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PURINERGIC NEUROTRANSMISSION

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The purine nucleoside, adenosine, its nucleotides, adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine monophosphate (AMP), and the pyrimidine nucleotide, uridine triphosphate (UTP) (Fig. 15.1), play a critical role in central and peripheral nervous system homeostasis and function as extracellular messengers to regulate cell function. The effects of adenosine and the nucleotides are mediated by activation of distinct P1 (adenosine) and P2 (ATP) cell-surface receptors present on neurons, astrocytes, and microglia, as well as other cells that are present in the central nervous system (CNS) under different conditions (e.g., macrophage infiltration). These receptors are generically known as *purinergic receptors* (1).

Adenosine, ADP, and ATP, and, to a lesser extent, UTP are well-known intracellular constituents, intimately involved in all aspects of cell function acting as enzyme cofactors, sources of energy, and building blocks for DNA. Thus, the factors regulating their availability in the extracellular space as chemical messengers have been an area of active research and considerable debate since the late 1970s (2).

ATP can be released as a cotransmitter together with acetylcholine, norepinephrine, glutamate, γ-aminobutyric acid (GABA), calcitonin gene–related peptide, vasoactive intestinal peptide, and neuropeptide Y (3). ATP is available on demand, and the body can synthesize its own weight in ATP per day (4). Even though extracellular ATP levels can reach millimolar concentrations in the extracellular local environment after release or cellular perturbation (1), these concentrations are miniscule compared with the overall steady-state nucleotide content of the cell. Once released, in addition to interacting directly with P2 receptors, ATP can be hydrolyzed by a family of approximately 11 ectonucleotidases that metabolize ATP, ADP, diadenosine polyphosphates such as Ap4A, Ap5A (Fig. 15.2), and nicotinamide-adenine dinucleotide (5). Ecto-ATPases hydrolyze ATP to ADP, ectoapyrases convert both ATP and ADP to AMP, and ecto-5′-nucleotidase converts AMP to adenosine. The activities of ectoapyrase and ecto-5′-nucleotidase can change with cellular dynamics (6), and in guinea pig vas deferens, soluble nucleotidases are released together with ATP and norepinephrine (7), a finding representing a potential mechanism to limit the actions of extracellular ATP by enhancing its inactivation. The metabolic pathways linking ATP, ADP, AMP, and adenosine and the potential for each of these purines to elicit distinct receptor-mediated effects on cell function form the basis of a complex, physiologically relevant, purinergic cascade comparable to those involved blood clotting and complement activation (8) (Fig. 15.3).

The extracellular effects of ATP on the various members of the P2 receptor family are terminated either by receptor desensitization or by dephosphorylation of the nucleotide, leading to the formation of ADP, AMP, and adenosine. These latter compounds have their own receptor-mediated functional activities, some of which are antagonistic to one another. For instance, ATP antagonizes ADP actions on platelet aggregation, whereas adenosine-elicited CNS sedation contrasts with the excitatory actions of ATP on nerve cells (9). In the broader framework of ATP-modulated proteins (or ATP-binding cassette proteins), ATP-sensitive potassium channels (K_{ATP}) undergo activation when intracellular ATP levels are reduced (10,11). Thus, as P2 receptor–mediated responses decrease because of ATP hydrolysis to adenosine, P1-mediated responses and K_{ATP}-mediated responses are enhanced. In addition to activating the as yet uncloned platelet $P2_T$ receptor, ADP also enhances its own availability. Activation of A_1 and A_{2A} receptors can inhibit ATP availability (1), and activation of hippocampal A_{2A} and A_3 receptors can desensitize A_1 receptor–mediated inhibition of excitatory neurotransmission (12). The transfer of purines transfer from one cell to another in the context of cellular adenylate charge (13) reflects another means by which purines can modulate cellular communication, in terms of both information transfer and alteration of the target environment. ATP also functions as a substrate for synaptic ectokinases, which modulate the phosphorylation state of the synaptic membrane (14) and, consequently, the intrinsic properties of the synapse. Once in the extracellular space, ATP thus has the ability to function as a pluripotent modulator of synaptic function.

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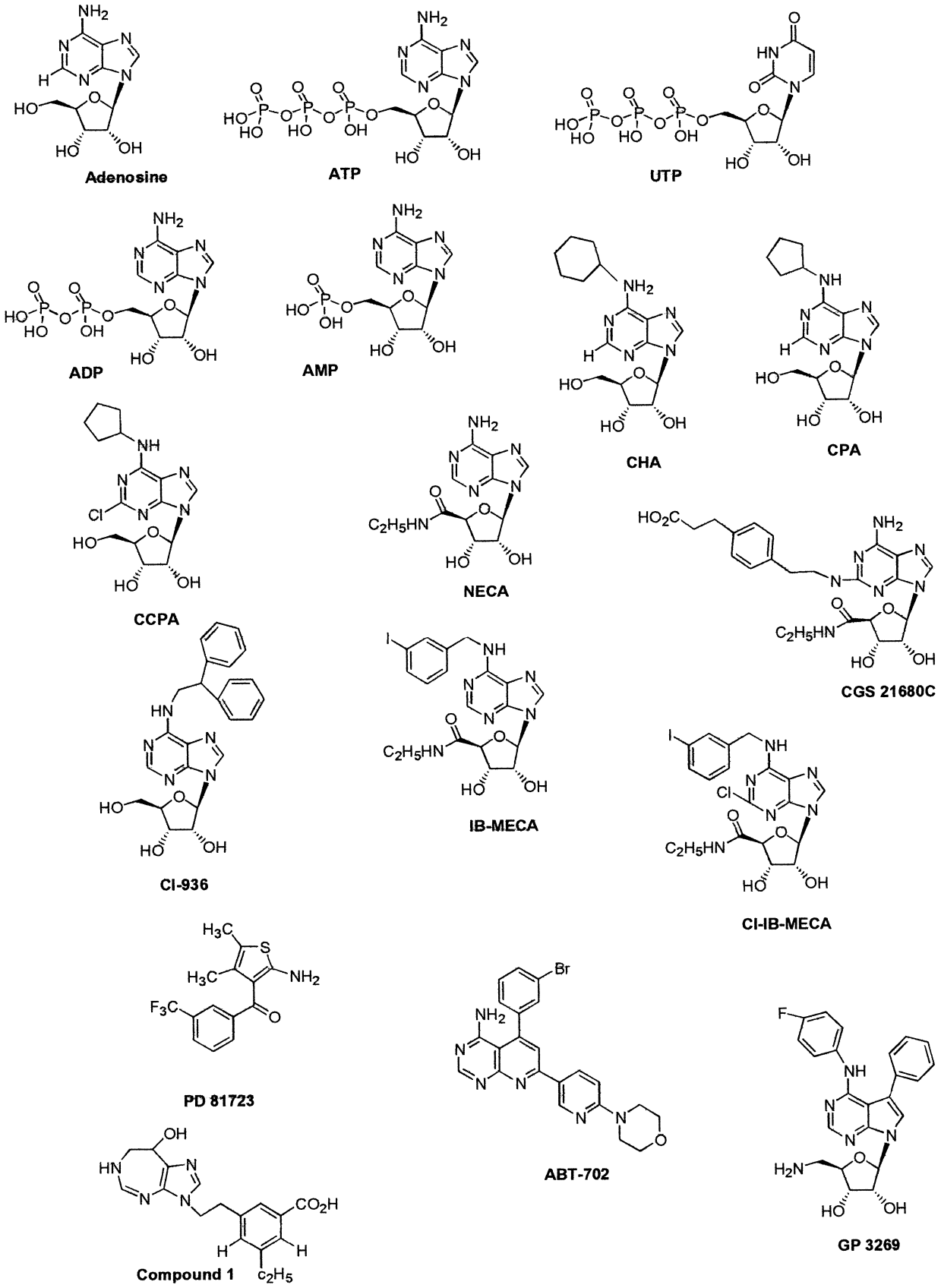

FIGURE 15.1. Structures of P1 and P2 agonists and modulators of adenosine availability.

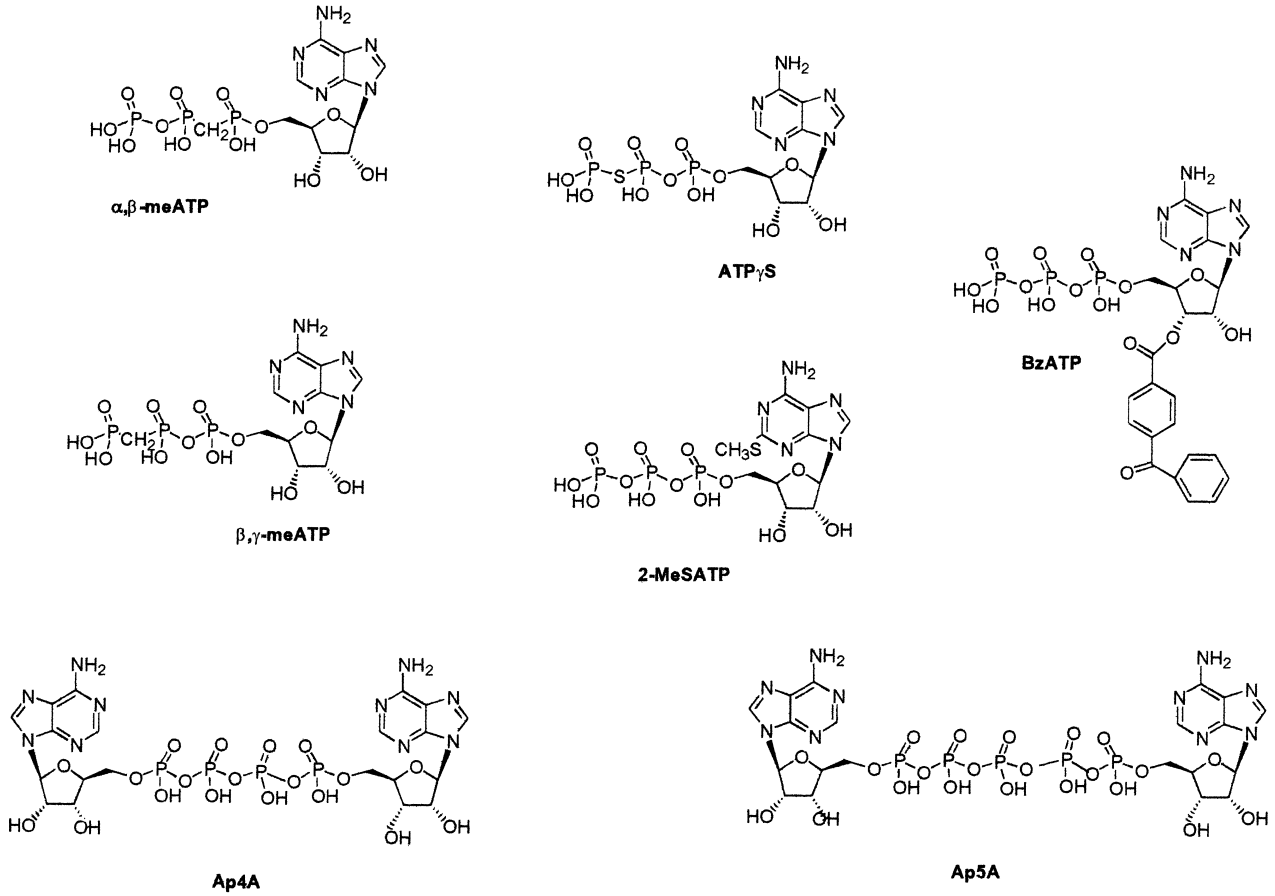

FIGURE 15.2. Structures of P2 receptor agonists.

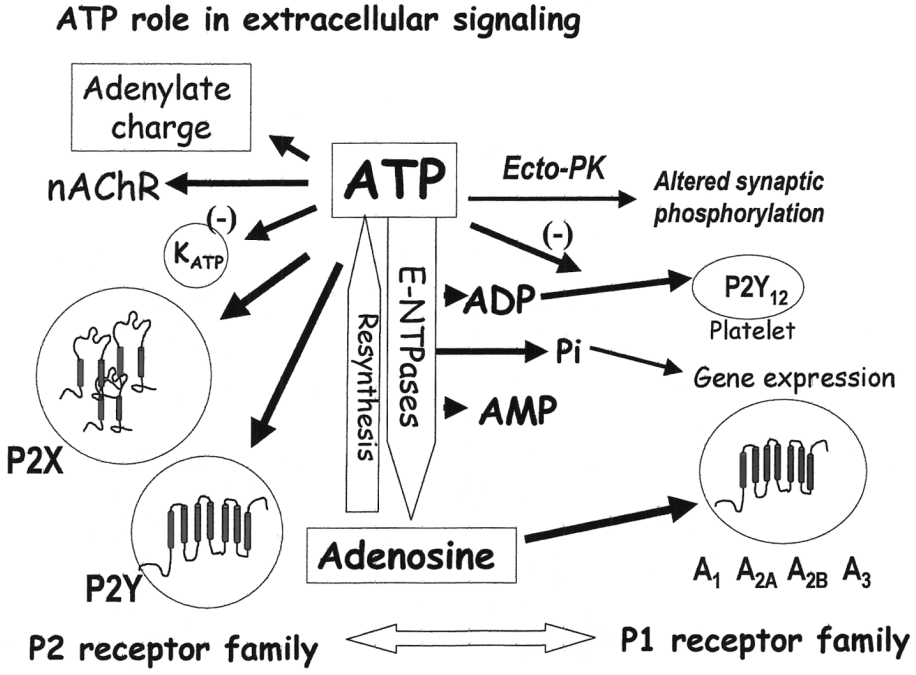

FIGURE 15.3. The purinergic cascade. ATP is released into the extracellular milieu from nerves or cells, where they can interact to form a purinergic cascade. ATP acts at a variety of P2 receptors (see text) and is sequentially degraded to ADP and AMP by ectonucleotidase activity. ADP interacts with P_{2T} receptors. AMP gives rise to adenosine, which can interact with the various P1 receptors (A_1, A_{2A}, A_{2B}, A_3). Adenosine can also be formed by intracellular 5′-nucleotidase activity. Adenylate charge indicates the transfer of energy in the form of adenine nucleosides or nucleotides from one cell to another (see ref. 13). KATP is an ATP-modulated potassium channel.

The corresponding role of UTP in terms of functional synaptic signaling is less well understood (15), and although high concentrations of exogenous uracil have been shown to modulate dopaminergic systems in the CNS (16), data on the existence of a ''uridine receptor'' equivalent to the P1 receptor are limited (17).

Extracellular adenosine levels at rest are in the range of 30 to 300 nM (18), and they subserve a physiologic role in tissue homeostasis as reflected by the CNS stimulant actions of caffeine, a natural methylxanthine that acts as an antagonist to counteract the sedative actions of endogenous adenosine, and the role of the nucleoside as an endogenous hypnotic (19). Adenosine also acts as an autocrine homeostatic agent or, as conceptualized by Newby (20), a ''retaliatory metabolite,'' to regulate the tissue energy supply-and-demand balance in response to changes in blood flow and energy availability and thus to conserve tissue function under adverse conditions. Reduced oxygen or glucose availability resulting from tissue trauma, such as during stroke, epileptogenic activity, and reduced cerebral blood flow, leads to ATP breakdown and the formation of ADP, AMP, and adenosine.

Under basal conditions, extracellular levels of adenosine are tightly regulated by ongoing metabolic activity. Bidirectional nucleoside transporters and the enzymes adenosine deaminase (ADA) and adenosine kinase (AK) regulate adenosine removal from the extracellular space (21). Numerous studies have shown that inhibition of AK is physiologically more relevant in increasing extracellular adenosine levels than inhibition of ADA or adenosine transport. AK inhibitors are also more effective in enhancing the neuroprotective actions of endogenous adenosine than inhibitors of ADA or adenosine transport (8,21). Selective AK inhibitors, such as GP 3269 and ABT-702 (Fig. 15.1), and ADA inhibitors, such as compound 1 (Fig. 15.1), are effective site- and event-specific agents that can locally enhance levels of adenosine in areas of tissue trauma and thus have the potential to avoid the potential cardiovascular side effects associated with a general elevation of extracellular levels of the purine (8). However, data have shown that *in vivo* administration of AK inhibitors (22), even at single doses close to where these agents show efficacy in animal models of epilepsy and pain, results in brain microhemorrhaging that can lead to minicerebral infarcts and cognitive impairment. Based on this finding, the AK approach to selective modulation of endogenous adenosine function does not appear to have a sufficient therapeutic window in CNS tissues to be a viable drug discovery target.

Adenosine has both presynaptic and postsynaptic effects on neurotransmission processes (12), whereas ATP has excitatory actions in a variety of neuronal systems including rat trigeminal nucleus, nucleus tractus solitarius, dorsal horn, and locus ceruleus. The nucleotide also functions as a fast transmitter in guinea pig celiac ganglion and rat medial habenula (9). Electrophysiologic studies on P2X and neuronal nicotinic receptor (nAChR)–mediated responses suggested that these two ligand-gated ion channels (LGICs) interacted with one another, with each receptor containing a inhibitory binding site for agonists active at the corresponding receptor and resulting in functional cross-talk between the systems (23). Recombination of nAChR α-subunits with P2X receptor subunits to form functional receptor constructs has also been reported (24), a finding further suggesting that heterooligimerization between these two different classes of LGICs may occurs and represents a molecular basis for the cross-talk hypothesis. This finding also adds a layer of further complexity to an already complex situation in understanding the precise subunit composition of functional P2X receptors. Dimerization of the A_1 subtype of the P1 (adenosine) G-protein–coupled receptor (GPCR) also occurs (25), a finding consistent with the emerging view that functional homooligimerization and heterooligimerization of a variety of GPCRs is the norm rather than the exception (26).

P1 AND P2 RECEPTORS

Four distinct P1 receptors sensitive to adenosine and 12 P2 receptors sensitive to ADP, ATP, and UTP have been cloned and characterized (1), thus providing a diversity of discrete cellular targets through which adenosine, ADP, ATP, and UTP can modulate tissue function (Table 15.1). The four adenosine-sensitive P1 receptors are designated A_1, A_{2A}, A_{2B}, and A_3. Functional P2 receptors are divided into ionotropic P2X receptors, a family of eight LGICs ($P2X_1$ to $P2X_8$), and the metabotropic P2Y family, which consists of the $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, and $P2Y_{13}$ GPCRs. The missing numbers in the P2Y family sequence are proposed receptors that have been subsequently found to lack functional responses, are species variants, or have been inadvertently assigned to the P2 receptor family (1,8).

Adenosine (P1) Receptors

All four P1 GPCRs—A_1, A_{2A}, A_{2B}, and A_3 (Table 15.1)—are heterogeneously distributed in a variety of mammalian tissues including heart, smooth muscle, kidney, testis, platelets, leukocytes, and adipocytes. in addition to the CNS. The A_1 receptor is widely distributed in the CNS and is functionally coupled to inhibition of cyclic AMP (cAMP) formation, stimulation of potassium conductance, inhibition of N-channel–mediated calcium conductance, stimulation of phospholipase C production, and modulation of nitric oxide production (1,12). Selective agonists for the A_1 receptor are all adenosine analogues and include cyclohexyl (CHA; A_1 K_i = 1 to 5 nM), cyclopentyl (CPA; A_1 K_i = 0.6 nM), and 2-chlorocyclopentyl (CCPA; K_i = 0.6 nM) adenosine (27) (Fig. 15.1). Agonist effects at the

TABLE 15.1. P2X RECEPTORS[a]

	Agonist Rank Order Potency	Antagonist Rank Order Potency
$P2X_1$	2-MeSATP ≥ ATP ≥ α,β-meATP > BzATP	Ip_5I >> suramin, PPADS > MRS 2220
$P2X_2$	2-MeSATP > ATP >> α,β-meATP	Suramin, PPADS ≥ TNP-ATP
$P2X_3$	2-MeSATP > ATP > BzATP	TNP-ATP >> suramin, PPADS
$P2X_4$	ATP > 2-MeSATP > α,β-meATP	TNP-ATP >> suramin
$P2X_5$	ATP > 2-MeSATP >> α,β-meATP	Suramin, PPADS
$P2X_6$	ATP > 2-MeSATP >> α,β-meATP	Suramin
$P2X_7$	BzATP > ATP > UTP >> α,β-meATP	KN-62 >> suramin, PPADS
$P2X_8$	ATP = 2meSATP> α,β-meATP > ATPγS	Suramin, PPADS

[a]Functional heteromers composed of $P2X_{1/5}$, $P2X_{2/3}$, $P2X_{2/6}$, and $P2X_{2/6}$ subunits have been described.

A_1 receptor are selectively blocked by the 8-substituted xanthines, cyclopentylxanthine (Fig. 15.3) (CPX; K_i = 0.46 nM) and CPT (K_i = 24 nM), and by nonxanthines such as N-0861 (K_i = 10 nM). The A_1 receptor shows distinct species pharmacology (8). Like other GPCRs, it can be allosterically modulated (28) by compounds such as PD 81,723 (Fig. 15.1) that, although not directly interacting with the agonist binding site of the receptor, stabilize an agonist-preferring conformation of the A_1 receptor independent of G-protein interactions (29).

The A_2 receptor exists in two distinct molecular and pharmacologically subtypes, both of which are linked to activation of adenylate cyclase (1). The A_{2A} receptor has high affinity for adenosine, may also use N- and P-type Ca^{2+} channels as signal transduction mechanisms, and is localized in the striatum, nucleus accumbens, and olfactory tubercle regions of mammalian brain. The lower-affinity A_{2B} receptor is more ubiquitously distributed throughout the CNS (1,30). The adenosine analogue, CGS 21680C (Fig. 15.1) (K_i A_{2A} = 15 nM), is the present agonist of choice for the A_{2A} receptor with the xanthine antagonists KF 17837 (Fig. 15.4) (K_i A_{2A} = 24 nM) and CSC 8-(3-chlorylstyryl) caffeine (K_i A_{2A} = 9 nM) and nonxanthines such as SCH 58261 (K_i A_{2A} = 2.3 nM) and ZM 241385 (K_i A_{2A} = 0.3 nM), being up to 6,800-fold selective for the A_{2A} receptor (27). Like the A_1 receptor, the A_{2A} receptor also shows species-dependent pharmacology (8). The A_{2B} receptor has been cloned and is widely distributed in brain and peripheral tissues (1,30). However, its functional characterization has proven difficult because of a paucity of selective ligands. Responses more potently elicited by the nonselective adenosine agonist, NECA (Fig. 15.1) and not by selective A_1-, A_{2A}-, or A_3-receptor agonists can be attributed to A_{2B}-receptor activation. Enprofylline (Fig. 15.3) is a selective, albeit weak, A_{2B}-receptor antagonist (30).

The A_3 receptor was the first P1 receptor identified by cloning rather than by pharmacologic characterization and is linked to inhibition of adenylate cyclase and elevation of cellular inositol 1,4,5-triphosphate (IP_3) levels and intracellular Ca^{2+}. It also shows distinct species-dependent pharmacology, especially in regard to xanthine antagonist blockade of the rat A_3 receptor (1,31,32), and it shows widespread distribution with low levels in brain. IB-MECA (Fig. 15.1) (K_i = 1nM) and its 2-chloro analogue (Cl^- IB-MECA; K_i = 0.3 – 0.7 nM) are potent and selective A_3-receptor agonists (27). The human, but not the rat, A_3 receptor is selectively blocked by xanthines (31), such as DBXRM (K_i A_3 = 229 nM), and by nonxanthines such as MRS 1191, MRS 1220, L-249313, L-268605, and MRE-1008-21M (Fig. 15.4). A_3 receptors are involved in mast cell function, eosinophil apoptosis, and the phenomenon known as preconditioning that occurs during ischemic reperfusion of the heart that protects against myocardial infarction (1,33).

P2 (ATP and UTP) Receptors

P2 receptors were originally classified on the basis of the rank-order potency of agonists structurally related to ATP. Most of these putative receptors (with the exception of the P_{2T} receptor) have been subsequently cloned and functionally characterized in various heterologous expression systems (1). However, their functional characterization in native tissues and in animals has been limited by a paucity of potent, selective, and bioavailable ligands, both agonists and antagonists. All known P2-agonist ligands are analogues of ATP, UTP, and ADP and, irrespective of their degree of chemical modification, show varying degrees of susceptibility to extracellular degradation and differences in intrinsic activity (27,34). The selectivity and potency of these agonists are thus very much dependent on the tissue preparation and species used and also on the experimental protocol. Indeed, few studies exist in which a systematic evaluation of the relative selectivity of P2-receptor agonists has been determined. BzATP (Fig. 15.2), which is widely used as a selective agonist for the $P2X_7$ receptor (EC_{50} = 18 μM), is, however, far more potent at transfected rat and human $P2X_1$ (EC_{50} = 1.9 nM) and $P2X_3$ (EC_{50} = 98 nM) receptors (35) and thus cannot be used, *a priori,* in defining a $P2X_7$-receptor–mediated response.

P2 receptors are present on excitable tissues, such as neu-

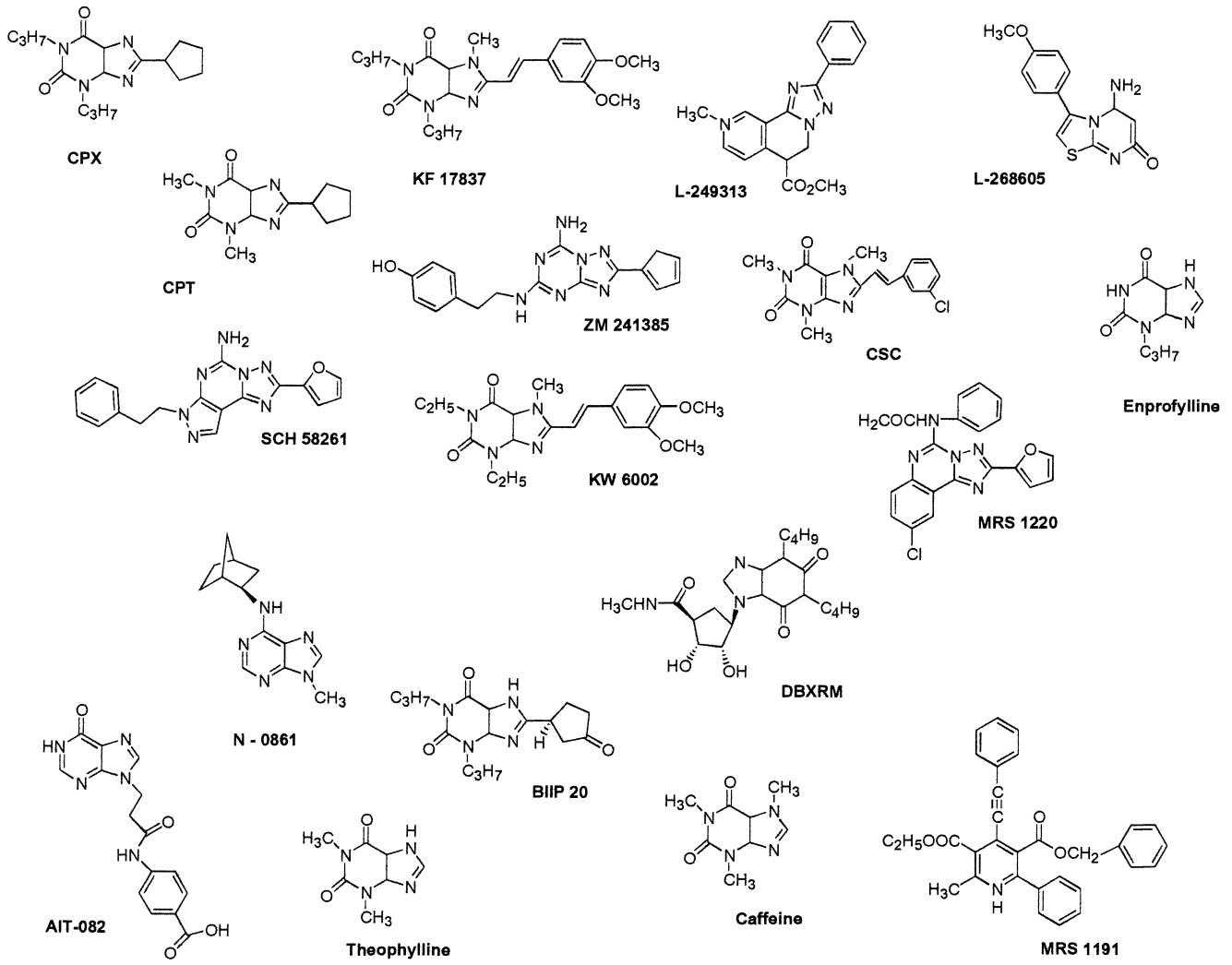

FIGURE 15.4. Structures of P2 receptor antagonists.

rons, glia, and smooth muscle cells (1), and can be grouped into three classes based on agonist effects (36). Group 1, comprising the $P2X_1$ and $P2X_3$ receptors, has high ATP affinity for ATP (EC_{50} = 1 μM) and is rapidly activated and desensitized. Group 2 includes the $P2X_2$, $P2X_4$, $P2X_5$, and $P2X_6$ receptors that have lower ATP affinity (EC_{50} = 10 μM), have a slow desensitization profile, and exhibit sustained depolarizing currents. The only receptor in Group 3 is the $P2X_7$ LGIC, which has low ATP affinity (EC_{50} = 300 – 400 μM) and shows little or no desensitization on agonist exposure.

P2X Receptors

P2X receptors are ATP-gated LGICs formed from various P2X subunits that share a common motif of two transmembrane-spanning regions (2TM). Like the amiloride-sensitive epithelial Na^+ channel, P2X receptor subunits have a large extracellular domain with both the N- and C-termini located intracellularly (1,37). A functional P2-receptor channel consists of multimeric combinations of the various P2X subunits to form a nonselective pore permeable to Ca^{2+}, K^+, and Na^+ that mediates rapid (approximately 10-millisecond) neurotransmission events. Available evidence indicates that functional P2X receptors are trimeric, in contrast to the typical pentameric structure of other LGICs (38). In addition to putative $P2X_1$ to $P2X_7$ homomers, $P2X_{1/5}$, $P2X_{2/3}$, and $P2X_{4/6}$ functional heteromers have been identified (1,39). $P2X_5$ and $P2X_6$ receptors do not appear to exist in homomeric form, but rather as heteromers with other P2X receptor subtypes. Unlike other LGICs, such as nAChRs, the 5-hydroxytryptamine ($5\text{-}HT_3$) receptor, little is known regarding the agonist (ATP) binding site on P2X receptor constructs or of ancillary sites that may modulate receptor function.

The utility of current P2-receptor antagonists, such as PPADS, DIDS, reactive blue-2, and suramin (Fig. 15.5) (27), is limited by their lack of selectivity for different P2

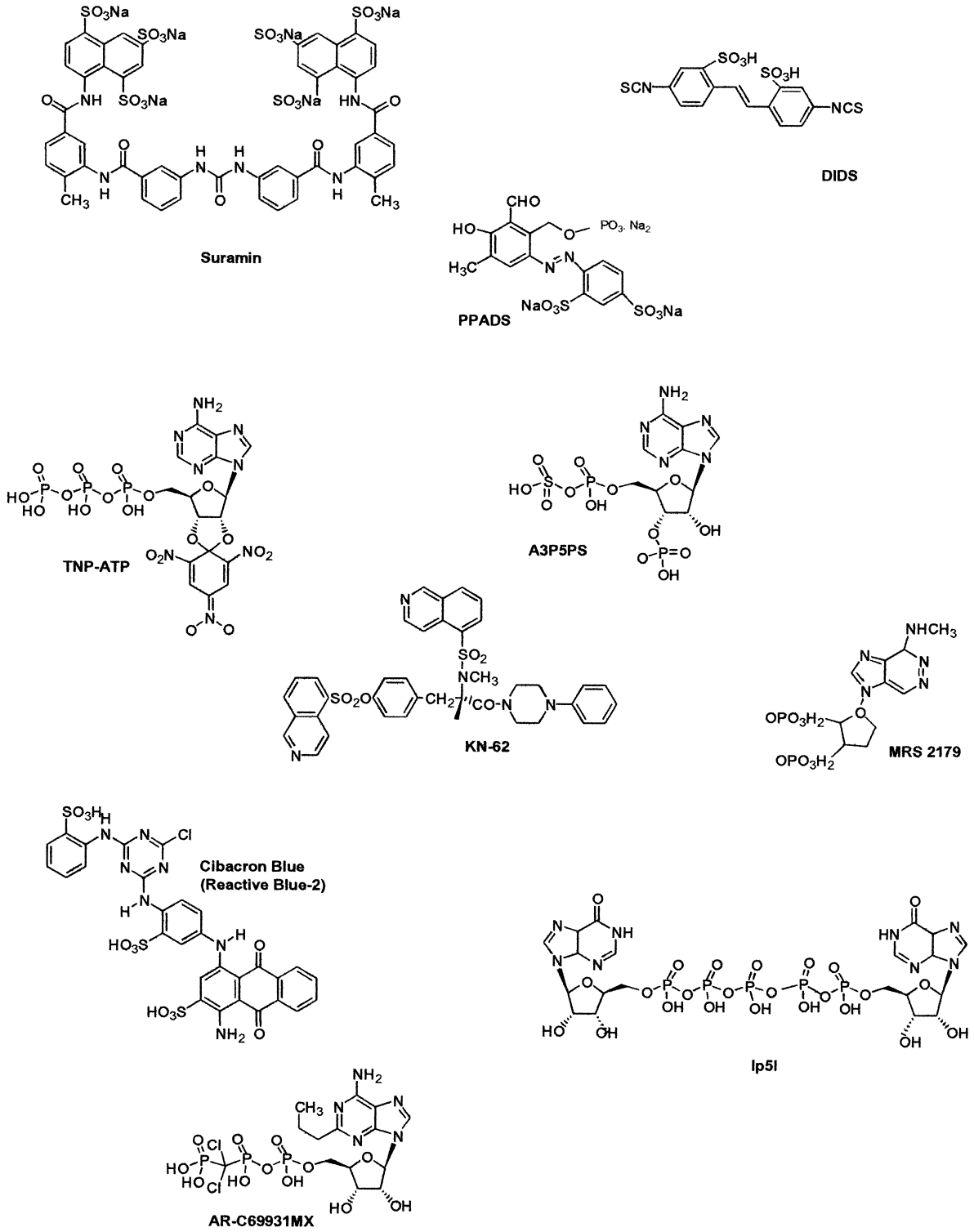

FIGURE 15.5. Structures of P1 receptor antagonists.

receptors and other proteins (34) or by their limited potency and bioavailability. These compounds can also inhibit the ectonucleotidases responsible for ATP breakdown, thus confounding receptor characterization (40). Radioligand-binding assays for P2 receptors are also far from robust; available ligands binding to cell lines lack any type of P2 receptor (41). The use of high throughput screening techniques to identify novel ligands thus depends on functional fluorescence assays such as FLIPR (fluorescence imaging plate redder) rather than binding.

Among the newer P2X-receptor antagonists (Fig. 15.5) are the following: TNP-ATP, a noncompetitive, reversible allosteric antagonist at $P2X_1$ and $P2X_3$ receptors with nanomolar affinity (42) that also has weak activity at $P2X_4$ and $P2X_7$ receptors; Ip5I, a potent, selective $P2X_1$ antagonist ($K_i < 100$ nM) antagonist (43); KN-62, a potent (IC_{50} = 9 to 13 nM), noncompetitive antagonist of the human $P2X_7$ receptor that is inactive at the rat $P2X_7$ receptor (44). The ATP analogue, A3P5PS (Fig. 15.5), is a partial agonist–competitive antagonist at the turkey erythrocyte $P2Y_1$ receptor (27), with the derivative, MRS 2179 being a full $P2Y_1$-receptor antagonist (IC_{50} = 330 nM). AR-C 69931-MX (Fig. 15.5) is a potent, selective antagonist at the ADP-sensitive $P_{2T}/P2Y_{AC}$ receptor involved in platelet aggregation that is currently in clinical trials as a novel antithrombotic agent (45).

$P2X_1$ receptors are activated by 2MeSATP, ATP, and αβ-meATP (Fig. 15.2), and they exhibit rapid desensitization kinetics (Table 15.2) (1). $P2X_1$ subunits are present in the dorsal root, in trigeminal and celiac ganglia, and in spinal cord and brain. The $P2X_2$ receptor is activated by 2MeSATP and ATPγS but is insensitive to αβ-meATP and βγ-meATP (Fig. 15.2). It is present in brain, spinal cord, superior cervical ganglia, and adrenal medulla. $P2X_{2\text{-}1}$, $P2X_{2\text{-}2}$, $P2X_{2\text{-}3R}$, and $P2X_{2\text{-}3}$ receptors are splice variants of the $P2X_2$ receptor that have been localized, among other places, to the cochlear endothelium, an area in the ear associated with sound transduction (46). $P2X_1$ and $P2X_2$ receptors can be blocked by PPADS and suramin (1). The $P2X_3$ receptor has a rank order of activation in which 2MeSATP >> ATP > αβ-meATP and is localized to a subset of sensory neurons that includes the dorsal root, trigeminal, and nodose ganglia (1). It has similar properties to the $P2X_1$ subtype including αβ-meATP sensitivity and rapid desensitization kinetics. $P2X_2$ and $P2X_3$ subunits can form a functional heteromeric $P2X_{2/3}$ receptor *in vitro* (39) that combines the pharmacologic properties of $P2X_3$ (αβ-meATP sensitivity) with the kinetic properties of $P2X_2$ (slow desensitization). $P2X_4$ receptors are activated by 2MeSATP and are only weakly activated by αβ-meATP. The rat and human homologues of the $P2X_4$ receptor differ in their sensitivity to suramin and PPADS; the human $P2X_4$ receptor is weakly sensitive, and the rat $P2X_4$ receptor is insensitive to these P2X-receptor antagonists (1). The $P2X_4$ receptor is present in rat hippocampus, superior cervical ganglion, spinal cord, bronchial epithelium, adrenal gland, and testis, as well as human brain. The agonist profile for the $P2X_5$ receptor is ATP > 2MeSATP > ADP with αβ-meATP being inactive. This receptor does not exhibit rapid desensitization kinetics but is blocked by suramin and PPADS. Message for the $P2X_5$ receptor is present in the central horn of the cervical spinal cord, in trigeminal and dorsal root ganglia neurons, and in the brain in the mesencephalic nucleus of the trigeminal nerve. The $P2X_6$ receptor is present in the superior cervical ganglion, cerebellar Purkinje cells, spinal motoneurons of lamina IX of the spinal cord, superficial dorsal horn neurons of lamina II, and trigeminal, dorsal root, and celiac ganglia (1). $P2X_4$ and $P2X_6$ subunits form functional heteromers *in vitro* (39).

The $P2X_7$ receptor, also known as the $P_{2Z}/P2Z$ receptor before it was cloned (47), is present in the superior cervical ganglion and spinal cord, mast cells, and macrophages (48). Cerebral artery occlusion results in an increase in $P2X_7$ immunoreactivity in the stroke-associated penumbral region (49). The $P2X_7$ receptor has a long (240 amino acid) intracellular C-terminal region that allows the receptor to form

TABLE 15.2. CLASSIFICATION OF P2Y RECEPTORS

	Agonist Rank Order Potency	Antagonist Rank Order Potency
$P2Y_1$	2-MeSADP > 2-MeSATP > HT-AMP > ADP > ADPβS > ATP > α,β-meATP > UTP inactive	MRS2179 > isoPPADS > A3′P5′P ≥ PPADS suramin
$P2Y_2$	ATP = UTP (100) > ATPγS = Ap_4A	
$P2Y_4$	UTP ≥ UTPγS > ATP	PPADS > reactive blue 2 > suramin > ATP (human)
$P2Y_6$	UDP >> UTP ≥ 2-MeSADP	Suramin > PPADS
$P2Y_{11}$	ATP > ADP >>> UTP	
$P2Y_{12}$	ADP	AR-C 69931MX = CT5054 >>> ATP
$P2Y_{13}$	ADP	2Me5ADP >> ATP

a large nonselective cytolytic pore on prolonged or repeated agonist stimulation (1,48). Exposure of the $P2X_7$ receptor to ATP for brief periods (1 to 2 seconds) results in transient pore opening that mediates cell-to-cell communication. Prolonged receptor activation triggers cytolytic pore formation with the initiation of an apoptotic cascade involving caspase-1 (interleukin 1β convertase) and an associated release of IL-1β from macrophages (48). The $P2X_7$ receptor is partially activated by saturating concentrations of ATP and is fully activated by the ATP analogue BzATP (Fig. 15.2). The ability to form a cytolytic pore was considered unique for the $P2X_7$ receptor, but other P2X receptors such as P2X and P2X show the same phenomenon on prolonged exposure to ATP, a finding indicating that the intracellular C-terminal tail is not a prerequisite for cytolytic pore formation (50,51).

P2Y Receptors

P2Y receptors are GPCRs activated by purine or pyrimidine nucleotides (1,52). The seven mammalian functional subtypes, $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, and $P2Y_{13}$, have been cloned and are coupled to Gq_{11}. Receptor activation results in stimulation of phospholipase C and IP_3 activation and subsequent release of calcium from intracellular stores. The P_{2T} receptor, present in platelets and preferentially sensitive to ADP, has been cloned, as the $P2Y_{12}$ receptor.

The $P2Y_1$ receptor is preferentially activated by adenine nucleotides, with 2MeSATP the most potent. UTP and UDP are inactive at this receptor. Suramin, PPADS, cibacron blue, A3P5PS, and MRS 2179 (Fig. 15.5) are competitive antagonists at this receptor (1,27). The $P2Y_2$ receptor is activated by both ATP and UTP; nucleotide diphosphates are inactive (1,52). Antagonists such as suramin are less efficacious at the $P2Y_2$ receptor. UTP is the preferred agonist for the $P2Y_4$ receptor, with ATP and the nucleotide diphosphates inactive. Diphosphates are more active at the $P2Y_6$ receptor than triphosphates, and this has led to the classification of the $P2Y_6$ receptor as a UDP-preferring receptor. The $P2Y_{11}$ receptor is unique among other P2Y receptors in that only ATP serves as an agonist for this receptor (53). The $P2Y_{12}$ and $P2Y_{13}$ receptors are ADP-selective receptors.

Diadenosine polyphosphates including Ap4A and Ap5A (Fig. 15.2) comprise another group of purine-signaling molecules that modulate cell function by activation of cell-surface P2 receptors (1,54). Whereas an Ap4A receptor that modulates neurotransmitter release and amphetamine-elicited Ap4A release has been pharmacologically characterized in nervous tissue, it is unclear whether diadenosine polyphosphate actions involve distinct receptor subtypes or reflect activation of known P2 receptors. Receptors for the diadenosine polyphosphates have not yet been cloned.

MITOCHONDRIAL PURINE RECEPTORS?

In addition to functioning as the key source of ATP within the cell, mitochondria play a key role in the apoptotic cascade as the source of the cytochrome C that is released after changes in mitochondrial transition pore function elicited by members of the bcl-2 family of cell death proteins (55). The ability of the $P2X_7$ receptor to initiate an apoptotic cascade by activation of caspase-1 (48) and the key role of mitochondria in various degenerative diseases (56,57) raise the question whether intracellular P2 receptors are present on the outer mitochondrial membrane and may provide a direct mechanism for ATP to influence mitochondrial function.

THERAPEUTIC POTENTIAL OF PURINES IN NERVOUS TISSUE

Adenosine potently inhibits the release of the neurotransmitters dopamine, GABA, glutamate, acetylcholine, serotonin, and norepinephrine and acts through presynaptic A_1 receptors (12). Adenosine acts preferentially on excitatory versus inhibitory neurotransmitter release, a finding suggesting a degree of physiologic specificity in modulating brain function. Adenosine also directly modulates postsynaptic neuronal excitability by activating A_1 and A_{2A} receptors resulting in hyperpolarization of the postsynaptic membrane.

Over the past 2 decades, many studies have provided evidence of involvement of purines in the actions of various CNS-active drugs including antipsychotics, antidepressants, anxiolytics, and cognition enhancers. These studies have come from experiments in which the effects of known CNS drugs representative of these therapeutic classes were examined for their ability to modulate adenosine-mediated responses in the CNS, or, alternatively, they studied the effects of various P1 ligands, both agonists or antagonists, on the effects of such prototypic CNS agents. In many instances, only single, somewhat high, concentrations of an isolated compound, or limited numbers of compounds, were used to generalize to a complete class of psychotherapeutic agents, often with no negative control data, thus limiting the value of the data (58).

For P2 receptors, the absence of ligands, agonists, and antagonists has limited the functional characterization of the various receptor subtypes. The delineation of a role for P2 receptors in CNS disorders has been postulated largely on the basis of *in situ* localization of the mRNAs encoding the different P2 receptor subtypes or of immunohistochemical studies.

Transgenic Models of P1 and P2 Receptor Function

For both P1 and P2 receptors, the use of mice either deficient in, or overexpressing, a targeted receptor can poten-

tially provide a unique means to assess the role of the given receptor, the phenotype of which will provide information on the role of the receptor. Although this approach is not always straightforward because some phenotypes are fatal and, in others, a knockout of one receptor leads to compensation in associated receptor systems such that the resultant mouse phenotype is atypical, knockouts can be helpful in the absence of selective antagonists or antisense probes.

P1 receptor knockouts show altered cardiovascular function (A_1) and reduced exploratory activity, aggressiveness, hypoalgesia, and high blood pressure (A_{2A}) (59). P2 knockouts are associated with decreased male fertility ($P2X_1$) (60), decreased nociception and bladder hyporeflexia ($P2X_3$) (61), decreased platelet aggregation and bleeding time ($P2Y_1$) (62), and reduced chloride secretion ($P2Y_2$) (63). A preliminary report on a $P2X_7$ knockout has appeared (64).

PURINERGIC THERAPEUTICS

Three distinct classes of compound can modulate P1 and P2 receptor function: (a) conventional agonist, partial agonist and antagonist ligands; (b) allosteric modulators of receptor function; and (c) modulators of the endogenous systems that regulate the extracellular availability of ATP, adenosine, UTP, and their respective nucleotides. This last group includes the various ecto-ATPases that catalyze the degradation of nucleotides (5), ADA, AK, and the bidirectional member transporter systems that remove adenosine from the extracellular environment (21,65). From data on AK effects in brain tissue (22), it appears that modulation of endogenous adenosine levels by inhibition of AK is not a viable drug discovery approach.

Efforts over the last 25 years to develop directly acting P1-receptor agonists and antagonists as therapeutic agents (8) have proven less than successful because of a combination of the choice of disease states in which other therapeutic modalities are clearly superior (58) and side effects are associated with global receptor modulation. Partial agonists, allosteric modulators, and novel modulators of ATP metabolism may prove clinically useful agents with improved therapeutic indices (65).

Stroke and Ischemia

Extracellular adenosine levels are markedly increased after hypoxia and focal ischemia, a finding providing additional evidence that the purine acts as a homeostatic neuroprotective agent (8). Adenosine-receptor agonists such as CHA reduce stroke-related cell death and hippocampal neurodegeneration, whereas adenosine antagonists exacerbate ischemic brain damage by enhancing glutamate release. The neuroprotective effects of adenosine are mediated by several P1 receptors: A_1-receptor activation stabilizes neuronal membrane potential, inhibits neuronal excitability and glutamate release (8,12), and thus prevents initiation of the stroke cascade (66).

Adenosine also hyperpolarizes astrocyte membranes limiting extracellular glutamate and potassium accumulation and modulates local cerebral blood flow and local inflammatory responses, such as platelet aggregation, neutrophil recruitment, and adhesion acting through the A_{2A} receptor (67). A_3-receptor agonists have biphasic effects on cell survival. At nanomolar concentrations, they are neuroprotective and inhibit apoptosis, but at micromolar concentrations they are neurotoxic (31).

mRNA for the $P2X_7$ receptor is up-regulated on microglial cells in the ischemic penumbral region 24 hours after middle cerebral artery occlusion in the rat (49), a finding indicating that cytolytic pore formation and inflammatory cytokine release are associated with neural trauma and neurodegeneration. Antisense to the $P2X_7$ receptor or selective receptor antagonists may represent a novel approach to the treatment of stroke.

Epilepsy

Seizure activity is associated with rapid and marked increases in CNS adenosine concentrations in animals (68), as well as in patients with epilepsy with spontaneous-onset seizures (69). Seizure activity induced by a variety of chemical and electrical stimuli in animal models is reduced by adenosine and related agonists (68) acting through A_1receptors. In electrically kindled seizure models, adenosine agonists reduce seizure severity and duration without significantly altering seizure threshold. These anticonvulsant effects are blocked by doses of methylxanthines that, when given alone, have no observable effect on seizure activity (68), a finding leading to the hypothesis that adenosine functions as an endogenous anticonvulsant.

Neurodegeneration: Alzheimer's Disease and Parkinson's Disease

The nerve cell death that follows excessive glutamate release and changes in calcium homeostasis after ischemia and hypoxia may reflect an acute manifestation of more subtle, long-term changes associated with apoptotic and necrotic cell death in Alzheimer's disease (AD) and Parkinson's disease (PD). Adenosine antagonists including caffeine, theophylline, and BIIP 20 (Fig. 15.4) are potent CNS stimulants (8,18), and they can enhance cognition in animal models by blocking the actions of endogenous adenosine. Certain compounds acting by purinergic mechanisms, such as BIIP 20 and propentofylline, have been examined in the clinic for their efficacy in cognitive disorders. Although provocative clinical data have been generated, neither compound showed sufficiently robust efficacy in larger AD trials

to warrant continuation. However, aged patients with rheumatoid arthritis who consume large quantities of antiinflammatory agents such as indomethacin show an inverse correlation for the incidence of AD, a finding highlighting the pivotal role of inflammation in disease origin. Adenosine agonists and AK inhibitors have marked antiinflammatory activity (67), inhibiting free radical production, and thus they may be effective in maintaining cell function in AD, in addition to modulating cytotoxic events.

Trophic factors in nervous tissue act to ensure neuronal viability and regeneration. Withdrawal of nerve growth factor, which exerts a tonic cell death–suppressing signal, leads to neuronal death. Polypeptide growth factors linked to receptor tyrosine kinases, such as fibroblast growth factors, epidermal growth factor, and platelet-derived growth factor, are increased with neural injury (70). ATP can act in combination with various growth factors to stimulate astrocyte proliferation and to contribute to the process of reactive astrogliosis, a hypertrophic-hyperplastic response typically associated with brain trauma, stroke and ischemia, seizures, and various neurodegenerative disorders. In reactive astrogliosis, astrocytes undergo process elongation and express glial fibrillary acidic protein, an astrocyte-specific intermediate filament protein with an increase in astroglial cellular proliferation. ATP increases glial fibrillary acidic protein and activator protein-1 (AP-1) complex formation in astrocytes and mimics the effects of basic fibroblast growth factor (70). Both ATP and guanosine triphosphate induce trophic factor (nerve growth factor, neurotrophin-3, fibroblast growth factor) synthesis in astrocytes and neurons. The effects of guanosine triphosphate are, however, not consistent with any known P2-receptor profile. Nonetheless, these studies have focused research on the hypoxanthine analogue, neotrofin (AIT-082) (Fig. 15.1), which up-regulates neurotrophin production and enhances working memory and restores age-induced memory deficits in mice (71). This compound has shown positive effects in early phase II trials for AD.

In 1974, Fuxe showed that methylxanthines such as caffeine could stimulate rotational behavior and could potentiate the effects of dopamine agonists in rats with unilateral striatal lesions Conversely, adenosine agonists blocked the behavioral effects of dopamine (72). Anatomic links between central dopamine and adenosine systems are well established; adenosine A_{2A} receptors are highly localized in striatum, nucleus accumbens, and olfactory tubercle, brain regions that also have high densities of dopamine D1 and D2 receptors. mRNAs for adenosine A_{2A} receptors and dopamine D2 receptors are co-localized in GABAergic-enkephalin striatopallidal neurons in the basal ganglia (Fig. 15.6) that form an ''indirect'' pathway from the striatum to the globus pallidus that originates from striatal GABA-enkephalinergic neurons. Through GABAergic relays, this pathway interacts with a glutaminergic pathway from the subthalamic nucleus that can activate the internal segment of the pars reticulata, which, turn, through a pars reticulata–thalamic GABAergic pathway, inhibits the thalamic-cortical glutaminergic pathway. Dysfunction of this pathway may underlie the movement disorders seen in Huntington chorea and PD. A direct pathway originating in striatal GABAergic–substance P–dynorphinergic neurons inhibits the internal segment of the pars reticulata to disinhibit the ascending thalamic glutaminergic pathway and to activate the cortex (Fig. 15.6). The balance between the direct (cortical activating) and indirect (cortical inhibiting) striatal dopaminergic pathways provides a tonic regulation of normal motor activity. These studies indicate that striatal adenosine A_{2A} receptors may play a pivotal role in neurologic disorders involving basal ganglia dysfunction such as PD. The A_{2A} agonist, CGS 21680, given intrastriatally, attenuates the rotational behavior produced by dopamine agonists in unilaterally lesioned rats. Mechanistically, radioligand-binding studies have shown an increased efficacy of CGS 21680 in reducing the binding affinity of supersensitive D2 receptors, a finding supporting the increased sensitivity of animals with supersensitive dopamine receptors to CGS 21680 treatment. Repeated administration of the dopamine antagonist, haloperidol can up-regulate the density of both D2 and A_{2A} receptors in rat striatum.

Adenosine A_1 receptor activation can reduce the high-affinity state of striatal dopamine D1 receptors, the A_1 receptor agonist, and CPA blocking D1-receptor–mediated locomotor activation in reserpinized mice (72). The nonselective adenosine agonist, NECA, can attenuate the perioral dyskinesias induced by D1-receptor activation in rabbits. Acting through striatal A_{2A} and A_1 receptors, adenosine directly modulates dopamine-receptor–mediated effects on striatal GABA-enkephalinergic neurons and striatal GABA–substance P neurons (Fig. 15.6). These adenosine agonist–mediated effects are independent of G-protein coupling and may involve an intramembrane modulatory mechanism involving receptor heterooligimerization (26).

The dynamic interactions between dopaminergic and purinergic systems in striatum suggest that dopaminergic dysfunction may be indirectly ameliorated by adenosine receptor modulation. Selective adenosine A_{2A} receptor antagonists such as KF 17837 and KW 6002 (Fig. 15.4) have shown positive effects in 1-methyl-4-[henyl-1,2,3,6-tetrahydropyridine–lesioned marmosets and cynomolgus monkeys, well characterized animal models of PD, enhancing the effects of L-dopa (73,74). KW-6002 has successfully completed human phase I trials. More recently, a 30-year longitudinal study of 8,004 Japanese-American man enrolled in the Honolulu Heart Program showed an inverse association of the incidence of PD with caffeinated coffee consumption. In men who drank no coffee, the incidence of PD was 10.4 per 10,000 person-years, and it was 1.9 per 10,000 person-years in men drinking at least 28 oz of coffee per day (75).

Adenosine agonists can mimic the biochemical and be-

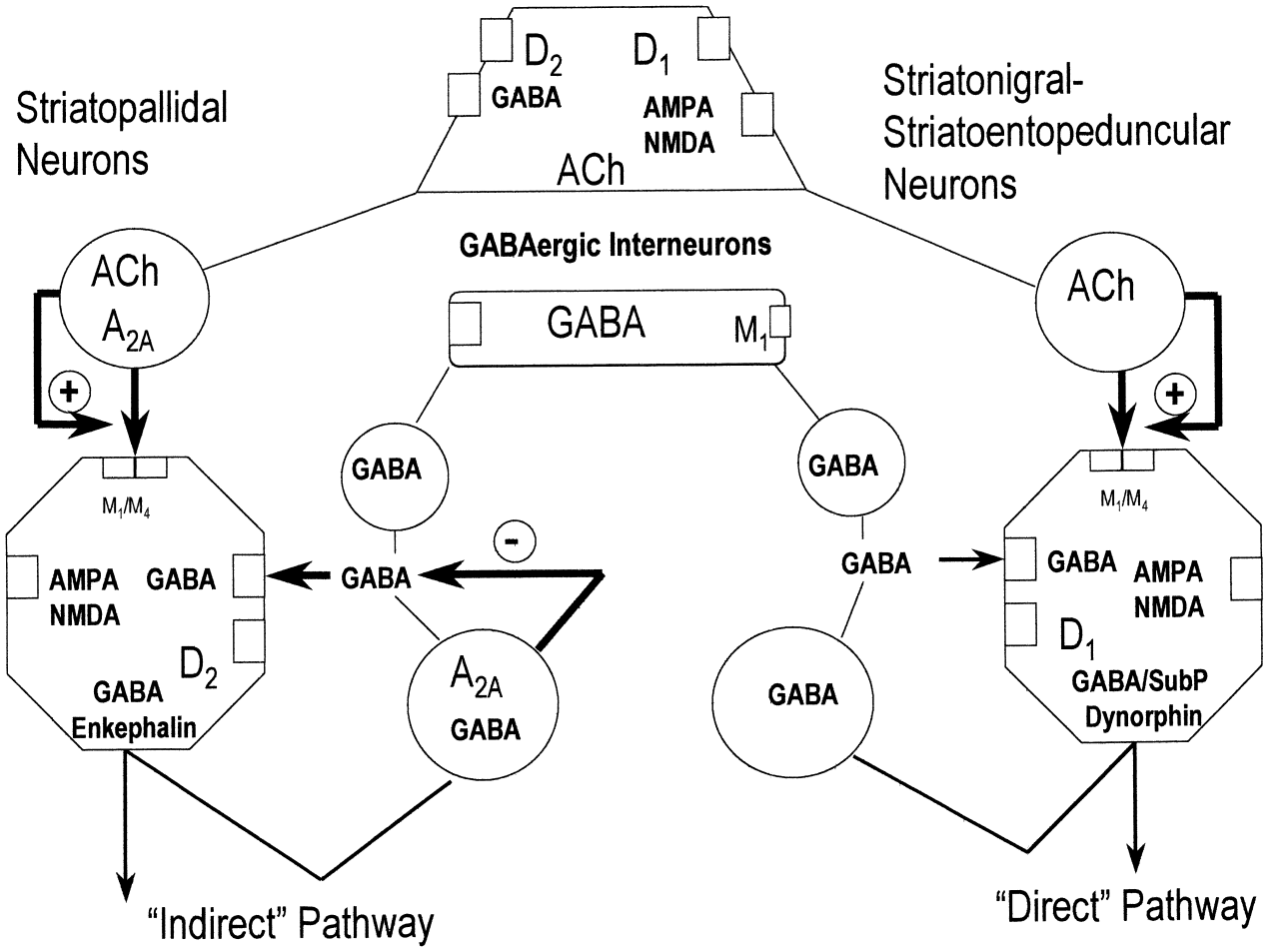

FIGURE 15.6. Dopamine–adenosine (ADO) interactions in the substantia nigra. An indirect pathway dopaminergic pathway arises from the striatal GABA-enkephalinergic dopaminergic neurons on which both dopamine D1 and adenosine A_{2A} receptors are co-localized. Through a GABAergic interneuron originating in the external globus pallidus, the indirect pathway connects to a glutaminergic pathway arising in the subthalamic nucleus. This, in turn, can activate the internal segment of the pars reticulata and, through another GABA pathway, inhibit ascending glutaminegic neurons arising from the thalamus that innervate the cortex. The direct pathway arises from striatal GABA–substance P–dynorphinergic neurons that, through a GABAergic relay, inhibit the internal segment of the pars reticulata to disinhibit the ascending thalamic-cortical glutaminergic pathway. The balance between the direct (activating) and indirect (inhibitory) striatal dopaminergic pathways can then tonically regulate normal motor activity. Dopaminergic inputs arising from the substantia nigra pars compacta can facilitate motor activity, inhibiting the indirect pathway by activation of D2 receptors and activating the direct pathway by D1 receptor activation. (Adapted from Svenningsson P, Le Moine C, Fisone G, et al. Distribution, biochemistry and function of striatal adenosine A_{2A} receptors. *Prog Neurobiol* 1999;59:355–396; and Richardson PJ, Kase H, Jenner PG. Adenosine A_{2A} receptor antagonists as new agents for the treatment of Parkinson's disease. *Trends Pharmacol Sci* 1997;18:338–344.)

havioral actions of dopamine antagonists in animal models by activation of A_{2A} receptors (9,72), a process that inhibits dopamine synthesis and attenuates dopamine transductional processes. CGS 21680, like typical and atypical neuroleptics, can reverse apomorphine-induced loss of prepulse inhibition (76). These actions involve a decrease in dopaminergic neurotransmission, with adenosine receptor agonists acting as functional dopamine antagonists. Adenosine agonists have a behavioral profile similar to that of dopamine antagonists in a conditioned avoidance response paradigm (77), in which they potently disrupting avoidance responding without significantly impairing escape behavior. They also produce catalepsy at the same dose levels effective in attenuating conditioned avoidance response, a property shared by typical neuroleptic agents such as haloperidol. CI-936, an A_{2A} agonist (Fig. 15.1), entered clinical trials in the mid 1970s as a novel antipsychotic agent, but its development was discontinued for unstated reasons.

Sleep

The hypnotic and sedative effects of adenosine are well known, as are the central stimulant activities of the various xanthine adenosine antagonists including caffeine (18). Direct adenosine administration into the brain elicits an EEG profile similar to that observed in deep sleep, an increase in rapid eye movement (REM) sleep with a reduction in REM sleep latency resulting in an increase in total sleep. In contrast, caffeine suppresses REM sleep and decreases total sleep time. Microdialysis studies have shown that extracellular adenosine concentrations are increased in basal forebrain in direct proportion to periods of sustained wakefulness and decline during sleep, a finding indicating that adenosine functions as a endogenous sleep regulator (19). Infusion of the A_{2A} agonist, CGS 21680, into the subarachnoid space associated with the ventral surface of the rostral basal forebrain, an area designated the prostaglandin D_2–sensitive sleep-promoting zone, increased slow-wave and paradoxical sleep, effects that were blocked by the A_{2A} antagonist, KF 17837 (78). The A_1-selective agonist, CHA, suppressed slow-wave and paradoxical sleep before eliciting an increase in low-wave sleep.

Pain

The role of purines in pain perception is well established (79–81), and both P1 agonists and P2X antagonists may represent novel approaches to nociception. ATP application to sensory afferents results in neuronal hyperexcitability and the perception of intense pain (79). These pronociceptive effects are mediated by $P2X_3$ and $P2X_{2/3}$ receptors present on sensory afferents and in the spinal cord. The nucleotide also induces nociceptive responses at local sites of administration and can facilitate nociceptive responses to other noxious stimuli, such as substance P. P2 receptor antagonists such as suramin and PPADS, even though they are limited in their *in vivo* effects, reduce nociceptive responses in animal models of acute and persistent pain (1,79). ATP is released from certain cell types (e.g., sympathetic nerves, endothelial cells, visceral smooth muscle) in response to trauma (1,8,79), and $P2X_3$-receptor expression is up-regulated in sensory afferents and spinal cord after damage to peripheral sensory fibers. $P2X_3$-receptor knockout mice have reduced nociceptive responses (61). The effects of adenosine are opposite effects to those of ATP (80), a finding suggesting that the nociceptive effects of ATP can be autoregulated by adenosine production from the nucleotide. Adenosine, adenosine-receptor agonists, and AK inhibitors inhibit nociceptive processes in the brain and spinal cord. When given intrathecally, these agents have analgesic activity in a broad spectrum of animal models (e.g., mouse hot plate, mouse tail flick, rat formalin, mouse abdominal constriction, rat neuropathic pain models), effects that are blocked by systemic or intrathecal administration of adenosine antagonists. Adenosine A_1-receptor agonists modulate acutely evoked and inflammation-evoked responses of spinal cord dorsal horn nociceptive neurons and can also inhibit pain behaviors elicited by spinal injection of substance P and the glutamate agonist, *N*-methyl-D-aspartate (NMDA). Glutamate is a key mediator of the abnormal hyperexcitability of spinal cord dorsal horn neurons (central sensitization) associated with clinical pain states. A_1 agonists can inhibit the spinal cord release of glutamate and can also reduce cerebrospinal fluid levels of substance P in rat, another key mediator of nociceptive responses. Adenosine has both presynaptic and postsynaptic effects on transmission from primary afferent fibers to neurons of the substantia gelatinosa of the spinal dorsal horn (12,80,81), and it involves both peripheral and supraspinal mechanisms. Adenosine agonists such as CHA and NECA, were 10- to 1,000-fold more potent in inhibiting acetylcholine-induced writhing in mice when these agents were administered intracerebroventricularly than orally, a finding indicating a supraspinal site of action. The ability of adenosine to inhibit peripheral neurotransmitter (12), and inflammatory processes (67), may block peripheral sensitization, a key feature of the pain resulting from tissue injury and inflammation.

Adenosine agonists are also active in human pain states (81). Spinal administration of the A_1 agonist, R-PIA, relieved allodynia in a patient with neuropathic pain without affecting normal sensory perception, whereas adenosine infusion at doses without effect on the cardiovascular system improved pain symptoms and reduced spontaneous pain and ongoing hyperalgesia and allodynia in patients with neuropathic pain. Low-dose infusion of adenosine during surgical procedures reduced the requirement for volatile anesthetic and also for postoperative opioid analgesia (82). AK inhibitors, such as CP 3269 and ABT-702 (Fig. 15.1), are effective analgesic agents in animal pain models by effects that can be blocked by xanthine adenosine antagonists.

CHALLENGES IN THE DEVELOPMENT OF CNS-SELECTIVE THERAPEUTIC AGENTS

The field of purinergic molecular biology and pharmacology has exploded as more is learned about the cellular targets through which ATP, ADP, AMP, and adenosine (and UTP) produce their effects on mammalian tissues. A clear historical delineation between the P1 and P2 fields is that in the former, more than 20 years of pharmacology and medicinal chemistry resulted in the identification of receptor selective ligands before the receptors were cloned. In contrast, definitive evidence for the existence of the P2-receptor family resulted from both pharmacologic and cloning studies. The latter have resulted in the identification of a remarkable diversity of receptors responsive to ATP, unfortunately in the absence of selective, bioavailable ligands, especially antagonists, that will allow a clearer understanding of P2-re-

ceptor function in normal and pathologic states. Evidence of the oligomerization of GPCRs and the emerging data on P2X heteromers both within the P2-receptor family and with other LGICs, such as nAChRs, suggest that the dynamics and the actual composition of systems targeted by purinergic receptors are potentially very complex (83).

Early efforts to develop therapeutics based on the modulation of P1-receptor–mediated processes met with limited success. Only adenosine has been approved for use as a cardiac imaging agent and for the treatment of supraventricular tachycardia, acute systemic uses that avoid some of the side effects seen with long-acting adenosine agonists. Similarly, the unexpected *in vivo* effects of AK inhibitors suggest that this is not a viable approach to the discovery of new drugs. The use of the adenosine antagonist theophylline for the treatment of asthma and the widespread use of caffeine as a CNS stimulant represent other P1-targeted therapeutics. The evaluation of A_{2A} antagonists as indirect dopamine agonists for use in PD (73–75) is an intriguing and novel approach to treating this neurodegenerative disorder, although the side effect liabilities are unknown at present.

In contrast, the highly discrete localization of $P2X_3$ receptors to sensory nociceptive neurons (79) has led to an intensive effort to identify $P2X_3$ antagonists as novel analgesic agents. Similarly, the discrete localization of other P2 receptors and evidence from mouse knockout studies suggest that selective agonists and antagonists for these receptor subtypes may represent very novel therapeutic agents as well as research tools to understand target function.

A caveat in the drug discovery process, as in all life's endeavors, is that the less that is known regarding the functional liabilities of a molecular target, the more attractive it is as drug target. In the area of purinergic medications, the identification of new ligands in combination with a broader-based evaluation of compound efficacy and side effect liability will greatly assist in the prioritization of therapeutic targets that are amenable to modulation by purinergic ligands (57). Finally, the renewed interest in mitochondria as cellular organelles that have function beyond energy production (56) represents an additional level of molecular targeting for P1- and P2-receptor ligands that may have benefit in treating human disease states, especially those involving apoptosis (55).

ACKNOWLEDGMENTS

I would like to thank Mike Jarvis for his contributions to the previous CD-ROM version of this chapter. Because of space limitations, it is not possible to cite primary literature sources exhaustively. The reader is referred to reference 1 for a more comprehensive bibliography.

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