

# Historical review: ATP as a neurotransmitter

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**Purinergic signalling is now recognized to be involved in a wide range of activities of the nervous system, including neuroprotection, central control of autonomic functions, neural–glial interactions, control of vessel tone and angiogenesis, pain and mechanosensory transduction and the physiology of the special senses. In this article, I give a personal retrospective of the discovery of purinergic neurotransmission in the early 1970s, the struggle for its acceptance for ~20 years, the expansion into purinergic cotransmission and its eventual acceptance when receptor subtypes for ATP were cloned and characterized and when purinergic synaptic transmission between neurons in the brain and peripheral ganglia was described in the early 1990s. I also discuss the current status of the field, including recent interest in the pathophysiology of purinergic signalling and its therapeutic potential.**

## Early history

The diverse range of physiological actions of ATP was recognized relatively early. For example, in 1929, Drury and Szent-Györgyi [1] demonstrated the potent extracellular actions of ATP and adenosine on the heart and coronary blood vessels. The published follow-up studies of the cardiovascular actions of purines during the next 20 years were reviewed by Green and Stoner in 1950 in a book entitled 'Biological Actions of the Adenine Nucleotides' [2]. In 1948, Emmelin and Feldberg [3] demonstrated that intravenous injection of ATP into cats caused complex effects that affected both peripheral and central mechanisms. Injection of ATP into the lateral ventricle produced muscular weakness, ataxia and a tendency of the cat to sleep. Application of ATP to various regions of the brain produced biochemical or electrophysiological changes [4]. Holton presented the first hint of a transmitter role for ATP in the nervous system by demonstrating the release of ATP during antidromic stimulation of sensory nerves supplying the rabbit ear artery [5]. Buchthal and Folkow recognized a physiological role for ATP at the neuromuscular junction, finding that acetylcholine (ACh)-evoked contraction of frog skeletal muscle fibres was potentiated by exposure to ATP [6]. Furthermore, presynaptic modulation of ACh release from the neuromuscular junction by purines in the rat was reported by Ginsborg and Hirst [7]. An extensive review

describing the physiological significance, pharmacological action and therapeutic use of adenylyl compounds in humans was published in 1957 by Boettge *et al.* [8]. Subsequently, an influential hypothesis was proposed by Berne [9], who postulated that adenosine was the physiological mediator of the coronary vasodilatation associated with myocardial hypoxia. An alternative hypothesis was proposed later [10], namely that ATP released from endothelial cells during hypoxia and shear stress acted on endothelial P2 receptors, resulting in the release of nitric oxide (NO) and subsequent vasodilatation; adenosine participated only in the longer-lasting component of reactive hyperaemia (increased blood flow). Details of various early studies that reported extracellular roles of ATP are summarized in a historical review published in 1997 [11]. In this article, I review the later research, in which I have been an active participant.

## 'Non-adrenergic, non-cholinergic neurotransmission'

After completing studies of noradrenaline (NA)- and ACh-mediated responses of the guinea-pig taenia coli in Edith Bülbiring's laboratory at the Department of Pharmacology, Oxford [12], I moved to Melbourne, Australia. There, I set up the sucrose gap apparatus in my laboratory for recording continuous correlated changes in electrical and mechanical activity of smooth muscle [13]. Graeme Campbell and Max Bennett were working with me, and one day in 1962 we decided to look at the direct response of the smooth muscle of the guinea-pig taenia coli after blocking the responses of the two classical neurotransmitters ACh and NA. We expected to see contractions in response to direct stimulation of smooth muscle, perhaps associated with depolarization, but remarkably we obtained rapid hyperpolarizations and associated relaxations in response to single electrical pulses. This was an exciting moment and we all felt instinctively that this unexpected result was going to be important. Later we, and others, showed that tetrodotoxin, which had just been discovered in Japan and which blocked nerve conduction but not muscle responses, abolished these hyperpolarizations and we realized that we were looking at inhibitory junction potentials (IJPs) in response to a non-adrenergic, non-cholinergic (NANC) inhibitory neurotransmitter [14,15]. At about the same time, Martinson and Muren [16] in Sweden recognized the existence of NANC inhibitory neurotransmission in the cat stomach. A detailed study of the mechanical responses of the taenia coli to stimulation of intramural NANC and sympathetic nerves was carried

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out while I was on sabbatical leave at the School of Pharmacy in London, working with Mike Rand [17].

### The 'purinergic' hypothesis

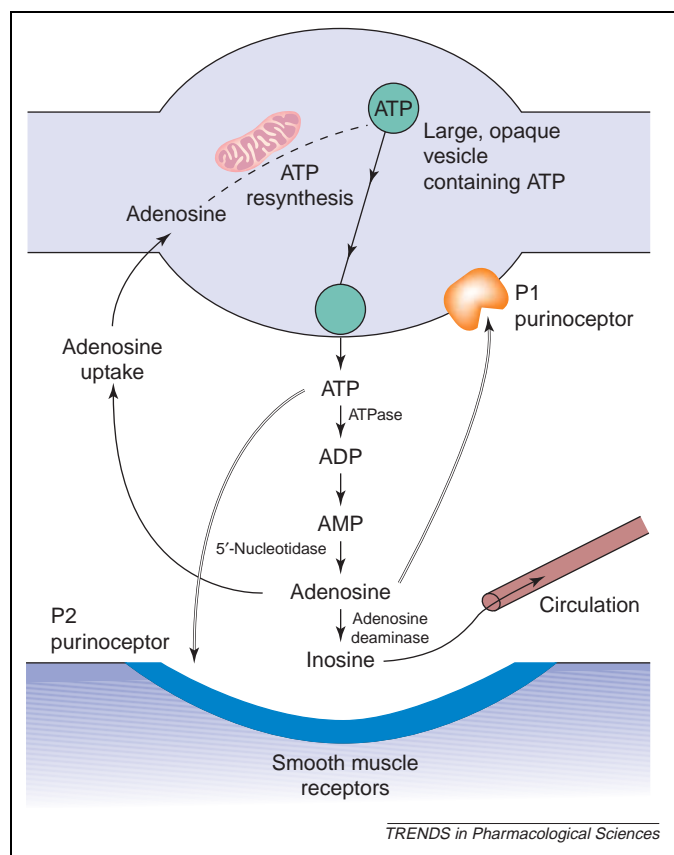
Several years later, after many experiments, we published a study that suggested that the NANC transmitter in the guinea-pig taenia coli and stomach, rabbit ileum, frog stomach and turkey gizzard was ATP [18]. The experimental evidence included: mimicry of the NANC nerve-mediated response by ATP; measurement of the release of ATP during stimulation of NANC nerves with luciferin-luciferase luminometry; histochemical labelling of subpopulations of neurons in the gut with quinacrine, a fluorescent dye known to label selectively high levels of ATP bound to peptides; and the later demonstration that the slowly degradable analogue of ATP,  $\alpha,\beta$ -methylene ATP ( $\alpha,\beta$ -meATP), which produces selective desensitization of the ATP receptor, blocked the responses to NANC nerve stimulation. Soon after, evidence was presented for ATP as the neurotransmitter for NANC excitatory nerves in the urinary bladder [19]. The term 'purinergic' was proposed in a short letter to *Nature* in 1971 and evidence for purinergic transmission in a wide variety of systems was presented in *Pharmacological Reviews* [20] (Figure 1). This concept met with considerable resistance for many years. I believe that this was partly because biochemists

felt that ATP was established as an intracellular energy source involved in various metabolic cycles and that such a ubiquitous molecule was unlikely to be involved in extracellular signalling. However, ATP was one of the biological molecules to first appear and, therefore, it is not surprising that it should have been used for extracellular, in addition to intracellular, purposes early in evolution [21]. The fact that potent ectoATPases were described in most tissues in the early literature was also a strong indication for the extracellular actions of ATP. Purinergic neurotransmission is now generally accepted [22,23] and a volume of '*Seminars in Neuroscience*' was devoted to purinergic neurotransmission in 1996 [24].

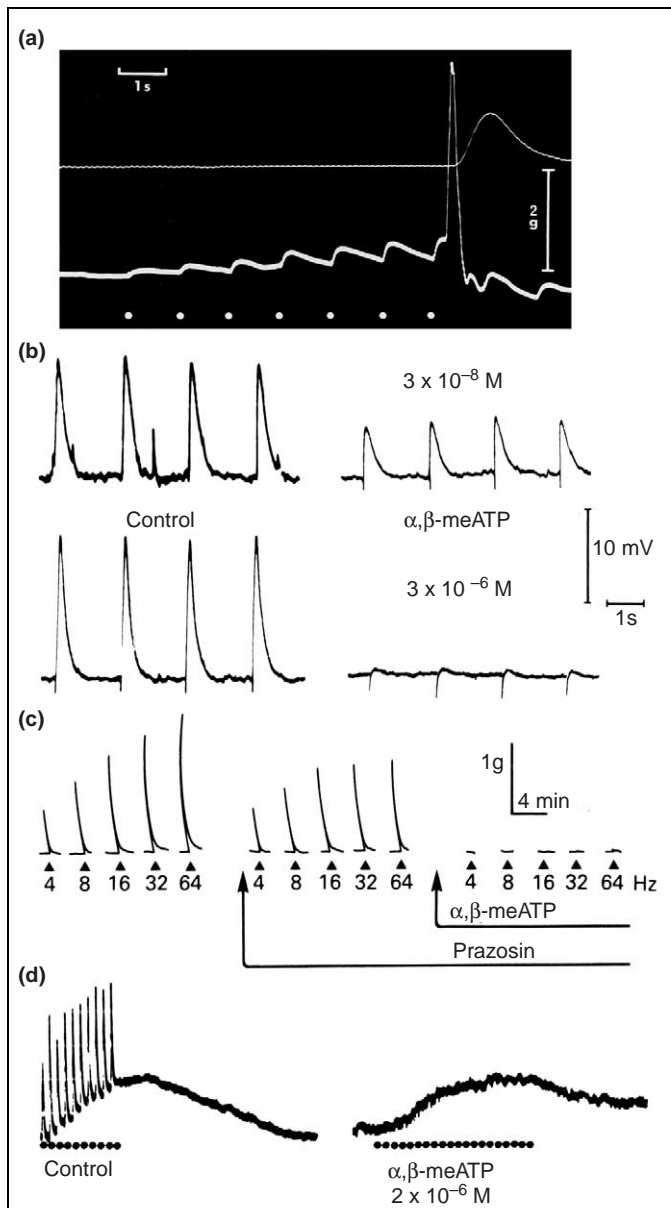
### Purinergic cotransmission

Another concept that has had a significant influence on our understanding of purinergic transmission is cotransmission. I wrote a Commentary in *Neuroscience* in 1976 entitled: 'Do some nerves release more than one transmitter?' [25]; this challenged the single neurotransmitter concept, which became known as 'Dale's Principle', even though Dale himself never defined it as such. The commentary was based on hints about cotransmission in the early literature describing both vertebrate and invertebrate neurotransmission and, more specifically, with respect to purinergic cotransmission, on the surprising discovery by Che Su, John Bevan and me, while I was on sabbatical leave at University of California, Los Angeles in 1971, that ATP was released from sympathetic nerves supplying the taenia coli and from NANC inhibitory nerves [26]. Ironically, when Mollie Holman and I published the first electrophysiological study of sympathetic neuromuscular transmission in the vas deferens [27], we recorded excitatory junction potentials (EJPs) that were not blocked by adrenoceptor antagonists (Figure 2a). It was not until >20 years later, when Peter Sneddon joined my laboratory in London, that we showed that the EJPs were blocked by  $\alpha,\beta$ -meATP (Figure 2b) [28], a compound shown by Kasakov and Burnstock to be a selective desensitizer of P2X receptors [29]. This clearly supported the earlier demonstration of sympathetic cotransmission in the vas deferens in the laboratory of Dave Westfall [30], following the report of sympathetic cotransmission in the cat nictitating membrane [31]. Purinergic cotransmission was later described in the rat tail artery [32] (Figure 2d) and in the rabbit saphenous artery [33] (Figure 2c). NA and ATP are now well established as cotransmitters in sympathetic nerves (Figure 3a), although the proportions of these transmitters vary in different tissues and species, during development and aging and in different pathophysiological conditions [34].

ACh and ATP are cotransmitters in parasympathetic nerves that supply the urinary bladder [35]. Subpopulations of sensory nerves have been shown to use ATP in addition to substance P and calcitonin gene-related peptide; it seems likely that ATP cooperates with these peptides in 'axon reflex' activity. The current consensus of opinion is that ATP, vasoactive intestinal polypeptide and NO are cotransmitters in NANC inhibitory nerves, but that they vary considerably in proportion in different



**Figure 1.** Purinergic neuromuscular junction depicting the synthesis, storage, release and inactivation of ATP. ATP, stored in vesicles in nerve varicosities, is released by exocytosis to act on postjunctional P2 purinoceptors on smooth muscle. ATP is broken down extracellularly by ATPases and 5'-nucleotidase to adenosine, which is taken up by varicosities to be resynthesised and restored in vesicles. Adenosine acts prejunctionally on P1 purinoceptors to modulate transmitter release. If adenosine is broken down further by adenosine deaminase to inosine, it is removed by the circulation. Modified, with permission, from [20].



**Figure 2.** (a) Excitatory junction potentials (EJPs) in response to repetitive stimulation (white dots) of adrenergic nerves in the guinea-pig vas deferens. The upper trace records the tension whereas the lower trace records the electrical activity of the muscle measured extracellularly by the sucrose gap method. Note both the summation and the facilitation of successive junction potentials. At a critical depolarization threshold an action potential is initiated that results in contraction. Reproduced, with permission, from [95]. (b) The effect of various concentrations of  $\alpha, \beta$ -methylene ATP ( $\alpha, \beta$ -meATP) on EJPs recorded from guinea-pig vas deferens (intracellular recordings). The control responses to stimulation of the motor nerves at 0.5 Hz are shown on the left. After at least 10 min in the continuous presence of the indicated concentration of  $\alpha, \beta$ -meATP, EJPs were recorded using the same stimulation parameters. Both concentrations of  $\alpha, \beta$ -meATP resulted in reduced responses to nerve stimulation. Reproduced, with permission, from [28]. (c) Contractions produced in the isolated saphenous artery of the rabbit following neurogenic transmural stimulation (0.08–0.10 ms; supramaximal voltage) for 1 s at the frequencies indicated (triangles). Nerve stimulations were repeated in the presence of 10  $\mu$ M prazosin ( $\alpha_1$ -adrenoceptor antagonist) added before desensitization of the P2 purinoceptors with 10  $\mu$ M  $\alpha, \beta$ -meATP as indicated by the arrows. Reproduced, with permission, from [33]. (d) Intracellular recording of the electrical responses of single smooth muscle cells of the rat tail artery to field stimulation of sympathetic motor nerves (the pulse width was 0.1 ms at 0.5 Hz, indicated by the circles) in the absence (control) and presence of  $\alpha, \beta$ -meATP.  $\alpha, \beta$ -meATP totally abolished the fast depolarizations; however, slow depolarization persisted, although at a reduced level, in the presence of  $\alpha, \beta$ -meATP. Reproduced, with permission, from [32].

regions of the gut [36]. More recently, ATP has been shown to be a cotransmitter with NA, 5-hydroxytryptamine, glutamate, dopamine and  $\gamma$ -amino butyric acid (GABA) in the central nervous system (CNS) [37]. ATP and NA act synergistically to release vasopressin and oxytocin, which is consistent with ATP cotransmission in the hypothalamus. ATP, in addition to glutamate, is involved in long-term potentiation in hippocampal CA1 neurons that are associated with learning and memory [38].

### Purine receptors

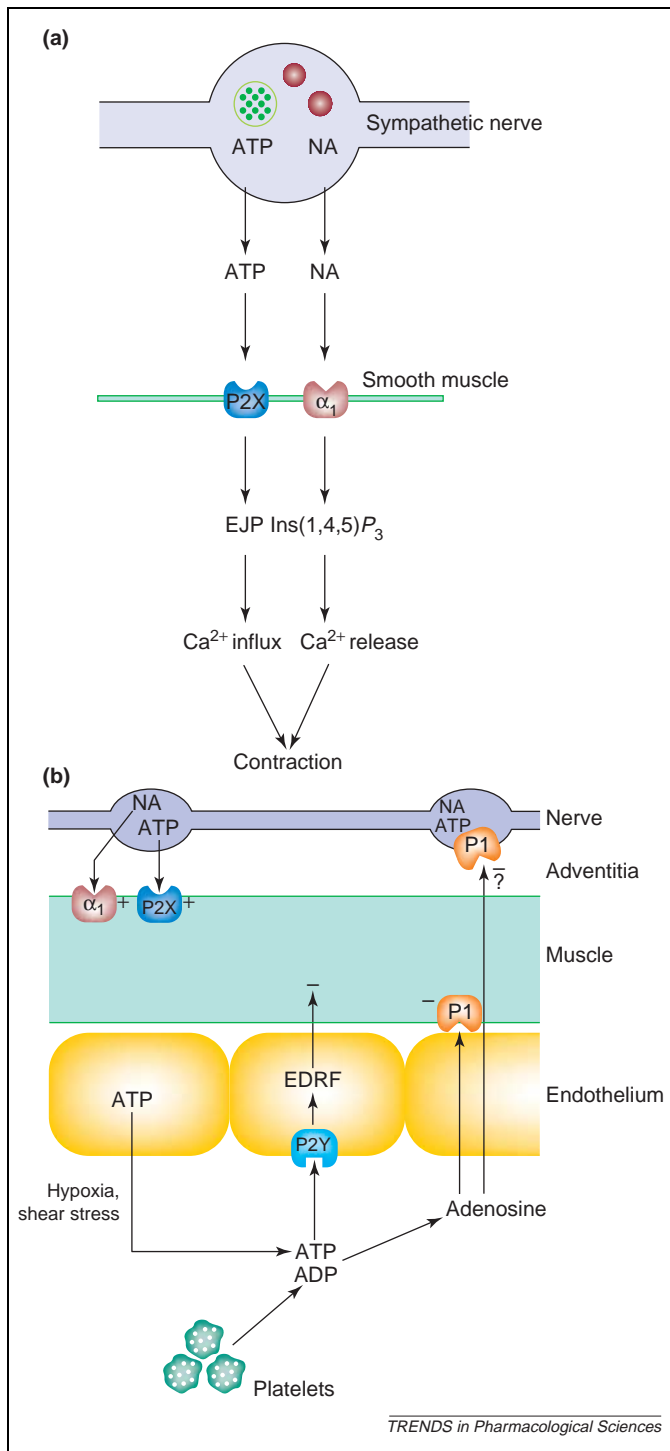
Implicit in the purinergic neurotransmission hypothesis was the presence of purinoceptors. A basis for distinguishing two types of purinoceptor, identified as P1 and P2 for adenosine, and ATP and ADP, respectively, was recognized [39]. This helped resolve some of the ambiguities in earlier reports, which were complicated by the breakdown of ATP to adenosine by ectoenzymes, so that some of the actions of ATP were directly on P2 receptors, whereas others were due to the indirect action of adenosine on P1 receptors.

At about the same time, two subtypes of P1 (adenosine) receptor were recognized [40] but it was not until 1985 that a pharmacological basis for distinguishing two types of P2 receptors (P2X and P2Y) was proposed by Charles Kennedy and I [41]. A year later, two further P2 receptor subtypes were named, a P2T receptor that was selective for ADP on platelets and a P2Z receptor on macrophages [42]. Further subtypes followed, perhaps the most important of which being the P2U receptor, which could recognize pyrimidines such as UTP in addition to ATP [43]. However, to provide a more manageable framework for newly identified nucleotide receptors, Abbracchio and I [44] proposed that purinoceptors should belong to two major families: a P2X family of ligand-gated ion channel receptors and a P2Y family of G-protein-coupled receptors. This was based on studies of transduction mechanisms and the cloning of nucleotide receptors: first, P2Y receptors were cloned with the help of Eric Barnard [45] and from the laboratory of David Julius, and a year later P2X receptors were cloned by the groups of Alan North and David Julius [46,47]. This nomenclature has been widely adopted and currently seven P2X subtypes and eight P2Y receptor subtypes are recognized [48,49] (Table 1). Four subtypes of P1 receptor have been cloned and characterized [50].

It is now widely recognized that purinergic signalling is a primitive system [21] that is involved in many non-neuronal and neuronal mechanisms, in both short-term and long-term (trophic) events [51,52], including exocrine and endocrine secretion, immune responses, inflammation, mechanosensory transduction, platelet aggregation and endothelial-mediated vasodilatation, and in cell proliferation, differentiation, migration and death in development and regeneration, respectively.

### ATP release and degradation

There is clear evidence for exocytotic vesicular release of ATP from nerves, and the concentration of nucleotides in vesicles are claimed to be up to 1000 mM. It was generally assumed that the main source of ATP acting on purinoceptors was damaged or dying cells. However, it is now



**Figure 3.** (a) Cotransmission in sympathetic nerves. ATP and noradrenaline (NA) from terminal varicosities of sympathetic nerves can be released together. With NA acting via the postjunctional  $\alpha_1$ -adrenoceptor to release cytosolic  $\text{Ca}^{2+}$ , and ATP acting via the  $\text{P2X}_1$ -gated ion channel to elicit  $\text{Ca}^{2+}$  influx, both contribute to the subsequent response (contraction). Modified, with permission, from [96]. (b) The interactions of ATP released from perivascular nerves and from the endothelium. ATP is released from endothelial cells during hypoxia (or in response to shear stress) to act on endothelial  $\text{P2Y}$  receptors, leading to the production of endothelium-derived relaxing factor (EDRF) (nitric oxide) and subsequent vasodilation (-). ADP released from aggregating platelets also acts on endothelial  $\text{P2Y}$  receptors to stimulate vasodilation. By contrast, ATP released as a cotransmitter with noradrenaline (NA) from perivascular sympathetic nerves at the adventitia-muscle border produces vasoconstriction (+) via  $\text{P2X}$  receptors on the muscle cells. Adenosine, produced following rapid breakdown of ATP by ectoenzymes, produces vasodilation by a direct action on the muscle via  $\text{P1}$  receptors, and acts on the perivascular nerve terminal varicosities to inhibit transmitter release. Reproduced, with permission, from [97]. Abbreviations: EJP, excitatory junction potential;  $\text{Ins}(1,4,5)\text{P}_3$ , inositol (1,4,5)-trisphosphate.

recognized that ATP release from many cells is a physiological or pathophysiological response to mechanical stress, hypoxia, inflammation and some agonists [53]. There is debate, however, about the ATP transport mechanisms involved. There is compelling evidence for exocytotic release from endothelial and urothelial cells, osteoblasts, astrocytes, mast and chromaffin cells, but other transport mechanisms have also been proposed, including ATP-binding-cassette transporters, connexin hemichannels and plasmalemmal voltage-dependent anion channels.

Much is now known about the ectonucleotidases that break down ATP released from neurons and non-neuronal cells [54]. Several enzyme families are involved, including: ecto-nucleoside triphosphate diphosphohydrolases (E-NTPDases), of which NTPDase1, 2, 3 and 8 are extracellular; ectonucleotide pyrophosphatase (E-NPP) of three subtypes; alkaline phosphatases; ecto-5'-nucleotidase; and ecto-nucleoside diphosphokinase (E-NDPK). NTPDase1 hydrolyses ATP directly to AMP and hydrolyses UTP to UDP, whereas NTPDase2 hydrolyses ATP to ADP and 5'-nucleotidase hydrolyses AMP to adenosine.

### Physiology and pathophysiology of neurotransmission

The purinergic neurotransmission field is expanding rapidly; there is increasing interest in the physiology and pathophysiology of this neurosignalling system and therapeutic interventions are being explored. The first clear evidence for nerve-nerve purinergic synaptic transmission was published in 1992 [55–57]. Synaptic potentials in the coeliac ganglion and in the medial habenula in the brain were reversibly antagonised by the anti-trypanosomal agent suramin. Since then, many articles have described either the distribution of various  $\text{P2}$  receptor subtypes in the brain and spinal cord (Figure 4) or electro-physiological studies of the effects of purines in brain slices, isolated nerves and glial cells [58]. Synaptic transmission has also been demonstrated in the myenteric plexus and in various sensory, sympathetic, parasympathetic and pelvic ganglia [59]. Adenosine produced by the ectoenzymatic breakdown of ATP acts through presynaptic  $\text{P1}$  receptors to inhibit the release of excitatory neurotransmitters in both the peripheral nervous system and the CNS. Purinergic signalling is also implicated in higher order cognitive functions, including learning and memory in the prefrontal cortex.

### Neuroprotection

In the brain, purinergic signalling is involved in nervous tissue remodelling following trauma, stroke, ischaemia or neurodegenerative disorders [58]. The hippocampus of chronic epileptic rats shows abnormal responses to ATP that are associated with increased expression of  $\text{P2X}_7$  receptors. Neuronal injury releases fibroblast growth factor, epidermal growth factor and platelet-derived growth factor. In combination with these growth factors, ATP can stimulate astrocyte proliferation, contributing to the process of reactive astrogliosis and to hypertrophic and hyperplastic responses.  $\text{P2Y}$  receptor antagonists have been proposed as potential neuroprotective agents in the cortex, hippocampus and cerebellum. Blockade of  $\text{A}_{2\text{A}}$  ( $\text{P1}$ ) receptors antagonises tremor in Parkinson's disease

**Table 1. Characteristics of receptors for purines and pyrimidines<sup>a,b</sup>**

Receptor	Main distribution	Agonists <sup>c</sup>	Antagonists <sup>c</sup>	Transduction mechanisms
<b>P1 (adenosine)</b>				
A <sub>1</sub>	Brain, spinal cord, testis, heart, autonomic nerve terminals	CCPA, CPA	DPCPX, N0840, MRS1754	G <sub>i1</sub> , G <sub>i2</sub> and G <sub>i3</sub> , ↓cAMP
A <sub>2A</sub>	Brain, heart, lungs, spleen	CGS21680	KF17837, SCH58261	G <sub>s</sub> , ↑cAMP
A <sub>2B</sub>	Large intestine, bladder	NECA (nonselective)	Enprofylline, MRE2029F20	G <sub>s</sub> , ↑cAMP
A <sub>3</sub>	Lung, liver, brain, testis, heart	IB-MECA, CI-IB-MECA, DBXRM, VT160	MRS1220, L268605, MRS1191	G <sub>i2</sub> , G <sub>i3</sub> and G <sub>q/11</sub> , ↓cAMP, ↑Ins(1,4,5)P <sub>3</sub>
<b>P2X</b>				
P2X <sub>1</sub>	Smooth muscle, platelets, cerebellum, dorsal horn spinal neurons	α,β-meATP = ATP = 2-meSATP (rapid desensitization)	TNP-ATP, IP <sub>5</sub> I, NF023, NF449	Intrinsic cation channel (Ca <sup>2+</sup> and Na <sup>+</sup> )
P2X <sub>2</sub>	Smooth muscle, CNS, retina, chromaffin cells, autonomic and sensory ganglia	ATP ≥ ATP <sub>γ</sub> S ≥ 2-meSATP ≫ α, β-meATP (pH and zinc sensitive)	Suramin, iso-PPADS, RB2, NF770	Intrinsic ion channel (particularly Ca <sup>2+</sup> )
P2X <sub>3</sub>	Sensory neurons, NTS, some sympathetic neurons	2-MeSATP ≥ ATP ≥ α,β-meATP ≥ Ap <sub>4</sub> A (rapid desensitization)	TNP-ATP, PPADS	Intrinsic cation channel
P2X <sub>4</sub>	CNS, testis, colon	ATP ≫ α,β-meATP, CTP, ivermectin	A317491, NF110	
P2X <sub>5</sub>	Proliferating cells in skin, gut, bladder, thymus, spinal cord	ATP ≫ α,β-meATP, ATP <sub>γ</sub> S	TNP-ATP (weak), BBG (weak)	Intrinsic ion channel (particularly Ca <sup>2+</sup> )
P2X <sub>6</sub>	CNS, motor neurons in spinal cord	–(does not function as homomultimer)	Suramin, PPADS, BBG	Intrinsic ion channel
P2X <sub>7</sub>	Apoptotic cells in, for example, immune cells, pancreas, skin	BzATP > ATP ≥ 2-meSATP ≫ α, β-meATP	–	Intrinsic cation channel and a large pore with prolonged activation
<b>P2Y</b>				
P2Y <sub>1</sub>	Epithelial and endothelial cells, platelets, immune cells, osteoclasts	2-MeSADP > 2-meSATP = ADP > ATP, MRS2365	MRS2179, MRS2500	G <sub>q</sub> /G <sub>11</sub> ; PLC-β activation
P2Y <sub>2</sub>	Immune cells, epithelial and endothelial cells, kidney tubules, osteoblasts	UTP = ATP, UTP <sub>γ</sub> S, INS37217	Suramin > RB2, ARC126313	G <sub>q</sub> /G <sub>11</sub> and possibly G <sub>i</sub> ; PLC-β activation
P2Y <sub>4</sub>	Endothelial cells	UTP ≥ ATP, UTP <sub>γ</sub> S	RB2 > suramin	G <sub>q</sub> /G <sub>11</sub> and possibly G <sub>i</sub> ; PLC-β activation
P2Y <sub>6</sub>	Some epithelial cells, placenta, T cells, thymus	UDP > UTP ≫ ATP, UDPβS	MRS2578	G <sub>q</sub> /G <sub>11</sub> ; PLC-β activation
P2Y <sub>11</sub>	Spleen, intestine, granulocytes	ARC67085 > BzATP ≥ ATP <sub>γ</sub> S > ATP	Suramin > RB2, NF157	G <sub>q</sub> /G <sub>11</sub> and G <sub>s</sub> ; PLC-β activation
P2Y <sub>12</sub>	Platelets, glial cells	2-MeSADP ≥ ADP ≫ ATP	CT50547, ARC69931MX, INS49266, AZD6140, PSB0413	G <sub>i</sub> (G <sub>o</sub> ); inhibition of adenylyl cyclase
P2Y <sub>13</sub>	Spleen, brain, lymph nodes, bone marrow	ADP = 2-meSADP ≫ ATP and 2-meSATP	MRS2211	G <sub>i</sub> /G <sub>o</sub>
P2Y <sub>14</sub>	Placenta, adipose tissue, stomach, intestine, discrete brain regions	UDP glucose = UDP-galactose	–	G <sub>q</sub> /G <sub>11</sub>

<sup>a</sup>Abbreviations: Ap<sub>4</sub>A, diadenosine tetraphosphate; BBG, Brilliant blue green; BzATP, 2'- and 3'-O-(4-benzoyl-benzoyl)-ATP; CCPA, chlorocyclopentyl adenosine; CI-IB-MECA, 2-chloro-N<sup>6</sup>-(3-iodobenzyl)-N-methyl-5'-carbamoyladenosine; CPA, cyclopentyl adenosine; CTP, cytosine triphosphate; DBXRM, N-methyl-1,3-dibutylxanthine-7-β-D-ribofuronamide; DPCPX, 1,3-dipropyl-8-cyclopentylxanthine; IB-MECA, N<sup>6</sup>-(3-iodobenzyl)-N-methyl-5'-carbamoyladenosine; Ins(1,4,5)P<sub>3</sub>, inositol (1,4,5)-trisphosphate; Ip<sub>5</sub>I, di-inosine pentaphosphate; α,β-meATP, α,β-methylene ATP; 2-MeSADP, 2-methylthio ADP; 2-MeSATP, 2-methylthio ATP; NECA, 5'-N-ethylcarboxamido adenosine; PLC, phospholipase C; PPADS, pyridoxal-phosphate-6-azophenyl-2', 4'-disulfonic acid; RB2, reactive blue 2; TNP-ATP, trinitrophenyl-substituted ATP.

<sup>b</sup>Table updated and reprinted from [94].

<sup>c</sup>See Chemical names.

whereas ATP–MgCl<sub>2</sub> is being explored for the treatment of spinal cord injuries.

### CNS control of autonomic function

Functional interactions seem likely to occur between purinergic and nitrenergic neurotransmitter systems; these interactions might be important for the regulation of hormone secretion and body temperature at the hypothalamic level and for cardiovascular and respiratory control at the level of the brainstem [60,61]. The nucleus

tractus solitarius (NTS) is a major integrative centre of the brain stem involved in reflex control of the cardiovascular system, and stimulation of P2X receptors in the NTS evokes hypotension. P2X receptors expressed in neurons in the trigeminal mesencephalic nucleus might be involved in the processing of proprioceptive information.

### Neuron–glia interactions

ATP is an extracellular signalling molecule between neurons and glial cells. ATP released from astrocytes

## Chemical names

**ARC126313:** 5-(7-chloro-4*H*-1-thia-3-aza-benzo[*f*]-4-yl)-3-methyl-6-thioxo-piperidin-2-one  
**ARC67085:** 2-propylthio- $\beta,\gamma$ -dichloromethylene-d-ATP  
**ARC69931MX:** *N*<sup>6</sup>-(2-methylthioethyl)-2-(3,3,3-trifluoropropylthio)- $\beta,\gamma$ -dichloromethylene ATP  
**A317491:** 5-(((3-phenoxybenzyl)[(1*S*)-1,2,3,4-tetrahydro-1-naphthalenyl]amino)carbonyl)-1,2,4-benzenetricarboxylic acid  
**AZD6140:** 3-{7-[2-(3,4-difluoro-phenyl)-cyclopropylamino]-5-propylsulfanyl [1-3]triazolo[4,5-*d*]pyrimidin-3-yl}-5-(2-hydroxy-methoxy)-cyclopentane-1,2-diol  
**CGS21680:** 2-[*p*-(2-carboxyethyl)phenyl-ethylamino]-5'-N-ethylcarboxamidoadenosine  
**CT50547:** N1-(6-ethoxy-1,3-benzothiazol-2-yl)-2-(7-ethoxy-4-hydroxy-2,2-dioxo-2*H*-2)6benzo [4,5] [1,3]thiazolo[2,3-*c*] [1,2,4]thiadiazin-3-yl)-2-oxo-1-ethanesulfonamide  
**INS37217:** P(1)-(uridine 5')-P(4)-(2'-deoxycytidine 5')tetraphosphate, tetrasodium salt  
**INS49266:** 6-phenylurea-2',3'-phenylacetaldehyde acetal ADP  
**KF17837:** 1,2-dipropyl-8-(3,4-dimethoxystyryl)-7-methylxanthine  
**KN04:** N-[1-[N-methyl-*p*-(5-isoquinolinesulfonyl)benzyl]-2-(4-phenylpiperazine)ethyl]-5-isoquinoline-sulfonamide  
**KN62:** 1-[N,O-bis(5-isoquinolinesulfonyl)-N-methyl-L-tyrosyl]-4-phenylpiperazine  
**L268605:** thiazolopyrimidine  
**MRE2029F20:** N-benzo [1,3]dioxol-5-yl-2-[5-(2,6-dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-1-methyl-1*H*-pyrazol-3-yl-oxyl]-acetamide  
**MRS1191:** 3-ethyl-5-benzyl-2-methyl-4-phenylethynyl-6-phenyl-1,4-( $\pm$ )-dihydropyridine-3,5-dicarboxylate  
**MRS1220:** 9-chloro-2-(2-furyl) [1,2,4]triazolo[1,5-*c*]quinazolin-5-phenylacetamide  
**MRS1754:** N-(4-cyanophenyl)-2-[4-(2,3,6,7-tetrahydro-2,6-dioxo-1,3-dipropyl-1*H*-purin-8-yl)-phenoxy]acetamide

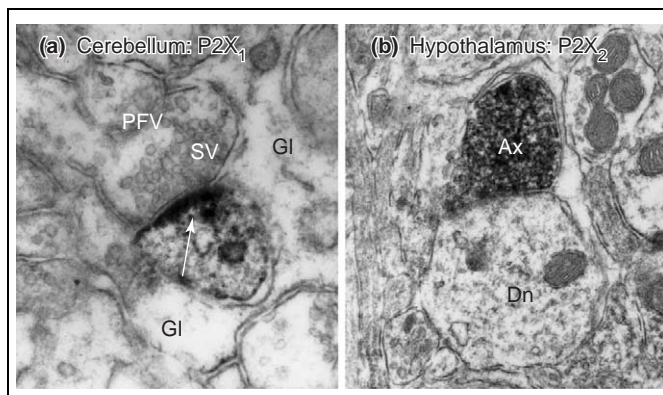
**MRS2179:** *N*<sup>6</sup>-methyl 2'-deoxyadenosine-3',5'-bisphosphate  
**MRS2211:** 3,5-diethyl-2-methyl-4-(trans-2-(4-nitrophenyl)viny)-6-phenyl-1,4-dihydropyridine-3,5-dicarboxylate  
**MRS2365:** (1'*S*,2'*R*,3'*S*,4'*R*,5'*S*)-4-[[6-amino-2-methylthio-9*H*-purin-9-yl)-1-diphosphoryloxymethyl]bicyclo[3.1.0]hexane-2,3-diol  
**MRS2427:** carbamic acid, [(1*S*)-2-(4-benzoyl-1-piperazinyl)-1-[[4-[[4-methylphenyl)sulfonyl]oxy]phenyl)methyl]-2-oxoethyl]-,phenylmethyl ester (9Cl)  
**MRS2500:** 2-iodo-N6-methyl-(N)-methanocarba-20-deoxyadenosine-30,50-bisphosphate  
**MRS2578:** 1,4-di-[[3-isothiocyanato phenyl]-thioureido]butane  
**N0840:** *N*<sup>6</sup>-cyclopentyl-9-methyladenine  
**NF023:** 8,8'-(carbonyl-bis(imino-3,1-phenylenecarbonylimino))-bis(naphthalene-1,3,5-trisulfonic acid)-hexasodium salt  
**NF110:** 4,4',4'',4'''-(carbonylbis(imino-5,1,3-benzenetriylbis(carbonylimino))) tetrakisbenzenesulfonic acid, tetrasodium salt  
**NF157:** 8,8'-(carbonylbis[imino-3,1-phenylenecarbonylimino(4-fluoro-3,1-phenylene)carbonylimino])bis-1,3,5-naphthalenetrisulfonic acid.hexasodium  
**NF449:** 4,4',4'',4'''-(carbonylbis(imino-5,1,3-benzenetriylbis(carbonylimino)))tetrakis-benzene-1,3-disulfonic acidoctasodium salt  
**NF770:** 7,7'-(carbonylbis(imino-3,1-phenylenecarbonylimino-3,1-(4-methyl-phenylene)carbonylimino))bis(1-methoxy-naphthalene-3,6-disulfonic acid) tetrasodium salt  
**PSB0413:** 2-propylthioadenosine-5'-adenylic acid (1,1-dichloro-1-phosphonomethyl-1-phosphonyl) anhydride  
**SCH58261:** 5-amino-7-(phenylethyl)-2-(2-furyl)-pyrazolo[4,3-*e*]1,2,4-triazolo[1,5-*c*]pyrimidine  
**VT160:** proprietary information; chemical name not available

might be important in triggering cellular responses to trauma and ischaemia by initiating and maintaining reactive astrogliosis, which involves striking changes in the proliferation and morphology of astrocytes and microglia. Some of the responses to ATP released during brain injury are neuroprotective, but at higher concentrations ATP contributes to the pathophysiology initiated after trauma [62]. Multiple P2X and P2Y receptor subtypes are expressed by astrocytes, oligodendrocytes and microglia [63]. ATP and basic fibroblast growth factor (bFGF) signals merge at the mitogen-activated protein kinase (MAPK) cascade, which underlies the synergistic interactions of ATP and bFGF in astrocytes. ATP can activate P2X<sub>7</sub> receptors in astrocytes to release glutamate, GABA and ATP, which regulate the excitability of neurons.

Microglia, immune cells of the CNS, are also activated by purines and pyrimidines to release inflammatory cytokines such as interleukin 1 $\beta$  (IL-1 $\beta$ ), IL-6 and tumour necrosis factor  $\alpha$  (TNF- $\alpha$ ). Thus, although microglia might have an important role against infection in the CNS, overstimulation of this immune reaction might accelerate the neuronal damage caused by ischaemia, trauma or neurodegenerative diseases. P2X<sub>4</sub> receptors induced in spinal microglia gate tactile allodynia after nerve injury [64] whereas P2X<sub>7</sub> receptors mediate superoxide production in primary microglia and are upregulated in a transgenic mouse model of Alzheimer's disease, particularly around  $\beta$ -amyloid plaques [65].

## Purine transmitter and receptor plasticity

The autonomic nervous system shows marked plasticity: that is, the expression of cotransmitters and receptors show dramatic changes during development and aging, in nerves that remain after trauma or surgery and in disease conditions. There are several examples where the purinergic component of cotransmission is increased in pathological conditions [51]. The parasympathetic purinergic nerve-mediated component of contraction of the human bladder is increased to 40% in pathophysiological



**Figure 4.** Electronmicroscopic immunohistochemistry of P2X receptors of rat brain. (a) A P2X<sub>1</sub> receptor-positive postsynaptic dendritic spine of a Purkinje cell (arrow) in the rat cerebellum (magnification:  $\times 97\,000$ ). Modified, with permission, from [98]. (b) A P2X<sub>2</sub> receptor-labelled presynaptic axon profile adjacent to an unlabelled dendrite in the neuropil of the rat supraoptic nucleus (magnification:  $\times 43\,000$ ). Modified, with permission, from [99]. Abbreviations: Ax, axon; Dn, dendrite; Gl, neuroglial process; PFV, parallel fibre varicosity; SV, synaptic vesicles.

conditions such as interstitial cystitis, outflow obstruction, idiopathic instability and also some types of neurogenic bladder [35]. ATP also has a significantly greater cotransmitter role in sympathetic nerves supplying hypertensive compared with normotensive blood vessels [66]. Upregulation of P2X<sub>1</sub> and P2Y<sub>2</sub> receptor mRNA in the hearts of rats with congestive heart failure has been reported and there is a dramatic increase in expression of P2X<sub>7</sub> receptors in the kidney glomerulus in diabetes and hypertension [67].

#### Dual purinergic neural and endothelial control of vascular tone and angiogenesis

ATP and adenosine are involved in the mechanisms that underlie local control of vessel tone in addition to cell migration, proliferation and death during angiogenesis, atherosclerosis and restenosis following angioplasty [68]. ATP, released as a cotransmitter from sympathetic nerves, constricts vascular smooth muscle via P2X receptors, whereas ATP released from sensory-motor nerves during 'axon reflex' activity dilates vessels via P2Y receptors. Furthermore, ATP released from endothelial cells during changes in flow (shear stress) or hypoxia acts on P2Y receptors in endothelial cells to release NO, which results in relaxation (Figure 3b). Adenosine, following breakdown of extracellular ATP, produces vasodilatation via smooth muscle P1 receptors.

#### Pain and purinergic mechanosensory transduction

The involvement of ATP in the initiation of pain was recognized first in 1966 and later in 1977 using human skin blisters [69,70]. A major advance was made when the P2X<sub>3</sub> ionotropic receptor was cloned in 1995 and shown later to be localized predominantly in the subpopulation of small nociceptive sensory nerves that label with isolectin IB4 in dorsal root ganglia (DRG), whose central projections terminate in inner lamina II of the dorsal horn [71]. In 1996, I proposed a unifying 'purinergic' hypothesis for the initiation of pain by ATP acting via P2X<sub>3</sub> and P2X<sub>2/3</sub> receptors associated with causalgia (a persistent burning pain following injury to a peripheral nerve), reflex sympathetic dystrophy, angina, migraine, pelvic and cancer pain [72]. This has been followed by an increasing number of published reports expanding on this concept for acute, inflammatory, neuropathic and visceral pain [74–76]. P2Y<sub>1</sub> receptors have also been demonstrated in a subpopulation of sensory neurons that colocalize with P2X<sub>3</sub> receptors.

A hypothesis was proposed that purinergic mechanosensory transduction occurred in visceral tubes and sacs, including ureter, bladder and gut, where ATP, released from epithelial cells during distension, acted on P2X<sub>3</sub> homomultimeric and P2X<sub>2/3</sub> heteromultimeric receptors on subepithelial sensory nerves, thus initiating impulses in sensory pathways to pain centres in the CNS [77] (Figure 5). Subsequent studies of bladder [78], ureter [79], gut [80], tongue and tooth pulp [52] have produced evidence in support of this hypothesis. P2X<sub>3</sub> receptor knockout mice were used to show that ATP released from urothelial cells during distension of the bladder act on P2X<sub>3</sub> receptors on subepithelial sensory nerves to initiate

both nociceptive and bladder-voiding reflex activities [81]. In the distal colon ATP released during moderate distension acts on P2X<sub>3</sub> receptors on low-threshold intrinsic subepithelial sensory neurons to influence peristalsis, whereas high-threshold extrinsic subepithelial sensory fibres respond to severe distension to initiate pain (Figure 5b).

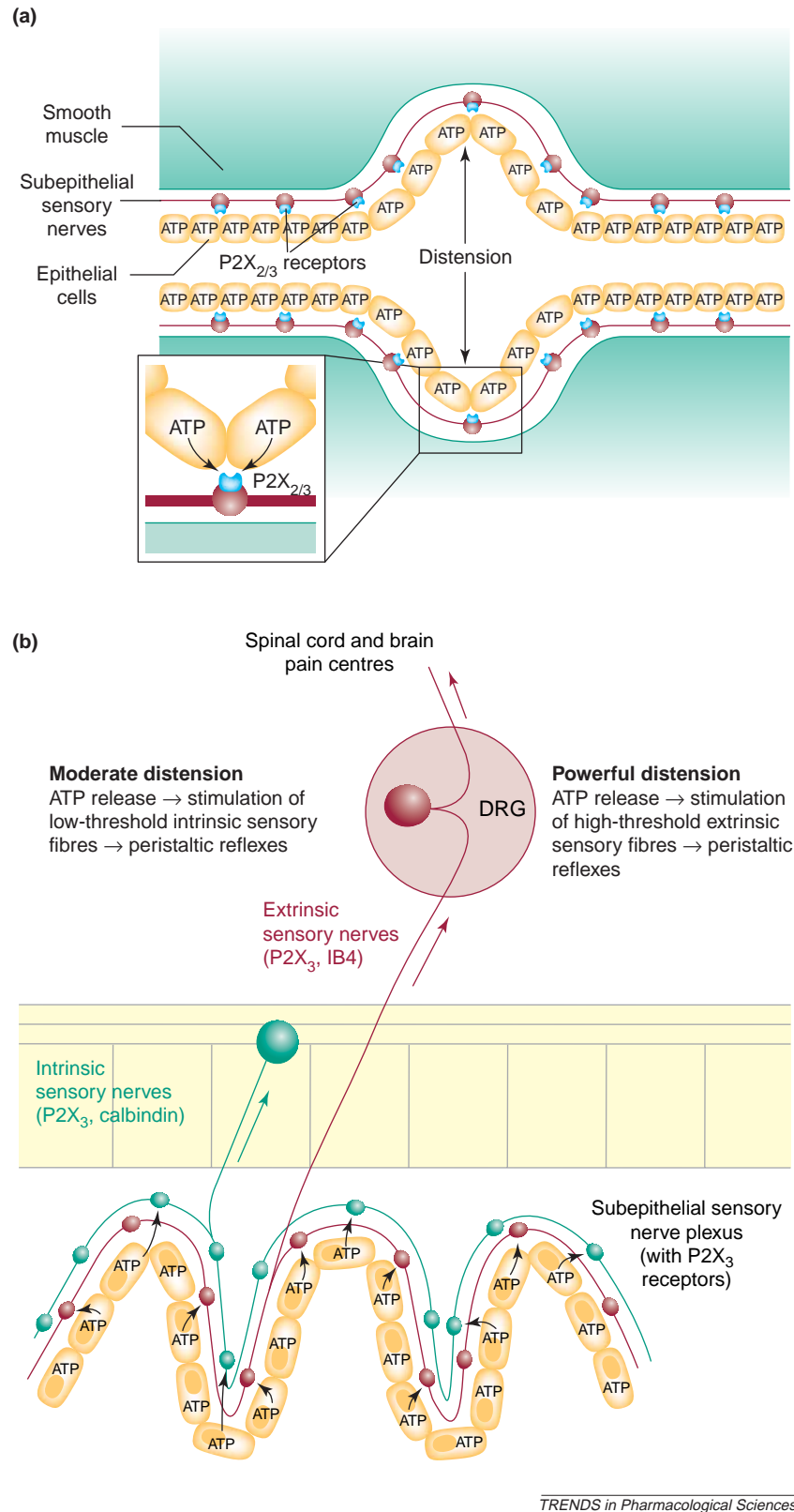
ATP is also a neurotransmitter released from the spinal cord terminals of primary afferent sensory nerves to act at synapses in the central pain pathway [73,76]. Using transverse spinal cord slices from postnatal rats, excitatory postsynaptic currents have been shown to be mediated by P2X receptors activated by synaptically released ATP, in a subpopulation of <5% of the neurons in lamina II, a region known to receive major input from nociceptive primary afferents.

There is an urgent need for selective P2X<sub>3</sub> and P2X<sub>2/3</sub> receptor antagonists that do not degrade *in vivo*. Pyridoxal-phosphate-6-azophenyl-2',4'-disulfonic acid (PPADS) is a nonselective P2 receptor antagonist but has the advantage that it dissociates ~100–10 000 times more slowly than other known antagonists. The trinitrophenyl-substituted nucleotide TNP-ATP is a selective and potent antagonist at both P2X<sub>3</sub> and P2X<sub>2/3</sub> receptors. A317491 is a potent and selective non-nucleotide antagonist of P2X<sub>3</sub> and P2X<sub>2/3</sub> receptors and it reduces chronic inflammatory and neuropathic pain in the rat [82]. Antisense oligonucleotides have been used to downregulate the P2X<sub>3</sub> receptor and in models of neuropathic (partial sciatic nerve ligation) and inflammatory (complete Freund's adjuvant) pain, inhibition of the development of mechanical hyperalgesia was observed within 2 days of treatment [83]. P2X<sub>3</sub> receptor double-stranded-short interfering RNA (siRNA) also relieves chronic neuropathic pain and opens up new avenues for therapeutic pain strategies in humans [76]. Tetramethylpyrazine, a traditional Chinese medicine, used as an analgesic for dysmenorrhoea, is claimed to be a P2X receptor antagonist and it inhibited significantly the first phase of nociceptive behaviour induced by 5% formalin and attenuated slightly the second phase in the rat hindpaw pain model [84]. Antagonists of P2 receptors are also beginning to be explored in relation to cancer pain [85].

#### Special senses

##### Eye

P2X<sub>2</sub> and P2X<sub>3</sub> receptor mRNA is present in the retina and receptor protein expressed in retinal ganglion cells [86]. P2X<sub>3</sub> receptors are also present on Müller cells, which release ATP during Ca<sup>2+</sup> wave propagation. ATP, acting via both P2X and P2Y receptors, modulates retinal neurotransmission, affecting retinal blood flow and intraocular pressure. Topical application of diadenosine tetraphosphate has been proposed for the lowering of intraocular pressure in glaucoma [87]. The formation of P2X<sub>7</sub> receptor pores and apoptosis is enhanced in retinal microvessels early in the course of experimental diabetes, suggesting that purinergic vasotoxicity might have a role in microvascular cell death, a feature of diabetic retinopathy [88]. The possibility has been raised that alterations in sympathetic nerves might underlie some of the



**Figure 5.** (a) The hypothesis for purinergic mechanosensory transduction in tubes (e.g. ureter, vagina, salivary and bile ducts and gut) and sacs (e.g. urinary and gall bladders, and lung). It is proposed that distension leads to the release of ATP from the epithelium lining the tube or sac, which then acts on P2X<sub>2/3</sub> receptors on subepithelial sensory nerves to convey sensory (nociceptive) information to the CNS. Modified, with permission, from [77]. (b) A novel hypothesis about purinergic mechanosensory transduction in the gut. It is proposed that ATP released from mucosal epithelial cells during moderate distension acts preferentially on P2X<sub>3</sub> receptors on low-threshold subepithelial intrinsic sensory nerve fibres (labelled with calbindin), contributing to peristaltic reflexes. ATP released during extreme distension also acts on P2X<sub>3</sub> receptors on high-threshold extrinsic sensory nerve fibres [labelled with isolectin B4 (IB4)] that send messages via the dorsal root ganglia (DRG) to pain centres in the CNS. Modified, with permission, from [100].



complications observed in diabetic retinopathy; ATP is well established as a cotransmitter in sympathetic nerves, which raises the potential for the use of P2 receptor antagonists in glaucoma. P2Y<sub>2</sub> receptor activation increases salt, water and mucus secretion and thus represents a potential treatment for dry eye conditions [89].

### Ear

Both P2X and P2Y receptors have been identified in the vestibular system. ATP regulates fluid homeostasis, cochlear blood flow, hearing sensitivity and development, and thus might be useful in the treatment of Ménière's disease, tinnitus and sensorineural deafness. ATP, acting via P2Y receptors, depresses sound-evoked compound action potentials in the auditory nerve and the distortion product otoacoustic emission, the latter being a measure of the active process of the outer hair cells [90]. P2X splice variants are found on the endolymphatic surface of the cochlear endothelium, an area associated with sound transduction. Sustained loud noise produces an upregulation of P2X<sub>2</sub> receptors in the cochlea, particularly at the site of outer hair cell sound transduction. P2X<sub>2</sub> receptor expression is also increased in spiral ganglion neurons, indicating that extracellular ATP acts as a modulator of auditory neurotransmission that is adaptive and dependent on the noise level [91]. Excessive noise can irreversibly damage hair cell stereocilia, leading to deafness. Data suggest that the release of ATP from damaged hair cells is required for Ca<sup>2+</sup> wave propagation through the support cells of the organ of Corti and also involving P2Y receptors [92]; this might constitute the fundamental mechanism to signal the occurrence of hair cell damage.

### Nasal organs

The olfactory epithelium and vomeronasal organs contain olfactory receptor neurons that express P2X<sub>2</sub>, P2X<sub>3</sub> and P2X<sub>2/3</sub> receptors [93]. It is suggested that the neighbouring epithelial supporting cells or the olfactory neurons themselves can release ATP in response to noxious stimuli, acting on P2X receptors as an endogenous modulator of odour sensitivity. Enhanced sensitivity to odours was observed in the presence of P2 receptor antagonists, suggesting that low-level endogenous ATP normally reduces odour responsiveness. It was suggested that the predominantly suppressive effect of ATP on odour sensitivity might be involved in reduced odour sensitivity that occurs during acute exposure to noxious fumes and might be a novel neuroprotective mechanism.

### Concluding remarks

The purinergic transmission hypothesis underwent strong resistance after it was first proposed in 1972, but following the cloning and characterization of purinoceptor subtypes and the recognition of purinergic synaptic transmission in the brain and autonomic ganglia in the early 1990s it gained wide recognition. There is now much interest in the physiology and pathophysiology of purinergic cotransmission and purinergic interactions between neurons and glial cells in both the CNS and the periphery. There is hope

that useful therapeutic interventions for a variety of neurological disorders, including neurodegenerative diseases, pain, migraine and diseases of the special senses, will be developed in the not too distant future.

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