0022-3565/00/2953-0862$03.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
JPET 295:862–869, 2000

Vol. 295, No. 3
900036/864752
Printed in U.S.A.

Perspectives in Pharmacology

P2 Purinergic Receptors: Modulation of Cell Function and Therapeutic Potential[1]

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Accepted for publication August 24, 2000 This paper is available online at http://www.jpet.org

In addition to its key role in cellular metabolism where it acts as a ubiquitous enzyme cofactor and as the key source of the cellular energy unique to phosphate bond formation, the purine nucleotide ATP (Fig. 1) also functions as a potent extracellular messenger producing its effects via the activation of a family of distinct cell surface receptors, the P2 receptor family (Ralevic and Burnstock, 1998). The pyrimidine nucleotide UTP (Fig. 1) as well as the dinucleotides ADP and UDP also modulate cell function via P2 receptor activation. Thus ATP, UTP, ADP, and UDP play key roles in a diversity of tissue functions that include fast excitatory neurotransmission, developmental processing, pulmonary function, nociception, auditory and ocular function, the apoptotic cascade, astroglial cell function, metastasis formation, bone and cartilage disease, and platelet aggregation/hemostasis (Burnstock, 2000; Williams and Jarvis, 2000).

P2 receptors exist as two distinct families: the P2X ligand-gated ionotropic channel (LGIC) family that is involved in fast excitatory neurotransmission and the P2Y metabotropic, heptahelical G-protein coupled receptor (GPCR) family. P2X receptors respond more rapidly than P2Y receptors as they act via ion channels (Burnstock, 2000). The P2X subunit motif is 2-transmembrane and is related to the sodium-selective FNaC channel (FMRFamide-gated sodium channel). Unlike the latter, functional P2X receptors, which current evidence indicates are composed of P2X subunits as trimeric homomers and heteromers, do not discriminate in their cation permeability (North and Surprenant, 2000).

Eight members of the P2X receptor family with a number of splice variants and approximately 11 P2Y receptors have been cloned and expressed (Ralevic and Burnstock, 1998). Of these, seven P2X receptors ($P2X_{1–7}$) and six P2Y receptors ($P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, and $P2Y_{12}$) are molecularly distinct entities that can elicit functional responses (Table 1). The remainder are either species variants, lack a functional response, or, like the $P2Y_7$ receptor, have been misassigned to the P2 family. $P2Y_2$, $P2Y_4$, and $P2Y_6$ receptors are uracil nucleotide (pyrimidine)-sensitive. The ADP-sensitive P_{2T} receptor present on platelets that has been designated as the $P2Y_T$ receptor has been cloned as the $P2Y_{12}$ receptor.

Pharmacological characterization of P2 receptors has generally been based on their rank order of activation by agonists related to ATP and UTP since the majority of available P2 receptor antagonists are relatively weak and only marginally selective for one P2 receptor subtype over another. These antagonists also interact with other ATP recognition sites and with other receptor classes and signal transduction systems (Bhagwat and Williams, 1997).

Based on agonist efficacy and also desensitization characteristics, P2X receptors have been grouped into three distinct classes (Dubyak et al., 1996). Group 1 includes $P2X_1$ and $P2X_3$ receptors with high affinity for ATP (EC_{50} = 1 μM) that are rapidly activated and desensitized; group 2 includes $P2X_2$, $P2X_4$, $P2X_5$, and $P2X_6$ receptors that have lower affinity for ATP (EC_{50} = 10 μM) and show slow desensitization and sustained depolarizing currents; and group 3 is represented by the $P2X_7$ LGIC that has very low ATP affinity (EC_{50} = 300–400 μM), shows little or no desensitization, and in addition to functioning as an ATP-gated ion channel, can also function as a nonselective ion pore (Di Virgilio et al.,

Received for publication July 17, 2000.
[1] Space limitations have precluded citation of all the relevant primary literature for which the authors apologize to their colleagues.

ABBREVIATIONS: LGIC, ligand-gated ionotropic channel; E-NTPase, ectonucleoside triphosphate diphosphohydrolase; CNS, central nervous system; K_{ATP}, ATP-sensitive potassium channels; CamK-II, calcium-calmodulin dependent protein kinase-II; IP_5I, diinosine pentaphosphate; TNP-ATP, 2′(3′)-*O*-(2,4,6-trinitrophenyl)adenosine 5′-triphosphate; BzATP, 2′- and 3′-*O*-(4-benzoylbenzoyl)adenosine 5′-triphosphate; β,γ-MeATP, β,γ-methyleneadenosine 5′-triphosphate; DRG, dorsal root ganglia; GFAP, glial fibrillary acidic protein; UUI, urge urinary incontinence; FGF, fibroblast growth factor; MGC, multinucleated giant cell; IL, interleukin; Ap_4A, P^1,P^4-di(adenosine 5′)-tetraphosphate; PPADS, pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid; DIDS, 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid.

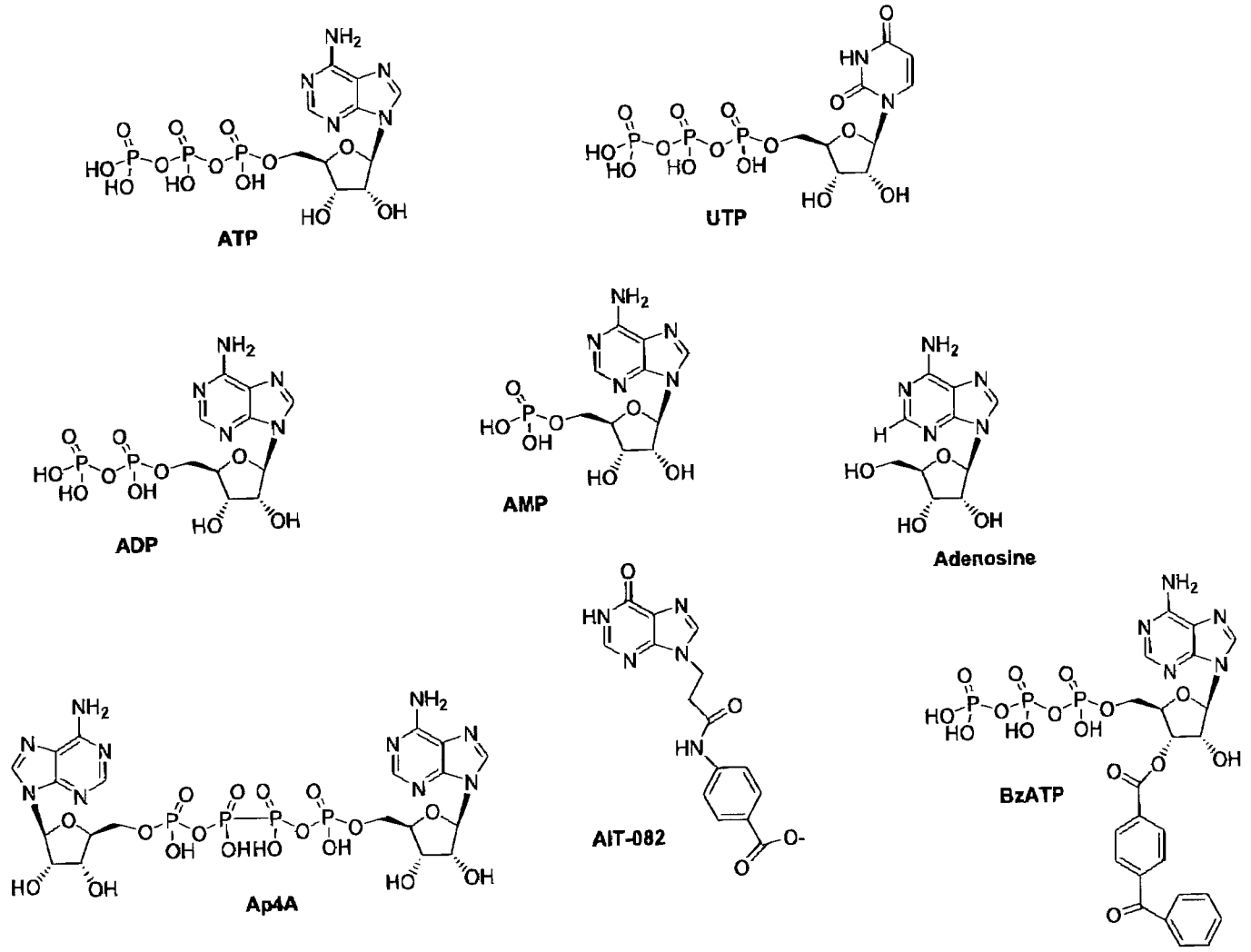

Fig. 1. Structures of P2 agonists, adenosine, and AIT-082.

1999), a phenomenon that is also seen with other P2X receptors (Williams and Jarvis, 2000).

ATP Availability, P2 Receptor Dynamics, and the Purinergic Cascade

Under normal physiological conditions, ATP is co-released with a number of neurotransmitters including acetylcholine, norepinephrine, glutamate, γ-aminobutyric acid, and neuropeptide Y (Burnstock, 1999). In tissue(s) undergoing hypoxia/ischemic insult or other trauma, ATP release is markedly increased. Once released, ATP is degraded to ADP, AMP, and adenosine by a family of approximately 11 ectonucleotidases (ectonucleoside triphosphate diphosphohydrolases; E-NTPases; Zimmerman, 1999a), thus limiting the extracellular actions of the nucleotide(s) by enhancing its removal, as well as producing the pharmacologically active nucleoside, adenosine. The E-NTPases, as well as P2 receptors, are dynamic cellular entities. For example, in myeloid leukocytes, P2Y receptors and the E-NTPases, ecto-apyrase and ecto-5′-nucleotidase, undergo stage-specific transient expression (Dubyak et al., 1996), while in guinea pig vas deferens, soluble E-NTPases are released together with ATP and norepinephrine (Todorov et al., 1997) and act to limit the extracellular effects of ATP. Studies on the role of altered E-NTPase activities in disease pathophysiology are at an early stage, but it is becoming increasingly evident that the E-NTPases represent novel drug targets.

While enzyme-catalyzed hydrolysis results in the inactivation of ATP, the breakdown products of the purine nucleotide are themselves pharmacologically active, some having opposing effects to one another, and form a purinergic cascade (Williams and Jarvis, 2000; Fig. 2). ATP can antagonize ADP actions on $P2Y_T/P2Y_{12}$ receptor-mediated platelet aggregation, while the sedative effects of adenosine in the CNS activity contrast with the excitatory actions of ATP on nerve cells. Adenosine (P1) receptor activation can inhibit ATP release. ATP-sensitive potassium channels (K_{ATP}) are activated when intracellular ATP levels are reduced. Thus, as P2 receptor responses become attenuated following ATP hydrolysis to adenosine, P1-mediated responses and K_{ATP}-mediated responses are enhanced. While UTP and UDP are active at $P2Y_2$, $P2Y_4$, and $P2Y_6$ receptors (Communi and Boeynaems, 1997), evidence for the physiological role of uracil has been limited. There is however an emerging body of evidence suggesting the existence of uracil/"U1" receptors equivalent to adenosine/P1 receptors (Kardos et al., 1999).

TABLE 1
P2 receptor nomenclature and pharmacology

Receptor Subunit	Rank Order of Agonist Activity	Antagonists	Signal Transduction
$P2X_1$	2-MeSATP > ATP > α,β-meATP	TNP-ATP, TNP-GTP, IP_5I	$I_{Na/K/Ca^{2+}}$
$P2X_2$	2-MeSATP > ATP α,β-meATP inactive	None	$I_{Na/K}$
$P2X_3$	2-MeSATP > ATP > α,β-meATP	TNP-ATP, TNP-GTP, IP_5I	$I_{Na/K/Ca^{2+}}$
$P2X_4$	ATP > 2-MeSATP >> α,β-meATP	None	$I_{Na/K}$
$P2X_5$	ATP > 2-MeSATP > ADP	None	$I_{Na/K/Ca^{2+}}$
$P2X_6$	ATP > 2-MeSATP > ADP	None	$I_{Na/K/Ca^{2+}}$
$P2X_7$	ATP > 2-MeSATP > ADP	KN-62[a] Brilliant blue G	$I_{Na/K}$, pore formation
Receptor Subtype	**Agonists**	**Antagonists**	**Signal Transduction**
$P2Y_1$	2-MeSATP > ATP > ADP (UTP inactive)	MRS 2216	PLCβ/IP_3 Ca^{2+}
$P2Y_2$	4-thioUTP > UTP = ATP >> 2-MeSATP	Suramin	PLCβ/IP_3 Ca^{2+}
$P2Y_4$	UTP = UDP > ATP = ADP	Reactive blue-2	PLCβ/IP_3 Ca^{2+}
$P2Y_6$	UDP > UTP > ATP	None	PLCβ/IP_3 Ca^{2+}
$P2Y_{11}$	ATP > 2-MeSATP > ADP	None ATP	PLCβ/IP_3 Ca^{2+} Adenylate cyclase IP_3 Ca^{2+}
$P2Y_T$/$P2Y_{12}$	ADP	AR-C 69931 MX Compound 1	Adenylate cyclase

2-MeSATP, 2-methylthioadenosine 5′-triphosphate; PLCβ, phospholipase Cβ; IP_3, inositol 1,4,5-trisphosphate; α,β-meATP, α,β-methylene adenosine 5′-triphosphate; TNP-GTP, 2′(3′)-*O*-(2,4,6-trinitrophenyl)guanosine 5′-triphosphate.
[a] Allosteric modulator.

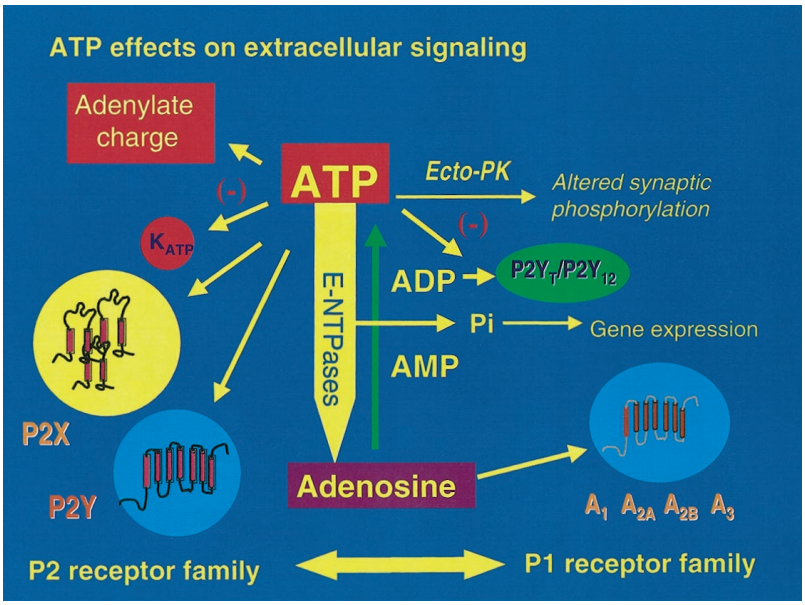

Fig. 2. ATP effects on cellular communication. ATP is released into the extracellular space where it forms the basis of a purinergic cascade. ATP acts directly on P2X and P2Y receptors (see text) and is degraded to ADP and AMP by E-NTPase activity. ADP interacts with $P2Y_T$/$P2Y_{12}$ receptors, and ATP can block this response. AMP gives rise to adenosine, which activates the various P1 receptors (A_1, A_{2A}, A_{2B}, A_3). Decreases in ATP lead to activation of K_{ATP} channels. ATP can also signal between cells on the basis of adenylate charge (see Williams and Jarvis, 2000) and also by acting as a substrate for ecotokinase activity (Redegeld et al., 1999). P1 and P2 receptors can modulate the function responses to one another (see text). ATP is reformed from adenosine by uptake and subsequent rephosphorylation involving adenylate kinase. UTP can undergo metabolism similar to that of ATP, and evidence is emerging for the existence of uridine (U) receptors (see text). UTP interacts with $P2Y_2$ and $P2Y_4$ receptors, while UDP is the most potent agonist at the $P2Y_6$ receptor. Phosphate can also function as a signal for gene induction, e.g., osteopontin.

P2 Receptor Ligands

All known P2 receptor agonists are ATP or UTP analogs substituted in the polyphosphate side chain to improve stability to enzymatic degradation. Substitutions at the 2- and N^6-positions on the purine ring confer receptor-subtype selectivity (Jacobson and Knutsen, 2001).

Putative P2 receptor antagonists include PPADS, DIDS, suramin, and dyes like reactive blue-2 (Fig. 3) that, as noted, lack potency and selectivity for P2 receptors (Bhagwat and Williams, 1997) and are not especially bioavailable, limiting their use as in vivo research tools. Pharmacological characterization of these antagonists has also been confounded by the use of different tissues, species, and assay systems and also by their ability to inhibit endogenous E-NTPase activity, thus potentiating the actions of endogenous ATP. The suramin analogs NF023 and XAMR 0721 (Fig. 3) are selective antagonists at rat P2X receptors with reduced effects on E-NTPase activity (Bhagwat and Williams, 1997).

The search for newer ligands, both agonists and antagonists, using high-throughput screening approaches has also been limited by a lack of reliable binding assays (Williams and Jarvis, 2000), although functional fluorescent imaging (FLIPR) in cell lines transfected with rat and human P2 receptors has proven to be useful (Bianchi et al., 1999). Among newer P2 antagonists (Fig. 3; Williams and Jarvis, 2000; Jacobson and Knutsen, 2001) are TNP-ATP, a potent (1 nM) noncompetitive, reversible antagonist at $P2X_1$ and $P2X_3$ receptors, MRS 2216, a full $P2Y_1$ receptor antagonist (IC_{50} = 210 nM), and diinosine pentaphosphate (IP_5I), a $P2X_1$ receptor antagonist (K_i = 3 nM). AR-C 69931 MX is a potent (IC_{50} = 0.4 nM), selective $P2Y_T$/$P2Y_{12}$ receptor antagonist that blocks ADP-induced platelet aggregation. Compound 1 is a

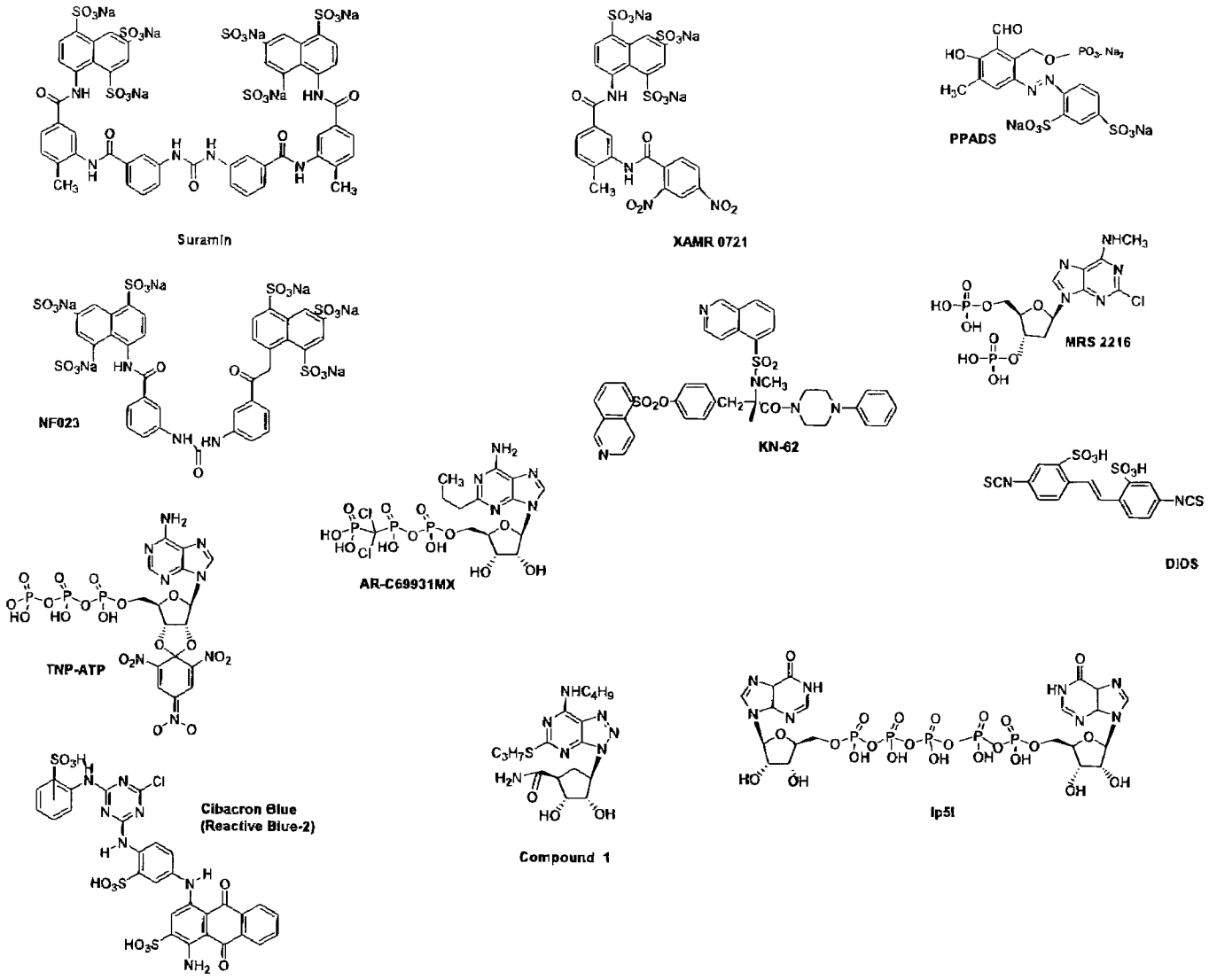

Fig. 3. P2 receptor antagonists and allosteric modulators.

nonphosphorylated $P2Y_{12}$ antagonist. KN-62, a calcium-calmodulin dependent protein kinase-II (CamK-II) inhibitor, is also a potent (IC_{50} = 9–13 nM) noncompetitive $P2X_7$ receptor antagonist being 70 to 100 times more potent at human $P2X_7$ receptors than at CamK-II. Brilliant blue G is another potent (IC_{50} rat = 10 nM; human = 200 nM) selective, noncompetitive antagonist of $P2X_7$ receptors. Avermectin is a positive allosteric modulator of $P2X_4$ receptors. BzATP (Fig. 1), which is widely used as a selective $P2X_7$ receptor agonist (EC_{50} = 18 μM), is 4 to 5 orders of magnitude more potent at functional $P2X_1$ (EC_{50} = 1.9 nM) and $P2X_3$ (EC_{50} = 98 nM) receptors (Bianchi et al., 1999), raising the possibility that many cellular responses sensitive to BzATP that have been ascribed to $P2X_7$ receptors may involve $P2X_1$ or $P2X_3$ receptors.

The discovery of these antagonists/allosteric modulators indicates that P2X receptors are equal in their complexity to other LGICs. The symmetrical structures of suramin and its analogs, the P2X antagonist IP_5I and the dinucleotide polyphosphate P2 agonists, e.g., Ap_4A (Fig. 1) etc. (Miras-Portugal et al., 1999), may suggest that bidentate ligand interactions occur such that functional P2 receptors, both P2X and P2Y, may require ligand interactions with two ATP recognition sites for activation, a possibility that has yet to be explored in any detail (Jacobson and Knutsen, 2001).

P2 Receptor Function

The use of mice deficient in a targeted receptor (knockouts) is a useful way in which to assess the functional role of the receptor in the absence of selective antagonists or antisense probes. P2 receptor knockouts are associated with decreased male fertility ($P2X_1$; Mulryan et al., 2000), decreased nociception and bladder hyporeflexia ($P2X_3$; Cockayne et al., 2000), decreased platelet aggregation and bleeding time ($P2Y_1$; Fabre et al., 1999; Leon et al., 1999), and reduced chloride secretion ($P2Y_2$; Cressman et al., 1999).

Neurotransmission. ATP is a cotransmitter with norepinephrine in sympathetic nerves, with acetylcholine in parasympathetic nerves supplying the bladder, and in nonadrenergic, noncholinergic (NANC) inhibitory enteric nerves (Burnstock, 1999). The nucleotide has both excitatory and sedative effects in the CNS with both P2X and P2Y receptors

being widely distributed in the central and peripheral nervous systems.

In the auditory system, ATP, acting via P2Y receptors, depresses sound-evoked gross compound action potentials in the auditory nerve and the distortion product otoacoustic emission, the latter a measure of the active process of the outer hair cells. Both P2X and P2Y receptors have been identified in the vestibular system. $P2X_2$ receptor splice variants are present in the cochlea. $P2X_{2\text{-}1}$ and $P2X_{2\text{-}3R}$ receptors are found in the rat (Housley, 2000), and $P2X_{2\text{-}1}$, $P2X_{2\text{-}2}$, and $P2X_{2\text{-}3}$ receptors are found in the guinea pig. In the rat, P2X splice variants are found on the endolymphatic surface of the cochlear endothelium, an area associated with sound transduction (Housley, 2000). P2Y receptors are present in the marginal cells of the stria vascularis, a tissue involved in regulating the ionic and electrical gradients of the cochlea. While little is currently known regarding the pharmacology of hearing and vestibular function, ATP may regulate fluid homeostasis, cochlear blood flow, hearing sensitivity, and development and thus may be useful in the treatment of Ménière's disease, tinnitus, and sensorineural deafness (Housley, 2000).

In the eye, ATP, acting via both P2X and P2Y receptors, modulates retinal neurotransmission affecting retinal blood flow and intraocular pressure. The ATP analog β,γ-MeATP has greater efficacy in reducing intraocular pressure (40%) than muscarinic agonists like pilocarpine (25%) or β-adrenoceptor blockers (30%; Pintor et al., 2000). In the ocular mucosa, $P2Y_2$ receptor activation increases salt, water, and mucus secretion and thus represents a potential treatment for dry eye disease (Yerxa, 2000). In the retinal pigmented layer, $P2Y_2$ receptor activation promotes fluid absorption and may be involved in retinal detachment.

Epilepsy. Microinjection of ATP analogs into the prepiriform cortex induces generalized motor seizures similar to those seen with *N*-methyl-D-aspartate and bicuculline (Knutsen and Murray, 1997). $P2X_2$, $P2X_4$, and $P2X_6$ receptors are expressed in the prepiriform cortex, suggesting that a P2X receptor antagonist may have potential as an antiepileptic (Collo et al., 1997).

Pain. ATP, given systemically, elicits pain responses, and endogenous ATP may contribute to the pain associated with causalgia, reflex sympathetic dystrophy, angina, migraine, lumbar, pelvic, and cancer pain (Burnstock, 2000). The nucleotide is also a key mediator of neurogenic inflammation via its actions on P2 receptors on neutrophils, macrophages, and monocytes, activation of which results in cytokine production and release (Dubyak and El Motassim, 1993).

$P2X_3$ receptors are selectively localized to sensory pathways in trigeminal, nodose, and dorsal root ganglia (DRG) and represent unique targets for novel analgesic agents that function as $P2X_3$ receptor antagonists. Nonspecific P2 receptor antagonists, e.g., suramin, are antinociceptive and $P2X_3$ receptor knockout mice have reduced nociceptive responses (Cockayne et al., 2000).

Neonatal capcasicin treatment reduces $P2X_3$ mRNA in the DRG and abolishes ATP-mediated acute nociceptive responses (Burnstock, 2000). $P2X_2$ receptor immunoreactivity is also present in the DRG and in lamina II of the dorsal horn of the spinal cord; $P2X_3$ receptors are colocalized with the vanilloid VR-1 receptor (Guo et al., 1999). ATP is also involved in the conduction of innocuous mechanical stimuli and in a tissue culture system of tooth-pulp afferent (nociceptive) and muscle stretch receptor (non-nociceptive) rat sensory neurons, acting via distinct P2X receptors; ATP has both nociceptive and non-nociceptive roles (Cook et al., 1997).

For visceral pain, a purinergic mechanosensory transduction mechanism has been proposed (Burnstock, 1999) where distention of tubes, such as the ureter, gut, salivary, and bile ducts and sacs like the urinary and gall bladder causes ATP release from the lining epithelial cells to act on $P2X_3$ receptors located on the subepithelial sensory nerve plexus to relay nociceptive signals to the CNS.

Changes in cell volume, e.g., in liver epithelium following exposure to insulin and uptake of amino and bile acids, also result in ATP release that then acts as an autocrine regulator to facilitate cell volume recovery, thus linking cellular hydration state to P2 pathways involved in cellular homeostasis (Roman and Fitz, 1999).

When administered together with nitric oxide (NO), ATP, probably acting as adenosine following hydrolysis, mimics the effects of the inhalation anesthetic, enflurane (Fukunaga, 1997) and also reduces the amount of inhalation anesthetic required for anesthesia.

Trophic Actions. In nervous tissue, trophic factors ensure neuronal viability and regeneration. Neural injury increases tyrosine kinase-linked polypeptide growth factors like fibroblast growth factor (FGF), epidermal growth factor, and platelet-derived growth factor (Neary et al.,1996). In combination with these growth factors, ATP can act to stimulate astrocyte proliferation, contributing to the process of reactive astrogliosis, a hypertrophic/hyperplastic response that is associated with brain trauma, stroke/ischemia, seizures, and neurodegenerative disorders.

In reactive astrogliosis, astrocytes undergo process elongation and express GFAP (glial fibrillary acidic protein), an astrocyte specific intermediate filament protein. ATP can increase GFAP and AP-1 complex formation in astrocytes (Neary et al.,1996) in a manner similar to that seen with bFGF. In addition, ATP as well as GTP can induce trophic factor (nerve growth factor, NT-3, FGF) synthesis in astrocytes and neurons. The effects of GTP are inconsistent with any known P2 receptor. However, a synthetic purine, Neotrofin (AIT-082; Fig. 1), up-regulates neurotrophin production, enhances working memory, and restores age-induced memory deficits in mice (Rathbone et al., 2000). This compound has also shown positive acute effects in Alzheimer's disease patients. P2 receptor-mediated effects on trophic factor production are unlikely to be limited to nervous tissue.

Neurourology. Urinary bladder function is regulated by sympathetic and parasympathetic input. ATP mimics the effects of parasympathetic nerve stimulation, resulting in bladder contraction (Burnstock et al., 1978; Dean and Downie, 1978) via activation of P2X receptors in the smooth muscle of urinary bladder detrusor muscle that is involved in bladder emptying. Detrusor malfunction results in urge urinary incontinence (UUI), a major health problem in the aging female population.

Micturition involves urethral relaxation where ATP functions as a cotransmitter together with NO. NO mediates the first stage of relaxation (Pinna et al., 1998) with ATP mediating the second phase of the voiding response. Serosal ATP release occurs in rabbit bladder due to the hydrostatic pressure changes associated with bladder filling (Yoshimura and

de Groat, 1997). Muscarinic receptors, through which the anticholinergics oxybutynine chloride (Ditropan; ALZA, Palo Alto, CA) and tolterodine (Detrol; Pharmacia, Kalamazoo, MI) produce their clinically beneficial effects on UUI, mediate 15% of the neurogenic contraction in rat urinary bladder. Another 50% is mediated by P2X receptors present in bladder urothelium (Hashimoto and Kokubun; 1995), suggesting that P2X receptor antagonists may be potentially useful in the treatment of UUI.

In male rat genitalia, antibodies to $P2X_1$ and $P2X_2$ subunits show immunoreactivity in the membranes of the smooth muscle layer of the vas deferens, suggesting an involvement in ejaculation (Lee et al., 2000). In male $P2X_1$ receptor knockout mice, fertility is reduced by 90% without affecting copulatory performance. This is due to a decreased sperm count in the ejaculate due to a 60% reduction in the sensitivity of the vas deferens to sympathetic nerve stimulation (Mulryan et al., 2000). The remaining 10% in fertility rate suggests that other, non-$P2X_1$ receptor-mediated components are involved in vas deferens smooth muscle tone.

In the body of the penis, strong $P2X_1$ with weaker $P2X_2$ immunoreactivity is present in the smooth muscle of blood vessels and the corpus cavernosum, suggesting a participation in erectile function (Lee et al., 2000). ATP effects on cavernosal smooth muscle depend on the basal tone, relaxing corporal smooth muscle at high basal tension and contracting it at low tension (Wu et al., 1993). Both adenosine and ATP show an enhanced sensitivity in penile tissue responses in diabetic males, although in diabetic rats, ATP-induced relaxation is decreased, while the effects of adenosine, which are thought to be mediated via potassium channels, are enhanced (Gür and Öztürk, 2000).

Hemostasis. ADP is a potent platelet recruiting factor and induces platelet aggregation via interaction with two P2 platelet receptors, a $P2Y_1$ receptor linked to phospholipase C pathways and calcium influx that is involved in shape changes and transient aggregation and the $P2Y_T$/$P2Y_{12}$ receptor that is negatively coupled to adenylate cyclase that mediates degranulation and sustained aggregation. In support of two ADP-sensitive P2 receptors, in $P2Y_1$ knockout mice, ADP was still able to inhibit platelet adenylate cyclase activity (Leon et al., 1999). A $P2X_1$ receptor is also present on platelets that modulates calcium influx. However, it has not been shown to have any functional significance. Hydrolysis of ADP by the E-NTPase, CD39 inhibits platelet aggregation by removing ADP and by forming adenosine, which also inhibits aggregation (Zimmerman, 1999b). ATP is a competitive ADP antagonist at platelet P2Y receptors and stimulates production of PGI_2 and NO, which can also inhibit platelet aggregation and act as vasodilators. Exogenous ATP thus acts to localize thrombus formation to areas of vascular damage, controlling the relationship between hemostasis, thrombosis, and fibrinolysis. In turn, CD39 appears to act together with the extracellular nucleotides released as a result of tissue damage to modulate blood fluidity and platelet activation (Zimmerman, 1999b). $P2Y_1$ knockout mice show increased bleeding time and are resistant to thromboembolism (Fabre et al., 1999). ATP stimulates granulocyte differentiation via activation of the $P2Y_{11}$ receptor, suggesting that selective $P2Y_{11}$ agonists may have potential in the treatment of neutropenia and leukemia, either alone or in combination with granulocyte-colony stimulating factor.

AR-C 69931 MX is a systemically active, synthetic $P2Y_{12}$ receptor antagonist with a safer side effect profile than aspirin and superior antithrombotic properties compared with GPIIb/IIIa antagonists. An orally active $P2Y_{12}$ antagonist derived from Compound 1 (Fig. 3) is entering phase I trials as an antithrombotic agent.

Bone Function. ATP released in response to shear stress (Burnstock, 1999, 2000) may function as mechanotransducer in skeletal tissue acting as osteoblast mitogens, potentiating the effects of growth factors on these bone cells (Dixon and Sims, 2000). P2X and P2Y receptors are present on osteoclasts with P2Y receptors only being present on osteoblasts. ATP, but not adenosine, stimulates the formation of osteoclasts and their resorptive actions in vitro (Morrison et al., 1998) and can inhibit osteoblast-dependent bone formation. The bisphosphonate clodronate, which is used in the treatment of Paget's disease and tumor-induced osteolysis, may act via osteoclast P2 receptors (Dixon and Sims, 2000). Modulation of P2 receptor function may have potential in the treatment of osteoporosis, rheumatoid arthritis, periodontitis, and osteopenia.

Apoptosis. The $P2X_7$ (P_{2Z}) receptor can function as a nonselective ion pore in mast cells, platelets, macrophages, and lymphocytes (Dubyak and El Moatassim, 1993; Di Virgilio et al.,1999). $P2X_7$ receptor activation triggers apoptosis to facilitate embryogenesis and to remove cancerous or infected cells from tissues.

Multinucleated giant cells (MGCs) are formed when monocytes fuse with one another in granulomatous inflammation. Interferon-γ and lipopolysaccharide stimulate MGC formation by up-regulating $P2X_7$ receptor expression (Humphreys and Dubyak, 1996) and also decrease E-NTPase activity, increasing the susceptibility of MGCs to the cytolytic actions of extracellular ATP.

ATP also induces cytolysis via $P2X_7$ receptors in macrophages infected with mycobacterium by both apoptotic and necrotic pathways (Lammas et al., 1997). This novel antimicrobial activity of ATP was thought to have potential use in the treatment of tuberculosis. However, in $P2X_7$ receptor knockout mice this receptor was not essential for the antimicrobial effects of ATP (Sikora et al., 1999). The $P2X_7$ receptor is present in the superior cervical ganglion and spinal cord, and cerebral artery occlusion causes an increase in $P2X_7$ immunoreactive cells in the penumbral region around the infarct (Collo et al., 1997).

In macrophages, ATP, acting via $P2X_7$ receptors, stimulates the release and maturation of IL-1β (Di Virgilio et al., 1999). In stratified epithelium, $P2X_5$ receptors are associated with proliferating and differentiating cells, while $P2X_7$ receptors label apoptotic cells (Groschel-Stewart et al., 1999). $P2X_5$ and $P2X_7$ receptor agonists may thus have potential in the treatment of psoriasis, scleroderma, basal cell carcinoma, and for restenosis following angioplasty.

Cancer. Exogenous ATP has positive effects in the treatment of cancer and cancer cachexia (Rapaport, 1997), effects attributed to inhibition of gluconeogenesis, inhibition of the acute-phase response, and decreased production of the proinflammatory cytokines, IL-1 and IL-6. In a nonrandomized clinical trial, infusion of ATP for 96 h at 28-day intervals at doses of 50 μg/kg/min to patients with advanced non-small-cell lung cancer increased ATP pools in red blood cells, inhibited weight loss, reduced cachexia, and improved survival

(Rapaport, 1997). In a subsequent randomized trial (Agteresch et al., 2000) in patients with advanced (stage IIIB or IV) non-small-cell lung cancer, intravenous ATP reduced weight loss (−1.0 kg to 0.2 kg/month) and reversed decreases in serum albumin, elbow flexor muscle strength, and quality of life measures. Positive effects on body weight, muscle strength, and albumin concentration were especially marked in cachectic patients.

In cystic fibrosis transmembrane conductance regulator (CFTR) homozygous and heterozygous nude mice, breast tumor implantability was decreased, an effect ascribed to elevated blood ATP levels. Similarly, ATP reduced breast tumor cell growth in vitro, supporting the concept of ATP as an antitumor agent (Abraham et al., 1996). Another P2X receptor, P2XM, which is homologous with the $P2X_6$ receptor subunit, is induced by the tumor oncogene p53 and is implicated in soft tissue tumor genesis (Nawa et al., 1999).

Gastrointestinal Tract Function. ATP and adenosine are potent stimulants of fluid and electrolyte (chloride) secretion in colon and gallbladder and in the pancreatic and bile ducts (Burnstock, 1999; Roman and Fitz, 1999), effects that appear to primarily involve $P2Y_2$ receptor activation.

ATP also functions in the gastrointestinal tract as a paracrine signaling molecule; ATP released from hepatocytes activates P2 receptor signaling pathways in neighboring hepatocytes and biliary cells. ATP may also be involved as a paracrine mediator in hepatobiliary coupling, a process coordinating the hepatocyte and ductular components of bile formation, and may thus be clinically useful in increasing bile flow and treating prolonged cholestasis (Roman and Fitz, 1999).

Diabetes. ATP stimulates pancreatic insulin release via a glucose- dependent, P2Y receptor-mediated mechanism (Loubatieres-Mariani et al., 1997) and also modulates insulin secretion by interactions with ATP-sensitive potassium channels in islet β-cells. ADP can antagonize the ATP inhibition of these channels by binding to the second nucleotide binding site on the associated sulfonylurea receptor (SUR; Nichols et al., 1996), thus activating K_{ATP} channels and inhibiting insulin secretion.

Cardiopulmonary Function. ATP is a mediator of vagal reflexes in the heart and lung (Burnstock, 2000). In anesthetized rats, P2X receptors have been implicated in evoking a Bezold-Jarisch response (hyperventilation, bradycardia, hypotension, apnea). ATP and UTP, acting via $P2Y_2$ receptors, stimulate chloride secretion in airway epithelium and mucin glycoprotein release from epithelial goblet cells (Stutts and Boucher, 1999), enhancing mucociliary clearance and reflecting a potential treatment for cystic fibrosis and chronic bronchitis. In controlled clinical studies, UTP, used in preference to ATP as an $P2Y_2$ receptor agonist since it does not form cardiovascularly active metabolites like adenosine, dose dependently stimulated mucociliary clearance and sputum expectoration in smokers, nonsmokers, and patients with chronic bronchitis. E-NTPase-resistant analogs of UTP like INS 365 (Yerxa, 2000), in addition to being used in cystic fibrosis and chronic bronchitis, may act as adjunctive agents to enhance the effectiveness of antibiotics used in the treatment of respiratory infections and thus reduce the amounts used, potentially avoiding antibiotic resistance phenomena. $P2Y_2$ knockout mice show reduced chloride secretion (Cressman et al., 1999).

ATP may also have a direct role in asthma via its actions on bronchial innervation. The nucleotide triggers a reflex bronchoconstriction via activation of a P2X receptor on vagal C fibers, and both ATP and UTP can potentate IgE-mediated mast cell histamine release, effects involving P2Y receptors (Schulman et al., 1998).

P2 Receptor-Based Therapeutics

Advances in the molecular biology of the P2 receptor family are now being used concurrently to identify novel ligands that have the potency, selectivity, and bioavailability to characterize P2 responses in animals and intact tissues and to better understand the role of this receptor family in tissue function and disease.

It will be important as new approaches to human therapeutics target P2 receptors that the multiplicity of actions associated with ATP—especially in regard to molecular targets distinct from the P2 receptor family—are factored into the functional responses observed in model systems with ATP and its analogs. Thus members of the ATP binding cassette protein family, E-NTPases (Zimmerman, 1999a), ATP-modulated potassium channels, and enzymes that utilize ATP for their function, e.g., ecto-protein kinases (Redegeld et al., 1999), are all potential targets for ligands that interact with P2 receptors.

At the present time, knowledge regarding the binding site(s) for ATP on both P2X and P2Y receptors is limited, as is knowledge related to the sites at which allosteric modulators like KN-62 and avermectin interact. Emerging data on P2X heteromers (North and Surprenant, 2000) and their functional interactions with other LGICs, e.g., neuronal nicotinic cholinergic receptors (Searl et al., 1998), add an additional layer of complexity in understanding P2 receptor function and also in identifying compounds that can be used as lead structures for drug discovery. Nonetheless, as more is learned about the potential use of P2 receptors in human disease using knockout and imaging techniques, it is imperative that new molecules are found to assess the pharmacological relevance of these targets in nontransgenic animal models.

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