

# Potential therapeutic targets in the rapidly expanding field of purinergic signalling

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**ABSTRACT** – The concept of a purinergic signalling system, using purine nucleotides and nucleosides as extracellular messengers, was first proposed over 30 years ago. After a brief historical review and update of purinoceptor subtypes, this article focuses on the diverse physiological roles of adenosine triphosphate, adenosine diphosphate, uridine triphosphate and adenosine. These molecules mediate short-term (acute) signalling functions in neurotransmission, secretion and vasodilation, and long-term (chronic) signalling functions in development, regeneration, proliferation and cell death. Plasticity of purinoceptor expression in pathological conditions is frequently observed, including an increase in the purinergic component of parasympathetic nervous control of the human bladder in interstitial cystitis and outflow obstruction, and in sympathetic cotransmitter control of blood vessels in hypertensive rats. The antithrombotic action of clopidogrel (Plavix), a P2Y<sub>12</sub> receptor antagonist, has been shown to be particularly promising in the prevention of recurrent strokes and heart attacks in recent clinical trials (CAPRIE and CURE). The role of P2X<sub>3</sub> receptors in nociception and a new hypothesis concerning purinergic mechanosensory transduction in visceral pain will be considered, as will the therapeutic potential of purinergic agonists or antagonists for the treatment of supraventricular tachycardia, cancer, dry eye, bladder hyperactivity, erectile dysfunction, osteoporosis, diabetes, gut motility and vascular disorders.

**KEY WORDS:** purinoceptors, interstitial cystitis, thrombosis, visceral pain, cancer, osteoporosis, peripheral vascular disease, cystic fibrosis, Parkinson's disease, kidney failure

## Purinergic signalling: history and receptor subtypes

A seminal paper by Drury and Szent-Györgi in 1929 described the potent actions of purine nucleotides and nucleosides, adenosine triphosphate (ATP) and

adenosine on the heart and blood vessels<sup>1</sup>. A landmark paper by Pamela Holton in 1959 showed that, during antidromic stimulation of sensory nerves, ATP was released to the rabbit ear artery in sufficient amounts to produce changes in vascular tone<sup>2</sup>. Then, in 1970, Burnstock *et al.* found evidence for the role of ATP as a neurotransmitter in nonadrenergic, noncholinergic (NANC) nerves supplying the gut<sup>3</sup>, and, in 1972, the word 'purinergic' was coined and the purinergic-neurotransmission hypothesis was put forward<sup>4</sup> (Fig 1). This concept met with considerable resistance for many years because ATP had been established as an intracellular energy source involved in various metabolic cycles, and it was thought that such a ubiquitous molecule was unlikely to be involved in selective extracellular signalling. However, the concept is now widely accepted. Later, it was established that ATP is a cotransmitter with classical transmitters in both the peripheral and the central nervous systems, and that purines are powerful extracellular messengers to non-neuronal cells, including exocrine and endocrine, secretory, endothelial, bone, immune and inflammatory cells<sup>5</sup>.

Implicit in the purinergic hypothesis is the presence of purinoceptors. A basis for distinguishing P1 (adenosine) from P2 (ATP/adenosine diphosphate (ADP)) receptors was proposed by Burnstock in 1978<sup>6</sup>. 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, while others were due to indirect action via P1 receptors. Four subtypes of P2 receptors were cloned, namely A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub> and A<sub>3</sub>, and, in 1985, Burnstock and Kennedy proposed a basis for distinguishing between two types of P2 purinoceptor, P2X and P2Y, based largely on pharmacological criteria<sup>7</sup>. In the early 1990s, studies of transduction mechanisms and cloning of both P2Y and P2X receptors (Fig 2) were carried out, which led Abbraccio and Burnstock to put forward a new nomenclature system in 1994<sup>10</sup>, which is now widely accepted. They proposed that there are two families of P2 purinoceptors, namely P2X ionotropic ligand-gated ion-channel receptors and P2Y metabotropic G-protein-coupled receptors. This framework has allowed a logical expansion as

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new receptors are identified. Currently, seven subtypes of P2X receptors and six subtypes of P2Y receptors are clearly recognised, and their distribution in the body and pharmacological properties have been defined<sup>11</sup>. The P2X<sub>1</sub> receptor is prominent in contractile smooth muscle cells but is not detectable in proliferating smooth muscle cells, in which P2Y receptor expression is substantially increased.

Purinergic signalling is rapid in synaptic neurotransmission, neuromuscular transmission leading to contraction or relaxation of smooth muscle, and exocrine or endocrine secretion. However, there are now many examples of purinergic signalling regulating long-term events such as cell proliferation, differentiation, migration and death, and in the course of development, regeneration and wound healing<sup>12-14</sup>. Both P2X and P2Y receptors play prominent roles in embryonic development, including the development of the nervous system, cartilage in limb buds, the mesonephros, retina, myotubes and neuromuscular junctions<sup>15</sup>.

## Pathophysiology and therapeutic developments

There is increasing interest in the therapeutic potential of purinergic compounds in a wide range of diseases, in relation to both P1 receptors<sup>16-19</sup> and P2 receptors<sup>12-14,20</sup>. A number of purine-related compounds have been patented<sup>21</sup>.

The autonomic nervous system shows marked plasticity. Dramatic changes occur in the expression of cotransmitters and receptors during development and ageing, in nerves that remain after trauma or surgery and in disease conditions. There are several pathological conditions in which the cotransmission of purinergic components is increased<sup>12</sup>.

## Nervous system

ATP is a cotransmitter in many nerve types, probably reflecting the early evolutionary presence of purinergic signalling. There is now evidence for the action of ATP as a cotransmitter with

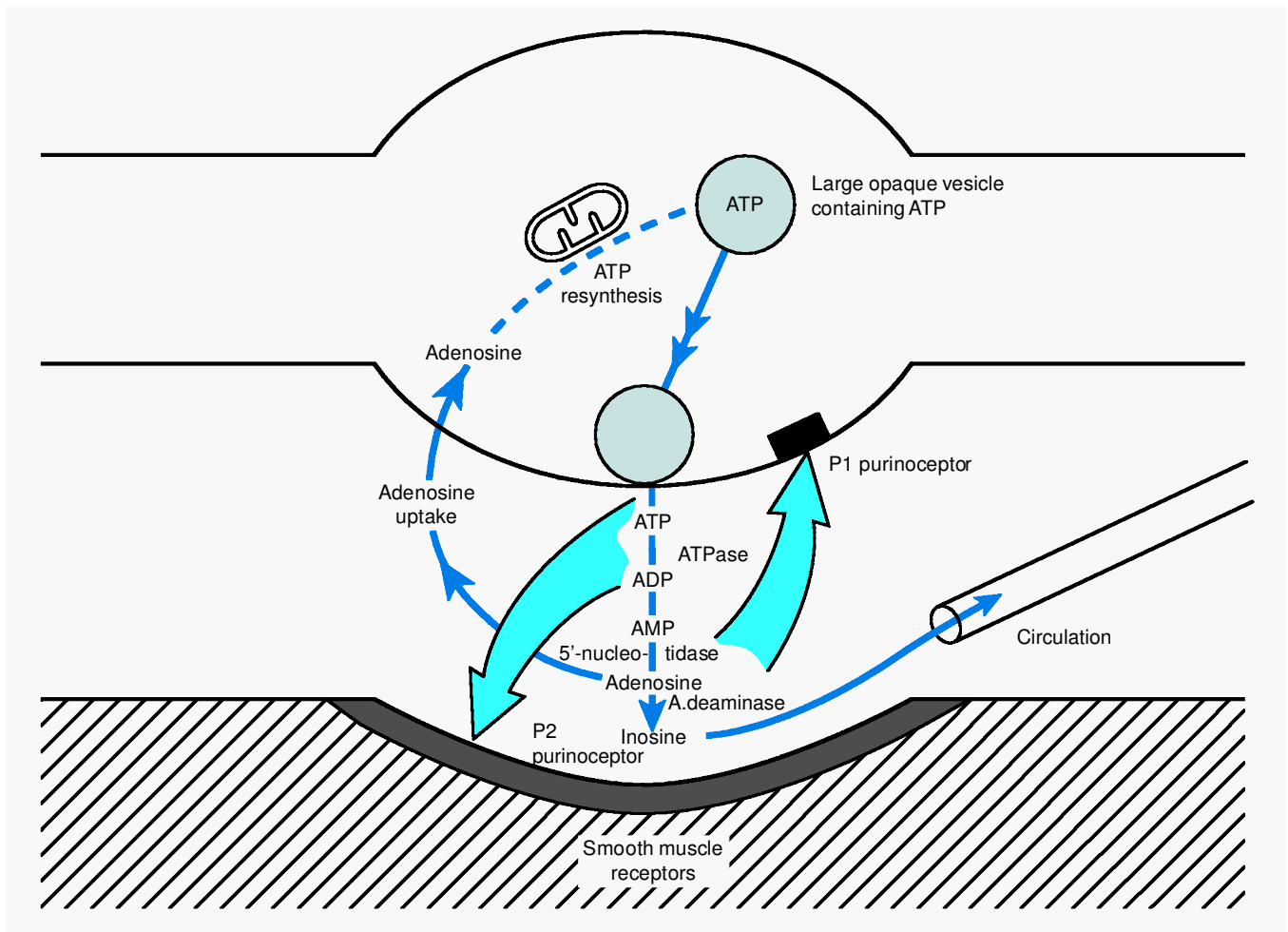


Fig 1. Schematic representation of purinergic neuromuscular transmembrane depicting the synthesis, storage, release and inactivation of ATP, and autoregulation via prejunctional adenosine (P1) receptors. Modified from Burnstock 1972<sup>4</sup>. Reproduced with permission from the American Society for Pharmacology and Experimental Therapeutics.

noradrenaline (NA) and neuropeptide Y (NPY) in sympathetic nerves, with acetylcholine and vasoactive intestinal peptide (VIP) in some parasympathetic nerves, with nitric oxide (NO) and VIP in enteric NANC inhibitory nerves, and with calcitonin gene-related peptide and substance P in sensory-motor nerves<sup>5</sup>. There is also evidence for the cotransmission of ATP with  $\gamma$ -aminobutyric acid in retinal nerves, and with glutamate, 5-hydroxytryptamine (serotonin) or dopamine in nerves in the brain. In sympathetically innervated tissues, such as the vas deferens or blood vessels, ATP produces fast responses mediated by P2X receptors, followed by a slower component mediated by G-protein-coupled  $\alpha$ -adrenoceptors. Similarly, in the parasympathetic nerves supplying the urinary bladder, ATP provokes a fast transient response via P2X receptors, while the slower component is mediated by G-protein-coupled muscarinic receptors. There are considerable differences between the proportion of cotransmitters in nerves supplying different regions of the gut or vasculature, and between species. The first clear evidence for nerve-nerve purinergic synaptic transmission was published in two papers in *Nature* in 1992<sup>22,23</sup>. Synaptic potentials in the coeliac ganglion and in the medial habenula in the brain were reversibly antagonised by suramin, a P2X antagonist. Since then, many articles have described either the distribution of various P2 receptor subtypes in the brain and spinal cord or electrophysiological studies of the effects of purines in brain slices, isolated nerves and glial cells. Synaptic transmission has also been found in the myenteric plexus and in various sensory, sympathetic and pelvic ganglia<sup>24</sup>. Adenosine, produced by the ectoenzymatic breakdown of ATP<sup>25</sup>, acts through presynaptic P1 receptors to inhibit the release of excitatory neurotransmitters in both the peripheral and the central nervous systems<sup>26</sup>. P2Y receptors are expressed on both nonmyelinating and myelinating Schwann cells, and ATP causes proliferation of glial cells, whereas adenosine inhibits proliferation.

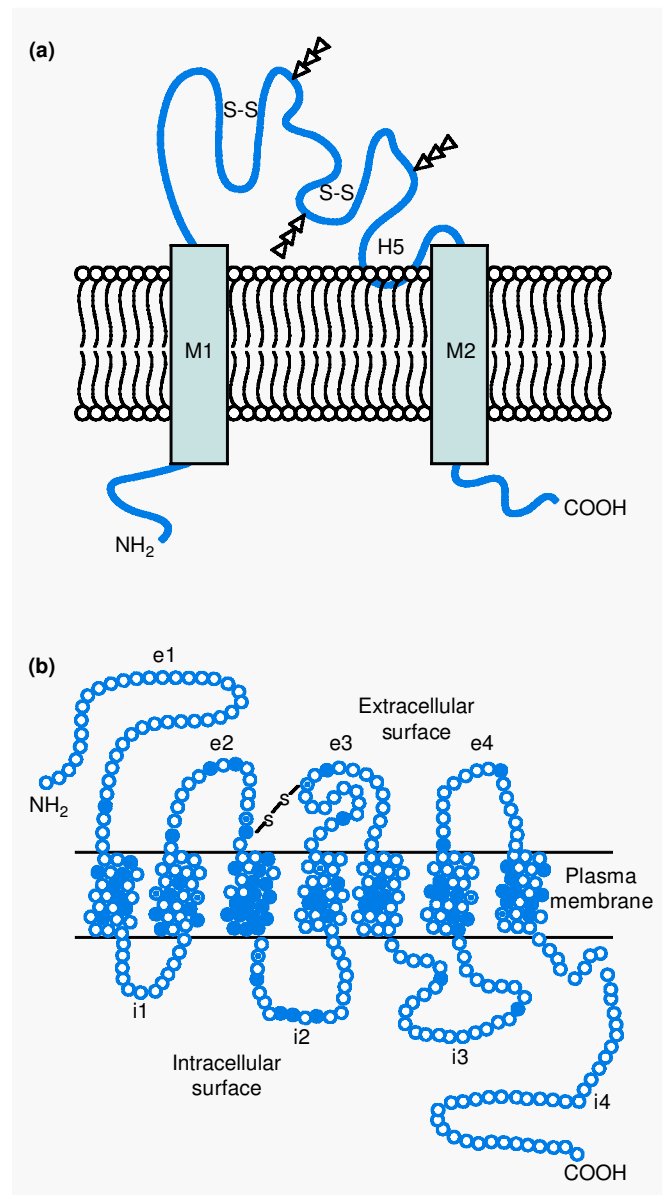
Agonists and antagonists of adenosine and ATP are being explored as therapeutic agents for a number of neurological conditions. For example, microinjection of ATP analogues into the prepiriform cortex induces generalised motor seizures<sup>27</sup>. P2X<sub>2</sub>, P2X<sub>4</sub> and P2X<sub>6</sub> receptors are expressed in the prepiriform cortex, suggesting that a P2X receptor antagonist may have potential as a neuroleptic agent.

**Fig 2. (a) Diagram depicting the transmembrane topology for P2X receptor protein showing both N-terminus and C-terminus in the cytoplasm.** Two putative membrane-spanning segments (M1 and M2) traverse the lipid bilayer of the plasma membrane and are connected by a hydrophilic segment of 270 amino acids. This putative extracellular domain is shown containing two disulphide-bonded loops (S-S) and three N-linked glycosyl chains (triangles). From Brake *et al.*<sup>8</sup>; reproduced with permission from *Nature*, [www.nature.com](http://www.nature.com) **(b) Schematic diagram of the sequence of the P2Y<sub>1</sub> receptor showing its differences from P2Y<sub>2</sub> and P2Y<sub>3</sub> receptors.** Filled circles represent amino acid residues that are conserved among the three receptors. Modified from Barnard *et al.*<sup>9</sup>; reproduced with permission from Elsevier Science.

ATP, given systemically, elicits pain responses, and endogenous ATP may contribute to the pain associated with causalgia, reflex sympathetic dystrophy, angina, migraine and pelvic and cancer pain<sup>28</sup>.

P2X<sub>3</sub> receptors are selectively localised on sensory neurons in trigeminal, nodose and dorsal root ganglia (DRG), and the terminals of these nociceptive neurons in the skin and visceral organs represent unique targets for novel analgesic agents that function as P2X<sub>3</sub> receptor antagonists. Nonspecific P2 receptor antagonists, eg suramin, are antinociceptive, and P2X<sub>3</sub> receptor-knockout mice have reduced nociceptive inflammatory responses<sup>29</sup>.

In nervous tissue, trophic factors ensure neuronal viability and regeneration. Neuronal injury releases fibroblast growth factor, epidermal growth factor and platelet-derived growth factor<sup>30</sup>. In combination with these growth factors, ATP can stimulate astrocyte proliferation, contributing to the process of



reactive astrogliosis, a hypertrophic/hyperplastic response associated with brain trauma, stroke, ischaemia, seizures and neurodegenerative disorders. Adenosine modulates long-term synaptic plasticity in the hippocampus, and it attenuates long-term potentiation, which is facilitated by P1 receptor antagonists. Adenosine-related compounds might prove helpful in the treatment of memory disorders and impaired intellectual performance related to caffeine intake<sup>31</sup>. Inhibitors of adenosine kinase, a key intracellular enzyme that regulates intracellular and extracellular concentrations of adenosine, have demonstrated efficacy in animal models of epilepsy, cerebral ischaemia, sleep apnoea, pain and inflammation<sup>32</sup>. A<sub>2A</sub> receptor antagonists are being investigated for the treatment of Parkinson's disease<sup>14</sup>.

### Cardiovascular system

Adenosine and ATP are very much involved in the local control of vessel tone<sup>33</sup> (Fig 3), as well as in cell migration, proliferation and death during angiogenesis, atherosclerosis and restenosis following angioplasty<sup>35</sup>. ATP released as a cotransmitter from sympathetic nerves constricts vascular smooth muscle via P2X receptors, while ATP released from sensory-motor nerves during 'axon-reflex' activity dilates vessels via P2Y receptors. Further, ATP released from endothelial cells during changes in flow (shear stress) or hypoxia acts on P2Y receptors in these cells to release nitric oxide (NO), resulting in relaxation. Adenosine, produced by the breakdown of extracellular ATP, causes vasodilation via P1 receptors. P2X receptors are also present on endothelial cells, and appear to be associated with cell adhesion and permeability.

Adenosine was identified early, and is in current use to reverse supraventricular tachycardia. There have been very promising recent developments concerning purinergic antithrombotic drugs. Platelets have been shown to express both P2Y and P2X purinoceptors<sup>36,37</sup>. Recent 'mega' clinical trials (CAPRIE<sup>38</sup> and CURE<sup>39</sup>) have provided clear evidence that the purinergic antithrombotic drugs clopidogrel and ticlopidine, which are antagonists to the platelet P2Y<sub>12</sub> receptor, reduce the risks of recurrent strokes and heart attacks, especially when combined with aspirin. Patents have been lodged for the application of P1 receptor subtype agonists and antagonists in myocardial ischaemia-reperfusion injury, cerebral ischaemia and stroke<sup>17</sup>.

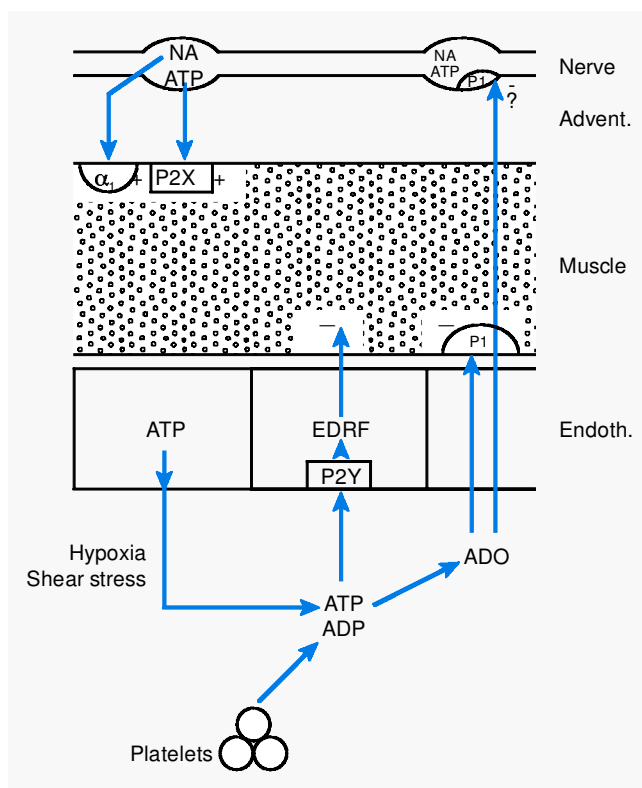
ATP plays a significant cotransmitter role in sympathetic nerves supplying hypertensive blood vessels. Upregulation of P2X<sub>1</sub> and P2Y<sub>2</sub> receptor mRNA in the hearts of rats with congestive heart failure has been reported<sup>40</sup>. Further therapeutic targets for P2 receptor agonists and antagonists include congestive heart failure, hypertension, stroke and angina.

### Respiratory system

In Type II alveolar cells, ATP and uridine triphosphate (UTP) stimulate P2Y receptor-mediated surfactant secretion and transepithelial chloride secretion; there are abnormalities in this mechanism in cystic fibrosis<sup>41</sup>. Nucleotides also increase mucus secretion from goblet cells and increase the ciliary beat

frequency of airway epithelial cells. Purinergic compounds are being explored for the treatment of cystic fibrosis, to improve the clearance of secretions from the bronchi in chronic obstructive pulmonary disease (COPD) and for sputum expectoration in smokers<sup>41,42</sup>. Pulmonary hypertension can be a problem in patients with COPD which also has other causes; it is a life-threatening condition, and intravenous ATP infusion produces a significant decrease in mean pulmonary arterial pressure and pulmonary vascular resistance without changing the mean systemic arterial pressure<sup>43</sup>.

The use of theophylline, an adenosine-receptor antagonist, as an antiasthmatic agent has focused attention on the development of novel P1 receptor antagonists as asthmatic medications<sup>44</sup>. ATP may also have a direct role in asthma through its actions on bronchial innervation. Nucleotides trigger a reflex bronchoconstriction by activating a P2X receptor on vagal C fibres, and both ATP and UTP can potentiate IgE-mediated mast-cell histamine release, which involves P2Y receptors<sup>45</sup>.



**Fig 3. A schematic representation of the interactions of ATP released from perivascular nerves and from the endothelium (Endoth.).** ATP is released from endothelial cells during hypoxia to act on endothelial P2Y receptors, leading to the production of endothelium-derived relaxing factor (EDRF) (NO) and subsequent vasodilation (-). In contrast, ATP released as a cotransmitter with noradrenaline (NA) from perivascular sympathetic nerves at the adventitia (Advent.)-muscle border produces vasoconstriction (+) via P2X receptors on the muscle cells. Adenosine (ADO), resulting from rapid breakdown of ATP by ectoenzymes, produces vasodilation by direct action on the muscle via P1 receptors, and acts on the perivascular nerve terminal varicosities to inhibit transmitter release. From Burnstock 1987<sup>34</sup>, reproduced with permission from Karger, Basel.

## Gastroenterology

Purinergic signalling plays a major role in different activities of the gut<sup>46</sup>. ATP is a cotransmitter in NANC nerves responsible for the inhibitory phase in peristalsis, it participates in synaptic transmission in the myenteric and submucosal ganglia, and it is involved in vascular control of the gastrointestinal tract and in the control of mucosal secretion.

A limited number of studies have been conducted to date on changes in purinergic signalling in the diseased gut. ATP and adenosine have been implicated in the development of gastric ulcers, Hirschsprung's and Chagas' diseases, ischaemia and colonic tumours<sup>46</sup>.

Intrinsic and extrinsic sensory neurons in both the myenteric and submucous plexuses of the gut show positive immunoreactivity for P2X<sub>3</sub>. It is proposed<sup>47</sup> that during moderate distension, low-threshold intrinsic enteric sensory fibres may be activated via P2X<sub>3</sub> receptors by ATP released from mucosal epithelial cells, leading to reflex propulsion of material down the gut. In contrast, during substantial (colic) distension associated with pain, higher-threshold extrinsic sensory fibres may be activated by ATP released from the mucosal epithelia; these fibres pass messages through the dorsal root ganglia to pain centres in the central nervous system<sup>47</sup>. P2X<sub>3</sub> receptor expression is increased in human inflammatory bowel disease, suggesting a potential new therapeutic target in the treatment of dysmotility and pain<sup>48</sup>.

## Urogenital system

There is a substantial presence of purinoceptors in the kidney, including subtypes involved in the regulation of renin secretion, glomerular filtration and the transport of water, ions, nutrients and toxins. ATP and adenosine have been used to protect kidneys from renal ischaemic–reperfusion injury, and are being explored for the treatment of chronic renal failure and transplantation-induced erythrocytosis<sup>49</sup>.

In the normal human bladder, atropine will block at least 95% of parasympathetic nerve-mediated contraction, indicating that its innervation is predominantly cholinergic; purinergic signalling is responsible for the atropine-resistant component of contraction<sup>50</sup>. There are a number of examples of the purinergic component of cotransmission increasing in pathological conditions<sup>12,50</sup>. One is that purinergic nerve-mediated contraction of the human bladder is increased to 40% in pathophysiological conditions such as interstitial cystitis, outflow obstruction, idiopathic instability and possibly also neurogenic bladder. Purinergic signalling also appears to play a role in afferent sensation from the bladder. ATP is released from urothelial cells when the bladder is distended. Sensory-nerve recording has indicated that P2X<sub>3</sub> receptors are involved in mediating the nerve responses to bladder distension, providing mechanosensory feedback involving both the micturition reflex and pain<sup>51</sup>. This, too, might be a potential target for pharmacological manipulation in the treatment of detrusor instability.

Normal penile erectile function depends on a delicate balance between contracting and relaxing factors in the corpus

cavernosum smooth muscle fibres, which are modulated by signalling from both nerves and endothelial cells. Evidence has accumulated to support a pivotal role for NANC neurotransmitters. NO plays a central role in mediating cavernosal smooth muscle relaxation, but other neurotransmitters can modulate this action and may play a role in erectile dysfunction. ATP potentially relaxes cavernosal smooth muscle strips *in vitro*, an action pharmacologically consistent with P2Y receptors. Indeed, P2Y receptors are present on both cavernosal smooth muscle cells and endothelial cells, and ATP is released from a subpopulation of the cavernosal nerves. It appears that smooth muscle relaxation is caused both by ATP acting directly on the cavernosal smooth muscle cells and indirectly, mediated by NO released from the endothelial cells. ATP-mediated cavernosal relaxation is impaired in diabetes mellitus (independent of NO), implying that purinergic signalling may be involved in the pathophysiology of erectile dysfunction<sup>52</sup>.

The potential role of P2X<sub>3</sub> receptors in mechanosensory transduction has already been mentioned in relation to the bladder. However, there is increasing evidence that this is not an isolated phenomenon and that ATP released from the epithelial linings of other organs, such as the ureter, gut and bile duct, following distension may act on P2X<sub>3</sub> receptors on afferent nerves in the subepithelial plexuses to provide sensory feedback and, in the case of the ureter, renal colic pain (Fig 4). P2X<sub>3</sub> receptors have been found on the suburothelial nerve plexus, and both human and guinea-pig ureters release ATP in a pressure-dependent fashion when distended<sup>54</sup>. This ATP release is abolished when the urothelium is removed, and sensory-nerve recordings during ureteral distension have demonstrated purinergic involvement, suggesting that specific P2X<sub>3</sub> antagonists may be able to alleviate renal colic<sup>51</sup>.

## Musculoskeletal system

Several reports implicate purinergic signalling in bone development and remodelling. Both P2X and P2Y receptors are present on osteoclasts, while only P2Y receptors are present on osteoblasts. ATP, but not adenosine, stimulates the formation of osteoclasts and their resorptive actions *in vitro*, and can inhibit osteoblast-dependent bone formation. The bisphosphonate clodronate, which is used in the treatment of Paget's disease and tumour-induced osteolysis, may act through osteoclast P2 receptors. A recent study has shown that very low (nM) concentrations of ADP acting through P2Y<sub>1</sub> receptors turn on osteoclast activity<sup>55</sup>. Modulation of P2 receptor function may have potential in the treatment of osteoporosis<sup>56</sup>.

## Immune system and inflammation

ATP and adenosine are released at sites of inflammation. ATP is involved in the development of inflammation through a combination of actions: release of histamine from mast cells, provoking production of prostaglandins, and the production and release of cytokines from immune cells<sup>57</sup>. In contrast, adenosine exerts anti-inflammatory actions.



In addition to the roles of purines in inflammation, they have a broad range of functions carried out through purinergic receptors on immune cells, including killing intracellular pathogens by inducing apoptosis of host macrophages, chemoattraction and cell adhesion<sup>58</sup>. Purinergic compounds may turn out to be useful for the treatment of neurogenic inflammation, rheumatoid arthritis and periodontitis<sup>59</sup>.

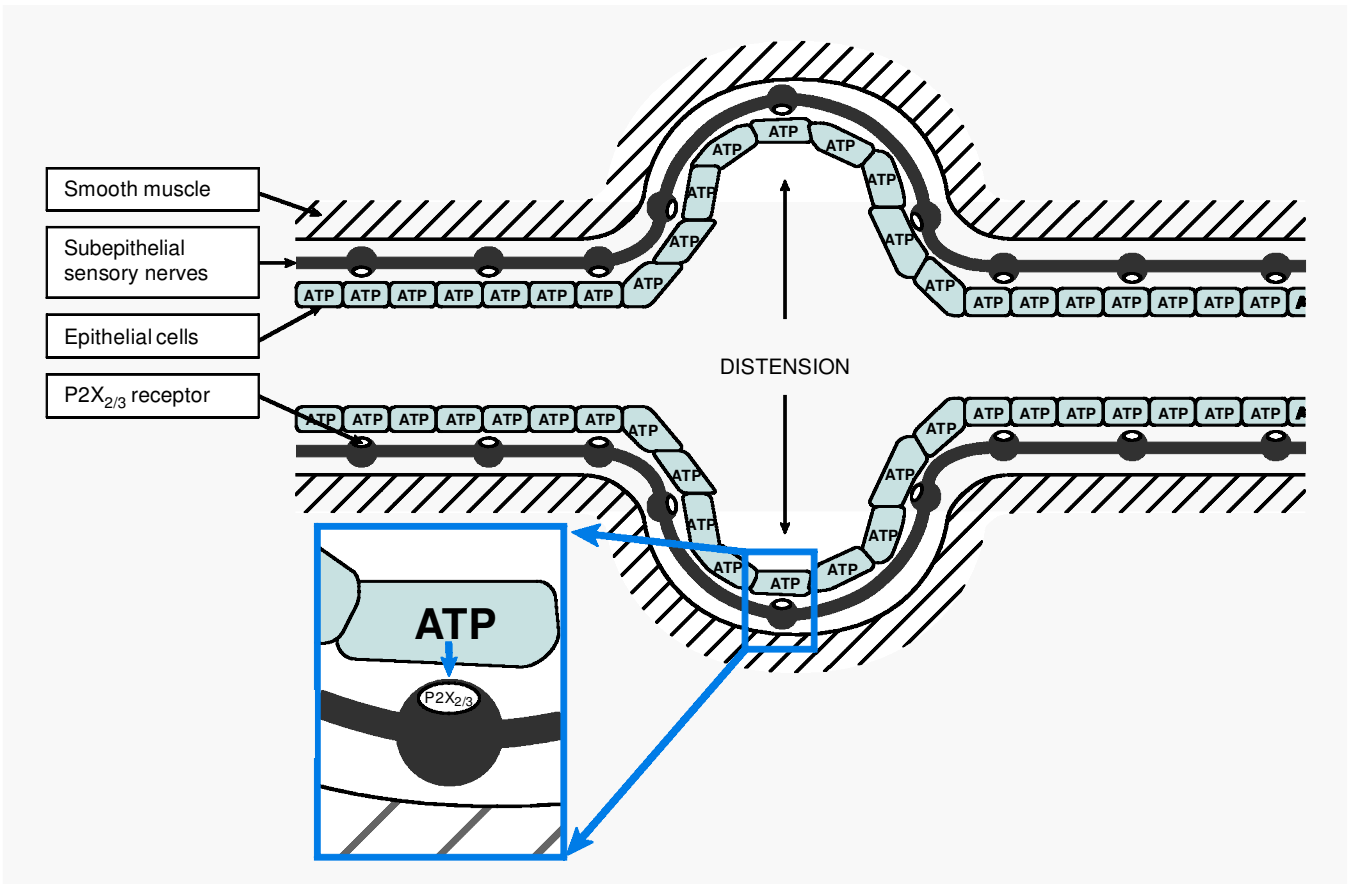
### Oncology

The anticancer activity of adenine nucleotides was first described by Rapaport in 1983<sup>60</sup>. Intraperitoneal injection of ATP into tumour-bearing mice resulted in significant anticancer activity against several fast-growing aggressive carcinomas<sup>13</sup>. ATP inhibits the growth of murine colonic adenocarcinoma and human pancreatic carcinoma in mice as well as inhibiting the associated weight loss. Growth of prostate-cancer cells *in vitro* is inhibited by up to 90% by ATP via P2 receptors, although it is not yet clear which subtype mediates this effect and whether it is a directly antiproliferative effect or a proapoptotic effect<sup>61</sup>. Phase I clinical trials have shown that ATP infusion in patients with advanced cancer is feasible, but is limited by chest tightness

and dyspnoea, probably due to conversion to adenosine<sup>13</sup>. A phase II trial has been carried out in patients with non-small cell lung cancer, showing that intravenous ATP administered for 96 hours at 4-week intervals reduced weight loss<sup>13</sup>. A combination of interferon- $\gamma$  and ATP is being explored for the treatment of acute myeloid leukaemia. Further clarification of the mechanisms mediating the dramatic antineoplastic effects of ATP on breast, ovarian and colorectal carcinoma<sup>62</sup> may yield new purinergic agents that are more specific, better tolerated and more appropriate for human trials.

### Diabetes

P2Y receptors are present on pancreatic  $\beta$ -cells and are involved in insulin secretion. ATP stimulates pancreatic insulin release through a glucose-dependent P2Y receptor-mediated mechanism<sup>63</sup>, and also modulates insulin secretion through interactions with ATP-sensitive potassium channels in islet  $\beta$ -cells. The potential role of purinergic compounds as novel treatments for diabetes, especially type II, has yet to be fully explored.



**Fig 4.** Schematic representation of the hypothesis for purinergic mechanosensory transduction in tubes (eg ureter, vagina, salivary and bile ducts and gut) and sacs (eg 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 central nervous system. From Burnstock 1999<sup>53</sup>; reproduced with permission from Cambridge University Press.

## Special senses

In the eye, ATP, acting via both P<sub>2</sub>X and P<sub>2</sub>Y receptors, modulates retinal neurotransmission, affecting retinal blood flow and intraocular pressure. The ATP analogue  $\beta,\gamma$ -methylene ATP is more effective in reducing intraocular pressure (40%) than muscarinic agonists such as pilocarpine (25%) and  $\beta$ -adrenoceptor blockers (30%), raising the potential for the use of purinergic agents in glaucoma. P<sub>2</sub>Y<sub>2</sub> receptor activation increases salt, water and mucus secretion, and thus represents a potential treatment for dry eye conditions<sup>41</sup>. In the pigmented layer of the retina P<sub>2</sub>Y<sub>2</sub> receptor activation promotes fluid absorption, and may be involved in retinal detachment.

In the auditory system ATP, acting via P<sub>2</sub>Y receptors, depresses sound-evoked gross 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<sup>64</sup>. P<sub>2</sub>X splice variants are found on the endolymphatic surface of the cochlear endothelium, an area associated with sound transduction. Both P<sub>2</sub>X and P<sub>2</sub>Y receptors have been identified in the vestibular system. ATP may regulate fluid homeostasis, cochlear blood flow, hearing sensitivity and development, and thus may be useful in the treatment of Ménière's disease, tinnitus and sensorineural deafness.

## Future developments

Although in its infancy, the clinical manipulation of purinergic signalling is no longer speculative. Several clinically relevant pharmacological interventions are already part of day-to-day practice. However, one of the main reasons why we do not yet have more purinergic therapies in our formularies is the current sparsity of receptor-subtype-specific agonists and antagonists that are effective *in vivo*. In addition to the development of selective agonists and antagonists for the different P<sub>2</sub> receptor subtypes, therapeutic strategies are likely to include agents that control the expression of P<sub>2</sub> receptors, inhibitors of extracellular breakdown of ATP and enhancers or inhibitors of ATP transport. Investigating the interactions of purinergic signalling with other established signalling systems will also be important. The development of new investigative tools and recent advances in the understanding of cell biology mean that progress is being made at an ever-increasing rate.

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## Key Points

**Purinergic signalling is widespread and acts through three families of receptors: P<sub>1</sub> receptors for adenosine; P<sub>2</sub>X ligand-gated ion-channel receptors and P<sub>2</sub>Y G-protein-coupled receptors for ATP, ADP and UTP**

**P<sub>2</sub>Y<sub>12</sub> receptors on platelets mediate ADP-induced aggregation; the P<sub>2</sub>Y<sub>12</sub> receptor antagonist clopidogrel (Plavix) is a very promising antithrombotic agent, especially when combined with aspirin**

**P<sub>2</sub>X<sub>3</sub> receptors are located in nociceptive sensory nerves, and selective antagonists are being developed against the initiation of visceral pain**

**P<sub>2</sub>Y<sub>2</sub> agonists and antagonists, which modulate mucus secretion, are being developed to treat cystic fibrosis, chronic obstructive pulmonary disease and 'dry eye' conditions**

**Purinergic agonists and antagonists are being developed to modulate cell proliferation, migration, differentiation and death for treating tumours, osteoporosis and vascular conditions such as hypertension, atherosclerosis and restenosis, and to regulate angiogenesis**

**Adenosine is being used successfully as a diagnostic tool and for reversing supraventricular tachycardia. The antiasthmatic drug theophylline acts through antagonism of the adenosine receptor, and adenosine-receptor antagonists are being explored for the treatment of Parkinson's disease**

**The therapeutic potentials of purinergic compounds in kidney failure, glaucoma, osteoporosis, wound healing, detrusor instability, erectile function and arthritis are also being considered**

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