Eur J Med Chem (1997) 32, 183–193

Invited review

P2 purine and pyrimidine receptors: emerging superfamilies of G-protein-coupled and ligand-gated ion channel receptors

SS Bhagwat*, M Williams

Neurological and Urological Diseases Research, Pharmaceutical Products Division, Abbott Laboratories, Abbott Park, IL 60064-3500, USA

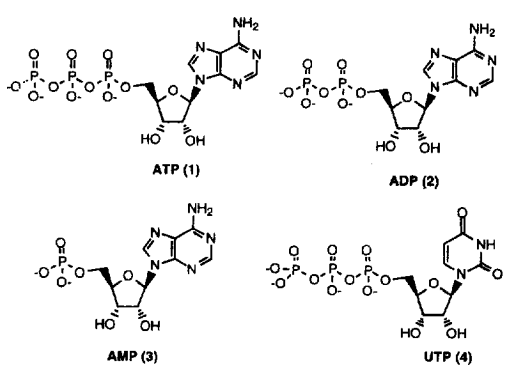
(Received 26 December 1996)

Summary — Against a background of considerable skepticism and limited efforts in medicinal chemistry, the pivotal role of adenosine triphosphate (ATP) and related nucleotides as neurotransmitters and/or neuromodulators has been firmly established in the past decade. At least 15 receptor subtypes have been cloned, seven from the ligand gated ion channel (LGIC) P2X superfamily and eight from the G-protein-coupled receptor (GPCR) P2Y superfamily. All agonist pharmacophores for the P2 receptor superfamily with the exception of some recently described theobromine analogs are modified forms of the natural ligands ATP, adenosine diphosphate (ADP) or uridine triphosphate (UTP). On the other hand, antagonist ligands represent a diverse group of compounds. These include: the 2'- and 3'-phosphate analogs of ATP, eg, A3P5PS; the antitrypanosomal agent, suramin, the suramin analogs, XAMR0721, NF023 and BSt101; PPADS (pyridoxal phosphate-6-azaphenyl-2',4'-disulfonic acid); DIDS (4,4'-diisothiocyanotostilbene-2,2'-disulfonate) and a series of isothiocyanato sulfonates related to DIDS that includes β-INS; and a series of chromophores that include, reactive red 2, trypan blue, Cibachrome blue, Evans blue and the desmethyl derivative of Evans blue, NH01. Such agents demonstrate varying degrees of potency and selectivity, in general limiting their usefulness in characterizing P2 receptor function especially at the in vivo level. With an increasing number of potential receptor targets being identified by cloning techniques, the use of modern medicinal chemistry technologies (eg, combinatorial chemistry, computer-assisted molecular design) together with the high throughput screening of compound libraries and natural product sources offers a considerable opportunity to advance the therapeutic potential for P2 purinoceptor targeted molecules in the immediate future. In addition, the identification of allosteric modulators of P2 receptor function like PIT (2,2'-pyridylisatogen tosylate) and *d*-tubocurarine potentially provides a more subtle approach to modulation of receptor function that is comparable to the effects (and therapeutic promise) of benzodiazepines in modulating GABA-A receptor function.

P2 receptor / purine receptor / pyrimidine receptor

Introduction

The potential role(s) of adenosine triphosphate (ATP, **1**) and the related nucleotides adenosine diphosphate (ADP, **2**), adenosine monophosphate (AMP, **3**) as well as the pyrimidine, uridine triphosphate (UTP, **4**) as neuroeffector agents has gained increased recognition over the past decade [1–8]. The neurotransmitter role of ATP has been clearly delineated from its role as an intracellular building block and cellular energy source [1, 6, 9] as the nucleotide has been shown to modulate CNS, immune and systemic function [6, 8]. ATP and related nucleotides act via a family of discrete cell surface receptors, the P2 receptor superfamily, 15 of which have been cloned as of the time of writing (tables I and II; [10–12]. Another 12 or so are rumored to reside as expressed sequence tags (ESTs) in various normal and diseased human genomic databases. ATP and UTP can function as trophic factors, alone or in combination with other pleiotropic agents like basic fibroblast growth factor (bFGF) to alter DNA synthesis, cell differentiation and influence apoptotic processes [13].



*Correspondence and reprints

Table I. P2X receptor classification based on functional cloning.

Receptor	*Tissue of origin, GenBank Accession number*	*Pharmacological profile (agonist based)*	*Transduction system(s)*
$P2X_1$	Rat vas deferens, *X80477*	2-MeSATP ≥ ATP > α,β meATP	$I_{Na/K/Ca}$
	Urinary bladder, human *X 83688*	ATP > α,β meATP	
	Mouse, *X84896*		$I_{Na/K/Ca}$
$P2X_2$	Rat PC12 cell, *U14414*	2-MeSATP > ATP, α,β meATP inactive	$I_{Na/K/Ca}$
$P2X_{2-1}$	Rat cochlea, short form, *L43511*		
$P2X_3$	Rat dorsal root ganglion, *X90651/X91167*	2-MeSATP > ATP > α,β meATP	$I_{Na/K}$
$P2X_4$	Rat hippocampus, *X91200*	ATP > 2-MeSATP > α,β meATP	$I_{Na/K}$
	Rat DRG, *X87763*	ATP > 2-MeSATP > α,β meATP	
	Rat brain, *U32497*		
	Rat/human brain, *X93565*		
	Rat endocrine tissue, *U47031*		
$P2X_5$	Rat celiac ganglia, *X92069*	ATP > 2-MeSATP > ADP	
$P2X_6$	Rat brain, rat cervical ganglion, *X92070*	ATP > 2-MeSATP > ADP	
$P2X_7$	Mouse macrophage, *X95882*	BzATP > ATP > UTP	$I_{Na/K}$ or I_{ca}

I = LGIC current; Bz ATP = 2'- and 3'-*O*-(4-benzoylbenzoyl) ATP.

A potential physiological role for ATP was first described in the 1920s [14] although the now seminal 1929 paper of Drury and Szent-Gyorgyi [15] examining the effects of purines on cardiac function is more widely cited. It was not until the 1950s, however, that a neurotransmitter role for the nucleotide was described in the context of the release of ATP following antidromic nerve stimulation [16]. ATP was subsequently identified as a potential candidate for non-adrenergic, non-cholinergic (NANC) transmission processes in the autonomic nervous sytem [17] leading, in 1972, to Burnstock's now seminal, yet at that time, highly controversial, proposal of the concept of purinergic transmission [18]. Based on additional physiological and pharmacological data, two families of purinergic receptor were proposed [19], the P1, activated by adenosine (ADO), that was subsequently shown to consist of four distinct receptor subtypes termed A_1, A_{2A}, A_{2B} and A_3 [20, 9], and the P2 superfamily, activated by ATP and related nucleotides, that is currently comprised of at least 15 subtypes [12].

Phylogenetically, ATP is an effector agent in a variety of lower organisms that include amoebae, sea anemone, leech, snail, marine molluscs, starfish, elasmobranchs and teleosts [21] while its hydrolysis product, ADO, has been described as a melanin-dispersing agent in catfish and guppys [22].

Current interest in ATP as a neurotransmitter has been driven to a major extent by the cloning of the various members of the P2 receptor superfamily [12] although an extensive body of pharmacological and physiological data amassed in the past 20 years has been pivotal in enabling the identification and characterization of these clones [1, 23].

In addition to a neurotransmitter role mediated via P2 receptor activation, ATP, when hydrolysed by synaptic ectonucleotidases, results in the formation of ADP, AMP and ADO, molecules that have discrete receptor activity in their own right [24]. In addition, there is considerable potential for crosstalk between the various purinergic receptors. ATP antagonizes the effects of ADP on platelet aggregation [25] and also modulates ADO interactions at the A_1 receptor [26, 27]. An additional facet of the neurotransmitter role of ATP is that its release, as well as degradation and resynthesis (the latter potentially in cells different

Table II. P2Y receptor classification based on functional cloning.

Receptor	*Tissue of origin, GenBank Accession number*	*Pharmacological profile (agonist based)*	*Transduction system(s)*
$P2Y_1$	Chick brain, *X73268*	2-MeSATP > ATP > ADP, UTP inactive	$PLCb/IP_3/Ca^{2+}$
		2-MeSATP > ATP > ADP, UTP inactive	
	Turkey brain, *U09842*	2-MeSATP ≥ 2Cl-ATP ≥ ATP, α,β meATP inactive	$PLCb/IP_3/Ca^{2+}$
	Mouse insulinoma cell, *U22829*	2-MeSATP > ATP >> UTP	
	Rat insulinoma cell, *U22830*		$PLCb/IP_3/Ca^{2+}$
	Human placenta, *Z49205*		
	Bovine endothelium, *X87628*		$PLCb/IP_3/Ca^{2+}$
	Human HEL cell, U42029		
	Human brain		
	Human prostate and ovary		
$P2Y_2$	Mouse NG-108 -1 5, *L14751*	ATP = UTP >> 2-MeSATP	$PLCb/IP_3/Ca^{2+}$
	Human CT/43 cells, *U07225*	ATP = UTP >> 2-MeSATP	
	Rat lung, *U09402*	ATP = UTP	$PLCb/IP_3/Ca^{2+}$
	Rat pituitary, L46865		$PLCb/IP_3/Ca^{2+}$
	Wistar Kyoto rat, U56839		
$P2Y_3$	Chick brain, X98283	ADP > UTP > ATP = UDP	$PLCb/IP_3/Ca^{2+}$
$P2Y_4$	Human placenta, X91852	UTP = UDP > ATP = ADP	$PLCb/IP_3/Ca^{2+}$
	Rat brain, *X91852*		
$P2Y_5$	Human activated T cells, *P32250/ L06109*	ATP > ADP > 2-MeSATP >> UTP, α,β meATP	
$P2Y_6$	Aortic smooth muscle, D63665	UDP > 5-Br-UTP > UTP > ADP > 2-MeSATP > ATP	$PLCb/IP_3/Ca^{2+}$
	Human placenta, X97058		
$P2Y_7$	HEL cells, *U41070*	ATP > ADP = UTP	
XL-P2Y	Xenopus neural plate	ATP = UTP = ITP = CTP = GTP	$PLCb/IP_3/Ca^{2+}$

PLC = phospholipase C; IP_3 = inositol-1,4,5 trisphosphate.

from that from which ATP is released) may contribute to changes in the cellular 'energy charge' [28] providing yet another potential nuance to the messenger role of ATP.

ATP is a short-lived mediator of fast excitatory neurotransmission acting via P2X receptors [29, 30] while the hydrolysis product, ADO, is a potent and selective inhibitor of excitatory transmitter release via the activation of presynaptic ADO A_1 receptors [31]. The extent to which these various receptor-mediated mechanisms are physiologically functional depends on both the intrinsic ectonucleotidase activity [32] and the array of purinergic receptors and cell types present in and around a given tissue or organ. For instance, in inflammatory conditions, the recruitment of macrophages and leukocytes provides an additional source of purinergic receptors usually not seen under normal conditions [33]. Furthermore, recent data have shown that P2 receptors can be dynamically regulated. In leukocytes, proinflammatory cytokines like γ-interferon down regulate $P2X_1$ and $P2Y_2$ receptors and can upregulate the $P2X_7$ receptor [34].

ATP thus has a significant potential, both on its own, and as a source of other purine neuromodulators, to act as a multifunctional effector agent in a cascade-like manner to elicit effects that are both complex and potentially mutually antagonistic or synergistic.

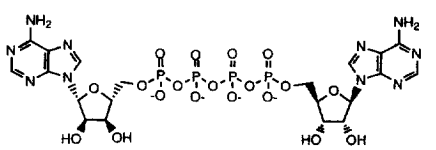
P2 receptor diversity

Nomenclature

Following from the classification of purinergic receptors into P1 and P2 families [19], additional, albeit limited, pharmacological studies using agonist ligands derived from ATP led to the description of P2X and P2Y [35] and P_{2T} and P_{2Z} receptors [36]. Up until 1994, various different descriptors were used to describe P2 receptors including P2, $P2_X$, P_{2X}, etc, with both capital and lower case letters used interchangeably to designate different receptors. In addition, letters were ascribed to newly pharmacologically defined receptors in a somewhat random manner such that the x, y and z receptors were followed by t, u, n and d [37]. The confusion surrounding P2 receptor nomenclature has been addressed by the International Union of Pharmacology (IUPHAR) Purinergic Receptor Nomenclature Committee [20, 38] resulting in a recommended nomenclature based on the division of P2 receptors into ligand gated ion channel (LGIC; P2X) and G-protein-coupled receptor (GPCR; P2Y) families, respectively (tables I and II; [39, 40]) and on structural relationships based on receptor cloning [12]. This nomenclature describes the two P2 receptor families as P2X and P2Y with the number of the actual receptor as a subscript, eg, $P2X_1$. Receptors that have not been cloned are represented in italics, eg, $P2_D$, $P2_T$, as recommended by the main IUPHAR Receptor Nomenclature Committee [41]. Newly cloned P2 receptors in each of the two families are numbered in the sequence in which they are identified, based on their structural relationships to other P2 receptors and, obviously, signal transduction pathways [12, 40]. Finally, with the identification and characterization of the $P2Y_2$ receptor which is equally sensitive to ATP and UTP [42], of UTP- and UDP-, but not ATP-sensitive P2 receptors [43–45] and the cloning of two P2 receptors, $P2Y_4$ and $P2Y_6$, that are uniquely UTP- and UDP-sensitive, respectively [46, 47], it is now clear that there are distinct pyrimidinergic receptors. Thus the term P2 'purinoceptor' is no longer to be used. Instead, the P2 designation is now used to describe both purinergic and pyrimidinergic receptors, eg, P2 receptor [38].

P2 receptors

Seven P2X receptors, $P2X_{1\text{-}7}$ have been cloned and eight P2Y receptors, $P2Y_{1\text{-}8}$ identified [12]. The $P2Y_2$ receptor, previously designated as P_{2U} or $P2_U$, as noted above is activated by both ATP and UTP but not by diphosphate nucleotides [42]. The $P2Y_4$ receptor is sensitive to UTP but only weakly activated by ATP, ADP or UDP and is unique in being the only P2 receptor identified to date that lacks an RGD integrin binding domain [48]. The $P2Y_6$ receptor is selectively activated by UDP [45]. The $P2X_1$ receptor was cloned from rat vas deferens [49] and PC12 cells [50]. This receptor is similar in sequence to *RP-2*, a gene expressed in apoptotic thymocytes that appears to play a role in programmed cell death [50]. RP-2 may code for a P2 receptor. The P_{2Z} receptor, an ATP-gated ion pore sensitive to 2'- and 3'-*O*-(4-benzoylbenzoyl) ATP [51] that is involved in macophage apoptosis [52] has been cloned as the $P2X_7$ receptor [53]. The P_{2D} or Ap4A receptor is a GPCR activated by diadenosine polyphosphates [54] like Ap4A **5**. This ATP dimer has been described as an 'alarmone' [55], a molecule mediating cellular responses to stress via effects on gene transcription and translation. *Ap4A* binding sites have been identified in the nervous and cardiovascular systems [54, 56]. However, Ap4A is equipotent with ATP at the $P2Y_2$ receptor [57] and can also block platelet aggregation (P_{2T} receptor [58]) indicating that this nucleotide is not selective for a single class of P2 receptor. The existence of a distinct Ap4A (or P_{2D}) receptor is thus controversial and the receptor has yet to be cloned. The P_{2T} receptor that is present on platelets has also to be cloned.



Ap4A (5)

While all members of the P2Y receptor family are related in structure to other 7-transmembrane (7TM) GPCRs [59], the generalized structure of P2X receptor subunits is very different from that of other LGICs, consisting of two transmembrane-spanning regions [11]. This structural motif is related to both the epithelial amiloride-sensitive sodium channel (ENaC) and the FMRFamide-gated sodium channel (FNaC) found in *Helix aspersa* [60]. ENaCs are related to the *Caenorhabditis elegans* genes *deg-1*, *mec-4* and *mec-10* that code for the degenerins [61]. The expression of the various P2X cDNAs varies from tissue to tissue with some tissues expressing one subunit and others several types of P2X cDNA. Functional P2X receptors in mammalian tissues can thus exist as both homo- and hetero-multimers [11] with their pharmacological diversity and function being defined on the basis of their consituent subunits. P2X receptors may potentially exist as pentamers

[62]. As noted by Buell et al [8], the physiology/pharmacology of native P2X receptors has yet to be related to the cDNAs identified to date and other receptor subunits may also exist. Nonetheless, it appears likely that the P2X receptor family may evidence a complexity similar to that seen with central benzodiazepine [63], glutamate [64] and nicotinic ion channels [65] where selective ligands have been crucial in defining receptor function and where the precise molecular basis of receptor function is still in the process of evolving.

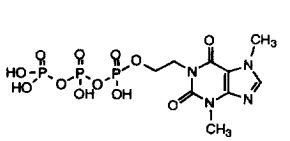
P2 receptor function to date has been structurally inferred or based on tissue localization. For instance, the localization of the $P2X_3$ receptor in the rat DRG and its presence in small nociceptive neurons using anti-peripherin staining [66] supports a role for ATP in pain perception [67, 68]. Similarly, the presence of the novel $P2X_{2s}$ receptor in the cochlea [69] may indicate a role in hearing or vestibular function, specifically, balance.

P2 receptor chemistry

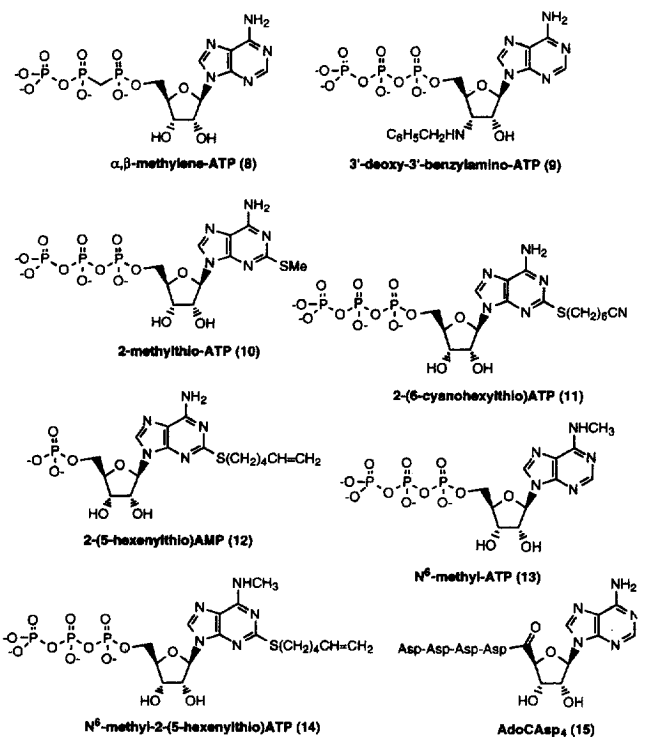
Medicinal chemistry efforts targeted at developing novel pharmacophores for the P2 receptor family have been limited [70], with the major work originating from the laboratories of Cusack [71] and Jacobson [72, 73].

Agonists

With the exception of a novel series of recently described theobromine analogs [74] that includes **6**, all known agonist ligands for the P2 receptor family are essentially variations on the parent purine (or pyrimidine) nucleotide pharmacophore [6, 70, 75]. SAR efforts over the past 20 years have predominately focused on increasing the stability of the polyphosphate side-chain to prevent ectonucloetidase actions using various bioisostere strategies. More recently, however, synthetic work has focused on extrapolating structure–activity relationships from the the P1 receptor [76] to the P2 area. However, the agonist activity of these newer nucleotide analogs can be confused with the ability of compounds like ARL 67156 **7**, that inhibit ectonucleotidase activity [77] and thus can alter the rank order agonist potency of ATP analogs as well as that of endogenous ATP [32]. A major issue in determining and comparing the activity of the various P2 receptor ligands is the wide variety of tissue test systems and varying experimental protocols that have been used to examine the effects of P2 receptor ligands. In many instances, the selectivity and efficacy of a compound differs depending on: a) the tissue system used; b) the experimental protocol; and c) the source and age of the compound, thus providing a confusing picture for the development of the required structure–activity relationships for the various receptors. This is further compounded by the polypharmic activities of the few P2 antagonist ligands identified to date. The rigorous evaluation of the biological properties of these molecules has shown that these compounds have the potential to confuse the recognition requirements of the receptor with its associated signal transduction elements (see below).



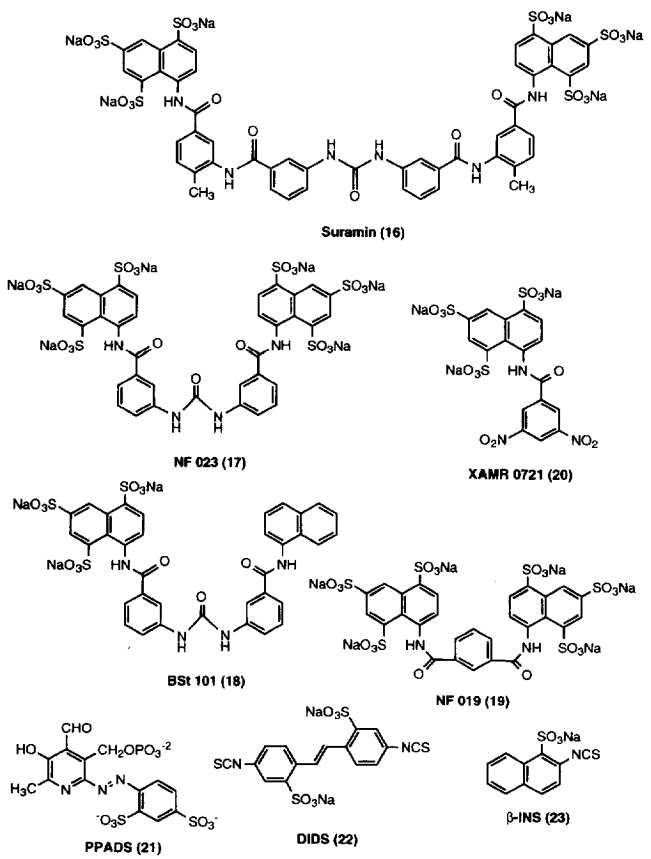
Bioisoteric substitution of the phosphoester bridging oxygens with methylene or dihalomethylene groups or the introduction of a terminal thio group increases ligand stability [70]. α,β-meATP **8** is a P2X receptor agonist at all receptors except the $P2X_2$. 3'-Deoxy-3'-benzylamino ATP **9** is also a potent P2X agonist. Alkylthio chain substitution at the 2-thio position of adenine nucleotides enhances P2Y receptor activity as in 2-methylthio ATP (2-MeSATP, **10**) and the 2-(6-cyanohexyl) analog **11**. While AMP is



inactive at P2Y receptors, 2-thioether AMP analogs such as 2-hexylthio-AMP, **12**, are 16 000 times more potent than ATP at turkey erythrocyte P2Y receptors [78]. This retention of affinity appears to result from the addition of a long chain at a site distal to the triphosphate group that reduces nucleotidase activity allowing the 2-thioether to potentially act as a secondary anchor for binding [70]. These analogs are inactive at P2X receptors. N^6-Modification can increase P2Y selectivity. N^6-Methyl ATP **13** is equipotent with ATP at *Iaenia coli* P2Y receptors and inactive at both vascular P2Y and smooth muscle P2X receptors. The N^6-methyl-2-(5-hexenylthio) analog of ATP **14** is active at P2Y receptors but inactive at P2X receptors. Replacement of the labile phosphate groups in ATP with peptide residues like aspartate, glutamate and γ-carboxyglutamate results in compounds like AdoCAsp4, **15**, which is a weak agonist at C6 glioma cell P2 receptors [79].

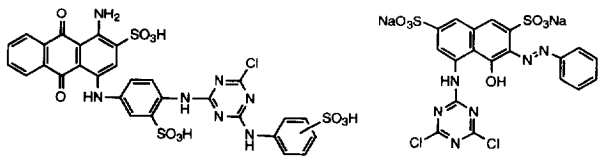
Antagonists

Suramin **16**, a polysulfonated aromatic antitrypanocidal drug, is a competitive P2 receptor antagonist with a K_d of 4 μM in rat vas deferens [80]. However, the compound also interacts with bFGF and NMDA receptors, ectonucleotidases, various protein kinases and proteases, and G-protein subunits [81–83]. Simplification of the structure of suramin has resulted in NF023 (**17**), BSt101(**18**), NF 019 (**19**) and XAMR0721 (**20**). Characterization of these compounds at the rat vas deferens P2 receptor [80] has shown that NF023 and BSt101 retain antagonist activity (K_d = 1 – 5 μM) while further reductions in size lead to a loss of receptor activity. Although XAMR 0721 is a somewhat weaker entity (K_d = 515 μM), it has greater selectivity in that it lacks ectonucleotidase inhibitory activity. PPADS (pyridoxalphosphate-6-azaphenyl-2',4'-disulfonic acid, **21**) is another widely used P2 receptor antagonist. The compound is approximately ten times more selective for P2X than for P2Y receptors [10] but has been recently reported as a non-specific antagonist of IP_3 channels [84]. While DIDS (4,4'-diisothiocyanotostilbene-2,2'-disulfonate, **22**) is a P2 receptor antagonist with a K_d in the rat vas deferens of 3 μM [85], it is also an inhibitor of anion transport and ectonucleotidase activity [86]. β-INS **23** is a DIDS analog with greater P2X receptor activity and reduced ectonucleotidase inhibitory activitiy [85]. An SAR analysis of DIDS indicates that increasing the size of the isothiocynate (eg, naphthalene versus benzene ring) increased the potency as in the case of the suramin analogs. The position of the isothiocynate did not appear to affect the P2 antagonist activity within the series.



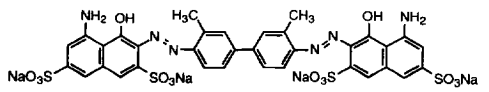
A series of dyes including Cibachron blue 3GA (**24**) (also known as reactive blue 2), reactive red 2 (**25**), trypan blue (**26**), Evans blue (**27**) and brilliant blue (**28**) represent other compounds that have been used as putative P2 receptor antagonists [1, 10]. Many of these dyes are impure with considerable batch to batch variability and data generated on their activity as P2 receptor anatgonists must be interpreted with caution. For instance, Cibachron blue 3GA can also antagonise responses to GABA and glutamate [87]. NH01 (**29**) is a desmethyl derivative of Evans blue that is one of the more potent P2X receptor antagonists yet described (K_d = 800 nM; [88]). A3P5PS (**30**) is a recently described competitive antagonist of the $P2Y_1$ receptor [89]. ARL 66096 (**31**) and ARL 67085 (**32**) are ATP biososteres that are potent and selective antagonists of the P_{2T} receptor [90, 91]. Efforts to modify UTP to obtain more stable analogs have yet to result in reports of novel compounds [92].

In general, as more is learned regarding the various classes of P2 receptor antagonist identified to date, it would appear that many have the potential to interact with P2 receptor signal transduction mechanisms. Thus rather than being selective receptor antagonists, they may be functional antagonists of the receptor activation process. The identification of novel phar-

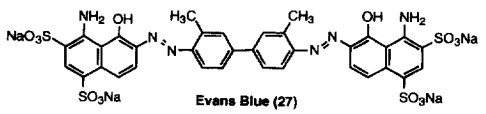


Cibachron Blue 3GA (24) (Reactive Blue-2)

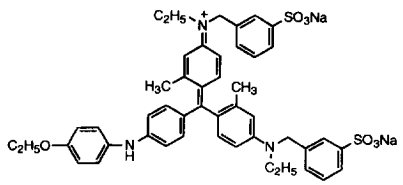
Reactive Red-2 (25)



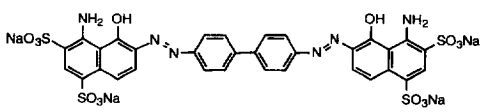
Trypan Blue (26)



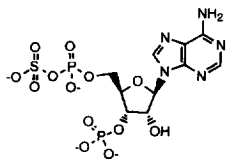
Evans Blue (27)



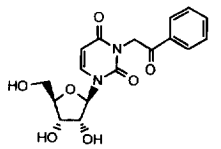
Brilliant Blue G (28)

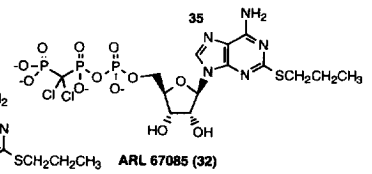


NH 01 (29)

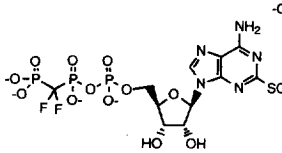


A3P5PS (30)

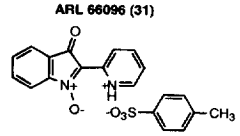




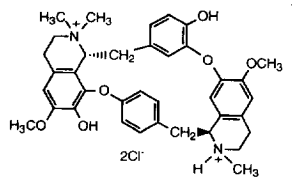
ARL 67085 (32)



ARL 66096 (31)



2,2'-Pyridylisatogen tosylate (PIT, 33)



d-tubocurarine chloride (34)

macophores that are truely selective for the various P2 receptors that can be used to unambiguously characterize the various members of the receptor superfamily is absolutely critical in advancing the field.

Allosteric modulators

Allosteric binding sites on NMDA, $GABA_A$/benzodiazepine (BZ) and nicotinic LGICs are well known, and in the case of the $GABA_A$/BZ channel complex have resulted in important therapeutic agents with unusual efficacy and safety profiles as compared to directly acting agents. Thus BZs like clonazepam and diazepam are safe and effective anxiolytics acting by modulating $GABA_A$ receptor effects on chloride conduction. In contrast, directly acting GABA agonists with a good safety profile have yet to be identified. Allosteric modulators of P2 receptor function include PIT (2,2'-pyridylisatogen, **33**), originally described as a direct P2 receptor antagonist [93] that enhances $P2Y_1$ receptor responses [94] and *d*-tubocurarine (**34**) which increases radioligand binding to $P2X_4$ receptors [95].

P2 receptor characterization

The search for novel pharmacophores for the P2 receptor superfamily and their subsequent characterization is highly dependent on the ability to utilize high throughput binding and functional assays. To date, only agonist radioligands have been identified for P2 receptors, that have the potential to label binding sites for ATP in tissue in a highly non-specific manner. For instance, cell lines lacking functional P2 responses can bind the currently available agonist ligands with high affinity. Transfection of cells that are null for a given P2 receptor with human P2 receptor cDNA adds little in the way of additional specific radioligand binding (MF Jarvis et al, unpublished data) suggesting that the current ligands have the potential to bind to a multitude of cellular proteins that recognize ATP. This severely limits data interpretation [10, 96] making expressed human P2 receptors using functional reporter systems critical to dissecting out ligand function. [^{3}H] α, β meATP and [^{35}S]ATPγS have been used as ligands for P2X receptors and [^{32}P]BzATP and [^{35}S]dATPγS for P2Y receptors [1, 3, 10]. [^{35}S]ATPγS can label recombinant $P2X_1$ and $P2X_2$ receptors [97]. In contrast, while [^{35}S]dATPγS has been used to label P2Y receptors [98], the ligand does not bind to cells expressing a functional $P2Y_3$ receptor [99]. The usefulness of these radioligands is further compounded by their potential lability [10]. Improved radioligand binding assays are critical to the use of high throughput screening to identify novel, non-purine, non-nucleotide pharmacophores using chemical and combinatorial library compound sources [100]. In their absence, ligands interacting with P2 receptors can be assessed using functional assay systems like the fluo-

rescence imaging plate reader (FLIPR) using cell lines transiently or stably transfected with human cDNA expressing the various classes of P2 receptor.

P2Y receptors, like other GPCRs, are coupled via G proteins to phospholipase C activation, adenylate cyclase modulation and Ca^{2+} translocation processes [3, 10]. P2 receptor activation can also result in the inhibition of guanylate cyclase and nitric oxide synthase (NOS) activity [1, 3]. P2X receptors can modulate cell function by gating Na^+, K^+ and Ca^{2+} permeability [3, 10]. UTP-sensitive P2 receptors modulate Ca^{2+} influx via a protein kinase C mechanism [101] and can also stimulate Ca^{2+} release from internal stores [102]. In the renal epithial cell line, MDCK, P2 receptor-mediated phospholipase A2 activation results in arachidonic acid release [103].

Pyrimidinergic transmission?

The recognition of UTP and UDP as distinct neuromodulators acting via their own P2 receptors, the $P2Y_4$ and $P2Y_6$ subtypes, raises the possible existence of pyrimidine nucleoside receptors analogous in structural requirements to the P1 receptor family. Uridine effects on cell systems have been described [104] and a series of uridine analogs of **35** have been claimed as hypnotic, anxiolytic, muscle relaxant and antiepileptic agents [105]. At high concentrations, uridine has been reported to have effects on dopaminergic function in the CNS [106, 107]. Efforts to date to identify [^{3}H]uridine binding sites in mammalian brain have been unsuccessful (MF Jarvis, unpublished data).

Therapeutic applications of P2 receptor ligands

Cystic fibrosis

ATP and UTP can promote airway defense mechanisms in cysticfibrosis (CF) by stimulating mucin secretion, activating chloride channel function and increasing ciliary beat frequency [108, 109]. UTP is currently in phase II clinical trials as a treatment for CF, the pyrimidine nucleotide being preferred to ATP to avoid the bronchoconstrictor and cardiac depressant effects of the ADO formed from the hydrolysis of ATP. UTP alone, or in combination with the diuretic, amiloride, restores normal function to the peripheral airways of the lung in CF patients.

Cancer

ATP is an effective and long-lasting inhibitor of human tumor cell growth, an effect that appears to involve the permeabilization of the tumor cell membrane via the opening of Ca^{2+} and Na^+ channels [110]. These effects of ATP are broad in scope and include cytostatic and cytotoxic effects, anticachexic actions, modulation of tumor blood flow and enhancement of superoxide production. The duration of the cytotoxic effects of ATP appears to involve the ability of the nucleotide to faciliate its own localized release [110, 111] and may be mechanistically related to $P2X_7$ receptor-mediated apoptosis [52]. In vitro, ATP can transform leukemia cells into white blood cells and has been proposed as an adjunct therapy to retinoic acid in the treatment of breast and prostate cancer [112]. Exercise-related reductions in tumor volume are associated with increases in tumor energy charge [113]. A phase II trial of ATP for the treatment of small cell lung cancer showed increased weight gain, improved performance status and quality of life [114].

Trophic and apoptotic effects

P_2 receptor agonists can promote astrocyte maturation and the proliferation of rat mesengial cells [13]. ATP can act synergistically with bFGF to enhance mitotic processes, activating MAP kinase to modulate early response gene expression (eg, *c-jun*, *c-fos*, AP-1, etc) and cell growth, differentiation and stress responses [13]. The apoptotic role of ATP has been most clearly defined for the macrophage P_{2Z}/P2X receptor [52] and is involved in thymocyte phagocytosis [115]. The structural homology between the $P2X_2$ receptor and the thymocyte apoptotic RP-2 receptor [44] further supports a critical role for P2 receptors in regulating events related to programmed cell death.

Pain

ATP is a physiological mediator of nociceptive processing [116, 117]. However, the selective destruction of the rat DRG $P2X_3$ receptor by the nociceptive compound, capcasin [66] suggests a pivotal role for ATP in pain processing. Antagonists selective for this P2 receptor thus may represent novel, non-opioid analgesic agents [67, 68]. As with other systems, there is considerable potential for ATP interactions with ADO in modulating pain processing mechanisms. Depending on the route of administration, ADO can elicit pain responses [118] or attenuate the actions of other nociceptive transmitters [119]. Preliminary studies in human, have shown that ADO is a potent and long-acting analgesic agent [120, 121] effects that may be extended to the potential treatment of migraine [122].

Anesthesia

ATP has been used as an adjunct to inhalation anesthetics [123] reducing the doses required for the latter

to produce their effects. This has the potential to provide an improved safety margin, decreasing the risks associated with overdosage and toxicity and improving post-surgical recovery time. In over 1000 patients undergoing facial surgery, ATP elicits controlled hypotension, decreases the potential for surgical bleeding, induces analgesia without respiratory depression and blunts autonomic responses. These effects of ATP are quite remarkable although it is presently unclear as to whether the ADO formed from ATP is the mediator of the actions ascribed to ATP as ADO has been reported to have similar actions in the surgical setting [124].

Diabetes

Both P1 and P2 receptors can modulate islet cell insulin secretion from pancreatic β cells [125]. ATP stimulates insulin secretion by activating a $P2Y_1$ receptor and can potentiate the effects of tolbutamide. ADO has biphasic actions on insulin secretion and can also modulate glucagon release. Intracellular levels of ATP and ADP link blood glucose concentrations to insulin release via interactions with pancreatic ATP-sensitive K^+ channels (K_{ATP}) comprising a small inward rectifier K^+ channel (Kir 6.0) and the sulfonylurea receptor (SUR; 126). When glucose concentrations are low, ADP levels increase relative to those of ATP and activate pancreatic K_{ATP} channels leading to β cell hyperpolarization and a decrease in insulin release. This action of ADP occurs via binding to SUR [126]. At levels that induce insulin release, glucose can also increase Ap4A levels in β cells by 30–70-fold resulting in an Ap4A-mediated inhibition of K_{ATP} channel activity [127].

Septic shock

The involvement of purinergic mechanisms in modulating inflammatory responses is an area undergoing exponential growth [33]. 2-MeSATP can block endotoxin-stimulated TNF-α and interleukin-1 (IL-1) release in a mouse model of septic shock [128] and prevents endotoxin lethality. 2-MeSATP can also attenuate LPS-induced expression of inducible macrophage NOS [129]. The therapeutic utility of these responses given the complexity of the septic shock response remains to be determined. A neutrophil P_{2U}-like receptor is involved in chemotaxis and it has been suggested that ATP and UTP are potential inflammatory mediators [130].

Thrombosis

The ATP bioisostere, ARL 67085 (**32**) is an ADP receptor (P_{2T}) antagonist [91] that is 30 000-fold selectivity for the P_{2T} receptor. It has antithrombotic activity in dog with minimal cardiovascular liability. The compound was reportedly safer than fibrinogen receptor antagonists and superior in efficacy to aspirin [91]. Ap4A analogs are also effective anti-platelet agents [58].

Miscellaneous

The involvement of P2 receptor-mediated mechanisms in lymphocyte cell adhesion [131] via L-selectin shedding [132], chondrocyte and osteoblast function [133, 134], the latter by the potentiation of PDGF effects [135], and in reproductive [136], auditory [69] and renal cell [137] function has also been described. ATP can also act as an autocrine regulator of cell volume [138]. While the fast transmitter role of ATP [29, 30] has yet to be linked to a distinct CNS function, the distribution of $P2X_4$ and $P2X_6$ receptors in brain tissue has been taken as evidence [8] that ATP-receptor-mediated events are more prevelant in the CNS. The potential role of ATP as a mediator in communication between the nervous and immune systems [8] is supported by the potential role of mast cell P2 receptors in the medial habenula may be involved in stress and neural-endocrine interactions [139].

Purinergic receptors and drug discovery

The identification of potent and selective P2 receptor agonists and antagonists as well as novel allosteric modulators of these receptors will aid in defining their physiological/pharmacological function and represents a critical element in both the understanding of P2 receptor function in disease processes and tissue injury and in the discovery of new therapeutic agents acting via these receptors.

While the concept of purinergic neurotransmission and neuromodulation has, after many years of controversy, become a major growth area of biomedical research especially in the areas of immune and nervous system function, it is of additional interest to consider ATP in the context of its role as a modulator of ATP-modulated potassium inward rectifier channel (Kir 6.0) function [140] and as a modulator of the ABC (ATP binding cassette, [141]) family of proteins. The complexity of the role of ATP (and potentially UTP) as a modulator of a wide variety of fundamental cell processes suggests that purines (and pyrimidines) are key elements in the processes of cellular homeostasis and tissue survival. The intriguing results related to the role of ATP as a modulator of tumor growth [110] that extend way beyond the apparent half life of this labile, energy-rich molecule, indicate that its effects on cell function are potentially unlike those of

more conventional neuroeffector agents. While this provides obvious opportunities for drug discovery, it also underlines the challenge to the medicinal chemist in terms of designing novel and selective modulators of the various members of the P2 receptor family. With the advent of the 21st century, it appears that ATP (cancer) and UTP (cystic fibrosis) have the potential to become drugs early in the next century for disease states where no effective treatments currently exist. With such utility for the endogenous agonists of the P2 receptor family, it is difficult not to envisage a promising future for other more novel compounds acting through P2 receptor mechanisms as important drugs for the future.

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