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Perspective

Adenosine Receptors: Targets for Future Drugs

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Introduction

The profound hypotensive, sedative, antispasmodic, and vasodilatory actions of adenosine were first recognized over 5 decades ago.[1] During the intervening years the number of biological roles proposed for adenosine and for precursor adenine nucleotides have increased considerably. Extracellular adenosine receptors and adenine nucleotide receptors have been identified. The adenosine receptors appear linked in many cells to adenylate cyclase, while nucleotide receptors probably control ion fluxes. A variety of adenosine analogues and ATP analogues have been introduced in recent years for the study of these receptor functions. Some of the adenosine analogues are shown in Chart I. Alkylxanthines, such as caffeine and theophylline, are the best known antagonists of adenosine receptors. Recently some extremely potent xanthine antagonists have been developed. Some of the adenosine antagonists are depicted in Chart II. There do not appear to be any satisfactory specific ATP-receptor antagonists at the present time.

Adenosine perhaps represents a general regulatory substance, since no particular cell type or tissue appears uniquely responsible for its formation. In this regard, adenosine is unlike various endocrine hormones. Nor is there any evidence for storage and release of adenosine from nerve or other cells. Thus, adenosine is unlike various neurotransmitter substances. The presence of purinergic nerves has been proposed in peripheral systems,[2] but ATP rather than adenosine has been considered the neurotransmitter in such nerves.

The significance of the metabolic pathways involved in formation and inactivation of adenosine remains an important but poorly resolved question. Certainly, the most obvious pathway for formation of adenosine involves ATP → ADP → AMP as precursors. Formation of adenosine via this ATP pathway appears to increase considerably in cells under conditions of high-energy demands.[3] Adenosine formed intracellularly from this ATP pathway must then reach extra cellular receptors, perhaps by diffusion, perhaps by a transport mechanism. The ATP pathway can also occur extracellularly, and this may in some tissues be a very important route to adenosine. Thus, ATP released from nerve cells either alone or with a neurotransmitter can be hydrolyzed to adenosine by extracellular phosphatases and 5′-nucleotidases. Other pathways to adenosine may ultimately prove just as important as the ATP pathways, but at the present time the ATP pathway is considered to be the major route to adenosine.

A variety of routes for "inactivation" of adenosine are present in cells. These include enzymes, such as adenosine deaminase, adenosine kinase, and *S*-adenosylhomocysteinase, and uptake processes. The function of these pathways appears to be the maintenance of low levels of endogenous adenosine. Because of this, intracellular levels

Chart I

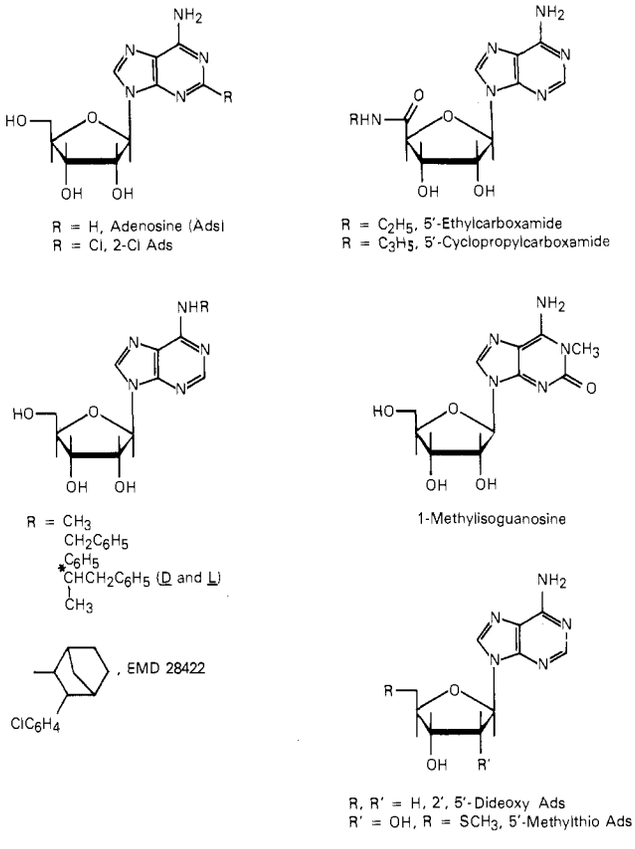

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Table I. Biological Roles for Adenosine

system	response to adenosine	blockade by theophylline	ref[a]
vasculature	vasodilation	yes	5, 6
kidney	vasoconstriction	yes	7
heart	negative chronotropic, inotropic effects	yes	8
smooth muscle	relaxation[b]	yes	9, 10
striated muscle	relaxation, centrally mediated	yes	11
adipocytes	inhibition, lipolysis	yes	12, 13
	stimulation, glucose oxidation	?	14
platelets	inhibition, aggregation	yes	5, 15, 16
adrenals	stimulation, steroidogenesis	yes	17
lymphocytes	inhibition, function	yes	18
pancreas	potentiation, glucagon release	yes	19
mast cells	potentiation, histamine release	yes	20
neurons	inhibition	yes	21
	inhibition, transmitter release	yes	7, 22, 23, 24
brain	sedation	yes	25

[a] Only a few representative lead references are provided. [b] Adenosine can cause contraction in trachea, which is reversed by xanthines.[26]

Chart II

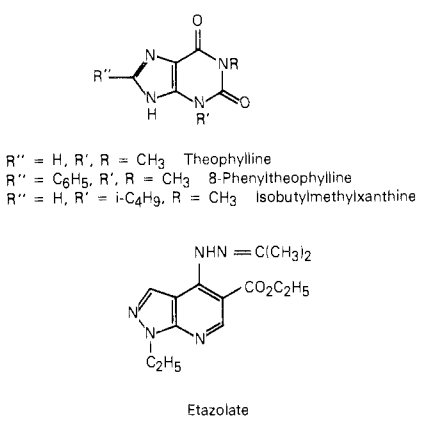
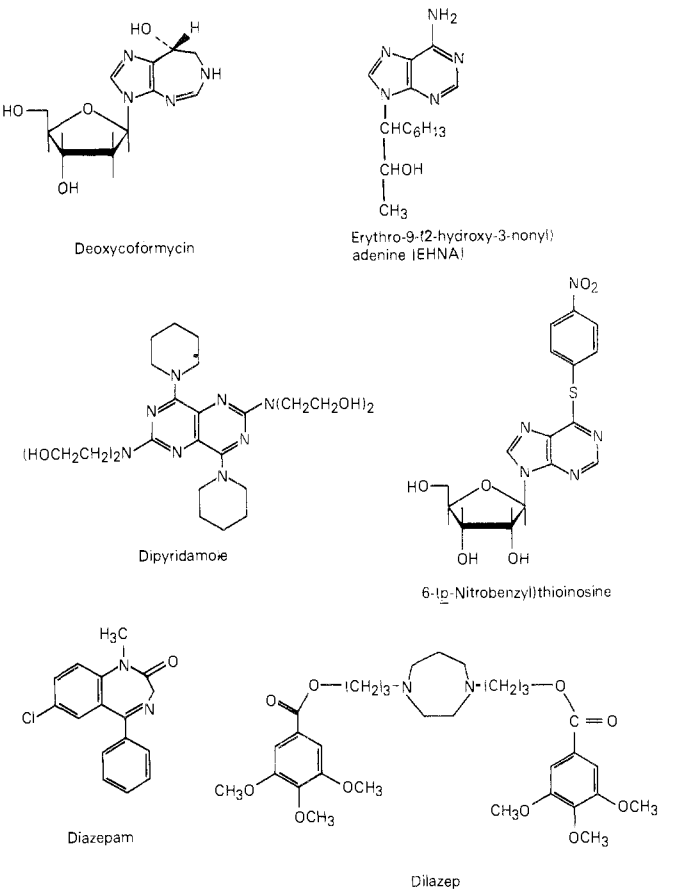


Chart III



of adenosine would appear likely to remain at 1–2 μM or less under most physiological conditions. Extracellularly, adenosine levels probably also never rise under normal conditions above 1–2 μM. The enzyme adenosine kinase, of course, represents a route for incorporation of adenosine into pools of adenine nucleotides. The relative importance of the catabolic and anabolic pathways for adenosine in different cell types has not been well defined. It seems likely that variations in the importance of different routes will pertain. A variety of agents have been developed for manipulation of the pathways for adenosine formation and inactivation. Some of these are shown in Chart III.

Adenosine might be compared as a physiological regulator to the prostaglandins: In both cases the enzymes involved in the metabolic formation are ubiquitous and appear to be responsive to alterations in the physiological state of the cell. Receptors for adenosine, like those for prostaglandins, are proving to be very widespread. Finally, both prostaglandins and adenosine appear in many instances to be involved with the regulation of functions involving calcium ions. Prostaglandins, of course, derive from membrane precursors, while adenosine derives from cytosolic precursors.

The present article will attempt to overview possible physiological roles for adenosine, pathways involved in formation and inactivation of adenosine, the nature of adenosine receptors, profiles of agonists and antagonists, the development of radioactive ligands for adenosine receptors, and strategies for the investigation and exploitation of adenosine functions with agents and tools that specifically affect this complex of reactions and actions. Comprehensive reviews on metabolism and physiology of adenosine are available.[3,4] In keeping with the objectives of the perspective series, the present article does not at-

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tempt to be comprehensive.

Physiological Roles for Adenosine

Although adenosine can affect a variety of physiological functions (see Table I), particular attention has been directed over the years toward actions which might lead to clinical applications. Preeminent has been the cardiovascular effects of adenosine which lead to vasodilation and hypotension but which also lead to cardiac depression. The antilipolytic, antithrombotic, and antispasmodic actions of adenosine have also received some attention. The antilipolytic action appears due to inhibition of adipocyte adenylate cyclase by adenosine. The antithrombotic action appears due to activation of platelet adenylate cyclase by adenosine and a resultant inhibition of platelet aggregation. The antispasmodic and vasodilatory actions appear linked to smooth-muscle depressant effects of adenosine. Such depressant effects are perhaps linked to activation of muscle adenylate cyclase. Adenosine stimulates steroidogenesis in adrenal cells, again probably via activation of adenylate cyclase. Adenosine has inhibitory effects on neurotransmission and on spontaneous activity of central neurons. The mechanisms are unknown but certainly involve calcium and may involve presynaptic inhibition of adenylate cyclase. The reversal of the inhibitory effects of adenosine on cholinergic and noradrenergic transmitter release by calcium ions has led to adenosine and analogues being referred to as calcium antagonists. Finally, the bronchoconstrictor action of adenosine and its antagonism by xanthines, such as theophylline, represents an important area of research. This effect of adenosine and the vasoconstriction elicited by adenosine in the kidney are very poorly understood. Adenosine affects various other cellular functions, and the mechanisms involved in most of these actions have not been well defined. In addition to regulatory roles, adenosine and certain analogues can be cytotoxic agents. This action may be due to irreversible inhibition of *S*-adenosylhomocysteinase.[27]

Many questions and challenges clearly remain to be resolved before the physiological regulatory functions mediated by ATP and/or adenosine are understood. These include the source and disposition of ATP/adenosine, as well as the localization and nature of the receptors and the mechanisms involved in their action.

Source and Disposition of Adenosine

Relationship to ATP. There are relationships between ATP and adenosine, both with regard to formation and disposition and in some tissues with regard to actions. Certainly, the pathway considered at present to be the most important for the formation of adenosine might be called the "ATP pathway". This pathway is operative in many cells under conditions of energy deficit.[3] Under such conditions, 5′-AMP levels increase as levels of ATP decrease due to excessive energy demands. Endo-5′-nucleotidase, thought to be freed from inhibition by ATP, hydrolyzes 5′-AMP to adenosine. Adenosine can then cross biological membranes either actively or passively to reach extracellular receptors. The ATP pathway can also lead to adenosine from ATP released into extracellular space. For example, ATP coreleased with a neurotransmitter, such as acetylcholine or norepinephrine, can either act at ATP receptors or can be hydrolyzed to adenosine by ecto-ATPases and 5′-nucleotidases. Similarly, ATP might be released as a transmitter itself to act on receptors and/or be hydrolyzed to adenosine. In either case the resultant adenosine can then activate adenosine receptors. It should be noted that 5′-nucleotidase is primarily an ecto-enzyme. Inhibitors of ecto-ATPases and ecto-5′-nucleotidase, such as α,β-methyleneadenosine diphosphate and 5′-GMP, have been used to investigate the involvement of "released" ATP and metabolism to adenosine in physiological or biochemical responses (vide infra). Potent and selective inhibitors of ATPases and 5′-nucleotidases would provide valuable tools for further definition of the role of ATP pathways to the physiological formation and functions of adenosine in different tissues.

A second possible cellular pathway to adenosine which does not involve ATP can be proposed, namely, the "methylation pathway". Biological methylations in intact cells lead to the generation of *S*-adenosylhomocysteine, which is converted by the enzyme *S*-adenosylhomocysteinase to adenosine and homocysteine. It should be noted that this reaction is readily reversible. Exogenous adenosine plus homocysteine can lead to prodigous intracellular levels of *S*-adenosylhomocysteine.[28,29] Under such conditions, biological methylations with various *S*-adenosylmethionine-requiring enzymes will be completely blocked. The methylation pathway, unlike the ATP pathway, can serve not only to generate adenosine but can, in the presence of homocysteine, serve to maintain very low levels of the nucleoside within the cell. Other enzymes whose function may be to maintain low levels of intracellular adenosine are adenosine deaminase and adenosine kinase (vide infra). It has been suggested that irreversible interactions with *S*-adenosylhomocysteine may represent the molecular loci for the cytotoxicity of adenosine and adenosine analogues.[27] Undoubtedly, *S*-adenosylhomocysteinase represents one of the cellular proteins which bind adenosine. There have been extensive studies on adenosine analogues, such as 3-azaadenosine, which can inhibit *S*-adenosylhomocysteinase.[30] The resultant ac-

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cumulation of analogues of S-adenosylhomocysteine causes an inhibition of methylation pathways within the cell.

Inactivation of ATP is normally thought to involve hydrolysis of phosphate bonds ultimately leading to adenosine, while inactivation of adenosine is thought to be primarily the function of two enzymes. These are adenosine deaminase and adenosine kinase. Both have K_m values in the low micromolar range.

Adenosine deaminase converts adenosine to an inactive metabolite, inosine. However, it should be noted that inosine has been shown to interact with benzodiazepine receptors and so is not an entirely "inactive" metabolite.[31] Inosine is further metabolized by phosphorylases to hypoxanthine. Hypoxanthine also interacts with benzodiazepine receptors, as does adenosine itself. The relationship of adenosine and inosine to the actions of benzodiazepines has attracted considerable attention. At present, no conclusions are warranted. It is clear that certain sites on the benzodiazepine–GABA–receptor ionophore complex show similarities to adenosine receptors. For example, caffeine is not only an adenosine receptor antagonist but also blocks binding of diazepam to its receptor sites.[32] The affinity of caffeine and of inosine and adenosine for these sites is increased by GABA. Caffeine can antagonize nearly all of the pharmacological effects of benzodiazepines,[33] and diazepam can antagonize caffeine-induced seizures.[34] Whether these effects involve direct antagonistic effects at diazepam binding sites requires further research. Benzodiazepines can, conversely, interact with adenosine sites associated with the high-affinity adenosine uptake system.[35] The relevance of this to the pharmacology of benzodiazepines is unclear.[36] Recently a potent inhibitor of adenosine uptake, namely, dipyridamole, was shown to have relatively high affinity for benzodiazepine binding sites.[37] Finally, certain N^6-substituted adenosine analogues, namely, N^6-[2-(4-chlorophenyl)bicyclo[2.2.2]octyl]adenosine (EMD 28422), can increase the apparent levels of benzodiazepine receptors in brain membranes.[38] Clearly, the interrelationship of the sedative and muscle-relaxant actions of benzodiazepines and adenosine deserves further investigation. A naturally occuring adenosine analogue, namely, 1-methylisoguanosine, is a sedative and muscle relaxant, presumably through actions mediated by adenosine receptors, but it is also active as an antagonist of diazepam binding.[37]

Potent inhibitors for adenosine deaminase are available, namely, deoxycoformycin which is an irreversible inhibitor and *erythro*-9-(2-hydroxy-3-nonyl)adenine (EHNA) which is a reversible inhibitor.[39,40] The use of such inhibitors often appears to cause in some cases sufficient accumulation of adenosine to trigger a biological response. It is interesting that EHNA is a central depressant. Conversely, the use of exogenous adenosine deaminase has proven a useful technique for probing the role of adenosine in a particular response.[41,42] For example, if a response to ATP involves a requisite hydrolysis to adenosine, then the presence of adenosine deaminase will prevent any accumulation of adenosine, thereby blocking or reducing the response. Many adenosine analogues are not substrates for adenosine deaminase.[43] These include 2-chloroadenosine, adenosine 5′-carboxamides, N^6-phenyladenosine and related N^6-substituted analogues, 5′-(methylthio)-adenosine, 2′,5′-dideoxyadenosine, and 8-bromoadenosine.

The other enzyme involved in the inactivation of adenosine, namely, adenosine kinase, converts adenosine to 5′-AMP, which then enters into the metabolic pool of adenine nucleotides. Inhibitors of this pathway are available, for example, 5′-iodotubercidin,[44] but have not been widely used as research tools to study the physiological significance of the kinase inactivation pathway. Certainly, more potent and specific inhibitors are needed. Adenosine kinase may be involved in facilitated uptake of adenosine by converting adenosine to 5′-AMP.[45] However, facilitated uptake of adenosine appears to occur even in cells with very low levels of adenosine kinase.[46] The enzyme accepts a relatively wide range of substrates but of course will have no effect on analogues such as the adenosine 5′-alkylcarboxamides or 2′,5′-dideoxyadenosine, which lack the requisite 5′-hydroxyl group.

Another class of enzymes that might be important to the inactivation of adenosine are the phosphorylases which would convert adenosine to adenine. Little is known of the possible significance of this pathway. It is a major pathway for the metabolism of inosine, leading to hypoxanthine.

In addition to the enzymatic inactivations, which occur primarily within the cell, adenosine is subject to a high-affinity uptake system which removes it from the extracellular compartment and, hence, from access to extracellular adenosine receptors. Such an uptake system may actually be "tightly coupled" to sites of extracellular generation of adenosine and to the enzymes involved in intracellular inactivation of adenosine. With a tightly coupled system, an uptake inhibitor might potentiate an adenosine response, while having little or no effect on total uptake of exogenous adenosine. Potent inhibitors of the adenosine-uptake system include dilazep, dipyridamole, lidoflazine, hexobendine, papaverine, and certain nucleosides, such as 6-(*p*-nitrobenzyl)thioinosine.[47-50] Such compounds potentiate adenosine responses, and the re-

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sultant shifts in adenosine dose–response relationships provide strong evidence for the extracellular localization of adenosine receptors.[50,51] Furthermore, an uptake blocker such as dipyridamole should potentiate responses to ATP only if hydrolysis to adenosine and interaction with an adenosine receptor is involved.[52] However, the uptake blockers should be used cautiously, since most have other biological activities, including activity as phosphodiesterase inhibitors.[53] A number of adenosine analogues, for example, 2-chloroadenosine and the adenosine 5′-carboxamides, are not good substrates for the adenosine uptake system.[54,55] The uptake system may be reversible and may serve under some conditions to transport adenosine out of cells, but this requires further study. Recently, there has been considerable interest in the inhibition of adenosine uptake systems by psychoactive agents, particularly benzodiazepines[35] (vide supra). However, it appears unlikely that such compounds owe their central antianxiety and anticonvulsant activity to blockade of adenosine uptake, since their affinities for specific sites associated with the GABA–receptor–chloride channel complex are manyfold higher than their affinities for adenosine uptake sites.[36]

The relationship of the localization of extracellular ATP and adenosine receptors to the source of the stimulatory adenine derivative is an important question. In some cases, receptors may be on the same cell from which ATP and/or adenosine originate and may provide a mechanism for feedback modulation of function. In other cases, the receptors may be on a target or distant cell. A variety of approaches to these questions have described, particularly in neuromuscular preparations, where ATP has been proposed to play a role as a neurotransmitter or neuromodulator. One approach involves labeling of adenine nucleotide pools with radioactive adenine or adenosine and studying the release of radioactivity during studies on evoked responses.[56] Release of ATP also can be monitored directly in such preparations.[57] An inherent problem using labeled tissue is that released radioactivity, usually in the form of adenosine, inosine, and hypoxanthine, can come from many cellular compartments and only the sum total can be ascertained. Often the primary question, namely, the origin of adenosine and the involvement of ATP, will require selective manipulations of activation of different cell types, a difficult endeavor.

Adenosine and ATP Receptors

Definitions and Generalities. Two classes of purine receptors in membranes of peripheral cells have been proposed,[2] one preferring adenine nucleotides, the other preferring adenosine. These were termed the P_2- and P_1-purinergic receptors, respectively. The adenine nucleotide (P_2) receptors (ATP receptors) probably control ion fluxes, while the adenosine (P_1) receptors in many instances control adenylate cyclase activity. In most smooth-muscle preparations, the activation of the ATP receptors causes contracture and activation of adenosine receptors relaxation. In such preparations a contraction initiated by ATP would lead ultimately to a relaxation as the ATP was hydrolyzed to adenosine. It has now been recognized that there are not one but at least two classes of extracellular receptors involved in the action of adenosine. One of these has a high affinity for adenosine and at least in some cells couples to adenylate cyclase in an inhibitory manner. These have been termed the A_1 receptors by Van Calker et al.[58] and the R_i receptors by Londos et al.[17] The other class of receptors has a lower affinity for adenosine and in many cell types couples to adenylate cyclase in a stimulatory manner. These have termed the A_2 receptors or R_s receptors. All of the above terms (P_1, P_2, A_1, A_2, R_i, R_s) are currently in use and ultimately some resolution of terminologies must be sought. In the present overview, the adenine nucleotide (P_2) receptors are referred to as ATP receptors, and the two classes of adenosine receptors are referred to as A_1-adenosine and A_2-adenosine receptors.

Characterization of the ATP receptor and the adenosine receptors has now been possible with a variety of structural analogues. Certain of the biologically active ATP analogues are proving particularly valuable, since they are either resistant to hydrolysis by ATPases[59] or as in the case of 8-Br-ATP yield on hydrolysis an adenosine analogue which is inactive at adenosine receptors.[60] Adenosine analogues resistant to metabolism or uptake systems are available. These are particularly valuable, since their apparent potencies will be less affected by metabolic removal from the effector system. The adenosine analogues exhibit differing rank orders of potencies at A_1- and A_2-adenosine receptors, providing a simple method of categorizing a physiological response with respect to the nature of the adenosine receptor (vide infra). The blockade of adenosine receptors but not ATP receptors by theophylline, caffeine, and other xanthines provides another method of categorizing a response with respect to the involvement of adenosine and/or ATP receptors. However, in certain systems adenosine receptors with low sensitivity to theophylline have been described.[61,62] The presence of an inhibitor of adenosine uptake systems, such as dipyridamole, can result in an apparent increase in the potency of theophylline as an adenosine antagonist.[63,64] This rather remarkable effect deserves further study. Antagonism of responses to ATP, ADP, or AMP by inhibitors of ecto-ATPases or nucleotidases[40,65] or by the presence of adenosine deaminase[65] can provide evidence for a requisite hydrolysis to adenosine, followed by activation of adenosine receptors, rather than direct activation of ATP receptors. Unfortunately, no truly specific antagonists of ATP receptors are available. Apamin, quinidine, and 2′,2′-dipyridylisatogen have been suggested as ATP antagonists but do not appear to have the requisite specificity for these receptors.[66–68] An (arylazido)aminoproprionyl-

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Table II. Structure-Activity for Adenosine Analogues[a]

effect	relative potency[b]	ref
vasodilation (reduction in blood pressure)	Ado 5'-cyclopropyl(ethyl)carboxamide > 2-Cl-Ado > L-N^6-(Ph-*i*-Pr)-Ado > D-N^6-(Ph-*i*-Pr)-Ado	5, 6, 78, 79
cardiac depression	L-N^6-(Ph-*i*-Pr)-Ado >> D-N^6-(Ph-*i*-Pr)-Ado	80
muscle relaxation		
ileum	Ado, N^6-Me-Ado, 1-Me-Ado	9
aorta	Ado, N^6-Me-Ado >>> 1-Me-Ado	9
striated	2-Cl-Ado > 1-Me-isoguanosine >>> Ado	11
platelet aggregation (inhibition)	Ado 5'-ethylcarboxamide > 2-Cl-Ado > L-N^6-(Ph-*i*-Pr)-Ado	5, 15
lymphocyte-induced cytolysis (inhibition)	2-Cl-Ado > N^6-Bzl-Ado, N^6-Ph-Ado	18
adipocyte lipolysis (inhibition)	L-N^6-(Ph-*i*-Pr)-Ado, N^6-Ph-Ado, 2-Cl-Ado > Ado 5'-ethylcarboxamide > D-N^6-(Ph-*i*-Pr)-Ado, N^6-Bzl-Ado	13, 80
adrenal and Leydig cell (stimulation steroidogenesis)	Ado 5'-ethylcarboxamide > L-N^6-(Ph-*i*-Pr)-Ado	17
neurotransmitter release or synaptic transmission (inhibition)		
olfactory cortex	N^6-cycloheptyl-Ado > 2-Cl-Ado > N^6-Bzl-Ado	81
hippocampus	L-N^6-(Ph-*i*-Pr)Ado >> D-N^6-(Ph-*i*-Pr)-Ado	82
vas deferens	Ado 5'-cyclopropylcarboxamide > L-N^6-(Ph-*i*-Pr)-Ado > 2-Cl-Ado > D-N^6-(Ph-*i*-Pr)-Ado	23, 83
ileum	Ado 5'-cyclopropylcarboxamide > L-N^6-(Ph-*i*-Pr)-Ado > 2-Cl-Ado > D-N^6-(Ph-*i*-Pr)-Ado	23
central neurons (depression)	2-Cl-Ado, 2-F-Ado, Ado > 2-NH_2-Ado, 2-(*p*-MeOPh)-Ado > N^6-Ph-Ado, inosine > adenine. *8*-Br-Ado inactive	21
behavior (depression)	N^6-cyclohexyl-Ado, L-N^6-(Ph-*i*-Pr)-Ado >> D-N^6-(Ph-*i*-Pr)-Ado	25, 80

[a] Only a few lead references are provided. Compounds separated by commas were nearly equipotent. [b] Abbreviations used are: Ado, adenosine; Cl, chloro; Ph, phenyl, *i*-Pr, isopropyl; Me, methyl; Bzl, benzyl; Br, bromo; NH_2, amino; *p*-MeOPh, *p*-methoxyphenyl.

adenosine triphosphate has recently been proposed as a photoaffinity antagonist of ATP receptors.[69]

At present, ATP receptors do not appear to be linked to adenylate cyclase; instead, they seem to be involved with regulation of certain ion channels.[2,66] Most but perhaps not all adenosine receptors appear linked to adenylate cyclase. However, at present only a limited number of actions of adenosine can be stated with *assurance* to involve cyclic AMP systems. One is the potent inhibition of lipolysis in adipocytes by adenosine and certain adenosine analogues (Table I). In this case the adenosine receptor is of the A_1-receptor category and is inhibitory to the cyclase system. Another action is the inhibition of aggregation of platelets by adenosine and adenosine analogues (Table I). In this case, the adenosine receptor is of the A_2-receptor category and is stimulatory to the cyclase system. A third is the stimulation of steroidogenesis by adenosine in adrenal or Leydig cells (Table I). This response also involves an adenosine receptor of the A_2-category, which is stimulatory to adenylate cyclase. Conversely, at present very few actions of adenosine can be stated with assurance *not* to involve cyclic AMP systems. One such action is the stimulation of glucose oxidation in adipocytes by adenosine and analogues.[14] This action does *not* appear to involve cyclic AMP systems.

Further studies will be needed to firmly establish the role of cyclic AMP to most actions of adenosine. If cyclic AMP is involved in an adenosine-mediated response, then there should be a correlation of the physiological response with an alteration in cyclic AMP levels, in the degree of activation of cyclic AMP dependent protein kinase and in protein phosphorylation patterns relevant to the physiological response. The profile of potencies and effectiveness of adenosine analogues should correlate with respect to the physiological response and to the alterations in cyclic AMP systems. Similarly, the rank order of potencies for blockade of adenosine responses by various xanthines should correlate physiologically and biochemically. Furthermore, if the effect of adenosine is suspected to involve elevation of cyclic AMP then (i) the response should be mimicked by cyclic AMP analogues which directly activate the cyclic AMP dependent protein kinases, (ii) the response should be mimicked by the diterpene forskolin, a general activator of the enzyme adenylate cyclase,[70] and (iii) the response should be potentiated or mimicked by phosphodiesterase inhibitors. If the effect of adenosine is suspected to involve receptor-mediated reductions in cyclic AMP levels, then cyclic AMP analogues, forskolin, and phosphodiesterase inhibitors should oppose the response, and the response should be reduced by prolonged pretreatment with a so-called islet-activating protein from *Bordetella* bacteria. Treatment of cells with this protein has been shown to reduce the inhibitory effect of adenosine on cardiac adenylate cyclase.[71]

Adenosine and adenosine analogues have received some attention as so-called "calcium antagonists".[72] Indeed, the inhibitory effects of adenosine on neurotransmitter release and smooth muscle or cardiac function can be competitively overcome by extracellular calcium.[72-74] Further studies will be required to delineate the mechanisms involved and the possible role of cyclic AMP in systems where adenosine acts as an apparent calcium antagonist. Prostaglandins have also been proposed as being involved in the action of adenine nucleotides or adeno-

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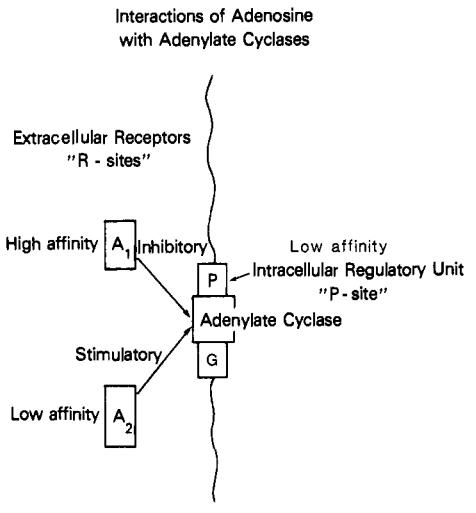

Figure 1. Schematic representation of possible interactions of adenosine with adenylate cyclase. The receptor-mediated actions require the presence of a guanyl nucleotide binding subunit (depicted as G), while the P-site-mediated action occurs intracellularly at the catalytic subunit or a closely associated protein (depicted as P). It is unknown whether any cells contain both A_1- and A_2-adenosine receptors as is depicted in this figure.

sine,[2,59,75] but the possible mechanisms involved are poorly defined.

Adenosine Receptors

Structure–Activity Profiles. There are extensive structure–activity profiles with respect to adenosine interactions with cyclic AMP systems[12,17,76,77] and more limited profiles with respect to physiological responses. A few of the latter are summarized in Table II. The adenylate cyclase system has been extensively studied, and as mentioned, two classes of receptors have been proposed. One is a high-affinity receptor inhibitory to adenylate cyclase (A_1) which is present in adipocytes and heart and brain cells;[13,17,58,71] affinity constants for adenosine and analogues are in the low nanomolar range. The other is more ubiquitous in occurrence and is a low-affinity receptor (A_2); affinity constants for adenosine and analogues in the low micromolar range.[76,77] Virtually all adenylate cyclases appear to have a third adenosine-sensitive site associated with them, namely, the inhibitory P site.[84] This is not a receptor site but rather an intracellular site, perhaps directly associated with the catalytic subunit of the enzyme.[85-87] The affinity constants for adenosine and analogues for the inhibitory P site are in the low to high micromolar range. The physiological significance of this site is unknown, since it seems unlikely that free adenosine levels in intact cells ever reach high micromolar concentrations. The three sites of interaction of adenosine with adenylate cyclase are presented schematically in Figure 1. Profiles of activity of adenosine analogues at the two classes of receptors and at the P site differ markedly. Indeed, it appears that only a limited number of compounds need to be used in order to characterize the nature of an adenosine response as mediated by the A_1 receptor, A_2 receptor, or P site (see Table III).

Table III. Selected Agonists and Antagonists for Characterization of Adenosine-Sensitive Sites Involved in Control of Adenylate Cyclase[a]

agent	affinity constant,[c] μM: A_1 receptor, inhibitory	A_2 receptor, stimulatory	P site, inhibitory
Agonists			
adenosine	0.01	5–10	20
2-Cl-Ado	0.01	5–10	inact
Ado 5′-ethylcarboxamide	0.1	0.5–2	inact
L-N^6-(Ph-*i*-Pr)-Ado	0.003	30	inact
D-N^6-(Ph-*i*-Pr)-Ado	0.2	100	inact
N^6-cyclohexyl-Ado	0.003	30	inact
N^6-Ph-Ado	0.003	50	inact
N^6-Bzl-Ado	0.2	80	inact
N^6-Me-Ado	0.1	80	inact
2′,5′-d_2-Ado	inact	inact	2
8-Br-Ado	inact	inact	inact
Antagonists			
5′-(MeS)-Ado	?	10	inact
8-phenyltheophylline	0.2	0.2	inact
3-isobutyl-1-methylxanthine	2	5	inact
theophylline	10	10	inact
caffeine	30	30	inact

[a] The literature contains a range of values, and the "affinity" constants should be considered as merely representative and may differ considerably at subclasses of these sites and under different conditions (see text). [b] Abbreviations used are: Ado, adenosine; Cl, chloro; Phe, phenyl; *i*-Pr, isopropyl; Bzl, benzyl; Me, methyl; d_2, dideoxy; Br, bromo; MeS, methylthio. [c] inact = inactive.

A_1-Adenosine Receptor. The most potent adenosine analogues in this class of receptors—inhibitory to adenylate cyclase—are the N^6-substituted compounds. L-N^6-(Phenylisopropyl)adenosine is much more potent—from 30- to 200-fold—than the D diastereomer. N^6-Phenyladenosine is much more potent than N^6-benzyladenosine. The 5′-ethylcarboxamide analogue is less potent than adenosine or 2-chloroadenosine. The following is the rank order of potencies for inhibition of catecholamine stimulation of adenylate cyclase systems in adipocytes[12,13] by adenosine analogues: N^6-cyclohexyladenosine > L-N^6-(phenylisopropyl)adenosine, N^6-phenyladenosine > 2-chloroadenosine, adenosine > N^6-methyladenosine, N^6-benzyladenosine, D-N^6-phenylisopropyladenosine, adenosine 5′-ethylcarboxamide. While A_1-adenosine receptors represent one class, there may well be subdivisions. Indeed, adenosine 5′-cyclopropylcarboxamide has been reported to be *more* potent than the N^6-substituted adenosine at certain presynaptic receptors which in other ways appear to fit the

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A_1 definition.[23] These are receptors inhibitory to release of norepinephrine in the vas deferens and of acetylcholine in the ileum.

A_2-Adenosine Receptor. The most potent analogues in this class of receptors—stimulatory to adenylate cyclase—are the adenosine 5′-ethylcarboxamide and 5′-cyclopropylcarboxamide. Both are at least 10-fold more potent than adenosine or 2-chloroadenosine. N^6-Substitution usually decreases the potency of adenosine for this class of receptors. The L-N^6-(phenylisopropyl)adenosine is only *slightly* more potent—3-fold or less—than the D isomer. N^6-Phenyladenosine is only *slightly* more potent than N^6-benzyladenosine. The following is the rank order of potencies for stimulation of adenylate cyclase systems in human fibroblasts:[76] Adenosine 5′-ethylcarboxamide > 2-chloroadenosine, adenosine > N^6-phenyladenosine, N^6-benzyladenosine > L-N^6-(phenylisopropyl)adenosine, N^6-cyclohexyladenosine > D-N^6-(phenylisopropyl)-adenosine. It should be noted that 5′(methylthio)-adenosine is a relatively potent competitive antagonist of adenosine in the fibroblast system.[76] While A_2-adenosine receptors represent one class, there may well be subdivisions. Indeed, the A_2-receptors in liver show a very high affinity for adenosine 5′-ethylcarboxamide, much higher than, for example, the A_2 receptors in adrenal or Leydig cells.[17]

There are a number of analogues of adenosine which are inactive at both A_1- and A_2-adenosine receptor controlled adenylate cyclase systems. These include 8-bromoadenosine, inosine, and adenine. 8-Bromoadenosine is probably inactive because it exits as the syn conformer. Activation of the A_2 receptor appears to require binding to the anti conformer of adenosine and adenosine analogues.[76]

Adenosine-Receptor Antagonists

The Xanthines. The best known antagonists of adenosine receptors are alkylxanthines, such as theophylline and caffeine (Table III). As yet no selective xanthine antagonists for either A_1- or A_2-adenosine receptors have been developed. Structure–activity correlations for xanthines have been studied most thoroughly for inhibition of adenosine-stimulated cyclic AMP formation in human fibroblasts.[88] The reversal by various xanthines of adenosine-inhibited adenylate cyclase in adipocytes has been studied only to a limited extent.[12] An 8-phenyl substituent confers very high affinity at both A_1 and A_2 receptors. 1,3-Dibutyl- and 1,3-dipropylxanthines are more potent than theophylline (1,3-dimethylxanthine). The following is the rank order of potencies of some xanthines vs. both A_1- and A_2-adenosine receptor mediated effects on adenylate cyclases: 8-Phenyltheophylline > 1,3-dibutylxanthine > 3-isobutyl-1-methylxanthine > theophylline > caffeine > theobromine. The inhibition constant for 8-phenyltheophylline is about 0.2 μM, while the constant for theophylline is about 10 μM. The xanthines have no effect on P-site inhibition of adenylate cyclase. Many of the xanthines have other biological activities, in particular, inhibitory effects on phosphodiesterases.[89] Certain xanthines, for example, 1-isoamyl-3-isobutylxanthine, are much more potent as phosphodiesterase inhibitors than as adenosine antagonists.[90] Their activity as phosphodiesterase inhibitors complicates the use of xanthines as selective adenosine antagonists. Recently, 8-(*p*-sulfophenyl)theophylline has been introduced as a polar adenosine antagonist which should penetrate cells only to a limited extent.[19,91] Unlike 8-phenyltheophylline, this polar derivative is not particularly potent, being comparable in potency to theophylline itself. The xanthines are not the only class of adenosine anatagonists (see Chart II), but none of the other antagonist types have been systematically studied. Hopefully, some of these may exhibit selectivity with regard to antagonism of responses mediated by either A_1 or A_2 receptors. One of these compounds, etazolate (SQ 20009), not only antagonizes binding of adenosine analogues to brain membranes[92] (vide infra) but enhances binding of diazepam.[93] Etazolate is an active anxiolytic agent, which points again to possible interrelationships of adenosine and benzodiazepines.[94]

P Site

Adenosine not only inhibits adipocyte adenylate cyclase via an extracellular receptor but also via the intracellular site which was termed the P site.[84] The P site has relatively low affinity for adenosines even under optimal assay conditions—usually 2 mM manganese. The inhibition constants for adenosine is about 10–20 μM under the optimal conditions. The significance of this intracellular site is, therefore, unclear, since adenosine levels cannot be expected to greatly exceed 1–2 μM in cells. Unlike the adenosine receptors, the P site will accommodate virtually no alterations in the purine ring.[84-87] 2-Chloroadenosine has very low activity, while N^6-substituted adenosines are completely inactive. 8-Bromoadenosine, inosine, and adenine are inactive. 2-Fluoroadenosine is somewhat more potent than adenosine. The most potent P-site inhibitor is 2′,5′-dideoxyadenosine, which is about 10-fold more potent than adenosine. Certain other ribose-modified analogues are active, such as adenine arabinoside, adenine xylofuranoside, and 2′-deoxyadenosine, while others such as the 5′-methylthio and 5′-cyclopropylcarboxamide analogues are inactive. 2′-Deoxyadenosine 3′-phosphate is about as active as adenosine. Other nucleotides, such as ATP, ADP, and 5′-AMP, are inactive at the P site. Inhibitors of adenosine uptake, such as dipyridamole, antagonize P-site inhibition of adenylate cyclase by exogenous adenosine by preventing uptake of the riboside into the cell. It should be stressed that theophylline has no effect on P-site inhibition of adenylate cyclases.

Factors Affecting Potencies of Adenosine-Receptor Agonists and Antagonists

Adenosine might profitably be omitted from any list of nucleosides to be used for characterization of adenosine-receptor mediated responses. This is because, unlike many of the analogues, uptake and metabolism are major complications when adenosine is used in pharmacological studies. Furthermore, unlike the analogues, adenosine acts at both adenosine receptors and at P sites.

For characterization of an adenosine-mediated physiological response, the adenosine analogues listed in Table III should be considered. However, the apparent potencies of the adenosine analogues and of xanthines with regard to interaction with adenylate cyclase systems shows considerable range in different systems. This is undoubtedly

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due in part to experimental variables, such as the presence or absence of uptake inhibitors, phosphodiesterase inhibitors, and other agents, the use of intact cells vs. homogenates or membrane fractions, and so forth. However, the variability probably also reflects biological variables, such as levels of spare receptors, extent of metabolic or physical inactivation, desensitization, and so forth. To what extent differences reflect additional subclasses of adenosine receptors will require further study. It does appear that additional subclasses of adenosine receptors exist in different cells and require pharmacological definition with adenosine analogues.

Differences in apparent potencies of xanthines as adenosine antagonists may likewise in part reflect experimental variables. Indeed, xanthines in certain systems appear to be much more potent adenosine antagonists in the presence than in the absence of an adenosine uptake inhibitor.[63,64] However, clearly in some systems, adenosine receptors are relatively insensitive to xanthines.[61,62] Development of antagonists specific to A_1- or A_2-adenosine receptors would represent a major breakthrough in this research field. Activity of xanthines as phosphodiesterase inhibitors, calcium-releasing agents, and inhibitors of other enzymes certainly complicates correlations of physiological activity with blockade of specific adenosine receptors. Recently, the central behavioral stimulant activity of theophylline, caffeine, theobromine, and two other xanthines was shown to correlate with their potencies as adenosine antagonists.[25]

Development of Radioactive Ligands for Adenosine Receptors

It is obvious that in 5 decades much progress has been made with regard to providing the knowledge and tools for delineation of mechanisms involved in physiological responses to ATP and adenosine. During the past decade a wide range of adenosine analogues and xanthines have been developed for definition of the nature of adenosine receptors involved in physiological functions (Charts I and II). In addition, relatively specific agents which inhibit either high-affinity uptake of adenosine or metabolism by adenosine deaminase or adenosine kinase have been developed (Chart III). However, one invaluable methodology was absent as recently as 1979. This was a satisfactory ligand or methodology for studying binding parameters at adenosine receptors, a technique which has proven so useful for other receptors, for example, adrenergic, dopaminergic, and opiate receptors. A binding protocol for adenosine receptors would be invaluable for probing the characteristics of receptor subtypes, the tissue distribution of subtypes, and correlations of binding profiles with effects on adenylate cyclase and physiological responses.

Early efforts to develop a binding protocol for adenosine receptors had utilized radioactive adenosine itself. Adenosine was found to bind to a variety of sites, both in soluble and membrane fractions. The soluble sites a priori would not be expected to show characteristics of ectoreceptors and they did not.[95] Binding sites for adenosine in membranes from fat cells[96] or arteries[97] also did not show characteristics entirely consonant with those expected of either the A_1- or A_2-adenosine receptors. For example, adenine and 2′,5′-dideoxyadenosine were potent antagonists in fat cell membranes. Theophylline was virtually inactive as an antagonist in either fat cell or arterial membranes. Binding sites for adenosine in guinea pig cerebral cortical membranes appeared in many respects to correspond to adenosine receptors.[98,99] 2-Chloroadenosine and theophylline were potent antagonists of binding. However, more than one class of binding site appeared to be present, since theophylline only partially antagonized the specific binding of adenosine. In contrast, in rat brain membranes, with similar assay conditions, theophylline was a very weak inhibitor of the specific binding of adenosine, requiring a concentration of about 2 mM for 50% displacement.[100] 2-Chloroadenosine was not any more potent than adenine as an antagonist of adenosine binding in rat brain membranes.

It appeared likely that the most satisfactory ligand for the adenosine receptor would be an analogue which was not subject to metabolism by adenosine deaminase or to high-affinity transport systems and which had an affinity for adenosine receptors greater than adenosine itself. In 1980, four laboratories reported the development of protocols for the characterization and investigation of binding of radioactive adenosine analogues to brain membranes: These ligands were 2-chloro[^{3}H]adenosine,[101-103] N^6-cyclohexyl[^{3}H]adenosine,[91] and L-N^6-(phenylisopropyl)[^{3}H]adenosine.[104] The profile of antagonism by adenosine analogues of binding of these ligands to brain membranes was consonant with interactions primarily at an A_1-adenosine receptor. Furthermore, affinity constants for ligands and adenosine analogues were in the low nanomolar range, as would be expected for an A_1 receptor. Pretreatment of membranes with adenosine deaminase or the copresence of adenosine deaminase during binding assays appeared necessary to remove endogenous adenosine and thereby prevent its competitive binding to receptors. However, in one study, binding of 2-chloro[^{3}H]-adenosine was reported for rat cerebral cortical membranes in the absence of exogenous adenosine deaminase.[101,102] Under such conditions, adenine and inosine were reported to be potent and effective antagonists of binding of the 2-chloro[^{3}H]adenosine, a result not consonant with structure–activity correlations at adenosine receptors and in marked contrast to the binding results of adenosine ligands from the other laboratories. However, in the main, the data obtained with various ligands in different laboratories were in agreement. Some of the data have been tabulated in Table IV.

N^6-Cyclohexyl[^{3}H]adenosine has been used fairly extensively as a ligand for A_1-adenosine receptors. It exhibits a very high affinity and a very high ratio of specific to nonspecific binding. Binding of this analogue has now been investigated in brain membranes from mouse,[25] rat,[105] guinea pig,[91] and cattle,[91,106,107] and in rat testes mem-

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Table IV. Antagonism of Binding of Radioactive Adenosine Receptor Ligands by Adenosine Analogues and Xanthines[a]

antagonist	N^6-cyclohexyl[^{3}H]adenosine				L-N^6-(phenylisopropyl)-[^{3}H]adenosine		2-chloro[^{3}H]-adenosine	
	guinea pig brain[b]	rat brain[c]	bovine brain[d]	rat testes[c]	rat brain[e]	rat fat[f]	rat brain[g]	rat brain[h]
				K_i, nM				
L-N^6-(phenylisopropyl)adenosine	3.3	1.6	0.25	1.4	23	5	0.6	
N^6-cyclohexyladenosine	5	1.6	2.5	1.4		7		
2-chloroadenosine	8		25		920	9	1.3	10
adenosine 5'-cyclopropylcarboxamide	8						5.5	
D-N^6-(phenylisopropyl)adenosine	130	125	8	110	900	50	15	
				K_i, μM				
8-phenyltheophylline	0.4		0.2				0.5	
isobutylmethylxanthine	8	3	7	5	0.8	20	2	0.05
theophylline	13	9	20	20	2	22	4.5	0.15
caffeine	80	40	130	60	25	60	15	

[a] Values are taken from or estimated from the cited references in footnotes *b-h*. [b] Reference 91. [c] Reference 105. [d] References 106 and 107. [e] Reference 104. [f] Reference 108. [g] Reference 103. [h] Reference 101.

branes.[105] In all cases, the data and profiles of antagonism of binding with adenosine analogues and xanthines are consonant with binding to an A_1-adenosine receptor. Recently, N^6-cyclohexyl[^{3}H]adenosine was used for radioautographic study of the distribution of A_1 receptors in rat brain.[109,111] The potencies of various adenosine analogues and xanthines vs. binding of N^6-cyclohexyl-[^{3}H]adenosine in membranes of rat brain and rat testes are in remarkably good accord.[105] Binding of N^6-cyclohexyl[^{3}H]adenosine in membranes of bovine brain[91] is somewhat different: There appear to be two sets of specific binding sites, one of which has significantly higher affinities for adenosine analogues than in other species. The affinities of adenosine analogues for the specific binding sites are reduced by guanyl nucleotides, such as GppNHp and GTP, while affinities of the xanthines are unaltered.[91,103,106,107] These results are consonant with an association of the specific binding sites with adenylate cyclase in brain membranes. Sodium ions also decrease binding of N^6-cyclohexyl[^{3}H]adenosine.[106] The binding sites for N^6-cyclohexyl[^{3}H]adenosine have been solubilized from bovine brain membranes.[107] Such solubilized sites display unaltered affinities for the various adenosine analogues and for the xanthines. Various anxiolytics anatagonize the binding of 2-chloro[^{3}H]adenosine to brain membranes.[93]

Densities of specific binding sites for the various adenosine ligands have been found to be higher in brain than in other tissues. In rat brain, the densities of specific binding sites for N^6-cyclohexyl[^{3}H]adenosine were about 9 pmol per gram wet weight of brain, while being about 2 pmol per gram wet weight in testes and less than 0.3 pmol per gram wet weight in heart, lung, kidney, adrenal, stomach, small intestine, pancreas, fat, thyroid, muscle, vas deferens, ovary, and submandibular gland.[105] Regional distribution of binding sites for 2-chloro[^{3}H]adenosine in brain was relatively uniform.[103] There was only about a 2-fold difference between the lower values in the cortex, hypothalamus, spinal cord, medulla, and pons and the higher values detected in the thalamus, cerebellum, and hippocampus. Specific binding sites for 2-chloro[^{3}H]-adenosine were about 3-fold lower in testes than in brain, while being fully 20-fold lower in liver, kidney, and heart. The function of the adenosine receptors detected in testes is unknown but is under active investigation.

Availability of radioactive ligands, such as N^6-cyclohexyl[^{3}H]adenosine, for one class of adenosine receptors—the A_1 (high affinity) receptor—makes possible a variety of studies correlating binding parameters at this adenosine receptor with physiological responses in various tissues. However, a ligand for the A_2 (low affinity) receptor is still needed. One attractive candidate as a ligand for A_2-adenosine receptors is either adenosine 5'-cyclopropylcarboxamide or adenosine 5'-ethylcarboxamide. These analogues of adenosine are the most potent known agonists at A_2-adenosine receptors. A preliminary report suggested that binding of [^{3}H]adenosine 5'-ethylcarboxamide to rat striatal membranes is to both A_2- and A_1-adenosine receptors, with the majority of specific binding being to A_2 receptors.[111]

The 8-phenyl-1,3-dialkylxanthines appear to be the best antagonist candidates for binding to adenosine receptors because of their remarkably high potencies as adenosine antagonists. 1,3-Diethyl-8-[^{3}H]phenylxanthine was prepared, and its binding to brain membranes was investigated.[91] The compound proved to be an excellent ligand for what appeared to be A_1-adenosine receptors in bovine forebrain membranes. 1,3-Diethyl-8-[^{3}H]phenylxanthine bound primarily to a lower affinity site in guinea pig brain membranes. This site had virtually no affinity for a variety of adenosine analogues. The significance of this xanthine binding site in guinea pig brain membranes is unknown. However, such results suggest that binding studies with 1,3-diethyl-8-[^{3}H]phenylxanthine in different species and tissues must be evaluated carefully. Further efforts are in progress with regard to other more satisfactory and selective xanthine ligands for adenosine receptors.

Initial efforts to develop a ligand for the P site associated with adenylate cyclase in brain membranes were not successful.[86] 2',5'-Dideoxy[^{3}H]adenosine did bind with relatively high affinity to specific binding sites in rat brain membranes, and profiles for inhibition of binding of 2',5'-dideoxy[^{3}H]adenosine by various adenosine analogues and the lack of inhibition of binding by theophylline were in many respects consonant with those expected for P sites. However, the most potent antagonist of binding, namely, 5'-(methylthio)adenosine, has no affinity for P sites. Furthermore, densities of specific binding sites for 2',5'-dideoxy[^{3}H]adenosine and levels of adenylate cyclase ac-

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Table V. Tools for the Investigation of the Role of Receptors for ATP or Adenosine in Physiological Functions

1.	Structure-activity correlations for responses to adenosine analogues: Profile with adenosine, 2-chloroadenosine, N^6-cyclohexyladenosine, L- and D-N^6-(phenylisopropyl)adenosine, N^6-benzyladenosine, N^6-phenyladenosine, adenosine 5'-cyclopropyl(ethyl)carboxamide, 2',5'-dideoxyadenosine, 5'-(methylthio)adenosine, 8-bromoadenosine, inosine, adenine.
2.	Structure-activity correlations for alteration in physiological function or in blockade of adenosine responses by alkylxanthines: Profile with 8-phenyltheophylline, 1,3-dipropylxanthine, 3-isobutyl-1-methylxanthine, theophylline, caffeine, 1,7-dimethylxanthine, theobromine, isocaffeine.
3.	Structure-activity correlations for responses to ATP analogues: Profile with ATP, 2-Cl-ATP, 8-Br-ATP, 2'-(MeS)-ATP, ITP, GTP.
4.	Alteration in function by exogenous adenosine deaminase: Reduction in adenosine-mediated but not ATP-mediated response.
5.	Alteration in function or responses by inhibition of adenosine deaminase: Potentiation of effects of endogenous adenosine by 2'-deoxycoformycin or *erythro*-9-(2-hydroxy-3-nonyl)adenine.
6.	Alteration in function or responses by inhibitors of ecto-ATPases and nucleotidases: Blockade of conversion of ATP to adenosine with α,β-methyleneadenosine diphosphate.
7.	Alteration in function or responses by inhibitors of adenosine uptake: Potentiation of adenosine-mediated but not ATP-mediated responses by dipyridamole, dilazep, hexobendine, 6-(*p*-nitrobenzyl)thioguanosine, papaverine.
8.	Control of intracellular levels of adenosine due to action of *S*-adenosylhomocysteine: Reduction of intracellular adenosine levels with homocysteine.
9.	Characterization of membrane receptors with radioactive ligands. Comparison of structure-activity for binding of [^{3}H]adenosine analogues and [^{3}H]alkylxanthines with physiological or biochemical responses.

[a] See text for discussion.

tivity in different brain membranes showed no correlations. Thus, it appears likely that the specific binding of 2',5'-dideoxy[^{3}H]adenosine is not to P sites but is instead to some theophylline-insensitive site to which adenosine also binds with high affinity. The significance of such sites is unknown. It was suggested that they might be intracellular membrane sites and might serve a role in "facilitated" uptake of adenosine into cells.

Future Strategies for Investigation of Adenosine Functions

It is apparent that 1980 represents a turning point in research on the physiological functions of adenosine. The advent of binding assays for adenosine receptors of the A_1 high-affinity class provides a new approach to the investigation of the nature, distribution, and control of such functional receptors. Further research on radioligands and binding assays for A_2-adenosine receptors, P sites, and ATP receptors must now assume a high priority. A wide range of research tools for the investigation of physiological functions for ATP and adenosine have been delineated and profitably used to advance our knowledge in this field. Some of these tools and approaches are listed in Table V. Undoubtedly, their diligent use will provide not only further definition of physiological functions controlled by the adenine nucleotides and nucleosides, but will reveal further classes of receptors and further complexities.

Communications to the Editor

Inhibitors of Gastric Acid Secretion: 3,4-Diamino-1,2,5-thiadiazole 1-Oxides and 1,1-Dioxides as Urea Equivalents in a Series of Histamine H_2-Receptor Antagonists

Sir:

The discovery of burimamide by Black and associates[1] provided the first example of a specific antagonist of the histamine H_2 receptor. This prototype, although of low intrinsic inhibitory activity, constituted a lead for the development of the more potent inhibitors metiamide[2] and cimetidine.[3] The clinical efficacy of the latter as a gastric antisecretory drug stimulated a search for agents with improved potency, longer duration of action, and a lower potential for side effects.

Recently, highly potent nonimidazole H_2 inhibitors, such as ranitidine[4] and tiotidine,[5] have been described. Structural comparison of these drugs reveals three fundamental units: a substituted heterocycle joined by a 2-thiabutyl connecting chain to an acyclic end group or "urea equivalent" such as cyanoguanidine or diaminonitroethene.

This report describes a new class of histamine H_2 receptor antagonists wherein 3,4-diamino-1,2,5-thiadiazole oxides function as the "urea equivalent". Representative examples (**1a–f**)[6] are presented to illustrate structure–activity relationships within the series and for comparison with reference drugs (Table I).

Chemistry. Carmack and co-workers[7] described the

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