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COMMENTARY

Purinergic and Pyrimidinergic Receptors as Potential Drug Targets

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ABSTRACT. In the last decade, the field of purinergic pharmacology has continued to grow as the complexity of the receptor families and the various enzymes involved in purine metabolism have been defined in molecular terms. A major theme that has emerged from these studies is the functional complexity of the interactions between P1 and P2 receptors, based upon the dynamic interrelationship between ATP and adenosine as extracellular signaling molecules. It is now clear that ATP and its degradation products (particularly ADP and adenosine) form a complex cascade for the regulation of cell-to-cell communication that can function to attenuate the consequences of tissue trauma (e.g. ischemia) that involve alterations in cellular energy charge and depletion of ATP stores. In addition to the P2 receptor family, alterations in cellular ATP stores can also affect the function of other receptors, e.g. K_{ATP} channels, and mitochondrial function. The discovery of pyrimidine-preferring (UTP/UDP) P2Y receptors has also raised the possibility that the corresponding nucleoside, uracil, may function as a signaling molecule. BIOCHEM PHARMACOL **59**;10:1173–1185, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. purines; receptors; ligand-gated ion channels; heptahelical receptors

Studies of the modulation of cellular function by purines and pyrimidines have increased exponentially over the past decade as a large family of discrete molecular targets has been identified using molecular biological and pharmacological tools [1].

The concept of purinergic transmission is irrevocably associated with the seminal work of Burnstock, who originally proposed the concept in 1972 based on a considerable body of physiological and pharmacological data that originated in the 1920s. Despite a general lack of acceptance based on the dogma that a high energy phosphate-containing intracellular molecule such as ATP and its main metabolite, adenosine (ADO, Fig. 1), were not logical choices as intercellular messenger molecules, Burnstock subsequently proposed the existence of distinct P1 (adenosine) and P2 (ATP) receptors. In 1994, P2 receptors were divided further into two structural classes: P2X, a superfamily of LGICs†, and P2Y, a heptahelical GPCR superfamily [1]. Four P1 receptors and eleven P2 receptors now have been described [1, 2]. The physiological function(s) of these receptors and their role in tissue homeostasis and pathophysiology are being elucidated, using receptor localization and pharmacological as well as genomic approaches including receptor knockouts.

In addition to directly affecting P2 receptor function, ATP (Fig. 1) also modulates the function of other LGICs [3], and thus has a potentially complex and multifunctional role in modulating cellular and tissue function that can be conceptualized as a purinergic cascade [4] (Fig. 2). ATP also is metabolized rapidly by a family of extracellular ectoATPases [5, 6] to yield ADP (Fig. 1) and adenosine, which, in turn, interact with platelet P_{2T} and P1 receptors, respectively, to produce their own effects on cellular function, some of which are opposite to those produced by ATP. Thus, ATP functions as an excitatory transmitter in the CNS [7], whereas adenosine inhibits CNS excitability [8].

Historically, ligands selective for the P1 receptor family were developed in the complete absence of information regarding the molecular structure of these receptors [9]. For the P2 receptor family, however, a diversity of receptors responsive to ATP has been identified, preceding the identification of potent and selective ligands, which are in the process of being identified.

However, drugs that produce their effects by direct modulation of purine receptor function are few in number. Adenosine itself is used for the treatment of supraventricular tachycardia and for cardiac imaging, situations where its short half-life (1–10 sec) is advantageous. The adenosine antagonist, theophylline is used for the treatment of asthma, but has a very narrow window of efficacy before it elicits CNS stimulation [10]. Newer therapeutic targets under exploration for both P1 and P2 receptor ligands include inflammation, pain, congestive heart failure, myo-

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† *Abbreviations*: LGIC, ligand gated ion channel; GPCR, G protein coupled receptor; GABA, γ-aminobutyric acid; cAMP, cyclic AMP; PLCβ, phospholipase Cβ; CamK-II, calcium calmodulin kinase II; DA, dopamine; NO, nitric oxide; IP_3, inositol 1,4,5-triphosphate; AK, adenosine kinase; COX, cyclooxygenase; REM, rapid eye movement; and UUI, urge urinary incontinence.

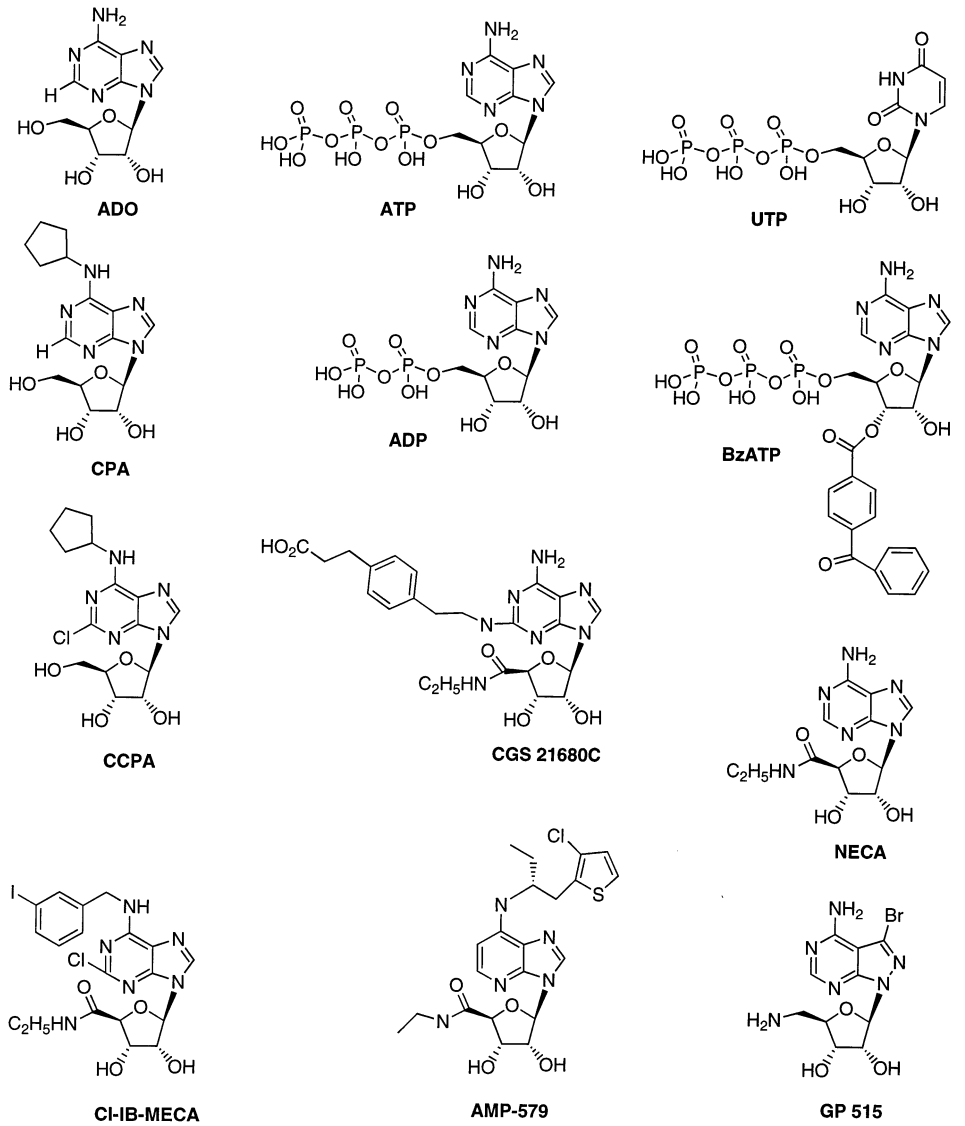

FIG. 1. Structures of P1 and P2 receptor agonists. Abbreviations: ADO, adenosine; CPA, cyclopentyl adenosine; CCPA, 2-chlorocyclopentyl adenosine; Cl-IB-MECA, 2-chloro-N^6-(3-iodobenzyl)-adenosine-5′-*N*-methyluronamide; BzATP, 2′ and 3′-*O*-(4-benzoylbenzoyl)-ATP; and NECA, 5′-N^6-ethylcarboamidoadenosine.

cardial ischemia, stroke, sleep apnea, and Parkinson's disease [4, 11, 12].

P1 AND P2 RECEPTOR DYNAMICS: THE PURINERGIC CASCADE

ATP is co-released with acetylcholine, norepinephrine, glutamate, GABA, and neuropeptide Y [13]. Once released, it is degraded via ectonucleotidase activity, limiting its extracellular actions by enhancing its removal. These enzymes and some P2 receptors are dynamic entities. In myeloid leukocytes, P2Y receptors and the ectonucleotidases ecto-apyrase and ecto-5′-nucleotidase undergo stage-specific transient expression [14], whereas in stroke, $P2X_7$ receptors are up-regulated [15]. Soluble nucleotidases are released from guinea pig vas deferens neurons along with ATP and norepinephrine [16], serving to limit the effects of the released ATP.

ATP inactivation is, however, an inaccurate term, as the products of ATP breakdown—ADP, AMP, and adenosine—form a purinergic cascade [4] (Fig. 2), the products of which have distinct activities, some of which, as mentioned previously, are antagonistic to the actions of ATP. The nucleotide antagonizes the actions of ADP on platelet aggregation (P_{2T} receptor), whereas the sedative and anticonvulsant actions of adenosine [4] contrast with the excitatory actions of ATP on nerve cells [7]. In the broader framework of ATP-modulated proteins, decreased ATP levels lead to activation of ATP-sensitive potassium channels (K_{ATP}) [3]. Thus, as P2 receptor-mediated responses are attenuated via ATP hydrolysis to adenosine, P1 receptor-mediated and K_{ATP}-mediated responses are enhanced.

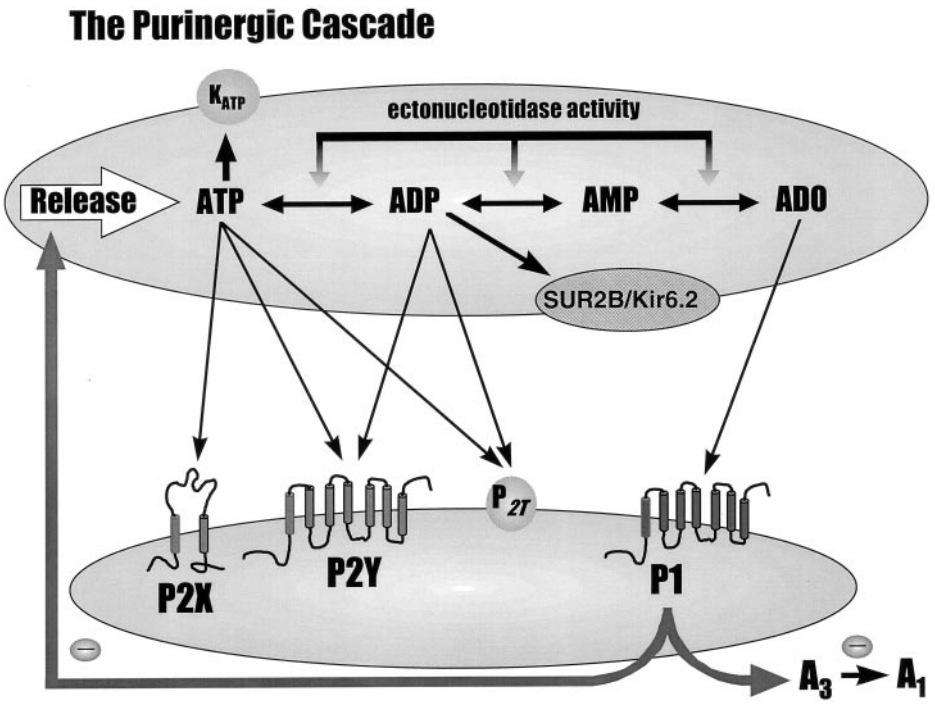

FIG. 2. The purinergic cascade. ATP, ADP, or adenosine (ADO) are released from nerves or cells into the extracellular milieu where they can interact to form a purinergic cascade. ATP acts at a variety of P2 receptors (see text) and is degraded sequentially to ADP and AMP by ectonucleotidase activity. ADP is the preferred agonist for P_{2T} receptors and $P2Y_1$ receptors. AMP gives rise to ADO, which can interact with the various P1 receptors (A_1, A_{2A}, A_{2B}, A_3). ADO activation of P1 receptors can lead to inhibitory feedback on ATP release, which can lead to increased activation of K_{ATP} channels, as well as receptor modulation. For example, activation of the A_3 receptor produces a down-regulation in A_1 receptor activity in the hippocampus. ADO also can be formed by intracellular 5′-nucleotidase activity. See text for further discussion. The P_{2T} receptor, indicated by a circle, has not been defined yet in molecular terms.

Whereas UTP (Fig. 1) and UDP are agonists at certain P2 receptors [17], it is only recently that there has been a focus on the physiological properties of uracil [18], even though there is currently no molecular evidence for the existence of uracil (? U1) receptors [4].

Purines As Neuromodulators

Extracellular levels of ATP and adenosine are increased in response to tissue trauma, e.g. ischemia or hypoxia [19]. Extracellular adenosine is a potent inhibitor of dopamine, GABA, glutamate, acetylcholine, serotonin, and norepinephrine release, acting primarily via presynaptic A_1 receptors [8] with preferential effects on excitatory as opposed to inhibitory neurotransmitter release [20, 21]. Postsynaptically, adenosine modulates neuronal excitability by hyperpolarizing the postsynaptic membrane [8]. ATP is a fast excitatory neurotransmitter in nervous tissue [7], with P2X and P2Y receptors being widely distributed on neurons, astroglia, microglia, and oligodendroglia [1, 15].

P1 Receptors

Four P1 receptors, A_1, A_{2A}, A_{2B}, and A_3 (Table 1), have been defined pharmacologically and cloned [1] and are members of the heptahelical GPCR superfamily that are heterogeneously distributed in mammalian tissues, activated by adenosine, and antagonized by xanthines (Fig. 3), including the psychomotor stimulant caffeine [10]. A_1 receptor activation leads to inhibition of cAMP formation and N-channel-mediated calcium conductances, stimulation of potassium conductances and phospholipase C pro-

TABLE 1. P1 receptor subtypes and pharmacology

Receptor	Agonists	Antagonists	Transductional system
A_1	CCPA > CPA	CPX	Decrease cAMP Increase IP_3
A_{2A}	CGS 21680C > NECA	SCH 58261, ZM 241385, CSC, KF 17837, KW6002	Increase cAMP
A_{2B}	NECA	Enprofylline, CPX, MRS 1595	Increase cAMP
A_3	Cl-IB-MECA > IB-MECA	MRS 1220, L-249313, L-268605	Decrease cAMP

See text and Figs. 1 and 3 for information on specific compounds.

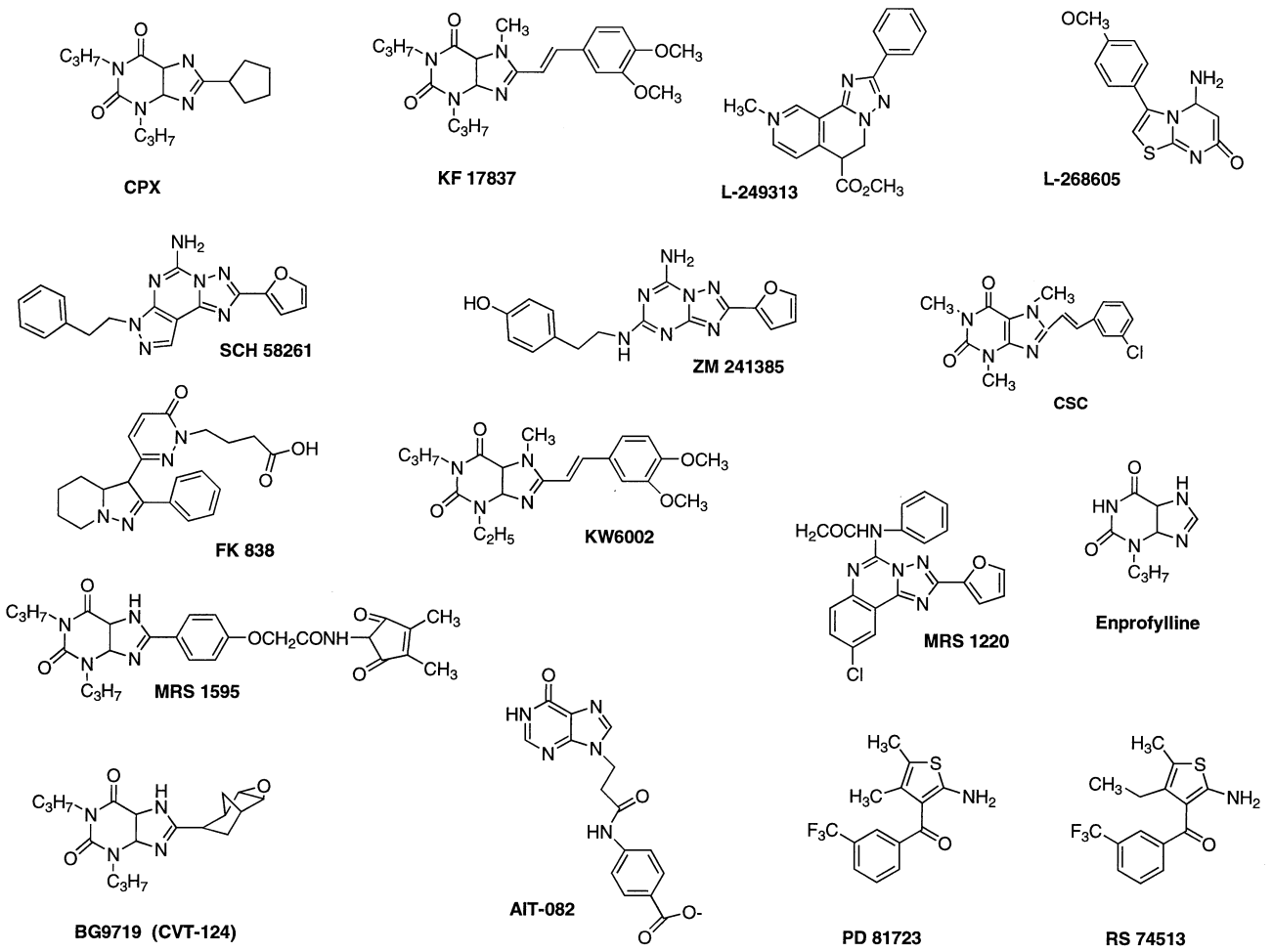

FIG. 3. Structures of P1 receptor antagonists. Abbreviations: CPX, cyclopentylxanthine; and CSC, 8-(3-chlorylstyryl)caffeine.

duction, and modulation of NO production. Cyclopentyl (CPA; K_i = 0.6 nM for A_1) and 2-chlorocyclopentyl (CCPA; K_i = 0.6 nM) adenosine (Fig. 1) are 780- and 1500-fold selective for the A_1 receptor versus other P1 receptors [9]. Cyclopentylxanthine (CPX; K_i = 0.46 nM) is 740-fold selective for the A_1 receptor (Fig. 3). A_1 receptors can be modulated allosterically by thiophenes such as PD 81723 and RS 74513 (Fig. 3), which stabilize an agonist-preferring conformation of the A_1 receptor independent of G-protein interactions [22].

The A_{2A} receptor has high affinity for adenosine, is functionally coupled to activation of adenylate cyclase, and also may utilize N- and P-type Ca^{2+} channels for signal transduction. This receptor is highly localized in the CNS to striatal, nucleus accumbens, and olfactory tubercule regions. A lower affinity A_{2B} receptor that also stimulates adenylate cyclase activity is distributed more ubiquitously throughout the CNS and periphery. CGS 21680C (Fig. 1) is a prototypic A_{2A} receptor agonist. The xanthine antagonists, KF 17837 and CSC (Fig. 3) are 108- and greater than 3000-fold selective for A_{2A} receptors versus other members of the P1 receptor family [9]. SCH 58261 and ZM 241385 (Fig. 3) are novel and potent A_{2A} receptor antagonists. Agonists and antagonists for the A_{2B} receptor have proven difficult to identify. Enprofylline (Fig. 3), although weak, has been described as an A_{2B} antagonist [23], although newer compounds such as MRS 1595 (Fig. 3) [24] that have high affinity (K_i = 19 nM) at human A_{2B} receptors will provide better tools for understanding A_{2B} receptor function. In the interim, responses mediated by the non-selective adenosine agonist NECA (Fig. 1) but not by other P1 receptor agonists can be attributed to A_{2B} receptor activation [4].

The A_3 receptor is linked to adenylate cyclase inhibition and elevation of cellular IP_3 levels and intracellular Ca^{2+}. All P1 receptors show varying degrees of species-dependent pharmacology; however, human A_3 receptors are sensitive to xanthine blockade, whereas rat receptors are not [25]. Cl-IB-MECA (Fig. 1) is a potent A_3 receptor agonist. MRS 1220, L-249313, and L-268605 (Fig. 3) are newer non-xanthine A_3 receptor antagonists [9, 25].

The P2 Receptor Family

ATP, acting via P2 receptors, plays a critical role in fast excitatory neurotransmission, tissue development, nocicep-

TABLE 2a. P2X receptor subtypes and pharmacology

Receptor subunit	Rank order of agonist activity	Antagonists	Signal transduction
$P2X_1$	BzATP ≫ 2-MeSATP > ATP > α,β-MeATP	TNP-ATP, TNP-GTP	$I_{Na/K/Ca^{2+}}$
$P2X_2$	2-MeSATP > ATP > α,β-MeATP (inactive)	PPADS	$I_{Na/K}$
$P2X_3$	BzATP > 2-MeSATP > ATP > α,β-MeATP	TNP-ATP, TNP-GTP Other antagonists*	$I_{Na/K/Ca^{2+}}$
$P2X_4$	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	$I_{Na/K/Ca^{2+}}$
$P2X_7$	BzATP ≫ ATP^{4-}	KN-62, PPADS	$I_{Na/K}$, pore formation

*NH 01 and NF023 are selective antagonists for rat P2X receptors with reduced ectonucleotidase activity (see text).

tion, apoptosis, platelet aggregation, astroglial cell function, and metastasis formation [1]. The P2X ionotropic LGIC receptor family is involved in fast excitatory neurotransmission [7], whereas P2Y metabotropic receptors are members of the GPCR superfamily [2].

Seven P2X receptors ($P2X_{1-7}$) and eleven P2Y receptors have been identified (Table 2) [1]. Of the latter, only five—$P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, and $P2Y_{11}$—are distinct molecular entities that elicit functional responses (Table 2b), with the remainder being species variants or proteins lacking ATP-mediated functional activity [2]. The $P2Y_2$, $P2Y_4$, and $P2Y_6$ receptors are uracil nucleotide (pyrimidine) sensitive [17]. The P2X receptor subunit motif is two transmembrane spanning regions, being related to the FMRFamide-gated sodium channel (FNaC channel). However, P2X receptors do not discriminate in their cation permeability [1]. The subunit stoichiometry of the P2X receptor is controversial, with tri-, tetra- and pentameric structures having been proposed [26, 27]. Functional heteromers also exist, the best characterized of which is the $P2X_{2/3}$ [28]. In addition to their structural/signal transduction classification, P2 receptors also can be grouped into three major classes based on agonist sensitivity [29]. Group 1 includes $P2X_1$ and $P2X_3$ receptors that have high affinity for ATP (EC_{50} = 1 μM) and are rapidly activated and desensitized. Group 2 includes $P2X_2$, $P2X_4$, $P2X_5$, and $P2X_6$ receptors that have a lower affinity for ATP (EC_{50} = 10 μM) and show a slow desensitization and sustained depolarizing currents. Group 3 is represented by the $P2X_7$ LGIC, which has very low affinity for ATP (EC_{50} = 300–400 μM), shows little or no desensitization, and in addition to functioning as an ATP-gated ion channel, also functions as a non-selective ion pore [30]. The ability to form non-selective ion pores also is shared by other P2X receptors following prolonged agonist exposure [31, 32].

P2 receptor agonists are typically analogs of ATP and UTP (Fig. 1) with bioisosteric substitutions in the polyphosphate side chain to improve stability [9, 33]. Substitutions in the 2- and N^6-positions on the purine ring can modify receptor selectivity [33]. Putative P2 receptor antagonists include suramin, PPADS, and dyes such as reactive blue-2 (Cibacron Blue) (Fig. 4).These compounds are, however, only marginally selective for one type of P2 receptor over another and also interact with other nucleotide (ATP) binding sites and modulate the activity of other receptors [33]. Antagonists that inhibit ectonucleotidase activity potentiate the half-life of endogenous ATP, thus confusing receptor characterization [34]. The search for selective P2 receptor antagonists using high throughput screening has been hampered by a lack of reliable binding assays. However, functional fluorescent imaging assays in cell lines

TABLE 2b. P2Y receptor subtypes and pharmacology

Receptor subtype	Agonists	Antagonists	Signal transduction
$P2Y_1$	2-MeSATP > ATP > ADP (UTP inactive)	MRS 2179	PLCβ/IP_3 Ca^{2+}
$P2Y_2$	4-thioUTP > UTP = ATP ≫ 2-MeSATP	None	PLCβ/IP_3 Ca^{2+}
$P2Y_4$	UTP = UDP > ATP = ADP	None	PLCβ/IP_3 Ca^{2+}
$P2Y_6$	UTP > ADP = 2-MeSATP > ATP	None	PLCβ/Ip_3 Ca^{2+}
$P2Y_{11}$	ATP > 2-MeSATP > ADP	None	PLCβ/IP_3 Ca^{2+} Adenylate cyclase

See text and Figs. 1 and 4 for information on specific compounds. The nomenclature for P2 receptors has evolved in a somewhat haphazard manner reflecting both the complexity of this superfamily and the limited pharmacological tools available for receptor characterization. Thus, a P_{2X}, P_{2Y}, P_{2T} and P_{2Z} nomenclature was followed by the identification of various pharmacologically defined receptors designated P_{2D}, P_{2U}, P_3, P_4, $P2_{Ap4A}$, etc. [1]. Since ATP was known to produce its receptor-mediated effects via either ion channels or G protein-coupled receptors, P2 receptors were then divided into two main classes: P2X, which are ligand-gated ion channels, and P2Y, which are GPCRs. With the cloning of the members of the P2X and P2Y families, the previous nomenclature systems have been replaced with $P2X_n$ and $P2Y_n$ designations [1]. For the P2X receptor family, these receptors are sequentially numbered 1 through 7 ($P2X_1$–$P2X_7$). For the P2Y family, receptors designated $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, and $P2Y_{11}$ have been cloned and shown to have ATP-sensitive functional activity. This unusual numbering reflects the fact that at least six other putative P2Y receptors have been identified based on putative sequence homology, which are either non-mammalian homologues or receptors for which nucleotides are not the preferred agonists [1, 4].

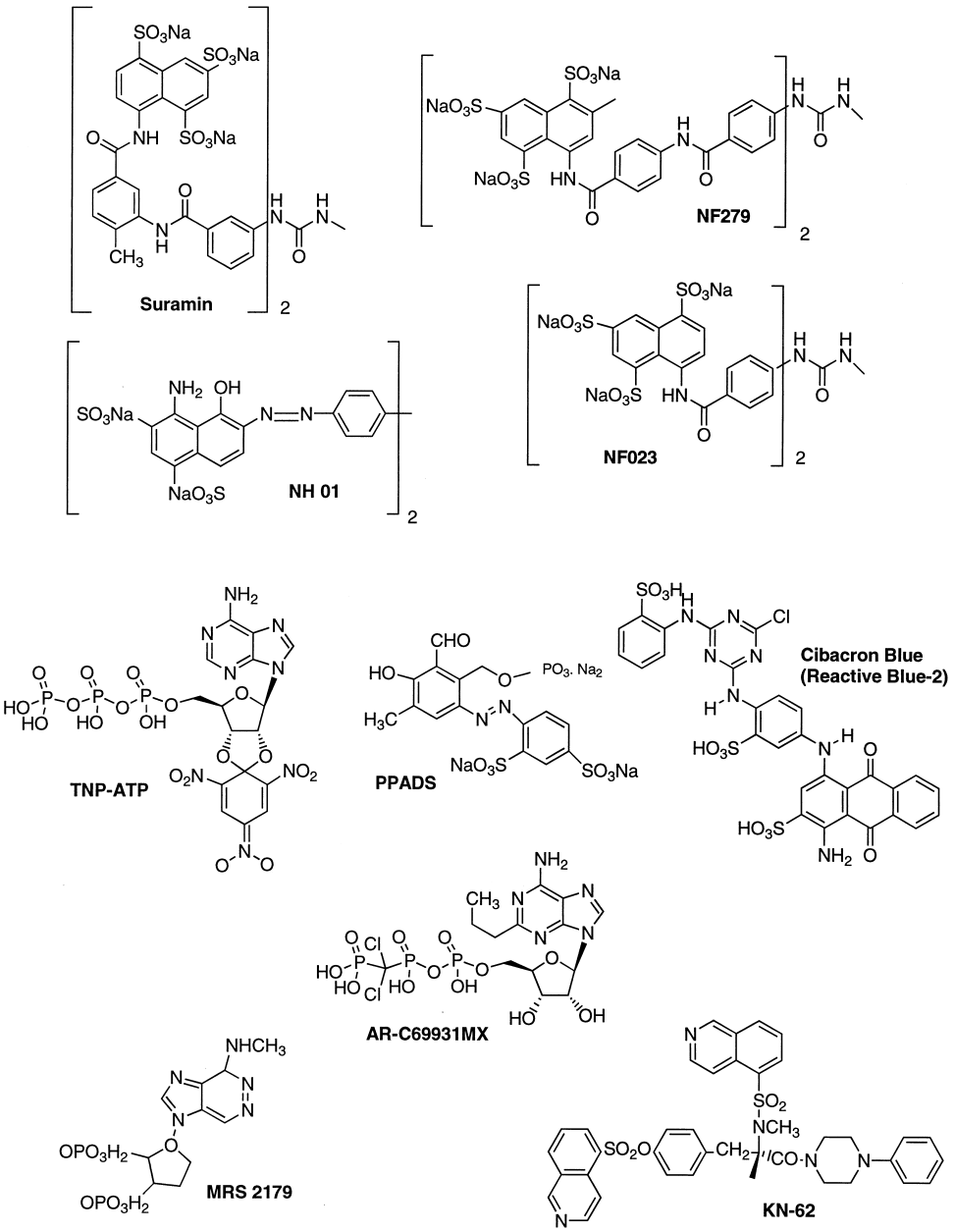

FIG. 4. Structures of P2 receptor antagonists. Abbreviations: TNP-ATP, 2′,3′-O-(2,4,6-trinitrophenyl)-ATP; and PPADS, pyridoxal phosphate-6-azophenyl-2′-4′-disulphonic acid.

transfected with P2 receptors provide an alternative approach [35].

NH 01 and the truncated suramin analogs, NF023 and NF279 (Fig. 4) are selective rat P2X receptor antagonists with reduced ectonucleotidase activity [33]. TNP-ATP is a potent (K_i = 1 nM), reversible antagonist of $P2X_1$, $P2X_3$, and $P2X_{2/3}$ receptors [36]. Whereas it originally was described as a noncompetitive antagonist, TNP-ATP competitively blocks the non-desensitizing $P2X_{2/3}$ receptor. TNP-GTP is equiactive, indicating that adenine is not critical for activity, whereas phosphate removal reduces potency (TNP-ATP > TNP-ADP ≫ TNP-AMP). MRS 2179 is a full, potent (IC_{50} = 330 nM) antagonist for the turkey erythrocyte $P2Y_1$ receptor [37] (Fig. 4). AR-C69931MX is a selective antagonist for the ADP-sensitive $P_{2T}/P2Y_{AC}$ receptor involved in platelet aggregation (IC_{50} = 0.4 nM) [38]. KN-62, a CamK-II inhibitor, has potent $P2X_7$ antagonist activity (IC_{50} = 9–13 nM), being 70–100 times more potent at the $P2X_7$ receptor than CamK-II [39]. KN-62 is active at the human $P2X_7$ receptors but inactive at the rat homolog [40].

P2 receptor ligands have not always been examined for selectivity in a broad spectrum of assays, such that their selectivity is assay- and species-specific. BzATP (Fig. 1), a compound that is widely used as a selective $P2X_7$ receptor agonist (EC_{50} = 18 μM), is four to five orders of magnitude more potent at functional $P2X_1$ (EC_{50} = 1.9 nM) and $P2X_3$ (EC_{50} = 98 nM) receptors [35], suggesting that many

BzATP responses ascribed to $P2X_7$ receptors may be mediated by $P2X_1$ or $P2X_3$ receptors.

THERAPEUTIC ASPECTS OF P1 AND P2 RECEPTOR FUNCTION

Compounds that produce their effects via purinoceptor systems, including P1 or P2 receptors, comprise three distinct classes: (i) conventional agonist, partial agonist, or antagonist ligands; (ii) allosteric modulators of receptor function; and (iii) modulators of the enzyme systems regulating the extracellular availability of ATP, adenosine, UTP, and their respective nucleotides. This latter group includes the various ecto-ATPases [5, 6], adenosine deaminase, AK, and the bidirectional transporter systems that remove adenosine from the extracellular environment [16, 41, 42].

The development of directly acting P1 receptor agonists and antagonists as therapeutic agents [12] has been largely unsuccessful due to the choice of disease states in which other therapeutic modalities exist (e.g. hypertension) and to the side-effects associated with global P1 receptor modulation. However, newer P1 antagonists, e.g. KW6002 [43] (for Parkinson's disease) and FK 838 [44] and BG9719 [45] (Fig. 3), the latter two for congestive heart failure, are being examined currently in the clinic.

Partial agonists with enhanced tissue specificity [42], allosteric modulators [22], or modulators of adenosine and ATP metabolism may be clinically useful agents with improved therapeutic indices [41, 42]. Compounds targeted at P2 receptors are in early stages of development, with the P_{2T} receptor antagonist AR-C69931MX (Fig. 4) [38] and UTP (Fig. 1) [46] being the most advanced.

Epilepsy

Rapid increases in brain adenosine occur during seizure activity [47, 48], and adenosine agonists, acting via A_1 receptors, reduce seizure activity induced by a variety of chemical and electrical stimuli without altering the seizure threshold [47]. These anticonvulsant effects of adenosine are blocked by doses of methylxanthines that, when given alone, have no effect on seizure activity. Agents that limit adenosine metabolism enhance the anticonvulsant effects of adenosine. Thus, AK inhibitors block chemically induced seizures in mice and rats [49, 50], being more effective than inhibitors of adenosine deaminase or transport. Microinjection of ATP analogs into the prepiriform cortex, where $P2X_2$, $P2X_4$, and $P2X_6$ receptors are present, induces generalized motor seizures similar to those seen with *N*-methyl-d-arginine or bicuculline [47]. Thus, a P2X receptor antagonist may be a potentially novel approach to anticonvulsant therapy.

Auditory and Ocular Function

P2 and P2 receptors are present in the vestibular system, and novel $P2X_2$ receptor splice variants are present in rat and guinea pig cochlea [51]. The latter are present on the endolymphatic surface of the rat cochlear endothelium, an area associated with sound transduction [52]. P2Y receptors are present in the marginal cells of the stria vascularis, which is involved in generating the ionic and electrical gradients of the cochlea. ATP has the potential to regulate fluid homeostasis, hearing sensitivity, and development. Perilymphatic ATP depresses the sound-evoked gross compound action potential of the auditory nerve and the distortion product otoacoustic emission, the latter a measure of the active process of the outer hair cells [53]. A_1 and A_3 receptors are also present in the auditory system, and cisplatin-induced ototoxicity is accompanied by their up-regulation in the cochlea [54]. Introduction of the A_1 agonist, *R*-phenylisopropyladenosine (*R*-PIA) into the cochlea attenuates the deleterious effects of repeated exposure to high-intensity, high-frequency noise [55].

Parkinson's Disease and Schizophrenia

Nearly 30 years ago, the methylxanthine adenosine antagonist, caffeine [10] was found to stimulate rotational behavior and to potentiate the effects of DA agonists in rats with unilateral striatal lesions [56]. Subsequently, adenosine agonists were found to block the behavioral effects of DA, acting via A_{2A} receptors and thus acting as an indirect DA antagonist. Adenosine A_{2A} receptor mRNA and DA D_2 receptors are co-localized in GABAergic–enkephalin striatopallidal neurons in the basal ganglia [56]. The modulatory influence of adenosine on dopaminergic neurotransmission is enhanced following increased DA receptor sensitivity. Chronic haloperidol treatment can up-regulate D_2 and A_{2A} receptors in the rat striatum. In mouse A_{2A} receptor knockouts, exploratory motor activity is reduced [57]. Caffeine further reduces this activity, an effect opposite to the psychomotor stimulant effects of this adenosine antagonist. The A_{2A} receptor antagonists, KF 17837 and KW6002, potentiate the antiparkinsonian effects of l-dopa in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated marmosets without producing dyskinesia [44, 58]. All known DA antagonists selectively disrupt the conditioned avoidance response, and activity in this paradigm is correlated with clinical efficacy in treating schizophrenia. Adenosine agonists, like DA antagonists, disrupt the conditioned avoidance response without impairing escape behavior [59]. A selective A_1 adenosine agonist, CI-936, was advanced to the clinic over a decade ago as a novel antipsychotic, but was terminated for unknown reasons.

Pain

ATP is involved in the processing of nociceptive sensory information and may be directly involved in the pain associated with causalgia, reflex sympathetic dystrophy, angina, migraine, and cancer [60]. ATP is also a mediator of neurogenic inflammation, acting via P2 receptors on neutrophils, macrophages, and monocytes to induce cytokine

and prostaglandin release [61]. Peripheral administration of ATP produces pain at the site of administration [62] and facilitates nociceptive responses to other noxious stimuli [63, 64]. These effects are blocked by the P2 antagonists suramin and PPADS (Fig. 4) [62, 64, 65]. ATP released in response to tissue trauma can activate $P2X_3$ receptors that initiate and contribute to the peripheral and central sensitization associated with cutaneous and visceral nociception [63, 64]. $P2X_3$ homomeric and $P2X_{2/3}$ heteromeric receptors are highly localized on sensory ganglia [66, 67] and can be regulated by extracellular Ca^{2+} concentrations [68]. $P2X_3$ receptor expression is up-regulated in sensory afferents and spinal cord following damage to peripheral sensory fibers [69], whereas neonatal capsaicin treatment reduces $P2X_3$ message in the dorsal root ganglion [1] and abolishes ATP-mediated acute nociceptive responses [70]. $P2X_3$ receptor antagonists thus may have potential as novel analgesics.

Adenosine has opposite effects to ATP, inhibiting nociceptive processes in the brain and spinal cord [71]. Given intrathecally, adenosine agonists and AK inhibitors provide pain relief in a broad spectrum of animal models of pain [41, 72], including neuropathic pain [73, 74] and pain elicited by spinal injection of substance P and the glutamate agonist *N*-methyl-D-aspartate [75]. Spinal administration of the A_1 agonist, *R*-PIA relieved allodynia in a neuropathic pain patient without affecting normal sensory perception [76], whereas infusion of adenosine at doses without effects on cardiovascular responses improved pain symptoms [77], reducing spontaneous pain, ongoing hyperalgesia, and allodynia in patients with neuropathic pain [78]. Administered with NO, ATP, probably acting via adenosine formation, as well as AK inhibitors mimic the effects of the inhalation anesthetic enflurane [79, 80] and reduce the amount of inhalation anesthetic required for anesthesia.

Inflammation

Adenosine is released at sites of inflammation and exerts anti-inflammatory effects via multiple mechanisms [81]. Adenosine inhibits neutrophil rolling and adhesion to vascular endothelium, decreases oxygen free radical production by neutrophils via A_{2A} receptor activation, and also exerts effects on endothelial cell permeability, reducing bradykinin- and histamine-induced vascular leakage, via activation at A_1 and A_{2A} receptors. Adenosine also inhibits production of the pro-inflammatory cytokine, tumor necrosis factor-α by macrophages *in vitro* (A_{2A}, A_{2B}, and A_3 receptors) and suppresses tumor necrosis factor-α mRNA expression and plasma levels *in vivo*. The purine also inhibits production and gene expression of the matrix metalloprotease collagenase (matrix metalloprotease-1), but not tissue inhibitor of metalloproteases-1 or stromelysin, on synoviocytes (A_{2B} receptor). An AK inhibitor, GP 515 (Fig. 1) [82] improved survival in a murine septic shock model and a rat model of bacterial peritonitis, suggesting that the anti-inflammatory actions of adenosine do not suppress normal immune responses to infection. AK inhibitors also decreased carrageenan-induced pleurisy and paw edema, and air pouch and dermal neutrophil accumulation after local injection of inflammatory mediators. A_1 receptor agonists and AK inhibitors also inhibit pleural and peritoneal inflammation in rats [41, 81].

The anti-inflammatory actions of nonsteroidal anti-inflammatory drugs have been reported recently [83] to be independent of the inhibition of COX, since the anti-inflammatory effects of aspirin, sodium salicylate, and sulfasalazine were all maintained in COX-2 and NFκB knockout mice. Adenosine levels in air pouch exudates from aspirin-treated mice were increased 17-fold versus controls (224 vs 13 nM). The anti-inflammatory effects of aspirin were reversed by the addition of the A_{2A} receptor antagonist 3,7-dimethyl-1-propargylxanthine (DMPX) or the adenosine catabolic enzyme, adenosine deaminase. It was proposed that adenosine acts as an anti-inflammatory autacoid independent of inhibition of either COX-1 or COX-2 or of NFκB p105.

P2 receptors also can contribute to inflammation. Local application of ATP produces a localized inflammatory response [84], whereas the potency of ATP agonists to elicit nociceptive responses increases during inflammation.

Sleep

Adenosine has profound hypnotic and sedative effects [84], whereas adenosine antagonists such as caffeine [10] and theophylline are central stimulants. Direct administration of adenosine into the brain elicits an electroencephalographic profile indicative of deep sleep, e.g. an increase in REM sleep with a reduction in REM sleep latency, resulting in an increase in total sleep [85]. In contrast, caffeine suppresses REM sleep and decreases total sleep time. Extracellular adenosine concentrations are increased in the basal forebrain in proportion to periods of sustained wakefulness and decline during sleep, suggesting that the purine functions as an endogenous sleep regulator [86]. Infusion of the A_{2A} agonist, CGS 21680C into the subarachnoid space of the rostral basal forebrain, an area involved in sleep promotion, increases slow wave and paradoxical sleep, effects blocked by the A_{2A} antagonist, KF 17837 [87].

Trophic Actions of ATP and Neurodegenerative Disorders

Trophic factors ensure neuronal viability and regeneration and are increased following neural injury [88]. ATP can act synergistically with growth factors to stimulate astrocyte proliferation, contributing to the process known as reactive astrogliosis, a hypertrophic/hyperplastic response associated with brain trauma, stroke/ischemia, seizures, and neurodegenerative disorders.

In reactive astrogliosis, astrocytes undergo process elongation and express the intermediate filament protein, glial fibrillary acidic protein, with an increase in astroglial cellular proliferation. ATP, like basic fibroblast growth

factor, increases astrocyte glial fibrillary acidic protein and AP-1 complex formation [88]. ATP and GTP induce trophic factor (nerve growth factor, neurotrophin-3, fibroblast growth factor) synthesis in astrocytes and neurons, an effect that is not consistent with the profile of any known P2 receptor. The hypoxanthine analog, AIT-082 (Fig. 3) up-regulates neurotrophin production, enhances working memory, and restores age-induced memory deficits in mice [89] and currently is showing promise in Phase II clinical trials for Alzheimer's disease.

The $P2X_7$ or P_{2Z} receptor is a unique member of the P2X receptor family that functions as a non-selective ion pore in mast cells, platelets, macrophages, and lymphocytes [29]. $P2X_7$ receptor activation triggers apoptosis and stimulates the release and maturation of interleukin-1β from macrophages by activating interleukin-1β convertase. $P2X_7$ receptors are present in the microglia within the superior cervical ganglia and spinal cord, and cerebral artery occlusion increases $P2X_7$ immunoreactive cells in the stroke penumbra [15].

ATP also induces $P2X_7$ receptor-mediated cytolysis in macrophages infected with *Mycobacterium* via both apoptotic and necrotic events [90]. This antimicrobial activity of ATP may have potential use in the treatment of tuberculosis and also may provide a more basic understanding of $P2X_7$ receptor-mediated apoptotic events than can be derived from more complex mammalian cell systems.

Neurourology

The urinary bladder is controlled by both sympathetic and parasympathetic nervous system input. ATP mimics the effects of parasympathetic stimulation, resulting in bladder contraction [91, 92] via activation of P2X receptors present in the smooth muscle of the urinary bladder detrusor muscle involved in bladder emptying [93]. Detrusor malfunction can lead to UUI, a major health problem in the aging female population.

Micturition involves urethral relaxation, in which ATP functions as a co-transmitter with NO. NO mediates the first stage of relaxation [94] and ATP the second, acting via P2 receptors. P2X receptors are also present in the bladder urothelium, and serosal ATP release occurs in the rabbit bladder as a result of the hydrostatic pressure changes associated with bladder filling [95]. Muscarinic receptors mediate 15% of rat urinary bladder neurogenic contraction, and another 50% is mediated by P2X receptor mechanisms [96]. Muscarinic antagonists such as oxybutynine and tolteridine are the mainstay treatment for UUI and have typical muscarinic side-effects. P2X receptor antagonists may be potentially improved agents for the treatment of UUI, provided that their side-effect profile is acceptable.

Cancer

The cytolytic actions of ATP have potential utility in the treatment of cancer and cancer cachexia [97]. Unpublished Phase II trials indicated that ATP administration via a 96-hr infusion of 50 mg/kg/min every 28 days resulted in an expansion of red blood cell ATP pools that was correlated with cytostatic and cytotoxic effects on tumor growth, inhibition of cachexia, improvements in organ function, analgesia, enhancement of superoxide production, and modulation of blood flow. While these trials reportedly had positive effects on quality of life outcomes, they were discontinued for unknown reasons. ATP also functions as a cytotoxic agent in patients with cystic fibrosis [98].

Diabetes

ATP stimulates pancreatic insulin release via a glucose-dependent mechanism involving $P2Y_1$ receptors [99]. P2Y receptor agonists thus may have potential as antidiabetic agents. ATP, acting via a P2Y-like receptor, evokes a redistribution of the glucose transporters GLUT1 and GLUT4 from the plasma membrane to microsomal membranes in cardiomyocytes [100]. ATP also modulates insulin secretion by interactions with ATP-sensitive potassium channels in islet β-cells. ADP antagonizes the ATP inhibition of these channels by binding to the second nucleotide binding site on the associated sulfonylurea receptor, thus activating K_{ATP} channels and inhibiting insulin secretion [101].

Pulmonary, Renal, and Cardiovascular Function

ATP and UTP, acting via the $P2Y_2$ receptor, potently stimulate chloride secretion in airway epithelium and mucin glycoprotein release from epithelial goblet cells [46]. UTP is a potent and selective modulator of mucociliary transport in the lung with potential utility in the treatment of chronic obstructive pulmonary diseases. UTP was selected because it has the advantage in use over ATP in that its breakdown product, uracil, unlike adenosine, has limited ancillary pharmacology at the doses of UTP used [18]. UTP is being developed as an inhalation formulation to enhance mucociliary clearance for the potential treatment of cystic fibrosis and chronic bronchitis.

ATP also may play a role in asthma via its actions on bronchial innervation. Thus, the nucleotide, as well as adenosine, triggers a reflex neurogenic bronchoconstriction. The effects of ATP involve activation of P2X receptors on vagal C fibers [102].

In the cardiovascular system, adenosine acts as a potent coronary vasodilator, slows heart rate and atrioventricular node conductance, and antagonizes the stimulatory actions of β-adrenoceptor agonists [103]. The purine is also an endogenous cardioprotective agent that is released during ischemia and inhibits neutrophil activation and the effects of the associated inflammatory sequelae on the endothelium, thus reducing infarct size and preserving endothelial function [104]. AMP-579 (Fig. 1) is a weakly selective A_1 receptor agonist currently in Phase II as a cardioprotectant [105]. Adenosine also mediates the protective phenomenon

known as preconditioning via activation of both A_1 and A_3 receptors, acting via a K_{ATP} channel to promote cardiomyocyte survival under ischemic conditions [104, 106].

Activation of adenosine A_1 receptors in the kidney increases Na^+ reabsorption in proximal and distal tubules [107]. Selective A_1 receptor blockers, e.g. BG 9719 [43] and FK 838 [42], are diuretics that produce natriuresis with minimal effects on kaliuresis, renal blood flow, and glomerular filtration rate and may be useful in the treatment of congestive heart failure and acute renal failure [108]. Adenosine agonists inhibit renin release, whereas antagonists increase renin release, leading to the concept of an "adenosine-brake hypothesis," the physiological relevance of which is being evaluated [108]. ATP also can influence renal tubular transport, acting via $P2Y_2$ receptors to stimulate transport in proximal and distal tubules and via a P2X receptor to inhibit transport [107].

Other Targets for ATP Action

In identifying potent and selective P2 receptor ligands for the potential treatment of human disease states, it will be important to recognize the multiplicity of actions associated with ATP, especially in regard to molecular targets other than P2 receptors. In addition to elucidating the structural requirements to identify compounds interacting with the various P2 receptors, it will be critical to establish how these differ from the requirements for recognition by members of the ATP binding cassette protein family [109], ectonucleotidases [5, 6], ATP-modulated potassium channels [3], and other enzymes that utilize ATP for their function.

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