Neurotransmission by ATP: New insights, novel mechanisms

Parakashtha Ghildyal and Rohit Manchanda*

Biomedical Engineering Group, BJM School of Bioscience and Bioengineering, IIT-Bombay, Powai, Mumbai 400 076

Received 5 April 2002

Purines have long been known for their roles in extracellular signaling. One of the most interesting functions to come to light recently has been the involvement, particularly of adenosine 5'-triphosphate (ATP), as a neurotransmitter in the central and the sympathetic nervous system. ATP is stored in and released from synaptic nerve terminals, like other neurotransmitters, and is known to act post-synaptically via specific rapidly-conducting, ligand-gated ion channels, the P2X receptors. Another interesting feature is the discovery that ATP is widely found to be a "co-transmitter" at the same synapses in combination with other neurotransmitters such as noradrenaline, acetylcholine, and GABA, altering our picture of the biophysics and biochemistry of neurotransmission at these synapses. We describe here these and other aspects of neurotransmission by ATP being investigated vigorously today, including recent findings on P2X receptors and those on the synaptic inactivation of ATP by ecto-ATPase. We conclude by pointing out possible pharmacological and clinical implications of neurotransmission by ATP.

Introduction

ATP, the purine ubiquitously responsible for the storage and provision of metabolic energy in biological organisms, has sprung a few intriguing surprises on the community of biochemists, molecular biologists, and physiologists in recent years. One is that in addition to its extensively investigated intracellular biochemical functions, it has proved to have extracellular physiological activity of several kinds. A second surprise is that provision of energy by cleavage of the high-energy bond of ATP may not be required for many of these newly discovered extracellular functions.

It is now accepted that ATP has diverse extracellular functions and physiological roles to play in different tissues and cell types. Table 1 provides a short list of some of the currently demonstrated and proposed extracellular activities of ATP.

Perhaps the most interesting and unexpected extracellular function of ATP to emerge in recent years has been its postulated release from nerve terminals and action upon target cells in order to transmit signals across synapses, i.e. as a synaptic neurotransmitter. In mediating this function, ATP joins the ranks of neurotransmitter molecules such as the better known acetylcholine (ACh), noradrenaline (NA), serotonin (5-HT), dopamine (DA), and gamma-amino butyric acid (GABA). It was in the late sixties and early seventies, with the discovery and elucidation of "purinergic" nerves, that the possible role of ATP as a neurotransmitter was propounded. Over the ensuing three decades, neurotransmitter functions of ATP have been the subject of intensive scientific scrutiny.

Lately, a further intriguing aspect of this role was propounded. This is the possibility of ATP being released from nerves as a "co-transmitter", i.e. as one of two, or more, substances that mediate neurotransmission concomitantly. The co-transmitters may act independently of each other on target cells, and may modulate the actions of each other noticeably. A co-transmitter role for ATP was first suggested in the autonomic nervous system along with NA and ACh in sympathetic and parasympathetic neurotransmission respectively. More recently, co-transmission by ATP has also been proposed in the central nervous system, prompting the suggestion that it might be of importance in modulating neurophysiological function, extending possibly to mental health and disease.

In this article, we review the evidence for neurotransmission by ATP and its possible functional significance. We shall emphasize those aspects of neurotransmission by ATP that are receiving the most vigorous attention today, namely, (i) novel features of the co-transmitter function of ATP, (ii) the structure and function of the membrane receptors transducing the ATP signal (i.e. the purinergic receptors), and (iii) the enzymatic inactivation of ATP in the synapse.

*Author for correspondence
E-mail: rmanch@cc.iitb.ac.in; Fax: 91-022-5723840
neurons could contain and release more than one neurotransmitter. These findings led Geoffrey Burnstock to suggest neurotransmission by "purinergic nerves" in addition to adrenergic and cholinergic nerves in the enteric nervous system, a postulate that has been confirmed and strengthened since 28,29.

Areas of investigation that offer the most promising avenues for further research will be highlighted.

Early Developments

Ever since its discovery some seventy years ago, in 1929, researchers had started attributing physiological roles to ATP and its non-phosphorylated derivatives. Szent-Gyorgyi and Drury had observed cardioinhibitory and hypotensive actions of AMP. In 1933, Gillespie showed that ATP and its non-phosphorylated derivatives caused relaxation of the guinea pig ileum (in which ATP had the greatest potency among the purines) and coronary vasodilatation in cats and rabbits (in which adenosine was most potent) 8,23,26. These pharmacological studies and other studies conducted subsequently indicated the presence of different receptors for adenosine on the one hand (A$_1$ and A$_2$ adenosine receptors, later to be rechristened as the P1 purinergic receptors) and its phosphorylated derivatives on the other (the family of P$_2$ receptors) 6,27. In the 1950's, it was demonstrated that ATP was released from sensory nerves, strengthening the idea that ATP might act as a neurotransmitter. Subsequently, investigations into the innervations received by the intestines revealed the existence in these tissues of "non-adrenergic, non-cholinergic" (NANC) nerves. Some of these NANC nerves were found to contain ATP as the sole neurotransmitter. These findings led Geoffrey Burnstock to suggest neurotransmission by "purinergic nerves" in addition to adrenergic and cholinergic nerves in the enteric nervous system, and a postulate that has been confirmed and strengthened since 28,29.

Purinergic Transmission and Co-transmission

Before considering the features of purinergic neurotransmission and co-transmission in detail, it is necessary to satisfy ourselves that ATP does function as a neurotransmitter at synapses. There is broad agreement on the criteria that need to be satisfied for a substance to be accepted as a neurotransmitter 22, as follows:

(i) The proposed neurotransmitter must be synthesized and stored within the neurons in question.
(ii) Electrical stimulation of the nerve should result in the release of the putative neurotransmitter.
(iii) The released chemical must act on a demonstrable postsynaptic receptor to produce its biological effects.
(iv) External application of an agonist should produce postsynaptic responses similar to those brought about by the putative neurotransmitter. Conversely, receptor antagonists should inhibit the actions of the neurotransmitter in a dose-dependant manner.
(v) The presence of an effective mechanism for the removal

### Table 1: Physiological actions of extracellular ATP

<table>
<thead>
<tr>
<th>Actions of ATP</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibition of platelet aggregation</td>
<td>Soslau &amp; Youngprapakorn, 1997$^1$</td>
</tr>
<tr>
<td>Modulation of the immune system</td>
<td>Sperlagh et al., 2000$^2$; Surprenant et al., 1995$^3$</td>
</tr>
<tr>
<td>Cytotoxic and apoptotic effects</td>
<td>di Virgilio, 2000$^4$; Persechini et al., 1998$^5$</td>
</tr>
<tr>
<td>Sound transduction and control of cochlear blood flow</td>
<td>Housley &amp; Thorne, 2000$^6$; Vlajkovic et al., 1998$^7$</td>
</tr>
<tr>
<td>Long-term (trophic) effects in the development and the regeneration of the nervous system</td>
<td>Raleigh et al., 1998$^8$.</td>
</tr>
<tr>
<td>Generation and transmission of nociception</td>
<td>Ding et al., 2000$^9$; Irmich et al. 2001$^{10}$, Ralevic et al., 1998$^8$; Cook &amp; McCleskey, 1997$^{11}$; Kennedy &amp; Leff, 1995$^{12}$</td>
</tr>
<tr>
<td>Endothelium dependent vasodilatation</td>
<td>Sneddon, 1999$^{13}$; Meghji et al., 1995$^{14}$</td>
</tr>
<tr>
<td>Neurotransmission in sympathetic and central nervous system</td>
<td>Robertson et al., 2001$^{15}$; Dunn et al., 2001$^{16}$; Liang et al., 2000$^{17}$</td>
</tr>
<tr>
<td>Semen emission</td>
<td>Burton et al., 2000$^{18}$</td>
</tr>
<tr>
<td>Control of respiration</td>
<td>Thomas et al., 2001$^{19}$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Areas of investigation</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sema emission</td>
<td>Ding et al., 2000$^9$; Irmich et al. 2001$^{10}$, Ralevic et al., 1998$^8$; Cook &amp; McCleskey, 1997$^{11}$; Kennedy &amp; Leff, 1995$^{12}$</td>
</tr>
<tr>
<td>Generation and transmission of nociception</td>
<td>Sneddon, 1999$^{13}$; Meghji et al., 1995$^{14}$</td>
</tr>
<tr>
<td>Neurotransmission in sympathetic and central nervous system</td>
<td>Robertson et al., 2001$^{15}$; Dunn et al., 2001$^{16}$; Liang et al., 2000$^{17}$</td>
</tr>
<tr>
<td>Control of respiration</td>
<td>Thomas et al., 2001$^{19}$</td>
</tr>
</tbody>
</table>
inactivation) of the neurotransmitter following its release from the nerve should be demonstrable.

The evidence for the neurotransmitter function of ATP has been growing in recent years principally along 3 lines. (i) In those nerves and innervated organs where the evidence for the transmitter function of ATP has been strongest historically (e.g. the sympathetic neuromuscular junction of the mammalian vas deferens), the evidence relating to the criteria for a neurotransmitter has been further strengthened and consolidated. (ii) There has been a broadening of the spectrum of organs and tissues in which ATP is suggested to be a neurotransmitter (most strikingly, in the central nervous system i.e., CNS), and also of the transmitters along with which it is suggested to act as a co-transmitter. (iii) There has been an inquiry into the modulatory functions, often subtle, that ATP may perform when acting as a co-transmitter, such as increasing or decreasing the efficacy of its paired transmitter. 

Co-transmission by ATP in the mammalian vas deferens

In organs such as the rodent vas deferens and small arteries that receive sympathetic motor innervation, the first four of the criteria listed above for the establishment of a neurotransmitter role for ATP had been fairly persuasively satisfied by the mid-nineties. The reader may consult recent reviews for an account of this body of work. The most compelling evidence so far has been obtained for the synaptic potentials (in these organs known as the excitatory

![Diagram of synaptic transmission](image)

Fig. 1—Sympathetic excitatory co-transmission by noradrenaline (NA) and ATP in the mammalian vas deferens [The structures shown are not to scale; the neuronal varicosity can be about 1 µm in diameter, while the synaptic cleft (separation between varicosity and smooth muscle membrane) can be only ~20-50 nm wide]
junction potentials, or EJPs), and for the "twitch" or rapid phase of the usually biphasic neurogenic contractions of these organs (the second, slower phase being mediated by NA, see Fig. 2). Briefly, these responses have been shown, using a battery of physiological and pharmacological approaches, to be mediated by neuronally released ATP and not by NA.

In an interesting recent study on the vas deferens, the post synaptic target of ATP, i.e. the P2X1 receptor, was eliminated by creating P2X1 deficient ("knockout") mice. The vasa deferentia from these mice showed a lack of electrical and contractile response to purinergic agonists like ATP, α,β-methylene ATP and 1-β,γ-methylene ATP, while responses to NA were left undiminished. Although the copulatory behaviour in these P2X1-deficient mice remained unaltered, the fertility rates dropped by around 90%. Histological studies showed that the drop in fertility was not due to sperm dysfunction but a reduced sperm count in the ejaculate. These novel findings suggest that P2X1 receptor antagonists could prove to be putative targets for the development of a non-hormonal male contraceptive. Conversely, agents potentiating the release or the action of ATP at these sites might find use in certain forms of male infertility. Thus, modulation of the effects of ATP at various synaptic sites may have not only physiological but also therapeutic significance.

Although there is now a wealth of information on aspects such as release of ATP from nerves and purinergic receptors (see section below), information on the inactivation of synaptically released ATP was relatively slow in coming. Nevertheless, in the last decade or so, a considerable amount of insight has been gained into this aspect as well, and the available evidence is consistent with a neurotransmitter function of ATP. These insights have been facilitated by the recent availability of relatively specific inhibitors of synaptic ATPase, which has made it possible to explore more directly than before the role of this ATPase in modulating neurotransmission. The electrophysiological studies of Sneddon and colleagues, for instance, show that the presumptive ATPase inhibitor ARL 67156 significantly potentiates the synaptic potentials of the guinea-pig vas deferens (the excitatory junction potentials, or EJPs) when applied alone, and restores the amplitude of EJPs pre-inhibited by the P2X antagonist NF023. This study constitutes the most convincing evidence to date for a physiological role.

<table>
<thead>
<tr>
<th></th>
<th>A. BIPHASIC CONTRACTIONS</th>
<th>B. EJPs</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td></td>
<td>2 g</td>
</tr>
<tr>
<td>RESERPINE TREATMENT</td>
<td></td>
<td>20 sec</td>
</tr>
<tr>
<td>α1 ANTAGONIST (PRAZOSIN)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P2 ANTAGONIST (SURAMIN)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 2—Schematic illustration of the effects of reserpine, α1 antagonist and P2 antagonist on neurogenic contractile and electrical responses of guinea pig vas deferens [Reserpine and prazosin, which interfere with noradrenergic mechanisms, cause reduction only in the tonic contraction, but do not abolish the twitch contractions; EJP's are not significantly affected. The P2 antagonist inhibits the twitch contractions as well as EJPs, while tonic contractions remain unaltered. Thick horizontal bar indicates the period of repeated stimulation at 8-10 Hz for ~20 sec to elicit biphasic contractions (shown only for the control). Thin vertical lines under EJPs indicate single brief stimuli (duration ~20-100 μs) delivered to the sympathetic nerve. *:Twitch contraction caused by action of ATP; **:Tonic contraction caused by action of noradrenaline]
of synaptic ATPase in modulating the earliest signals generated in the target organ by purinergic transmission, i.e. the synaptic potentials. In another study, ARL 67156 was found to significantly increase the purinergic phase of the neurogenic contractions of the guinea pig urinary bladder. Furthermore, this potentiated response was abolished in the presence of PPADS (a P_{2X} blocker)\textsuperscript{35}. From these results it could be concluded that post-junctional actions of ATP are normally attenuated by enzymatic degradation and inhibitors of the ecto-ATPase would potentiate these actions. Enzyme inhibition studies have also been carried out using other compounds like Reactive Blue\textsuperscript{36} and Evans Blue\textsuperscript{37}, but these have had limited success due to the cross reactivity of these compounds with the P_{2X} Receptors themselves.

**Transmission and Co-transmission by ATP in other tissues, e.g. CNS**

Until the early to the mid-nineties, the most extensive documentation for neurotransmission by ATP was in the G-I tract\textsuperscript{13} and at the sympathetic neuroeffector junction in smooth muscle\textsuperscript{39,42}. Of late, this documentation has been extended to include a range of other tissues and organs, such as those of the endocrine system\textsuperscript{43} and the CNS\textsuperscript{44}, including brain areas such as the locus coeruleus\textsuperscript{30}, the hippocampus\textsuperscript{45} and the cerebellum\textsuperscript{46}. An especially interesting role to emerge is that in pain perception and modulation, involving multiple types of purinergic receptors\textsuperscript{9,47}. In vivo studies on animals and humans have shown that administration of ATP and adenosine at low concentrations may lower the requirement for post-operative opioid administration for pain relief\textsuperscript{37}.

Another new facet to emerge is that while earlier work indicated co-transmission by ATP mainly in conjunction with catecholaminergic neurotransmitters\textsuperscript{39,44}, recent studies, including those on the CNS, have widened the range of associated transmitters to include amino acids such as GABA, glycine and glutamate (see Dunn, Zhong and Burnstock, 2001\textsuperscript{16}; Robertson et al., 2001\textsuperscript{44} for reviews). Indeed, studies on purinergic co- transmission in the CNS have given rise to interesting insights on how transmitters might interact to produce novel patterns of post-synaptic activation, unobtainable by either transmitter alone. In the hippocampus and cultured spinal neurons, for example, where GABA and ATP may be employed as co-transmitters\textsuperscript{16,44,50}, GABA opens membrane Cl\textsuperscript{−} channels which drive the post-synaptic neuron to hyperpolarized (i.e., more negative) potentials. Although this effect may be viewed from a conventional standpoint as inhibition, the concomitant release and action of ATP transforms the picture. ATP acts on P_{2X} receptors primarily to increase Ca\textsuperscript{2+} permeability and Ca\textsuperscript{2+} influx in the target cell. This influx should also produce, normally, depolarization of membrane potential; however, the simultaneous action of GABA may “clamp” membrane potential at hyperpolarized values such that the depolarizing influence of P_{2X} receptor activation is precluded. The prevailing hyperpolarization increases the driving force for Ca\textsuperscript{2+} through P_{2X} receptor-ion channels, thus augmenting Ca\textsuperscript{2+} influx due to purinergic action (see Fig. 3). In this manner, the biochemical excitatory effects of Ca\textsuperscript{2+} may occur independently of electrical excitation via depolarization; indeed, the former may be augmented while the latter is suppressed owing to the synchronous actions of the co-transmitters on the post-synaptic membrane.

**Membrane Receptors for ATP**

As for all neurotransmitters, specialized membrane-bound receptors transduce the signal carried by ATP. The class of membrane receptors that respond to purines are generally known as “purinergic receptors”, of which the two main are P_{1} and P_{2} receptors. Several subtypes have now been characterized. These are depicted in Fig. 4. However, only a subset of the purinergic receptors serve to transduce the signals carried by ATP as a neurotransmitter. These are the P_{2X} receptors. All the other purinergic receptors are metabotropic receptors i.e. they transmit signals via the biochemical pathways. As opposed to this, P_{2X} receptors are ligand-gated ion channels. Upon binding ATP, the P_{2X} receptor-ion channel complex increases its permeability to cations, within a few milliseconds. This allows the P_{2X} receptors to transmit signals much faster than its metabotropic counterparts. Owing to this property, the P_{2X} receptors are widely utilised in fast synaptic transmission in the CNS and the peripheral nervous system, and offer today a particularly alluring territory for research (for reviews on the other subtypes, see Collo et al. (1997)\textsuperscript{45}; Dunn et al. (2001)\textsuperscript{16}; Stone et al. (2000)\textsuperscript{46}; Bhagwat & Williams (1997)\textsuperscript{47}; Ralevic & Burnstock (1998)\textsuperscript{3}).

**P_{2X} Receptors**

P_{2X} receptors are ligand-gated ion channels, of which the best-known representatives in the nervous system are the nicotinic cholinergic and glutamatergic NMDA receptors. These receptors also function as
ion channels that increase their permeability to specific ions following the binding of the neurotransmitter or of agonists to their binding sites.

Perhaps the most intriguing fact to emerge about \( P_{2\times} \) receptors in recent years is that, despite these similarities with other neurotransmitter receptors, they form an entirely novel class of ligand-gated ion channel receptors, distinct from the two “superfamilies” known earlier to exist. These superfamilies are, a), the “cys-loop” family, which includes the Ach-nicotinic, 5-HT, GABA and Gly receptors; and b), the “glutamate” family, comprising of the NMDA, AMPA and kainate receptors. Whilst members of the “cys-loop” family have five homologous subunits, those of the glutamate family have four. \( P_{2} \) receptors, however, are thought to possess only three homologous subunits, and they also seem to differ in their subunit composition and structure from the other families.

The \( P_{2\times} \) receptors possess several other features that are singular and distinguish them from those of the other receptor families. First, each subunit expresses only two transmembrane domains, while the glutamate and “cys-loop” families have three and four membrane spanning regions, respectively, per subunit. Second, more than 70% of each \( P_{2\times} \) receptor subunit lies in the extracellular space, a fraction much larger than that found for receptor subunits in the other families. This extracellular domain bears the ATP-binding site and the sites for antagonists and modulators. Finally, the overall topography of the \( P_{2\times} \) receptor subunits, i.e. two transmembrane regions, two short amino and carboxy terminals and a large extracellular loop, presents intriguing parallels with proteins that have physiological functions entirely unrelated to those of ligand-gated, receptor-operated ion channels. As a particularly interesting example, this topography is strikingly similar to that proposed for the ecto-ATPase, the enzyme involved in the breakdown of extracellular ATP. Moreover, the \( P_{2\times} \) receptor bears a close similarity to two kinds of non ligand-gated ion channels, namely the inward rectifier potassium channel and the epithelial sodium channel.
**Gating properties of \( P_{2X} \) Receptors**

In keeping with the plethora of receptor subtypes characterized for other kinds of receptors, it now seems that there may also be a large number of \( P_{2X} \) receptors serving different physiological functions. No less than seven \( P_{2X} \) receptor subunits (\( P_{2X1-7} \)), at the last count, have been proposed to exist\(^{56} \). Permutations and combinations of these subunits may give rise to a wide variety of receptor subtypes. It is thought that three (possibly four) \( P_{2X(1-7)} \) subunits are assembled to form the homo/heteromeric ATP-gated \( P_{2X} \) receptor\(^{1,3,2} \). Furthermore, it is suggested that within each of these subunits there may be the possibility of alternative splicing at the mRNA level. We may, therefore, be faced with a bewilderingly large repertoire of \( P_{2X} \) subtypes, possibly with subtle functional differences between them. Fortunately, however, all of them share certain important features in common; one is that \( P_{2X} \) receptors without exception bring about rapid changes in transmembrane electrical conductance (typically on the order of a few tens of milliseconds). They are permeable to \( Ca^{2+} \), and to a lesser extent, to \( Na^+ \) and \( K^+ \). Thus, activation of \( P_{2X} \) receptors can result in depolarization of the membrane potential and elevation of the intracellular \( [Ca^{2+}] \), both of which events can have profound physiological consequences.

Most studies on the gating and kinetic properties of \( P_{2X} \) receptors have been done on recombinant receptors and they have been distinguished from each other on the basis of their pharmacological profiles and receptor kinetics using electrophysiological techniques and by measuring \( Ca^{2+} \) influx\(^{35,54} \). One of the main stumbling blocks in the identification and detailed study of \( P_{2X} \) receptors in native tissues has been the absence of specific receptor antagonists and radio-ligands. For example, pyridoxal-phosphate-6-azophenyl-2',4'-disulfonic acid (PPADS)\(^{56} \) and the erstwhile antihelmintic agent suramin\(^{55} \) have been used experimentally as putative \( P_{2X} \)-specific antagonists despite the fact that they lack the desirable degree of selectivity and specificity. These compounds have been reported to have very specific antagonist activity at some recombinant \( P_{2X} \) receptor subtypes like the \( P_{2X1} \), \( P_{2X2} \) and \( P_{2X3} \) while they are only partial antagonists at other \( P_{2X} \) receptor subtypes\(^{53} \). Furthermore, they may also have significant ecto-ATPase inhibitory activity\(^{58} \), which complicates the interpretation of their effects on physiological responses. The main challenge for the next few years is the synthesis or discovery of specific antagonists.

**Inactivation of ATP**

We conclude our description of purinergic transmission by briefly discussing the inactivation of ATP at the synapse. The activity of neurotransmitters at synapses is usually terminated by specialized mechanisms, such as enzymatic degradation (e.g. of...
acetylcholine by acetylcholine esterase at the somatic neuromuscular junction) and reuptake (e.g., of noradrenaline at sympathetic synapses). For ATP, as in the case for acetylcholine, there is thought to be a specific enzyme responsible for the rapid degradation (within a few milliseconds) of ATP in the synapse following its release from the nerve terminal. The enzyme thought to be involved is the extracellular ecto-ATPase (EC 3.6.1.3)\(^6\).

The idea of an ecto-ATPase for the removal of extracellular ATP was conceived almost 40 years ago, even before the extracellular actions of ATP were proved beyond doubt. Today, its presence has been shown in a variety of tissues and cell types including neurons, glia, smooth muscles and endothelial cells\(^1,3,4,6,2\). At synapses, ecto-ATPase hydrolysates the nerve released ATP shortly after it acts on P\(\alpha\) receptors, degrading it into ADP and inorganic phosphate. Subsequently ADP is degraded into AMP and adenosine by 5'-nucleotidases. Thus, ecto-ATPase limits the time for which ATP persists in the vicinity of the purinergic receptors.

The ecto-ATPases are an entirely different class of enzymes when compared to the better-known intracellular mitochondrial F1-F0 ATPase, surface membrane Na-K ATPase or the sarcoplasmic ATPase. The basis of distinction and characterization of the ecto-ATPases (EC 3.6.1.3) rest on the following features: (i) Activation by Ca\(^{2+}\) and Mg\(^{2+}\) ions; (ii) Nonspecific hydrolysis of different nucleoside tri- and di-phosphates (ATP, however, being the preferred substrate); (iii) Insensitivity to inhibitors of intracellular ATPases (like ouabain, oligomycin, vanadate, etc.)

The ecto-ATPases are glycoproteins present on the extracellular side of the plasma membrane with their active sites uniquely oriented towards the extracellular space in contrast to the other known ATPases\(^5,6\). The biochemical and molecular characterization of the ecto-ATPases as yet remain incomplete, due to the problems associated with obtaining the purified form of the enzyme, i.e., low abundance and its extreme lability during protein isolation procedures.

The evidence for the presence of a degradation enzyme in the synaptic cleft, till lately, was only indirect. It was suggested that the presence of ATP degradation products like ADP, AMP and adenosine in the synaptic cleft stemmed from the sequential breakdown of ATP by the action of an ecto-ATPase\(^2,4\). Similarly, the presence of an ecto-ATPase was indicated by the effect of temperature drop on the synaptic potentials produced by ATP\(^4\). It was observed that cooling from 35°C to 25°C prolonged the ATP evoked EJP significantly, whilst potentials generated by a,β-meATP, an enzymatically stable, non-degradable analog of ATP, were left unaffected.

Over the past few years there has been more direct evidence for the presence and role of an ecto-ATPase in the breakdown of synaptic ATP. The enzyme has been isolated from many tissues and organs, cloned and studied for its activity and substrate specificities\(^5,6\). However, compounds that specifically target the ecto-ATPase and inhibit it are yet to be found. The discovery of such compounds would be central to the study of purinergic transmission since they could help discriminate between the effects of ATP and its metabolites and also to potentiate the actions of ATP, analogous to the way in which cholinesterase inhibitors (e.g., neostigmine) have been crucial to the understanding of cholinergic transmission. There are many difficulties in obtaining specific inhibitors of the ecto-ATPase. The principal difficulty is the structural similarity between the ATP-binding domains of the ecto-ATPase and the P\(\alpha\)-purinoreceptor. Compounds found to block the enzyme are also found to bind to the purinoreceptor to some degree. Suramin is widely used as an agonist of the P\(\alpha\) receptors; studies now show that suramin also binds and inhibits the ecto-ATPase of the guinea pig bladder\(^3,6\). Similarly vital dyes like Trypan Blue, Evans Blue and Reactive Red 2 are found to antagonize the P\(\alpha\) purinoreceptor as well as inhibit the enzyme\(^3,5,6\).

**Recent Developments**

A curious feature of the presence and action of ecto-ATPase has been discovered in recent years, viz., that a second form of the ecto-ATPase may be contained within presynaptic neurotransmitter vesicles\(^6,7\). This enzyme has been termed the “soluble” or the “releasable” ATPase (r-ATPase). Interestingly, stimulation-evoked release of the r-ATPase occurs from the vesicles into the synapse, and it is suggested that the r-ATPase thus released may participate in the hydrolysis of the ATP released concomitantly during neurotransmission, complementing the activity of the synaptic, membrane-bound enzyme. Inactivation of neurotransmitter has so far been known to be mediated either by an enzyme present in the synaptic cleft (as in the case of ACh), or by reuptake of transmitter (as for catecholamines and...
Beyond Physiology

As described above, purinergic neurotransmission has been implicated in the functioning of both the peripheral and the central nervous systems. The question inevitably arises as to whether these physiological findings might lead to applications in the understanding and amelioration of certain disorders. In recent years, a number of studies have suggested that this extension might indeed be possible. For example, an especially interesting role for ATP and related purines to emerge is that in transduction of painful stimulus by nociceptive terminals and modulation of the afferent pain signals at spinal synapses involving multiple types of purinergic receptors\(^9,11,22\). As mentioned above, in vivo studies on animals and humans have shown that administration of ATP and adenosine at low concentrations may lower the requirement for postoperative opioid administration for pain relief via a "pain gating" mechanism\(^7,22\). In the vas deferens, the role of purinergic transmission in contractility has been unequivocally demonstrated (see Section under neurotransmission at synapses, where such mechanisms had not previously been suspected or invoked. An additional singular feature of purinergic neurotransmission is the purinergic P\(_{2X}\) receptor, which forms a third class of neurotransmitter receptors. The topology of the P\(_{2X}\) receptor is unique, with only two transmembrane domains, making it one of the simplest mammalian neurotransmitter-gated ion channels, and the only one with a topology similar to non-neurotransmitter gated ion channels. Thus, consideration of purinergic mechanisms may offer us new standpoints for exploring and analyzing transmission at synapses, where such mechanisms had not previously been suspected or invoked.

Conclusions

It may have surprised many that a molecule like ATP is employed by the nervous system for neurotransmission. However, a close look at the properties of this molecule reveals that the choice of ATP as an extracellular signalling molecule may be teleologically sound, as it turns out to possess the properties desirable in a neurotransmitter. For example, its near absence in the extracellular milieu ensures that small quantities of neuronally released ATP will constitute a distinct recognizable signal (provided highly specific receptors for ATP are present post-synaptically). The small size of this molecule allows rapid diffusion, hence enabling its use as a fast synaptic neurotransmitter. Finally, its high charge density renders it readily labile, providing for efficient regulation of its extracellular activities.

In this review, we have seen how signalling in the nervous system by ATP is mediated and modulated by the concerted actions of the P\(_{2X}\) receptors and the ecto-ATPase. While interfering with the receptors or enzyme might be of potential use in disorders such as urinary incontinence, certain types of male infertility, hypertension and pain perception\(^9,32,70,71\), one of the main stumbling blocks in this development will be the lack of specific agonists and antagonists for these molecules.

In this review we have discussed the new insights being gained into the mechanisms of neural signaling by ATP, strengthening the idea that it may function as a neurotransmitter. An intriguing outfall of these studies is that some of these mechanisms are so novel as to influence our conception of the process of neurotransmission at broader levels. For example, the presence of the transmitter-hydrolyzing enzyme, the ecto-ATPase, in synaptic vesicles along with the neurotransmitter ATP itself suggests novel mechanisms for modulating neurotransmitter activity.

References
GHILDYAL & MANCHANDA: NEUROTRANSMISSION BY ATP