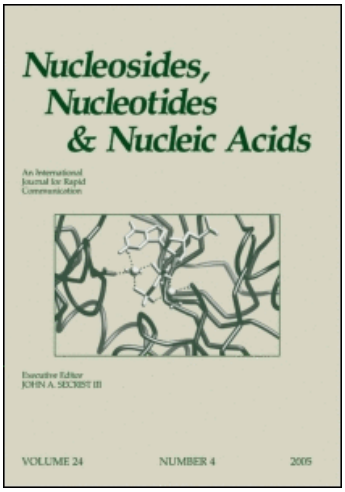

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PHARMACOLOGICAL IMPLICATIONS OF A MULTIPLICITY OF ADENOSINE ACTIONS IN THE CNS.

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ABSTRACT. Adenosine (50 nM - 50 μM) in brain extracellular space acts on two major classes of receptors present on virtually every cell. Specificity of action may be achieved by altering brain adenosine levels and by using partial agonists and/or drugs that affect more than one biochemical target.

Formation and levels of adenosine in the CNS

Adenosine is formed either from adenine nucleotides or by breakdown from S-adenosyl homocysteine (AdoHcy). There is good evidence that the latter pathway is of importance in the resting heart, but formation from AMP apparently is more important following cardiac work and/or hypoxia (1, 2). In the CNS formation from adenine nucleotides is thought to dominate, but firm evidence is lacking. However, it is clear that the bulk of adenosine release in slices from cortical tissue derives from newly formed intracellular adenosine and not from breakdown of extracellular adenine nucleotides (3, 4). Thus, even though release of adenine nucleotides may be important in cellular signalling it is not a quantitatively important source of extracellular adenosine in the CNS (4).

In vivo studies of adenosine levels.

It is notoriously difficult to accurately determine the levels of adenosine *in vivo*. Thus, the only method to adequately determine the basal adenosine levels appears to be freeze-blowing (5, 6). This technique obviously precludes any attempt

to localize the nucleoside regionally or determine relative intra- and extracellular levels. Therefore microdialysis has been widely used to determine the biologically relevant extracellular level of adenosine (7-12). In these initial studies the levels were found to be close to 1 μM. However, it was eventually realized that this may be an overestimate because the implantation of the microdialysis probe appears to induce a marked trauma (7, 13, 14). When adequate time is taken to allow the trauma to subside and the measurements are carried out in awake animals, the levels of adenosine, inosine and hypoxanthine between the 24th and the 52nd hour are 50, 110 and 590 nM, respectively. However, the concentration of adenosine was markedly (about 7-fold) elevated by combined uptake (dipyridamole, lidoflazine and NBMPR) and adenosine deaminase (deoxycoformycin) inhibitors. Inosine and hypoxanthine concentrations were unaffected. This suggests that a substantial part of the adenosine might be lost between the bulk of the extracellular space and the site of sampling i.e. by uptake followed by deamination in glial cells. Thus, the resting adenosine level in the extracellular space of the rat striatum probably is between 40 and 1000 nM.

Even though trauma following the implantation of the fiber may have led to an overestimate of the basal adenosine level in several previous studies there is no evidence that this should have caused any overestimation of the levels of adenosine after hypoxia or ischemia. In fact, our results rather indicate that acute trauma will decrease the response to a transient ischemia (15). Therefore, it seems reasonable to conclude that the levels of adenosine may well reach 50 μM after ischemia (9, 11, 12, 15). Thus, the best estimates indicate that the levels of adenosine in extracellular fluid may vary 1000-fold under physiological and pathophysiological conditions.

Adenosine acting on A_1 receptors.

Adenosine acts on adenosine A_1 receptors already in submicromolar concentrations. We recently determined the potency of adenosine as a ligand at A_1 adenosine receptors using DDT MF2 cells (16). The potency of adenosine as a ligand at the low affinity form of the adenosine receptor was some 10 times lower than that of cyclohexyladenosine. This indicates that its potency at the high affinity form that mediates the biological effects is likely to be in the low nmolar range. This is however not what we find (see Table 1). Thus, even though adenosine has

TABLE 1.

Potency of several adenosine analogues on binding to A_1-receptors in the membranes of DDT MF2 cells or to intact cells compared to their potency as inhibitors of isoprenaline stimulated cyclic AMP accumulation.

	Membrane binding		Binding to intact cells	cAMP (IC_{50})
	K_H (nM)	K_L (nM)	K_D (nM)	(nM)
CPA	0.4	41	58	0.8
CHA	0.8	93	77	1.5
R-PIA	1.7	150	140	2.5
NECA	2	360	440	19
2-chloro ado	2.5	640	800	39
Ado	---	---	1630	800

the potential of interacting with the A_1 receptors already at very low concentrations it seems as if its actual potency is much less, possibly indicating that the interaction between the receptor and the signal transduction system is not quite as straightforward as is commonly assumed.

Association with G-proteins.

Both adenosine A_1 and A_2 receptors appear to be coupled to G-proteins. In the case of the A_1 receptor the coupling has been particularly well studied since this receptor has been purified. If its purification is achieved by means of an agonist affinity column it is recovered as a G-protein coupled complex (17), whereas purification on an antagonist affinity column yielded a receptor that is uncoupled from the G-protein (18). It is also well known that binding of agonist ligands to adenosine A_1 receptors is strongly affected by GTP (19, 20). In intact cells where the level of GTP is high, the binding to A_1 receptors by agonists conforms with the binding in membranes that have been treated with high concentrations of GTP (16, 21). By the use of quantitative autoradiography it can be demonstrated that virtually all receptors of the A_1 class in the CNS are associated with G-proteins since all high affinity binding of agonists is abolished by GTP (22).

TABLE 2 Actions of adenosine A_1 receptors.

Mediated via G_α-subunits	
	Inhibition of Ca-channels (N-type)
	Activation of K^+-channels (several)
	Inhibition of adenylate cyclase??
Mediated via $G_{\beta,\gamma}$-subunits	
	Inhibition of adenylate cyclase?
	Inhibition of PI-turnover?
	Inhibition of PC-turnover?
	Activation of phospholipase A2?
Indirect effects	
	Actions on Ca-activated K^+-channels
	Enhancement of PI-turnover??
	Several voltage dependent channels

In the rat hippocampal slice preparation, there are several effects of adenosine that are mediated via A_1 receptors (23-25). So far it has not proven possible to distinguish between these receptors with either agonists or antagonists. However, using *in vivo* treatment with PTX it was found that some effects of adenosine were virtually abolished, whereas other effects were essentially unchanged (26), suggesting that there could be differences in the coupling between G-protein(s) and adenosine receptor(s). Possibly a single class of adenosine A_1 receptor has the ability to interact with a multitude of different G-proteins not all of which are present in all membranes possessing the A_1 receptor. Alternatively there is a family of adenosine A_1 receptors that differ less in regions binding to adenosine than in the parts that determine the interaction with G-protein(s). Clearly adenosine A_1 receptors can elicit a wide variety of intracellular effects (Table 2).

Possibilities for specific agonists and or antagonists.

It is an important question if this in itself means that there are differences between the receptors that can be utililized pharmacologically to distinguish between receptors that produce one or the other effect. A final answer to this question cannot be given until the adenosine receptors are all cloned and expressed. However, a few guesses can be made based on our knowledge of other receptors.

Work with chimeric receptors and with site-directed mutagenesis on adrenoceptors and acetylcholine receptors has revealed that it is possible to alter the way that a receptor interacts with G-proteins without markedly affecting the binding characteristics of either agonists or antagonists (27). Conversely there is good evidence that a single molecular form of a receptor is able to interact with several different G-proteins. Therefore it is unlikely that it will be possible to e.g. develop an adenosine receptor antagonist that is selectively able to antagonize, say, adenosine effects on potassium channels and not affect adenosine effects on adenylate cyclase.

It is, however, quite likely that the efficacy of coupling between the adenosine A_1 receptor and the G-protein could be different so that it would appear that agonists are better at causing some effects than others. In the terminology of classical pharmacology we could easily envisage the situation where the number of spare receptors is larger for e.g. the presynaptic effects of adenosine than for the postsynaptic. Given the well-known association between the number of spare receptors and the potency of partial agonists it does seem possible to develop compounds that show selectivity in their ability to activate adenosine A_1 mediated effects. Thus the stoichiometry of the adenosine receptor - G-protein - effector system may be very important in determining the relative agonist potency as discussed e.g. by Kenakin (28, 29).

In addition it is clearly possible to alter the relative agonist potency if the agonists are not very selective for one receptor type. An important case in point is NECA, a compound that often shows an anomalous behaviour in description of relative agonist potency. It must then be borne in mind that this compound is a good agonist both on A_1 and on A_2 receptors (e.g. 31). This means that if a given cell possesses both A_1 and A_2 receptors, a situation that is by no means uncommon, then the potency of NECA both as an A_1 and as an A_2 agonist will be underestimated relative to compounds that are pure agonists. So far this has only resulted in considerable confusion in the adenosine receptor literature. However, it is possible that the deliberate development of mixed agonists may provide a selectivity that cannot be achieved by compounds that selectively interact with one type of receptor.

Adenosine A_2 receptors.

There is good reason to believe that most of the effects due to stimulation of A_2 receptors depend on the activation of G_s. Hence, one may expect either stimulation of adenylate cyclase or possibly activation of a Ca-channel to be the result of A_2 receptor activation. It has been proposed that there are two types of adenosine A_2 receptors: A_{2a} and A_{2b} (30). The A_{2a} subtype exhibits a somewhat higher affinity for agonists including NECA and can also be studied by adenylate cyclase assays in broken cell preparations (32, 33). By contrast, the low affinity A_{2b} receptor, which shows a very wide distribution among cell types, cannot be studied functionally in broken cell preparations.

CGS 21680, a recently developed analogue of NECA and 2-phenylamino-adenosine, the two previously most selective compounds, exhibits a considerable selectivity for the high affinity A_{2a} receptor (34, 35). This is very clearly demonstrated using autoradiography (36, 37). However, this compound has the ability to activate also other types of receptors. It was recently shown that CGS 21680 may activate A_1 receptors, albeit with a 100-fold lower potency than A_{2a} receptors (38). We have recently studied its ability to activate A_2 receptors of a typical A_{2b} subtype, namely those located on T-leukemia cells. We found that even though the compound is virtually inactive under normal circumstances it does have the ability to activate cAMP formation provided that forskolin is present (39). However, it never could stimulate the cAMP formation to the same degree as NECA. Other adenosine agonists, including CV 1808 and CV 1674, are also less efficacious agonists than NECA. They are, however, not necessarily less potent. Thus, in this system, as well as in other A_2 receptor mediated systems, many adenosine analogues appear to act as partial agonists. Even so they do not have the ability to antagonize the actions of the full agonist. This suggests that there exist hitherto unexplored complications in the coupling between A_2 receptors and the adenylate cyclase. Furthermore, partial agonists may show a more selective effect than full agonists and perhaps the two compounds CGS 21680 and CV 1808 achieve selectivity in this way.

A_2 receptor-mediated effects are enhanced by agents that activate PkC and/or Ca.

It has long been known that agents such as noradrenaline and histamine can enhance the cAMP response due to activation of adenosine, prostaglandin or VIP

receptors. This effect is dependent upon Ca and calmodulin (40, 41). But it has also been shown that protein kinase C activation by phorbol esters may enhance the cAMP response not only in the brain (42-45), but also in Jurkat cells (46, 47). Therefore the magnitude of the adenosine A_2 receptor-mediated effects are strongly dependent upon the simultaneous presence of stimuli that activate PkC and/or Ca-mechanisms within the cell. A drug that affects not only the A_2 receptor, but also these other receptors may therefore give a more selective response.

Therapeutic possibilities.

There are several possibilities. Some of these which have been tried in practice are summarized below:

1) Concentrations of adenosine are low. Adenosine itself or adenosine analogues may be used.

   Diagnosis and treatment in supraventricular tachycardia (48).
   Controlled hypotension (49).
   Anti-platelet effects in extracorporeal circulation (50).

2) Adenosine levels are high and may be detrimental--antagonists.

   Ischemia, NSAID use etc. in the kidney. Drugs that antagonize the vasoconstrictor and anti-diuretic action of adenosine--including xanthine derivatives--may be beneficial(51).

3) Adenosine production is high and this is beneficial--possible use of uptake inhibitors.

   E.g. Ischemic brain damage. Drugs that further increase adenosine levels, particularly in penumbral zones could have a therapeutic potential (11, 52-54).

Conclusions.

Adenosine is present in brain in concentrations sufficient to activate receptors and all the cells in the central nervous system possesses at least one type of adenosine receptor. Hence, a very wide variety of biological effects are possible

and have been demonstrated. Hopes are slim that each of these multiple effects are mediated by receptors that exhibit sufficient structural differences that they can be used to develop drugs that selectively interact with them. It is, however, possible that there are sufficient differences in the efficacy of coupling that drugs that are partial agonists may prove to be relatively selective in their actions. Furthermore, drugs that interact not only with adenosine receptors but also with other targets may be functionally more selective than drugs that selectively interact with one type of adenosine receptor. Finally, there are very interesting possibilities to develop drugs that interact with the formation and/or elimination of adenosine.

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