionais da condução intra-auricular (Konings et al, 1994).

A distribuição de múltiplos circuitos de reentrada pelas aurículas, formando um gradiente de frequências (dominantes na AE, junto às VP) capazes de manter a FA, e a evidência de espirais (rotores) com condução *fibrilhatória* para o restante tecido auricular contribuiram também para o reforço dos microcircuitos de reentrada de alta frequência como um dos mecanismos da FA (Mandapati et al, 2000; Jalife, 2002). Recentemente, num modelo experimental de indução de FA na insuficiência cardíaca, foram demonstrados circuitos de microreentrada associados a zonas de fibrose intersticial na parede posterior da AE, adjacentes às VP, como mecanismo subjacente à FA (Tanaka et al, 2007). Em doentes com FA têm sido descritas áreas de fibrose auricular mais extensas e com distribuição diferente das encontradas numa população com ritmo sinusal (Luo et al, 2006; Everett & Olgin, 2007), atribuindo-se à fibrose um papel no substrato para a manutenção de FA, devido ao aumento significativo na heterogeneidade da condução eléctrica auricular (Guerra et al, 2006). Embora os mecanismos responsáveis pelo desenvolvimento da fibrose não estejam esclarecidos, admite-se que o sistema renina-angiotensina-aldosterona, a expressão aumentada de TGFβ1, a inflamação e o *stress* oxidativo possam ser factores indutores do seu aparecimento (Everett & Olgin, 2007; Lip & Patel, 2007).

A demonstração de focos arritmogénicos localizados nas VP reabilitou a teoria focal, atribuindo à taquicardia das VP um papel principal (*trigger*) na indução de episódios recorrentes de FA

(Haissaguerre et al, 1998). As características eléctricas das VP, com PRE mais curtos que na AE, permitindo uma actividade repetitiva rápida, potenciais de acção mais curtos e com velocidade de fase 0 e velocidade de condução mais lentas, que favorecem os fenómenos de reentrada, têm conduzido à hipótese de que os focos arritmogénicos possam desempenhar também um papel relevante na manutenção da FA (Adragão et al, 2002; Ehrlich et al, 2003; Fynn & Kalman, 2004). A presença nas VP de prolongamentos musculares partindo da AE contribui provavelmente para atrasos locais na condução dando origem a potenciais fraccionados e potenciando circuitos de reentrada (Glatter & Chiamvimonvat, 2005). Além das VP, estão identificadas outras estruturas com actividade focal repetitiva rápida, como a parede posterior da AE, o SC, as veias cavas superior e inferior, o ligamento de Marshall, o septo interauricular ou a crista terminalis, que, menos frequentemente, podem contribuir para episódios recorrentes de FA (Haissaguerre et al, 1998; Shah et al, 2003; Wit & Boyden, 2007).

Tem sido sugerido que doentes sem evidência de cardiopatia com episódios transitórios de FA mas sem evolução para FA *persistente* apresentam maior probabilidade de actividade focal como mecanismo subjacente à arritmia, enquanto nos casos com patologia cardíaca e dilatação auricular as condições seriam mais favoráveis à existência de múltiplos circuitos de reentrada (Nattel & Opie, 2006). No entanto, estes mecanismos não se excluem mutuamente e admite-se que possam coexistir no mesmo doente (Fuster et al, 2006).

A complexidade dos diferentes factores e mecanismos que contribuem para os episódios de FA torna muito difícil a caracterização do substrato responsável pelo início e perpetuação da arritmia, particularmente no que se refere à interacção dinâmica entre fenómenos *trigger*, condução *fibrilhatória*, microcircuitos, ondas de activação e circuitos de reentrada múltiplos. A definição da melhor estratégia na abordagem terapêutica da FA está limitada pelos aspectos controversos que permanecem em debate na fisiopatologia da FA, nomeadamente, em relação à importância das variações na electrofisiologia das aurículas e VP condicionando vulnerabilidade

para a ocorrência de FA e ao papel que os fenómenos de remodelagem auricular causados pela FA desempenham no substrato da arritmia.

1.4 Propriedades Electrofisiológicas e Substrato da Fibrilhação Auricular

A função mecânica do coração depende de mecanismos electrofisiológicos, numa sequência adequada de fenómenos envolvendo propriedades cardíacas fundamentais, como o automatismo, a condutibilidade e a excitabilidade. Embora as células cardíacas sejam passíveis de despolarização espontânea, são as que têm uma capacidade de gerar estimulos de frequência mais elevada que se designam de células marca-passo, e que formam uma rede que se estende a todo o coração incluíndo o SC e as VP (Tomaselli & Roden, 2005). Estas células iniciam potenciais de acção espontaneamente que se propagam através do coração num processo que depende de estruturas especializadas de condução (*gap-junctions* e conexinas), e que é influenciado pelo SNA, pelo envelhecimento, pela arquitectura celular do miocárdio, pela isquemia e hipóxia, pela fibrose, pela dilatação auricular, por fármacos antiarrítmicos e por mutações genéticas que envolvem conexinas e/ou proteínas estruturais (Ehrlich & Nattel 2005; Eijsbouts et al, 2003; Koura et al, 2002; Platonov 2007).

O potencial de acção consiste numa variação rápida e transitória da diferença de potencial transmembrana consequente a uma estimulação eficaz, seguida de retorno a um valor basal - o potencial de repouso -. Durante grande parte do potencial de acção não há resposta da célula qualquer que seja a intensidade do estímulo - período refractário absoluto ou efectivo -. No entanto, no segmento terminal do potencial de acção é possível obter uma resposta despolarizante e iniciar outro potencial de acção, através um estímulo de maior intensidade - período refractário relativo - (figura 2).

O potencial de repouso da membrana celular é determinado por correntes de potássio I_{K1}, que asseguram a saída de iões K^+ da célula, mantendo o potencial transmembranar com valores

negativos (-90 mV) (figura 2). Quando a célula é despolarizada (fase 0), os canais de sódio voltagem-dependentes abrem, permitindo uma súbita mobilização dos iões Na^+ a favor do gradiente electroquímico (corrente de sódio I_{Na}). Esta fase do potencial de acção é determinante na velocidade de condução. A despolarização celular promove uma progressiva inactivação dos canais de sódio e a abertura dos canais de cálcio. A fase de "plateau" (fase 2) resulta da actividade de várias correntes, que representam o influxo e efluxo de iões - corrente de activação rápida I_{KR}, corrente retardada de efluxo de activação lenta I_{KS} e o canal de cálcio tipo $L_{ICa.L}$ -. Diferentes correntes intervêm nas fases de repolarização precoce (fase 1) (I_{to}, I_{Kur}) e tardia (fase 3) (I_{Ks}, K_{Kr}, I_{K1}) com vista a restaurar o potencial de repouso e permitir novo ciclo de excitabilidade celular (Schram et al, 2002; Rubart & Zipes, 2008). Os efeitos cardíacos da estimulação vagal são mediados pela corrente de influxo de potássio I_{KACh}, que é activada pela acetilcolina, causando encurtamento do potencial de acção e hiperpolarização da membrana celular (Nattel et al, 2008).

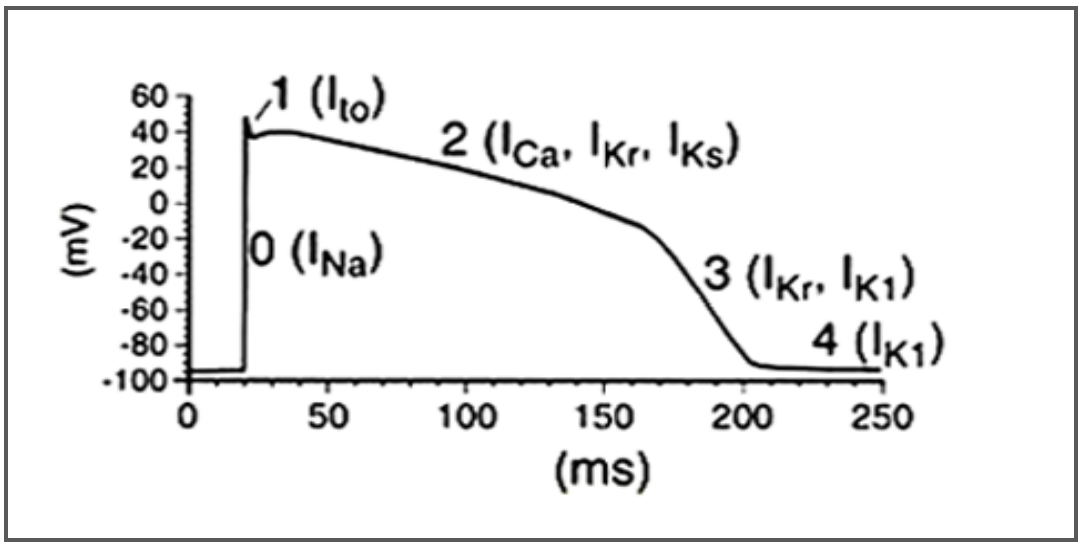

Figura 2 – Potencial de acção de célula miocárdica com as respectivas fases e correntes de efluxo e influxo de iões. *I_{Na}=corrente influxo de sódio ; I_{to}=corrente transitória de efluxo de potássio; I_{Ca}=corrente influxo de cálcio; I_{Kr}=corrente de activação rápida de potássio; I_{Ks}=corrente de activação lenta de potássio; I_{K1}=corrente retificadora de potássio. (adaptado de Melo et al, Latin-American Journal of Pacemaker and Arrhythmia, March 2009:23-26)*

A despolarização é transmitida célula a célula de forma rápida e coordenada através de proteínas estruturais transmembranares - as conexinas -, sendo as Cx40, Cx43 e Cx45 as de maior expressão nas aurículas (Van der Velden et al, 2002; Duffy et al, 2006). Modificações na distribuição das conexinas contribuem para alterações da condução eléctrica auricular e para a presença do substrato necessário ao início e manutenção de FA (Van der Velden et al, 2002; Kanagaratnam et al, 2004).

Tal como já referido, os mecanismos de manutenção e interrupção da FA ainda não estão bem determinados, mas a demonstração de que os PRE auriculares e o aumento da sua dispersão espacial proporcionam um substrato electrofisiológico para a reentrada de múltiplas ondas de propagação tem contribuído para o interesse crescente do estudo destas propriedades eléctricas do tecido auricular e da sua importância no início e na manutenção dos episódios de FA. Diferentes trabalhos têm sugerido que a ocorrência de FA depende de múltiplos factores que modificam as propriedades eléctricas das auriculas, admitindo-se que a idade, a doença cardiovascular subjacente, os fármacos com efeitos antiarrítmicos, a actividade do SNA e a própria FA possam contribuir para a vulnerabilidade para perpetuar ou terminar a FA (Brembilla et al, 2004; Markides & Schilling, 2003; Chen PS, 2006). O desenvolvimento do substrato da FA pode ser mediado pelo encurtamento do potencial de acção, com redução heterogénea dos PRE e da velocidade de condução auriculares, que favorecem a ocorrência de mecanismos de reentrada (Nattel, 2002).

A actividade ectópica focal, descrita sobretudo nas VP, resulta do aumento do automatismo e da ocorrência de pós-potenciais precoces ou tardios e pode desencadear actividade auricular repetitiva e FA (Alessie et al, 2001; Shimizu & Centurion, 2002; Chen et al, 2001). A base iónica deste fenómeno é complexa e relaciona-se com as correntes $I_{Ca,L}$ e $I_{Na,}$ no caso dos pós-potenciais precoces, e com níveis de Ca^{2+} intracelular diastólicos anormais nos pós-potenciais tardios (Tomaselli & Roden, 2005). A propagação destes impulsos rápidos no tecido auricular pode iniciar circuitos de natureza ondulatória (*wavelets*), cujo número e manutenção são

determinados pela velocidade de condução e refractariedade locais ("wavelenght"=*velocidade de condução X PRE*) e pela superfície das aurículas onde ocorre a propagação, parecendo ser este um factor essencial para a manutenção da FA ao permitir a coexistência de múltiplos circuitos de reentrada (Alessie et al, 2001; Efimov & Fedorov, 2005). As descargas causadas por alterações do automatismo ou actividade *trigger* podem dever-se a níveis elevados de catecolaminas séricas, actividade autonómica, hipertrofia cardíaca, estiramento auricular, isquemia, hipóxia, sobrecarga de pressão-volume auricular, distúrbios ácido-base e electrolíticos, inflamação ou toxicidade (álcool, digitálico) (Kozluk, 2006; Marchlinksi, 2008). De acordo com o conceito subjacente ao "triângulo de Coumel", estes aspectos da electrofisiologia celular representam o substrato arrítmico, que, para desenvolver FA, requer factores iniciadores (actividade focal) e a modulação das propriedades electrofisiológicas resultante sobretudo da influência do SNA (Farré & Wellens, 2004). Além disso, na presença de alterações no tecido auricular a FA pode persistir, levando a uma sequência de modificações envolvendo as correntes iónicas e as propriedades electrofisiológicas, designada de remodelagem eléctrica auricular, que facilita não só a recorrência como a perpetuação da arritmia (Wijffels et al, 1995; Workman et al, 2008).

Os requisitos electrofisiológicos para iniciar um circuito de reentrada incluem a heterogeneidade da velocidade de condução e dos PRE de duas ou mais zonas ligadas entre si, a ocorrência de extrassistolia e o bloqueio unidireccional de uma das vias (figura 3). Este mecanismo é traduzido no aumento do automatismo e da actividade *trigger* que associados à diminuição da duração do potencial de acção e à redução heterogénea dos PRE e da velocidade condução contribuiem para o início da FA e para o substrato eléctrico necessário à sua manutenção (Nattel, 2002) (figura 4).

Figura 3 – Condições electrofisiológicas relacionadas com o mecanismo de reentrada.
A - via com velocidade de condução mais lenta; B - via com bloqueio unidireccional devido a maior refractariedade; C - manutenção de circuito de reentrada

Quando o estímulo resultante de uma extrassístole surge durante o PRE de uma das vias de condução, a propagação do impulso pode fazer-se através de outro componente do circuito, activando, por condução retrógada, a via que antes se encontrava refractária. Estes circuitos de reentrada activam ambas as aurículas, numa onda de propagação em espiral, com distribuição espacial variável, influenciada pela presença de obstáculos anatómicos e funcionais (de acordo com a refractariedade e velocidade de condução de diferentes áreas), podendo fragmentar-se e formar novas frentes de activação (Mandapati et al, 2000; Shimizu & Centurion, 2002). Assim, para uma determinada superfície auricular, o número de circuitos independentes é maior para valores de PRE mais curtos e velocidade de condução mais baixa, criando condições para manter a FA.

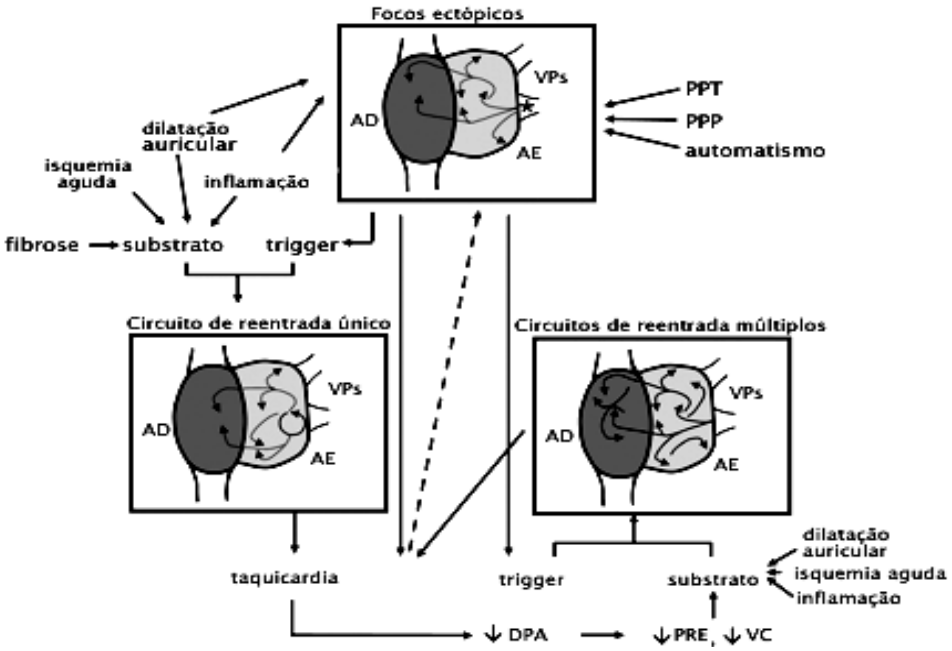

Figura 4 – Mecanismos fisiopatológicos associados à vulnerabilidade para fibrilhação auricular.
PPT=pós-potenciais tardios; PPP=pós-potenciais precoces; DPA=duração do potencial de acção; PRE=período refractário efectivo; VC=velocidade de condução; VPs=veias pulmonares; AE=aurícula esquerda; AD=aurícula direita.
(adaptado de Nattel et al. Circ Arrhythm Electrophysiol 2008; 1:62-73)

As alterações do potencial de acção influenciam a refractariedade local e a velocidade de condução, identificadas como as variáveis electrofisiológicas mais frequentemente alteradas na FA, podendo ser determinantes nos mecanismos de reentrada (Bosch & Nattel, 2002; Jacquemet et al, 2005). A dispersão da refractariedade auricular, resultante da distribuição espacial heterogénea dos PRE, tem sido aceite como capaz de promover o substrato para múltiplos circuitos de reentrada, representando um importante marcador de vulnerabilidade para a ocorrência de FA (Zhen et al, 2002; Soylu et al, 2003; Oliveira et al, 2007; Oliveira et al, 2008). Semelhante irregularidade na condução auricular pode facilitar a reentrada e formar um substrato para FA mantida (Verheule et al, 2004; Everett & Olgin, 2007). O mapeamento endocárdico auricular em ritmo sinusal permite identificar electrogramas prolongados e fraccionados que reflectem uma condução lenta e anisotropismo e parecem associar-se ao

maior risco de evolução para FA *persistente* (Nakao et al, 2002; Pytkowski et al, 2008). Além disso, doentes com FAP têm intervalos de condução intra-auricular aumentados, obtendo-se um prolongamento mais acentuado da condução auricular em resposta a um extra-estímulo auricular, quando comparados com um grupo controlo (Oliveira et al, 2009). A presença de atraso e heterogeneidade da condução em determinados locais das aurículas, verificado nomeadamente na parede posterior da AE, pode ser explicada com base na condução anisotrópica, que depende da arquitectura e configuração celulares e da expressão e distribuição das *gap junctions* (Alessie et al, 2001; Koura et al, 2002). As alterações da condução eléctrica auricular podem ser também atribuídas à presença de infiltrado inflamatório e fibrose intersticial, associados a maior risco de ocorrência de FA, sobretudo na presença de insuficiência cardíaca (Frustaci et al, 1997; Akar et al, 2004; Efremidis & Bramos, 2008). Embora a actividade focal das VP possa promover o início de FA, atrasos segmentares numa condução auricular não homogénea podem funcionar como substrato de reentrada e aumentar a susceptibilidade para a manutenção de FA (Woon et al, 2008; Roberts-Thomson RC et al, 2008; Oliveira et al, 2009).

Da base científica dos mecanismos subjacentes ao substrato da FA fazem parte as alterações da electrofisiologia celular nomeadamente ao nível das correntes iónicas, das *gap junctions* através das conexinas, do metabolismo do cálcio intracelular e/ou da expressão genética. A compreensão da base celular e da complexidade da dinâmica dos fenómenos moleculares, iónicos e electrofisiológicos subjacentes à génese e manutenção da FA, bem como a sua relação com as flutuações do fluxo autonómico, constituem na actualidade uma área de importância crescente na investigação dos mecanismos arritmogénicos desta arritmia.

1.5 Mecanismos moleculares e alterações electrofisiológicas na remodelagem aurícular

O processo complexo de adaptação fisiopatológica das aurículas à actividade fibrilhatória envolve aspectos funcionais e estruturais e tem sido designado por remodelagem desde a sua

descrição por Wijffels e colaboradores (Wijffels et al, 1995). Num modelo experimental de FA mantida com *pacing* de alta frequência, estes autores demonstraram que após vários períodos de arritmia de curta duração, os ciclos dos electrogramas auriculares durante a FA e os PRE podiam ser encurtados de modo reprodutível, com efeitos evidentes após 24 horas de estimulação, atingindo a máxima redução da refractariedade auricular dois dias após o início da arritmia e permitindo que 90% dos animais apresentassem FA *persistente* decorridas 2-3 semanas. Na mesma altura, outro grupo concluia que o *pacing* auricular rápido ou a manutenção de FA causava uma redução no PRE auricular em cerca de 15% (Morillo et al, 1995). Entretanto, trabalhos com registo do potencial de acção em células auriculares de modelos animais e no Homem, permitiram identificar a redução das correntes iónicas do cálcio (I_{Ca}), do sódio (I_{Na}) e do potássio (I_{TO}) com o *pacing* auricular rápido, condicionando alterações na morfologia e duração do potencial de acção e redução dos PRE (Yue et al, 1997; Bosch et al, 1999; Bosch & Nattel, 2002). A novidade deste conceito decorria do facto da FA ser responsável pelas alterações das correntes iónicas e propriedades electrofisiológicas, criando as condições para se auto-perpetuar (*AF begets AF*) (Wijffels et al, 1995).

São vários os mecanismos que podem contribuir para aumentar a actividade ectópica e circuitos de reentrada no processo de remodelagem auricular. Assim, há evidência do aumento da actividade focal ectópica, possivelmente relacionada com pós-potenciais associados a modificações no cálcio intracelular, (Zhou et al, 2002) e à diminuição da corrente de influxo do sódio que poderá perturbar a velocidade de condução de modo não uniforme (Zhang et al, 2005; Nattel et al, 2007). As modificações nos canais de cálcio, devidas à frequência auricular elevada durante os periodos de FA, levam ao aumento do cálcio intracelular para protecção do miócito e têm sido aceites como um dos factores mais importantes na remodelagem eléctrica (Tieleman et al, 1997; Brundel et al, 2002). Estão incluídos nestes mecanismos de remodelagem o aumento da actividade $I_{K1,}$ a diminuição da actividade da corrente I_{CaL} e da síntese do RNA_m envolvendo

as proteínas dos canais iónicos e das conexinas, podendo influenciar as propriedades electrofisiológicas das auriculas (Brundel et al, 2001; Nao et al, 2003; Dobrev et al, 2005; Gollob 2008), nomeadamente condicionando o encurtamento do potencial de acção (Daoud et al, 1996; Fareh et al, 1998; Neuberger et al, 2006) e dos PRE auriculares (Nattel, 2002; Gaborit et al, 2005) (figuras 5 e 6). As correntes de potássio $I_{K1,}$ I_{TO}, I_{Ksus}, I_{Kur}, $I_{KATP,}$ e I_{KACh}, que têm um papel relevante no potencial de repouso e na repolarização, estão também alteradas, admitindo-se a possibilidade de influenciarem a manutenção da arritmia (Zhang et al, 2005; Choen & Naccarelli, 2008; Voigt et al, 2008). Outros estudos mostraram, também, a redução da corrente I_{KACh} que interfere no potencial de repouso e na excitabilidade celular e que é mais intensa nas VP (Ehrlich et al, 2004) e das correntes $I_{TO,}$ I_{Ksus}, I_{Kur} e I_{KATP} que podem influenciar a morfologia do potencial de acção, estando a última implicada nas alterações electrofisiológicas relacionadas com a isquémia auricular (Van Wagoner, 2003; Zhang et al, 2005; Wu et al, 2005, Nattel et al, 2008; Ehrlich JR, 2008). No entanto, um estudo envolvendo a corrente I_p (bomba sódio-potássio) não mostrou diferenças na FA (Workman et al, 2008).

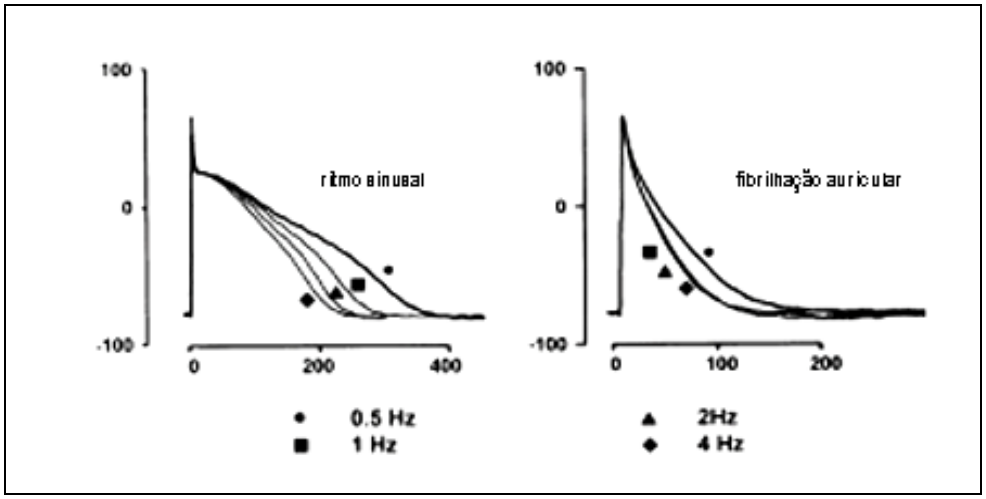

Figura 5 – Representação esquemática mostrando as diferenças na duração e morfologia dos potenciais de acção registados durante *pacing* auricular rápido a 0.5, 1, 2 e 4Hz num indívíduo sem história de FA (à esquerda) e num doente com FAP (à direita). *mV=milivolts; ms=milisegundos (adaptado de Bosch et al, Cardiovascular Research 1999; 44:121-131)*

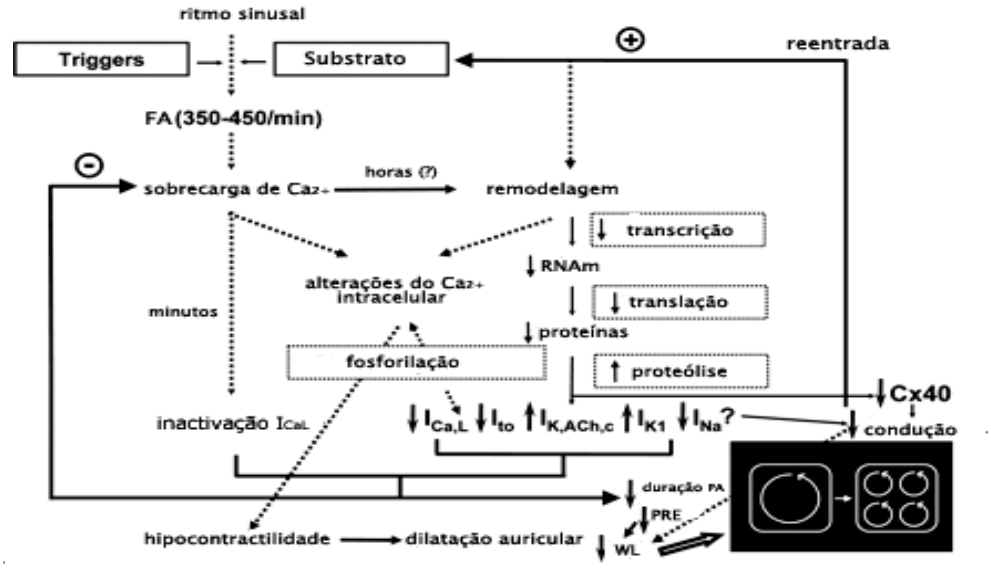

Figura 6 – Representação dos mecanismos moleculares subjacentes à remodelagem auricular. A sobrecarga de Ca^{2+} resultante de FA condiciona diminuição da corrente I_{CaL} e da expressão proteíca dos canais iónicos e conexinas, em particular Cx40. A inactivação I_{CaL} causa diminuição da duração do potencial de acção, dos PRE e do comprimento de onda no tecido auricular, favorecendo circuitos de reentrada mais pequenos e em maior número. *PA=potencial de acção;; WL=comprimento de onda ("wavelength") (adaptado de Nattel et al. Circ Arrhythm Electrophysiol 2008 ; 1:62-73*).

Algumas observações têm considerado a hipótese da própria FA condicionar remodelagem a nível molecular (Nao et al, 2003; Dobrev et al, 2005; Gollob 2008). A diminuição significativa da corrente de Ca^{2+} do tipo L (I_{CaL}) e da corrente transitória de saída de potássio (I_{TO}), observada na sequência de remodelagem eléctrica induzida com *pacing* auricular rápido no modelo do cão é explicada pela redução dos níveis de proteínas dos canais (Brundel et al, 2002). Doentes com FA persistente têm mostrado uma redução do gene Ca^{2+}-ATPase sarcoplasmático (Lai et al, 1999) e do RNA_m, e expressão dos genes de diversos canais de potássio (Brundel et al, 2002). A diminuição da corrente I_{TO} é acompanhada da redução da expressão proteíca e do RNA mensageiro das subunidade Kv4.3 que forma o canal iónico (Nattel et al, 2008). O influxo do potássio depende da corrente I_{K1}, que é formada por subunidades Kir2, cuja expressão ao nível do RNA mensageiro se encontra também aumentada na FA (Gaborit et al, 2005). A expressão

proteíca das subunidades do canal iónico subjacente à corrente I_{KACh} diminui em doentes com FA, enquanto que o aumento da actividade da corrente $I_{KACh,c}$, que promove a FA no contexto da remodelagem auricular, se deve à fosforilação da proteína quinase C (Voigt et al, 2008; Nattel et al, 2008). Também foram documentadas alterações da expressão do canal de sódio a nível do RNA mensageiro e da Cx40 contribuindo para alterações da condução eléctrica auricular na FA (Brundel et al, 2001; Gollob et al, 2006).

Em resposta à FA e ao *pacing* auricular rápido ocorrem também alterações na função e estrutura mitocondrial que contribuem para aumentar o *stress* oxidativo que, por sua vez, pode influenciar a actividade de diferentes canais de potássio, envolvendo-se também nos fenómenos de remodelagem (Dudley et al, 2005; Van Wagoner, 2008). A expressão do glutatião endógeno, potente antioxidante que protege as células dos radicais livres de oxigénio, está diminuida em doentes com FA submetidos a cirurgia cardíaca (Carnes et al, 2007). Além disso, o *stress* oxidativo pode induzir inflamação e em conjunto contribuirem para a remodelagem estrutural através da proliferação de fibroblastos, fibrose intersticial, acumulação de colagénio, dilatação e hipertrofia (Burashnikov A, 2008). A relevância clínica desta interacção complexa "*stress* oxidativo-inflamação" na fisiopatologia da FA permanece por definir. As perturbações da condução no tecido auricular em resultado da remodelagem eléctrica parecem envolver o aparecimento de mais áreas dispersas de fibrose e também anomalias na comunicação intercelular por alterações da quantidade, distribuição e função das conexinas (Gaspo et al, 1997; Luo et al, 2007; Duffy & Wit, 2008).

No caso de se verificar manutenção da arritmia, o fenómeno da remodelagem é progressivo, tornando a conversão a ritmo sinusal tanto mais difícil quanto maior a duração da FA e contribuindo significativamente na evolução de FAP para as formas *persistente* e *permanente* (Choen & Naccarelli, 2008). Quando se restabelece o ritmo sinusal, há uma tendência para reverter gradualmente todo o processo, podendo ocorrer normalização do meio electrofisiológico quando o ritmo sinusal se mantem estável (remodelagem inversa) (Everett TH et al, 2000). No

entanto, a normalização da actividade auricular pode obter-se 24 horas após cardioversão, quando o período de FA foi inferior a 2 semanas, ou depois de 1 mês, nos casos de FA com duração superior a 6 semanas (Manning et al, 1994).

Além das modificações nas correntes iónicas transmembranares a remodelagem auricular devida à FA mantida leva a que ocorram alterações estruturais e contrácteis do tecido cardíaco, com perda de contractilidade, risco de formação de trombos, alteração da arquitectura celular, dilatação e fibrose das aurículas (remodelagem estrutural) (Van Gelder et al, 2006; Everett & Olgin, 2007). Os factores que contribuem para a remodelagem estrutural incluem a angiotensina II, que favorece a hipertrofia do miócitos e a formação de fibrose, e a sobrecarga hemodinâmica, que promove a activação de metaloproteinases da matriz com dilatação auricular e fibrose intersticial, particularmente no contexto de hipertensão arterial, disfunção valvular e/ou insuficiência cardíaca (Van Wagoner, 2008).

A dilatação auricular aumenta a superfície de tecido auricular capaz de acomodar circuitos de reentrada, tornando-se um marcador determinante do número de ondas de activação e, consequentemente, da manutenção da FA (Nattel et al, 2008). Tem sido sugerido que, depois de estabelecida a remodelagem estrutural, a influência das propriedades electrofisiológicas na perpetuação da FA seria menos relevante, e, portanto, menor a eficácia dos fámacos antiarrítmicos que actuam através da modulação da actividade dos canais iónicos, e maior o papel de intervenções com vista a reduzir a remodelagem estrutural, *stress* oxidativo e inflamação, como os inibidores da enzima de conversão da angiotensina, antagonistas dos receptores da angiotensina e estatinas (Cha et al, 2004; Chen & Tan, 2007; Van Wagoner, 2008, Savelieva & Camm, 2008).

O tipo de remodelagem depende da duração da FA e da sobrecarga hemodinâmica, com respostas adaptativas a nível genómico, iónico e electrofisiológico, que ocorrem em minutos ou horas e que são reversíveis, e alterações associadas à FA *persistente* ou *permanente*,

envolvendo fibrose e alterações anatómicas, habitualmente, irreversíveis (Casaclang-Verzosa et al, 2008).

Diversos estudos têm sugerido que o início, manutenção e interrupção da FA são dependentes de múltiplos factores que modificam as propriedades eléctricas das aurículas, tornando complexa a definição do contributo dos diferentes mecanismos envolvidos na fisiopatologia desta arritmia.

A actividade do SNA tem sido intensamente estudada e considerada como tendo um papel potencial na modulação das condições necessárias ao substracto electrofisiológico auricular para a ocorrência de FA, mas também podendo apresentar alterações resultantes da própria FA, num processo designado por remodelagem autonómica, que envolve alterações heterogéneas da inervação simpática cardíaca que se correlacionam com modificações das propriedades electrofisiológicas facilitando os episódios recorrentes e a perpetuação da FA (Olshansky, 2005; Chen et al, 2005; Arora & Kadish, 2008; Lu et al, 2008).

1.6 Sistema nervoso autónomo e substrato electrofisiológico para a fibrilhação auricular

A nível cardiovascular, a disfunção autonómica, pode associar-se a diversos distúrbios patológicos, envolvendo incapacidade de adaptação cardiovascular e/ou anomalias na resposta areflexos cardiovasculares, que incluem a taquicardia sinusal em repouso, a síndrome de taquicardia postural ortostática, a hipotensão ortostática, a hipertensão arterial, a síncope neurocardiogénica ou as arritmias cardíacas.

No que se refere à susceptibilidade para arritmias, tem sido estabelecida uma relação entre alterações da actividade autonómica e as propriedades electrofisiológicas cardíacas, representadas pelo automatismo, excitabilidade e condutibilidade e envolvendo o encurtamento dos PRE e o início de circuitos de reentrada no miocárdio que podem favorecer o aparecimento de taquidisritmias, nomeadamente em doentes com patologia cardíaca subjacente (Simantirakis

et al, 2001; Martins & Arnar, 2000). Por outro lado, situações clínicas que se associam a alterações no equilíbrio simpático-parassimpático, como o enfarte do miocárdio, a insuficiência cardíaca, a síndrome de apneia obstrutiva do sono, hipertensão arterial, *diabetes mellitus*, alcoolismo, epilepsia, ou doença cerebrovascular apresentam uma maior incidência de arritmias cardíacas (Bloomfield et al, 2001; Aytemir et al, 2007; Yiu & Tse, 2008; Perciaccante et al, 2006; Bär et al, 2008; Lathers et al, 2008; Micieli & Cavallini, 2000).

Desde os trabalhos de Coumel que se atribui ao SNA um papel modulador na fisiopatologia da FA, encontrando-se associado à recorrência de episódios da arritmia, em particular, no que se refere às situações clínicas de FA *vagal,* descrita em jovens sem cardiopatia subjacente, com episódios paroxísticos que tendem a surgir no período nocturno, em repouso ou em fase pós-prandial, e de FA *adrenérgica*, desencadeada pelo exercício ou por situações de *stress* (Coumel, 1992). No entanto, tem sido aceite que a proporção de doentes que integra este grupo específico de etiologia da FA é muito pequena, permanecendo controversa a importância da contribuição relativa dos componentes simpático e parassimpático nos mecanismos electrofisiológicos envolvidos na génese da FA e sua relação directa com o início espontâneo, manutenção e interrupção da arritmia (Fuster et al, 2006; Chen PS, 2006; Chen J et al, 2006).

As propriedades electrofisiológicas relacionadas com a vulnerabilidade auricular para a ocorrência de FA podem ser influenciadas pela actividade simpática e parassimpática, com libertação excessiva de neurotransmissores que diminuem a refractariedade auricular, e que, dependendo da sua concentração, podem induzir despolarizações rápidas focais (Patterson et al, 2005; Chen P, 2007; Zhou et al, 2007). A estimulação vagal reduz a FC e a velocidade de condução no tecido auricular, encurta o potencial de acção e a refractariedade de modo não uniforme, provavelmente devido a um gradiente de distribuição dos plexos ganglionares no miocárdio auricular, criando condições para o aparecimento de arritmias auriculares de reentrada (Thai, 2001; Hirose et al, 2002; Chevalier et al, 2005). Por outro lado, a estimulação simpática ao

aumentar a velocidade de condução através da modificação da fase 0 do potencial de acção, ao diminuir o tempo de condução auriculo-ventricular, ao reduzir a refractariedade auricular e ao induzir actividade *trigger* com despolarizações rápidas repetitivas e taquiarritmias auriculares parece actuar como factor pró-arrítmico (Coumel, 1996; Chen, 2006). Embora a estimulação vagal e simpática diminua os PRE auriculares, admite-se que a actividade vagal tenha mais influência na susceptibilidade para FA, devido ao impacto significativo na heterogeneidade e dispersão da refractariedade (Zhang & Mazgalev, 2008). Por outro lado, o aumento da inervação simpática, descrito em situações como o *pacing* rápido mantido em modelos animais, na insuficiência cardíaca e no pós-enfarte do miocárdio, ao ocorrer de forma não homogénea, também poderá contribuir para uma maior dispersão da refractariedade, circuitos de reentrada e perpetuação da FA (Chang et al, 2001; Hamabe et al, 2003; Jiang et al, 2007). Sharifov e colaboradores, mostraram no cão que a infusão de isoprenalina e adrenalina na artéria do nódulo sinusal induzia FA em 21% dos animais, enquanto com administração de acetilcolina induzia FA em 100% dos casos, sendo a indução e maior duração da FA mediada pela acetilcolina facilitada pelo efeito da isoprenalina, o que não só mostra o papel do vago na génese da FA mas, também, sugere um papel potencial para o efeito combinado simpático-vagal (Sharifov et al, 2004). Também outros autores sugerem que as variações bruscas de tónus autonómico podem estar na base de *descargas* simpático-vagais indutoras de FA (Chen & Tan, 2007; Korantzopoulos et al, 2009). De facto, tem sido mostrado que o efeito combinado da estimulação simpática e da actividade vagal resulta no substracto adequado para o início da FA (Amar et al, 2003; Sharifov et al, 2004; Patterson et al, 2005). Estudos recentes têm sugerido que, além da influência determinante nas situações de FA isolada, o SNA possa desempenhar também um papel importante na génese da FA em presença de cardiopatia subjacente, nomeadamente na insuficiência cardíaca (Hamabe et al, 2003; Ogawa et al, 2007). Além disso, vários autores têm avaliado a actividade autonómica que precede episódios de FAP tendo documentado variações significativas no tónus simpático e parassimpático, quer em doentes

com FA *isolada*, quer associada a cardiopatia estrutural. De acordo com Bettoni, a ocorrência de FAP depende de variações do tónus autonómico, com um aumento da actividade adrenérgica seguido duma fase de predomínio vagal, sem diferenças entre os grupos com e sem cardiopatia (Bettoni & Zimmermann, 2002). Em doentes medicados com amiodarona, a análise espectral da variabilidade da FC após cardioversão eléctrica de FA com conversão a ritmo sinusal, mostrou que o aumento da razão LF/HF caracterizava os casos com recorrência precoce de FA (Lombardi et al, 2001). Num estudo da variabilidade da FC após cirurgia de revascularização miocárdica, o valor da razão LF/HF aumentou progressivamente minutos antes do início da FA em resultado do aumento do LFe da redução do HF (Dimmer et al, 1998). No entanto, mais recentemente, o aparecimento de FA no pós-operatório de cirurgia cardíaca foi associado ao aumento do tónus parassimpático e simpático no período de 2 horas que antecedeu a arritmia (Amar et al, 2003). Em jovens com coração estruturalmente normal e episódios nocturnos de FA, registou-se um aumento isolado da actividade vagal nos minutos que precederam o início da arritmia (Herweg et al, 1998). Em contraste, outro estudo mostrou apenas predomínio simpático, sem activação parassimpática, antes de episódios de FA ocorrendo durante o sono (Coccagna et al, 1997). Também, em doentes com FAP na ausência de cardiopatia, foi detectada activação simpática antes dos episódios diurnos acompanhada por um aumento progressivo do LF e do HF nos episódios nocturnos (Tomita et al, 2003). Numa população com FAP envolvendo doentes com e sem patologia cardíaca, a maioria dos episódios eram caracterizados por um predomínio de modulação simpática nos 30 minutos precedendo a arritmia, enquanto em 30% dos episódios, na sua maioria nocturnos, se detectava apenas um aumento da actividade vagal (Lombardi et al, 2004). Neste estudo, as alterações do balanço simpático-parassimpático desapareciam imediatamente após a interrupção da FA.

Deste modo, as flutuações do tónus autonómico e a actividade reflexa envolvendo variações bruscas do SNA podem ser determinantes nos episódios de FA, admitindo-se que em muitos

doentes a ocorrência de “descargas” simpático-vagais possa ser particularmente pro-fibrilhatória (Chen & Tan, 2007; Korantzopoulos et al, 2009).

Apesar da evidência de que ocorrem alterações importantes do controlo autonómico cardíaco antes do início espontâneo de FA, permanecem controversos os dados obtidos nos diversos trabalhos que têm procurado avaliar a dinâmica da actividade autonómica associada ao início da FA. Os diferentes resultados podem ser atribuídos, de entre outras, às limitações da metodologia de análise da variabilidade da FC. De facto, as técnicas utilizadas têm aplicado métodos de análise no domínio do tempo e no domínio da frequência aos períodos de ritmo sinusal estável, quase sempre excluíndo variações RR dependentes de ectopias, e em segmentos com duração variando entre 5 minutos e 2 horas, o que torna dificil a caracterização adequada dos fenómenos dinâmicos e complexos da actividade autonómica

A actividade ectópica das VP é capaz de desencadear FA e contribuir para a sua manutenção através de descargas focais rápidas com condução *fibrilhatória* (Haissaguerre et al, 1998; Chen S et al, 1999; Sueda et al, 2001), cuja modulação tem sido, pelo menos parcialmente, atribuída ao SNA (Vaquero et al, 2008). Num modelo animal, a isoprenalina aumentou o automatismo e induziu actividade *trigger,* que foi possível inibir com propranolol (Arora et al, 2003; Zhou et al, 2002). As VP apresentam uma estrutura anatómica com múltiplas fibras musculares que se prolongam dos segmentos proximais para a AE, numa organização tridimensional complexa que envolve a distribuição de grupos celulares heterogéneos até à porção distal, tendo sido documentados localmente actividade *trigger*, aumento do automatismo e fenómenos de reentrada, apontados como desempenhando um papel importante nos mecanismos da arrítmogenicidade das VP (Chen J & Chen A, 2006). Estudos em tecido embrionário humano e em modelos animais têm identificado a presença de células P, células de transição e células de Purkinge com actividade automática nas VP, estando igualmente demonstrada a ocorrência de despolarizações espontâneas de fase 4 e circuitos de microreentrada em resultado da condução

decremental atribuída à mudança brusca de orientação das fibras e de fenómenos de anisotropismo (Perez-Lugones et al, 2003; Arora et al, 2003; Chen J & Chen A, 2006).

A inervação autonómica cardíaca intrínseca compreende pelo menos 7 plexos ganglionares, que parecem actuar numa rede interactiva, e que apresentam maior concentração nas aurículas e VP (plexos auricular dorsal direito, auricular direito ventral, coronário direito, coronário esquerdo, dorsal esquerdo, médio dorsal e auricular esquerdo ventral), sendo a parede posterior da AE e a zona da junção VP esquerdas-AE, particularmente rica em terminações autonómicas (Pauza et al, 2000; Hou et al, 2007; Arora & Kadish, 2008) (figura 7). Apesar dos coxins de gordura epicárdica serem mais ricos em terminações vagais do que em fibras simpáticas, pois exibem um predomínio de efeitos vagais quando estimulados, a sua eliminação cirúrgica ou por radiofrequência afecta ambos os componentes da inervação cardíaca (Zhang & Mazgalev, 2008). A estimulação eléctrica de plexos ganglionares associa-se à libertação de neurotransmissores, ao aumento da actividade *trigger* nas VP e indução de FA (Schauerte et al, 2001; Scherlag et al, 2005; Zhou et al, 2007), e a estimulação combinada de nervos simpáticos e fibras vagais também induz actividade focal *trigger* e pós-potenciais precoces (Patterson et al, 2005), o que sugere um papel potencial do SNA na arritmogénese das VP.

Recentemente, Arora e colaboradores demonstraram que as VP, o AAE e a parede posterior da AE têm características de refractariedade diferentes em resposta à modulação autonómica, nomeadamente com maior encurtamento dos PRE nas VP e parede posterior da AE em resultado da estimulação vagal (Arora et al, 2007). Nesse estudo, a heterogeneidade dos PRE foi mais acentuada durante estimulação vagal combinada com propranolol, sugerindo a influência de interacções simpático-vagais nas condições electrofisiológicas necessárias aos circuitos de reentrada e FA. Além disso, as variações regionais na diminuição dos PRE foram correlacionadas com o padrão de distribuição da expressão de I_{KAch} na AE. A estimulação eléctrica dos plexos ganglionares tem, igualmente, sido associada à libertação de

neurotransmissores, aumento da excitabilidade nas VP e indução de FA, apontando para a possibilidade de que a modificação da estimulação autonómica cardíaca poderia contribuir para o tratamento da FA (Schauerte et al, 2001; Scherlag et al, 2005; Zhou et al, 2007; Chen & Tan, 2007).

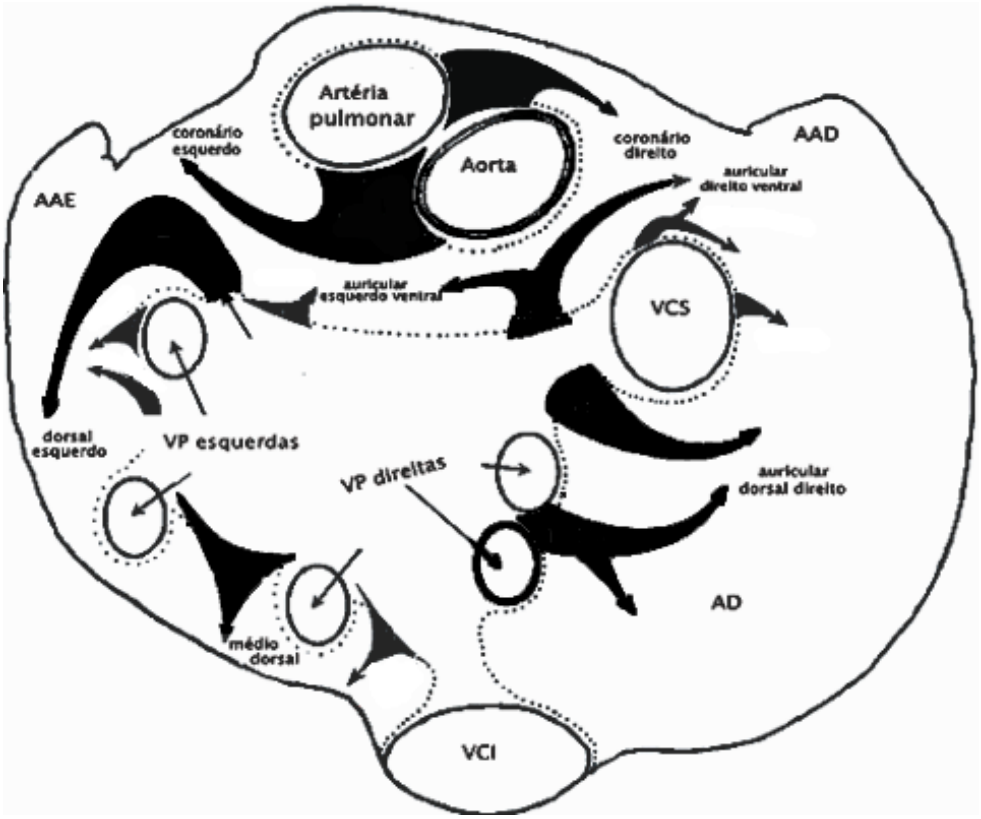

Figura 7 - Esquema da localização e direcção dos nervos epicárdicos com acesso aos plexos cardíacos a nível auricular e das VP. Estão identificados os plexos ganglionares coronário esquerdo, coronário direito, auricular esquerdo ventral, auricular direito ventral, dorsal esquerdo, dorsal direito e médio dorsal. *AAE=apêndice auricular esquerdo, AAD=apêndice auricular direito; AD=aurícula direita; VP=veias pulmonares*. (*adaptado de Pauza et al. Anat Rec 2000; 259 (4):353-82*)

A evidência de que a actividade focal rápida proveniente das VP pode ser eliminada pelo isolamento eléctrico destas estruturas e, assim, prevenir os episódios de FA tem fomentado o desenvolvimento de técnicas ablativas de utilização crescente, com recurso a diferentes cateteres e formas de energia, tendo por objectivo permitir uma maior eficácia no

restabelecimento e manutenção do ritmo sinusal. Recentemente, meta-análises comparando a terapêutica ablativa com o tratamento baseado em fármacos antiarrítmicos mostraram superioridade do isolamento circunferencial das VP na abordagem da FA (*paroxística* e *persistente*), com taxas de sucesso de cerca de 75% no 1º ano de *follow-up* pós-procedimento (Noheria et al, 2008; Nair et al, 2009). O isolamento eléctrico das VP tem sido considerado como a técnica padrão no tratamento ablativo da FA. No entanto, a modificação do substracto arrítmico pode justificar, de acordo com a situação clínica, diferentes estratégias de ablação. A identificação da presença de focos arritmogénicos fora da zona das VP implica o seu mapeamento e a respectiva eliminação (Higa et al, 2006). A actividade dominante de alta-frequência causada por microcircuitos de reentrada pode causar condução *fibrilhatória*, partindo da área anatómica do antro das VP para o restante miocárdio auricular, com fragmentação dos electrogramas e formação de ondas de activação, sendo necessária uma linha de ablação mais ampla, de forma a envolver uma área extensa em torno dos ostia das VP e eliminar esses circuitos que mantêm a FA (Jalife, 2003; Mansour, 2006). Oral e colaboradores, num estudo com 188 doentes, mostraram que a ablação de FA baseada somente no isolamento eléctrico das VP foi mais eficaz no tratamento da FA *adrenérgica* que na FA *vagal*, sugerindo a importância de inervação simpática das VP e seu papel nos episódios de FAP (Oral T et al, 2004).

A importância do envolvimento das VP na fisiopatologia da FA pode ficar a dever-se especificamente à elevada densidade de terminações nervosas adrenérgicas e colinérgicas na junção PV-AE, evidenciando o papel potencial do SNA nos episódios de FA (Chevalier et al, 2005; Tan et al, 2006; Pokushalov, 2008).

A execução de lesões lineares com o objectivo de compartimentalizar as aurículas, diminuindo a superfície fibrilhatória para impedir a perpetuação da FA, tem sido também utilizada, nomeadamente no âmbito da cirurgia cardíaca, com relatos de taxas de sucesso entre 63% e 93% (Cox, 2004; Sie et al, 2004; Chiappini et al, 2004; Melo et al, 2008). Por outro lado, a hiperactividade associada à libertação dos neurotransmissores colinérgicos e adrenérgicos ao

nível dos plexos ganglionares cardíacos, com repercussões importantes na electrofisiologia das aurículas e VP, tem levado alguns centros a incluir na estratégia de intervenção terapêutica na FA a ablação dos plexos ganglionares com resultados prometedores (Platt et al, 2004; Nadmanee et al, 2004; Scherlag et al, 2006; Pokushalov, 2008; Scanavacca et al, 2009). De facto, o atingimento dos plexos ganglionares pelas lesões obtidas com aplicações de energia de radiofrequência foi proposto como um dos mecanismos de sucesso na ablação do antro das VP, podendo abolir a actividade *trigger* das VP em 95% dos casos (Nakagawa et al, 2003; Scherlag et al, 2006). Igualmente, a eliminação de áreas onde se obtêm reflexos vagais durante a aplicação de radiofrequência (causando atenuação parassimpática na variabilidade da FC) ou de zonas de miocárdio com espectro de altas-frequências (*ninhos de FA*), que parecem relacionar-se com a inervação vagal, tem sido associada a sucesso na ablação da FA (Pappone et al, 2004; Scanavacca et al, 2006; Pachón et al, 2007). No entanto, no cão, os efeitos da desinervação vagal obtidos com a ablação por radiofrequência de zonas de gordura epicárdica (junto às VP direitas e entre a veia cava inferior e a AE) apenas se mantiveram durante 4 semanas, sugerindo que os efeitos da intervenção podem ser reversíveis, possivelmente devido a ablação incompleta, reinervação da rede neuronal ou aumento da sensibilidade cardíaca devido a um processo de remodelagem local envolvendo o aumento e densidade dos receptores autonómicos (Oh et al, 2006). No Homem, os resultados da desinervação vagal parcial mostraram redução, aumento ou mesmo efeito neutro, na incidência de FA no pós-operatório de cirurgia cardíaca, o que evidencia a necessidade de melhorar a informação nos aspectos metodológicos desta abordagem terapêutica (Melo et al, 2004; Cummings et al, 2004; Alex et al, 2005).

A FA representa uma das situações mais heterogéneas em arritmologia, envolvendo múltiplas causas, factores de risco, *triggers* e mecanismos de manutenção da arritmia que interagem entre si num substrato electrofisiológico complexo, que tem sido amplamente associado à influência do

SNA. A multiplicidade de variáveis que influenciam a interacção da actividade autonómica e tecido auricular podem explicar as dificuldades da análise e correlação entre os aspectos clínicos da FA e dados resultantes de modelos experimentais. Nas últimas décadas, a evolução do conhecimento alcançada na compreensão da fisiopatologia da FA contribuiu de modo relevante para implementar novas formas de abordagem terapêutica desta situação clínica comum, com impacto clínico e epidemiológico importante. O desenvolvimento de modelos de investigação básica em colaboração com técnicas da experiência clínica que permitam interpretar fenómenos no âmbito da biologia molecular e da electrofisiologia auricular associados ao papel da actividade autonómica na génese e manutenção da FA poderá representar um contributo para a prevenção e tratamento desta arritmia. A investigação continuada nesta vertente fundamental da abordagem da FA permitirá melhorar a compreensão dos múltiplos mecanismos funcionais que constituem o substrato electrofisiológico da génese e manutenção desta arritmia e da remodelagem autonómica, de forma a desenvolver metodologias de prevenção e tratamento com maiores níveis de eficácia e segurança

1.7 Avaliação funcional autonómica do controlo cardiovascular

O SNA integra o sistema nervoso periférico e tem por função a regulação funcional contínua dos diferentes sistemas viscerais do organismo e adaptação aos estímulos internos e externos com vista à manutenção da homeostasia (Goldstein, 2001). É composto pelo sistema nervoso simpático, sistema nervoso parassimpático ou vagal e pelo sistema nervoso entérico, que asseguram o controlo autonómico da respiração, da actividade cardíaca e circulatória, da temperatura corporal, da musculatura lisa, das glândulas endócrinas e exócrinas (Appenzeller, 1999). O nível de actividade do SNA é regulado pelo centro integrador, que agrupa núcleos desde a medula espinal, tronco cerebral e hipotálamo até ao sistema límbico no córtex cerebral, processando a informação aferente com origem nos receptores sensoriais (Jänig & Häbler,

1995). Os sistemas simpático e parassimpático têm associadas fibras aferentes que transmitem informação da condição funcional dos diferentes orgãos até aos centros integradores a nível central. As respostas da actividade autonómica são rápidas e controlam os diferentes orgãos efectores através de vias eferentes específicas numa organização funcional baseada no conceito de arco reflexo (Spyer KM, 2001).

A complexidade inerente à dinâmica permanente do sistema de integração e de manutenção da homeostasia característicos da actividade do SNA torna díficil o seu estudo funcional. Os objectivos da avaliação autonómica implicam identificar o tipo e grau de disfunção, a sua origem e repercussões funcionais e, por último, saber se tem natureza primária ou secundária. (Mathias & Bannister, 2001; Cheshire & Kuntz, 2009).

O estudo da função autonómica utiliza diferentes manobras, na maioria dos casos efectuadas em regime ambulatório com provas não-invasivas e indolores. De uma forma geral, estas provas classificam-se em cardiovasculares, que englobam três grupos principais - fisiológicos, bioquímicos e farmacológicos -, de função sudomotora, de função gastrointestinal, de função renal e/ou tracto urinário, de função sexual, de função respiratória e de função ocular.

Os testes fisiológicos do grupo das provas cardiovasculares incluem o teste de *tilt* ou de ortostatismo passivo, a manobra de Valsalva, o teste do ortostatismo activo, testes pressores - frio, exercício isométrico e *stress* mental-, o teste da respiração profunda, a massagem do seio carotídeo e a ingestão líquida (Hilz M & Dutch, 2009).

A FC, a PA e o débito vascular são variáveis cardiovasculares de extrema importância afectadas por factores nervosos, hormonais e locais. A regulação reflexa do aparelho cardiovascular é modulada continuamente por circuitos com origem em sinais aferentes a partir de receptores sensoriais localizados em zonas especializadas que enviam informação até ao núcleo do tracto solitário, que depois de integrada e processada origina activação das vias eferentes simpática e parassimpática. As lesões associadas a disfunção autonómica podem ocorrer a diferentes níveis, envolvendo qualquer estrutura do arco reflexo - vias aferentes, centros de integração e

suas conexões com a rede autonómica central, vias eferentes e órgãos efectores -, e condicionar variações da FC e/ou PA, cujos padrões podem ser registados para proceder a análise da variabilidade do sinal.

A documentação de hipotensão ortostática (diminuição mantida da PA sistólica >20 mmHg e/ou da PA diastólica >10 mmHg em ortostatismo) não acompanhada pelo aumento da FC é um sinal de disfunção autonómica. A sua avaliação é feita pelo teste de ortostatismo passivo (teste de *tilt*), pelo teste de ortostatismo activo ou pela manobra de Valsalva.

No teste de *tilt* coloca-se o indivíduo na posição ortostática a 60º-70º por intermédio de uma mesa basculante, obtendo-se, na maior parte dos laboratórios a monitorização contínua e análise em tempo real das variações da PA e da FC durante diferentes períodos de tempo, de acordo com o objectivo e protocolo do estudo. A análise do comportamento autonómico implica a comparação com os valores observados num período dito basal ou de repouso.

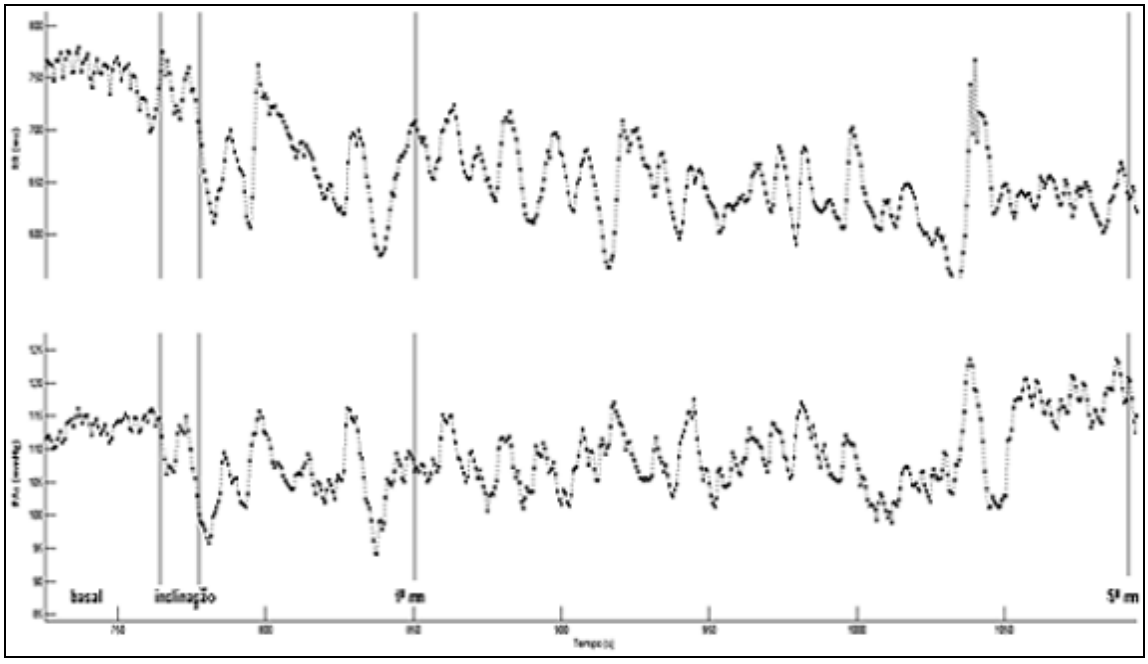

Figura 8 – Registo contínuo da evolução da PA sistólica (PAs) e FC num indivíduo normal durante o teste de ortostatismo passivo a 60º *(Unidade de Sistema Nervoso Autónomo, Instituto de Fisiologia, Faculdade de Medicina de Lisboa)*

Num indivíduo normal, o teste de *tilt* evoca apenas alterações ligeiras de PA mas, na presença de disfunção autonómica habitualmente ocorre descida acentuada da PA por deficiente adaptação do baroreflexo (Mathias CJ & Bannister R, 2001). Em termos gerais, uma prova normal apresenta na fase inicial de adaptação (1-2 minutos) uma diminuição ligeira da PA sistólica (≤ 10 mmHg), um aumento de PA diastólica (5-10 mmHg) e um aumento da FC mediada pela actividade simpática (cerca de 10 bpm) (Hilz et al, 1993; Wieling et al, 1997).

Em indivíduos normais, em simultâneo com a variação da PA, pode observar-se um ligeiro aumento da FC (figura 8), que quando ausente e na presença de uma diminuição de PA indica a presença de disfunção baroreflexa (figura 9). A velocidade e a duração da queda da PA vão depender do grau de disfunção autonómica e da forma como os mecanismos não neuronais são recrutados.

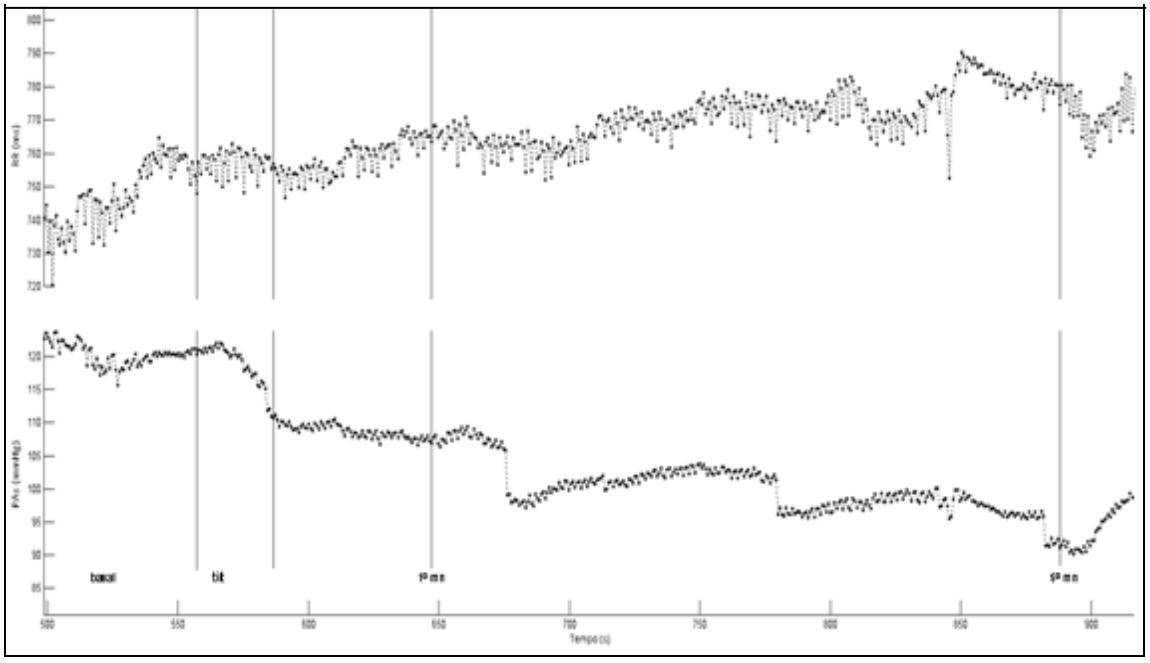

Figura 9 – Registo contínuo da evolução da PA sistólica (PAs) e FC num doente com Atrofia Multisistémica durante o teste de ortostatismo passivo a 60º *(Unidade de Sistema Nervoso Autónomo, Instituto de Fisiologia, Faculdade de Medicina de Lisboa)*

A manobra de Valsalva permite avaliar as alterações da PA e da FC decorrentes do aumento de 40 mmHg na pressão intratoráxica. É uma forma de avaliar o baroreflexo, sendo constituída por 4 fases: duas mecânicas (fases I e III) e duas reflexas (fases II e IV) (figura 10). Uma forma de analisar esta prova é através do índice de Valsalva (*Valsalva ratio*), que relaciona as variações da PA com as variações de FC e que é calculado pela razão das variações da FC nas fases II (máxima subida) e IV (máxima descida), devendo ser superior a 1 num indivíduo com um teste normal. Outros autores consideram outros valores de normalidade específicos para a idade e sexo (Low PA, 2003; Mosqueda-Garcia R, 1995).

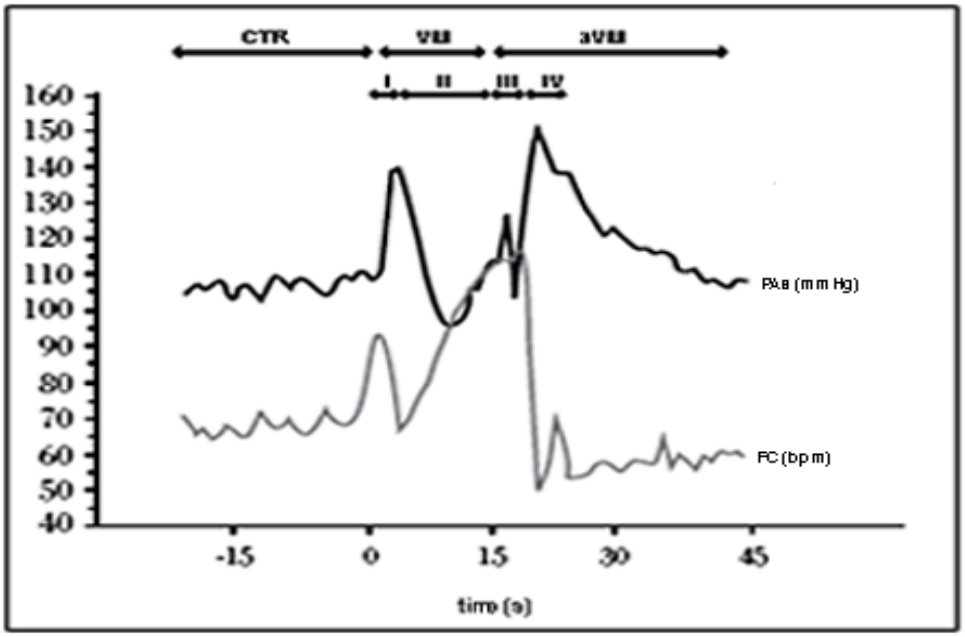

Figura 10 – Flutuações da PA sistólica (linha superior) e da FC (linha inferior) durante as várias fases da manobra de Valsalva (I, II, III and IV) e em 3 períodos de análise: CTR (controlo basal), VM (15 s da manobra de Valsalva) e aVM (pós-Valsalva). Regista-se uma subida da FC no início da inspiração profunda antes da manobra.(a*daptado de Xavier et al.* Rev Port Cardiol 2008; 27 (4): 435-441)

Os testes pressores têm como objectivo provocar um aumento da PA através do incremento das eferências simpáticas como consequência da estimulação de diferentes tipos de aferências. Os testes pressores ao frio (*cold pressure test*) e de contração isométrica (*hand grip*) pertencem a esta categoria.

No teste pressor ao frio ocorre a estimulação nociceptora quando o indivíduo coloca a mão até ao punho em gelo fundente a 4ºC durante 1-3 minutos, observando-se, em condições normais, uma elevação gradual de PA acompanhada por uma subida discreta da FC (figura 11). Nos primeiros 30 segundos deste teste a PA sistólica aumenta devido ao aumento da FC e do débito cardíaco. Apesar de não existirem ainda valores consensuais de subida de PA e FC, considera-se que uma prova é normal se for observado um aumento de PA sistólica de 20mmHg e um aumento da FC de 10bpm (Hilz & Dutch, 2009)

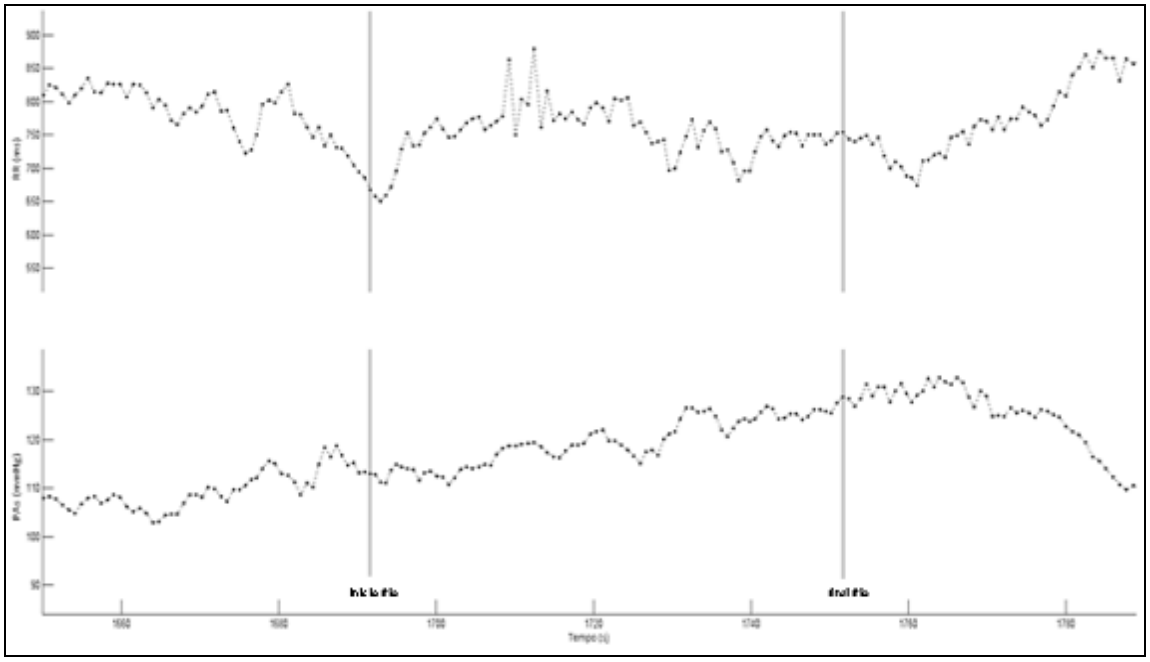

Figura 11 – Registo contínuo da evolução da PA sistólica (PAs) e FC num indivíduo normal durante o teste pressor do frio *(Unidade de Sistema Nervoso Autónomo, Instituto de Fisiologia, Faculdade de Medicina de Lisboa)*

O teste da contração isométrica pressupõe a realização de pressão manual mantida durante 2-3 minutos, correspondente a um terço da força máxima efectuada numa contração voluntária. Num indivíduo normal observa-se um aumento da PA sistólica e da PA diastólica, acompanhado da subida ligeira da FC (figuras 12 e 13) (Melrose, 2005). Um aumento da PA diastólica ≥16 mmHg representa uma prova normal enquanto uma prova anormal se traduz por um aumento da PA diastólica ≤10 mmHg (Appenzeller, 1999).

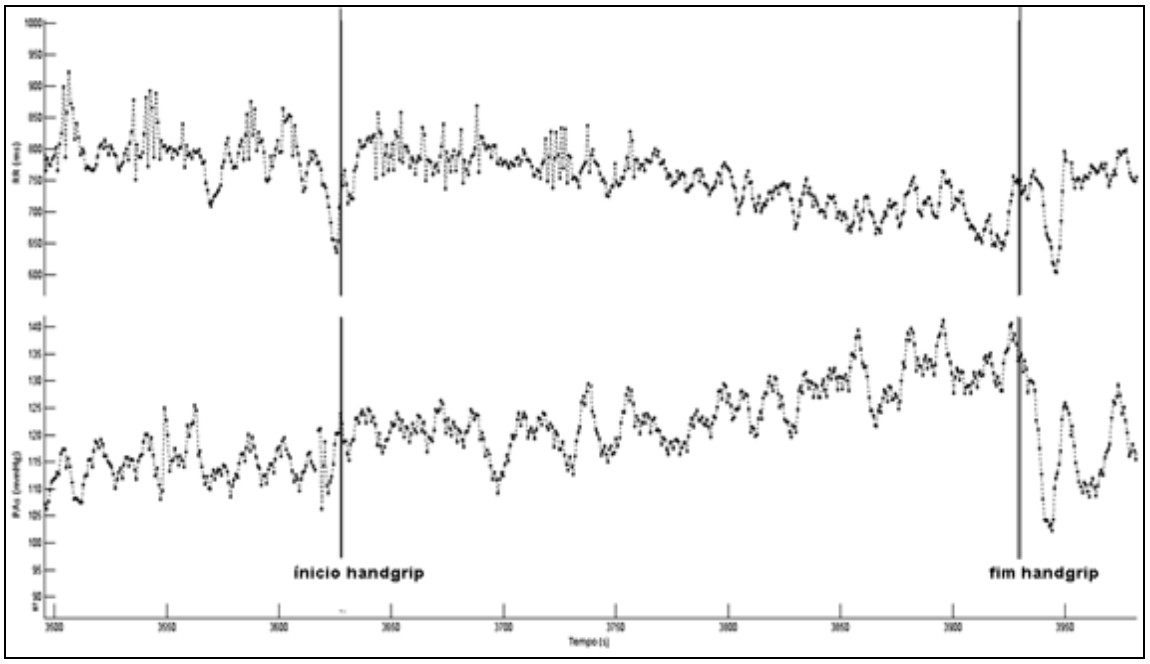

Figura 12 – Registo contínuo da evolução da PA sistólica (PAs) e FC num indivíduo normal durante o teste de contracção manual isométrica (*handgrip*) *(Unidade de Sistema Nervoso Autónomo, Instituto de Fisiologia, Faculdade de Medicina de Lisboa)*

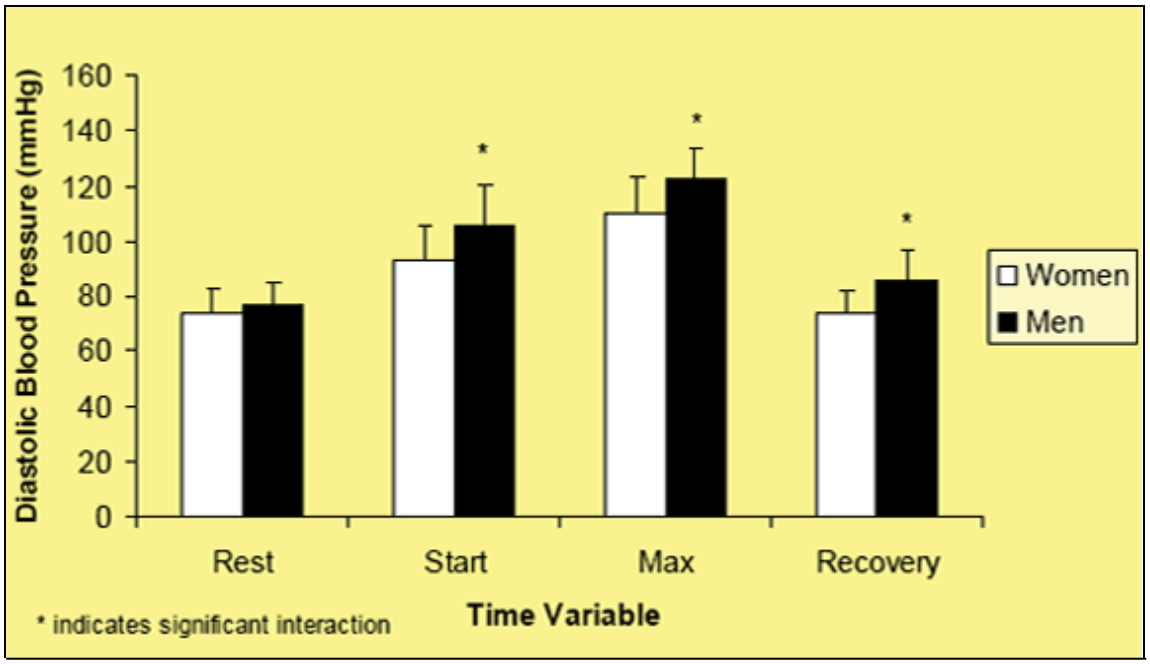

Figura 13 – Evolução da PA diastólica em indivíduos normais (n=31) durante o teste de contracção manual isométrica (*handgrip*) *(Adaptado de Melrouse D. Journal of Exercise Physiology August 2005; 8(4):29-35)*

A prova da respiração profunda (*deep-breath*) baseia-se no fenómeno da arritmia respiratória sinusal em que ocorre um aumento de FC durante a inspiração e uma diminuição desta durante a expiração. Nesta prova, e após um período de repouso, é pedido ao indivíduo que respire profundamente a uma frequência de 1 ciclo em cada 10 segundos durante 1 minuto (6 ciclos/minuto) (figura 14). Para avaliação deste teste autonómico calcula-se a diferença expiração-inspiração (E-I) ou o índice expiração-inspiração (E/I) entre as FC máxima e mínima durante a manobra. Os valores obtidos vão depender da idade, sexo, peso, terapêutica e esforço respiratório, pelo que valores de referência únicos poderão ser controversos. Apesar disso, considera-se uma prova como normal se as diferenças E-I forem superiores a 15 bpm. (Low PA, 2003; Persson & Solders, 1983; Hilz & Dutch, 2009).

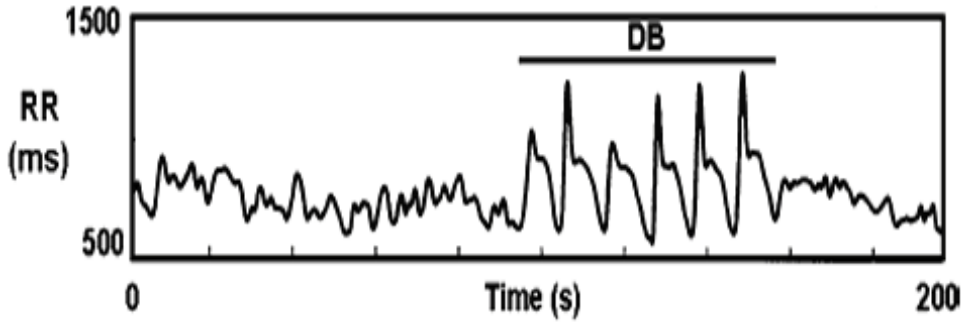

Figura 14 – Evolução dos intervalos RR em indivíduos normais (n=14) durante o teste de respiração profunda (*deep-breath*) *(Adaptado de Ducla-Soares. Exp Physiol 2007; 92(4): 677–686)*

Até ao momento não dispomos dum teste que permita fazer uma avaliação autonómica global. A maior parte das provas são dirigidas à análise das variações da PA e da FC, as primeiras relacionadas com alterações simpáticas, principalmente de natureza vascular, e as segundas com modificações do tónus cardíaco vagal. A interpretação dos valores obtidos deverá ser contextualizada, de acordo com a situação clínica do doente, com outros testes diagnósticos e com os critérios de normalidade que, até ao momento, são definidos para cada laboratório de estudo do SNA, justificando-se, pelas implicações clínicas e na investigação uma uniformização adequada.

Nos últimos anos a interpretação dos resultados da avaliação autonómica tem beneficiado da aplicação de diferentes metodologias de análise a uma característica dos sinais fisiológicos, a sua variabilidade, que se encontra alterada na presença de disfunção autonómica. Assim, o estudo da variabilidade de diferentes sinais envolve três dimensões analíticas: o domínio do tempo, o domínio da frequência e o domínio do tempo-frequência.

No primeiro caso, utilizam-se métodos de natureza estatística para comparar alterações na variabilidade da FC, avaliando-se o desvio padrão dos intervalos RR retirados de um registo electrocardiográfico. A razão entre o desvio padrão dos intervalos RR e o intervalo entre duas ondas R obtido durante o período de registo, o índice pNN50, que corresponde á proporção de

diferenças entre intervalos RR consecutivos que são superiores a 50ms, ou o índice RMSSD (a raiz quadrada da média do quadrado das diferenças entre RR sucessivos) são os parâmetros mais usados já que têm mostrado resultados reprodutíveis não influenciados pela FC média em repouso (Hilz & Dutch, 2009).

Relativamente às metodologias utilizadas no domínio da frequência, utilizam habitualmente a transformada rápida de Fourier que decompõe um sinal nas frequências que o constituem. Assim, em termos de avaliação autonómica aplicada à variabilidade da FC foram definidas duas bandas principais: a das baixas frequências (*low frequencies* - LF -, 0.04-0.4 Hz para o Homem), relacionada principalmente com a actividade simpática, e a de altas frequências (*high frequencies* - HF -, 0.4-0.15Hz para o Homem), indicadora de actividade parassimpática e da frequência respiratória (Malik M, 1996). Como índice de tónus autonómico global definiu-se a razão LF/HF. Assim, durante um aumento da actividade simpática, o índice LF/HF apresenta valores superiores em relação ao valor controlo, enquanto se houver predomínio da actividade parassimpática, o valor do índice descerá por comparação ao controlo (Malliani et al, 1994; Malik M, 1996; Hilz & Dutch, 2009). Esta técnica de processamento de sinal tem sido, também, extendida à variabilidade da PA, o que introduziu modificações no cálculo do índice LF/HF que deverá relacionar a variação das alterações de LF observadas na PA com as alterações de HF observadas na FC (Malliani et al, 1994).

No entanto, a aplicação da transformada de Fourier apresenta algumas limitações pois exige um sinal estável com registo entre 3-5 minutos e não correlaciona as alterações de frequência com o tempo em que elas ocorrem. A introdução de técnicas para estudo no domínio do tempo-frequência permitiu ultrapassar algumas destas limitações. Neste âmbito, a aplicação da transformada discreta de *wavelets* tem-se revelado de grande utilidade, já que permite uma decomposição no domínio tempo-frequência, com respectiva caracterização de sinais dinâmicos com flutuações intermitentes, sendo possível localizar e quantificar alterações na actividade

autonómica em pequenos períodos de tempo (Postolache et al, 2003; Ducla-Soares et al, 2008). Deste modo, as alterações precoces decorrentes da aplicação de manobras autonómicas podem ser avaliadas, tal como demonstrado em trabalhos do nosso grupo (Postolache et al, 2003; Ducla-Soares et al, 2007; Oliveira et al, 2007; Xavier et al, 2008).

Estão disponíveis diferentes tipos de funções de *wavelets* aplicáveis a sinais biológicos, sendo mais utilizada em análise autonómica cardiovascular a do tipo Daubechie, proposta como sendo mais adequada para o perfil da FC e PA (Postolache et al, 2003).

Outra forma de avaliação global do balanço simpático-parasimpático é através do índice da sensibilidade do baroreflexo. De facto, alterações da sensibilidade do baroreflexo contribuem para a redução da actividade parassimpática e aumento da actividade simpática que se associam ao desenvolvimento e progressão de diferentes patologias cardiovasculares (La Rovere, et al, 2001). Neste âmbito, a metodologia clássica envolve o cálculo do índice de sensibilidade do baroreflexo, que é avaliado como o declive à curva de regressão entre sequências de variação da PA sistólica e as variações simultâneas observadas nos intervalos RR em resposta a fármacos vasoactivos, à manobra de Valsalva e à técnica de estimulação com pressão negativa ou positiva a nível cervical (La Rovere, et al, 2001). No entanto, a análise da evolução espontânea da PA e FC em registo contínuo de 24 horas permitiu estabelecer valores normais de sensibilidade do baroreflexo de 7.6±2.0ms/mmHg quando ocorrem aumentos da PA e de 6.4±1.5ms/mmHg quando a PA desce (Parati G, et al, 1988).

Estão disponíveis diferentes metodologias para avaliação do baroreflexo sendo, o método sequencial, pela sua simplicidade, um dos de maior aplicação na actualidade. Este método avalia a relação entre variações espontâneas dos intervalos RR e da PA sistólica numa análise de registo contínuo, que inclui a identificação de rampas de 3 ou mais ciclos cardíacos consecutivos em que ocorre aumento (sequências positivas) ou redução (sequências negativas) progressivos ($\geq$1mmHg) da PA sistólica (Di Rienzo, et al, 2001).

Têm sido também aplicados métodos espectrais, que analizam a relação (em termos de ganho) dos componentes oscilatórios específicos da FC e PA sistólica, valorizando níveis de coerência elevados na banda de frequências médias (0.07-0.14 Hz, no Homem) com os quais se obtêm níveis de correlação elevados com os valores da análise da sensibilidade do baroreflexo (Robbe et al, 1987).

Apesar da importância atribuída ao impacto de mecanismos envolvendo a actividade simpática e parassimpática na fisiopatologia da FA permanece escassa a informação relativa ao comportamento autonómico durante testes fisiológicos evocando respostas cardiovasculares no âmbito da FAP.

Capítulo II.

Objectivos

A análise dos dados disponíveis da investigação clínica e em modelos animais sugere que há características electrofisiológicas do tecido auricular favoráveis à ocorrência de FAP que podem ser moduladas pelo SNA. No entanto, permanece por esclarecer a relação precisa da actividade autonómica com os vários factores que intervêm na fisiopatologia da FAP.

Numa perspectiva mais específica, é importante: a) avaliar o impacto da actividade simpática e parassimpática, e sua importância relativa, nas propriedades electrofisiológicas mais relacionadas com o início e manutenção da FAP; b) determinar se esta população apresenta alterações do SNA fora dos períodos de arritmia, dado que o reconhecimento e a valorização de alterações da regulação autonómica cardíaca constituem factores essenciais para a compreensão e melhor controlo da FAP; c) estudar a influência do SNA nos fenómenos de remodelagem auricular, cujos mecanismos envolvem, para além das alterações estruturais, fenómenos dinâmicos ainda pouco conhecidos nos domínios molecular, iónico, celular, eléctrico e neuro-hormonal.

O objectivo dos trabalhos incluídos nesta investigação, que incluem o estudo da actividade autonómica e propriedades electrofisiológicas em doentes com FAP e em modelos animais de FA, é o de contribuir para a compreensão da influência autónomica na génese, manutenção e interrupção da FAP, incidindo para isso no seguinte conjunto de questões:

I) com estudos em humanos

1. Importância relativa das propriedades electrofisiológicas auriculares na vulnerabilidade para indução e manutenção de FA e sua relação com a modulação da actividade do SNA.

1.1. qual o impacto do valor dos PRE e dispersão da refractariedade na inducibilidade de FA?

1.2. a heterogeneidade dos PRE e as alterações da condução nas aurículas são factores igualmente determinantes no início e na manutenção da FA?

1.3. a modulação aguda autonómica influencia o substrato electrofisiológico auricular na FAP com evolução clínica de longo-prazo?

2. Comportamento da actividade simpática e parassimpática durante manobras provocativas da função autonómica em doentes com FAP.

2.1. as respostas autonómicas durante o teste de ortostatismo passivo, esforço isométrico, respiração profunda e teste pressor ao frio são diferentes do normal em doentes com FAP?

2.2. os reflexos autonómicos com predomínio de activação vagal ocorrem mais frequentemente durante o teste de inclinação em doentes com FAP quando comparados com uma população com síncope neurocardiogénica?

2.3. haverá diferenças, em relação ao comportamento normal, nas respostas da sensibilidade do baroreflexo na FAP isolada?

II) com estudos em modelos animais

3. Papel da actividade autonómica nos fenómenos electrofisiológicos, vulnerabilidade para FA e na expressão genética das proteínas dos canais iónicos e conexinas do tecido auricular.

3.1. quais os efeitos agudos da estimulação vagal e simpática, de forma isolada ou combinada, na refractariedade e conductibilidade das aurículas e VP e o seu impacto na indução de FA?

3.2. que sequência temporal e tipo de alterações ocorrem na codificação dos genes de canais iónicos e conexinas das aurículas induzidas por *pacing* auricular de alta-frequência ou por estimulação autonómica intensa e contínua - simpática, parassimpática ou combinada?

Com a combinação das respostas a estas diferentes questões, integradas no contexto dum substrato arrítmico que se reconhece como complexo, esperamos contribuir para a compreensão da fisiopatologia duma condição clínica que representa um desafio considerável na actualidade, pela associação com múltiplos mecanismos, pela prevalência crescente, morbilidade e impacto na sociedade.

Capítulo III.

Importância relativa das propriedades electrofisiológicas auriculares na vulnerabilidade para indução e manutenção de fibrilhação auricular e sua relação com a modulação da actividade do Sistema Nervoso Autónomo

Dispersão da Refractariedade Auricular como Substrato Electrofisiológico da Vulnerabilidade Auricular em Doentes com Fibrilhação Auricular Paroxística

Mario Oliveira, M. Nogueira da Silva, Ana T. Timoteo, Joana Feliciano, Lidia Sousa, Sofia Santos, Fernando Marques, Rui Ferreira. Rev Port Cardiol 2007; 26 (7-8):1-12

Inducibility of atrial fibrillation during electrophysiologic evaluation is associated with increased dispersion of atrial refractoriness.

Mario Oliveira, M. Nogueira da Silva, Ana T. Timoteo, Joana Feliciano, Lidia Sousa, Sofia Santos, Luis Silva-Carvalho, Rui Ferreira. Int J Cardiol 2009; 136:130-135

Influência da Estimulação e Bloqueio do Sistema Nervoso Autónomo na Refractariedade Auricular em Doentes com Fibrilhação Auricular Paroxística
Idiopática

Mario Oliveira, M. Nogueira da Silva, Joana Feliciano, Ana T. Timoteo, Fernando Marques, Sofia Santos, Isabel Rocha, Luis Silva-Carvalho, Rui Ferreira. Rev Port Cardiol 2009; 28(6):655-670

Effects of acute autonomic modulation on atrial conduction delay and local electrograms duration in paroxysmal atrial fibrillation

Mário Oliveira, M. Nogueira da Silva, Pedro Cunha, Ruben Ramos, Fernando Marques, Sofia Santos, Isabel Rocha, Luis Silva-Carvalho, Rui Ferreira. Int J Cardiol 2010, Mar 16. doi:10.1016/j.ijcard.2010.02.006

Dispersão da Refractariedade Auricular como Substrato Electrofisiológico da Vulnerabilidade Auricular em Doentes com Fibrilhação Auricular Paroxística

MÁRIO MARTINS OLIVEIRA, MD, FESC, NOGUEIRA DA SILVA, MD , ANA TERESA TIMÓTEO, MD, FESC , JOANA FELICIANO, MD , LIDIA DE SOUSA, MD, SOFIA SANTOS, AP , FERNANDO MARQUES, AP , RUI FERREIRA, MD

Serviço de Cardiologia, Hospital de Santa Marta, Lisboa, Portugal

Rev Port Cardiol 2007; 26 (7-8):

RESUMO

A remodelagem eléctrica auricular tem sido associada ao aumento da heterogeneidade da refractariedade auricular, referida como podendo desempenhar um papel importante nos episódios recorrentes de fibrilhação auricular (FA).

Objectivo: avaliar a dispersão da refractariedade auricular (disp_A) e sua relação com a vulnerabilidade para a indução de FA (vuln_FA) em doentes com FA paroxística.

População e métodos: Estudo efectuado em 36 doentes - 22 homens; 53±13 anos (43-76) - com >1 ano de evolução de FA paroxística (sem cardiopatia estrutural: n=20, hipertensão arterial: n=14, prolapso valvular mitral: n=1, estenose valvular pulmonar operada: n=1). O estudo electrofisiológico foi realizado com consentimento informado, após suspensão da terapêutica antiarrítmica e sem sedação. A refractariedade auricular foi analizada em 5 locais (aurícula direita alta, aurícula direita lateral baixa, septo interauricular alto, seio coronário proximal e seio coronário distal) durante pacing contínuo a 600 ms com introdução de extra-estimulo. A disp_A foi calculada como a diferença entre o período refractário efectivo mais longo e o mais curto. A vuln_FA foi definida como a possibilidade de induzir FA com extra-estímulos auriculares ou pacing auricular incremental. O protocolo incluiu a análise de actividade eléctrica focal através de ectopias supraventriculares em salvas (espontâneas ou com manobras

ABSTRACT

Enhanced Dispersion of Atrial Refractorines as an Electrophysiological Substrate for Vulnerability to Atrial Fibrillation in Patients with Paroxysmal Atrial Fibrillation

Atrial electrical remodeling plays a part in recurrence of atrial fibrillation (AF). It has been related to an increase in heterogeneity of atrial refractoriness that facilitates the occurrence of multiple reentry wavelets and vulnerability to AF. Aim: To examine the relationship between dispersion of atrial refractoriness (Disp_A) and vulnerability to AF induction (A_Vuln) in patients with clinical paroxysmal AF (PAF).

Methods: Thirty-six patients (22 male; age 55±13 years) with ≥1 year of history of PAF (no underlying structural heart disease - n=20, systemic hypertension - n=14, mitral valve prolapse - n=1, surgically corrected pulmonary stenosis - n=1), underwent electrophysiological study (EPS) while off medication. The atrial effective refractory period (AERP) was assessed at five different sites - high (HRA) and low (LRA) lateral right atrium, high interatrial septum (IAS), proximal (pCS) and distal (dCS) coronary sinus - during a cycle length of 600 ms. AERP was taken as the longest S1-S2 interval that failed to initiate a propagation response. Disp_A was calculated as the difference between the longest and shortest AERP. A_Vuln was defined as the ability to induce AF with 1-2 extrastimuli or

provocativas). Foram considerados doentes com indução de FA (grupo A; n=25) e sem indução de FA (grupo B; n=11), e comparados os valores da disp_A. Foi analizada também a disp_A e vuln_FA nos doentes com evidência de actividade focal repetitiva. Usámos regressão logística para determinar a associação com vuln_FA para as seguintes variáveis: idade, hipertensão arterial, tamanho da aurícula esquerda, função ventricular esquerda e hipertrofia ventricular esquerda (avaliados por ecocardiografia), duração da história de FA, documentação de flutter auricular e disp_A. Resultados: Induziu-se FA em 25 doentes (71%). O grupo A tinha valores de disp_A significativamente superiores aos do grupo B (105±78 ms vs 49±20 ms; p=0,01). A disp_A foi >40 ms em 50% dos doentes sem vuln_FA e em 91% dos doentes com vuln_FA (p=0,05). Foi demonstrada actividade focal em 14 casos (39%) - 57% com vuln_FA -. A disp_A foi de 56±23 ms neste grupo e de 92±78 ms nos restantes (p=0,07). Com análise univariada, a disp_A foi a única variável com valor predizente para vuln_FA (p=0·05). *Conclusão:* O aumento da disp_A em doentes com FA paroxística é um marcador importante de vuln_FA, podendo representar um substracto electrofisiológico de menor relevância na FA dependente de actividade focal.

Palavras-Chave
Dispersão da refractariedade; Vulnerabilidade auricular; Fibrilhação auricular.

with incremental atrial pacing (600-300 ms) from the HRA or dCS. The EPS included analysis of focal electrical activity based on the presence of supraventricular ectopic beats (spontaneous or with provocative maneuvers). The patients were divided into group A - AF inducible (n=25) and group B - AF not inducible (n=11). Disp_A was analyzed to determine any association with A_Vuln. Disp_A and A_Vuln were also examined in those patients with documented repetitive focal activity. Logistic regression was used to determine any association of the following variables with A_Vuln: age, systemic hypertension, left ventricular hypertrophy, left atrial size, left ventricular function, duration of PAF, documented atrial flutter/tachycardia and Disp_A.
Results: There were no significant differences between the groups with regard to clinical characteristics and echocardiographic data. AF was inducible in 71% of the patients and noninducible in 29%. Group A had greater Disp_A compared to group B (105±78 ms vs. 49±20 ms; p=0.01). Disp_A was >40 ms in 50% of the patients without A_Vuln and in 91% of those with A_Vuln (p=0.05). Focal activity was demonstrated in 14 cases (39%), 57% of them with A_Vuln. Disp_A was 56±23 ms in this group and 92±78 ms in the others (p=0.07). Using logistic regression, the only predictor of A_Vuln was Disp_A (p=0.05). *Conclusion:* In patients with paroxysmal AF, Disp_A is a major determinant of A_Vuln. Nevertheless, the degree of nonuniformity of AERP appears to be less important as an electrophysiological substrate for AF due to focal activation.

Key words
Dispersion of atrial refractoriness; Atrial vulnerability; Atrial fibrillation

INTRODUÇÃO

A fibrilhação auricular (FA) é a arritmia mantida mais comum na prática clínica. A sua prevalência aumenta com a idade, variando entre 0,4% na população geral e 5% acima dos 65 anos de idade, e com a cardiopatia estrutural, esperando-se um risco 4.5 e 5.9 vezes superior de

INTRODUÇÃO

Atrial fibrillation (AF) is the most common sustained arrhythmia in clinical practice. Its prevalence increases with age, ranging between 0.4% in the general population and 5% in those aged over 65 and with structural heart disease: the risk for AF is 4.5 and 5.9 times greater in men

FA para homens e mulheres, respectivamente, na presença de insuficiência cardíaca congestiva [1-3]. São reconhecidas as complicações associadas à FA e o seu impacto desfavorável na qualidade de vida, morbilidade e mortalidade [4,5]. A abordagem clínica da FA permanece um desafio, não só pela sua importância epidemiológica e custos inerentes para a sociedade, mas também pelas limitações na compreensão dos mecanismos fisiopatológicos subjacentes à génese desta arritmia. Apesar dos desenvolvimentos recentes nas estratégias terapêuticas da FA, não está integralmente esclarecida a sequência de fenómenos responsáveis pelos episódios recorrentes de FA. A remodelagem eléctrica auricular, que se associa à diminuição do período refractário efectivo (PRE) de modo heterogéneo, tem sido aceite como factor facilitador de vulnerabilidade auricular para a ocorrência de FA espontânea e indutível [6]. Sabe-se também que doentes com FA indutível têm maior risco de episódios recorrentes de FA, mesmo após isolamento eléctrico das veias pulmonares [7,8]. A demonstração de que o PRE e o aumento da sua dispersão espacial proporcionam um substrato electrofisiológico para a reentrada de múltiplas ondas de propagação ("wavelets") tem contribuído para o interesse crescente do estudo destas propriedades eléctricas do tecido auricular e sua importância no início e manutenção dos episódios de FA [9-11]. A vulnerabilidade para a indução de FA pode ser resultante do substrato necessário para o início de FA na presença de factores "trigger", dado que o risco de FA parece aumentar à medida que diminuem os períodos refractários auriculares e aumenta a heterogeneidade espacial da refractariedade [12]. Além disso, a dispersão da refractariedade pode ser um dos mecanismos que conduzem à perpetuação ou cessação da FA consoante o grau de homogeneidade eléctrica do tecido auricular e consequente número de "wavelets" [13,14]. No presente estudo, avaliámos se a dispersão da refractariedade auricular influência a vulnerabilidade para a indução de FA em doentes com história clínica de FA paroxística.

POPULAÇÃO E MÉTODOS

Foram estudados 36 doentes consecutivos (22 homens e 14 mulheres) com idade média de

and women respectively in the presence of congestive heart failure [1-3]. The complications associated with AF are well known, as is its negative impact on quality of life, morbidity and mortality [4, 5]. The clinical approach to AF remains a challenge, not only because of its epidemiologic importance and the resulting costs to society, but also because of our limited understanding of the pathophysiologic mechanisms underlying its genesis. Despite recent developments in therapeutic strategies, the sequence of events that lead to recurrent episodes of AF is still unclear. Atrial electrical remodeling, which is associated with shortened effective refractory period (ERP) and increased dispersion, is accepted as a factor in the vulnerability of the atrium to spontaneous and inducible AF [6]. It is also known that patients with inducible AF are at greater risk for recurrence, even after electrical isolation of the pulmonary veins [7, 8]. The fact that increased spatial dispersion of ERP provides an electrophysiological substrate for multiple reentry wavelets has stimulated interest in the study of the electrical properties of atrial tissue and their importance in triggering and maintaining AF [9-11]. Vulnerability to AF induction may result from the existence of a suitable substrate together with the presence of triggering factors, since the risk for AF seems to grow in proportion to the shortening of atrial refractory periods and to increased spatial heterogeneity of refractoriness [12]. Moreover, dispersion of refractoriness may be one of the mechanisms that lead to the maintenance or cessation of AF, depending on the degree of electrical homogeneity of the atrial tissue and hence the number of wavelets [13, 14]. In the present study, we examined whether dispersion of atrial refractoriness influences vulnerability to AF induction in patients with a history of paroxysmal AF.

METHODS

Thirty-six consecutive patients were studied (22 men and 14 women), mean age 55±13 years, referred for 1 year of history of episodes of paroxysmal AF documented on electrocardiogram and/or Holter monitoring, despite antiarrhythmic drugs. They included 20 patients with no evidence of underlying structural heart

53±13 anos, referenciados por episódios de FA paroxística com 1 ano de evolução clínica, documentada em electrocardiogramas e/ou registo de Holter, apesar de terapêutica farmacológica antiarrítimica. Foram incluídos 20 doentes sem evidência de cardiopatia estrutural, 14 com história de hipertensão arterial, 1 com prolapso valvular mitral e 1 com estenose valvular pulmonar operada. A clínica de episódios recorrentes de FA tinha uma duração variando entre 1 e 8 anos. Foram excluídos os doentes com evidência de doença do nódulo sinusal, portadores de pacemaker definitivo, disfunção tiróideia ou com FA mantida durante o período de monitorização electrocardiográfica intrahospitalar que antecedeu o estudo electrofisiológico (EEF). A amiodarona foi suspensa dois meses antes do EEF e substituida por fármaco de semi-vida mais curta. Todos os outros antiarrítmicos foram interrompidos cinco semi-vidas antes do procedimento. O protocolo do estudo foi aprovado pela Comissão de Ética do Hospital de Santa Marta. O EEF foi efectuado após autorização obtida em documento de consentimento informado.

PROTOCOLO DO ESTUDO ELECTROFISIOLÓGICO

O EEF foi efectuado após jejum de 6 horas e sem sedação. Não se detectaram anomalias do ionograma sérico. Para registo e estimulação eléctrica, utilizámos electrocateteres multipolares 6F (pólos com intervalos de 2 mm; Daig Co), introduzidos por via percutânea através das veias femural e jugular interna. Um cateter quadripolar foi colocado no apêndice auricular direito e posicionado, durante o protocolo, na aurícula direita lateral-baixa ou no septo interauricular alto, outro foi utilizado para registo em posição hisiana e um cateter decapolar foi colocado ao longo do seio coronário, até à posição mais distal *(figura 1)*.

Os electrogramas e ECGs foram registados num polígrafo de 32 canais (Bard Lab System), com frequência de resposta de 50 a 500 Hz e gravados em sistema de disco óptico para posterior análise.

O PRE foi avaliado em cinco locais diferentes: aurícula direita alta (a nível do apêndice auricular), aurícula direita lateral baixa, septo

disease, 14 with systemic hypertension, one with mitral valve prolapse and one with surgically corrected pulmonary stenosis. Duration of history of recurrent AF varied between 1 and 8 years. Patients with evidence of sinus node disease, permanent pacemaker, thyroid dysfunction or sustained AF during the in-hospital electrocardiographic monitoring that preceded the electrophysiological study (EPS), were excluded. Amiodarone therapy was suspended two months prior to EPS and replaced by a drug with a shorter half-life; all other antiarrhythmics were suspended five half-lives before the procedure. The study protocol was approved by the Ethics Committee of Hospital de Santa Marta and written informed consent was obtained before the EPS was performed.

ELECTROPHYSIOLOGICAL STUDY PROTOCOL

The EPS was performed after 6 hours fasting and without sedation. No serum electrolyte abnormalities were detected. For recording and electrical stimulation, 6F multipolar catheters with 2 mm interelectrode spacing were used (Daig Corp.), introduced percutaneously via the femoral and internal jugular veins. A quadripolar catheter was placed in the right atrial appendage and, during the protocol, was positioned in the low lateral right atrium or the high interatrial septum; another was used for recording in the paraHissian position, while a decapolar catheter was placed along the coronary sinus up to the most distal position *(Figure 1)*.

The electrograms and ECGs were recorded on a 32-channel polygraph (Bard LabSystem) with a frequency response of 50 to 500 Hz and saved onto optical disc for later analysis.

ERP was assessed at five different sites: high right atrium (at the atrial appendage), low lateral right atrium, high interatrial septum, proximal coronary sinus and distal coronary sinus. In stable conditions, an extrastimulus (S2) was introduced during continuous pacing with a cycle length of 600 ms. The initial coupling interval was 100 ms shorter than the baseline pacing cycle, and was then reduced in 10-ms steps until ERP was reached.

Programmed atrial stimulation (600 ms cycle protocols, delivering up to 2 extrastimuli) and

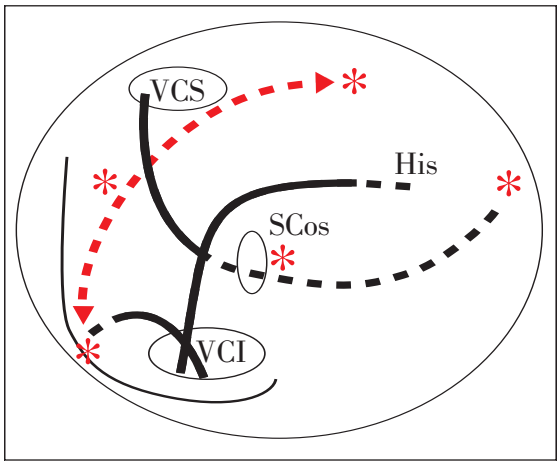

Figura 1. Esquema do posicionamento dos cateteres na aurícula direita e seio coronário (de acordo com a incidência antero-posterior). As setas representam o movimento do cateter multipolar da aurícula direita lateral-baixa para o apêndice auricular direito e septo interauricular alto. Os asteriscos assinalam os 5 locais de medição dos períodos refractários efectivos. VCS=veia cava superior; His=posição hisiana; SCos=ostium do seio coronário; VCI=veia cava inferior

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Figure 1. Diagram of positioning of catheters in the right atrium and coronary sinus (anteroposterior view). The arrows represent the movement of the multipolar catheter from the low lateral right atrium to the right atrial appendage and high interatrial septum. The asterisks indicate the 5 sites at which effective refractory periods were measured. VCS: superior vena cava; His: paraHissian position; SCos: coronary sinus ostium; VCI: inferior vena cava

interauricular alto, seio coronário proximal e seio coronário distal. Em condições estáveis, introduziu-se um extra-estimulo (S2) durante pacing contínuo com ciclo de 600 ms. O intervalo de acoplamento inicial foi 100 ms inferior ao do ciclo basal de pacing, decrescendo depois em intervalos de 10 ms até atingir o PRE.

Procedemos a protocolos de estimulação auricular programada (ciclo de 600 ms até 2 extraestímulos) e de pacing incremental (pacing incremental contínuo, 600 a 300 ms, durante 5s), a partir do bipolo distal, situado na auricular direita alta e seio coronário distal (output com o dobro da amplitude do limiar de pacing naquele ponto e uma largura de impulso de 2 ms). Nos casos com indução de FA mantida durante > 5 minutos sem conversão espontânea, efectuou-se cardioversão eléctrica externa. Nas situações com indução recorrente de FA mantida o protocolo foi terminado após o número máximo de 3 choques.

O maior intervalo de acoplamento (S1-S2) não seguido de propagação do impulso foi considerado como o PRE naquele ponto. A dispersão da refractariedade auricular foi calculada como a diferença entre o período

incremental pacing (continuous incremental pacing, 600-300 ms, for 5 s) were then performed using the distal bipole situated in the high right atrium and the distal coronary sinus, with an output of the pacing threshold at that point and with a pulse width of 2 ms. When sustained AF was induced for >5 minutes without spontaneous conversion, external cardioversion was performed. In cases of recurrent induction of sustained AF, the protocol was ended after a maximum of three shocks.

The longest coupling interval (S1-S2) not followed by a propagation response was taken as the ERP at that point. Dispersion of atrial refractoriness was calculated as the difference between the longest and shortest ERP at the different sites assessed. Atrial vulnerability was defined as the ability to induce AF with a duration of ≥10 s with 1-2 extrastimuli or incremental atrial pacing during stimulation of the right high atrium or the distal coronary sinus. The protocol included analysis of focal electrical activity based on the presence of supraventricular ectopic beats, spontaneous or with provocative maneuvers (handgrip, Valsalva maneuver, carotid sinus massage, or rapid atrial pacing).

STATISTICAL ANALYSIS

Continuous variables were expressed as means ± standard deviation and compared, and categorical variables were expressed as frequencies and percentages. Comparisons between groups were made using the unpaired Student's t test for continuous variables and the chi-square test for categorical variables. Logistic regression was used to determine any association of atrial vulnerability for AF induction with the following variables: age, gender, systemic hypertension, left atrial size by M-mode echocardiography, left ventricular function and left ventricular hypertrophy (both assessed by echocardiography), duration of history of AF, documented atrial flutter/tachycardia and atrial ERP dispersion. Results with a p value of <0.05 were considered significant. The statistical package used was SPSS version 12.0 for Windows (SPSS Inc., Chicago, IL, USA).

refractário efectivo mais longo e o mais curto obtidos nos diferentes locais avaliados. A vulnerabilidade auricular foi definida como a possibilidade de induzir FA com duração ≥10s, utilizando 1-2 extra-estímulos auriculares ou pacing auricular incremental durante estimulação da aurícula direita alta ou seio coronário distal. O protocolo incluiu a análise de actividade eléctrica focal através de ectopias supraventriculares em salvas, de início espontâneo ou com manobras provocativas (handgrip, manobra de Valssalva, massagem do seio carotídeo, pacing auricular rápido).

ANÁLISE ESTATÍSTICA

As variáveis contínuas foram expressas sob a forma de média±desvio padrão e comparadas, e as variáveis categóricas em frequências e percentagens. As comparações intergrupos foram efectuadas pelo teste t de Student (não emparilhado) para as variáveis contínuas e com o «2 para as variáveis categóricas. Usámos a análise de regressão logística para determinar a associação com vulnerabilidade auricular para

RESULTS

AF was inducible in 71% of the patients (group A, n=25) and noninducible in 29% (group B, n=11). There were no significant differences between the groups with regard to clinical characteristics and echocardiographic data *(Table I)*. Mean ERP values increased progressively from the right atrium to the proximal and distal coronary sinus, with those in the coronary sinus being significantly higher (Table II). No significant differences were found between the two groups with regard to ERP at the five sites assessed. Group A had a significantly higher dispersion of atrial refractoriness compared to group B (105±78 ms vs. 49±20 ms; p=0.01) *(Figure 2)*. Dispersion was >40 ms in 50% of the patients without AF vulnerability and in 91% of those with vulnerability (p=0.05). Using logistic regression, the only predictor of AF vulnerability was dispersion of atrial ERP (odds ratio=??; p=0.05).

Focal activity was demonstrated in 14 cases (39%), 57% of them with AF vulnerability. Atrial refractoriness dispersion was 56±23 ms in this group and 92±78 ms in the others (p=0.07).

Tabela 1
Características clínicas da população

	total (n=36)	grupo A (n=25)	grupo B (n=11)
Idade, anos	53±13	56±13	52±15
Sexo masculino	61%	60%	63,6%
FA idiopática	56%	56%	54,5%
Número prévio de AA	1.6±0,8	1.6±0,9	1.6±0.7
AE ≥22 mm/m2	36,1%	36%	36,3%
Hipertrofia VE	13.9%	12%	18%
FEj VE <50%	11.1%	12%	9,1%
Clinica de FAp (anos)	2.3±2.0	2.0±1.6	2.6±2.2
FLA/TA	11.1%	12%	9,1%

FA=fibrilhação auricular; AA=antiarrítmicos; AE=aurícula esquerda; VE=ventrículo esquerdo; FEj=fracção de ejecção; FAp=fibrilhação auricular paroxística; FLA/TA=flutter auricular/ taquicardia auricular. p=NS

Table 1
Características clínicas da população

	Total (n=36)	**Group A (n=25)**	**Group B (n=11)**
Age, years	53±13	56±13	52±15
Male	61%	60%	63.6%
Idiopathic AF	56%	56%	54.5%
Previous no. of AA	1.6±0.8	1.6±0.9	1.6±0.7
LA ≥22 mm/m2	36.1%	36%	36.3%
LV hypertrophy	13.9%	12%	18%
LV EJ <50%	11.1%	12%	9.1%
History of PAF, years	2.3±2.0	2.0±1.6	2.6±2.2
AFL/AT	11.1%	12%	9.1%

FA=fibrilhação auricular; AA=antiarrítmicos; AE=aurícula esquerda; VE=ventrículo esquerdo; FEj=fracção de ejecção; FAp=fibrilhação auricular paroxística; FLA/TA=flutter auricular/ taquicardia auricular. p=NS

Tabela 2
Períodos refractários efectivos auriculares em 5 locais

PRE (ms)	Grupo A (n=25)	Grupo B (n=11)	p
AD alta	212±18	216±24	NS
AD lateral-baixa	208±20	218±24	NS
SIA alto	228±31	231±39	NS
SC proximal	266±35*	245±35*	NS
SC distal	294±90*	242±34*	NS

Resultados expressos como média ±desvio padrão. AD=auricular direita; SIA=septo interauricular; SC=seio coronário. * p<0.01 (SC proximal e SC distal vs. AD alta e AD lateral-baixa)

indução de FA para as seguintes variáveis: idade, sexo, hipertensão arterial, tamanho da aurícula esquerda (ecocardiografia modo-M), função ventricular esquerda e hipertrofia ventricular esquerda (avaliados por ecocardiografia), duração da história de FA, documentação de flutter auricular e dispersão dos PRE auriculares. Considerámos estatisticamente significativos os resultados com valor <0,05. O programa estatístico

utilizado foi o SPSS versão 12.0 para o Windows (SPSS Inc., Chicago, IL, USA).

RESULTADOS

Com o protocolo utilizado, induziu-se FA em 71% dos doentes (grupo A, n=25). Nos restantes casos não se induziu FA (grupo B, n=11). Não havia diferenças estatísticamente significativas entre os grupos relativamente às características clínicas e ecocardiográficas *(Tabela I)*. Os valores médios do PRE aumentaram progressivamente da auricula direita para o seio coronário proximal e distal, sendo os PRE no seio coronário significativamente mais altos (*Tabela II*). Não se registaram diferenças entre os 2 grupos relativamente aos valores de PRE nos cinco locais avaliados. O grupo A tinha valores de dispersão da refractariedade auricular significativamente superiores aos do grupo B (105±78 ms vs 49±20 ms; p=0,01) *(figura 2)*. A dispersão da refractariedade auricular foi >40 ms em 50% dos doentes sem vulnerabilidade para indução de FA e em 91% dos doentes com vulnerabilidade para indução de FA (p=0,05). Na análise de regressão logística a dispersão dos PRE auriculares foi o único factor predizente de

Table 2
Atrial effective refractory periods at 5 sites

ERP (ms)	Group A (n=25)	Group B (n=11)	p
High RA	212±18	216±24	NS
Low lateral RA	208±20	218±24	NS
High IAS	228±31	231±39	NS
Proximal CS	266±35*	245±35*	NS
Distal CS	294±90*	242±34*	NS

Results expressed as means ± standard deviation. RA: Right atrium; IAS: Interatrial septum; CS: Coronary sinus. * p<0.01 (proximal CS and distal CS vs. high RA and low lateral RA)

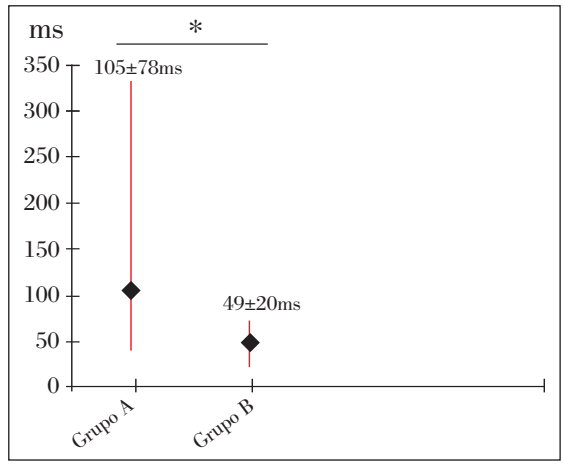

Figura 2. Dispersão dos períodos refractários efectivos auriculares de acordo com a vulnerabilidade para indução de fibrilhação auricular (grupo A - com vulnerabilidade para indução de fibrilhação auricular; grupo B - sem vulnerabilidade para indução de fibrilhação auricular). Resultados expressos como média ±desvio padrão. As linhas representam os valores médio, máximo e minímo para cada grupo. * p=0.01.

Figure 2. Dispersion of atrial refractoriness in terms of vulnerability to AF induction (group A -AF inducible, group B - AF not inducible). Results expressed as means ± standard deviation. The lines represent mean, maximum and minimum values for each group. * p=0.01.

DISCUSSION

The aim of the present study was to examine the impact of dispersion of atrial refractoriness on vulnerability to AF induction in a population with clinical paroxysmal AF. Assessment of dispersion of atrial refractoriness was based on nonuniformity of effective refractory periods measured at different points. The results show that ERP dispersion is a major determinant of atrial vulnerability, with higher values leading to a greater likelihood of AF induction. On the other hand, in patients with documented focal activity, spontaneous or with provocative maneuvers,

vulnerabilidade para indução de FA (odds ratio= ; p=0,05)

Foi demonstrada actividade focal em 14 casos (39%), dos quais 57% tinham vulnerabilidade para indução de FA. A dispersão da refractariedade auricular foi de 56±23 ms no grupo com documentação de actividade focal e de 92±78 ms nos restantes doentes (p=0,07).

DISCUSSÃO

No presente estudo, procurou-se avaliar o impacto da dispersão da refractariedade auricular na vulnerabilidade para indução de FA numa população com história clínica de FA paroxística. O conceito de dispersão da refractariedade auricular baseia-se na não uniformidade dos PRE avaliados em diferentes locais. Os resultados mostraram que a dispersão dos PRE é um factor determinante da vulnerabilidade auricular, facilitando a indução de FA na presença de valores mais altos da dispersão da refractariedade. Por outro lado, nos doentes com demonstração de actividade focal, espontânea ou durante manobras provocativas, registou-se uma tendência para a dispersão da refractariedade auricular ser menor, traduzindo assim um papel de menor relevância deste fenómeno electrofisiológico como substrato para a inducibilidade de FA neste grupo. De facto, a presença de focos ectópicos, localizados sobretudo nas veias pulmonares, tem sido associada a episódios de FA dependentes de actividade "trigger", responsável não só pelo início mas também pela manutenção da condução fibrilhatória [15,16]. A sugestão de que nalguns doentes a actividade focal ("trigger" ou automática) possa ser o mecanismo dominante na FA é um argumento contra a necessidade da ocorrência de múltiplas ondas de reentrada auriculares [17]. Recentemente, em humanos, verificou-se que os PRE eram menores nas veias pulmonares do que na aurícula esquerda, permitindo condições favoráveis para o início de FA, nomeadamente por facilitarem ectopias locais muito precoces [16,18]. Além disso, há evidência experimental de que, em certos casos, a FA possa ser mantida também por pequenos circuitos cuja frequência seja dominante ("rotors") [19,20]. Nestas condições, o aumento da dispersão espacial da refractariedade parece ser

dispersion of refractoriness tended to be less, and so appears to be less important as an electrophysiological substrate for AF in this group. The presence of ectopic foci, particularly in the pulmonary veins, has been associated with episodes of AF that depend on a trigger mechanism that not only originates but also maintains fibrillation [15, 16]. The suggestion that focal activity, whether triggered or automatic, may be the dominant mechanism in AF in some patients could be used to counter the view that multiple atrial reentry wavelets are always required [17]. It has recently been demonstrated that ERPs in humans were shorter in the pulmonary veins than in the left atrium, thus favoring initiation of AF by facilitating very early local ectopic beats [16, 18]. Furthermore, there is experimental evidence that, in some cases, AF may also be maintained by small circuits (rotors) with a dominant frequency [19, 20]. In such cases, increased spatial dispersion of refractoriness would appear to be a less important factor in vulnerability to AF. The presence of a more complex substrate, involving trigger factors and changes in electrophysiological properties arising from atrial remodeling, combined with other factors such as autonomic nervous system effects and structural alterations, make analysis of the pathophysiology of AF a considerable challenge, given our limited understanding of the mechanisms underlying this condition. However, although different explanations have been put forward for the genesis of recurrent AF, dispersion of atrial refractoriness has been consistently associated with vulnerability to initiation and maintenance of AF. In this study, we were able to identify significant differences in dispersion of refractoriness in terms of vulnerability to AF induction, even with similar clinical characteristics and echocardiographic data.

ATRIAL REFRACTORY PERIODS

Shortened ERP has been described as an important electrophysiological alteration in maintenance of AF [21]. It has been shown that AF can lead to reduced atrial ERP without significant changes in conduction rate [22, 23]. Shortened ERP can result in shorter wavelength, which depends on ERP and conduction rate,

menos importante como factor determinante da vulnerabilidade auricular para FA. A presença dum substrato mais complexo, envolvendo factores "trigger" e alterações das propriedades electrofisiológicas condicionadas pelo fenómeno de remodelagem auricular, combinados com variáveis como os efeitos do sistema nervoso autónomo e as alterações estruturais, tornam a análise da fisiopatologia da FA um desafio considerável, devido às limitações na compreensão dos mecanismos subjacentes a esta condição clínica. No entanto, apesar de diferentes conceitos para explicar os mecansimos subjacentes à génese dos episódios recorrentes de FA, a dispersão da refractariedade auricular tem sido consistentemente associada com a vulnerabilidade para o início e manutenção de FA. Neste estudo, foi possível identificar diferenças significativas na dispersão da refractariedade auricular de acordo com a vulnerabilidade para indução de FA, apesar de características clínicas e ecocardiográficas semelhantes.

PERÍODOS REFRACTÁRIOS AURICULARES

O encurtamento dos PRE auriculares tem sido descrito como uma das alterações electrofisiológicas importantes na manutenção da FA [21]. Está demonstrado que a FA pode conduzir à diminuição dos PRE auriculares sem modificação significativa da velocidade de condução [22,23]. O encurtamento do PRE pode levar a um menor comprimento de onda ("wavelenght"), que depende do PRE e velocidade de condução, criando condições para manter a FA [24]. No nosso estudo, à semelhança do descrito por outros autores [25,26], o PRE aumentou gradualmente nos diferentes locais analisados da direita para a esquerda, com valores mais baixos na aurícula direita alta, lateral baixa e septo interauricular quando comparados com as determinações no seio coronário proximal e distal. Facto possivelmente causado pela distribuição não uniforme das terminações vagais nervosas, que parecem influenciar de forma mais acentuada o PRE no apêndice auricular direito que na aurícula esquerda [27]. No presente trabalho, a vulnerabilidade auricular não se relacionou com os valores absolutos do PRE mas sim com a

establishing conditions for sustained AF [24]. In our study, as in others [25, 26], ERP increased gradually at the different sites assessed from right to left, with lower values in the high right atrium, low lateral atrium and interatrial septum than those in the proximal and distal coronary sinus. This may be due to nonuniform distribution of vagal nerve endings, which appear to have a stronger effect on ERP in the right atrial appendage than in the left atrium [27]. In the present study, atrial vulnerability did not correlate with absolute values of ERP but with dispersion of refractoriness. These findings are in agreement with those of Fareh et al., in which duration of and vulnerability to AF correlated with heterogeneity of refractoriness but not with ERP or wavelength [12].

It is generally accepted that heterogeneity of atrial electrophysiological properties can lead to multiple reentry circuits, facilitating spontaneous initiation and induction of AF [13, 28]. Experimental studies also suggest that ERP dispersion in atrial tissue may be an important factor in maintaining AF [29, 30]. Reentry phenomena in the atria as a substrate for initiation of AF has been associated with shortened ERP and increased dispersion of refractoriness. It is also possible that the surface of the left atrium plays a part in this process, since a sufficient mass of atrial tissue may be required to reach the critical number of propagation waves needed to initiate and maintain AF. Our results show that, although there were no significant differences between the groups with regard to left atrial size or absolute values of ERP, dispersion of refractoriness is a determinant of vulnerability to AF.

STUDY LIMITATIONS

The fact that no control group without a history of paroxysmal AF was included means that no comparison of electrophysiological characteristics was possible between the patients studied and a population without the arrhythmia. However, the aim of the study was to examine the relationship between the extent of dispersion of atrial refractoriness and vulnerability to AF induction, which means it would be hard to apply to patients undergoing EPS without clinical AF. Another limitation is that the protocol did not include assessment of ERPs in the pulmonary veins or different sites of the left atrium, and so

dispersão da refractariedade. Estes resultados estão de acordo com o estudo de Fareh, onde a duração e vulnerabilidade da FA se correlacionavam com a heterogeneidade dos PRE auriculares mas não com o PRE ou "wavelenght" [12].

Tem sido amplamente aceite que a heterogeneidade das propriedades electrofisiológicas auriculares pode permitir a ocorrência de múltiplos circuitos de reentrada facilitando o início espontâneo e a indução de FA (13,28). Estudos experimentais sugeriram também que a dispersão dos PRE do tecido auricular pode ser um factor importante na capacidade de manter a FA [29,30]. A presença de fenómenos de reentrada nas aurículas como substrato para o início de episódios de FA tem sido associada ao encurtamento do PRE com aumento da dispersão da refractariedade. Não é também de excluir que a superfície auricular esquerda desempenhe um papel neste âmbito uma vez que pode ser necessária uma massa de tecido auricular suficiente para permitir um número crítico de ondas de propagação essenciais para iniciar e manter a FA. Os nossos resultados mostram que apesar de não se registarem diferenças significativas entre os grupos no que se refere às dimensões da auricula esquerda ou do valor absoluto dos PRE, a dispersão da refractariedade mantem-se um marcador de vulnerabilidade auricular para FA.

LIMITAÇÕES DO ESTUDO

A não inclusão dum grupo controlo sem história de FA paroxística, torna impossível a comparação das propriedades electrofisiológicas estudades entre os doentes considerados e uma população sem a arritmia. No entanto, o objectivo do estudo foi avaliar a relação entre o grau de dispersão de refractariedade auricular e a vulnerabilidade para a indução de FA, tornando-se mais difícil a sua aplicabilidade a doentes submetidos a EEF sem evidência clínica de FA. Outra das limitações do estudo, está relacionada com o facto do protocolo não incluir a avaliação dos PRE das veias pulmonares e diferentes locais da aurícula esquerda, não dispondo assim de informação completa para comparação dos PRE da aurícula direita (mais curtos na população estudada) com os das veias pulmonares (aceites

there is insufficient information to compare ERPs in the right atrium (shorter in the population studied) with those in the pulmonary veins (which are known to be shorter than those of the left atrial roof). These considerations should be taken into account in future studies. However, in a recent study comparing ERPs along the coronary sinus and the right and left atria, the values for the right atrial appendage were significantly lower than those of the left atrium. The study also showed that ERPs at the distal coronary sinus reflect those assessed in left atrial tissue [31]. Finally, in our investigation ERP and dispersion of atrial refractoriness were measured from a baseline cycle, without considering the duration of the monophasic action potential or the properties of atrial conduction. Consequently, the results do not represent all the alterations associated with electrical remodeling, which is known to be a component in the pathophysiology of AF that is extremely difficult to assess in humans.

CONCLUSIONS

The present study shows that enhanced dispersion of atrial refractoriness favors AF induction in a population with paroxysmal AF, identifying a group with greater atrial vulnerability from among patients with similar clinical and echocardiographic characteristics. In the subgroup of patients with AF due to focal activation, dispersion of refractoriness appears to be less important as an electrophysiological substrate for vulnerability to AF induction. These results strengthen the importance of dispersion of atrial refractoriness as a risk marker in AF.

como mais curtos que os do tecto da auricula esquerda). A importância destes aspectos deverá ser considerada em estudos futuros. Apesar disso, num trabalho recente, comparando os PRE ao longo do seio coronário, aurículas direita e esquerda, os resultados obtidos no apêndice auricular direito eram significativamente inferiores aos medidos em diferentes locais da aurícula esquerda. Neste estudo, foi também demonstrado que o PRE a nível do seio coronário distal reflecte o PRE avaliado no tecido auricular esquerdo [31]. Finalmente, baseámos a nossa investigação nos PRE e dispersão da refractariedade auricular medidos a partir dum ciclo de base, sem considerar a duração do potencial de acção monofásico e as propriedades de condução auricular. Assim, os resultados não representam todas as alterações associadas à remodelagem das propriedades electrofisiológicas, fenómeno reconhecido como um componente da fisiopatologia da FA de muito díficil avaliação em humanos.

CONCLUSÕES

O presente estudo mostra que o aumento da dispersão refractariedade auricular favorece a indução de FA numa população com FA paroxística, permitindo, em doentes com características clínicas e ecocardiográficas semelhantes, identificar um grupo com maior vulnerabilidade auricular. No subgrupo de doentes com identificação de FA dependente de actividade focal, a dispersão da refractariedade parece representar um substrato electrofisiológico de menor relevância de vulnerabilidade para a indução de FA. Estes resultados vêm consolidar a importância da dispersão dos PRE auriculares como marcador do risco na FA.

Pedidos de separatas para:
Dr Mário Oliveira

Serviço de Cardiologia, Hospital de Santa Marta
Rua Santa Marta, 1169-024, Lisboa, Portugal
Fax: 351213560368 , Telefone:351.213594311
e-mail: mmoliveira@clix.pt

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Inducibility of atrial fibrillation during electrophysiologic evaluation is associated with increased dispersion of atrial refractoriness

Mario Oliveira [a,*], M. Nogueira da Silva [a], Ana T. Timoteo [a], Joana Feliciano [a], Lidia Sousa [a], Sofia Santos [a], Luis Silva-Carvalho [b], Rui Ferreira [a]

[a] *Cardiology Department, Santa Marta Hospital, Lisbon, Portugal*
[b] *Institute of Physiology, Faculty of Medicine of Lisbon, Portugal*

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Abstract

The impact of atrial dispersion of refractoriness (Disp_A) in the inducibility and maintenance of atrial fibrillation (AF) has not been fully resolved. Aim: To study the Disp_A and the vulnerability (A_Vuln) for the induction of self-limited (<60 s) and sustained episodes of AF. Methods and results: Forty-seven patients with paroxysmal AF (PAF): 29 patients without structural heart disease and 18 with hypertensive heart disease. Atrial effective refractory period (ERP) was assessed at five sites - right atrial appendage and low lateral right atrium, high interatrial septum, proximal and distal coronary sinus. We compared three groups: group A - AF not inducible ($n=13$); group B - AF inducible, self-limited ($n=18$); group C - AF inducible, sustained ($n=16$). Age, lone AF, hypertension, left atrial and left ventricular (LV) dimensions, LV systolic function, duration of AF history, atrial flutter/tachycardia, previous antiarrhythmics, and Disp_A were analysed with logistic regression to determine association with A_Vuln for AF inducibility. The ERP at different sites showed no differences among the groups. Group A had a lower Disp_A compared to group B (47±20 ms vs 82±65 ms; $p=0.002$), and when compared to group C (47±20 ms vs 80±55 ms; $p=0.008$). There was no significant difference in Disp_A between groups B and C. By means of multivariate regression analysis, the only predictor of A_Vuln was Disp_A ($p=0.04$). Conclusion: In patients with PAF, increased Disp_A represents an electrophysiological marker of A_Vuln. Inducibility of both self-limited and sustained episodes of AF is associated with similar values of Disp_A. These findings suggest that the maintenance of AF is influenced by additional factors.

Keywords: Dispersion of refractoriness; Atrial vulnerability; Non-sustained and sustained atrial fibrillation

1. Introduction

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia in the general practice setting. Its prevalence increases with age, from 0.4% in the general population to more than 5% over the age of 65 [1,2], and it is recognized as a potentially dangerous arrhythmia, with impact on both life expectancy and quality of life [3,4]. AF remains a considerable clinical challenge, in part due to our limitations in understanding the electrophysiological mechanisms underlying the condition. Despite the amount of recent information on management and therapeutic strategies on AF, we still have limited knowledge regarding the mechanisms of arrhythmia recurrence and progression to sustained AF. In fact, paroxysmal AF, defined as recurrent, self-terminating within 7 days of onset, progresses to persistent AF in over 18% of patients, even if there is no sign of underlying structural heart disease [5,6].

Electrical remodelling of the atrial tissue, which is associated with shortening of the atrial refractory period in a heterogeneous way, is known to be related with atrial vulnerability for the occurrence of spontaneous and inducible

* Corresponding author.
E-mail address: m.martinsoliveira@gmail.com (M. Oliveira).

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AF and to favour the maintenance and perpetuation of the arrhythmia [7]. Patients with inducible AF are at an increased risk of AF recurrence, even after pulmonary vein isolation [8,9]. Atrial effective refractory periods (ERP) and its spatial dispersion heterogeneity have been accepted to promote AF re-initiation and to provide a substrate for the re-entry of multiple wavelets to enhance the ability of the disorder to sustaining itself [10,11]. Also, an increase in the electrical homogeneity or a decrease in the dispersion of refractoriness may contribute to decrease the number of wavelets and lead to the AF termination [12]. Studies have shown that spatial dispersion of refractoriness is involved in the maintenance of AF [13,14]. Increased dispersion of atrial refractoriness and shortening of wavelength have been also correlated with initiation and maintenance of AF after its induction in a pacing-induced model of AF in the pig [15]. Nevertheless, there is lack of data concerning the impact of the degree of the non-uniformity of ERP on the vulnerability for the inducibility and for the persistence of AF among humans.

In the present study, we investigated whether the dispersion of atrial refractoriness influences the vulnerability for the induction of AF in patients with paroxysmal AF (PAF). Additionally, we evaluated the relationship between the magnitude of atrial refractoriness dispersion and inducibility of self-limited and self-sustained AF.

2. Methods

2.1. Patient population

The study consisted of 47 patients referred to our institution, with ≥1 year duration of clinical history of PAF, despite antiarrhythmic therapy. PAF was documented with electrocardiograms and/or Holter recordings. Patients with evidence of sick sinus syndrome, failure to remain in stable sinus rhythm while in-hospital monitoring before the electrophysiological study (EPS), permanent pacemaker implanted, bronchopulmonary disease and pregnancy or thyroid dysfunction were not included in the study. Prior to the EPS, all antiarrhythmic drugs were withdrawn for at least 5 half-life times. Patients under amiodarone stopped treatment 2 months before the EPS. The study protocol was approved by the local ethics. All subjects were required to give written informed consent.

The study protocol was performed according to the ethical guidelines of the Declaration of Helsinki.

2.2. Electrophysiological protocol

All patients underwent EPS in a non-sedated postabsorptive state. No serum electrolyte disturbances were found.

Electrical programmed stimulation and recording of electrograms were performed by using 6F catheter electrodes inserted percutaneously into the femoral and internal jugular veins. A quadripolar electrode catheter (2-mm-spaced; Daig Co) was positioned in the right atrial appendage (RAA), and moved to the low right posterolateral atrium (LRA) and high interatrial septum (IAS), a second quadripolar electrode catheter (2-mm-spaced; Daig Co) was inserted into the His bundle area (HBE), and a 2-mm-spaced decapolar electrode catheter (Daig Co) was advanced into the coronary sinus (CS). All bipolar electrograms were recorded using a multichannel electrophysiological recorder (Bard Lab System) with a frequency response of 50–500 Hz onto optical disks for later analysis. Twelve-lead surface ECGs were also simultaneously recorded. Hard copies of the electrograms were printed at a recording speed of 100 mm/s.

As a measure of local refractoriness, ERP were assessed in each patient at five different sites (RAA, LRA, IAS, proximal and distal CS). Under stable conditions, a programmed electrical stimulation using a single premature stimulus (S2) was delivered, while pacing continuously at a basic drive cycle length of 600 ms. Stimulation was performed with impulses of 2 ms duration at twice the diastolic threshold. A premature beat was introduced in late diastole, beginning at a coupling interval of 100 ms less than the basic cycle length. The coupling interval of the premature stimulation was decreased by 10 ms steps until the ERP was reached. The ERP were taken as the longest S1–S2 intervals that failed to initiate a propagation response. Dispersion of refractoriness was obtained in all patients as the difference between the longest and the shortest ERP at the five stimulation sites.

All patients underwent programmed bipolar stimulation (drive-train cycle length of 600 ms using S2–S3 extra-stimuli) and incremental pacing protocols (short-term of burst pacing range from 600 to 300 ms) during sinus rhythm by pacing from the distal electrode pairs positioned at the RAA and distal CS catheters. AF was defined as a rapid atrial rhythm (rate >350 beats/min) characterized by variability of the beat-to-beat cycle length, polarity, configuration and amplitude of the recorded atrial electrograms and lasting more than 5 cycles [16]. The concept of atrial vulnerability was based on the ability to induce AF with 1–2 extra-stimuli or with incremental atrial pacing during electrical stimulation from the RAA or distal CS. If AF was induced, an external electrical cardioversion was performed after ≥5 min of continuous AF without spontaneous termination. In patients requiring external cardioversion, a maximum of 3 shocks was delivered. The patients were separated into group A — AF not inducible, group B — AF inducible, self-limited (<60 s), and group C — AF inducible, self-sustained, terminated by therapeutic intervention.

2.3. Statistical analysis

The results are presented as mean value±standard deviation. Categorical variables are expressed as frequencies and percentages. Student's *t* test and repeated ANOVA were utilised for the analysis of continuous variables (overall comparison). The Chi-square test was used to evaluate the

Table 1
Clinical characteristics of the patients.

Characteristic	All patients (n=47)	Group A (n=13)	Group B (n=18)	Group C (n=16)
Age, years	56±14	57±13	54±15	57±14
Male gender	47%	47%	50%	44%
History of hypertension	36%	38.5%	33.3%	44%
Number of previous AA	1.7±0.8	1.6±0.9	1.4±0.8	2.0±0.5
LA≥22 mm/m^2	36%	38.5%	38.9%	31%
LV hypertrophy	12.7%	15.3%	11.1%	12.5%
LVEF<50%	11.1%	15.4%	11.1%	6.3%
Duration of AF (years)	2.3±1.9	2.0±1.5	1.9±2.0	3.0±2.2
AFL/AT	11.1%	15.4%	5.6%	12.5%

AA=antiarrhythmics; LA=left atrium (M-mode measurements); LV=left ventricle; LVEF=left ventricular ejection fraction; AF=atrial fibrillation; AFL=atrial flutter; AT=atrial tachycardia.
None of the variables differed significantly between the groups.

differences in categorical variables. Logistic regression analysis was used to assess the relation of variables with atrial vulnerability for the induction of AF. We tested the following variables for all patients: age, sex, diagnosis of lone PAF, history of systemic hypertension, left atrial dimension, left ventricular ejection fraction, presence or absence of left ventricular hypertrophy, duration of clinical paroxysmal AF, number of previous antiarrhythmics, documentation of atrial flutter, and dispersion of atrial ERP. For all tests a value of $p<0.05$ was considered statistically significant. Data were analyzed using GraphPAD Instruments (GraphPad Software, Inc., California, USA).

3. Results

3.1. Patient characteristics

Forty-seven patients (47% male) with a mean age of 56±14 years (range, 18 to 76 years) were subjected to this study. The average duration of the history of PAF was 2.3±1.9 years (median 1 year; range, 1 to 8 years).The population included 29 patients without structural heart disease and 18 with hypertensive heart disease. AF was inducible in 72% of the patients and non-inducible in 28%. The patients with atrial vulnerability for arrhythmia induction showed self-limited AF in 53% and self-sustained AF requiring electric intervention in 47%. Group A included 13 patients (8 men and 5 women with a mean age of 57±13 years). These patients had a 2.0±1.5 years of clinical history of PAF, refractory to 1.6±0.9 antiarrhythmic drugs. Group B consisted of 18 patients (11 men and 7 women with a mean age of 54±15 years), who had 1.4±0.8 years of PAF, refractory to 1.9±2.0 antiarrhythmic drugs. Group C was composed of 16 patients (10 men and 6 women with a mean age of 57±14 years), who had 2.0±0.5 years of history of paroxysmal AF episodes, refractory to 3.0±2.2 antiarrhythmic drugs. There were no significant differences regarding clinical and echocardiographic data among the three groups (Table 1).

Table 2
Effective refractory periods (ERP) measured at five atrial sites.

ERP (ms)	Group A (n=13)	Group B (n=18)	Group C (n=16)
RAA	216±24	204±9	215±22
LRA (lateral)	218±24	217±24	210±14
IAS (high)	232±40	235±40	220±15
pCS	245±33*	270±24*	260±40*
dCS	242±34*	340±126*	256±35*

Data are expressed as mean±SD. RAA=right atrial appendage; LRA=low right atrium; IAS=interatrial septum; pCS=proximal coronary sinus; dCS=distal coronary sinus. $p>0.05$ for comparisons between the groups. * $p<0.01$ for comparisons between ERP in the pCS and dCS vs RAA and LRA.

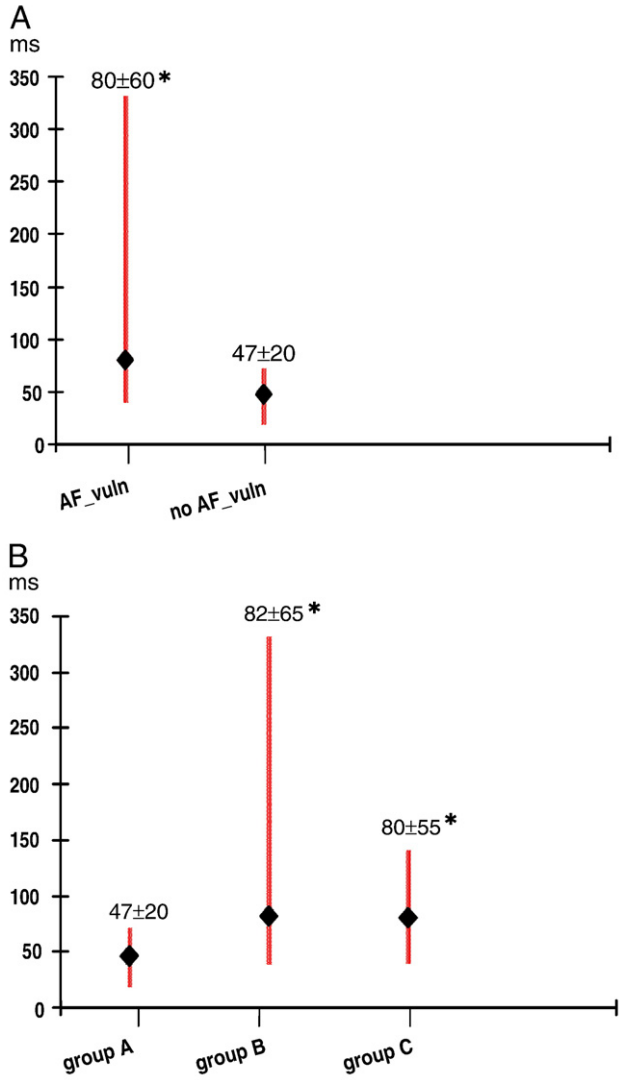

Fig. 1. The line graphs summarize the dispersion of effective atrial refractory periods for all the groups. Data are expressed as mean±SD. The lines represent the mean, minimum and maximum values for each group. A. AF_vuln=patients with vulnerability for AF induction (n=34); no AF_vuln=patients without AF induction (n=13). B. group A=AF not inducible (n=13); group B=AF inducible, self-limited (<60 s) (n=18); group C=AF inducible, self-sustained (n=16). Asterisks represent statistical significance (AF_vuln vs no AF_vuln, p=0.01; group A vs group B, p=0.002; group A vs group C, p=0.008).

3.2. Atrial refractoriness

ERP values measured at the CS (proximal and distal) were significantly higher when compared with the other evaluated sites (Table 2). The mean ERP increased progressively from the RAA, LRA, and IAS to the proximal and distal CS (212±18 ms, 213±20 ms, 228±31 ms, 256±33 ms and 273±73 ms respectively; ERP at the RAA vs distal CS, $p<0.01$). There were no significant differences among the ERP values measured in the three groups of patients at any of the five sites that were assessed (Table 2). However, dispersion of ERP was significantly higher in the group of 34 patients who had atrial vulnerability for the induction of AF compared with those who remained in sinus rhythm during the EPS (80±60 ms vs 47±20 ms; $p=0.01$) (Fig. 1). Moreover, the group A had a significant lower dispersion of the mean ERP compared to the group B (47±20 ms vs 82±65 ms; $p=0.002$) and when compared to the group C (47±20 ms vs 80±55 ms; $p=0.008$). There was no significant difference in dispersion of ERP between the patients with AF lasting ≤60 s and those with self-sustained AF (Fig. 1). By means of multivariate logistic regression, the only predictor of atrial vulnerability for the induction of AF was dispersion of atrial ERP ($p=0{\cdot}04$).

4. Discussion

4.1. Major findings

This study was designed to evaluate the impact of the degree of dispersion of atrial ERP on the vulnerability for the induction and maintenance of AF. The results have demonstrated that ERP dispersion values are determinants of atrial vulnerability. Increased dispersion of refractoriness facilitated AF induction, but the ability to sustain AF may be influenced by other factors in addition to the degree of the non-uniformity of local ERP. In fact, despite a greater dispersion of refractoriness in both groups with inducible AF, the ERP dispersion was similar in patients with inducibility of self-limited AF and in those patients who have induction of AF lasting ≥5 min. This suggests that the electrophysiological substrate that creates the conditions for the maintenance of AF is more complex, probably resulting from the combination of different underlying mechanisms in addition to the magnitude of atrial refractoriness dispersion. It is more likely that multiple variables, including the effects of autonomic nervous system, catecholamines, presence of stretched segments of the atria, ischemia and electrolyte imbalance, conduction abnormalities and other electrophysiological characteristics, contribute to the maintenance of AF.

4.2. Atrial refractory periods

Shortening of the atrial ERP has been reported as one of the main underlying electrophysiological changes in patients with sustained AF [17]. Nevertheless, in our study the ERP measurements at different sites where not different between groups. Previous studies have demonstrated that AF lead to a decrease in atrial ERP, without a significant change in conduction velocity [18,19]. A shorter ERP can create a shorter wavelength (ERP × conduction velocity), which significantly contributes to the maintenance of AF [20]. In our study, the ERP was gradually prolonged from the right to the left side, with higher determinations in the proximal CS and distal CS when compared with the RAA, LRA and IAS. Those patients with induction of AF showed a larger dispersion of refractoriness due to a marked difference between ERP at the right atrium and those obtained along the CS. These findings are in accordance with other authors, who reported shorter ERP in the high right atrium when compared with distal CS in patients with AF [7,21]. This may be explained by a non-uniform distribution of vagal nerve endings, which seems to cause greater changes in refractory period in the RAA than in left atrium [22].

It is widely accepted that heterogeneity of electrophysiological properties may play a major role in favouring re-entry waves, and hence the initiation of AF [13,23]. Experimental studies also suggested that ERP dispersion is an important factor in determining the ability to sustain AF [24,25]. Our results showed that dispersion of refractoriness is a suitable indicator of atrial vulnerability for the induction of AF. However, dispersion of atrial refractoriness was not significantly increased in patients with self-sustained AF when compared with the group with inducible non-sustained AF.

4.3. Self-limited vs self-sustained atrial fibrillation

AF is generally considered to be maintained by multiple re-entrant wavelets of excitation that propagate in different directions around the atrial myocardium [26,27]. The maintenance of AF seems to depend on the presence of a sufficient number of small wavefronts while undergoing fractionation, collisions and coalescence over the atrial surface. To allow multiple re-entrant wavelets to propagate resulting in a self-perpetuating activity, a critical mass of excitable atrial tissue must exist [28]. Re-entry within the atria is associated with shortening of the ERP with increased dispersion of refractoriness, thereby providing a substrate for initiation of AF. The concept of dispersion of refractoriness is based on the non-uniformity of local atrial refractory periods. This results in the coexistence of regions of the atria with relatively short ERP in close proximity to areas with much longer ERP, instead of a gradual transition. Also, re-entrant wavelets must never encounter refractory tissue left over by a previous wavelet, otherwise the wavelets will be extinguished and the arrhythmia will not be sustained. Thus, non-uniform changes in refractoriness are associated with an increased frequency of induction of AF. However, when the dispersion of refractoriness is too large, re-entrant wavelets may be extinguished due to a slower recovery of adjacent

atrial myocardium. If this tissue cannot recover before the wavelet arrives, the critical number of existing wavelets, essential for the AF maintenance, will not be achieved. Moreover, a larger number of circulating wavelets can exist on the surface of larger atria. So, to accommodate the maximum number of wavelets in a constant area, several factors including atrial size, velocity of conduction and dispersion of atrial refractory periods need to be adequately combined.

Recently, in human studies, the presence of shorter ERP in the pulmonary veins, when compared to left atrial refractoriness, was considered to provide a favourable milieu for the initiation of AF and possibly to sustain fibrillatory activity [29,30]. In addition, experimental evidence suggests that certain cases of AF are maintained by small re-entrant dominant frequency sources (rotors) [31,32]. Despite different concepts to explain the perpetuation of AF, dispersion of atrial refractoriness has been consistently associated with vulnerability to the initiation and maintenance of AF.

4.4. Study limitations

First, the lack of a control group without history of AF makes the comparison of the dispersion of ERP between our patients and a population without the arrhythmia impossible. However, the aim of this study was to assess the relationship between the intensity of atrial refractoriness dispersion and the vulnerability for the induction of self-limited and self-sustained AF in a population with PAF. Second, the protocol did not include measurements of the ERP from the pulmonary veins and different left atrial sites. Therefore, it is not possible to compare ERP to the right atrium and pulmonary veins in this population. Nevertheless, in previous studies, the distal CS ERP was accepted as reflecting the ERP of the local left atrial tissue [21,33]. Finally, although the number of subjects included in the study allowed for the identification of significant differences in the dispersion of atrial refractoriness, the resulting sample was relatively small, representing only a subpopulation of patients with PAF and absent or minimal structural heart disease. Thus, further studies in a larger group may be needed to confirm these findings.

5. Conclusions

Increased atrial ERP dispersion enhances the propensity for the inducibility of AF during electrophysiologic evaluation. Nevertheless, patients with vulnerability for the induction of AF lasting less than 1 min and those with inducibility of self-sustained AF had similar and significant increases in atrial dispersion of refractoriness. These results emphasize the importance of the dispersion of ERP as an electrophysiological marker of vulnerability for the induction of AF, and suggest that the maintenance of AF induced during EPS is influenced by additional factors beyond the degree of the non-uniformity of ERP.

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Effects of acute autonomic modulation on atrial conduction delay and local electrograms duration in paroxysmal atrial fibrillation

Mário Oliveira [a,*], Nogueira da Silva [a], Pedro Cunha [a], Ruben Ramos [a], Fernando Marques [a], Sofia Santos [a], Isabel Rocha [b], Luis Silva-Carvalho [b], Rui Ferreira [a]

[a] Cardiology Department, Santa Marta Hospital, Lisbon, Portugal
[b] Institute of Physiology, Faculty of Medicine, Lisbon, Portugal

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ABSTRACT

Slowed atrial conduction may contribute to reentry circuits and vulnerability for atrial fibrillation (AF). The autonomic nervous system (ANS) has modulating effects on electrophysiological properties. However, complex interactions of the ANS with the arrhythmogenic substrate make it difficult to understand the mechanisms underlying induction and maintenance of AF.

Aim: To determine the effect of acute ANS modulation in atrial activation times in patients (P) with paroxysmal AF (PAF).

Methods and results: 16P (9 men; 59±14 years) with PAF, who underwent electrophysiological study before AF ablation, and 15P (7 men; 58±11 years) with atrioventricular nodal reentry tachycardia, without documentation or induction of AF (control group). Each group included 7P with arterial hypertension but without underlying structural heart disease. The study was performed while off drugs. Multipolar catheters were placed at the high right atrium (HRA), right atrial appendage (RAA), coronary sinus (CS) and His bundle area (His). At baseline and with HRA pacing (600 ms, shortest propagated S2) we measured: i) *intra-atrial conduction time* (IACT, between RAA and atrial deflection in the distal His), ii) *inter-atrial conduction time* (interACT, between RAA and distal CS), iii) *left atrial activation time* (LAAT, between atrial deflection in the distal His and distal CS), iv) *bipolar electrogram duration at four atrial sites (RAA, His, proximal and distal CS)*. In the PAF group, measurements were also determined during handgrip and carotid sinus massage (CSM), and after pharmacological blockade of the ANS (ANSB). AF was induced by HRA programmed stimulation in 56% (self-limited — 6; sustained — 3), 68.8% (self-limited — 6; sustained — 5), and 50% (self-limited — 5; sustained — 3) of the P, in basal, during ANS maneuvers, and after ANSB, respectively (p = NS). IACT, interACT and LAAT significantly lengthened during HRA pacing in both groups (600 ms, S2). P with PAF have longer IACT ($p<0.05$), a higher increase in both IACT, interACT ($p<0.01$) and electrograms duration ($p<0.05$) with S2, and more fragmented activity, compared with the control group. Atrial conduction times and electrograms duration were not significantly changed during ANS stimulation. Nevertheless, ANS maneuvers increased heterogeneity of the local electrograms duration. Also, P with sustained AF showed longer interACT and LAAT during CSM.

Conclusion: Atrial conduction times, electrograms duration and fractionated activity are increased in PAF, suggesting a role for conduction delays in the arrhythmogenic substrate. Acute vagal stimulation is associated with prolonged interACT and LAAT in P with inducible sustained AF and ANS modulation may influence the heterogeneity of atrial electrograms duration.

1. Introduction

Atrial fibrillation (AF) is the most common cardiac arrhythmia in clinical practice. It has been recognized as a growing problem, with a prevalence ranging from 1% in the general population to more than 5% over the age of 65 [1,2]. The complex pathophysiology of AF has not been clearly elucidated, due to limitations in studying the mechanisms that lead to the initiation and maintenance of this arrhythmia. Clinical and experimental works have provided new insights into a better understanding of AF, suggesting an important contribution of multiple depolarization wavelets, single dominant reentry circuits, focal sources of electrical activity, and different forms of atrial remodeling to the creation of electrophysiologic substrate for both the recurrence and progression to sustained AF [3–5]. Patients with established AF have regions of slowed conduction facilitating the functional substrate for the occurrence of reentry circuits within the atria [6].

* Corresponding author. Cardiology Department, Santa Marta Hospital, Rua Santa Marta, 1169-024 Lisbon, Portugal. Tel.: +351 213594311; fax: +351 213560368.
E-mail address: m.martinsoliveira@gmail.com (M. Oliveira).

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Furthermore, atrial remodeling causes changes in atrial refractoriness and atrial conduction that may promote AF [7]. Autonomic nervous system (ANS) activity is believed to play an important role in AF pathogenesis [8,9]. The onset of AF is often preceded by fluctuations in autonomic balance that are recognized as modulators in mediating AF [10,11]. Also, a number of electrophysiological properties related with vulnerability for AF may change as a result of vagal or sympathetic activation [12,13]. Vagal stimulation reduces velocity of the conduction in the atrial tissue and shortens the atrial effective refractory periods (ERP) heterogeneously, whereas sympathetic stimulation can increase atrial conduction velocity, favor trigger activity and uniformly reduce atrial refractoriness. However, complex interactions of the ANS with the arrhythmogenic substrate make it difficult to understand its influence in the mechanisms underlying induction and maintenance of AF. The present study was performed to assess the effects of acute ANS modulation in atrial conduction times and the duration of atrial local electrograms in patients with paroxysmal AF (PAF).

2. Methods

2.1. Patient groups

The study included a group of 16 patients (9 men and 7 women with a mean age of 59 ± 14 years) with ≥1 year duration of clinical history of PAF, documented with electrocardiograms and/or Holter recordings, despite antiarrhythmic therapy, referred to our institution for AF ablation, and a control group of 15 patients (7 men and 8 women with a mean age of 58 ± 11 years), with clinically documented supraventricular tachycardia (all with electrophysiological diagnosis of atrioventricular nodal reentry tachycardia). None of these patients had a history of AF or induction of AF during the electrophysiological study (EPS) performed before ablation. Each group included 7 patients with arterial hypertension, but without underlying structural heart disease assessed with transthoracic echocardiography.

Patients with previous myocardial infarction or angina, heart failure, evidence of sick sinus syndrome, failure to remain in stable sinus rhythm while in-hospital monitoring before the EPS, permanent pacemaker implanted, bronchopulmonary disease, sleep apnea, and pregnancy or thyroid dysfunction were excluded. Prior to the EPS, all antiarrhythmic drugs were withdrawn for at least 5 half-life times. Patients under amiodarone stopped treatment 2 months before the EPS. The study protocol was approved by the local ethics and performed according to the ethical guidelines of the Declaration of Helsinki. All subjects were required to give written informed consent.

2.2. Electrophysiological protocol

All patients underwent EPS in a non-sedated post-absorptive state. No serum electrolyte disturbances were found. Atrial electrical stimulation and recording of electrograms were performed by using 6F bipolar catheter electrodes inserted percutaneously into the femoral and internal jugular veins. Quadripolar electrode catheters (2-mm-spaced; DaigCo) were positioned in the high anterior wall of the right atrium (HRA), right atrial appendage (RAA), His bundle area (HBE), and a decapolar catheter with 2 mm interelectrode distance and 5 mm space between each electrode pair was advanced into the coronary sinus (CS) as distal as possible. Stability of the electrode catheters was maintained by fluoroscopic monitoring. Surface ECG leads I, II, V1, and V5 and four intracardiac electrograms (RAA, HBE, CS proximal and CS distal) were displayed on an oscilloscope and a multichannel electrophysiological recorder (Bard Lab System) with a frequency response of 50–500 Hz used onto optical disks for later analysis.

Intra-atrial conduction time (IACT), the interval from the RAA to the atrial electrogram at the HBE, interatrial conduction time (interACT), the interval from the RAA to the atrial electrogram at the distal part of the CS, left atrial activation time (LAAT), the interval from the atrial electrogram at the HBE to the atrial electrogram at distal CS, and local wave duration from different atrial sites (RAA, HBE, proximal and distal CS) were obtained during sinus rhythm, at baseline drive-train stimulation (S1–S1, cycle length of 600 ms) and at the earliest propagated extra-stimulus (S2) during S1 pacing at the HRA. The maximal prolongation of the atrial electrograms during S2 was represented by the % of increase compared to baseline at each recording site.

Stimulation was performed with impulses of 2 ms duration at twice the diastolic threshold. All atrial electrograms were recorded at a fixed gain setting (accompanied by a 0.2 mV = 0.3 mm calibration signal) and remained almost consistent and reproducible at each recording site in each patient. The duration of the local electrograms was measured from the beginning of the earliest electrical activity that deviated from the stable baseline value to the last point of the atrial electrogram at which the baseline value was crossed [14]. Fragmented activity was defined as a disorganised atrial electrogram, with multiple deflections, resulting in a prolonged duration of the activation complex greater than or equal to 150% of the electrogram duration of basic beats [15].

Table 1
Baseline patient clinical characteristics and left atrial size.

Characteristic	PAF group ($n=16$)	Control group ($n=15$)
Age, years	59 ± 14	58 ± 11
Male gender	56%	47%
Body mass index	27 ± 5	28 ± 6
History of hypertension	44%	47%
Heart rate, bpm	65 ± 9	63 ± 8
Systolic blood pressure, mm Hg	132 ± 20	126 ± 21
Diastolic blood pressure, mm Hg	85 ± 11	81 ± 12
LA M-mode, mm	43 ± 3	40 ± 3
History of palpitations; years	2.5 ± 2.0	3.0 ± 2.0

PAF = paroxysmal atrial fibrillation; LA = left atrium (M-mode measurements in parasternal view).
None of the variables differed significantly between the groups.

In the PAF group, measurements were made also during ANS stimulation maneuvers and after pharmacological ANS blockade (atropine 0.04 mg/kg + propranolol 0.15 mg/kg). Sympathetic stimulation was achieved by 3 min of static, intermittent handgrip (HG) of submaximal intensity until fatigue set in, and vagal activity was induced by right carotid sinus massage (CSM), with pressure applied at the point of strongest pulse at the level of the cricoid cartilage (for 10 s at 10-s intervals in 3-min periods). In the absence of a response, CSM was repeated on the left side. Continuous ECG and blood pressure monitoring, together with spectral analysis of RR intervals in the frequency domain (Task Force Monitor 3040; CNSystems), were used to confirm ANS stimulation or blockade. The frequency spectrum was divided into three components: very low frequency (VLF) (0–0.04 Hz), low frequency (LF) (0.04–0.15 Hz) and high frequency (HF) (0.15–0.4 Hz). HF values, attributed to vagal modulation, are affected by mechanical stimulation of the carotid sinus, while LF values mainly reflect sympathetic activity and increase during HG. Intravenous administration of propranolol and atropine resulted in total suppression of HF and LF activity, thus enabling assessment of the intrinsic electrophysiological properties [16].

All patients underwent programmed bipolar stimulation (drive-train cycle length of 600 ms using S2–S3 extra-stimuli delivered after eight paced beats) and incremental pacing protocols (short-term of burst pacing range from 600 to 300 ms) during sinus rhythm, by pacing from the distal electrode pair positioned at the HRA. AF was defined as a rapid atrial rhythm (rate >350 beats/min) characterized by variability of the beat-to-beat cycle length, polarity, configuration and amplitude of the recorded atrial electrograms and lasting more than 5 cycles [17]. AF was considered not inducible; inducible, self-limited (< 60 s) or inducible, sustained, terminated by therapeutic intervention [18].

2.3. Statistical analysis

Categorical variables are expressed as frequencies and percentages. Continuous variables were expressed as means ± standard. Student's *t* test was used to compare all paired data in the same group. Comparisons between groups were made using the unpaired Student's *t* test, repeated ANOVA for continuous variables (overall comparison) or Mann–Whitney's test as appropriate. The chi-square test with Yates correction was used for categorical variables. A value of $p<0.05$ was considered statistically significant. Data were analyzed using GraphPAD Instruments version 3.05 (GraphPad Software, Inc., California, USA).

3. Results

Table 1 shows the clinical characteristics and the left atrial size (evaluated by M-mode echocardiography) of the patients with and without PAF. There were no significant differences between the groups.

Table 2
Comparison of the atrial conduction times between groups.

	SR	600 ms	S2
IACT (ms)			
PAF group	ψ34 ± 15	52 ± 19*	§102 ± 45**
Control group	24 ± 13	50 ± 22*	63 ± 19*
InterACT (ms)			
PAF group	82 ± 19	118 ± 22**	§176 ± 52**
Control group	73 ± 20	115 ± 20**	126 ± 22**
LAAT (ms)			
PAF group	53 ± 15	68 ± 17*	77 ± 58*
Control group	49 ± 18	58 ± 19*	70 ± 18*

SR = sinus rhythm; 600 ms = drive-train stimulation with a cycle length of 600 ms; S2 = the earliest propagated extra-stimulus; IACT = intra-atrial conduction time; interACT = interatrial conduction time; LAAT = left atrial activation time; PAF = paroxysmal atrial fibrillation; control = no clinical history of atrial fibrillation nor induction of atrial fibrillation. *$p<0.05$ (vs. measurement in SR); **$p<0.01$ (vs. measurement in SR); ψ$p<0.05$ (between groups); §$p<0.01$ (between groups).

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AF was induced during programmed stimulation in 56% (self-limited — 6; sustained — 3), 68.8% (self-limited — 6; sustained — 5), and 50% (self-limited — 5; sustained — 3) of the PAF group in basal, during ANS maneuvers, and after ANS blockade, respectively (p = NS).

In baseline, mean P-wave duration was 108 ± 14 ms in patients with PAF and 96 ± 20 ms in patients without AF (p = 0.05). Baseline IACT, during sinus rhythm, was longer in the PAF group, compared to the control group, without significant differences in interACT and LAAT between groups (Table 2). IACT, interACT and LAAT significantly lengthened in both groups during HRA pacing (drive-train with a cycle length of 600 ms and during premature stimulation). Patients with PAF showed a greater prolongation in both IACT and interACT with the earliest propagated extra-stimulus (p<0.01) (Table 2).

Electrogram duration showed significant differences at the RAA, when comparing both groups during baseline sinus rhythm and with S2 (Fig. 1). Also, patients with PAF had greater prolongation of electrogram wave duration measured at the RAA and distal CS during the earliest propagated S2 (73 ± 35% vs. 11 ± 8% at the RAA and 13 ± 3%

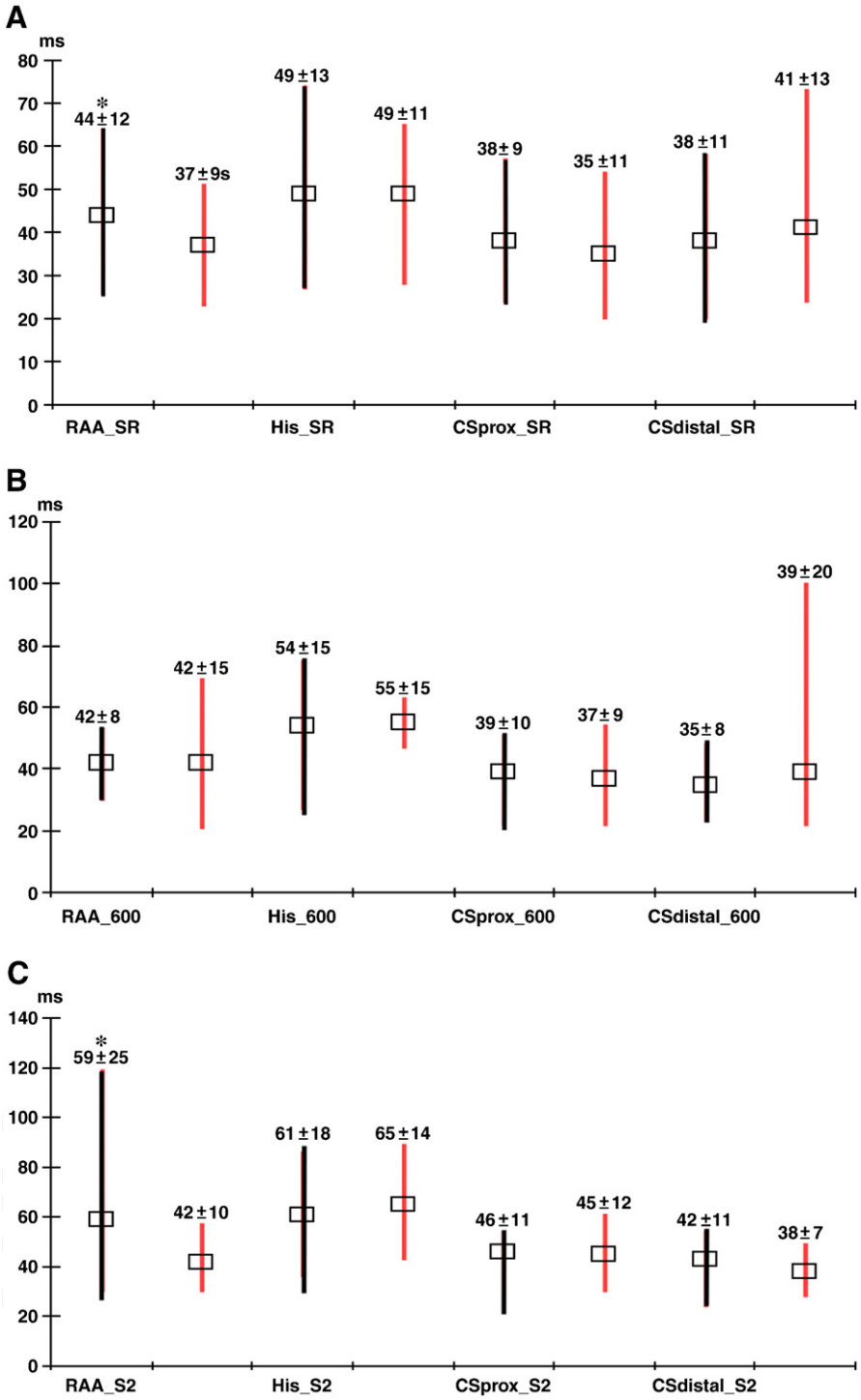

Fig. 1. Electrogram duration measured in sinus rhythm (SR) and during high right atrium pacing with a drive-train stimulation (600 ms) and with the earliest propagated extra-stimulus (S2). Comparison between the group with paroxysmal atrial fibrillation (black line) and the group without atrial fibrillation (red line). A: Baseline values during SR (*p<0.05, at RAA site). B: Values during 600 ms cycle length pacing (p = NS). C: Values during the earliest propagated S2 (*p<0.05, at RAA site). Values expressed in milliseconds, mean ± standard deviation. The lines represent mean, maximum and minimum values. RAA = right atrial appendage; His = His position; CSprox = proximal coronary sinus; CSdistal = distal coronary sinus. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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Table 3
Atrial conduction times during autonomic modulation in paroxysmal atrial fibrillation patients.

	PAF group ($n=16$)	AF non-inducible ($n=5$)	AF inducible ($n=11$)	AF self-limited ($n=6$)	AF sustained ($n=5$)
IACT_baseline	34±15	34±18	35±15	35±19	35±10
IACT_hand-grip	29±11	32±15	28±10	27±13	29±7
IACT_carotid sinus massage	30±14	29±7	29±11	30±9	30±13
IACT_ANS blockade	28±13	25±13	30±14	29±5	30±13
interACT_baseline	82±19	77±24	83±19	80±20	86±20
interACT_hand-grip	84±20	83±18	84±22	80±26	89±17
interACT_carotid sinus massage	80±21	76±28	81±18	71±17*	95±5*
interACT_ANS blockade	72±14	64±16	80±8	74±20	77±9
LAAT_baseline	53±15	44±7	54±19	53±10	57±19
LAAT_hand-grip	55±17	51±11	57±19	52±21	62±16
LAAT_carotid sinus massage	52±16	45±7**	55±14	47±7**	65±14**
LAAT_ANS blockade	44±12	38±11	42±14	45±13	48±11

PAF = paroxysmal atrial fibrillation; AF = atrial fibrillation; IACT = intra-atrial conduction time; interACT = interatrial conduction time; LAAT = left atrial activation time; ANS = autonomic nervous system. Values expressed in milliseconds, mean ± standard deviation. *$p<0.05$; **$p<0.01$.

vs. 1 ± 6% ms at the distal CS, for PAF patients and control group, respectively; $p<0.05$). Fragmented atrial activity was identified in 43.8% of the PAF group and in 6.7% of the control group ($p=0.03$).

3.1. Conduction parameters during acute autonomic modulation

Table 3 summarizes the results of the atrial conduction intervals during HG and CSM maneuvers, and after ANS blockade among patients with PAF. Atrial conduction times were not significantly changed during ANS stimulation. However, patients with inducible sustained AF had longer interACT and LAAT during CSM.

Despite longer electrograms in RAA and His during CSM when compared to baseline recordings, there were no significant differences in the mean duration of the measured electrograms during autonomic stimulation or after autonomic blockade (Fig. 2). Nevertheless, we observed an increased heterogeneity of the atrial wave duration, with significant differences between the recording sites, appearing during ANS maneuvers, and abolished after ANS blockade (Fig. 2). Representative intracardiac

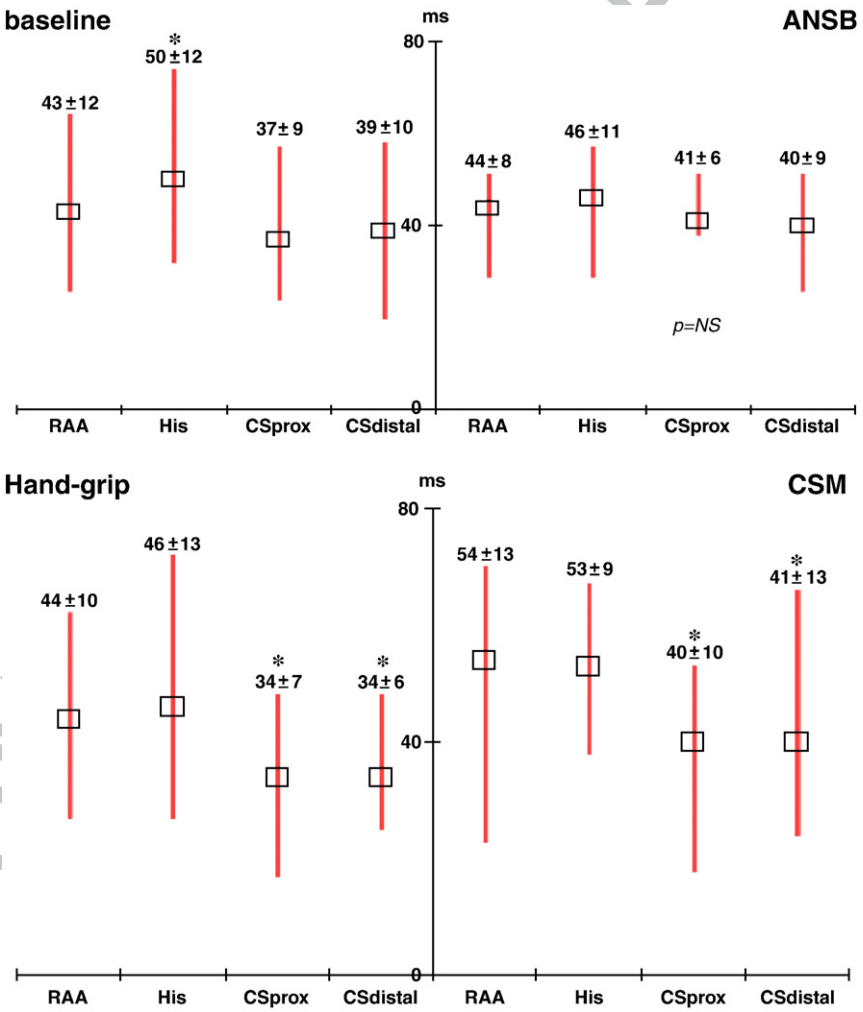

Fig. 2. Electrogram duration measured during sinus rhythm in baseline, with handgrip, carotid sinus massage (CSM) and after pharmacological blockade of autonomic activity (ANSB). *$p<0.05$ compared to other sites. Values expressed in milliseconds, mean ± standard deviation. The lines represent mean, maximum and minimum values. RAA = right atrial appendage; His = His position; CSprox = proximal coronary sinus; CSdistal = distal coronary sinus.

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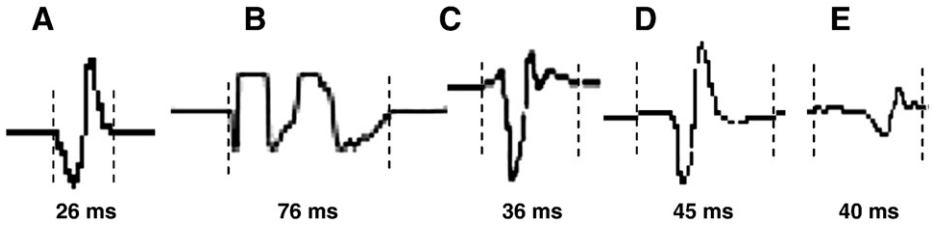

Fig. 3. Electrograms obtained from the right atrial appendage in a 72 years old woman with paroxysmal atrial fibrillation. Sinus rhythm (A), with the earliest propagated extra-stimulus (B), during handgrip (C) and carotid sinus massage (D), and after pharmacological autonomic blockade (E).

electrograms, obtained from the RAA during sinus rhythm, with S2, HG and CSM, and after ANSB are shown in Fig. 3.

4. Discussion

Although the triggers for AF initiation appear to be located in the pulmonary veins, established AF has been associated with conduction disturbances and heterogeneous reduction of ERP, that facilitate the occurrence of multiple reentry circuits within the atria, probably contributing to the electrophysiological substrate required for the presence of AF [3,6,18].

While prolongation of atrial conduction is a frequent finding in patients with AF [19,20], the influence of autonomic activity in the atrial conduction intervals and local wave duration is incompletely explored. The present study characterized the IACT, the interACT and electrogram duration measured in different atrial recording sites in response to an extra-stimulus with a short coupling interval and during acute modulation of the ANS. There were no differences between the baseline characteristics of PAF patients and controls. However, the group with history of PAF showed longer IACT and RAA electrograms in baseline, compared with control patients. Furthermore, they showed significant atrial conduction delays and greater prolongation of atrial wave duration during early premature impulses delivered at the HRA. Also, fragmented atrial activity was identified in more patients with PAF than in control patients. These findings are consistent with previous studies who demonstrated greater delays in intra-atrial or inter-atrial conduction, and a higher incidence of atrial fragmentation in patients with PAF [6,20–22]. In fact, the presence of marked conduction delay during an atrial premature beat with a short coupling interval, combined with longer and fractionated electrograms is an important electrophysiological finding, compatible with the necessary conditions for the occurrence and maintenance of local reentry circuits. Focal repetitive activity, most frequently originated from pulmonary veins, plays an important role in the initiation of AF, particularly when combined with abnormal atrial impulse conduction, which appears to be pre-requisite for the maintenance of AF [23,24]. Heterogeneity of atrial conduction delay and the presence of local fragmented potentials have long been associated with the substrate for AF [25–27]. The greater prolongation of atrial activation times and of local wave duration with the earliest propagated extra-stimulus, showed in our results, might contribute to explain why the mean coupling interval was significantly shorter for pulmonary veins discharges initiating AF than for discharges that did not in a recent study by Arentz et al. [28]. Therefore, a combination of atrial premature complexes with short coupling intervals and delayed activation of the atria may act as one component of the arrhythmogenic substrate for the vulnerability to PAF.

4.1. Autonomic modulation of conduction parameters in PAF patients

The supporting evidence of the impact of ANS activity in the electrophysiological properties of the atria and its role in the initiation and maintenance of AF has been mostly studied in experimental preparations. Little is known about the effects of acute stimulation or blockade of the ANS in atrial conduction and electrogram duration during electrophysiological evaluation of patients with PAF. In our data, obtained from patients with clinical history of PAF, the interACT and LAAT were significantly prolonged during CSM in the group with inducible sustained AF, supporting the notion that the substrate of AF is associated with conduction abnormalities of the atria, which can be more pronounced during vagal stimulation and contribute to the maintenance of AF. Although conduction abnormalities in PAF have been associated with increased age, atrial dilation and stretch, fibrosis, changes in the expression levels of connexins and electrophysiological remodeling [2,3,5–7,24], acute autonomic modulation seems to influence atrial conduction properties in patients with PAF.

It has been known that vagal stimulation shortens the atrial ERP and increases dispersion of atrial refractoriness [8,9,12,13]. Although both vagal and sympathetic stimulations could produce significant reductions on ERP, vagal stimulation appears more arrhythmogenic in promoting AF [29]. One reason could be related with the lengthening of atrial conduction time during vagal activity, that results in a pronounced wavelength shortening (ERP x conduction velocity), which would promote AF maintenance.

In the present study, we evaluated atrial electrograms duration during autonomic stimulation and after pharmacological autonomic blockade. Electrograms duration increased slightly in RAA and His during CSM, but no significant differences were obtained during acute autonomic modulation. However, when compared to baseline recordings, differences in atrial wave duration between the recording sites became more pronounced during ANS maneuvers and were abolished after ANS blockade. Although the impact of autonomic stimulation appears to be modest in the induction of AF (68.8% during ANS maneuvers vs. 50% after ANS blockade), it is possible that the electrogram duration heterogeneity and local conduction delay produced by autonomic modulation contribute to the initiation and maintenance of AF. Vagal stimulation has been found to result in a large regional heterogeneity of atrial electrograms, and there is evidence that the appearance of complex fractionated atrial electrograms during activation of the intrinsic cardiac autonomic neural elements reflects a change in the local electrophysiological properties [30,31]. In fact, differential areas of conduction velocity and dispersion of electrogram characteristics may provide a substrate for functional reentry, creating a suitable environment for AF [32,33].

All patients underwent programmed bipolar stimulation (drive-train cycle length of 600 ms using S2–S3 extra-stimuli delivered after eight paced beats) and incremental pacing protocols (short-term of burst pacing range from 600 to 300 ms) during sinus rhythm, by pacing from the distal electrode pair positioned at the HRA.

There have been limited data on autonomic influences in the characteristics of electrograms during sinus rhythm. Guo et al., in a canine model, found that vagal stimulation shortened the electrogram duration in ischemic myocardium zone in the right atrium, whereas sympathetic stimulation did not alter electrogram duration [34]. In a previous analysis of atrial electrograms during sinus rhythm in patients with PAF, electrograms with ≥ 4 deflections and duration ≥ 40 ms were associated with a parasympathetic response during AF ablation [35]. The explanation for this finding was related with local

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effects of acetylcholine in atrial tissue, causing conduction block between adjacent fiber bundles. Recently, in a different study, complex fractionated atrial electrograms, representing slow conduction areas or pivoting points in reentry circuits, were induced by local application of varying concentrations of acetylcholine or by injecting acetylcholine into the anterior right ganglionated plexi, providing evidence that ANS activity may induce changes in local atrial conduction [36].

There is a great need for experimental and clinical studies to better understand the relationship between the dynamic changes in atrial electrogram morphology and autonomic innervation and its role in the maintenance of AF.

5. Study limitations

Although it was possible to identify slight changes in atrial activation times and wave duration during acute autonomic modulation, the study included a small number of patients. However, all patients acted as their own controls to enable comparison of the parameters during stimulation and after blockade of the ANS. Although obtaining high density recordings by using multipolar catheters with better spatial resolution from several simultaneous right and left atrial sites could give more precise results, allowing a better comprehension of the problem, transseptal punctures for the use of left atrial catheters were not justifiable in a preliminary investigative study in humans. Another concern is that despite the confirmation of HG and CSM effects based on frequency domain spectral analysis, direct stimulation of sympathetic and parasympathetic nerves would have improved the results.

6. Conclusions

The presented study demonstrated that atrial conduction times, electrograms duration and fractionated activity are increased in patients with PAF when compared with control patients, suggesting that conduction abnormalities in the atria contribute to the arrhythmogenic substrate for AF. Also, acute vagal stimulation prolonged interACT and LAAT in patients with inducible sustained AF and ANS modulation influenced the heterogeneity of atrial electrograms duration in the recording sites. These should be taken into consideration in future studies in order to better understand the dynamic phenomena involved in the onset and perpetuation of AF episodes.

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The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology [37].

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Influência da Estimulação e Bloqueio do Sistema Nervoso Autónomo na Refractariedade Auricular em Doentes com Fibrilhação Auricular Paroxística Idiopática [51]

Mário Martins Oliveira, Nogueira da Silva, Joana Feliciano, Ana Timóteo, Fernando Marques, Sofia Santos, Isabel Rocha, Luís Silva-Carvalho, Rui Ferreira

Serviço de Cardiologia, Hospital de Santa Marta, Lisboa, Portugal
Unidade de Sistema Nervoso Autónomo, Instituto de Fisiologia, Faculdade de Medicina de Lisboa, Lisboa, Portugal

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RESUMO

A diminuição dos períodos refractários (PRE) e o aumento da dispersão da refractariedade (Disp_A) auriculares são marcadores de vulnerabilidade para recorrência de fibrilhação auricular (FA).
Objectivo: Estudar os efeitos da estimulação e bloqueio do Sistema Nervoso Autónomo (SNA) nos PRE e Disp_A em doentes (D) com ≥1 ano de evolução clínica de FA paroxística idiopática (FAp).
Métodos e Resultados: 10D (6 homens, 55±14 anos), submetidos a estudo electrofisológico sem sedação, após suspensão de antiarrítmicos. Os PRE foram avaliados em cinco locais (apêndice auricular direito - AAD -, aurícula direita lateral baixa - AD -, seio coronário proximal e distal - SCp e SCd - e septo interauricular alto - SIA -) durante *pacing* com ciclo básico de 600 ms e analisados em condições basais, com massagem do seio carotídeo (MSC) e *handgrip* (HG), e após bloqueio autonómico (BSNA) (atropina 0.04 mg/kg + propranolol 0.15 mg/kg). Os intervalos RR foram de 853±68 ms, 724±73 ms, 928±131 ms e 856±81 ms, respectivamente em basal, HG, MSC e após BSNA (basal *versus* HG, p<0,05). A pressão arterial (PA) sistólica aumentou durante HG (126±8 mmHg *versus* 135±10 mmHg, p<0,05).

ABSTRACT

Effects of stimulation and blockade of the autonomic nervous system on atrial refractoriness in patients with lone paroxysmal atrial fibrillation

Heterogeneous shortening of the atrial effective refractory period (AERP) and increased dispersion of refractoriness (disp_A) predispose to recurrent episodes of atrial fibrillation (AF).
Aim: To evaluate the effects of stimulation and blockade of the autonomic nervous system (ANS) on atrial refractoriness in patients with ≥1 year clinical history of lone paroxysmal AF (PAF).
Methods: Ten patients (6 men, aged 55±14 years) underwent electrophysiological study while off medication. AERP was assessed at 5 sites – right atrial appendage (RAA) and low lateral right atrium (LRA), high interatrial septum (IAS), proximal (pCS) and distal (dCS) coronary sinus in basal conditions, during handgrip (HG) and carotid sinus massage (CSM), and after ANS blockade (ANSB) (atropine 0.04 mg/kg + propranolol 0.15 mg/kg). The AERP was taken as the longest S1-S2 interval that failed to initiate a response. Disp_A was calculated as the difference between the longest and shortest

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Não se registaram diferenças significativas da PA durante MSC e BSNA. Os PRE foram de 208±15 ms, 212±22 ms, 252±43 ms, 256±37 ms e 246±31 ms, no AAD, AD, SIA, SCp e SCd, respectivamente (AAD *versus* SIA e SCp, p<0,05). Com MSC, os PRE diminuiram na AD e, após BSNA, houve aumento significativo no SCd. A Disp_A variou entre 70±39 ms em basal, 71±34 ms com HG, 75±46 ms com MSC e 54±37 ms após BSNA (p<0,05 para BSNA vs basal, HG e MSC). Os D com indução de FA tinham maior disp_A (70±15 ms *versus* 44±20 ms, p<0,05) e maior diminuição do PRE no AAD durante HG (11±9% *versus* 2±4%, p=0,02), sem diferenças relativamente aos PRE basais.

Conclusões: Em D com FAp ocorrem alterações dos PRE durante estimulação do SNA, enquanto o BSNA aumenta a refractariedade no SCd e diminui a Disp_A. No grupo com FA indutível, a Disp_A é maior e os PRE são mais curtos no AAD durante estimulação simpática. Estes dados reforçam a complexidade da influência autonómica nas alterações da refractariedade relacionadas com vulnerabilidade para FA.

Palavras-Chave

Fibrilhação auricular paroxística; Bloqueio autonómico; Estimulação autonómica; Refractariedade auricular.

AERP. Results: RR intervals were 853±68 ms, 724±73 ms, 928±131 ms and 856±81 ms in basal conditions, HG, MSC and ANSB respectively (p<0.05 for basal vs. HG). Systolic blood pressure (BP) increased significantly during HG (from 126±8 mmHg to 135±10 mmHg, p<0.05), but there were no significant differences in BP values during CSM and ANSB. The AERPs were 208±15 ms, 212±22 ms, 252±43 ms, 256±37 ms and 246±31 ms, in RAA, LRA, IAS, pCS and dCS respectively (RAA vs. IAS and pCS, p<0.05). AERPs decreased significantly in LRA during CSM, and increased in dCS after ANSB. Disp_A was 70±39 ms in basal conditions, 71±34 ms during HG, 75±46 ms with CSM, and 54±37 ms after ANSB (p<0.05 for ANSB vs. all others). Patients with inducible AF had greater disp_A (70±15 ms vs. 44±20 ms, p<0.05) and a larger reduction of AERP in RAA during HG (11±9% vs. 2±4%, p=0.02), with no significant differences in basal AERP.

Conclusion: In patients with PAF, ANS stimulation alters AERP, whereas ANSB increases AERP in dCS and decreases disp_A. Patients with inducible AF show greater disp_A and shorter AERP in RAA during sympathetic stimulation. These findings highlight the complexity of the influence of the ANS on alterations in refractoriness related to vulnerability to AF.

Key words

Paroxysmal atrial fibrillation; Autonomic blockade; Autonomic stimulation; Atrial refractoriness.

INTRODUÇÃO

A fibrilhação auricular (FA) é a arritmia cardíaca mantida mais comum na prática clínica. A sua prevalência varia entre aproximadamente 1% na população geral e 5% acima dos 65 anos, vindo a aumentar com o envelhecimento da população[1-3]. O reconhecimento do impacto desfavorável que representa para a qualidade de vida, morbilidade e mortalidade[4,5], combinado com o esforço crescente no desenvolvimento da compreensão dos mecanismos subjacentes

INTRODUCTION

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia in clinical practice. Its prevalence ranges between 1% in the general population and 5% in those aged over 65 years, and is increasing with aging populations[1-3]. Recognition of the negative impact of AF on quality of life, morbidity and mortality[4,5] has led to increasing efforts to improve understanding of the mechanisms underlying the pathophysiology, of this arrythmia.

à fisiopatologia desta condição clínica tem constituído um desafio clínico considerável. A FA paroxística (caracterizada por ter duração <7 dias e ser, habitualmente, de terminação espontânea) é considerada idiopática em cerca de metade dos casos[6]. Apesar das limitações associadas ao completo esclarecimento da sequência de fenómenos responsáveis pela recorrência de FA, tem sido sugerido que, em particular na FA idiopática, o sistema nervoso autónomo (SNA) possa desempenhar um importante papel como modulador na génese, manutenção e interrupção de episódios de FA paroxística[7-10]. A demonstração de que os períodos refractários efectivos auriculares (PRE) e o aumento da sua dispersão espacial proporcionam um substrato electrofisiológico para a reentrada de múltiplas ondas de propagação (*wavelets*), tem contribuído para o interesse do estudo destas propriedades eléctricas e sua importância nos episódios de FA. A refractariedade auricular encurta de modo heterogéneo com a estimulação vagal, enquanto a estimulação simpática aumenta a actividade *trigger*, facilitando a ocorrência de despolarizações rápidas repetitivas, e reduzindo a refractariedade auricular de modo uniforme[7-9,11]. Deste modo, as flutuações do tónus autonómico podem ser determinantes na ocorrência FA paroxística. A remodelagem eléctrica, resultante da exposição do tecido auricular a frequências rápidas, contribui também para o encurtamento dos PRE, com aumento da dispersão espacial da refractariedade, condicionado uma maior vulnerabilidade para recorrência e perpetuação de FA[12-13]. Permanece por esclarecer a influência do SNA na refractariedade auricular em humanos com história clínica prolongada de episódios recorrentes de FA. No presente estudo, avaliámos os efeitos da estimulação e bloqueio do SNA nos PRE e dispersão da refractariedade auricular em doentes com FA paroxística idiopática, com evolução clínica superior a 1 ano.

POPULAÇÃO E MÉTODOS

Foram estudados 10 doentes (6 homens e 4 mulheres) com idade média de 55±14 anos (entre 25 e 66 anos), referenciados para estudo electrofisiológico (EEF) e terapêutica ablativa de FA, por episódios recorrentes de FA paroxística com ≥1 ano de evolução clínica, documentada

Paroxysmal AF (defined as lasting less than seven days, and usually terminating spontaneously) is considered idiopathic (lone) in around half of cases[6]. Despite limitations in understanding of the sequence of phenomena responsible for recurrence of AF, it has been suggested that the autonomic nervous system (ANS) may play an important role in the genesis, perpetuation and termination of episodes of paroxysmal, particularly lone, AF[7-10]. Evidence that atrial effective refractory periods (AERPs) and increased spatial dispersion of refractoriness provide an electrophysiological substrate for multiple reentry wavelets has aroused interest in studying these electrical properties and their importance in episodes of AF. Vagal stimulation leads to heterogeneous shortening of atrial refractoriness, while sympathetic stimulation acts as a trigger mechanism, by promoting repeated rapid depolarizations and reducing atrial refractoriness in a uniform manner[7-9,11]. Fluctuations in autonomic tone may thus be a determining factor in paroxysmal AF. The electrical remodeling resulting from the exposure of atrial tissue to rapid frequencies also contributes to shortening of AERPs, with increased spatial dispersion of refractoriness, which leads to greater vulnerability to recurrence and perpetuation of AF[12,13]. The influence of the ANS on atrial refractoriness in humans with a long clinical history of recurrent AF remains to be clarified. This study set out to evaluate the effects of stimulation and blockade of the ANS on AERPs and dispersion of refractoriness in patients with ≥1 year clinical history of lone paroxysmal AF.

METHODS

Ten patients (6 men and 4 women), mean age 55±14 years(25-66), were assessed following referral for electrophysiological study (EPS) and ablation therapy due to ≥1 year clinical history of recurrent episodes of paroxysmal AF, documented by electrocardiography and/or Holter monitoring, despite antiarrhythmic drug therapy. None of the patients showed evidence of underlying heart disease on physical examination, chest X-ray, myocardial ischemia testing or echocardiography. Patients with hypertension, sinus node disease, permanent pacemaker, obstructive pulmonary disease,

em electrocardiogramas e/ou registo de Holter, apesar de terapêutica farmacológica antiarrí-tmica. Nenhum dos doentes apresentava evidência de cardiopatia subjacente após exame objectivo clínico, radiografia do tórax, prova de isquemia miocárdica e ecocardiograma. Foram excluídos casos com hipertensão arterial, doença do nódulo sinusal, portadores de *pacemaker* definitivo, doença pulmonar obstrutiva, apneia do sono, diabetes *mellitus*, disfunção tiróideia ou com FA durante o período de monitorização electrocardiográfica intrahospitalar que precedeu o EEF.

A história clínica de FA paroxística tinha uma duração variando entre 1 e 5 anos (2.6±2.0 anos), com recurso prévio a diferentes antiarrítmicos (2±0.7/doente). A medicação antiarrítmica foi suspensa cinco semi-vidas antes do procedimento. No caso da amiodarona, interrompeu-se a terapêutica dois meses antes, substituindo-se por fármaco de semi-vida mais curta. Não havia utilização de outra medicação do foro cardiovascular. O protocolo do estudo foi efectuado de acordo com as recomendações da Declaração de Helsínquia e aprovado pela Comissão de Ética do Hospital de Santa Marta. O EEF foi realizado após autorização obtida em termo de consentimento informado.

Estudo electrofisológico

O EEF foi efectuado após jejum de seis horas, em ambiente com controlo de temperatura (21º C) e sem sedação. Não se detectaram anomalias do ionograma sérico. Para registo de electrogramas e estimulação eléctrica, utilizámos electrocateteres multipolares 6F (pólos com intervalos de 2 mm; Daig Co), introduzidos por via percutânea através das veias femural e jugular interna. Um cateter quadripolar foi colocado no apêndice auricular direito (AAD) e, durante o protocolo, posicionado na aurícula direita lateral-baixa (AD lateral), no septo interauricular alto (SIA) ou apex do ventrículo direito. Um segundo cateter quadripolar foi utilizado para registo em posição hisiana e um cateter decapolar (*Daig Co*) foi colocado ao longo do seio coronário (SC), até à posição mais distal. Os electrogramas e o ECG foram registados num polígrafo de 32 canais (*Bard Lab System*), com frequência de resposta de 50 a 500 Hz e gravados em sistema de disco óptico.

sleep apnea, diabetes, or thyroid dysfunction were excluded, as were those with AF during in-hospital electrocardiographic monitoring prior to EPS.

Their clinical history of paroxysmal AF varied between 1 and 5 years (2.6±2.0), with use of different antiarrhythmics (2±0.7/patient). Antiarrhythmics were suspended five half-lives before the procedure. Amiodarone therapy was suspended two months prior to EPS and replaced by a drug with a shorter half-life. No patient was taking any other cardiovascular medication. The study protocol was carried out in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Hospital de Santa Marta. Informed consent was obtained before EPS was performed.

Electrophysiological study

EPS was performed after 6 hours fasting in a temperature-controlled environment (21º C) and without sedation. No serum electrolyte abnormalities were detected. For recording and electrical stimulation, 6F multipolar catheters with 2 mm interelectrode spacing were used (Daig Corp.), introduced percutaneously via the femoral and internal jugular veins. A quadripolar catheter was placed in the right atrial appendage (RAA) and, during the protocol, was positioned in the low lateral right atrium (LRA), the high interatrial septum (IAS) or the right ventricular apex. A second quadripolar catheter was used for recording in the para-Hisian position, while a decapolar catheter (Daig Corp.) was placed along the coronary sinus (CS) up to the most distal position. The electrograms and ECGs were recorded on a 32-channel polygraph (Bard LabSystem) with a frequency response of 50 to 500 Hz and saved onto optical disc.

The EPS included assessment of basal conduction intervals and sinus and atrioventricular node function, exclusion of accessory pathways, and inducibility of supraventricular arrhythmias that could degenerate into AF, using the standard methodology as described in the literature[14-16].

AERPs were assessed at five different sites: RAA, LRA, IAS, and proximal and distal CS in basal conditions, during ANS stimulation maneuvers and after pharmacological ANS blockade (atropine 0.04 mg/kg + propranolol 0.15 mg/kg). Sympathetic stimulation was

As etapas do EEF incluíram a avaliação dos intervalos de condução basais, da função dos nódulos sinusal e aurículo-ventricular, exclusão de via(s) acessória(s) e inducibilidade de arritmias supraventriculares com possibilidade de degenerar em FA, segundo metodologia habitual, descrita em detalhe na literatura[14-16].

Os PRE foram avaliados em cinco locais diferentes: AAD, AD lateral, SIA, SC proximal e SC distal em condições basais, durante manobras provocativas da actividade do SNA e após obtenção de bloqueio autonómico farmacológico (atropina 0.04 mg/kg + propranolol 0.15 mg/kg). A estimulação da actividade simpática foi efectuada com recurso a três minutos de *hand-grip* (HG) estático, intermitente, de intensidade submáxima até à fadiga e a actividade vagal evocada através de massagem do seio carotídeo (MSC) direito, com pressão mantida no ponto de maior percepção do pulso carotídeo, ao nível da cartilagem cricóide (durante 10s, alternando com intervalos de 10s, em períodos de três minutos). Na ausência de resposta, a MSC era repetida à esquerda. Para confirmação da estimulação e bloqueio da actividade autonómica, utilizámos monitorização contínua do ECG e pressão

achieved by 3 minutes of static, intermittent handgrip (HG) of submaximal intensity until fatigue set in, and vagal activity was induced by right carotid sinus massage (CSM), with pressure applied at the point of strongest pulse at the level of the cricoid cartilage (for 10 s at 10-s intervals in 3-min periods). In the absence of a response, CSM was repeated on the left side. Continuous ECG and blood pressure (BP) monitoring, together with spectral analysis of RR intervals in the frequency domain (Task Force Monitor 3040i; CNSystems), were used to confirm ANS stimulation or blockade. The frequency spectrum was divided into three components: very low frequency (VLF) (0-0.04 Hz), low frequency (LF) (0.04-0.15 Hz) and high frequency (HF) (0.15-0.4 Hz). HF values, attributed to vagal modulation, are affected by mechanical stimulation of the carotid sinus, while LF values mainly reflect sympathetic activity and increase during HG (Figure 1). Intravenous administration of propranolol and atropine resulted in total suppression of HF and LF activity, thus enabling assessment of the intrinsic electrophysiological properties (*Figure 1*).

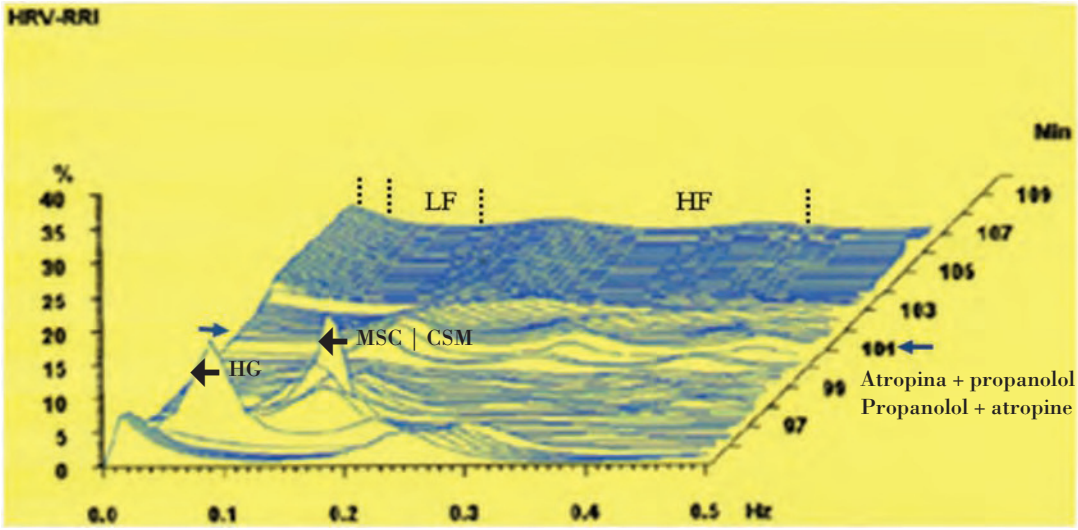

Figura 1. Imagem do registo parcial da monitorização contínua da variabilidade dos intervalos RR obtida por análise espectral (Task Force Monitor, CNSystems) nas fases pré e pós-bloqueio autonómico farmacológico. As setas assinalam as manobras de hand-grip (HG), massagem do seio carotídeo (MSC) e a administração de propanolol e atropina. LF=baixa frequência (0.04-0.15 Hz), HF=alta-frequência (0.15-0.40 Hz).

Figure 1. Part of a recording during continuous monitoring of RR interval variability obtained by spectral analysis (Task Force Monitor, CNSystems) before and after autonomic blockade. The arrows indicate handgrip (HG), carotid sinus massage (CSM) and administration of propanolol and atropine. LF: low frequency (0.04-0.15 Hz), HF: high frequency (0.15-0.40 Hz).

arterial e análise espectral dos intervalos RR no domínio da frequência (*Task Force Monitor 3040i;CNSystems*). O espectro das frequências foi dividido em três componentes básicos: muito baixa frequência (VLF) – de 0 a 0,04 Hz, baixa frequência (LF) – de 0,04 a 0,15 Hz e alta frequência (HF) – de 0,15 a 0,4 Hz. Os valores superiores a 0,15 Hz, atribuídos à modulação pelo vago, são influenciados pela estimulação mecânica do seio carotídeo, enquanto a banda LF, se relaciona, sobretudo, com a actividade simpática aumentando durante a manobra de HG (*Figura 1*). Após administração e.v. de propranolol e atropina registou-se abolição total da actividade dos componentes HF e LF permitindo o estudo de propriedades electrofisiológicas intrínsecas (*Figura1*).

Em condições estáveis, introduziu-se um extra-estímulo (2 ms de duração com o dobro da amplitude do limiar de captura) durante *pacing* contínuo com ciclo de 600 ms. O intervalo de acoplamento inicial foi 100 ms inferior ao do ciclo basal de *pacing*, decrescendo em intervalos de 10 ms até atingir o PRE. Para avaliação da vulnerabilidade para indução de FA, procedemos a estimulação auricular programada (ciclo de 600 ms até dois extra--estímulos) e *pacing* incremental (contínuo, 600 a 300 ms, durante 5 s), a partir do bipolo distal, situado no AAD e SC distal. O maior intervalo de acoplamento não seguido de propagação do impulso foi considerado como o PRE naquele ponto. A dispersão da refractariedade auricular foi calculada como a diferença entre o PRE mais longo e o mais curto obtidos nos diferentes locais avaliados. A vulnerabilidade auricular foi definida como a possibilidade de induzir FA com duração ≥10 s[17].

Análise estatística

As variáveis contínuas foram expressas sob a forma de média±desvio padrão, em termos absolutos ou como percentagem de variação em relação aos valores basais, e as variáveis categóricas em frequências e percentagens. As comparações intergrupos foram efectuadas pelo teste t de Student (não emparelhado) e análise de variância ANOVA seguida pelo teste de Dunnet para as variáveis contínuas. Para a comparação de variáveis contínuas sem distribuição normal usámos o teste de Mann-Whitney. O teste

In stable conditions, an extrastimulus (2 ms at twice the amplitude of the capture threshold) was introduced during continuous pacing with a cycle length of 600 ms. The initial coupling interval was 100 ms shorter than the basal pacing cycle, and was then reduced in 10-ms steps until the AERP was reached. To assess vulnerability to AF induction, programmed atrial stimulation (600 ms cycle, delivering up to 2 extrastimuli) and incremental pacing (continuous incremental pacing, 600-300 ms, for 5 s) were then performed using the distal bipole situated in the RAA and distal CS. The longest coupling interval not followed by a propagation response was taken as the AERP at that site. Dispersion of atrial refractoriness was calculated as the difference between the longest and shortest AERP at the different sites assessed. Atrial vulnerability was defined as the ability to induce AF with a duration of ≥10 s[17].

Statistical analysis

Continuous variables were expressed as means ± standard deviation and compared in absolute terms or as a percentage variation from basal values, with categorical variables expressed as frequencies and percentages. Comparisons between groups were made using the unpaired Student's t test and ANOVA followed by Dunnett's test for continuous variables. The Mann-Whitney test was used to compare continuous variables with a non-normal distribution, and the chi-square test with Yates correction was used for categorical variables. Results with $p<0.05$ were considered significant. The statistical package used was GraphPad version 3.05 (GraphPad Software Inc., California, USA).

RESULTS

Values of basal conduction intervals and for sinus and atrioventricular node function are shown in *Table I*. Mild disturbance of atrioventricular supra-Hisian conduction or slight prolongation of infra-Hisian conduction were documented in two patients. The presence of accessory pathways was excluded in all cases. A self-limited counterclockwise cavo-tricuspid isthmus-dependent atrial flutter was induced in one patient and was successfully ablated the

Table I. **Demographic characteristics, basal intervals, and sinus and atrioventricular node function.**

Patient	Age	Gender	Supra-His	Intra-His	Infra-His	cSNRT	WP	AVNERP	IntraA	InterA
1	52	M	98	20	40	256	350	320	28	78
2	75	M	94	20	55	258	375	240	50	108
3	57	F	90	20	65	258	360	250	40	92
4	69	F	124	18	45	368	560	550	54	88
5	43	M	78	19	45	0	340	360	24	103
6	54	M	70	18	45	240	300	320	22	72
7	23	M	56	14	40	242	260	300	60	66
8	70	F	100	19	45	440	420	440	20	118
9	60	M	118	15	44	440	400	390	36	56
10	55	F	48	13	55	172	280	280	38	67

AVNERP: atrioventricular node effective refractory period (at pacing cycle length of 600 ms); cSNRT: corrected sinus node recovery time; Infra-His: infra-Hisian conduction; InterA: interatrial conduction in sinus rhythm; IntraA: intra-atrial conduction in sinus rhythm; Intra-His: intra-Hisian conduction; Supra-His: supra-Hisian conduction; WP: Wenckebach point. Age expressed in years, other data in milliseconds.

de $\chi 2$, com correcção de Yates, foi utilizado para as variáveis categóricas. Considerámos estatisticamente significativos os resultados com $p<0,05$. O programa estatístico utilizado foi o GraphPAD na versão 3.05 (*GraphPad Software,Inc.,California, USA*).

RESULTADOS

Os valores dos intervalos de condução basais e da avaliação da função dos nódulos sinusal ou aurículo-ventricular estão descritos na *Tabela I*. Documentou-se ligeira perturbação da condução aurículo-ventricular suprahisiana ou prolongamento ligeiro da condução infrahisiana em dois doentes. Excluiu-se a presença de via acessória em todos os casos. Num doente, foi induzido *flutter* auricular anti-horário istmo cavo-tricúspide dependente auto-limitado, submetido a ablação com sucesso no final da avaliação electrofisiológica.

electrophysiological study protocol.

The RR intervals were 853±68 ms, 724±73 ms, 928±131 ms and 856±81 ms respectively in basal conditions, during HG and CSM, and after pharmacological ANS blockade (basal vs. HG, $p<0.05$) (Figure 2A). Systolic BP increased with HG and CSM, reaching statistical significance during HG (from 126±8 mmHg to 135±10 mmHg, $p<0.05$), with no differences observed after ANS blockade (*Figure 2B*). There were no statistically significant changes in diastolic BP during ANS stimulation or blockade compared to basal values. Double product (heart rate x blood pressure) increased significantly during HG, showing highly significant differences between the various stages of the protocol (CSM vs. HG, $p<0.001$; CSM and HG vs. ANS blockade, $p<0.01$). No patient presented significant ventricular pauses or falls in BP of ≥50 mmHg during CSM. Supraventricular ectopic beats, isolated or in salvos, were recorded in 70% of patients (spontaneous in 5, during HG in 3, and during CSM in 2), without triggering AF.

Tabela I. **Características demográficas, intervalos basais e avaliação da função do nódulo sinusal e nódulo aurículo-ventricular.**

Doente	idade	sexo	AH	H	HV	TRNSc	PW	PRENAV	intraA	interA
1	52	M	98	20	40	256	350	320	28	78
2	75	M	94	20	55	258	375	240	50	108
3	57	F	90	20	65	258	360	250	40	92
4	69	F	124	18	45	368	560	550	54	88
5	43	M	78	19	45	0	340	360	24	103
6	54	M	70	18	45	240	300	320	22	72
7	23	M	56	14	40	242	260	300	60	66
8	70	F	100	19	45	440	420	440	20	118
9	60	M	118	15	44	440	400	390	36	56
10	55	F	48	13	55	172	280	280	38	67

M=masculino; F=feminino; AH=condução suprahisiana; H=condução intrahisiana; HV=condução infrahisiana; TRNSc=tempo de recuperação do nódulo sinusal corrigido; PW=ponto de Wenckbach; PRENAV=período refractário efectivo do nódulo aurículo-ventricular (ciclo base de 600 ms); ntraA=condução intraauricular em ritmo sinusal; interA=condução interauricular em ritmo sinusal (idade expressa em anos, restantes dados em milisegundos)

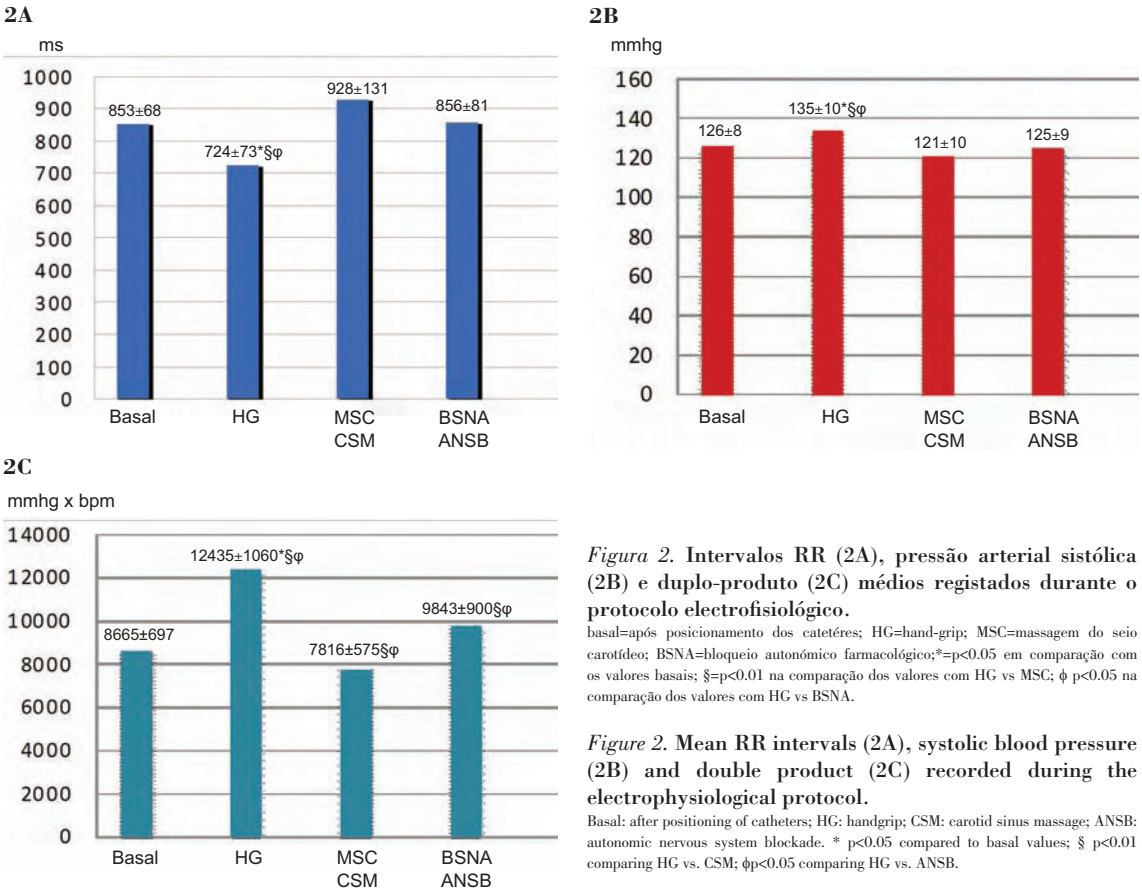

Figura 2. **Intervalos RR (2A), pressão arterial sistólica (2B) e duplo-produto (2C) médios registados durante o protocolo electrofisiológico.**
basal=após posicionamento dos catetéres; HG=hand-grip; MSC=massagem do seio carotídeo; BSNA=bloqueio autonómico farmacológico;*=p<0.05 em comparação com os valores basais; §=p<0.01 na comparação dos valores com HG vs MSC; ϕ p<0.05 na comparação dos valores com HG vs BSNA.

Figure 2. **Mean RR intervals (2A), systolic blood pressure (2B) and double product (2C) recorded during the electrophysiological protocol.**
Basal: after positioning of catheters; HG: handgrip; CSM: carotid sinus massage; ANSB: autonomic nervous system blockade. * p<0.05 compared to basal values; § p<0.01 comparing HG vs. CSM; ϕp<0.05 comparing HG vs. ANSB.

Os intervalos RR foram de 853±68 ms, 724±73 ms, 928±131 ms e 856±81 ms, respectivamente em basal, durante HG, MSC e após bloqueio autonómico farmacológico (basal *versus* HG, p<0,05) (*Figura 2A*). Ocorreu variação da pressão arterial sistólica com as manobras de HG e MSC, atingindo significado estatístico durante HG (126±8 mmHg *versus* 135±10 mmHg, p<0,05), sem registo de diferenças após bloqueio autonómico (*Figura 2B*). A pressão arterial diastólica não teve diferenças estatisticamente significativas durante estimulação ou bloqueio do SNA quando comparada com o basal. O duplo-produto (frequência cardíaca x pressão arterial) aumentou significativamente com a manobra de HG e mostrou diferenças muito significativas na comparação entre as diferentes fases do protocolo (MSC *versus* HG, p<0,001; MSC e HG *versus* bloqueio autonómico, p<0,01). Nenhum doente apresentou pausas ventriculares significativas ou queda da pressão arterial ≥50 mmHg durante MSC. Documentaram-se ectopias supraventriculares, isoladas ou em salvas, em 70% dos doentes (5 com ectopias

Atrial refractoriness

In basal conditions, AERP increased progressively from RAA and LRA to IAS and proximal and distal CS (RAA vs. IAS and proximal CS, p=0.01) (*Table II*). HG was not associated with significant changes in AERP (*Table II*). During CSM, AERP decreased significantly in LRA compared to basal values (212±22 ms vs. 188±21 ms, p<0.05) (*Table II*). A significant increase of AERP in the distal CS was recorded after ANS blockade (246±31 ms vs. 265±37 ms, p<0.05) (*Table II*). Dispersion of refractoriness varied between 70±39 ms in basal conditions, 71±34 ms with HG, 74±46 ms with CSM, and 54±37 ms after ANS blockade (ANS blockade vs. basal, HG and MSC, p<0.05) (*Figure 3A*).

Vulnerability to atrial fibrillation induction

AF was induced in 50% of the patients with this protocol. There were no statistically significant differences between patients with and

espontâneas, 3 durante HG e 2 durante MSC), sem desencadearem episódios de FA.

Refractariedade auricular

Em condições basais, os PRE aumentaram progressivamente do AAD e AD lateral para o SIA, SC proximal e distal (AAD *versus* SIA e SC proximal, p=0,01) (*Tabela II*). A manobra de HG não se associou a modificação significativa dos PRE (*Tabela II*). Durante a MSC, os PRE diminuiram significativamente na AD lateral, quando comparado com os valores basais (212±22 ms *versus* 188±21 ms, p<0,05) (*Tabela II*). Após bloqueio autonómico, registou-se aumento significativo dos PRE no SC distal (246±31 ms *versus* 265±37 ms, p<0,05) (*Tabela II*). A dispersão da refractariedade variou entre 70±39 ms no registo basal, 71±34 ms com HG, 74±46 ms com MSC e 54±37 ms após bloqueio autonómico (bloqueio do SNA *versus* basal, HG e MSC, p<0,05) (*Figura 3A*).

Tabela II. **Períodos refractários efectivos avaliados em cinco localizações auriculares em condições basais, durante *hand-grip* (HG), massagem do seio carotídeo (MSC) e após bloqueio autonómico farmacológico (BSNA).**

PRE (ms)	Basal	HG	MSC	BSNA
AAD	208±15	206±34	205±16	213±17
AD lateral-baixa	212±22	213±23	188±21*	212±15
SIA alto	§252±43	234±40	232±48	241±41
SCp	§256±37	238±28	243±28	240±20
SCd	246±31	242±32	247±43	§264±35*

PRE=período refractário efectivo; AAD=apêndice auricular direito; AD=aurícula direita; SIA=septo interauricular; SCp=seio coronário proximal; SCd=seio coronário distal (valores expressos em milissegundos, média±desvio padrão). * p<0.05 na comparação com o PRE basal; § p<0.05 na comparação do PRE na mesma condição.

Vulnerabilidade para indução de fibrilhação auricular

Com o protocolo utilizado, induziu-se FA em 50% dos doentes. Não havia diferenças estatísticamente significativas entre os grupos relativamente às características clínicas (*Tabela III*), nem nos valores de PRE obtidos nos cinco locais avaliados (*Tabela IV*). No entanto, com a manobra de HG obteve-se uma maior redução dos PRE nos doentes com vulnerabilidade para indução de FA (11±9% *versus* 2±4%, p=0,02). O grupo com FA indutível tinha valores de dispersão da refractariedade auricular em condições basais significativamente superiores aos do grupo sem indução de FA (70±15 ms *versus*

Table II. **Effective refractory periods assessed at 5 atrial sites in basal conditions, during handgrip (HG) and carotid sinus massage (CSM) and after autonomic nervous system blockade (ANSB).**

AERP (ms)	Basal	HG	CSM	ANSB
RAA	208±15	206±34	205±16	213±17
Low lateral RA	212±22	213±23	188±21*	212±15
High IAS	§252±43	234±40	232±48	241±41
pCS	§256±37	238±28	243±28	240±20
dCS	246±31	242±32	247±43	§264±35*

AERP: atrial effective refractory period; dCS: distal coronary sinus; IAS: interatrial septum; pCS: proximal coronary sinus; RAA: right atrial appendage; RA: right atrium. Values expressed in milliseconds, mean ± standard deviation. * p<0.05 compared to basal AERP; § p<0.05 comparing AERPs under the same conditions.

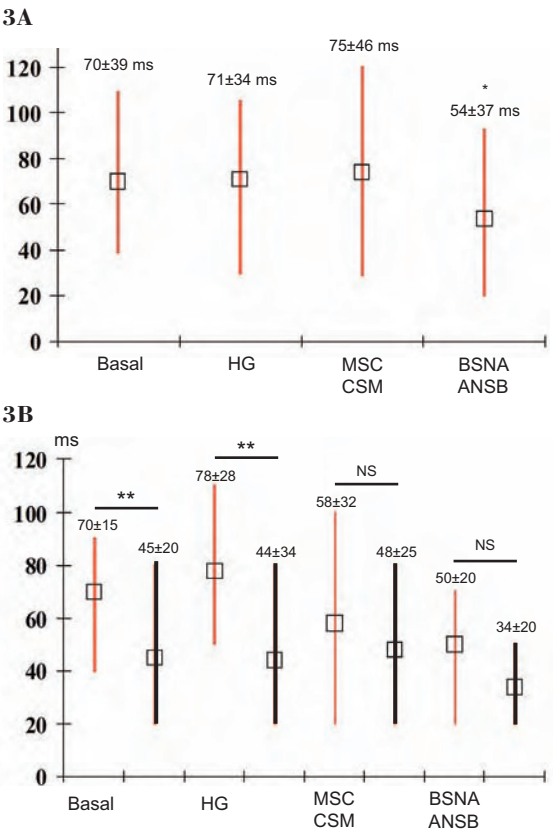

Figura 3. **Dispersão da refractariedade auricular em condições basais, durante hand-grip (HG), massagem do seio carotídeo (MSC) e após bloqueio autonómico farmacológico (BSNA) . 3A – valores obtidos no total dos doentes estudados (n=10). 3B – comparação entre o grupo com indução de fibrilhação auricular (linha vermelha) e o grupo sem indução de fibrilhação auricular (linha preta).** (valores expressos em milissegundos, média±desvio padrão. As linhas representam a média, valores máximos e mínimos em cada fase do protocolo). *=p<0.05 na comparação com basal, HG e MSC; **=p<0.05 na comparação entre os 2 grupos; ns=não significativo.

Figure 3. **Dispersion of atrial refractoriness in basal conditions, during handgrip exercise (HG) and carotid sinus massage (CSM), and after autonomic nervous system blockade (ANSB). 3A: Values for all patients studied (n=10). 3B: Comparison between the group with (red line) and the group without atrial fibrillation induction (black line).**

Values expressed in milliseconds, mean ± standard deviation. The lines represent mean, maximum and minimum values at each stage of the protocol. * p<0.05 compared to basal conditions, HG and CSM; ** p<0.05 comparing the two groups; NS: non-significant

45±20 ms; p<0,05). A diferença na dispersão dos PRE manteve-se durante a estimulação com HG, perdendo o significado estatístico com a MSC e após bloqueio autonómico (*Figura 3B*). A dispersão da refractariedade auricular foi >40 ms em 40% dos doentes sem vulnerabilidade para indução de FA e em 100% dos doentes com FA indutível (p=0,08).

Tabela III. **Características clínicas e tamanho da auricula esquerda (ecocardiograma modo M, incidência paraesternal) nos doentes com e sem vulnerabilidade para indução de fibrilhação auricular (FA) durante o protocolo do estudo electrofisiológico.**

	com indução de FA (n=5)	sem indução de FA (n=5)	p
idade (anos)	56±12,1	52,5±17,6	ns
sexo masculino	4(80%)	3(60%)	ns
AE (modo M;mm)	42,8±2,2	40,6±3,1	ns
AE >22mm/m^2	3(60%)	1(20%)	ns
duração da FA (anos)	2.3±2.0	2.0±1.9	ns
nº prévio de antiarrítmicos	1.7±0.9	1.6±0.8	ns

AE=auricula esquerda; ns=não significativo.

DISCUSSÃO

Este estudo foi efectuado para avaliar o impacto da estimulação autonómica, através de manobras provocativas como a MSC e o HG, e do bloqueio farmacológico dos receptores do SNA nos valores dos PRE e dispersão da refractariedade, em doentes com história clínica de longa duração consistindo em episódios recorrentes de FA paroxística. Estudámos ainda a possibilidade da vulnerabilidade para indução de FA, durante o EEF, se associar a respostas diferentes nas modificações da refractariedade auricular relacionadas com o SNA. Os resultados

without AF induction in clinical characteristics (*Table III*) or in AERP values at the five sites assessed (*Table IV*). However, there was a greater reduction in AERP during HG in patients with vulnerability to AF induction (11±9% vs. 2±4%, p=0.02). Those with inducible AF had significantly higher values for dispersion of atrial refractoriness in basal conditions than those without (70±15 ms vs. 45±20 ms, p<0.05). The differences in dispersion remained during stimulation with HG, but failed to reach statistical significance with CSM or after ANS blockade (Figure 3B). Dispersion of refractoriness was >40 ms in 40% of the patients without vulnerability to AF induction and in 100% of those with inducible AF (p=0.08).

Table III. **Clinical characteristics and left atrial size (assessed by M-mode echocardiography in parasternal view) of patients with and without vulnerability to AF induction during electrophysiological study.**

	with AF induction (n=5)	without AF induction (n=5)	p
Age (years)	56±12.1	52.5±17.6	NS
Male	4 (80%)	3 (60%)	NS
LA (M-mode; mm)	42.8±2.2	40.6±3.1	NS
LA >22 mm/m2	3 (60%)	1 (20%)	NS
Duration of AF (years)	2.3±2.0	2.0±1.9	NS
No. of previous antiarrhythmics	1.7±0.9	1.6±0.8	NS

LA: left atrium; NS: non-significant

Tabela IV. **Comparação dos períodos refractários efectivos avaliados em cinco localizações auriculares em condições basais, durante *hand-grip* (HG), massagem do seio carotídeo (MSC) e após bloqueio autonómico farmacológico (BSNA) nos doentes com (grupo I, n=5) e sem (grupo II, n=5) vulnerabilidade para indução de fibrilhação auricular.**

	Basal		HG		MSC		BNSA	
PRE (ms)	**grupo I**	**grupo II**	**grupo I**	**grupo II**	**grupo I**	**grupo II**	**grupo I**	**grupo II**
AAD	208±11	215±29	184±27*	217±32	202±22	210±29	210±12	205±25
AD lateral-baixa	206±23	207±21	208±28	227±55	203±33	195±17	210±14	210±17
SIA alto	247±28	226±46	230±22	208±47	243±15	218±50	245±9	215±22
SCp	250±28	252±34	238±29	246±38	228±46	246±38	245±8	230±26
SCd	250±26	244±50	243±13	252±55	243±35	240±46	255±14	221±36

PRE=período refractário efectivo; AAD=apêndice auricular direito; AD=aurícula direita; SIA=septo interauricular; SCp=seio coronário proximal; SCd=seio coronário distal (valores expressos em milissegundos, média±desvio padrão).
* p=0.05 na comparação com o PRE do grupo II e correspondendo a uma diminuição significativa do PRE durante hand-grip (12±9% no grupo I vs 2±4% no grupo II, p=0.02).

DISCUSSION

The aim of this study was to evaluate the effects of autonomic stimulation through HG and CSM maneuvers, and of pharmacological ANS blockade on AERP and dispersion of refractoriness, in patients with a long clinical

Table IV. **Comparison of effective refractory periods assessed at 5 atrial sites in basal conditions, during handgrip (HG) and carotid sinus massage (CSM) and after autonomic nervous system blockade (ASNB) in patients with (group I, n=5) and without (group II, n=5) vulnerability to AF induction.**

	Basal		HG		CSM		ANSB	
AERP (ms)	**group I**	**group II**	**group I**	**group II**	**group I**	**group II**	**group I**	**group II**
RAA	208±11	215±29	184±27*	217±32	202±22	210±29	210±12	205±25
Low lateral RA	206±23	207±21	208±28	227±55	203±33	195±17	210±14	210±17
High IAS	247±28	226±46	230±22	208±47	243±15	218±50	245±9	215±22
pCS	250±28	252±34	238±29	246±38	228±46	246±38	245±8	230±26
dCS	250±26	244±50	243±13	252±55	243±35	240±46	255±14	221±36

AERP: atrial effective refractory period; dCS: distal coronary sinus; IAS: interatrial septum; pCS: proximal coronary sinus; RAA: right atrial appendage; RA: right atrium. Values expressed in milliseconds, mean ± standard deviation.

* p=0.05 compared to AERP in group II, corresponding to a significant decrease in AERP during handgrip (12±9% in group I vs. 2±4% in group II, p=0.02).

mostram que, nestas condições, a estimulação vagal se associa a uma diminuição dos PRE na AD lateral, sem impacto significativo noutros locais auriculares avaliados ou na dispersão da refractaridade. Por outro lado, o aumento do tónus simpático durante HG não se associou a modificação significativa dos PRE e o bloqueio autonómico, não só aumentou significativamente os PRE a nível do SC distal, reflectindo, de acordo com Chen et al, a refractariedade no tecido auricular esquerdo[18], como diminuiu, de forma marcada, a dispersão da refractariedade em comparação com os valores basais. Estes dados reforçam o papel do SNA como factor modulador das variações dinâmicas da refractariedade auricular.

Influência autonómica na electrofisiologia das aurículas

Tem sido reconhecido que a actividade do SNA pode condicionar alterações nas propriedades electrofisiológicas das aurículas envolvendo os PRE, a velocidade de condução e o automatismo[7]. Em doentes com FA paroxística, apesar da sequência de mecanismos subjacentes aos episódios desta arritmia permanecer por esclarecer, a não uniformidade dos PRE em diferentes locais é apontada como um dos aspectos mais importantes da vulnerabilidade para FA, associando-se, de forma consistente, à ocorrência de FA espontânea ou induzida[19-21]. Além disso, a heterogeneidade da refractariedade auricular apresenta maior correlação com a vulnerabilidade para FA do que o comprimento de onda (*wavelenght*), que depende do PRE e da velocidade de condução[20]. Deste modo, o conceito de dispersão da refractariedade auricular baseada na distribuição espacial não uniforme dos PRE pode representar um factor

history of recurrent paroxysmal AF. We also assessed whether vulnerability to AF induction during EPS was associated with different ANS-related responses in terms of atrial refractoriness. The results show that vagal stimulation was associated with shortening of AERPs in the lateral RA, with no significant impact at the other sites assessed or in dispersion of refractoriness. On the other hand, increased sympathetic tone during HG was not associated with significant change in AERPs, whereas ANS blockade not only significantly increased AERPs at the distal CS, reflecting the refractoriness of left atrial tissue according to Chen et al. (18), but also markedly decreased dispersion of refractoriness compared to basal conditions. These findings highlight the role of the ANS as a modulator of dynamic variations in atrial refractoriness.

Autonomic influence on atrial electrophysiology

It is known that ANS activity can affect atrial electrophysiological properties and cause changes in AERP, conduction velocity and automatism[7]. Although the sequence of mechanisms underlying episodes of paroxysmal AF has yet to be fully clarified, it has been suggested that nonuniformity of AERPs at different sites is one of the most important factors in vulnerability to AF, since it is consistently associated with spontaneous or induced AF [19-21]. In addition, atrial refractoriness heterogeneity shows a stronger correlation with vulnerability to AF than wavelength, which depends on AERP and conduction velocity[20]. Thus, nonuniform spatial dispersion of atrial refractoriness may be a key factor in the pathophysiology of AF.

Various authors have suggested that autonomic activity, both sympathetic and para-

chave na fisiopatologia da FA.

Diferentes autores têm sugerido que a actividade autonómica, quer simpática quer parassimpática, pode desempenhar um papel importante como modulador na recorrência de episódios de FA paroxística[7-9,22]. A estimulação vagal reduz significativamente a refractariedade auricular, de modo heterogéneo, criando condições para o aparecimento de arritmias auriculares de reentrada e FA[7-9], enquanto a actividade simpática reduz os PRE, sem efeito significativo nos índices de heterogeneidade[7-9,23]. Estudos com base na análise da variabilidade da frequência cardíaca têm mostrado que modificações significativas no balanço simpático--vagal precedem o início de FA espontânea[22,24,25]. Portanto, as flutuações do tónus autonómico podem ser determinantes na caracterização do substrato electrofisiológico para a ocorrência de episódios de FA.

Períodos refractários efectivos auriculares e dispersão da refractariedade

No nosso estudo, à semelhança do descrito por outros autores[26], o PRE aumentou gradualmente nos diferentes locais analisados da direita para a esquerda, com valores mais baixos no AAD, AD lateral e SIA quando comparados com as determinações no SC proximal e distal. Facto possivelmente causado pela distribuição não uniforme das terminações vagais nervosas, que parecem influenciar de forma mais acentuada o PRE no AAD que na aurícula esquerda[27].

As manobras provocativas do SNA utilizadas envolveram o HG, considerado como um teste simples, facilmente reprodutível, com elevada sensibilidade e especificidade para estimulação da actividade simpática[28] e a MSC, aplicada frequentemente como método de avaliação neuro-autonómica capaz de evocar uma resposta reflexa parassimpática[29]. Apesar de condicionarem alterações ligeiras da frequência cardíaca e pressão arterial sistólica (com significado estatístico para o HG), associaram-se a uma grande amplitude na variação dos valores do duplo-produto (HG *versus* MSC, $p<0.001$) e a monitorização da variabilidade RR no gráfico da análise espectral no domínio da frequência permitiu confirmar os efeitos destas manobras e do bloqueio farmacológico com propranolol e atropina na actividade autonómica (*Figura 1*).

sympathetic, may play an important role in modulating recurrence of paroxysmal AF[7-9,22]. Vagal stimulation significantly reduces atrial refractoriness in a heterogeneous manner, creating the conditions for the appearance of reentrant atrial arrhythmias and AF[7-9], while sympathetic activity shortens AERP, with no significant effect on heterogeneity[7-9,23]. Studies based on analysis of heart rate variability have shown that significant changes in the sympatho-vagal balance precede the onset of spontaneous AF[22,24,25]. Thus, fluctuations in autonomic tone may be crucial in characterizing the electrophysiological substrate for episodes of AF.

Atrial effective refractory periods and dispersion of refractoriness

As reported by other authors[26], the AERP increased progressively from right to left at the different sites assessed in our study, with lower values in the RAA, LRA and IAS than those recorded in the proximal and distal CS, possibly due to the nonuniform distribution of vagal nerve terminals, which appear to affect AERP more in the RAA than in the left atrium[27].

The maneuvers used for ANS stimulation were handgrip, which is considered a simple, reproducible test with high sensitivity and specificity for sympathetic stimulation[28], and carotid sinus massage, which is frequently used in neuroautonomic assessment since it produces a parasympathetic reflex response[29]. Although both maneuvers produced slight changes in heart rate and systolic BP (with statistical significance in the case of HG), there were considerable differences in double product (HG vs. MSC, $p<0.001$), and assessment of RR variability by spectral analysis in the frequency domain confirmed the effect on autonomic activity of these maneuvers and of pharmacological blockade with propranolol and atropine (*Figure 1*).

In patients with ≥1 year clinical history of paroxysmal AF, alterations in electrophysiological properties resulting from atrial remodeling are to be expected, particularly heterogeneous shortening of AERPs, which contributes to perpetuation of the condition[30,31]. Even so, vagal stimulation significantly reduced AERPs. However, only ANS blockade had a significant impact on refractoriness in the distal CS, with a marked decrease in dispersion. These results

Num grupo de doentes com FA paroxística e evolução clínica ≥1 ano são esperadas alterações das propriedades electrofisiológicas, como consequência do fenómeno de remodelagem auricular, associando-se, em particular, a uma diminuição heterogénea dos PRE que contribui para perpetuar a situação clínica[30,31]. Ainda assim, a estimulação vagal permitiu obter uma redução significativa dos PRE na AD. No entanto, apenas o bloqueio autonómico influenciou de modo significativo o valor da refractariedade ao nível do SC distal, associando-se a uma marcada diminuição da dispersão da refractariedade. Estes resultados podem traduzir um efeito protector da utilização de fármacos com acção simpaticolítica e vagolítica, face ao aumento da refractariedade em tecido auricular esquerdo combinado com a redução da variabilidade dos PRE. Assim, em situações de FA paroxística com clínica recorrente de longa duração, a estimulação autonómica, em particular da actividade vagal, parece manter um papel importante na redução da refractariedade auricular. Num estudo recente, Lu et al demonstraram que a inervação cardíaca autonómica tem um impacto crucial na fase aguda da remodelagem eléctrica induzida por *pacing* auricular de alta frequência, sendo possível prevenir a redução dos PRE e a indução de FA com bloqueio do SNA[32]. No presente trabalho, o bloqueio autonómico permitiu evidenciar modificações nos PRE e dispersão da refractariedade que podem ter impacto na vulnerabilidade para episódios de FA em situações de evolução clínica de longo prazo.

Refractariedade auricular e vulnerabilidade para indução de fibrilhação auricular

Os resultados mostram que a heterogeneidade dos PRE é um factor determinante da vulnerabilidade auricular, associando-se a indução de FA à presença de valores basais mais altos da dispersão da refractariedade, apesar de valores absolutos semelhantes para os PRE nos diferentes locais analizados. Estes dados estão de acordo com o estudo de Fareh, onde a a vulnerabilidade para FA se correlacionava com a heterogeneidade dos PRE auriculares mas não com o PRE ou *wavelenght*[20]. No presente trabalho, verificou-se uma tendência para o tamanho da aurícula esquerda ser maior nos doentes com indução de FA mas não houve diferenças estatisticamente

may indicate the protective effect of drugs with sympatholytic and vagolytic action. Thus, in patients with a long clinical history of recurrent paroxysmal AF, autonomic stimulation, particularly of vagal activity, appears to play an important role in reducing atrial refractoriness. A recent study by Lu et al. demonstrated that the intrinsic cardiac autonomic nervous system plays a critical role in acute atrial electrical remodeling induced by rapid atrial pacing, and that it is possible to prevent AERP reduction and AF induction with ANS blockade[32]. In the present study, autonomic blockade enabled changes in AERP and dispersion of refractoriness to be documented that may influence vulnerability to AF in patients with a long clinical history of such episodes.

Atrial refractoriness and vulnerability to atrial fibrillation induction

Our results show that AERP heterogeneity is a determining factor in atrial vulnerability, and that AF induction is associated with greater basal dispersion of refractoriness, despite similar absolute AERP values at the different sites assessed. This finding is in agreement with the study by Fareh et al., in which vulnerability to AF correlated with AERP heterogeneity but not with AERP or wavelength[20]. The present study found a tendency for the left atrium to be larger in patients with AF induction, but there were no statistically significant differences in the clinical characteristics of the two groups. The larger reduction in AERP observed at the RAA in the group vulnerable to AF induction, together with increased dispersion of refractoriness in response to sympathetic stimulation, may reflect greater susceptibility to arrhythmias following ectopic beats or extrastimuli with shorter coupling intervals, which demonstrates the possible role of this electrophysiological phenomenon as a substrate for AF inducibility.

STUDY LIMITATIONS AND CONCLUSIONS

The size of the sample did not allow for subgroup analysis based on the presence of spontaneous supraventricular ectopic beats or on the strength of response to autonomic

significativas nas características clínicas dos dois grupos. A documentação duma redução mais acentuada dos PRE no AAD, combinada com a maior dispersão da refractariedade em resposta à estimulação simpática no grupo com vulnerabilidade para indução de FA, pode representar uma maior susceptibilidade para a ocorrência de arritmias na sequência de ectopias ou extra-estímulos com intervalos de acoplamento mais curtos, traduzindo assim um papel potencial deste fenómeno electrofisiológico como substrato para a inducibilidade de FA.

LIMITAÇÕES DO ESTUDO E CONCLUSÕES

A dimensão da amostra não permitiu a análise estatística em subgrupos tendo em conta a presença de ectopias supraventriculares espontâneas ou o grau de intensidade da resposta às manobras autonómicas. Além disso, a não inclusão de doentes com FA paroxística de início recente limita as conclusões do estudo a situações com história clínica de longa duração. No entanto, trata-se dum grupo restrito de doentes com FA paroxística idiopática, incluído em protocolo de avaliação clínica e electrofisiológica de selecção numa fase inicial do programa de ablação de FA, que mostrou um comportamento homogéneo no que concerne à influência da activação e bloqueio autonómico nos aspectos avaliados da refractariedade auricular. Outra limitação está relacionada com a não inclusão do estudo da refractariedade das veias pulmonares, não dispondo assim de informação completa para comparação dos PRE dos cinco locais avaliados com os das veias pulmonares, aceites como mais curtos que os da aurícula esquerda[33,34]. No futuro, a importância destes aspectos deverá ser considerada para melhor compreensão dos fenómenos electrofisiológicos envolvidos no início, manutenção e interrupção dos episódios de FA. Finalmente, apesar da confirmação dos efeitos do HG e MSC com base na frequência cardíaca, pressão arterial e análise espectral da variabilidade da frequência, o recurso a outras manobras provocativas da actividade autonómica, como o teste do frio, a respiração profunda (*deep-breathing*) ou a manobra de Valsalva teriam contribuído para melhorar os resultados da estimulação do tónus simpático e/ou parassimpático.

maneuvers. Furthermore, the fact that patients with paroxysmal AF of recent onset were not included means that the study's conclusions can only be applied to cases with a long clinical history of the condition. Nevertheless, this group of patients with lone paroxysmal AF undergoing a clinical and electrophysiological protocol as the initial selection stage of an AF, ablation program, showed similar behavior in terms of the effect of autonomic stimulation and blockade on the aspects of atrial refractoriness studied. Another limitation is that pulmonary vein refractoriness was not studied, and thus no information is available to compare AERPs at the five sites assessed with those of the pulmonary veins, which are known to be shorter than those of the left atrium[33,34]. These points should be taken into consideration in any future study in order to better understand the electrophysiological phenomena involved in the onset, perpetuation and termination of AF episodes. Finally, although the effects of HG and CSM were confirmed on the basis of heart rate, blood pressure and spectral analysis of frequency variations, use of other maneuvers to stimulate autonomic activity, such as cold pressor test, deep breathing or Valsalva maneuver, would have improved the results of stimulation of sympathetic and/or parasympathetic tone.

In conclusion, patients with a long clinical history of lone paroxysmal AF show slight changes in atrial refractoriness during ANS stimulation, but maintain significant shortening of AERP in the right atrium with vagal stimulation. Increased AERP in the distal CS, associated with decreased dispersion of refractoriness during autonomic blockade, suggests a possible role for pharmacological or non-pharmacological intervention in changes to the electrophysiological substrate related to vulnerability to AF.

Em conclusão, nos doentes com FA paroxística idiopática de longa evolução clínica ocorrem ligeiras alterações da refractariedade auricular durante estimulação do SNA, mantendo encurtamento significativo dos PRE na AD com estimulação vagal. O aumento dos PRE no SC distal associado à menor dispersão da refractariedade resultante do bloqueio autonómico sugere um papel potencial para medidas terapêuticas de âmbito farmacológico ou não farmacológico nas alterações do substrato electrofisiológico relacionado com vulnerabilidade para FA.

Pedido de separatas:
Address for reprints:

MÁRIO OLIVEIRA
Cardiology Department
Santa Marta Hospital
Rua Santa Marta
1169-024 Lisboa - Portugal
Fax: 351 213 560 368
Phone: 351 213 594 311
e-mail: m.martinsoliveira@gmail.com

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Capítulo IV.

Comportamento da actividade simpática e parassimpática durante manobras provocativas da função autonómica em doentes com fibrilhação auricular paroxística

Alterações da actividade autonómica durante o teste de inclinação em doentes com fibrilhação auricular paroxística: análise com *wavelets*

Mário Oliveira, M. Nogueira da Silva, Ana Timóteo, Joana Feliciano, Sofia Silva, Rita Xavier, Isabel Rocha, Luis Silva-Carvalho, Rui Ferreira. Rev Port Cardiol 2009; 28 (3):243-257

Autonomic outflow during provocative maneuvers in paroxysmal atrial fibrillation

Mário Oliveira, M. Nogueira da Silva, Ana Timóteo, Sérgio Laranjo, Rita Xavier, Vera Geraldes, Luis Silva-Carvalho, Rui Ferreira, Isabel Rocha.
Clinical Autonomic Research (submetido)

Incidência de "Falsos-positivos" em Idosos com Fibrilhação Auricular Paroxística Submetidos a Teste de Inclinação

Mário Oliveira, Joana Feliciano, Ana Timóteo, M. Nogueira da Silva, Eduardo Antunes, Sofia Silva, Sandra Alves, Luis Silva-Carvalho, Rui Ferreira. Rev Port Cardiol 2008; 27 (11): 1383-1394

Baroreflex sensitivity during orthostatic stress in patients with lone paroxysmal atrial fibrillation

Mário Oliveira, Sérgio Laranjo, Cristiano Tavares, Victor Vaz-da-Silva, Vera Geraldes, Sofia Silva, Rita Xavier, Rui Ferreira, Isabel Rocha. Autonomic Neuroscience (submetido)

Alterações da actividade autonómica durante o teste de inclinação em doentes com fibrilhação auricular paroxística: análise com *wavelets* [22]

MÁRIO MARTINS OLIVEIRA, NOGUEIRA DA SILVA, ANA TERESA TIMÓTEO, JOANA FELICIANO, SOFIA SILVA, RITA XAVIER, ISABEL ROCHA, LUÍS SILVA-CARVALHO, RUI FERREIRA

Serviço de Cardiologia, Hospital de Santa Marta, Lisboa, Portugal
Instituto de Fisiologia da Faculdade de Medicina de Lisboa, Lisboa, Portugal

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RESUMO

O sistema nervoso autónomo (SNA) é reconhecido como factor modulador da fibrilhação auricular paroxística (FAp). Durante o *stress* ortostático são esperadas alterações do controlo da variabilidade da frequência cardíaca (VFC), contribuindo para a manutenção da homeostasia cardiovascular. A análise da VFC com *wavelets* tem sido proposta para obter informação no domínio da frequência, permitindo identificar ao longo do tempo as frequências envolvidas nesse processo dinâmico e respectivo contributo nas variações transitórias do sinal.
Objectivo: estudar o perfil de resposta autonómica durante o teste de inclinação passivo (TT) em doentes (D) com FAp e em indivíduos saudáveis (N) através da análise da VFC com transformada de *wavelets*.
Métodos: 21D com FAp (9 homens, 58±14 anos) - 15D sem evidência de cardiopatia e 6 com hipertensão arterial - e 21N (7 homens, 48±12 anos) submetidos a TT, depois de um período de repouso em posição supina e enquanto em ritmo sinusal. Não foram usados agentes farmacológicos provocativos. Obteve-se monitorização contínua do ECG e pressão arterial (Task Force Monitor; CNSystems). As *wavelets* foram aplicadas aos intervalos R-R para cálculo dos valores de valores de LF (baixa-frequência; 0,04-0,15 Hz), HF (alta-frequência; 0,15-0,60 Hz) e razão LF/HF (equilíbrio simpático-

ABSTRACT

Alterations in autonomic response during head-up tilt testing in paroxysmal atrial fibrillation patients: a wavelet analysis

The autonomic nervous system (ANS) is known to be an important modulator in the pathogenesis of paroxysmal atrial fibrillation (PAF). Changes in ANS control of heart rate variability (HRV) occur during orthostatism to maintain cardiovascular homeostasis. Wavelet transform has emerged as a useful tool that provides time-frequency decomposition of the signal under investigation, enabling intermittent components of transient phenomena to be analyzed.
Aim: To study HRV during head-up tilt (HUT) with wavelet transform analysis in PAF patients and healthy individuals (normals).
Methods: Twenty-one patients with PAF (8 men; age 58±14 yrs) were examined and compared with 21 normals (7 men, age 48±12 yrs). After a supine resting period, all subjects underwent passive HUT (60º) while in sinus rhythm. Continuous monitoring of ECG and blood pressure was carried out (Task Force Monitor, CNSystems). Acute changes in RR-intervals were assessed by wavelet analysis and low-frequency power (LF: 0.04-0.15 Hz), high-frequency power (HF: 0.15-0.60 Hz) and LF/HF (sympathovagal) were calculated for 1) the last 2 min of the supine period; 2) the 15 sec of tilting movement (TM); and 3) the 1st

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vagal) calculados nas seguintes fases: 1) últimos 2 min de posição supina (basal); 2) 15 s do movimento contínuo até à posição de ortostatismo (TM); 3) 1º min (TT1) e 2º min (TT2) de ortostatismo a 60º. Dados expressos como média±erro *standard*.
Resultados: Os intervalos RR foram idênticos para os 2 grupos. A pressão arterial basal foi semelhante, registando-se, durante o ortostatismo, uma subida mantida nos D e uma descida, seguida de elevação e subsequente recuperação nos N. Na fase basal, os valores de LF, HF e LF/HF para os D com FAp foram 632±162 ms², 534±231 ms² e 1,95±0,39, respectivamente, e para os N 1058±222,6 ms², 789±243,6 ms² e 2,4±0,36, respectivamente (FAp vs N, p=NS). Para os 15 s do TM, os valores de LF, HF e LF/HF para os D com FAp foram 747±277 ms², 387±94 ms² e 2,9±0,6, respectivamente, e para os N 1316±315 ms², 698±148 ms² e 2,8±0,6, respectivamente (p<0,05 para LF e HF). No TT1, os valores de LF, HF e LF/HF para os D com FAp foram 1243±432 ms², 302±88 ms² e 7,7±2,4, respectivamente, e para os N 1992±398 ms², 333±76 ms² e 7,8±0,98, respectivamente (p<0,05 para LF). No TT2, os valores de LF, HF e LF/HF para os D com FAp foram 871±256 ms², 242±51 ms² e 4,7±0,9, respectivamente, e para os N 1263±335,4 ms², 317±108 ms² e 8,6±0,68, respectivamente (p<0.05 para LF e LF/HF). Na análise do padrão dinâmico das diferentes frequências nos D com FAp, os valores de LF e HF não variaram significativamente durante o TM, nem em TT2, enquanto a relação LF/HF não se modificou significativamente durante o TM, mas aumentou em TT1 e TT2.
Conclusão: Nos D com FAp há alterações da VFC na adaptação ao ortostatismo, com valores representativos de LF e HF sem variações significativas, inferiores aos dos N. Estes dados sugerem que a análise com transformada de *wavelets* pode melhorar a compreensão da regulação autonómica cardíaca e reforça a presença de anomalias do SNA na FAp

Palavras-Chave
Fibrilhação auricular paroxística; Sistema nervoso autónomo; Variabilidade da frequência cardíaca; Teste de mesa basculante; Transformada de *wavelets*

(TT1) and 2nd (TT2) min of HUT. Data are expressed as means ± SEM.
Results: Baseline and HUT RR-intervals were similar for the two groups. Supine basal blood pressure was also similar for the two groups, with a sustained increase in PAF patients, and a decrease followed by an increase and then recovery in normals. Basal LF, HF and LF/HF values in PAF patients were 632±162 ms², 534±231 ms² and 1.95±0.39 respectively, and 1058±223 ms², 789±244 ms² and 2.4±0.36 respectively in normals (p=NS). During TM, LF, HF and LF/HF values for PAF patients were 747±277 ms², 387±94 ms² and 2.9±0.6 respectively, and 1316±315 ms², 698±148 ms² and 2.8±0.6 respectively in normals (p<0.05 for LF and HF). During TT1, LF, HF and LF/HF values for PAF patients were 1243±432 ms², 302±88 ms² and 7.7±2.4 respectively, and 1992±398 ms², 333±76 ms² and 7.8±0.98 respectively for normals (p<0.05 for LF). During TT2, LF, HF and LF/HF values for PAF patients were 871±256 ms², 242±51 ms² and 4.7±0.9 respectively, and 1263±335 ms², 317±108 ms² and 8.6±0.68 respectively for normals (p<0.05 for LF/HF). The dynamic profile of HRV showed that LF and HF values in PAF patients did not change significantly during TM or TT2, and LF/HF did not change during TM but increased in TT1 and TT2.
Conclusion: Patients with PAF present alterations in HRV during orthostatism, with decreased LF and HF power during TM, without significant variations during the first minutes of HUT. These findings suggest that wavelet transform analysis may provide new insights when assessing autonomic heart regulation and highlight the presence of ANS disturbances in PAF.

Key words
Paroxysmal atrial fibrillation; Autonomic nervous system; Heart rate variability; Head-up tilt; Wavelet transform

INTRODUÇÃO

A fibrilhação auricular (FA) é a perturbação persistente do ritmo cardíaco mais comum na prática clínica. A sua prevalência aumenta com a idade, variando entre 0,4% na população geral e 5% acima dos 65 anos de idade, com impacto desfavorável na qualidade de vida, morbilidade e mortalidade[1-5]. A abordagem da FA permanece um desafio, não só pela sua importância epidemiológica e custos para a sociedade, mas também pelas limitações na compreensão da sequência dos mecanismos fisiopatológicos subjacentes à sua génese. A contribuição relativa das principais teorias associadas à FA, a origem focal e a hipótese dos múltiplos circuitos de reentrada, permanece por esclarecer[6,7]. O sistema nervoso autónomo (SNA) tem sido reconhecido como um modulador na fisiopatologia da FA, encontrando-se muitas vezes associado à recorrência de episódios paroxísticos, podendo contribuir também para a manutenção e interrupção da FA[8-10]. As propriedades electrofisiológicas relacionadas com a vulnerabilidade auricular para a ocorrência de FA espontânea podem ser influenciadas pela actividade simpática e parassimpática[8]. A estimulação vagal reduz a velocidade de condução no tecido auricular, encurta o potencial de acção e a refractariedade de modo não uniforme, criando condições para o aparecimento de arritmias auriculares de reentrada[9,10]. Por outro lado, a estimulação simpática reduz a refractariedade auricular e induz actividade *trigger* com despolarizações rápidas repetitivas e taquiarritmias auriculares[8-10]. Deste modo, as flutuações do tónus autonómico podem ser determinantes nos episódios de FA. Durante o *stress* ortostático ocorrem modificações rápidas na actividade do SNA de modo a assegurar a homeostasia cardiovascular[11]. A análise espectral da frequência cardíaca tem permitido a avaliação, de forma indirecta, do controlo mediado pela actividade simpático-vagal[12]. A análise com *wavelets* tem sido utilizada no estudo de sinais biológicos como uma modalidade de aplicação flexível, permitindo a decomposição no domínio do tempo e frequência, de modo a avaliar o comportamento das várias frequências em fenómenos transitórios e intermitentes[13,14]. No presente estudo, utilizámos a análise com transformada de *wavelets* para caracterizar e

INTRODUCTION

Atrial fibrillation (AF) is the most common persistent cardiac arrhythmia encountered in clinical practice. Its prevalence increases with age, ranging from 0.4% in the general population to 5% in those aged over 65, with an adverse impact on quality of life, morbidity and mortality[1-5]. Management of AF is still a challenge, not only because of its epidemiological importance and burden on society, but also due to our limited understanding of the pathophysiological mechanisms underlying its genesis. The extent to which the main theories – focal origin and multiple reentrant circuits – explain pathogenesis of AF remains to be determined[6,7]. The autonomic nervous system (ANS) is known to be an important modulator in the pathophysiology of AF, and is often associated with recurrent paroxysmal episodes; it may also play a role in the perpetuation and termination of AF[8-10]. The electrophysiological properties linked to atrial vulnerability to spontaneous AF can be affected by sympathetic and parasympathetic activity[8]. Vagal stimulation reduces conduction velocity in atrial tissue and shortens action potentials and refractoriness in a nonuniform manner, creating the conditions for the development of reentrant atrial arrhythmias[9,10]. Sympathetic stimulation reduces atrial refractoriness and triggers repeated rapid depolarizations and atrial tachyarrhythmias[8-10]. Fluctuations in autonomic tone can thus lead to episodes of AF. During orthostatic stress there are rapid changes in ANS activity in order to maintain cardiovascular homeostasis[11]. Spectral analysis of heart rate enables indirect assessment of control mediated by sympathovagal activity[12]. Wavelet analysis has been used as a flexible way of studying biological signals that provides time-frequency decomposition, from which the behavior of various frequencies in transient and intermittent phenomena can be analyzed[13,14]. The present study uses wavelet transform analysis to characterize and quantify ANS activity in modulating RR intervals during tilt testing in patients with paroxysmal AF (PAF) and in healthy individuals (normals).

quantificar a actividade do SNA na modulação dos intervalos RR durante o teste de inclinação em doentes com FA paroxística (FAp) e em indivíduos saudáveis (N).

POPULAÇÃO E MÉTODOS

O estudo incluiu 21 doentes (58±14 anos, 42,8% do sexo masculino) com FAp (>1 episódio sintomático/mês; duração da história clínica de 6-80 meses) e 21 voluntários saudáveis (48±12 anos, 33,3% do sexo masculino). Nos doentes com FAp, 15 não apresentavam evidência de cardiopatia após avaliação clínica, ecocardiográfica e prova de isquémia miocárdica e 6 tinham diagnóstico de hipertensão arterial, controlada com inibidores da enzima de conversão da angiotensina, antagonistas dos receptores da angiotensina II ou bloqueadores dos canais do cálcio. A FAp estava documentada em electrocardiogramas e registo(s) de Holter. Nenhum dos indivíduos apresentava história de síncope ou enfarte do miocárdio, insuficiência cardíaca congestiva ou diabetes *mellitus*. Antes do teste de inclinação, os antiarrítmicos foram suspensos no período correspondente a ≥5 semi-vidas. Nos casos sob amiodarona, o estudo foi efectuado 2 meses após suspensão do fármaco. No dia da realização do teste de inclinação, não foi permitido o consumo de álcool, tabaco ou substâncias com propriedades anticolinérgicas. O teste de inclinação decorreu no período compreendido entre as 12 e as 15 horas, após 4 horas de jejum, em ambiente calmo, com controlo de temperatura e humidade, sem recurso a punção venosa. Todos os participantes se encontravam em ritmo sinusal aquando da realização do estudo. A monitorização contínua electrocardiográfica e da tensão arterial foi obtida com equipamento *Task Force Monitor (CNSystems, Graz, Áustria)*. Os testes foram efectuados após aprovação pela Comissão de Ética do Hospital de Santa Marta e após consentimento informado.

Protocolo do Teste de Inclinação

Os indivíduos foram colocados numa mesa basculante automática, com uma placa de suporte para os membros inferiores e seguros com faixas para impedir a queda no caso da ocorrência de tonturas ou síncope. Foram dadas instruções no sentido da manutenção duma respiração com

METHODS

The study included 21 patients (age 58±14 years, 42.8% male) with PAF (>1 symptomatic episode/month; clinical history 6-80 months) and 21 healthy individuals (age 48±12 years, 33.3% male). Of the PAF patients, 15 showed no evidence of heart disease on clinical assessment, echocardiography or myocardial ischemia testing; six had hypertension, controlled by angiotensin-converting enzyme inhibitors, angiotensin receptor blockers or calcium channel blockers. PAF was documented by electrocardiogram and Holter monitoring. None of the subjects had a history of syncope, myocardial infarction, congestive heart failure or diabetes. Prior to tilt testing, antiarrhythmic drugs were discontinued for ≥5 half-lives. Amiodarone was suspended two months before testing. On the day of testing, the subjects were required to abstain from alcohol, tobacco and substances with anticholinergic properties. Tilt testing was performed between noon and 3 pm, after 4 hours fasting, in a quiet environment with controlled temperature and humidity, without venous puncture. All participants were in sinus rhythm at the time of the test. Continuous monitoring of electrocardiogram and blood pressure was carried out using a Task Force Monitor (CNSystems, Graz, Austria). The tests were approved by the Ethics Committee of Hospital de Santa Marta and the participants gave their informed consent.

Tilt test protocol

The subjects were placed on an automatic tilt table with a foot plate support and secured with straps to prevent falls in the event of dizziness or syncope. They were instructed to maintain normal breathing rate and rhythm. After an initial 15-minute stabilization period in the supine position, the table was tilted at a constant speed, reaching 60º in 15 seconds.

Wavelet analysis was applied to the RR intervals obtained by continuous monitoring, and values for low-frequency power (LF: 0.04-0.15 Hz), high-frequency power (HF: 0.15-0.60 Hz) and LF/HF (reflecting sympathovagal balance) were calculated for four periods: 1) the last two minutes of the supine period (basal value used as control); 2) the 15 seconds of tilting movement to orthostatism at 60º (TM); 3) the first minute

frequência e ritmo normais. Após uma fase inicial de estabilização com duração de 15 minutos, em posição supina, procedeu-se à inclinação progressiva da mesa a uma velocidade constante, atingindo 60º em 15 segundos.

A análise de *wavelets* foi aplicada aos intervalos RR, obtidos da monitorização contínua, e os valores de LF (baixa-frequência; 0.04-0.15 Hz), HF (alta-frequência; 0.15-0.60 Hz) e razão LF/HF (equilíbrio simpático-vagal) calculados para 4 períodos: 1) os últimos 2 minutos da fase de posição supina (basal, utilizado como controlo); 2) os 15 segundos do movimento contínuo até à posição de ortostatismo a 60º (*TM*); 3) o 1º minuto de adaptação ao ortostatismo (*TT1*); 4) o 2º minuto de ortostatismo (*TT2*). Os valores obtidos da variabilidade dos intervalos RR foram comparados entre os doentes com FAp e os N.

Aquisição e processamento do sinal

Os dados foram adquiridos a 1kHz e analizados no domínio do tempo-frequência, com recurso à transformada discreta de *wavelets*. Seleccionámos a *wavelet* do tipo Daubechies 12, devido ao facto da sua morfologia se assemelhar ao padrão presente nas séries de informação contida no sinal avaliado[16]. Os dados foram processados em ambiente *Origin (OriginLab, Origin Lab Scientific Graphing and Analysis Software, Origin Lab Corporation, Northampton, MA, USA)*. Foi implementado um sistema de detecção RR "pico-a-pico" e reconstruídas curvas de evolução para os intervalos RR, computorizadas em ambiente *Matlab (MathWorks, Natick, MA, USA)*. O sinal obtido foi tratado e analizado segundo metodologia e formulação matemática descrita em publicação prévia[13].

Análise estatística

As variáveis contínuas foram expressas sob a forma de média±desvio padrão (características dos doentes) ou média±erro padrão (valores na análise com *wavelets*) e as variáveis categóricas expressas em frequências e percentagens. As comparações para as variáveis contínuas dos valores de LF, HF e LF/HF intergrupos foram efectuadas pelo teste t de *Student* não emparelhado e, no mesmo grupo, durante o TT, pelo teste t de *Student* emparelhado. Para as variáveis categóricas foi utilizado o teste de $\chi 2$. Considerámos estatisticamente significativos os resultados com valor <0,05. O programa

of adaptation to orthostatism (TT1); and 4) the second minute of orthostatism (TT2). The values for RR-interval variability in PAF patients and normals were compared.

Signal acquisition and processing

The data were acquired at 1 kHz and analyzed in the time-frequency domain using discrete wavelet transform. The Daubechies 12 wavelet transform was chosen since its shape resembles the pattern of the information contained in the signal being assessed[16]. The data were processed in the Origin environment (Origin Lab Corporation, Northampton, MA, USA). A peak-to-peak system was used to determine RR intervals and to reconstruct evolution curves, processed in the Matlab environment (MathWorks, Natick, MA, USA). The signals were processed and analyzed using the same methodology and mathematical formulation as described in a previous publication[13].

Statistical analysis

Continuous variables were expressed as means ± standard deviation (patient characteristics) or means ± standard error (wavelet analysis data) and categorical variables as frequencies and percentages. The continuous variables of LF, HF and LF/HF were compared between groups using the unpaired Student's t test and within the same group during tilt testing by the paired Student's t test. The chi-square test was used for categorical variables. Results with $p<0.05$ were considered statistically significant. The program used was GraphPAD Instruments (GraphPad Software, Inc., California, USA).

RESULTS

Population characteristics

Table I shows the clinical characteristics of the study population, comparing the PAF group with the normals. The PAF group had a higher mean age than the normal group, with no significant differences in gender distribution or in mean, systolic or diastolic blood pressure.

estatístico utilizado foi o *GraphPAD Instruments (GraphPad Software, Inc., California, USA).*

RESULTADOS

Características da população

O *Quadro I* mostra as características clínicas da população e compara o grupo da FAp com os N. Os doentes com FAp apresentavam uma idade média ligeiramente superior à do grupo dos N, sem diferenças significativas relativamente à distribuição por sexos e valores da pressão arterial sistólica, diastólica e média.

Quadro I. **Características clínicas da população estudada**

Característica	grupo FAp (n=21)	grupo N (n=21)	p
Idade, anos	58±14 (20-78)	48±12 (28-75)	<0,05
Sexo masculino	42,8%	33,3%	NS
Frequência cardíaca basal, bpm	60±7	61±8	NS
Pressão arterial sistólica, mmHg	124±18	120±11	NS
Pressão arterial diastólica, mmHg	81±12	77±8	NS

* dados expresssos com media±desvio padrão;.bpm=batimentos por minuto
FAp=fibrilhação auricular paroxística; N=voluntários saudáveis

Teste de inclinação

Frequência cardíaca e pressão arterial

Todos os participantes completaram o protocolo sem sintomas ou ocorrência de FA. Durante o teste de inclinação, os N mostraram uma descida não significativa da pressão arterial, seguida de retorno aos valores basais e ligeira subida. Este padrão associou-se a uma ligeira variação da frequência cardíaca (*Quadro II e Figura 1*). No grupo da FAp, a pressão arterial

Figura 1. **Comparação da evolução dos intervalos RR durante as fases do teste de inclinação em doentes com fibrilhação auricular paroxística e indivíduos saudáveis** *(FAp vs N, p=NS). basal=posição supina; TM=durante o movimento basculante (15 segundos); TT1=durante o 1º minuto de ortostatismo a 60º; TT2=durante o 2º minuto de ortostatismo a 60º; FAp=grupo com fibrilhação auricular paroxística; N=grupo de voluntários saudáveis.*

Figure 1. **Comparison of changes in RR intervals during different stages of tilt testing in patients with paroxysmal atrial fibrillation and in healthy individuals (PAF vs. N, p=NS).** *PAF: group with paroxysmal atrial fibrillation; N: group of healthy individuals; basal: supine position; TM: during tilting movement (15 seconds); TT1: during 1st minute of orthostatism at 60º; TT2: during 2nd minute of orthostatism at 60º.*

Table I. **Clinical characteristics of the study population**

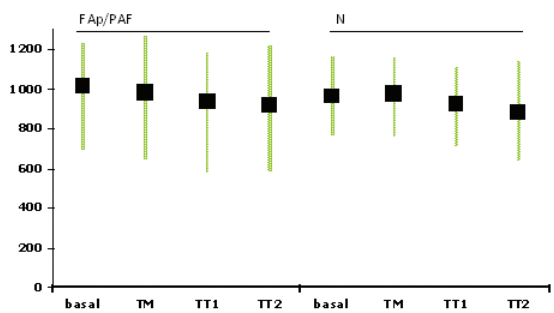
Characteristic	PAF group	Normal group	p
	(n=21)	(n=21)	
Age, years	58±14 (20-78)	48±12 (28-75)	<0.05
Male	42.8%	33.3%	NS
Basal heart rate, bpm	60±7	61±8	NS
Systolic blood pressure, mmHg	124±18	120±11	NS
Diastolic blood pressure, mmHg	81±12	77±8	NS

* Data expressed as means ± standard deviation. bpm: beats per minute; PAF: paroxysmal atrial fibrillation

Tilt test

Heart rate and blood pressure

All participants completed the protocol without symptoms or occurrence of AF. During the tilt test, the normal group showed a non-significant fall in blood pressure, followed by a return to basal values and a slight increase. This pattern was associated with a slight variation in heart rate (*Table II and Figure 1*). In the PAF group, mean blood pressure rose significantly during TM, TT1 and TT2 ($p<0.01$ compared to basal values), accompanied by a significant increase in heart rate ($p<0.001$, comparing TT1 and TT2 with basal values). Two types of blood pressure profile were identified: a gradual sustained increase in PAF patients and a decrease followed by an increase and then recovery in the normals (*Figure 2*). No differences were observed between the groups in heart rate response for the periods analyzed (*Figure 1*). Basal blood pressure was similar in the two groups (*Table II* and *Figure 2*). However, systolic and diastolic blood pressure rose during the tilting movement in the PAF group, resulting in a significant increase in mean blood pressure (*Table II* and *Figure 2C*).



média aumentou significativamente durante as fases TM, TT1 e TT2 (p <0,01; comparando com os valores basais), acompanhada dum aumento significativo da frequência cardíaca (p <0,001; comparando os valores em TT1 e TT2 com os basais). Foram identificados dois tipos de perfil dinâmico na pressão arterial: um aumento gradual mantido nos doentes com FAp e uma descida, seguida por elevação e recuperação nos N (*Figura 2*). Não se registaram diferenças entre os 2 grupos relativamente à resposta da frequência cardíaca nos períodos analisados (*Figura 1*). Em condições basais, a pressão arterial era semelhante nos 2 grupos (*Quadro II e Figura 2*). No entanto, a pressão arterial sistólica e diastólica aumentaram durante o movimento de elevação da mesa basculante no grupo com FAp, condicionando a elevação significativa de pressão arterial média (*Quadro II e Figura 2C*).

Quadro II . **Alterações nos intervalos RR e pressão arterial durante o teste de inclinação**

	Basal	TM	TT1	TT2
Intervalos RR (ms)				
FAp	1014±167	980±180	933±180§	919±179§
N	987±119	974±130	948±120*	902±127*
Pressão arterial sistólica (mmHg)				
FAp	124±18	129±19	135±17*	135±12*
N	120±10	115±13	115±14	117±9
Pressão arterial diastólica (mmHg)				
FAp	81±12	86±12*	95±13*	96±10*
N	77±8	77±9	78±11	85±8**
Pressão arterial media (mmHg)				
FAp	96±13	101±13*	109±14*	109±10*
N	92±10	88±10	89±10	90±9

FAp=grupo com fibrilhação auricular paroxística; N=grupo de voluntários saudáveis. Dados expressos com média ±desvio padrão
* p ≤0,01; ** p = 0,02; § p < 0,001, quando comparado com os valores basais.

Table II. Changes in RR intervals and blood pressure during tilt testing

	Basal	TM	TT1	TT2
RR intervals (ms)				
PAF patients	1014±167	980±180	933±180§	919±179§
Normals	987±119	974±130	948±120*	902±127*
Systolic blood pressure (mmHg)				
PAF patients	124±18	129±19	135±17*	135±12*
Normals	120±10	115±13	115±14	117±9
Diastolic blood pressure (mmHg)				
PAF patients	81±12	86±12*	95±13*	96±10*
Normals	77±8	77±9	78±11	85±8**
Mean blood pressure (mmHg)				
PAF patients	96±13	101±13*	109±14*	109±10*
Normals	92±10	88±10	89±10	90±9

Data expressed as means ± standard deviation
* p≤0.01; ** p=0.02; § p<0.001, compared to basal values.

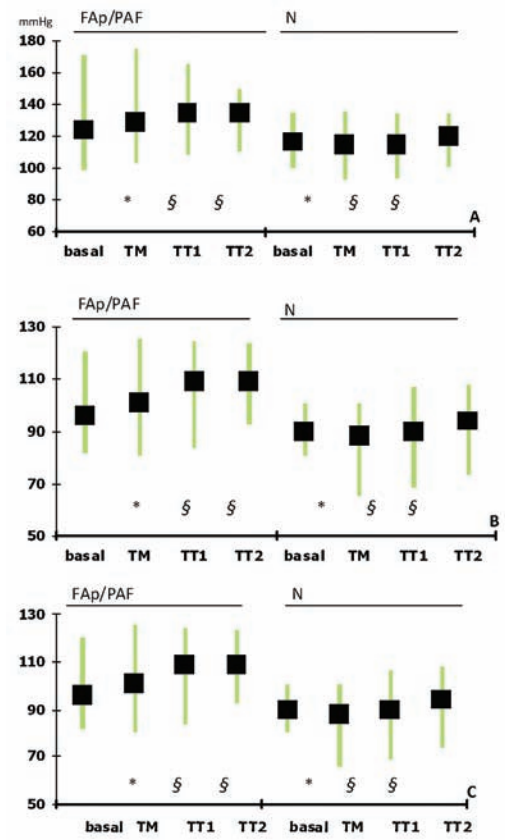


Figura 2. **Comparação de evolução da pressão arterial sistólica (A), diastólica (B) e média (C) durante as fases do teste de inclinação em doentes com fibrilhação auricular paroxística e indivíduos saudáveis.** *FAp=grupo com fibrilhação auricular paroxtística; N=grupo de voluntários saudáveis; basal=posição supina (FAp vs N, p=NS); TM=durante o movimento basculante (FAp vs N, p<0.02); TT1=durante o 1º minuto de ortostatismo a 60º (FAp vs N, p<0.001); TT2=durante o 2º minuto de ortostatismo a 60º (FAp vs N, p<0.001);*=p<0.02;§=p<0.01.*

Figure 2. **Comparison of changes in systolic (A), diastolic (B) and mean (C) blood pressure during different stages of tilt testing in patients with paroxysmal atrial fibrillation and in healthy individuals.**

PAF: group with paroxysmal atrial fibrillation; N: group of healthy individuals; basal: supine position (PAF vs. N, p=NS); TM: during tilting movement (PAF vs. N, p<0.02); TT1: during 1st minute of orthostatism at 60º (PAF vs. N, p<0.001); TT2: during 2nd minute of orthostatism at 60º (PAF vs. N, p<0.001);=p<0.02; §=p<0.01.*

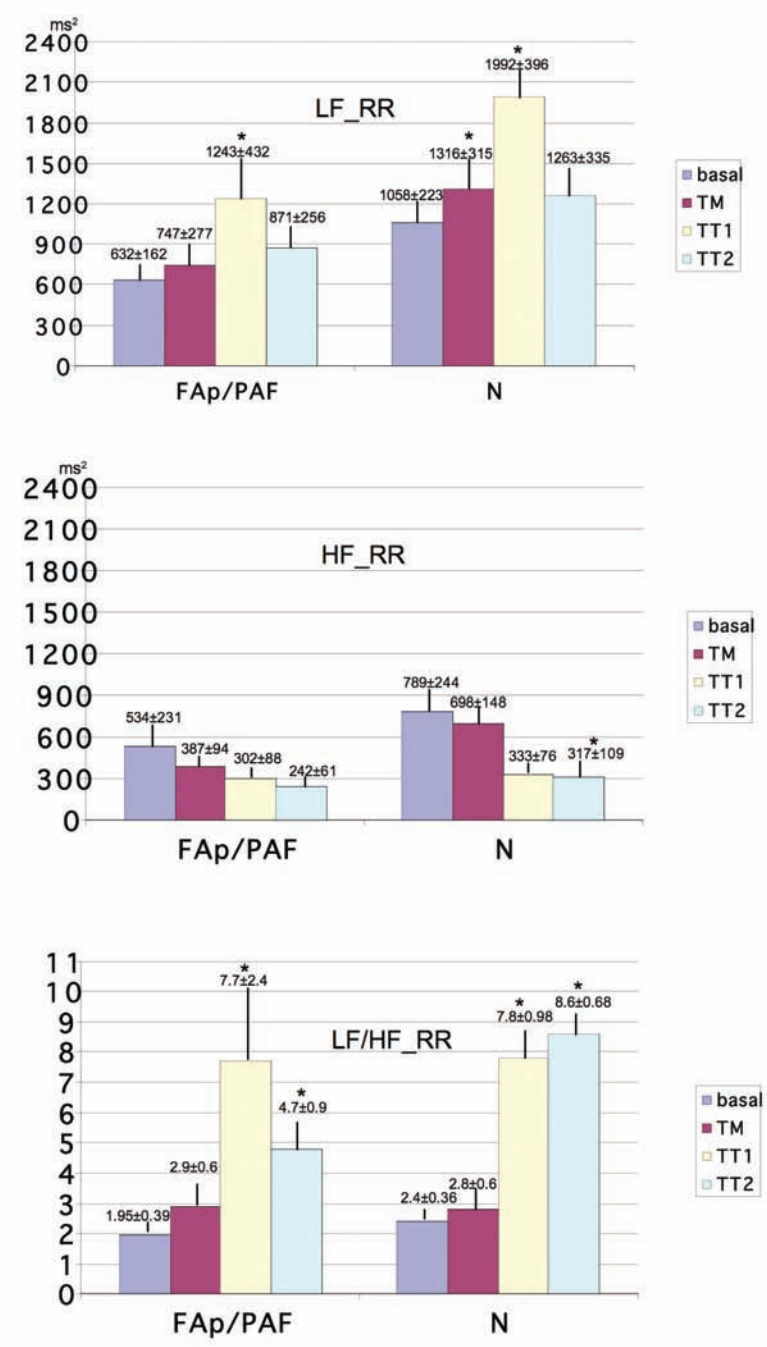


Figura 3. **Evolução das modificações nos parâmetros de actividade autonómica obtidos dos intervalos RR durante o teste de inclinação.** *FAp=grupo com fibrilhação auricular paroxística; N=grupo de voluntários saudáveis. LF_RR=banda LF (baixa-frequência); HF_RR=banda HF (alta-frequência); LF/HF_RR=razão LF/HF (balanço simpático-vagal); basal=posição supina (FAp vs N, p=NS); TM=durante o movimento basculante (FAp vs N, p<0.05 para LF_RR e HF_RR); TT1=durante o 1º minuto de ortostatismo a 60º (FAp vs N, p<0.05 para LF_RR); TT2=durante o 2º minuto de ortostatismo a 60º (p<0.05 para LF_RR e LF/HF_RR). *=p<0.05 quando comparado com basal. Dados expressos como média±erro padrão.*

Figure 3. **Changes in parameters of automic activity obtained from RR intervals during tilt testing.**
*PAF: group with paroxysmal atrial fibrillation; N: group of healthy individuals; LF_RR: low frequency band; HF_RR: high frequency band; LF/HF_RR: LF/HF ratio (sympathovagal balance); basal: supine position (PAF vs. N, p=NS); TM: during tilting movement (PAF vs. N, p<0.05 for LF_RR and HF_RR); TT1: during 1st minute of orthostatism at 60º (PAF vs. N, p<0.05 for LF_RR); TT2: during 2nd minute of orthostatism at 60º (p<0.05 for LF_RR and LF/HF_RR). *=p<0.05 compared to basal values. Data expressed as means ± standard error.*

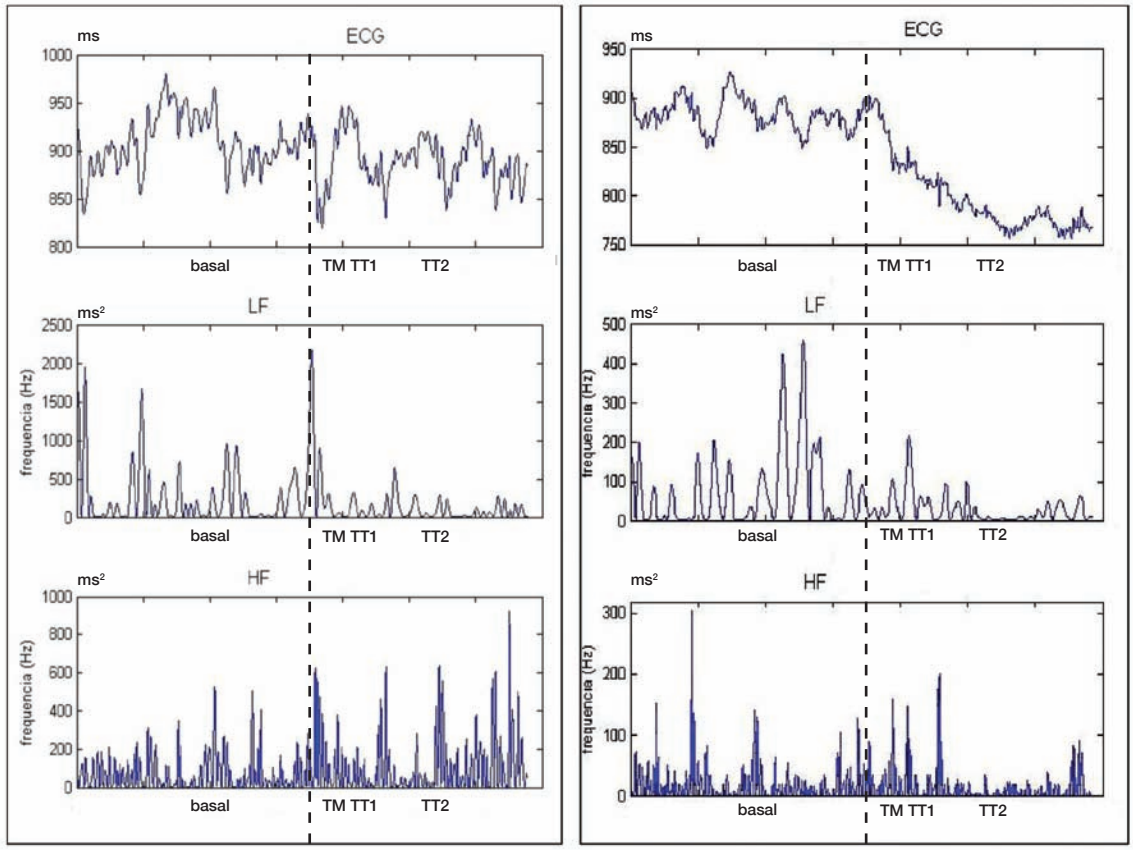


Figura 4. **Variações dos intervalos RR durante o teste de inclinação analizadas pela transformada de *wavelets*. Os quadros mostram a relação dos intervalos RR (em cima) com as flutuações na actividade do sistema nervoso autónomo. À esquerda, mulher com 65 anos de idade, saudável. À direita, homem com 67 anos de idade e história de fibrilhação auricular paroxística. A linha a tracejado assinala o início do movimento de inclinação.**
basal=posição supina; TM=durante o movimento basculante (15 segundos); TT1=durante o 1º minuto de ortostatismo a 60º; TT2=durante o 2º minuto de ortostatismo a 60º.

Figure 4. **Changes in RR intervals during tilt testing by wavelet transform analysis, showing the relationship of RR intervals (top) to fluctuations in ANS activity; left: a healthy 65-year old woman; right: a 67-year old man with a history of paroxysmal atrial fibrillation. The dotted line marks the beginning of the tilting movement.**
Basal: supine position; TM: during tilting movement (15 seconds); TT1: during 1st minute of orthostatism at 60º ; TT2: during 2nd minute of orthostatism at 60º

Análise da variabilidade da frequência cardíaca com transformada de *wavelets*

Os valores da variabilidade da frequência cardíaca basais, na posição supina, foram semelhantes nos 2 grupos. A análise com *wavelets* mostrou uma subida significativa dos valores na banda LF durante os períodos TM e TT1 (comparando com o basal), e uma descida significativa na banda HF em TT2 nos N, enquanto no grupo com FAp, o aumento significativo de LF ocorreu apenas em TT1, sem modificações significativas na banda HF (*Figura 3*). No período TM, os valores de LF e HF foram significativamente inferiores no grupo com FAp (746,8±277 ms² *vs* 1311±317 ms² e 387±94 ms² *vs* 694±147 ms²; p<0,05). Nos valores de LF/HF,

Analysis of heart rate variability using wavelet transform

Basal heart rate variability in the supine position was similar in the two groups. Wavelet analysis showed a significant increase in the LF band during TM and TT1 (compared to basal values) and a significant decrease in the HF band in TT2 in the normal group, while in the PAF group a significant increase in LF occurred only in TT1, with no significant change in HF (*Figure 3*). During TM, LF and HF values were significantly lower in the PAF group (746.8±277 ms² vs. 1311±317 ms² and 387±94 ms² vs. 694±147 ms² respectively; p<0.05). A significant increase in LF/HF was seen in both groups (from 2.9±0.6 following orthostatism to 4.76±0.9 during TT2

verificou-se um aumento significativo em ambos os grupos (de 2,9±0,6, após ortostatismo, até 4,76±0,9, durante TT2, nos doentes com FAp, e de 2,8±0,4 até 8,6±0,6 nos N; p<0,05 em TT2) (*Figura 3*). A *Figura 4* mostra a evolução dos intervalos RR durante o teste de inclinação, com as respectivas alterações do SNA, resultantes da análise com a transformada de *wavelets*, numa doente com FAp e num indivíduo saudável.

DISCUSSÃO

Este estudo foi desenhado para comparar, com recurso à transformada de *wavelets*, a actividade do SNA envolvida na adaptação aguda da variabilidade da frequência cardíaca, durante o ortostatismo passivo, utilizando o teste de inclinação em doentes com FAp e em N. Os resultados mostraram que, apesar de valores de frequência cardíaca e pressão arterial semelhantes em condições basais, os doentes com FAp têm resposta diferente da actividade do SNA durante o período inicial de adaptação ao ortostatismo.

A transformada de Fourier tem sido o método mais usado na análise da variabilidade da frequência cardíaca. Nesta técnica, os resultados representam o poder espectral dos vários componentes do sinal analizado, distribuídos por 4 bandas (*ultra-low frequency, very-low frequency*, LF e HF), duas das quais aceites como traduzindo actividade autonómica: a banda HF, reflectindo actividade parasimpática, e a banda LF, mais relacionada com a modulação simpática. Consequentemente, a razão LF/HF tem sido utilizada como um indicador do balanço simpático-vagal[17,18]. No entanto, a análise com a transformada de Fourier é aplicada quando o sinal é estável, com uma duração de, pelo menos, 5 minutos. Com esta metodologia, a necessidade de calcular os coeficientes médios representativos num número suficiente de intervalos RR não permite detectar, com precisão, modificações súbitas do tónus autonómico, tornando impossível determinar o momento em que ocorre uma alteração na actividade do SNA. Por seu lado, a transformada de *wavelets*, surgiu como um método de grande utilidade, que permite a decomposição no domínio tempo-frequência, com respectiva caracterização de sinais dinâmicos com flutuações intermitentes, tornando possível

in PAF patients, and from 2.8±0.4 to 8.6±0.6 in the normals; p<0.05 in TT2) (*Figure 3*). *Figure 4* shows the changes in RR intervals during the tilt test and respective ANS fluctuations based on wavelet transform analysis, in a patient with PAF and a healthy individual.

DISCUSSION

This study was designed to compare the activity of the ANS associated with acute changes in heart rate variability during the passive orthostatism of tilt testing in PAF patients and healthy individuals using wavelet transform analysis. The results show that, although basal heart rate and blood pressure values are similar, patients with PAF have a different ANS response during the initial period of adaptation to orthostatism.

Fourier transform is the method most often used to analyze heart rate variability. The results of this technique represent the spectral power of the various components of the signal being analyzed, divided into four bands (ultralow, very low, low and high frequency), the latter two being accepted as representing autonomic activity: HF reflecting parasympathetic activity and LF related more to sympathetic modulation. The LF/HF ratio has therefore been used as an indicator of sympathovagal balance[17,18]. However, Fourier transform analysis is used when the signal is stationary for at least five minutes. Since this method requires mean representative coefficients to be calculated from a large number of RR intervals, it does not detect sudden changes in autonomic tone with precision, and hence cannot determine the moment at which a change in ANS activity occurs. Wavelet transform analysis has proved to be a very useful method that provides time-frequency decomposition, characterizing dynamic signals with intermittent fluctuations, making it possible to identify and quantify changes in ANS balance[19]. It has been used to analyze short-term data to determine the contribution of LF and HF bands to dynamic changes in a particular biological signal[15]. Its ability to provide time and spectrum data simultaneously is particularly useful, and it has thus become accepted as a method to explore cardiovascular signals in the time-frequency domain[20-22]. Various types of wavelet functions

localizar e quantificar alterações no equilíbrio do SNA[19]. Tem sido aplicada na análise de curta duração, com identificação da contribuição das bandas LF e HF nas modificações dinâmicas dum determinado sinal biológico[15]. Torna-se particularmente útil pela capacidade de disponibilizar, simultaneamente, informação temporal e espectral. Assim, tem sido aceite como método para o estudo de sinais cardiovasculares no domínio do tempo e frequência[20-22]. Estão disponíveis vários tipos de funções de *wavelets* aplicáveis a sinais biológicos. Neste estudo, seleccionámos o tipo Daubechie, proposto como o mais adequado para o perfil da frequência cardíaca[15,16].

Efeitos fisiológicos do teste de inclinação

Durante o ortostatismo, ocorrem alterações do controlo autonómico na variabilidade da frequência cardíaca, que contribuem para a manutenção da homeostasia cardiovascular. O estudo das diferentes técnicas não-invasivas para avaliar a actividade do SNA, baseadas no domínio da frequência, tem demonstrado que a análise dos intervalos RR constitui um dos métodos mais utilizados na investigação clínica dos mecanismos subjacentes à regulação cardiovascular[23].

O teste de inclinação tem sido usado como estímulo fisiológico para provocar o aumento do tónus simpático e diminuição da actividade vagal[24]. Tem 2 fases de adaptação: uma resposta cardiovascular precoce, observada nos primeiros 30 segundos após ortostatismo, seguida por um período de estabilização, composto por uma fase inicial de adaptação, nos primeiros 2 minutos, e uma segunda fase, relacionada com o ortostatismo mais prolongado, com duração superior a 5 minutos[15,25]. Os mecanismos de curta-duração na adaptação ao *stress* ortostático são mediados pelas vias neurais do SNA. Assim, os nossos resultados mostram, através da aplicação da transformada de *wavelets* nas 4 fases estudadas, as modificações da actividade do SNA subjacentes à variabilidade da frequência cardíaca.

Durante o teste de inclinação, o grupo N mostrou um aumento significativo do tónus simpático durante a fase TM, enquanto nos doentes com FAp esse aumento foi mais tardio, ocorrendo apenas na fase TT1. No entanto, o balanço simpático-vagal foi semelhante nos dois grupos devido à menor diminuição da actividade

are available for application to biological signals. In this study, we chose the Daubechies function, which is recommended as the most appropriate for heart rate profiles[15,16].

Physiological effects of tilt testing

Changes in autonomic control of heart rate variability occur during orthostatism to maintain cardiovascular homeostasis. Studies of different noninvasive techniques to assess ANS activity based on the frequency domain have demonstrated that analysis of RR intervals is one of the most useful methods for clinical research into the mechanisms underlying cardiovascular regulation[23].

Tilt testing is used as a physiological stimulus to induce an increase in sympathetic tone and a decrease in vagal activity[24]. The test reveals two stages of adaptation: an early cardiovascular response, seen in the first 30 seconds of orthostatism, followed by a period of stabilization consisting of an initial adaptation phase in the first two minutes, and a second phase related to more prolonged orthostatism, lasting more than five minutes[15,25]. The short-term mechanisms of adaptation to orthostatic stress are mediated by ANS neural pathways. Through the application of wavelet transform analysis to the four periods studied, our results show the changes in ANS activity underlying heart rate variability.

During tilt testing, the normal group showed a significant increase in sympathetic tone during TM, while this increase occurred later in PAF patients, during TT1. Nevertheless, the sympathovagal balance was similar in the two groups due to a less marked decrease in vagal activity in the normals (*Figure 3*). The persistence of significant differences in sympathetic tone during TM, TT1 and TT2 resulted in a higher LF/HF ratio in the normal group during the second minute of orthostatism. These differences were associated with higher blood pressure in the PAF group, despite similar heart rate. These findings may be related to progressive adaptation of cardiovascular autonomic receptors caused by some degree of autonomic dysfunction. Sustained changes in ANS tone can result in regulatory dysfunction of receptors, leading to greater sensitivity in cardiovascular responses to ANS activity[26-28]. Thus, the delayed increase in sympathetic tone, as shown by analysis of RR intervals, may reflect

vagal nos N (*Figura 3*). A manutenção das diferenças significativas relativamente ao tónus simpático durante TM, TT1 e TT2 condicionaram uma maior relação LF/HF no grupo N no 2º minuto de ortostatismo. Estas diferenças ocorreram associadas a valores de pressão arterial mais elevada no grupo com FAp, apesar da frequência cardíaca semelhante. Estes resultados podem relacionar-se com uma adaptação progressiva dos receptores autonómicos cardiovasculares devida a algum grau de disfunção autonómica. De facto, alterações mantidas no tónus do SNA podem condicionar disfunção da regulação dos receptores, conduzindo a uma maior sensibilidade nas respostas cardiovasculares à actividade do SNA[26-28]. Portanto, o atraso no aumento do tónus simpático, traduzido na análise dos intervalos RR, pode reflectir algum grau de disfunção autonómica, enquanto os valores de pressão arterial significativamente mais elevados, sem diferenças na frequência cardíaca, durante o teste de inclinação, podem resultar de alterações na sensibilidade do baroreflexo ou duma maior sensibilidade cardiovascular às respostas do SNA. Trabalhos em modelos experimentais têm atribuído as dificuldades na regulação da frequência cardíaca durante variações da pressão arterial a alterações na actividade cardíaca parassimpática, na função dos receptores muscarínicos cardíacos ou a disfunção do reflexo baroreceptor[29-32].

Uma análise complementar na interpretação destes resultados pode ser concordante com os trabalhos de Akselrod e colaboradores, onde se demonstra que a hipertensão essencial se associa a alterações do controlo autonómico cardiovascular, que podem estar já presentes numa fase muito inicial da doença, mesmo antes de se registarem valores elevados da pressão arterial[33]. Num estudo recente, o controlo da hipertensão arterial com trandolapril e/ou irbesartan não condicionou diferenças nos valores das frequências dos componentes espectrais da variabilidade da frequência cardíaca, mantendo-se a resposta fisiológica à mudança postural durante o teste de inclinação[34]. A importância deste aspecto da actividade autonómica é valorizada pelo facto de registos prospectivos recentes apontarem a hipertensão arterial como a patologia mais associada a todos os tipos de FA[35,36].

Os resultados do presente estudo, evidenciam

autonomic dysfunction, while the significantly higher blood pressure values during tilt testing, with no differences in heart rate, may result from changes in baroreflex sensitivity or from heightened cardiovascular sensitivity to ANS responses. Studies in experimental models have attributed difficulty in regulating heart rate during blood pressure variations to changes in parasympathetic cardiac activity or in cardiac muscarinic receptor function, or to baroreceptor reflex dysfunction[29-32].

A complementary analysis of our results may show a link with the work of Akselrod et al., who demonstrated that essential hypertension is associated with changes in cardiovascular autonomic control which may be present at a very early stage of the disease, even before blood pressure is elevated[33]. In a recent study, control of hypertension with trandolapril and/or irbesartan did not result in changes in frequency of the spectral components of heart rate variability, physiological response to postural change during tilt testing with patients maintaining[34]. The importance of this aspect of autonomic activity is highlighted by the fact that recent prospective surveys have indicated that hypertension is the pathology most strongly associated with all types of AF[35,36].

The results of the present study show that patients with paroxysmal AF have an abnormal ANS response to orthostatism. The physiological significance of the complex interaction between adrenergic and vagal activity is a constant challenge in current research into AF, and further studies are needed, not only to help clarify the mechanisms underlying PAF but also to identify potential indicators of the clinical course of this pathology.

Study limitations

The study protocol did not include assessment of heart rate, blood pressure or heart rate variability beyond the first two minutes of orthostatism and thus no analysis of autonomic activity during prolonged orthostatic stress is possible. Nevertheless, it is accepted that the rapid fluctuations that occur during the initial stages of tilt testing are mediated by ANS activity[11,19]. Another limitation is the fact that the sample size did not enable the study population to be divided into sufficiently large subgroups according to age or concomitant hypertension for

a presença dum padrão anormal na actividade do SNA na resposta ao ortostatismo em doentes com FA paroxística. O significado fisiológico desta complexa interacção entre actividades adrenérgica e vagal representa um desafio permanente no actual panorama da investigação no âmbito da FA, justificando-se estudos futuros que possam contribuir não só para a compreensão dos mecanismos subjacentes à FAp mas também para identificar potenciais indicadores da evolução clínica associados a esta patologia.

Limitações do estudo

No protocolo do estudo, não considerámos a avaliação da frequência cardíaca, pressão arterial ou variabilidade da frequência cardíaca para além dos dois primeiros minutos de ortostatismo. Nesse sentido, a comparação da actividade autonómica durante o ortostatismo prolongado não pode ser incluída nesta discussão. No entanto, tem sido aceite que as flutuações rápidas que ocorrem neste período inicial do teste de inclinação são mediadas pela actividade do SNA[11,19]. Outra limitação, prende-se com a amostra analisada, que, pela sua dimensão, não permitiu uma subdivisão em grupos de faixas etárias diferentes ou com diagnóstico concomitante de hipertensão arterial em número adequado, de modo a avaliar o impacto destas variáveis no tipo de resposta autonómica. Apesar de não se poder excluir alguma influência nos resultados devida à diferença na idade média dos dois grupos, as alterações descritas do balanço simpático-vagal durante o ortostatismo passivo, determinadas pela idade, incluem a redução da razão LF/HF nos idosos (em comparação com o aumento LF/HF nos jovens)[18]. No presente trabalho, o grupo com FAp manteve o aumento da razão LF/HF durante o ortostatismo, tal como o grupo dos N, registando-se um atraso no aumento do tónus simpático, que manteve valores inferiores aos N durante todo o protocolo. Por outro lado, num estudo sobre a evolução da variabilidade da frequência cardíaca com a faixa etária, não se encontraram diferenças significativas entre a população com idades compreendidas entre os 40 e os 59 anos[37].

the purpose of determining the impact of these variables on autonomic response. Some influence on the results due to the difference in the mean age of the two groups cannot be excluded; age-related changes in the sympathovagal balance during passive orthostatism include lower LF/HF ratios in the elderly (in contrast to higher LF/HF in the young)[18]. In the present study, the PAF group showed increased LF/HF ratios during orthostatism, as in the normal group, but with a delayed increase in sympathetic tone, which showed lower values than the normal group throughout the protocol. However, in a study of changes in heart rate variability according to age, no significant differences were found in populations aged between 40 and 59 years[37].

CONCLUSIONS

Patients with paroxysmal atrial fibrillation present alterations in autonomic control of heart rate during the passive orthostatism of tilt testing, with lower LF and HF values than healthy individuals, without significant variations during the initial stages of the test. This strongly suggests abnormal ANS activity in PAF and highlights the importance of wavelet transform analysis in detecting acute short-term fluctuations in heart rate variability, by providing a more accurate dynamic assessment of cardiovascular autonomic function in clinical practice.

CONCLUSÕES

Os doentes com FAp apresentam alterações do controlo autonómico da frequência cardíaca durante o ortostatismo passivo associado ao teste de inclinação, com valores representativos de LF e HF inferiores aos dos N, sem variações significativas no período inicial do teste de inclinação. Estas modificações, reforçam a presença dum comportamento anormal do SNA na FAp e evidenciam a importância da análise com transformada de *wavelets* na detecção de flutuações agudas e transitórias da variabilidade da frequência cardíaca, permitindo uma avaliação dinâmica, mais adequada, da função autonómica cardiovascular na prática clínica.

Pedido de Separatas:
Address for reprints:

MÁRIO OLIVEIRA
Serviço de Cardiologia
Hospital de Santa Marta
Rua de Santa Marta
1169-024 Lisboa - Portugal
Fax: 351213560368
Telefone: 351.213594311
E-mail:mmoliveira@clix.pt

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Autonomic outflow during provocative maneuvers in paroxysmal atrial fibrillation

AUTHORS

Mário Oliveira, MD, FESC, FHRS*, M. Nogueira da Silva, MD *, A. Teresa Timóteo, MD, FESC, Sérgio Laranjo, MD **, Rita Xavier, AP **, Vera Geraldes, AP **, L. Silva-Carvalho, MD, PhD ** , Rui Ferreira, MD *, Isabel Rocha, PhD **

* Department of Cardiology, Santa Marta Hospital, Lisbon, Portugal

** Institute of Physiology, Faculty of Medicine of Lisbon, Portugal and Instituto de Medicina Molecular, Lisbon, Portugal

Running Title: ***AUTONOMIC TESTS IN ATRIAL FIBRILLATION***

Author for correspondence

Prof Isabel Rocha

Instituto de Fisiologia, Faculdade de Medicina de Lisboa

Av Prof Egas Moniz, 1649-028 Lisbon - Portugal

Fax: (+ 351) 217 999 436

Phone (+351) 217 999 435

E-mail: isabelrocha@fm.ul.pt

ABSTRACT

Autonomic dysfunction may play a role in paroxysmal atrial fibrillation (PAF). **Aim:** to assess the acute changes in RR-intervals (RRI) and blood pressure (BP) variability in PAF patients (P) during standard autonomic tests. **Methods:** 16P with PAF (8 men; 55±17 years) and 16 healthy individuals (HI) (8 men; 54±10 years) while on sinus rhythm. RRI and BP were assessed and LF (low-frequency power), HF (high-frequency power) and LF/HF calculated in response to head-up tilt (HUT), isometric handgrip (HG), cold pressure test (CPT) and deep breathing (DB). **Results:** In baseline RRI and BP were similar for both groups. HI showed larger mean RRI and maximum RRI during DB, with no differences between groups in the other tests. During HUT, two BP profiles were observed: an increase in PAF P, and a decrease followed by an increase and further recovery in HI. HG and CPT provoked a progressive increase of BP with a delayed pattern in PAF P. DB elicited a respiratory modulation of systolic and diastolic BP in both groups, but a significant decrease occurred only in HI. PAF P showed significantly lower LF in HUT, and decreased HF in basal and during tilting movement in RRI variability analysis, without differences regarding systolic BP variability. LF of BP variability increased earlier in PAF P, with higher values during the second minute of HG. During the CPT, LF for BP variability analysis increased significantly only in HI. There were no differences between groups for RRI variability during DB. **Conclusions:** P with PAF present modified cardiovascular responses during maneuvers evoking sympathetic outflow. Wavelets analysis may provide a new insight into the assessment of cardiovascular regulation and underscore the presence of autonomic disturbances in PAF.

Key words: atrial fibrillation, autonomic tests, heart rate variability, blood pressure variability, wavelet transform

Introduction

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia in clinical practice. Its prevalence increases with age, with impact on both life expectancy and quality of life (1-4). The occurrence of paroxysmal AF (PAF), defined as self-terminating episodes with <7 days duration, ranges between 28,5% and 30% of all AF cases and is more frequent in younger patients without underlying heart disease (5-6). AF physiopathology remains a considerable challenge, in part due to our limitations in understanding the precise mechanisms underlying the initiation and maintenance of this arrhythmia. The relative contribution of the multiple wavelet hypothesis proposed by Moe (7) and the focal sources of electrical activity described by Haissaguerre (8) to explain AF remains unknown. The autonomic nervous system (ANS) has been recognized as an important modulator in the pathogenesis of PAF, but the mechanisms linking ANS activity with AF are incompletely understood (9-11). A number of electrophysiological properties, related with atrial vulnerability for AF, may change as a result of the pro-arrhythmic effects of vagal or sympathetic activation (12-14). Vagal stimulation reduces conduction velocity in the atrial tissue and shortens the action potential duration and the atrial effective refractory periods in a non-uniform way, facilitating re-entrant atrial arrhythmias (9,13). Sympathetic stimulation can favour reduction of atrial refractoriness and induction of trigger activity such as rapid repetitive activations and atrial tachyarrhythmias (10,13,15). Therefore, fluctuations of autonomic tone and functional alterations of ANS function may be determinant for the occurrence of PAF episodes (9,10). Provocative maneuvers such as head-up tilt (HUT), isometric handgrip (HG), cold pressure test (CPT) and deep breathing (DB) evoke rapid short-term adjustments in autonomic control of heart rate (HR) and blood pressure (BP) that can be used to evaluate sympathetic and parasympathetic outflows indirectly (16-18). Spectral analysis of HR and BP recordings using Fast Fourier Transform (FFT) has been shown to provide indirect estimates of sympathovagal cardiovascular control (19). In fact, a range of low frequencies (LF) and high frequencies (HF) has been established, the first one reflecting predominantly sympathetic activity, whereas the second is related with parasympathetic outflow (20). However, FFT requires a stationary signal with a duration of, at least, five minutes, which makes it unsuitable for the analysis of the short transient changes that occur during the very first period of time after the beginning of an autonomic provocative manoeuvre. The impossibility of correlating the changes in frequency with the time of their occurrence in a

signal constitutes another limitation of FFT analysis, that can be overcome by applying wavelet transform, which allows a time-scale representation of data even during abrupt changes of signal (18,21).

In the present study, we used the wavelet transform to analyze HR and BP recordings and characterize ANS modulation of cardiovascular activity recorded during standard autonomic tests (HUT, HG, CPT, and DB) in patients with lone PAF.

Subjects and Methods

Subjects

The study enrolled 16 patients (8 females and 8 males, age 55±17 years) affected by ≥1 year of PAF episodes (range 1-5 years), and 16 healthy volunteers (HI) (8 females and 8 males, mean age 54±10 years). PAF was documented with electrocardiograms (ECG) and/or Holter recordings. Patients with PAF were diagnosed as having lone AF (without clinical or echocardiographic evidence of cardiopulmonary disease). None of these subjects had history of syncope, myocardial infarction, congestive heart failure, diabetes mellitus, hypertension or sleep apnea. Prior to autonomic evaluation, all antiarrhythmic drugs were withdrawn for at least 5 half-life times. Patients under amiodarone ceased treatment 2 months before the test. Alcohol, tobacco and medication with anticholinergic properties were not allowed on the day of the tests. Some subjects were unable to perform all the tests adequately and in some recordings simultaneous HR and BP analysis was not available, so these results have been withdrawn from the study. Hence, for each group, the HUT was validated in 16 cases (55±17 years and 54±10 years, for PAF and HI, respectively, p=NS), HG test was validated in 12 cases (54±15 years and 53±11 years, for PAF and HI, respectively, p=NS) and the CPT and DB tests in 8 of the 16 subjects (50±16 years and 51±15 years, for PAF and HI, respectively, p=NS). All tests were performed in a quiet environment, with controlled temperature and humidity, during the morning, following four hours of fasting. ECG and BP were continuously monitored by a Task Force Monitor (*CNSystems, Graz, Austria*). Studies were approved by the local ethics committee and performed after informed consent.

Autonomic Testing protocol

After a resting period of 15 minutes in the supine position, subjects were placed on a tilt table with a foot plate support, secured with snug restraints to prevent falling if syncope occurred, and tilted head-up to a level of 60º at a constant speed (within 15 seconds) for 7 minutes. Then the electrical table returned to the horizontal position. A period of rest (15 minutes) in the supine position was allowed between each provocative maneuver to guarantee a stable condition. All testes were performed during controlled breathing. To achieve sympathetic activation, 3 minutes of static HG exercise at 30% of maximum voluntary contraction was performed, followed by right hand immersion in ice-cold water (4º C) for 1 minute. Finally, subjects were instructed to breathe deeply at a rate of 6 breaths per minute, guided by a metronome for a period of 1 minute, to measure parasympathetic function indirectly.

Data analysis

Wavelet analysis using wavelet Daubechies 12 (Db 12) was implemented on RR intervals (RRI) and BP derived from continuous monitoring of ECG and arterial BP, respectively (see signal acquisition and processing below). For each autonomic test, data analysis was done as follows.

Head-up tilt. The RRI and systolic BP data analysis was performed on four periods: 1) the last 2 minutes of the resting period (basal, used as control); 2) during the 15 seconds of the change from supine to standing position (TT), 3) at 60º, during the 1st-minute of tilt adaptation (TA1); and 4) during the 2nd-minute of tilt adaptation (TA2). In each of these 1-min-periods, data analysis was performed in periods of 10 seconds. The period of 10 seconds that had the largest change from maximum to minimum was the one chosen to compare with the control values.

Handgrip. The analysis of RRI and diastolic BP was done in the last 3 minutes of the resting period just prior to the test and during the 3 minutes of sustained isometric exercise. In each of these 3-min-periods, analysis was performed in periods of 10 seconds. The period of 10 seconds that had the largest change from maximum to minimum was chosen to compare with the baseline values.

Cold pressure test. Cardiovascular variables were analyzed during two periods: the last 2 minutes of the resting period just prior to the test and during 1 minute, divided into six epochs of 10 seconds. The period of 10 seconds with the larger change from maximum to minimum was the one chosen to compare with baseline.

Deep breathing. Data analysis was performed on two periods: the last minute of the resting period before the test and during 1 minute of DB, divided into six epochs of 10 seconds. The period of 10 seconds with the largest change from maximum to minimum was chosen to compare with baseline.

Signal acquisition and processing

Data were acquired at 1Kz and analysed on the time-scale domain using the discrete wavelet transform Db12. Db12 was selected because its shape resembles the type of feature present in the time series of the signal information (21). Data were then processed under MatLab (MathWorks, Natick, MA, USA) and Origin environments (OriginLab, Origin Lab Scientific Graphing and Analysis Software, Origin Lab Corporation, Northampton, MA, USA) as described by Ducla-Soares et al (18).

Statistical analysis

Continuous variables were expressed as mean ± SEM, unless otherwise indicated, and categorical variables as frequencies and percentages. Normality of the distributions of the continuous variables was analyzed with the Kolmogorov-Smirnov test. Two-way ANOVA analysis of variance with repeated measures was used to compare data during provocative maneuvers and between groups. The chi-square test with Yates correction was used for categorical variables. A value of $p<0.05$ was considered statistically significant. Data were analyzed using GraphPAD Instruments version 3.05 (*GraphPad Software, Inc., California, USA*).

Results

All participants completed the testing protocol without any symptoms or AF initiation. No significant differences in age, sex ratio, baseline arterial BP and HR were found between the control group and the group of patients with PAF (table I).

Head-up tilt

heart rate and blood pressure changes

In PAF patients, HR rose on TA1 and TA2 periods when compared with baseline values, but no significant differences were observed between the two groups on HR response during all analyzed periods (Figure 1A). During HUT, HI showed an initial fall in BP, followed by a recovery and a further increase, accompanied by a slight decrease in HR (figure 1B). In PAF patients group, a significant increase of BP was observed in TT, TA1 and TA2 periods when compared with basal values, with BP values significantly higher during orthostatism, compared to HI (figure 1B). Thus, two BP-response profiles were observed: a sustained increase in PAF patients, and a decrease followed by an increase and further recovery in control subjects.

heart rate and blood pressure variability

In PAF patients, wavelet analysis of the RRI did not change for the LF and HF bands during the four periods of HUT, whereas HI showed significant variations for LF power during TA1 and for HF power during TA2, compared to basal (table 2). Also, LF values were higher for HI in all analyzed periods and HF values were higher in basal and during TA1. There were no differences in the LF/HF ratio pattern between groups, except for the TT period. The application of wavelets to systolic BP signal showed no significant differences between both groups for all the periods (table 2). Figure 2 shows an example of acute changes on RR intervals during HUT analyzed by wavelet transform in an HI and a patient with PAF.

Handgrip

heart rate and blood pressure changes

Figure 3 shows the RRI and BP values during HG testing. The RRI did not change significantly in the two groups. In basal, BP was similar for both groups. Compared to baseline, there were significant variations of systolic BP for HI during the 3-minutes HG analysis ($p < 0.0001$), whereas PAF patients showed a significant increase of systolic BP only during the third minute of HG ($p<0.01$). Diastolic BP increased significantly for both groups during the 3-minutes HG testing ($p<0.01$).

blood pressure variability

In basal there were no differences between PAF patients and HI. Wavelet results of the diastolic BP are represented in table 3. In PAF patients the LF band increased significantly since the first minute of HG testing, while in HI a significant variation of LF occurred only during the third minute of HG. Also, the LF/HF ratio changed significantly during the 3-minute HG in the PAF group, whereas HI showed no significant variations compared to basal. LF values were higher for PAF patients during the second minute of HG. There were no differences between groups when comparing HF and LF/HF ratio values.

Cold Pressure Test

heart rate and blood pressure changes

Figure 4 shows the RRI and BP values during CPT. RRI did not change significantly during the test. In basal, BP values were not statistically different between groups. Compared to baseline, there was a significant sustained increase of systolic BP for HI since the first 10-seconds epoch, whereas PAF patients showed a significant increase of systolic BP only after the fourth 10-seconds epoch. Also, diastolic BP increased significantly since the second 10-seconds epoch in HI, and only after the fifth 10-seconds epoch in PAF patients.

blood pressure variability

Wavelet analysis of the systolic BP during the CPT is represented in table 4. There were no differences between PAF patients and HI when comparing LF, HF and LF/HF results in baseline and during the period of 10 seconds with the larger change from maximum to minimum during 1 minute of testing. However, in HI, the LF band changed significantly during the CPT when compared to baseline.

Deep breathing

heart rate and blood pressure changes

Figure 5 represents the RRI and BP values during DB. Mean RRI decreased slightly for both groups but did not change significantly from baseline to DB. However, the maximum RRI were significantly higher for both

groups during the test. HI showed mean RRI and maximum RRI larger than PAF patients during DB ($p=0.03$ and $p<0.01$, respectively). There were no differences for BP values between groups. Compared to baseline, systolic BP and diastolic BP decreased significantly for HI ($p<0.05$), whereas PAF patients showed no significant changes during DB.

heart rate variability

Wavelet analysis of the RRI during DB is shown in table 5. There were no differences between PAF patients and HI when comparing LF, HF and LF/HF results in baseline and during the period of 10 seconds with the larger change from maximum to minimum during 1 minute of testing. There was a marked increase in the LF values due to a shift of the frequency range of the parasympathetic-mediated changes to the LF band at this respiratory rate (6 breaths per minute).

Discussion

The main purpose of the present study was to analyse, by using wavelet transform applied to continuous RRI and BP signals, the autonomic behaviour of PAF patients during four classical autonomic testing maneuvers: HUT, HG, CPT and DB. Our results show that, despite similar clinical characteristics and no differences of mean HR and BP in basal conditions, patients with PAF have significant differences in the analysis of the ANS outflow and BP responses in the initial period of adaptation after orthostasis, in the HG isometric test, and during the CPT. when compared to HI. Furthermore, the DB testing was associated with significant differences in RRI and BP changes between the groups, despite eliciting the same pattern for the parasympathetic activation.

In a previous study, we have shown that wavelet transform is a suitable mathematical tool to evaluate acute autonomic changes in HI (18). Wavelet analysis is able to reflect a precise, temporally localized, shift in ANS equilibrium, and has been widely accepted for performing time-frequency decomposition of cardiovascular signals (18, 19, 22,23). The wavelet transform methodology has been previously used by our group in short duration analysis, allowing the visualization in time of the contribution of LF and HF to the observed dynamic

changes of non-stationary cardiovascular signals, being able to reflect a precise, temporally localized, shift in ANS equilibrium (18, 24,25).

In the present study, we selected the Daubechie type of wavelet function because it has been proposed as the most suitable for BP and HR signal profiles (18,21), and we studied the contribution of two ranges of frequencies, LF- band [0,038-0,15 Hz] and HF-band [0,15-0,40 Hz] (20). On wavelets analysis, LF and HF keep their physiological significance, encountered on FFT applications: LF [low frequencies] reflects mainly sympathetic activity, while HF [high frequencies] is a measure of parasympathetic outflow and respiration. Beyond LF and HF, the LF/HF ratio is also an important relation as it indicates the sympatho-vagal balance (20, 21, 26).

Effect of provocative maneuvers on RRI, BP, and cardiovascular variability

Head-up tilt

HUT is a physiological stimulus that is associated with increased sympathetic tone and withdrawal of vagal activity (27). Classically, HUT has two phases of adaptation: an earlier cardiovascular response, that can be observed during the first 15 seconds after orthostasis, followed by a stabilization period, composed by an early adaptation occurring in the first 2 minutes of HUT, and a second period related to prolonged orthostasis that lasts more than 5 minutes (18, 28). The rapid short-term adjustments to orthostatic stress are mediated by ANS neural pathways (16). In this population, no differences on baseline HR and BP values, age or sex distribution were found between the PAF group and the HI. Our results show the modifications of autonomic outflow that underlie the changes of HR and BP during the adaptation period of orthostatic challenge. Compared to HI, PAF patients showed a different BP pattern, with a sustained increase, causing significantly higher values during orthostatism. This contrasts with the significant lower values in the LF band of the RRI variability combined with lower HF values when changing from supine to standing position, and no differences in the systolic BP variability analysis (figure 1, table 2). During the adaptation phase after orthostatism, only the HI group revealed a significant increase of the LF component and a significant parasympathetic withdrawal as observed by Ducla-Soares et al (18). PAF individuals showed a trend to an increase of the sympathetic tone with a reduction in parasympathetic activity, obtaining a significant increase in the LF/HF ratio for the first

and second minutes after tilting. Nevertheless, this pattern clearly occurred with a lower power compared to HI, reflecting less HR variability (table 2). Therefore, these results revealed that the autonomic response to passive tilting is impaired in PAF patients, indicating an imbalance in the cardiac influence of autonomic activity, with reduced LF and HF components. In a previous study, no significant cardiovascular autonomic dysfunction was found in patients with PAF compared to normal subjects (29). However, the study was limited to the measure of HR response to standing, DB and Valsalva maneuver, in addition to baroreflex sensitivity test and 24-hour HR variability. Our protocol analysed a time-frequency decomposition of HR and systolic BP to define a pattern of autonomic activation during the adaptation phases to orthostatic stimulus and confirmed that patients with lone PAF may have dysfunction of the cardiac autonomic responses to HUT when compared with normal controls. The differences observed in HR variability, when compared to HI, may also reflect the phenomenon of autonomic remodeling, which is characterized by nerve sprouting and a heterogeneous increase in atrial sympathetic innervation, caused by AF (30). These observations could be related with a progressive adaptation of the autonomic cardiovascular receptors due to a certain degree of ANS dysfunction. In fact, abnormalities of the autonomic tone can lead to an up-regulation of the cardiovascular receptors, which may be related with a heightened susceptibility to autonomic cardiovascular responses (31,32. Also, a complementary view in the interpretation of these data may be in concordance with previous studies showing that essential hypertension is linked to basic alterations in cardiovascular autonomic control, with specific abnormalities being already present at a very early stage of the disease, perhaps even before any sign of increased BP can be observed (33). In a recent prospective survey, systemic hypertension was found to be the most common disease associated with all types of AF (34). Therefore, changes of ANS responses detected during HUT, might also be looked as a potential indicator for the development of hypertension in the future.

Pressor stimuli – handgrip and cold pressure

Sustained HG isometric exercise and the cutaneous CPT elevate BP by stimulating sympathetic activity. Our results show that during HG the highest diastolic BP raised more than 10 mmHg compared to baseline, following a similar pattern in both groups despite the differences observed in the LF band of the diastolic BP

variability. The LF band and the LF/HF ratio increased significantly in the group with PAF since the first minute of HG testing, whereas in HI a significant variation of LF occurred only during the third minute of HG without significant changes in the LF/HF ratio. Furthermore, there was a delayed increase of systolic BP in the PAF group, showing a significant raise of systolic BP only during the third minute of HG. In a previous study by Van den Berg et al, diastolic BP increasing in PAF patients during HG was in accordance with the normal values of the laboratory (3). Nevertheless, the authors did not evaluate the ANS outflow during the test. The explanation for this discrepancy between systolic and diastolic BP profile is speculative because the effects of static HG on regional hemodynamics are only partially understood. It may be related with the involvement of regional changes in blood volume and local factors affecting the vascular responses. Recently, it has been suggested that sympathetic vasoconstrictor nerves and nitric oxide are linked with respect to the support of the vasodilator response (35).

In the CPT, used to activate peripheral receptors of pain and temperature by immersion of one hand and arm in ice-cold water, the sympathetic activation induces an increase of HR and BP, but systolic BP has been accepted as the more reliable measurement (18,36). In our study, there was a significant sustained increase of BP for both groups, but with a delay in PAF patients, observed in diastolic and systolic BP. This could be related with the significant increase of the LF band without changes in HF recorded only in the HI group. The data suggest a slightly attenuated response in PAF patients compared with control subjects. In a previous study, we demonstrated a direct relation between changes in systolic BP and sympathetic outflow in healthy subjects (18). Thus, cardiovascular responses during provocative maneuvers associated with tests evoking sympathetic outflow seem to be modified in patients with PAF.

Deep breathing

Parasympathetic function may be evaluated by the analysis of RRI variability during normal and DB (18). At a respiratory rate of 6 breaths per minute the processing of the RRI signal shows a shift of vagal-mediated changes from the HF band to the LF range of frequencies. Accordingly, the parasympathetic outflow should be coincidental with the LF power when the frequency of deep-breathing is carefully controlled (37). Deep breathing is known to reduce BP in HI (38). In our population, although there were no differences between

PAF patients and HI when comparing LF, HF and LF/HF results in baseline and during DB, HI showed larger mean RRI and maximum RRI, and a significant decrease of both systolic and diastolic BP. As described by Van den Berg et al, the vagal reactivity to DB may be marginally depressed in PAF patients (3). Thus, beyond the evidence of heightened atrial sympathetic innervation caused by AF, parasympathetic tone is likely to be remodeled in AF. Studies in humans and animal models showed that muscarinic acetylcholine receptors are remodeled in AF. In a canine model of AF induced by heart failure and in humans with AF, I_{KACh}, an inwardly-rectified K^+ current carried by channel subunits activated by acetylcholine and believed to be the principle mediator of parasympathetic effects on the heart, was downregulated and mRNA expression levels of both G-protein regulated inward-rectifier K^+ channels and muscarinic acetylcholine receptors were reduced (39,40).

We here present a report on acute changes of ANS outflow in patients with PAF submitted to standard autonomic tests. The application of the wavelet transform function allowed a time-frequency analysis of the RRI and BP signals with information regarding the transient fluctuations in specific frequency bands. The role of the ANS in AF is complex and difficult to evaluate. The described changes of cardiovascular responses, associated with modifications of autonomic control in tests evoking sympathetic outflow, may play a role both in the genesis of AF and in facilitating recurrent AF episodes in relation with autonomic remodeling. Further studies are needed to confirm these findings and determine the clinical relevance of autonomic activity and cardiovascular responses in AF recurrence.

Study limitations

The resulting sample size with PAF was relatively small and not all patients performed the four tests. However, the results were obtained in a group with lone AF who underwent autonomic evaluation off medication and the number of subjects included in the study allowed for the identification of significant differences in the analysed parameters when comparing HI with PAF patients.

The study did not include measurements beyond the 2-minutes of orthostatic stress. Therefore, comparison of the autonomic activity occurring during prolonged standing can not be discussed. Nevertheless, it has been accepted that the rapid short-term adjustments in the first two minutes of HUT are mediated by the neural

pathways of the ANS (Wieling & Lieshout, 1993). Also, these results might not necessary apply to autonomic tests performed according to different protocols.

Finally, cardiovascular responses to these tests include factors such as motivation, individuals variation in cold exposure tolerability, regulation of sympathetic and parasympathetic outflows, noradrenalin reuptake, adrenoceptor number and affinities and baroreflex sensivity. Therefore, the physiological meaning of the interaction between the complex mechanisms underlying responses to provocative maneuvers and AF still represents a challenge that needs to be further explored.

Conclusions

The present data indicate that wavelets analysis may provide a new insight into the assessment of autonomic cardiovascular regulation and suggest that patients with lone PAF present a modified autonomic control of HR and BP during tests evoking sympathetic outflow, combined with significant changes in the cardiovascular responses associated with both autonomic systems.

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Table 1. Clinical characteristics of the subjects

Characteristic	PAF group (n=16)	HI group (n=16)
Age, years	55±17	54±10
Male gender	50%	50%
Heart rate, bpm	60±10.0	58±7.3
Systolic blood pressure, mmHg	125±18.3	115±8.3
Diastolic blood pressure, mmHg	81±11.5	75±8.5

*PAF=paroxysmal atrial fibrillation; HI=healthy individuals; bpm=beats per minute. * Data expressed as mean ± SD or %*

Table 2 – Changes in heart rate and blood pressure variability during head-up tilt

	Basal	**TT**	**TA1**	**TA2**
a) RRI				
LF values (ms^2)				
PAF	298±73.7	337±87.1	567±131.7	513±200.6
HI	657±143.3§	648±170.6§	1128±255.2*§	813±153.1§
HF values (ms^2)				
PAF	150±26.3	133±37.8	171±55.1	101±26.3
HI	349±85.1§	478±118.1§	240±43.7	156±39.6*
LF/HF ratio				
PAF	2,4±0.55	4.4±1.28	9.0±2.79*	10.3±3.90*
HI	2.6±0.55	1.4±0.29§	7.5±2.16*	10.6±3.09*
b) SBP				
LF values ($mmHg^2$)				
PAF	4.6±0.62	6.6±1.46	10.4±2.20*	11.1±2.5*
HI	3.6±0.86	5.7±2.54	15.6±3.26*	16.0±4.4*

Basal=supine position; TT=tilting movement; TA1=1st minute of orthostatism; TA2=2nd minute of orthostatism; RRI=RR intervals; SBP=systolic blood pressure; PAF=paroxysmal atrial fibrillation group; HI=healthy individuals group. ** p < 0.05 within the same group and when compared with the basal;§ p < 0,05 between PAF and HI for the same period of analysis; Data expressed as mean±SEM.*

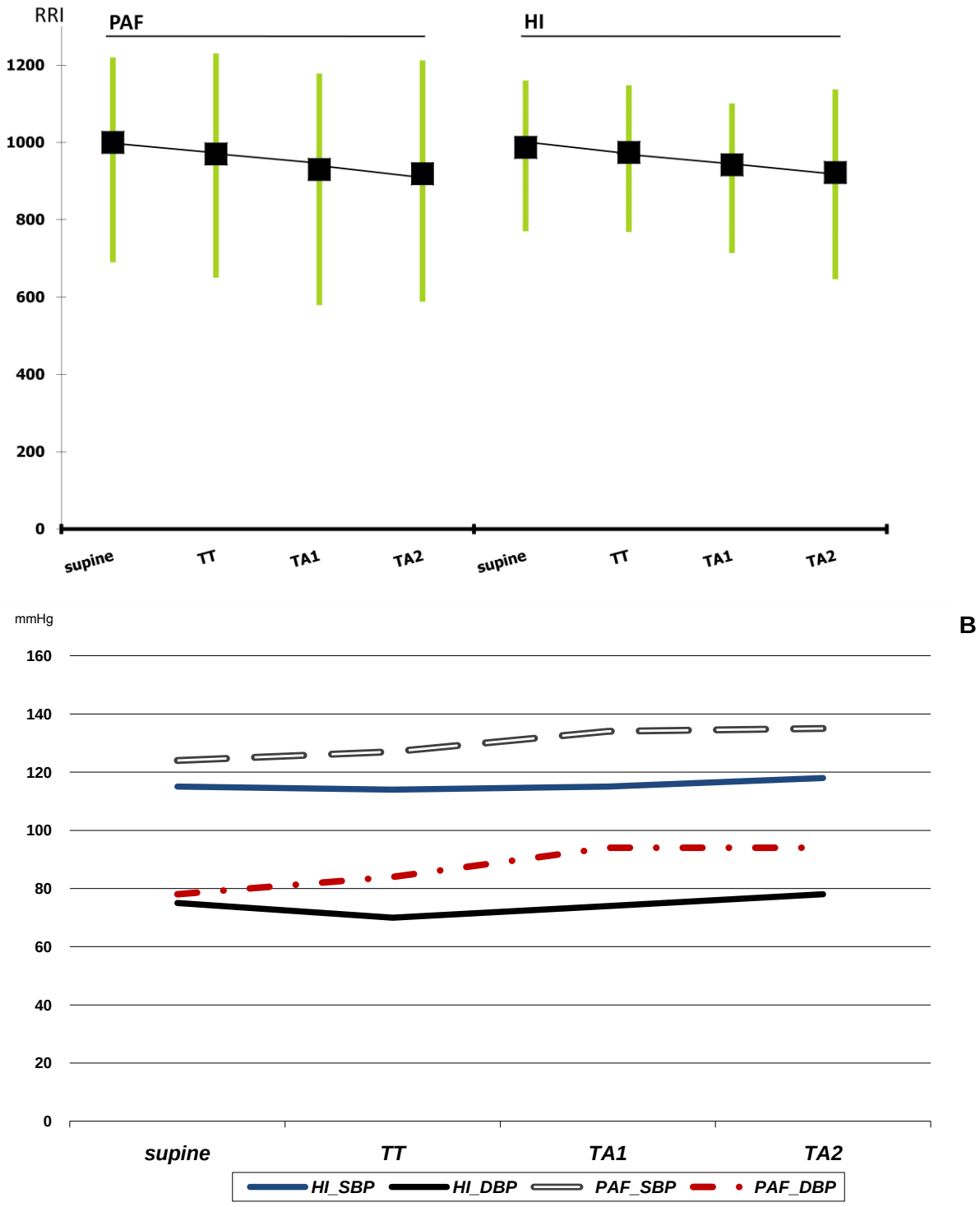


Figure 1 - Comparison of changes in RR intervals (A), systolic and diastolic blood pressure (B) during head-up tilt testing in patients with paroxysmal atrial fibrillation (PAF) and in healthy individuals (HI). *RRI=RR intervals; N_SBP=systolic blood pressure in HI; N_DBP=diastolic blood pressure in HI; PAF_SBP=systolic blood pressure in the PAF group; PAD_DBP=diastolic blood pressure in the PAF group.TT=tilting movement; TA1=1st minute of tilting; TA2=2nd minute of tilting; p=NS for RRI response between groups; BP comparison between groups: in supine (PAF vs. HI, p=NS); during TT (PAF vs. HI, p<0.02); during TA1 (PAF vs. HI, p<0.001); during TA2: (PAF vs.HI, p<0.001).*

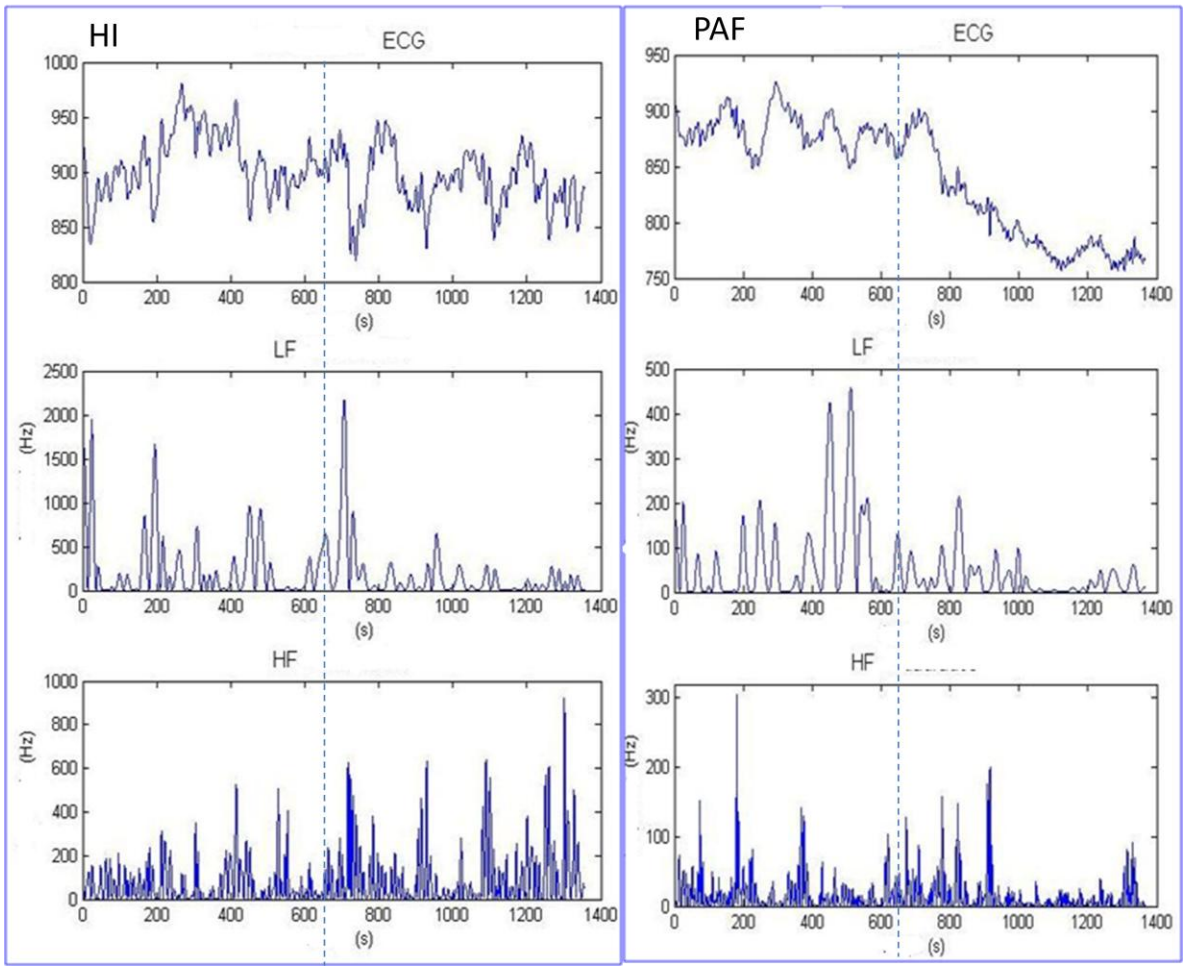


Figure 2– Changes on RR intervals during head-up tilt analyzed by wavelet transform.
This figure shows LF and HF changes evoked by tilt testing on a 65 years-old normal subject (HI) (left) and a 67 years-old patient with paroxysmal atrial fibrillation (PAF) (right). On top panels is well shown that, after tilting, the PAF patient is unable to adapt and restore the baseline values of heart rate when compared with the observed changes on the HI. This is well correlated with the modifications observed in LF and HF as well as with the scaling differences which indicate a lower power for both LF and HF of the PAF patient.

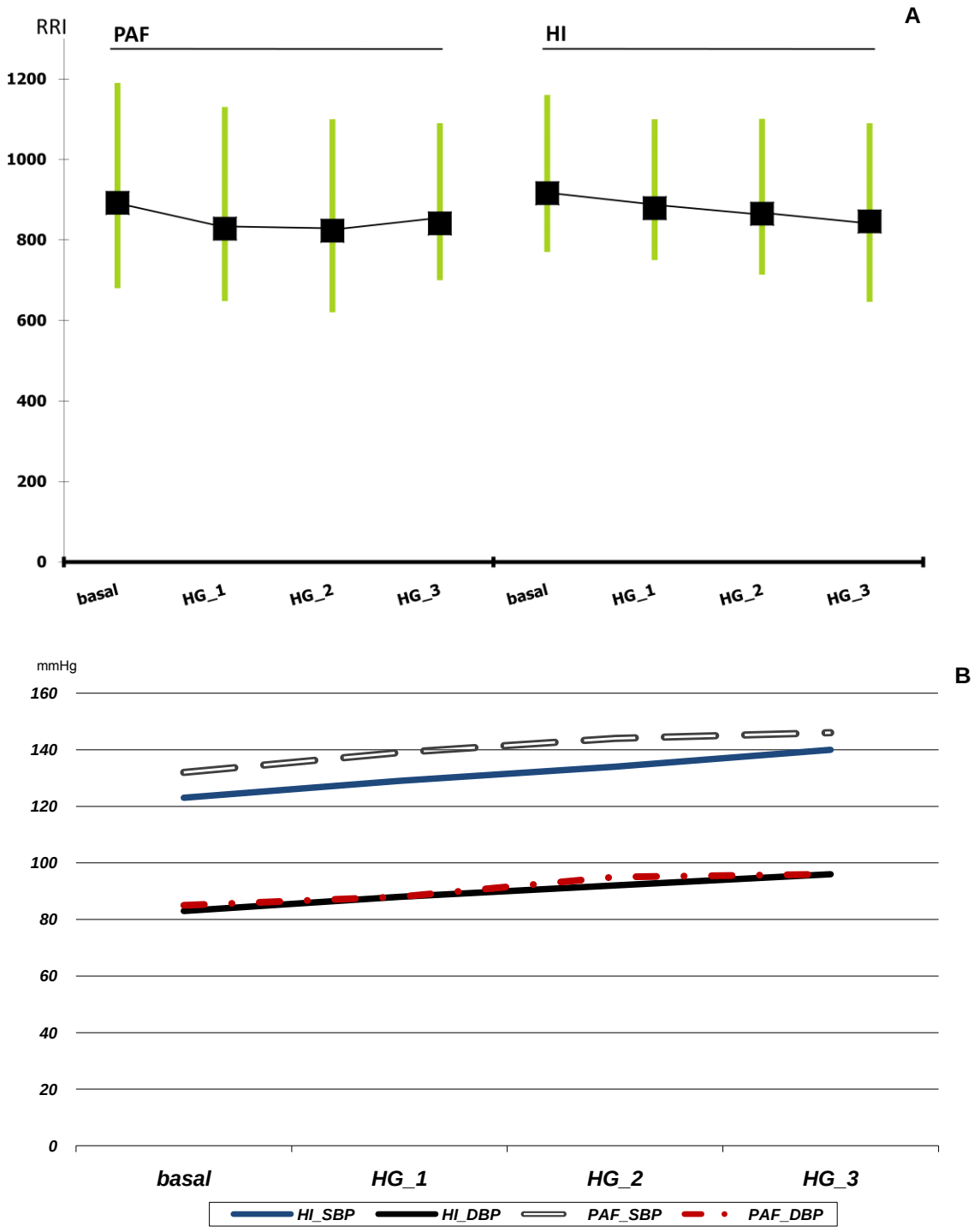


Figure 3 - Comparison of changes in RR intervals (A), systolic and diastolic blood pressure (B) during handgrip testing in patients with paroxysmal atrial fibrillation (PAF) and in healthy individuals (HI). *RRI=RR intervals; N_SBP=systolic blood pressure in HI; N_DBP=diastolic blood pressure in HI; PAF_SBP=systolic blood pressure in the PAF group; PAD_DBP=diastolic blood pressure in the PAF group. HG_1=1st minute of handgrip; HG_2=2nd minute of handgrip; HG_3=3rd minute of handgrip. p=NS for RRI response during HG within each group; p<0.01 for SBP response in PAF patients during HG_3; p<0.01 for systolic BP response during HG_1, HG_2 and HG_3 in HI; p≤0.01 for diastolic BP during HG_1, HG_2 and HG_3in both groups. p=NS for the comparison of RRI and BP between groups.*

Table 2 – Changes in blood pressure variability during the handgrip maneuver

	Basal	HG_1	HG_2	HG_3
DBP				
LF values ($mmHg^2$)				
PAF	3.06±0.7	11.4±2.0*	§17.2±7.4*	10.3±2.5*
HI	4.41±0.8	6.91±1.8	§4.21±0.7	12.4±4.0*
HF values ($mmHg^2$)				
PAF	0.98±0.4	4.72±2.6	5.66±4.0	1.22±0.8
HI	0.46±0.1	0.45±0.1	0.48±0.2	0.60±0.2
LF/HF ratio				
PAF	7.66±2.6	30.9±13.6*	25.8±8.9*	35.1±16.1*
HI	12.4±2.7	22.6±6.0	27.9±10.8	22.4±5.2

Basal=resting period; *HG_1=1st minute of handgrip; HG_2=2nd minute of handgrip; HG_3=3rd minute of handgrip;* DBP=diastolic blood pressure; PAF=paroxysmal atrial fibrillation group; HI=healthy individuals group. ** $p < 0.05$ within the same group and when compared with the basal;§ $p < 0,05$ between PAF and HI for the same period of analysis; Data expressed as mean±SEM.*

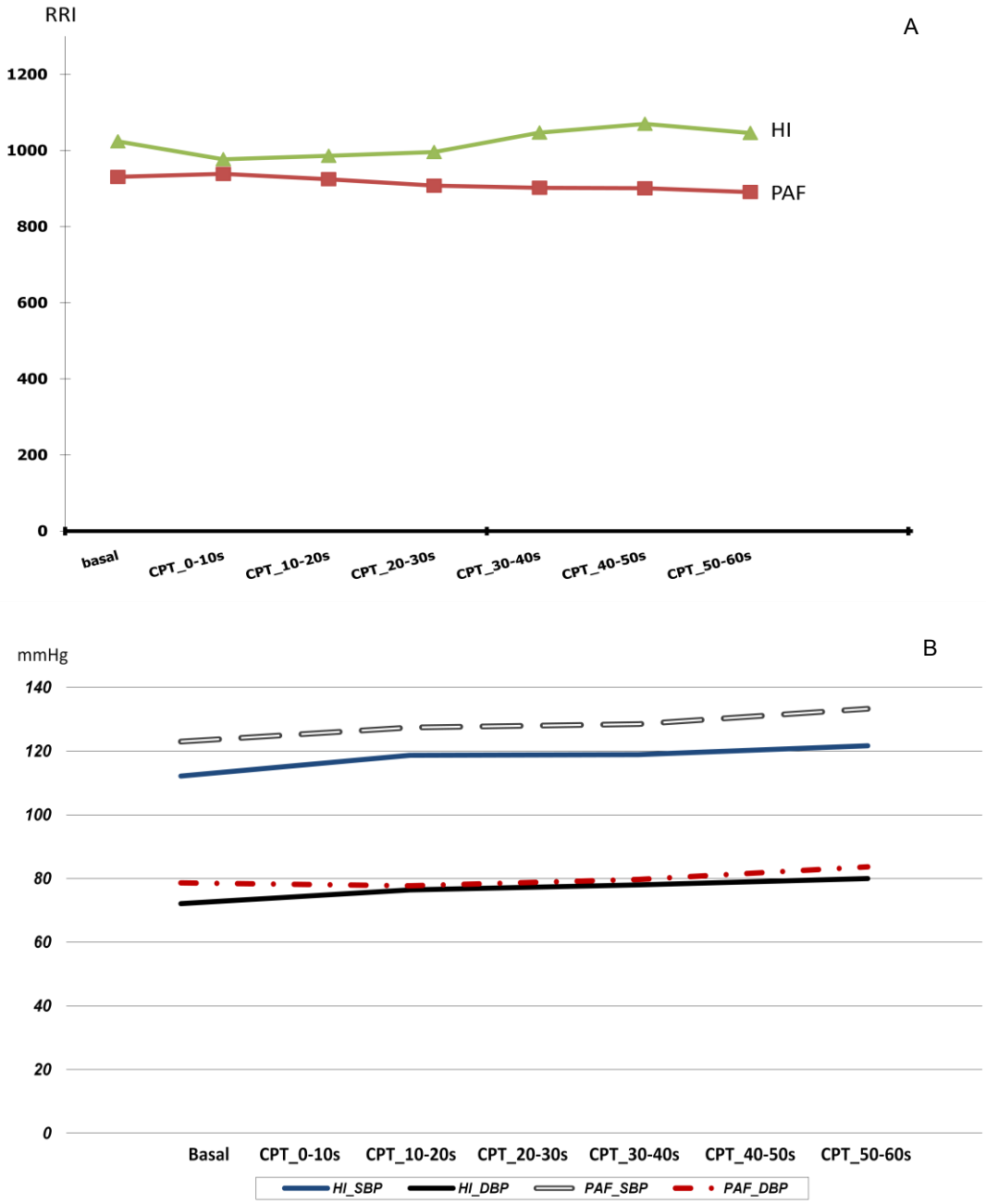
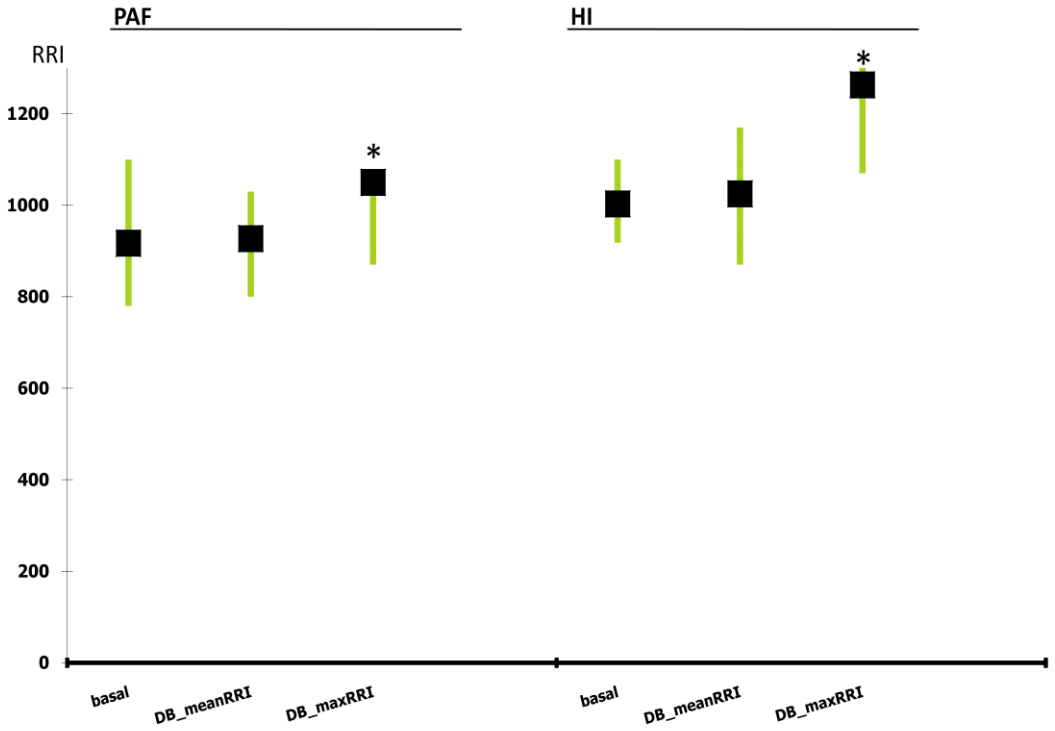


Figure 4 - Comparison of changes in RR intervals (A), systolic and diastolic blood pressure (B) during the cold pressure test in patients with paroxysmal atrial fibrillation (PAF) and in healthy individuals (HI). *RRI=RR intervals; N_SBP=systolic blood pressure in HI; N_DBP=diastolic blood pressure in HI; PAF_SBP=systolic blood pressure in the PAF group; PAD_DBP=diastolic blood pressure in the PAF group. CPT=cold pressure test (divided into six epochs of 10 seconds from 0 to 60 seconds). p=NS for RRI response during cold pressure testing within each group; p<0,05 for BP response after 40 seconds of testing in PAF patients; p<0.05 for BP response since the first 10s of the test in HI.*

Table 4 – Wavelet analysis of systolic blood pressure changes during cold pressure test

	Basal	CPT_max
SBP		
LF values ($mmHg^2$)		
PAF	6.03±2.0	12.9±3.9
HI	2.71±0.3	8.91±1.3*
HF values ($mmHg^2$)		
PAF	1.41±0.4	1.47±0.3
HI	0.99±0.2	1.39±0.4
LF/HF ratio		
PAF	4.44±1.0	11.8±5.0
HI	3.83±0.9	12.5±5.3

Basal=resting period; CPT_max= the period of 10 seconds with the larger change from maximum to minimum*; S*BP=systolic blood pressure; PAF=paroxysmal atrial fibrillation group; HI=healthy individuals. ** p <0.05, within the same group and compared with baseline. Data expressed as mean±SEM.*



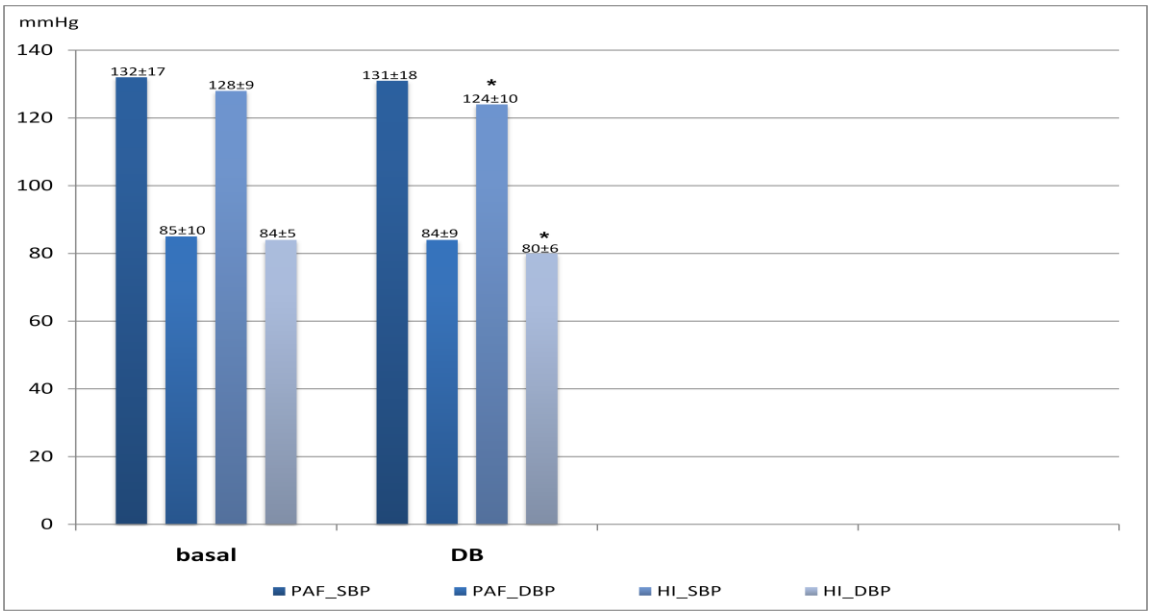
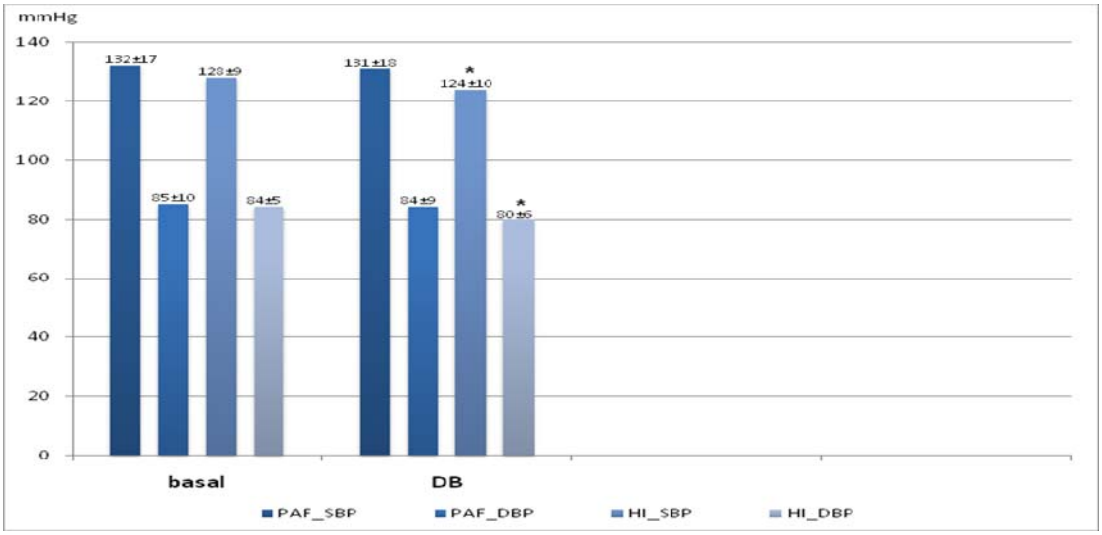


Figure 5 - Comparison of changes in mean RR intervals (A), systolic and diastolic blood pressure (B) during the deep breathing test in patients with paroxysmal atrial fibrillation (PAF) and in healthy individuals (HI). *RRI=RR intervals; N_SBP=systolic blood pressure in HI; N_DBP=diastolic blood pressure in HI; PAF_SBP=systolic blood pressure in the PAF group; PAD_DBP=diastolic blood pressure in the PAF group. DB=deep breathing.* p <0.05 within the same group and compared with baseline; p<0.05 for the comparison of mean RRI and maximum RRI during DB, between PAF and HI groups.*

Table 5 – Wavelet analysis of RR intervals during deep breathing

	Basal	DB_R6
LF values (ms^2)		
PAF	496.6±162.4	6301.2±1822.5 *
HI	1341±458.9	11607±3504.3*
HF values (ms^2)		
PAF	219.6±63.3	432.6±247.7
HI	531.6±204.9	1658.3±763.7

Basal=resting period; DB_R6= deep breathing at 6 breaths per minute. PAF=paroxysmal atrial fibrillation group; HI=healthy individuals. In the processing of the RR intervals, the parasympathetic band (HF range in basal) is shifted to the frequency range of the LF band (at a respiratory rate of 6 breaths per minute). ** p <0.01 within the same group and compared with HF values in baseline. Data expressed as mean±SEM. p=NS between groups.*



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Incidência de "Falsos-positivos" em Idosos com Fibrilhação Auricular Paroxística Submetidos a Teste de Inclinação [97]

Mário Martins Oliveira, Joana Feliciano, Ana Teresa Timóteo, Nogueira da Silva, Eduardo Antunes, Sofia Silva, Sandra Alves, Luís Silva-Carvalho, Rui Ferreira

Serviço de Cardiologia, Hospital Santa Marta Hospital, Lisboa, Portugal
Instituto de Fisiologia da Faculdade de Medicina de Lisboa, Lisboa, Portugal

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RESUMO

O sistema nervoso autónomo é reconhecido como desempenhando um papel importante na modulação dos mecanismos fisiopatológicos da fibrilhação auricular paroxística (FAp). No entanto, o padrão clínico da FAp "mediada pelo vago" tem sido descrito sobretudo em jovens sem cardiopatia. As respostas neurocardiogénicas que ocorrem durante o *stress* ortostático são atribuídas a reflexos autonómicos com predomínio da activação vasovagal.

Objectivo: avaliar numa população de idosos com FAp, qual a susceptibilidade para desencadear mecanismos reflexos associados a síncope neurocardiogénica.

População e Métodos: estudámos os resultados do teste de inclinação passivo (TT) em 34 doentes (D) com ≥65 anos (62% do sexo feminino, 72±7 anos), referenciados por FAp (≥1 ano de episódios recorrentes) (grupo FAp), comparados com 34D da mesma faixa etária (53% do sexo feminino, 74±6 anos), avaliados no contexto de investigação etiológica de síncopes de repetição (grupo Sc). Em 19D do grupo FAp não havia evidência de cardiopatia subjacente, 11D tinham história de hipertensão arterial, com dilatação auricular esquerda ligeira (≤45 mm) e 4D tinham o diagnóstico de doença das coronárias, sem enfarte do miocárdio prévio, submetidos a revascularização miocárdica e com prova de isquemia negativa. Nenhum caso apresentava diabetes *mellitus*, insuficiência cardíaca congestiva ou antecedentes de síncope. No

ABSTRACT

False Positive Responses to Head-up Tilt Testing in Elderly Patients with Paroxysmal Atrial Fibrillation

The autonomic nervous system (ANS) plays a role as a modulator in the pathogenesis of paroxysmal atrial fibrillation (PAF). The clinical pattern of vagally mediated PAF has been observed mainly in young patients. Neurocardiogenic responses during orthostatic stress are related to autonomic reflexes in which the vagal influence predominates.

Aim: To evaluate the susceptibility of elderly patients with PAF to activation of vasovagal syncope mechanisms.

Methods: We performed passive head-up tilt testing (HUT) in 34 patients (62% women, aged 72±7 years), with ≥ 1 year of clinical history of PAF - 19 without structural heart disease, 11 with hypertensive heart disease and 4 with coronary artery disease (who had no previous myocardial infarction, had undergone myocardial revascularization, and had no documented ischemia) (PAF group), and compared the results with those obtained in a group of 34 age-matched patients (53% women, aged 74±6 years), who underwent HUT due to recurrent syncope (Sc group). In this group, 21 had no documented heart disease and none had a clinical history of AF. There was no diabetes, congestive heart failure or syncope in the PAF group. After a supine resting period, the subjects were tilted at 70º for 20 minutes while in sinus rhythm. No

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grupo Sc, 21D não apresentavam cardiopatia associada (p=NS) e não havia história clínica de FA. Efectuámos TT a 70º, enquanto em ritmo sinusal, durante 20 minutos, após período de repouso em posição supina, sem recurso a agentes provocativos. Obteve-se monitorização contínua electrocardiográfica e da pressão arterial (PA) (*Task Force Monitor, CNSystems*). O TT foi considerado positivo quando ocorreu síncope/pré-síncope com bradicardia e/ou hipotensão arterial, e as respostas classificadas como cardioinibitória, vasodepressora ou mista. Considerou-se hipotensão ortostática a queda da PA sistólica >20 mmHg ou da PA diastólica >10 mmHg nos primeiros 3 minutos de ortostatismo.
Resultados: Ocorreram respostas positivas em 7D (vasodepressora em 5D e mista em 2D) - 20,5% do total e 26,3% do grupo sem cardiopatia - do grupo FAp, e em 8D (vasodepressora em 6D e mista em 2D) do grupo Sc (p=NS). Em 3D do grupo FAp (num caso imediatamente após síncope vasodepressora), registaram-se períodos auto-limitados de FA (duração de 15 a 33 segundos) durante o TT. Não houve diferenças estatisticamente significativas para a distribuição por sexo, idade e cardiopatia subjacente. Nenhum D apresentou síncope neurocardiogénica cardioinibitória ou hipotensão ortostática.
Conclusão: Em idosos com FAp, regista-se um número significativo de falsos positivos durante a fase passiva do TT, traduzindo uma provável hiperreactividade vasovagal apesar da faixa etária. Estes dados podem contribuir para a compreensão dos mecanismos autonómicos e alterações subjacentes à FAp.

Palavras-Chave

Fibrilhação auricular paroxística; Sistema nervoso autónomo; Teste de mesa basculante; Síncope neurocardiogénica

provocative agents were used to complement the HUT. ECG and blood pressure were continuously monitored (Task Force Monitor, CNSystems). The test was considered positive when syncope or presyncope occurred with bradycardia and/or arterial hypotension. Abnormal responses were classified as cardioinhibitory, vasodepressor or mixed.
Results: HUT was positive in seven patients of the PAF group - vasodepressor response in five and mixed in two (20.5% of the total; 26.3% of those without heart disease) - and in eight patients (vasodepressor in six and mixed in two) of the Sc group (p=NS). During HUT, three patients of the PAF group had short periods of self-limited PAF (in one, after vasodepressor syncope). There were no differences in gender distribution, age or heart disease. No cardioinhibitory responses or orthostatic hypotension were observed.
Conclusion: In elderly patients with PAF, a significant number of false positive results during passive HUT may be expected, suggesting increased vasovagal reactions despite aging. This suggests that ANS imbalances may be observed in this population.

Key words

Paroxysmal atrial fibrillation; Autonomic nervous system; Head-up tilt; Neurocardiogenic syncope

INTRODUÇÃO

A fibrilhação auricular (FA) é a perturbação persistente do ritmo cardíaco mais comum na prática clínica. A sua prevalência aumenta com a idade, variando entre 0,5% na população geral e 5% acima dos 65 anos de idade, com

INTRODUCTION

Atrial fibrillation (AF) is the most common sustained cardiac rhythm disturbance in clinical practice. Its prevalence increases with age, ranging from 0.5% in the general population to 5% in those aged over 65, and has adverse

impacto desfavorável na qualidade de vida, morbilidade e mortalidade [1-5]. Em registos europeus recentes, envolvendo largos milhares de doentes, a idade média da população estudada foi superior a 65 anos sendo a hipertensão arterial a patologia mais frequentemente associada à FA [2,5-7]. A compreensão dos mecanismos fisiopatológicos subjacentes ao início e manutenção da FA permanece um desafio, estando por esclarecer qual a sequência de fenómenos responsáveis pelos episódios recorrentes de FA. Estima-se que a FA paroxística (FAp) represente entre 25-62% dos casos de FA tratados no âmbito dos cuidados primários de saúde e em ambiente hospitalar [6,8].

A contribuição relativa das principais teorias associadas à FA, a relacionada com a origem focal e a hipótese dos múltiplos circuitos de reentrada, permanece por esclarecer [6,7]. O sistema nervoso autónomo (SNA) tem sido reconhecido como um importante modulador na fisiopatologia da FA, encontrando-se muitas vezes associado à recorrência de episódios paroxísticos [9-11]. A estimulação vagal interfere significativamente em propriedades electrofisiológicas relacionadas com vulnerabilidade para a FA, como a velocidade de condução, a duração do potencial de acção e a refractariedade no tecido auricular, criando condições para o aparecimento de arritmias auriculares. Flutuações bruscas da actividade autonómica podem ser determinantes na ocorrência de FAp, estando descritas, por diversos autores, alterações significativas do tónus simpático e/ou vagal precedendo episódios de FA [9,11,12]. No entanto, o padrão clínico da FAp "mediada pelo vago" tem sido descrito sobretudo em adultos jovens, sem cardiopatia associada [10,13]. Por outro lado, também as respostas neurocardiogénicas que ocorrem durante o *stress* ortostático são atribuídas a reflexos autonómicos com predomínio da activação vasovagal, estando demonstrado, com recurso a análise espectral dos intervalos RR, o aumento da influência vagal na síncope induzida durante o teste de inclinação [14]. É escassa a informação relativa à incidência "falsos--positivos", condicionando síncopes vasovagais, em doentes com FAp. No presente estudo, utilizámos o teste de inclinação passivo (TT) para avaliar a susceptibilidade para a indução de síncope associada a mecanismos neurocardiogénicos em idosos com FAp.

effects on quality of life, morbidity and mortality [1-5]. In recent European registries including many thousands of patients, the mean age of the population studied was over 65, and hypertension was the pathology most frequently associated with AF [2, 5-7]. Understanding the pathophysiological mechanisms underlying the onset and maintenance of AF is a challenge, and the sequence of phenomena responsible for recurrent episodes of AF is poorly understood. It is estimated that paroxysmal AF (PAF) accounts for 25-62% of cases of AF treated in primary health care and hospital environments [6, 8].

The relative contributions of the main mechanisms thought to be behind AF - focal origin and multiple reentry circuits - have yet to be determined [6, 7]. The autonomic nervous system (ANS) is known to be an important modulator in the pathogenesis of AF, and is often associated with recurrent paroxysmal episodes [9-11]. Vagal stimulation significantly affects the electrophysiological properties associated with susceptibility to AF, such as conduction velocity, action potential duration and atrial refractoriness, creating the conditions for atrial arrhythmias. Sudden fluctuations in autonomic activity can be a determining factor in PAF, various authors having described significant alterations in sympathetic and/or vagal tone prior to episodes of AF [9, 11, 12]. However, the clinical pattern of vagally mediated PAF has been observed mainly in young adults without associated heart disease [10, 13]. At the same time, neurocardiogenic responses during orthostatic stress are related to autonomic reflexes in which the vagal influence predominates, and increased vagal activity in tilt-induced syncope has been demonstrated through spectral analysis of RR intervals [14]. There is little information on the incidence of false positive responses leading to vasovagal syncope in patients with PAF. In the present study, we used passive head-up tilt testing (HUT) to evaluate the susceptibility of elderly patients with PAF to induction of syncope related to activation of vasovagal syncope mechanisms.

METHODS

The study included 34 patients aged ≥65 years (72±7 years, 62% female) with a clinical history of PAF (≥1 symptomatic episode/month,

POPULAÇÃO E MÉTODOS

O estudo incluiu 34 doentes com idade igual ou superior a 65 anos (72±7 anos, 62% do sexo feminino) e história clínica de FAp (≥1 episódio sintomático/mês; duração da história clínica de 12-60 meses). Considerámos como grupo controlo 34 doentes consecutivos da mesma faixa etária (74±6 anos, 53% do sexo feminino), submetidos a TT, sem agentes provocativos, para estudo etiológico de síncopes recorrentes.

A FAp estava documentada em electrocardiogramas e registo(s) de Holter e foi considerada "idiopática" após exclusão de doença cardiovascular em avaliação clínica, ecocardiográfica e prova de isquémia miocárdica. Assim, em 19 doentes não havia evidência de cardiopatia subjacente, 11 tinham diagnóstico de hipertensão arterial (com dilatação auricular esquerda ligeira; ≤45 mm no ecocardiograma modo-M) e 4 de doença das coronárias, sem enfarte do miocárdio prévio, submetidos a revascularização miocárdica e com prova de isquemia negativa. Foram excluídos do estudo os doentes com características clínicas da FAp sugerindo predomínio vagal, nomeadamente com episódios nocturnos ou em repouso, ocorrendo no período pós-prandial ou, se informação disponível, quando precedidos por bradicardia sinusal. Nenhum dos indivíduos tinha diabetes *mellitus*, insuficiência cardíaca congestiva ou antecedentes de síncope. No grupo controlo, 21 doentes não apresentavam cardiopatia associada e não havia história clínica de FA. Nos doentes com hipertensão arterial manteve-se a terapêutica antihipertensiva, com excepção para os beta-bloqueantes. Foram excluídos do estudo doentes portadores de *pacemaker*. Antes do TT, os antiarrítmicos foram suspensos no período correspondente a ≥5 semi-vidas. Nos casos sob amiodarona, o TT foi efectuado 1 mês após substituição do fármaco por outro antiarrítmico. No dia da realização do TT, não foi permitido o consumo de álcool, tabaco ou substâncias com propriedades anticolinérgicas. O TT decorreu no período compreendido entre as 12 e as 14 horas, após 4 horas de jejum, em ambiente calmo, com controlo de temperatura e humidade, sem recurso a cateterização venosa. Todos os participantes se encontravam em ritmo sinusal aquando da realização do TT. Os testes foram efectuados após aprovação pela Comissão de Ética do Hospital de

over a period of 12-60 months), and a control group of 34 consecutive age-matched patients (74±6 years, 53% female) undergoing HUT, without provocative agents, to study the etiology of recurrent syncope.

PAF was documented by electrocardiography and Holter monitoring and was considered idiopathic if cardiovascular disease was excluded by clinical and echocardiographic assessment and myocardial ischemia testing. Nineteen patients had no evidence of structural heart disease, 11 had hypertension with mild left atrial dilatation (≤45 mm on M-mode echocardiogram) and four had coronary artery disease (who had no previous myocardial infarction, had undergone myocardial revascularization, and had no documented ischemia). Patients with clinical characteristics of PAF suggesting vagal predominance, namely episodes at night or at rest, post-prandial, or preceded by documented sinus bradycardia, were excluded from the study. None of the PAF group had diabetes, congestive heart failure or a history of syncope. In the control group, 21 patients had no associated heart disease and none had a clinical history of AF. In patients with hypertension, antihypertensive therapy was maintained with the exception of beta-blockers. Patients with pacemakers were excluded. Antiarrhythmics were discontinued for at least five half-lives prior to HUT. In the case of amiodarone, HUT was performed one month after this drug was replaced by a different antiarrhythmic. On the day of testing, patients were instructed not to consume alcohol, tobacco or substances with anticholinergic properties. HUT was performed between 12 and 2 pm after four hours of fasting, in a calm environment with controlled temperature and humidity, and without intravenous cannulation. All patients were in sinus rhythm at the time of testing. The tests were carried out following approval from the Ethics Committee of Hospital de Santa Marta and with the informed consent of the participants.

Tilt test protocol

The subjects were placed on an automated tilt table with foot plate support and secured with straps to prevent falls in the event of dizziness or syncope. Following an initial 15-minute stabilization phase with patients in the supine position, the table was tilted at a constant speed, reaching 70º in 15 seconds. A 20-minute period

Santa Marta e depois da obtenção de consentimento informado.

Protocolo do Teste de Inclinação

Os indivíduos foram colocados numa mesa basculante automática, com uma placa de suporte para os membros inferiores e seguros com faixas para impedir a queda em caso de tonturas ou síncope. Após uma fase inicial de estabilização com duração de 15 minutos, em posição supina, procedeu-se à inclinação progressiva da mesa a uma velocidade constante, atingindo 70º em 15 segundos. Foi considerada para análise do estudo a fase de 20 minutos de ortostatismo passivo sem recurso a fármacos provocativos. A monitorização contínua electrocardiográfica e da pressão arterial (PA) foi obtida com equipamento Task Force Monitor (*CNSystems, Graz, Áustria*). O TT foi considerado positivo quando ocorreu síncope/pré-síncope com bradicardia e/ou hipotensão arterial, e as respostas classificadas como cardioinibitória (frequência cardíaca <40 bpm ou assistolia >3 segundos com queda da PA), vasodepressora (queda rápida da PA e diminuição da frequência cardíaca inferior a 10% da frequência cardíaca máxima monitorizada) ou mista (queda da PA e redução da frequência cardíaca para valores >40 bpm, <40bpm durante <10 segundos ou com assistolia <3 segundos). Considerou-se hipotensão ortostática a queda da PA sistólica >20 mmHg ou da PA diastólica >10 mmHg nos primeiros 3 minutos de ortostatismo.

Análise estatística

As variáveis contínuas foram expressas sob a forma de média±desvio padrão e as variáveis categóricas expressas em frequências e percentagens. As comparações para as variáveis contínuas foram efectuadas pelo teste t de Student. Para as variáveis categóricas foi utilizado o teste de $\chi 2$. Considerámos estatisticamente significativos os resultados com valor <0.05. O programa estatístico utilizado foi o GraphPAD Instruments (GraphPad Software, Inc., California, USA).

RESULTADOS

Características da população

O *Quadro I* mostra as características clínicas da população e compara o grupo da FAp com o grupo controlo. Os doentes com FAp apresentavam

of passive orthostatism, without use of provocative agents, was used in the analysis. Continuous electrocardiographic and blood pressure (BP) monitoring was carried out using a Task Force Monitor (CNSystems, Graz, Austria). The test was considered positive when syncope or presyncope occurred with bradycardia and/or hypotension, and the responses were classified as cardioinhibitory (heart rate [HR] <40 bpm or asystole >3 seconds with fall in BP), vasodepressor (rapid BP fall and HR decrease of less than 10% of maximum monitored HR), or mixed (BP fall and HR decrease to >40 bpm, HR <40 bpm for <10 seconds, or asystole <3 seconds). Orthostatic hypotension was diagnosed on the basis of a fall in systolic BP of >20 mmHg or in diastolic BP of >10 mmHg within three minutes of orthostatism.

Statistical analysis

Continuous variables are expressed as means ± standard deviation and categorical variables as frequencies and percentages. Continuous variables were compared using the Student's t test, and categorical variables by the chi-square test. Results with $p<0.05$ were considered statistically significant. The statistical program used was GraphPad Instruments (GraphPad Software, Inc., California, USA).

RESULTS

Population characteristics

Table I gives the clinical characteristics of both the PAF and control groups. The patients with PAF showed a slightly lower baseline heart rate than the group with a history of syncope, but there were no significant differences in gender distribution, associated heart disease or systolic and diastolic BP.

Tilt test

BP values in the supine position and during the stabilization phase of orthostatism were similar in the two groups (*Table II*). The PAF group had lower HR immediately before tilting and in the first minute of passive orthostatism. The test was positive in seven patients of the PAF group (20.5% of the total and 26.3% of those with idiopathic AF) and in eight patients of the control group (23.5% of the total and 28.5% of those

uma frequência cardíaca basal tendencialmente mais baixa que o grupo com história clínica de síncope, sem diferenças significativas relativamente à distribuição por sexos, presença de cardiopatia associada e valores da PA sistólica e diastólica.

Teste de Inclinação

Os níveis de PA na posição supina e na fase de adaptação ao ortostatismo foram semelhantes nos dois grupos (*Quadro II*). Relativamente à frequência cardíaca, registaram-se valores mais baixos no grupo da FAp, imediatamente antes da elevação da mesa basculante e no primeiro minuto de ortostatismo passivo. O TT foi positivo em 7 doentes do grupo da FAp (20,5% do total e 26,3% do grupo com FA idiopática) e em 8 doentes do grupo de controlo (23,5% do total e 28,5% do grupo sem cardiopatia subjacente) (p=NS). As respostas neurocardiogénicas obtidas foram do tipo vasodepressora em 71,4% e mista em 28,6%, e do tipo vasodepressora em 75% e mista em 25% dos casos, no grupo da FAp e no grupo com história de síncope, respectivamente (p=NS) (*Figura 1*). Nenhum doente apresentou resposta cardioinibitória ou hipotensão ortostática. O tempo decorrido até à sincope/pré-síncope foi semelhante nos dois grupos (15,8±8 vs 16±9 minutos, grupo FAp e grupo controlo, respectivamente; p=NS). Em 3 casos do grupo com FAp (8,8%), registaram-se períodos auto-limitados de FA durante o TT, com duração variando entre 15 e 33 segundos. A *Figura* 2 mostra o registo dum episódio de FA que ocorreu imediatamente após síncope neurocardiogénica mista. Nos outros dois casos com FA não ocorreu síncope durante o TT.

without structural heart disease) (p=NS). The neurocardiogenic responses obtained in the PAF group were vasodepressor in 71.4% and mixed in 28.6%, while in the syncope group they were vasodepressor in 75% and mixed in 25% (p=NS) (*Figure 1*). No patient presented a cardioinhibitory response or orthostatic hypotension. The time elapsed before occurrence of syncope or presyncope was similar in the two groups (15.8±8 vs. 16±9 minutes respectively for the PAF and control group; p=NS). During HUT, three patients of the PAF group (8.8%) had periods of self-limited AF lasting between 15 and 33 seconds. *Figure 2* shows the tracing for an episode of AF that occurred immediately after mixed-type neurocardiogenic syncope. Syncope did not occur during the test in the other two cases of AF.

DISCUSSION

This study shows that in a selected population of elderly patients with PAF but no clinical history of syncope, the rate of neurocardiogenic responses during head-up tilt testing, performed in sinus rhythm without provocative agents, is similar to that observed in an elderly population with no history of AF undergoing the same HUT protocol for etiological study of recurrent syncope.

The recording of PAF episodes associated with vasovagal syncope has contributed to the debate on the ANS alterations linked to spontaneous onset of AF [13, 15-17]. Significant

Quadro I. Características clínicas dos doentes do estudo

Table I. Clinical characteristics of the study population

Características	*Characteristic*	grupo FAp *PAF group* (n=34)	grupo Síncope *Syncope group* (n=34)	p
Idade, anos	*Age, years*	72±7	74±6	NS
Sexo feminino	*Female*	62%	53%	NS
Cardiopatia associada	*Associated heart disease*	56%	62%	NS
FC basal, bpm	*Baseline HR, bpm*	68±10	74±10	0,05
PA sistólica basal, mmHg	*Baseline systolic BP, mmHg*	127±15	129±13	NS
PA diastólica basal, mmHg	*Baseline diastolic BP, mmHg*	81±11	80±14	NS

* Dados expressos com ±= media±desvio padrão; FC=frequência cardíaca.bpm=batimentos por minuto; PA=pressão arterial; mmHg=milímetros de mercúrio; NS=não significativo

Data expressed as mean ± standard deviation. HR: heart rate; BP: blood pressure; NS: non-significant

Quadro II. Pressão arterial e frequência cardíaca em posição supina e após ortostatismo

Table II. Blood pressure and heart rate in supine position and after orthostatism

		Supina *Supine*	Ortostatismo (início) *Orthostatism (initial)*	Ortostatismo (5 min.) *Orthostatism (after 5 min.)*
Frequência cardíaca (bpm)	*Heart rate (bpm)*			
Grupo FAp	*PAF group*	62±13*	63±10*	63±12
Grupo Síncope	*Syncope group*	71±10	73±11	70±10
Pressão arterial sistólica (mmHg)	*Systolic blood pressure (mmHg)*			
Grupo FAp	*PAF group*	127±16	125±17	126±13
Grupo Síncope	*Syncope group*	128±13	126±15	127±19
Pressão arterial diastólica (mmHg)	*Diastolic blood pressure (mmHg)*			
Grupo FAp	*PAF group*	83±12	87±15	82±10
Grupo Síncope	*Syncope group*	80±10	84±10	82±13

FAp=fibrilhação auricular paroxística; bpm=batimentos por minuto; mmHg=milímetros de mercúrio,* p<0.05, grupo FAp vs grupo Síncope. Dados expressos como média±desvio padrão.

*Data expressed as means ± standard deviation. PAF: paroxysmal atrial fibrillation; * p<0.05: PAF group vs. syncope group.*

DISCUSSÃO

Este estudo mostra que, numa população seleccionada de idosos com FAp, sem história clínica de síncope, a taxa de respostas neurocardiogénicas, durante o TT efectuado em ritmo sinusal, sem recurso a agentes provocativos, é semelhante à verificada numa população de idosos submetida a um mesmo protocolo de TT, para estudo etiológico de síncopes recorrentes, sem história de FA.

O registo de episódios de FAp associados à ocorrência de síncope vasovagal, tem contribuído para a discussão e interesse relacionados com as alterações do SNA ligadas ao início espontâneo de FA [13,15-17]. De facto, têm sido descritas modificações significativas da actividade autonómica precedendo episódios de FAp, que envolvem quer o tónus simpático quer parassimpático [9-12]. No que respeita ao papel da actividade vagal, a sua importância parece ser maior em populações mais jovens, sem cardiopatia associada [10,13]. As respostas neurocardiogénicas que ocorrem durante o ortostatismo são também devidas a reflexos autonómicos com predomínio da activação vasovagal, estando demonstrado o aumento da influência vagal na síncope induzida durante o TT [14]. Um estudo sobre a actividade autonómica durante o TT, concluiu que, apesar do mecanismo autonómico responsável pela síncope vasovagal ser semelhante em jovens e idosos, as respostas do

changes in autonomic activity have been described preceding PAF episodes, involving both sympathetic and parasympathetic tone [9-12]. Vagal activity appears to play a more important role in younger populations without associated heart disease [10, 13]. Neurocardiogenic responses during orthostatic stress are related to autonomic reflexes, mainly vasovagal activation, and increased vagal influence has been demonstrated in tilt-induced syncope [14]. A study on autonomic activity during HUT concluded that, although the autonomic mechanism responsible for vasovagal syncope was similar in young and elderly patients, the sympathetic and parasympathetic responses to orthostatism were weaker in the elderly group, which would explain the higher frequency of responses associated with vasodilatation, i.e. vasodepressor or mixed, and the low incidence of cardioinhibitory syncope [18]. The results of the present study show that during HUT without provocative agents, neurocardiogenic mechanisms produce a rate of positive tests in patients with PAF that is similar to that observed in patients with recurrent syncope. In addition, the recording of self-limited episodes of AF, associated with neurocardiogenic syncope in one case, leads to the assumption that autonomic modulation may also play a role in triggering PAF under these conditions.

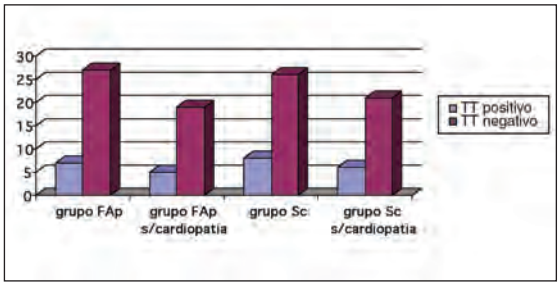
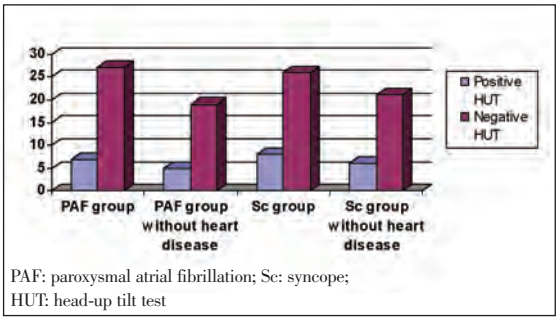


Figura 1. Resultados do teste de inclinação passivo (sem fármacos provocativos). Incidência de respostas positivas neurocardiogénicas (grupo com fibrilhação auricular paroxística *versus* grupo com síncopes, p=NS; grupo com FAp sem cardiopatia associada *versus* grupo com síncopes sem cardiopatia associada, p=NS). FAp=fibrilhação auricular paroxística; Sc=síncopes; TT=teste de inclinação



PAF: paroxysmal atrial fibrillation; Sc: syncope;
HUT: head-up tilt test

Figure 1. Results of passive tilt testing (without provocative agents): incidence of positive neurocardiogenic responses (paroxysmal atrial fibrillation group vs. syncope group, p=NS; PAF group without associated heart disease vs. Sc group without associated heart disease, p=NS).

simpático e do parassimpático ao ortostatismo eram de menor intensidade no grupo dos idosos, o que explicaria a ocorrência de mais respostas associadas a vasodilatação, como a vasodepressora e a mista, e a baixa incidência de síncope cardioinibitória [18]. Os resultados do presente trabalho, mostram que durante o TT, sem recurso a agentes provocativos, os mecanismos neuro-cardiogénicos condicionam uma incidência de testes positivos em doentes com FAp sobreponível à registada na população com síncopes recorrentes. Por outro lado, a documentação de episódios auto-limitados de FA, num dos doentes associada mesmo a síncope neurocardiogénica, pressupõe que a modulação autonómica também possa contribuir para o aparecimento de FAp naquelas condições.

Respostas neurocardiogénicas durante o teste de inclinação

O TT tem sido utilizado de forma crescente como técnica de grande relevância na determinação etiológica da síncope, através da indução de reacções vaso-vagais, contribuindo, em particular, para a compreensão dos mecanismos fisiopatológicos da síncope neurocardiogénica. Está demonstrado que doentes com este tipo de síncope, apresentam alterações do equilíbrio neurovegetativo, que podem ser reproduzidas durante o TT [19]. A maioria dos estudos refere valores de especificidade na ordem dos 90% para o TT, efectuado com angulações de 60º e 70º, na ausência de agentes provocativos [20]. Neste sentido, a utilização clínica de diferentes protocolos, tem mostrado que a sensibilidade do TT sem agentes provocativos é baixa, diminuindo nos idosos e com

Neurocardiogenic responses during tilt testing

HUT is increasingly used to determine the etiology of syncope through induction of vasovagal reactions, contributing in particular to an understanding of the pathophysiological mechanisms of neurocardiogenic syncope. It has been demonstrated that patients with this type of syncope present alterations in neurovegetative balance, which can be reproduced during HUT [19]. Most studies report specificity of around 90% for HUT performed at 60º and 70º, without provocative agents [20]. Moreover, use of different protocols in clinical practice has shown that the sensitivity of drug free HUT is low, and is lower still in the elderly and with protocols of shorter duration [21, 22]. Thus, HUT without provocative agents or intravenous cannulation, for a period of 20 minutes, should be highly specific in the elderly for induction of vasovagal mechanisms, enabling more accurate identification of asymptomatic individuals or those with abnormal responses. In the present study, one in five of the elderly patients in the PAF group with no history of syncope presented a neurocardiogenic response during short-duration passive HUT. These results indicate that vasovagal reflexes are relatively easily activated in this population. Despite previous findings of a relationship between PAF and syncope associated with reflex mechanisms [23, 24], to our knowledge there is no information on the incidence of false positives during passive HUT in elderly patients referred due to a history of PAF. Our results suggest that such reflexes are more often induced in cases of idiopathic PAF, which may be partly explained by

protocolos de menor duração [21,22]. Assim, a análise do TT, sem fármacos provocativos ou recurso a punção venosa, durante 20 minutos, numa população de idosos, deverá permitir uma elevada especificidade na indução de mecanismos vaso-vagais, permitindo descriminar com precisão entre indivíduos com respostas anormais e assintomáticos. No presente trabalho, num grupo de idosos com FAp, sem história clínica de síncope, 1 em cada 5 apresentou respostas neurocardiogénicas, num TT passivo de curta duração. Estes dados apontam para uma relativa facilidade na activação de reflexos vaso-vagais nesta população. Tanto quanto é do nosso conhecimento, apesar do registo prévio da relação entre FAp e síncope associada a mecanismos reflexos [23,24], não há informação relativa à incidência de "falsos--positivos" durante o TT passivo em idosos referenciados por história clínica de FAp. Os dados apontam para a possibilidade duma maior indução destes reflexos nos casos de FAp idiopática, o que pode atribuir-se, em parte, à ausência de alterações do equilíbrio simpático--parassimpático associadas a efeitos da medicação ou à hipertensão arterial e doença das coronárias. Num estudo anterior, que envolveu doentes numa faixa etária mais baixa, obtiveram-se 25% de TT positivos em doentes com FAp, somente após administração de isoprenalina [16]. No presente trabalho, avaliámos a susceptibilidade para a indução de síncope associada a mecanismos neurocardiogénicos sem recurso a agentes provocativos, numa população com história recorrente de FAp, sem características clínicas de predomínio vagal. Os resultados sugerem que um número significativo de idosos com FAp apresenta reacções vaso-vagais durante um TT passivo. Num estudo de van den Berg et al, a incidência de reacções vasovagais durante o TT em doentes com FAp foi de 13%, sem diferenças significativas entre os doentes com FAp "vagal" e FAp "não-vagal" [17].

No nosso estudo, 8.8% dos doentes tiveram períodos de FA auto-limitados durante o TT, num dos casos associados a síncope vasodepressora (*Figura 2*). A ocorrência de FA no contexto de TT está também documentada por outros autores, nomeadamente após recurso a fármacos provocativos [16,24]. Assim, os resultados obtidos apontam para a presença de alterações reflexas da actividade do SNA na resposta ao ortostatismo em idosos com FAp, com indução de FA num

the sympathetic-parasympathetic imbalance associated with the effects of medication, hypertension or coronary artery disease. A previous study involving younger patients obtained a positive HUT in 25% of patients with PAF only after administration of isoprenaline [16]. Our study evaluated susceptibility to induction of neurocardiogenic syncope without use of provocative agents in a population with a history of recurrent PAF but no clinical characteristics of vagal predominance. The results indicate that a significant proportion of elderly patients with PAF present vasovagal reactions during passive HUT. In a study by van den Berg et al., the incidence of such reactions was 13%, with no significant differences between those with vagal versus nonvagal PAF [17].

In our study, 8.8% of the patients had periods of self-limited AF during the test, in one case associated with vasodepressor syncope (*Figure 2*). AF in the context of HUT has also been reported by other authors, particularly with the use of provocative agents [16, 24]. These results indicate reflex changes in ANS activity in response to orthostatic stress in elderly patients with PAF, leading to AF induction in some of them, which could be due to greater autonomic influence and/or cardiovascular sensitivity to changes in ANS function. PAF associated with a neurocardiogenic response would thus be plausible in the context of a stronger vagal response causing a greater heterogeneous shortening of atrial refractory periods, thus increasing susceptibility to spontaneous AF. This reflex response may occur as a result of greater sympathetic stimulation, which is considered a modulating factor in triggering mechanisms and automatism, with an effect on atrial tachyarrhythmias [25]. However, the complexity of the mechanisms underlying these autonomic reflex phenomena makes it difficult to determine the influence of the ANS in the pathophysiology of PAF.

Study limitations

The study was based on analysis of a 20-minute HUT without provocative agents, and so the results cannot be generalized to different protocols. However, the aim was not to assess the diagnostic accuracy of HUT in determining the etiology of syncope, but to take advantage of the high level of specificity expected from a short

subgrupo de doentes, que poderá atribuir-se a uma maior intensidade na modulação autonómica e/ou a maior sensibilidade cardiovascular às alterações da função do SNA. Neste sentido, a ocorrência de FAp associada à resposta neurocardiogénica seria possível no contexto duma resposta vagal de maior intensidade, capaz de causar maior impacto na redução heterogénea dos períodos refractários do tecido auricular, aumentando a vulnerabilidade para FA espontânea. Esta resposta reflexa pode surgir na sequência duma maior estimulação simpática, considerada factor modulador da actividade *trigger* e do automatismo, com influência nas taquiarritmias auriculares [25]. No entanto, a complexidade de mecanismos subjacentes a estes fenómenos autonómicos reflexos torna difícil a compreensão da influência do SNA na fisiopatologia da FAp.

Limitações do estudo

O estudo baseou-se na análise de 20 minutos de TT, sem recurso a fármacos provocativos. A discussão dos resultados não pode por isso generalizar-se a diferentes protocolos. No entanto, o objectivo não era avaliar a acuidade diagnóstica do TT no âmbito da avaliação etiológica da síncope, mas sim valorizar a elevada especificidade esperada num exame de curta duração e sem fármacos. A escolha dum grupo controlo com história de síncopes recorrentes constitui uma limitação do estudo, face à importância da comparação dos resultados do TT drug-free test. The fact that the control group had a history of recurrent syncope was another limitation, given the importance of comparing passive HUT results to those in an elderly population without associated pathology, but it was not possible to obtain a sufficiently large number of age-matched healthy volunteers to undergo tilt testing. A further limitation concerns the difference observed in heart rate between the two groups under baseline conditions and during the initial stage of orthostatism, which suggests that the PAF patients may still have been under a degree of pharmacological influence despite suspension of their antiarrhythmic therapy. This difference was no longer significant when the groups were compared in the post-orthostatic stabilization period (*Table II*), and all neurocardiogenic responses occurred after prolonged orthostatism. Finally, techniques to study ANS activity would enable patterns of the sympathetic-parasympathetic balance to be documented, not only those preceding neurocardiogenic syncope but also episodes of AF. It would therefore be useful to analyze cardiovascular signals such as RR intervals and BP values in future studies, which would contribute to our understanding of the influence of ANS activity prior to episodes of PAF.

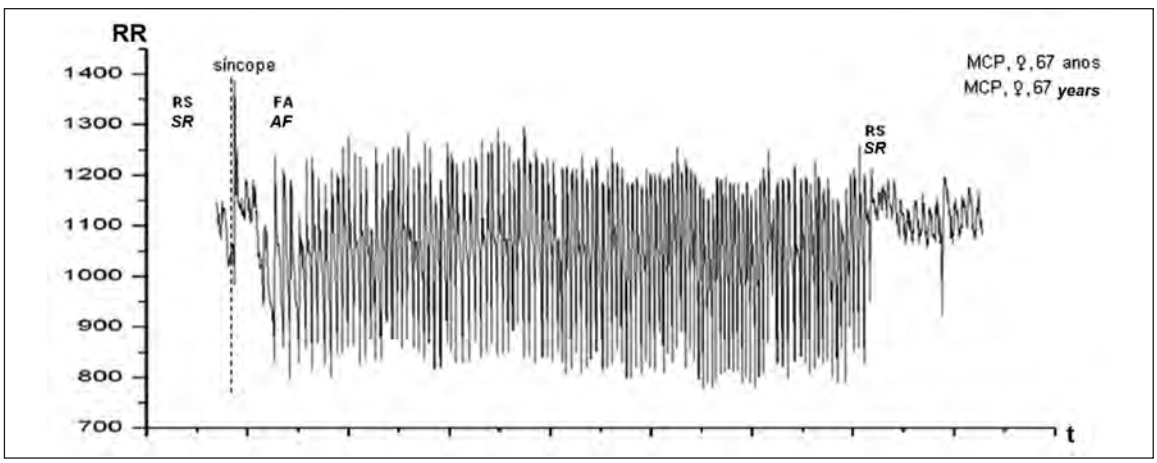


Figura 2. Episódio de fibrilhação auricular auto-limitada, imediatamente após síncope neurocardiogénica mista durante teste de inclinação passivo em idoso com fibrilhação auricular paroxística, sem história clínica de síncope. RS=ritmo sinusal; FA=fibrilhação auricular

Figure 2. Episode of self-limited atrial fibrillation, immediately after mixed-type neurocardiogenic syncope during passive tilt test in an elderly patient with paroxysmal atrial fibrillation and no history of syncope.
SR: sinus rhythm; AF: atrial fibrillation

passivo com uma população de idosos sem patologia associada. No entanto, não foi possível obter um número significativo de voluntários saudáveis nesta faixa etária para realização de TT. Outra limitação, diz respeito à diferença verificada na frequência cardíaca dos 2 grupos em condições basais e na fase inicial do ortostatismo, que sugere alguma influência farmacológica nos doentes com FAp, apesar da suspensão terapêutica dos antiarrítmicos. Essas diferenças deixam de ser significativas quando se comparam os grupos na fase de estabilização pós-ortostatismo (*Quadro II*). Além disso, as respostas neurocardiogénicas ocorreram sempre após ortostatismo prolongado. Finalmente, o recurso a técnicas para estudo da actividade do SNA, permitiria documentar padrões do balanço simpático-parassimpático, não só precedendo as síncopes neurocardiogénicas, como também os episódios de FA. Nesse sentido, justifica-se o recurso a metodologia de análise de sinais cardiovasculares, como os intervalos RR e a PA, em estudos futuros, que possam contribuir para a compreensão da influência do SNA precedendo episódios de FAp.

CONCLUSÃO

Em idosos com FAp, um número significativo doentes apresenta resultados falsos-positivos durante o TT sem recurso a fármacos provocativos, podendo associar-se, nalguns casos, à indução de episódios auto-limitados de FA, o que sugere uma incidência aumentada de reacções vaso-vagais apesar da faixa etária. Estes dados estão de acordo com a possibilidade desta população poder apresentar respostas inadequadas da actividade autonómica durante o *stress* ortostático.

CONCLUSION

A significant number of elderly patients with PAF present false positive results during HUT without provocative agents, including induction of self-limited episodes of AF in some cases, suggesting increased vasovagal reactions despite aging. These results support the possibility that this population may present inappropriate autonomic responses during orthostatic stress.

Pedidos de separatas para:
Address for reprints:

MÁRIO OLIVEIRA
Serviço de Cardiologia, Hospital de Santa Marta
Rua de Santa Marta
1169-024 Lisboa, PORTUGAL
Fax: +351 213560368
Tel: +351 213594311
e-mail: mmartinsoliveira@gmail.com

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Baroreflex sensitivity during orthostatic stress in patients with lone paroxysmal atrial fibrillation

Running title: Baroreflex sensitivity in PAF patients

Key words: paroxysmal atrial fibrillation, baroreflex sensitivity, orthostatic stress

Mário Oliveira, MD [1,2], Sérgio Laranjo, MD [2], Cristiano Tavares, MSc[2], Victor Vaz-da-Silva., MSc [2], Vera Geraldes, MSc [2], Sofia Silva, BSc [1], Rita Xavier, MSc [2], Rui Ferreira, MD [1] & Isabel Rocha, PhD [2]

[1] Department of Cardiology, Santa Marta Hospital & [2] Unit of Autonomic Nervous System, Instituto de Medicina Molecular, Faculty of Medicine of Lisbon, Lisbon, Portugal

For correspondence
Mário Oliveira, MD
Unit of Autonomic Nervous System
Instituto de Medicina Molecular, Faculdade de Medicina de Lisboa
Av Prof Egas Moniz, 1649-028 Lisbon, Portugal
tel: (+351) 21 7999435; fax: (+351) 21 7999436
E-mail:m.martinsoliveira@gmail.com

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ABSTRACT

Abnormal neural control of atria has been considered to be one of the mechanisms of paroxysmal atrial fibrillation (PAF) pathogenesis. The baroreflex has an important role in cardiovascular regulation and may be used as an index of autonomic function. The aim of this study was to evaluate baroreflex control of heart rate during upright tilt (HUT) in patients (P) with lone PAF. Thirty four P with PAF (16 men; 54±15years) and 34 healthy individuals (HI) (11 men; 48±12years) underwent estimation of baroreflex sensitivity (BRS) in supine and during HUT while on sinus rhythm. ECG and BP were continuously monitored and analyzed in the supine position (4 min; baseline), in the initial phase of tilt adaptation (1 min; TA1), and during the early phase of orthostasis (between 1^{st}- 5^{th} min; TA5). BRS was determined by the sequence method, and the number of BP ramps, the BRS slope and the baroreflex effectiveness index (BEI) taken as parameters. RR intervals and supine BP were similar for both groups. During HUT, two BP profiles were observed: a sustained increase in PAF P, and a decrease followed by an increase and further recovery in HI. Compared to HI, PAF P showed lower average BEI values and lower BRS slope. The number of systolic BP ramps increased significantly in HI during TA5, while PAF P showed no variation. When compared to HI, PAF patients show an impairment of baroreflex function during rest and the initial phase of postural stress. These findings underscore the presence of baroreflex disturbances in lone PAF.

Introduction

Atrial fibrillation (AF) is the most common type of cardiac arrhythmia in clinical practice, often associated with a negative impact on both life expectancy and quality of life (Wolf at al, 1996; Chugg et al, 2001; Van den Berg et al, 2001). Approximately 30% of all AF cases present with paroxysmal AF (PAF), defined as self-terminating episodes of the arrhythmia with <7 days duration (Nieuwlaat et al, 2005; Fuster et al, 2006). Comprehension of AF physiopathology remains limited, partly due to the complex interaction between the various mechanisms underlying its initiation and maintenance. Lone AF is related to electrophysiological phenomena involving the contribution of multiple wavelets that propagate and interact in a random fashion over the atrial surface and focal sources of electrical activity (triggers), possibly influenced by combined sympathovagal discharges (Chen and Tan, 2007; Krummen and Narayan, 2009). The autonomic nervous system (ANS) has been implicated as an important modulator in the pathogenesis of PAF, but the mechanisms linking changes in autonomic activity with this arrhythmia are incompletely understood (Coumel, 1994; Chen and Tan, 2007; Tai, 2001; Olshansky, 2005). In fact, electrophysiological properties related with atrial vulnerability for AF may change as a result of the effects of vagal or sympathetic activation (Chen and Tai, 2007; Tai, 2001; Olshansky, 2001; Furukawa et al, 2009). Also, fluctuations of autonomic tone have been demonstrated in relation with the occurrence of AF episodes (Bettonni and Zimmerman, 2002; Lombardi et al, 2004). The baroreceptor reflex is an important homeostatic mechanism playing a major role in the short-term regulation of the cardiovascular system that may be used as an index of autonomic function (DelColle et al, 2007). It is uncertain if patients with PAF have a specific dysautonomic condition. Little is known on the relation between PAF and baroreflex modulation. This study aimed at investigating whether patients with lone PAF exhibit abnormal baroreceptor reflex control during rest and upright tilt (HUT).

Subjects and Methods

Subjects

We studied 34 patients (18 females and 16 males, mean age 54±15 years) with a history of ≥1 year of PAF (range 1-5 years), and 34 healthy volunteers (HI) (22 females and 12 males, mean age 48±12 years). PAF was documented with electrocardiograms (ECG) and/or Holter recordings. All patients were diagnosed as having lone PAF (absence of clinical or echocardiographic evidence of cardiovascular disease). The exclusion criteria were previous syncope, myocardial infarction, congestive heart failure, renal failure, diabetes mellitus, hypertension and sleep apnea. Prior to baroreflex sensitivity (BRS) evaluation, all antiarrhythmic drugs were withdrawn for at least 5 half-life times. Patients under beta-blockers or with recent episodes of AF (in the previous 48 hours) were excluded from the study. Alcohol, tobacco and medication with anticholinergic properties were not allowed on the day of the study. According to previous studies, the protocol of BRS analysis was evaluated in supine and during HUT (Berend et al, 2001; Cooper & Hainsworth, 2002; Taneyama & Goto, 2009). HUT was performed in a quiet environment, with controlled temperature and humidity, during the morning, following four hours of fasting. ECG and blood pressure (BP) were continuously monitored by a Task Force Monitor (*CNSystems, Graz, Austria*). Briefly, after a resting period of 15 minutes in the supine position (0º), subjects were placed on a tilt table with a footboard, secured with snug restraints to prevent falling if syncope occurred, and tilted head-up using an electronically operating system to a level of 60º at a constant speed (within 15 seconds). Subjects were instructed to breathe normally. Continuous RR intervals (RRI) and non-invasive BP was obtained and data analysis performed on three periods: 1) the last 4 minutes of the supine resting period (0º, baseline); 2) during the first minute of tilt adaptation at 60º (TA1); and 3) during the early phase of adaptation to orthostasis, between the first and fifth minute of HUT (TA5). Systolic BP (SBP) and RRI were used for subsequent analysis with the sequence method baroreflex sensitivity (BRS) software (15,16). The study complied with the Declaration of Helsinki was approved by the local ethics committee, and performed after informed consent.

Arterial baroreflex function

The estimation of the BRS was based on the analysis of beat-to-beat series of SBP, scanned in order to identify ramps of three or more consecutive heart beats with a progressive increase or decrease, of at least 1 mmHg, regardless of the possible occurrence of concomitant RRI changes. The algorithm identified sequences, defined as SBP ramps followed by concordant pulse interval variations of ≥ 5ms, coupled with 0-, 1-, and 2-beat lags, with each sequence being included only once (DiRienzo et al, 2001; Iacoviello et al, 2008).

For each sequence, the average regression slope of the linear interrelationship between SBP and the following RRI values was calculated and considered reliable when the correlation coefficient was greater than 0.80. The mean slope of all regression lines in a stationary phase was used as a marker of BRS. For each period selection considered for analysis, the baroreflex effectiveness index (BEI), which reflects the number of times the arterial baroreflex is being in controlling the heart rate response to blood pressure fluctuations, was defined as the ratio between the number of BRS sequences detected and the total number of SBP ramps observed (Di Rienzo et al, 2001).

Statistical analysis

Continuous variables are expressed as mean ± standard error of the mean (SEM), unless otherwise specified. Categorical variables are given as frequencies and percentages. Normality of the distributions of the continuous variables was analyzed with the Kolmogorov-Smirnov test. Two-way ANOVA analysis of variance with repeated measures was used to compare data along the test and between groups. The chi-square test was used for categorical variables. A value of $p<0.05$ was considered statistically significant. Data were analysed using GraphPAD Instruments version 3.05 (*GraphPad Software, Inc., California, USA*).

Results

All participants completed the testing protocol without any symptoms or AF initiation. No significant differences in age, gender distribution, body mass index, baseline arterial BP and heart rate were found between the control group and the group of patients with PAF (table I).

a) heart rate and blood pressure changes

Heart rate rose on TA1 and TA5 periods when compared with baseline values, but no significant differences were observed between the two groups on heart rate response during all analyzed periods (Figure 1). In PAF patients group, an increase of BP was observed in TA1 and TA5 periods when compared with baseline values, with BP values significantly higher during orthostatism, compared to HI (Figure 2). During HUT, HI showed an initial non-significant fall in BP, followed by a recovery and a further increase, whereas PAF patients had a progressive sustained increase (Figure 3).

b) *baroreflex sensitivity*

Figure 4 shows the mean number of SBP ramps per minute, the mean BRS and BEI values during the different periods of the HUT protocol (baseline, TA1, and TA5). Although there were no significant differences for the comparison of the number of SBP ramps between the groups (Figure 4A), the HI group showed a significant increase in the number of SBP ramps per minute increased significantly along the HUT, whereas the PAF patients showed no changes in the number of ramps recorded during the test.

Spontaneous BRS during rest was similar in HI and PAF patients. After orthostatism, a significant decrease of the BRS slope with respect to baseline was observed in HI (19.1±1.6 mmHg/ms vs. 15.7±1.7 mmHg/ms, $p<0.05$) and in the PAF patients (17.8±1.9 mmHg/ms vs. 12.1±1.8 mmHg/ms, $p<0.01$), and then remained stable for both groups (15.7±1.7 mmHg/ms vs. 15.1±2.0 mmHg/ms, p=NS; 12.1±1.8 mmHg/ms vs. 11.3±1.6 mmHg/ms, p=NS; for HI and PAF, respectively), with lower values during TA1 and TA5 for the PAF patients compared to HI ($p<0.05$) (figure 4B). In the PAF group, BEI values were significantly lower than in the HI group in baseline (in supine position) and after tilting (TA1 and TA5).

Discussion

The baroreceptor reflex is one of the most relevant physiological mechanisms mediating short-term or rapid cardiovascular autonomic control, assuming particular importance during postural

changes to reduce variations in BP (Del Colle at al, 2007; Di Rienzo, 2001; Iacoviello et al, 2008; Eckberg and Sleight, 1992). The main purpose of the present study was to analyse BRS in PAF patients during a passive short HUT, by using the spontaneous sequence method applied to continuous RRI and BP signals. The sequence method provides assessment of BP variability leading to baroreceptor activation by calculating SBP ramps, and offers information regarding BRS, by measuring the magnitude of the reflex changes in RRI in relation to the variations in the SBP (Di Rienzo, 2001). Furthermore, the baroreflex index (BEI) indicates the baroreflex function by quantifying the number of times the arterial baroreflex respond to progressive SBP changes with concordant RRI modulation changes.

Our results reveal that, despite similar clinical characteristics and no differences of mean HR and BP between groups in baseline conditions, patients with PAF demonstrated a significant reduction in their ability to regulate BP response to tilt, with a progressive sustained increase pattern of SBP. Also, the group of PAF patients showed a reduced input to arterial baroreflex as indicated by a lower BEI compared to HI, without significant changes in the number of SBP ramps. Finally, in the PAF group a greater reduction of the BRS slope was observed since the first minute of HUT. Therefore, PAF patients have significant differences in the BRS and BP responses during the initial period of adaptation after orthostasis, when compared to HI.

In the HI group there was a significant increase in SBP ramps along the five-minute HUT, whereas the PAF patients showed no changes in the number of SBP ramps during the test. This SBP pattern represents a frequent stimulation to the arterial baroreflex and may be considered an expression of BP variability. However, recently it has been suggested that the occurrence of SBP ramps is a phenomenon somewhat unrelated with the baroreflex (Di Rienzo, 2001). Also, Waki et al found that within the normal physiological range of BP, the rate of the ramp change in BP did not affect BRS in the anesthetized rat (Waki et al, 2008). Thus, the role of SBP ramps dynamic pattern as a marker of adaptation to short-term orthostatism remains uncertain.

Although the spontaneous BRS slope values were similar for both groups in the supine position, and the response to short-term HUT was a significant decrease in BRS, PAF patients seemed to have a larger reduction in BRS (figure 4B). Conflicting results on BRS assessment in patients with PAF have been previously reported (Lok and Lau, 1998; Van den Berg et al, 2003, Styczkiewicz et al, 2007). According to studies looking to “autonomic mediated” AF by using

linear regression between SPB and RRI and cross spectral analysis, the BRS did not differ from the remaining AF patients nor the controls (Lok and Lau, 1998; Van den Berg et al, 2003). However, other study reported reduced spontaneous BRS in patients with symptomatic PAF (Styczkiewicz et al, 2007). There is, however, lack of information regarding the arterial baroreflex function during postural stress in PAF.

The simultaneous reduced BRS and BEI during HUT represents a marker of arterial baroreflex dysfunction in patients with PAF. However, our data cannot clarify whether these findings represent an epiphenomenon. Studies performed in healthy subjects also have found discrepancy between the results obtained with different protocols to determine BRS during HUT. A reduction of the BRS slope has been observed using spontaneous time-domain (sequence) BRS methods (Berend et al, 2006; Bahjaoui et al, 1998), while in other studies BRS was unaltered from baseline (Cooper and Hainsworth, 2002; Samniah et al, 2004). Pitzalis et al (2003), in a study performed in healthy subjects with unexplained syncope and control subjects, found discrepancies between the results of resting spontaneous BRS (ms/mmHg) and the BEI assessed at rest using the sequence method.

The postural baroreceptor unloading evokes adjustments in sympathetic and parasympathetic outflow from the central nervous system to the cardiovascular effector mechanisms with an increase in both heart rate and vasoconstriction, making ANS activity one of the main determinants of BRS (Berend et al, 2006; Bahjaoui et al, 1998; Cooper and Hainsworth, 2002; Samniah et al, 2004). In fact, in a previous study using the sequence technique in rats, cardiac autonomic blockade by atropine and propranolol significantly reduced the number of sequences (Stauss et al, 2006).

Recently, using the wavelet transform methodology, we addressed the acute changes of HR and BP variability in PAF patients during the initial phase of tilt adaptation (Oliveira et al, 2008). Our findings showed that compared to normal subjects, PAF patients presented diminished HR variability, without differences regarding SBP variability during HUT, suggesting the presence of ANS disturbances in cardiovascular regulation of PAF patients. Previous experiments, obtaining separate values of the gain of the arterial and cardiopulmonary components of the baroreflex suggested that the arterial component remains unchanged during tilt while the cardiopulmonary contribution decreases (Mullen et al, 1997). These data may provide a

contribution to explain a sustained increase in BP in the PAF group despite reduced BRS slope and BEI.

Although validated in the assessment of baroreflex control (Watkins et al, 1995; Oka et al, 2003; Iellamo et al, 1996), the contribution of the BRS slope and BEI analysis based on the spontaneous sequence method in clinical practice need to be explored in further studies, in order to clarify the relationship between alterations in the baroreflex function and PAF episodes.

Study limitations

The study did not include measurements beyond the 5-minutes of passive orthostatic stress. Therefore, comparison of the baroreflex function during active standing or prolonged HUT can not be discussed. Nevertheless, it has been accepted that the rapid short-term adjustments in the first two minutes of HUT are mediated by the neural cardiocirculatory control (Wieling and Karemaker, 1999). Also, these results might not necessary apply to postural tests performed according to different protocols. Another limitation of the method is related with the differences between BES slope and BEI patterns, witch makes more complex a comprehensive explanation of the baroreflex function in PAF. However, there is some information regarding discrepant behavior of BEI and BRS slope, indicating that these variables can provide data on different aspects of baroreflex control (Di Rienzo et al, 2001; Reyes del Paso et al, 2004), and thus, it is possible that analysis of short-term protocols may benefit from the complementary nature of these indexes. Finally, cardiovascular responses to HUT include a complex interaction between factors such as individual motivation, central neural influences, sympathetic and parasympathetic outflows, adrenoceptor number and affinities, and modulation of humoral substances.

In conclusion, in HI, arterial baroreflex induces BRS sequences in response to the majority of the BP ramps, whereas patients with PAF show an overall impairment in baroreflex function during rest and in the initial phase of orthostatism. These findings suggest the presence of baroreflex disturbances in PAF and underscore the complex interaction between the mechanisms underlying responses to postural stress.

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Disclosures

There is no conflict of interests.

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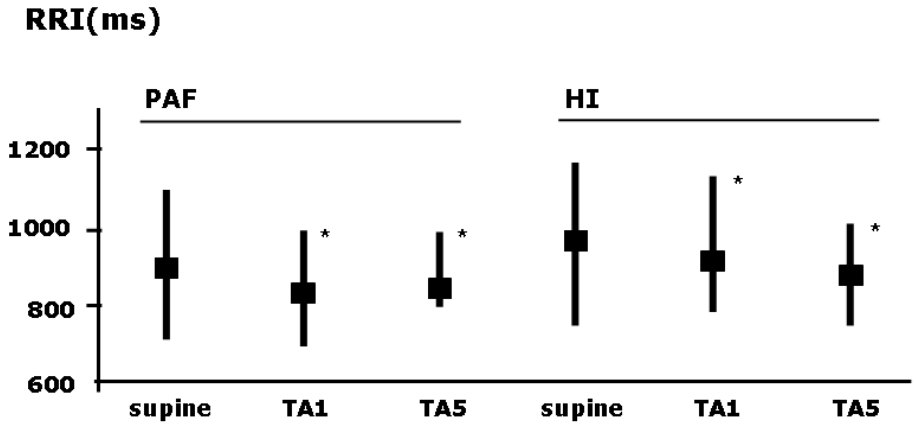
Table 1. Clinical characteristics of patients with paroxysmal atrial fibrillation and the control group

Characteristic	PAF group (n=34)	HI group (n=34)	p-value
Age, years	54±15	48±12	NS
Male gender, %	47.0	35.3	NS
BMI	26.9±3.6	25.0±2.7	NS
Heart rate, bpm	67±4	63±5	NS
Systolic blood pressure, mmHg	125±14.0	116±9.5	NS
Diastolic blood pressure, mmHg	80±12.0	75±9.5	NS

*PAF=paroxysmal atrial fibrillation; HI=healthy individuals; BMI=body mass index; bpm=beats per minute. * Data expressed as mean ± SD or %; p=NS*

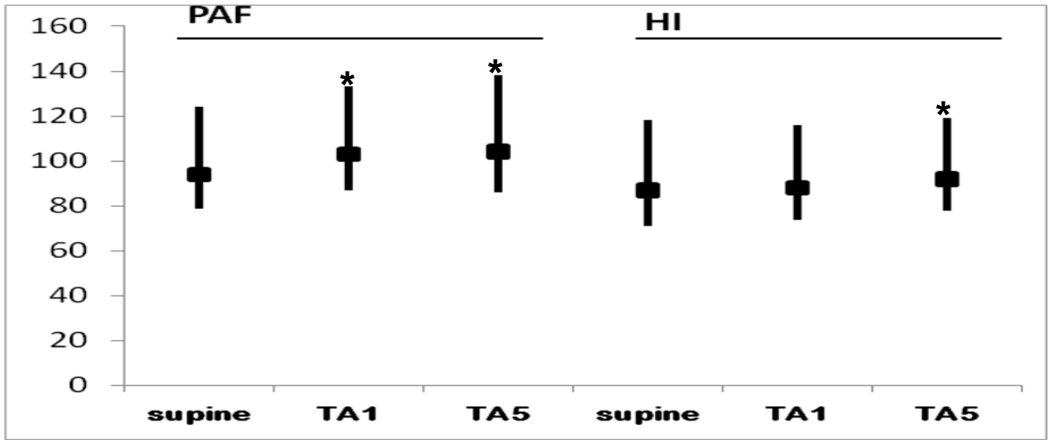
Figure Legends

Figure 1 - **Comparison of changes in RR intervals during head-up tilt testing in patients with paroxysmal atrial fibrillation (PAF) and healthy individuals (HI)**



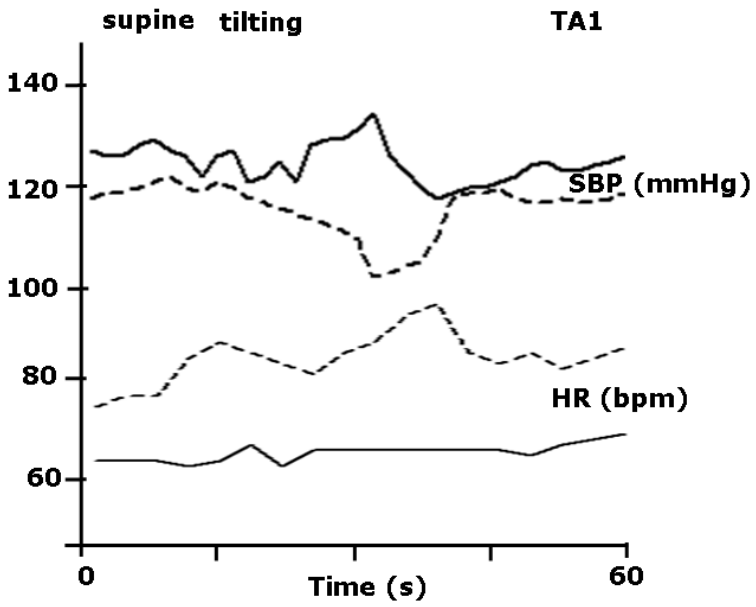
RRI=RR intervals. TA1=1st minute of tilting; TA5=1st-5th minute of tilting; p=NS for RRI response between groups

Figure 2 – Modifications of systolic, diastolic and mean blood pressure during head-up tilt of paroxysmal atrial fibrillation patients (PAF) and healthy individuals (HI)



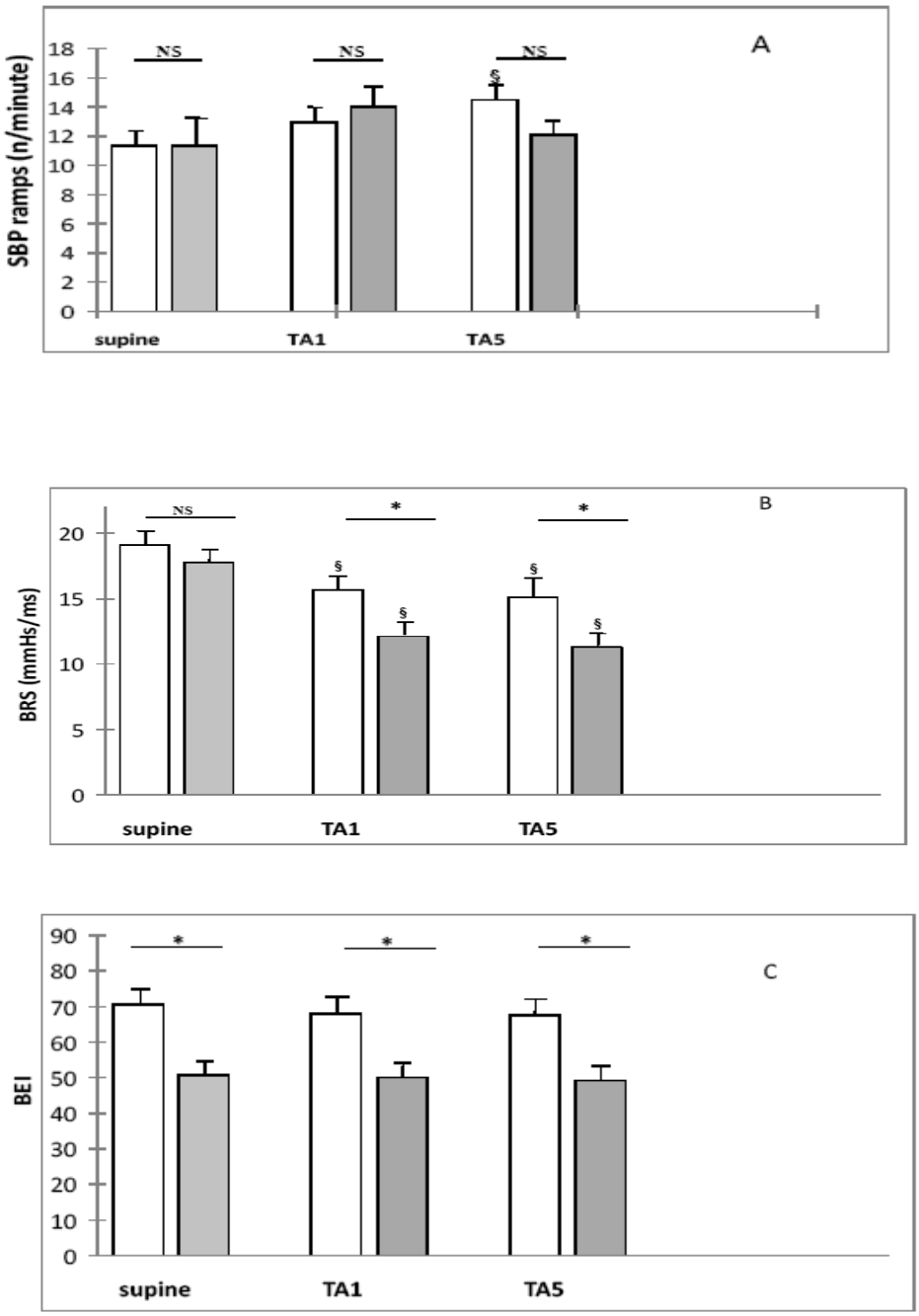
*TA1=1st minute of tilting; TA5=1st-5th minute of tilting; BP comparison between groups: in supine (PAF vs. HI, p=NS); during TA1 (PAF vs. HI, p<0.01); during TA5: (PAF vs. HI, p=0.001). *=p<0.05 compared with the baseline. Data expressed in mmHg.*

Figure 3 - **Systolic blood pressure and heart rate changes during the first minute of head-up tilt.**



This figure shows changes evoked by tilt testing on a 45 years-old normal subject (dashed lines) and a 46 years-old patient with paroxysmal atrial fibrillation (solid lines). On top is shown the systolic blood pressure (SBP) and on bottom the heart rate (HR). TA1=first minute of tilt adaptation

Figure 4 - Mean values of systolic blood pressure ramps, baroreflex sensitivity and baroreflex effectiveness index in paroxysmal atrial fibrillation patients (gray bar) and healthy subjects (white bar)



SBP=systolic blood pressure; BRS=baroreflex sensitivity; BEI=baroreflex effectiveness index; supine=last four minutes of the baseline position (0º), TA1= first minute of tilt adaptation (60º), TA5= between the first and fifth minute of head-up tilt (60º). *= *$p<0.05$ for the comparison between groups. §=0.02 compared with baseline values.*

Capítulo V.

Papel da actividade autonómica nos fenómenos electrofisiológicos e vulnerabilidade para fibrilhação auricular e na expressão genética das proteínas dos canais iónicos e conexinas no modelo animal

Acute vagal modulation of atrial and pulmonary veins electrophysiology increases vulnerability to atrial fibrillation

Mário Oliveira, M. Nogueira da Silva, Vera Geraldes, Rita Xavier, Sérgio Laranjo, Vitor Silva, Gabriela Postolache, Rui Ferreira, Isabel Rocha.

Experimental Physiology (aceite para publicação)

Sympathetic-parasympathetic interactions on acute electrophysiological modulation and atrial fibrillation inducibility

Mário Oliveira, Gabriela Postolache, Vera Geraldes, Vitor Silva, Sérgio Laranjo, Cristiano Tavares, M. Nogueira da Silva, Rui Ferreira, Isabel Rocha.

Autonomic Neuroscience (submetido)

Time course of ionic and gap junctional remodeling induced by short-term rapid pacing in rat atria

Mário Oliveira, Gabriela Postolache, Vera Geraldes, João P. Gomes, Alexandra Nunes, Vitor Silva, Sérgio Laranjo, Rui Ferreira, Isabel Rocha.

Clinical Science (submetido)

Alterations in atrial ion channel and connexin gene expression induced by autonomic stimulation: a potential substrate for atrial fibrillation

Mário Oliveira, Gabriela Postolache, Vera Geraldes, João P. Gomes, Alexandra Nunes, Cristiano Tavares, Sérgio Laranjo, M. Nogueira da Silva, Rui Ferreira, Isabel Rocha.

Heart Rhythm (submetido)

Acute vagal modulation of atrial and pulmonary veins electrophysiology increases vulnerability to atrial fibrillation

Mário Oliveira, MD, FESC, FHRS**
M. Nogueira da Silva, MD*
Vera Geraldes, AP**
Rita Xavier, BioEng**
Sérgio Laranjo, MD**
Vitor Silva, Eng**
Gabriela Postolache, PhD **
Rui Ferreira, MD*
Isabel Rocha, PhD**

* Department of Cardiology, Santa Marta Hospital, Lisbon, Portugal
** Institute of Physiology, Faculty of Medicine, Lisbon, Portugal & Unit of Autonomic Nervous System, Instituto de Medicina Molecular, Lisbon, Portugal,

Running Title: Electrophysiological changes and vagally-mediated atrial fibrillation

Keywords: atrial fibrillation, cardiovascular physiology, autonomic nervous system

Abstract

Vagal activity is thought to influence atrial electrophysiological properties and play a role in the initiation and maintenance of atrial fibrillation (AF). We evaluated the effects of acute vagal stimulation on atrial conduction, atrial and pulmonary veins (PV) refractoriness and inducibility of AF. An open-chest epicardial approach was performed in New Zealand white rabbits with preserved autonomic innervation. Atrial electrograms were obtained with 4 unipolar electrodes placed epicardial along the atria (n=22), and an electrode adapted to the proximal left PV (n=10). Cervical vagus nerve was stimulated with bipolar platinum electrodes (20Hz). Epicardial activation was recorded in sinus rhythm, and effective refractory periods (ERP), dispersion of refractoriness and conduction times from high-lateral right atrium (RA) to high-lateral left atrium (LA) and PV, assessed at baseline and during vagal stimulation. Burst pacing (50Hz, 10s), alone or combined with vagal stimulation, was applied to the right (RAA) and left (LAA) atrial appendages, and PV to induce AF. **Results:** At baseline, ERP were lower in PV than in LA and LAA, but did not differ significantly from RA and RAA, and there was a significant delay in the conduction time from RA to PV compared to the activation time from RA to LA ($p<0.01$). During vagal stimulation: a) ERP decreased significantly in all sites, without significant differences in dispersion of refractoriness; b) the atrial conduction times changed from 39±19 ms to 49±9 ms (RA to PV, p=NS) and from 14±7 ms to 28±12 ms (RA to LA, p=0.01). AF induction was reproducible in 50% of cases at 50Hz and in 82% at 50Hz + vagal stimulation ($p<0.05$). During vagal stimulation, AF cycle length decreased in all sites, and AF duration changed from 1.0±0.9s to 14.0±10.0s ($p<0.01$), with documentation of PV tachycardia in 3 cases. In 70% of the animals inducible AF ceased immediately after interruption of vagal stimulation. **Conclusions:** In an intact heart rabbit, vagal activity prolongs interatrial conduction and shortens atrial and PV ERP, contributing to the vulnerability for the induction and maintenance of AF. This model may be useful in the assessment of the autonomic influence in the mechanisms underlying AF.

Introduction

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia in clinical practice, with impact on both life expectancy and quality of life (Camm, 1997; Benjamin et al, 1998; Van Den Berg et al, 2001). AF pathophysiology remains a considerable challenge, due to our limitations in understanding the precise mechanisms underlying the initiation and maintenance of this arrhythmia. Experimental work has provided an important contribution to improve comprehension of complex AF mechanisms. The relative contribution of the multiple wavelet hypothesis proposed by Moe and co-workers (1964) and the focal sources of electrical activity described by Haissaguerre et al (1998) in explaining AF remain unknown. Nevertheless, phenomena that are able to influence electrophysiological properties of the atria can have a role in AF pathogenesis (Nattel, 2003).

The autonomic nervous system (ANS) has been strongly recognized as an important modulator in mediating AF (Tai, 2001; Chen et al, 2006; Chen and Tan, 2007). Variations of autonomic tone may be important in the genesis of paroxysmal AF. A number of electrophysiological properties, related with atrial vulnerability for AF, may change as a result of vagal or sympathetic activation. Vagal stimulation reduces velocity of the conduction in the atrial tissue and shortens the action potential duration and atrial effective refractory periods (ERP) non-uniformly, facilitating reentrant arrhythmias (Chen et al, 2006; Nattel et al, 2000), and sympathetic stimulation can favour reduction of atrial refractoriness and induction of trigger activity (Nattel et al, 2000). However, the specific contribution of each division of the ANS to the genesis of AF remains unclear. Sharifov et al (2004), reported that direct infusion of acetylcholine into the sinus node artery could induce AF in 100% of dogs, but infusion of isoproterenol and adrenaline induced AF only in 21% of the animals. Sympathetic outflow does not

promote AF as much as vagal stimulation, probably because the effects of sympathetic stimulation are more spatially homogeneous in the atria than those resulting from vagal activity (Olshansky, 2005). It has been recognized that vagal activity might predispose patients to develop paroxysmal AF as it has been described (Coumel et al, 1978) that vagal overactivity is a precipitating cause of several short daily attacks of an arrhythmia which are usually not completely nocturnal, and that vagal nerve stimulation can facilitate the induction of AF (Hirose, 2002). Characterization and quantification of ANS electrophysiological effects are complex and difficult to define. Several animal models have been developed to understand the origin and possible mechanisms underlying AF. Nevertheless, an important part of these experimental studies were carried out in isolated heart models. In the present study, we used the intact anesthetized rabbit heart, with preserved autonomic innervation, in order to investigate the effects of vagal stimulation on atrial conduction velocity and refractoriness, and its relevance in the context of vulnerability for the induction and maintenance of AF.

Methods

Ethical Issues

All the experimental procedures were performed in accordance with the Portuguese and European law on animal welfare and were also approved by the Ethics Committee of the Faculty of Medicine of Lisbon.

Anaesthesia and Surgical Protocol

Twenty two New Zealand white rabbits of both sexes (weight 3.9-5.0 kg) were anaesthetized with sodium pentobarbital (60 mg/kg, iv). A tracheal cannula was inserted below the larynx, via a tracheostomy, to allow ventilation with O_2-enriched room air through a positive-pressure ventilator (Harvard Apparatus, UK). Neuromuscular

blockade was achieved with vecuronium (0.05mg/kg/h, i.v.), in order to assure strict control of respiratory parameters such as rate and volume. Ventilation was adjusted to ensure an end-tidal CO_2 of 5-7%. The adequate levels of anaesthesia were maintained with a 20% solution of the anaesthetic and assessed by observing the presence of the withdrawal reflex after pinching a paw before the injection of vecuronium and, from the observed changes on arterial blood pressure (BP) and heart rate after the same type of noxious stimulation after the neuromuscular blockade. Femoral artery and vein were cannulated for BP monitoring (Lectromed, UK) and for intravenous drug and saline administration, respectively. Rectal temperature was monitored and maintained at 38±1°C throughout the experiment with a heating blanket (Harvard Apparatus). The urinary bladder was cannulated and drained.

A midline sternectomy was performed and the pericardium opened in order to expose the heart and prepare an epicardial approach. After thoracotomy, an end-expiratory pressure of 1-2 cmH_2O was maintained. The ECG was recorded with the use of bipolar percutaneous electrodes placed in the four limbs of the animal. BP, ECG, heart rate, ventilatory rate and end-tidal CO_2 were continuously monitored (PowerLab, ADInstruments). At the end of the experiment the animal was killed with an overdose of the anaesthetic.

Experimental protocol

a) Electrical stimulation of the vagus nerve

The right cervical vagus nerve was identified and isolated via a midline neck incision and prepared to be electrically stimulated with a bipolar silver electrode. As a physiological test, the vagus nerve was shortly stimulated (20 Hz, 0.2 ms, 150-200μA) to evoke a sinus rate decreasing of 50%.

b) Electrophysiological protocol

Atrial electrograms were recorded by four unipolar electrodes custom-made (125-µm Ag–AgCl Teflon-isolated wire), inserted into a patch of nylon network at inter-electrode distances of 4 mm (figure 1). This electrode-array was placed epicardially and distributed along the atria, from the high-lateral right atrium (RA) to the high-lateral left atrium (LA). In ten animals, a fifth electrode (twin coaxial cable) was adapted to the left pulmonary veins (PV), near the area of insertion in the LA, for electrograms recording and pacing. Atrial epicardial activation was recorded during normal sinus rhythm, and the conduction time between RA and LA, and between RA and PV measured in basal conditions and during vagal stimulation. The recorded signals were amplified, filtered at 0.5 to 300 Hz (Neurolog, Digitimer, UK) and digitized (PowerLab, ADInstruments) (figure 2).

Bipolar electrical stimulation was performed with a teflon-coated silver wire electrode (0.1 mm diameter), positioned with a micromanipulator (WPI, M330), and with a coaxial electrode adapted to the PV for the assessment of ERP and induction of AF. A multi 8-channel, programmable stimulator (Master8, AMPI, Israel) was used for pacing. As a measure of local refractoriness, ERP were assessed in each animal at four different sites (RA, right atrial appendage, left atrial appendage and LA). ERP of the PV was also measured in 10 animals. Under stable conditions, a programmed electrical stimulation using a single premature stimulus was delivered, while pacing continuously at a basic drive cycle length of 300 ms (or 20 ms below the RR interval if basal heart rate >200 bpm). Stimulation was performed with impulses of 2 ms duration at twice the diastolic threshold. A premature beat was introduced in late diastole, beginning at a coupling interval of 100 ms less than the basic cycle length. The coupling interval of the premature stimulation was decreased by 10 ms steps until the ERP was reached. ERP

was defined as the longest S1-S2 interval that failed to initiate a propagation response, and measured in basal conditions and during vagal stimulation. Dispersion of atrial refractoriness was calculated as the difference between the longest and the shortest ERP at the stimulation sites. The AF cycle length was measured in all sites with online calipers at a paper speed of 100 mm/s by averaging 5 consecutive cycles before the termination of AF.

The concept of vulnerability for AF induction was evaluated with bursts (50Hz, 10s, supraliminar intensity), performed with a bipolar electrode placed at the right and left atrial appendages, alone or combined with vagal stimulation. AF was defined as a fast atrial rhythm characterized by a fluctuating beat-to-beat cycle length, polarity, configuration and amplitude of the recorded atrial electrograms and lasting more than 5 cycles (15).

Statistical Analysis

Results are shown as mean±SD. Categorical variables are expressed as frequencies and percentages. Normality of the distributions of the continuous variables was analyzed with the Kolmogorov-Smirnov test. Student's *t* test was used to assess all paired data in the same group. Comparisons between groups were made using the unpaired Student's *t* test or nonparametric analysis with the Mann-Whitney's test, as appropriate. The Chi-square test, with Yates correction, was used to evaluate the differences in categorical variables. All statistical tests were 2-sided, and a probability value <0.05 was required for statistical significance. Data were analyzed using GraphPAD Instat, version 3.05 (GraphPad Software, Inc., California, USA).

Results

In stable conditions, after all the surgical procedures and under neuromuscular blockade, heart rate ranged between 175 bpm and 222 bpm (195±29 bpm, n=22), the systolic BP between 60 mmHg and 90 mmHg (82±15 mmHg, n=22), and the P-wave between 38 ms and 60 ms (53±8 ms, n=22). Vagal stimulation at cervical level for 5s elicited a pronounced sinus bradycardia, with occurrence systemic hypotension (<60 mmHg) in all animals.

a) Effects of vagal stimulation on atrium electrophysiological parameters

The conduction times, from the RA to the LA were, on basal conditions, of 14±7 ms (10-30 ms), which increased to 28±10 ms (20-35 ms) by stimulation (20 Hz, 0.2 ms, 150-200μA) of the right vagus nerve (n=22, $p<0.05$) (figure 3). In baseline conditions, during normal sinus rhythm, there was a significant delay of the conduction between the RA and the PV, slightly influenced by vagal stimulation (figure 3).

Table I shows the ERPs obtained in basal and during vagal activation. In basal conditions, there was a significant increase of atrial refractoriness from the RA, right-atrial appendage (RAA) and PV to the LA and left atrial appendage (LAA) (table I). Vagal stimulation produced a reproducible reduction in the atrial ERP assessed in all sites (table I).

The dispersion of ERP based only on atrial measurements was significantly lower than the dispersion of ERP calculated with atrial and PV refractoriness values (33±14 ms vs. 64±25 ms, $p<0.05$). Compared to baseline, vagal stimulation did not affect significantly the dispersion of refractoriness (33±14 ms vs. 38±17 ms for atrial sites, p=NS; 64±25 ms vs. 67±28 ms for all sites, p=NS).

b) Effects of vagal stimulation on atrial fibrillation inducibility

The inducibility and the duration of AF were significantly increased by vagal stimulation (figure 4). AF was induced in 50% of the rabbits with rapid atrial bursts delivered to the RAA or PV, but could not be induced by LAA high-rate pacing alone. During vagal activation, AF was consistently induced in 100%, 60% and 20% by pacing the RAA, PV and LAA, respectively (RAA vs LAA, $p<0.05$). The mean AF cycle length measured under control conditions and during vagus stimulation is represented in table II. Cycle lengths during AF induced by 50 Hz pacing combined with vagal stimulation decreased significantly in all atrial sites and PV. On three occasions, AF was initiated by a single extra-stimulus combined with vagal stimulation during ERP measurements. PV tachycardia (cycle length 80-90 ms) was induced by high-rate PV pacing combined with vagal stimulation in 3 animals (figure 5).

The duration of the inducible AF changed from 1.0±0.8s, with 50Hz rapid stimulation alone, to 14.0±10.0s, in response to 50Hz rapid stimulation combined with vagal stimulation ($p<0.01$) (figure 4). AF lasted >10 seconds in 45.5% of the rabbits (only during vagal stimulation) and terminated immediately after the cessation of vagal stimulation in 7 out of those 10 cases. These animals had similar interatrial and atrial to PV activation times. However, they showed the shortest ERP in the RA, with higher dispersion of atrial refractoriness, at baseline and also during vagal stimulation (table III).

Discussion

The present experimental *in vivo* model allowed the study of the effects of vagus nerve stimulation on cardiac electrophysiological properties and on the vulnerability for AF. We have characterized the influence of direct acute stimulation of the right cervical

vagal trunk on electrical conduction and refractoriness of the atria and PV. We have also confirmed a role of vagal activity on the vulnerability for the initiation and duration of AF.

Main findings

This model, with preserved autonomic innervation, shows a significant activation delay, during sinus rhythm, from the atria to the PV. In fact, the mean conduction time from the RA to PV where about three times more than the RA to LA interval measured at baseline conditions (figure 3). Also, a significant increase of baseline atrial ERP was observed from the RA to the LA, suggesting heterogeneity of the electrophysiological properties of the atria. In addition, ERP of the PV were significantly lower than those measured in the LA and LAA. In this preparation, vagal activity was important in obtaining significant reproducible changes in atrial activation times, atrial and PV ERP, frequency of AF induction, mean AF cycle length and duration of the induced AF. Moreover, we demonstrated significant differences on atrial dispersion of refractoriness and RA ERP in the group of animals with AF lasting >10 s.

Animal models and mechanisms of atrial fibrillation

Various animal models have been developed to explore the mechanisms underlying AF. The classic hypothesis of multiple circuit reentry with variable spatial orientation, focal activity, and single rotors with fibrillatory conduction that remains the framework for our understanding of AF, were established based on experimental studies in animals. However, the development of *in situ* models that allow electrophysiological studies and a contribution to the comprehension of the conditions associated with the generation of arrhythmias remains a major challenge.

In dogs, micro-reentry, abnormal automaticity and triggered activity have been observed as possible mechanisms for the occurrence of atrial arrhythmias (Friedman et al, 1996;

Hocini et al, 2002). Hayashi and co-workers (1998), used efferent cervical vagal stimulation to induce AF by bursts of atrial pacing in anesthetized open-chest dogs and evaluate the efficacy of class Ic and III antiarrhythmic drugs to terminate AF. Other investigators have used versions of this model to explore the ability of different antiarrhythmics in terminating induced AF (Goldberger & Pavelec, 1986; David et al, 1990). Nevertheless, there is still a lack of data regarding the use of experimental models with preserved autonomic innervation, to assess the fundamental aspects of the electrophysiological properties of the atria and PV underlying the vulnerability for development of AF. Despite the amount of recent information on management strategies in respect of AF, the effects of vagal activation in the arrhythmia recurrence and progression to a sustained condition remain poorly understood. In fact, it has been accepted that the ANS contributes as a modulator of the initiation, perpetuation, ventricular response rate, and termination of AF, but its precise role remains controversial (Olshansky, 2005). The importance of the ANS in AF is supported by animal experiments and recent clinical studies showing that vagal denervation enhances the efficacy of circumferential PV ablation in preventing AF recurrence (Chan & Tan, 2007). Abnormal atrial electrophysiology and higher vagal reflex activity may play an important role in the genesis of AF (Chen et al, 1999).

Decreased atrial ERP and its spatial dispersion heterogeneity have been accepted to promote AF re-initiation and to provide a substrate for the reentry of multiple wavelets to enhance the ability of the disorder to sustain itself (Nattel et al, 2000). In previous studies, sympathetic stimulation was much less effective than vagal stimulation in promoting AF and heterogeneity in atrial refractoriness (Liu et al, 1997; Chen et al, 1999). Vagus nerve stimulation has been associated with atrial ERP shortening and facilitation of AF inducibility in dogs (Arora et al, 2008).

Our present data, using the intact heart rabbit model, support the importance of vagal activity in determining significant changes of atrial refractoriness and its contribution for the induction and cycle length of AF. The calculated dispersion of atrial refractoriness has not been significantly modified by vagal stimulation. This may explain the inability to sustain AF despite maintenance of vagal activation. In fact, reproducible induction of AF during vagal stimulation was obtained in all animals with a significant increase in the duration of the episodes, but the longest duration of AF was 45 s, lasting >10 seconds only in 45.5% of the rabbits. Interestingly, those animals with longer AF duration showed higher dispersion of atrial refractoriness and shortest RA ERP, despite similar conduction activation times. This emphasizes the role of refractoriness properties as important markers of vulnerability to AF. We were able to obtain conditions that decrease the wavelength (by decreasing the conduction velocity and ERPs of the atrial tissue), which permits multiple wavelets and promotes AF.

Effects of vagal stimulation on conduction times, refractoriness and vulnerability to atrial fibrillation

Electrophysiological abnormalities necessary for the genesis of AF include conduction disturbances, shortening of the ERP, and increase in atrial dispersion of refractoriness (Fuster et al, 2001). Vagal tone may influence the refractoriness and conduction velocity in the atrial tissue, contributing to the vulnerability for the occurrence of AF (Chen et al, 2007). However, the assessment of autonomic fluctuations before the onset of paroxysmal AF, measured with heart rate variability, has shown conflicting results (Chen et al, 2006).

In the present study, the electrophysiological characterization of the recognized effects of vagal stimulation in atrial conduction and refractoriness properties was clearly shown. We have provided a consistent method to obtain reproducible changes in

electrophysiological properties of the atria, facilitating AF induction in the presence of vagal stimulation. Oh et al (2006), using a canine model, demonstrated that the effects of vagal stimulation on atrial ERP and AF inducibility were significantly changed immediately after epicardial fat pad ablation, suggesting that it is possible to achieve temporary suppression of the vagal tone in the atria (Oh et al, 2006). Recently, in a study with dogs, sustained AF could be induced easily during vagal nerve stimulation *in vivo* (Lemola et al, 2008). Elvan et al, also used burst stimulation and cervical vagal stimulation to induce and maintain AF in open-chest dogs (Elvan et al, 1995). Their study suggested that atrial radiofrequency catheter ablation markedly attenuated vagally induced shortening of ERP and reduced the inducibility of AF. In our rabbit model, we have shown that vagal activation was able to decrease the atrial and PV cycle length during AF and increase significantly the duration of the induced AF episodes. Vagal stimulation increased significantly the interatrial activation times, without changes in the conduction velocity between atrial and PV activation. This suggests nonuniformity of vagal responses, with evidence of regional differences in conduction delay, and supports the concept that the presence of anisotropic conduction properties and slow conduction at the area of PV-LA junction may contribute to promote re-entry formation and thus play a role as a substrate for the maintenance of AF (Kumagai et al, 2004). Also, the vagal effect in the degree of ERP decreasing was slightly higher in the RA, LA and PV.

Vagal activity seemed to directly influence the termination of AF, since in 70% of those animals with AF duration lasting >10s the arrhythmia stopped immediately after cessation of vagal stimulation. So, despite the inability to demonstrate a significant impact in the dispersion of the ERP, vagal activation decreased the conduction velocity

in the atria and evoked reductions of the ERP, contributing to the induction, duration and termination of AF.

Limitations of the study

Although programmed electrical stimulation with a single premature stimulus has been widely used for the study of atrial refractoriness properties, we believe that further studies may be needed to confirm these findings, using different electrophysiological techniques, because the current method may be of relatively low resolution. Also, the low tendency of the rabbit atria to fibrillate and maintain the arrhythmia due to its small size might contribute to the inability to sustain AF in this preparation. Although brief episodes of AF in the rabbit reflect an evidence of arrhythmic vulnerability, the precise mechanisms underlying AF maintenance may be different in humans. Further experimental studies using more accurate methods may be needed to elucidate the impact of autonomic innervation in the dynamics of recurrent sustained AF episodes.

Conclusions

The acute stimulation of parasympathetic activity exerts a reduction of atrial conduction velocity and decreases ERP in multiple atrial sites and in the PV. This experimental preparation displays also the impact of vagal stimulation in enhancing the propensity for the inducibility of AF, demonstrating an important role in the vulnerability for the occurrence of AF and contributing to the duration of AF episodes, particularly in those animals with higher dispersion of refractoriness. Our data suggest that this experimental vagal stimulation model may provide a useful method into the assessment of the ANS influence in the pathophysiology of AF.

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Tables

Table 1 - Atrial effective refractory periods measured at baseline conditions and during vagal stimulation

	Baseline	vagal stimulation	%_Δ	p
RA	86±17 ms	60±20 ms	-33±20%	<0.01
RAA	79±18 ms	60±22 ms	-21±33%	<0.05
LAA	105±24 ms*	76±25 ms	-20±17%	<0.05
LA	102±27 ms*	76±23 ms	-26±15%	<0.01
PV	65±40 ms	38±28 ms	-31±36%	<0.01

(RA=right atrium; RAA=right atrial appendage; LAA=left atrial appendage; LA=left atrium; left pulmonar veins). *=p<0.01 when compared to high lateral RA, RAA and PV in baseline (%_Δ=percentage of variation of the average effective refractory periods).

Table II. Mean atrial and pulmonary veins cycle lengths measured under control conditions and following vagal stimulation

	RA	RAA	LA	LAA	PV
AFCL control (ms)	154±35	170±23	173±16	171±16	152±25
AFCL vagal (ms)	83±34*	77±21**	86±35**	110±48§	71±17**

AFCL=atrial fibrillation cycle length; RA= high-lateral right atrium; RAA=right atrial appendage; LA= high-lateral left atrium; LAA=left atrial appendage; PV=proximal segment of pulmonary veins.*p=0.002; **p<0.0001; §p<0.05 (for the comparison between control conditions and under vagal stimulation). p=NS for measurements obtained in the same condition.

Table III - characteristics, atrial refractoriness and atrial conduction times in the group of rabbits with atrial fibrillation lasting >10 seconds

	group AF <10s	Group AF >10s	p
P wave duration (ms)	35±9	50±14	NS (0.08)
Baseline heart rate (bpm)	196±13	208±28	NS
Baseline systolic BP (mmHg)	78±11	75±15	NS
Weight (kg)	4,4±0,3	4,3±0,4	NS
Atrial dispersion of ERP (ms)	22±15	42±5	0.03
Atrial + PV dispersion of ERP	73±38	60±26	NS
Atrial dispersion of ERP vagal	28±15	48±11	0.03
Atrial+PV dispersion of ERP vagal	72±40	63±13	NS
RA ERP baseline (ms)	97±12	70±10	<0.05
RA ERP vagal	80±20	40±10	<0.05
RAA ERP baseline	67±15	80±11	NS
RAA ERP vagal	53±15	64±27	NS
LAA ERP baseline	102±21	107±6	NS
LAA ERP vagal	75±24	88±19	NS
LA ERP basal	105±35	100±27	NS
LA ERP vagal	80±56	73±16	NS
PV ERP basal	75±51	58±38	NS
PV ERP vagal	41±33	34±30	NS
Interval RA-LA baseline (ms)	10±7	17±4	NS
Interval RA-LA vagal	26±9	31±16	NS
Interval RA-PV basal	55±50	40±20	NS
Interval RA-PV vagal	58±20	51±8	NS

BP=blood pressure; PV=left pulmonary veins; ERP=effective refractory period; vagal=during cervical vagal nerve stimulation. Atrial + PV dispersion includes ERP measurements from high-lateral right (RA) and left (LA) atria, right (RAA) and left (LAA) atrial appendages, and PV.

Figure Legends

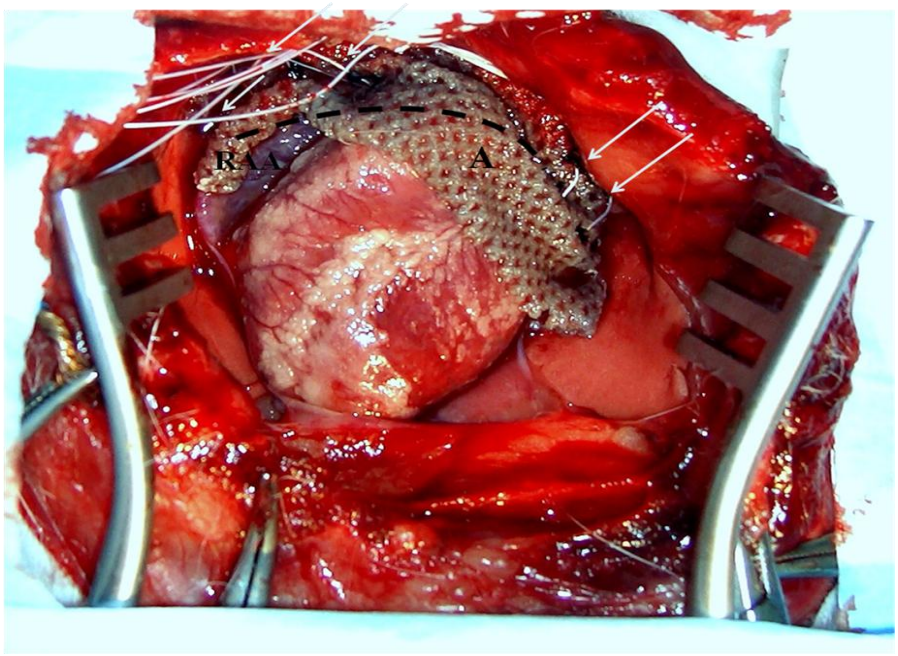

Figure 1 – Open-chest preparation of the intact rabbit heart with nylon network (A) supporting 4-unipolar electrodes (arrows) to record electrograms along the atria (from the high-lateral right atrium to the high-lateral left atrium. RAA=right atrial appendage.

Figure 2 – Continuous recording of blood pressure (BP), ECG (electrocardiogram, "DI-like" lead), atrial and left pulmonary veins epicardial electrograms during sinus rhythm (RA1=high-lateral right atrium; RA2 – right atrial appendage; LA1- left atrial appendage; LA2 – high-lateral left atrium; PV – pulmonary veins)

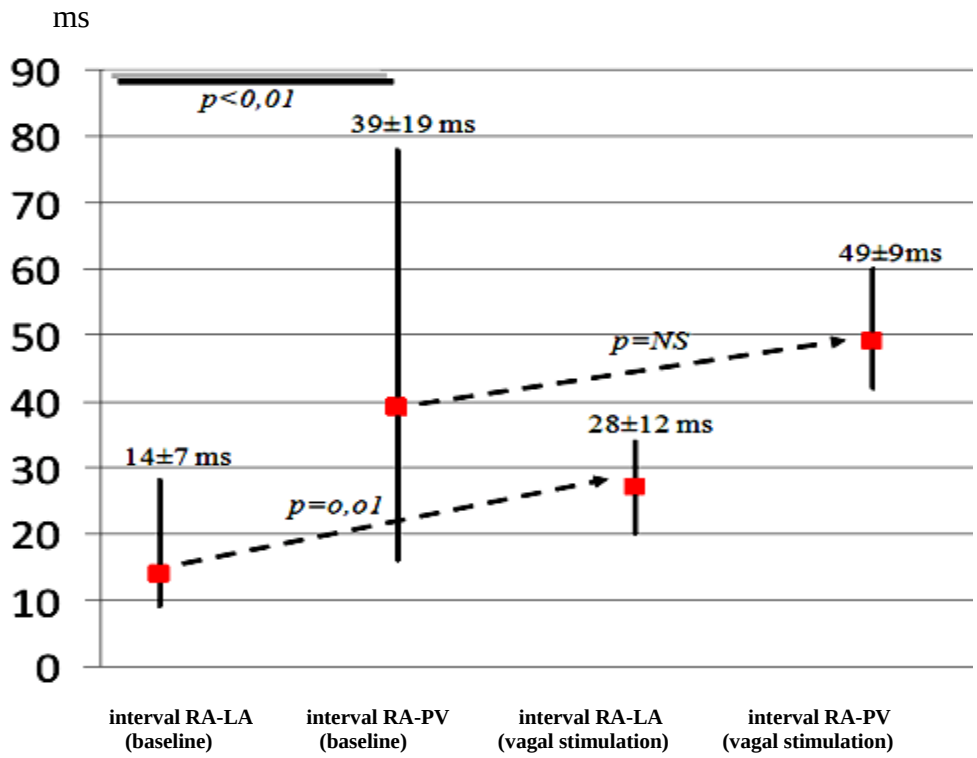

Figure 3 – Atrial conduction times between the high-lateral right atrium (RA) and the high-lateral left atrium (LA), and between the RA and left pulmonary veins (PV) near the area of insertion in the LA in baseline conditions and during right cervical vagal stimulation

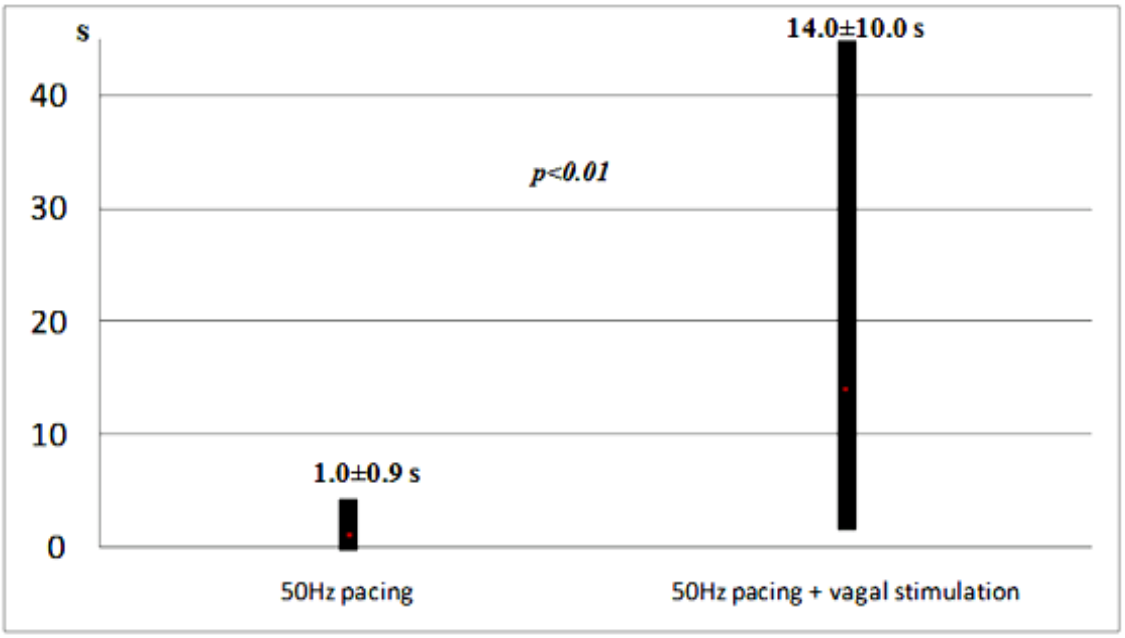

Figure 4 – Duration of the inducible atrial fibrillation episodes with rapid atrial stimulation alone (50Hz pacing) and in response to rapid atrial stimulation combined with vagal activation (50Hz pacing + vagal stimulation)

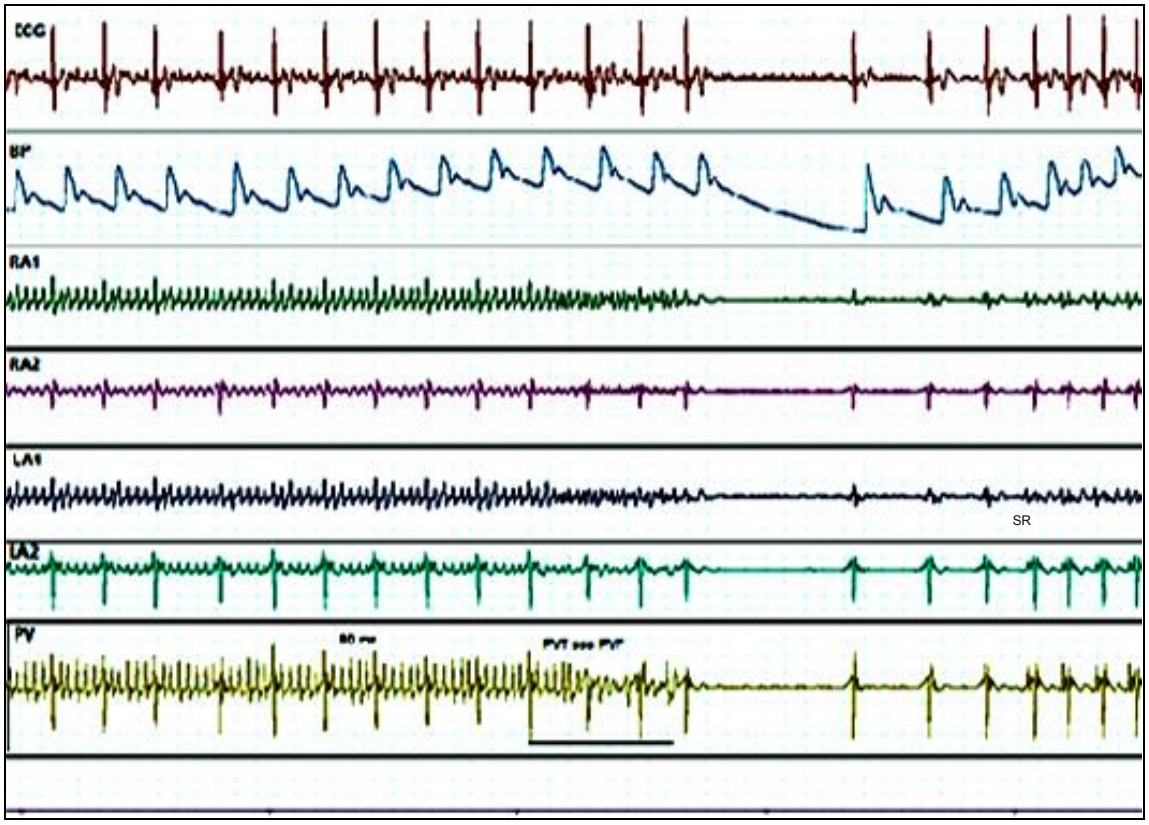

Figure 5 – Example of a recording with pulmonary veins tachycardia (PVT) induced by pulmonary veins stimulation (50Hz, 10s) combined with vagal activation, showing the transition to pulmonary vein fibrillation (PVF) followed by termination of the arrhythmia. PVT=pulmonary veins tachycardia; PVF=pulmonary veins fibrillation; SR=sinus rhythm. (ECG=electrocardiogram "DI-like" lead; BP=blood pressure; RA1=high-lateral right atrium; RA2=right atrial appendage; LA1=left atrial appendage; LA2= high-lateral left atrium; VP=pulmonary veins).

Sympathetic-parasympathetic interactions on acute electrophysiological modulation and atrial fibrillation inducibility

Mário Oliveira[1], Gabriela Postolache[2], Vera Geraldes[2], Vitor Silva[2], Sérgio Laranjo[2], Cristiano Tavares[2], Nogueira da Silva[1], Rui Ferreira[1], Isabel Rocha[2]

[1] Electrophysiology Laboratory, Cardiology Department, Santa Marta Hospital, Lisbon, Portugal

[2] Autonomic Nervous System Unit, Physiology Institute, Institute of Molecular Medicine, Faculty of Medicine, Lisbon, Portugal

***Running title**: autonomic effects in atrial electrophysiology and atrial fibrillation*

***Keywords:** atrial fibrillation inducibiliy, autonomic nervous system, rabbit heart, atrial refractoriness, electrical conduction*

Abstract

Atrial fibrillation (AF) is a complex disease with multiple mechanisms requiring a better comprehension to explain the interaction between autonomic nervous system (ANS), electrophysiological properties of the atria and pulmonary veins (PV), and vulnerability for AF. We evaluated the effects of acute vagal (vagus_stim) and sympathetic stimulation (symp_stim) on atrial conduction, atrial and PV refractoriness and inducibility of AF in an *in vivo* rabbit model with preserved autonomic innervation. An open-chest epicardial approach was performed in 17 New Zealand white rabbits, anaesthetised and artificially ventilated. Electrograms were obtained with 4 unipolar electrodes placed epicardial along the atria, and an electrode adapted to the proximal segment of PV. Cervical vagus nerve and thoracic sympathetic trunk were stimulated with bipolar electrodes. Epicardial activation was recorded in sinus rhythm, and effective refractory periods (ERP) and conduction times from high-lateral right atrium (RA) to high-lateral left atrium (LA) and PV quantified at baseline, during vagus_stim, symp_stim or dual ANS stimulation (n=8). Burst pacing (50Hz, 10s), alone or combined with vagus_stim, symp_stim or dual autonomic stimulation (n=8), was performed in the right (RAA) and left (LAA) atrial appendages, and PV to test for AF inducibility. Results: At baseline, ERP were higher in the LAA and there was a non significant delay in the conduction from RA to PV, compared to the mean activation time from RA to LA. With vagus_stim or dual autonomic stimulation, ERP decreased significantly in all sites, and the interatrial activation times changed from 20±4 ms to 30±10 ms ($p<0.05$) and 31±11 ms ($p<0.05$), respectively. Symp_stim, resulted in a significant decrease of ERP in the LAA, and a reduction of the interatrial interval to 16±6 ms ($p<0.05$). AF inducibility ranged from 35% to 53% with 50Hz pacing, 65% to 76 % during vagus_stim or symp_stim, and 75% to 100% with dual ANS stimulation ($p<0.05$). The duration of AF increased significantly with 50Hz stimulation combined with symp_stim and/or vagus_stim. In 2/3 of the animals with longer inducible AF, the arrhythmia ceased immediately after vagus_stim interruption. Conclusions: In a full innervated heart rabbit *in vivo*, acute autonomic stimulation shortens atrial and PV ERP, and change interatrial velocity and conduction between atria and PV. It also increases the likelihood for the induction of AF and the duration of AF episodes. This experimental model may provide a valuable method into a better understanding of the ANS interactions with AF pathophysiology.

Introduction

Atrial fibrillation (AF) is a complex disease with multiple possible mechanisms that require a better comprehension to explain the interaction between the phenomena underlying initiation, maintenance and termination of the arrhythmia. Lone AF is probably related to an electrophysiological substrate, involving the contribution of focal sources of automatic activity, often in the form of rapidly firing ectopic foci inside the pulmonary veins (PV), and multiple wavelets that propagate and interact in a random fashion over the atrial surface (Moe GK et al, 1959; Haissaguerre M et al, 1998; Mandapati R et al, 2000; Allessie M et al, 2002). It is now recognized that autonomic nervous system (ANS) may influence electrophysiological properties of the atria and act as an important modulator in the pathophysiology of AF (Nattel, 2003; Chen J et al, 2006; Chen & Tan, 2007). In fact, autonomic tone can favour reentrant arrhythmias and trigger activity (Coumel IP et al, 1996; Nattel 2003; Chen J et al, 2006). Clinical studies suggest that both sympathetic and parasympathetic innervations are important in the generation of AF (Coumel IP, 1996; Tomita T et al, 2003; Lombardi F et al, 2004). Also, experimental work has provided an important contribution to improve comprehension of the arrhythmogenic influence of sympathetic and vagal mechanisms in the genesis of AF (Liu & Nattel, 1997; Schauerte P et al, 2001, Tomita T et al, 2003; Sharifov O et al, 2004; Oh et al, 2006). In dog experiments, it has been suggested that the occurrence of atrial arrhythmias was caused by alterations in local autonomic outflow to the PV and superior vena cava (Schauerte P et al, 2001). Activation of both sympathetic and vagal systems in dogs was capable of reducing atrial effective refractory periods (ERP) and favour the occurrence of reentry circuits (Arora R et al, 2008). In addition, it has been shown that a heterogeneous distribution of autonomic innervation throughout the atrium represents a substrate mediating AF occurrence, particularly in settings where there are no anatomic abnormalities (Arora R et al, 2008). Also, stimulation of ganglionated plexi, that modulates autonomic innervation, results in the local release of parasympathetic and sympathetic neurotransmitters, which may lead to increased automaticity, shortening of the action potential duration and of the ERP (Scherlag BJ et al, 2006). Direct autonomic nerve recordings in canine models has shown that simultaneously sympatho-vagal discharges are the most common triggers of paroxysmal atrial tachycardia and AF after a period of high-rate left atrial pacing (Tan A et al, 2008). Although the ANS is known to be important in AF pathogenesis, the precise interplay among all of these complex features remains unclear. In fact, the specific contribution of each division of the ANS to the genesis of AF remains unclear.

Several animal models have been developed to characterize the electrophysiological effects of ANS stimulation and understand the complex mechanisms underlying AF. Nevertheless, an important part of these experimental studies were carried out in isolated heart models. There is a great need to better understand the relationship between acute autonomic activation *in vivo* and vulnerability for the initiation and maintenance of AF, in part because there is a potential adjunctive role to play in AF ablation, especially when targeting selectively areas of highest neural innervation (Schaurte P et al, 2000; Melo et al, 2004; Darge et al 2009).

In the present study, we used the intact anesthetized rabbit heart, with full preserved autonomic innervation, in order to investigate the effects of either sympathetic or parasympathetic nerve stimulation on conduction times and refractoriness of the atria and PV, and the relevance of neuromodulation on the vulnerability for the induction and maintenance of AF.

Methods

Experimental Procedures

a) animal model preparation

Seventeen New Zealand white rabbits of both sexes (weight 2.6-4.9 kg) were anaesthetized with sodium pentobarbital (60 mg/kg, i.v.). A tracheal cannula was inserted below the larynx, via a tracheostomy, to allow ventilation with O_2-enriched room air through a positive-pressure ventilator (Harvard Apparatus, UK). Neuromuscular blockade was achieved with vecuronium bromide (Norcuron, 0.05 mg/kg/h, i.v.), in order to assure strict control of respiratory parameters such as rate and volume. Ventilation was adjusted to ensure an end-tidal CO_2 of 5-7%. The adequate level of anaesthesia was maintained with a 20% solution of the anaesthetic and assessed by observing the presence of the pedal reflex before the injection of vecuronium bromide and from the changes on arterial blood pressure (BP) and heart rate. The right femoral artery and vein were cannulated for BP monitoring (Sensonor transducer and Lectromed apparatus, UK) and for intravenous drug and 0.9% saline administration, respectively. Rectal temperature was maintained at 38±1◦C throughout the procedure with an electric warming blanket with temperature control (Harvard Apparatus). The ECG was recorded with the use of bipolar subcutaneous electrodes placed in the four limbs of the animal. BP, ECG, heart rate, ventilatory rate and end-tidal CO2 were continuously monitored during the experiment (PowerLab, ADInstruments). Briefly, the anaesthetized and paralyzed rabbit was fixed on ventral recumbency to expose the dorsal side of the thorax. After preparation of the skin surface, an incision was done over

the thoracic vertebrae and the subcutaneous dorsal fat pushed aside. The tendons of cervical and thoracic part of trapezius and rhomboid muscles, and the semispinal and external intercostalis muscles were removed in the region of the C6-T2 vertebrae, using the distal end of the electrocautery as a blunt dissector. The spinal thoracal muscle and the musculus longissimus thoracis were pushed aside with a retractor. A 2-mm drill bite was used to create a hole in the T1 vertebra and open the spinal dura mater. Then, rabbits were placed in dorsal recumbency. The right cervical vagus nerve was identified via a midline neck incision, isolated with silk sutures and prepared to be electrically stimulated with a bipolar silver electrode. The animals were slightly right rotated in order to insert a bipolar electrode into the spinal canal to stimulate the thoracic outflow of the sympathetic trunk at T1 level, which mainly includes preganglionic fibers to the stellate ganglion (Craven J, 2008). The electrodes for stimulation of the sympathetic and vagus nerves were connected to a multi-channel programmable stimulator (Master8, AMPI, Israel). As a physiological test, the vagus nerve was shortly stimulated (20Hz, pulse width 2 ms) and the stimulation amplitude adjusted to yield a sinus rate decrease of ≥50%, and the sympathetic outflow tested (3 Hz, pulse width 1 ms) to obtain heart rate and BP increasing following stimulation. The vagal and sympathetic stimulation (2-8 mA and 2-10 mA, respectively) was performed using a transadmitance device with a 90 V battery as power supply.

In addition to heart rate and BP monitoring, power spectral analysis of series of 30 seconds stable recorded R-R intervals was evaluated using a Fast Fourier Transform (FFT) under MatLab software (MathWorks, Natick, MA, USA) and Origin environments (OriginLab, Origin Lab Corporation, Northampton, MA, USA), as described in previous work (Moguilevski et al, 1996; Aso et al, 1998; Postolache et al, 2006), to confirm ANS stimulation. The frequency spectrum was divided into three components: very low frequency (VLF; 0.01-0.1 Hz), low frequency (LF; 0.1-0.3Hz) and high frequency (HF; 0.3-1Hz). HF values were attributed to vagal modulation, while LF values reflected sympathetic activity.

A midline sternectomy was performed and the pericardium opened in order to expose the heart and prepare an epicardial approach of the atria and PV. After thoracotomy, an end-expiratory pressure of 1-2 cmH_2O was maintained. A four electrode-array was placed epicardially and distributed along the atria, from the high-lateral right atrium (RA) to the high-lateral left atrium (LA), to obtain continuous recording of local electrograms, and one bipolar electrode (twin coaxial cable) was adapted to the PV, near the area of insertion in the left atrium roof, for electrograms recording and pacing. The recorded signals

were amplified, filtered at 0.5 to 300Hz (Neurolog, Digitimer, UK) and digitized at a frequency of 1kHz (PowerLab, ADInstruments) (figure 1). At the end of the experiment the animal was killed with an overdose of anaesthetic.

All the experimental procedures were approved by the Institutional Animal Care and Ethics Committee of the Faculty of Medicine of University of Lisbon and complied with the Portuguese and European law on animal welfare.

b) Electrophysiological protocol

Epicardial activation of the atria was recorded during normal sinus rhythm, and the conduction times from RA to LA, and between RA and PV measured in baseline conditions and during either vagal or sympathetic stimulation or both sympathetic and vagal stimulation.

Bipolar electrical stimulation was performed with a teflon-coated silver wire electrode (0.1 mm diameter), positioned with a micromanipulator (WPI, M330), and with a coaxial electrode adapted to the PV for the assessment of ERP and induction of AF. A multi 8-channel, programmable stimulator (Master8, AMPI, Israel) was used for pacing. As a measure of local refractoriness, ERP were assessed in each animal at three sites (right atrial appendage, left atrial appendage and PV). Under stable conditions, a programmed electrical stimulation using a single premature stimulus was delivered, while pacing continuously for approximately 10 seconds at a basic drive cycle length of 300 ms (or 20 ms below the RR interval if basal heart rate >200 bpm). Stimulation was performed with impulses of 1 ms duration at twice the diastolic threshold. A premature beat was introduced, beginning at a coupling interval of 150 ms less than the basic cycle length, and decreased in steps of 10 ms until the ERP was reached. As the S1-S2 intervals approached the ERP, decrements were reduced to 5 ms. ERP was defined as the longest S1-S2 interval that fail to initiate a propagation response, and measured in baseline conditions after animal preparation, during either vagal or sympathetic stimulation, and with combined autonomic activation.

The vulnerability for the induction of AF was evaluated with stimuli trains (50Hz, 1-ms pulse duration) lasting 10 seconds, delivered via a bipolar electrode placed at the PV, right atrial appendage (RAA) and left atrial appendage (LAA), alone or combined with ANS stimulation. A maximum of up to 3 trains were emitted in each atrial site and PV. The average duration of the observed AF was calculated for each condition. AF was defined as a fast irregular atrial rhythm characterized by a fluctuating beat-to-beat

cycle length, polarity, configuration and amplitude of the recorded atrial electrograms and lasting more than 5 cycles (Hirose et al, 2002).

Statistical Analysis

Data were analyzed using GraphPAD Instat, version 3.05 (GraphPad Software, Inc., California, USA). Normality of the distributions of the continuous variables was analyzed with the Kolmogorov-Smirnov test. Student's *t* test and Anova test were used to assess the differences induced by sympathetic or vagal stimulation on measured physiological parameters. The Chi-square test, with Yates correction, was used to evaluate the differences in categorical variables. All statistical tests were 2-sided, and a probability value <0.05 was required for statistical significance. The results are shown as mean±SD or percentage.

Results

In stable conditions, after all the surgical procedures and under neuromuscular blockade, the mean BP was 79±17 mmHg (range 71 to 87 mmHg) and the mean heart rate 211±37 (range 193 to 229 bpm). BP, heart rate, electrocardiogram, bipolar electrograms from PV and signals from four electrode-array placed epicardially along the atria were continuously monitored. Figure 1 shows raw data of signals recorded in baseline conditions. Vagal stimulation produced a significant decrease in mean heart rate (from 214±30 bpm to 100±31 bpm, p<0.0001) and mean BP (from 79±23 mmHg to 58±20 mmHg, p=0.007) (figure 2). Sympathetic preganglionic neurons stimulation induced a small increased in heart rate (from 210±34 bpm to 237±37 bpm, p=0.036) and mean BP (from 75±13 to 91±19 mmHg, p=0.007) (figure 3). After cessation of autonomic stimulation, heart rate and BP showed a gradual return to prestimulation values.

The power spectrum density of recorded R-R intervals in anaesthetized, paralyzed, with open chest rabbits showed a pattern of frequency bands related with sympathetic and parasympathetic outflow to the heart in baseline conditions (figure 4). FFT was applied for stationary part of R-R intervals to evaluate the cardiac response to sympathetic and parasympathetic stimulation. Sympathetic stimulation produced an increase in LF component and LF/HF ratio in comparison with baseline condition (figure 5). Vagal stimulation augmented heart rate variability (the amplitude of PSD resulted from FFT analysis of 30 seconds R-R signal represented in figure 5 are 1000 times higher).

Effects of autonomic nerve stimulation on the electrophysiological parameters

The conduction times between the RA and LA increased with vagal stimulation from 20±4 ms to 30±10 ms ($p<0.05$) and decreased to 16±6 ms with sympathetic stimulation ($p<0.05$). Enhancing ANS outflow to the heart by simultaneous stimulation of cervical vagus and sympathetic trunk significantly changed the conduction times between RA and LA (from 21±5 ms to 31±11 ms, $p=0.03$, $n=8$) (figure 6). The conduction time between RA and PV significantly decreased with sympathetic stimulation (from 24±6 ms to 17±8, $p<0.01$), and increased with vagal stimulation ($p<0.05$), but no significant changes had been produced when dual ANS stimulation was performed (figure 6). Table I shows the ERPs obtained in basal and with autonomic activation. In baseline conditions, atrial refractoriness was higher in the LA (83±16ms vs 69±16 ms in RAA and 59±16 ms in PV, Anova test, $p <0.01$). Vagal and simultaneous vagal and sympathetic stimulation produced a reproducible reduction of refractoriness in the atria and PV. Sympathetic stimulation resulted in a significant reduction of refractoriness only in the LAA. During vagal stimulation, the mean ERPs of the PV were significantly shorter, compared to those at the LAA (Anova test, $p=0.042$). With autonomic activation, the ERPs of the LAA decreased in a higher extent than those measured in the other sites.

Effects of Autonomic Nerve Stimulation on Atrial Fibrillation Inducibility

The inducibility and the duration of AF were significantly influenced by autonomic stimulation (table II). High frequency pacing (50 Hz, 1ms) at RAA, LAA and PV induced AF in 41%, 35% and 53%, respectively. During vagal stimulation, the ability to consistently induce AF increased to 76%, 65% and 76% by 50 Hz pacing the RAA, LAA, and PV, respectively ($p<0.05$ for RAA). Stimulation of vagus nerve resulted in spontaneous occurrence of atrial tachyarrhtmias or reproducible AF induction with a single extrastimulus in the RAA in 4 out of 17 animals. The duration of AF was significantly increased by vagal stimulation with RAA (5.8±2.5 s vs. 1.8±1.2s, $p<0.01$) and VP pacing (3.6±1.6 s vs. 1.7±0.7s, $p<0.05$). AF inducibility increased with sympathetic stimulation (71%, 65% and 76%, for RAA, LAA and PV 50Hz pacing, respectively), but statistical significance was not reached. With sympathetic activation short runs of AF were induced with an extrastimulus in the atria or PV in 2 animals. There was also spontaneous premature depolarizations arising from the PV in 2 cases, and in one rabbit ventricular fibrillation was induced during 50 Hz pacing in the RAA. AF duration increased significantly with PV pacing combined with sympathetic stimulation (9.6±3.7 s vs. 1.7±0.7 s, $p<0.05$).

By combining simultaneous sympathetic and parasympathetic stimulation, AF became reproducible induced with RAA and LAA high-rate pacing in 87,5%, 100%, and 75% of the animals, by pacing the RAA, LAA, and PV, respectively ($p<0.05$ vs. baseline for RAA and LAA pacing, n=8).

During simultaneous sympathetic and parasympathetic activation, induced AF episodes were longer in all pacing sites (table II). AF lasted >10 seconds in 37.5% of the rabbits (only during vagal or combined autonomic stimulation) and terminated immediately after cessation of vagal stimulation in 4 out of those 6 cases.

Discussion

The present experimental *in vivo* heart rabbit model allows the study of the effects of acute ANS stimulation on cardiac electrophysiological properties and on the vulnerability to AF. We have provided a consistent method to obtain, reproducible changes on electrophysiological properties of the atria and PV in the presence of direct vagal and sympathetic trunk stimulation. We have also confirmed a role of autonomic activity on the vulnerability for the initiation and duration of AF.

Main findings

This model, with preserved autonomic innervation, shows a significant activation delay of interatrial conduction during vagal stimulation, and enhanced conduction times during sympathetic activation. In normal sinus rhythm, there was a slight prolongation (not statistically significant) of the mean conduction interval between RA and PV, when compared to interatrial conduction, possibly related with a delay in the PV-LA junction, which seems to display a great degree of decremental conduction and significant heterogeneity in conduction properties (Kumagai et al, 2004; Erlich & Nattel, 2005). This activation times decreased during sympathetic stimulation, but were not affected by acute vagal activation.

The LAA ERPs were significantly higher than those assessed at the PV or RAA, suggesting heterogeneity of the electrophysiological properties in both atria and PV. Acute vagal stimulation decreased markedly atrial and PV refractoriness, whereas sympathetic activation showed a significant reduction only of the LAA ERPs. The heterogeneous effects of autonomic modulation in the conduction and refractoriness of the atria and PV evidence possible regional differences in the innervation of the heart. These could influence the electrophysiological substrate for AF, which has been previously

related with conduction disturbances and reduction of the ERPs, that facilitate the occurrence of multiple reentry circuits within the atria (Erlich & Nattel, 2005; Pytkowsky et al, 2008).

In this normal heart preparation, vagal and sympathetic activity was important to obtain significant reproducible changes in atrial activation times, atrial and PV ERP, but also to increase the propensity of the rabbit atria to fibrillate and influence the duration of AF episodes.

Animal models and insights into vulnerability for atrial fibrillation

Studies in various animal models have provided important knowledge about mechanisms underlying AF. The classic hypothesis of multiple simultaneous wavelets with variable spatial orientation, focal activity, and single rotors with fibrillatory conduction, that still the framework for our understanding of AF, were established based on these experimental animal studies. However, the development of *in vivo* models that allow electrophysiological evaluation and a contribution to the comprehension of the autonomic conditions associated with the generation of AF remains a major challenge. In dogs, both micro-reentry, abnormal automaticity and triggered activity have been observed as possible mechanisms for the occurrence of atrial arrhythmias (Friedman et al, 1996; Hocini et al, 2002). Hayashi and co-workers (1998), used efferent cervical vagi stimulation to induce AF by bursts of atrial pacing in anesthetized open-chest dogs. Vagus nerve stimulation has been associated with atrial ERP shortening and facilitation of AF inducibility in dogs (Arora et al, 2008; Lemola et al, 2008). A different study showed that these vagal effects were significantly changed immediately after epicardial fat pad ablation (Oh et al, 2006). In the canine model with chronic rapid pacing, sympathetic stimulation can lead to rapid repetitive activations in the isolated canine PV and contribute to AF initiation (Chen YJ et al, 2000). Also, stimulation of both sympathetic and parasympathetic elements modulates electrophysiological properties and increase propensity to AF in dogs (Sharifov et al, 2004; Hou et al, 2007).

There is still lack of data regarding the use of different experimental models with preserved autonomic innervation to assess the fundamental aspects of the electrophysiological properties of the atria and PV underlying the vulnerability for developing AF. Despite the amount of recent information on ANS innervation to the genesis of AF episodes, and its potential role in management strategies to attain a successful treatment of AF, the effects of acute vagal and sympathetic activation in the arrhythmia initiation and maintenance remain not yet completely understood (Olshansky, 2005; Das MK et al, 2008).

The importance of the ANS in complexity of mechanisms underlying AF is supported by animal experiments and recent clinical studies showing that vagal denervation enhances the efficacy of circumferential PV ablation in preventing AF recurrence (Chan & Tan, 2007). Abnormal atrial and PV electrophysiology and higher autonomic reflex activity may play an important role in the pathophysiology of AF (Chen et al, 1999; Chen & Tan, 2007). In previous studies, sympathetic stimulation was much less effective than vagal stimulation in influencing atrial refractoriness and promoting AF (Liu & Nattel, 1997; Chen et al, 1999). Our present data, using the intact normal heart rabbit model, which exhibits very little propensity to AF under baseline conditions, confirm the importance of potential effects resulting from the interaction between vagal and sympathetic activity, in determining significant changes of conduction and refractoriness of the atria and PV, and its contribution to increase inducibility and duration of AF. Also, vagal activity seemed to directly influence the termination of AF, since in 2/3 of the animals with longer AF duration the arrhythmia was immediately interrupted after cessation of vagal stimulation. Little is known about how AF terminates spontaneously. One would predict that reversal of the autonomic changes that accompanied AF initiation would favor AF termination. Tomita et al (2003) demonstrated that the autonomic fluctuations preceding AF onset returned to normal values immediately following AF termination. Studies in animals have accumulated evidence for the impact of cardiac ganglionated plexi in terminating paroxysmal AF (Schauerte P et al, 2000; Scherlag et al, 2006). Recently, patients undergoing percutaneous AF ablation, including selective vagal denervation, showed a reduction in AF recurrences, (Pappone et al, 2004; Nakahawa et al, 2004; Scanavacca et al, 2006). In this context, the concept that cardiac denervation could take place in preventing AF represents an exciting topic that needs to be clarified.

We were able to reproduce electrical conditions that decrease the wavelength (by decreasing the conduction velocity and ERPs), allowing the substrate for multiple reentry circuits, facilitating the occurrence of AF. This emphasizes the role of both sympathetic and parasympathetic nervous systems in mediating AF, and reinforces the importance of atrial and PV electrophysiological properties as markers of vulnerability for AF. The influence of ANS in the pathogenesis of paroxysmal AF has been supported by clinical and animal studies. Despite conflicting results of heart rate variability analysis performed before spontaneous episodes of AF, paroxysmal AF seems related to fluctuations in autonomic tone, possibly with an interaction between adrenergic drive and modulation of vagal activity (Huang et al, 1998; Fioranelli et al, 1999; Lombardi et al, 2004; Chen et al, 2006). Also, it has been

suggested that sympathetic and parasympathetic discharges are synergistic in facilitating the occurrence of AF (Tan AY et al, 2008). Nevertheless, further studies are necessary to better understand the role of dual autonomic activity on the trigger and substrate of atrial arrhythmogenesis.

Limitations of the study

One of the limitations of the study is the assessment of refractoriness based in three sites of the atria and PV. In fact, the use of a single electrode to evaluate ERPs in baseline and during different autonomic conditions, following a multisite technique would have a relatively low spatial resolution, taking a considerable amount of time that would be impractical in order to maintain stable this *in vivo* preparation. Also, although programmed electrical stimulation with a single stimulus has been widely used for the study of refractoriness properties, we believe that further studies, using different methods may be needed to confirm these findings. However, in our study the documented electrophysiological changes and the impact on AF inducibility occurred immediately after autonomic stimulation, suggesting a clear relation between the evoked effects of autonomic modulation and the obtained results. Finally, the low tendency of the rabbit atrium to fibrillate and maintain the arrhythmia due to its small size might contribute to the inability to sustain AF in this *in vivo* normal heart. Although brief episodes of AF in the rabbit reflect arrhythmic vulnerability, the mechanisms underlying AF maintenance may be different in humans.

Conclusions

This fully innervated *in vivo* rabbit heart preparation provides a useful tool to investigate the effects of direct parasympathetic and sympathetic activation nerves on the electrophysiological properties of the atria and PV. Vagal activity exerts a reduction in interatrial conduction velocity and decreases ERP in the atria and PV, whereas sympathetic stimulation enhances interatrial velocity and conduction between atria and PV, but reduces refractoriness only in the LAA. This study displays also evidence that autonomic stimulation have a significant influence in the vulnerability for the inducibility of AF, contributing to the duration of AF episodes. Our data suggest that this innervated heart preparation is a valuable model to study the autonomic interactions with AF pathophysiology.

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Figures

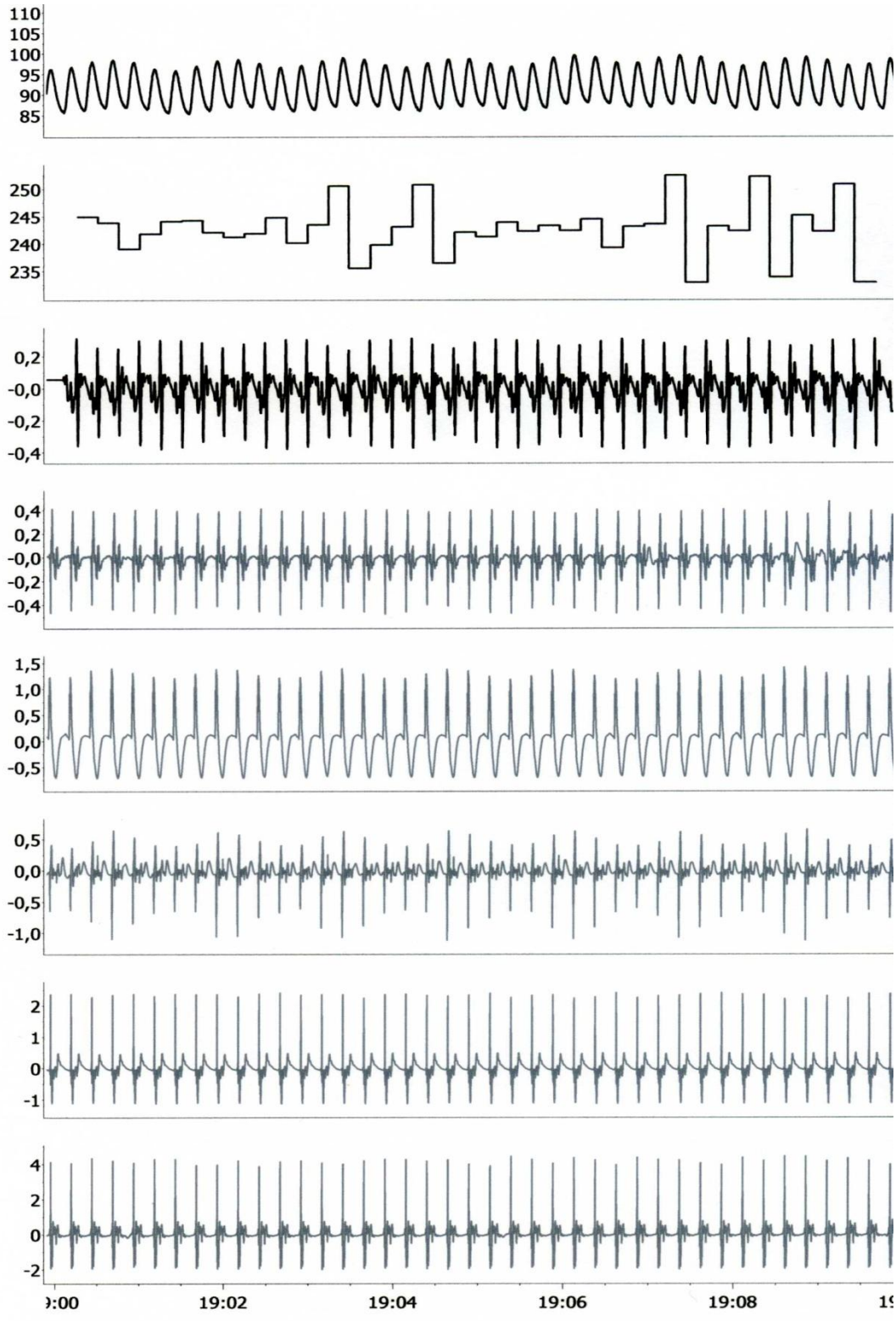

Figure 1 – Raw data on baseline condition.

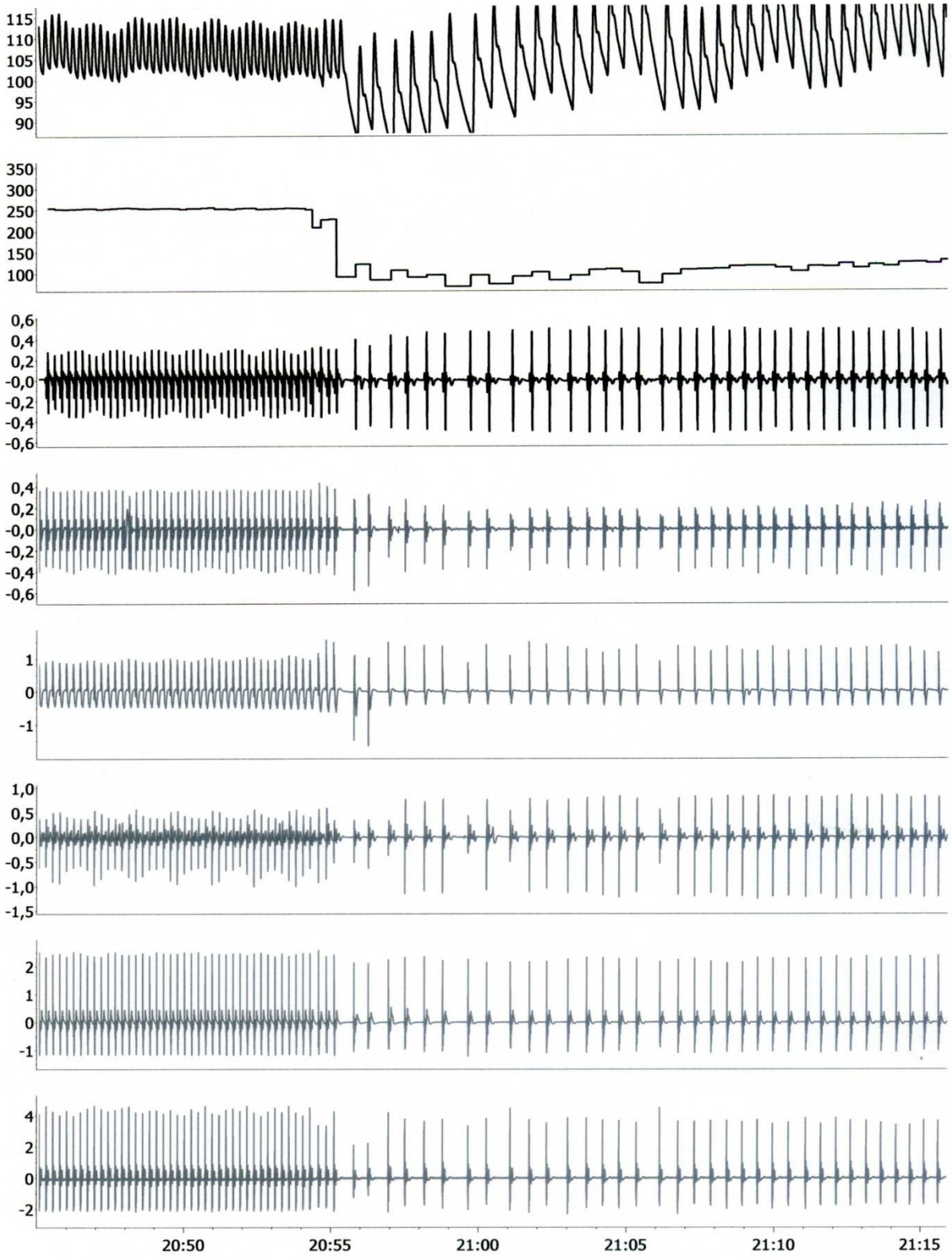

Figure 2 – Raw data showing vagal stimulation.

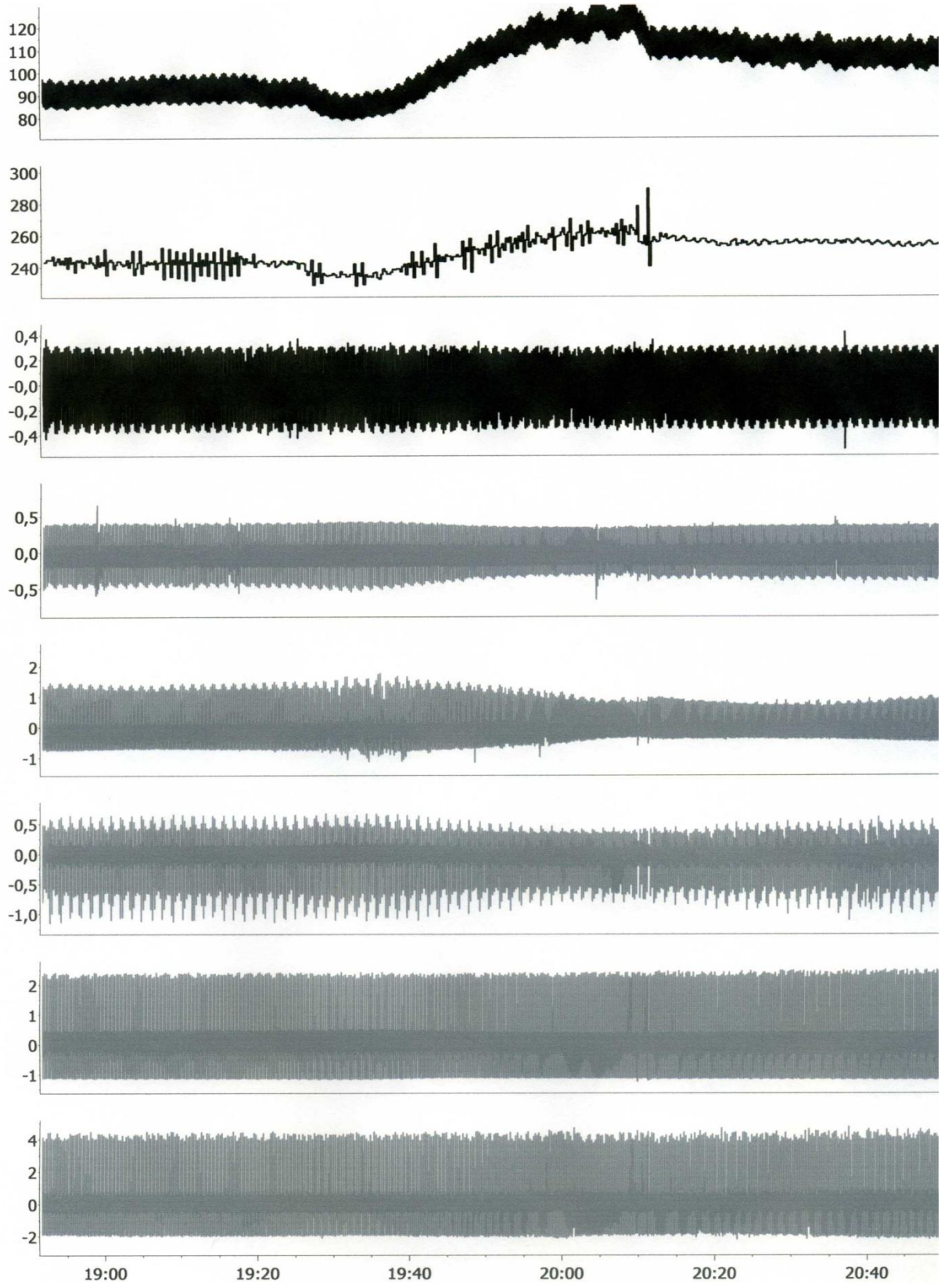

Figure 3 - Raw data showing the effects of sympathetic preganglionic stimulation.

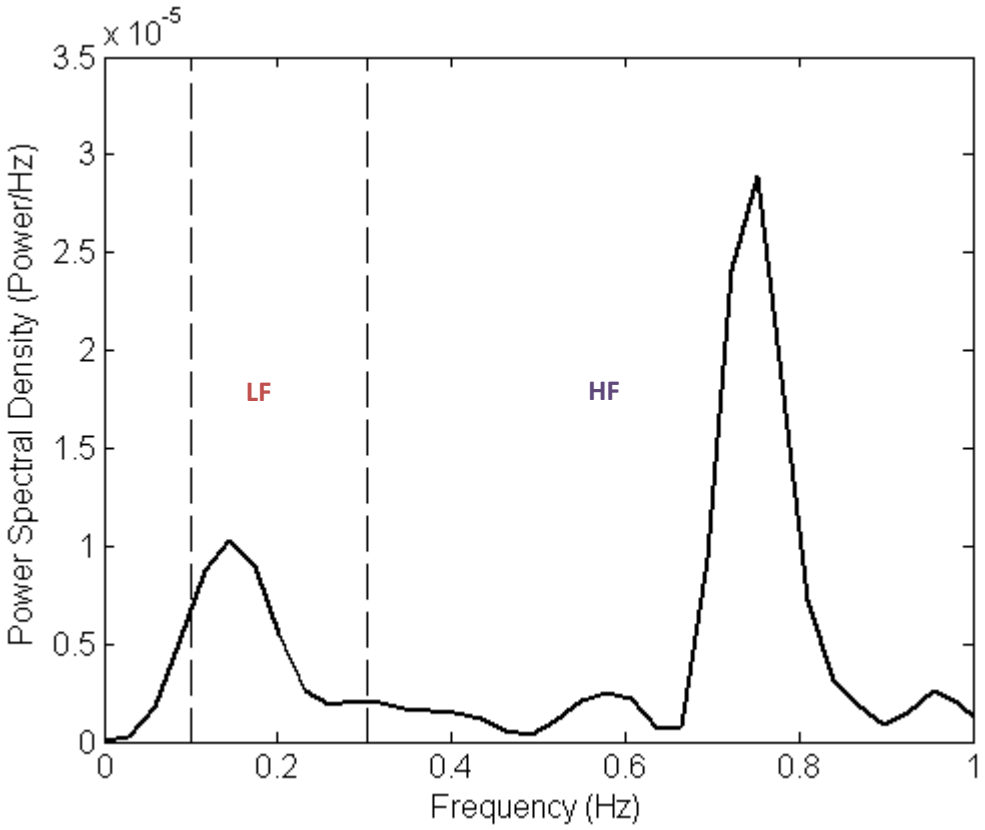

Figure 4 – Power Spectrum Density of R-R signal recorded in baseline conditions. FFT spectrum of 30s R-R signal from anesthetized paralyzed rabbit with open chest and artificially respiratory ventilation. The VLF component (0.0-0.1 Hz) is lower than in longer analyzed R-R signal but the some pattern of energy distribution related with LF and HF component in FFT spectrum is observed.

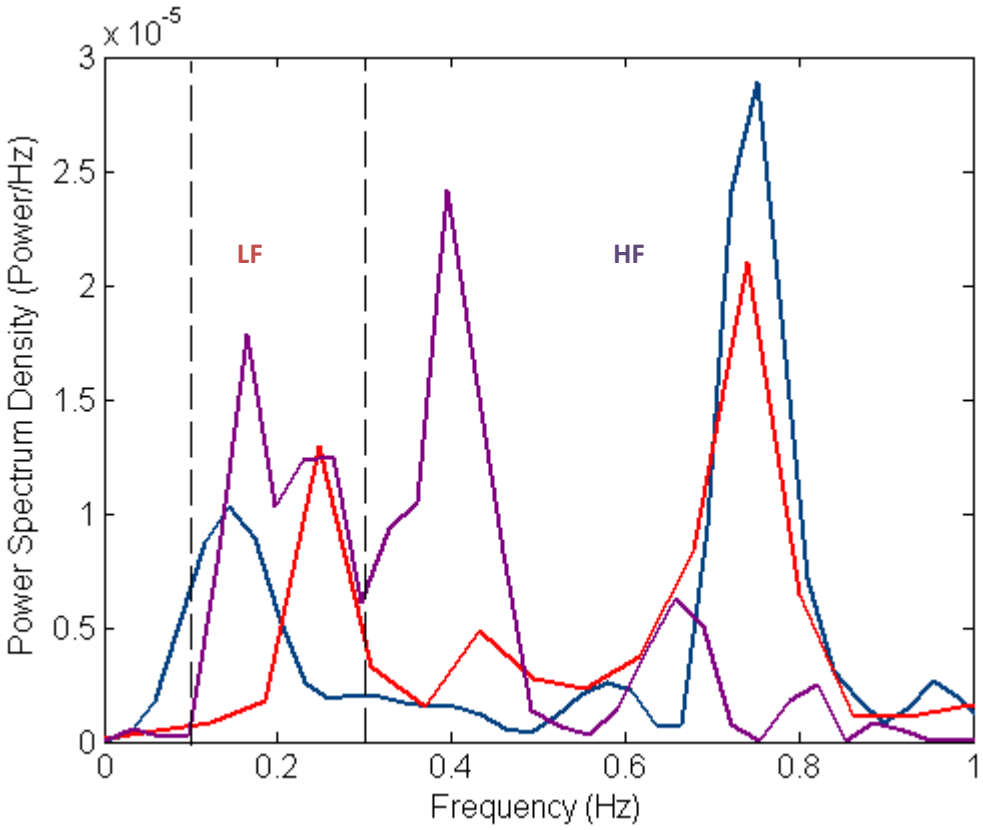

Figure 5 - Changes in FFT spectrum in rabbits with sympathetic stimulation and vagal stimulation. The dominant oscillation in HF band is moved toward LF oscillation. During vagal stimulation variability in the cardiac signal showed a higher power (the represented amplitude of PSD resulted from FFT analysis of 30 seconds R-R signal are 1000 times higher). Baseline signal (red line); R-R signal response with sympathetic stimulation (violet line); R-R signal response with vagal stimulation (blue line).

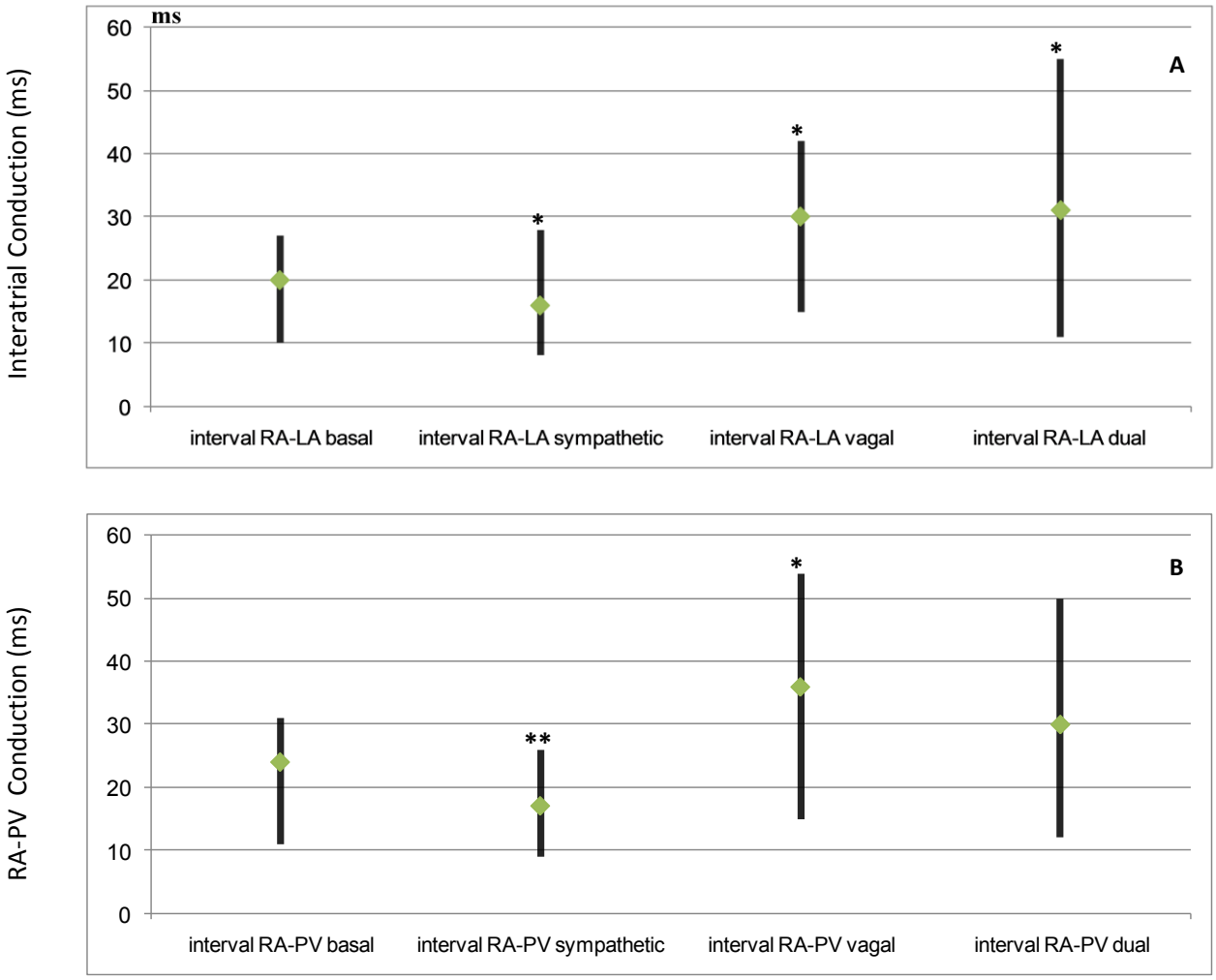

Figure 6 - Conduction time (CT) in baseline conditions, during cervical vagal stimulation, sympathetic thoracic stimulation, and simultaneous dual autonomic stimulation. A. CT between the high-lateral right atrium (RA) and the high-lateral left atrium (LA), and B. CT between the RA and left pulmonary veins (PV) near the area of insertion in the LA roof. * *p<0.05; ** p<0.01*

Table 1 - Effective refractory periods measured at baseline conditions and during autonomic stimulation

	baseline (n=17)	vagal (n=17)	%_Δ	sympathetic (n=17)	%_Δ	vagal + sympathetic (n=8)	%_Δ
RAA (ms)	69±16	48±17**	30.4	64±15	7.3	51±20*	26.1
LAA (ms)	83±16§	53±21***	36.6	56±23**	31.7	51±23***	37.8
PV (ms)	59±16	41±17**$^{\Psi}$	30.5	49±19	16.9	40±18**	32.2

RAA=right atrial appendage; LAA=left atrial appendage; PV=pulmonar veins; %_Δ=percentage of variation of the average effective refractory periods). *=p<0.05 vs. baseline; **=p<0.01; ***=p<0.001; §=p<0.01, when compared to RAA and PV in baseline; $^{\Psi}$=p<0.05 vs. baseline and vs. LAA during vagal stimulation.

Table 2 - Atrial fibrillation inducibility and duration with 50Hz pacing at the right atrial appendage (RAA), left atrial appendage (LAA) and pulmonary veins (PV), alone (baseline) or combined with autonomic stimulation

	baseline			sympathetic			vagal			vagal + sympathetic		
	RAA	LAA	PV	RAA	LAA	PV	RAA	LAA	PV	RAA	LAA	PV
AF inducibility	41%	35%	53%	71%	65%	76% §	76% *	65%	76% §	87,5% *	100% *§	75%
AF duration average (s)	1.8±1.2	2.6±1.4	1.7±0.7	1.8±0.8	2.4±1.6	9.6±3.7 *	5.8±2.5 *	3.3±1.6	3.6±1.6 *	3.5±1.4 *	6.0±1.4 *	3.7±1.1 *

** p<0.05, compared with baseline 50Hz pacing (t test); §=p<0.05, comparing the inducibility rates between RAA, LAA and PV pacing (Anova test)*

Time course of ionic and gap junctional remodeling induced by short-term rapid pacing in rat atria

Mário Oliveira[1,2], Gabriela Postolache[2], Vera Geraldes[2], João Paulo Gomes[3], Alexandra Nunes[3], Vitor Silva[2], Sérgio Laranjo[2], Nogueira da Silva[1], Rui Ferreira[1], Isabel Rocha[2]

[1] Cardiology Department, Santa Marta Hospital, Lisbon, Portugal

[2] Autonomic Nervous System Unit, Physiology Institute, Institute of Molecular Medicine, Faculty of Medicine, Lisbon, Portugal

[3] Instituto Ricardo Jorge, Lisbon, Portugal

Keywords: *ion channels; connexins; atrial remodeling; experimental model*

Running title: *atrial remodeling induced by short-term rapid pacing*

Abstract

Gap junctions and ion channels have been implicated in the remodeling phenomena due to atrial fibrillation (AF). The time course of the mechanisms underlying the remodeling process remains unclear. We addressed the time-dependence relation between short-term rapid atrial pacing and mRNA levels of ion channels and connexins (Cx). **Methods:** Gene expression levels for K^+, Na^+, Ca^{2+} and Cl^- channels, Cx40 and Cx43 were investigated after 30 minutes (P30m), 2 hours (P2h) and 4 hours (P4h) of 50Hz atrial pacing in Wistar rats (n=45). The control group underwent all steps of the experiment except pacing stimulation (n=45) or only the surgical procedures (n=5, baseline). Right (RA) and left (LA) atrial mRNA amounts of KCND2, KCND3, KCNA5, KCNJ3, KCNJ6, SCN5A, CACNA1, CFTR, Cx40 and Cx43 genes were quantified with a two-step Real Time PCR assay. LA and RA were collected for each group. **Results:** a decrease was observed in the Cx40 and Cx43 expression from both atria after P30m. Compared with controls, there was an increase of the Cx43/([Cx43+Cx40]) ratio in LA, and a decrease in RA after P30m and P4h. After P30m: SCN5A, KCND2 and CACNA1 expression decreased in both atria; KCNJ3 decreased only in LA; and KCND3 decreased in RA. With P2h: SCNA5 and CACNA1 levels decreased in LA; and KCND3 decreased in RA. P4h induced a reduction of SCNA5 and CFTR expression in both atria, a decrease of KCNJ3, KCNJ6, KCND2, KCND3, KCNA5 in RA, a decrease of CACNA1 only in LA, and an increase of KCND2 and KCNA5 in LA. LA KCNJ6 and LA KCND3 expression did not change significantly. **Conclusion:** Extensive changes in the gene expression of Cx and ion channel occur after high-rate pacing in rat atria, with different patterns in RA and LA. These may be at least partially responsible for AF-induced remodeling.

Introduction

Atrial fibrillation (AF) is the most common cardiac arrhythmia seen in clinical practice, often responsible for substantial morbidity and an increased risk of mortality (1,2). Paroxysmal AF occurs in intermittent episodes that terminate spontaneously in less than seven days, usually lasting less than 24 hours (3). Although the mechanisms underlying AF recurrence remain incompletely elucidated, clinical and experimental studies have provided new insights into the comprehension of this arrhythmia. The functional substrate that promotes progression to persistent AF has been related to electrophysiological changes in the atria, with AF facilitating the production of abnormal impulse generation, shortening of refractoriness, enhanced dispersion of the atrial effective refractory periods, and regions of slowed conduction, that are currently associated with the complex remodeling process (4-6). These electrical mechanisms can be explained by the occurrence of heterogeneous changes of connexin and ion channel expression (5-9). It appears that the molecular alterations within the atrial tissue in AF are directed primarily at protecting the myocardial cells from stressors. However, such phenomena modify electrophysiological changes properties in a way that promotes the maintenance of AF (6).

Long-lasting rapid pacing has been used mostly in instrumented animal models to produce a sustained atrial tachyarrhythmia and induce electrophysiological remodeling (10-13). Clarification of the early changes and time course of the molecular mechanisms underlying ionic and gap-junctional remodeling is presumed to be an important issue regarding our understanding of AF pathophysiology. This study examined the effects of high-rate atrial pacing on the gene expression levels of major ion channels and connexins in the rat atria.

Methods

Experimental Procedures

a) animal model preparation

A total of 95 Wistar rats (11 to 14 weeks-old) were used in the present study. Of these, 45 rats were monitored after experimental instrumentation, kept in sinus rhythm and used as controls (30 minutes, 2 and 4 hours) and 5 rats underwent only the surgical procedures

(baseline). The other animals underwent the same experimental conditions and 30 minutes (P30m), 2 (P2h) and 4 (P4h) hours of high-rate atrial pacing (fifteen animals per period). Rats were anaesthetized with sodium pentobarbitone (60 mg/Kg), and additional doses applied as needed. The right femoral artery and vein were cannulated for arterial blood pressure monitoring (Sensonor transducer, Lectromed apparatus, UK) and fluid injections, respectively. The trachea was cannulated below the larynx, via a tracheostomy, to perform mechanical ventilation. The surface ECG was obtained (Neurolog, Digitimer Ltd, UK) with the use of three needle bipolar electrodes inserted into the limbs. Heart rate was derived from the ECG (Lectromed, UK). After induction of neuromuscular blockade with vecuronium bromide (Norcuron, 0.05 mg/kg/h), in order to assure strict control of respiratory parameters, such as rate and volume, the animals were ventilated with O2-enriched air, using a positive-pressure ventilator (Harvard Apparatus, UK). Ventilation was regulated to maintain end-tidal CO_2 between 4.5 and 5 % (ADC gas analyser, UK). Body temperature was maintained at 36.5-38ºC throughout the procedure using a servo-controlled heating blanked (Harvard Apparatus, UK). Briefly, the anaesthetized and paralyzed animal was placed in a dorsal position. A midline sternectomy was performed to expose the heart. After thoracotomy, an end-expiratory pressure of 1-2 cmH_2O was maintained.

All the experimental procedures were approved by the Institutional Animal Care and Ethics Committee of the Faculty of Medicine of University of Lisbon and in accordance with the European laws on animal welfare and with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

b) ***atrial stimulation protocol***

Bipolar electrical stimulation was performed at the right atrial appendage with a teflon-coated silver wire electrode (0.1 mm diameter), positioned with a micromanipulator (WPI, M330). A multi 8-channel, programmable stimulator (Master8, AMPI, Israel) was used for continuous pacing. Stimulation was performed with 1-millsecond rectangular pulses, using a constant current at twice the diastolic threshold. The stimulation frequency was set at

50Hz. After predetermined durations of stimulation (P30m, P2h and P4h), the right (RA) and left (LA) atria were carefully excised and considered for evaluation. The control group was instrumented identically (baseline), and monitored (30 minutes, 2 and 4 hours), but without pacing. At the end of experiment the animals were killed with an overdose of anaesthetic and separate samples of the RA and LA were collected, harvested and saved in 500µl *RNA later* (4ºC overnight, and then -20ºC) for RNA quantification.

For each experimental condition, we investigated gene expression quantification for a set of ten genes: SCN5A (encoding an I_{Na} cardiac sodium channel, responsible for the fast depolarization upstroke of the action potential); KCND2 and KCND3 (encoding the alpha-subunits of the voltage-gated potassium channels Kv4.2 and Kv4.3, conducting the fast transient outward current of the cardiac action potential); KCNA5 (encoding Kv1.5, a voltage-gated potassium channel, which influences the I_{Kur} repolarization current); KCNJ3 and KCNJ6 genes (encoding inwardly rectifier K^+ channels, which includes the muscarinic potassium channel I_{KACh}); CACNA1 (encoding L-type Ca^{2+} channel); and CFTR (encoding a chloride ion channel). We also studied the gene expression levels for GJA1 and GJA5 (connexin 43 and connexin 40, respectively), gap-junction proteins expressed in the atrial myocardium and responsible for the rapid cell-to-cell transmission of the action potential (8,14). The ratio of Cx43 to total Cx (Cx43/[Cx40+Cx43]) was considered as a marker directly related to propagation velocity (14).

RNA extraction and generation of pools

Total RNA from each atrium, previously stabilized with RNA later (Qiagen, Valencia, CA, USA), was extracted by using the RNeasy Fibrous Tissue Mini Kit (Qiagen, Valencia, CA, USA) according to the package insert. Briefly, 350 µl of RLT buffer (containing 2-Mercaptoethanol) and ethanol were added to the sample, which was then applied to an RNeasy mini column. In order to remove residual DNA, an on-column DNase digestion using 30 Kunitz U of RNase-free DNase (Qiagen, Valencia, CA, USA) was performed. This step was performed twice to ensure complete DNA removal. RNA was eluted by adding 50 µl of RNase-free water and quantified by O.D. measured at A_{260}. RNA purity was estimated

from the ratio of absorbance readings at A_{260} and A_{280}. The RNA extraction method yielded up to 30 μg from each sample. All RNA samples were stored at -80ºC in RNase-free tubes.

Reverse Transcription (RT) and Quantitative Real-Time PCR

cDNA was generated from 300 ng of each RNA pool samples using TaqMan RT Reagents (Applied Biosystems, Foster City, CA) and random hexamers, as previously described (15). Each RT reaction resulted in ~30 μg of cDNA. We used the two-step real-time polymerase chain reaction (RT-PCR) assay to avoid differences in RT reaction efficiency for each gene in a sample because 3′ specific primers (used for one-step RT-PCR) may have unequal hybridization efficiency. Using random hexamers, even if some differences in the overall RT efficiency between samples occur, the relative amount of cDNA among the different genes remains the same for the respective sample. This is important for gene comparability and for the reproducibility of the final RT-PCR. In order to select the endogenous control for the real-time assays, the uniformity of the expression levels of housekeeping genes was assayed. Briefly, cDNA samples representing all predetermined pacing conditions were diluted to a concentration of 10 ng/μl and subjected to RT-PCR analysis as described below. For the β-actin gene, the obtained threshold cycle (cT) values varied only slightly (less than one cT) between samples, validating the use of this gene as an endogenous control (data not shown). Quantification of expression for the 10 genes under study was achieved using the ABI 7000 SDS (Applied Biosystems), the SYBR Green chemistry, and the standard curve method for relative quantification, using reagents and thermocycling as previously described (15).

Primers for each of the 10 genes (sequence available under request) were designed using Primer Express Software (Applied Biosystems). We used DNA standard curves (as chromosomal DNA represents equal amounts of each gene), which is essential in order to simultaneously compare the expression of all genes at each experimental condition. These DNA standard curves were made using eight serial dilutions of DNA (starting at 20 ng of total DNA). Each plate included all pooled groups representing the predetermined experimental conditions, and three standard curves (two target genes and one control

gene). Besides standard curves, each RT-PCR plate contained two replicates of each sample cDNA, and negative controls. The latter included no template controls and RNA only controls to ensure the absence of contaminating genomic DNA. For all experiments, the amount of target and control gene was determined from the respective standard curve by conversion of the mean cT values. Normalization was obtained by dividing the quantity of the target gene by the quantity of the control gene (β-*actin*). The specificity of the amplified products was verified by analysis of the dissociation curves generated by the ABI 7000 software based on the specific melting temperature for each amplicon.

Statistical Analysis

Data are presented as mean±standard deviation for continuous variables. Relative quantitation of gene expression levels was made using β-actin as endogenous control to normalize the quantity of cDNA target. Data are presented as mean ± standard deviation for continuous variables. The possible relation between gene expression and atrial high-rate pacing was analyzed using bivariate correlations (Pearson). One way ANOVA was first completed and then, if appropriate, comparison between variations in gene expression during atrial rapid pacing group was done using Student's t-test. Values of $p < 0.05$ were considered statistically significant.

Results

In stable baseline conditions, after all the surgical procedures and under neuromuscular blockade, the mean HR was 316±12 bpm and mean systolic BP was 112±5 mmHg. With high-frequency atrial pacing, HR was 386±11 bpm, 400±12 bpm and 407±11 bpm after P30m, P2h and P4h, respectively. Mean BP values were 111±6 mmHg, 112±11 mmHg and 111±9 mmHg after P30m, P2h and P4h, respectively.

Effects of high-rate pacing on atrial connexin gene expression

Connexin (Cx)43 and Cx40 mRNA expression data from RA and LA during high-rate pacing are presented in Figure 1A and 1B, respectively. The mRNA displayed a significant reduced expression of Cx43 and Cx40 in both atria after P30m, with no significant changes

at P2h and P4h for Cx43 and an increase of LA mRNA Cx40 at P4h. Compared to controls, Cx43/([Cx43+Cx40]) ratio was higher in LA with P30m and P4h, and was lower in RA with P30m and P4h (table I).

Effects of high-rate pacing on atrial ion channels gene expression

Inward rectifying K+ (Kir) channels. Analysis of the expression of KCNJ3 and KCNJ6 genes showed that KCNJ3 changed significantly compared to baseline, decreasing in RA after 2 and 4 hours of rapid pacing (t test, $p < 0.0001$), and in LA after 30 minutes (figure 2A). In contrast, mRNA expression of KCNJ6 change significantly only in RA after P4h (figure 2B).

Voltage-gated potassium (Kv) channels. KCNA5, KCND2 and KCND3 expression levels changed significantly with this short-term high-rate pacing protocol. KCNA5 expression levels changed in both atria after P4h, with a significant reduction in RA and an increase in LA, compared to the control rats (figure 3). KCND2 expression showed decreased levels after P30m in both atria, with no significant changes in P2h rats. A different pattern was observed after 4 hours of pacing, with a down-regulation in RA and an up-regulation in LA (figure 3). KCND3 expression levels changed significantly in all pacing conditions, but only in RA, with greatest alterations produced by P2h (t test, $p < 0.001$) (figure 4).

L-type Ca^{2+} ion channel. A significant reduction of the CACNA1 expression was seen in both atria after P30m ($p < 0.001$ vs. control). However, after P2h and P4h the mRNA expression levels decreased significantly only in LA (figure 5A).

Voltage-gated sodium and chloride ion channels. There were significant changes in the expression levels of SCN5A and CFTR from both atria. Rapid atrial pacing induced a SCN5A decrease in both atria after P30m and P4h, but only in LA with P2h (figure 5B). Compared to the control group, molecular expression of CFTR changed significantly only after 4 hours of high-rate pacing, showing increased levels in RA and decreased levels in LA (t-test, $p<0.05$ for RA and $p<0.001$ for LA) (figure 6).

Discussion

Long-term rapid atrial pacing has been shown to be a useful model in the study of AF (11,16), but little is know regarding the initial changes and the time course of the early molecular mechanisms behind atrial tachycardia-induced ionic and gap-junctional remodeling. Our results show that the present experimental model allows the study of the effects of short-term high-rate atrial pacing on gene expression levels of major atrial connexins and ion channels. The extensive significant changes in these gene expression profiles, obtained in a large cohort of rats after direct high-rate atrial pacing, emphasizes the potential role of basic mechanisms on the acute modification of electrophysiological properties related to the remodeling process of the atria.

Main findings

In this study, we have demonstrated that significant changes in the mRNA levels of genes encoding atrial ion channels, including Na^+, L-type Ca^{2+}, inward rectifier, transient outward and voltage-gated K^+ currents, and Cx43, underlie the early phases of the atrial tachycardia-induced remodeling process. Compared to control-operated animals, mRNA expression levels of SCNA5, KCND2, KCND3, KCNJ3 and CACNA1 channels reduced significantly after P30m, and preceded those of KCNJ6, KCNA5 and CFTR, while the Cx43 and Cx40 expression levels showed an early significant decrease in both atria, followed by an increase of LA Cx40 expression levels after P4h, and no detectable changes for Cx43.
Atrial high-rate pacing induced heterogeneous alterations of ion channel and connexin gene expression, regarding the time course and intensity of the changes, but also in relation with differential alterations in RA and/or LA involvement, and direction of the responses of the mRNA expression levels in two of the analyzed ion channels.

Connexin gene expression levels in atria. Atrial myocytes are electrically coupled through gap junctions that are made up of proteins from the connexin family. Cx40 and Cx43, the most abundant myocyte connexins, are critical elements underlying the gap junction function, contributing to propagation of atrial activation (8,14). Although Cx40 and Cx43 are both expressed in atrial myocytes of the mouse heart, Cx43 was suggested to be the main

connexin in the rat atria (17,18). Cx43 is distributed homogeneously throughout the RA and LA, and reaches levels similar to Cx40 in the RA, making similar contributions to gap-junctional conductance (19,20). It has been accepted that altered distribution of Cx40 and Cx43 in the atria may contribute to abnormal atrial conduction and, thereby, to substrate changes required for the initiation and maintenance of AF (8,14,21,22). Both initiating triggers and changes in electrophysiologic properties contribute to the vulnerability for the recurrence of AF episodes. AF itself promotes an arrhythmogenic substrate, by shortening atrial refractoriness, enhance spatial dispersion of refractory periods and induce abnormal impulse conduction (3-5). Alterations in connexin and ion channel expression have been associated with atrial remodeling phenomena, and probably represent the initial functional substrate related with the acute electrical changes induced by tachycardia (5,22,23). A key advance in understanding the mechanisms of AF has been the identification of ion channels and gap junctions as molecular determinants of abnormal atrial electrical activity, and how these are modified in AF.

In the present study, samples of both atria showed significant changes in Cx43 and Cx40 gene expression levels, with an early reduction after 30 minutes of continuous rapid atrial pacing. However, after 4 hours of pacing the mRNA expression for Cx40 increased significantly in LA. In previous studies, quantification of connexins has mostly been confined to small pieces removed from both atria or to RA samples (22-25). Although it has often been assumed that these fragments are representative of the rest of the atria, it is likely that changes in quantity and relationships of the different connexins and the response to rapid-pacing are regionally dependent. In our study, mRNA quantification analysis was performed in samples containing the entire RA and LA. We were able to demonstrate that atrial high-rate pacing induced changes in both atria, with a different pattern for the LA Cx40 expression. Although in previous studies Cx40 was considered the dominant connexin for atrial impulse conduction, the propagation velocity in the atria has been associated with the interaction between Cx40 and Cx43 expression, with the ratio of Cx43 to total Cx (Cx43/[Cx40+CX43]) being directly related to the velocity of conduction (14,26). Kanagaratnam et al, found a stronger correlation between atrial conductivity and the

relative immunodetectable signal of the two connexins than that with Cx40 alone, suggesting that the relative expression may be an important factor (26). Also, Cx43 has also been reported to regulate Cx40, a relationship that may play a role in atrial conduction if one of these connexins changes during remodeling (27). In our study, when compared with controls, the group of rats with P30m and P4h had a higher value of the Cx43/([Cx43+Cx40]) ratio for the LA, and a lower value for the RA, suggesting that remodeling induced by atrial high-frequency pacing may influence differently the conduction velocity in each atria.

It has been suggested that in cell lines in which Cx40 and Cx43 are naturally coexpressed the Cx43:Cx40 mRNA ratio predicts the Cx43:Cx40 protein ratio (28). Therefore, the alterations induced by short-term high-rate pacing, may represent another mechanism of gap-junctional remodeling, either through a reduction of Cx43 and Cx40 proteins quantity, distribution or function, influencing heterogeneously the gap-junctional communication within the atria and, thus, leading to atrial conduction slowing and reentry (22,25,29). The initial mechanisms involving the complex features of atrial remodeling in AF makes understanding the functional significance of gene expression of these proteins a difficult challenge.

Ion channels gene expression levels in atria. The effects of few hours of AF on the ion channel gene expression levels and atrial electrophysiological characteristics have not been well clarified. However, there is evidence that alterations in the gene expression of ion channels, induced by rapid pacing, can provide gradual changes of atrial refractoriness (25). Molecular mechanisms of electrical remodeling have been implicated in the promotion of AF (5,6,26,30). At the functional level, alterations in the distribution and density of ion channels influencing the atrial ERP depend on modified gene expression (31,32).

We addressed the hypothesis that the gene expression levels of major ion channels in the atria can be affected by short-term atrial rapid pacing. Our results show that the mRNA levels of genes encoding a number of ion channels playing a central role in the regulation of action potential duration and atrial refractoriness, including the KCNJ3 and KCNJ6, the

KCND2 and KCND3, KCNA5, SCN5A, CACNA1 and CFTR, may be extensively influenced after atrial high-rate pacing of short duration (range 0.5-4 hours). The observed reduction in gene expression of these channels is probably one of the initial mechanisms contributing to the remodeling process.

Reductions in atrial refractoriness and conduction velocity will facilitate the occurrence of AF, but the underlying ionic mechanisms are not well known. Ionic changes that have been produced with atrial tachycardia at a rate in the range of that of AF include decreased transient outward current (I_{to}), L-type Ca^{2+} current (I_{Ca}) and Na^{+} current (I_{Na}), and increased inward rectifier K^{+} currents (5,33). Nevertheless, on a celular basis, changes in K^{+} currents are inconsistent, and the molecular mechanisms that cause these changes in ion channel function are less known (33-35). In previous studies, the mRNA levels of the ultra-rapid delayed rectifier ($K_{V1.5}$) and inward rectifier ($K_{v3.1}$) were significantly decreased in patients with AF (31,32). However, there are also reports of no changes in the gene expression encoding the protein of the subunit $K_{v1.5}$ or in the density of this current (36). Changes in the expression of these channels would be expected to alter resting membrane potential and action potential repolarization; however, the consequences of these changes are not fully understood.

In our study, differential alterations in the time course, direction and atrial involvement occurred among the gene expression of ultra-rapid delayed rectifier, transient outward current channels, and inward rectifying K^{+} channels, suggesting that a complex pattern of behavior in the molecular mechanisms response to atria tachycardia may explain the heterogeneous reduction in atrial refractoriness related with electrical remodeling. Yamashita et al, also found differential changes in the time course, duration, and direction of mRNA levels of these K^{+} channels induced by short-term rapid atrial pacing in rat atrial appendages (25).

At the functional level, L-type Ca^{2+}-current down-regulation and decreased L-type Ca^{2+}-current density are believed to be major determinants of the shortening of ERP (37). High atrial rate causes calcium overload which may ultimately lead to irreversible cell damage.

To prevent dangerous calcium overload, atrial myocytes decrease L-type Ca^{2+} current by downregulation of L-type Ca^{2+} channel and shortens atrial refractoriness (33).

Chloride channels are known to be distributed nonuniformly, depending on region of the heart (38). Cl^- currents may influence membrane potential and impulse formation, and may play a role in cardiac arrhythmogenesis (39-41). However, there is lack of data concerning the genetic expression of CFTR in the context of ionic remodeling of the atria. The impact of the observed CFTR reduction in the electrophysiological substrate of AF is not clear. In the present study, CFTR expression changed in opposite direction in RA and LA after 4 hours of high-rate pacing.

Since I_{Na} is a major determinant of conduction velocity, our data point to a significant reduction of the mRNA expression levels for SCNA5 as a potentially important mechanism contributing to the arrhythmogenic substrate in tachycardia-induced remodeling.

Atrial tachycardia–induced remodeling, accepted as an AF-promoting condition, can produce a wide range of significant heterogeneous alterations even in short periods of time in a number of atrial ion channel and Cx gene expression that may have a potential role in the creation of a functional electrophysiological substrate suitable for AF. It is a significant challenge to determine the clinical impact if these early specific changes in the promotion of AF maintenance. Such knowledge would be useful to develop effective therapeutic approaches.

Study limitations

Despite being accepted as an AF-promoting condition, animal models of tachycardia-induced remodeling do not clearly replicate pathophysiological events in humans. Therefore, the results obtained with these experimental conditions cannot be directly extrapolated to the molecular mechanisms related with AF recurrence in humans. Although rapid pacing was delivered in the right atrial appendage the gene expression analysis by mRNA quantification was investigate in both atria, making difficult to exclude a potential influence of the local of stimulation in the distribution of the alterations in gene expression levels. Another concern is that the time periods analyzed over this study are insufficient to

clarify the remodeling of global ionic and gap-junctional gene expression, but they add information concerning the early stages of this process. Last, despite the changes obtained in the mRNA levels of a selected number of genes, offering insights into the dynamic transcriptional regulation that accompanies atrial remodeling, it is not possible to establish a clear relationship with the functional ion current densities. There are numerous possible mechanisms influencing the molecular regulation of the genetic protein expression process, making difficult to draw conclusions regarding the modulation of distribution, density and functional properties of ion channels and connexins in atrial remodeling.

Conclusions

The present study has provided evidence that short-term high-rate atrial pacing induces a wide range of alterations in the expression levels of genes encoding Cx43 and Cx40, and major ion channels in atrial tissue. High-rate pacing produced differential alterations in the time course, intensity, direction and atrial involvement among the ion channels and connexin gene expression. These extensive and heterogeneous alterations may be responsible for AF-induced electrical remodeling. This heart preparation may be a valuable model for our understanding of the pathways involved in the interaction between the molecular remodeling and acute electrophysiological changes in AF.

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Table I – Mean values for the ratio of connexin 43 to total mRNA levels (Cx43/[Cx43+Cx40])

	Ctr	P_30m	Ctr	P_2h	Ctr	P_4h
RA	0.98960±0.0002	**0.98872±0.0001**	0.99720±0.0001	0.99411±0.0001	0.99352±0.0001	**0.98877±0.0001**
		p<0.05		ns		**p<0.05**
LA	0.98939±0.0001	**0.99287±0.0001**	0.99523±0.0002	0.99434±0.0001	0.98318±0.0001	**0.98414±0.00001**
		p<0.05		ns		p<0.05

Ctr =control rats; P_30m, P_2h, P_4h (30 minutes, 2 hours and 4 hours atrial high-frequency pacing); RA=right atrium; LA=left atrium; ns=non significant

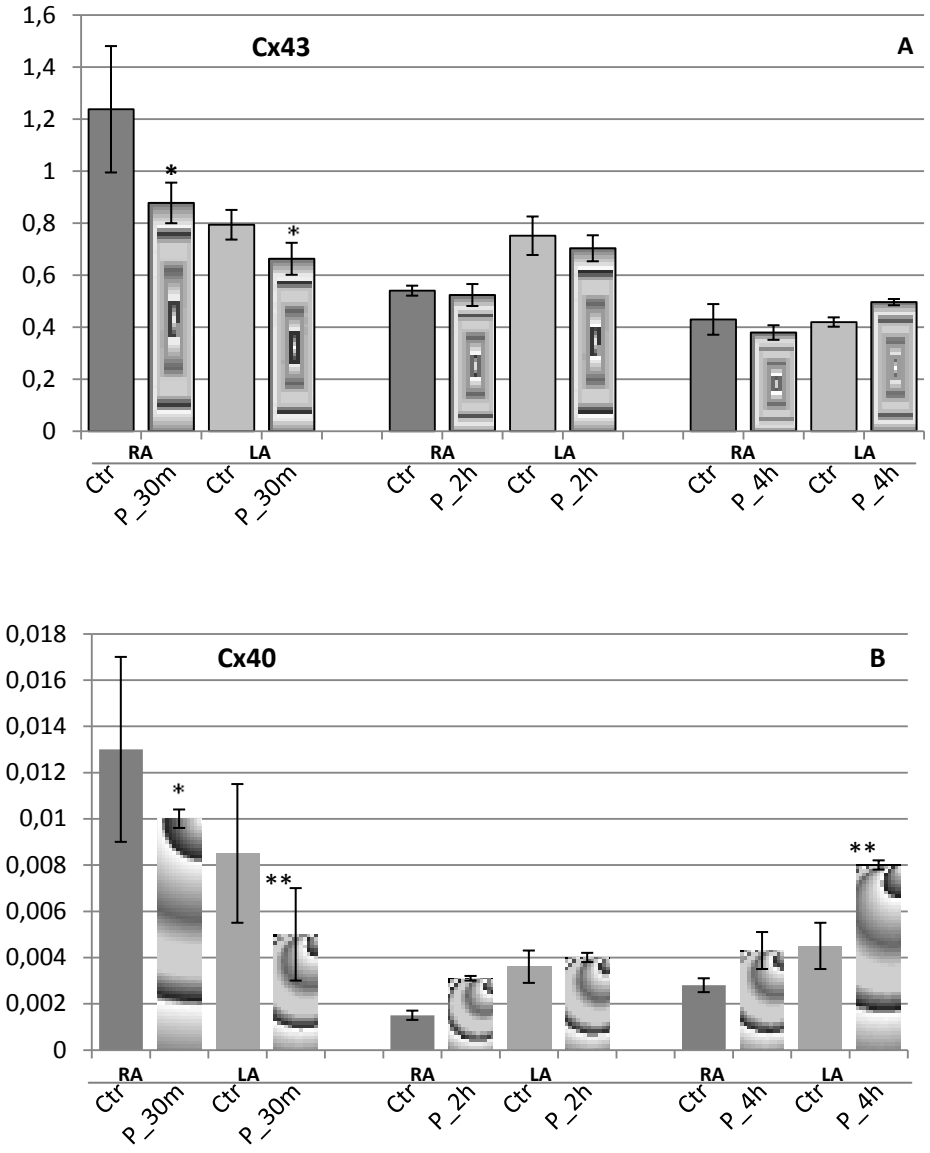

Figure 1. Changes in mRNA for connexins 43 and 40 induced by atrial high-frequency pacing. Gray bars are fold differences in right (RA) and left (LA) atrial mRNA for Cx43 (figure 1A) and Cx40 (figure 1B) in control rats (Ctr); striped bars are fold differences of mRNA for Cx43 (figure 1A) and Cx40 (figure 1B) after 30 minute, 2h and 4h pacing (P_30m, P_2h, P_4h). ** p<0.05; **p<0.01*

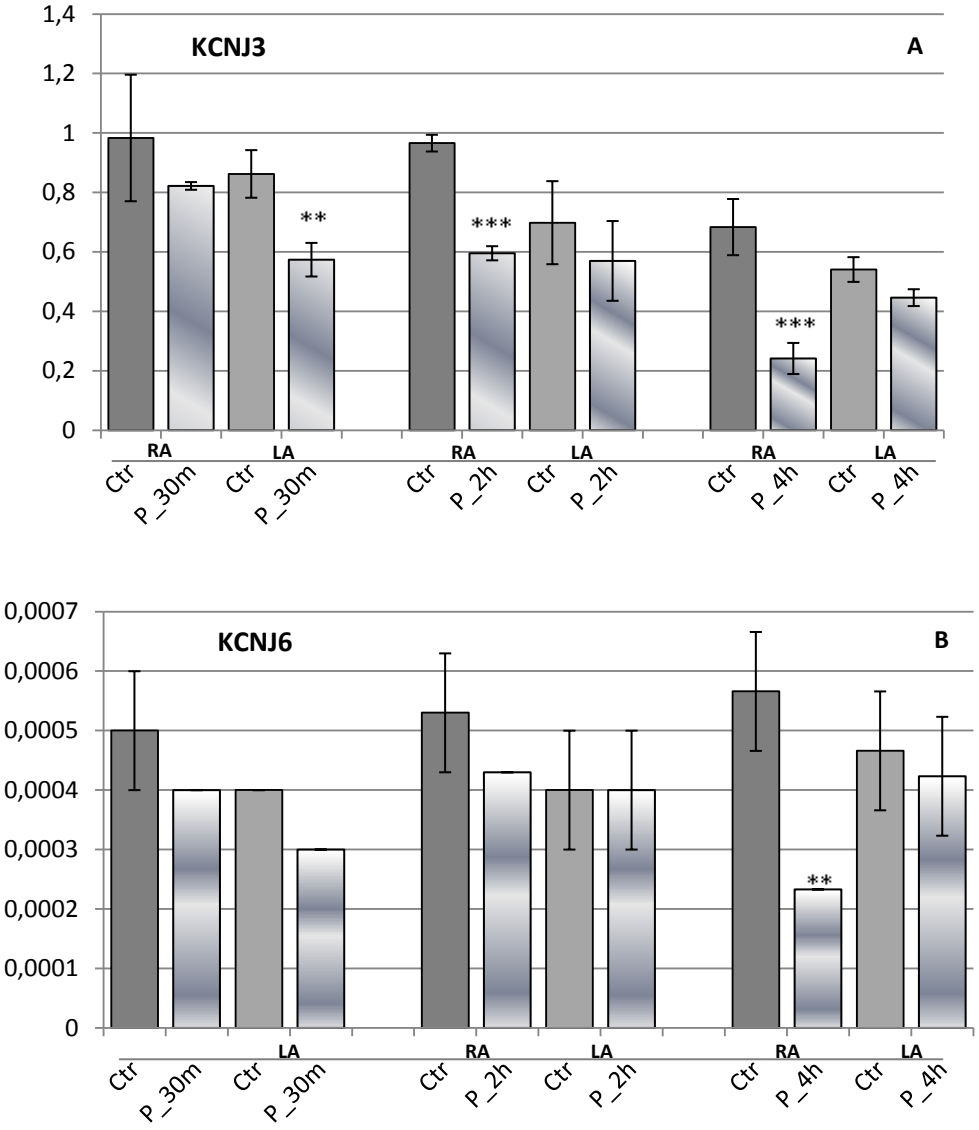

Figure 2. Changes in mRNA for KCNJ3 (A) and KCNJ6 (B) induced by atrial high-frequency pacing. Gray bars are fold differences in control rats for each experimental condition (Ctr); striped bars are fold differences after 30 minute, 2h and 4h pacing (P_30m, P_2h, P_4h). Data are represented for right (RA) and left (LA) atrial samples. *** p<0.01; ***p<0.001*)

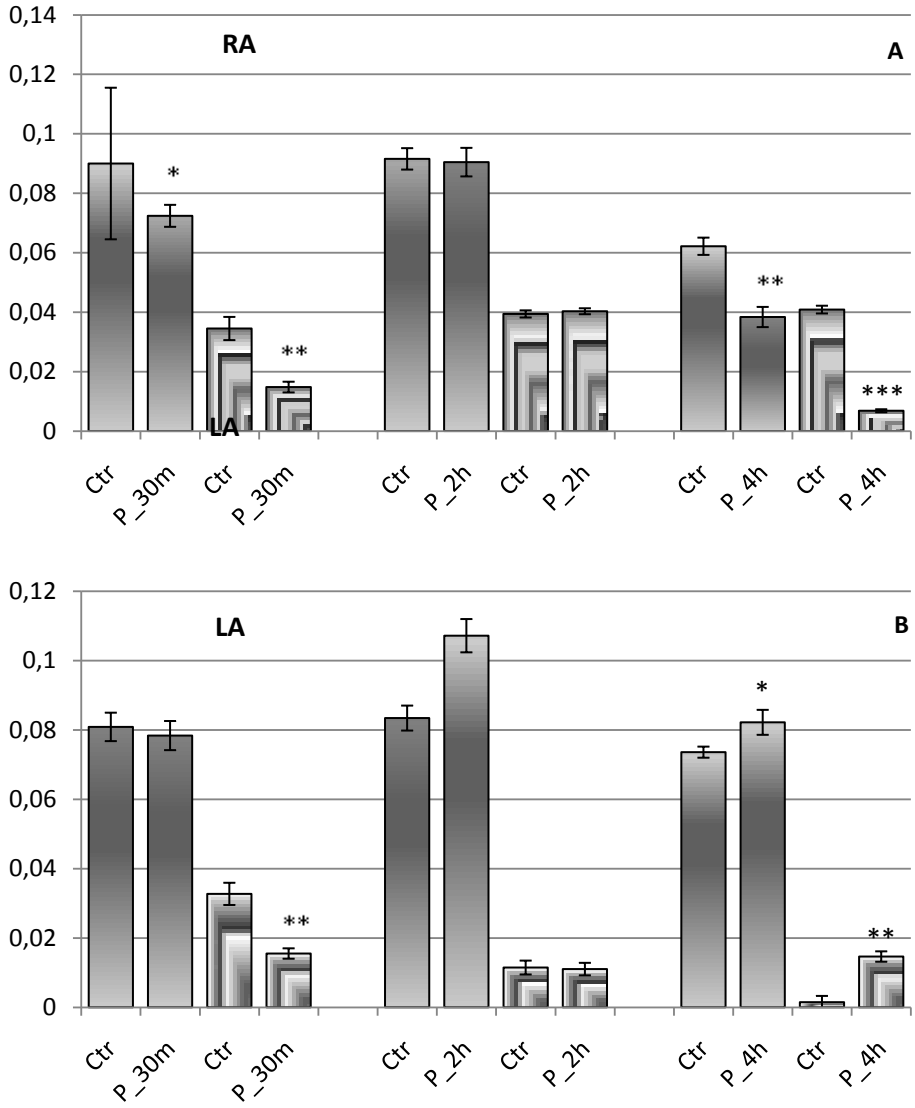

Figure 3. Changes in KCNA5 and KCND2 gene expression induced by atrial high-frequency pacing. Gray bars are fold differences in mRNA KCNA5 levels in right atrium (RA) (figure 3A) and left atrium (LA) (figure 3B). Striped bars are fold differences of mRNA KCND2 levels in RA (figure a) and LA (figure b) after 30 minute, 2h and 4h pacing (P_30m, P_2h and P_4h). ** p<0.05; **p<0.01; ***p<0.001*

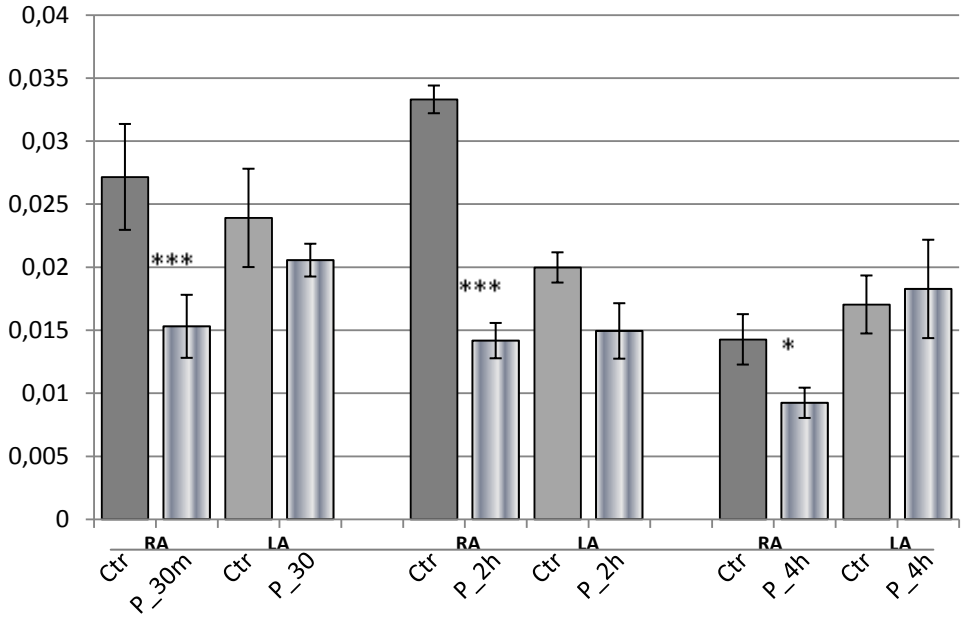

Figure 4. Changes in KCND3 gene expression induced by atrial high-frequency pacing.
Gray bars are fold differences in control rats for each experimental condition (Ctr); striped bars are fold differences after 30 minute, 2h and 4h pacing (P_30m, P_2h, P_4h). Data are represented for right (RA) and left (LA) atrial samples. ** p<0.05; ***p<0.001*)

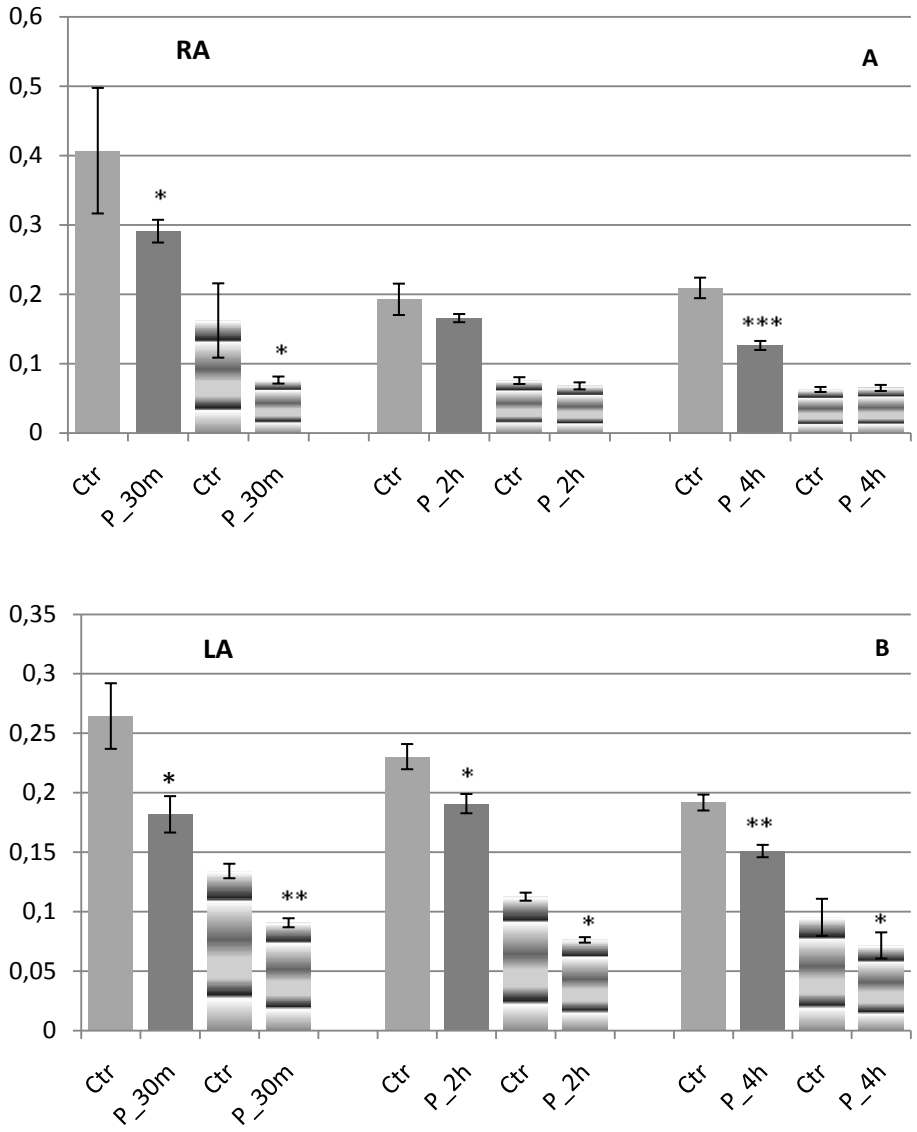

Figure 5. Changes in SCN5A and CACNA1 gene expression induced by atrial high-frequency pacing. Gray bars are fold differences in mRNA SCNA5A levels in right atrium (RA) (figure 5A) and left atrium (LA) (figure 5B). Striped bars are fold differences of mRNA CACNA1 levels in RA (figure 5A) and LA (figure 5B). Ctr=control rats for each experimental condition; P_30m=30 minute pacing; P_2h=2h pacing; P_4h=4h pacing. ** p<0.05; **p<0.01; ***p<0.001*

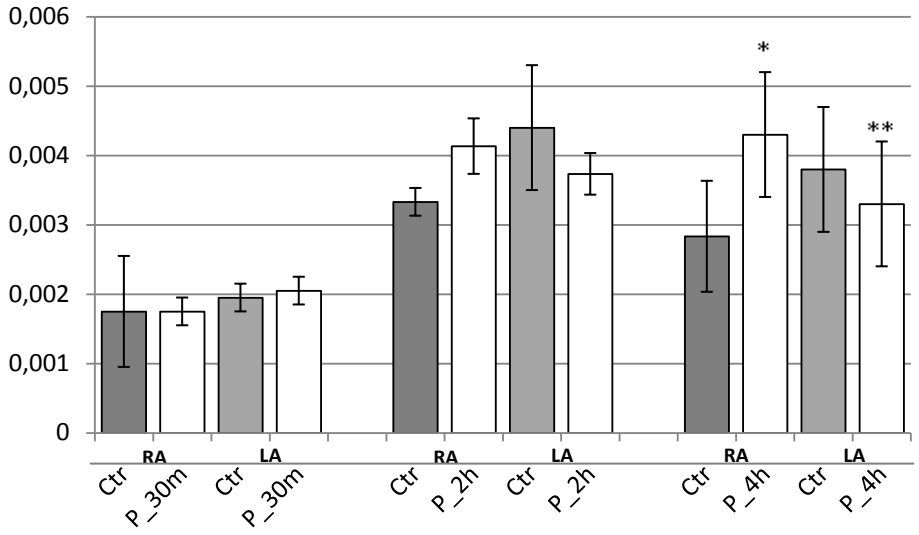

Figure 6. Changes in mRNA for CFTR induced by atrial high-frequency pacing.
Gray bars are fold differences in control rats for each experimental condition (Ctr); white bars are fold differences after 30 minute, 2h and 4h pacing (P_30m, P_2h, P_4h). Data are represented for right (RA) and left (LA) atrial samples. ** p<0.05; **p<0.01*)

Alterations in atrial ion channel and connexin expression induced by autonomic stimulation: a potential substrate for atrial fibrillation

Mário Oliveira[1,2], Gabriela Postolache[2], Vera Geraldes[2], João Paulo Gomes[3], Alexandra Nunes[3], Cristiano Tavares[2], Sérgio Laranjo[2], M. Nogueira da Silva[1], Rui Ferreira[1], Isabel Rocha[2]

[1] Cardiology Department, Santa Marta Hospital, Lisbon, Portugal

[2] Autonomic Nervous System Unit, Physiology Institute, Institute of Molecular Medicine, Faculty of Medicine, Lisbon, Portugal

[3] Instituto Ricardo Jorge, Lisbon, Portugal

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Keywords: *autonomic nervous system stimulation, gene expression, atrial tissue, ion channels, connexins*

Abstract

The autonomic nervous system (ANS) influences atrial and pulmonary veins electrophysiology, but the underlying molecular mechanisms remain unclear. This study examined the mRNA expression of ion channels and connexins in response to experimental sympathetic (S) and parasympathetic (PS) modulation in a rat model. We investigated gene expression levels for K^+, Na^+, Ca^{2+}, and Cl^- channels, and connexins (Cx40, Cx43) after continuous S, PS, and dual autonomic stim. Experiments were performed in 4 groups of Wistar rats (n=60): control; thoracic S stim; cervical PS stim; simultaneous S and PS stim. Right (RA) and left (LA) atrial mRNA for KCND2, KCND3, KCNA5, KCNJ3, KCNJ6, SCN5A, CACNA1, CFTR, Cx40 and Cx43 genes was quantified with a two-step Real Time *PCR* assay in baseline, and after 2h and 4h of stim. **Results:** LA Cx43 expression increased after S stim, and decreased in LA and RA with PS and dual autonomic stim. S stim increased SCN5A, KCND2, KCND3, KCNA5, KCNJ3 and CACNA1 expression in both atria. PS stim produced a decrease in LA KCNA5, and SCN5A, with an increase in RA SCN5A expression. With dual stim the RA showed an increase in SCN5A and CACNA1, and a decrease in KCND3 and KCNJ3 expression, whereas LA had an increase in CACNA1, with a decrease in KCND3 and KCNA5. Cx40 and CFTR expression levels did not change. LA had larger expression changes after 2h of ANS stim, while RA had higher changes after 4h. **Conclusion:** ANS stim induces extensive and early changes in gene transcription in rat atria, with heterogeneous patterns in RA and LA.

Introduction

Atrial fibrillation (AF) is the most common cardiac arrhythmia in clinical practice, causing substantial morbidity and an increased risk of mortality (1,2). Although the complex mechanisms underlying AF remain incompletely elucidated, clinical and experimental studies have provided new insights into the comprehension of this arrhythmia. The functional substrate of AF has been related to electrophysiological changes, with the occurrence of multiple depolarization wavelets, single dominant reentry circuits, focal sources of electrical activity, and different forms of atrial remodeling (3,4). Electrophysiological properties of the atria and pulmonary veins are influenced by the autonomic nervous system (ANS), and governed by heterogeneity of connexin and ion channel expression (5). Abnormal impulse generation, shortening of refractoriness, enhanced dispersion of the atrial effective refractory periods (ERP), and regions of slowed conduction contribute to the susceptibility to AF (3,4,6), and may change as a result of ANS activity (7,8). On a molecular level, the dynamic balance of inward and outward ion currents through a variety of membrane protein channels, as well as properties and distribution of connexins may affect different electrophysiologic parameters (9,10). AF is also accompanied by a remodeling process related with alterations in the electrophysiology, ionic currents and connexins within the atrial tissue (4,11,12). The importance of ANS in the pathogenesis of paroxysmal AF has been supported by clinical studies and animal experiments (7,8,12). However, molecular mechanisms involved with the cardiac inputs of ANS activity remain unclear. This study examined the effects of sympathetic (S) and parasympathetic (PS) stimulation on the gene expression levels of major ion channels and connexins in the rat atria.

Methods

Experimental Procedures

a) animal model preparation

Experiments were performed in 60 Wistar rats (aged 11 to 20 weeks), anaesthetized with sodium pentobarbitone (60 mg/Kg, intraperitoneal). The right femoral artery and vein were cannulated for blood pressure (BP) monitoring (Sensonor transducer, Lectromed, UK) and fluid injections, respectively. The trachea was cannulated below the larynx to allow mechanical ventilation. The electrocardiogram (ECG) was recorded (Neurolog, Digitimer Ltd, UK) with the use of three bipolar electrodes inserted into the limbs. Heart rate (HR) was derived from the ECG. After induction of neuromuscular blockade with vecuronium bromide (Norcuron, 0.05 mg/kg/h, i.v.), in order to assure strict control of respiratory parameters, such as rate and volume, the animal was ventilated with O_2-enriched air, using a positive-pressure ventilator (Harvard Apparatus, UK). Ventilation was regulated to maintain end-tidal CO_2 between 4.5 and 5 % (ADC gas analyser, UK). An adequate level of anaesthesia was maintained with a 20% solution of the anaesthetic, by ensuring the absence of a withdrawal reflex before the neuromuscular blockade and from the changes on BP and HR thereafter. Body temperature was maintained at 36.5-38ºC using a servo-controlled heating blanked (Harvard Apparatus, UK). BP, ECG, HR, ventilatory rate and end-tidal CO_2 were continuously monitored during the experiment (PowerLab, ADInstruments).

Briefly, the anaesthetized and paralyzed animal was placed on ventral recumbency. After preparation of the skin surface, an opening was made over the thoracic vertebrae. The tendons of cervical and thoracic part of trapezius and rhomboid muscles, and the semispinal and external intercostalis muscles were removed in the region of the C6-T2 vertebrae, using the distal end of the electrocautery as a blunt dissector. The spinal thoracal muscle and the musculus longissimus thoracis were pushed aside with a retractor. A 2-mm drill bite was used to create a hole in the T1 vertebra and open the spinal dura mater. Then, rats were placed in a dorsal position. The right cervical vagus

nerve was identified via a midline neck incision, and prepared to be electrically stimulated with a bipolar silver electrode. Animals were slightly right rotated in order to insert a bipolar electrode into the spinal canal to stimulate the thoracic outflow of the sympathetic trunk at T1 level, which mainly includes preganglionic fibers to the stellate ganglion (13). A midline sternectomy was performed to expose the heart. After thoracotomy, an end-expiratory pressure of 1-2 cmH_2O was maintained.

b) ***autonomic nerve stimulation***

The bipolar electrodes for stimulation of the sympathetic and vagus nerves were connected to a multi-channel programmable stimulator (Master8, AMPI, Israel). As a physiological test, the vagus nerve was shortly stimulated (20Hz, pulse width 2 ms) and the stimulation amplitude adjusted to yield a steady sinus rate decrease of $\geq$50%, and the sympathetic outflow tested (6Hz, pulse width 1 ms) to obtain HR and BP increasing. The vagal and sympathetic stimulation (2-6 mA and 2-10 mA, respectively) was performed using a device with a 90 V battery as power supply, which allowed stimulation over a range of strengths. The selected frequency and stimulus strengths were used throughout the study protocol.

The animals were classified in four groups and considered for evaluation in baseline, after 2 hours, and after 4 hours for each experimental condition: the control group (n=15) was submitted to all steps of the procedure except ANS stim; the S group (n=15) received continuous thoracic S trunk stimulation; the PS group (n=15) underwent continuous right cervical vagus stimulation; and the dual autonomic group (n=15) received simultaneous S and PS stimulation. Autonomic stimulation was performed for periods of 2 and 4 hours.

In addition to HR and BP monitoring, power spectral analysis of series of 30 seconds stable recorded R-R intervals was evaluated by Fast Fourier Transformation (FFT) (MathWorks, Natick, MA, USA), as described in previous work (14) to confirm ANS stimulation. The frequency spectrum was divided into two major components: low

frequency (LF; 0.1-0.6 Hz) and high frequency (HF; 0.6-1.5 Hz). HF values were attributed to vagal modulation, while LF values reflected sympathetic activity (15). At the end of experiment, the animals were killed with an overdose of anaesthetic and samples of the right and left atria were collected, harvested and saved (500µl *RNA later,* 8ºC overnight, and then -20ºC) for RNA quantification. For each experimental condition, we investigated gene expression levels for SCN5A (the gene encoding the cardiac sodium channel), KCND2 and KCND3 (encoding the alpha-subunits of the voltage-gated potassium channels Kv4.2 and Kv4.3, conducting the fast transient outward current of the cardiac action potential), KCNA5 (that encodes Kv1.5, a voltage-gated potassium channel expressed in atria), KCNJ3 and KCNJ6 genes (encoding inwardly rectifier K^+ channels), CACNA1 (encoding L-type Ca^{2+} channel), and CFTR (encoding a chloride ion channel). We also studied the gene expression levels for connexin (Cx)43 and Cx40, and the levels of Cx43 expression relative to total connexin signal (Cx43/[Cx40+Cx43]).

All the experimental procedures were approved by the Institutional Animal Care and Ethics Committee of the Faculty of Medicine of University of Lisbon and in accordance with the Portuguese and European laws on animal welfare and with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

RNA extraction and generation of pools

Total RNA from each atrium, previously stabilized with RNA later (Qiagen, Valencia, CA, USA), was extracted using the RNeasy Fibrous Tissue Mini Kit (Qiagen, Valencia, CA, USA). Briefly, 350 µl of RLT buffer (containing 2-Mercaptoethanol) and ethanol were added to the sample, which was then applied to an RNeasy mini column. In order to remove residual DNA, an on-column DNase digestion using 30 Kunitz U of RNase-free DNase (Qiagen, Valencia, CA, USA) was performed. RNA was eluted by adding 50 µl of RNase-free water and quantified by O.D. measured at A_{260}. RNA purity was

estimated from the ratio of absorbance readings at A_{260} and A_{280} nm. The RNA extraction method yielded up to 30 μg from each sample. All RNA samples were stored at -80ºC in RNase-free tubes.

Reverse Transcription (RT) and Quantitative Real-Time PCR

cDNA was generated from 300 ng of each RNA pool using TaqMan RT Reagents (Applied Biosystems, Foster City, CA) and random hexamers, as previously described (16). Each RT reaction resulted in ~30 μg of cDNA. We used the two-step RT-PCR assay to avoid differences in RT reaction efficiency for each gene in a sample because 3´ specific primers (used for one-step RT-PCR) may have unequal hybridization efficiency. Using random hexamers, even if some differences in the overall RT efficiency between samples occur, the relative amount of cDNA among the different genes remains the same for the respective sample. This is important for gene comparability and for the reproducibility of the final RT-PCR.

In order to select the endogenous control for the real-time assays, the uniformity of the expression levels of housekeeping genes was assayed. cDNA samples representing all experimental conditions from each of the experiments were diluted to a concentration of 10 ng/μl and subjected to real-time PCR analysis as described below. For the β-*actin* gene, the obtained threshold cycle (cT) values varied only slightly (less than one cT) between samples, validating the use of this gene as an endogenous control (data not shown). Quantification of expression for the 10 genes under study was achieved using the ABI 7000 SDS (Applied Biosystems), the SYBR Green chemistry, and the standard curve method for relative quantification, using reagents and thermocycling as previously described (16). Primers for each of the 10 genes (sequence available under request) were designed using Primer Express (Applied Biosystems), according to software guidelines. We used DNA standard curves (as chromosomal DNA represents equal amounts of each gene), which is essential in order to simultaneously compare

the expression of all genes at each experimental condition. These DNA standard curves were made using eight serial dilutions of DNA (starting at 20 ng of total DNA). Besides standard curves, each real-time PCR plate contained two replicates of each sample cDNA, and negative controls. The latter included no template controls and RNA only controls to ensure the absence of contaminating genomic DNA. For all experiments, the amount of target and control gene was determined from the respective standard curve by conversion of the mean cT values. Normalization was obtained by dividing the quantity of the target gene by the quantity of the control gene (β-*actin*). The specificity of the amplified products was verified by analysis of the dissociation curves generated by the ABI 7000 software based on the specific melting temperature for each amplicon.

The results were based on four independent groups (control, S, PS, and dual ANS stimulation) where each biological replicate is represented by pools of five rats for each condition, yielding a total of 15 animals per each autonomic stimulation arm of the protocol.

Statistical Analysis

Data are presented as mean±standard deviation for continuous variables. The possible relation between gene expression during S, PS and S+PS stimulation was analyzed using bivariate correlations (Pearson). Student's *t* tests for unpaired data or one way Anova was used to compare variation in gene expression during S, PS and dual combined autonomic stimulation. A p value<0.05 was considered statistically significant.

Results

In stable conditions, after all the surgical procedures and under neuromuscular blockade, vagal stimulation produced a significant decrease in HR (from 317±13 bpm to 149±20 bpm, p<0.0001) and BP (from 112±5 mmHg to 84±6 mmHg, p=0.004) (table

1). S stimulation induced a small increased in HR (from 337±17 bpm to 378±20 bpm, p=0.1) and BP (from 97±7 to 120±8 mmHg, p<0.05) (table 1). Dual autonomic simultaneous stimulation did not change BP or HR significantly.

Effects of autonomic nerve stimulation on atrial connexin gene expression

Cx43 mRNA expression results from RA and LA during all experimental conditions are presented in figure 1. S stimulation decreased Cx43 expression in LA samples after 2 hours stimulation but increased it significantly after 4 hours (figure 1B). Compared to baseline and the control group, total Cx43 expression levels from RA samples did not change significantly with S stimulation (figure 1A). In the PS stimulation group, expression of the Cx43 was significantly reduced only in LA after 2 hours, and in both atria after 4 hours. Simultaneous combined ANS stimulation induced a significant reduction of Cx43 levels in both atria after 4 hours. The larger increase of LA Cx43 gene expression was obtained with 4 hours of S stimulation. In the PS stimulation group, expression of the Cx43 was significantly reduced only in LA after 2 hours, and in both atria after 4 hours. Simultaneous combined ANS stimulation induced a significant reduction of Cx43 levels in both atria after 4 hours. The larger increase of LA Cx43 gene expression was obtained with 4 hours of S stimulation.

Cx43 expression relative to total connexin signal (Cx43/[Cx40+Cx43]) decreased in both atria after 2 and 4 hours of PS and dual ANS stimulation, whereas increased only in the LA after 4 hours of S stimulation (table 2). Cx40 expression levels did not change significantly under the given autonomic experimental conditions.

Effects of autonomic nerve stimulation on atrial ion channels gene expression

Inward rectifying K+ (Kir) channels. Analysis of the expression of KCNJ3 and KCNJ6 genes showed significant changes only in KCNJ3 compared to baseline, increasing in both atria after 4 hours of S stimulation, and decreasing in RA after 2 hours of vagal or dual ANS stimulation (table 3).

Voltage-gated potassium (Kv) channels. KCND2, KCND3 and KCNA5 expression levels changed significantly in both atria (table 3). KCND2 expression showed increased levels after 2h of S stimulation, whereas KCND3 and KCNA5 levels increased only after 4 hours of S stimulation. Vagal stimulation induced a significant reduction in the KCNA5 expression in RA and LA after 2 hours, followed by an increase after 4 hours of continuous stimulation. With 4 hours of vagal stimulation, there was a significant increase of KCND3 expression levels in the LA and a decrease of KCND2 in the RA. Dual ANS stimulation induced a significant decrease of KCND3 expression in RA and LA. LA KCNA5 decreased significantly after 2 hours, but increased in both atria after 4 hours stimulation.

L-type Ca^{2+}, voltage-gated sodium, and chloride ion channels. There were significant changes in the expression levels of CACNA1 and SCN5A from in different experimental conditions. Expression of CACNA1 increased in both atria after 4 hours of S, and after 2 hours of dual ANS stimulation (table 3). Vagal stimulation caused an opposite pattern in the expression levels of CACNA1 in RA and LA after 4 hours, with a decrease and increase, respectively. S stimulation induced SCN5A increase in LA and in both atria after 2 hours and 4 hours, respectively. Vagal and combined ANS stimulation, increased SCN5A gene expression levels in RA and LA after 4 hours (figure 2). Molecular expression of CFTR did not change significantly under the given autonomic experimental conditions.

The ion channel and connexin expression of the LA were mainly changed by 2 hours of S or PS stimulation, while RA had higher changes after 4 hours (figure 3).

Discussion

Our results show that this experimental model allows the study of the effects of short-term ANS stimulation on gene expression levels of atrial connexins and ion channels. The significant changes in gene transcription obtained after direct vagal and S trunk

stimulation emphasize the important modulatory effect of ANS input to remodeling of a potential atrial substrate.

Main findings

Changes in atrial gene transcription occur early in response to cardiac ANS stimulation being associated with significant heterogeneous alterations in the mRNA levels of genes encoding ion channels, including Na^+, L-type Ca^{2+}, inward rectifier, transient outward and voltage-gated K^+ currents, and Cx43. S stimulation induced Cx43 expression changes mainly in LA samples, producing a significant increase after 4 hours, without significant changes in the RA, whereas PS and dual ANS stimulation reduced Cx43 expression in both atria. KCNJ3 increased in both atria after S stimulation and decreased in RA after dual ANS stimulation, without significant changes induced by vagal stimulation. KCND2, KCND3 and KCNA5 expression increased in both atria after S stimulation, whereas KCNA5 decreased with vagal stimulation and showed a double pattern of variation with dual ANS stimulation. KCND3 also decreased with dual ANS stimulation. Furthermore, there were significant changes in the expression of SCN5A and CACNA1 from both atria in different ANS stimulation conditions. Cx40 and CFTR mRNA expression levels did not change significantly under the given experimental protocol.

Connexin gene expression levels in atria. Cx40 and Cx43 are critical elements underlying the function of gap junctions, contributing to propagation of atrial activation (17,18). The vulnerability to AF is determined by the presence of an arrhythmogenic substrate and initiating triggers. This functional substrate is formed by reduced ERP, enhanced spatial dispersion of refractoriness, and/or abnormal atrial impulse conduction (3,6). Initiating triggers of AF most frequently originate from the pulmonary veins (3,19). Alterations in Cx and ion channel expression have been associated with atrial remodeling phenomena and propensity for the recurrence or perpetuation of AF (4,20). Gap

junctions are clusters of transmembrane channels, composed by integral membrane subunits called connexins. These proteins underlie the electrical coupling of cardiac myocytes, with the predominant connexins expressed in the atria being Cx40 and Cx43 (17,20). Altered distribution of Cx40 and Cx43 in the atria may contribute to abnormal atrial conduction and, thereby, to substrate changes required for the maintenance of AF (17,18,20), yet the functional consequences of this co-expression remain poorly understood. In the present study, LA samples showed significant changes in Cx43 mRNA expression levels, with a reduction after continuous PS activation, and an increase after S stimulation. It has been suggested that in cell lines in which Cx40 and Cx43 are naturally coexpressed the Cx43:Cx40 mRNA ratio predicts the Cx43:Cx40 protein ratio (21). Although previous studies indicated that Cx40 is the dominant Cx for atrial impulse conduction, the propagation velocity in the atria has been associated with interactions between Cx40 and Cx43 expression, with the ratio of Cx43 to total Cx (Cx43/[Cx40+CX43]) being directly related to the velocity of impulse conduction in humans (22). Kanagaratnam et al, found a stronger correlation between conduction velocity and the relative immunodetectable signal of the two connexins than that with Cx40 alone, suggesting that the relative expression may an important factor (22). Therefore, if no significant changes occur in Cx40, conduction velocity may be influenced by the levels and distribution of Cx43. Also, a recent study showed that Cx40 and Cx43 are equally abundant in atrial tissue and make similar contributions to gap-junctional conductance (23). The alterations observed in atrial Cx43 expression after ANS stimulation may contribute to reduce the velocity of conduction during vagal or dual autonomic activation and increase velocity with S stimulation. Moreover, the heterogeneous effects of autonomic stimulation in Cx43 expression, with changes obtained mainly in the LA, could influence the electrical substrate for the occurrence of re-entrant circuits.

The remodeling process caused by AF changes the electrophysiology of the cells and the gap-junctional communication within the atria (24). These phenomena are

accompanied by alterations in the expression levels of Cx43 and Cx40, causing an heterogeneous distribution pattern of gap-junctions (24,25). However, the multiple mechanisms involving the complex features of remodeling make understanding the role of the gene expression of these proteins in AF difficult. Also, the contribution of ANS activity in modulating Cx gene expression and its potential impact in the functional arrhythmogenic substrate remains unclear.

Ion channels gene expression levels in atria. Molecular and electrical remodeling of ion channels, determining action potential duration, has been proposed as a major mechanism in AF (11,26). At the functional level, alterations in the distribution and density of ion channels influencing the atrial ERP depend on modified gene expression (27). Although no data are available in the literature regarding protein gene expression changes in the atria due to ANS stimulation, we addressed the hypothesis that ion channel expression can be affected by short-term ANS activation. Our results show that the mRNA levels of genes encoding a number of ion channels central to atrial refractoriness, including the KCNJ3, KCND2 and KCND3, KCNA5, SCN5A, and CACNA1, may be significantly influenced by ANS. S stimulation induced an up-regulation of these ion channels, which are known to modify the action potential duration and atrial refractoriness (26-28), while PS stimulation induced a down-regulation only for KCNA5 of both atria, and for RA CACNA1. The observed increase in gene expression of K^+ channels can explain the shortening of atrial ERP obtained by S activation in animal models (7,29). Reductions in atrial refractoriness will facilitate the occurrence of AF, but the underlying ionic mechanisms are not well known. In previous studies, the mRNA levels of the ultra-rapid delayed rectifier ($K_{V1.5}$) were significantly decreased in patients with persistent AF (30,31). However, in a canine model, no changes could be found in the density of this current (32). This discrepancy between alterations in protein expression and ERP in patients with AF may suggest a compensatory mechanism that serves to prolong the initially reduced ERP.

Theoretically, action potential duration and ERP of the atrial tissue can be shortened by an increase in K^+ channel gene expression and activity, a decrease in L-type Ca^{2+} channel gene expression and activity, or a combination of both. L-type Ca^{2+}-current down-regulation and decreased L-type Ca^{2+}-current density are central to the shortening of the ERP (27,33). The effects of autonomic stimulation in the atria are unusual in that S and PS stimulation do not have opposing actions but instead both shorten the ERP. Therefore, one may hypothesize that a significant increase in genes encoding atrial K^+-currents during S stimulation, combined with a decrease of the CACNA1 expression levels in the RA related with PS activation, and an up-regulation of SCNA5 induced by S, PS, and dual ANS stimulation can represent a probable explanation for the heterogeneous reduction in atrial refractoriness related with ANS activity. In AF this may be potentiated by significant neural remodeling, characterized by heterogeneous increase of sympathetic innervation and extensive nerve sprouting (7). By inducing significant heterogeneous changes in a number of atrial ion channels and Cx43 gene expression, the continuous activation of ANS may be relevant in experimental studies investigating the creation of a functional electrophysiological substrate suitable for AF. In contrast to the well-defined alterations of cellular and molecular electrophysiology underlying chronic AF, the initial changes favoring the maintenance of atrial arrhythmias and their time course are currently unknown, although they might be interesting targets for future therapeutic interventions. Recently, Lu et al, in a canine model demonstrated that ablation of the main ganglionated plexi in the atria reversed the effects of ERP shortening and eliminated AF inducibility, suggesting that intrinsic cardiac ANS plays a critical role in the acute stages of atrial electrical remodeling (34).

Clinical Implications

ANS has been recognized as an important modulator of the cardiac electrophysiological properties related with vulnerability for AF (7,8,12). We have

shown in a specific model that continuous short-term stimulation of S and PS (alone or combined) induces significant heterogeneous changes in the gene expression levels of K^+, Na^+ and Ca^{2+} channels, and Cx43. Alterations in the distribution and density of ion channels depend on modified gene expression and are determinants of changes in ERP, dispersion of atrial refractoriness and conduction velocity and thus development of an AF substrate (27). Therefore, given the clinical importance of AF, these observations may provide incentives for future research in pharmacological and interventional strategies to prevent functional changes of atrial ion channels and connexins related with disturbances of ANS cardiac regulation pathways.

Study limitations

The S thoracic outflow was stimulated from within the spinal cord. Therefore, this would have incorporated both cardiac and vascular efferent pathways. Although power spectral analysis of HR signal reflected cardiac S activity, this was not considered as direct heart stimulation. Another concern is that despite the changes obtained in a selected number of genes, the present data cannot be extrapolated to human AF. It should also be pointed out that the molecular species underlying rat ion channels distribution is to be likely different from that existing in human atria. Finnaly, there can be multiple possible mechanisms on a molecular level involved in the regulation of the genetic protein expression, making difficult to draw conclusions regarding the modulation of distribution, density and functional properties of ion channels and connexins by ANS stimulation.

Conclusions

The present study has provided evidence that short-term ANS stimulation influences extensively the expression levels of genes encoding Cx43 and major ion channels in atrial tissue. S, PS and dual ANS stimulation had heterogeneous effects in the investigated Cx and ion channel expression, with different patterns in LA and RA. The

molecular changes of the LA were mainly changed by 2 hours of S or PS stimulation, while RA had higher changes after 4 hours. This may be a valuable model to investigate the S-PS interactions on the molecular basis of acute changes in the electrophysiological properties of the atria.

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Table 1. – Blood pressure and heart rate in different experimental conditions of autonomic stimulation

Experimental condition	baseline BP (bpm)	baseline HR (mmHg)	stimulation BP (bpm)	stimulation HR (mmHg)
vagal stimulation	112 ± 5	317 ± 13	84 ± 6*	149±20*
sympathetic stimulation	97 ± 7	337 ± 17	120 ± 8 **	378±20§
dual ANS stimulation	97 ± 6	374 ± 21	103 ± 7	395 ± 16

*(abbreviations are as defined in text). * p<0.001, ** p<0.05, § p=0.1, when compared to baseline, immediately before autonomic stimulation, within the same group.*

Table 2. - Mean differences in atrial [Cx43/Cx43+Cx40]x100

	Ctr	S 2h	Ctr	S 4h
RA	0.99546	0.99038	0.99658	0.99205
LA	0.99621	0.99573	0.99173	0.99181*

	Ctr	PS 2h	Ctr	PS 4h
RA	0.99546	0.98728*	0.99658	0.989147*
LA	0.99621	0.98590*	0.99173	0.989345*

	Ctr	S+PS 2h	Ctr	S+PS 4h
RA	0.99546	0.99147*	0.99658	0.99249*
LA	0.99621	0.99022*	0.99173	0.98570*

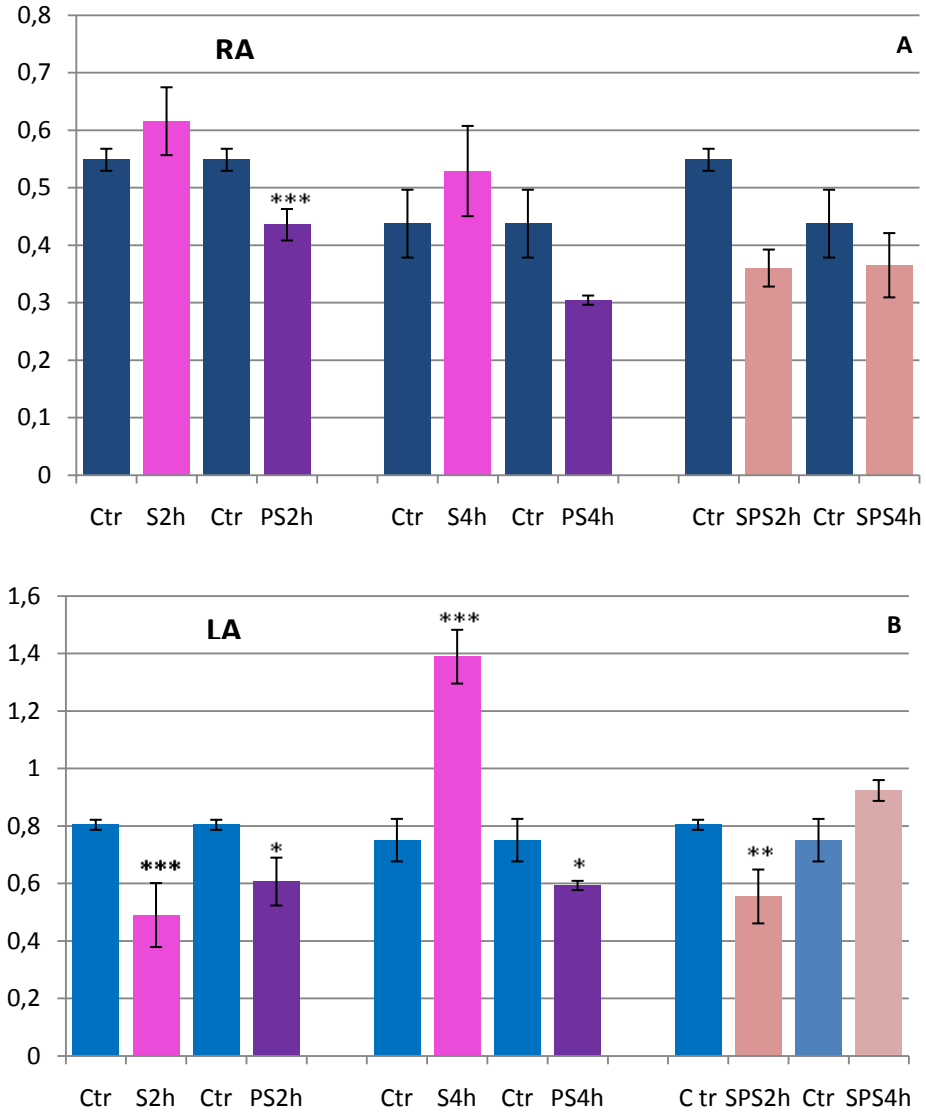
Cx=connexin; RA=right atrium; LA=left atrium; Ctr=control; S 2h, S 4h=2 and 4 hours of sympathetic stimulation; PS 2h, PS 4h=2 and 4 hours of parasympathetic stimulation; S+PS 2h, S+PS 4h=2 and 4 hours of sympathovagal stimulation. ***** *p<0.05*

Table 3. Mean variations in atrial expression levels of mRNA encoding K^+ and Ca^{2+} ion channels induced by autonomic stimulation

	S2h		PS2h		S4h		PS4h		S_PS2h		S_PS4h	
	RA	**LA**	**RA**	**LA**	**RA**	**LA**	**RA**	**LA**	**RA**	**LA**	**RA**	**LA**
KCNA5	-0.0491	-0.0634	-0.0415	-0.0320	+0.0314	+0.0626	+0.045	+0.069	+0.004	-0.0445	+0.0177	+0.0254
	ns	**ns**	**p<0.001**	**p<0.001**	**p<0.001**	**p<0.001**	**p<0.001**	**p<0.001**	**ns**	**p<0.001**	**p<0.001**	**p<0.001**
KCND3	+0.0005	-0.0030	-0.0019	+0.0174	+0.0052	+0.0137	-0.002	+0.0033	-0.009	-0.008	-0.001	-0.010
	ns	**ns**	**ns**	**ns**	**p<0.05**	**p<0.001**	**ns**	**p<0.05**	**p<0.001**	**p<0.001**	**ns**	**p<0.001**
KCND2	+0.0125	+0.0132	+0.002	+0.0149	+0.112	+0.0132	-0.004	+0.004	+0.004	+0.003	-0.006	+0.0014
	p<0.001	**p<0.001**	**ns**	**ns**	**p<0.001**	**p<0.001**	**p<0.01**	**ns**	**ns**	**ns**	**p<0.05**	**ns**
KCNJ3	-0.353	-0.0992	-0.885	-0.187	+0.1348	+0.2441	+0.1550	+0.0415	-0.211	-0.067	-0.070	+0.066
	p<0.001	**ns**	**p<0.001**	**ns**	**p<0.01**	**p<0.001**	**ns**	**ns**	**p<0.01**	**ns**	**ns**	**ns**
KCNJ6	+0.0001	+0.0001	+0.0007	+0.0003	+0.0004	+0.0001	+0.0007	+0.0008	+0.0002	+0.0002	-0.000	-0.0002
	ns	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**
CACNA1	+0.212	+0.2056	+0.033	+0.002	+0.1414	+0.1126	-0.1742	+0.0416	+0.096	+0.0674	+0.124	+0.043
	p<0.001	**p<0.001**	**ns**	**ns**	**p<0.001**	**p<0.001**	**p<0.001**	**p<0.001**	**p<0.001**	**p<0.01**	**p<0.001**	**ns**

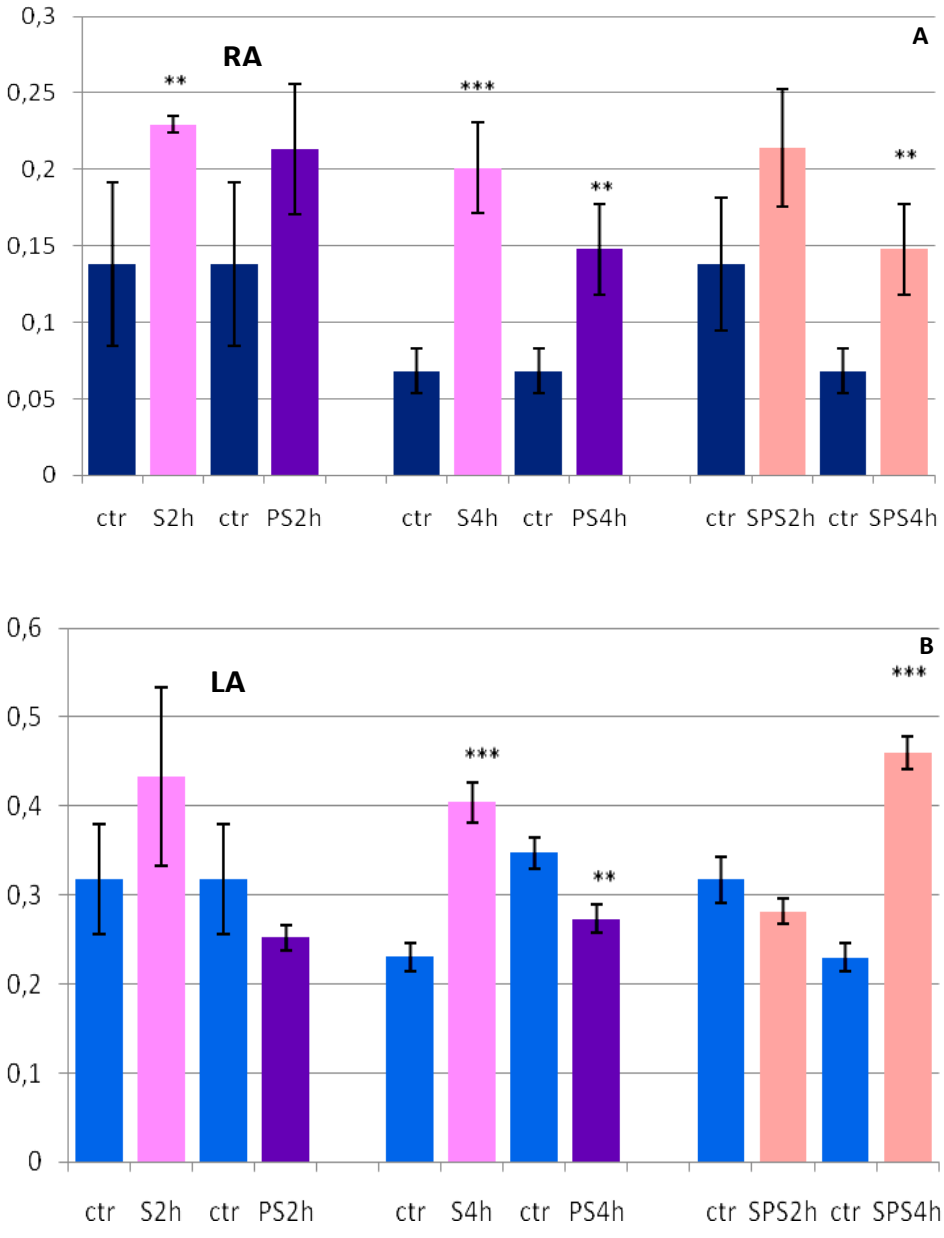
(Ion channels abbreviations are as defined in text). RA=right atrium; LA=left atrium; S 2h, S 4h=2 and 4 hours of sympathetic stimulation; PS 2h, PS 4h=2 and 4 hours of parasympathetic stimulation; S+PS 2h, S+PS 4h=2 and 4 hours of sympathovagal stimulation

Figure 1. - Atrial mRNA levels for Cx43



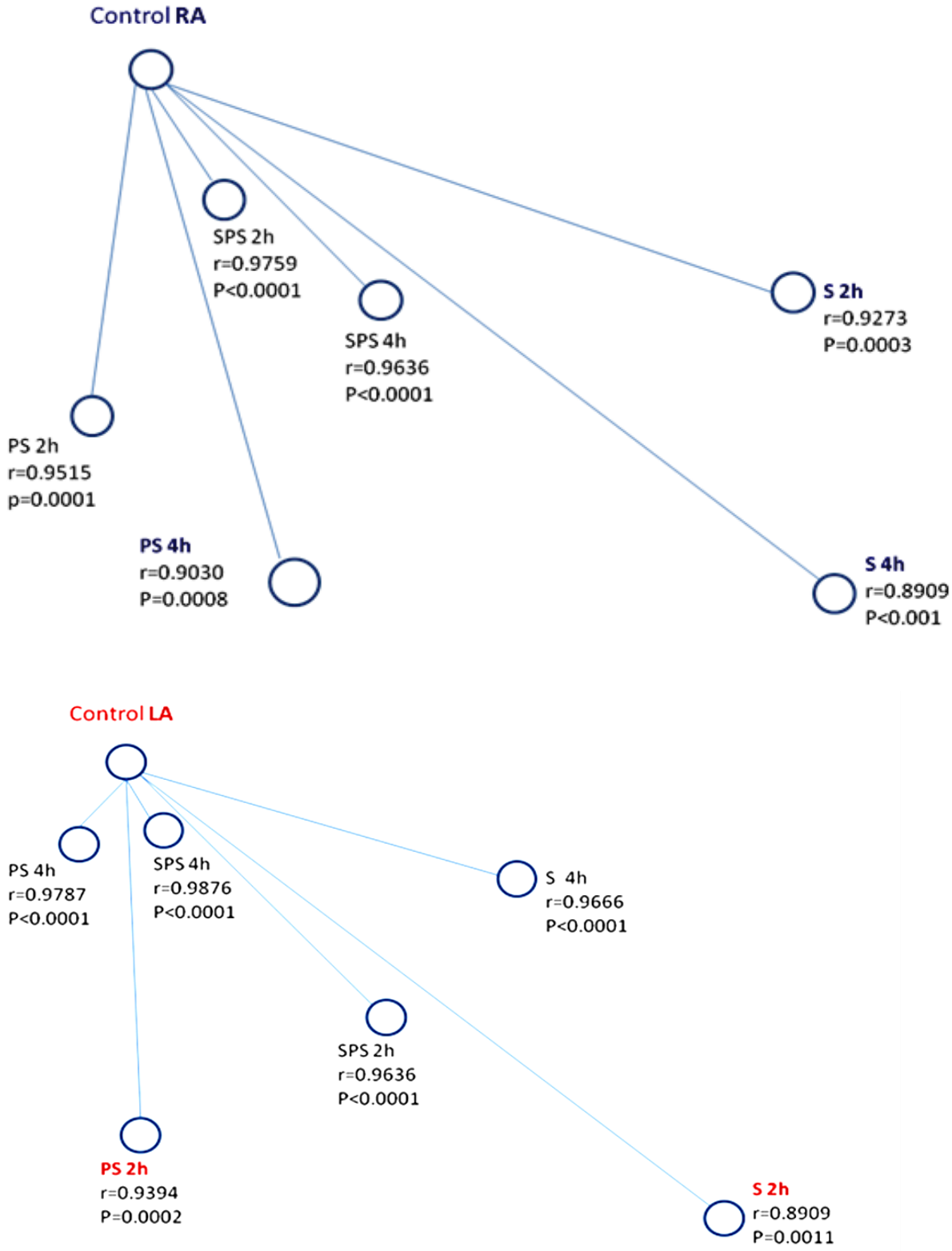
*Bars are fold differences in right (RA, figure 1A) and left (LA, figure 1B) atrial mRNA for Cx43 in control rats (Ctr) and after 2h and 4h of sympathetic stimulation (S2h, S4h), parasympathetic stimulation (PS2h, PS4h), and sympathovagal stimulation (SPS2h, SPS4h). * p<0.05; **p<0.01; *** p <0.001*

Figure 2. - Atrial mRNA levels for SCNA5



*Bars are fold differences in right (RA, figure 2A) and left (LA, figure 3B) atrial mRNA for SCNA5 in control rats (Ctr) and after 2h and 4h of sympathetic stimulation (S2h, S4h), parasympathetic stimulation (PS2h, PS4h), and sympathovagal stimulation (SPS2h, SPS4h).**p<0.01; *** p <0.001*

Figure 3. - Representation of statistical differences on ion channel and connexins mRNA levels compared to control rats



RA=right atrium; LA=left atrium; S 2h, S 4h=2 and 4 hours of sympathetic stimulation; PS 2h, PS 4h=2 and 4 hours of parasympathetic stimulation; S+PS 2h, S+PS 4h=2 and 4 hours of sympathovagal stimulation.

Capítulo VI.

Discussão geral e conclusões

Discussão geral e conclusões

O tema proposto nesta dissertação, envolvendo o estudo de bases moleculares e electrofisiológicas da influência do SNA na génese e manutenção da FAP, teve como objectivos a compreensão da importância das propriedades electrofisiológicas das aurículas na vulnerabilidade para FA e sua relação com a modulação autonómica; a caracterização das alterações da actividade simpática e parassimpática durante manobras provocativas autonómicas em doentes com FAP; e o estudo, em modelos animais, do papel da estimulação autonómica nos fenómenos electrofisiológicos das aurículas e VP, na vulnerabilidade para FA e na expressão genética das proteínas dos canais iónicos e conexinas do tecido auricular.

Modulação da actividade do SNA e propriedades electrofisiológicas auriculares na FAP

Apesar dos desenvolvimentos no conhecimento dos diversos mecanismos que contribuem para a ocorrência de episódios de FA, a compreensão da dinâmica e regulação dos fenómenos electrofisiológicos subjacentes à vulnerabilidade para FA permanece um importante desafio, estando por esclarecer as alterações funcionais que se relacionam com o início e manutenção da arritmia. A FA envolve aspectos fisiopatológicos complexos que resultam da integração de vários fcatores: as propriedades eléctricas do tecido auricular como a refractariedade e velocidade de condução, a actividade focal proveniente sobretudo das VP capaz de iniciar e manter a FA, e a existência de múltiplos circuitos reentrada que induzem a formação de ondas de activação auricular e, que podem perpetuar a arritmia através da sua fragmentação, colisão e coalescência (Jacquemet V, 2005; Chou CC & Chen PS, 2008). O valor dos PRE e a velocidade de condução local ao condicionarem o *comprimento de onda* (ou seja, a distância percorrida pela frente de despolarização durante o período refractário funcional) dos diferentes circuitos de reentrada, definem o número de circuitos que podem coexistir numa determinada massa auricular, e por isso, são capazes de influenciar o início e manutenção da FA (Biffi M et al, 2002; Byrd G et al, 2005). Os mecanismos subjacentes à recorrência de FA incluem alterações das propriedades electrofisiológicas das aurículas induzidas pela actividade fibrilhatória e resultantes dum

processo adaptativo complexo designado por remodelagem eléctrica que, mercê da redução dos PRE e da dispersão da refractariedade associados a alterações da velocidade de condução no tecido auricular podem constituir o substrato para a "auto-perpetuação" da arritmia, contribuindo para a sua recorrência e persistência (Wijfeels M et al, 1995; Alessie M 1998; Van Wagoner 2003; Duffy & Wit, 2008).

Os resultados obtidos neste trabalho mostram que em doentes com história clínica de FAP e com características clínicas e ecocardiográficas semelhantes, a vulnerabilidade para indução de FA relaciona-se com o aumento da dispersão da refractariedade auricular, não dependendo dos valores absolutos dos PRE avaliados em diferentes locais das aurículas. Deste modo, a importância que tem sido atribuída ao encurtamento dos PRE auriculares no processo fisiopatológico da FAP pode ter um impacto inferior à resultante da dispersão da refractariedade local. Por outro lado, este substrato electrofisiológico parece ter menor relevância no subgrupo com demonstração de actividade focal repetitiva, espontânea ou induzida por manobra provocativas, o que pode traduzir a importância de focos de alta-frequência como mecanismo de indução e manutenção da FA.

A diminuição heterogénea dos PRE e o aumento da dispersão da refractariedade auricular têm sido referidos como capazes de promover o substrato para a ocorrência de múltiplos circuitos de reentrada, associando-se a um maior risco de perpetuação da FA (Zhen et al, 2002; Soylu et al, 2003; Oliveira et al, 2006). Os nossos dados sugerem, no entanto, que sendo a dispersão da refractariedade um marcador de vulnerabilidade para indução de FA, não representa uma condição determinante para a manutenção da arritmia, admitindo-se, da análise dos estudos electrofisiológicos efectuados, que a capacidade para desenvolver FA mantida seja influenciada por outros factores, envolvendo mecanismos complexos como as alterações da condução eléctrica no tecido auricular e os efeitos da modulação do SNA. Apesar do reconhecimento de que as perturbações da condução auricular são um achado frequente em doentes com FAP (O'Donnell D et al, 2002; Pytkowski M et al, 2008), a influência da actividade autonómica nos electrogramas auriculares e nos intervalos de condução eléctrica auricular não são completamente conhecidos. De facto, o trabalho desenvolvido permitiu identificar actividade

auricular fragmentada e atrasos da condução, com heterogeneidade na duração dos electrogramas e nos PRE auriculares em doentes com FAP. Os valores dos PRE, da dispersão da refractariedade, a duração dos electrogramas e os intervalos de condução eléctrica nas aurículas foram significativamente modificados por manobras de estimulação aguda e bloqueio farmacológico autonómicos, com impacto no substrato arritmogénico associado ao início e manutenção da FA.

O SNA tem sido aceite como tendo um papel potencial nas alterações das propriedades electrofisiológicas auriculares em doentes com FA (Olshansky B, 2005; Chen & Tan, 2007), havendo evidência crescente de que a compreensão dos mecanismos subjacentes aos efeitos do SNA no substracto arrítmico da FAP possa contribuir para desenvolver estratégias terapêuticas que incluam a modulação autonómica (Zhang Y & Mazgalev T, 2008; Darge A, 2009).

Os nossos resultados reforçam o papel potencial e a complexidade da influência das flutuações do tónus do SNA como factor modulador das variações dinâmicas das propriedades electrofisiológicas auriculares que se relacionam com maior susceptibilidade para o início e manutenção de episódios de FAP.

Regulação cardiovascular durante manobras provocativas da função autonómica em doentes com FAP: comportamento do tónus simpático e parassimpático e do baroreflexo

A importância que tem sido atribuída ao SNA como potencial modulador dos mecanismos fisiopatológicos da FAP reforça a necessidade duma melhor compreensão da dinâmica das respostas simpática e parassimpática com recurso a manobras utilizadas para avaliação autonómica cardiovascular. No trabalho aqui apresentado, a actividade do SNA de doentes com FAP foi comparada com a de voluntários saudáveis durante manobras provocativas da função autonómica, como o ortostatismo passivo, esforço isométrico (*handgrip*), resposta pressora ao frio (*cold pressor test*) e respiração profunda (*deep breathing*). Os resultados mostram que, apesar de valores de FC e PA semelhantes em condições basais, o grupo com FAP tem diferentes padrões de resposta, na FC, PA sistólica ou PA diastólica nas provas efectuadas, com

documentação de diferenças significativas da influência autonómica na FC e PA, na fase inicial de adaptação ao ortostatismo, no esforço isométrico e durante o teste pressor ao frio.

As manobras utilizadas têm sido aceites como testes clássicos para avaliação laboratorial da actividade do SNA em situações clínicas associadas a disfunção autonómica, como a atrofia multisistémica, a polineuropatia amiloidótica, a hipertensão arterial, a diabetes mellitus, ou a insuficiência cardíaca congestiva, de entre outras (Ducla-Soares et al, 1993; Sullivan & Feldman 2005; Akselrod et al, 1997; Sousa & Saraiva, 2003; Folino et al, 2005). No entanto, é escassa a informação relativa ao comportamento autonómico durante testes provocativos no âmbito da FAP. Em 2 estudos envolvendo a análise da resposta da FC ao ortostatismo, exercício isométrico, respiração profunda e manobra de Valsalva não se encontraram alterações sugestivas de disfunção autonómica cardiovascular em doentes com FAP (Lok & Lau, 1998; Van der Berg et al, 2003). No nosso trabalho, o recurso à transformada de *wavelets,* como método de análise contínua da FC e PA, permitiu decompor os sinais no domínio do tempo-frequência em segmentos de curta duração, tornando possível localizar e quantificar a contribuição das bandas LF e HF, reflectindo, respectivamente, as alterações dos fluxos simpático e parassimpático na actividade do SNA. Considerámos, também, a razão LF/HF, aceite como um importante indicador do balanço simpático-vagal (Malliani, 2000; Postolache et al, 2003, Ducla-Soares e tal, 2007).

Nas manobras associadas à estimulação do tónus simpático (ortostatismo, exercício isométrico e frio cutâneo) os doentes com FAP mostraram diferenças do grupo controlo relativamente ao padrão de resposta da PA, com um aumento progressivo, atingindo níveis mais elevados durante o teste de ortostatismo, e com atraso na elevação da PA durante o exercício isométrico e frio cutâneo. Na adaptação ao ortostatismo, em que as alterações da FC e PA são mediadas pelo SNA, a análise da variabilidade da FC mostrou uma subida significativa do componente LF, com redução dos valores na banda HF no grupo de indivíduos saudáveis, tal como observado por Ducla-Soares (2007). Por seu lado, os doentes com FAP tiveram valores de LF mais baixos durante as fases consideradas nos dois minutos iniciais de ortostatismo, e valores de HF significativamente inferiores em posição supina e durante o movimento de elevação da mesa

basculante, mantendo um aumento significativo da razão LF/HF, em resultado da tendência para elevação do tónus simpático associada à diminuição da actividade parassimpática. Os valores mais baixos registados nas bandas LF e HF, reflectem atenuação da variabilidade da FC no grupo com FAP, podendo traduzir algum grau de disfunção autonómica cardíaca, enquanto a PA mais elevada, sem diferenças na FC, pode resultar duma maior sensibilidade cardiovascular às respostas do SNA. A diminuição da variabilidade da FC pode, no entanto, ser uma manifestação da presença de remodelagem autonómica resultante da FA, descrita em doentes e modelos experimentais, que tem vindo a ser associada a distribuição heterogénea da inervação cardíaca, com aumento significativo da inervação simpática auricular e maior facilidade na recorrência de FA (Gould et al, 2006; Che-Ming et al, 2001; Chen & Tan, 2007; Lu Z et al, 2008).

Presentemente, os mecanismos exactos subjacentes ao processo de remodelagem autonómica permanecem por esclarecer. Tem sido sugerido que anomalias mantidas do tónus do SNA podem conduzir a uma adaptação progressiva de receptores cardiovasculares, levando a fenómenos de regulação ascendente (*up-regulation*), que aumentam a sensibilidade às flutuações da actividade autonómica (Delahaye et al, 2001; Harris & Matthews, 2004; Olshansky 2005). Deste modo, de acordo com trabalhos prévios de Askelrod e colaboradores (1997, 2004), a resposta exagerada da PA, com um comportamento anormal da actividade simpática e parassimpática, pode ser interpretada como uma evidência de alterações atribuídas ao controlo autonómico cardiovascular, presentes mesmo antes de se registarem valores elevados de PA, e traduzir um marcador potencial de evolução para hipertensão arterial, considerada a situação clínica que mais frequentemente se associa a todos os tipos de FA (Levy et al, 1999; Alan et al, 2001; Nieuwlaat et al, 2005).

Nos doentes com FAP, o atraso registado na subida da PA sistólica e diastólica com o teste de frio cutâneo associou-se ao aumento não significativo da banda LF na análise da variabilidade da PA sistólica, enquanto no grupo controlo este aumento foi significativo ocorrendo com elevação da PA desde os primeiros 10 segundos. Por outro lado, no exercício isométrico, houve também atraso na resposta da PA sistólica com evolução da PA diastólica semelhante ao grupo dos

indivíduos saudáveis, mas com elevação mais precoce da banda LF e da razão LF/HF na análise da variabilidade da PA diastólica. Num estudo de Van den Berg e colaboradores (2001), o comportamento da PA diastólica durante o exercício isométrico manual também foi considerado normal. No entanto, estes autores não avaliaram a actividade autonómica durante o teste. A discrepância que observámos no perfil da PA sistólica e a variabilidade da PA diastólica é um indicador da complexidade dos mecanismos de controlo cardiovascular.

Os efeitos do exercício isométrico na hemodinâmica regional são apenas parcialmente conhecidos, sendo aceite que o tipo de resposta vascular e as alterações na banda LF da variabilidade da PA resultam da acção do sistema nervoso simpático, do volume sanguíneo e da concentração de factores com influência vasomotora em processos de auto-regulação locais (Hughson et al, 1995; Gary et al, 2008). Além disso, trabalhos experimentais mostraram que, em ratos hipertensos, a elevação da PA não ocorre em paralelo com alterações da variabilidade da PA, podendo haver hipertensão arterial mantida apesar de redução paradoxal da actividade simpática na variabilidade da PA (Zhan et al, 2005; Nagai et al, 2003).

A respiração profunda (6 ciclos/minuto) é frequentemente utilizada como forma de estimular o fluxo parassimpático, causando aumento do intervalo RR e diminuição da PA (Janomägi et al, 2003; Ducla-Soares et al, 2007). Embora durante a respiração profunda não se identificassem diferenças entre os doentes com FAP e o grupo controlo relativamente aos valores das bandas LF e HF, apenas os indivíduos saudáveis tiveram diminuição significativa da PA, com intervalos RR máximo e RR médio maiores que o grupo de FAP. À semelhança dos resultados de Van den Berg (2001), e apesar da demonstração do aumento da actividade vagal, os doentes com FAP apresentaram uma resposta cardiovascular mais atenuada. Constatamos assim que a população com FAP evidencia alterações no comportamento cardiovascular em testes provocativos autonómicos que evocam actividade simpática e parassimpática.

Uma situação clínica relativamente comum, que tem sido associada a alterações na regulação do SNA, é a síncope reflexa neurocardiogénica. Apesar de se admitir estar subjacente à interacção complexa de mecanismos envolvendo a actividade simpática e parassimpática, a

modulação cortical e a integração bulbar, diversos estudos indicam que resulta de alterações na regulação cardiovascular pelo SNA, que ocorrem de forma episódica em resposta a um factor desencadeador, e que podem ser reproduzidas durante o teste de *tilt* (Kaufmann, 1997; Barón-Esquivias et al, 2003; Pachon et al, 2005; Folino et al, 2007). As respostas neurocardiogénicas registadas com este teste são atribuídas a reflexos autonómicos com predomínio da activação vagal imediatamente antes da síncope induzida pelo *stress* ortostático (Suzuki et al, 2003). Sendo escassa a informação relativa à incidência de síncopes vasovagais numa população com FAP, a demonstração duma taxa de respostas neurocardiogénicas em idosos com FAP, sem história clínica de síncope, semelhante à duma população da mesma faixa etária, com síncopes recorrentes e sem história de FA, pode traduzir vulnerabilidade para síncope, associada a mecanismos reflexos neurocardiogénicos em idosos com FAP. A importância da actividade vagal e da susceptibilidade para respostas vasovagais tem sido mais referida em jovens com FA idiopática, enquanto a influência simpática se parece associar sobretudo à ocorrência de FAP em doentes idosos com cardiopatia subjacente (Ringdahl 2000; Siotia et al, 2004; Chen & Tan, 2007). No entanto, os resultados que obtivémos em idosos com FAP (associada a cardiopatia em 44% dos casos), mostram que um número significativo tem reacções vaso-vagais, com indução de síncope e/ou FA auto-limitada, durante um teste de inclinação passivo. Dado que a maioria dos estudos refere valores de especificidade na ordem dos 90% para o teste de inclinação com angulações de 60º ou 70º, sem recurso a agentes provocativos (Lamarre-Cliche & Cusson, 2001; Brignole et al, 2004), o protocolo que utilizámos, de curta duração e sem punção venosa, deverá permitir uma elevada especificidade na indução de reflexos neurovegetativos, permitindo identificar com precisão indivíduos com respostas inadequadas da actividade autonómica durante o *stress* ortostático prolongado.

Finalmente, a avaliação do comportamento do baroreflexo na fase de adaptação ao ortostatismo passivo em doentes com FAP mostrou também diferenças significativas quando comparado com um grupo de indivíduos saudáveis. O baroreflexo é um dos mecanismos homeostáticos de regulação cardiovascular que asseguram o controlo rápido da PA, sendo usado como um

marcador de função autonómica (Eckberg & Sleight 1992; Colle et al, 2007). A informação disponível relativamente à função do baroreflexo em doentes com FAP é limitada e com resultados discrepantes. Estudos com avaliação da sensibilidade do baroreflexo em doentes com FAP, baseados no recurso à regressão linear entre PA sistólica e intervalos RR ou com técnicas de análise espectral, não encontraram diferenças significativas com o grupo controlo (Lok & Lau, 1998; Van den Berg et al, 2003). Mais recentemente, Styczkiewicz e colaboradores (2007) mostraram que a sensibilidade do baroreflexo espontâneo se encontrava reduzida em doentes com FAP. Os nossos resultados, obtidos em posição supina e durante o ortostatismo (que implica respostas adaptativas rápidas dependentes do baroreflexo), permitiram evidenciar que, apesar de características clínicas semelhantes às do grupo de voluntários saudáveis, os doentes com FAP apresentaram uma função do baroreflexo diminuída, quando avaliada pela sensibilidade do baroreflexo e pelo índice de eficácia do baroreflexo, com consequentes diferenças na resposta da PA.

Sendo a actividade do SNA um componente determinante do baroreflexo na regulação cardiovascular, a demonstração neste capítulo de anomalias da actividade autonómica durante as diversas manobras provocativas utilizadas para avaliação do comportamento autonómico, e de alterações da função do baroreflexo na adaptação ao ortostatismo, vem consubstanciar a presença de respostas inadequadas dos mecanismos que envolvem o SNA no panorama complexo da regulação cardiovascular na FAP.

Efeito da estimulação autonómica no substracto electrofisiológico e vulnerabilidade para FA e na expressão genética de canais iónicos e conexinas das aurículas no modelo animal

O crescendo de informação recente envolvendo a influência da inervação autonómica cardíaca na génese da FAP, bem como o impacto potencial da desinervação autonómica selectiva nas estratégias de tratamento ablativo da FA, tem evidenciado o papel do SNA como importante modulador nos mecanismos subjacentes à FA (Olshansky, 2005; Das MK et al, 2008;

Scanavacca & Sosa, 2009). No entanto, os efeitos da activação aguda vagal e/ou simpática no início e manutenção da arritmia não estão completamente esclarecidos.

Neste capítulo desenvolvemos modelos experimentais *in vivo* com inervação autonómica preservada, utilizando o coração do coelho para estudo do impacto da estimulação aguda simpática e parassimpática nas propriedades electrofisiológicas das aurículas e VP, e sua influência na inducibilidade e duração dos episódios de FA, e o coração do rato para avaliar os efeitos do *pacing* auricular de alta-frequência e da modulação autonómica na expressão genética de canais iónicos e conexinas das aurículas.

É sabido que, apesar de nenhum modelo experimental ser considerado como representativo da FA "clínica", têm sido o cão e a cabra os modelos autonómicos mais frequentemente utilizados no âmbito do estudo dos mecanismos da FA (Nattel et al, 2005; Nishida et al, 2009). Neste trabalho, baseámos a escolha dos modelos na maior facilidade de acesso e na experiência da sua aplicação em diferentes protocolos de investigação em fisiologia. Além disso, no coelho, que na investigação de mecanismos de FA tem sido usado sobretudo em preparações de coração isolado, foi desenvolvido um protocolo experimental mantendo intacta a inervação autonómica. Caracterizámos o papel da estimulação directa do nervo vago cervical direito e do tronco simpático torácico na condução eléctrica e parâmetros da refractariedade auricular e das VP, e mostrámos o potencial da actividade vagal isolada e da estimulação autonómica combinada no aumento da vulnerabilidade para FA e na duração da arritmia. Além disso, a cessação da estimulação vagal pareceu influenciar directamente a interrupção dos episódios de FA, o que constitui um dado importante para a compreensão dos fenómenos que condicionam a manutenção e terminação espontânea na FAP.

Os resultados apontam para um atraso da condução entre as aurículas e as VP, durante as condições basais em ritmo sinusal, possivelmente relacionado com a propagação do impulso a nível da junção AE-VP, que parece desempenhar um papel na condução decremental e na heterogeneidade da condução. Estudos prévios, demonstraram que podem ocorrer intervalos de condução mais longos ou mesmo bloqueio da condução ao nível da junção AE-VP (Hamabe et al, 2002; Po et al, 2005). De facto, a sobreposição de tecido auricular e das VP numa distribuição

complexa de fibras, descrita naquela zona de transição, condiciona alterações heterogéneas da condução que facilitam fenómenos de reentrada locais (Hocini, et al 2002; Erlich & Nattel, 2005).

Apesar do prolongamento consistente da condução interauricular durante a estimulação vagal ou simpática e vagal combinada, os resultados obtidos no tempo de activação entre a AD e as VP mostraram uma resposta menos marcada, com uma variação que não atingiu significado estatístico na avaliação do impacto da estimulação simultânea simpática e vagal (capítulo III), o que sugere um padrão não uniforme na resposta ao aumento da actividade autonómica, com impacto diferente nos atrasos de condução eléctrica auricular.

Por outro lado, a estimulação do tronco simpático torácico diminuiu significativamente os tempos de condução interauricular e entre a AD e VP. Deste modo, o balanço do tónus simpático/parassimpático influencia a capacidade de condução rápida na transição AE-VP, podendo desempenhar um papel facilitador na indução de FA por actividade *trigger* das VP e na manutenção da FA por focos de alta frequência ou por fenómenos de reentrada local. Recentemente, Furukawa e colaboradores (2009) mostraram, no cão, que a estimulação vagal não influencia a condução AAE-VP, enquanto outros autores, usando o mesmo modelo animal, concluíram que a estimulação vagal cervical provoca um atraso na condução da AE à VP superior esquerda, sugerindo um papel deste fenómeno como substrato funcional para circuitos locais de reentrada (Yuemei et al, 2008). Segundo Furukawa (2009), durante a estimulação simpática farmacológica ocorre aumento da velocidade de condução AAE-VP na presença de remodelagem auricular, mas não no coração normal. No mesmo estudo, o efeito vagal na redução da refractariedade auricular e das VP facilitou a indução e manutenção de FA no cão com coração normal, mas não na presença de remodelagem auricular. Por outro lado, durante a estimulação da actividade simpática diminuem os PRE da AD e VP de forma significativa, aumentando a inducibilidade de FA somente após remodelagem auricular (Horikawa et al, 2007; Furukawa et al, 2009). Ao contrário do nosso estudo, aqueles autores compararam os resultados dos PRE e condução com um grupo controlo e não no mesmo animal em condições basais e durante estimulação autonómica. Além disso, a bradicardia com a estimulação vagal foi menos intensa que no nosso protocolo e a activação simpática foi obtida com administração de

isoproterenol. No modelo experimental que estudámos, mostrámos ainda os efeitos da estimulação directa simultânea de nervos simpáticos e parassimpáticos em propriedades electrofisiológicas auriculares e das VP, e na inducibilidade e duração da FA.

Assim, os dados do presente trabalho permitem confirmar que, num coração normal com inervação autonómica intacta, os valores de PRE são mais baixos nas VP e AD, sendo reduzidos nas aurículas e VP durante estimulação vagal ou estimulação simpático-vagal simultânea e no AAE com estimulação simpática. O impacto significativo da actividade autonómica na condução eléctrica e na refractariedade das aurículas e VP pode justificar o aumento da taxa de inducibilidade de FA com *pacing* de alta-frequência nestes locais durante estimulação aguda vagal ou autonómica combinada, e valoriza o papel do SNA como factor facilitador da ocorrência de FA.

No coração do coelho, que em condições basais tem muito baixa propensão para FA, não obtivemos aumento significativo da dispersão da refractariedade, variável que tem sido referida como um marcador importante de vulnerabilidade para FA (Rahme et al, 1999; Soylu et al, 2003). Estes resultados podem explicar a impossibilidade de manutenção da FA no coelho apesar da estimulação autonómica. É provável que as alterações que ocorrem na dispersão dos PRE auriculares traduzam parte do processo de remodelagem eléctrica (Fareh et al, 1998; Fynn et al, 2001), difícil de demonstrar com condições de estimulação autonómica aguda. No entanto, o subgrupo de animais em que se induziu FA de maior duração com activação vagal apresentava PRE mais curtos na AD e maior dispersão de refractariedade auricular em condições basais e durante estimulação vagal.

Este modelo experimental permitiu evidenciar que as alterações electrofisiológicas das aurículas e VP, resultantes da modulação autonómica aguda, são heterogéneas e podem ter um papel importante no substrato eléctrico da FA, sendo os efeitos da interacção simpático-vagal aparentemente determinantes nas propriedades que influenciam a inducibilidade e duração dos episódios de FA. De facto, os efeitos da activação simultânea simpática e parassimpática têm sido relacionados com a capacidade de modular as propriedades electrofisiológicas e de

aumentar a vulnerabilidade para FA no modelo do cão (Sharifov et al, 2004; Scherlag & Po, 2006; Hou et al, 2007; Tan et al, 2008).

Não obstante o desenvolvimento de vários modelos animais no âmbito da investigação básica para explorar os mecanismos subjacentes à FA, o estudo das modificações das propriedades electrofisiológicas em animais com inervação autonómica preservada tem sido menos utilizado. De facto, apesar da importância atribuída ao SNA com o factor modulador da ocorrência e perpetuação da FA, tem permanecido controverso o papel da actividade simpática e parassimpática no início, manutenção e interrupção da FA (Olshansky, 2005; Chen et al, 2006; Zhang & Mazgalev, 2008). A evidência de que a actividade autonómica reflexa se associa a alterações electrofisiológicas significativas das aurículas e a demonstração recente de que a desinervação vagal pode aumentar a eficácia da terapêutica ablativa da FA reforçam a necessidade de estudos que permitam esclarecer qual o verdadeiro impacto do SNA na génese da FAP (Chen et al, 1999; Siotia & Muthusamy, 2004; Chen & Tan, 2007). Apesar da estimulação simpática ser referida como tendo menos repercussão que a actividade vagal na refractariedade auricular e no início e manutenção da FA (Liu & Nattel, 1997; Chen el al, 1999; Tai et al, 2002; Zhang & Mazgalev, 2008), podemos sugerir que a interacção simpático-vagal pode ser sinérgica e ter um papel importante nos mecanismos subjacentes à génese da FA, representando uma área de investigação com grande potencial no âmbito da fisiopatologia e tratamento desta arritmia.

A desinervação autonómica selectiva tem sido objectivo de estudos recentes (Schauerte et al, 2000; Melo et al, 2004; Oh et al, 2006; Scanavacca et al, 2006; Yamada et al, 2009). No entanto, apesar da evidência científica de que o SNA pode ser determinante na fisiopatologia da FA, a eficácia de técnicas ablativas das terminações nervosas autonómicas permanece por estabelecer. Além disso, a possibilidade do bloqueio farmacológico autonómico ter um efeito modulador das propriedades electrofisiológicas auriculares, podendo alterar o substrato relacionado com a vulnerabilidade para FA, justifica o interesse no desenvolvimento de novos fármacos, específicos para a obtenção de bloqueio autonómico cardíaco, que contribuam para

implementar estratégias terapêuticas de modulação autonómica. As técnicas de neuro-modulação, recorrendo a processos de estímulo-resposta envolvendo a medula espinhal, nervos aferentes, músculos e respiração têm sido propostas como método alternativo de influenciar a regulação autonómica em diferentes situações clínicas, representando uma área de investigação no âmbito das neurociências e bioengenharia, com potencial para aplicação terapêutica (Eckert & Horstkotte, 2009; Ardell & Foreman, 2009; Foreman & Qin, 2009).

A possibilidade do recurso a um modelo animal com inervação autonómica intacta, permitindo avaliar de forma reprodutível as variações das propriedades electrofisiológicas cardíacas e a dinâmica da interacção dos efeitos da actividade simpática e parassimpática no substrato arritmogénico da FA pode representar um contributo para a melhor compreensão dos mecanismos neurais associados ao início, recorrência, manutenção e interrupção de FA.

Uma das vertentes que tem despertado interesse crescente na fisiopatologia da FA é a que diz respeito aos fenómenos subjacentes ao processo de remodelagem, que ocorre no âmbito da adaptação das aurículas à actividade fibrilhatória, e que envolve alterações eléctricas e estruturais que aumentam a vulnerabilidade para a recorrência e manutenção da arritmia. As alterações das conexinas e dos canais iónicos têm sido apontadas como estando na base das modificações electrofisiológicas que ocorrem no tecido auricular durante a remodelagem (Nattel et al, 2008; Workman et al, 2008; Duffy & Wit, 2008).

Neste trabalho, utilizámos o coração do rato como modelo experimental para estudar a dinâmica da evolução temporal das alterações iniciais da expressão genética das conexinas e canais iónicos das aurículas na remodelagem induzida por *pacing* auricular de alta-frequência, aceite como método de reprodução de taquidisritmias auriculares e de indução de remodelagem electrofisiológica (Wood et al, 1998; Gaspo et al, 1999; Willems et al, 2002; Laurent et al, 2008), e um protocolo de estimulação autonómica de curta-duração para avaliar o impacto da actividade simpática e/ou parassimpática na expressão genética de canais iónicos e conexinas.

O conhecimento actual relativamente à precocidade e sequência das alterações a nível molecular, bem como ao papel do SNA nestes processos é limitado. Os resultados obtidos

mostraram que, no presente modelo, é possível induzir modificações significativas dos níveis de RNAm relativos à expressão genética de canais iónicos importantes (Na^+, K^+, Ca^{2+} e Cl^-) e das conexinas (Cx43 e Cx40), com *pacing* auricular de alta-frequência ou durante estimulação autonómica. Estas alterações ocorrem precocemente e de forma heterogénea, exibindo diferentes padrões de resposta na AD e AE.

Ao contrário do que acontece no Homem, no coração do rato é a Cx43 a que se apresenta em maior quantidade nas aurículas (Polontchouk et al, 2001). A estimulação vagal ou autonómica combinada induziu redução significativa da expressão genética da Cx43 nas duas aurículas, enquanto no *pacing* de alta-frequência se obteve redução precoce na expressão da Cx43 e Cx40. A heterogeneidade regional na distribuição das conexinas a nível do tecido auricular tem sido associada a alterações na velocidade de condução com implicações no substrato arrítmico, facilitando nomeadamente a ocorrência e manutenção de circuitos de reentrada (Gollob, 2008). Deste modo, a estimulação contínua do SNA também pode condicionar modificações a nível da condução eléctrica auricular, tal como as descritas no processo de remodelagem devida a taquidisrimias auriculares.

As alterações dos canais iónicos em resposta á actividade auricular fibrilhatória têm impacto na refractariedade, excitabilidade e condução auriculares (Yamashita et al, 2000; Nattel, 2008). Durante *pacing* de alta-frequência os níveis de expressão genética modificaram-se mais precocemente em relação com os canais SCN5A, KCND2, KCNJ3 e CACNA1, observando-se alterações significativas envolvendo a expressão genética de todos os canais estudados nas primeiras 4 horas de estimulação. Estes resultados permitem estabelecer uma sequência temporal para as modificações nos níveis de RNAm, com diferenças na comparação com estudos prévios no Homem e no cão, que sugerem a ocorrência de alterações significativas mas na presença de FA de maior duração (Van Gelder et al, 1999; Barth et al, 2005; Cardin et al, 2007).

Uma vez que a nível funcional as modificações na distribuição e densidade dos canais iónicos dependem da expressão genética (Brundel et al, 2001), podemos sugerir que as alterações significativas obtidas com estimulação autonómica contínua de curta-duração terão repercussões

importantes nas propriedades electrofisiológicas auriculares. A estimulação simpática induziu aumento significativo biauricular dos níveis de RNAm dos KCND2, KCND3, KCNA5, KCNJ3, CACNA1 e SCNA5, enquanto com estimulação vagal ou simpático-vagal foi possível induzir alterações significativas de perfil heterogéneo nos níveis de expressão de KCND2, KCND3, KCNA5, KCNJ3, CACNA1 e SCNA5, que, relativamente aos genes KCND3 e KCNA5 foram sobreponíveis às observadas após *pacing* de alta-frequência.

As variações na expressão proteica de canais iónicos subjacentes às correntes de Na^+ (I_{Na}), de Ca^{2+} do tipo L (I_{CaL}), e correntes transitória de saída de K^+ (I_{TO}), rectificadora de K^+ ultra-rápida (I_{kur}), rectificadora do influxo de K^+ (K_{ir}) e da conexina 43 induzidas pela actividade simpática, vagal ou simpático-vagal, por vezes com intensidade e padrões de resposta diferentes, poderão influenciar de modo significativo (e heterogéneo) a condução eléctrica, a refractariedade e o potencial de acção a nível das aurículas, podendo desempenhar um papel facilitador para a ocorrência de FA (Lai et al, 1999; Grammer et al, 2001; Yamashita et al, 2003; Darbar et al, 2008). Assim, mesmo durante curtos períodos de estimulação é possível modificar a expressão genética de vários canais iónicos e conexinas determinantes da electrofisiologia do tecido auricular, admitindo-se que, além dos fenómenos de remodelagem resultantes da FA mantida, também a actividade do SNA, nomeadamente a interacção dinâmica simpático-parassimpático, possa ter um papel potencial na evolução do substrato electrofisiológico relacionado com a maior susceptibilidade para FA.

A FA é uma entidade clínica multifactorial de grande complexidade, com importantes consequências clínicas e aspectos dinâmicos controversos, apresentando-se com características diferentes de indivíduo para indivíduo. A perspectiva da identificação e melhor compreensão dos mecanismos subjacentes às modificações dinâmicas das propriedades electrofisiológicas das aurículas e VP e sua relação com as alterações complexas da actividade autonómica na vulnerabilidade para a ocorrência e manutenção de FA, constitui um importante desafio, e representa, na actualidade, um dos temas de interesse crescente na investigação translacional, envolvendo diferentes disciplinas científicas de pesquisa básica, aplicada, biomédica e clínica,

numa visão multidisciplinar do conhecimento, cada vez mais importante na obtenção de resultados com benefícios potencialmente inovadores na abordagem desta arritmia.

CONCLUSÕES

1. Na avaliação electrofisiológica em doentes com história clínica de FAP superior a 1 ano:
   a) os tempos de condução auricular, a duração dos electrogramas e a actividade eléctrica fraccionada estão aumentados. Os valores elevados da dispersão da refractariedade auricular são um marcador de risco para a indução de FA e permitem, em indivíduos com características clínicas e ecocardiográficas semelhantes, identificar um grupo com maior vulnerabilidade auricular, parecendo ter menor relevância como substrato electrofisiológico nos casos com demonstração de FA dependente de actividade focal. Além disso, a dispersão da refractariedade tem valores semelhantes em doentes com indução de FA auto-limitada (< 1 minuto) ou mantida, sugerindo que a perpetuação de FA é influenciada por factores adicionais além do grau de heterogeneidade dos PRE.
   b) a estimulação aguda autonómica influencia os intervalos de condução auricular, a refractariedade auricular e a heterogeneidade da duração dos electrogramas auriculares, contribuindo para o substrato arritmogénico da FA, enquanto o bloqueio autonómico farmacológico aumenta a refractariedade a nível do SC distal, diminui a dispersão da refractariedade auricular e reduz a heterogeneidade da duração dos electrogramas auriculares, o que sugere um papel protector potencial resultante da combinação de efeitos simpaticolítico e vagolítico.

2. Na avaliação não invasiva da actividade autonómica em doentes com FAP isolada:
   a) a análise com transformada de *wavelets* permite evidenciar alterações agudas significativas do controlo autonómico da FC durante o período inicial do teste de inclinação, e no controlo da PA no teste de esforço isométrico manual e na resposta pressora ao frio, sugerindo que a FAP se associa à modificação das respostas na regulação autonómica cardiovascular.
   b) estão presentes alterações da função do baroreflexo arterial em posição supina e na fase inicial do ortostatismo, evidenciadas na análise da sensibilidade do baroreflexo pelo

método sequencial e pelo índice de eficácia do baroreflexo.

c) o teste de inclinação em idosos induz um número significativo de reacções reflexas vaso-vagais com resultados falsos-positivos, podendo associar-se à indução de episódios auto-limitados de FA, o que reforça a possibilidade de ocorrerem respostas inadequadas da actividade autonómica durante o *stress* ortostático com tradução clínica.

3. No estudo dos efeitos do SNA no substracto electrofisiológico e vulnerabilidade para FA no modelo do coração de coelho *in vivo* com inervação autonómica preservada:
   a) a estimulação vagal aguda associa-se ao aumento da condução interauricular e diminuição dos PRE das aurículas e VP, enquanto a estimulação simpática aumenta a velocidade de condução interauricular e entre as aurículas e VP, diminuindo os PRE somente no AAE.
   b) a estimulação autonómica aguda permite aumentar significativamente a inducibilidade e duração da FA, sendo maior o impacto resultante da combinação simpático-vagal. Os nossos dados suportam que o desenvolvimento deste modelo experimental pode fornecer informação importante no estudo da influência da interacção da actividade autonómica com os mecanismos fisiopatológicos da FA.

4. Na análise do impacto do *pacing* de alta-frequência e da modulação aguda do SNA na expressão genética de canais iónicos e conexinas das aurículas no coração do rato:
   a) foi possível induzir alterações extensas nos níveis dos genes que codificam as conexinas 40 e 43 e vários canais iónicos (relacionados com correntes do Na^+, K^+, Ca^{2+} e Cl^-) no tecido auricular com *pacing* auricular de alta-frequência, mesmo quando de curta-duração (30 minutos a 4 horas). Estas modificações precoces são heterogéneas na sequência temporal, direcção da variação, intensidade e tipo de envolvimento auricular e podem traduzir parte dos fenómenos subjacentes à remodelagem auricular.
   b) demonstrámos pela primeira vez que a estimulação autonómica contínua de curta-duração influencia a expressão proteíca dos genes que codificam a conexina 43 e vários canais dos iónicos a nível auricular, com efeitos heterogéneos resultantes da estimulação

simpática, vagal ou simpático-vagal, e diferentes padrões de resposta na AD e AE. As alterações observadas na AE foram mais marcadas após 2 horas de estimulação simpática ou parassimpática, enquanto na AD essas alterações foram mais evidentes após 4 horas de estimulação simpática ou parassimpática. Esta preparação experimental pode ser desenvolvida como um modelo para estudo da interacção entre a actividade autonómica e as bases moleculares das alterações agudas verificadas nas propriedades electrofisiológicas auriculares em resultado da estimulação autonómica, no âmbito da génese da FA e dos processos complexos de remodelagem auricular.

RESUMO

Bases Moleculares e Electrofisiológicas da Influência da Actividade Autonómica na Génese e Manutenção da Fibrilhação Auricular Paroxística

As propriedades electrofisiológicas auriculares são uma componente fundamental do substrato arrítmico da FA, contribuindo para a ocorrência de circuitos de reentrada, com múltiplas ondas de propagação, através de actividade ectópica, da redução heterogénea dos PRE e da velocidade de condução na superfície auricular. A demonstração no Homem e em modelos experimentais da importância de focos arritmogénicos, localizados sobretudo nas VP, e da evidência de espirais (*rotores*) com condução *fibrilhatória* para o restante tecido auricular, permitiu reforçar o papel das características eléctricas das aurículas e VP na indução e manutenção de episódios de FAP. As alterações da refractariedade local e da velocidade de condução, dependem dos processos de transporte nos canais iónicos ao nível da membrana celular, da distribuição e função das junções de hiato (conexinas) e da arquictectura celular. Além disso, a FA *per si* pode causar uma sequência de modificações, num processo complexo de adaptação fisiopatológica das aurículas à actividade fibrilhatória envolvendo as correntes iónicas e as propriedades electrofisiológicas, designado por remodelagem auricular, que facilita não só a recorrência, como a perpetuação da arritmia.

Diferentes estudos têm sugerido que o início, manutenção e interrupção da FA são dependentes de múltiplos factores que modificam as propriedades eléctricas das aurículas e VP, tornando complexa a definição do contributo dos diferentes mecanismos envolvidos na fisiopatologia desta arritmia. O SNA tem sido considerado como tendo um papel potencial na modulação das condições necessárias ao substracto electrofisiológico auricular para a ocorrência de FA, sugerindo-se ter influência na arritmogénese das aurículas e VP, mas podendo também apresentar alterações das funções autonómicas resultantes da própria FA, num processo de remodelagem autonómica. A compreensão da base celular e da dinâmica complexa dos fenómenos moleculares, iónicos e electrofisiológicos subjacentes à génese da FA, bem como a sua relação com as flutuações da actividade autonómica, constituem uma área de importância

crescente na investigação dos mecanismos arritmogénicos da FA. A FA representa uma das situações mais heterogéneas em arritmologia, envolvendo múltiplas causas e factores de risco, *triggers* e mecanismos de manutenção da arritmia num substrato electrofisiológico que tem sido associado à influência do SNA. A interacção complexa da actividade autonómica e coração pode explicar as dificuldades da análise e correlação entre os aspectos clínicos da FA e dados resultantes de modelos experimentais.

A inervação autonómica cardíaca intrínseca compreende pelo menos 7 plexos ganglionares, que parecem actuar numa rede interactiva, e que apresentam maior concentração de terminações nervosas na parede posterior da AE e na zona de inserção das VP esquerdas. Apesar da evidência de que o SNA pode influenciar o substrato arritmogénico criando condições facilitadoras para a ocorrência de FA, tem sido aceite que a proporção de doentes que se integra num grupo específico de FA de etiologia neurogénica é pequena, permanecendo controversa a importância da contribuição relativa dos sistemas simpático e parassimpático nas alterações electrofisiológicas e dos canais iónicos envolvidos na FA, e sua relação directa com o início, manutenção e interrupção da arritmia. Têm sido documentadas flutuações do tónus autonómico e da actividade reflexa envolvendo variações bruscas do SNA que culminam em episódios de FAP, admitindo-se que a ocorrência de “descargas” simpático-vagais possa ser particularmente pró-fibrilhatória. Estudos recentes têm mesmo sugerido que, além da influência nas situações de FA isolada, o SNA possa desempenhar também um papel importante na ocorrência de FA em presença de cardiopatia subjacente. No entanto, permanecem controversos os dados relativos à dinâmica da actividade autonómica associada à génese da FAP.

A nível cardiovascular, a disfunção da regulação autonómica pode associar-se a diversos distúrbios patológicos, envolvendo incapacidade de adaptação cardiovascular e/ou, anomalias de reflexos cardiovasculares, que incluem a taquicardia sinusal em repouso, síndrome de taquicardia postural ortostática, hipotensão ortostática, hipertensão arterial, síncope neurocardiogénica ou arritmias cardíacas. No entanto, o papel atribuído aos reflexos arteriais e cardiopulmonares na mediação das alterações autonómicas observadas na FA permanece ainda pouco claro. A possibilidade de que a hiperactividade autonómica ao nível dos plexos

ganglionares cardíacos, com repercussões importantes na electrofisiologia das aurículas e VP, possa ter um papel na génese dos episódios de FAP tem levado alguns centros a incluir a ablação dos plexos ganglionares na estratégia terapêutica da FA. O atingimento dos plexos ganglionares pelas lesões obtidas com energia de radiofrequência tem sido proposto como um dos mecanismos de sucesso na ablação do antro das VP. Também a eliminação de áreas onde se obtêm reflexos vagais durante as aplicações de radiofrequência parece associar-se a maior sucesso no tratamento ablativo da FA. Por outro lado, os resultados da desinervação vagal parcial mostraram redução, aumento ou mesmo efeito neutro na incidência de FA no pós-operatório de cirurgia cardíaca, o que evidencia a necessidade de melhorar a informação nos aspectos metodológicos desta abordagem terapêutica.

O desenvolvimento de modelos de investigação básica em colaboração com técnicas da experiência clínica que permitam interpretar fenómenos no âmbito da biologia molecular e electrofisiologia auricular associados ao papel da actividade autonómica na génese e manutenção da FA, poderá representar um contributo para a prevenção e tratamento desta arritmia. Neste trabalho procurámos contribuir para uma visão global e abrangente da influência autónomica na fisiopatologia da FAP numa perspectiva translacional. Abordámos a importância relativa de aspectos da electrofisiologia auricular na vulnerabilidade para indução e manutenção de FA e sua relação com a modulação da actividade do SNA, bem como o comportamento da actividade simpática e parassimpática durante manobras provocativas da função autonómica em doentes com FAP, e estudámos o papel da estimulação autonómica em propriedades electrofisiológicas das aurículas e VP, na vulnerabilidade para FA e na expressão genética de proteínas dos canais iónicos e conexinas no modelo animal.

Os resultados dos estudos electrofisiológicos numa população de 50 doentes com FAP mostraram que, apesar de mais de 1 ano de episódios recorrentes de arritmia, em cerca de 30% dos casos não se induziu FA com o protocolo de estimulação auricular. Os valores médios dos PRE aumentaram progressivamente da AD para o SC proximal e distal, sendo significativamente mais altos no SC ($p<0,01$), sem diferenças entre os grupos com e sem indução de FA relativamente aos valores de PRE medidos nos diferentes locais. No entanto, nos doentes com

indução reprodutível de FA os valores de dispersão da refractariedade eram significativamente superiores (105±78 ms vs 49±20 ms; p=0,01), sendo >40 ms em mais de 90% dos casos (p=0.05). Por outro lado, não houve diferença significativa da dispersão da refractariedade entre os grupos com indução de FA auto-limitada e FA mantida justificando intervenção terapêutica (82±65 ms vs. 80±55 ms, p=NS). A análise com regressão logística, para determinar a associação de variáveis clínicas, ecocardiográficas e electrofisiológicas com vulnerabilidade para FA, mostrou que a dispersão da refractariedade auricular foi o único marcador de risco com valor predizente para inducibilidade de FA (p <0.05).

Noutro estudo, em que se evidenciou aumento dos intervalos de condução eléctrica auricular, da duração dos electrogramas e da actividade fraccionada em doentes com FAP (FAP vs. controlo, p<0.05), apesar de ligeiras variações, não houve diferenças significativas na taxa de inducibilidade de FA durante modulação autonómica (56% em basal, 69% com manobras provocativas e 50% após bloqueio autonómico farmacológico, p=NS). Os resultados não mostraram alterações dos tempos de condução auricular durante estimulação autonómica, mas no grupo com indução de FA mantida houve um aumento significativo da condução interauricular (p<0.05) e do tempo de activação auricular esquerdo (p<0.01) induzido pela estimulação vagal. Além disso, ocorreu um aumento da heterogeneidade na duração dos electrogramas auriculares durante as manobras provocativas, abolido após bloqueio farmacológico do SNA. Mostrámos também que, nestes doentes, os PRE respondem de forma heterogénea à modulação autonómica, diminuindo na AD durante estimulação vagal, mostrando maior redução no AAD durante estimulação simpática em doentes com indução de FA, e aumentando no SC distal após bloqueio autonómico. A dispersão da refractariedade auricular diminuiu significativamente após bloqueio farmacológico do SNA, sugerindo um papel protector potencial na modificação do substrato electrofisiológico relacionado com a vulnerabilidade para FA.

Os resultados dos estudos da regulação cardiovascular durante manobras provocativas da função autonómica numa população de 90 doentes com FAP mostraram que durante a fase inicial do ortostatismo passivo, apesar de valores de PA basais semelhantes ao grupo de

voluntários saudáveis, a PA tem uma subida mantida nos doentes, sem diferenças relativamente à FC. Na análise da variabilidade dos intervalos RR com transformada de *wavelets,* que permite a decomposição no domínio tempo-frequência, com respectiva caracterização e localização de alterações no equilíbrio do SNA, os doentes da FAP, quando comparados com o grupo controlo, tiveram valores de LF (relacionados com a modulação simpática) mais baixos em posição supina e durante os dois primeiros minutos de *stress* ortostático. Na banda HF (reflectindo actividade parasimpática), os valores foram mais baixos em posição supina e durante o movimento basculante da mesa de inclinação e a relação LF/HF (utilizada como um indicador do balanço simpático-vagal) foi também mais baixa durante o movimento de inclinação e no 2º minuto de ortostatismo. Durante a fase inicial de adaptação ao ortostatismo não ocorreram diferenças significativas relativamente à variabilidade da PA sistólica.

A análise com *wavelets* durante outras manobras provocativas que evocam alterações do fluxo autonómico simpático, evidenciou diferenças significativas no comportamento da banda LF da variabilidade da PA durante esforço isométrico e na resposta pressora ao frio, comparando com o grupo de indivíduos saudáveis. Tendo em consideração a importância da actividade do SNA como um dos factores determinantes do baroreflexo, estudámos a função do baroreflexo arterial, em posição supina e durante o período de adaptação ao ortostatismo, utilizando o método sequencial e considerando como parâmetros o número de rampas da PA sistólica, a sensibilidade do baroreflexo e o índice de eficácia do baroreflexo. Demonstrámos que os doentes com FAP apresentam uma função do baroreflexo diminuída, com consequentes diferenças na resposta da PA. Sendo consensual que respostas neurocardiogénicas durante o teste de inclinação são mais frequentes em jovens e atribuídas a reflexos autonómicos com predomínio da activação vasovagal, estudámos um grupo de idosos com FAP sem antecedentes de síncope, com o objectivo de avaliar a susceptibilidade para desencadear mecanismos reflexos associados a síncope neurocardiogénica. Durante o teste de inclinação a 70º, sem fármacos provocativos, ocorreram respostas positivas (vasodepressora ou mista) em 20,5% do grupo com FAP (26,3% do subgrupo sem cardiopatia associada) e em 23,5% do grupo controlo (28,5% do subgrupo sem cardiopatia associada), com síncopes recorrentes de etiologia desconhecida e sem história de

FA (p=NS), sendo o tempo decorrido até à síncope/pré-síncope semelhante nos dois grupos (15,8±8 vs. 16±9 minutos, FAP e controlo, respectivamente; p=NS). Em 3 casos com FAP (8,8%) registaram-se também períodos auto-limitados de FA durante o teste de inclinação, sugerindo que a modulação autonómica também possa contribuir para o aparecimento de FAP naquelas condições.

Os trabalhos que avaliaram o impacto da estimulação autonómica aguda no substrato electrofisiológico e vulnerabilidade para FA, realizados em 50 coelhos com inervação cardíaca preservada, permitiram validar este modelo *in vivo* no estudo da interacção do SNA com a fisiopatologia da FA. Os resultados mostraram que, em condições basais, os PRE das VP, AD e AAD são inferiores aos medidos na AE e AAE, registando-se um atraso da condução auricular na activação da AD para as VP (variável durante estimulação autonómica), com uma taxa de inducibilidade de FA entre 35% e 50%, de acordo com o local de estimulação rápida, e uma duração média de 1.0 a 2.6 s. Durante estimulação vagal, o intervalo de condução interauricular aumentou significativamente, os PRE diminuiram nos locais auriculares avaliados, sem aumento na dispersão da refractariedade, e a taxa de indução e a duração de FA variaram de 65% a 100% ($p < 0.05$), e de 3.6 s a 14.0 s ($p < 0.01$), respectivamente. Documentaram-se episódios de taquicardia das VP em 8% das experiências. Em 70% dos episódios de FA com duração >10s a arritmia cessou imediatamente após interrupção da estimulação vagal. Com estimulação simpática, os intervalos de condução auricular diminuiram, os PRE tiveram redução significativa no AAE, e a inducibilidade e duração de FA variaram entre 65% e 76% (p=NS) e 1.8 s a 9.6 s ($p < 0,05$), respectivamente. A estimulação simpático-vagal aumentou o tempo de condução interauricular, diminuiu os PRE nos locais avaliados e aumentou a taxa de inducibilidade e duração de FA para 75% a 100% ($p < 0.05$) e 3.5 s a 6.0 s ($p < 0.05$), respectivamente.

O estudo da expressão genética de canais iónicos e conexinas auriculares, efectuado em 150 ratos submetidos a *pacing* auricular de alta-frequência ou estimulação autonómica de curta-duração, mostrou uma diminuição da expressão genética da Cx43 e Cx40 em ambas as aurículas aos 30 mn de *pacing*. Aos 30 mn de *pacing* rápido ocorreu diminuição significativa da

expressão do RNAm relacionado com SCN5A, KCND2 e CACNA1 em ambas as aurículas, do KCNJ3 na AE e do KCND3 na AD. Após 2 horas de *pacing* os níveis de expressão de SCNA5 e CACNA1 diminuíram na AE, e os do KCND3 diminuíram na AD. Às 4 horas de *pacing* auricular rápido a redução da expressão genética foi significativa para o SCNA5 e CFTR em ambas as aurículas, para os genes KCNJ3, KCNJ6, KCND3 e KCNA5 na AD e para o CACNA1 na AE, enquanto na AE se obteve um aumento da expressão genética de KCNJ6 e KCND3. Nestas condições experimentais não houve alterações significativas dos níveis de RNAm que codificam o KCNJ6 e o KCND3 na AE.

Com estimulação autonómica contínua de curta-duração induziram-se também alterações significativas na expressão proteica da conexina 43 e de canais iónicos do tecido auricular. A expressão da Cx43 aumentou na AE com estimulação simpática e diminuiu em ambas as aurículas com estimulação vagal ou simpático-vagal. Não se obteve variação significativa na expressão auricular da Cx40. A estimulação simpática aumentou os níveis de SCN5A na AE e os de KCND2 na AE e AD após 2 horas, e aumentou os de SCN5A, KCND2, KCND3, KCNA5, KCNJ3 e CACNA1 em ambas as aurículas após 4 horas. A estimulação vagal diminuiu a expressão de KCNA5 nas duas aurículas e de KCNJ3 na AD após 2 horas, e induziu aumento biauricular da expressão de KCNA5, com variações significativas heterogéneas do KCND2, KCND3, SCN5A e CACNA1 após 4 horas. Decorridas 2 horas de estimulação simpático-vagal combinada, aumentou a expressão de CACNA1 e diminuiu a de KCND3 na AD e AE, enquanto diminuiu a de KCNA5 na AE e a de KCNJ3 na AD. Às 4 horas de estimulação simpático-vagal, houve aumento da expressão de CACNA1 na AD e de SCN5A e KCNA5 nas duas aurículas, e diminuição do KCND3 na AE e do KCND2 na AD. Não se obtiveram alterações significativas dos níveis de expressão do gene CFTR. As variações da expressão genética induzidas pela estimulação autonómica foram de maior intensidade na AE após 2 horas e na AD após 4 horas. As modificações heterogéneas, obtidas precocemente na expressão genética de conexinas e de vários canais iónicos podem ter um papel relevante no substrato subjacente às alterações electrofisiológicas iniciais que ocorrem no processo de remodelagem atribuído à FA.

A investigação continuada nesta vertente fundamental da abordagem da fisiopatologia da FAP permitirá melhorar a compreensão dos múltiplos mecanismos funcionais que constituem a base electrofisiológica da génese e manutenção desta arritmia, e dos fenómenos complexos da remodelagem, de forma a desenvolver metodologias de prevenção e tratamento com maiores níveis de eficácia e segurança.

SUMMARY

Molecular and Electrophysiological Basis of Autonomic Activity Influence in the Genesis and Maintenance of Paroxymal Atrial Fibrillation

Atrial electrophysiological properties have been considered as a major determinant on the development of the atrial fibrillation (AF) substrate by influencing the occurrence of reentry circuits, multiple propagation wavelets, rapid ectopic activity, and the heterogeneous shortening of the effective refractory periods (ERP) and conduction velocity. In the last decade, demonstration of arrhythmogenic foci, mostly from the pulmonary veins (PV), spiral waves and rotors with fibrillatory conduction, in humans and experimental models, enhanced the role of the electrical characteristics of the atria and PV in the propensity for the induction and maintenance of paroxysmal AF (PAF) episodes. Local changes of refractoriness and electrical conduction are related with the expression, distribution and functional properties of ion channels and gap-junctions (connexins) and tissue architecture. Moreover, AF *per si* induces complex pathophysiological changes in ionic currents and electrophysiological properties as a consequence of fibrillatory activity. This process, known as remodeling, contributes to the recurrence and perpetuation of the arrhythmia. Several studies suggested that initiation, maintenance and interruption of AF result from a complex interaction of multiple mechanisms influencing the electrical properties of the atrial and PV. Autonomic nervous system (ANS) has been considered as a modulating factor in the arrhythmogenic substrate for the occurrence of AF, probably affected by the AF itself in a process of autonomic remodeling that tends to perpetuate

the conditions to sustain the arrhythmia. Understanding the celular basis and the dynamics of molecular, ionic and electrophysiological phenomena underlying the pathogenesis of AF, and its relation with changes in the ANS activity, represents an interesting and challenging research field. In fact, AF is one of the most common and heterogeneous arrhythmias in clinical practice, associated with multiple etiologies, risk factors, trigger and maintenance mechanisms, probably interacting with the ANS in a complex functional substrate. The intrinsic cardiac nervous system includes at least 7 ganglionated plexi, in an atrial neural network with numerous epicardial nerve fibers, having distinct patherns of distribution, with higher densitiy at the posterior wall of the left atrium (LA) and ostia of the PV. Although ANS influences the arrhythmogenic substrate of AF, it has been accepted that only a small proportion of patients with PAF has “pure” neurogenic mechanisms causing the arrhythmia. The relative contribution of sympathetic and parasympathetic activity in electrophysiological and ion channels alterations contributing to AF is still incompletely understood. Previous studies have shown fluctuations of the autonomic flow with acute changes associated with AF episodes, suggesting that sympathovagal discharges can precede the development of arrhythmia. Recently, ANS activity has been considered has an important modulator also in AF associated with underlying structural heart disease. Nevertheless, it is difficult to determine the mechanisms mediating the impact of ANS in PAF.

Autonomic dysfunction of cardiovascular regulation results from combined (or isolated) abnormalities of sympathetic or parasympathetic activity, and may be associated with innapropriated sinus tachycardia, postural orthostatic tachycardia syndrome, orthostatic hypotension, hypertension, reflex neurocardiogenic syncope and cardiac arrhythmias. However, the impact of arterial and cardiopulmonary reflexes in mediating autonomic disturbances in PAF remains unknown. According to several authors, the hypothesis that cardiac autonomic hyperactivity at the ganglionated plexi can be responsable for important variations in the electrophysiology of the atria and PV that facilitate AF episodes will include selective ablation targeting of these ganglionated plexi into the intervention strategies of AF. One of mechanisms of sucess in PV antrum ablation is the elimination of neural fibers and vagal reflexes. On the other

hand, the results of partial vagal denervation after cardiac surgery have shown reduction, increase or a neutral effect on AF incidence.

There is a need to develop the field of translational research and clinical investigation in order to understand the molecular and electrophysiological phenomena related with the effects of autonomic activity in the genesis and maintenance of PAF. With this investigation, we aimed to contribute to a translational approach of the influence of ANS in the pathophysiology of PAF. We studied patients with PAF regarding the relative impact of atrial conduction and refractoriness characteristics in the induction and maintenance of AF, its relation with acute autonomic modulation, and the sympathetic and parasympathetic activity during autonomic provocative maneuvers. We also used animal models to investigate the effects of acute ANS stimulation in the electrophysiological properties of the atria and PV, in the vulnerability for AF, and in the gene expression of ion channels and connexins of the atrial tissue.

The results of the electrophysiological studies in 50 patients with PAF showed that, despite > 1 year of arrhythmia reccurrences, AF could not be induced with the atrial stimulation protocol in 30% of the cases. The mean values of ERP increased from the right atrium (RA) to the proximal and distal coronary sinus (CS) ($p < 0.01$), without differences between the groups with and without AF induction. However, patients with reproducible AF induction had a higher dispersion of refractoriness (105±78 ms vs. 49±20 ms; $p=0.01$), > 40 ms in more than 90% ($p=0.05$). Also, there were no differences between groups with induced self-limited AF (lasting <60 s) and self-sustained AF regarding the dispersion of ERP (82±65 ms vs. 80±55 ms, p=NS). The logistic regression analysis of multiple clinical, echocardiographic and electrophysiological variables showed that dispersion of refractoriness was the only marker with predictive value for the inducibility of AF ($p < 0.05$). Another study, demonstrated slowed atrial conduction, increased electrograms duration and fragmented activity in PAF patients (PAF vs. control, $p < 0.05$), with no significant differences in AF inducibility rates during acute autonomic modulation (56% in basal, 69% during provocative maneuvers and 50% after pharmacological autonomic blockade, p=NS). Atrial conduction times did not change during autonomic stimulation, but in the group with self-

sustained AF the interatrial conduction ($p<0.05$) and the LA activation times ($p<0.01$) increased significantly during vagal activation. There was also an increase in the heterogeneity of atrial electrograms duration during provocative maneuvers, abolished by autonomic blockade. In these patients, acute autonomic modulation induced heterogeneous responses in ERPs, with a decrease in RA during vagal stimulation, a pronounced decrease in RA appendage during sympathetic stimulation in patients with AF inducibility, and an increase in distal CS after pharmacological autonomic blockade. Autonomic blockade decreased dispersion of atrial refractoriness, suggesting a protective effect in the electrophysiological substrate related with AF vulnerability.

The studies of cardiovascular regulation during provocative maneuvers of autonomic function performed in 90 PAF patients showed a sustained increase in blood pressure (BP) values, with no differences regarding heart rate (HR), during the initial phase of passive orthostasis, despite baseline BP similar to healthy individuals (HI). Analysis of HR variability with wavelets transform, a metodology that allows a time-frequence decomposition of the signal, demonstrated lower LF values in the supine position and during the first 2 minutes of orthostatic stress, lower values for the HF band in the supine position and during the tilting movement, and lower LF/HF ratio during the tilting movement and in the second minute of orthostatism. However, there were no significant differences regarding systolic BP variability in the adaptation phase of orthostatism. Compared to HI,, the characterization of other provocative maneuvers evoking changes in autonomic flow, showed significant differences in the LF band of BP variability during hand grip and with the cold pressure test. Considering the importance of ANS activity as a determinant factor of arterial baroreflex, we evaluated the baroreflex function during rest and in the initial phase of postural stress using the sequential method to study the number of systolic BP ramps, the baroreflex sensitiviy, and the baroreflex effectiveness index. PAF patients showed an impairment of baroreflex function, with differences in BP response, compared to HI. These findings underscore the presence of baroreflex disturbances in lone PAF.

Neurocardiogenic responses during head-up tilting are due to autonomic reflexes, related with predominant vasovagal activation, and are more likely to occur in young subjects. We studied a group of elderly patients with PAF, without history of syncope, to evaluate the susceptibility to induce reflex mechanisms causing neurocardiogenic syncope. During head-up tilting (70º) without provocative agents, positive vasodepressive or mixed results occurred in 20.5% of the PAF group (26.3% among the subgroup without underlying heart disease) and in 23.5% of the group with syncopes of unknown etiology, without history of AF (28.5% among the subgroup without underlying heart disease) (p=NS). The time to syncope/pre-syncope was the same for both groups (15.8±8 vs. 16±9 minutes, PAF and control group, respectively; p=NS). In three patients with PAF (8.8%), we also observed short periods of self-limited AF during head-up tilting test, suggesting that autonomic modulation may trigger AF episodes in that stress condition.

The studies regarding the effects of acute autonomic stimulation in the electrophysiological substrate and vulnerability for AF were determined in 50 rabbits with preserved cardiac innervation. The results were useful to validate this *in vivo* model for further understanding of the biological role of ANS interaction with the pathophysiology of AF. In baseline conditions, we showed that the ERPs of the PV, RA and RA appendage are shorter than those measured in the LA and LA appendage, with a delay in the atrial activation from RA to PV (influenced by autonomic stimulation), and an inducibility rate of AF ranging between 35% and 50%, with a mean duration between 1.0 s and 2.6 s. During vagal stimulation, the interatrial conduction times increased significantly, the ERPs were reduced in all sites, without increasing the dispersion of refractoriness, and the induction and duration of AF ranged between 65% and 100% ($p < 0.05$), and from 3.6 s to 14.0 s ($p < 0.01$), respectively. We also documented PV tachycardia episodes in 8% of the experiments. In 70% of the AF episodes lasting more than 10 s the arrhythmia ceased immediately after interruption of vagal stimulation. With sympathetic stimulation, the atrial conduction times decreased, ERPs shortened significantly at the LA appendage, and the inducibility rate and duration of AF ranged between 65% and 76% (p=NS), and between 1.8 s and 9.6 s ($p < 0.05$), respectively. Simultaneous sympathovagal stimulation increased the mean

interatrial conduction interval, shortened the ERP in all sites, and increased the inducibility and duration of AF from 75% to 100% ($p < 0.05$) and from 3.5 s to 6.0 s ($p < 0.05$), respectively.

In the studies of gene expression of atrial ion channels and connexins, performed in 150 rats submitted to high-rate atrial pacing or short-term continuous autonomic stimulation, we demonstrated a reduction in the expression of connexin (Cx) 43 and Cx40 in both atria after 30 minutes of pacing. After 30 minutes of rapid pacing, there was a significant reduction in SCN5A, KCND2 and CACNA1 mRNA expression in both atria, KCNJ3 in LA, and KCND3 in RA. After 2 hours of pacing, the expression levels of SCNA5 and CACNA1 decreased in LA, and the KCND3 decreased in RA. After 4 hours, there was a significant reduction of SCNA5 and CFTR in both atria, KCNJ3, KCNJ6, KCND3 and KCNA5 in RA and CACNA1 in LA, while KCNJ6 e KCND3 expression levels increased in LA. With these experimental conditions there were no changes in the mRNA levels of KCNJ6 and KCND3 genes in LA.

Continuous short duration stimulation of cardiac autonomic innervation also induced significant alterations in the atrial Cx43 and ion channel gene expression levels. Cx43 increased in the LA after direct thoracic sympathetic trunk stimulation and decreased after vagal or sympathovagal stimulation in both atria. There were no changes in the atrial expression of Cx40. With sympathetic stimulation, SCN5A expression increased in LA and KCND2 increased in LA and RA after 2 hours, and the SCN5A, KCND2, KCND3, KCNA5, KCNJ3 and CACNA1 levels increased in both atria after 4 hours. With vagal stimulation, KCNA5 expression decreased in RA and LA and KCNJ3 decreased in RA after 2 hours, whereas KCNA5 expression increased in both atria, with demonstration of significant and heterogeneous variations of KCND2, KCND3, SCN5A and CACNA1 after 4 hours. After 2 hours of sympathovagal stimulation, the mRNA expression of CACNA1 increased and the KCND3 expression decreased in both atria, while KCNA5 decreased in LA and KCNJ3 decreased in RA. After 4 hours, there was an increase of CACNA1 expression in RA and of SCN5A and KCNA5 in both atria. There was also a decrease of KCND3 expression in LA and KCND2 in RA. There were no significative changes in the expression levels of the CFTR gene.

With sympathetic or vagal stimulation, LA showed larger ion channel and connexin expression changes after 2 hours, while RA had higher changes after 4 hours. These early heterogeneous modifications obtained in the gene expression of Cx43 and a number of major ion channels may have a role in the functional substrate underlying the initial changes in the electrophysiological properties of the atria related with the process of atrial remodeling.

Further translational research is need to contribute to a better understanding of the multiple mechanisms with implications in the electrophysiological basis of the PAF pathophysiology, and in the development of the remodeling phenomena, in order to define strategies for development of long-term successful and safe strategies for the prevention and treatment of PAF.

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"I have tremor cordis on me: My heart dances; But not for joy; not joy"

William Shakespeare