REVIEW

Purinergic Signalling: Pathophysiology and Therapeutic Potential

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The article begins with a review of the main conceptual steps involved in the development of our understanding of purinergic signalling, including non-adrenergic, non-cholinergic (NANC) neurotransmission; identification of ATP as a NANC transmitter; purinergic cotransmission; recognition of two families of purinoceptors [P1 (adenosine) and P2 (ATP/ADP)]; and, later, cloning and characterisation of P1 (G protein-coupled), P2X (ion channel) and P2Y (G protein-coupled) receptor subtypes. Further studies have established the involvement of ATP in synaptic neurotransmission in both ganglia and in the central nervous system; long-term (trophic) purinergic signalling in cell proliferation, differentiation and death occurring in development and regeneration; and short-term purinergic signalling in neurotransmission, neuromodulation and secretion. ATP is released from most cell types in response to gentle mechanical stimulation and is rapidly degraded to adenosine by ecto-nucleotidases. This review then focuses on the pathophysiology of purinergic signalling in a wide variety of systems, including urinogenital, cardiovascular, airway, musculoskeletal and gastrointestinal. Consideration is also given to the involvement of purinoceptors in pain, cancer and diseases of the central nervous system. Purinergic therapeutic approaches for the treatment of some of these diseases are discussed.
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Introduction

The emphasis in this article is on the growing interest in the pathophysiology and therapeutic potential of purinergic signalling.[1–8] However, it begins with a review of the discovery and main conceptual steps that have contributed to our current understanding of purinergic signalling.

Basic Science − Conceptual Steps

Non-adrenergic, non-cholinergic neurotransmission

The sucrose-gap technique for recording correlated mechanical and electrical activity of smooth muscle was described[9] and applied to a guinea-pig taenia coli innervated smooth muscle preparation.[10,11] Experiments were carried out at Melbourne University in which neurotransmission mediated by the classical autonomic neurotransmitters acetylcholine (ACh) and noradrenaline (NA) were blocked by atropine and bretylium. We were expecting to see direct stimulation of the smooth muscle resulting in depolarisation and contraction. However, to our surprise and excitement, the response was hyperpolarisation to single pulses and relaxation. Tetrodotoxin, which was known to block nerve conduction but not affect smooth muscle activity, completely blocked the hyperpolarisations (**Fig. 1**), showing that they were inhibitory junction potentials in response to non-adrenergic, non-cholinergic (NANC) neurotransmission.[12] It was later shown that the response to NANC nerve stimulation was mediated

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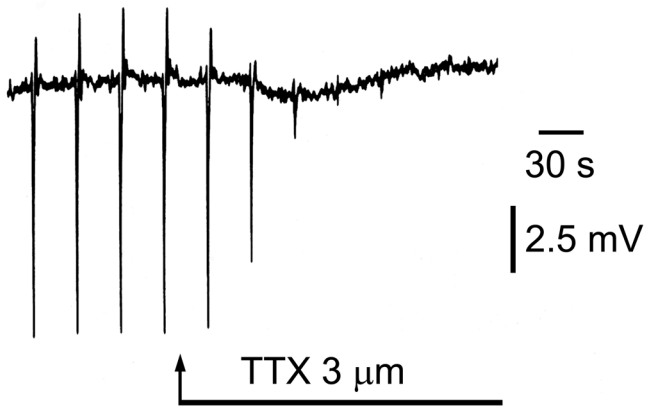

Fig. 1 Sucrose-gap recording of membrane potential changes in smooth muscle of guinea-pig taenia coli in the presence of atropine (0.3 μM) and guanethidine (4 μM). Transmural field stimulation (0.5 ms, 0.033 Hz, 8 V) evoked transient hyperpolarisations, which were followed by rebound depolarizations. Tetrodotoxin (TTX, 3 μM) added to the superfusing Kreb's solution (applied at arrow) rapidly abolished the response to transmural field stimulation, establishing these as inhibitory junction potentials in response to non-adrenergic, non-cholinergic neurotransmission. (Reproduced from Burnstock[98] with permission of Blackwell Publishing.)

by intrinsic inhibitory enteric neurones that were controlled by vagal and sacral parasympathetic nerves.[13]

Purinergic neurotransmission

We tried to identify the transmitter involved in NANC inhibitory neurotransmission in the gut and NANC excitatory neurotransmission in the urinary bladder by seeing if neuropeptides, monoamines or amino acids satisfied the criteria proposed by Eccles, but we were not successful. However, after reading two papers, one by Drury and Szent-Györgyi[14] that described extracellular actions of purines on the heart and blood vessels and a later paper by Pamela Holton[15] that showed release of ATP during antidromic stimulation of sensory nerves, in 1970, together with David Satchell, we showed that ATP satisfied the criteria for NANC neurotransmission in both gut and bladder.[16,17] In a review 2 years later, I invented the word "purinergic," ATP being a purine nucleotide, and came up with the purinergic hypothesis (**Fig. 2**).[18] Unfortunately, this concept met with almost universal opposition during the following 20 years. This was perhaps not surprising, because ATP was well established as an intracellular energy source, and it seemed unlikely that such a ubiquitous molecule would also be involved in extracellular signalling.[19,20]

Purinergic cotransmission

During a sabbatical leave at UCLA, we showed that ATP was released not only from NANC nerves in the taenia coli but also from sympathetic nerves supplying the smooth muscle.[21] This was followed by several other papers supporting the concept that ATP might be a cotransmitter with NA from sympathetic nerves,[22,23] and I published a Commentary in *Neuroscience* entitled: "Do some nerve cells release more than one transmitter?" which challenged what had been known as Dale's Principle, that one nerve only released one transmitter.[24] This was later followed by convincing evidence that the excitatory junction potentials recorded in smooth muscle of the vas deferens in response to sympathetic stimulation[25,26] were due to release of ATP as a cotransmitter with NA.[27,28] Parasympathetic nerves supplying the urinary bladder were shown to utilize ATP as a cotransmitter with ACh.[17,29] The cotransmitter concept was also initially resisted, but it is now well established that every nerve, in both the peripheral and central nervous system (CNS), utilises ATP as a cotransmitter (**Table 1**).[30]

Purinergic receptors

In 1978, I recognised from hints in the literature and some simple experiments that there were different receptor families for adenosine (called P1 receptors) and for ATP and ADP (called P2 receptors).[31] P1, but not P2 receptors, were antagonised by methylxanthines. P2 receptors were later subdivided into P2X and P2Y families, based on pharmacology.[32] However, widespread acceptance of purinergic signalling did not come until the early 1990s, when the receptors to purines and pyrimidines were cloned and characterised. First, four P1 receptor subtypes were identified: A_1, A_{2A}, A_{2B} and A_3.[33,34] In 1993, we cloned a G protein-coupled ATP receptor, which we named $P2Y_1$,[35] at about the same time as David Julius and his colleagues in San Francisco cloned a $P2Y_2$ receptor.[36] The first two P2X ion channel receptors were cloned and characterised the following year.[37,38] Later, a formal division into P2X ionotropic and P2Y metabotropic receptor families was proposed.[39]

A major conceptual advance was made when the crystal structure of P2X4 receptors was presented (**Fig. 3**),[40] thereby enabling medicinal chemists to start development of selective P2X receptor agonists and antagonists for therapeutic purposes. Cloning of P2 receptors led to studies of immunohistochemical localization of purinoceptors, and this showed that most non-neuronal cells, as well as neurones, expressed multiple P2 receptors.[41] More recent studies have shown that P2X purinoceptors are expressed in primitive animals, such as amoeba and *Schistosoma*, that have comparable molecular structures to those identified in mammals, indicating that ATP was likely one of the earliest extracellular messengers.[42]

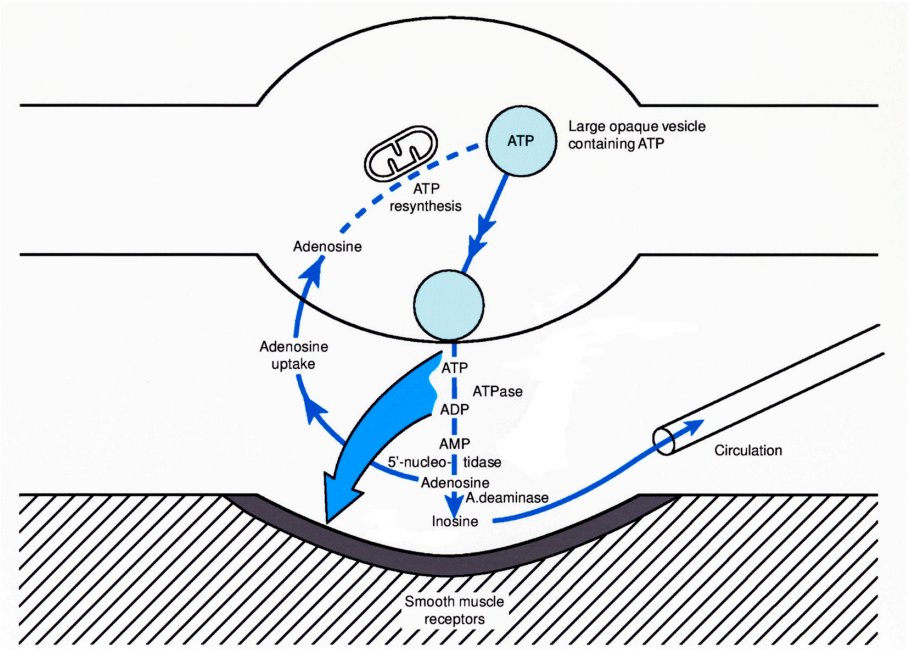

Fig. 2 Purinergic neuromuscular transmission depicting the synthesis, storage, release and inactivation of ATP. ATP stored in vesicles in nerve varicosities is released by exocytosis to act on postjunctional receptors for ATP on smooth muscle. ATP is broken down extracellularly by ATPases and 5′-nucleotidase to adenosine, which is taken up by varicosities to be resynthesised and reincorporated into vesicles. Adenosine is broken down further by adenosine deaminase (A.deaminase) to inosine and hypoxanthine and removed by the circulation. (Reproduced from Burnstock[18] with permission from the American Society for Pharmacology and Experimental Therapeutics.)

Table 1 Summary of cotransmitters in the peripheral and central nervous systems (Modified from Abbracchio et al[99] with permission from Elsevier)

	Cotransmitters	References
Peripheral nervous system	ATP + NA + NPY	28, 100
Sympathetic nerves	ATP + ACh +VIP	101
Parasympathetic nerves	ATP + CGRP + SP	102
Sensory-motor	ATP +NO +VIP	103,104
NANC enteric nerves	ATP + ACh	105,106
Motor nerves (in early development)		
Central nervous system	ATP + ACh	107
Cortex, caudate nucleus	ATP + NA	108, 109
Hypothalamus, locus coeruleus	ATP + GABA	110
Hypothalamus, dorsal horn, retina	ATP + DA	111
Mesolimbic system	ATP + glutamate	112, 113
Hippocampus, dorsal horn		

NPY, neuropeptide Y; ACh, acetylcholine; VIP, vasoactive intestinal peptide; CGRP, calcitonin gene-related peptide; SP, substance P; NO, nitric oxide; GABA, γ-aminobutyric acid; DA, dopamine

Purinergic synaptic transmission

Purinergic synaptic neurotransmission was reported between neurones in ganglia[43,44] and in the brain.[45] This was a conceptual step, which was particularly influential to neuroscientists interested in the CNS.

Short- and long-term (trophic) purinergic signalling

In addition to short-term purinergic signalling in neurotransmission, neuromodulation, secretion and platelet aggregation, it is now recognised that long-term (trophic) purinergic signalling is involved in cell proliferation, dif-

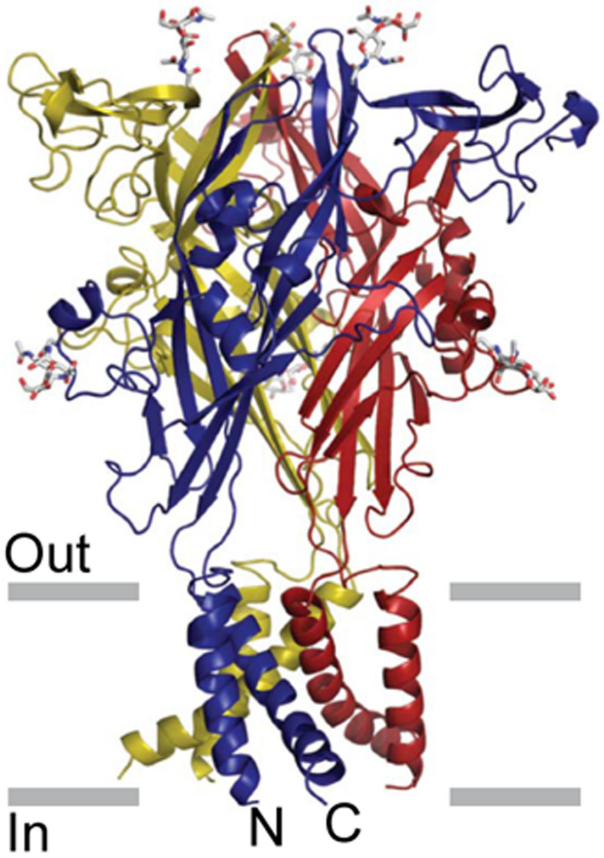

Fig. 3 The architecture of the P2X4 receptor. Stereoview of the homotrimeric ΔzfP2X4 structure viewed parallel to the membrane. Each subunit is depicted in a different colour. *N*-acetylglucosamine and glycosylated asparagine residues are shown in stick representation. The grey bars suggest the boundaries of the outer (Out) and inner (In) leaflets of the membrane bilayer. (Reproduced from Kawate et al[40] with permission from the Nature Publishing Group.)

ferentiation and death during development and regeneration,[46] for example in vascular[47] and bone[48] remodelling, and in cancer.[49] There is evidence that ATP acts synergistically with growth factors to produce trophic signalling, for example, during neurite extension following transplantation of the myenteric plexus into the brain[50] and in stem cell differentiation.[51,52]

ATP release and breakdown

It is now recognised that ATP is released, without causing any damage, from many cell types, although for many years it was assumed that the main source of ATP acting on purinoceptors was damaged or dying cells. Endothelial and urothelial cells, astrocytes, macrophages, osteoblasts, odontoblasts and many other cell types have been reported to release ATP in response to gentle mechanical disturbance, hypoxia and some agents.[53,54] Purinergic mechanosensory transduction occurs in a variety of physiological and pathophysiological events, including visceral pain.[55,56] The mechanism of ATP transport from cells appears to be a combination of connexin and pannexin-1 hemichannels and vesicular exocytosis. Much is now known about the ectoenzymes involved in the breakdown of released ATP into ADP, AMP, adenosine, inosine and hypoxanthine.[57,58] The enzymes involved include ectonucleoside triphosphate diphosphohydrolases, ecto-nucleotide pyrophosphatase/phosphodiesterases, alkaline phosphatases, 5′-nucleotidase and monoamine oxidase.

Pathophysiology and Therapeutic Potential

Bladder disorders

In contrast to laboratory animals, in which the purinergic component of parasympathetic cotransmission supplying the bladder is about 50%, in healthy human bladder it is only about 2%, even though receptors for ATP are expressed by the smooth muscle. However, in pathological conditions such as interstitial cystitis, outflow obstruction and neurogenic bladder, the purinergic component increases to up to 40% and is therefore a target for therapeutic treatment.[3] Botulinum neurotoxin type A is widely used to treat bladder incontinence and acts by inhibiting the release of ATP and ACh from both parasympathetic nerves and urothelial cells.[59,60]

Cardiovascular disorders

ADP acting via $P2Y_1$ and $P2Y_{12}$ receptors mediates platelet aggregation, and so $P2Y_{12}$ receptor antagonists clopidogrel and related compounds are widely used for the treatment of thrombosis and stroke.[61,62]

There are reports that in spontaneously hypertensive rats there is a significantly greater cotransmitter role for ATP in sympathetic nerves supplying blood vessels.[4] Long-term purinergic control of proliferation of smooth muscle and endothelial cells via P1 and P2Y receptors during vascular remodelling in restenosis following angioplasty and in atherosclerosis has been reported, and therapeutic possibilities are being explored.[47]

There is increased expression of P2X1 and $P2Y_2$ receptors in the hearts of rats with congestive heart failure.[63]

Diabetes

α-Cells in pancreatic islets express P2X7 receptors. In streptozotocin-induced diabetes, these P2X7 receptor-expressing cells replace the space no longer occupied by insulin-containing β-cells.[64] Purinergic compounds are being explored for the treatment of diabetes.[65]

Osteoporosis

The involvement of purinergic signalling in bone development and regeneration has been reported, and therapeutic approaches are being explored for osteoporosis, in particular treatment with P2X7 receptor antagonists.[47,66]

Kidney failure

There is rich expression of purinoceptor subtypes in the kidney, both in the glomerulus and tubules. Increased expression of P2X7 receptors in diabetes, hypertension, polycystic kidney disease and glomerulonephritis is opening up novel purinergic possibilities for the treatment of kidney failure.[67–69]

Erectile dysfunction and in vitro fertilization (IVF)

Abnormalities in purinergic signalling may be involved in the pathophysiology of erectile dysfunction associated with diabetes and prostate enlargement and might be a target for therapy.[70–72]

Treatment of sperm with extracellular ATP improves the *in vitro* fertility rate for IVF.[73,74]

Gut disorders

A variety of different activities in the gut involve purinergic signalling, including peristalsis, synaptic transmission in both myenteric and submucous plexuses, vascular tone, mucosal secretion and pain.[75] Exploratory studies for purinoceptors as therapeutic targets for gut disorders are in progress,[76] including P2X3 receptor antagonists for inflammatory bowel disease.[77]

Cystic fibrosis

A long-lasting $P2Y_2$ receptor agonist has been developed and has been in clinical trials for the treatment of cystic fibrosis. It has been accepted on the Japanese drug market for the treatment of dry eye.[78]

Hyperventilation

Cells of the neuroendocrine bodies, which line the lungs at intervals in the epithelium, release ATP in response to distension, which then acts via P2X3 receptors to activate vagal sensory fibres originating in the nodose ganglion.[79,80] This appears to be a mechanism underlying reflex responses to hyperventilation and noxious gases.

HIV/AIDS

Recent papers have implicated purinergic signalling in HIV/AIDS and may offer novel therapeutic approaches.[81–83]

Pain

The involvement of purinergic signalling in the initiation of pain was proposed in an article in the *Lancet* in 1996.[84] It was proposed that P2X3 receptors, expressed on nociceptive nerve endings,[85] were stimulated by ATP released as a cotransmitter from sympathetic nerves during causalgia and reflex sympathetic dystrophy. It was also suggested that ATP was released from endothelial cells in the microvasculature supplying heart, skeletal muscle and cerebral vessels during angina, ischemia and migraine and from tumour cells during cancer pain. Later, purinergic mechanosensory transduction was identified (**Fig. 4**)[55] and its involvement in the initiation of visceral pain proposed.[86,87] Kazu Inoue and colleagues showed that there was increased expression of P2X4 receptors on microglia in neuropathic pain, and this pain was reduced by antagonists to the P2X4 receptor and in P2X4 receptor knockout mice.[88,89] Later, antagonists to P2X7 and P2Y receptors expressed on microglia were also shown to reduce neuropathic pain.[86] P2X3 and P2X7 receptors are being targeted for the treatment of arthritis.[90,91]

Disorders of the CNS

Investigations of the roles of purinergic signalling have been reported in disorders of the CNS, including injury due to accidents, surgery, stroke and ischemia, neurodegenerative diseases (such as Alzheimer's, Parkinson's and Huntington's diseases), multiple sclerosis, epilepsy and neuropsychiatric disorders (including schizophrenia, depression and anxiety).[56,92,93]

Cancer

It was recognised early that ATP was effective against cancer.[94] More recent studies have shown that $P2Y_1$ and $P2Y_2$ receptors mediate proliferation in most tumours, that P2X5 receptors mediate differentiation and therefore are anti-proliferative, and that P2X7 receptors lead to apoptotic death of tumour cells (**Fig. 5**).[5,49] The therapeutic potential of $P2Y_1$ and $P2Y_2$ receptor antagonists and P2X5 and P2X7 receptor agonists is being explored.

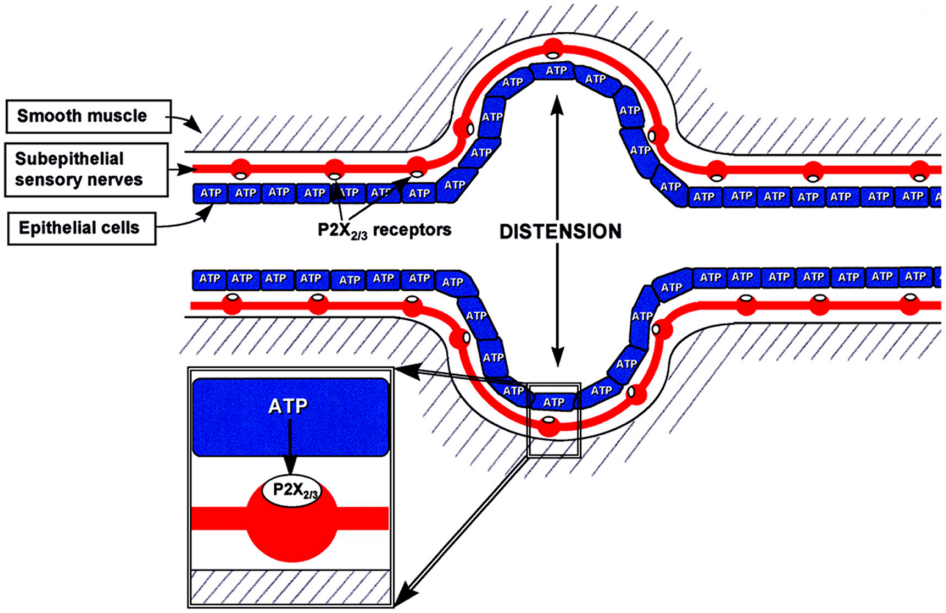

Fig. 4 Schematic hypothesis for purinergic mechanosensory transduction in tubes (e.g., ureter, vagina, salivary and bile ducts, gut) and sacs (e.g., urinary and gall bladders and lung). It is proposed that distension leads to release of ATP from epithelium lining the tube or sac, which then acts on P2X3 and/or P2X2/3 receptors on subepithelial sensory nerves to convey sensory/nociceptive information to the CNS. (Reproduced from Burnstock[55] with permission from Blackwell Publishing.)

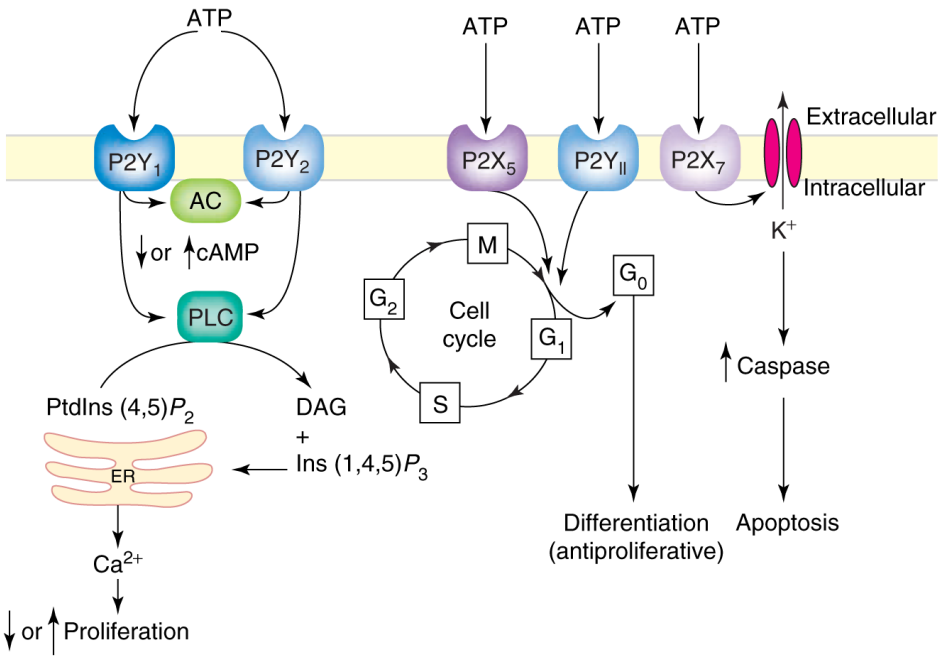

Fig. 5 Schematic of the different mechanisms by which P2 receptor subtypes might alter cancer cell function. $P2Y_1$ and $P2Y_2$ receptors could affect the rate of cell proliferation through altering the intracellular levels of cAMP by modulating adenylyl cyclase (AC) or by increasing intracellular calcium levels through the phospholipase C (PLC) pathway. P2X5 and $P2Y_{11}$ receptor activation might switch the cell cycle from proliferation into a state of differentiation. The P2X7 receptor activates the apoptotic caspase enzyme system. PtdIns (4, 5)P_2, phosphatidylinositol 4,5-bisphosphate; DAG, diacylglycerol; ER, endoplasmic reticulum. (Reproduced from White and Burnstock[49] with permission from Elsevier.)

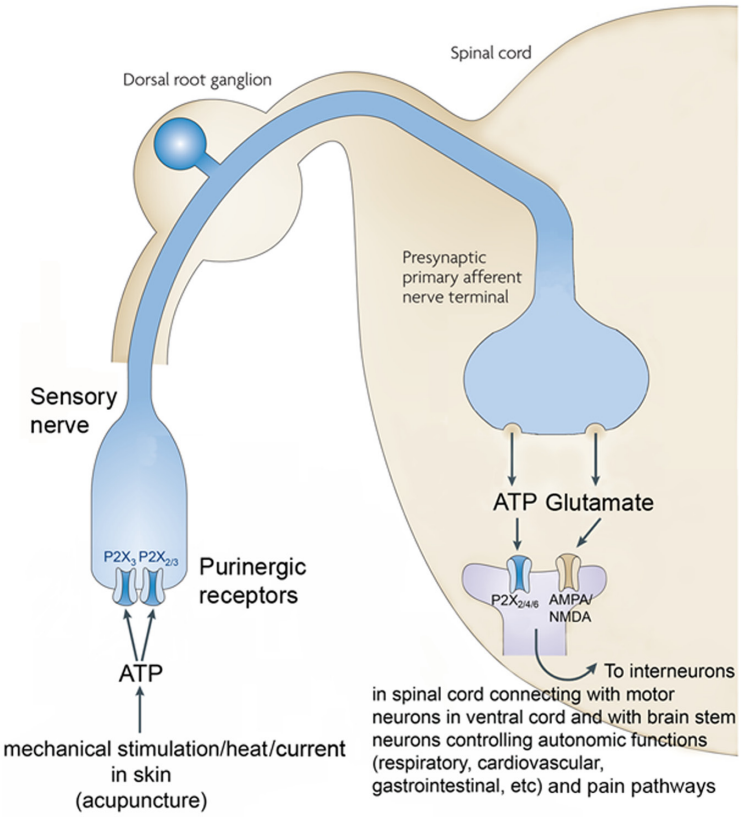

Fig. 6 Schematic hypothesis of purinergic signalling in acupuncture. AMPA, 2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)propanoic acid; NMDA, *N*-methyl-D-aspartate. (Reproduced from Burnstock[95] with permission from Elsevier.)

Diseases of the special senses

Purinergic signalling is much involved in the physiology of the eye, ear, nasal organs and tongue. Consequently, purinergic therapeutic strategies are being developed for glaucoma, retinal detachment, diabetic retinopathy, Ménière's disease, tinnitus, sensorineural deafness, noxious odour damage and taste defects.[1]

Acupuncture

Acupuncture has been used over many years to treat a wide variety of diseases and to alleviate pain. The traditional Chinese view is that manipulation of the flow of meridian energy (qi) is the underlying mechanism for acupuncture. However, it seems more likely that the nervous system is involved. It has been proposed that purinergic signalling is a major factor in the physiological mechanism responsible for the effects of acupuncture.[95] It is suggested that mechanical stimulation caused by twisting needles in the skin and tongue, heat, electrical currents or laser treatment leads to release of ATP from keratinocytes and mast cells that accumulate at acupuncture sites. ATP then initiates activity via P2X3 receptors on sensory nerves supplying the skin that relay messages through interneurones to the brain stem where they modulate the activity of motor neurones that control autonomic function (**Fig. 6**). Sensory messages will also modulate the pain pathways leading to the conscious pain centres in the cortex.

Concluding Comments

The clinical manipulation of purinergic signalling is in its infancy. Therapeutic strategies to deal with pathological disorders include the design of selective P1 and P2 receptor subtype agonists and antagonists, as well as of inhibitors of extracellular ATP breakdown and of ATP transport enhancers and inhibitors. Medicinal chemists are developing small-molecule purinergic drugs that are orally bioavailable and stable *in vivo*.[3,6,96,97] There has been a remarkable growth in the number of papers published about purinergic signalling via ATP since 1972 (**Fig. 7**).

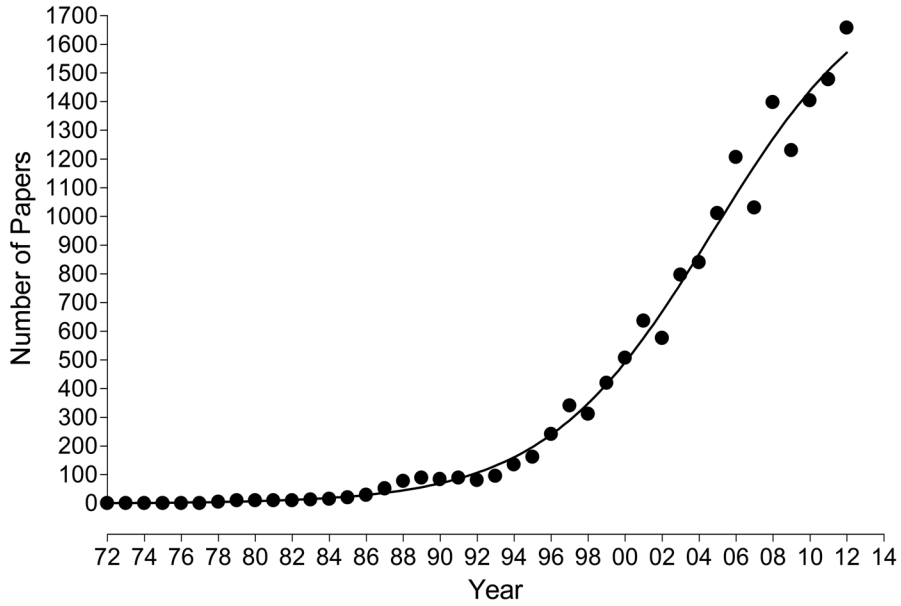

Fig. 7 Graph showing the number of papers published on P2 purinergic signalling between 1972 and the end of 2012. (Reproduced and updated from Burnstock[20] with permission from John Wiley & Sons.)

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