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Minireview

ATP – a fast neurotransmitter

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ATP receptor-mediated responses in peripheral and central neurones have many characteristics which suggest that ATP may act as a fast neurotransmitter. While the receptors underlying these responses have properties which are similar to other ligand-gated ion channels which mediate fast neurotransmision, the nature of their calcium permeability and the rapid breakdown of ATP to adenosine may confer unique properties on ATP mediated synaptic transmission. The evidence that ATP acts as a fast neurotransmitter is reviewed and the properties of ATP and its receptor channels are discussed in terms of synaptic transmission.

ATP receptor; Patch clamp; Synaptic transmission; Neurotransmitter

1. INTRODUCTION

Our recent demonstration of ATP-mediated synaptic currents in slices of the rat medial habenula [1] confirmed an accumulation of indirect evidence, that ATP acts as a fast transmitter in the mammalian brain. A few months before our report, two other groups reported ATP-mediated synaptic currents measured in mammalian neurones cultured from coeliac ganglia [2,3]. Although there is considerable evidence for ATP acting as a transmitter or cotransmitter at neuroeffector junctions in the periphery (for reviews see [4,5]) these three reports provided the first direct evidence that ATP acts as a fast transmitter between neurones. This review will address the action of ATP as a fast transmitter between neurones and the features of ATP and its receptor/channels which may give such a synapse properties different from other synapses in the central nervous system.

2. HISTORICAL BACKGROUND

ATP is present in all mammalian neurones due to its well known role as a major energy carrier for cellular metabolism. The ubiquitous nature of the molecule and the fact that it is rapidly hydrolysed to adenosine by ectonucleotidases in the extracellular space, have made other functions difficult to identify. Various reports have, however, been suggestive of a neurotransmitter role for ATP. In 1978, White [6] developed a technique for specific detection of ATP release using the ATP-induced light response of firefly luciferin and luciferase. He was able to detect release of ATP from rat brain synaptosomes, which resembled neurotransmitter release in that it was potassium and calcium sensitive. In the following years he and his colleagues were able to demonstrate similar release of ATP from synaptosomes taken from specific brain areas, particularly corpus striatum and cortex. They also presented evidence that most of the cortical release of ATP was not correlated with acetylcholine release as seems often to be the case in the periphery.

The first direct evidence for extracellular actions of ATP in the central nervous system came from Jahr and Jessel [7] who recorded inward currents in response to ATP application in a subpopulation of dorsal horn neurones. Earlier in the same year Krishtal and colleagues reported large inward currents in response to ATP application in neurones from a wide variety of sensory ganglia [8]. These early reports were followed by evidence of both membrane ionic current responses and changes in neurone action potential firing patterns measured in a variety of preparations. These included: increases in excitability of a specific subpopulation of *spinal cord* neurones when ATP was applied locally in vivo in cats [9,10] or rats [11]; neurone depolarisation recorded with intracellular electrodes and increased frequency of spontaneous action potentials recorded extracellularly in slices of rat *locus coeruleus* [12]; a range of effects in rat *hippocampal slices* from inward currents and increased frequency of glutamatergic EPSPs [13], to no effect on orthodromic spikes [14], to a decrease in extracellularly measured unit spikes [15] and finally increased release of ATP in response to tetanic, but not low frequency stimulus [16]. Recently, using extracellu-

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lar recordings, Day and colleagues have reported an activation of vasopressin secreting *supraoptic neurones* in response to stimulation of the vagus nerve in anaesthetised rats which was blocked by the P_2 ATP receptor antagonist suramin [17]. Thus although many studies have been published in the last decade which are suggestive of a neuromodulatory or neurotransmitter role for ATP, most of the parameters measured relate to the properties of complex neural networks. These often have multiple interacting features, always with pre- and postsynaptic components and sometimes with possible adenosine mediated effects included. Therefore, although suggestive, no definite conclusion could be made from these studies as to whether ATP acts as a fast synaptic transmitter in the brain.

3. RECEPTOR PHARMACOLOGY

Before dealing with the evidence for and implications of ATP as a fast neurotransmitter it is useful to describe the basic aspects of ATP receptor pharmacology. Purinoceptors are generally classified as P_1 or P_2 receptors [18]. P_1 receptors are adenosine receptors and are blocked by methylxanthines. These can be clearly distinguished from P_2 receptors which are ATP receptors. The P_2 receptors are not antagonized by methylxanthines but are blocked by the trypanocidal agent suramin originally characterized by Dunn and Blakely [19]. Within both receptor groups there are clearly multiple receptor subtypes (for a review see [20]). The ATP receptors have been sub-divided into P_{2T}, P_{2X}, P_{2Y} and P_{2Z} [21] and even within this classification it is quite possible that there is significant heterogeneity. Of these subtypes the P_{2X} and P_{2Y} receptors are most likely to be involved in neurotransmission postsynaptically. There is, so far, little evidence as to whether P_2 receptors are located presynaptically whereas adenosine P_1 receptors mediate inhibition of transmitter release [4].

Largely from work in the periphery, P_{2X} and P_{2Y} receptors have been defined according to their relative sensitivity to different agonists. By this method of defining the receptors, P_{2X} receptors have an agonist sensitivity order of α,β-methylene ATP > ATP > 2-methylthio-ATP while the order for P_{2Y} receptors is reversed. P_{2Y} receptors will not be further considered here, though it is interesting to note that the ligand gated ion channels in ganglia [22,23,24], and locus coeruleus [12,25] have some aspects of the pharmacology of P_{2Y} receptors leading Illes and Nörenberg [26] to suggest that neuronal P_{2Y} receptors should be subdivided into $P_{2Y\alpha}$ (ligand-gated ion channels) and $P_{2Y\beta}$ (G-protein coupled receptors). We would suggest a functional definition such that P_{2X} receptors are ligand-gated ion channel receptors while the P_{2Y} receptors are members of the family of G-protein coupled receptors. Pharmacological differences would then lead to subtypes within these classes. These two receptor types are thus likely to mediate quite different forms of synaptic transmission. The ion channels formed by the ATP receptors (P_{2X}, by our definition) which have been studied so far are non-selective cation channels [5,23,25] and so are likely to have characteristics similar to several other receptor-channels mediating fast excitatory synaptic transmission. In contrast, synaptic transmission mediated by P_{2Y} receptors is likely to be neuromodulatory, reflecting the slower and more diverse functions performed by the G-protein coupled receptors.

4. IONIC AND CONDUCTANCE PROPERTIES OF ATP RECEPTOR/CHANNELS

Both single channel and whole cell currents carried via ATP-gated ion channels have been studied in various preparations. This work was recently well reviewed [5] and the details of the characteristics of the conductance under different conditions described. We will only include a very brief summary of the points which might be important for ATP-mediated synaptic transmission.

The ligand gated ion channels, activated from the extracellular surface by ATP have been shown to be cation selective and to show marked inward rectification such that very little outward current flows at positive potentials [23,24]. In autonomic ganglion cells [22,23,3] the channels show very little selectivity between cations allowing all small monovalent cations (e.g. Na^+, K^+, Cs^+) to pass approximately equally. The Ca^{2+} permeability is also high in many cases [24,27,28,29] and it is interesting to note that in sympathetic ganglion neurones the ATP response (and Ca^{2+} influx) is potentiated by micromolar concentrations of Zn^{2+} [24]. Despite a high permeability to calcium, calcium ions also partially block the flow of sodium through the channel, decreasing the apparent conductance at both the whole cell and single channel level [28,29,30,31]. In this respect the ATP receptor is like the NMDA receptor. However, NMDA channels are blocked in a voltage-dependent manner by magnesium ions so that calcium influx through NMDA channels is dependent on the membrane voltage. In contrast, calcium influx through ATP receptor-channels would not be expected to be voltage dependent and so may occur even in the absence of cell depolarization. Moreover, the channels show strong inward rectification [23,27,28] and both the rectification and the driving force for calcium will result in maximal influx of calcium at more negative membrane potentials. The significance of these properties in terms of synaptic transmission will be discussed below.

5. ATP AS A FAST TRANSMITTER BETWEEN NEURONES CULTURED FROM GUINEA PIG COELIAC GANGLIA

The first synaptic currents reported to be mediated

by ATP were in cultured coeliac ganglion cells. Two groups [2,3] demonstrated that synaptic currents between these cells in culture were blocked by suramin and mimicked by ATP and α, β-methylene-ATP. These results demonstrated clearly that ATP could act as a transmitter between neurones. Moreover, Silinsky and colleagues [23] recently reported evidence that ATP receptor mediated synaptic currents also occur in intact ganglia.

6. ATP AS A FAST TRANSMITTER IN THE CENTRAL NERVOUS SYSTEM

Previously the only well characterised fast excitatory transmitter in the mammalian brain had been glutamate. Using the whole cell patch clamp technique in fresh brain slices [1], we were able to record both miniature and evoked synaptic currents which were blocked by the competitive ATP receptor antagonist suramin and also by the desensitising ATP receptor agonist α,β-methylene ATP (Fig. 1). This suggested that ATP was the transmitter mediating these synaptic currents. Supporting this conclusion further, we used a combination of agonist-evoked whole cell currents and bath application of other receptor antagonists to eliminate the possibility that any of the known fast transmitters other than ATP, could be mediating the suramin sensitive currents. The same cells also featured glutamate and GABA-mediated synaptic currents which could be distinguished both kinetically and pharmacologically from the ATP-mediated currents.

7. IMPLICATIONS OF ATP RECEPTOR/CHANNEL PROPERTIES FOR SYNAPTIC TRANSMISSION IN THE BRAIN

Subsynaptic ATP receptor/channels in the habenula nucleus probably have similar properties to those studied in other preparations. If this is the case, ATP would act synaptically as a fast excitatory transmitter. The likelihood that they have significant calcium permeability would have particular implications for ATP-mediated synaptic transmission as can be seen by comparison with the role of NMDA receptors in glutamate-mediated synaptic transmission.

The *N*-methyl-D-aspartate subtype of glutamate receptor/channel (NMDA receptor/channel) which is present in many central excitatory synapses has attracted much interest because of its role in both excitotoxicity and a form of synaptic plasticity known as long term potentiation (LTP) which is a model for the processes of learning and memory in the brain. Both these phenomena depend on the fact that NMDA receptor-channels are permeable to calcium. However, the NMDA receptor is blocked in a voltage-dependent manner by extracellular Mg^{2+} ions and therefore Ca^{2+} influx through NMDA channels is voltage-dependent, occurring only if the cell is depolarised. In contrast, the current through ATP receptor channels is not blocked

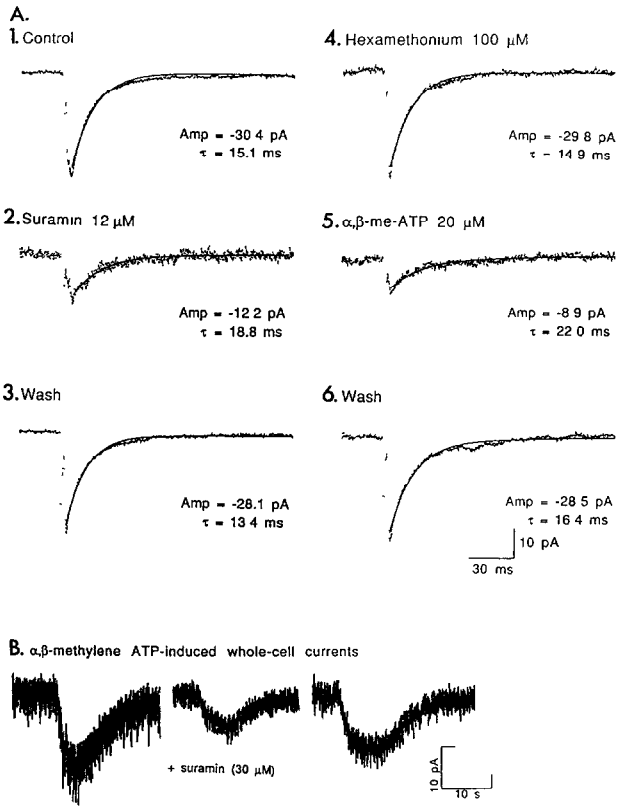

Fig. 1. Pharmacology of ATP receptor-mediated currents in the medial habenula. (A) Control currents (1) and block of currents by the P_2 receptor antagonist suramin (2) followed by washout of the suramin (3). Application of the nicotinic antagonist hexamethonium had no effect on these synaptic currents. (4) After wash-out of hexamethonium the desensitizing P_2 receptor agonist α,β-methylene ATP was shown to block the currents partially (5) followed by recovery on washout (6). (B) Block of α,β-methylene ATP induced whole-cell currents by suramin in another medial habenula cell. The traces show, from left to right, a control response to pressure application of α,β-methylene ATP (300μM) followed by application of α,β-methylene ATP (300μM) plus suramin (30μM) and recovery of the response to α,β-methylene ATP alone (Methods are described in [1]; figure adapted from Fig. 2 of [1]).

by Mg^{2+} and thus calcium dependent phenomena could, in this case, be mediated directly by an excitatory response, even if concurrent inhibitory activity prevented depolarisation. This would be the first synaptic mechanism discovered in the brain in which calcium could enter the cell without prior depolarisation. In addition to these postsynaptic effects, the calcium permeability of ATP receptors could result in presynaptic enhancement of transmitter release, if presynaptic ATP receptors are present. Such an effect would be very transient, as ATP is rapidly hydrolysed.

8. IMPLICATIONS OF THE RAPID BREAKDOWN OF ATP TO ADENOSINE FOR SYNAPTIC TRANSMISSION IN THE BRAIN

The fact that hydrolysis of ATP, rapidly results in the

formation of adenosine at the synapse brings forward another interesting possibility. In the mammalian central nervous system (CNS), adenosine has been shown to be an inhibitory neuromodulator, both pre- and postsynaptically (for reviews see [32,33]). Presynaptically, adenosine has been shown to inhibit release of various transmitter substances, including glutamate and GABA while postsynaptically its inhibitory actions are mediated via adenylate cyclase causing the opening of potassium channels. Thus if the neurones in the medial habenula also have adenosine receptors, it is likely that release of ATP may result first in fast but transitory excitation, followed by a delayed and longer lasting inhibition. This would be a novel phenomenon, with release of an active substance having a specific transient effect and that same molecule on metabolism to another active substance causing subsequent effects via completely different mechanisms.

9. CONCLUSIONS

It seems clear that ATP receptors mediate neuronal synaptic transmission, at least in cultured coeliac ganglia and in the rat medial habenula nucleus . Although the role of these synapses is not yet clear it is interesting in the light of the properties of the channels and of ATP itself to speculate on possible unique features of ATP receptor-mediated synaptic transmission. It may be that ATP-mediated synaptic currents have a key role to play in allowing local influx of calcium into the cell. This would occur even during intense activity of inhibitory inputs such that depolarisation of the membrane was completely prevented. Furthermore ATP could act as a dual transmitter, causing brief excitation followed by inhibition both pre and postsynaptically due to its breakdown product, adenosine.

REFERENCES

[1] Edwards, F.A., Gibb, A.J. and Colquhoun, D. (1992) Nature 359, 144–146.
[2] Silinsky, E.M., Gerzanich, V. and Vanner, S.M. (1992) Br. J. Pharmacol. 106, 762–763.
[3] Evans, R.J., Derkach, V. and Surprenant, A. (1992) Nature 357, 503–505.
[4] Hoyle, C.H.V. and Burnstock, G. (1991) in: Adenosine in the Nervous System, (Stone, T.W. ed.) Academic Press, London.
[5] Bean, B. (1992) Trends Pharmacol. Sci. 13, 87–90.
[6] White, T.D. (1978) J. Neurochem. 30, 329–336.
[7] Jahr C.E. and Jessel, T.M. (1983) Nature 304, 730–732.
[8] Krishstal, O.A., Marchenko, S.M. and Pidoplichko, V.I. (1983) Neurosci. Lett. 35, 41–45.
[9] Fyffe, R.E.W. and Perl, E.R. (1984) Proc. Natl. Acad. Sci. USA 181, 6890–6893.
[10] Salter, M.W. and Henry, J.L. (1985) Neuroscience 115, 815–825.
[11] Sun, M-K.,Wahlstedt, C. and Reis, D.J. (1992) Eur. J. Pharmacol. 224, 93–96.
[12] Tschöpl, M., Harms, L., Nörenberg, W. and Illes, P. (1992) Eur. J. Pharmacol. 213, 71–77.
[13] Inoue, K., Nakazawa, K., Fujimori, K., Wotano, T., Takanaka, A. (1992) Neurosci. Lett. 134, 215–218.
[14] Stone, T.W. and Cusack, N.J. (1989) 97, 631–635.
[15] Di Cori, S. and Henry, J.L. (1984) Br. Res. Bull. 13, 199–201.
[16] Wierasko, A., Goldsmith, G. and Seyfried, T.N. (1989) Brain Res. 485, 244–250.
[17] Day, T.A., Sibbald, J.R. and Khanna, S. (1993) Brain Res. (in press)
[18] Burnstock, G. (1978) in: Cell Membrane Receptors for Drugs and Hormones: A Multidisciplinary Approach, (L. Bolis and R.W. Straub eds.) pp. 107–118, Raven Press, New York.
[19] Dunn, P. and Blakely, A.G.H. (1988) Br. J. Pharmacol. 93, 243–245.
[20] Hoyle C.H. (1992) Autonomic Neuroeffector Mechanisms (Burnstock G. and Hoyle C.H. eds) pp. 367–407.
[21] Burnstock, G. and Kennedy, C. (1985) Gen. Pharmacol. 16, 433–440.
[22] Fieber, L.A. and Adams, D.J. (1991) J. Physiol. 434, 239–256,
[23] Silinsky, E.M. and Gerzanich, V. (1993) J. Physiol. 464, 197–212.
[24] Cloues, R., Jones, S. and Brown, D.A. (1993) Pflügers Arch. (in press).
[25] Shen, K.-Z. and North, R.A. (1993) J. Neurosci. 13, 894–899.
[26] Illes, P. and Nörenberg, W. (1993) Trends Pharmacol. Sci. 14, 50–54.
[27] Bean,B.P., Williams, C.A. and Ceelen, P.W. (1990) J. Neurosci. 10, 11–19.
[28] Krishtal, O.A., Marchenko, S.M. and Obukhov, A.G. (1988) Neuroscience 27, 995–1000.
[29] Nakazawa, K., Fujimori, K., Takanaka, A. and Inoue, K. (1991) 434, 647–660.
[30] Neuhaus, R., Reber, B.F.X. and Reuter, H. (1991) J. Neurosci. 11, 3984–3990.
[31] Ueno, S., Harata, N., Inoue, K. and Akaike, N. (1992) J. Neurophysiol. 68, 778–785.
[32] Greene, R.W. and Haas, H.L. (1991) Prog. Neurobiol. 36, 329–341.
[33] Daval, J.-L., Nehlig, A. and Nicolas, F. (1991) Life Sciences 49,1435–1453.