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Review

Purine and pyrimidine receptors

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Abstract. Adenosine 5′-triphosphate (ATP), in addition to its intracellular roles, acts as an extracellular signalling molecule via a rich array of receptors, which have been cloned and characterised. P1 receptors are selective for adenosine, a breakdown product of ATP, produced after degradation by ectonucleotidases. Four subtypes have been identified, A_1, A_{2A}, A_{2B} and A_3 receptors. P2 receptors are activated by purines and some subtypes also by pyrimidines. P2X receptors are ligand-gated ion channel receptors and seven subunits have been identified, which form both homomultimers and heteromultimers. P2Y receptors are G protein-coupled receptors, and eight subtypes have been cloned and characterised to date.

Keywords. Cloned receptors, gene regulation, G protein-coupled receptors, heteromultimers, ion channel receptors, P1 receptor, P2X receptor, P2Y receptor.

Early studies

A seminal paper describing the potent actions of adenine compounds was published by Drury and Szent-Györgyi in 1929 [1]. Many years later, ATP was proposed as the transmitter responsible for non-adrenergic, non-cholinergic transmission in the gut and bladder, and the term 'purinergic' was introduced by Burnstock in 1972 [2]. Early resistance to this concept appeared to stem from the fact that ATP was recognized first for its important intracellular roles in many biochemical processes, and the intuitive feeling was that such a ubiquitous and simple compound was unlikely to be utilized as an extracellular messenger, although powerful extracellular enzymes involved in its breakdown were known to be present.

Implicit in the concept of purinergic neurotransmission was the existence of postjunctional purinergic receptors, and the potent actions of extracellular ATP on many different cell types also implicated membrane receptors. Purinergic receptors were first defined in 1976 [3]. Two years later a basis for distinguishing two types of purinoceptors, identified as P1 and P2 (for adenosine and ATP/ADP, respectively), was proposed [4]. At about the same time, two subtypes of the P1 (adenosine) receptor were recognised [5, 6], but it was not until 1985 that a proposal suggesting a pharmacological basis for distinguishing two types of P2 receptors (P2X and P2Y) was made [7]. In 1993, the first G protein-coupled P2 receptors were cloned [8, 9], and a year later two ion-gated receptors were cloned [10, 11]. In 1994 Abbracchio and Burnstock [12], on the basis of molecular structure and transduction mechanisms, proposed that purinoceptors should belong to two major families: a P2X family of ligand-gated ion channel receptors and a P2Y family of G protein-coupled receptors. This nomenclature has been widely adopted and currently seven P2X subtypes and eight P2Y receptor subtypes are recognized, including receptors that are sensitive to pyrimidines as well as purines [13–15].

It is widely recognized that purinergic signalling is a primitive system involved in many non-neuronal as

well as neuronal mechanisms, including exocrine and endocrine secretion, immune responses, inflammation, pain, platelet aggregation and endothelial-mediated vasodilatation [16, 17]. Cell proliferation, differentiation and death that occur in development and regeneration are also mediated by purinergic receptors [18, 19].

P1 receptors

In 1989, complementary DNAs (cDNAs) encoding two different P1 receptor subtypes (A_1 and A_2) were isolated [20], and, shortly after, the A_3 subtype was identified [21]. Four different P1 receptor subtypes, A_1, A_{2A}, A_{2B} and A_3, have been cloned and characterised [22–25]. All are members of the rhodopsin-like family of G protein-coupled receptors. Their N-termini are relatively short (7–13 residues in length), as are their C-termini (32–120 residues). In the transmembrane domains (TMI-TMVII), human adenosine receptors share 39–61% sequence identity with each other and 11–18% identity with P2Y receptors. Each of the four human P1 receptor genes contains an intron within the coding region, located immediately after the end of the third transmembrane domain. Polymorphisms have been observed in the A_1 and the A_{2A} receptors.

P1 receptors couple principally to adenylate cyclase. A_1 and A_3 are negatively coupled to adenylate cyclase through the $G_{i/o}$ protein α-subunits, whereas A_{2A} and A_{2B} are positively coupled to adenylate cyclase through G_s [26]. The human A_{2B} receptor has also been observed to couple through $G_{q/11}$ to regulate phospholipase C activity, and the A_3 receptor may interact directly with G_s.

A number of P1 subtype-selective agonists and antagonists have been identified (see Table 1). All of the known P1 receptor agonists are closely related to adenosine in structure, with few modifications permitted. The most selective agonist for the A_1 subtype is CCPA (2-chloro-N^6-cyclopentyladenosine). CGS 21680 is the most selective A_{2A} agonist, while NECA (5′-*N*-ethylcarboxamidoadenosine) is the most potent A_{2B} receptor agonist. Cl-IB-MECA is 11-fold selective for the human and about 1400-fold selective for the rat A_3 receptor. Methylxanthines such as caffeine and theophylline are weak P1 receptor antagonists. DPCPX (8-cyclopentyl-1,3-dipropylxanthine) is an A_1 receptor antagonist with sub-nanomolar affinity. The most selective A_{2B} receptor antagonist is MRS1751. MRE 3008F20 is the most selective human A_3 receptor antagonist.

The diverse physiological effects mediated by the different P1 receptor subtypes, particularly modulation of the cardiovascular, immune and central nervous systems, have been confirmed by transgenic knockout mice [27, 28]. Null mice have been generated for each of the A_1, A_{2A} and A_3 receptors, and in all knockout animals generated, the P1 receptors in question do not appear to play a critical role during development. Knockout mice have yet to be described for the A_{2B} receptor subtype. In contrast to knockout studies, overexpression of either A_1 or A_3 subtypes in transgenic mice has a cardioprotective effect [29]. P1 and P2Y receptors are frequently expressed in the same cells.

P2X receptors

Molecular structure

The first cDNAs encoding P2X receptor subunits were isolated in 1994. Members of the family of ionotropic $P2X_{1-7}$ receptors show a subunit topology of intracellular N- and C-termini possessing consensus binding motifs for protein kinases; two transmembrane-spanning regions (TM1 and TM2), the first involved with channel gating and the second lining the ion pore; a large extracellular loop, with 10 conserved cysteine residues forming a series of disulfide bridges; a hydrophobic H5 region close to the pore vestibule, for possible receptor/channel modulation by cations and an ATP-binding site, which may involve regions of the extracellular loop adjacent to TM1 and TM2. The $P2X_{1-7}$ receptors show 30–50% sequence identity at the peptide level [14, 30–32]. The stoichiometry of $P2X_{1-7}$ receptors is thought to involve three subunits, which form a stretched trimer or a hexamer of conjoined trimers [14, 33].

The pharmacology of the recombinant P2X receptor subtypes expressed in oocytes or other cell types is often different from the pharmacology of P2X-mediated responses in naturally occurring sites [34]. Several contributing factors may account for these differences. First, heteromultimers as well as homomultimers are involved in forming the trimer ion pore [33]. For example, heteromultimers are clearly established for $P2X_{2/3}$ receptors. $P2X_{1/2}$, $P2X_{1/5}$, $P2X_{2/6}$, $P2X_{4/6}$ and more recently $P2X_{1/4}$ heteromultimers have also been identified (see later). $P2X_7$ does not form heteromultimers, and $P2X_6$ will not form a functional homomultimer. Second, spliced variants of P2X receptor subtypes might play a part. For example, a splice variant of $P2X_4$ receptor, while it is non-functional on its own, can potentiate the actions of ATP through the full-length $P2X_4$ receptors [35].

The P2X subunit proteins are 384 ($P2X_4$) to 595 ($P2X_7$) amino acids long [32]. Each has two hydrophobic regions. All the P2X receptor subunits have

consensus sequences for *N*-linked glycosylation. The $P2X_7$ subunit has a much longer COOH terminus than the other subunits, and this contains an additional hydrophobic domain (residues 510–530).

There are seven genes for P2X receptor subunits. $P2X_4$ and $P2X_7$ subunit genes are located close to the tip of the long arm of chromosome 12 [14]. $P2X_4$ and $P2X_7$ subunits are among the most closely related pairs in amino acid sequences. $P2X_1$ and $P2X_5$ genes are also very close together on the short arm of chromosome 13. The remaining genes are on different chromosomes. The genes vary considerably in size (e.g., $mP2X_3$: 40 kb; $hP2X_6$: 12 kb). The full-length forms have 11–13 exons, and all share a common structure, with well-conserved intron/exon boundaries. Many spliced forms of the receptor subunits (or fragments) have been described. Several full-length non-mammalian vertebrate sequences have been identified. There are no reports of homologous sequences from invertebrate species, although there is considerable functional evidence that extracellular ATP and other nucleotides can directly gate ion channels in invertebrates [31–32].

Recent advances have been made by the preparation of knockout mice for $P2X_1$, $P2X_2$, $P2X_3$, $P2X_4$ and $P2X_7$ receptors, and transgenic mice that overexpress the $P2X_1$ receptor.

P2X receptor subtypes

$P2X_1$ receptors

A cDNA encoding the $P2X_1$ receptor was isolated by direct expression in *Xenopus* oocytes, beginning with a cDNA library made from rat vas deferens [11]. Human and mouse cDNAs have also been cloned and expressed. The homomeric $P2X_1$ receptor is a cation-selective channel that shows little selectivity for sodium over potassium. It has a relatively high permeability to calcium.

A major property of the $P2X_1$ receptor is the mimicry of the agonist actions of ATP by α,β-methylene ATP (α,β-meATP), which distinguishes $P2X_1$ and $P2X_3$ receptors from the other homomeric forms. 2′,3′-O-(benzoyl-4-benzoyl)-ATP (BzATP) is also an effective agonist. $P2X_1$ receptors are blocked by suramin and pyridoxal-phosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS), but there are newer antagonists that are more $P2X_1$-selective (see Table 1). A valuable antagonist at $P2X_1$ receptors is 2′,3′-*O*-(2,4,6-trinitrophenyl)-ATP (TNP-ATP), which has an IC_{50} (mean inhibitory concentration) of about 1 nM.

Desensitization means the decline in the current elicited by ATP during the continued presence of ATP [14, 31]. In some P2X receptors this decline occurs in milliseconds (fast desensitization: $P2X_1$, $P2X_3$), and in others it occurs 100–1,000 times more slowly (slow desensitization: $P2X_2$, $P2X_4$). Recovery from desensitization is extremely slow. Treatment with apyrase allows $P2X_1$ receptors to recover from desensitization. Adenoviral expression of a $P2X_1$ receptor-green fluorescent protein construct in vas deferens shows the receptor to be localized in clusters, with larger ones apposing nerve varicosities [36].

$P2X_2$ receptors

The rat $P2X_2$ receptor cDNA was isolated from a library constructed from nerve growth factor-differentiated PC12 cells by testing pools for functional expression in *Xenopus* oocytes [10]. The human receptor cDNA was amplified from pituitary gland [37]. There are no agonists currently known that are selective for $P2X_2$ receptors. However, $P2X_2$ receptors are potentiated by protons and by low concentrations of zinc and copper. There are no selective antagonists for $P2X_2$ receptors. The $P2X_2$ receptor is generally described as non-desensitizing, compared with the $P2X_1$ and $P2X_3$ receptors.

When oocytes are injected with RNAs encoding $P2X_2$ receptors, and also the α3- and β4-subunits of nicotinic receptors, they show responses to both ATP and acetylcholine; these can be selectively antagonized with appropriate receptor blockers [30, 32]. However, with concomitant application of both agonists, the resultant current is less than the expected sum of the two independent currents. Such occlusion of the currents indicates an interaction between the two receptors.

Heteromeric $P2X_{1/2}$ receptors

$P2X_1$ and $P2X_2$ receptor subunits have been coexpressed in defolliculated *Xenopus* oocytes and the resultant receptors studied under voltage clamp conditions [38–40]. Coexpression yielded a mixed population of homomeric and heteromeric receptors with a subpopulation of novel pH-sensitive P2X receptors showing identifiably unique properties that indicate the formation of heteromeric $P2X_{1/2}$ ion channels. It has been claimed that trimeric $P2X_{1/2}$ receptors incorporate one $P2X_1$ and two $P2X_2$ subunits.

$P2X_3$ receptors

$P2X_3$ receptor subunit cDNAs were isolated from rat dorsal root ganglion cDNA libraries [41, 42], and later from a human heart cDNA library [43] and from a zebrafish library [44]. The mimicry of ATP by α,β-meATP makes these receptors similar to $P2X_1$ and distinct from the other homomeric forms. 2-Methylthio ATP is as potent as or more potent than ATP at $P2X_3$ receptors. Diadenosine pentaphosphate is a full agonist. The antagonists suramin, PPADS and TNP-ATP do not readily distinguish between $P2X_1$ and

$P2X_3$ receptors, but NF023 is about 20 times less effective at $P2X_3$ than $P2X_1$ receptors. $P2X_3$ receptors are prominently expressed on nociceptive sensory neurons [45] (see Table 1).

Heteromeric $P2X_{2/3}$ receptors

Direct association between $P2X_2$ and $P2X_3$ receptor subunits has been shown by coimmunoprecipitation [42, 46–50]. $P2X_{2/3}$ heteromeric channels can be defined on the basis of a sustained current elicited by α,β-meATP. However, they share some properties with homomeric $P2X_2$ receptors; they are potentiated by low pH, and they do not desensitize within the time course of a few seconds. The $P2X_{2/3}$ heteromer shares with the homomeric $P2X_3$ receptor the high sensitivity to block by TNP-ATP, as well as PPADS and suramin. Diinosine pentaphosphate is a much more potent blocker at $P2X_1$ and $P2X_3$ homomers than at the $P2X_{2/3}$ heteromer and is therefore useful for distinguishing between $P2X_3$ and $P2X_{2/3}$ receptors [51]. $P2X_{2/3}$ receptors have been identified in subpopulations of sensory neurons, sympathetic ganglion cells and brain neurons.

$P2X_4$ receptors

cDNAs for the rat $P2X_4$ receptor were isolated independently from superior cervical ganglion (SCG) and brain [52–54]. Human, mouse, chick cDNA and *Xenopus* cDNAs have also been isolated. Homomeric $P2X_4$ receptors are activated by ATP but not by α,β-meATP. The most useful distinguishing feature of ATP-evoked currents at $P2X_4$ receptors is their potentiation by ivermectin. Cibacron blue and zinc also potentiate currents at the $P2X_4$ receptor.

When the application of ATP is of short duration, $P2X_4$ receptors operate as cation-selective channels; the calcium permeability is relatively high. When the application of ATP is continued for several seconds, the $P2X_4$ receptor channel becomes increasingly permeable to larger organic cations such as N-methyl-D-glucamine (NMDG). Desensitization at $P2X_4$ receptors is intermediate between that observed at $P2X_1$ and $P2X_2$.

The rat $P2X_4$ receptor is unusual among the P2X receptors in its relative insensitivity to blockade by the conventional antagonists suramin and PPADS. Currents evoked by ATP at the mouse $P2X_4$ receptor are actually increased by PPADS and suramin, probably because of their ectonucleotidase inhibitory activity [45] (see Table 1).

Heteromeric $P2X_{1/4}$ receptors

Co-injection of $P2X_1$ and $P2X_4$ subunits into *Xenopus* oocytes showed that both subunits were present in trimeric complexes of the same size [55]. Voltage clamp experiments revealed functional P2X receptors with kinetic properties resembling homomeric $P2X_4$ receptors and a pharmacological profile similar to homomeric $P2X_1$ receptors. Preliminary results show that the $P2X_1$ receptor from the vas deferens and the $P2X_4$ receptor from salivary gland form complexes of the same size as the recombinant trimeric complexes expressed in oocytes.

$P2X_5$ receptors

The $P2X_5$ receptor cDNA was first isolated from cDNA libraries constructed from rat coeliac ganglion and heart [45, 56]. A $P2X_5$ receptor was also cloned from embryonic chick skeletal muscle [57], and a bullfrog $P2X_5$ receptor has been isolated from larval skin. The only human cDNAs reported are missing exon 10 ($hP2X_{5a}$) or exons 3 and 10 ($P2X_{5b}$) [30, 32]. A feature of the currents elicited by ATP in cells expressing the rat $P2X_5$ receptor is their small amplitude, compared with the currents observed with $P2X_1$, $P2X_2$, $P2X_3$ or $P2X_4$ receptors expressed under similar conditions. The currents otherwise resemble those seen at $P2X_2$ receptors: they show little desensitization, are not activated by α,β-meATP and are blocked by suramin and PPADS at concentrations similar to those effective at $P2X_2$ receptors. $P2X_5$ messenger RNA (mRNA) is highly expressed in developing skeletal muscle [45] (see Table 1).

Heteromeric $P2X_{1/5}$ receptors

$P2X_1$ and $P2X_5$ subunits can be coimmunoprecipitated, and the defining phenotype of this heteromer is a sustained current evoked by α,β-meATP, which is not seen for either of the homomers when expressed separately [36, 45, 58–60]. Cells expressing the heteromeric receptor are more sensitive to ATP than those with homomeric receptors; concentrations as low as 3 or 10 nM evoke measurable currents. The sensitivity to the antagonist TNP-ATP is intermediate between the sensitive homomeric $P2X_1$ receptor and the insensitive homomeric $P2X_5$ receptor.

$P2X_6$ receptors

The rat $P2X_6$ receptor was cloned from SCG cDNA [36, 45] and from rat brain [61]. The human equivalent was isolated from peripheral lymphocytes as a p53-inducible gene. This was originally designated P2XM to reflect its abundance in human and mouse skeletal muscle. The $P2X_6$ receptor appears to be a 'silent' subunit, in the sense that no currents are evoked by ATP when it is expressed as a homomultimer in oocytes or HEK293 cells. It appears that the $P2X_6$ subunit is only functionally expressed as a heteromultimer.

Table 1. Characteristics of purine-mediated receptors[a] (modified and reproduced from [45], with permission from Elsevier).

Receptor		Main distribution	Agonists*	Antagonists	Transduction mechanisms
P1 (adenosine)	A_1	brain, spinal cord, testis, heart, autonomic nerve terminals	CCPA, CPA, S-ENBA, CVT-510	DPCPX, N-0840, MRS1754, N-0840, WRC-0571	$G_{i/o}$ ↓cAMP
	A_{2A}	brain, heart, lungs, spleen	CGS 21680, HE-NECA, CVT-3146	KF17837, SCH58261, ZM241385, KW 6002	G_S ↑cAMP
	A_{2B}	large intestine, bladder	NECA (non-selective)	enprofylline, MRE2029-F20, MRS17541, MRS 1706	G_S ↑cAMP
	A_3	lung, liver, brain, testis, heart	IB-MECA, 2-Cl-IB-MECA, DBXRM, VT160	MRS1220, L-268605, MRS1191, MRS1523, VUF8504	$G_{i/o}$ $G_{q/11}$ ↓cAMP ↑IP_3
P2X	$P2X_1$	smooth muscle, platelets, cerebellum, dorsal horn spinal neurons	α,β-meATP = ATP = 2-MeSATP, L-β,γ-meATP (rapid desensitisation),	TNP-ATP, IP_5I, NF023, NF449	intrinsic cation channel (Ca^{2+} and Na^+)
	$P2X_2$	smooth muscle, CNS, retina, chromaffin cells, autonomic and sensory ganglia	ATP≥ATPγS≥2-MeSATP>>α,β-meATP (pH + zinc-sensitive)	suramin, isoPPADS, RB2, NF770, NF279	intrinsic ion channel (particularly Ca^{2+})
	$P2X_3$	sensory neurones, NTS, some sympathetic neurons	2-MeSATP≥ATP≥α,β-meATP≥Ap_4A (rapid desensitisation)	TNP-ATP, PPADS, A317491, NF110, Ip_5I, phenol red	intrinsic cation channel
	$P2X_4$	CNS, testis, colon	ATP>>α,β-meATP, CTP, Ivermectin potentiation	TNP-ATP (weak), BBG (weak), phenolphthalein	intrinsic ion channel (especially Ca^{2+})
	$P2X_5$	proliferating cells in skin, gut, bladder, thymus, spinal cord	ATP>>α,β-meATP, ATPγS	suramin, PPADS, BBG	intrinsic ion channel
	$P2X_6$	CNS, motor neurons in spinal cord	(does not function as homomultimer)	–	intrinsic ion channel
	$P2X_7$	apoptotic cells in, for example, immune cells, pancreas, skin	BzATP>ATP≥2-MeSATP>>α,β-meATP	KN62, KN04, MRS2427, O-ATP Coomassie brilliant blue G	intrinsic cation channel and a large pore with prolonged activation
P2Y	$P2Y_1$	epithelial and endothelial cells, platelets, immune cells, osteoclasts	2-MeSADP= ADPβS>2-MeSATP = ADP>ATP, MRS2365	MRS2179, MRS2500, MRS2279, PIT	G_q/G_{11}; PLC-β activation
	$P2Y_2$	immune cells, epithelial and endothelial cells, kidney tubules, osteoblasts	UTP=ATP, UTPγS, INS 37217, INS 365	suramin > RB2, AR-C126313	G_q/G_{11} and possibly G_i; PLC-β activation
	$P2Y_4$	endothelial cells	UTP≥ATP, UTPγS, INS 37217	RB2 > suramin	G_q/G_{11} and possibly G_i; PLC-β activation
	$P2Y_6$	some epithelial cells, placenta, T cells, thymus	UDP>UTP>>ATP, UDPβS, IDP	MRS2578	G_q/G_{11}; PLC-β activation
	$P2Y_{11}$	spleen, intestine, granulocytes	AR-C67085MX>BzATP≥ATPγS>ATP	suramin > RB2, NF157, 5′-AMPS	G_q/G_{11} and G_S; PLC-β activation
	$P2Y_{12}$	platelets, glial cells	2-MeSADP≥ADP>>ATP	CT50547, AR-C69931MX, INS49266, AZD6140, PSB0413, ARL66096, 2-MeSAMP	$G_{i/o}$; inhibition of adenylate cyclase
	$P2Y_{13}$	spleen, brain, lymph nodes, bone marrow	ADP=2-MeSADP>>ATP=2-MeSATP	MRS2211, 2-MeSAMP	$G_{i/o}$
	$P2Y_{14}$	placenta, adipose tissue, stomach, intestine, discrete brain regions	UDP glucose = UDP-galactose	–	G_q/G_{11}

*Abbreviations: BBG, Brilliant Blue Green; BzATP, 2'-&3'-*O*-(4-benzoyl-benzoyl)-ATP; cAMP, cyclic AMP; CCPA, chlorocyclopentyl adenosine; CPA, cyclopentyl adenosine; CTP, cytosine triphosphate; IP_3, inosine triphosphate; Ip_5I, di-inosine pentaphosphate; 2-MeSADP, 2-methylthio ADP; 2-MeSATP, 2-methylthio ATP; NECA, 5'-*N*-ethylcarboxamido adenosine; PLC, phospholipase C; RB2, reactive blue 2

Heteromeric $P2X_{2/6}$ receptors

$P2X_2$ and $P2X_6$ receptors have been found to coimmunoprecipitate after expression in HEK293 cells [46]. Oocytes expressing this combination have subtly different responses to ATP than oocytes expressing only $P2X_2$ receptors [62]. The most convincing of these differences is the fact that at pH 6.5 the inhibition of the current by suramin is clearly biphasic; one component has the high sensitivity of homomeric $P2X_2$ receptors, whereas the other component is less sensitive. $P2X_{2/6}$ receptors are prominently expressed by respiratory neurons in the brain stem.

Heteromeric $P2X_{4/6}$ receptors

$P2X_4$ and $P2X_6$ receptors form a heteromeric channel when coexpressed in oocytes [63]. The subunits can be coimmunoprecipitated from oocytes and HEK293 cells. The principal functional evidence for coexpression is that currents elicited by ATP are larger in oocytes 5 days after injection of mRNAs for $P2X_4$ and $P2X_6$ than after injection of $P2X_4$ alone. However, the phenotype of the heteromer differs only in minor respects from that of $P2X_4$ homomers. $P2X_{4/6}$ receptors are prominent in adult trigeminal mesencephalic nucleus and in hippocampal CA1 neurons [14].

$P2X_7$ receptors

A chimeric cDNA encoding the rat $P2X_7$ receptor was first constructed from overlapping fragments isolated from SCG and medial habenula; full-length cDNAs were subsequently isolated from a rat brain cDNA library [64]. Human [65] and mouse [66] cDNAs were cloned from monocyte and microglial cells, respectively. The main feature of the $P2X_7$ receptor is that in addition to the usual rapid opening of the cation-selective ion channel, with prolonged exposure to high concentrations of ATP it becomes permeable to larger cations (eg NMDG) and later undergoes a channel to pore conversion to allow the passage of large dye molecules such as ethidium and YO-PRO-1. This often leads to cell death. Evidence for $P2X_7$ receptor activation includes inward currents and increase in $[Ca^{2+}]_i$; other end points involve uptake of YO-PRO-1 or similar fluorescent dyes which bind to nucleic acid and structural changes in the cell such as membrane blebbing.

BzATP is a potent agonist at the $P2X_7$ receptor. There are five main types of blockers (see Table 1): ions (calcium, magnesium, zinc, copper and protons), the suramin analog NF279, Brilliant Blue G, which is most effective at rat $P2X_7$ receptors, oxidized ATP and KN-62 which is selective for the human $P2X_7$ receptor.

ATP or BzATP induces remarkable changes in the appearance of HEK293 cells transfected with the rat $P2X_7$ receptor. After continuous application of BzATP (30 μM) for about 30 s, the plasma membrane begins to develop large blebs, and after 1 or 2 min, these become multiple and sometimes coalesce. The time to the appearance of the first bleb can be delayed by removal of extracellular sodium. Blebs are usually preceded by the appearance of smaller vesicles (<1 μm diameter), which are shed from the cell and appear to release inflammatory cytokines.

P2Y receptors

Molecular structure

The first P2Y receptors were cloned in 1993 [8, 9]. Since then several other subtypes have been isolated by homology cloning and assigned a subscript on the basis of cloning chronology ($P2Y_4$, $P2Y_6$, $P2Y_{11}$). The long-awaited G_i-coupled ADP receptor ($P2Y_{12}$) of platelets was finally isolated by expression cloning in 2001 [67], while $P2Y_{13}$ and $P2Y_{14}$ receptors were characterized later during a systematic study of orphan receptors [68, 69]. At present, there are eight accepted human P2Y receptors: $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$ and $P2Y_{14}$ [15, 70] (see Table 1). The missing numbers represent either non-mammalian orthologs, or receptors having some sequence homology to P2Y receptors, but for which there is no functional evidence of responsiveness to nucleotides. In particular p2y3 may be a chicken ortholog of $P2Y_6$ [71], while p2y8 and tp2y could be the *Xenopus* and turkey orthologs of $P2Y_4$, respectively. p2y7 is a leukotriene B_4 receptor [72]. p2y5 and p2y10 are considered orphan receptors. A p2y8 receptor was cloned from the frog embryo, which appears to be involved in the development of the neural plate [73]. p2y9 was reported to be a novel receptor for lysophosphatidic acid, distant from the Edg family [74]. $P2Y_{15}$ was recently introduced to designate the orphan receptor GPR80/GPR99 on the basis that it would be a receptor for AMP [75]. But it is now firmly established that it is actually a receptor for α-ketoglutarate, as underlined in a report by the IUPHAR P2Y Subcommittee [76].

In contrast to P2X receptors, P2Y receptor genes do not contain introns in the coding sequence, except for the $P2Y_{11}$ receptor. Site-directed mutagenesis of the $P2Y_1$ and $P2Y_2$ receptors has shown that some positively charged residues in TM3, 6 and 7 are crucial for receptor activation by nucleotides [77].

From a phylogenetic and structural (i.e., protein sequence) point of view, two distinct P2Y receptor subgroups characterised by a relatively high level of sequence divergence have been identified [15]. The first subgroup includes $P2Y_{1,2,4,6,11}$ and the second subgroup encompasses the $P2Y_{12,13,14}$ subtypes (see dendrogram in Fig. 1).

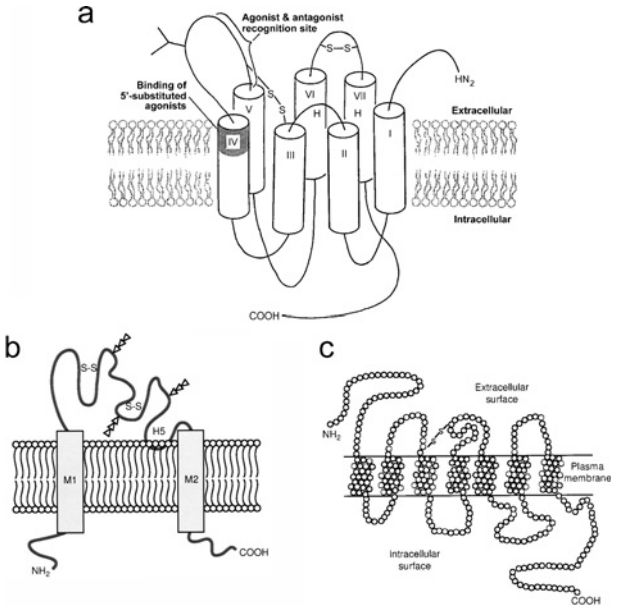

Figure 1. Membrane receptors for extracellular ATP and adenosine. The P1 family of receptors for extracellular adenosine are G protein-coupled receptors that signal by inhibiting or activating adenylate cyclase (*a*). The P2 family of receptors bind extracellular ATP or ADP, and comprise two types of receptors (P2X and P2Y). The P2X family of receptors are ligand-gated ion channels (*b*), and the P2Y family are G protein-coupled receptors (*c*). a from [13] reproduced with permission from the American Society for Pharmacology and Experimental Therapeutics; b, from [10] reproduced with permission from Nature; c modified from [125] and reproduced with permission from Elsevier Science.

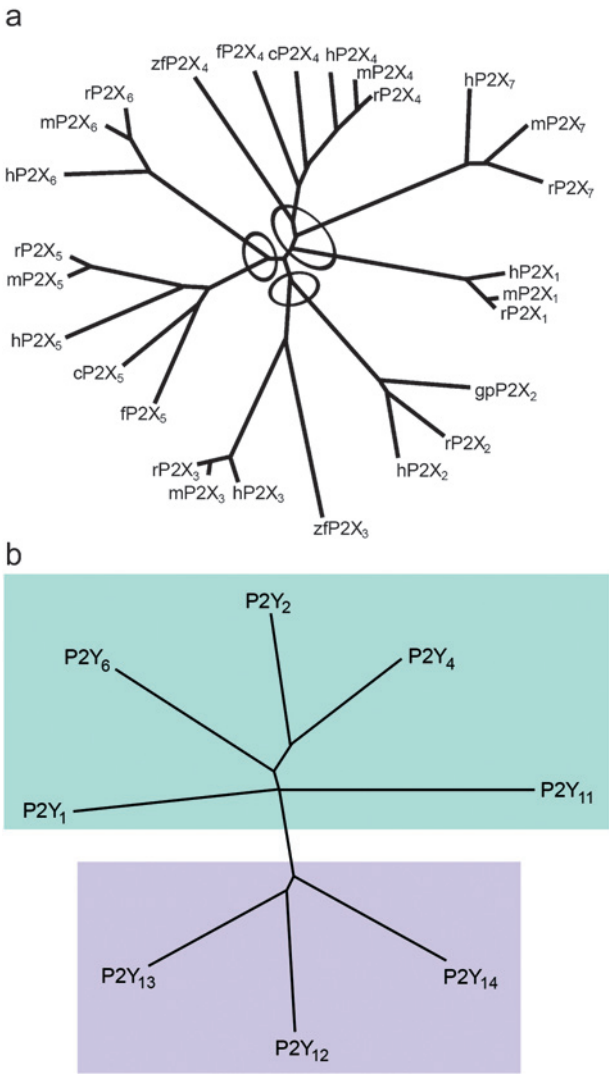

Figure 2. (*a*) Dendrogram to show relatedness of 29 P2X receptor subunits. Full-length amino acid sequences were aligned with Clustal W using default parameters. The dendrogram was constructed with TreeView. h, Human (*Homo sapiens*); r, rat (*Rattus norvegicus*); m, mouse (*Mus musculus*); gp, guinea pig (*Cavia porcellus*); c, chicken (*Gallus gallus*); zf, zebrafish (*Danio rerio*); bf, bullfrog (*Rana catesbeiana*); x, claw-toed frog (*Xenopus laevis*); f, fugu (*Takifugu rubripes*). The ellipses indicate the apparent clustering by relatedness into subfamilies. (Reproduced from [14], with permission from the American Physiological Society.) (*b*) A phylogenetic tree (dendrogram) showing the relationships among the current members of the P2Y receptor family (human $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$ and $P2Y_{13}$ receptors) and the human UDP-glucose receptor (here indicated as the $P2Y_{14}$ receptor). The P2Y receptors can be divided into two subgroups shown with green and lilac backgrounds. Sequences were aligned using CLUSTALX, and the tree was built using the TreeView software. (Reproduced from [70], with permission from Elsevier.

Many of the P2Y receptor subtypes are still lacking potent and selective synthetic agonists and antagonists (see Table 1). However, ADPβS is a potent agonist of $P2Y_1$, $P2Y_{12}$ and $P2Y_{13}$ receptors. 2-Alkylthio ethers appear to provide high potency at $P2Y_1$ and $P2Y_2$ subtypes when bonded to a variety of alkyl or alkylaryl groups. Notably, 2-methylthio ADP (2-MeSADP) is a potent agonist (EC_{50} – mean effective concentration – in nM) at $P2Y_1$, $P2Y_{12}$ and $P2Y_{13}$ receptors. P2Y receptor-mediated responses occur in non-neuronal and non-muscular cell types, as well as in the nervous system, involved in both short- and long-term signalling [16].

Second messenger systems and ion channels

$P2Y_1$, $P2Y_2$, $P2Y_4$ and $P2Y_6$ receptors couple to G proteins to increase inositol triphosphate (IP_3) and cytosolic calcium. Activation of the $P2Y_{11}$ receptor by ATP leads to a rise in both cAMP and in IP_3, whereas activation by UTP produces calcium mobilization without IP_3 or cAMP increase. The $P2Y_{13}$ receptor can simultaneously couple to G_{16}, G_i and, at high concentrations of ADP, to G_s. The activation of several P2Y receptors is commonly associated with stimulation of several mitogen-activated protein kinases (MAPKs), in particular extracellular signal-regulated protein kinase 1/2 [15].

In recent years, G protein-coupled receptors in neurons and other excitable cells have been found to modulate the activity of voltage-gated ion channels in the cell

membrane through certain actions of activated G proteins. Such actions are now well-established in closing (or in certain cases opening or potentiating) various classes of K^+ channels [78] and voltage-gated Ca^{2+} channels [79]. There have been several demonstrations of ion channel responses upon activation of native P2Y receptors in brain neurons [80, 81]. For example ATP (or UTP, or their products ADP or UDP) present at synapses, plus ATP diffusing from astrocytes, activates P2Y receptors on distinct subsets of brain neurons, regulating their activities by the coupling of those receptors to specific ion channels. While ion channel couplings of P2Y receptors are primarily of importance in neurons, they have in a few cases been detected also in various other tissues, e.g., in cardiac muscle cells [82]. Among the channels with which the SCG cell membrane is well endowed are two types of voltage-gated channels, which are important in receptor-based regulation of neuronal activity, the Ca^{2+} channel of the N-type and the M-current K^+ channel [83].

P2Y receptor subtypes

$P2Y_1$ receptors

Human, rat, mouse, cow, chick, turkey and *Xenopus* $P2Y_1$ receptors have been cloned and characterised. In most species, ADP is a more potent agonist than ATP and their 2-methylthio derivatives are even more potent; UTP, UDP, CTP and GTP are inactive [84]. At present, the most potent and selective agonist known is the *N*-methanocarba analog of 2-MeSADP, MRS2365 (EC_{50} of 0.4 nM) [85]. The most effective antagonists to display selectivity for the $P2Y_1$ receptor are MRS2179, MRS2279 and MRS2500 (see Table 1). Site-directed mutagenesis studies on the human $P2Y_1$ receptor have shown that amino acid residues in TM3, 6 and 7 are critical determinants in the binding of ATP [86]. Four cysteine residues in the extracellular loops, which are conserved in P2Y receptors, are essential for proper trafficking of the human $P2Y_1$ receptor to the cell surface [87]. $P2Y_1$ mRNA was highest in various regions of the human brain, prostate gland and placenta, and was also detected at varying levels in other organs [16, 88]. In addition, in post-mortem brain sections from sufferers of Alzheimer's disease the $P2Y_1$-like immunoreactivity in the hippocampus and entorhinal cortex was localised to neurofibrillary tangles, neuritic plaques and neuropil threads [89].

$P2Y_1$ receptor knockout mice have been generated [90, 91]. These mice are viable, with no apparent abnormalities affecting their development, survival and reproduction. Platelet counts are normal, but shape change is abolished. Transgenic mice overexpressing the $P2Y_1$ receptor specifically in the megakaryocytic/platelet lineage have also been generated using the promoter of the tissue-specific platelet factor 4 gene [92]. This led to a phenotype of platelet hyper-reactivity *in vitro*.

$P2Y_2$ receptors

$P2Y_2$ receptors have been cloned and pharmacologically characterised from human, rat, mouse, canine and porcine cells or tissues [93]. $P2Y_2$ receptors are fully activated by equivalent concentrations of ATP and UTP, whereas ADP and UDP are much less effective agonists. The γ-thiophosphate, UTPγS, has been shown to be a potent hydrolysis-resistant agonist of $P2Y_2$ receptors, as is the recently developed $P2Y_2$ receptor agonist INS 37217 (Up_4dC) [15]. Suramin acts as a weak competitive antagonist of human and rat $P2Y_2$ receptors. AR-C126313 and the related aminotetrazole derivative AR-C118925, flavanoids and tangeretin have been claimed recently to be effective antagonists. $P2Y_2$ receptors can directly couple to $PLC\beta_1$ via $G\alpha_{q/11}$ protein to mediate the production of IP_3 and diacylglycerol, second messengers for calcium release from intracellular stores and protein kinase C (PKC) activation, respectively.

Expression of $P2Y_2$ receptor mRNA has been detected in many tissues [16]. $P2Y_2$ receptor activation increases the synthesis and/or release of arachidonic acid, prostaglandins and nitric oxide [94, 95]. $P2Y_2$ receptor expression in smooth muscle cells is upregulated by agents that mediate inflammation, including interleukin-1β, interferon-γ and tumor necrosis factor-α [96], and $P2Y_2$ receptor upregulation has been shown to promote nucleotide-induced activation of PKC, cyclooxygenase and MAPK [97]. $P2Y_2$ receptor activation increases Cl^- secretion and inhibits Na^+ absorption in epithelial cells [98]. A $P2Y_2$ receptor knockout mouse has been produced that is defective in nucleotide-stimulated ion secretion in airway epithelial cells [99]. $P2Y_2$ receptors have been shown to inhibit bone formation by osteoblasts [100] and N-type calcium currents in neurons [101].

$P2Y_4$ receptors

Human, rat and mouse $P2Y_4$ receptors have been cloned and characterized. UTP is the most potent activator of the recombinant human $P2Y_4$ receptor [102]. GTP and ITP are about 10 times less potent than UTP. In contrast, the recombinant rat and mouse $P2Y_4$ receptors are activated equipotently by ATP and UTP [103]. Up_4U (INS365) and dCp_4U (INS37217) are agonists of the human $P2Y_4$ receptor. Reactive Blue 2 at a concentration of 100 μM effectively blocks rat $P2Y_4$ receptors, but only partially blocks human $P2Y_4$ receptors. Suramin is a weak antagonist at the $P2Y_4$ receptor. The structural determinants of agonism versus antagonism by ATP

are located in the N-terminal domain and the second extracellular loop.

In human and mouse, $P2Y_4$ mRNA and protein was most abundant in the intestine, but was also detected in other organs [16]. $P2Y_4$-null mice have apparently normal behaviour, growth and reproduction, but the chloride secretory response of the jejunal epithelium to apical UTP and ATP is abolished [104].

$P2Y_6$ receptors

The mouse, rat and human $P2Y_6$ receptors are selective for UDP [105]. UDPβS and Up_3U are selective agonists of the $P2Y_6$ receptor and more stable to degradation [106]. INS48823 is also a potent $P2Y_6$ agonist. A 1,4-di-(phenylthioureido) butane derivative (MRS2578) has been shown to selectively inhibit UDP-induced phospholipase C activity through both human and rat $P2Y_6$ receptors. A unique feature of the $P2Y_6$ receptor is its slow desensitization and internalization. A wide tissue distribution of $P2Y_6$ mRNA and protein has been demonstrated, with the highest expression in spleen, intestine, liver, brain and pituitary [16].

$P2Y_{11}$ receptors

Among P2Y receptors, the human $P2Y_{11}$ has a unique profile [15]. It is the only P2Y receptor gene that contains an intron in the coding sequence. The potency of its natural agonist ATP is relatively low, and it is dually coupled to PLC and adenylyl cyclase upon stimulation. ATPγS is a more potent agonist than ATP. The $P2Y_{12}$ antagonist AR-C67085MX acts as a potent agonist at the $P2Y_{11}$ receptor. Suramin behaves as a competitive antagonist of the $hP2Y_{11}$ receptor [107]. The $hP2Y_{11}$ gene differs from other P2Y genes by the presence in the coding sequence of a 1.9 Kb intron that separates an exon encoding the first six amino acid residues from a second exon encoding the remaining part of the protein [108].

$P2Y_{12}$ receptors

The human, rat and mouse $P2Y_{12}$ receptors have been identified and characterised [15]. ADP is the natural agonist of this receptor. There is also direct transduction by the $P2Y_{12}$ receptor in neurons through the N-type Ca^{2+} channel. The $P2Y_{12}$ receptor is heavily expressed in the megakaryocyte/platelet lineage, where it is the molecular target of the active metabolite of the antiplatelet drug clopidogrel [109]. Potent direct competitive $P2Y_{12}$ antagonists also exist, including the 5′-triphosphate derivative AR-C69931MX compound, named cangrelor. The $P2Y_{12}$ receptor has also been shown to be expressed in sub-regions of the brain, glial cells, brain capillary endothelial cells, smooth muscle cells and chromaffin cells [16]. $P2Y_{12}$ knockout mice have been generated which display the phenotype of clopidogrel-treated animals [110, 111], i.e., prolonged bleeding time, inhibition of platelet aggregation to ADP and resistance to arterial thrombosis in various models.

$P2Y_{13}$ receptors

The human, mouse and rat $P2Y_{13}$ receptors have been identified and characterised [15]. ADP and Ap_3A are naturally occurring agonists of the $P2Y_{13}$ receptor. The $P2Y_{13}$ receptor is primarily coupled to a $G_{i/o}$ protein. Cangrelor, which was previously believed to be a selective antagonist of the $hP2Y_{12}$ receptor, is also an antagonist of the human and rat $P2Y_{13}$ receptors. Recently, MRS2211, a derivative of PPADS, was shown to selectively antagonise the human $P2Y_{13}$ receptor. The $P2Y_{13}$ receptor is strongly expressed in the spleen, followed by placenta, liver, heart, bone marrow, monocytes, T cells, lung and various brain regions [112]. $P2Y_{13}$-null mice have been generated recently, but no phenotype has been characterised to date.

$P2Y_{14}$ receptors

From a phylogenetic and structural point of view, the $P2Y_{14}$ receptor (previously known as GPR105 or UDP-glucose receptor) is 47 % identical to the $P2Y_{12}$ and $P2Y_{13}$ receptors. The gene for this receptor has been found in human chromosome 3q24–3q25, where a cluster of other related G protein-coupled receptors, consisting of $P2Y_1$, $P2Y_{12}$, $P2Y_{13}$ receptors and the orphan receptors GPR87, GPR91 and H963, have been found [70]. The $P2Y_{14}$ receptor couples to the $G_{i/o}$ family of G proteins and is activated by UDP-glucose as well as UDP-galactose, UDP-glucuronic acid and UDP-*N*-acetylglucosamine but not by uridine or adenine nucleotides [113]. At present, no selective antagonists are available. $P2Y_{14}$ mRNA is widely distributed in the human body. Both chemoattractant and neuroimmune functions have been claimed for the $P2Y_{14}$ receptor.

Receptor dimerisation and cross-talk

It is now recognised that interactions between G protein-coupled receptors can take place through the formation of oligomers, or downstream of the receptor through the action of second messengers. The former process is commonly referred to as receptor dimerisation. The latter process is known as receptor cross-talk.

There is evidence that the human $P2Y_1$ receptor forms homodimers [114, 115]. An example of dimerisation involving P2Y receptors with non-P2Y receptors is rat $P2Y_1$ and adenosine A_1 receptors coexpressed in HEK293 cells [116]. It has also been shown that the

$P2Y_1$ and A_1 receptors are co-localised in neurons of the rat cortex, hippocampus and cerebellum [117]. The formation of oligomers by P2Y receptors is likely to be widespread and to greatly increase the diversity of purinergic signalling. P2X receptors are often expressed in the same cells as P2Y receptors. Thus, there is the possibility of bidirectional cross-talk between these two families of nucleotide-sensitive receptors [118, 119]. For example, the $P2X_1$ receptor may have a priming role in activation of $P2Y_1$ receptors during platelet stimulation.

Gene activation regulated by P2Y receptors

There is a small amount of definitive information on gene transcription control by identified G_q- or $G_{i/o}$-linked P2Y receptors. For example, when stably expressed in 1321N1 cells, the $P2Y_2$ receptor was found to signal through the p38 MAPK cascade to phosphorylate the cAMP response element-binding transcription factor, which then mediated cis-activation of target genes, including the anti-apoptotic bcl-2 and bcl-xl genes [120]. UTP incubation also upregulated expression of a range of genes for neurotrophins and neuropeptides and induced proliferation of the astrocytoma cells. The possibility that ATP, released as a cotransmitter, is involved in regulation of gene transcription has been explored using the neuromuscular junction (NMJ) of skeletal muscles [121, 122]. Functional post-synaptic $P2Y_1$ and $P2Y_2$ receptors, co-localised at the NMJs with the nicotinic ACh receptors (AChRs), have been demonstrated in mammalian, chicken and amphibian muscles. Exposure to 2-MeSADP or to UTP each produces an activation of the genes of the multiple subunits of the AChR and also of the acetylcholinesterase catalytic subunit gene. There is a total block of the $P2Y_1$-coupled action at the gene promoters by the specific $P2Y_1$ antagonist MRS 2179.

Concluding comments

Receptors to purines and pyrimidines, including heteromultimers as well as homomultimers, are remarkably rich; up to 25 receptors have been currently identified, with the possibility that further P2Y receptor subtypes may still be discovered. This wide diversity of receptor subtypes may reflect the primitive nature of this signalling system [123].

Many cells express multiple P1 and P2 receptor subtypes, but the mechanisms underlying the interactions of the physiological events mediated by these receptor subtypes needs resolution [124]. For example, there is evidence that some receptors mediate short-term signalling, while others mediate long-term (trophic) signalling. Some receptors only appear to be activated in pathological conditions, while other receptors respond differently to low and high concentrations of endogenous agonists. There is increasing interest in P1 and P2 receptor dimerisation and cross-talk between nucleoside and nucleotide receptors and receptors to other signalling systems.

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