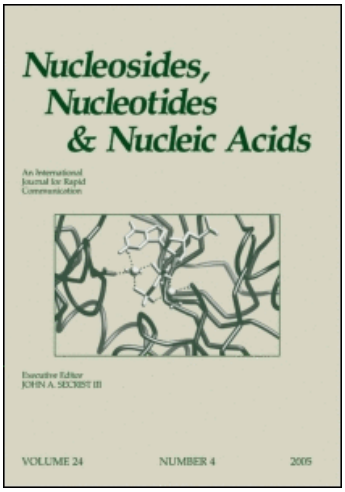

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ADENOSINE RECEPTORS IN ISOLATED TISSUE PREPARATIONS

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Abstract

Adenosine receptors in isolated tissues from guinea-pigs have been investigated using agonists and antagonists. The receptor mediating decreases in rate and force of contraction of the atria is of the A_1 sub-type. Responses of smooth muscle preparations to adenosine and its analogues are complex involving relaxation mediated by both A_2 receptors an by non-receptor mechanisms. Contractions mediated by an A_1 receptor can also be detected.

Introduction

In the search for sub-types of receptors and for selective ligands binding studies using cell membranes offer advantages of throughput and economy. A limitation with ligand binding techniques however, is their inability to assess efficacy, therefore it is not possible to determine whether a novel ligand is an agonist or an antagonist. CNS tissue is commonly used in ligand binding studies because of the high density of receptors present. There is always the possibility that receptors in peripheral tissues have different characteristics from those in the CNS. For these reasons, there is a need to develop isolated tissue preparations, which will allow the assessment of both affinity and efficacy, and which may also help to predict the peripheral effects of selective ligands *in vivo*.

The sub-classification of adenosine receptors has relied upon ligand binding studies and on the measurement of cyclic nucleotide levels in certain cell types[1,2,3]. In our studies we have attempted to understand the complexity of the responses evoked by adenosine and

its analogues in isolated tissues and to classify the adenosine receptors present. The isolated tissue preparations which have been used in these studies are the atria, trachea and aorta from guinea-pigs.

Guinea-pig atria

In the guinea-pig atria, adenosine and its analogues evoke negative inotropic and chronotropic responses[4,5]. For both of these responses the order of agonist potency is: 5'-N-ethylcarboxamide adenosine (NECA) $\geqslant$ N^6-cyclohexyladenosine (CHA) = R(-)N^6-phenylisopropyl adenosine (R-PIA) > 2-chloroadenosine > S(+)-PIA. Ribose modified adenosine analogues such as 2'5'dideoxyadenosine and 9-B-D-arabinofuranosyl adenine have little effect on atrial function indicating the presence of an R-type adenosine receptor. The high degree of stereoselectivity (100 fold) between the diastereo-isomers R- and S-PIA support the classification of the atrial receptor as A_1. The similar or slightly greater potency of NECA than of R-PIA is at variance with the classical order of R-PIA > NECA at A_1 receptors although the difference in potency between the analogues is small in binding studies using guinea-pig tissue[6]. These latter considerations have led to the suggestion that the atrial receptor may be a novel "A_3" subtype[7].

Studies using antagonists in atrial preparations have been performed using a number of agonists and experimental conditions. The affinity of an antagonist does not vary significantly when different adenosine receptor agonists (R-PIA, NECA, 2-chloroadenosine) are used[8] indicating that they all act at the same site. A factor which can complicate the interpretation of agonist dose-response data and cause an underestimation of antagonist affinity in the atrium is an increase in the basal force or rate of contraction upon addition of the antagonist[9]. This increase can be avoided in many cases by the addition of adenosine deaminase, to remove the negative inotropic or chronotropic effect of endogenous adenosine (Table 1). With some antagonists, increases in rate or force persist even in the presence of adenosine deaminase. This indicates that an effect other than blockade of the effects of endogenous adenosine is occurring, and this can be compensated for by the use of pharmacologic resultant analysis[10].

TABLE 1 Effect of 1,3-dipropyl-8-cyclopentyl xanthine (DPCPX) on atrial rate in the absence and presence of adenosine deaminase

Conditions	Initial Rate	Rate after DPCPX	pA_2
Adenosine as agonist	157±8	212±6*	8.03
2-chloroadenosine as agonist plus adenosine deaminase	203+8	210±7	8.36

* $P<0.005$

TABLE 2 Comparison of apparent affinity of adenosine antagonists assessed in isolated atria from guinea-pigs and from R-PIA binding studies using guinea-pig cerebral cortex membranes

Antagonist	pA_2	pKi Cortex
Theophylline	4.9	4.9
8-phenyltheophylline (8PT)	6.3	5.9
8-p-sulphophenyl theophylline	4.9	4.7
XAC	7.4	7.6
PD115199	6.4	6.7
DPCPX	8.4	8.1

We have assessed the affinity of a range of standard antagonists in the atria using 2-chloroadenosine as the agonist in the presence of adenosine deaminase. The apparent affinities of these compounds (Schild analysis) are in good agreement with pKi values derived from the displacement of tritiated R-PIA from guinea-pig cerebral cortex membranes (Table 2). The highest antagonist affinity in both the isolated tissue and the binding assay was observed for DPCPX (Fig 1). Thus, these studies with antagonists strongly suggest that the adenosine

TABLE 3 Comparison of apparent affinity for 8-substituted xanthines in atria from the rat and the guinea-pig

Antagonist	pA_2 g.pig	pA_2 rat
8PT	6.30	7.16
XAC	7.22	7.82
XCC	7.40	7.93

receptor in the guinea-pig atrium is similar to that in cerebral cortex membranes from the same species and is of the A_1 sub-type.

A marked species difference has been noted in the affinity of adenosine antagonists for A_1 receptors in CNS binding studies[6]. This difference also pertains in atria from the two species[11] with affinity in the rat being higher than in the guinea-pig (Table 3).

Guinea-pig aorta and trachea

The major response evoked by adenosine and its analogues in isolated smooth muscle preparations is one of relaxation. In studies using the aorta and the trachea we have used sub-maximal concentrations of phenylephrine and carbachol, respectively, to evoke a standardized level of contractile tone[9]. The order of agonist potency in relaxing these two pre-contracted isolated smooth muscle preparations is NECA > 2-chloroadenosine > R-PIA = CHA > S-PIA, with only a small difference in potency between R- and S-PIA. This order implies the presence of an A_2 sub-type of adenosine receptor[12,13].

There are some unusual features of the relaxant responses evoked by adenosine and 2-chloroadenosine in the aorta and trachea. The maximal relaxation evoked by these agonists is greater than that evoked by NECA. In addition, the time course of responses evoked by high concentrations of 2-chloradenosine is slower than that to NECA and adenosine. A number of experiments have been performed using the isolated aorta preparation

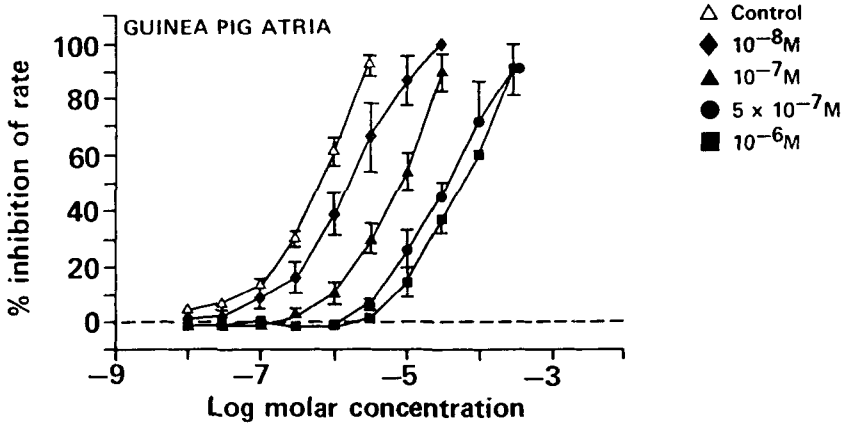

FIGURE 1 Effect of DPCPX on negative chronotropic responses to 2-chloroadenosine

in order to investigate the mechanisms of the enhanced maximum response to adenosine[14]. Surprisingly, treatment of the tissue with the adenosine transport inhibitor nitrobenzyl-thio-inosine (NBMPR 10μM) or with the adenosine deaminase inhibitor erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA 10μM), reduces the maximal response evoked by adenosine (Fig 2). The adenosine receptor antagonist 8PT (10μM) is less effective as an antagonist of high than of low concentrations of adenosine (Fig 2). These results imply that high concentrations of adenosine have an additional intracellular effect to evoke vascular smooth muscle relaxation. Since EHNA reduces maximal responses to adenosine it is likely that a deaminated metabolite is responsible for this effect. Inosine can also evoke relaxation of the aorta which is inhibited by NBMPR and which is insensitive to 8PT (Fig 3).

Since 2-chloroadenosine is resistant to deamination, it is highly unlikely that an analogue of inosine accounts for its enhanced maximum response. The enhanced maximum response persists in the presence of dipyridamole and EHNA. An alternative explanation for this effect is that 2-chloroadenosine has greater efficacy at the aortic adenosine receptor than either NECA or adenosine. This possibility has been

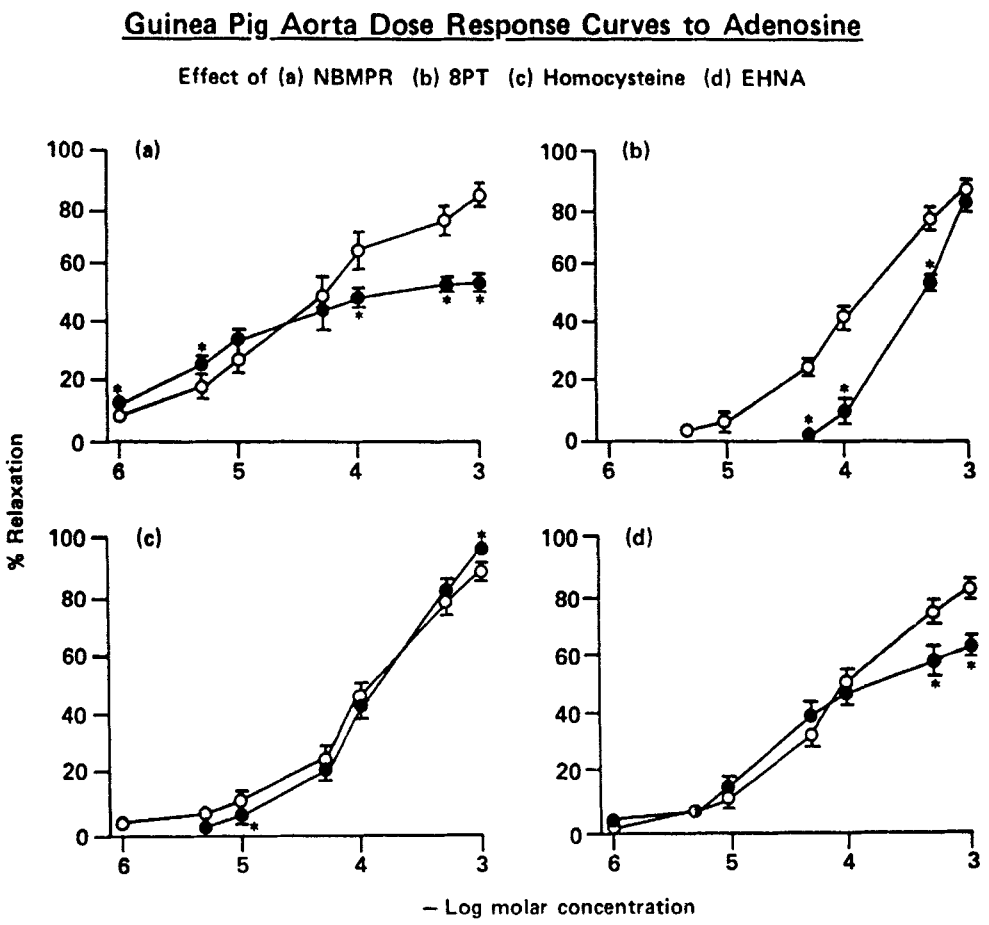

FIGURE 2

investigated by superimposing dose-response curves to 2-chloroadenosine on a pre-existing maximal response to adenosine (in the presence of EHNA and dipyridamole). Under these conditions, 2-chloroadenosine evokes a further relaxation but the dose-response curve is not displaced to the right of a control 2-chloroadenosine curve. This indicates that the enhanced maximum response to 2-chloroadenosine is not the result of enhanced efficacy relative to adenosine, but to an additional action at a site which is not occupied by adenosine. Further evidence for an additional action of 2-chloroadenosine has been obtained from studies in aortic rings which have been contracted by a high extracellular

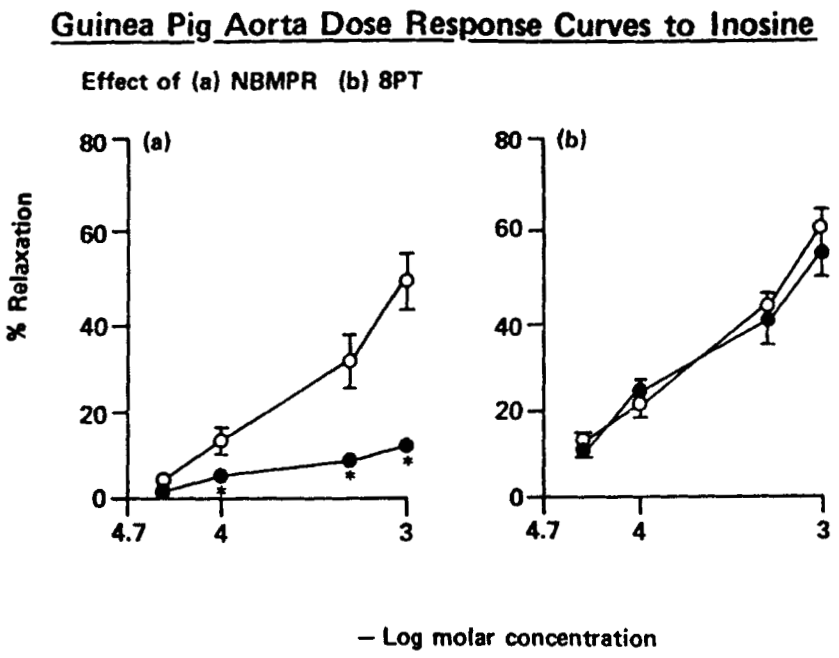

FIGURE 3

potassium concentration (80mM). Adenosine cannot relax contractions evoked by these high potassium concentrations, whereas 2-chloroadenosine evokes a relaxation at high concentrations. The relaxation of the potassium contracted aorta by 2-chloroadenosine is not inhibited by antagonists such as DPCPX (5μM) or 8PST (100μM), in fact these antagonists enhance this relaxant response. The mechanism underlying this additional effect of 2-chloroadenosine requires further investigation.

Although the major response of the aorta to adenosine and to its analogues is relaxation, small contractile responses can also be evoked by agonists such as R-PIA and CHA[15]. These contractile responses are variable in magnitude between different aortic preparations. An A_1 receptor selective concentration of DPCPX (0.5μM) abolishes these contractions which indicates the presence of a small population of A_1 receptors in the aorta mediating this response.

TABLE 4 Comparison of apparent affinity of antagonists assessed in the isolated aorta and in NECA binding studies using PC_{12} cell membranes

Antagonist	pA_2 Aorta	pKi: PC_{12}
Theophylline	5.0	4.9
8PT	6.5	6.5
XAC	7.7	8.0
PD115199	6.6	8.4
PD113297	7.1	7.8
DPCPX	6.7	7.0
CGS 15943A	8.1	9.2

Given the complexity of the response to purinergic agonists seen in smooth muscle preparations, great care is required in the choice of experimental conditions for studies which assess antagonist affinity. In our studies we have used adenosine as the agonist in the presence of dipyridamole and EHNA in order to restrict the purines action to cell surface adenosine receptors. Using these experimental conditions, the apparent affinity of a range of standard antagonists is in good agreement with pKi values derived from tritiated NECA binding studies using rat PC_{12} cell membranes (Table 4).

The antagonist with the highest affinity in both systems was CGS 15943A although it must be noted that a non-linear Schild plot was obtained[16]. The similar order of pA_2 and pKi values for these antagonists supports the concept that the aortic receptor is of the A_2 sub-type. (It should be noted that there is no evidence for a species difference in affinity of A_2 receptors from rat and guinea-pig tissue[17]).

One anomaly in this series of antagonists is PD115199 which has a significantly higher affinity in the PC_{12} cell than in the aorta. This result suggests that the aortic receptor may not be of the conventional A_{2a} type. The A_{2a} selective agonist 2-phenylamino adenosine is only

weakly effective in the aorta. These results suggest that the aortic A_2 receptor may be similar to the A_{2b} site identified in brain cells, but further studies are required to confirm or refute this suggestion.

Conclusions

The adenosine receptor in the guinea-pig atrium which mediates decreases in the rate and force of contraction appears to be similar to the A_1 receptor identified in CNS tissue from this species. The receptor responsible for mediating the major component of the relaxant response of smooth muscle preparations is similar in many respects to the A_2 adenosine receptor identified in other systems. There is also evidence for a small population of A_1 adenosine receptors which mediate contraction. There are, however, additional non-receptor sites in smooth muscle at which adenosine and its analogues can mediate relaxation. Although these additional sites of action are unlikely to be of physiological significance, their presence can complicate assessment of the affinity of novel A_2 receptor ligands in smooth muscle preparations.

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