



Erythrocyte Acetylcholinesterase is a Signaling Receptor



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Abstract

The acetylcholinesterase (AChE), is an enzyme located in the erythrocyte membrane that motivate continually questions, about its physiological function. The aim of this mini review is highlight the receptor behavior of AChE in human red blood cells for chemical signals associated with nitric oxide mobilization and efflux from erythrocytes. Data from the experimental models used are presented. Consequently, key participative proteins in the steps of the signal transduction pathways, in dependence of AChE receptor, are suggested to be further considered as potential therapeutic targets.

Keywords: Erythrocyte; Acetylcholinesterase; signaling ; Acetylcholine; Receptor

Abbreviations: RBCs: Red Blood Cells; AChE: Acetylcholinesterase; ACh: Acetylcholine; AC: Adenylyl cyclase; cAMP: cyclic Adenosine Monophosphate (cAMP)

Mini Review

The need to characterize the composition, structure and function of the red blood cells (RBCs) membrane, the presence of the acetylcholinesterase (AChE) enzyme, which kinetically resembled the brain esterase, but markedly different from the cholinesterase found in serum was evidenced [1]. Human erythrocyte AChE enzyme activity is the highest one in the mammalian scale [2,3]. The presence of AChE far away of the neuronal system raises questions about its function beyond the enzyme action. Herzand Kaplan in their revision paper pointed that after *in vitro* hemolysis, acetylcholinesterase activity can be recovered in the erythrocyte membrane [4]. Also the tendency to couple ion transport mechanisms across erythrocyte membrane with AChE was explored with failure [4]. Later was reported AChE enzyme activity as a biomarker of human red blood cell aging and of RBC membrane integrity [5,6]. The blood of healthy humans presents a wide variety of a gradient scale with different aged classes from older to younger erythrocytes each one showing variable amount of RBCs resulting from its natural life span of 120 days. During this period, exovesicles enriched with AChE are released from erythrocyte membrane to blood circulation resting the older erythrocyte with lower enzyme activity [5,7,8]. Considering gender the human female has higher AChE enzyme activity than the men matched in age [9].

Higher human erythrocyte AChE enzyme activity were verified in patients with glaucoma, essential hypertension, and

ALS [10]. These pathologies are inflammatory vascular diseases characterized by high concentration in blood of inflammatory molecules, reactive oxygen species, and reactive nitrogen species, [10]. The RBCs AChE enzyme activity data obtained in those above mentioned vascular diseases reinforce the Das's statement of erythrocyte AChE be considered a biomarker of inflammation [11]. Das verified also, that in blood samples of inflammatory diseases, with high enzyme activity of erythrocyte AChE, lower plasma acetylcholine (ACh) levels were obtained [11].

Acetylcholine molecule acting in the parasympathetic system and in the neuron muscular junction, was considered as a neurotransmitter during several decades. Further was also recognized also as an anti-inflammatory agent through the interplay between immune and neuronal systems, the nominated "cholinergic anti-inflammatory pathway" [12-14]. This consists in the activation of adrenergic neurons in the spleen that liberate nor- epinephrine near the T cell capable to secrete acetylcholine. This non-neuronal ACh acts on $\alpha 7$ subunit-containing nicotinic acetylcholine receptors expressed on macrophages which after binding induce suppression on the synthesis and on the secretion of inflammatory cytokines. Macrophages act as an interface between the brain and the immune system [15]. The activation of afferent vagus nerve by endotoxin or pro-inflammatory cytokines stimulates hypothalamic-pituitary-adrenal anti-inflammatory responses conducted by the efferent vagus nerve.

Back to erythrocyte membrane enzyme, AChE has the particularity to be inhibited by high concentrations of ACh meaning by its own natural substrate, [10,16]. So, different types of enzyme complexes may be presented namely, active, inactive and less active ones according the amount of ACh existent in the experimental medium [16,17]. The unusual AChE kinetic behavior a non-neuronal environment and the discover of nitric oxide (NO) produced in the lumen of rabbit aortic endothelium cells under the presence ACh, raised us the question if there is NO inside erythrocytes [18]. NO was observed, in erythrocytes with added ACh, by fluorescence microscopy [19]. Human erythrocyte suspensions, in presence of ACh, were loaded with the permeable non fluorescent probe diamino fluoresceine-2 diacetate (DAF-2Da). Intra erythrocyte fluorescence intensity of triazolofluorescein (DAF-2T) was visualized as a result from the reaction between NO and the 4,5- diamino fluorescein. We concluded that ACh, in a dose-dependent way, is able to induce NO mobilization inside the erythrocyte [19].

Acetylcholine is known to be present in human blood circulation being produced by T lymphocytes and endothelial cells [20]. The circulating ACh induces vasodilation or vasoconstriction in dependence of integrity of the endothelium, via the NO synthesized and released to smooth muscle [21]. Also the NO released from endothelial cells can move to the lumen of the vessels where in the blood circulation is captured by free hemoglobin or scavenged by erythrocyte present [22]. The erythrocyte NO-heme-hemoglobin adduct or nitrosylhemoglobin is formed at high tissues oxygen tension, for example, when deoxygenated blood enters into the vascular bed of pulmonary circulation [23]. After, NO is transferred to the thiol group of cysteine β 93 of Hb forming S- nitrosohemoglobin (SNOHb) considered as a NO reservoir molecule [23]. Low tissue oxygen tension is perceived by erythrocytes with occurrence of structural allosteric transitions in SNOHb which favor the transfer of its NO to the thiol group of band 3 protein, allowing the NO efflux from erythrocytes to the capillaries spreading those tissues [24].

In *in vitro* studies with erythrocytes obtained from healthy donors under the presence of ACh it was evidenced increased of NO efflux, erythrocyte deformability (EE) and decreased erythrocyte aggregation and hemoglobin oxygen affinity [25]. Other *in vitro* study with blood samples taken from patients with vascular diseases such as hypertension, hypercholesterolemia and kidney transplant, showing lower EE than healthy persons that was ameliorate by the presence of ACh [24]. A negative association between EE values and NO efflux from erythrocytes was showed in blood samples of those patients [26].

The NO efflux measurements from erythrocytes are based on the ACh signal transduction pathway through the AChE-ACh active enzyme complex (active conformation), associated with G α iprotein, adenylyl cyclase (AC) inhibition , band 3 protein phosphorylation by protein tyrosine kinase (PTK), protein kinase C (PKC) activation, phosphodiesterase-3 (PDE3)

activation, and low level of adenosine triphosphate (cAMP) molecules [17,27-31]. Lower NO efflux from erythrocytes were obtained in presence of velnacrine and timolol, which are strong and moderate AChE inhibitors respectively, [27,31,32].

Erythrocyte S-nitrosoglutathione (GSNO) is other NO reservoir molecule existing inside erythrocyte but able to be secreted by the binding of ACh or timolol to AChE forming active and less active enzyme complex respectively [33]. At variance GSNO efflux from erythrocyte is null in absence of AChE substrate or inhibitor [33]. Meanwhile, inside the erythrocytes the presence of the inactive complex AChE-VM showed higher GSNO concentration in relation to the active enzyme complex AChE-ACh [27,31]. At opposite GSNO levels inside erythrocytes were lower in presence of the less active enzyme complex AChE-timolol than in the case of the active enzyme complex AChE-ACh [33,34]. From all the data obtained in the above described studies we evidenced that erythrocyte AChE beyond its enzyme activity function as a receptor for signal molecules able to induce rescue or NO efflux from human erythrocytes and its mobilization from reservoir molecules. The NO derivative molecules inside them as nitrite, nitrate and peroxynitrite were also quantified in all mentioned studies.

The key protein molecules participants in the chemical signal transduction mechanism of NO under AChE receptor once identified were submitted to inhibitors or activators in *in vitro* models of hyper fibrinogenemia [35,36]. The acute phase protein fibrinogen binds to RBCs membrane CD47protein partner of the Rh macro-complex which is connected to the cytoskeleton via protein 4.2, and interacts also with the major RBC macro- -complex band 3 protein [37]. The presence of AChE-ACh and AChE-timolol complexes in RBCs induced the return to normal values the higher NO efflux obtained under high fibrinogen concentration [35,38-40]. Consequently this NO scavenged avoids the increase concentrations of the reactive nitrogen and oxygen species present in inflammatory conditions. Remembering that the inflammatory response is systemically associated with variable degree of endothelium dysfunction, high plasma ACh and fibrinogen concentrations, the above data are in accordance with others that consider acetylcholine as anti-inflammatory agent. Our studies performed *in vivo* with animal models of inflammation demonstrated the anti-inflammatory function of ACh [41].

The erythrocytes obtained from blood samples of patients with open glaucoma evidenced ex-vivo higher NO efflux values, internal GSNO levels and AChE enzyme activity values, than those assessed in erythrocytes of healthy persons [34,42]. With these data we can understand the therapeutic efficacy of forskolin in glaucoma patients [43]. Forskolin is AC activator, meaning synthesis of cAMP levels inside RBCs, that reverted the higher NO efflux from erythrocytes model of hyper fibrinogenemia [44]. Fibrinogen binding to RBCs augmented the NO efflux under lower levels of cAMP [36]. It will be necessary to confirm in glaucoma patients under forskolin therapy whether normalization of NO

efflux and lower nitrogen reactive species occur [34,44]. Also NO donors like nitroglycerin acts in treatment and prevention of endothelium dysfunction [45].

Conclusion

In conclusion the proteins therapeutic targets to control the RBCs bioavailability in NO (efflux or scavenge) in inflammatory vascular disease are AChE, AC, Gi, PTP, PTK, PKC, PI3K and PD3. Some activators and inhibitors of those proteins are already disposal for examples acetylcholine, velnacrine, timolol, adrenaline, forskolin, valsartan, wortmannin derivate and iloprost.

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