Ciba Foundation Symposium 198

P2 PURINOCEPTORS: LOCALIZATION, FUNCTION AND TRANSDUCTION MECHANISMS

1996

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P2 purinoceptors: historical perspective and classification

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Abstract. This article presents an overview that gives some historical perspective to the detailed papers at the cutting edge of P2 purinoceptor research that follow. I consider the proposal, first put forward by Abbracchio & Burnstock (Pharmacol Ther 64:445–475, 1994), that P2 purinoceptors should be regarded as members of two main families: a P2X purinoceptor family consisting of ligand-gated ion channels, and a P2Y purinoceptor family consisting of G protein-coupled receptors. The latest subclasses of these two families ($P2X_{1-4}$ and $P2Y_{1,5}$), identified largely on the basis of molecular cloning and expression, are tabled. Finally, I suggest some future directions for P2 purinoceptor research, including studies of the long-term (trophic) actions of purines, the evolution and development of purinoceptors and therapeutic applications.

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Early history

The first report about the potent actions of adenine compounds was published by Drury & Szent-Györgyi (1929), and the first hint that ATP might be a neurotransmitter appeared three decades later when ATP was shown to be released during antidromic stimulation of sensory nerves supplying the rabbit ear artery (Holton 1959). In the early 1960s, my colleagues and I in Melbourne and a group in Sweden proposed the existence of autonomic nerves supplying the gastrointestinal tract that were neither adrenergic nor cholinergic (Burnstock et al 1963, Martinson & Muren 1963). In the years that followed, strenuous efforts were made to identify the transmitter in non-adrenergic, non-cholinergic (NANC) nerves supplying the gut and the urinary bladder. Perhaps surprisingly, the substance that most satisfied the criteria at that time (Eccles 1964) was adenosine 5′-triphosphate (ATP) (Burnstock et al 1970), and the word 'purinergic' was coined and purinergic transmission proposed (Burnstock 1972).

Implicit in the concept of purinergic transmission was the existence of postjunctional receptors for ATP, although there was considerable confusion

in the literature about the variable effects of adenosine nucleotides and nucleosides on a wide variety of tissues (Burnstock 1976a). However, a step forward was taken in 1978 when, from a detailed analysis of the literature and some preliminary experiments, it was proposed (Burnstock 1978) that 'purinoceptors' could be subdivided into P1 (adenosine) purinoceptors which were coupled to adenylate cyclase and were competitively antagonized by low concentrations of methylxanthines, and P2 purinoceptors which were activated preferentially by ATP and ADP. Two of the most important implications of this purinoceptor subdivision were: (1) the importance of establishing whether in a particular situation ATP acts directly on P2 purinoceptors or via P1 purinoceptors after ectoenzymic breakdown to adenosine (Moody et al 1984); and (2) that during purinergic transmission, whereas ATP released from the nerve terminals acts on postjunctional P2 purinoceptors, adenosine generated from the extracellular breakdown of ATP acts largely via P1 purinoceptors on the nerve terminals to inhibit release of transmitter (De Mey et al 1979). Prejunctional modulation via P1 purinoceptors operates both as a negative feedback system in autoregulation in purinergic transmission and also to modulate the release of noradrenaline, acetylcholine (ACh) and other neurotransmitters (see Burnstock 1995).

In 1985, Burnstock & Kennedy proposed the first subdivision of P2 purinoceptors into P2X purinoceptors (which mediate vasoconstriction and contraction of visceral smooth muscle, with α,β-methylene ATP as a potent agonist) and P2Y purinoceptors (which mediate vasodilatation as well as relaxation of the smooth muscle of the gut, with 2-methylthioATP as a particularly potent agonist). Soon after, two further P2 purinoceptors were tentatively proposed (Gordon 1986): a P2T purinoceptor, which is ADP-selective involved in platelet aggregation; and a P2Z purinoceptor, which appears to be activated by ATP^{4-} and is prominent in macrophages, lymphocytes and mast cells. Another important landmark, following the seminal studies of Furchgott & Zawadzki (1980), was the recognition that P2Y purinoceptors on endothelial cells mediate vasodilatation via release of EDRF (endothelium-derived relaxing factor; De Mey & Vanhoutte 1981). This important discovery challenged the early hypothesis of Berne (1963) that adenosine is the local regulator of blood flow following hypoxia in heart and other vascular beds—it now seems likely that reactive hyperaemia is largely due to ATP, released from endothelial cells during hypoxia, acting on P2Y purinoceptors to release EDRF (now known to be nitric oxide, NO), resulting in vasodilatation; adenosine, produced following the breakdown of ATP, is likely to contribute to the later component of vasodilatation by direct action on P1 purinoceptors on vascular smooth muscle (Fig. 1, Hopwood et al 1989, Burnstock 1987, 1993a, Burnstock & Ralevic 1994).

The concept of co-transmission was put forward in 1976 in a review article entitled 'Do some nerve cells release more than one transmitter?' (Burnstock

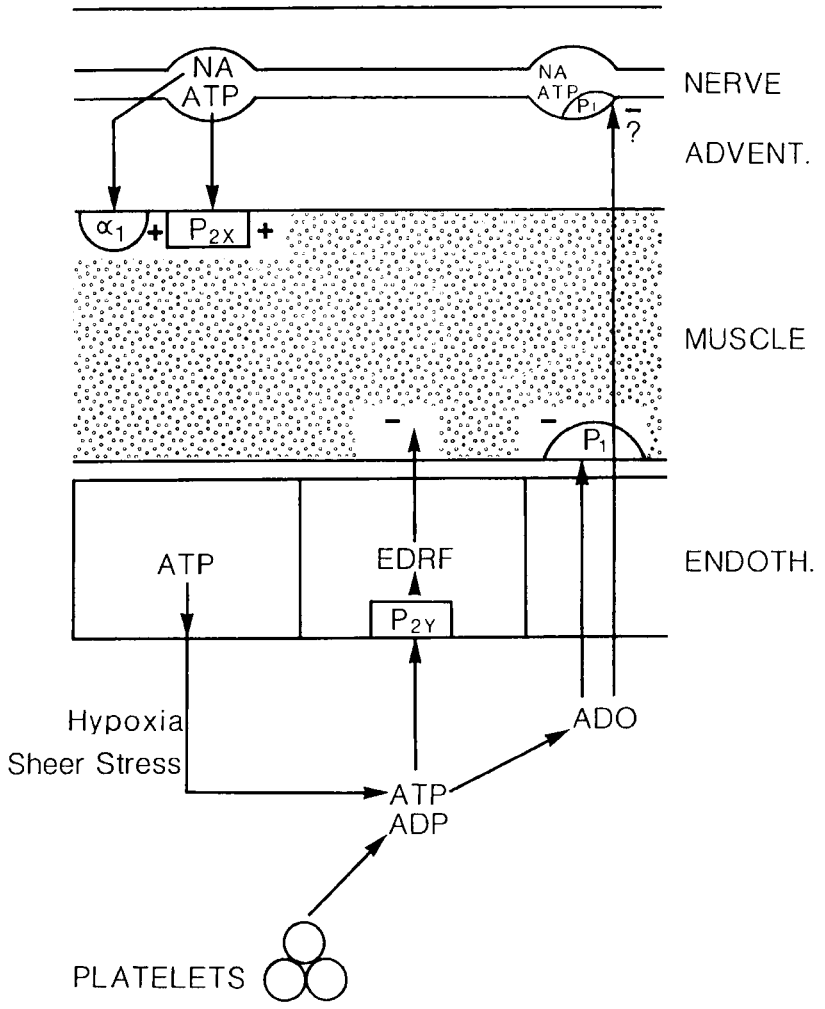

FIG. 1. A schematic representation of the interactions of ATP released from perivascular nerves and from endothelial cells (ENDOTH.). ATP is released from endothelial cells during hypoxia to act on endothelial P2Y purinoceptors leading to production of EDRF (NO) and subsequent vasodilatation (−). In contrast, ATP released as a co-transmitter with noradrenaline (NA) from perivascular sympathetic nerves at the adventitial (ADVENT.)–muscle border produces vasoconstriction (+) via P2X purinoceptors on the muscle cells. Different subclasses of P2Y purinoceptor are involved—both $P2Y_1$ and $P2Y_2$ (formerly termed $P2_U$) purinoceptors are present on most endothelial cells, while the subclass of P2Y purinoceptor found on some vascular smooth muscles has not been identified yet. Adenosine (ADO), resulting from rapid breakdown of ATP by ectoenzymes, usually produces vasodilatation by direct action on the muscle via P1 purinoceptors; it may also act via P1 purinoceptors on the perivascular nerve terminal varicosities to inhibit release of transmitter. (Figure modified from Burnstock 1987.)

1976b). This concept is now widely supported (Hökfelt et al 1986, Kupfermann 1991, Burnstock 1990b). It appears that ATP is a primitive transmitter and that it has been retained as a co-transmitter with other neurotransmitters in many different nerve types, albeit in proportions that vary between locations and species. For example, in sympathetic nerves ATP co-exists and is co-released with noradrenaline and neuropeptide Y; in some parasympathetic nerves with ACh; in some sensory-motor nerves with calcitonin gene-related peptide (CGRP) and substance P; and in NANC inhibitory nerves, together with NO and vasoactive intestinal peptide (VIP) (see Fig. 2).

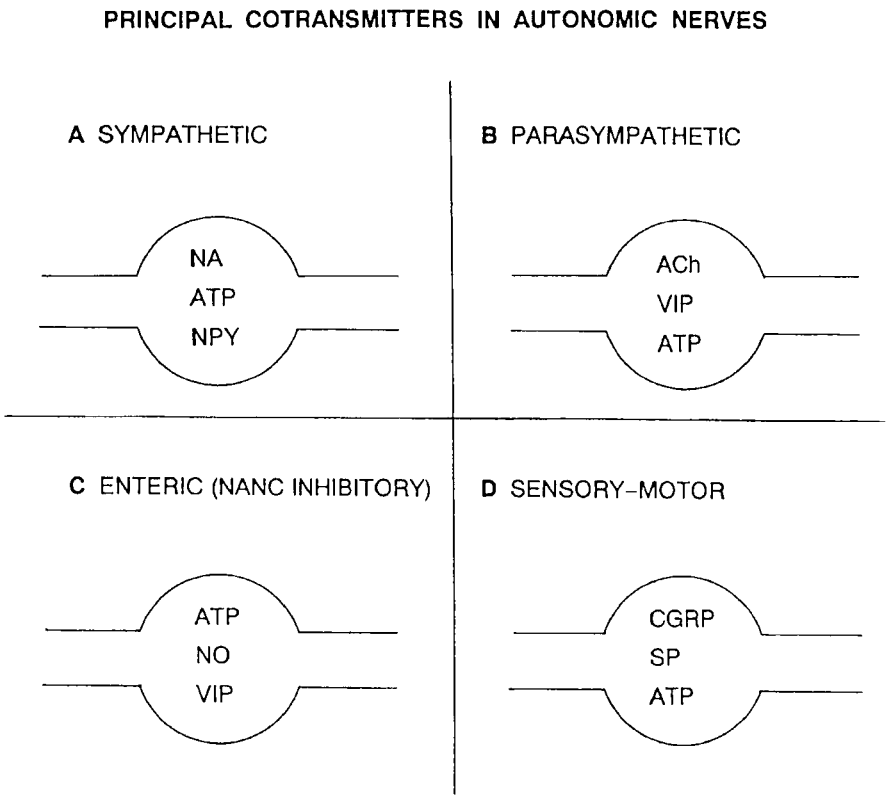

FIG. 2. Schematic representation of the chemical coding of autonomic nerves. The main transmitters found in terminal varicosities of sympathetic, parasympathetic, enteric (NANC inhibitory) and sensory–motor nerves are included, although the proportions of these transmitters varies considerably in different locations, species and at different stages of development and ageing. NA, noradrenaline; NPY, neuropeptide Y; ACh, acetylcholine; VIP, vasoactive intestinal polypeptide; CGRP, calcitonin gene-related peptide; SP, substance P.

In some recent studies, ATP has also been shown to be co-localized with γ-aminobutyric acid (GABA) in retinal nerves and perhaps released with glutamate in some hippocampal neurons (Perez & Bruun 1987, Wieraszko et al 1989, Kupfermann 1991). It is interesting that when excitatory junction potentials (EJPs) were first recorded in smooth muscle cells of the vas deferens in response to single pulses evoked by sympathetic nerves (Burnstock & Holman 1961), the authors were puzzled that depletion of noradrenaline with reserpine failed to block these responses (Burnstock & Holman 1962). It was about 15 years later before it was clear that we had been recording purinergic sympathetic co-transmission when, in response to sympathetic stimulation, EJPs were shown to be abolished by selective desensitization of the ATP receptor with α,β-methylene ATP in the vas deferens and rat tail artery (Kasakov & Burnstock 1983, Sneddon & Burnstock 1984a,b). Furthermore, local application of ATP, but not noradrenaline, mimicked EJPs in the vas deferens (Sneddon & Westfall 1984).

Expression of co-transmitters in autonomic nerves shows remarkable plasticity in development and ageing, in nerves that remain following trauma and surgery, under the influence of hormones, and in various disease situations (Burnstock 1990a). There are several examples which involve purinergic co-transmission. (1) In spontaneously hypertensive rats a significantly greater role for ATP compared with noradrenaline has been demonstrated in tail arteries (Vidal et al 1986), mesenteric arteries (Woolridge & van Helden 1990) and in various blood vessels *in vivo* (Bulloch & McGrath 1992). (2) Whereas the purinergic component of parasympathetic contraction of the rodent bladder is prominent compared to the cholinergic component (Burnstock et al 1978, Dean & Downie 1978, see Hoyle & Burnstock 1991), there has been debate about whether there is a purinergic component in the parasympathetic supply to the human bladder (Husted et al 1983, Sibley 1984, Hoyle et al 1989), even though the presence of P2 purinoceptors is well established (Hoyle et al 1989, Inoue & Brading 1991, Bo & Burnstock 1995). Interestingly, however, evidence for a substantial purinergic component in the responses to the urinary bladder of women with interstitial cystitis has been reported (Palea et al 1993) and increased purinergic responses demonstrated in the neurogenic bladder (Ruggieri et al 1990). (3) Whereas in the developing myotube, ATP acts as a co-transmitter with ACh with both transmitters opening ion channels (Kolb & Wakelam 1983, Hume & Honig 1986, Häggblad & Heilbronn 1988, Henning et al 1993), in the adult skeletal neuromuscular junction, the ATP released from adult nerves acts either as a postjunctional modulator potentiating the action of ACh or as a prejunctional modulator of ACh release via P1 purinoceptors after ectoenzymic breakdown of ATP to adenosine (Nagano et al 1992, Smith & Lu 1991, Lu & Smith 1991).

The concept of purinergic transmission was boosted when it was shown clearly that ATP was used as a fast transmitter between neurons in both

autonomic ganglia (Evans et al 1992, Silinsky et al 1992) and the medial habenula (Edwards et al 1992). Since then there have been several reports of the potent actions of ATP in the CNS (see Inoue et al 1992, Harms et al 1992, Ueno et al 1992, Wieraszko & Ehrlich 1994, Ergene et al 1994, Illes et al 1995) and convincing demonstrations of the autoradiographic localization of P2X purinoceptors in different regions of the brain (Bo & Burnstock 1994).

Current status of P2 purinoceptors

Knowledge of the structure and properties of P2 purinoceptors has lagged behind information for most other neurotransmitters. The general progress of information has involved structure–activity, pharmacology, quantitation of receptor expression, ligand binding and autoradiographic localization, transduction mechanisms involving second messenger systems and ion channels, and finally the molecular biology of the receptors with cloning and sequencing. Since the subdivisions of the P2 purinoceptor into P2X, P2Y, P2T and P2Z mentioned earlier, several subclasses have been proposed, including P2U purinoceptors where ATP and UTP are equipotent (O'Connor et al 1991) and a P2D purinoceptor, selective for diadenosine polyphosphates (Pintor & Miras-Portugal 1993). It was clearly shown that P2X purinoceptors involved ligand-gated cation channels, while P2Y purinoceptors involve G protein activation (Fig. 3, Dubyak 1991). More recently, the possibility that some P2Y purinoceptors act via G_i proteins to inhibit adenylate cyclase has been raised (Harden et al 1995) and also that there may be uridine nucleotide-selective G protein-linked receptors (Lazarowski & Harden 1994).

The first P2 purinoceptors to be cloned were G protein-coupled P2Y purinoceptors: $P2Y_1$ purinoceptors were isolated from chick brain (Webb et al 1993); and a P2U purinoceptor (later designated $P2Y_2$) from neuroblastoma cells (Lustig et al 1993). A year later two ligand-gated ion channel ATP receptors were reported—one from vas deferens (Valera et al 1994) and another from rat phaeochromocytoma PC12 cells (Brake et al 1994). The P2Y and P2U purinoceptors had the typical seven-transmembrane-domain structures (Fig. 4b), while the P2X purinoceptors consisted of two transmembrane domains with a large extracellular loop rich in cysteines (Fig. 4a).

In the recent paper from the subcommittee concerned with the nomenclature of P2 purinoceptors (Fredholm et al 1994), it was emphasized that the current purinoceptor subclassification, with so many letters of the alphabet being somewhat randomly added as new receptor subtypes were discovered, was unsatisfactory. They supported, in principle, a new system of classification proposed by Abbracchio & Burnstock (1994). In this proposal, it was suggested

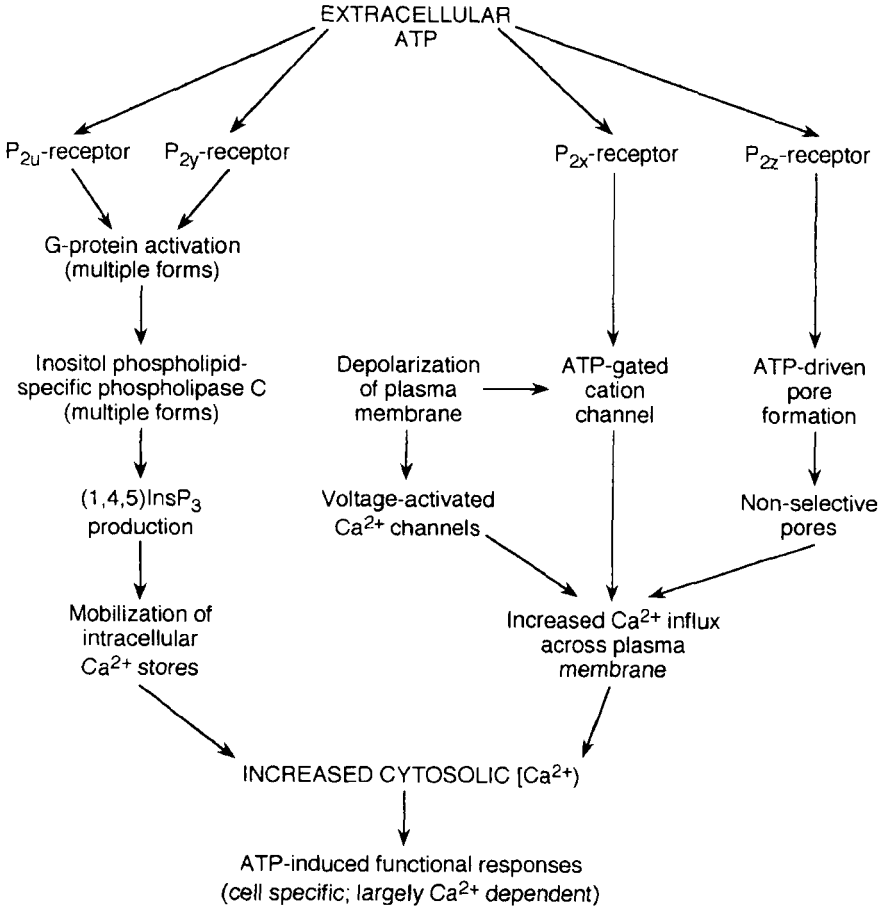

FIG. 3. Mechanisms underlying the increase in cystolic [Ca^{2+}] activated by different P2 purinoceptors for extracellular ATP. Extracellular ATP can interact with several P2 purinoceptor subtypes expressed in different cell types. Occupation of each of these receptor subtypes has been shown to induce rapid increases in cystolic [Ca^{2+}], with consequent activation of Ca^2-regulated cellular functions (e.g. contraction in muscle cells or exocytotic secretion in endocrine/neuroendocrine cells). Occupation of both P2Y and P2U purinoceptors primarily induces mobilization of Ca^{2+} sequestered in inositol 1,4,5-trisphosphate ($InsP_3$)-releasable, intracellular stores. Conversely, P2X and P2Z purinoceptors primarily increase Ca^{2+} influx, via a variety of channels and 'pores', across the plasma membrane. (From Dubyak 1991.)

that P2 purinoceptors should be placed in two major families, a P2X family consisting of ligand-gated cation channels and a P2Y family consisting of G protein-mediated receptors. They recognized that the P2Z purinoceptor, which opens non-selective pores, might represent a third family (Table 1). It

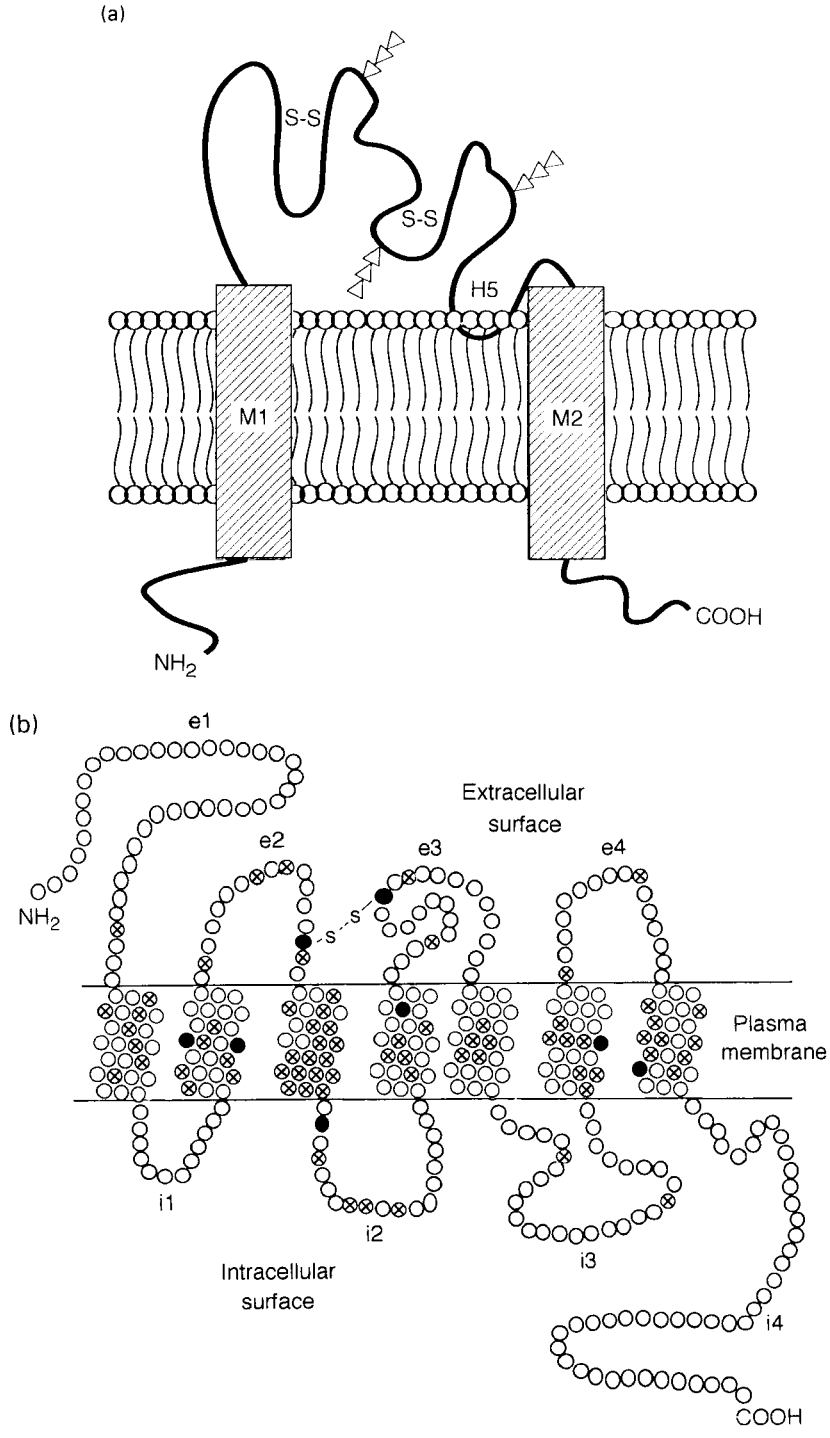
(a)
S-S
S-S
H5
M1
M2
COOH
NH2
(b)
e1
Extracellular
surface
e2
e3
e4
NH2
S
S
Plasma
membrane
i1
i2
i3
i4
Intracellular
surface
COOH

TABLE 1 Proposed subclassification of P2 purinoceptors

Name	*P2X purinoceptor family*	*P2Y purinoceptor family*	*P2Z*
Type	Ligand-gated channel	G protein-coupled	Non-selective pore
General agonist profile	α,β-meATP > β,γ-meATP > ATP ≈ 2-MeSATP ≈ ADP	2-MeSATP > ATP = ADP > α,β-meATP ⩾ β,γ-meATP	ATP^{4-}
Antagonists	α,β-meATP desensitization Suramin Selectively blocked by PPADS ANAPP3	Suramin Reactive blue 2	Oxidized ATP

α,β-meATP, α,β-methylene ATP; β,γ-meATP, β,γ-methylene ATP; 2-MeSATP, 2-methylthioATP; PPADS, pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid; ANAPP3, 3-*O*-3[*N*-(4-azido-2-nitrophenyl)amino] propriony! ATP. (Reproduced with permission from Abbracchio & Burnstock 1994.)

was pointed out that this classification brought ATP into line with most other neurotransmitters such as ACh, GABA, glutamate and 5-hydroxytryptamine (5-HT), where ligand-gated and G protein-mediated receptor subclassifications have already been established (Table 2, Burnstock 1995).

On the basis of the relative actions of a series of newly established purine analogues (Fischer et al 1993, Burnstock et al 1994, Bo et al 1994), an analysis of transduction mechanisms (Friel 1988, Dubyak 1991, Pirotton et al 1991, El-Moatassim et al 1992, Harden et al 1995) and on the structure of cloned receptors, Abbracchio & Burnstock tentatively named four subclasses of the

FIG. 4. Molecular structure of P2X and P2Y purinoceptors. (a) Model depicting a proposed transmembrane topology for $P2X_2$ protein is shown with both N- and C-termini 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 disulfide-bonded loops (S-S) and three *N*-linked glycosyl chains (triangles). The $P2X_2$ cDNA was sequenced on both strands using Sequenase (USB). (From Brake et al 1994.) (b) Schematic diagram of the sequence of $P2Y_1$ purinoceptor showing its differences from $P2Y_2$ and $P2Y_3$ purinoceptors. Crossed circles represent amino acid residues that are conserved between the three receptors; open circles represent residues that are not conserved; and filled circles represent residues that are known to be functionally important in other G protein-coupled receptors. (From Barnard et al 1994.)

TABLE 2 Comparison of fast ionotropic and slow metabotropic receptors for acetylcholine (ACh), γ-aminobutyric acid (GABA), glutamate and 5-hydroxytryptamine (5-HT) with those proposed for ATP

	Receptors	
Messenger	*Fast ionotropic*	*Slow metabotropic*
ACh	Nicotinic Muscle type Neuronal type	Muscarinic M1–M5
GABA	$GABA_A$	$GABA_B$
Glutamate	AMPA Kainate NMDA	$mGlu_1$–$mGlu_7$
5-HT	$5\text{-}HT_3$	$5\text{-}HT_{1A\text{-}F}$ $5\text{-}HT_{2A\text{-}C}$ $5\text{-}HT_4$ $5\text{-}HT_{5A\text{-}B}$ $5\text{-}HT_6$ $5\text{-}HT_7$
ATP	$P2X_{1-6}$	$P2Y_{1-7}$

AMPA, 2-(aminomethyl)phenylacetic acid; NMDA, *N*-methyl-D-aspartate.

P2X purinoceptor family and seven subclasses of the P2Y purinoceptor family. A number of further P2X and P2Y purinoceptor molecular sequences have subsequently been reported and the current situation is summarized in Tables 3 and 4. A new member of the P2X purinoceptor family has just been identified in our laboratory which is expressed by a subset of sensory neurons (Chen et al 1995). *In situ* hybridization and Northern blotting of the $P2X_3$ purinoceptor show that the channel transcript is present in a subset of rat dorsal root ganglion sensory neurons, some of which express nociceptor-associated markers; it is absent in other tissues we have tested, including sympathetic, enteric and CNS neurons. When expressed in *Xenopus* oocytes, the channel shows an ATP-dependent cation flux ($EC_{50} \approx 1.2\,\mu M$), and a rank order of potency of 2-methylthioATP > > ATP > α,β-methylene ATP > ATPγS > 2′-deoxy ADP > CTP ≈ ADP > > UTP ≈ β,γ-methylene ATP > GTP. $P2X_3$ is the only ligand-gated channel known to be expressed exclusively by a subset of sensory neurons. The remarkable selectivity of expression of the channel coupled with its sensory neuron-like pharmacology suggest that this channel may transduce ATP-evoked nociceptor activation. In Fig. 5, the primary sequence of the $P2X_3$ purinoceptor is aligned with the two other known members of the P2X purinoceptor family isolated from PC12 cells ($P2X_2$) and rat vas deferens ($P2X_1$).

TABLE 3 Classification of subtypes of P2X purinoceptor family on the basis of molecular and functional characteristics

P2 receptor subtype	*Tissue*	*Activity*	*Properties*	*References*	*Genbank/EMBL accession no.*
$P2X_1$	Vas deferens (rat)	2-MeSATP > ATP > α,β-meATP	$I_{Na/K/Ca}$	Valera et al 1994	X80477
$P2X_2$	PC12 cells (rat)	2-MeSATP > ATP (α,β-meATP inactive)	$I_{Na/K}$	Brake et al 1994	U14414
$P2X_2-1$ (short form)	Cochlea (rat)	ND	ND	Housley et al 1995	L43511
$P2X_3$	DRG cells (rat)	2-MeSATP > ATP > α,β-meATP	$I_{Na/K}$	Chen et al 1995a	—
	DRG cells (rat)	ATP > 2-MeSATP > α,β-meATP	$I_{Na/K/Ca}$	Lewis et al 1995	X91167
$P2X_4$	Hippocampus (rat)	ATP > 2-MeSATP > α,β-meATP	$I_{Na/K}$	Bo et al 1995	—
	DRG cells (rat)	ATP active, α,β-meATP inactive	$I_{Na/K/Ca}$	Buell et al 1996	—
	Neurons (rat)	ATP > > 2-MeSATP > CTP > α,β-meATP > dATP	$I_{Na/K/Ca}$	Soto et al 1996	X93565
	Neurons (rat)	—	—	Seguela et al 1996	U32497
$P2X_5$	Neurons (rat)	ATPγ5 ≥ ATP ≥ 2-MeSATP > > ADP (α,β-meATP inactive)	$I_{Na/K/Ca}$	Collo et al 1996	—
$P2X_6$	Neurons (rat)	2-MeSATP = 2-cl-ATP = ATP > ATPγ5 > > ADP (α,β-meATP inactive)	$I_{Na/K/Ca}$	Collo et al 1996	—
P2Z (or P2X-like)	Macrophage (mouse)	(i) BzATP > ATP > UTP (ii) ATP > UTP > BzATP	(i) $I_{Na/K}$ (ii) I_{Ca}	Nuttle et al 1993	—

ND, not determined; DRG, dorsal root ganglion; 2-MeSATP, 2-methylthioATP; a,β-meATP, α,β-methylene ATP; BzATP, 3′-O-(4-benzoyl)benzoyl ATP; dATP, 3′-deoxy ATP.

TABLE 4 Classification of subtypes of P2Y purinoceptor family on basis of molecular and functional characteristics

P2 receptor subtype	*Tissue*	*Activity*	*Properties*	*References*	*Genbank/EMBL accession no.*
$P2Y_1$	Brain (chick)	2-MeSATP > ATP > ADP (UTP inactive)	PLCβ/InsP_3/Ca^{2+}	Webb et al 1993	X73268
	Brain (turkey)	2-MeSATP > ADP > ATP (UTP inactive)	PLCβ/InsP_3/Ca^{2+}	Filtz et al 1994	U09842
	Brain (human)	—	—	Ayyanathan et al, unpublished	*U42029/30
	Insulinoma cells (mouse)	ND	ND	Tokuyama et al 1995	U22829
	Insulinoma cells (rat)	2-MeSATP > 2-Cl-ATP > ATP (α,β-meATP inactive)	PLCβ/InsP_3/Ca^{2+}	Tokuyama et al 1995	U22830
	Placenta (human)	ND	ND	Leon et al 1996	Z49205
	Endothelium (bovine)	2-MeSATP > ATP > > UTP	PLCβ/InsP_3/Ca^{2+}	Henderson et al 1995	X87628
$P2Y_2$	NG108-15 cells (mouse)	ATP = UTP > > 2-MeSATP	PLCβ/InsP_3/Ca^{2+}	Lustig et al 1993	L14751
	CT/43 cells (human)	ATP = UTP > > 2-MeSATP	PLCβ/InsP_3/Ca^{2+}	Parr et al 1994	U07225
	Lung (rat)	ATP = UTP	PLCβ/InsP_3/Ca^{2+}	Rice et al 1995	U09402
	Bone (human)	ND	ND	Bowler et al 1995	—
	Pituitary (rat)	ND	ND	Chen et al 1996	L46865
$P2Y_3$	Brain (chick)	ADP > UTP > ATP = UDP	PLCβ/InsP_3/Ca^{2+}	Webb et al 1996a	—
$P2Y_4$	Placenta (human)	UTP = UDP > ATP = ADP	PLCβ/InsP_3/Ca^{2+}	Communi et al 1995	—
	Brain (rat)	ND	ND	(to be confirmed)	—
$P2Y_5$	HEL cells (human)	ADP > ATP > UTP	ND	Kunapuli et al 1996	U41070
	Lymphocytes (chicken)	ATP > ADP > 2-MeSATP > > α,β-meATP = UTP†	ND	Webb et al 1996b	—
$P2Y_6$	Aortic smooth muscle (rat)	UTP > ADP = 2-MeSATP > ATP	PLCβ/Ins$P_3$$Ca^{2+}$	Chang et al 1995	D63665

ND, not determined; 2-MeSATP, 2-methylthioATP; 2-Cl-ATP, 2-chloroATP; α,β-meATP, α,β-methylene ATP; PLCβ, phospholipase Cβ, InsP_3, inositol 1,4,5-trisphosphate. *Direct submission to Genbank of long and short form of gene encoding the same receptor protein.
†Radioligand binding.

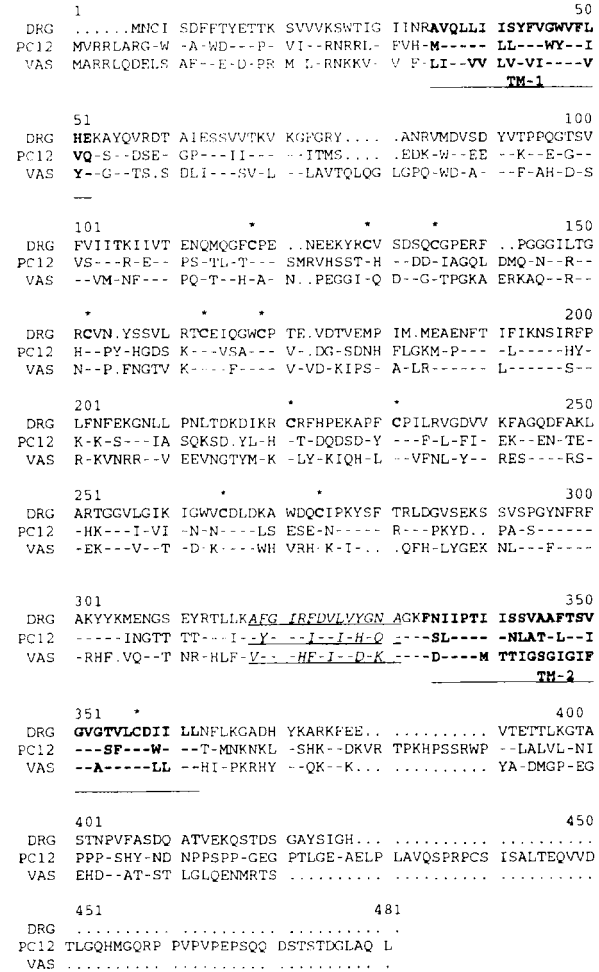

FIG. 5. Primary sequence of the $P2X_3$ purinoceptor (DRG) aligned with those of two other known members of the P2X purinoceptor family isolated from PC12 cells ($P2X_2$) and rat vas deferens ($P2X_1$). Dashes represent sequence identity, dots represent gaps. The putative transmembrane regions are bold and underlined, and the H5-related regions also present in K^+ channels are italicized and underlined. Note the conserved cysteine residues (bold) marked with asterisks. (From Chen et al 1995.)

Future directions

Long-term (trophic) actions of purines

While there are substantial studies of the short-term actions of purines in neural and neuromuscular transmission and secretion in a range of different cell types, there are relatively few studies on the long-term (trophic) actions of

TABLE 5 P2 purinoceptor antagonists

Compounds	*Receptor subtype claimed*	*Tissue*	*pA_2 value (if known)*	*References*	*Comments*
Quinidine	P2X	Taenia coli	—	Burnstock et al 1970	Low concentrations antagonize adrenergic responses; higher concentrations are needed to antagonize purinergic responses ($\approx 300\,\mu M$)
	P2Y	Urinary bladder	—	Burnstock et al 1972	
Imidazolines (phentolamine)	P2Y	Taenia coli	—	Sathell et al 1973	Effective only at high concentrations ($\approx 200\,\mu M$)
PIT	P2Y	Taenia coli	—	Hooper et al 1975	Causes direct relaxation of the muscle. At concentrations greater than 100 μM causes non-specific effects
			—	Spedding et al 1975	
ANAPP3	P2X	Vas deferens, urinary bladder	—	Hogaboom et al 1980	Irreversible antagonism occurs after photolysis due to covalent binding to ATP-binding sites. Binding occurs also to any ATP-metabolizing protein
			—	Westfall et al 1983	
Apamin	P2Y	Taenia coli	—	Brown & Burnstock 1981	Now best known as an inhibitor of K^+ channels
α,β-meATP	P2X	Urinary bladder, vas deferens	—	Kasakov & Burnstock 1983	Repeat administrations cause selective antagonism due to desensitization of purinoceptors. Very selective for P2X purinoceptors, but not competitive
				Meldrum & Burnstock 1983	
Reactive blue 2	P2Y	Colon, duodenum, caecum, portal vein, taenia coli	$pK_B = 5.0$	Kerr & Krantis 1979	Produces reasonably selective antagonism at P2Y purinoceptors at concentrations less than 50 μM
				Manzini et al 1985, 1986	
				Reilly et al 1987	
	P2X	Urinary bladder, vas deferens	—	Choo 1981	
				Bültmann & Starke 1994a	
	P2Z	Lymphocytes	—	Wiley et al 1993	
Suramin	P2X	Vas deferens, urinary bladder	$pA_2 = 4.6$–5.0	Dunn & Blakeley 1988	Fairly selective for P2 purinoceptors, but cannot discriminate between subtypes
				Hoyle et al 1990	Has diverse other biological activities (Voogd et al 1993)

(Continued)

	P2Y	Taenia coli	—	Hoyle et al 1990	
	P2T	Platelet	$pA_2 = 4.6$	Hourani et al 1992	
	P2Z	Lymphocytes	—	Wiley et al 1993	
XAMR0721	P2Y	Turkey erythrocytes	$K_i = 19\mu M$	van Rhee et al 1994	Benzamide congener of suramin. Claimed to be selective, but has not been tested on other receptors
PPADS	P2X	Vas deferens, urinary bladder, blood vessels	$pK_B = 6.0–6.5$	Lambrecht et al 1992 Ziganshin et al 1993, 1994 McLaren et al 1994	At present the drug of choice for selective antagonism at P2X purinoceptors
	P2Y	Turkey erythrocytes	—	Boyer et al 1994	
isoPPADS	P2X	Vas deferens, vagus nerve, sympathetic ganglia	$pK_B = 6.6$	Bültmann & Starke 1994a Trezise et al 1994a Khahk et al 1994 Connoly 1995	Nearly as effective as PPADS
P5P	P2X	Vas deferens, vagus nerve, sympathetic ganglia	$pK_i = 4.9$	Trezise et al 1994b Connoly 1995	Synthesis precursor of PPADS
DIDS	P2X	Vas deferens	$IC_{50} = 3.9\ \mu M$	Bültmann & Starke 1994b	Also inhibits anion transport (Cabantchik & Greger 1992)
	P2Z	Parotid acinar cells	—	Soltoff et al 1993	
Evans blue	P2X	Vas deferens	$pK_B = 5.9$	Bültmann & Starke 1993	
Trypan blue	P2X	Vas deferens	$pA_2 = 5.3$	Bültmann et al 1994	
Congo red	P2X	Vas deferens	$pK_B = 4.6$	Khakh et al 1994	
Brilliant blue	P2Z	Parotid acinar cells	—	Soltoff et al 1989	
	P2Y?	Vas deferens, urinary bladder	$pK_B = 4.5$	von Kügelgen et al 1994 Palea et al 1995	
TNP-ATP	P2Y?	Cochlear hair cells	—	Mockett et al 1994	
o-ATP	P2Z	Macrophages	—	Murgia et al 1993	
ARL 66096	P2T	Platelets	$pK_B = 8.7$	Humphries et al 1994	

PIT, 2,2′-pyridylisatogen tosylate; ANAPP3, 3-*O*-3[*N*-(4-azido-2-nitrophenyl) amino] proprionyl ATP; α,β-MeATP, α,β-methylene ATP; XAMRO721, 8-β,5-dinitrophenylene carbonylimino)-1,3,5-napthalene trisulfonate; PPADS, pyridoxalphosphate-6-azophenyl-2′4′-disulfonic acid; isoPPADS, pyridoxalphosphate-6-azophenyl-2′,5′-disulfonic acid; P5P, pyridoxal-5-phosphate; DIDS, 4,4′-diisothiocyanotostilbene-2,2′-disulfonate; ARL66096 (formerly FPL66096), 2-propylthio-D-β,γ-difluoromethylene ATP; TNP-ATP, 2′-(or 3′)-*O*-trinitrophenyl ATP; O-ATP, oxidized ATP.

purines (Burnstock 1993b, Neary et al 1996). However, there are an increasing number of reports about the roles of purines in embryonic development (Knudsen & Elmer 1987, Laasberg 1990), growth and cell proliferation (Gonzales et al 1990, Wang et al 1990, Malam-Souley et al 1993, Erlinge et al 1995). There is also recognition of the involvement of purines in programmed cell death or apoptosis (Zheng et al 1991, Murgia et al 1992, Avery et al 1992). Trophic roles for purines on the activation of glial and neuronal cells have recently been described (Rathbone et al 1992, Neary & Norenberg 1992, Abbracchio et al 1994), as have their interactions with growth factors (Neary et al 1994, Schäfer et al 1995, Abbracchio et al 1995).

The participation of purines in neuro-immune mechanisms (Steinberg & Silverstein 1987, El-Moatassim et al 1987, Murgia et al 1992) and in bone and cartilage resorption (Caswell et al 1992, Leong et al 1994, Yu & Ferrier 1993) is beginning to be explored.

Evolution and development of purinoceptors

ATP is one of the first molecules to appear in the evolution of biological systems as an energy source and as an essential component of nucleic acids (Sigel 1992). There are growing indications that ATP might also have been used early in evolution as an extracellular messenger (Burnstock 1975, 1977, 1979, Berlind 1977, Venter et al 1988, Hoyle & Greenberg 1988). For example, ATP has been reported to have potent effects on various activities of amoebae (Zimmerman 1962, Pothier et al 1987). Purine compounds elicit contractions of the pedal disc of the sea anemone (Hoyle et al 1989) and depolarize specific neurons in the nervous system of the leech (Backus et al 1994). Separate receptors for adenosine and ATP have been identified in molluscan preparations, including neurons of the snail (Yatani et al 1982), the bivalve *Mytilus* (Barraco & Stefano 1995), the heart of various marine molluscs (Hoyle & Greenberg 1988, Knight et al 1992) and proboscis muscle of *Buccinium* (Nelson & Huddart 1994). Purine compounds also relax the gastric ligament of the starfish (Hoyle & Greenberg 1988, Knight et al 1990).

The sensillae of the olfactory organ of the spiny lobster are excited by low concentrations of AMP, ADP or ATP, which act as chemoreceptors in feeding responses (Carr et al 1986, Zimmer-Faust et al 1988), and ATP stimulates the gorging responses in a variety of blood-sucking insects (Galun & Kabayo 1988, Liscia et al 1993). The effects of purine nucleosides and nucleotides have been studied in a number of preparations from lower vertebrates, including the elasmobranch and teleost fish intestine (Young 1988, Jensen & Holmgren 1985, Kitazawa et al 1990), the cardiovascular system of fish, amphibians, reptiles and birds (Flitney et al 1977, Colin et al 1979, Meghji & Burnstock 1984,

Knight & Burnstock 1995, Lennard & Huddart 1989), and fish chromatophores (Miyashita et al 1984, Namoto 1992). While there are reports about P2 purinoceptor activity in early development in a variety of tissues (e.g. Hilfer et al 1977, Smurs 1981, Levin et al 1981, Furukawa & Nomoto 1989, Laasberg 1990, Rathbone et al 1992, Zheng et al 1994, Abe et al 1995), few attempts have been made yet to characterize the purinoceptor subtypes involved (Erlinge et al 1995).

Therapeutic applications

There has been considerable interest in the therapeutic potential of adenosine acting via different subclasses of P1 purinoceptors for some time (see reviews by Daly 1982, Stone 1992, Williams 1993). Interest in the therapeutic potential of ATP acting through P2 purinoceptors is more recent (see Burnstock 1993b, Abbracchio & Burnstock 1994). It is remarkable that during the past year, serious explorations of a therapeutic role for P2 purinoceptors have been reported for cystic fibrosis (Boucher et al 1995), diabetes (Loubatiéres-Mariani et al 1995), immune and inflammatory disease situations (Di Virgilio et al 1995) and cancer (Rapaport 1993). Most recently, the discovery of a selective P2T antagonist (Humphries et al 1994) is leading to the development of an antithrombotic agent (Humphries et al 1995). Other conditions where there is interest in pursuing therapeutic goals involving P2 purinoceptors include bladder incontinence (Hoyle & Burnstock 1996), pulmonary hypertension (Rubino & Burnstock 1994), surfactant and mucosal secretion (Griese et al 1993), constipation and diarrhoea (Milner & Burnstock 1994), behavioural disorders such as epilepsy, depression and ageing-associated neurodegenerative diseases (Williams 1993), contraception and sterility (Foresta et al 1992), ischaemia (Phillis et al 1993), and wound healing (Wang et al 1990, Namiot et al 1993). Increasing interest in the therapeutic potential of purinoceptors reinforces the pressing need for the development of specific agonists and antagonists for all new purinoceptor subtypes as they become established and especially agents that will be effective *in vivo*. A summary of the various compounds claimed to be P2 purinoceptor antagonists through the years, with comments about their actions, is given in Table 5. The development and use of nucleoside transport inhibitors (Van Belle 1995) and ATPase inhibitors (Crack et al 1995) is also of considerable interest in therapeutic terms.

Final comment

There is clearly an exploding interest in the molecular identification, distribution and roles of different P2 purinoceptor subtypes and in their application to clinical medicine. The future of this field is intensely exciting and I look forward to much fruitful debate in the coming days.

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