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Purinergic signalling: from discovery to current developments

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New Findings

- **What is the topic of this review?**
  This is a personal historical review about the discovery and the main conceptual advances leading to our current understanding of purinergic signalling. The contributions of leading figures in the field are acknowledged. It includes the discovery of purinergic neuromuscular and synaptic transmission, cotransmission, the identification of P1 (adenosine), P2X nucleotide ion channel and P2Y nucleotide G protein-coupled receptors, the identity of ectonucleotidases and release of ATP from cells by mechanical stimulation and mechanosensory transduction.
- **What advances does it highlight?**
  It highlights the pathophysiology of purinergic signalling and recent therapeutic developments.

This lecture is about the history of the purinergic signalling concept. It begins with reference to the paper by Paton & Vane published in 1963, which identified non-cholinergic relaxation in response to vagal nerve stimulation in several species, although they suggested that it might be due to sympathetic adrenergic nerves in the vagal nerve trunk. Using the sucrose gap technique for simultaneous mechanical and electrical recordings in smooth muscle (developed while in Feldberg's department in the National Institute for Medical Research) of the guinea-pig taenia coli preparation (learned when working in Edith Bülbring's smooth muscle laboratory in Oxford Pharmacology), we showed that the hyperpolarizations recorded in the presence of antagonists to the classical autonomic neurotransmitters, acetylcholine and noradrenaline, were inhibitory junction potentials in response to non-adrenergic, non-cholinergic neurotransmission, mediated by intrinsic enteric nerves controlled by vagal and sacral parasympathetic nerves. We then showed that ATP satisfied the criteria needed to identify a neurotransmitter released by these nerves. Subsequently, it was shown that ATP is a cotransmitter in all nerves in the peripheral and central nervous systems. The receptors for purines and pyrimidines were cloned and characterized in the early 1990s, and immunostaining showed that most non-neuronal cells as well as nerve cells expressed these receptors. The physiology and pathophysiology of purinergic signalling is discussed.

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Non-adrenergic, non-cholinergic (NANC) transmission

I completed my PhD, supervised by J. Z. Young, about fish gut motility. It involved simple techniques of organ bath pharmacology and histology, and I needed to learn more sophisticated techniques. Wilhelm Feldberg kindly invited me to join his Department of Physiology at the Medical Research Institute, Mill Hill, to learn electrophysiology in 1957. Together with Ralph Straub, we developed the sucrose gap technique to record correlated changes in electrical and mechanical activity of smooth muscle (Burnstock & Straub, 1958). When Edith Bülbring heard about our results, she invited me to join her smooth muscle group in the Department of Pharmacology, Oxford University, where they had been finding microelectrode recording from spontaneously active smooth muscle cells difficult. There I studied the effect of the classical neurotransmitters, acetylcholine and noradrenaline (NA), on the guinea-pig taenia coli preparation, which was the experimental model of an innervated smooth muscle preparation favoured by her group (Burnstock, 1958*a*,*b*).

I was then appointed to be a Senior Lecturer in the Department of Zoology in Melbourne, Australia, after spending a year with Ladd Prosser at the University of Illinois on a Rockefeller Fellowship. I set up the sucrose gap apparatus and began a very enjoyable collaboration in the Department of Physiology with Mollie Holman, whom I had met in Oxford. One day, together with my first postgraduate student, Graham Campbell, and Max Bennett, a part-time electronic technician, we transmurally stimulated the taenia coli in the presence of atropine and after degeneration of sympathetic nerves with 6-hydroxydopamine. We expected to see direct stimulation of the smooth muscle, resulting in depolarization and contraction, but to our surprise the response was hyperpolarization in response to single pulses, and hyperpolarization and relaxation in response to a train of pulses (Fig. 1*A*). We felt that we were on to something important (see Burnstock, 2004), but the interpretation was debated internationally. At that time, in the early 1960s, I was fortunate in having a Japanese postdoctoral fellow working with me, whose friend in Japan was one of the authors of a paper about TTX

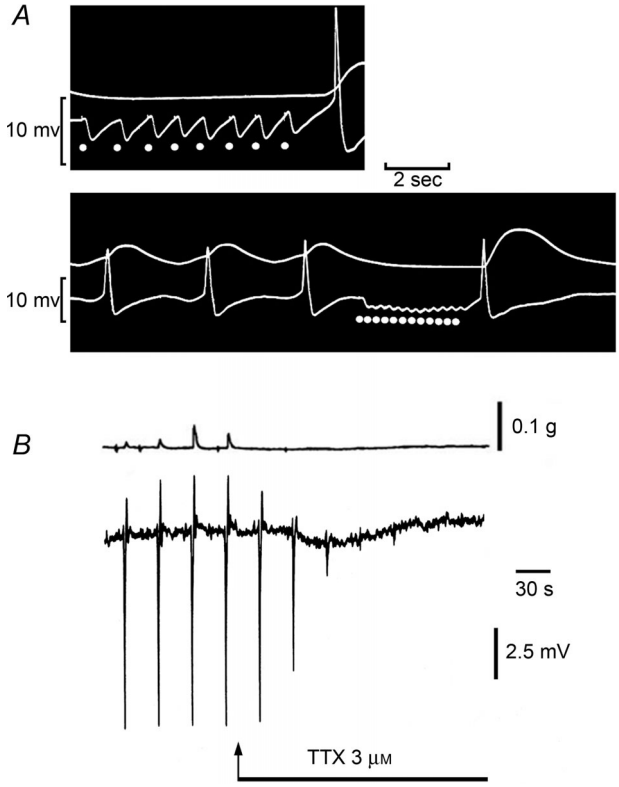

Figure 1. Changes in membrane potential and mechanical response recorded with a sucrose gap method
A, hyperpolarizations recorded in smooth muscle of the atropinized guinea-pig taenia coli in response to transmural stimulation of the intramural nerves remaining after degeneration of the adrenergic nerves by treatment of the animal with 6-hydroxydopamine (250 mg kg^{-1} I.P. for 2 successive days) 7 days previously. Upper trace shows responses to low-frequency stimulation (1 s^{-1}). Note the individual hyperpolarizations and rebound excitation (spike and contraction) following cessation of stimulation. Lower trace shows stimulation at higher frequencies, illustrating summed hyperpolarization and relaxation. [Reproduced from Burnstock (1972) with permission from the American Society for Pharmacology and Experimental Therapeutics.] *B*, transmural field stimulation (0.5 ms, 0.033 Hz, 8 V) of the taenia coli evoked transient hyperpolarizations in the presence of atropine (0.3 μM) and guanethidine (4 μM). Tetrodotoxin (TTX; 3 μM) added to the superfusing Krebs solution (applied at arrow) rapidly abolished the response to transmural field stimulation, establishing that the hyperpolarizations were inhibitory junction potentials in response to non-adrenergic, non-cholinergic (NANC) neurotransmission. [Reproduced from Burnstock (1986), reproduced with kind permission of Blackwell Publishing.]

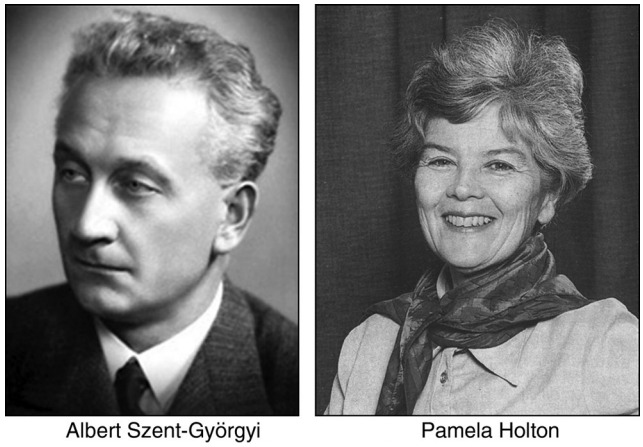

Figure 2. Photographs of Albert Szent-Györgyi and Pamela Holton

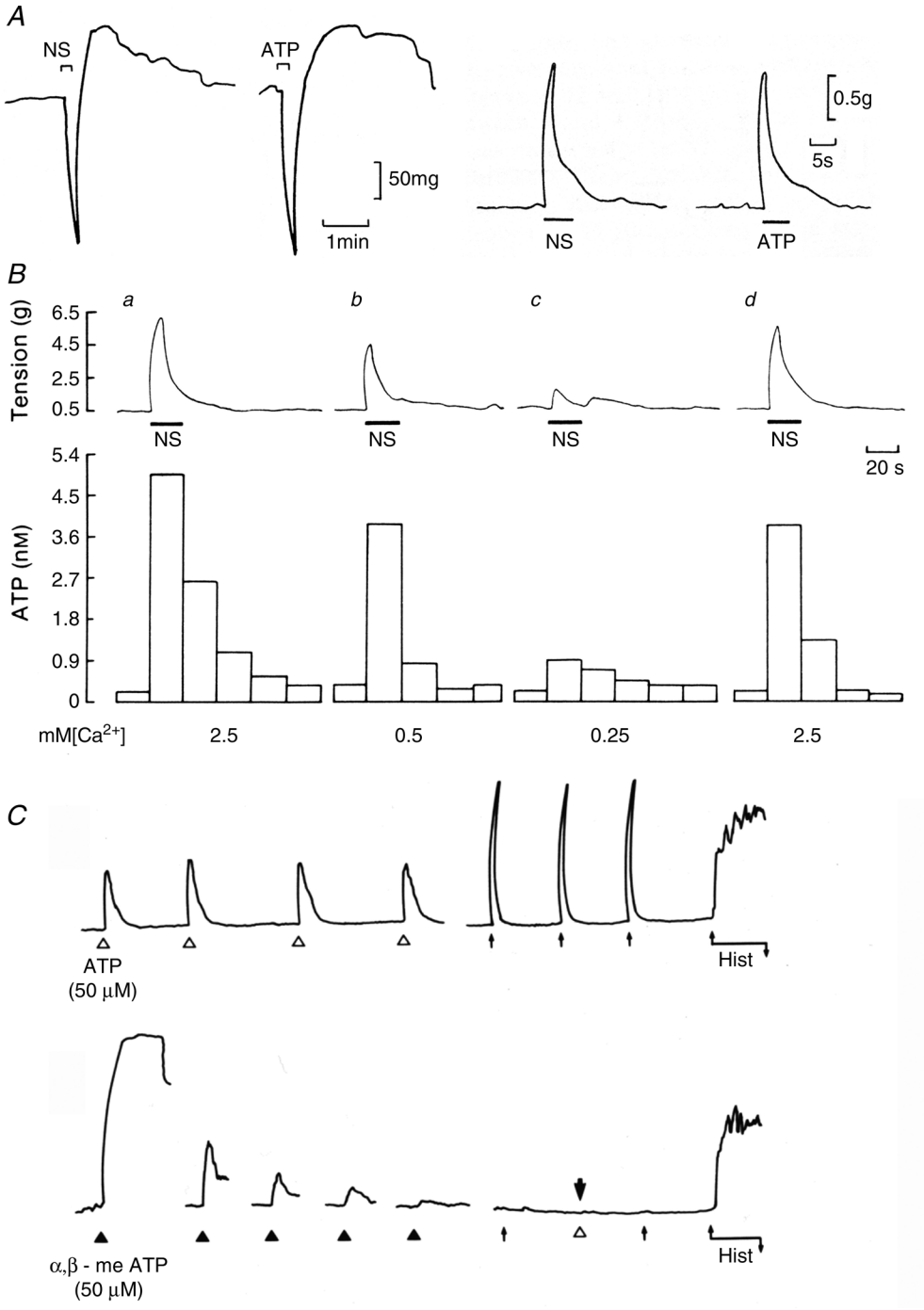

Figure 3. Evidence that ATP satisfied the criteria for its establishment as a neurotransmitter in the guinea-pig taenia coli and urinary bladder
A, left-hand side shows responses of the guinea-pig taenia coli to NANC inhibitory nerve stimulation (NS; 1 Hz, 0.5 ms pulse duration, for 10 s at supramaximal voltage) mimicked by ATP (2×10^{-6} M). Atropine (1.5×10^{-7} M), guanethidine (5×10^{-6} M) and sodium nitrite (7.2×10^{-4} M) were present. [From Burnstock & Wong (1978), reproduced with kind permission of the Nature Publishing Group.] *A*, right-hand side shows a comparison of the NANC contractile responses of the guinea-pig bladder strip to intramural nerve stimulation (NS; 5 Hz, 0.2 ms pulse duration and supramaximal voltage) mimicked by exogenous ATP (8.5 μM). Atropine (1.4 μM) and guanethidine (3.4 μM) were present throughout. [From Burnstock *et al.* (1978), reproduced with kind permission of the Nature Publishing Group.] *B*, effect of changing the calcium ion (Ca^{2+}) concentration on the release of ATP (measured with the firefly luciferin/luciferase technique) from the guinea-pig isolated bladder strip during stimulation of NANC nerves. Upper trace is a mechanical recording of changes in tension (in grams) during intramural nerve

extracted from the puffer fish. He sent us some TTX, which was known to block nerve conduction but not affect smooth muscle activity. It completely blocked the hyperpolarizations (Fig. 1*B*), so we realized that they were inhibitory junction potentials in response to NANC neurotransmission (Burnstock *et al.* 1964). Later, I worked together with Mike Rand, during a sabbatical visit to the School of Pharmacy in London, and we showed

stimulation (NS; 20 Hz, 0.2 ms pulse duration, supramaximal voltage for 20 s). Lower histograms show the concentration of ATP in consecutive 20 s fractions of the superfusate. The Ca^{2+} concentration in the superfusate varied as follows: 2.5 (normal Krebs; *a*) 0.5 (*b*), 0.25 (*c*) and 2.5 mM (*d*). The successive contractions were separated by 60 min intervals as indicated by the breaks in the mechanical trace. Atropine (1.4 μM) and guanethidine (3.4 μM) were present throughout. [From Burnstock *et al.* (1978), reproduced with kind permission of the Nature Publishing Group.] C, the effect of α,β-methylene ATP (α,β-meATP) receptor desensitization on the responses to nerve stimulation (↑), ATP (open triangles) and histamine (Hist). Atropine (1 μM) and guanethidine (3.4 μM) were present throughout. Top panel shows control responses. Bottom panel shows that α,β-me ATP desensitization, reached by five successive applications (filled triangles) at approximately 4 min intervals, completely abolished nerve-mediated (↑) and ATP-induced responses (open triangles); However, histamine-induced contraction was only slightly reduced. [Reproduced from Kasakov & Burnstock (1983), with permission.]

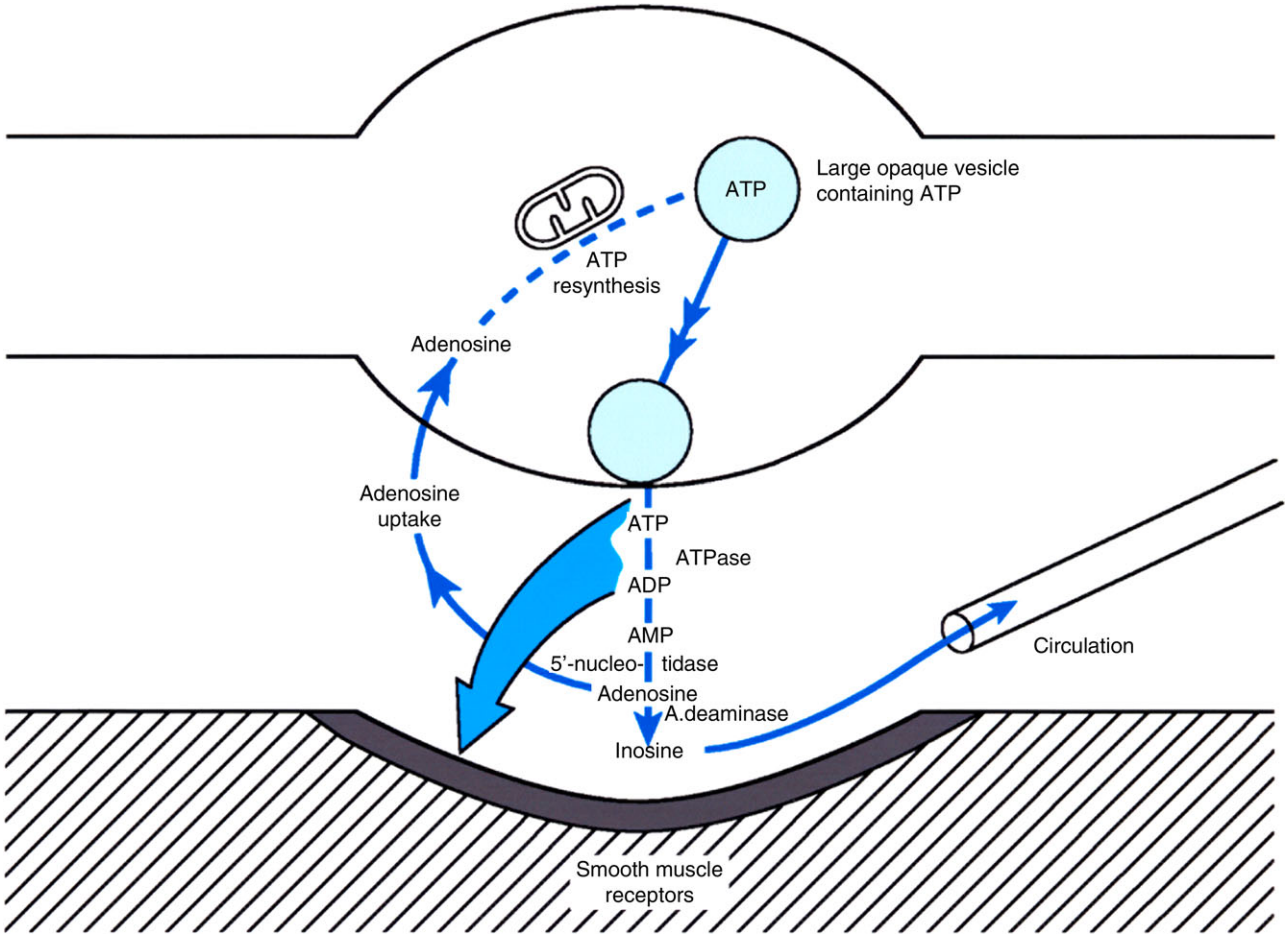

Figure 4. Purinergic neuromuscular transmission depicting the synthesis, storage, release and inactivation of ATP
Adenosine triphosphate, stored in vesicles in nerve varicosities, is released by exocytosis to act on postjunctional receptors for ATP on smooth muscle. The ATP is broken down extracellularly by ATPases and 5′-nucleotidase to adenosine, which is taken up by varicosities to be resynthesized and reincorporated into vesicles. Adenosine is broken down further by adenosine deaminase (A. deaminase) to inosine and hypoxanthine and removed by the circulation. [From Burnstock (1972), reproduced with permission from the American Society for Pharmacology and Experimental Therapeutics.]

that the NANC responses were mediated by intrinsic inhibitory neurones that were innervated by vagal and sacral parasympathetic nerves (Burnstock *et al.* 1966).

Purinergic transmission

The next question was whether we could identify the transmitter involved in NANC neurotransmission. We followed the advice of Sir John Eccles and Sir William Paton that several criteria need to be satisfied to identify a neurotransmitter, as follows: synthesis and storage in the nerve terminals; exogenous responses that mimic those to nerve stimulation; release during nerve stimulation by a Ca^{2+}-dependent mechanism; inactivation of the released transmitter by ectoenzymes or by an uptake mechanism; and identification of an antagonist that blocks both the response to nerve stimulation and the exogenously applied transmitter. Neuropeptides, monoamines and amino acids were explored, but none satisfied the criteria. However, I then read two papers, one by Drury & Szent-Györgyi (1929) that described extracellular actions of purines on the heart and blood vessels and a later paper by Pamela Holton (Holton, 1959) showing release of ATP during antidromic stimulation of sensory nerves supplying the rabbit ear artery (Fig. 2). David Satchell and I showed that ATP satisfied the criteria both for NANC

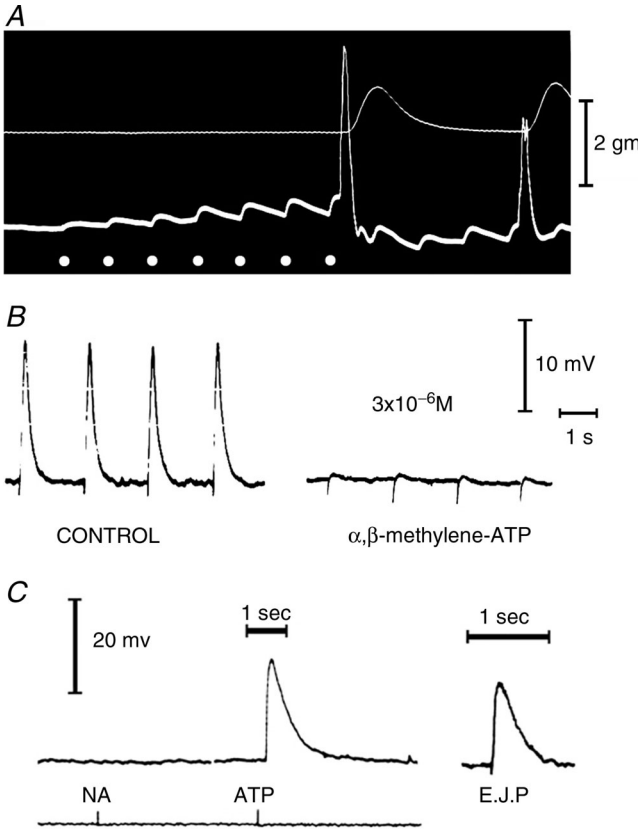

Figure 5. Evidence for ATP as a cotransmitter with noradrenaline in sympathetic nerves supplying the guinea-pig vas deferens
A, excitatory junction potentials (EJPs) in response to repetitive stimulation of sympathetic nerves (white dots) in the guinea-pig vas deferens. The upper trace records the tension, the lower trace the electrical activity of the muscle recorded extracellularly by the sucrose gap method. Note both summation and facilitation of successive junction potentials. At a critical depolarization threshold, an action potential is initiated, which results in contraction. [From Burnstock & Costa (1975), reproduced with permission of Chapman and Hall.]
B, the effect of α,β-methylene ATP (α,β-meATP) on EJPs recorded from the guinea-pig vas deferens (intracellular recording). 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 α,β-meATP, EJPs were recorded using the same stimulation parameters. The EJPs were abolished in the presence of α,β-meATP (3×10^{-6} M). [Reproduced from Sneddon & Burnstock (1984), with permission of Elsevier.]
C, spritzed ATP, but not noradrenaline (NA), mimicked the EJP recorded in the vas deferens [Reproduced from Burnstock & Verkhratsky (2012), with permission of Springer.]

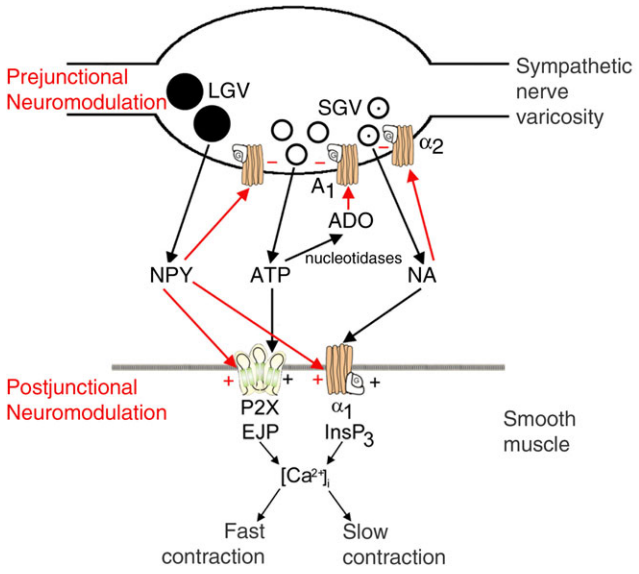

Figure 6. Schematic diagram of sympathetic cotransmission
Adenosine triphosphate released from small agranular vesicles and noradrenaline (NA) released from small granular vesicles (SGV) act on P2X and α_1-adrenoceptors on smooth muscle, respectively. ATP acting on inotropic P2X receptors evokes excitatory junction potentials (EJPs), increase in $[Ca^{2+}]_i$ and fast contraction, while occupation of metabotropic α_1-adrenoceptors leads to production of inositol trisphosphate ($InsP_3$), increase in $[Ca^{2+}]_i$ and slow contraction.
Neuropeptide Y (NPY) stored in large granular vesicles (LGV) acts on release both as a prejunctional inhibitory modulator of release of ATP and NA and as a postjunctional modulatory potentiator of the actions of ATP and NA. Nucleotidases are released from nerve varicosities, and are also present as ectonucleotidases to break ATP down to adenosine (ADO), which acts as a prejunctional modulator of ATP and NA release via A_1 receptors. Noradrenaline is also a prejunctional modulator via α_2-adrenoceptors. [Modified from Burnstock (2009*c*), and reproduced with permission from Elsevier.]

Table 1. Table showing cotransmitters in the peripheral and central nervous systems

	Cotransmitters
Peripheral nervous system	
Sympathetic nerves	ATP + NA + NPY
Parasympathetic nerves	ATP + ACh +VIP
Sensorimotor	ATP + CGRP + SP
NANC enteric nerves	ATP + NO + VIP
Motor nerves (in early development)	ATP + ACh
Central nervous system	
Cortex, caudate nucleus	ATP + ACh
Hypothalamus, locus coeruleus	ATP + NA
Hypothalamus, dorsal horn, retina	ATP + GABA
Mesolimbic system	ATP + DA
Hippocampus, dorsal horn	ATP + glutamate

Abbreviations: ACh, acetylcholine; ATP, adenosine triphosphate; CGRP, calcitonin gene-related peptide; DA, dopamine; GABA, γ-aminobutyric acid; 5-HT, 5-hydroxytryptamine; NA, noradrenaline; NANC, non-adrenergic, non-cholinergic; NO, nitric oxide; NPY, neuropeptide Y; SP, substance P; and VIP, vasoactive intestinal peptide. [Modified from Abbracchio *et al*. (2009), with permission.]

Table 2. Comparison of fast ionotropic and slow metabotropic receptors for ACh, GABA, glutamate and 5-HT with those proposed for ATP

Neurotransmitter	Receptors	
	Fast ionotropic (ligand-gated ion channels)	Slow metabotropic (G protein)
ACh	Nicotinic: Muscle type Neuronal type	Muscarinic: M1–M5
GABA	GABA A	GABA B
Glutamate	AMPA Kainate NMDA	$mGlu_1$ ↓ $mGlu_7$
5-HT	$5\text{-}HT_3$	$5\text{-}HT_{1A–F}$, $5\text{-}HT_{2A–C}$, $5\text{-}HT_4$, $5\text{-}HT_{5A–B}$, $5\text{-}HT_6$, $5\text{-}HT_7$
ATP	P2X: P2X1–P2X7	P2Y: $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$, $P2Y_{14}$

Abbreviations: AMPA, 2-(aminomethyl)phenylacetic acid; NMDA, *N*-methyl-D-aspartate. [Updated and reproduced from Burnstock (1996*b*) with permission from John Wiley and Sons.]

inhibitory neurotransmission in the gut and for NANC excitatory neurotransmission in the urinary bladder (Fig. 3; Burnstock *et al.* 1970). I invented the word 'purinergic', ATP being a purine nucleotide, in a review 2 years later, and launched the purinergic signalling hypothesis (Fig. 4; Burnstock, 1972).

This concept met with strong opposition during the following 20 years, perhaps 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. For example, when I left Australia for University College London in 1975, the Professor of Medicine referred to me at my farewell party as 'the inventor of the purimagine hypothesis'. Also at workshops at international meetings, two or three opponents were each given 10 min to explain their opposition to purinergic signalling, while I had 10 min to defend it. Von Euler gave me some exceptionally good advice when a scientist at one of these meetings said, 'I am going to devote my life to destroying the purinergic hypothesis'. Firstly, negative people vanish and secondly, if experiments are presented that are claimed to negate your hypothesis, be scrupulously objective in seeing if they fit your, or any other hypothesis.

Purinergic cotransmission

Von Euler's advice was relevant when, on sabbatical leave at University of California, Los Angeles with Che Su and John Bevan, we discovered that ATP was released from sympathetic nerves as well as from NANC nerves supplying smooth muscle of the taenia coli (Su *et al.* 1971). I was initially disconcerted by this new finding, but when the sun rose in the morning, I wondered whether ATP was being released as a cotransmitter with NA. I published a controversial Commentary in *Neuroscience* entitled: 'Do some nerve cells release more than one transmitter?' (Burnstock, 1976) after my arrival at University College London that challenged what was known as Dale's Principle (one nerve, one transmitter) formulated by Eccles (what Dale actually proposed was that the same transmitter was released from both central and peripheral terminals of primary sensory neurones). Mollie Holman and I recorded excitatory junction potentials in smooth muscle cells of the guinea-pig vas deferens in response to stimulation of sympathetic nerves in 1960 (Burnstock & Holman, 1960, 1961; Fig. 5*A*). We were surprised that the excitatory junction potentials were not abolished by adrenoceptor antagonists, given that NA was assumed to be the sole neurotransmitter in sympathetic nerves at that time. It was not until over 20 years later when Peter Sneddon joined my research group that we showed that α,β-methylene ATP, which desensitizes the nucleotide receptor (Kasakov & Burnstock, 1983), blocked the excitatory junction potentials (Sneddon & Burnstock, 1984; Fig. 5*B,C*). Thus, it was established that ATP was released as a cotransmitter with NA from sympathetic nerves (Fig. 6; Burnstock, 1990). The cotransmitter concept was also initially contested, but it is now well established that every nerve, in both the peripheral and

central nervous systems, uses ATP as a cotransmitter (Burnstock, 2012*c*; Table 1).

Purinergic receptors

Identification of the membrane receptors that respond to purine nucleotide and nucleoside messengers was the next conceptual step. In 1978, from hints in the literature and some simple experiments, I recognized that there were different receptor families for adenosine (called P1 receptors) and for ATP and ADP (called P2 receptors; Burnstock, 1978). The P1 receptors were antagonized by methylxanthines. Some of the earlier ambiguities in the literature were clarified. For example, while it was known early that there were ectoenzymes that rapidly degraded ATP to adenosine, it was unclear whether a response to ATP was mediated by P2 or by P1 receptors after breakdown to adenosine. This allowed us to update the original model of purinergic neurotransmission, where P2 receptors were the postjunctional receptors, while prejunctional P1 purinoceptors mediated autoregulatory negative feedback of transmitter release. In a Loewi-inspired experiment carried out in 1966, we showed that ATP mimicked the release of transmitter in the upper taenia coli preparation, while adenosine mimicked the response of the lower preparation to the perfusate after breakdown of ATP by ectoenzymes (Burnstock *et al.* 2010). The P2 receptors were subdivided into P2X and P2Y families based on pharmacology in 1985 (Burnstock & Kennedy, 1985).

However, the turning point for widespread acceptance of purinergic signalling came in the early 1990s when the receptors to purines and pyrimidines were cloned and characterized. Four P1 receptor subtypes were identified, i.e. A_1, A_{2A}, A_{2B} and A_3 (see Daly, 1985; Fredholm *et al.* 2001). In 1993, together with my old friend Eric Barnard, an expert in the cloning of nicotinic receptors, we cloned the first ATP receptor, which was a G protein-coupled receptor that we named $P2Y_1$ (Webb *et al.* 1993). At about the same time, David Julius and his colleagues in San Francisco cloned a $P2Y_2$ receptor (Lustig *et al.* 1993). The following year, the first two P2X ion channel receptors were cloned and characterized (Brake *et al.* 1994; Valera *et al.* 1994). These P2 receptors were later divided formally into P2X ionotropic and P2Y metabotropic receptor families (Abbracchio & Burnstock, 1994). This is compared with receptors to other neurotransmitters in Table 2. The G protein-coupled P1 and P2Y receptors typically had seven transmembrane domains and extracellular and intracellular C-terminals. However, the P2X ion channel was different from the other neurotransmitter ion channel receptors, with two transmembrane domains and inner N- and C-terminals (North, 1996; Fig. 7). Some of the scientists making important contributions to our knowledge of P1, P2X and P2Y receptors are shown in Figs S1, S2 and S3, respectively. Seven subtypes of the P2X receptor and eight subtypes of the P2Y receptor have been cloned and characterized and some receptor subtype-selective agonists and antagonists identified (see Tables 3 and 4). Three P2X receptor units form the cation pore, as either a homomultimer or a heteromultimer (Nicke

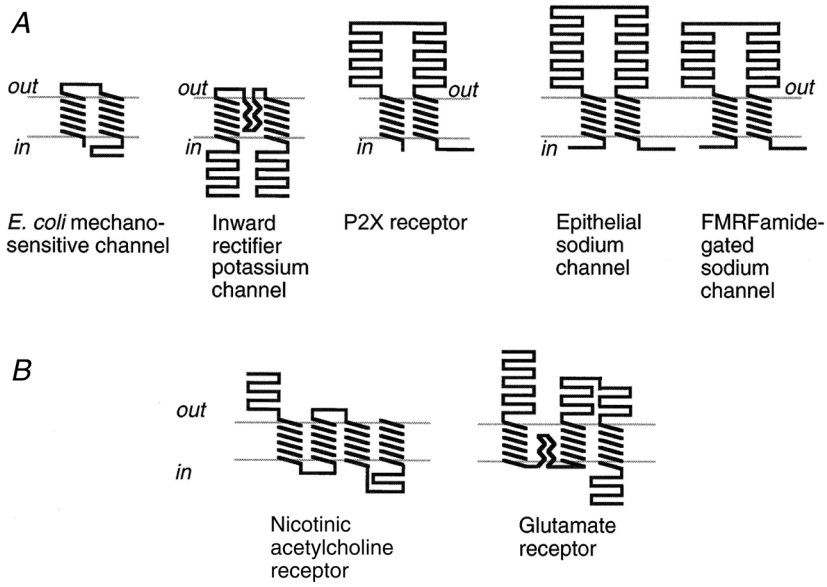

Figure 7. Structure of P2X receptors with respect to that of other channels
A, channel subunits that have two transmembrane domains. *B*, nicotinic and glutamate receptor families. [Reproduced from North (1996), with permission from Elsevier.]

Table 3. Characterization of P2X receptors

Receptor	Main distribution	Agonists	Antagonists
P2X1	Smooth muscle, platelets, cerebellum, dorsal horn spinal neurones	L-$\beta\gamma$-meATP $\geq$ α,β-meATP = ATP = 2-MeSATP	TNP-ATP, IP5I, NF023, NF449
		PAPET-ATP (rapid desensitization)	RO1, RO 0437626, NF279, MRS2159
P2X2	Smooth muscle, CNS, retina, chromaffin cells, autonomic and sensory ganglia	ATP $\geq$ ATPγS $\geq$ 2-MeSATP >> α,β-meATP, β,γ-CF2ATP (pH + zinc sensitive)	Suramin, RB2, NF770, isoPPADS, PSB-1011, NF778, aminoglycoside
P2X3	Sensory neurones, nucleus tractus solitarii, some sympathetic neurones	2-MeSATP $\geq$ ATP $\geq$ α,β-meATP = Ap_4A, PAPET-ATP (rapid desensitization)	TNP-ATP, isoPPADS, A317491, NF110, RN-1838, spinorphin, AF353
P2X4	CNS, testis, colon	ATP > α,β-meATP, CTP Ivermectin (potentiates)	TNP-ATP, BBG (weak antagonist), paroxetine, 5-BDBD, CORM 2, phenolphthalein
P2X5	Proliferating cells in skin, gut, bladder, thymus, spinal cord	ATPγS, Ap_4A, GTP	Suramin, PPADS, BBG
P2X6	CNS, motor neurones in spinal cord	(Does not function as homomultimer)	–
P2X7	Apoptotic cells in immune system, pancreas, skin etc.	BzATP > ATP $\geq$ 2-MeSATP > α,β-meATP	KN62, KN04, MRS2427, BBG, o-ATP, decavanadate, A-804598, RN-6189, AZD-9056, AZ10606120, A740003, A-438079, GSK-1370319

[Modified and updated from Burnstock (2003), with permission.]

Abbreviations: A317491, 5-[[[(3-phenoxyphenyl)methyl][(1S)-1,2,3,4-tetrahydro-1-naphthalenyl]amino]carbonyl]-1,2,4-benzenetricarboxylic acid; A-438079, 3-[[5-(2,3-dichlorophenyl)-1H-tetrazol-1-yl]methyl]pyridine hydrochloride; A740003, N-[1-[[(cyanoamino)(5-quinolinylamino)methylene]amino]-2,2-dimethylpropyl]-3,4-dimethoxybenzeneacetamide; A-804598, N-cyano-N″-[(1S)-1-phenylethyl]-N′-5-quinolinyl-guanidine; AF353, 5-(5-iodo-2-isopropyl-4-methoxy-phenoxy)-pyrimidine-2,4-diamine; Ap4A, diadenosine tetraphosphate; ATP, adenosine 5′-triphosphte; ATPγS, adenosine-5′-(γ-thio)-triphosphate; AZ10606120, N-[2-[[2-[(2-hydroxyethyl)amino]ethyl]amino]-5-quinolinyl]-2-tricyclo[3.3.1.13,7]dec-1-ylacetamide dihydrochloride; BBG, Brilliant blue green; 5-BDBD, 5-(3-bromophenyl)-1,3-dihydro-2H-benzofuro[3,2-e]- 1,4-diazepin-2-one; BzATP, 2′-&3′-O-(4-benzoyl-benzoyl)-ATP; β,γ-CF2ATP, α,β-difluoromethylene-ATP; CORM 2, carbon monoxide donor 2; CTP, cytidine triphosphate; GSK-1370319, N-[(2,4-dichlorophenyl)methyl]-1-methyl-5-oxo-L-prolinamide; GTP, guanosine-5′-triphosphate; IP5I, di-inosine pentaphosphate; isoPPADS, iso- pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid; KN04, N-[1-[N-methyl-p-(5-isoquinolinesulphonyl)benzyl]-2-(4-phenylpiperazine)ethyl]-5-isoquinoline-sulfonamide; KN62, 1-[N,O-bis(5-Isoquinolinesulfonyl)-N-methyl-L-tyrosyl]-4-phenylpiperazine; L-$\alpha\beta$-meATP, L-α,β-methylene ATP; L-$\beta\gamma$-meATP, L-β,γ-methylene ATP; 2-MeSATP, 2-methylthio ATP; MRS2159, pyridoxal-α5-phosphate-6-phenylazo-4′-carboxylic acid; NF023, 8,8′-[carbonylbis(imino-3,1-phenylenecarbonylimino)]bis-1,3,5-naphthalene-trisulfonicacid; NF110, 4,4′,4″,4‴-[carbonylbis[imino-5,1,3-benzenetriylbis(carbonylimino)]]tetrakisbenzenesulfonic acid; NF279, 8,8′-[carbonylbis(imino-4,1-phenylenecarbonylimino-4,1-phenylenecarbonylimino)]bis-1,3,5-naphthalenetrisulfonic acid; NF449, 4,4′,4″,4‴-[carbonylbis(imino-5,1,3-benzenetriyl-bis(carbonylimino))]tetrakis-1,3 benzenedisulfonic acid; NF770, 7,7′-(carbonylbis(imino-3,1-phenylenecarbonylimino-3,1-(4-methyl-phenylene)carbonylimino))bis(1-methoxy-naphthalene-3,6-disulfonic acid); NF778, 1-methoxy-3,5-disulfonic acid; PAPET, 2-[2-(4-aminophenyl)ethylthio]adenosine-5′-triphosphate; oATP, oxidised ATP; PSB-1011, disodium 1-amino-4-[3-(4,6-dichloro[1,3,5]triazine-2-ylamino)-4-sulfophenylamino]-9,10-dioxo-9,10-dihydroanthracene-2-sulfonate; RB2, reactive blue 2; RO1 (1*H*-benzoimidazole-2-carboxylic acid [1-(*R*)-1-(*S*)-cyclohexylmethyl-3-cyclopropyl-2-(*R*),3(*S*)-dihydroxy-propylcarbamoyl]-2-thiazol-4-yl-ethyl)-amide); RO 0437626, N-[(1R)-2-[[(1S,2R,3S)-1-(cyclohexylmethyl)-3-cyclopropyl-2,3-dihydroxypropyl]amino]-2-oxo-1-(4-thiazolylmethyl)ethyl]-1H-benzimidazole-2-carboxamide; TNP-ATP, 2′(3′)-O-(2,4,6-trinitrophenyl) ATP

et al. 1998; North, 2002; Burnstock, 2007*a*). An important conceptual advance was made when the crystal structure of the P2X4 receptor was presented (Kawate *et al.* 2009; Fig. 8).

The initial focus was about purinergic signalling in excitable tissues, but with employment of immunohistochemistry, it became clear that most non-neuronal cells in the body express multiple purinoceptor subtypes (Table 5). Some of the scientists involved in these discoveries are shown in Fig. S4. This raises questions about the different roles of subtypes and their interactions.

Table 4. Characteristics of P2Y receptors

Receptor	Main distribution	Agonists	Antagonists	Transduction mechanisms
$P2Y_1$	Epithelial and endothelial cells, platelets, immune cells, osteoclasts, brain	MRS2365 > 2-MeSADP = $Ap_5(\gamma B)$ >> ADPβS > ATP > 2-MeSATP = ADP	MRS2500 > MRS2279 > MRS2179, PIT, A3P5P	G_q/G_{11}; PLC-β activation
$P2Y_2$	Immune cells, epithelial and endothelial cells, kidney tubules, osteoblasts	2-Thio-UTP > UTP, MRS2698 $\geq$ ATP, INS 365 > INS 37217, UTPγS > Ap_4A > MRS 2768, Up_4-phenyl ester	AR-C126313 > suramin > Reactive Blue 2, PSB-716, MRS2576	G_q/G_{11} and possibly G_i/G_o; PLC-β activation
$P2Y_4$	Endothelial cells, placenta, spleen, thymus	2′-azido-dUTP > UTPγS, UTP $\geq$ ATP $\geq$ Ap_4A, Up_4U	ATP (human) > Reactive Blue 2 > suramin, MRS2577, PPADS	G_q/G_{11} and possibly G_i; PLC-β activation
$P2Y_6$	Airway and intestinal epithelial cells, placenta, T cells, thymus, microglia (activated)	MRS2693 > UDPβS, PBS0474 > INS48823, Up_3U, 3-phenacyl-UDP >> UDP > UTP >> ATP, α,β-meUDP	MRS2578 > Reactive Blue 2, PPADS, MRS2567, MRS2575 (human)	G_q/G_{11}; PLC-β activation
$P2Y_{11}$	Spleen, intestine, granulocytes	ATPγS > AR-C67085MX > BzATP $\geq$ ATP, NF546, NAD^+, $NAADP^+$	NF157 > suramin > Reactive Blue 2, 5′-AMPS, NF340, AMP-α-5,	G_q/G_{11} and G_S; PLC-β activation
$P2Y_{12}$	Platelets, glial cells	2-MeSADP $\geq$ ADP > ATP, ADPβS	AR-C69931MX > AZD6140, INS50589 > Reactive Blue 2 > 2-MeSAMP, AR-C66096, CT50547, PSB-0413, carba-nucleosides, MRS2395, AR-C67085	$G\alpha_i$; inhibition of adenylate cyclase
$P2Y_{13}$	Spleen, brain, lymph nodes, bone marrow, erythrocytes	ADP = 2-MeSADP > 2-MeSATP, ATP	AR-C69931MX > AR-C67085 > MRS2211, 2-MeSAMP	G_i/G_o
$P2Y_{14}$	Placenta, adipose tissue, stomach, intestine, discrete brain regions, mast cells	MRS2690 > UDP > UDP-glucose $\geq$ UDP-galactose, UDP-glucosamine		G_q/G_{11}

[Modified and updated from Burnstock (2003), with permission.]

Abbreviations: A3P5P, adenosine-3′-5′-bisphosphate; ADP, adenosine 5′-diphosphte; ADPβS, adenosine-5′-(β-thio)-diphosphate; 5′-AMPS, 5′-O-thiomnophosphate; Ap_4A, diadenosine tetraphosphate; Ap5($\beta\gamma$), adenosine pentaphosphate ($\beta\gamma$); AR-C126313, 2′-amino-2′-deoxy-2-thiouridine 5′-triphosphate; AR-C66096, 2-(propylthio)adenosine-5′-O-(β,γ-difluoromethylene)triphosphate; AR-C67085, [[[[(2R,3S,4R,5R)-5-(6-amino-2-propylsulfanylpurin-9-yl)-3,4-dihydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]oxy-hydroxyphosphoryl]-dichloromethyl]phosphonic acid; AR-C67085MX, [[[[(2R,3S,4R,5R)-5-(6-amino-2-propylsulfanylpurin-9-yl)-3,4-dihydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]oxy-hydroxyphosphoryl]-dichloromethyl]phosphonic acid; AR-C69931MX, [dichloro-[[[(2R,3S,4R,5R)-3,4-dihydroxy-5-[6-(2-methylsulfanylethylamino)-2-(3,3,3-trifluoropropylsulfanyl)purin-9-yl]oxolan-2-yl]methoxy-hydroxyphosphoryl]oxy-hydroxyphosphoryl]methyl]phosphonic acid; ATP, adenosine 5′-triphosphte; ATPγS, adenosine-5′-(γ-thio)-triphosphate; AZD6140, 3-[7-[[2-(3,4-difluorophenyl)cyclopropyl]amino]-5-propylsulfanyltriazolo[5,4-d]pyrimidin-3-yl]-5-(2-hydroxyethoxy)cyclopentane-1,2-diol; 2′-azido-dUTP, 2′-azido-deoxyuridine-5′-triphosphate; BzATP, 2′(3′)-O-(4-benzoylbenzoyl) adenosine 5′-triphosphate; CT50547, N1-(6-ethoxy-1,3-benzothiazol-2-yl-2-(7-ethoxy-4-hydroxy-2,2-dioxo-2H-2]6benzo-[4,5][1,3]thiazolo[2,3-c][1,2,4] thiadiazin-3-yl)-2-oxo-1-ethanesulfonamide; INS 365, [[[[(2R,3S,4R,5R)-5-(2,4-dioxopyrimidin-1-yl)-3,4-dihydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]oxy-hydroxyphosphoryl]oxy-hydroxyphosphoryl] [(2R,3S,4R,5R)-5-(2,4-dioxopyrimidin-1-yl)-3,4-dihydroxyoxolan-2-yl]methyl hydrogen phosphate; INS37217, P(1)-(uridine 5′)-P(4)- (2′-deoxycytidine 5′)tetraphosphate; INS48823, {[(3aR,4R,6R,6aR)-2-benzyl-6-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)-tetrahydro-2H-furo[3,4-d][1,3]dioxol-4-yl]methoxy}({[({[(2S,3R,4S,5S)-5-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)-3,4-dihydroxyoxolan-2-yl]methoxy}(hydroxy)phosphoryl)oxy](hydroxy)phosphoryl}oxy)phosphinic acid; INS50589, [(2S,3aR,4R,6R,6aR)-6-[6-(ethylcarbamoylamino)purin-9-yl]-2-[(E)-2-phenylethenyl]-3a,4,6,6a-tetrahydrofuro[4,3-d][1,3]dioxol-4-yl]methyl dihydrogen phosphate; 2-MeSADP, 2-methylthio ADP; 2-MeSAMP, 2-methylthio AMP 2-MeSATP, 2-methylthio ATP; α,β-meUDP, α,β-methylene UDP; MRS2179, [2-[(hydroxy-oxidophosphoryl)oxymethyl]-5-(6-methylaminopurin-9-yl)oxolan-3-yl] hydrogen phosphate; MRS2211, [(2Z)-2-[(2-chloro-5-nitrophenyl)hydrazinylidene]-4-formyl-6-methyl-5-oxopyridin-3-yl]methyl dihydrogen phosphate; MRS2279, [(1S,2R,4R)-4-[(2-chloro-6-methylaminopurin-9-yl)methyl]-2-(phosphonooxymethyl)cyclopentyl] dihydrogen phosphate; MRS2365, [[(1R,2R,3S,4R,5S)-4-[6-amino-2-(methylthio)-9H-purin-9-yl]-2,3-dihydroxybicyclo[3.1.0]hex-1-yl]methyl] diphosphoric acid mono ester; MRS2395, [[(1R,2R,3S,4R,5S)-4-[6-Amino-2-(methylthio)-9H-purin-9-yl]-2,3-dihydroxybicyclo[3.1.0]hex-1-yl]methyl] diphosphoric acid mono ester; MRS2500, [(1R,2S,5S)-4-(2-iodo-6-methylaminopurin-9-yl)-1-(phosphonooxymethyl)-2-bicyclo[3.1.0]hexanyl] dihydrogen phosphate; MRS2567, 1-isothiocyanato-4-[2-(4-isothiocyanatophenyl)ethyl]benzene; MRS2575, 1,4-phenylendiisothiocyanate; MRS2576, 1,2-diphenylethane diisothiocyanate; MRS2690, sodium (2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl [({[(2R,3S,4R,5R)-3,4-dihydroxy-5-(4-oxo-2-sulfanylidene-1,2,3,4-tetrahydropyrimidin-1-yl)oxolan-2-yl]methoxy}(sodiooxy)phosphoryl)oxy]phosphonate; MRS2578, 3-(3-isothiocyanatophenyl)-1-[4-[(3-isothiocyanatophenyl)carbamothioylamino]butyl]thiourea; MRS2693, (2R,3R,4S,5R)-1-(3,4-dihydroxy-5-(diphosphoryloxymethyl)-tetrahydrofuran-2-yl)-5-iodopyrimidine-2,4(1H,3H)-dione; MRS2698, [(2R,3S,4R,5R)-4-amino-3-hydroxy-5-(4-oxo-2-sulfanylidenepyrimidin-1-yl)oxolan-2-yl]methyl (hydroxy-phosphonooxyphosphoryl) hydrogen phosphate; MRS2768, uridine-5′-tetraphosphate δ-phenyl ester; $NAADP^+$, nicotinic acid adenine dinucleotide phosphate; NAD^+, nicotinamide adenine dinucleotide; NF157, 8-[[4-fluoro-3-[[3-[[3-[[2-fluoro-5-[(4,6,8-trisulfonaphthalen-1-yl)carbamoyl]phenyl]carbamoyl]phenyl]carbamoylamino]benzoyl]amino]benzoyl]amino]naphthalene-1,3,5-trisulfonic acid; NF340, N-[3,7-bis(trioxo-$l^{8}-sulfanyl)naphthalen-1-yl]-3-{[(5-{[3,7-bis(trioxo-$l^{8}-sulfanyl)naphthalen-1-yl]carbamoyl}-2-methylphenyl)carbamoyl]amino}-4-methylbenzamide; NF546, 4,4′-(carbonylbis(imino-3,1-phenylene-carbonylimino-3,1-(4-methyl-phenylene)-carbonylimino))-bis(1,3-xylene-α,α'-diphosphonic acid); PBS0474, 3-(2-oxo-2-phenylethyl)-uridine-5′-diphosphate; PIT, 2,2′-pyridylisatogen tosylate; PLC, phospholipase C; PPADS, pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid; PSB0413, 2-propylthioadenosine-5′-adenylic acid (1,1-dichloro-1-phosphonomethyl-1-phosphonyl) anhydride; PSB-716, 1-amino-4-(2-methoxyphenyl)-2-sulfoanthraquinone; 2-thio-UTP, 2-thio-uridine 5′-triphosphate; UDP, uridine 5′-diphosphate; UDPβS, uridine 5′-O-thiodiphosphate; Up4-phenyl ester, uridine tetraphosphate phenyl ester; Up_3U, diuridine triphosphate;Up_4U, diuridine tetraphosphate; UTP, uridine 5′-triphosphate; UTPγS, uridine 5′-O-3-thiotriphosphate

The molecular structure of ion channel receptors for ATP in primitive invertebrates, such as *Dictyostelium* and *Schistosoma*, as well as green algae, is remarkably similar to that for P2X receptors in mammals (Agboh *et al.* 2004; Fountain *et al.* 2007, 2008; Fountain & Burnstock, 2009), suggesting that ATP was one of the earliest extracellular messengers (see Burnstock & Verkhratsky, 2009). ATP signalling has also been identified in plants (see Demidchik *et al.* 2003, 2011; Kim *et al.* 2006; Clark & Roux, 2009).

Purinergic synaptic transmission in ganglia and brain

All the early studies were focused on purinergic neuromuscular transmission. However, purinergic synaptic neurotransmission was reported in 1992 between neurones in ganglia (Evans *et al.* 1992; Silinsky *et al.* 1992) and in the brain (Edwards *et al.* 1992).

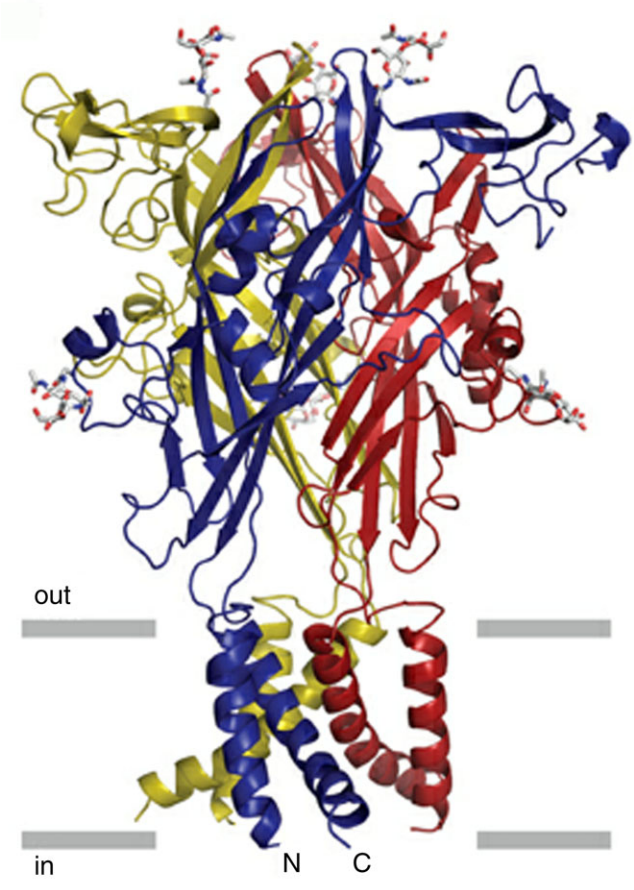

Figure 8. The architecture of P2X receptors
Stereoview of the homotrimeric P2X4 structure viewed parallel to the membrane. Each subunit is depicted in a different colour. *N*-acetyl-D-glucosamine and glycosylated asparagine residues are shown in stick representation. The grey bars suggest the boundaries of the outer (out) and inner leaflets (in) of the membrane bilayer. [Reproduced from Kawate *et al*. (2009), with permission from the Nature Publishing Group.]

Table 5. Principal P1 and P2 receptors expressed by non-neuronal cells

Smooth muscle	$P2X1$, $P2X2$, $P2X4$, $P2X7$, $P2Y_1$, $P2Y_2$
Cardiac muscle	$P2X1–6$, $P2Y_2$ (plus $P2X7$ and $P2Y_1$ in isolated ventricle myocytes)
Skeletal muscle	$P2X1–6$, $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$ (transiently expressed during development)
Osteoblasts	$P2X7$, $P2Y_1$, $P2Y_2$
Cartilage	$P2X2$, $P2Y_1$, $P2Y_2$, A_{2A}, A_{2B}
Keratinocytes	$P2X2$, $P2X3$, $P2X5$, $P2X7$, $P2Y_1$, $P2Y_2$, $P2Y_4$, A_{2B}
Fibroblasts	$P2X7$, $P2Y_1$, $P2Y_2$, A_{2A}
Adipocytes	$P2X1$, $P2Y_1$, $P2Y_2$, $P2Y_4$, A_1
Epithelial cells	$P2X4$, $P2X5$, $P2X6$, $P2X7$, $P2Y_1$, $P2Y_2$, $P2Y_6$, $P2Y_{11}$, A_1, A_{2A}, A_3
Hepatocytes	$P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{13}$, A_{2A}, A_3
Glial cells	$P2X1–7$, $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$, A_1, A_2
Sperm	$P2X2$, $P2X7$, $P2Y_2$, A_1
Endothelial cells	$P2X1$, $P2X4$, $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, A_1, A_{2A}
Erythrocytes	$P2X2$, $P2X4$, $P2X7$, $P2Y_1$
Platelets	$P2X1$, $P2Y_1$, $P2Y_{12}$, A_{2A}
Immune cells (lymphocytes, neutrophils, macrophages, basophils, mast cells, eosinophils, osteoclasts, microglia, dendritic cells)	$P2X1$, $P2X4$, $P2X7$, $P2Y_1$, $P2Y_2$, A_{2A}, A_3
Exocrine cells	$P2X1$, $P2X4$, $P2X7$, $P2Y_1$, $P2Y_2$, $P2Y_4$, A_1, A_{2A}
Endocrine cells	$P2X1–7$, $P2Y_2$, $P2Y_4$, A_1, A_{2A}, A_{2B}, A_3
Special senses	
Inner ear	$P2X1$, $P2X2$, $P2X3$, $P2X7$, $P2Y_2$, $P2Y_4$, A_1
Eye	$P2X2$, $P2X7$, $P2Y_2$, A_1, A_2, A_3
Tongue	$P2X2$, $P2X3$, $P2Y_1$, A_1
Olfactory organ	$P2X2$, $P2X4$, $P2Y_1$, $P2Y_2$, A_{2A}, A_3

[Reproduced from Burnstock (2012*a*) with permission from Wiley.]

This was an important conceptual step because most neuroscientists were interested in synaptic rather than neuromuscular transmission, and the purinergic concept reached many of them for the first time.

Short- and long-term (trophic) purinergic signalling

An important advance was the recognition that purines and pyrimidines are involved in long-term signalling of cell proliferation, differentiation and death during development and regeneration, as well as short-term purinergic signalling in neurotransmission, neuromodulation, secretion, chemoattraction and platelet

aggregation (see Abbracchio & Burnstock, 1998; Burnstock & Verkhratsky, 2010). In blood vessels, for example, ATP released from perivascular sympathetic nerves excites smooth muscle via P2X receptors, while ATP released from endothelial cells during shear stress produced by changes in blood flow and by hypoxia acts on endothelial P2Y and some P2X receptors to release nitric oxide, resulting in vasodilatation. This illustrates short-term dual control of vascular tone by purines (Fig. 9), and the leading scientists involved are shown in Fig. S5. In addition, ATP and adenosine mediate long-term signalling during embryonic development, angiogenesis, restenosis following angioplasty and atherosclerosis (see Burnstock, 2002, 2008*a*; Erlinge & Burnstock, 2008; Fig. 10).

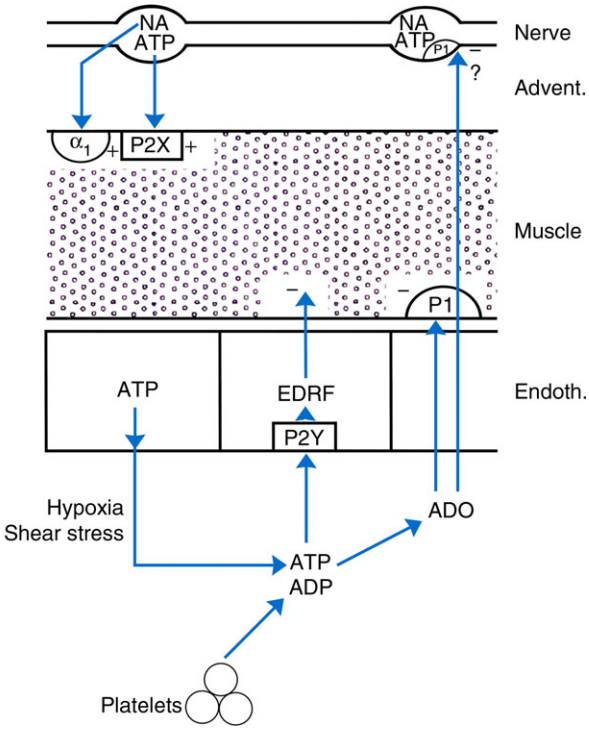

Figure 9. A schematic representation of short-term purinergic signalling, showing the interactions of ATP released from perivascular nerves and from the endothelium (Endoth.) controlling vascular tone
Adenosine triphosphate is released from endothelial cells during hypoxia to act mainly on endothelial P2Y receptors, leading to the production of endothelium-derived relaxing factor (EDRF; nitric oxide) and subsequent vasodilatation (−). 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 breakdown of ATP by ectoenzymes, later produces vasodilatation by direct action on the muscle via P1 receptors and acts on the perivascular nerve terminal varicosities to inhibit transmitter release. [From Burnstock (1987), reproduced with permission from S. Karger AG, Basel.]

Release of ATP and purinergic mechanosensory transduction

It was assumed for many years that the main source of ATP acting on purinoceptors was damaged or dying cells. However, it is now clear that ATP is released, without causing damage, from many cell types, including endothelial and urothelial cells, astrocytes, macrophages, osteoblasts and odontoblasts, in response to gentle mechanical disturbance, hypoxia and some agents (Bodin

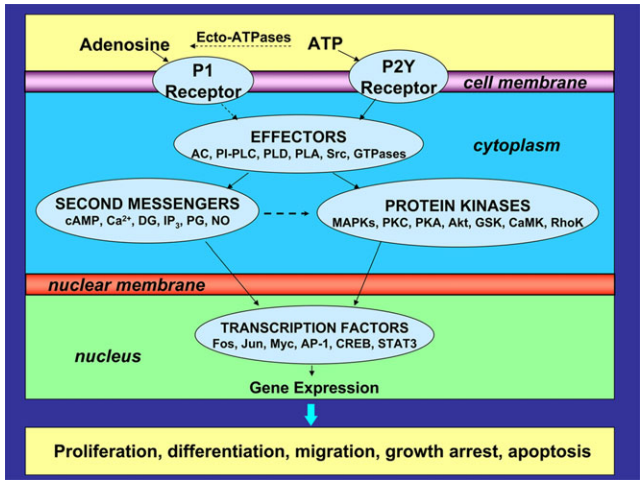

Figure 10. Schematic overview of purinergic signalling mechanisms that regulate long-term, trophic effects
Extracellular nucleotides and nucleosides bind to purinergic receptors coupled to signal-transducing effector molecules. Activation of the effectors leads to generation of second messengers and/or stimulation of protein kinases that regulate expression of genes needed for long-term, trophic actions. In some cases, P2X receptors, such as P2X7, are also coupled to protein kinase cascades and can mediate proliferation and apoptosis. Cell-specific and/or receptor subtype-specific differences are likely to account for variations in signalling pathways and functional outcomes. It should be noted that the list of elements is not meant to be all-inclusive. Other protein kinases, e.g. MEK, PI3K, are upstream of the listed kinases involved in purinergic signalling, while others are downstream, e.g. p70S6K. In addition, dashed arrows indicate that not all listed elements are activated by the upstream component, e.g. not all P1 receptors are coupled to all listed effectors. Abbreviations: AC, adenylyl cyclase; AP-1, activator protein-1; CaMK, calcium–calmodulin protein kinase; CREB, cyclic AMP response element binding protein; DG, diacylglycerol; GSK, glycogen synthase kinase; IP3, inositol trisphosphate; MAPKs, mitogen-activated protein kinases [including extracellular signal-regulated protein kinase (ERK), p38 MAPK and stress-activated protein kinase (SAPK)/c-Jun N-terminal kinase (JNK)]; MEK, MAPK/ERK kinase; NO, nitric oxide; PG, prostaglandin; PI3K, phosphoinositide 3-kinase; PI-PLC, phosphatidylinositol-specific phospholipase C; PKA, protein kinase A; PKC, protein kinase C; PLA, phospholipase A; PLD, phospholipase D; and STAT3, signal transducer and activator of transcription-3. [Modified from Burnstock (2007*b*), with permission from the American Physiological Society.]

& Burnstock, 2001; Lazarowski *et al.* 2011; Lazarowski, 2012). Scientists involved are shown in Fig. S6. This release underlies the purinergic mechanosensory transduction that is involved in a variety of physiological events, including bone remodelling and visceral pain (Burnstock, 1999, 2007*b*; Orriss *et al.* 2010). The mechanism of ATP transport from cells appears to be a combination of vesicular exocytosis and connexin and pannexin hemichannels (see Lazarowski, 2012).

Ectonucleotidases

Ectoenzymes are involved in the breakdown of released ATP into ADP, AMP, adenosine, inosine and hypoxanthine (see Zimmermann, 2006; Yegutkin, 2008). These enzymes include ectonucleoside triphosphate diphosphohydrolases, nucleotide pyrophosphatase/phosphodiesterases, alkaline phosphatases, 5′- nucleotidase and monoamine oxidase (see Fig. 11; Fig. S7 shows the leading figures in these studies).

Synergism between growth factors and purines during nerve regeneration and stem cell activation

Synergism between purines and trophic factors was shown in studies of the transplantation of the myenteric plexus into the brain (Tew *et al.* 1994, 1996). These studies were originally designed to explore enteric nerves as a possible source for replacement of missing messengers, such as dopamine, for Parkinson's disease, but the myenteric plexus was shown to cause a marked proliferation of nerve fibres in the corpus striatum. An analysis, using co-culture of striatal neurones with various elements of the myenteric plexus and enteric neurotransmitters, showed that a growth factor released by enteric glial cells

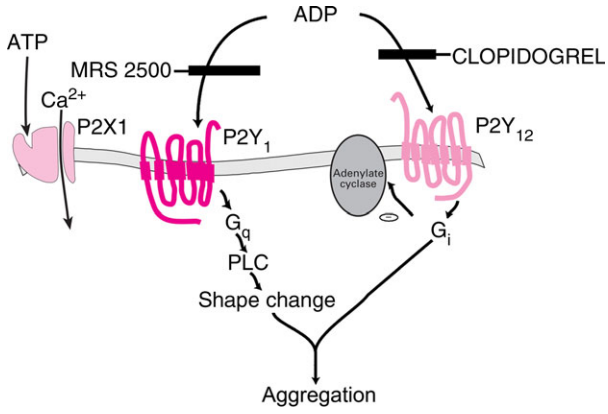

Figure 12. Three P2 receptor subtypes, P2X1, $P2Y_1$ and $P2Y_{12}$, are involved in ADP-induced platelet activation
Clopidogrel is a $P2Y_{12}$ receptor blocker that inhibits platelet aggregation and is in highly successful use for the treatment of thrombosis and stroke. A $P2Y_1$ receptor antagonist, MRS 2500, inhibits shape change. [Modified from Kunapuli & Daniel (1998), with permission from Portland Press Ltd.]

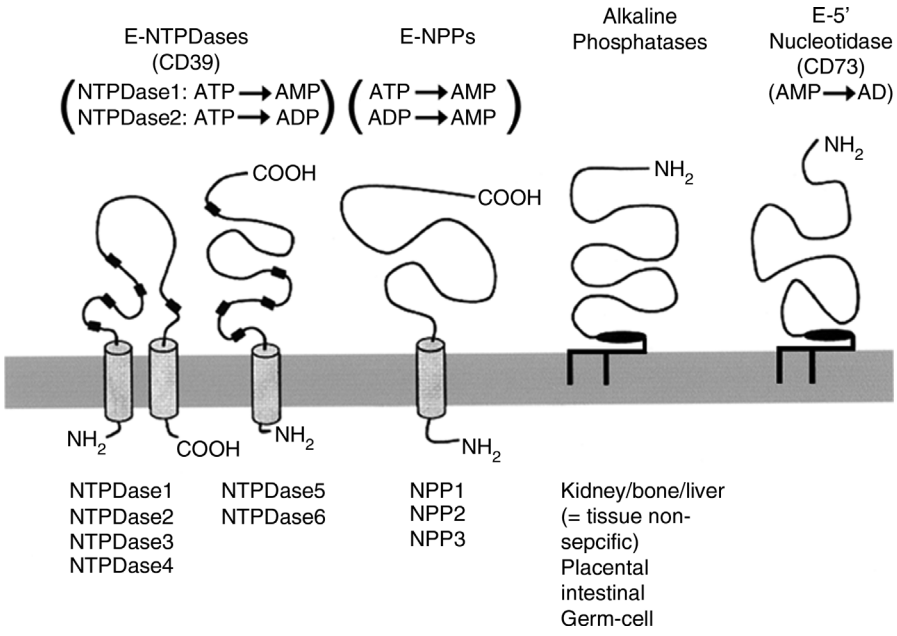

Figure 11. Predicted membrane topography of ectonucleotidases, consisting of the ectonucleoside triphosphate diphosphohydrolase (E-NTPDase) family (CD39), the ecto-nucleotide pyrophosphatase/phosphodiesterase family, alkaline phosphatases and ecto-5′-nucleotidase (CD73)
[Modified from Zimmermann (2001), with permission from Wiley-Liss, Inc.].

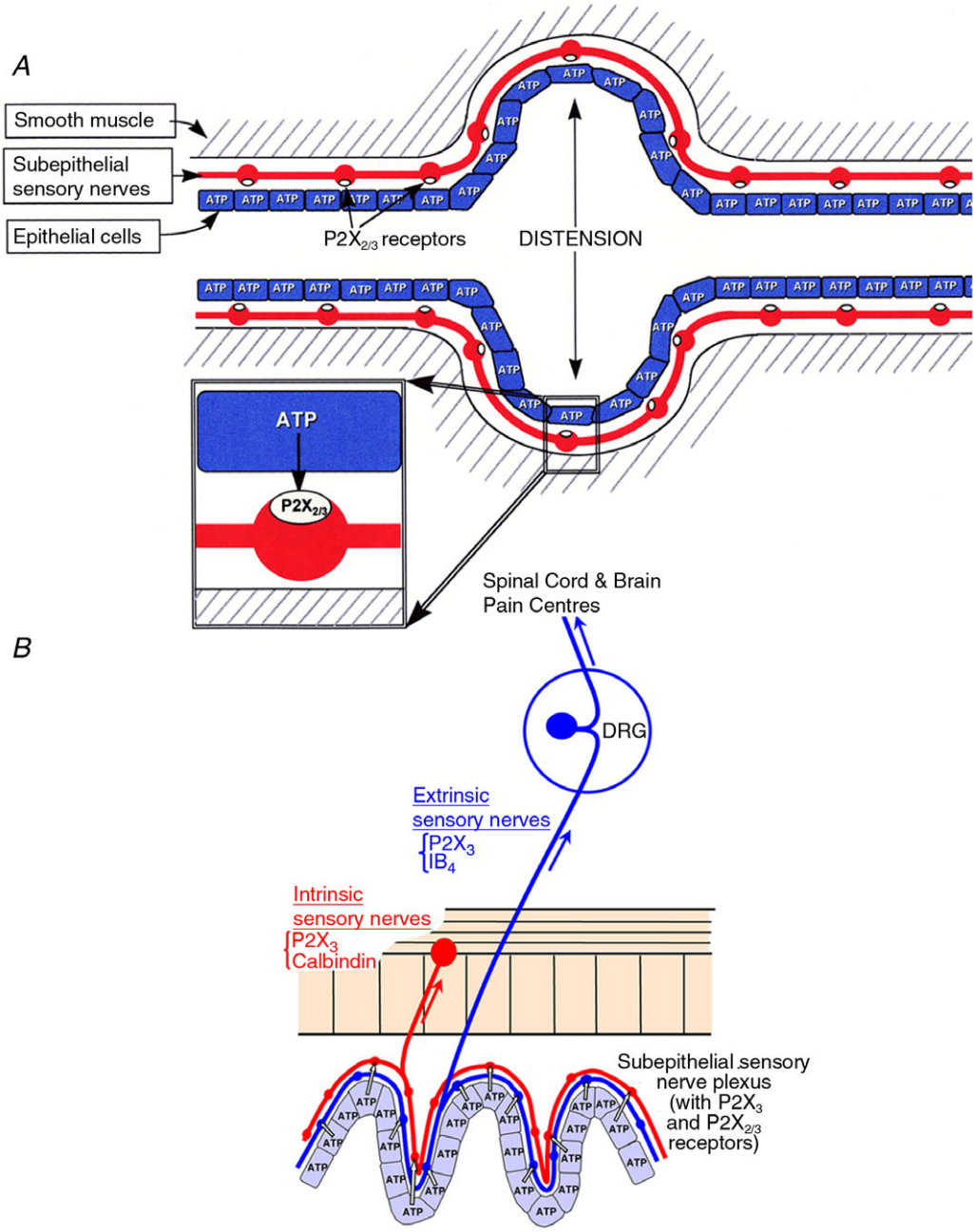

Figure 13. Purinergic mechanosensory transduction
A, schematic representation of the hypothesis for purinergic mechanosensory transduction in tubes (e.g. ureter, vagina, salivary duct, bile duct and gut) and sacs (e.g. urinary bladder, gall bladder and lung). It is proposed that distension leads to release 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. [From Burnstock (1999), reproduced with permission from Blackwell Publishing.] *B*, schematic diagram of 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 P2X3 and/or P2X2/3 receptors on low-threshold subepithelial intrinsic sensory nerve fibres (labelled with calbindin) to modulate enteric reflexes. The ATP released during extreme (colic) distension also acts on P2X3 and/or P2X2/3 receptors on high-threshold extrinsic sensory nerve fibres [labelled with isolectin B4 (IB_4)] that send messages via the dorsal root ganglia (DRG) to pain centres in the CNS. [From Burnstock (2001), reproduced with permission from Wiley.]

works synergistically with ATP (via adenosine) and NO released from NANC inhibitory nerves to promote nerve regeneration (Höpker *et al.* 1996). Later studies have extended this concept (Guarnieri *et al.* 2004; Neary & Zimmermann, 2009).

Stem cells express purinoceptors, and synergism between purines and growth factors appears to be important for stem cell differentiation (see Burnstock & Ulrich, 2011; Ulrich *et al.* 2012).

Purinergic pathophysiology and therapeutic potential

There is an increase in the ATP component of cotransmission in pathological conditions, including inflammation and stress. For example, while the purinergic component in parasympathetic nerves supplying the rodent bladder is about 50%, in the healthy human bladder it is minimal. However, in interstitial cystitis, outflow obstruction and neurogenic bladder dysfunction, the purinergic component increases

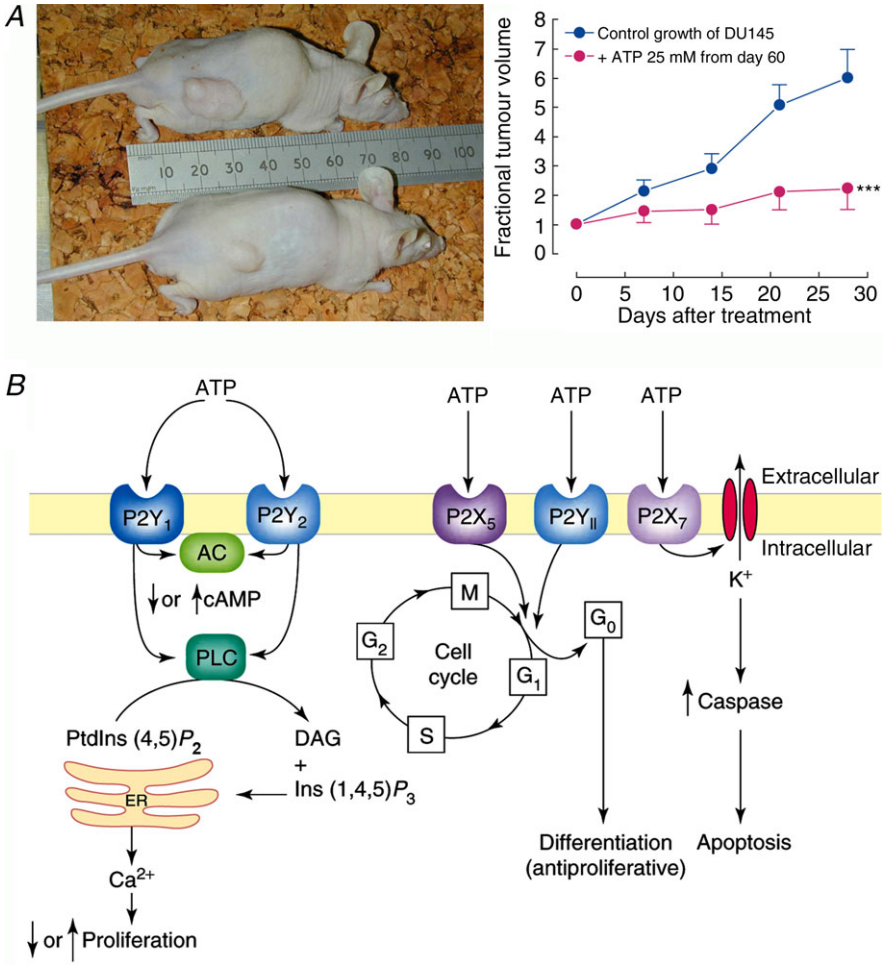

Figure 14. Reduction of cancer cell growth by nucleotides
A, left-hand panel shows the effect of ATP on the growth of implanted hormone refractory prostate cancer DU145 tumour cells *in vivo* after 60 days initial growth; the lower mouse received ATP treatment *versus* no treatment in the upper mouse. *A*, right-hand panel shows the effect of ATP on the fractional growth of DU145 tumour cells *in vivo* after 60 days initial growth. [Reproduced from Shabbir *et al.* (2008), with permission from Blackwell Publishing.] *B*, different mechanisms by which P2 receptor subtypes might alter cancer cell function. The $P2Y_1$ and $P2Y_2$ receptors affect the rate of cell proliferation by modulating adenylyl cyclase (AC) and altering the intracellular levels of cAMP, or by increasing the intracellular level of Ca^{2+} through the phospholipase C (PLC) pathway. P2X5 and $P2Y_{11}$ receptor activation mediates differentiation, which switches the cell cycle to antiproliferation. The P2X7 receptor activates the apoptotic cell death caspase enzyme system. Abbreviations: DAG, diacylglycerol; Ins(1,4,5)P_3, inositol (1,4,5)-trisphosphate; PtdIns(4,5)P_2, phosphatidylinositol (4,5)-bisphosphate. (Reproduced from White & Burnstock, 2006, with permission from Elsevier.)

to up to 40% (see Burnstock, 2011). There are also reports of a significantly greater cotransmitter role for ATP in sympathetic nerves supplying blood vessels in spontaneously hypertensive rats (Erlinge & Burnstock, 2008).

Platelet aggregation is mediated by ADP acting via $P2Y_1$ and $P2Y_{12}$ receptors. A major clinical contribution was made when clopidogrel, which is currently widely used against stroke and thrombosis, was found to block $P2Y_{12}$ receptors (Gachet, 2006; Fig. 12).

Soaking sperm in a solution containing ATP is being used to improve the effectiveness of *in vitro* fertilization (Rossato *et al.* 1999). Involvement of P2X receptors in most of the steps involved in spermatogenesis in developing testes is being explored as a therapeutic approach to contraception (Glass *et al.* 2001).

A hypothesis was presented in *The Lancet* proposing the involvement of purinergic signalling in the initiation of pain (Burnstock, 1996*a*). It was proposed that P2X3 receptors, expressed on nociceptive nerve endings, 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 the heart, skeletal muscle and cerebral vessels to activate nociceptive sensory nerve fibres during angina, ischaemia and migraine. There are high levels of ATP in tumour cells, and release from damaged cells may be involved in cancer pain. Later, purinergic mechanosensory transduction was identified (Burnstock, 1999), including its involvement in the initiation of visceral pain (Fig. 13; Burnstock, 2009*b*, 2012*b*). Kazu Inoue and colleagues showed that in neuropathic pain there was increased expression of P2X4 receptors on microglia, and neuropathic pain was reduced by antagonists to this receptor or after their removal (Tsuda *et al.* 2004). Later, antagonists to P2X7 and $P2Y_{12}$ receptors expressed on microglia were also shown to reduce neuropathic pain (Burnstock, 2009*b*). Leading figures who have worked on purinergic signalling and pain are shown in Fig. S8.

Purinergic signalling also occurs in bone development and regeneration, and therapeutic strategies are being developed for osteoporosis (Orriss *et al.* 2010) and also for kidney disease (Bailey *et al.* 2007; Taylor *et al.* 2009). There is evidence for a role for purines and pyrimidines in normal behaviour, including learning and memory, sleep and arousal, locomotion and feeding (see Burnstock *et al.* 2011). Investigations of the roles of purinergic signalling in disorders of the brain are also in progress. These include trauma following accidents, surgery, stroke and ischaemia, neurodegenerative diseases, such as Alzheimer's, Parkinson's and Huntington's, as well as multiple sclerosis, epilepsy and neuropsychiatric disorders, such as depression, anxiety and schizophrenia (see Burnstock, 2007*b*, 2008*b*; Burnstock *et al.* 2011). Leading scientists working in the CNS field are shown in Fig. S9 and those working on the special senses in Fig. S10.

It was recognized early that ATP was effective against cancer (Rapaport, 1983). Studies have extended these findings, showing that $P2Y_1$ and $P2Y_2$ receptors modulate proliferation in most tumours, that P2X5 receptors mediate differentiation resulting in antiproliferation, while P2X7 receptors mediate apoptotic death of tumour cells (Fig. 14; White & Burnstock, 2006; Shabbir & Burnstock, 2009; Burnstock & Di Virgilio, 2013).

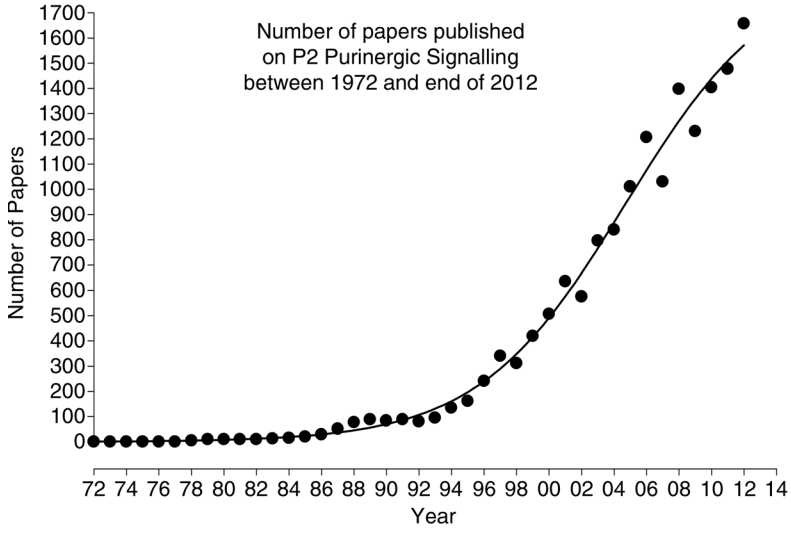

Figure 15. Graph showing the number of papers published on P2 purinergic signalling between 1972 and the end of 2012

It has been proposed that purinergic signalling is a major factor in the physiological mechanism responsible for the effects of acupuncture (see Burnstock, 2009*a*). It was suggested that the mechanical stimulation by twisting needles in the skin and tongue or heat or electrical currents leads to release of ATP from keratinocytes and from mast cells that accumulate in the region of acupuncture needles. The ATP then activates sensory nerves in the skin via P2X3 receptors that relay messages via interneurones to the brainstem, where they modulate the activity of motor neurones that control autonomic function and interrupt pain pathways leading to the conscious pain centres in the cortex.

Concluding comments

The remarkable growth of papers published about purinergic signalling via ATP since 1972 is shown in Fig. 15. Therapeutic approaches to pathological disorders include the development of selective P1 and P2 receptor subtype agonists and antagonists, inhibitors of extracellular ATP breakdown, and enhancers and inhibitors of ATP transport. Small molecule purinergic drugs that are orally bioavailable and stable *in vivo* are beginning to be developed by medicinal chemists (see Baqi *et al.* 2010; Gever *et al.* 2010; Burnstock, 2011; Burnstock & Kennedy, 2011). The leading medicinal chemists in this field are shown in Fig. S11.

Call for comments

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Additional information

Competing interests

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Supporting Information

The following supporting information is available in the online version of this article.

Figure S1. Photographs of leading figures in the P1 receptor field.

Figure S2. Photographs of leading figures in the P2X receptor field.

Figure S3. Photographs of leading figures in the P2Y receptor field.

Figure S4. Photographs of some leading scientists in the purinergic signalling of excitable tissues field.

Figure S5. Photographs of leading scientists involved in the vascular purinergic signalling field.

Figure S6. Photographs of leading scientists involved in ATP release mechanisms.

Figure S7. Photographs of leading figures in the ectonucleotidase field.

Figure S8. Photographs of leading figures in the purinergic signalling pain field.

Figure S9. Photographs of leading figures in the CNS purinergic signalling field.

Figure S10. Photographs of leading figures in the special senses purinergic signalling field.

Figure S11. Photographs of leading medicinal chemists in the purinergic signalling field.