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PURINE RECEPTORS IN MAMMALIAN TISSUES: PHARMACOLOGY AND FUNCTIONAL SIGNIFICANCE

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ADENOSINE AND ADENINE NUCLEOTIDES IN CELL FUNCTION

Purine nucleosides and nucleotides are major factors in cell function. They are intermediates in energy pathways, in cellular metabolic processes and are constituents of the cofactors necessary for enzymatic reactions (1) and cell replication (2). It has therefore been difficult to visualize that such chemicals also act in cellular communication.

The criteria used for evaluating whether a given compound is a neurotransmitter have been developed by historical precedent (3). They are based on knowledge of the prototypic neurotransmitter, acetylcholine (4). Adenosine has been identified as a neuromodulator using such classical criteria. This evaluation has lessened the enthusiasm for purine-related processes as targets for therapeutic agents for malfunctions of mammalian homeostatis. There is no evidence that specific anabolic processes form adenosine, distinct from those involved in its general metabolic functions. Also, the physiological factors regulating the extracellular availability of the nucleoside and its distribution make the development of a "purinergic" hypothesis of neuromodulation difficult (5). These issues are compounded by the paucity of dissimilar chemical entities that interact with adenosine receptors. Agonists are almost exclusively purine nucleosides (6), whereas with few exceptions, most antagonists are imidazopyrimidines (7–10). Studies of adenosine receptor–mediated events are thus limited, comparable to in-

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vestigations on adrenoceptors in which neither phentolamine, phenoxybenzamine, nor propranolol would be available as a selective receptor probe.

These caveats can also be applied to many of the recently discovered peptides thought to function as neuromodulators (11, 12). Potential physiological or pharmacological functions of the neuropeptides may result from optimistic conjecturing and may reflect, in part, a high technology bias, whereas the more negative view, in the case of adenosine, may reflect a "devil already known" component. The purine has marked effects on cardiovascular function, as was shown nearly 60 years ago (13). This fact, coupled with the limited usefulness of agonists as selective therapeutic agents, may lead to the conclusion that this neuromodulator has been judged and rejected. This review attempts to show that the therapeutic potential of adenosine-related compounds has yet to be objectively evaluated.

HISTORICAL PERSPECTIVE

Purine nucleosides and nucleotides are intercellular messengers. They function as chemoattractants in a variety of organisms and mammalian tissues (14). In 1929 Drury & Szent-Gyorgi (13) demonstrated the effects of purines in mammalian species. They discovered that adenosine caused bradycardia, coronary vasodilation, and blood pressure decreases. The short half-life of the nucleoside (16) confounded attempts to use adenosine as an antihypertensive agent (15). For the next three decades, basic research on "purinergic neurotransmission" concentrated on the physiological rather than the pharmacological. The purine has general vasodilatory actions (17, 18). In fact, it is a vasodilator in all vascular beds thus far studied, with the exception of the kidney (19) where it is a potent vasoconstrictor. Central administration of either adenosine or its nucleotide, ATP, can produce sedative and hypnotic effects (20, 21). The discovery of the second messenger cyclic AMP and the seminal biochemical studies of Sattin & Rall (22) gave further importance to adenosine as a neuromodulator. Adenosine is a potent activator of adenylate cyclase activity (6). However, the xanthines, theophylline, and caffeine (Figure 2), rather than potentiating the actions of adenosine, as their activity as phosphodiesterase (PDE) inhibitors would indicate, inhibited cyclic AMP formation (22). At this time Sattin & Rall (22) observed that the xanthines were acting as adenosine antagonists. Shortly after this observation, discrete cell-surface recognition sites, or receptors, were reported for the purine (23–25).

Modification of the xanthines also led to compounds such as 8-phenyltheophylline (8-PT; Figure 2) that were selective adenosine antagonists rather than PDE inhibitors (6, 9, 26). In 1980 the first of several radio-ligand-binding assays was reported (27), which increased interest (3, 6, 8, 28–30) in

the pharmacological role of adenosine. Concomitantly, the search for the chemical mediator of the phenomenon described by Burnstock and his coworkers (31, 32) as "non-adrenergic, non-cholinergic" neurotransmission led to the identification of ATP as a likely candidate. It was considered unlikely that a chemical even more essential to cellular function in terms of its energy-charge characteristics than adenosine (33) would serve a role as an intercellular mediator and would participate in, to some extent, a process wasteful of intracellular energy charge. However, continued efforts have led to the identification of two separate receptors sensitive to the nucleotide (32).

ADENOSINE RECEPTORS

Adenosine-mediated increases in cyclic AMP formation (22) were ascribed to the existence of a single receptor class susceptible to blockade by the xanthines. In the initial nomenclature for purinoceptors (31), those receptors sensitive to adenosine were termed P_1 and those sensitive to ATP were termed P_2 (Table 1). Additional studies indicated that the P_1 receptors could be further subdivided using appropriate concentrations of adenosine to modulate adenylate cyclase activity. Because an intact ribose group must be present on the purine molecule (Figure 1) to produce agonist activity, Londos & Wolff (23) termed these receptors R_i and R_a (Table 1), the subscripts of which refer to the inhibition (i) and activation (a) of adenylate cyclase activity. Independently, Van Calker et al (25) termed these same two adenosine receptors A_1 and A_2. A third adenosine recognition site, termed the P site, was also described (34; Table 1). The P site is located on the catalytic subunit of adenylate cyclase. It shows a preference for purines that have intact purine rings (hence the P designation) such as 2',5'-dideoxyadenosine (DDA; Figure 1) and is activated by relatively high concentrations of adenosine (6). However, the physiological significance of the P site is unknown. A_1 and A_2 receptors are linked to the catalytic subunit of adenylate cyclase by coupling proteins N_i and N_s, respectively (24).

Changes in cyclic AMP formation cannot always be related to events modulated by adenosine (5, 35, 36). The nucleoside probably influences cellular function by its actions on other second messenger systems such as calcium (37). Adenosine can also indirectly modulate phosphatidyl inositol turnover (38). Because of these findings, A_1 and A_2 receptors are currently classified based on their agonist pharmacology (39) rather than on their effects on cyclic AMP formation. Receptors of the A_1 subtype are preferentially activated by adenosine agonists (Figure 1) with substitutions in the N^6 position, such as cyclopentyladenosine (CPA; 40, 41), cyclohexyladenosine (CHA; 6), and phenylisopropyladenosine (PIA; 6). Analogs substituted in the 5'-position, most notably 5'*N*-ethylcarboxamido adenosine (NECA; 6, 40),

Table 1 Purinergic receptor classification criteria[a]

Receptor class	Subclasses	Agonist pharmacology	
P_1	$A_1(R_1)$	CPA ≥ CHA > R-PIA ≥ 2-CADO ≥ NECA > S-PIA > CV-1808	Receptor subtypes may exist
	$A_2(R_a)$	NECA > MECA = 2-CADO > CV1808 = R-PIA > CPA ≥ S-PIA	A_{2a} = high affinity subclass A_{2b} = lower affinity subclass
P_2		ATP > ADP > AMP ≥ Ado	
	P_{2x}	α-β-MeATP = β-α-MeATP > ATP = 2 MeSATP	
	P_{2y}	2 MeSATP >> ATP > α-β-MeATP = β-α-MeATP	
P		2'5' DDA >> Ado	

[a]For abbreviations see Figure 1 and text.

do show activity at A_2 receptors. NECA is not, however, A_2 selective (40). Rather, it is nonselective for either receptor subtype and may be considered to have A_2 activity solely because the A_1-selective compounds are inactive at A_2 receptors. A given biochemical or pharmacological event cannot be ascribed to A_2 activity merely because it is evoked by NECA. However, if both CHA and NECA are used and an additional response occurs with NECA, the event can probably be ascribed to A_2-receptor activation. Similarly, 2-chloroadenosine (2-CADO) is a relatively nonselective agonist (40), whereas 2-phenylaminoadenosine (CV 1808; Figure 1), the methyl analog of NECA, MECA, and aristeromycin (Figure 1), show A_2 selectivity with varying activity (40; M. Pankaskie, G. A. Stone & M. Williams, submitted for publication).

Both receptor subtypes can be blocked by the xanthine adenosine antagonists (6). 1,3-Dipropyl-8-(2-amino-4-chloro)phenylxanthine (PACPX; 7, 43; Figure 2) and the xanthine-peptide congener, XAC (10; Figure 2) are A_1 selective (9), whereas the triazoloquinazoline, CGS 15943A (Figure 3), is A_2 selective (44).

Both subclasses of adenosine receptor show stereoselectivity for the R- and

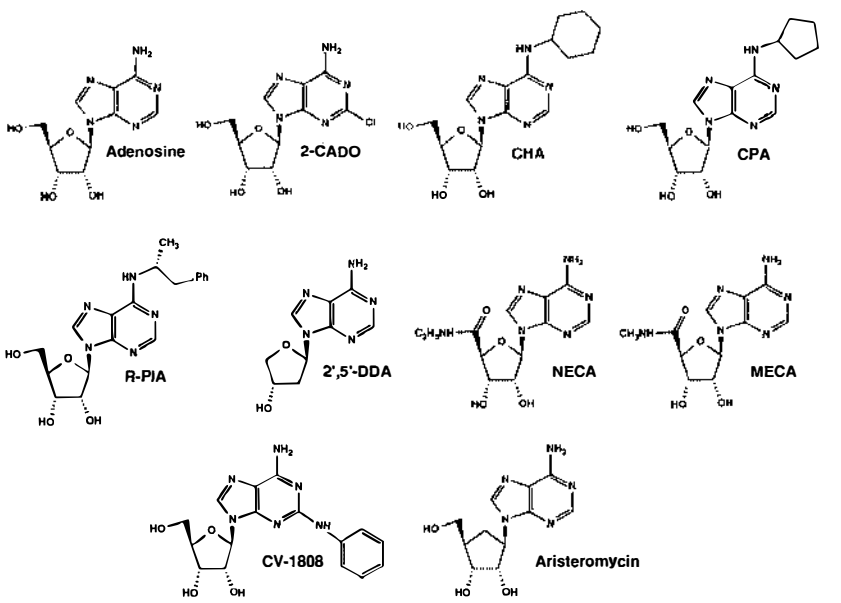

Figure 1 Adenosine agonists.

S-diastereomers of the amphetamine-derived adenosine analog, PIA (6). At the A_1 receptor, the R isomer has been reported to be up to 45-times more active than the S isomer. At the A_2 receptor the ratio of S-PIA to R-PIA is 10 or less. This ratio has been used as a criterion for the involvement of either receptor subclass (6, 28), but its magnitude appears to be dependent on the species used (45). Subclasses of both A_1 and A_2 receptors have been described, which are divided on the basis of a lack of interaction with adenylate cyclase (46, 47) or of agonist affinity (40, 47, 48). The designation A_3 has been used for an adenosine receptor present in cardiac tissue and nerve endings that is not cyclase linked (49).

Adenosine Receptor Ligands

CHA (27), CPA (41), and R-PIA (50) are routinely used to label A_1 receptors in CNS tissue, where dissociation constants (K_d) of 0.5–4.0 nM have been derived. Corresponding receptor densities (apparent B_{max} values) of 0.2–1.5 pmoles/mg protein have been observed (30; Table 2). High-affinity binding sites can only be demonstrated, however, when endogenous adenosine is removed using adenosine deaminase (ADA). These radioligands are less useful in peripheral tissues because of their relatively low specific activity (20–50 Ci/mmole) and because there are few such receptors in these tissues (8; Table 2). This problem has been circumvented by the use of iodinated

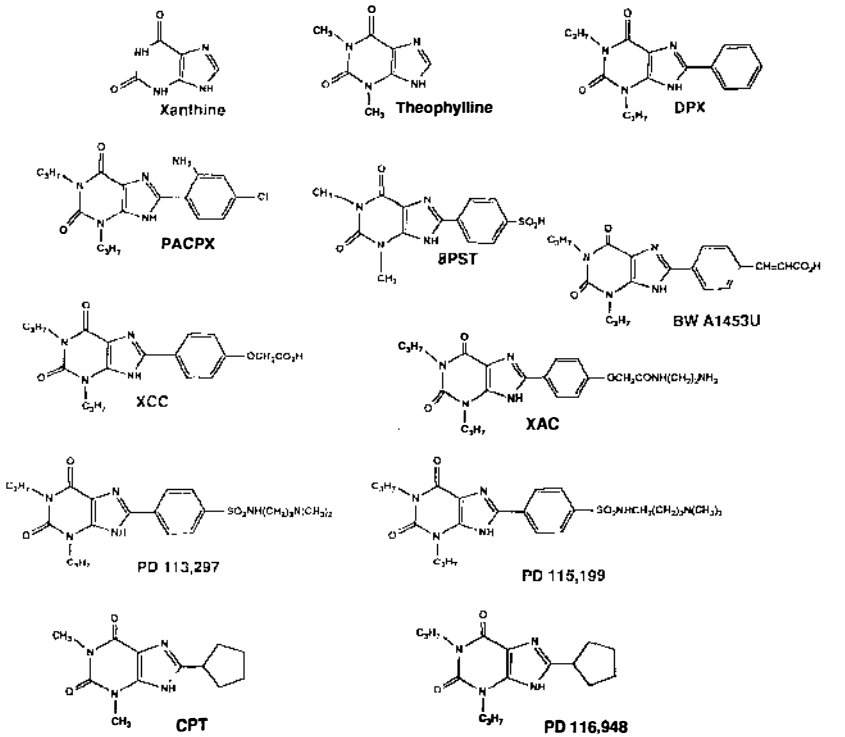

Figure 2 Xanthine adenosine antagonists.

forms of PIA (51), which have specific activities of up to 2200 Ci/mmole. 2-CADO has been used to label the A_1 (29) receptor; however, it is an unreliable ligand.

[^{3}H]NECA can be used to label A_2 receptors in striatal tissue from brain, after blocking binding to A_1 receptors using the alkylating agent *N*-ethylmaleimide (NEM; 52), NEM can uncouple the N_i protein from the receptor and dramatically reduce the affinity of the receptor for the ligand (53, 54) when cyclic AMP is the second messenger for the receptor. This approach lacks specificity, as NEM can also affect A_2-receptor affinity (40). An alternative method is to block the A_1 component of striatal NECA binding by adding low concentrations (50 nM) of the A_1-selective agonist, CPA (40). When this last method is used, NECA can bind selectively to A_2 receptors (40). Two of these sites have been identified. The first is a high-affinity site termed A_{2a} (40). It has a K_d value of approximately 4 nM (40, 45) and an apparent B_{max} value of 0.4–0.9 pmole/mg protein, depending on the species (45). A_{2b} (40) is a lower affinity site with K_d values of 13–245 nM and B_{max} values of 0.8–3.2 pmoles/mg protein, again dependent on the species (45).

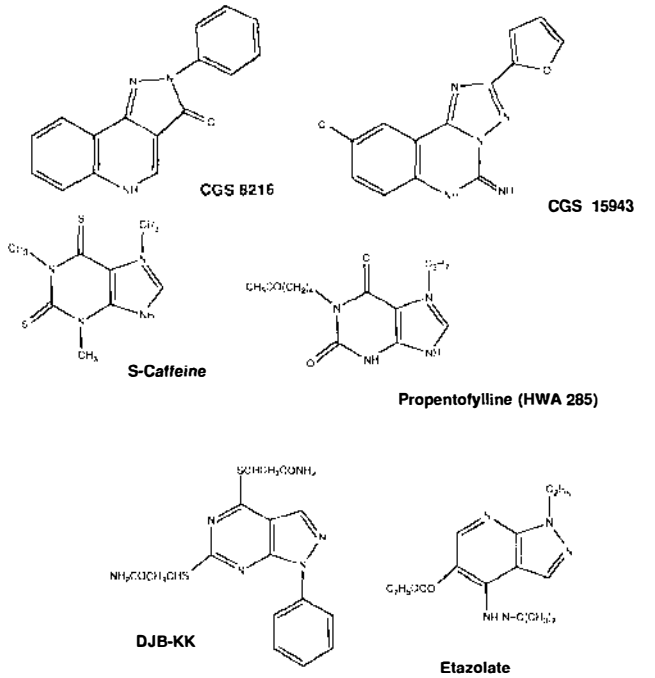

Figure 3 Atypical adenosine antagonists.

NECA may also label sites that are not adenosine receptors (40, 55). In the PC12 pheochromocytoma cell line, which is devoid of A_1 receptors, NECA binds only to A_2 receptors (56).

Use of antagonist radioligands for adenosine receptors has met with limited success. 1,3-Diethyl-8-phenylxanthine (DPX; Figure 2; 27), initially thought to label both A_1 and A_2 receptors (27), has unpredictable binding behavior. XAC and the related xanthine congeners XCC, PD 115,199, and PD 116,948 (Figure 2) have demonstrated useful binding characteristics (10, 57–59). Because of their high specific activity (100–150 Ci/mmole), these antagonists may aid assessing adenosine receptor pharmacology in peripheral tissues.

ATP RECEPTORS

Studies in peripheral tissues (51) provide the majority of evidence for the existence of ATP receptor–mediated processes. However, ATP-elicited responses not blocked by xanthines have been reported in the CNS (60). In many instances, the effects of ATP on cell function have been ascribed either to formation of adenosine from the nucleotide or to the possible chelation of calcium by the triphosphate side chain (47). The use of nonmetabolizable

Table 2 Characteristics of adenosine receptor binding in various mammalian tissue[a]

Tissue	Ligand	K_D (nM)	Bmax (fmole/mg protein)	Receptor	Reference
Rat brain	[^{3}H]CPA	0.5	416	A_1	41
Rat striatum	[^{3}H]NECA	3.5	1420	A_{2a}	40
	+ CPA	35.0	3600	A_{2b}	40
Rat brain	[^{125}I]HPIA	1.94	870	A_1	51
Guinea pig brain	[^{3}H]CHA	2.4	1230	A_1	237
Guinea pig ileum	[^{3}H]CHA	1.7	140	A_1	237
Rat mast cells	[^{3}H]Ado	28	16,400[b]	A_2	237
Guinea pig atrium	[^{3}H]NECA	4.3	127	A_1	238
Guinea pig ventricle	[^{3}H]NECA	3.2	50	A_1	238
Rat kidney membranes	[^{3}H]2-CADO	14900	2300	A_2	206
Bovine myocardium	[^{125}I]HPIA	1.1	31	A_1	51
Human neutrophils	[^{3}H]NECA	230	9310	A_2	218

[a]Abbreviations: CPA, Cyclopentyladenosine; NECA, 5'N-ethylcarboxamidoadenosine; CHA, Cyclohexyladenosine Ado, adenosine; and HPIA, Hydroxyphenylisopropyladenosine.
[b]Units in sites per cell.

analogs of ATP, including the α-β- and β-γ-methylene isosteres, provided definitive evidence of unique ATP recognition sites. These sites have recently (32) been subdivided into P_{2x} and P_{2y} subtypes on the basis of the actions of α-β-methylene ATP and 2-methylthio-ATP, respectively. Reliable antagonists for the P_2 receptor have proved difficult to identify. 3-O-3[*N*-(4-Azido-2-nitrophenyl)amino]proprionyl-ATP(ANAPP$_3$) has been a useful tool; it is a photolabile, irreversible blocker of P_2 receptors (61).

The stable ATP analog, [^{3}H]AppNHp, bound irreversibly to rat brain membranes with affinities of 10^{-9} M (62). [^{3}H]ATP has also been used to label ATP recognition sites in rabbit bladder membranes (63) that were distinct from ATPase sites. ANAPP$_3$ covalently labeled two recognition sites in guinea pig vas deferens smooth muscle (64). Because of the incubation conditions used to determine the covalent nature of [^{3}H]-ANAPP$_3$ binding, kinetic parameters could not be established. ANAPP$_3$ may be metabolized to adenosine (65).

ADENOSINE AVAILABILITY

The physiological factors controlling the availability of adenosine in mammalian tissues remain unclear. As with other mediators of cellular communication, adenosine can be removed from the extracellular environment by uptake

and degradation (deamination). The processes defining these events are well characterized (1, 66). The factors regulating the release of adenosine are, however, less clearly defined. In the heart, adenosine can be formed and released from hypoxic or ischemic myocardium (67, 68). The sources of cardiac adenosine are breakdown of either 5'-AMP or S-adenosylhomocysteine (SAH; 69–71). The possible contribution of a novel phosphoglycerol adenosine tetraphosphate (71a) has yet to be evaluated.

In the CNS and nonmammalian models of the synapse (such as Torpedo; 72), electrical stimulation and K^+ depolarization can cause purine release (73–75). Adenosine release has been described as a "neuronal nonexocytotic release" process involving carrier mediation (75, 76). The form of adenosine released also contributes to the confusion surrounding its availability. Cyclic AMP, 5'-AMP, and ATP have all been identified as potential sources for extracellular adenosine (77–80). Antisera to a levulinic acid derivative of adenosine have enabled the tentative mapping of adenosine-containing neurones in rat brain (81). Thc distribution of immunoreactivity is comparable to that seen for A_1 receptors (82). In hippocampus, 5'-nucleotidase is colocalized with A_1 receptors (82), which may be a finding of potential functional relevance. Broken cell preparations from brain tissue have an apparently unlimited capacity to produce adenosine (29, 46, 83); thus, tissues must be pretreated with ADA to detect high-affinity binding sites. Removal of this enzyme reduces specific binding (83), indicating that the purine is being continuously produced. The source of this adenosine, and its relationship to the physiological availability of the nucleoside, has not yet been determined. Overall, therefore, other than those in distress states such as hypoxia or ischemia (5, 84), the factors regulating the physiological availability of adenosine are poorly understood.

Another facet of this issue is the "purinergic inhibitory tone." Because xanthine adenosine antagonists can increase neurotransmitter release (85), cell firing (86), and behavioral activity (87), it is generally agreed that adenosine is normally present extracellularly (5) and that it may fulfill a homeostatic role in target tissues. In the heart, for instance, the release of adenosine during hypoxia prevents an excessive mechanical load on cardiac tissue while at the same time it dilates the coronary vasculature to increase oxygen supply to the tissue (88). Similarly, during epileptic fits, adenosine can be released in brain tissue (89) and may act as an endogenous anticonvulsant. Although speculative, the relationship between hypothalamic ADA-containing neurons and jaw movement is suggested to be related to aggressive attack behavior and, consequently, to survival (90). In all these situations, adenosine acts as a protective agent, or, as termed by Newby (84), a "retaliatory metabolite." An endogenous adenosine tone has also been reported at the neuromuscular junction (91).

Uptake or deamination terminates the actions of adenosine as a neuromodu-

lator (1, 66). High-affinity uptake mechanisms have been characterized (92). In rat brain a rapid-uptake system with a K_m value of 0.9 μM and a slower system with two components with K_m values of 1 and 5 μM have been described. Adenosine transport can be blocked in both directions such that many uptake inhibitors can prevent the purine from leaving the cell (92). Transport inhibitors such as dipyridamole (93) and, to a lesser extent, CV 1808 (Figure 1; 94) are effective vasodilators. Adenosine transport site can be labeled using either nitrobenzylthioinosine (NBI; 95), a ligand that is a selective adenosine uptake inhibitor, or dipyridamole (96). [^{3}H]NBI binds with high affinity (K_d = 50 pM) to mammalian brain tissue. Somewhat unexpectedly, [^{3}H]NBI does not require the removal of endogenous adenosine to facilitate its characterization. This difference may be due to the fact that NBI does not bind to the active site of the transporter mechanism but rather to a component protein. Also, dipyridamole is a relatively weak inhibitor of NBI binding (95), and higher concentrations of NBI than those used in binding are required for adenosine to depress cell firing (97). Inhibition of the catabolic enzyme, ADA, induces sleep (98, 99). Immunocytochemical studies of this enzyme (100) located a discrete plexus of ADA-containing neurons in the basal hypothalamus that innervate the striatum, amygdala, and cortex. A correlation between the regional distribution of ADA-immunoreactive neurons and NBI binding sites has been reported (101), suggesting that the systems are coinvolved in regulating the bioavailability of the purine.

SECOND MESSENGER SYSTEMS

Cyclic AMP

As indicated, cyclic AMP is the primary second messenger associated with adenosine receptor activation (6). The nucleoside is a ligand for the recognition site. In contrast to other cyclic nucleotide–modulated systems, cyclic AMP can also modulate the enzyme via direct interactions with the P site on the catalytic subunit (Table 1). In addition, it can contribute to the precursor pool of ATP used as a substrate by the enzyme. In intact slice preparations, the contribution of exogenous adenosine to this ATP pool is negligible (6) and cannot account for the increase in cyclic AMP associated with A_2 receptor activation. While cyclic AMP is a potent modulator of protein kinase activity (102), the physiological consequences of adenosine-related increases in cyclic AMP have yet to be demonstrated (29). The biphasic effects of R-PIA on glutamate release in cultured cerebellar neurons (103) in the absence and presence of pertussis toxin can be related to effects on both A_1 and A_2 receptors. In the absence of the toxin, R-PIA inhibits glutamate release. Pertussis toxin binds irreversibly to the N_i subunit (104) of adenylate cyclase.

This binding prevents A_1 receptor activation and reveals the involvement of an A_2 component in the adenosine agonist response and a concomitant increase in glutamate release. These toxin effects parallel similar effects on cyclic AMP formation.

Phosphatidylinositol Turnover

The recent surge of interest in the role of phospholipids as second messengers in the diacylglycerol–inositol triphosphate pathways (105) prompted evaluation of adenosine as an effector agent. While adenosine alone has no demonstrated effect, it can potentiate the effects of histamine (38).

Ion Fluxes

The effects of adenosine on ion movement in both central (30, 47, 73, 106) and peripheral (30, 107) tissues have been well documented. Adenosine has pre- and postsynaptic effects (30, 108–111), the former related to adenosine's indirect actions on the release of other neurotransmitters (see below) and the latter to its direct effects on cellular excitation. The ionic mechanisms involved reflect changes in calcium influx (30, 73, 112, 113), increases in potassium conductance (108, 109), and possibly regulation of sodium movement (113). Adenosine also has nonsynaptic effects in the hippocampal slice preparation (30, 114, 115) that probably result from an increase in K^+ conductance. Adenosine has an additional postsynaptic effect of long duration. It reduces neuronal excitability through calcium-dependent enhancement after hyperpolarizations (110). The effects of the purine on membrane conductance (106, 108, 113) are controversial (30). Synaptic transmission can be reduced by adenosine at concentrations that have no effect on membrane conductance potential (30). Presynaptic, rather than postsynaptic, actions, therefore, seem primarily responsible for the depressant actions of adenosine (114). The chronotropic and dromotropic actions of adenosine are also related to changes in K^+ conductance (107).

At the molecular level, direct interactions between adenosine and calcium have been reported (116, 117), although adenosine does not affect the positive inotropic actions of the dihydropyridine calcium agonist Bay K 8644 (118). Cyclic AMP does not appear to be an obligatory second messenger for the electrophysiological effects of adenosine (36). A third receptor subtype, termed A_3, may be involved in such effects (49).

Neurotransmitter Release

Adenosine is a potent and apparently ubiquitous modulator of neurotransmitter release. It has been reported to inhibit the release of aspartate, glutamate,

norepinephrine, GABA, dopamine, serotonin, and acetylcholine (for reviews see 30, 47, 119). Adenosine has been reported to have biphasic effects on acetylcholine (120), and on norepinephrine and dopamine release (121), that may be related to the receptor subtype activated (103, 120). The role cyclic AMP plays in these effects on transmitter release is unclear (3, 29, 30).

From a physiological, as opposed to pharmacological, vantage point, the effects of adenosine on transmitter release are confusing. The concentrations of adenosine required to change in vitro release are higher than those necessary to change electrophysiological parameters. This discrepancy has been discussed (30) in relation to the effects of potassium on the various release systems. As mentioned, adenosine is generally thought to act presynaptically and to regulate calcium flux (37, 116), affecting stimulus–secretion coupling (73). Obtaining definitive evidence for the direct effects of adenosine on calcium fluxes has been difficult (30). Silinsky (73) has alternatively suggested that receptor activation may reduce the affinity of a strategic component of the secretory apparatus for calcium. Irrespective of the molecular events involved, the effects of adenosine on transmitter release need evaluation within the context of autoreceptor function (122).

Receptor activation involves a neurotransmitter released presynaptically that can modulate its own release, usually by feedback inhibition. Assigning a role in this process to the apparently nonspecific actions of adenosine has led to a good deal of confusion and has significantly contributed to the skepticism surrounding the therapeutic value of an agent affecting purinergic systems. At a behavioral level, adenosine has sedative activity (3) because it inhibits the release of glutamate and aspartate, the major excitatory transmitters in the brain. Adenosine can also inhibit the release of GABA, the major inhibitory neurotransmitter in the brain. Adenosine's sedative effect can be reconciled to its inhibition of GABA release because it disinhibits GABA-related tonic influences. The localization of A_1 receptor to axon terminals of excitatory neurons (76) is consistent with this reasoning.

However, many studies use a reductionist approach, that is, tissues are usually prelabeled with an excess of a chosen radioactive transmitter (or a precursor) that may not be stored (or released) in a manner analogous to that of the endogenous substance. Because of this bias, only the substance chosen to be studied actually is. In vivo, however, the effects of caffeine on dopamine metabolism are regionally selective (123). Other in vivo studies using more sophisticated assay techniques to measure endogenous release will provide further evidence for the selectivity of adenosine.

Adenosine can also affect monoamine synthesis. It affects the activity of tyrosine hydroxylase (124), increasing the enzyme's activity in the PC12 pheochromocytoma cell line while changing cyclic AMP levels.

STRUCTURE–ACTIVITY RELATIONSHIPS AT ADENOSINE RECOGNITION SITES

Defining receptor site function depends almost exclusively on identifying chemicals that selectively interact with the receptor in question. The major impetus in adenosine-related research was the observation that caffeine, the most widely used psychoactive agent in the world (3, 125), was an adenosine antagonist (22). Although almost twenty years have since passed, studies of purine nucleosides are directed more to their actions as antimetabolites (126) than as neuromodulatory agents. Thus, without exception, all known adenosine agonists are purine nucleosides (6), whereas antagonists, with few exceptions, are imidazol [3,4d]-pyrimidines (9, 10, 26, 57).

Studies of these agonists and antagonists have led to a considerable knowledge of the structure–activity relationship (SAR) for adenosine receptors (127, 128). Using avidin-biotin conjugates (129), investigators have examined the receptor topography for the brain A_1 receptor. As already discussed, substitutions in the N^6, 2, and 5' positions on the purine ring confer receptor site selectivity (39) and make the compound more resistant to degradation and uptake (6). Examination of many agonist analogs led to the postulation of models for the brain A_1 receptor (130) and for the coronary receptor. The coronary receptor has an SAR different from that for either A_1 or A_2 receptors (131). Adenosine analogs substituted in the N^6 position (CHA, CPA, R-PIA; Figure 1) are A_1 selective; 5'-substituted (NECA, MECA) and 2-substituted analogs (CV 1808) are active at A_2 receptor. Compounds substituted in both the N^6 and 5' positions tend to still be A_1 selective (130), but with reduced activity. The observed lower intrinsic activity and efficacy of adenosine itself have generally been assumed (3, 6, 16, 29, 30) to be the result of the endogenous compound's susceptibility to metabolic degradation and uptake. This susceptibility may still be a major factor and certainly is in receptor binding assays that include ADA. However, several adenosine agonists differ in their efficacy, as assessed by their ability to increase A_2 receptor–mediated cyclic AMP formation (46, 48, 132). The order of activity is: NECA > 2-CADO > CPA ≥ N^6-methyladenosine = adenosine N'-oxide > adenosine > R-PIA ≥ CHA > S-PIA. The position of the substitutions does not appear to be consistently related to the efficacy of the analogs, which have been suggested (132) to be partial agonists. However, P site (Table 1) activation may contribute to the efficacy of the analogs. The analogs probably do not interact with A_1 and A_2 receptor subtypes (103), as the binding profiles of these compounds show (40).

Substitutions in the 8-position of the xanthine molecule (Figure 2) increase adenosine antagonist activity considerably (9, 10, 26, 43, 57) in some in-

stances and similarly decrease solubility (7). DPX, PACPX, and a series of 27 other xanthines exhibit receptor selectivity of the order: $A_1 > A_{2_b} > A_{2_a}$ (40). The activity of 8-cyclopentyltheophylline (CPT) when compared to that of CPA has led to the suggestion (40) that the cyclopentyl moiety of the xanthine and the purine both bind to the same region of the A_1 receptor. The xanthine therefore bound "backwards" compared to the nucleoside, with the pyrimidine ring of the xanthine corresponding to that of the purine (Figures 1 & 2). The functionalized congeners, XAC and XCC (Figure 2; 10), and PD 113,297 (Figure 2; 40; 133) are more soluble 8-substituted xanthines. PD 116,948 (Figure 2) is an A_1-selective antagonist, whereas PD 115,199, like NECA, is nonselective in its antagonist actions (59, 134). BW A1433U (Figure 2) is another potent xanthine antagonist (135).

Nonxanthine adenosine antagonists have also been described (Figure 3). CGS 8216, a pyrazoloquinoline that is a potent inverse agonist at the central benzodiazepine receptor (136), has weak adenosine antagonist activity (44). Subsequent chemical modification of this compound led to the identification of the triazoloquinazoline CGS 15943A (44), a potent A_2-selective adenosine antagonist with minimal benzodiazepine receptor interactions. The pyrazolopyridines etazolate and cartazolate also have adenosine antagonist activity (40, 129, 137), as does the pyrazolopyrimidine DJB-KK (138) (Figure 3). Two other xanthines, S-caffeine (139) and propentofylline (HWA 825; 140, 141), have somewhat unusual biological activity profiles. Their effects are opposite to those of the more classical xanthines. It has been speculated (140) that propentofylline may be the first xanthine adenosine agonist. It enhances the effects of adenosine (142).

PHYSIOLOGICAL IMPLICATIONS OF ADENOSINE RECEPTOR FUNCTION

Central Nervous System

Much of the receptor work on adenosine receptor function has been carried out using nervous tissue because this tissue has a higher density of these receptors than other tissues. Because of the ubiquitous distribution of the nucleoside and its receptors (82, 143) and the paucity of structurally dissimilar chemical entities to probe the adenosine receptor, adenosine's physiological role in CNS function has largely been defined by inference (29, 30).

ADENOSINE AND LOCOMOTOR ACTIVITY Adenosine agonists decrease spontaneous motor activity (3) when administered either peripherally or directly into the brain. Sedative effects can also be observed (3, 21, 142). While A_2 receptor activation has been linked to the sedative and locomotor effects of adenosine (144), the relative bioavailability of the various agonists

may also be a contributory factor (30). These effects of adenosine can be blocked by xanthines. However, adenosine at lower doses than those required to decrease locomotor performance can increase activity via mechanisms that cannot be blocked by caffeine (3).

ADENOSINE AND SLEEP Adenosine is a potent sedative, eliciting a hypnotic state in various species including mammals (20, 21, 99, 145, 146). In rats, adenosine agonists increase deep sleep duration (146) and may increase REM sleep. Administration of the ADA-inhibitors EHNA (erythrohydroxynonyladenine; 98) or deoxycoformycin (99) can induce sleeplike states. The adenosine analog, 1-methylisoguanosine (doridosine), does not affect sleep (147), suggesting receptor selectivity for adenosine. The central stimulant activity of the xanthines (3, 125) is consistent with a sedative and hypnotic action for endogenous adenosine. The ethanol-sensitive "long-sleep" mouse is more sensitive to the sedative and hypothermic actions of R-PIA than the ethanol-insensitive "short-sleep" mouse (148). Such differences that have been related to increases in both the affinity and density of A_1 receptors in the long-sleep mouse (149).

ADENOSINE AND ANXIETY Caffeine has well-documented anxiogenic activity (125, 150). Following the discovery of a central benzodiazepine receptor (151), considerable effort was expended in the search for an endogenous factor similar in function to the enkephalins at the opiate receptor (or receptors) that would be the endogenous anxiogenic and anxiolytic agent. Inosine, isolated from bovine brain, was found to be a weak inhibitor (IC_{50} = 10^{-3} M) of ligand binding to the benzodiazepine receptor. Isobutylmethylxanthine and 2-CADO were subsequently determined to be ligands for this receptor (151). The later identification of the β-carbolines (152) and various peptidic entities (153) that demonstrated activity similar to that of the benzodiazepines (IC_{50} values = 10^{-9} M) raised considerable debate about the physiological significance of the inosine interaction (151, 154, 155). Furthermore, no correlation could be demonstrated for the effects of a series of anxiolytics on benzodiazepine and A_1 receptor binding (137).

Despite these negative data, a considerable amount of circumstantial evidence still links purines to the antianxiety and other properties of the benzodiazepines. Xanthines can antagonize the effects of benzodiazepines on cell firing (47) and behavior (155). Purines, like the benzodiazepines, are effective muscle relaxants (156, 157). It has been suggested that the sedative effects of the benzodiazepines, but not their anxiolytic activity, involves some interaction with purine-related systems (155). The adenosine-uptake inhibitor, dipyridamole, can inhibit benzodiazepine and A_1 receptor binding equally (137, 158) and has benzodiazepine agonist activity in vivo (159). The

benzodiazepine antagonist, Ro 15-1788, can antagonize caffeine-induced seizures in mice (160). The effects of the prototypic anxiolytic, meprobamate, together with those of many other centrally active therapeutic agents (161), have been related to the ability of such compounds to weakly inhibit adenosine uptake (66). Benzodiazepine receptor antagonists cannot, however, inhibit the effects of diazepam on adenosine uptake (162). Given this series of relationships and the weak adenosine antagonist activity of the inverse agonist, CGS 8216 (136, 137), there may be similarities in the receptor topography and/or function of the central benzodiazepine receptor and the A_1 receptor.

ADENOSINE AND EPILEPSY Caffeine and theophylline have convulsant activity at high doses (125), whereas adenosine can prevent audiogenic- (21), kainate-, picrotoxin-, and mercaptoproprionic acid– (145) induced seizures. Adenosine levels in brain increase markedly during seizure activity (163), and as already noted, the purine may function as an endogenous anticonvulsant (89). R-PIA prolongs postictal depression and decreases the frequency of spiking following amydaloid-kindled and metrazole-induced (164, 165) seizures. The effects of the anticonvulsant carbamazepine have been related to both diazepam and adenosine processes although an SAR was absent (166). Chronic treatment with carbamazepine can up-regulate A_1 receptors (166). The barbiturate anticonvulsants have micromolar affinity for the A_1 receptor (167).

ADENOSINE AND ANALGESIA As with the other central processes in which adenosine may play a role, the association of adenosine with the mechanisms involved in analgesia is complex. Xanthines reduce morphine analgesia (168) and can elicit a "quasi-morphine withdrawal syndrome" (QMWS; 169), which involves an increase in norepinephrine turnover (170, 171). The α_2-adrenoceptor agonist clonidine can antagonize the QMWS phenomenon (172). Both adenosine and clonidine have been used to suppress responses to opiate withdrawal in humans (173) and animals (174). Xanthines reverse the respiratory depressant actions of morphine (175) and act as an analgesic adjuvant (176). This effect may be related to their ability to inhibit cyclooxygenase activity (177). Conflicting reports (168, 178) have suggested that xanthines have both analgesic and antinociceptive activity, as does adenosine (179). The analgesic actions of R-PIA can be blocked by theophylline but not by naloxone (180). Increases in adenosine receptor density have been found in morphine-dependent mice (181).

ADENOSINE AND DEPRESSION It has been suggested, on the basis of the effects of antidepressants on cyclic AMP formation and their ability to potentiate the effects of adenosine, that certain of the effects of anti-

depressants involve adenosine-related processes (182, 183). Chronic electroconvulsive therapy (ECT), used in the treatment of depression, increases A_1 receptor density in rat brain (184). However, neither chronic antidepressant (185) nor lithium (184) treatment affects the affinity or density of A_1 receptors. Several antidepressants are weak inhibitors of adenosine uptake (183).

ADENOSINE AND SELF-MUTILATION BEHAVIORS Large doses of caffeine can elicit a self-destructive behavior in rats (186) similar to that observed in Lesch–Nyhan syndrome (187), an X-linked disorder that results in a deficit in the purine-metabolizing enzyme hypoxanthine-guanine phosphoribosyltransferase (HRGTPase). While chronic caffeine intake elicits a similar behavioral profile, rather than decreasing HRGPTase activity, the xanthine can actually increase it (186). This action suggests that the enzyme changes are coincidental and that the primary lesion in this disorder is a hyperactivity of central dopaminergic systems, most notably at the level of the basal ganglia (188). A selective association between adenosine and dopamine occurs at the behavioral (189, 190) and anatomical (191) levels. This relationship is further emphasized by the demonstrated antipsychotic profile of adenosine agonists in animal models (192).

The relationship between α_2 adrenoceptor– and adenosine receptor– mediated events is further underlined by the fact that clonidine can induce self-mutilation (193). This effect is enhanced by caffeine and attenuated by adenosine (194).

Cardiovascular

Adenosine can regulate coronary blood flow (13, 18, 71) and has negative dromotropic, chronotropic, and inotropic effects on heart contractility (18, 107). These effects can be mediated directly through interactions with adenosine receptors. They can be indirectly mediated by either the inhibition of the release of other neurotransmitters affecting heart function or by the indirect antagonism of the myocardial actions of norepinephrine. The increase in coronary blood flow is mediated by A_2-receptor activation (195). The effects on cardiac rhythmicity that involve the suppression of impulse formation in the sinoatrial node and the blockade of impulse propagation in the atrioventricular node (107) involve an A_1 receptor (18, 107). Exogenously administered adenosine, due to its effects on cardiac conduction, has been successfully used in the treatment of supraventricular tachycardia (196). During hypoxia, ischemia, or reactive hyperemia, adenosine is freely available (13, 18). Thus, the observed effects of the purine on cardiac function are physiological rather than pharmacological. Purine effects on heart function have been directly compared with those of acetylcholine (197). Both compounds have negative ionotropic actions, shorten atrial transmembrane action poten-

tials, activate K^+ channels, and elicit antiadrenergic effects in Purkinje cell fibers. The negative chronotropic effects of adenosine, unlike those seen with acetylcholine, are indirect, however (197, 198). This indirect action, like that related to the antagonism of catecholamine actions at the adenylate cyclase level (18), has not been demonstrated in the intact animal (193). Adenosine infusion can markedly decrease mean arterial pressure without affecting heart rate (199). In light of adenosine's lack of selectivity for either receptor subtype, this finding suggests (195) that A_2 receptors are more sensitive to adenosine than A_1 receptors. However, the A_1 receptor–mediated effects of adenosine may offset the reflex stimulation of the heart when arterial blood pressure decreases. A vagal component of the effects of the purine on heart function also exists (8, 13) that does not appear to be mediated through reflex mechanisms.

Xanthines have been used as cardiotonics (200) although their solubility and efficacy as adenosine antagonists result in the concomitant inhibition of phosphodiesterase activity and the generation of arrhythmias. The vasodilatory actions of a series of adenosine analogs have been ascribed to interactions with an atypical A_2 receptor (201).

The possibility that the centrally observed actions of adenosine may be indirect and mediated via alteration in blood pressure or changes in the cerebral blood flow has been an issue of some concern (202). While peripheral actions can contribute to the central effects of the purine, changes in CNS function have been reported to occur at purine doses well below those that cause observable changes in blood pressure (3).

Renal

Adenosine, in contrast to its effects in the coronary vasculature, is a vasoconstrictor in the kidney (19) and has biphasic effects on renin release (203). At low concentrations, activating A_1 receptors inhibits renin release and at higher concentrations, activating A_2 receptors stimulates renin release. While both receptor subtypes are present on afferent arterioles (mediating constriction and dilation, respectively), only A_2 receptors are present on efferent arterioles, and these mediate dilation. Adenosine can increase sodium excretion (204) and sympathetic nervous activity, and it reduces the glomerular filtration rate (205). Adenosine is released from macula densa cells in response to an increased sodium load, which may function as the signal transducer to modulate renin release. Binding sites for [^{3}H]2-CADO have been identified in kidney membranes (206).

Pulmonary

Theophylline is one of the most widely used antiasthmatic agents, acting as a bronchodilator (207). This effect has been traditionally ascribed to inhibition

of phosphodiesterase activity, an effect that parallels the effects of β_2 adrenoceptor agonists and forskolin in increasing pulmonary cylic AMP levels (208). In rat mast cells and guinea pig lung tissue, adenosine potentiates the histamine release that results from an allergic challenge. In human basophils, however, adenosine inhibits mediator release (209, 210). This apparent dichotomy has been resolved by the work by Church & Holgate (211), who found that adenosine has biphasic effects on mediator release, which reflect the time of challenge after an allergic insult. Administration of adenosine to asthmatics causes bronchoconstriction but has no effect in normal subjects. Thus, the effects of theophylline as an antiasthmatic may be consistent with its actions as an adenosine antagonist. Examination of the agonist profile for the mediation of histamine release from rat mast cells (212) has provided evidence that a novel adenosine receptor, neither A_1 nor A_2, is involved in the mediation of autocoid release.

Adenosine and the Immune System

Adenosine can inhibit the mitogenic stimulation of lymphocytes by phytohemagglutinin and can inhibit the cytolytic actions of mouse lymphocytes (213). The purine modulates T-cell subset-specific antigen expression, facilitating T8 antigen expression and activating radioresistant suppressor activity (214). Defects in immune function have been linked to deficiencies in ADA activity. In Hodgkin's disease, both ADA and 5'-nucleotidase activity decrease (215). In acquired immune deficiency syndrome (AIDS), purine nucleoside phosphorylase and ADA activities are increased (216). Such an increase suggests that the cellular disorder in this disease probably results from abnormal lymphocyte differentiation. Adenosine can potently modulate neutrophil superoxide formation by interacting with A_2 receptors (217). It also prevents endothelial damage in cell cultures (218). The involvement of cyclic AMP in this effect is unclear (217). 2-CADO has immunosuppressant activity (219).

Gastrointestinal Function

Caffeine and theophylline can augment stress-related events (220). In rats restrained in the cold for 3 hr (221) or immobilized for 12 hr (222) a high incidence of gastric lesions occurs. CHA and R-PIA can also produce lesions in nonstressed animals. In studying the interaction of adenosine and clonidine in analgesia and self-mutilation and aggressive behavior, it was found that the α_2 adrenoceptor agonist, clonidine, could block the action of adenosine on gastric lesion formation. This stress-related phenomenon appears to be centrally mediated via A_1 receptors (221, 222). Adenosine receptors modulating gastric acid secretion are also present in the gastric fundus (223). The purine can also modulate pancreatic secretion (224).

Respiration

Adenosine can cause respiratory depression via a mechanism that can be blocked by aminophylline (225). When administered both centrally and peripherally, adenosine and related analogs can decrease inspiratory neural drive and prolong expiratory time. Aminophylline has been used to treat the paradoxical ventilatory response in infants, and i.v. adenosine can stimulate respiration (226) via a mechanism involving an increase in carotid-body chemoceptor discharge (227).

ADENOSINE RECEPTOR DYNAMICS

Treatment of animals with caffeine or theophylline can cause an up-regulation of A_1 receptors (228, 229), an effect associated with tolerance to the xanthines (230). Chronic carbamazepine (166) and opiate treatment (181) can increase receptor density. In contrast, in gerbils, transient ischemia can down-regulate A_1 receptors in the CA1 region of the hippocampus (231).

ADENOSINE UPTAKE

The physiological significance of the ability of various classes of psychotherapeutic agents to inhibit adenosine uptake has yet to be resolved, especially since the concentrations required to show significant effects are usually in the micromolar range. Nearly every class of centrally active therapeutic agents inhibits adenosine uptake (66). The side effects associated with the use of heterocyclic compounds (which can accumulate at high concentrations in brain bilayers) may be related to a relatively nonspecific effect on adenosine uptake.

ADENOSINE AND CELLULAR ENERGY CHARGES

The control of cerebral blood flow has been related to the redox state of NAD/NADH (232). However, cyclic AMP produced in response to A_2-receptor activation may be the primary mediator of the vasodilatory and vascular permeability changes (163, 233). In the context of the "retaliatory metabolite" hypothesis, and the production of adenosine under adverse conditions, some adenosine-related neuromodulatory events may involve an energy charge transfer (33). In hypoxia, purine release from brain slices is related to a decrease in energy charge (234).

THERAPEUTIC TARGETING OF ADENOSINE RECEPTOR MODULATORS

In considering the potential therapeutic applications of agents that either mimic or antagonize the actions of adenosine, it is clear that there are a variety of targets, many of which are attractive pharmacologically. Adenosine agonists may be effective as antihypertensive agents (8, 195), in the treatment of opiate withdrawal (174), as modulators of immune competence (215) and renin release (203), as antipsychotics (192), and as hypnotics (146). Conversely, antagonists may be useful as central stimulants (3), nootropics (141), cardiotonics (200), antistress agents (221), antiasthmatics (211), and in the treatment of respiratory disorders (225). As emphasized, this relative smorgasbord of therapeutic applications reflects the lack of tissue- and receptor-selective adenosine-receptor ligands. The demonstration of consistent species differences in adenosine-related systems (45, 235) further emphasizes the potential significance of adenosine in mammalian tissue function and the need for more selective agents (8).

Much research needs to be done to reach this goal. The consistent, but as yet undemonstrated, interactions between adenosine and opiate, α adrenoceptor, benzodiazepine receptor ligands, and, to a lesser extent, calcium channel–related processes further suggest that adenosine neuromodulation is not an epiphenomenon. In regard to the availability of endogenous adenosine under normal conditions, it has been suggested (29, 30, 236) that receptor density rather than purine availability is the primary factor determining responses to adenosine. This suggestion would be consistent with the selective activation of coronary A_2 receptors by nonselective agonists (195). Much of the work done in the past five years has, due to technical limitations, been confined to the A_1 receptor. The possibility that A_{2a}, A_{2b}, and P_2 receptors may play significant roles in tissue function cannot be discounted at this time.

Dunwiddie (30) has compared the role of adenosine as a neuromodulator with that of cyclic AMP in relation to protein kinase activation, where the location of the enzyme and its substrates regulates the final response to elevation of cyclic nucleotide levels. In any event, adenosine and related adenine nucleotides, like the peptides, represent unconventional neurotransmitters. They require new hypotheses to explain their role in tissue function (30). With the current rapid progress in the biological evaluation of adenosine as a neuromodulator, together with increased efforts in developing new chemical entities in this area, adenosine may be the first neuromodulator to aid significantly the understanding and treatment of human disease states where current therapy is either absent or limited.

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Literature Cited

1. Arch, J. R. S., Newsholme, E. A. 1978. The control of the metabolism and the hormonal role of adenosine. *Essays Biochem.* 14:82–123
2. Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K., Watson, J. D. 1983. *Molecular Biology of the Cell,* New York: Garland
3. Snyder, S. H. 1985. Adenosine as a neuromodulator. *Ann. Rev. Neurosci.* 8:103–24
4. Bradford, H. F. 1986. *Chemical Neurobiology,* pp. 158–59. New York: Freeman
5. Williams, M. 1984. Adenosine—a selective neuromodulator in the mammalian CNS? *Trends Neurosci.* 7:164–68
6. Daly, J. W. 1982. Adenosine receptors: target sites for drugs. *J. Med. Chem.* 25:197–207
7. Bruns, R. F., Daly, J. W., Snyder, S. H. 1983. Adenosine receptor binding: structure activity analysis generates extremely potent xanthine antagonists. *Proc. Natl. Acad. Sci. USA* 80:2077–80
8. Williams, M. 1985. Tissue and species differences in adenosine receptors and their possible relevance to drug development. In *Adenosine: Receptors and Modulation of Cell Function,* ed. V. Stefanovich, K. Rudolphi, P. Schubert, pp. 73–85. Oxford: IRL Press
9. Daly, J. W., Padgett, W., Shamin, M. T., Butts-Lamb P., Waters, J. 1985. 1,3-Dialkyl-8-(p-sulfophenyl)xanthines: potent water-soluble antagonists for A_1- and A_2 adenosine receptors. *J. Med. Chem.* 28:487–492
10. Jacobson, K. A., Kirk, K. L., Padgett, W. L., Daly, J. W. 1985. Functionalized congeners of 1,3-dialkylxanthines: preparation of analogs with high affinity for adenosine receptors. *J. Med. Chem.* 28:1334–40
11. Hanley, M. 1985. Peptide Binding Assays. In *Neurotransmitter Receptor Binding,* ed. H. I. Yamamura, S. J. Enna, M. J. Kuhar, pp. 91–102. New York: Raven. 2nd ed.
12. Schwartz, J. H. 1981. Chemical basis of synaptic transmission. In *Principles of Neural Science,* ed. E. R. Kandel, J. H. Schwartz, pp. 106–20. New York: Elsevier-North Holland
13. Drury, A. N., Szent-Gyorgi, A. 1929. The physiological activity of adenine compounds with especial reference to their actions upon the mammalian heart. *J. Physiol. London* 68:213–37
14. Williams, M. 1987. Purinergic receptors and central nervous system function. In *Psychopharmacology: A Third Generation of Progress,* ed. H. Meltzer. New York: Raven. In press
15. Honey, R. M., Ritchie, W. T., Thomson, W. A. R. 1930. The action of adenosine upon the human heart. *Quart. J. Med.* 23:485–89
16. Editorial. 1985. Adenosine revisited. *Lancet* 2:927–28
17. Berne, R. M., Knabb, R. M., Ely, S. W., Rubio, R. 1983. Adenosine in the local regulation of blood flow: a brief overview. *Fed. Proc.* 42:3136–42
18. Fredholm, B. B., Sollevi, A. 1986. Cardiovascular effects of adenosine. *Clin. Physiol.* 6:1–21
19. Osswald, H. 1983. Adenosine and renal function. In *Regulatory Function of Adenosine,* ed. R. M. Berne, T. W. Rall, R. Rubio, pp. 399–415. Boston: Nijhoff
20. Feldberg, W., Sherwood, S. L. 1954. Injections of drugs into the lateral ventricle of the cat. *J. Physiol. London* 123:148–67
21. Maitre, M., Ciesielski, L., Lehmann, A., Kempf, E., Mandel, P. 1974. Protective effect of adenosine and nicotinamide against audiogenic seizure. *Biochem. Pharmacol.* 23:2807–16
22. Sattin, A., Rall, T. W. 1970. The effect of adenosine and adenine nucleotides on the cyclic adenosine 3'5'-phosphate content of guinea pig cerebral cortex slices. *Mol. Pharmacol.* 6:13–23
23. Londos, C. Wolff, T. 1977. Two distinct adenosine-sensitive sites on adenyl-

ate cyclase. *Proc. Natl. Acad. Sci. USA* 74:5482–86
24. Londos, C., Wolff, J., Cooper, D. M. F. 1983. Adenosine receptors and adenylate cyclase interactions. See Ref. 19, pp. 17–32
25. Van Calker, D., Muller, M., Hamprecht, B. 1979. Adenosine regulates via two different types of receptors, the accumulation of cyclic AMP in cultured brain cells. *J. Neurochem.* 33:999–1005
26. Smellie, F. W., Davis, C. W., Daly, J. W., Wells, T. N. 1979. Alkylxanthines-inhibition of adenosine-elicited accumulation of cyclic AMP in brain slices and of brain phosphodiesterase activity. *Life Sci.* 24:2475–81
27. Bruns, R. F., Daly, J. W., Snyder, S. H. 1980. Adenosine receptors in brain membranes: binding of N^6-cyclohexyl[^{3}H]adenosine and 1,3-diethyl-8-[^{3}H]phenylxanthine. *Proc. Natl. Acad. Sci. USA* 77:5547–51
28. Stone, T. W. 1981. Physiological roles for adenosine and adenosine 5'-triphosphate in the nervous system. *Neuroscience* 6:523–45
29. Williams, M. 1984. Mammalian adenosine receptors. *Handb. Neurochem.* 6:1–28
30. Dunwiddie, T. V. 1985. The physiological roles of adenosine in the central nervous system. *Int. Rev. Neurobiol.* 27: 63–139
31. Burnstock, G. 1983. A comparison of receptors for adenosine and adenine nucleotides. See Ref. 19, pp. 49–62
32. Burnstock, G., Kennedy, C. 1985. Is there a basis for distinguishing two types of P_2-purinoceptor? *Gen. Pharmac.* 16:433–40
33. Atkinson, D. E. 1968. Energy charge of the adenylate pool as a regulatory parameter. *Biochemistry* 7:4030–34
34. Haslam, R. J., Davidson, M. M. L., Desjardins, T. V. 1978. Inhibition of adenylate cyclase by adenosine analogues in preparations of broken and intact human platelets. Evidence for the unidirectional control of platelet function by cyclic AMP. *Biochem. J.* 176:83–95
35. Dolphin, A. C., Archer, E. A. 1983. An adenosine agonist inhibits and a cyclic AMP analogue enhances the release of glutamate but not GABA from slices of rat dentate gyrus. *Neurosci. Lett.* 43:49–54
36. Dunwiddie, T. W., Fredholm, B. B. 1984. Adenosine receptors mediating inhibitory electrophysiological responses in rat hippocampus are different from receptors mediating cAMP formation. *Naunyn Schmiedebergs Arch. Pharmacol.* 326:294–301
37. Riberio, J. A., Sa-Almedia, A. M., Namordo, J. M. 1979. Adenosine and adenosine triphosphates decrease $^{45}Ca^{2+}$ uptake by synaptosomes stimulated by potassium. *Biochem. Pharmacol.* 28: 1297–300
38. Hollingsworth, E. B., De La Cruz, R. A., Daly, J. W. 1986. Accumulations of inositol phosphates and cyclic AMP in brain slices: synergistic interactions of histamine and 2-chloroadenosine. *Eur. J. Pharmacol.* 122:45–50
39. Hamprecht, B., Van Calker, D. 1985. Nomenclature of adenosine receptors. *Trends Pharmacol. Sci.* 6:153–54
40. Bruns, R. F., Lu, G. H., Pugsley, T. A. 1986. Characterization of the A_2 adenosine receptor labeled by [^{3}H]NECA in rat striatal membranes. *Mol. Pharmacol.* 29:331–46
41. Williams, M., Braunwalder, A., Erickson, T. E. 1986. Evaluation of the binding of the A-1 selective adenosine radioligand, cyclopentyladenosine (CPA), to rat brain tissue. *Naunyn Schmiedebergs Arch. Pharmacol.* 332:179–83
42. Deleted in proof
43. Schwabe, U., Ukena, D., Lohse, M. J. 1985. Xanthine derivatives as antagonists at A_1 and A_2 adenosine receptors. *Naunyn Schmiedebergs Arch. Pharmacol.* 330:212–21
44. Williams, M., Francis, J., Ghai, G., Psychoyos, S., Braunwalder, A., et al. 1987. Biochemical characterization of CGS 15943A, a novel, non-xanthine adenosine antagonist. *J. Pharmacol. Exp. Ther.* In press
45. Stone, G. A., Snowhill, E. W., Weeks, B., Williams, M. 1986. Species differences in adenosine A-2 receptor binding in mammalian striatal membranes. *Abstr. Soc. Neurosci.* 12:799
46. Premont, J., Perez, M., Blanc, G., Tarsin, J. P., Thierry, A. M., et al. 1979. Adenosine-sensitive adenylate cyclase in rat brain homogenates: kinetic characteristics, specificity, topographical, subcellular and cellular distribution. *Mol. Pharmacol.* 16:790–804
47. Phillis, J. W., Wu, P. H. 1981. The role of adenosine and its nucleotides in central synaptic transmission. *Prog. Neurobiol.* 16:187–93
48. Daly, T. W., Butts-Lamb, P., Padgett, W. 1983. Subclasses of adenosine re-

ceptors in the central nervous system: interaction with caffeine and related methylxanthines. *Cell. Mol. Neurobiol.* 3:69–80

49. Ribeiro, J. A., Sebastiao, A. M. 1986. Adenosine receptors and calcium: basis for proposing a third (A_3) adenosine receptor. *Prog. Neurobiol.* 26:179–209
50. Schwabe, U., Trost, T. 1980. Characterization of adenosine receptors in rat brain by (−)(^{3}H)N^6-phenylisopropyladenosine. *Naunyn Schmiedebergs Arch. Pharmacol.* 313:179–88
51. Linden, J., Hollen, C. E., Patel, A. 1985. The mechanism by which adenosine and cholinergic agents reduce contractility in rat myocardium. Correlation with cyclic adenosine monophosphate and receptor densities. *Circ. Res.* 56:728–35
52. Yeung, S.-M. H., Green, R. D. 1984. [^{3}H]-5'*N*-ethylcarboxamide adenosine binds to both Ra and Ri receptors in rat striatum. *Naunyn Schmiedebergs Arch. Pharmacol.* 325:218–25
53. Ukena, D., Poeschla, E., Hutteman, E., Schwabe, U. 1984. Effects of *N*-ethylmaleimide on adenosine receptors of rat fat cells and human platelets. *Naunyn Schmiedebergs Arch. Pharmacol.* 327:247–53
54. Fredholm, B. B., Lindren, E., Lindstrom, K. 1985. Treatment with *N*-ethylmaleimide selectively reduces adenosine receptor-mediated decreases in cyclic AMP accumulation in rat hippocampal slices. *Br. J. Pharmacol.* 86: 509–13
55. Barnes, E. M. Jr., Thampy, K. G. 1982. Subclasses of adenosine receptors in brain membranes from adult tissue and from primary cultures of chick embryo. *J. Neurochem.* 39:647–52
56. Williams, M., Abreu, M., Jarvis, M. F., Noronha-Blob, L. 1987. Characterization of adenosine receptors in the PC12 pheochromocytoma cell line using radioligand binding-evidence for A-2 selectivity. *J. Neurochem.* In press
57. Jacobson, K. A., Kirk, K. L., Padgett, W. L., Daly, J. W. 1986. A functionalized congener approach to adenosine receptor antagonists: amino acid conjugates of 1,3-dipropylxanthine. *Mol. Pharmacol.* 29:126–33
58. Ukena, D., Jacobson, K. A., Kirk, K. L., Daly, J. W. 1986. A [^{3}H]amine congener of 1,3-dipropyl-8-phenylxanthine: a new radioligand for A_2 adenosine receptors of human platelets. *FEBS Lett.* 199:269–74
59. Bruns, R. F., Lu, G. H., Pugsley, T. A. 1986. Adenosine receptor subtypes: binding studies. *Pfluegers Arch.* 407:54 (Suppl.)
60. Salter, M. W., Henry, T. L. 1985. Effects of adenosine 5'-monophosphate and adenosine 5'-triphosphate on functionally identified neurons in the cat spinal horn. *Neuroscience* 15:815–25
61. Fedan, J. S., Hogaboom, G. K., O'Donnell, J. P., Westfall, D. P. 1985. Use of photoaffinity labels as P_2-purinoceptor antagonists. In *Methods Used in Pharmacology. Methods Used in Adenosine Research*, ed. D. M. Paton, 6:279–92. New York: Plenum
62. Williams, M., Risley, E. A. 1980. Binding of ^{3}H-adenyl-5-imidodiphosphate (AppNHp) to rat brain synaptic membranes. *Fed. Proc.* 39:1009
63. Levin, R. M., Jacoby, R., Wein, A. J. 1983. High affinity, divalent ion-specific binding of ^{3}H-ATP to homogenate derived from rabbit urinary bladder. *Mol. Pharmacol.* 23:1–7
64. Fedan, J. S., Hogaboom, G. K., O'Donnell, J. P., Jeng, C. J., Guillory, G. 1985. Interaction of [^{3}H]arylazido aminopropronyl ATP ([^{3}H]ANAPP$_3$) with P_2-purinergic receptors in the smooth muscle of the isolated guinea pig vas deferens. *Eur. J. Pharmacol.* 108:49–61
65. Frew, R., Lundy, R. M. 1986. Arylazido aminopropionyl ATP (ANAPP$_3$): interaction with adenosine receptors in longitudinal smooth muscle of the guinea-pig ileum. *Eur. J. Pharmacol.* 123:395–400
66. Wu, P. H., Phillis, J. W. 1984. Uptake by central nervous tissues as a mechanism for the regulation of extracellular adenosine concentrations. *Neurochem. Int.* 6:613–32
67. Olsson, R. A., Snow, J. A., Gentry, M. K. 1978. Adenosine metabolism in canine myocardial reactive hyperemia. *Circ. Res.* 42:358–62
68. Rubio, R., Wiedmeier, V. T., Berne, R. M. 1974. Relationship between coronary flow and adenosine production and release. *J. Mol. Cell. Cardiol.* 6:561–66
69. Rubio, R., Berne, R. M., Dobson, J. G. Jr. 1973. Sites of adenosine production in cardiac and skeletal muscle. *Am. J. Physiol.* 225:938–53
70. Schraeder, J., Schutz, W., Bardenheuer, H. 1981. Role of S-adenosylhomocysteine hydrolase in aden-

osine metabolism in mammalian heart. *Biochem. J.* 196:65–70

71. Sparks, H. V., Bardenheuer, H. 1986. Regulation of adenosine formation by the heart. *Circ. Res.* 58:193–201

71a. Lawson, R., Mowbray, J. 1986. Purine nucleotide metabolism: The discovery of a major new oligomeric adenosine tetraphosphate derivative in rat heart. *Int. J. Biochem.* 18:407–13

72. Israel, M., Lesbats, B., Manaranche, R., Muenier, F. M., Franchon, P. 1980. Retrograde inhibition of transmitter release by ATP. *J. Neurochem.* 34:923–32

73. Silinsky, E. 1985. Processes by which purines inhibit transmitter release. In *Purines: Pharmacology and Physiological Roles*, ed. T. W. Stone, pp. 67–73. Deerfield Beach, Fla: VCH Publications

74. Fredholm, B. B., Hedqvist, P. 1980. Modulation of neurotransmission by purine nucleotides and nucleosides. *Biochem. Pharmacol.* 29:1633–43

75. Jonzon, B., Fredholm, B. B. 1985. Release of purines, noradrenaline and GABA from rat hippocampal slices by field stimulation. *J. Neurochem.* 44: 217–24

76. Goodman, R. R., Kuhar, M. J., Hester, L., Snyder, S. H. 1983. Adenosine receptors: autoradiographic evidence for their location on axon terminals of excitatory neurons. *Science* 220:967–68

77. Pons, F., Bruns, R. F., Daly, J. W. 1980. Depolarization-evoked accumulation of cyclic AMP in brain slices: the requisite intermediate adenosine is not derived from hydrolysis of released ATP. *J. Neurochem.* 34:1319–23

78. Newman, M. E., McIwain, H. 1977. Adenosine as a constituent of the brain and of isolated cerebral tissues, and its relationship to the generation of adenosine 3'5'-cyclic monophosphate. *Biochem. J.* 164:131–37

79. McDonald, W. F., White, T. D. 1984. Adenosine released from synaptosomes is derived from the extracellular dephosphorylation of released ATP. *Prog. Neuropsychopharmacol. Biol. Psychiatr.* 8:487–94

80. Marangos, P. J., Boulenger, T. P. 1985. Basic and clinical aspects of adenosinergic neuromodulation. *Neurosci. Biobehav. Rev.* 9:421–30

81. Braas, K. M., Newby, A. C., Wilson, V. S., Snyder, S. H. 1986. Adenosine containing neurons in the brain localized by immunocytochemistry. *J. Neurosci.* 6:1952–61

82. Goodman, R. R., Snyder, S. H. 1982. Autoradiographic localization of adenosine receptors in rat brain using [^{3}H]cyclohexyladenosine. *J. Neurosci.* 2:1230–41

83. Patel, J., Marangos, P. J., Stivers, J., Goodwin, F. K. 1982. Characterization of adenosine receptors in brain using N^6 cyclohexy[^{3}H]adenosine. *Brain Res.* 237:203–14

84. Newby, A. C. 1984. Adenosine and the concept of "retaliatory metabolites." *Trends Biochem. Sci.* 9:42–44

85. Harms, H. H., Wardeh, G., Mulder, A. H. 1978. Adenosine modulates depolarization-induced release of ^{3}H-noradrenaline from slices of rat brain neocortex. *Eur. J. Pharmacol.* 49:305–8

86. Fredholm, B. B., Dunwiddie, T. V., Bergman, B., Lindstrom, K. 1984. Levels of adenosine and adenine nucleotides in slices of rat hippocampus. *Brain Res.* 295:127–36

87. Carney, J. M., Logan, L., McMaster, S. B., Seale, T. W. 1985. Can we understand the CNS pharmacology of xanthines when we understand CNS adenosine systems? See Ref. 8, pp. 199–208

88. Berne, R. M. 1963. Cardiac nucleotides in hypoxia: possible role in regulation of coronary blood flow. *J. Physiol. London* 204:317–22

89. Dragunow, M., Goddard, G. V., Laverty, R. 1985. Is adenosine an endogenous anticonvulsant? *Epilepsia* 26:480–87

90. Nagy, J. I., Buss, M., Daddona, P. E. 1986. On the innervation of trigeminal mesencephalic primary afferent neurons by adenosine deaminase containing projections from the hypothalamus in the rat. *Neuroscience* 17:141–56

91. Sebastiao, A. M., Riberio, J. A. 1986. Enhancement of transmission at the frog neuromuscular junction by adenosine deaminase: evidence for an inhibitory role of endogenous adenosine on neuromuscular transmission. *Neurosci. Lett.* 62:267–70

92. Bender, A. S., Wu, P. H., Phillis, J. W. 1981. The rapid uptake and release of [^{3}H]-adenosine by rat cerebral cortical synaptosomes. *J. Neurochem.* 36:651–60

93. Moritaki, H. 1983. Possible mechanism of potentiation of the action of adenosine by some vasodilators. In *Physiology and Pharmacology of Adenosine Derivatives*, ed. J. W. Daly, Y. Kuroda, J. W. Phillis, H. Shimizu, M. Ui, pp. 197–207. New York: Raven

94. Taylor, D. A., Williams, M. 1982. Interaction of 2-phenylaminoadenosine

(CV 1808) with adenosine systems in rat tissues. *Eur. J. Pharmacol.* 86:35–42
95. Hammond, J. R., Clanachan, A. S. 1985. Species differences in the binding of [^{3}H]nitrobenylthioinosine to the nucleoside transport system in mammalian central nervous system membranes. Evidence for interconvertible forms of the binding site/transporter complex. *J. Neurochem.* 45:527–35
96. Marangos, P. J., Houston, M., Montgomery, P. 1985. [^{3}H]Dipyridamole: a new ligand probe for brain adenosine uptake sites. *Eur. J. Pharmacol.* 117:393–94
97. Sanderson, G. Schofield, C. N. 1986. Effects of adenosine uptake blockers and adenosine on evoked potentials of guinea-pig olfactory cortex. *Pfluegers Arch.* 406:25–30
98. Mendelson, W. B., Kuruvilla, A., Watlington, T., Goehl, K., Paul, S. M., Skolnick, P. 1983. Sedative and electroencephalographic actions of erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA): Relationship to inhibition of brain adenosine deaminase. *Psychopharmacology* 79:126–29
99. Radulovacki, M., Virus, R. M., Djuricic-Nedelson, M., Green, R. D. 1983. Hypnotic effects of deoxycorformycin in rats. *Brain Res.* 271:392–95
100. Nagy, J. I., Labella, F., Buss, M., Dadonna, P. E. 1984. Immunohistochemistry of adenosine deaminase: implications for adenosine neurotransmission. *Science* 224:166–68
101. Nagy, J. I., Geiger, J. D., Daddona, P. E. 1985. Adenosine uptake sites in rat brain: identification using [^{3}H]nitrobenzylthioinosine and co-localization with adenosine deaminase. *Neurosci. Lett.* 55:47–53
102. Browning, M. D., Huganir, R., Greengard, P. 1985. Protein phosphorylation and neuronal function. *J. Neurochem.* 45:11–23
103. Dolphin, A. C., Prestwich, S. A. 1985. Pertussis toxin reverses adenosine inhibition of neuronal glutamate release. *Nature* 316:148–51
104. Ui, M., Katada, T., Murayama, T., Kurose, T., Yajima, M., et al. 1984. Islet activating protein, pertussis toxin: a specific uncoupler of receptor-mediated inhibition of adenylate cyclase. *Adv. Cyclic Nucleotide Res.* 17:145–51
105. Berridge, M. J., Irvine, R. F. 1984. Inositol triphosphate, a novel second messenger in cellular signal transduction. *Nature* 312:315–21
106. Siggins, G. R., Schubert, P. 1981. Adenosine depression of hippocampal neurons in vitro: an intracellular study of dose-dependent actions on synaptic and membrane potential. *Neurosci. Lett.* 23:55–60
107. Bellardinelli, L., West, A., Crampton, R., Berne, R. M. 1983. Chronotropic and dromomotropic effects of adenosine. See Ref. 19, pp. 377–98
108. Segal, M. 1982. Intracellular analysis of a postsynaptic action of adenosine in the rat hippocampus. *Eur. J. Pharmacol.* 79:193–99
109. Trussell, L. O., Jackson, M. B. 1985. Adenosine-activated potassium conductance in cultured striatal neurons. *Proc. Natl. Acad. Sci. USA* 82:4857–61
110. Greene, R. W., Haas, H. L. 1985. Adenosine actions on CA_1 pyramidal neurones in rat hippocampal slices. *J. Physiol. London* 366:119–27
111. Schofield, C. N. 1978. Depression of evoked potentials in brain slices by adenosine compounds. *Br. J. Pharmacol.* 63:239–44
112. Kuroda, Y. 1985. Modulation of calcium channels through different adenosine receptors; ADO-1 and ADO-2. See Ref. 8, pp. 233–40
113. Proctor, W. R., Dunwiddie, T. V. 1983. Adenosine inhibits calcium spikes in hippocampal pyramidal neurons in vitro. *Neurosci. Lett.* 35:197–201
114. Dunwiddie, T. V., Haas, H. L. 1985. Adenosine increases synaptic facilitation in the in vitro rat hippocampus: evidence for a presynaptic site of action. *J. Physiol. London* 369:365–77
115. Schubert, P. 1985. Synaptic and nonsynaptic modulation by adenosine: a differential action on K- and Ca-fluxes. See Ref. 8, pp. 117–27
116. Phillis, J. W., Swanson, T. H., Barraco, R. A. 1984. Interactions between adenosine and nifedipine in the rat cerebral cortex. *Neurochem. Int.* 6:693–99
117. Murphy, K. M. M., Snyder, S. H. 1982. Adenosine receptor binding and specify receptors for calcium channel drugs. In *Calcium Entry Blockers Adenosine and Neurohumors: Advances In Coronary Vascular and Cardiac Control*, ed. G. F. Merrill, H. R. Weiss, pp. 295–306. Baltimore: Urban & Schwarzenberg
118. Bohm, M., Burmann, H., Meyer, W., Nose, M., Schmitz, W., Scholz, H. 1985. Positive inotropic effect of Bay K 8644: cAMP-independence and lack of inhibitory effect of adenosine. *Naunyn Schmiedebergs Arch. Pharmacol.* 329: 447–50

119. Stone, T. W. 1981. Physiological roles for adenosine and adenosine 5'-triphosphate in the nervous system. *Neuroscience* 6:523–45
120. Spignoli, G., Pedata, F., Pepeu, G. 1984. A_1 and A_2 adenosine receptors modulate acetylcholine release from brain slices. *Eur. J. Pharmacol.* 97:341–43
121. Ebstein, R. P., Daly, T. W. 1982. Release of norepinephrine and dopamine from brain vesicular preparations: effects of calcium antagonists. *Cell. Mol. Neurobiol.* 2:205–13
122. Carlsson, A. 1975. Receptor-mediated control of dopamine metabolism. In *Pre- and Postsynaptic Receptors,* ed. E. Usdin, W. E. Bunney, Jr., pp. 49–67. New York: Dekker
123. Govoni, S., Perkov, V., Montefusco, O., Missale, C., Battaini, F., et al. 1984. Differential effects of caffeine on dihydroxyphenylacetic acid concentration in various rat brain dopaminergic structures. *J. Pharm. Pharmacol.* 36: 458–60
124. Koboyashi, K., Kuroda, Y., Yoshioka, M. 1981. Change of cyclic AMP level in synaptosomes from cerebral cortex: increase by adenosine derivatives. *J. Neurochem.* 36:86–91
125. Snyder, S. H., Sklar, P. 1984. Behavioral and molecular actions of caffeine: focus on adenosine. *J. Psychiatr. Res.* 18:91–106
126. Robins, R. K., Revankar, G. R. 1985. Purine analogs and related nucleosides and nucleotides as antitumor agents. *Med. Res. Rev.* 5:273–96
127. Bruns, R. F. 1979. Adenosine receptor activation in human fibroblast: nucleoside agonists and antagonists. *Can. J. Physiol. Pharmacol.* 58:673–91
128. Bruns, R. F. 1981. Adenosine antagonism by purines, pteridines and benzopteridines in human fibroblasts. *Biochem. Pharmacol.* 30:325–33
129. Jacobson, K. A., Kirk, K. L., Padgett, W., Daly, J. W. 1985. Probing the adenosine receptor with adenosine and xanthine biotin conjugates. *FEBS Lett.* 184:30–35
130. Daly, J. W. 1985. Adenosine receptors in the central nervous system: structure activity relationships for agonists and antagonists. See Ref. 73, pp. 5–15
131. Kusachi, S., Thompson, R. D., Yamada, N., Daly, D. T., Olsson, R. A. 1985. Coronary Adenosine receptor: Structure of the N^6 aryl subregion. *J. Med. Chem.* 28:1636–43
132. Bazil, C. W., Minneman, K. P. 1986. An investigation of the low intrinsic activity of adenosine and its analogs at low affinity (A2) adenosine receptors in rat cerebral cortex. *J. Neurochem.* 47: 547–53
133. Bruns, R. F., Lu, G. H., Pugsley, T. A. 1985. Towards selective adenosine antagonists. See Ref. 8, pp. 51–58
134. Bruns, R. F., Fergus, J. H., Badger, E. W., Bristol, J. A., Santay, L. A., Hays, S. J. 1986. PD 115,199: Antagonist ligand for adenosine A_2 receptors. *Fed. Proc.* 45:801
135. Leighton, H. J., Daluge, S., Craig, R., Parmeter, L. L. 1986. Classification of purine receptors in electrically stimulated guinea pig ileum and guinea pig left atria using the potent purine receptor antagonist BW A1433U. *Pfluegers Arch.* 407:531 (Suppl.)
136. Czernik, A. J., Petrack, B., Kalinsky, H. J., Psychoyos, S., Cash, W. D., et al. 1982. CGS 8216: Receptor binding characteristics of a potent benzodiazepine antagonist. *Life Sci.* 30:363–72
137. Williams, M., Risley, E. A., Huff, J. R. 1981. Interaction of putative anxiolytic agents with central adenosine receptors. *Can. J. Physiol. Pharmacol.* 59:897–900
138. Davies, L. P., Chen Chow, S., Skerritt, J. H., Brown, D. J., Johnston, G. A. R. 1984. Pyrazolo[3,4-d]Pyrimidines as adenosine antagonists. *Life Sci.* 34: 2117–28
139. Fassina, G., Gaion, L. M., Caparrotta, L., Carponedo, F. 1985. A caffeine analogue (1,3,7-trimethyl-6-thioxo-2-oxopurine) with a negative inotropic and chronotropic effect. *Naunyn Schmiedebergs Arch. Pharmacol.* 330:222–26
140. Grome, J. J., Stefanovich, V. 1985. Differential effects of xanthine derivatives on local cerebral blood flow and glucose utilization in the conscious rat. See Ref. 8, pp. 453–57
141. Hindmarch, I., Subhan, Z. 1985. A preliminary investigation of "Albert 285" HWA 285 on psychomotor performance, mood and memory. *Drug Dev. Res.* 5:379–86
142. Fredholm, B. B., Lindstrom, K. 1986. The xanthine derivative 1-(5'-oxohexyl)-3-methyl-7-propyl xanthine (HWA 285) enhances the actions of adenosine. *Acta Pharmacol. Toxicol.* 58:187–92
143. Snowhill, E. A., Williams, M. 1986. Autoradiographic evaluation of the binding of [^{3}H]cyclohexyladenosine to aden-

osine A-1 receptor in rat tissues. *Neurosci. Lett.* 68:41–46
144. Barraco, R. A., Coffin, V. L., Altman, H. J., Phillis, J. W. 1983. Central effects of adenosine analogues on locomotor activity in mice and antagonism of caffeine. *Brain Res.* 272:392–95
145. Dunwiddie, T. V., Worth, T. 1982. Sedative and anticonvulsant effects of adenosine analogs in mouse and rat. *J. Pharmacol. Exp. Ther.* 220:70–76
146. Radulovacki, M., Virus, R. M., Djuricic-Nedelson, M., Green, R. D. 1984. Adenosine analogs and sleep in rats. *J. Pharmacol. Exp. Ther.* 228:268–74
147. Virus, R. M., Rapoza, D., Crane, R. C. 1985. Lack of effect of 1-methylisoguanosine on sleep in rats. *Neuropharmacology* 24:547–49
148. Proctor, W. R., Dunwiddle, T. V. 1984. Behavioral sensitivity to purinergic drugs parallel ethanol sensitivity in selectively bred mice. *Science* 224:519–21
149. Fredholm, B. B., Zahniser, N. R., Weiner, G. R., Proctor, W. R., Dunwiddle, T. V. 1985. Behavioral sensitivity to PIA in selectively bred mice is related to a number of A_1 adenosine receptors but not to cyclic AMP accumulation in brain slices. *Eur. J. Pharmacol.* 111:133–36
150. Charney, D. S., Galloway, M. P., Heninger, G. R. 1984. The effects of caffeine on plasma MPHG, subjective anxiety, autonomic symptoms and blood pressure in healthy humans. *Life Sci.* 35:135–44
151. Williams, M. 1983. Anxioselective anxiolytics. *J. Med. Chem.* 26:619–28
152. Braestrup, C., Nielsen, M., Olsen, C. E. 1980. Urinary and brain β-carboline-3-carboxylates as potent inhibitors of brain benzodiazepine receptors. *Proc. Natl. Acad. Sci. USA* 77:2288–92
153. Costa, E., Guidotti, A. 1985. Endogenous ligands for benzodiazepine recognition sites. *Biochem. Pharmacol.* 34:3399–403
154. Haefely, W., Kyburz, E., Gerecke, M., Mohler, H. 1985. Recent advances in the molecular pharmacology of benzodiazepine receptors and in the structure activity relationships of their agonists and antagonists. *Adv. Drug Res.* 14:165–322
155. Williams, M., Yokoyama, N. 1986. Anxiolytics, anticonvulsants and sedative-hypnotics. *Ann. Rep. Med. Chem.* 21:11–20
156. Turski, L., Schwarz, M., Turski, W. A., Ikonomidou, C., Sontag, K.-H. 1984. Effect of aminophylline on muscle relaxant action of diazepam and phenobarbitone in genetically spastic rats: further evidence for a purinergic mechanism in the action of diazepam. *Eur. J. Pharmacol.* 103:99–105
157. Bruns, R. F., Katims, J. J., Annau, Z., Snyder, S. H., Daly, T. W. 1983. Adenosine receptor interactions and anxiolytics. *Neuropharmacology* 22:1523–29
158. Davies, L. P., Cook, A. F., Poonian, A. M., Taylor, K. M. 1980. Displacement of [^{3}H]diazepam binding in rat brain by dipyridamole and by 1-methylisoguanosine, a natural marine product with muscle relaxant activity. *Life Sci.* 26:1089–97
159. Davies, L. P., Chow, S. C., Johnston, G. A. R. 1984. Interaction of purines and related compounds with photoaffinity-labeled benzodiazepine receptors in rat brain membranes. *Eur. J. Pharmacol.* 97:325–29
160. Albertson, T. E., Bowyer, J. F., Paule, M. G. 1982. Modification of the anticonvulsant efficacy of diazepam by Ro-15-1788 in the kindled amygdaloid seizure model. *Life Sci.* 31:1597–601
161. Phillis, J. W., Delong, R. E. 1984. A purinergic component in the central actions of meprobamate. *Eur. J. Pharmacol.* 101:295–97
162. Morgan, P. F., Lloyd, H. G., Stone, T. W. 1983. Inhibition of adenosine accumulation by a CNS benzodiazepine antagonist (Ro 15-1788) and a peripheral benzodiazepine receptor ligand (Ro 5-4864). *Neurosci. Lett.* 41:183–88
163. Winn, H. R., Morii, S., Berne, R. M. 1985. The role of adenosine in the autoregulation of cerebral blood flow. *Ann. Biomed. Eng.* 13:321–28
164. Rosen, J. B., Berman, R. F. 1985. Prolonged postictal depression in amygdaloid kindled rats by the adenosine analog, L-PIA. *Exp. Neurol.* 90:549–57
165. Burley, E. S., Ferrendelli, J. A. 1984. Regulatory effects of neurotransmitter on electroshock and pentylenetetrazol seizures. *Fed. Proc.* 43:2521–24
166. Marangos, P. J., Weiss, S. B. B., Montgomery, P., Patel, J., Narang, P. K., et al. 1985. Chronic carbamazepine treatment increases brain adenosine receptors. *Epilepsia* 26:493–98
167. Lohse, M. J., Klotz, K.-N., Jakobs, K. H., Schwabe, U. 1985. Barbiturates are selective antagonists at A_1 adenosine receptors. *J. Neurochem.* 45:1761–70
168. Ho, I. K., Lo, H. H., Way, E. L. 1973.

Cyclic adenosine monophosphate antagonism of morphine analgesia. *J. Pharmacol. Exp. Ther.* 185:336–46
169. Collier, H. O. J., Cuthbert, N. J., Francis, D. L. 1981. Character and meaning of quasi-morphine withdrawal phenomena elicted by methyl-xanthines. *Fed. Proc.* 40:1513–18
170. Reinhard, R. J. F. Jr., Galloway, M. P., Roth, R. H. 1983. Noradrenergic stimulation of serotonin synthesis and metabolism. II. Stimulation by 3-isobutyl-1-methylxanthine. *J. Pharmacol. Exp. Ther.* 226:764–69
171. Galloway, M., Roth, R. 1983. Clonidine prevents methylxanthine stimulation of norepinephrine metabolism in rat brain. *J. Neurochem.* 40:246–51
172. Grant, S. J., Redmond, D. E. 1982. Methylxanthine activation of noradrenergic unit activity and reversal by clonidine. *Eur. J. Pharmacol.* 85:1094–109
173. Gold, M. S., Pottash, A. C., Sweeney, D. R., Kleber, H. D. 1980. Opiate withdrawal using clonidine. A safe, effective and rapid nonopiate treatment. *J. Am. Med. Assoc.* 243:343–46
174. Collier, H. O. J., Plant, N. T., Tucker, J. F., Von Uexkull, A. 1984. Inhibition with adenosine derivatives of opiate withdrawal effects. *Br. J. Pharmacol.* 81:1–131
175. Arnanda, J. V., Thurman, T. 1970. Methylxanthines in apnea of prematurity. *Clin. Perinatol.* 6:87–108
176. Laska, E. M., Sunshine, A., Mueller, F., Elvers, W. B., Siegel, C., Rubin, A. 1984. Caffeine as an analgesic adjuvant. *J. Am. Med. Assoc.* 251:1711–18
177. Whorton, A. R., Collawn, J. B., Montgomery, M. E., Young, S. L., Kent, R. S. 1985. Arachidonic acid metabolism in cultured aortic endothelial cells. Effect of cAMP and 3-isobutyl-1-methylxanthines. *Biochem. Pharmacol.* 34:119–23
178. Gourley, D. R. H., Beckner, S. K. 1973. Antagonism of morphine analgesia by adenine, adenosine and adenine nucleotides. *Proc. Soc. Exp. Biol. Med.* 144:774–80
179. Yarbrough, B. V., McGuffin-Clineschmidt, J. 1981. In vivo behavioral assessment of central nervous system purinergic receptor. *Eur. J. Pharmacol.* 76:137–44
180. Ahlijanian, M. K., Takemori, A. E. 1985. Effects of (−)N^6(R-phenylisopropyl)-adenosine (PIA) and caffeine on nociception and morphine-induced analgesia, tolerance and dependence in mice. *Eur. J. Pharmacol.* 112:171–79
181. Ahlijanian, M. K., Takemori, A. E. 1986. Changes in adenosine receptor sensitivity in morphine-tolerant and -dependent mice. *J. Pharmacol. Exp. Ther.* 236:615–620
182. Sattin, A., Stone, T. W., Taylor, D. A. 1978. Biochemical and electropharmaceutical studies with tricyclic antidepressants in rat and guinea pig cerebral cortex. *Life Sci.* 23:2621–26
183. Phillis, J. W. 1984. Potentiation of the action of adenosine on cerebral cortical neurones by the tricyclic antidepressants. *Br. J. Pharmacol.* 83:567–75
184. Newman, M., Zohar, J., Kalian, M., Belmaker, R. H. 1984. The effects of chronic lithium and ECT on A_1 and A_2 adenosine receptor systems in rat brain. *Brain Res.* 291:188–92
185. Williams, M., Risley, E. A., Robinson, J. L. 1983. Chronic in vivo treatment with desmethylimipramine and mianserin does not alter adenosine A_1 radioligand being in rat cortex. *Neurosci. Lett.* 35:47–51
186. Minana, M. D., Portoles, M., Jorda, G., Grisolia, S. 1984. Lesch–Nyhan syndrome, caffeine model: increase of purine and pyrimidine enzymes in rat brain. *J. Neurochem.* 43:1556–60
187. Nyhan, W. L., Oliver, W. J., Lesch, M. 1965. A familiar disorder of uric acid metabolism and central nervous system function. *J. Pediatr.* 67:257–63
188. Lloyd, K. G., Hornykiewicz, O., Davidson, L., Shannak, K., Farley, I., et al. 1981. Biochemical evidence of dysfunction of brain neurotransmitters in the Lesch–Nyhan Syndrome. *N. Engl. J. Med.* 305:1106–11
189. Fredholm, B. B., Herrara-Marschitz, M., Jonzon, B., Lindstrom, K., Ungerstedt, U. 1983. On the mechanism by which methylxanthines enhance apomorphine induced rotation behavior in the rat. *Pharmacol. Biochem. Behav.* 19:535–41
190. Green, R. D., Proudfit, H. K., Yeung, S.-M. H. 1982. Modulation of striatal dopaminergic function by local injection of 5'-*N*-ethylcarboxamide adenosine. *Science* 218:58–61
191. Williams, M., Jarvis, M. J. 1987. Adenosine antagonists as potential therapeutic agents. *Pharmacol. Biochem. Behav.* In press
192. Heffner, T. G., Downs, D. A., Bristol, J. A., Bruns, R. F., et al. 1985. Antipsychotic-like effects of adenosine re-

ceptor agonists. *Pharmacologist* 27: 293
193. Ushijima, I., Katsuragi, T., Furukawa, T. 1984. Involvement of adenosine receptor activities in aggressive responses produced by clonidine in mice. *Psychopharmacology,* 83:335–39
194. Holloway, W. R. Jr., Thor, D. H. 1985. Interactive effects of caffeine, 2-chloroadenosine and haloperidol on activity, social investigation and play fighting in juvenile rats. *Pharmacol. Biochem. Behav.* 22:421–26
195. Evans, D. B., Schenden, J. A., Bristol, J. A. 1982. Adenosine receptors mediating cardiac depression. *Life Sci.* 30:2425–32
196. Dimarco, J. P., Sellers, T. D., Lerman, B. D., Greenberg, M. L., Berne, R. M., Belardinelli, L. 1985. Diagnostic and therapeutic use of adenosine in patients with supraventricular tachyarrhythmias. *J. Am. Coll. Cardiol.* 6:417–25
197. Rardon, D. P., Bailey, J. C. 1984. Adenosine attenuation of the electrophysiological effects of isoproterenol on canine cardiac purkinje fibers. *J. Pharmacol. Exp. Ther.* 228:792–98
198. Schutz, W., Freissmuth, M. 1985. Adenosine receptors in the heart: controversy about signal transmission. *Trends Pharmacol. Sci.* 6:310–11
199. Collis, M. G., Keddie, J. R., Pettinger, S. J. 1983. 2-Chloroadenosine lowers blood pressure in the conscious dog without reflex tachycardia. *Br. J. Pharmacol.* 80: 385 pp.
200. Lucchesi, B. R., Patterson, E. S. 1984. Antiarrhythmic drugs. In *Cardiovascular Pharmacology,* ed. M. Antonaccio, pp. 329–414, New York: Raven. 2nd ed.
201. Leung, E., Johnston, C. I., Woodcock, E. A. 1985. An investigation of the receptors involved in the coronary vasodilatory effect of adenosine analogues. *Clin. Exp. Pharmacol. Physiol.* 12:515–19
202. Barraco, R. A., Aggarawal, A. K., Phillis, J. W., Moron, M. A., Wu, P. H. 1986. Dissociation of the locomotor and hypotensive effects of adenosine analogues in the rat. *Neurosci. Lett.* 48:139–44
203. Itoh, S., Carretero, O. A., Murray, R. D. 1985. Possible role of adenosine in the macula densa mechanism of renin release in rabbits. *J. Clin. Invest.* 76:1412–17
204. Lang, M. A., Preston, A. S., Handler, J. S., Forrest, J. N. 1985. Adenosine stimulates sodium transport in kidney A6 epithelia in culture. *Am. J. Physiol.* 249:C330–36
205. Hall, J. E., Granger, J. P. 1986. Renal hemodynamics and arterial pressure during chronic intrarenal adenosine infusion in conscious dogs. *Am. J. Physiol.* 250:F32–39
206. Wu, P. H., Churchill, P. C. 1985. 2-chloro[^{3}H]-adenosine binding in isolated rat kidney membranes. *Arch. Int. Pharmacodyn.* 273:83–87
207. Cushley, M. J., Tattersfield, A. E., Holgate, S. T. 1983. Inhaled adenosine and guanosine on airway resistance in normal and asthmatic subjects. *Br. J. Clin. Pharmacol.* 15:161–65
208. Holgate, S. T., Lewis, R. A., Austen, K. F. 1980. Role of adenylate cyclase in immunologic release of mediators from rat mast cells and agonist and antagonist effects of purine- and ribose-modified adenosine analogs. *Proc. Natl. Acad. Sci. USA* 77:6800–4
209. Holgate, S. T., Mann, J. S., Cushley, M. J. 1984. Adenosine as a bronchoconstrictor mediator in asthma and its antagonism by methylxanthines. *J. Allergy Clin. Immunol.* 74:302–6
210. Marone, G., Findlay, S. R., Lichtenstein, L. M. 1979. Adenosine receptors on human basophils. Modulation of histamine release. *J. Immunol.* 123:1473–77
211. Church, M. K., Holgate, S. T. 1986. Adenosine and asthma. *Trends Pharmacol. Sci.* 7:49–50
212. Church, M. K., Hughes, P. J. 1985. Adenosine potentiates immunological histamine release from rat mast cells by a novel cyclic AMP-independent cell-surface action. *Br. J. Pharmacol.* 85:3–5
213. Marone, G., Ambrosio, G., Bonaduce, D., Genovese, A., Triggiani, M., Condorelli, M. 1984. Adenosine receptors on human inflammatory cells. *Int. Arch. Allergy Appl. Immunol.* 74:356–61
214. Moroz, C., Twig, S. 1985. The regulatory role of adenosine activated T-lymphocyte subset on the immune response in humans. II. Adenosine induced expression of T-8 antigen. *Biomed. Pharmacother.* 39:145–49
215. Giblett, E. R. 1985. ADA and PNP deficiencies: how it all began. *Ann. N. Y. Acad. Sci.* 451:1–8
216. Murray, J. L., Loftin, K. C., Munn, C. G., Reuben, J. M., Mansell, P. W. A., Hersh, E. M. 1985. Elevated adenosine deaminase and purine nucleoside phosphorylase activity in peripheral blood null lymphocytes from patients with ac-

quired immune deficiency syndrome. *Blood* 65:1318–23
217. Cronstein, B. N., Kramer, S. B., Weissman, G., Hirshhorn, R. 1983. Adenosine: a physiologic modulator of superoxide anion generation by human neutrophils. *J. Exp. Med.* 158:1160–77
218. Cronstein, B. N., Rosenstcin, E. D., Kramer, S. b., Weissmann, G., Hirschhorn, R. 1985. Adenosine: a physiologic modulator of superoxide anion generation by human neutrophils. Adenosine acts via an A_2 receptor on human neutrophils. *J. Immunol.* 135:1366–71
219. Samet, M. K. 1985. Inhibition of antibody production by 2-chloroadenosine. *Life Sci.* 37:225–33
220. Henry, J., Stephens, P. 1980. Caffeine as an intensifier of stress-indirect hormonal and pathophysiologic changes in mice. *Pharmacol. Biochem. Behav.* 13:719–24
221. Geiger, J. D., Glavin, G. B. 1985. Adenosine receptor activation in brain reduces stress-induced ulcer formation. *Eur. J. Pharmacol.* 115:185–90
222. Ushijima, I., Mizuki, Y., Yamada, M. 1985. Development of stress-induced gastric lesions involves central adenosine A_1-receptor stimulation. *Brain Res.* 339:351–55
223. Gerber, J. G., Nies, A. S., Payne, N. A. 1985. Adenosine receptors on canine parietal cells modulate gastric acid secretion to histamine. *J. Pharmacol. Exp. Ther.* 233:623–27
224. Yamagishi, F., Homma, N., Haruta, K., Iwatsuki, K., Chiba, S. 1985. Adenosine potentials secretion-stimulated pancreatic exocrine secretion in the dog. *Eur. J. Pharmacol.* 118:203–9
225. Wessburg, P., Hedner, J., Hedner, T., Persson, B., Jonason, J. 1985. Adenosine mechanisms in the regulation of breathing in the rat. *Eur. J. Pharmacol.* 106:59–67
226. Lagercrantz, H., Yamamoto, Y., Fredholm, B. B., Prabhakar, N., Euler, C. 1984. Adenosine analogues depress ventillation in rabbit neonatcs. Theophylline stimulation of respiration via adenosine receptors? *Pediatr. Res.* 18:387–90
227. McQueen, D. S., Ribeno, T. A. 1981. Effect of adenosine on carotid chemoreceptor activity in the rat. *Br. J. Pharmacol.* 74:129–36
228. Fredholm, B. B. 1982. Adenosine actions and adenosine receptor after one week treatment with caffeine. *Acta Physiol. Scand.* 115:283–86
229. Murray, T. F. 1982. Up-regulation of rat cortical adenosine receptors following chronic administration of theophylline. *Eur. J. Pharmacol.* 82:113–14
230. Chou, D. T., Khan, S., Forde, J., Hirsh, K. R. 1985. Caffeine tolerance: behavioral, electrophysiological and neurochemical evidence. *Life Sci.* 36:2347–58
231. Onodera, H., Kogure, K. 1985. Autoradiographic visualization of adenosine A_1 receptors in the gerbil hippocampus: changes in the receptor density after transient ischemia. *Brain Res.* 345:406–8
232. Dora, E. 1985. Effect of adenosine and its stabile analogue 2-chloroadenosine on cerebrocortical microcirculation and NAD/NADH redox state. *Pfluegers Arch.* 404:208–13
233. Li, Y.-O., Fredholm, B. B. 1985. Adenosine analogues stimulate cyclic AMP formation in rabbit cerebral microvessels via adenosine A_2-receptors. *Acta Physiol. Scand.* 124:253–59
234. Fredholm, B. B., Duner-Engstrom, M., Fastbom, J., Jonzon, B., Lindgren, E., et al. 1987. Interactions between the neuromodulator adenosine and classical transmitters. *Proc. 3rd Int. Symp. Adenosine. Munich, Fed. Republ. Germany:* Springer-Verlag. In press
235. Murphy, K. M. M., Snyder, S. H. 1982. Heterogeneity of adenosine A_1 receptor binding in brain tissue. *Mol. Pharmacol.* 21:250–57
236. Lee, K. S., Reddington, M., Schubert, P., Kreutzberg, G. 1983. Regulation of the strength of adenosine modulation in the hippocampus by a differential distribution of the density of A_1 receptors. *Brain Res.* 260:156–59
237. Marquardt, D. L., Wasserman, S. I. 1985. [^{3}H]Adenosine binding to rat mast cells—pharmacologic and functional characterization. *Agents Actions* 16: 453–61
238. Abreu, M. E., Valentine, H. L. 1986. Pharmacologic profile of adenosine receptor binding sites in guinea pig atrium and ventricle. *Pfluegers Arch.* 407:547 (Suppl.)