Adenosine: a Prototherapeutic Concept in Neurodegeneration

DAG K. J. E. von LUBITZ,[a,c] MARGARET F. CARTER,[a] MARK BEENHAKKER,[a] RICK C-S. LIN,[b] AND KENNETH A. JACOBSON[a]

[a]*Laboratory of Bioorganic Chemistry*
Molecular Recognition Section
National Institute of Diabetes and Digestive and Kidney Diseases/NIH
Bethesda, Maryland 20892
and
[b]*Department of Physiology and Biophysics*
Hahnemann University
Philadelphia, Pennsylvania 19102

ADENOSINE AND BRAIN: THE VIEWS AND THE VISTAS

Ten years ago, Newby introduced a new description of adenosine: "the retaliatory metabolite."[1] The theoretical notion that adenosine may protect against tissue injury[2] evolved rapidly into a practical demonstration of powerful neuroprotective effects of endogenous adenosine and its analogues.[3–5] Subsequent improvement in understanding both the effects of adenosine receptor stimulation and the pathological processes that accompany numerous neurological disorders ultimately led to proposals that adenosine-based therapies may be effective not only in stroke and seizures, but also in Alzheimer's, Huntington's and Parkinson's diseases, and a number of psychiatric pathologies.[5,6]

ADENOSINE AND BRAIN: THE FUNCTIONS

Endogenous Brain Adenosine and Pathologic Stress

Technical difficulties complicate the exact measurement of extracellular brain adenosine concentration.[4] Currently, the level of free adenosine level in the interstitial brain space of unanesthetized, freely moving animals is estimated at 50–300 nM.[4] More importantly, however, several laboratories have consistently reported that the amount of extracellular adenosine increases dramatically following cerebral metabolic stress caused by seizures, hypoxia, or ischemia.[4]

In focal ischemia (and probably global as well), the reduction of cerebral blood flow (CBF) correlates with the concomitant elevation of both adenosine and gluta-

[c]DvL is a Special Fellow of the Cystic Fibrosis Foundation, Washington, DC, and of Gilead Sciences, Foster City, CA.

mate.[7] However, while increased release of adenosine occurs at CBF values of 25 ml/100 g/min, further reduction of CBF (20 ml/100 g/min) is necessary to elevate concentration of the extracellular glutamate. Quite recently, Hoehn and White[8,9] showed that release of excitatory amino acids elicited by electrical field stimulation also results in the release of adenosine—an effect mediated in part by both *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methylisoxazole-4-proprionic acid (AMPA) receptors. It appears, therefore, that glutamate-mediated hyperexcitation of neurons (such as seen in cerebral ischemia) may provide an additional, and somewhat unexpected, stimulus for further increase in adenosine release. These observations indicate that, in view of the powerful inhibitory effect of adenosine on the release of several excitatory neurotransmitters (see below), it is quite likely that increase in the concentration of interstitial adenosine, which both precedes and accompanies massive intraischemic release of glutamate,[10–13] constitutes part of a mechanism whose operation provides a transient, endogenous protection of the brain against injury.[5]

Cerebral Receptors of Adenosine

Endogenous adenosine acts at three principal G-protein-associated receptor subtypes: A_1, A_2 and A_3.[14,15] Both the molecular structure and the nature of the effector coupling are known for all three subtypes.[16,17] Cerebral A_1 receptors are linked to several second messenger systems, and one of their characteristic responses to stimulation is inhibition of adenylate cyclase.[14] Activation of A_2 receptors stimulates adenylate cyclase,[14] whereas activation of A_3 receptors inhibits it, and also stimulates phosphoinositide metabolism.[18] Although their specific distribution varies,[19] all three adenosine receptor subtypes are found in the brain.[15,20] A_1 receptors are predominantly found in the hippocampus, IV–VI laminas of the cortex, striatum, amygdala, and superior colliculus, and appear to be codistributed with NMDA receptors.[21,22]

A_2 receptors, of which two subclasses (A_{2a} and A_{2b}) exist, abound on smooth muscle and endothelial cells of cerebral blood vessels, where they mediate vascular effects of adenosine.[23] High-affinity A_{2a} receptors are particularly well represented in the striatum and other dopamine-rich regions of the brain,[19] where they are colocalized with dopamine D_2 receptors, and exert profound modulatory effect on dopaminergic transmission.[24] Adenosine receptors on glial cells belong, most likely, to the low-affinity A_{2b} subclass.[4] Cerebral distribution of A_1 and A_2 receptors follows an intriguing pattern, *i.e.*, A_2 appear to be less abundant within regions where the density of A_1 sites is elevated, and vice versa. Differences in the anatomical distribution of A_1 and A_2 receptors may have striking behavioral consequences.[25] A_3 receptors are found throughout the brain but their density is much lower than that of either A_1 or A_2.[20] The cell type on which they are located is unknown.

Physiological Effects of Adenosine Receptor Stimulation

The principal function of adenosine in the brain is that of an inhibitory neuromodulator.[26,27] The inhibitory effects of adenosine are mediated mainly via both pre- and postsynaptic A_1 receptors.

Activation of presynaptic A_1 sites inhibits neuronal calcium uptake[28–30] and results in reduced release of several neurotransmitters, *e.g.*, acetylcholine, noradrenaline, dopamine, serotonin, and glutamate.[31–34]

Stimulation of both pre- and postsynaptic A_1 receptors causes activation of potassium[35–37] and chloride[38] conductances. The resultant elevation of the membrane potential and the depression of the membrane resistance[35,39] decrease neuronal excitability and firing rate.[35,40,41]

Apart from the involvement of adenosine A_2 receptors in regulation of CBF[23] adenosine A_2 receptors are responsible for accumulation of cyclic adenosine monophosphate (cAMP) in the brain.[4] The details of A_2 receptor involvement in neuronal physiology are still poorly understood, although existing evidence indicates that excitatory A_2 receptors are present in the hippocampus[42] and may be involved in potentiation of calcium-dependent neurotransmitter release[43,44] and in modulation of electrically evoked release of gamma-aminobutyric acid (GABA) in globus pallidus.[45] It is also known that in the striatum, A_2 receptors mediate control of gene expression in enkephalinergic neurons,[46] and that A_2 activation attenuates activity of the colocalized dopamine D_2 receptors through reduction of their affinity for D_2 agonists.[25,47,48] Finally, participation of A_2 receptors in generation of astrocytic edema has been also suggested.[49]

ADENOSINE AND NEUROPROTECTION: THE THEORETICALS

The first experimental confirmation of neuroprotective properties of adenosine analogues in cerebral ischemia has been provided by Evans *et al.*[52] and von Lubitz *et al.*[53,54] A variety of *in vitro* and *in vivo* models of hypoxic/ischemic models of neuronal injury have been used in most of the subsequent studies of neuroprotection afforded by adenosine, its analogues, and inhibitors of its uptake.[4] Moreover, the effect of these approaches has been also investigated in seizures[55] and in either clinical[56] or *in vitro* hypoglycemia.[57] Since pathophysiology of cerebral ischemia has been extensively reviewed,[58–60] for the purpose of the present review suffice to say that the arrest of brain blood supply results in a rapid depolarization of neuronal membranes,[61] massive release of excitatory neurotransmitters[11] and excitation of postsynaptic glutamate receptors (NMDA and non-NMDA[59]), followed by influx of calcium and its release from intracellular stores.[62] The latter process triggers a series of cascading events[60] that ultimately lead to neuronal demise.

From the preceding brief discussion of the effects of adenosine receptor stimulation it is apparent that adenosine analogues may be applicable in interrupting several ischemia-associated events, *e.g.*, membrane (hypoxic) depolarization, neurotransmitter release, hyperexcitation of NMDA receptors, and calcium influx.

Endogenous Adenosine and Hypoxic Depolarization

Rapid depolarization of neuronal membrane is one of the initial events evoked by either impaired or entirely interrupted supply of the cerebral blood flow.[63]

Moreover, duration of hypoxic depolarization may be the determining factor that dictates the subsequent fate of neurons, *i.e.*, their survival or death.[64]

Hypoxic depolarization is associated with enhanced influx of calcium through voltage-gated calcium channels,[59] and concomitant increase in neurotransmitter release. Since intraischemic liberation of endogenous adenosine precedes that of glutamate,[7] and since intraischemically released adenosine both significantly delays the onset of hypoxic depolarization[65] and reduces glutamate release,[66] it appears that adenosine-mediated protective processes take place already at the very beginning of the insult.

Adenosine A_1 Receptors and Excitatory Neurotransmitter Release

Significant reduction of intraischemic release of glutamate by the A_1 receptor agonist N^6-cyclopentyladenosine (CPA) and the A_1/A_2 agonist *N*-ethylcarboxamidoadenosine (NECA) has been demonstrated in the 4-vessel occlusion rat model of ischemia.[66] Reduction of glutamate release by the A_1 agonist N^6-cyclohexyladenosine (CHA) has been also reported in focal ischemia in the rat[3] and in forebrain ischemia in the gerbil (Marangos and von Lubitz, unpublished). However, while glycine levels were significantly attenuated by CHA in a study of global ischemia in rabbits,[67] the reduction of glutamate showed only a dose-dependent but statistically insignificant trend. Nonetheless, even if in the latter study glutamate release was affected only to a limited extent, the protective effect of adenosine agonist is still likely.

Glycine is necessary for activation of the ion-gated channel of the NMDA receptor which regulates calcium influx.[68] Moreover, several studies have showed that glycine antagonists and partial agonists have a neuroprotective effect.[69,70] Therefore, it is conceivable that, despite a variable effect on the liberation of glutamate, CHA-mediated reduction in glycine release may diminish the functional efficiency of the NMDA receptor-associated ion-gated channel, and thereby decrease the subsequent calcium overload.

Endogenous Adenosine and Glutamate Uptake Sites

Postischemic release of glutamate is comparatively brief and abates within approximately 30 min.[11] However, postischemic depression of CBF seen after severe ischemia (hypoperfusion stage) may result in secondary hypoxia.[71] Hence, a supplementary elevation in the extracellular glutamate concentration is also quite possible and may, unless astrocytic transport mechanisms remain intact, lead to exacerbation of the excitotoxic processes initiated by the primary event. Interestingly, Anderson *et al.*[72] have showed that even a brief (5-min) ischemia results in a prolonged upregulation of high-affinity excitatory amino acid (EAA) transport sites. At the same time, Schmidt *et al.*[73] have showed that a brief 10-min exposure to adenosine produces a significant increase in the density of high-affinity glutamate and aspartate uptake sites in rat hippocampal slices. Therefore, it is possible that intraischemic elevation of brain adenosine[74] may, apart from its effect on

neurotransmitter release, also result in a sustained upregulation of EAA transporters. Consequently, due to its control of both release and uptake of EAAs, endogenous adenosine may play an important role in prevention of excitotoxic damage following very brief ischemic periods, the absence of which has been noted by several authors.[75,76]

Postsynaptic Effects of Adenosine and Neurodegeneration

The intensity of excitatory synaptic input depends on the amount of NMDA-mediated influx of Ca^{2+} [77] which, in turn, increases membrane depolarization and acts as a synaptic amplifier. Since the evoked influx of calcium is tightly controlled by postsynaptic A_1 receptors even at low extracellular Ca^{2+} concentrations,[29,30,78] such control tends to attenuate calcium-mediated synaptic amplification.[4] Consequently, adenosine and its postsynaptic A_1 receptors regulate critical input frequencies required to operate postsynaptic NMDA receptors, as was recently demonstrated by Schubert and his colleagues.[30,79]

The additional, albeit indirect, benefit of reduced NMDA receptor-mediated depolarization elicited by interaction of adenosine with its A_1 receptors is the effect on voltage-sensitive K^+ currents.[35] Depolarization appears to block these currents and enhances neuronal excitability and firing rate.[80] Hence, vigorous activation of A_1 receptors by elevated concentrations of extracellular adenosine may counteract NMDA receptor-mediated depolarization, and drive the membrane potential toward voltage ranges at which depolarization-dependent block of potassium conductance is either less likely or does not occur.[4]

Apart from its enhancing effect on potassium conductance,[36,37,81] adenosine stimulates voltage-dependent Cl^- conductance as well.[38,82] It has been suggested that the opening of this conductance may diminish accumulation of intraneuronal Cl^- during repetitive firing[4] which, unless prevented, will eventually impair GABAergic inhibition.[83] Elevation in extracellular adenosine during periods of enhanced neuronal activity[41] may, therefore, assist in maintaining GABA-mediated inhibition, and constitute another functional aspect of the protective adenosine/adenosine receptor complex.

Adenosine A_2 Receptors and Neurodegeneration

The concept of A_2 receptor involvement in neurodegeneration has not been pursued with the same vigor as that of A_1 receptors. There is, however, indirect, evidence that A_2 receptors may play a pivotal role in neuronal death observed in the striatum, and possibly also in the substantia nigra. Contrary to general belief, it is the dorsolateral aspect of striatum rather than the hippocampal CA4 sector[75] that appears to be endowed with the highest sensitivity to ischemic insult.[84,85] Light microscopic evidence of neuronal impairment in the striatum is clearly discernible already 1 h after a very light ischemic episode, while acute ischemic damage in the hippocampal CA4 appears 6–12 h after the event.[84] Rapid, intraischemic release of dopamine and glutamate,[85,86] persistent elevation of cAMP,[87]

and eventual loss of dopamine D_2 receptors[86] precede morphologic damage of striatal neurons.

Globus *et al.*[85] have showed that, while increased concentration of intrastriatal dopamine alone has no adverse effect, elevated concentration of both dopamine and glutamate is associated with striatal vulnerability to ischemia. Since dopamine D_2 receptors attenuate the effect of glutamatergic stimulation,[88] it is possible that accelerated postischemic loss of D_2 receptors, rather than elevated concentration of both neurotransmitters per se may constitute one of the critical factors resulting in the apparent potentiation of glutamate-evoked damage. The most characteristic aspect of this damage is its containment to the medium-sized spiny neurons containing enkephalin and substance P,[84] *i.e.*, neurons receiving glutamatergic input from both substantia nigra and neocortex.[88] Moreover, the same medium-sized GABAergic enkephalin-containing neurons are also characterized by the highest density of adenosine A_2 receptors.[24]

Based on the existing evidence, and on the fact that stimulation of adenosine A_2 receptor decreases the affinity of D_2 receptors to agonist stimulation,[48] it is possible to construct a chain of conjectural events that may ultimately lead to the selective neuronal loss in the striatum. Most likely, the initial intraischemic surge of adenosine agitates high-affinity A_{2a} receptors located on enkephalin-containing GABAergic neurons. At the same time, the colocalized D_2 receptors which attenuate glutamatergic excitation supplied by cortical and nigro-striatal fibers[88] will be stimulated by dopamine, whose concentration also increases. However, the activated A_2 receptors decrease affinity of the colocalized D_2 sites to dopamine,[24] thereby diminishing the efficiency of their counterexcitatory effect. Ultimately, combination of A_2-D_2 interactions and postischemic loss of D_2 receptors[86] will result in a progressive shift toward unopposed glutamatergic hyperexcitation whose intensity will, eventually, attain the level sufficient to induce excitotoxic damage of enkephalin-containing GABAergic neurons.

Contrary to A_1 receptors, the time course of ischemia-induced adenosine A_2 receptor disappearance is unknown. However, cerebral ischemia causes elevation in striatal cAMP that persists for at least 4 h after the reperfusion.[87] Since stimulation of A_2 receptors leads to production of cAMP,[4,14] its prolonged postischemic presence may indicate that the functional A_2 receptors are preserved for several hours following the insult. Moreover, it was shown recently that A_2 receptor stimulation enhances ischemia-evoked release of glutamate and aspartate.[44] Thus, although the mechanism involved in this process is unknown, the sustained operation of A_2 receptors may amplify the damage to enkephalin-containing GABAergic neurons even further.

Allowing that this speculative sequence of events is correct, its repercussions on "downstream" damage caused by ischemia may be significant. Both global and prolonged forebrain ischemia cause damage in the substantia nigra as well as in the striatum and the hippocampus.[89,90] Hence, possible involvement of A_2 receptors in development of the rapid damage to the inhibitory enkephalin-containing neurons in the striatum may contribute to the subsequent loss of inhibitory input to the substantia nigra, and amplify the adverse effects of ischemia-associated hyperstimulation also in that region.

The pattern of striatal neuron loss in cerebral ischemia is very similar to that

observed in Huntington's chorea[84] and, although the postischemic fate of A_2 receptors is presently unknown, a significant decrease in their density was recently observed in striatal tissue of patients with Huntington's disease.[91] Since striatal adenosine A_2 receptors appear to play an important role in pathophysiology of basal ganglia associated with Huntington's and Parkinson's diseases,[25,46,92] drugs acting at these receptors may prove very useful in the treatment of these disorders. Involvement of A_2 receptors in neurodegenerative processes of different etiology is the subject of current, intensive studies at our laboratory.

Striatum apart, stimulation of adenosine A_2 receptors may result in an improved postischemic survival of neurons in other regions through, *e.g.*, improvement of postischemic CBF[93] or prevention of postischemic inflammatory processes.[94] Normalization of postischemic CBF may be obtained through A_2 receptor-mediated vasodilation[5,23,95] and through antithrombotic effects.[96,97] Moreover, since stimulation of A_2 receptors prevents activation of neutrophils, it may, through concomitant reduction in free radical release, diminish the damage to the endothelial lining of cerebral blood vessels.[98] Finally, stimulation of leukocyte A_2 receptors decreases their adherence to capillary walls, and appears to be involved in preventing postischemic "plugging" of cerebral capillaries.[99]

ADENOSINE AND NEUROPROTECTION: THE PRACTICALS

Effects of Acute Administration

The results of experimental studies of the neuroprotective effects of adenosine, its analogues, and agents affecting its turnover are the subject of several recent reviews.[3–5,100,101] Most of those studies concentrate on investigations of either forebrain or global cerebral ischemia, and use survival and/or neuropathology as the measures of outcome.

Due to their well-known physiological properties and their relevance in treatment of cerebral ischemia, A_1 receptors are the chief subject of the existing experimental work.[3,4] Significant neuroprotection has been reported in virtually all studies of focal (but see Roussel *et al*, 1991), global, and forebrain ischemia in which A_1 receptor agonists have been administered either shortly before or after the insult, whose duration ranged from 5 to 30 min.[3,4] However, since the maximum interval between pretreatment and ischemia was 15 min, and maximum postischemic delay did not exceed 30 min, the dimension of the therapeutic window within which acutely administered adenosine agonists are effective is uncertain. It is known, however, that rapid downregulation of A_1 receptors follows even a mild anoxic or ischemic episode,[103,104] and that 14–24 h after ischemia, A_1 receptors become dysfunctional.[4] Thus, since the strength of adenosine modulation depends on the density of A_1 receptors,[105] the therapeutic window for administration of A_1 analogues is probably not an extensive one.[4]

The veracity of neuroprotective effects of A_1 receptor agonists has been confirmed by studies in which A_1 antagonists have been used.[4] Uniformly, administration of antagonists has resulted in severe exacerbation of mortality,[106] and in amplified neuronal destruction.[4]

Contrary to the effects of A_1 receptor agonists, the results following acute administration of agents active at A_2 receptors is virtually unknown. Recently, however, Gao and Phillis[107] showed that pretreatment with a weakly selective A_2 antagonist CGS 15943 resulted in protection of the hippocampus against ischemic damage. Our own results (von Lubitz *et al.*, in preparation) indicate that A_2 antagonists administered prior to 10-min ischemia protect not only hippocampus but striatum as well.

Presently, only one report describes the effect of acute A_3 receptor stimulation on the outcome of forebrain ischemia.[50] The study shows that preischemic administration of a small dose (100 μg/kg) of a selective A_3 agonist, N^6 – (3-iodobenzyl)-adenosine-5′-methylcarboxamide (IB-MECA), results in an extensive hippocampal damage and a very high mortality (90%) within the initial 24 h after ischemia.

Despite their neuroprotective efficacy, the acute treatment with adenosine A_1 agonists is accompanied by two major side effects, *i.e.*, hypothermia and hypotension. Since hypothermia results in a significant reduction of postischemic neuronal damage,[107] it is possible that A_1 agonists mediate their neuron-sparing effect chiefly through the depression of brain temperature. However, both *in vitro* studies[108] and studies in which brain temperature has been carefully maintained[106] indicate that the protective effect is preserved also in the normothermic environment. Moreover, it must be remembered that, in the context of therapies aimed at stroke and brain ischemia, the comparatively mild hypothermic impact of A_1 receptor agonists may constitute a benefit rather than a hindrance.

Failure of cerebral perfusion pressure after ischemia is among the most critical factors that influence clinical recovery,[109,110] and hypotension and cardiodepression accompanying administration of A_1 agonists constitute potentially serious side effects of A_1 receptor-based therapies. Cardiovascular side effects of A_1 receptor agonists may be countered by coadministration of peripheral adenosine antagonists. However, von Lubitz and Marangos[111] have showed that, although concomitant postischemic administration of the A_1 receptor agonist CHA and the peripheral adenosine antagonist 8-P-sulphophenyladenosine (8-SPT) in gerbils resulted in a full normalization of CHA-evoked hypotension, the combined CHA/8-SPT treatment does not improve either survival or neurological impairment scores beyond those attained with CHA alone.

Effects of Chronic Administration

Among all disorders for which adenosine-based therapies have been envisaged, only stroke offers a target for their acute administration while most, if not all, other central nervous system (CNS) diseases require chronic, frequently even lifelong, exposure. However, very little is known about the chronic effects of agents acting at adenosine receptors in the context of neuronal pathologies. The pioneering study of Rudolphi *et al.*[112] showed that chronic treatment with caffeine—a nonspecific A_1/A_2 antagonist—resulted in protection against ischemic damage in gerbils (*i.e.*, the exactly opposite effect to that obtained with acute administration of another nonspecific antagonist, theophylline).[76] Von Lubitz *et al.*[106,112,113,115] have investigated the consequences of chronic administration of drugs acting at

adenosine receptors further, and have used the highly potent A_1 agonist CPA or antagonist 8-cyclopentyl-1,3-dipropylxanthine (CPX). The work of the latter authors has confirmed the results of Rudolphi and his colleagues,[76,112] and has also showed that while acute treatment with a selective A_1 receptor agonist is highly protective, chronic treatment with the same drug has a profoundly aggravating effect in several measures of postischemic recovery, *i.e.*, survival, neurological status, and preservation of ischemia-vulnerable brain regions. Treatment with A_1 receptor antagonists, on the other hand, produced a diametrically opposite effect, *i.e.*, acute administration enhanced, and chronic administration protected against the damage.[106] The same authors also showed that while acute treatment with adenosine A_3 receptor agonist enhanced ischemia-associated damage, chronic treatment was highly ameliorative.[50] Preliminary studies with agents acting at A_2 receptors indicate the same pattern of regimen-dependent reversal. Interestingly, regimen-dependency of the therapeutic outcome of adenosine-based treatment has been also described in NMDA-evoked seizures[50,113,116] and in the water maze model of learning and memory.[114]

ADENOSINE AND NEUROPROTECTION: THE PUZZLES AND THE PARADOXICALS

Despite numerous and convincing demonstrations of neuroprotective effects of endogenous adenosine, and despite highly alluring results of experimental treatment of cerebral ischemia with agents acting at all three adenosine receptor subtypes, a number of unsolved puzzles exists. We have already mentioned the fact that, although critical from the therapeutic point of view, time limits for efficient administration of acute adenosine therapies in stroke and cerebral ischemia are unknown. Glial response to the activation of their A_1 and A_2 receptors is also very poorly known, although there are indications that both glycogenolysis[117] and astrocytic edema[118] may ensue.

Degradation of endogenous adenosine contributes to the generation of highly destructive free radicals.[119] Since administration of free radical scavengers virtually eliminated production of superoxide species during and after cerebral ischemia,[119] therapies based upon elevation of endogenous adenosine may be less effective than those employing stimulation of adenosine receptors with appropriate analogues. Unquestionably, the problem requires a detailed and urgent examination. Finally, there is virtually no information on the interplay of individual adenosine receptor subtypes, although there are indications that such interplay may be critical for neuronal function and survival.[50]

The paradoxical effects of adenosine receptor-based therapies require further studies as well. The regimen-dependent nature of the outcome has been already mentioned. Prolonged stimulation by agonists or blockade by antagonists both *in vitro* and *in vivo* produces, respectively, either down- or upregulation of adenosine receptor density.[18,49,120] However, in some studies, no changes of either receptor density or ligand binding properties (K_d) were observed during prolonged exposure to selective A_1 agonists and antagonists, and to a nonselective A_1/A_2 antagonist theophylline *in vivo*.[106,115,116] On the other hand, Fastbom and Fredholm have

showed that prolonged exposure to theophylline upregulates adenosine receptors, and Shi *et al.*[122] have reported that chronic treatment with caffeine (a nonspecific A_1/A_2 antagonist) both upregulates A_1 receptors and results in very dramatic density shifts of some receptor types (*e.g.*, GABA, dopamine, noradrenaline), while having no effect on others (*e.g.*, NMDA). Finally, chronic caffeine-mediated upregulation of A_1 sites and its functional consequences were the most likely source of protection against ischemia reported by Rudolphi *et al.*[112]

Although the protective effect of chronically administered A_1 antagonists is easily explained when accompanied by receptor upregulation, the nature of the mechanisms behind ameliorative actions of a chronic antagonist regimen observed in absence of increased density of A_1 receptors remains entirely obscure. Changes in G-protein-mediated receptor-effector coupling have been proposed as a putative answer to the regimen-dependent shifts seen after chronic exposure to both nonselective and selective agonists and antagonists.[106,115,116] Significant alterations in $G_{S\alpha}$ and $G_{I\alpha}$ proteins that were unaccompanied by a corresponding change in their mRNAs have been seen in rat adipocytes following chronic treatment with A_1 receptor antagonist.[123] However, whether similar phenomena take place in the brain remains to be demonstrated.

The effect of acute stimulation of A_1 and A_3 receptors offers another paradox. While both receptors are negatively coupled to adenylate cyclase (*i.e.*, reduce its levels), acute preischemic activation of A_1 causes extensive neuroprotection. Acute activation of A_3 receptors, on the other hand, has an equally extensive but damaging result in cerebral ischemia,[50] although it is protective against NMDA-evoked seizures.[51] Moreover, chronic administration of A_3 receptor agonist protects equally well against cerebral ischemia and against chemically and electrically evoked seizures.[50,51]

Clearly, there are a number of questions that require additional, extensive studies. On the other hand, even if several aspects of adenosine action on a living cell, be it a neuron, a cardiac myocyte, or a nephron are unknown, Newby's "retaliatory metabolite" has already found its practical application in cardiology. Thus, under the name "Adenocard™," adenosine is now clinically used in treatment of supraventricular tachycardias, and it is not a premature hope that soon the concept of adenosine-based therapies will also find its application in treatment of the disorders of the brain.

REFERENCES

1. Newby, A. C. 1984. Adenosine and the concept of "retaliatory metabolites." TIPS **9:** 42–48.
2. Phillis, J. W. & P. H. Wu. 1981. The role of adenosine and its nucleotides in central synaptic transmission. Progr. Neurobiol. **16:** 187–239.
3. Miller, L. P. & C. Hsu. 1992. Therapeutic potential for adenosine receptor activation in ischemic brain injury. J. Neurotrauma **9**(Suppl. 2): S563–577.
4. Rudolphi, K. A., P. Schubert, F. E. Parkinson & B. B. Fredholm. 1992. Adenosine and brain ischemia. Cerebrovasc. Brain Metab. Rev. **4:** 346–369.
5. von Lubitz, D. K. J. E. & P. J. Marangos. 1992. Self-defense of the brain: adenosinergic strategies in neurodegeneration. *In* Emerging Strategies in Neuroprotection. P. J. Marangos and H. Lal, Eds. 151–186. Birkhauser. Boston.

6. MARANGOS, P. J. & J. P. BOULENGER. 1985. Basic and clinical aspects of adenosinergic neuromodulation. Neurosci. Biobehav. Rev. **9:** 421–430.
7. MATSUMOTO, K., R. GRAF, G. ROSNER, N. SCHIMADA & W. D. WEISS. 1992. Flow thresholds for extracellular purine catabolite elevation in rat focal ischemia. Brain Res. **579:** 309–314.
8. HOEHN, K. & T. D. WHITE. 1990. Role of excitatory amino acid receptors in K^+ and glutamate-evoked release of endogenous adenosine from rat cortical slices. J. Neurochem. **54:** 256–265.
9. HOEHN, K. & T. D. WHITE. 1990. *N*-methyl-D-aspartate, kainate and quisqualate release endogenous adenosine from rat cortical slices. Neurosci. **39:** 441–450.
10. BENVENISTE, H., J. DREJER, A. SCHOUSBOE & N. H. DIEMER. 1984. Elevation of the extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracerebral microdialysis. J. Neurochem. **43:** 1369–1374.
12. ANDINÊ, P., O. ORWÄR, I. JACOBSON, M. SANDBERG & H. HAGBERG. 1991. Changes in extracellular amino acids and spontaneous neuronal activity during ischemia and extended reflow in the CA1 of the rat hippocampus. J. Neurochem. **57:** 222–229.
13. PHILLIS, J. W., G. A. WALTER & R. E. SIMPSON. 1991. Brain adenosine and transmitter amino acid release from the ischemic rat cerebral cortex: effects of the adenosine deaminase inhibitor deoxycoformycin. J. Neurochem. **56:** 644–650.
14. VAN CALKER, D., M. MÜLLER & G. HAMPRECHT. 1979. Adenosine regulates via two different types of receptors the accumulation of cyclic AMP in cultured brain cells. J. Neurochem. **33:** 999–1005.
15. JACOBSON, K. A., P. J. M. VAN GALEN & M. WILLIAMS. 1992. Perspective, adenosine receptors: pharmacology, structure activity relationships, and therapeutic potential. J. Med. Chem. **35:** 407–422.
16. STILES, G. L. Adenosine receptors. J. Biol. Chem. **267:** 6451–6454.
17. JI, X-D., D. VON LUBITZ, M. E. OLAH, G. L. STILES & K. A. JACOBSON. 1994. Species differences in ligand affinity at central A_3 adenosine receptors. Drug Dev. Res. **33:** 51–59.
18. RAMKUMAR, V., J. R. BUMGARNER, K. A. JACOBSON & G. L. STILES. 1988. Multiple components of the A_1 adenosine receptor-adenylate cyclase system are regulated in rat cerebral cortex by chronic caffeine ingestion. J. Clin. Invest. **82:** 242–247.
19. JARVIS, M. F. & M. WILLIAMS. 1989. Adenosine in central nervous system function. *In* Adenosine and Adenosine Receptors. M. Williams, Ed. 423–474. Humana Press. Clifton.
20. JACOBSON, K. A., O. NIKODIJEVIĆ, D. SHI, C. GALLO-RODRIGUEZ, M. E. OLAH, G. L. STILES & J. W. DALY. 1993. A role for central adenosine A_3 receptors: mediation of behavioral depressant responses. FEBS Lett. **336:** 57–64.
21. COTMAN, C. W., D. T. MONAGHAN, O. P. OTTERSEN & J. STORM-MATHIESEN. 1987. Anatomical organization of excitatory amino acid receptors and their pathways. TINS **10:** 273–280.
22. DAVAL, J-L., D. K. J. E. VON LUBITZ, J. DECKERT, D. J. REDMOND & P. J. MARANGOS. 1989. Protective effect of cyclohexyladenosine on adenosine A_1 receptors, guanine nucleotide and forskolin binding sites following transient brain ischemia: a quantitative autoradiographic study. Brain Res. **491:** 212–226.
23. VAN WYLEN, D. G. L., V. M. SCIOTTI & H. R. WINN. 1991. Adenosine and the regulation of cerebral blood flow. *In* Adenosine and Adenine Nucleotides as Regulators of Cellular Function. J. W. Phillis, Ed. 191–202. CRC Press. Boca Raton.
24. FERRÉ S., K. FUXE, G. VON EULER, B. JOHANSSON & B. B. FREDHOLM. 1992. Adenosine-dopamine interactions in the brain. Neurosci. **51:** 501–512.
25. BARRACO, R. A., K. A. MARTENS, M. PARIZON & H. J. NORMILE. 1993. Adenosine A_{2a} receptors in the nucleus accumbens mediate locomotor depression. Brain Res. Bull. **31:** 397–404.
26. KOSTOPOULOS, G. K. & J. W. PHILLIS. 1977. Purinergic depression of neurons in different areas of the brain. Exp. Neurol. **55:** 719–724.

27. DUNWIDDIE, T. V. & B. J. HOFFER. 1980. Adenine nucleotides and synaptic transmission in the *in vitro* rat hippocampus. Br. J. Pharmacol. **69:** 59–68.
28. WU, P. H., J. W. PHILLIS & D. L. THIERRY. 1982. Adenosine receptor agonists inhibit K^+ evoked Ca^{2+} uptake by rat cortical synaptosomes. J. Neurochem. **39:** 700–708.
29. SCHUBERT, P., U. HEINEMANN & R. KOLB. 1986. Differential effect of adenosine on pre- and postsynaptic calcium fluxes. Brain Res. **376:** 382–386.
30. SCHUBERT, P., F. KELLER & K. A. RUDOLPHI. 1993. Depression of synaptic transmission and evoked NMDA Ca^{2+} influx in hippocampal neurons by adenosine and its blockade by LTP or ischemia. Drug Dev. Res. **28:** 399–405.
31. HARMS, H. H., G. WARDEH & A. H. MULDER. 1978. Adenosine modulates depolarization-induced release of ^{3}H-noradrenaline from slices of rat brain neocortex. Eur. J. Pharmacol. **49:** 305–308.
32. DOLPHIN, A. C. & E. R. ARCHER. 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.
33. CORADETTI, R., G. LO CONTE, F. MORONI, M. B. PASSANI & G. PEPEU. 1984. Adenosine decreases aspartate and glutamate release from rat hippocampal slices. Eur. J. Pharmacol. **104:** 19–26.
34. FREDHOLM, B. B. & T. V. DUNWIDDIE. 1988. How does adenosine inhibit transmitter release? TIPS **9:** 130–134.
35. HAAS, H. L. & R. W. GREENE. 1984. Adenosine enhances afterhyperpolarization and accommodation in hippocampal pyramidal cells. Pflüg. Arch. **402:** 144–247.
36. GREENE, R. W. & H. L. HAAS. 1991. The electrophysiology of adenosine in the mammalian central nervous system. Progr. Neurobiol. **36:** 329–341.
37. THOMPSON, S. M., H. L. HAAS & B. H. GÄHWILER. 1992. Comparison of the actions of adenosine at pre- and postsynaptic receptors in the rat hippocampus *in vitro*. J. Physiol. **451:** 347–363.
38. SCHUBERT, P., S. FERRONI & R. MAGER. 1991. Pharmacological blockade of Cl^- pumps or Cl^- channels reduces the adenosine mediated depression of stimulation train-evoked Ca^{2+} fluxes in rat hippocampal slices. Neurosci. Lett. **124:** 174–177.
39. GERBER, U., R. W. GREENE, H. L. HAAS & D. R. STEVENS. 1989. Characterization of inhibition mediated by adenosine in the hippocampus of the rat *in vivo*. J. Physiol. **417:** 567–578.
40. DUNWIDDIE, T. V. 1985. The physiological role of adenosine in the central nervous system. Int. Rev. Neurobiol. **27:** 64–139.
41. DUNWIDDIE, T. V. 1980. Endogenously released adenosine regulates excitability in the *in vitro* hippocampus. Epilepsia **21:** 541–548.
42. SEBASTIAO, A. M. & J. A. RIBEIRO. 1992. Evidence for the presence of A_2 adenosine receptors in the rat hippocampus. Neurosci. Lett. **138:** 41.
43. SPIGNOLI, G., F. PEDATA & G. C. PEPEU. 1984. A_1 and A_2 adenosine receptors modulate acetylcholine release from brain slices. Eur. J. Pharmacol. **97:** 341–342.
44. O'REGAN, M. H., R. E. SIMPSON, L. M. PERKINS & J. W. PHILLIS. 1992. The selective adenosine A_2 receptor agonist CGS 21680 enhances excitatory transmitter amino acid release from the ischemic rat cerebral cortex. Neurosci. Lett. **138:** 169–172.
45. MAYFIELD, R. D., F. SUZUKI & N. ZAHNISER. 1993. Adenosine A_{2a} receptor modulation of electrically evoked endogenous GABA release from slices of rat globus pallidus. J. Neurochem. **60:** 2334–2337.
46. SCHIFFMANN, S. N., P. HALLEUX, R. MENU & J-J. VANDERHAEGHEN. 1993. Adenosine A_{2a} receptor expression in striatal neurons: implications for basal ganglia pathophysiology. Drug Dev. Res. **28:** 381–385.
47. FERRÉ, S., P. SNAPRUD & K. FUXE. 1993. Opposing actions of an adenosine A_2 and a GTP analogue on the regulation of dopamine D_2 receptors in rat neostriatal membranes. Eur. J. Pharmacol. Mol. Pharmacol. Sect. **244:** 311–315.
48. FERRÉ, S., G. VON EULER, B. JOHANSSON, B. B. FREDHOLM & K. FUXE. 1991. Stimulation of high affinity adenosine A-2 receptors decreases the affinity of dopamine D-2 receptors in rat striatal membranes. Proc. Natl. Acad. Sci. USA **88:** 7238–7241.
49. ABBRACCHIO, M. P., G. P. FOGLIATTO, A. M. PAOLETTI, E. ROVATI & F. CATTABENI.

1992. Prolonged *in vitro* exposure of rat brain slices to adenosine analogues: selective desensitization of adenosine A_1 but not A_2 receptors. Eur. J. Pharmacol. Mol. Pharmacol. Sect. **227:** 317–324.

50. VON LUBITZ, D. K. J. E., R. C-S. LIN, P. POPIK, M. F. CARTER & K. A. JACOBSON. 1994. Adenosine A_3 receptor stimulation and cerebral ischemia. Eur. J. Pharmacol. In press.
51. VON LUBITZ, D. K. J. E., S. I. DEUTSCH, M. F. CARTER, R. C-S. LIN, J. MASTROPAOLO & K. A. JACOBSON. 1994. The effects of adenosine A_3 receptor stimulation on seizures in mice. Eur. J. Pharmacol. In press.
52. EVANS, M. C., J. H. SWAN & B. S. MELDRUM. 1987. An adenosine analogue, 2-chloroadenosine, protects against long term development of ischaemic cell loss in the rat hippocampus. Neurosci. Lett. **83:** 287–292.
53. VON LUBITZ, D. K. J. E., J. M. DAMBROSIA & O. KEMPSKI. 1986. Postischemic application of cyclohexyladenosine (CHA): improvement of survival and of preservation of selectively vulnerable areas in gerbil. Abstr. X Int. Congr. Neuropathol., Stockholm: 108.
54. VON LUBITZ, D. K. J. E., J. M. DAMBROSIA & D. J. REDMOND. 1989. Protective effect of cyclohexyl adenosine in treatment of cerebral ischemia in gerbils. Neuroscience **2:** 451–457.
55. DRAGUNOW, M. 1991. Adenosine and epileptic seizures. *In* Adenosine and Adenine Nucleotides as Regulators of Cellular Function. J. W. Phillis, Ed. 367–379. CRC Press. Boca Raton.
56. HVIDBERG, A., M. H. RASMUSSEN, N. J. CHRISTENSEN & J. HILSTED. 1994. Theophylline enhances glucose recovery after hypoglycemia in healthy man and in type I diabetic patients. Metabolism **43:** 776–781.
57. FREDHOLM, B. B., K. LINDSTROM & A. WALLMAN-JOHANSSON. 1994. Propentophylline and other adenosine transport inhibitors increase the efflux of adenosine following electrical and metabolic stimulation of rat hippocampal slices. J. Neurochem. **62:** 563–573.
58. RAICHLE, M. 1983. The pathophysiology of brain ischemia. Ann. Neurol. **13:** 9–10.
59. SIESJÖ, B. K. & F. BENGTSSON. 1989. Calcium fluxes, calcium antagonists, and calcium-related pathology in brain ischemia, hypoglycemia, and spreading depression: a unifying hypothesis. J. Cereb. Blood Flow Metab. **9:** 127– .
60. CHOI, D. W. 1992. Excitotoxic cell death. J. Neurobiol. **9:** 1261–1276.
61. HANSEN, A. J. 1985. Effect of anoxia on ion distribution in the brain. Physiol. Rev. **65:** 101–148.
62. BEAL, M. F. 1992. Mechanisms of excitotoxicity in neurologic disease. FASEB J. **6:** 3338–3344.
63. HANSEN, A. J. 1990. Ion homeostasis in cerebral ischemia. *In* Cerebral Ischemia and Resuscitation. A. Schurr & B. M. Rigor, Eds. 77–87. CRC Press. Boca Raton.
64. BALESTRINO, M., P. AITKEN & G. SOMJEN. 1989. Spreading depression-like hypoxic depolarization in CA1 and facia dentata of hippocampal slices: relationship to selective vulnerability. Brain Res. **497:** 102–107.
65. LEE, K. S. & T. LOWENKOPF. 1993. Endogenous adenosine delays the onset of hypoxic depolarization in the rat hippocampus *in vitro* via an action at A_1 receptors. Brain Res. **609:** 313–315.
66. SIMPSON, R. E., M. H. O'REAGAN, L. M. PERKINS & J. W. PHILLIS. 1992. Excitatory transmitter amino acid release from the ischemic rat cerebral cortex. Effects of adenosine agonists and antagonists. J. Neurochem. **58:** 1683–1690.
67. CANTOR, S. L., M. H. ZORNOW, L. P. MILLER & T. L. YAKSH. 1992. The effect of cyclohexyladenosine on the periischemic increases of hippocampal glutamate and glycine in the rabbit. J. Neurochem. **59:** 1884–1892.
68. KLECKNER, N. W. & R. DINGLEDINE. 1988. Requirement for glycine in activation of NMDA-receptors expressed in Xenopus oocytes. Science **241:** 835–837.
69. GILL, R. & G. N. WOODRUFF. 1990. The neuroprotective actions of kynurenic acid and MK-801 in gerbils are synergistic and not related to hypothermia. Eur. J. Pharmacol. **176:** 143–149.

70. VON LUBITZ, D. K. J. E., R. C-S. LIN, R. J. MCKENZIE, T. M. DEVLIN, P. SKOLNICK & R. T. MCCABE. 1992. A novel treatment of global cerebral ischemia with a glycine partial agonist. Eur J. Pharmacol. **219:** 153–158.
71. BENGTSSON, F. & B. K. SIESJÖ. 1990. Cell damage in cerebral ischemia: physiological, biochemical, and structural aspects. *In* Cerebral Ischemia and Resuscitation. A. Schurr & B. M. Rigor, Eds. 215–233. CRC Press. Boca Raton.
72. ANDERSON, K. J., B. NELLGÄRD & T. WIELOCH. 1993. Ischemia-induced upregulation of excitatory amino acid transporters. Brain Res. **622:** 93–98.
73. SCHMIDT, W., G. WOLF, K. GRUNGREIFF & K. LINKE. 1993. Adenosine influences the high affinity uptake of transmitter glutamate and aspartate under conditions of hepatic encephalopathy. Metab. Brain Dis. **8:** 73–80.
74. PHILLIS, J. W., M. H. O'REGAN & G. A. WALTER. 1988. Effects of deoxycoformycin on adenosine, inosine, hypoxanthine, xanthine, and uric acid release from the hypoxaemic rat cerebral cortex. J. Cereb. Blood Flow Metab. **8:** 733–741.
75. KIRINO, T. & K. SANO. 1984. Selective vulnerability in the gerbil hippocampus following transient ischemia. Acta Neuropathol. (Berlin) **62:** 201–208.
76. RUDOLPHI, K. A., M. KEIL & H. J. HINZE. 1987. Effect of theophylline on ischemically induced hippocampal damage in Mongolian gerbils: a behavioural and histopathological study. J. Cereb. Blood Flow Metab. **7:** 74–81.
77. HERRON, C. E., R. J. LESTER, E. J. COAN & G. L. COLLINGRIDGE. 1986. Frequency-dependent involvement of NMDA receptors in the hippocampus: a novel synaptic mechanism. Nature **322:** 265–268.
78. SCHUBERT, P. 1988. Physiological modulation by adenosine selective blockade of A_1 receptors with DPCPX enhances stimulus train-evoked neuronal Ca influx in rat hippocampal slices. Brain Res. **458:** 162–165.
79. SCHUBERT, P. & R. MAGER. 1991. The critical input frequency for NMDA-mediated neuronal Ca^{2+} frequency depends on endogenous adenosine. Int. J. Purine Pyrimidine Res. **2:** 11–25.
80. SEGAL, M., M. A. ROGAWSKI & J. L. BARKER. 1984. A transient potassium conductance regulates the excitability of cultured hippocampal and spinal neurons. J. Neurosci. **4:** 604–609.
81. TRUSSEL, L. O. & B. J. JACKSON. 1987. Dependence of an adenosine activated postassium current on GTP-binding protein in mammalian neurons. J. Neurosci. **7:** 3306–3316.
82. MAGER, R., S. FERRONI & P. SCHUBERT. 1990. Adenosine modulates a voltage-dependent chloride conductance in cultured hippocampal neurons. Brain Res. **532:** 58–62.
83. THOMPSON, S. M. & B. H. GÄHWILER. 1989. Activity dependent disinhibition. 1. Repetitive stimulation reduces IPSP driving force and conductance in rat hippocampus *in vitro*. J. Neurophysiol. **61:** 501–511.
84. CHESSELET, M-F., C. GONZALES, C-S. LIN, K. POLSKY & B-K. JIN. 1990. Ischemic damage in the striatum of adult gerbils: relative sparing of somatostatinergic and cholinergic interneurons contrasts with loss of efferent neurons. Exp. Neurol. **110:** 209–218.
85. GLOBUS, M. Y. T., R. BUSTO, W. D. DIETRICH, E. MARTINES, I. VALDES & M. D. GINSBERG. 1988. Intra-ischemia extracellular release of dopamine is associated with striatal vulnerability to ischemia. Neurosci. Lett. **91:** 36–40.
85. GLOBUS, M. Y. T., R. BUSTO, E. MARTINEZ, I. VALDES, W. D. DIETRICH & M. D. GINSBERG. 1991. Comparative effect of transient global ischemia on extracellular levels of glutamate, glycine, and γ-aminobutyric acid in vulnerable and nonvulnerable brain regions in the rat. J. Neurochem. **57:** 470–478.
85. CRAIN, B. J., W. D. WESTERKAM, A. H. HARRISON & J. V. NADLER. 1988. Selective neuronal death after transient forebrain ischemia in the Mongolian gerbil: a silver impregnation study. Neuroscience **27:** 387–402.
86. CHANG, C. J., H. ISHII, H. YAMAMOTO, T. YAMAMOTO & M. SPATZ. 1993. Effects of cerebral on regional dopamine release and D_1 and D_2 receptors. J. Neurochem. **60:** 1483–1490.
87. PRADO, R., R. BUSTO & M. Y. T. GLOBUS. 1992. Ischemia-induced changes in extracel-

lular levels of striatal cyclic AMP: role of dopamine neurotransmission. J. Neurochem. **59:** 1581–1584.
88. CEPEDA, N. A. BUCHWALD & M. S. LEVINE. 1993. Neuromodulatory actions of dopamine in the neostriatum are dependent upon the excitatory amino acid receptor subtypes activated. Proc. Natl. Acad. Sci. **90:** 9576–9580.
89. DIEMER, N. H. & E. SIEMKOWICZ. 1981. Regional neuronal damage after cerebral ischemia in normo- and hypoglycemic rats. Neuropathol. Appl. Neurobiol. **7:** 217–227.
90. ARAKI, T., H. KATO & K. KOGURE. 1989. Selective neuronal vulnerability following transient cerebral ischemia in the gerbil: distribution and time course. Acta Neurol. Scand. **80:** 548–553.
91. MARTINEZ-MIR, M. I., A. PROBST & J. M. PALACIOS. 1991. Adenosine A_2 receptors: selective localization in the human basal ganglia and alterations with disease. Neuroscience **42:** 697–706.
92. SCHIFFMANN, S. N. & J-J. VANDERHAEGHEN. 1993. Adenosine A_2 receptors regulate the gene expression of striatopallidal and striatonigral neurons. J. Neurosci. **13:** 1080–1087.
93. FORRESTER, T., A. M. HARPER, E. T. MACKENZIE & E. M. THOMPSON. 1979. Effect of adenosine triphosphate and some derivatives on cerebral blood flow and metabolism. J. Physiol. **296:** 343–355.
94. CRONSTEIN, B. N., R. I. LEVIN, J. BELANOFF, G. WEISSMANN & R. HIRSCHHORN. 1986. Adenosine: an endogenous inhibitor of neurtrophil-mediated injury to endothelial cells. J. Clin. Invest. **78:** 760–770.
95. SOLLEVI, A. 1986. Cardiovascular effects of adenosine in man: possible clinical implications, Progr. Neurobiol. **27:** 319–349.
96. BORN, G. V. R. & M. J. CROSS. 1963. The aggregation of blood platelets. J. Physiol. **168:** 178–195.
97. CUSACK, N. J. & S. M. O. HOURANI. 1991. Adenosine, adenine nucleotides, and platelet functions. *In* Adenosine and Adenine Nucleotides as Regulators of Cellular Function. J. W. Phillis, Ed. 121–131. CRC Press. Boca Raton.
98. CRONSTEIN, B. N. 1991. Purines and inflamation: neutrophils possess P_1 and P_2 purine receptors. *In* Adenosine and Adenine Nucleotides as Regulators of Cellular Function. J. W. Phillis, Ed. 133–140. CRC Press. Boca Raton.
99. GRISHAM, M. B., L. A. HERNANDEZ & D. N. GRANGER. 1989. Adenosine inhibits ischemia-reperfusion-induced leukocyte adherence and extravasation. Am. J. Physiol. **257:H** 1334–1339.
100. DRAGUNOW, M. & R. L. M. FAULL. 1988. Neuroprotective effects of adenosine. TIPS **9:** 193–194.
101. PHILLIS, J. W. 1990. Adenosine, inosine, and the oxypurines in cerebral ischemia. *In* Cerebral Ischemia and Resuscitation. A. Schurr & B. M. Rigor, Eds. 189–204. CRC Press. Boca Raton.
103. ROUSSEL, S., E. PINARD & J. SEYLAZ. 1991. Focal cerebral ischemia in chronic hypertension: no protection by (R)-phenylisopropyladenosine. Brain Res. **545:** 171–174.
103. LEE, K. S., W. TETZLAFFAND & G. W. KREUTZBERG. 1986. Rapid downregulation of hippocampal adenosine receptors following brief anoxia. Brain Res. **380:** 155–158.
104. ONODERA, H. & K. KOGURE. 1990. Calcium antagonist, adenosine A_1 and muscarine bindings in rat hippocampus after transient ischemia. Stroke **21:** 771–776.
105. LEE, K. S., P. SCHUBERT, M. REDDINGTON & G. W. KREUTZBERG. 1983. Regulation of strength of adenosine modulation in the hippocampus by a differential distribution of the density of A_1 receptors. Brain Res. **260:** 156–159.
106. VON LUBITZ, D. K. J. E., R. C-S. LIN, N. MELMAN, X-D. JI, M. F. CARTER, & K. A. JACOBSON. 1994. Chronic administration of adenosine A_1 receptor agonist or antagonist in cerebral ischemia. Eur. J. Pharmacol. **256:** 161–167.
107. GAO, Y. & J. W. PHILLIS. 1994. CGS 15943, an adenosine A_2 receptor antagonist, reduces cerebral ischemic injury in the Mongolian gerbil. Life Sci. **55:** 61–65.
107. BUSTO, R., W. D. DIETRICH, M. Y. T. GLOBUS & M. D. GINSBERG. 1989. The importance of brain temperature in cerebral ischemic injury. Stroke **20:** 1113–1114.

108. GOLDBERG, M. P., H. MONYER, J. H. WEISS & D. W. CHOI. 1988. Adenosine reduces cortical neuronal injury induced by oxygen and glucose deprivation *in vitro*. Neurosci. Lett. **89:** 323–327.
109. WAUQUIER, A., H. L. EDMONDS, JR. & G. H. C. CLINCKE. 1987. Cerebral resuscitation: pathophysiology and therapy. Neurosci. Biobehav. Rev. **11:** 287–306.
110. MILLER, J. T. 1983. Head injury and brain ischemia—implications for therapy. Br. J. Anaesth. **57:** 120–130.
111. VON LUBITZ, D. K. J. E. & P. J. MARANGOS. 1990. Cerebral ischemia in gerbils: postischemic administration of cyclohexyladenosine and 8-sulphophenyl-theophylline. J. Mol. Neurosci. **2:** 53–59.
112. RUDOLPHI, K. A., M. KEIL, J. FASTBOM & B. B. FREDHOLM. 1989. Ischaemic damage in gerbil hippocampus is reduced following upregulation of adenosine A_1 receptors by caffeine treatment. Neurosci. Lett. **103:** 275–280.
113. VON LUBITZ, D. K. J. E., I. A. PAUL & K. A. JACOBSON. 1993. Effects of N^6-cyclopentyl adenosine and 8-cyclopentyl-1,3 dipropylxanthine on *N*-methyl-D-aspartate induced seizures in mice. Eur. J. Pharmacol. **249:** 265–270.
114. VON LUBITZ, D. K. J. E., I. A. PAUL, R. T. BARTUS & K. A. JACOBSON. 1993. Effects of chronic administration of adenosine A1 receptor agonist and antagonist on spatial learning and memory. Eur. J. Pharmacol. **249:** 271–280.
115. VON LUBITZ, D. K. J. E., I. A. PAUL, X-D. JI, M. CARTER & K. A. JACOBSON. 1994. Chronic adenosine A_1 receptor agonist and antagonist: effect on receptor density and *N*-methyl-D-aspartate induced seizures in mice. Eur. J. Pharmacol. **253:** 95–99.
116. GEORGIEV, V., B. JOHANSSON & B. B. FREDHOLM. 1993. Long-term caffeine treatment leads to decreased susceptibility to NMDA-induced clonic seizures in mice without changes in adenosine A_1 receptor number. Brain Res. **612:** 271–277.
117. MAGISTRETTI, P. J., P. R. HOFF & J. L. MARTIN. 1986. Adenosine stimulates glycogenolysis in mouse cerebral cortex: a possible coupling mechanism between neuronal activity and energy metabolism. J. Neurosci. **6:** 2558–2562.
118. BOURKE, R. S., H. K. KIMELBERG & M. A. DAZE. 1978. Effects of inhibitors and adenosine on (HCO^-/CO_2) stimulated swelling and Cl^- in brain slices and cultured astrocytes. Brain Res. **154:** 196–202.
119. PHILLIS, J. W. 1994. Adenosine metabolites may provide a primary source of oxygen free radicals in ischemic/reperfused rat brain. Drug. Dev. Res. **31:** 308.
120. PARSONS, W. J. & G. L. STILES. 1987. Heterologous desensitization of inhibitory adenosine A_1 receptor adenylate cyclase system in rat adipocytes. J. Biol. Chem. **262:** 841–847.
121. RAMKUMAR, V., G. L. STILES, M. A. BEAVEN & H. ALI, JR. 1993. The A_3 adenosine receptor is the unique adenosine receptor which facilitates release of allergic mediators in mast cells. J. Biol. Chem. **268:** 16887–16890.
122. SHI, D., O. NIKODIJEVIĆ, K. A. JACOBSON & J. W. DALY. 1993. Chronic caffeine alters the density of adenosine, adrenergic, cholinergic, GABA, and serotonin receptors and calcium channels in mouse brain. Cell. Mol. Neurobiol. **13:** 247–261.
123. LONGBAUGH, J. P., J. DIDSBURY, A. SPIEGEL & G. STILES. 1989. Modification of the rat adipocyte A_1 adenosine receptor-adenylate cyclase system during chronic exposure to an A_1 receptor agonist: alterations in the quantity of $G_{S\alpha}$ and $G_{i\alpha}$ are not associated with changes in their mRNAs. J. Pharmacol. Exp. Ther. (Mol. Pharmacol.) **36:** 681–688.