

Minireview

ATP as a presynaptic modulator

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Abstract

There is considerable evidence that ATP acts as a fast transmitter or co-transmitter in autonomic and sensory nerves mostly through activation of ionotropic P2X receptors but also through metabotropic P2Y receptors. By analogy, the observations that ATP is released from stimulated central nervous system (CNS) nerve terminals and that responses to exogenously added ATP can be recorded in central neurons, lead to the proposal that ATP might also be a fast transmitter in the CNS. However, in spite of the robust expression of P2 receptor mRNA and binding to P2 receptors in the CNS, the demonstration of central purinergic transmission has mostly remained elusive. We now review evidence to suggest that ATP may also act presynaptically rather than solely postsynaptically in the nervous system. © 2000 Elsevier Science Inc. All rights reserved.

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Introduction

In the central nervous system (CNS), the use of isolated nerve terminals allowed to conclude that adenine nucleotides are released upon stimulation of CNS nerve terminals [1]. It was then shown by White [2,3] and by others [4,5] that ATP is the main adenine nucleotide released from nerve terminals of different brain areas. Thus, the release of ATP upon electrical stimulation of cortical [6], hippocampal [7,8], habenula [9] or hypothalamic preparations [10] may be derived from nerve terminals, although other CNS structures such as glial cells [11,12] and post-synaptic structures [13,14] may also contribute for ATP release. The existence of different cellular sources of ATP allows to hypothesise several possible roles for extracellular ATP in the CNS. The more likely role of released ATP may be to act as a neu-

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rotransmitter [15], by analogy with the transmitter role of ATP in the autonomic nervous system [16]. Other possible roles ascribed to ATP have been proposed in neuron-glia and glia-glia communication [e.g. 17,18] or as a trophic factor [19].

In this review, we will focus on the presynaptic role of ATP in the nervous system. We will start by considering the problems found in defining and classifying the presynaptic effects of ATP. We will then stress the mismatch between the abundant expression of ATP receptors (P2 receptors) and P2 receptor binding in the CNS with the general inability to demonstrate ATP-mediated transmission in the CNS. Finally, we will review the studies supporting a presynaptic role of ATP modulating the release of several neurotransmitters.

Problems found in defining and classifying ATP-mediated presynaptic response

One of the major problems in defining an ATP-mediated response lays in the difficulty to clearly exclude the involvement of adenosine in the presynaptic effects of ATP. This is particularly critical in studies performed in the CNS rather than in peripheral preparations, since the expression of adenosine A₁ receptor mRNA and of adenosine A₁ receptors are generally lower in peripheral tissues and more intense in most CNS regions [e.g. 20]. The activation of inhibitory A₁ receptors causes a profound inhibition of synaptic transmission and evoked neurotransmitter release through inhibition of calcium influx [21], at least in the CNS [see 22,23]. The conversion of ATP into adenosine is mediated by an ecto-nucleotidase pathway, which is present in most regions and cell types in the CNS [reviewed by 24]. The catalytic efficiency of the ecto-nucleotidase pathway is such that activation of adenosine A₁ receptors can occur within milliseconds after iontophoretical application of ATP [25]. Furthermore, the channelling organisation between ecto-nucleotidases and adenosine A₁ receptors, i.e. the ability of ATP-derived adenosine to activate A₁ receptors without equilibrating with the bio-phase [26–28], makes it mandatory to appropriately exclude the involvement of adenosine A₁ receptors in any presynaptic inhibitory effect of ATP [reviewed in 29,30]. The existence of this channelling process probably accounts for the proposed direct action of adenine nucleotides on adenosine A₁ receptors (27–31). Facilitatory effects of ATP may also be confounded by facilitatory effects of adenosine via activation of adenosine A_{2A} receptors [36,37]. Thus, the feed-forward inhibition of ecto-5'-nucleotidase, the last enzyme of the ecto-nucleotidase pathway [38,39], allows producing a burst-like formation of adenosine with the consequent activation of facilitatory A_{2A} receptors [26,27,30]. Activation of adenosine A_{2A} receptors facilitates calcium influx through voltage-sensitive calcium channels increasing the evoked release of neurotransmitters [reviewed in 30]. In this respect it is striking that in the studies reporting ATP-mediated facilitation of neurotransmitter release, the possible involvement of facilitatory adenosine A_{2A} receptors is never considered. It is important to stress that the use of P2 receptor antagonists does not allow to distinguish between P2- and adenosine (P1) receptor-mediated responses, since P2 antagonists (suramin, PPADS, Evans blue, cibacron blue 3GA) are all effective inhibitors of extracellular ATP catabolism [reviewed in 40].

ATP receptors are divided into two major classes: ionotropic P2X receptors (P2X_{1–7}) and metabotropic P2Y receptors (P2Y_{1,2,4,6,11}) [40,41]. This classification was initially based on pharmacological criteria [42] and later reinforced by receptor cloning and expression in heterologous systems [e.g. 43]. The involvement of P2Y and P2X receptors in ATP responses in native tissues is only tentative upon analysis of membrane currents and/or disruption of G

protein-mediated production of second messengers, since there are no selective agonists or antagonists for the two major P2 receptor classes, although some agonists/antagonists may be selective for some particular receptor subtype within a given class of receptors [reviewed in 40]. Furthermore, the observation that P2X receptors are oligomeric structures [44,45], not only homo-oligomeric structures, but also hetero-oligomeric structures [46,47], makes it more difficult to relate the physio-pharmacological properties of native P2 receptors with heterologously expressed P2X or mixtures of P2X receptor subunits. The former criteria of classifying P2X-mediated responses by α,β -methylene ATP induced-desensitisation of the response has also proved not to be adequate for all homomeric P2X receptors [40] and even less for heteromeric P2X receptors, which are still ill characterised. Direct measurement of presynaptic currents is only possible in exceptionally large nerve terminals [e.g. 48], and even if possible it is sometimes difficult to distinguish ionotropic responses from metabotropic responses tightly coupled to regulation of ion channels [49,50]. Only very few studies have provided convincing evidence, through the use of G protein modifiers, for the involvement of P2Y receptors in modulating neurotransmitter release [51–54]. Finally, it should be mentioned that a third class of purinergic receptors (P3 receptor) has been proposed to modulate transmitter release [55–57]. The distinctive characteristics of P3 receptors are to be nearly equally sensitive to ATP and adenosine and to be antagonised by xanthines [55–58]. It is not clear if this atypical P1/P2 pharmacology is due to a distinct molecular entity [see 59] or might result from the high activity of ecto-nucleotidases [25] and/or the channelling organisation of ecto-nucleotidases and A₁ receptors [26–28].

These considerations highlight the great care required to critically evaluate the conclusions on the involvement of P2X or P2Y receptors reached in several studies of presynaptic effects of ATP, and even in the claims of any P2 receptor involvement altogether.

ATP as a neurotransmitter in the CNS

The proposal of a transmitter role for ATP in the sensory system was first stated by Holton and Holton [60], but the acceptance of a transmitter role of ATP in the autonomic nervous system mostly stems from the persistent work of Geoffrey Burnstock since the 60' [16]. A co-transmitter role for ATP in sympathetic nerves, in sensory neurons, in some parasympathetic nerves and in non-adrenergic-non-cholinergic nerves is now well documented and has been the matter of several reviews [e.g. 61,62].

The acceptance of a transmitter role of ATP in the autonomic nervous system lead to the search of ATP-mediated responses in central neurons. It was first observed by Phillis' group [63] that ATP transiently facilitates cortical excitability, an effect not mimicked by other purines, but this action was attributed to the ability of ATP to chelate extracellular calcium. It was only nearly 10 years later that the first well documented depolarising effects of ATP in the CNS were reported in a subpopulation of dorsal horn neurons [64] and in caudal trigeminal nucleus [65]. Effects of exogenously added ATP were detected on other central neurons and have been tentatively classified as P2Y receptor-mediated, triggering intracellular calcium accumulation or modulating K⁺ channels or P2X receptor-mediated depolarising currents [reviewed in 40]. Not only neurons, but also astrocytes and microglial cells respond to ATP [see 40].

These ATP-mediated modifications of neuronal metabolism and excitability prompted the search for P2 receptors in the CNS. *In situ* hybridisation and northern blot studies indicate

the expression in the CNS of P2X₁ [66,67], P2X₂ [67], and P2X₄ and P2X₆ receptor mRNA [67–71], which are the most abundantly expressed P2 receptor subunit mRNAs [67,68]. The expression of P2X₃ receptor mRNA is restricted to dorsal root ganglia [72] and not detectable in other brain regions [67], whereas the P2X₅ mRNA expression was only found in mesencephalic nucleus of the trigeminal nerve [67]. The expression of P2Y₁ [73], P2Y₂ [74,75] and the R5 transcript of P2Y₄ [76], but not of P2Y₆ receptor mRNA [77,78], has also been detected in the CNS.

Comparatively fewer studies have investigated the location of P2 receptor proteins. Immunocytochemical studies show a widespread distribution of P2X₄ [79] and P2X₂ receptors in the brain [80,81], located both pre- and post-synaptically [82]. P2X₁ receptors have a more restricted location, being present in the perikarya of a distinct subpopulation of rat brainstem neurons [83] and in the cerebellum [84], and P2X₃ receptors appear only in central terminals of sensory neurons [85] and in the nucleus tractus solitarius [86]. Autoradiographic and membrane binding studies with [³H]α,β-methylene ATP, which mostly labels P2X₁ and P2X₃ receptors, reveal abundant high affinity labelling in different CNS areas [87–89]. Similar studies with thio-labelled ATP analogues ([³⁵S]deoxyATPαS, [³⁵S]ATPαS and [³⁵S]ATPγS) have claimed to label metabotropic P2Y receptors in the CNS [76,88,90,91].

The observation of evoked ATP release from CNS nerve terminals, the possibility of recording fast responses to exogenously added ATP, the molecular evidences for the strong expression of P2 receptor mRNA in CNS neurons and the robust labelling compatible with P2 receptor binding in the CNS lead to the acceptance of ATP as a fast neurotransmitter in the CNS [15] by analogy with the transmitter role of ATP in the autonomic system. However, it has been difficult to demonstrate the existence of ATP-mediated transmission in the CNS. With the exception of the medial habenula where a small proportion of the synapses use ATP as a fast neurotransmitter [92] and the suggestions for a transmitter role of ATP in the dorsal horn [93,94], in the locus coeruleus [95] and in the hippocampal CA1 area [96], most attempts to demonstrate ATP transmission in CNS synapses were unfruitful. Thus, there is currently a marked mismatch between the robust ATP release, mRNA expression and P2 receptor density in the CNS with the lack of attributable neuroactive roles for extracellular ATP in the CNS.

The widespread use of molecular genetics out of a well-defined physiological context has previously lead to situations in which our knowledge on the likely physiological role of a given neuroactive substance lags well behind our knowledge on the molecular characteristics of the receptors where this neuroactive substance acts. In the ionotropic receptor arena, it was wisely considered that, since ionotropic receptors are designed to mediate fast signals, they should be involved in fast transmission and there were no reasons to seek other possible roles. Thus, the knowledge of the molecular characteristics of ionotropic receptors and the study of their location would be enough to predict where and when a given ionotropic receptor would be active. But this strategy has previously failed in the case of two ionotropic receptor systems, the nicotinic acetylcholine receptor and the kainate subtype of glutamate receptors. In these two systems, like for the ATP/P2 receptor, there is a clear paradox between the abundant expression of receptor subunit mRNA and robust binding by selective ligands in the CNS and the inability to clearly identify transmission mediated by these receptors [97,98]. This lead to the proposal that, instead of being involved in fast neurotransmission,

kainate and nicotinic receptors would mainly behave as neuromodulatory systems [97,99]. Thus, a presynaptic locus of action of kainate and nicotinic receptors as modulators of neurotransmitter release [99,100] would reconcile a heavy expression and receptor density with their scarce involvement in fast transmission. We will now review the evidences supporting a role for P2 receptors in the modulation of neurotransmitter release, a role which would contribute to explain the contradiction between the great expression and P2 receptor density and the difficulty of ascribing a clear role for ATP as a fast transmitter in the CNS [15].

Modulation of neurotransmitter release by ATP in the periphery

Acetylcholine at the neuromuscular junction

The observations that cholinergic vesicles store ATP together with acetylcholine [4,101] and that ATP is released with acetylcholine at neuromuscular synapses [23,102,103] make ATP a likely transmitter or modulator in these cholinergic synapses. Initial studies in adult animals showed that ATP presynaptically inhibits the quantal content of evoked endplate potentials recorded from adult frog [104] and rat skeletal muscle fibres [105]. However, different groups concluded that these effects of ATP appear to be mediated by activation of inhibitory adenosine receptors upon extracellular catabolism of ATP into adenosine [23,106–109], with the exception of two reports, one suggesting a possible involvement of presynaptic P2 receptors [109] and the other describing unexpected results to justify a presynaptic inhibition mediated by ATP as such [110]. Changing the experimental conditions from low (0.2 Hz) frequency stimulation (to detect transmitter release via endplate potential recordings) into higher (≥ 5 Hz) frequency stimulation (to allow quantification of released acetylcholine as tritiated choline), adenine nucleotides also revealed to possess facilitatory effects on acetylcholine release [111–113] (Table 1). This ATP-mediated facilitation of acetylcholine release is readily observed at developing neuromuscular junctions [111,112]. At mature neuromuscular junctions, β,γ -imido ATP, the ATP analogue less prone to extracellular catabolism facilitated acetylcholine release through P2 receptor activation but a tonic role of P2 receptor activation could not be revealed [113]. Instead, endogenous ATP, released in higher amounts at higher frequencies of stimulation (≥ 5 Hz) is hydrolysed by ecto-nucleotidases leading to the preferential activation of facilitatory adenosine A_{2A} receptors [27,114]. Thus, it appears that the main inhibitory and facilitatory effects of released ATP at the neuromuscular junction are mediated by adenosine A_1 and A_{2A} receptors, according to the frequency of nerve stimulation. Presynaptic facilitatory and inhibitory effects of ATP have also been reported but their relative importance and physiological significance is not yet understood.

Acetylcholine at autonomic nerves

The release of ATP has been reported to occur at different cholinergic autonomic nerve terminals [115], the contracting smooth muscle also contributing for extracellular ATP accumulation [116]. Several studies (Table 1) showed that ATP on its own inhibited acetylcholine release [117–120]. The type of receptor(s) involved in ATP-induced inhibition of acetylcholine release has not been defined, with claims of atypical adenosine A_1 receptors [117–118], P3 receptors [120] or not-defined P2 receptors [119]. ATP can also facilitate evoked acetylcholine release from autonomic nerve endings [121]. This ATP-mediated facilitation of acetylcholine release was proposed to be mediated by P2X-like receptors [121], which is sup-

Table 1

Preparation	Measured effect	ATP effect	Proposed receptor	Reference
<i>Acetylcholine</i>				
frog NMJ	epp	inhibition	P1	104,105,106
frog NMJ	epp	inhibition	P2	110
rat NMJ	epp	inhibition	P1	104,105
rat NMJ	NT quantification	facilitation	P2	113
mouse NMJ	epp/mepp	inhibition	P2	109
xenopus NMJ	sepp/mepp	facilitation	P2	111,112
frog ganglion	epsc	inhibition	P2 atypical	119
chicken ganglion	presynaptic current	facilitation	P2X	48
guinea pig ileum	NT quantification	inhibition	P2	117,118
guinea pig ileum	NT quantification	facilitation	P2X	121
<i>Noradrenaline</i>				
rat vas deferens	NT quantification	inhibition	P3	57
mouse vas deferens	NT quantification	inhibition	P2Y	125
mouse vas deferens	Ca transients	inhibition	P2Y	135
rat/mouse vas deferens	NT quantification	inhibition	P2Y	125,128
rat tail artery	NT quantification	inhibition	P2Y	129
rat tail artery	NT quantification	inhibition	P3	55,56
rabbit ear artery	epj	facilitation	P2	136,137
rabbit saphenous artery	NT quantification	facilitation	P3	131
guinea pig saphenous	epj	inhibition	P2	138
rat atria	NT quantification	inhibition	P2	54
rat kidney	NT quantification	inhibition	P2	139
rat pancreas	NT quantification	inhibition	P2	140
guinea pig ileum	NT quantification	facilitation	P2X	121
rat iris	NT quantification	inhibition	P2Y	127
bovine chromaffin cells	NT quantification	inhibition	P2	141
	membrane capacitance	inhibition	P2Y	53
chick glanglia neurons	NT quantification	facilitation	P2Y	132,133
rat ganglia neurons	NT quantification	facilitation/inhibition	P2X/P2Y	52

Presynaptic effects of ATP on neurotransmitter release in the peripheral nervous system. epj, excitatory junction potential; epp, endplate potential; epsc, excitatory postsynaptic current; mepp, mini epp; NMJ, neuromuscular junction; sepp, spontaneous epp; NT, neurotransmitter.

ported by the observation that P2X receptor activation triggers an inward current in cholinergic nerve terminals of chicken ciliary ganglia [48]. In conclusion, ATP appears to be able to induce both inhibition and facilitation of acetylcholine release from smooth muscle nerve terminals, although only one of the first papers on ATP modulation of acetylcholine release has reported biphasic effects of an ATP analogue on the modulation of acetylcholine release from autonomic nerve endings [118].

Noradrenaline

Since a co-transmitter role for ATP was first recognised in the sympathetic system and there is a strong α_2 receptor-mediated automodulatory system, it was reasoned that P2 receptors might also fulfil an automodulatory role in noradrenergic nerve terminals [123]. Initial

studies showed that ATP [124] and ATP analogues inhibit the evoked release of noradrenaline, but it was not clear if this action involves the activation of either P2Y-like receptors [125–129], adenosine A₁ receptors [130] or a proposed mixed ATP/adenosine receptor, named P3 receptor [55–57,131]. Other studies (Table 1) showed that activation of P2-like receptors facilitates evoked release of noradrenaline, and this action was proposed to involve either ‘atypical’ P2Y-like receptors [132,133] or P2X receptors [121]. The proposal of P2Y receptor involvement was mostly based on the observation that the effects were mimicked by 2-methyl-thio-ATP, which was considered a selective P2Y receptor agonist [42], before the realisation that its catabolism by ecto-nucleotidases limits its potency as a P2X receptor agonist [reviewed in 134]. Also, the proposal of the involvement of P2X receptors relied on the effect of α,β -methylene ATP, the only tool thought to be selective for P2X receptors [42]. A recent study in the rat cervical ganglia showed, using G protein modifiers and direct measurement of intracellular free calcium concentration, that the presynaptic effect of ATP may be biphasic, acting via inhibitory P2Y-like receptors and via facilitatory P2X-like receptors [52], thus reconciling the different conclusions reached in previous studies in other sympathetic preparations (Table 1).

ATP as a presynaptic modulator in the CNS

Acetylcholine

The first study that distinguished between ATP- and adenosine-mediated inhibition of evoked acetylcholine release in the CNS was performed in rat cerebral cortical synaptosomes [142] (Table 2), confirming a previous suggestion that ATP might inhibit acetylcholine release from cortical slices [143]. An adequate presynaptic model was used (i.e. synaptosomes) [see 144], ATP analogues inhibited evoked acetylcholine release more potently than adenosine itself, and the effect of ATP was not prevented either upon blocking the extracellular formation of ATP-derived adenosine or upon removal of extracellular adenosine [142]. The same study showed (Table 2) that, in contrast to what occurs in cholinergic cortical nerve terminals, the ATP-mediated inhibition of acetylcholine release from rat hippocampal nerve terminals is mediated by adenosine [142]. However, the type of receptor(s) involved in the effect of ATP on acetylcholine release from rat cerebral cortical synaptosomes remains to be defined [142].

Noradrenaline

As observed for the modulation of noradrenaline release from peripheral preparations, ATP might also modulate in a biphasic manner the release of noradrenaline in the CNS (Table 2). Thus, it has been reported that P2Y-like receptor activation inhibits noradrenaline release from rat cerebral cortical [126] and hippocampal slices [145], and there is a hint that P2X receptor activation may facilitate noradrenaline release in the rabbit cerebral cortex [130].

Dopamine

The P2 receptor-control of dopamine release has been investigated in the striatum (Table 2). Using a microdialysis approach, it was concluded that ATP induces dopamine release [146,147] but it was not determined if this effect of ATP is on the nerve terminals. An opposite effect of ATP was observed in rat neostriatal slices where P2 receptor activation inhibits dopamine release [148].

Table 2

Brain preparation	Measured effect	ATP effect	Proposed receptor	Reference
<i>Acetylcholine</i>				
Rat cortex	NT quantification	inhibition	P2	142
Rat hippocampus	NT quantification	inhibition	P1	142
<i>Noradrenaline</i>				
Rabbit cortex	NT quantification	inhibition	P1	130
Rat cortex	NT quantification	inhibition	P2Y	126
Rat hippocampus	NT quantification	inhibition	P2	145
<i>Dopamine</i>				
Rat striatum	NT quantification	facilitation	P2	146,147
Rat striatum	NT quantification	inhibition	P2	148
<i>Serotonin</i>				
Rat cortex	NT quantification	inhibition	P2	149
Rat hippocampus	NT quantification	facilitation	P2X	150
<i>Peptidergic</i>				
Rat hypophysis	Ca/peptide quantification	facilitation	P2X ₂	152
Rat hypophysis	peptide quantification	inhibition	P2	153
<i>Glutamate</i>				
Rat hippocampus	epsp	inhibition	P1	27,28,156
Rat hippocampus	epsp	inhibition	P2Y/P3	157
Rat hippocampus	epsp	facilitation	ecto-protein kinase	158
Rat hippocampus	Ca/NT quantification	inhibition	P2Y	163
Rat hippocampus	epsc	inhibition	P2	162
Rat cortical slices	NT quantification	inhibition	P2	164
Rat spinal cord	epsc/mepsc	facilitation	P2X	159,160
Brain stem	mepsc/NT quantification	facilitation	P2X	161
<i>GABA</i>				
Rat hippocampus	ipsc	facilitation	P2	162
Rat hippocampus	NT quantification	no effect	—	165
Rat spinal cord	mipsc	facilitation	P2X ₂	166
<i>Glycine</i>				
Rat spinal cord	mipsc	facilitation	P2X ₂	166,167

Presynaptic effects of ATP on neurotransmitter release in the CNS. epsc, excitatory postsynaptic current; epsp, excitatory postsynaptic potentials; ipsc, inhibitory postsynaptic current; mepsc, mini epsc; mipsc, mini ipsc.

Serotonin

It was reported that activation of P2 receptors inhibits serotonin release from rat brain cortical slices [149]. As for the control of the release of other neurotransmitters, the effect of ATP may be biphasic (Table 2), since a likely presynaptic P2X receptor-mediated facilitation of serotonin release was observed in the rat hippocampus [150]. However, these studies in CNS preparations rely on a pharmacological rather than biochemical characterisation which, given the unavailability of selective tools to distinguish between P2Y and P2X receptors [40], only allow tentative conclusions. Also, the use of slices makes it difficult to distinguish between direct presynaptic P2 receptor modulation *versus* indirect effects, a

problem solved when using an isolated presynaptic model, i.e. synaptosomes [144] or neurite preparations [53,151].

Vasopressin

In the rat neurohypophysial terminals, the ATP-mediated modulation of the release of vasopressin was also studied by two different groups with opposite conclusions being reached (Table 2): in one of the studies a P2X-like-mediated facilitation was reported [152], whereas in the other a P2 receptor-mediated inhibition was observed [153].

Glutamate

The study of the effects of ATP on glutamatergic synaptic transmission has been hampered by the rapid metabolisation of ATP into adenosine [25,27,28], since adenosine causes an intense inhibition of excitatory synaptic transmission in different CNS areas [reviewed in 154]. This lead to the conclusion that if ATP has any effect on glutamatergic synaptic transmission, it is mediated by adenosine and should not involve P2 receptor activation [27,28,155,156; but see 157]. The analysis of the effects of ATP were further complicated by the proposal that the effects of ATP as such on plasticity-like phenomena in glutamatergic transmission were due to ATP-driven ecto-protein kinase activity [158]. However, more recent studies have convincingly demonstrated a P2X-like-mediated increase of glutamate release (Table 2), measured as an increase in frequency of mini excitatory postsynaptic currents in primary sensory afferents in the spinal cord [159,160] and in the brain stem [161]. Other electrophysiological studies in cultured rat hippocampal neurons have suggested that ATP may presynaptically inhibit glutamate release [162], a conclusion reinforced by the ability of ATP to inhibit the evoked release of glutamate in rat hippocampal neurons [163] and in cortical slices [164]. As was noted for modulation of noradrenaline and acetylcholine release from peripheral preparations, it appears that ATP may also biphasically modulate glutamate release in the CNS (Table 2).

GABA

The different effects of ATP reported in neurochemical or electrophysiological studies performed in slices or neuronal cultures could be due to opposite or compensating effects of ATP on glutamatergic and GABAergic systems. In rat cultured hippocampal neurons, ATP and ATP analogues enhance GABAergic transmission (Table 2), but not the effects of iontophoretically applied GABA, in a PPADS-sensitive manner [162]. However, the observation that ATP and ATP analogues failed to modify GABA release from superfused hippocampal synaptosomes [165], raises the question of whether the ATP-induced increase in GABAergic transmission reported in hippocampal neurons might be secondary to a decrease in ATP-evoked glutamate release [162]. In a neuronal culture from the spinal cord, the release of GABA, assessed by mini inhibitory postsynaptic currents or evoked inhibitory postsynaptic currents, is facilitated by ATP in 22% of the synapses, an effect proposed to be mediated by P2X₂ receptors [166]. The same study also reports that in 9% of glycinergic synapses in this spinal cord preparation were also under P2 receptor-mediated facilitatory influence [166] (Table 2).

Intracellular calcium

Since glutamatergic and GABAergic terminals account for nearly 90% of CNS nerve terminals, the monitorization of the effects of ATP on calcium influx in CNS nerve terminals should mainly reflect effects of ATP on GABAergic and/or glutamatergic nerve terminals.

Several studies have reported that ATP increases intracellular free calcium concentration [152,168]. This effect strictly depends on extracellular calcium and, thus, was proposed to be mediated by a P2X-like receptor.

Concluding remarks

It is now possible to set-up a general working hypothesis in which ATP may have biphasic presynaptic neuromodulatory effects: an inhibitory effect through activation of P2Y receptors and a facilitatory effect via activation of P2X receptors (Figure 1). This idea of ATP exerting a main role as a presynaptic neuromodulator still requires further evidences in the CNS. This demands the use of appropriate models to study presynaptic function, like the synaptosomes [144], although modulation through action potential propagation cannot be recorded, or neurite preparations [52,151], which cannot ascribe an effect to nerve terminals. It will also be necessary to exclude adenosine (A_1 , A_{2A} and the still ill-defined A_3) receptor involvement [29], and to use biochemical analysis to support a presynaptic localisation of P2 receptors using radioligands [e.g. 88] and immunological approaches [79–86], mainly using electron microscopy immunocytochemistry [82,84,86]. It will also be important to test whether the presynaptic role of ATP is indeed mediated by P2-receptor activation, or whether ATP is mostly acting as a phosphate donor for ecto-protein kinase modification of presynaptic proteins involved in the control of neurotransmitter release [see 158] (Figure 1).

The existence of an ATP-mediated auto-modulatory system allows ATP to join the signaling pattern of most neurotransmitters, such as glutamate, GABA, acetylcholine, noradrenaline or serotonin. These neurotransmitters possess both post-synaptic and pre-synaptic receptors [reviewed in 169]. It is interesting that purines display a double presynaptic neuromodulatory system: one mediated by ATP and another by adenosine. ATP is stored in vesicles and can be released by exocytosis, whereas adenosine is neither stored in vesicles nor released as a classical neurotransmitter, i.e. via exocytosis. So adenosine is mainly a neuromodulator whereas

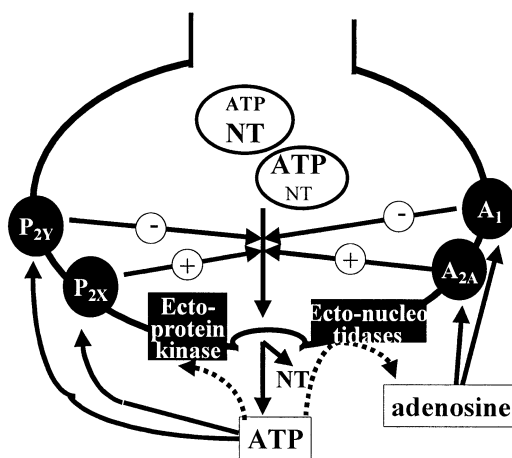


Fig. 1. Purinergic (ATP and adenosine) presynaptic modulation of neurotransmitter release.

ATP can also behave as a neurotransmitter, in conformity with the initial idea of Burnstock [16]. One may consider that these two different purinergic systems work in different time scales and are eventually not functionally interconnected. Thus, the high catalytic efficiency of extracellular ATP catabolism [24], would rapidly shut down ATP signalling, generating, upon further time-delayed catabolism, another signalling molecule, adenosine. An alternative hypothesis is that the activity of the two purinergic systems may be highly inter-dependent, and the ecto-nucleotidase cascade would assume a key role in balancing the action of these two neuromodulatory systems. This possibility has previously been addressed [26,30] and is further supported by the marked extracellular gradient of adenosine concentration at the synaptic level [37] and by the intense control of presynaptic ATP receptors by adenosine receptor activation (Diaz-Hernandez, Pereira, Pintor, Cunha and Miras-Portugal, unpublished observations). The critical role of ecto-nucleotidases in determining the relative importance of the two purinergic neuromodulatory systems is emphasised by the observation that different enzymatic activities are found in different preparations, with conversions of ATP into adenosine ranging from milliseconds to minutes [cf. 25, 170] and that ecto-nucleotidases are released upon stimulation of preparations, such as vas deferens [171,172] or vascular endothelial cells [173]. However, we were unable to detect the release of ecto-nucleotidase activities either from hippocampal slices or synaptosomes [28,174] and to date no molecular identified ecto-ATPase activity expressed in cell systems has been reported to be released (Zimmermann, personal communication). Another possible reason for the existence of two different purinergic neuromodulatory systems may reside in their different role, ATP being an auto-modulatory system (i.e. acting on the nerve terminal from where it is released), whereas adenosine would mostly fulfil an hetero-modulatory role (i.e. acting also in neighbouring neurons from where it is generated). It is hoped that as awareness may grow on the presynaptic role of ATP and on the strict need to distinguish ATP from adenosine-mediated presynaptic effects, it will be possible to start unveiling the relative roles and relation of these two presynaptic purinergic neuromodulatory systems.

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