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Evidence for an A_1-adenosine receptor in the guinea-pig atrium

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1 The purpose of this study was to determine whether the adenosine receptor that mediates a decrease in the force of contraction of the guinea-pig atrium is of the A_1- or A_2-sub-type.

2 Concentration-response curves to adenosine and a number of 5′- and N^6-substituted analogues were constructed and the order of potency of the purines was: 5′-N-cyclopropylcarboxamide adenosine (NCPCA) = 5′-N-ethylcarboxamide adenosine (NECA) > N^6cyclohexyladenosine (CHA) > L-N^6-phenylisopropyl adenosine (L-PIA) = 2-chloroadenosine > adenosine > D-N^6-phenylisopropyl adenosine (D-PIA).

3 The difference in potency between the stereoisomers D- and L-PIA was over 100 fold.

4 The adenosine transport inhibitor, dipyridamole, potentiated submaximal responses to adenosine but had no significant effect on those evoked by the other purines.

5 Theophylline antagonized responses evoked by all purines, and with D-PIA revealed a positive inotropic effect that was abolished by atenolol.

6 The results indicate the existence of an adenosine A_1-receptor in the guinea-pig atrium.

Introduction

Purine nucleosides and nucleotides exert negative inotropic and chronotropic effects on the mammalian heart (Burnstock, 1980). In the guinea-pig atrium, adenosine is a more potent negative inotropic agent than adenosine 5′-triphosphate (ATP), and theophylline is a competitive antagonist of the effects of both these agonists (Collis & Pettinger, 1982). The cell surface receptor mediating the negative inotropic response can therefore be classified as a P_1-purine receptor (Burnstock, 1978).

P_1-receptors have recently been divided into two types (A_1, A_2) on the basis of their effects on adenylate cyclase and by ligand binding studies (Van Calker, Muller & Hamprecht, 1979; Bruns, Daly & Snyder, 1980). Activation of the high affinity A_1-receptor inhibits adenylate cyclase, whereas the enzyme is stimulated by activation of the low affinity A_2-receptor (Van Calker *et al.*, 1979). Both receptor sub-types are antagonized by methylxanthines, but they can be distinguished by the relative potencies of certain adenosine analogues. The A_1-receptor exhibits marked stereospecificity, L-N^6-phenylisopropyladenosine (L-PIA) being 50 to 100 times more potent than its D-stereoisomer (Bruns *et al.*, 1980; Paton, 1981). The difference in potency between L-PIA and D-PIA at the A_2-receptor is at most 5 fold (Bruns *et al.*, 1980; Brown & Collis, 1982). Adenosine analogues substituted in the 5′ position, such as 5′-N-cyclopropylcarboxamide adenosine (NCPCA) and 5′-N-ethylcarboxamide adenosine (NECA), are about 50 times more potent than the N^6 substituted analogues, L-PIA and N^6-cyclohexyladenosine (CHA) at the A_2-receptor. However, there is little difference in potency between these compounds at the A_1-site (Bruns *et al.*, 1980; Paton, 1981).

In additon to these two sub-classes of cell surface adenosine receptor, an intracellular P-site at which adenosine, 2-chloroadenosine and certain ribose modified analogues (e.g. 9-β-D-xylofuranosyl adenosine) may interact has also been identified (Londos & Wolff, 1977). The 5′ and N^6 substituted analogues NECA, NCPCA, L-PIA, D-PIA and CHA are not agonists at this site (Daly, 1982). The P-site is insensitive to methylxanthines but its activation can be attenuated by purine transport inhibitors which reduce the access of exogenous purines into the cell (Brown & Collis, 1982).

The purpose of the present study was to investigate the nature of the adenosine receptor in the guinea-pig atrium and to determine to which sub-class it belongs.

Methods

Guinea-pigs of either sex (300–500 g) were killed and their hearts removed and placed in Krebs solution (37°C) (composition mM: NaCl 118.3, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25, Na-E.D.T.A. 0.26, glucose 5.5, bubbled with 95% O_2 and 5% CO_2). Left atria were mounted on punc-

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Table 1 The effect of theophylline (100 μM) on the response of the guinea-pig atrium to adenosine, its analogues and acetylcholine

	Control				*Theophylline (100 μM)*				
Agonist	*pD_2*	*Slope*	*Max. response***	n	*pD_2*	*Slope*	*Max. response (%)*	n	*log dose–ratio†*
Adenosine	5.40 ± 0.13	41 ± 4	97.6 ± 2.0	7	–	–	–		–
Adenosine (10 μM Dipyridamole)	6.95 ± 0.19	36 ± 4	102 ± 2.2	6	5.72 ± 0.10*	46 ± 3	98.2 ± 2.1	7	1.23 ± 0.13
2-Chloro-adenosine	6.52 ± 0.06	39 ± 2	100 (82.6 ± 1.8)δ	7	5.48 ± 0.07*	35 ± 3	100 (84.6 ± 1.6)δ	7	1.04 ± 0.13
NECA	7.23 ± 0.08	48 ± 4	102 ± 2.3	8	6.3 ± 0.06*	54 ± 3	101.5 ± 1.6	8	0.93 ± 0.12
NCPCA	7.28 ± 0.09	47 ± 3	101 ± 2.1	6	6.25 ± 0.14*	45 ± 4	98.3 ± 6.5	6	1.03 ± 0.14
L-PIA	6.62 ± 0.06	41 ± 3	95.1 ± 3.2	9	5.52 ± 0.10*	44 ± 5	96.8 ± 2.4	6	1.10 ± 0.13
D-PIA	4.46 ± 0.11	40 ± 4	‡	6	–	–	–	–	–
D-PIA (1 μM Atenolol)	4.67 ± 0.10	41 ± 2	‡	6	3.22 ± 0.12*	51 ± 11	1.9‡	6	1.45 ± 0.14
CHA	6.98 ± 0.09	39 ± 3	104.3 ± 3.0	6	5.80 ± 0.03*	46 ± 3	100.8 ± 4.9	6	1.18 ± 0.14
Acetylcholine	7.42 ± 0.08	52 ± 2	105.8 ± 3.7	5	7.42 ± 0.06	47 ± 5	96.8 ± 2.4	9	–

Values are expressed as mean ± s.e.mean of n determinations. For abbreviations, see text.
‡Maximum response not achieved due to poor solubility of D-PIA.
*Significantly different from control ($P < 0.001$).
†Standard error derived from pooled standard deviations from all groups.
δ% decrease in force of contraction.
**As % of 2-chloroadenosine (100 μM).

tate electrodes in 10 or 20 ml organ baths (37°C). The atria were attached to a force transducer (Pioden U.F.1) and changes in isometric tension recorded on a Devices MX2 polygraph. An initial tension of 1 g was applied to the tissues.

Atria were stimulated by square wave electrical pulses (4 Hz, 3 ms, 0.5–7 V) provided by a Grass S88 stimulator. Preliminary experiments demonstrated that the stimulus parameters used did not activate adrenergic or cholinergic nerves in the atrium as the force of contraction was not altered by β-adrenoceptor blockade (atenolol, 1 μM, $n = 4$) or by atropine (1 μM, $n = 6$).

An equilibration period of 45–60 min was allowed before experiments were started. Agonists were added cumulatively to the bath in volumes of 0.04–0.1 ml. A contact time of 1 min was allowed for each concentration; this was sufficient for the response to stabilize. All preparations were initially subjected to a maximally effective concentration (100 μM) of 2-chloroadenosine, and the response of subsequently applied drugs was related to that of 2-chloroadenosine on each preparation.

A 30 min contact time was allowed when tissues were exposed to dipyridamole, theophylline or atenolol. The effects of the synthetic agonists used required a prolonged period of washing for reversal. Consequently, control dose-response curves and those in the presence of dipyridamole or of theophylline were constructed in atria from different animals.

Drugs and compounds used

Acetylcholine chloride (Sigma), adenosine (Sigma), atenolol (Tenormin, I.C.I.), atropine (Sigma), 2-chloroadenosine (Sigma), dipyridamole (Persantin, Boehringer Ingelheim), L-N^6-phenylisopropyladenosine (Boehringer Mannheim) and theophylline (Sigma) were used. The following compounds were synthesized by J. Preston and R. Maisey, Pharmaceuticals Division, I.C.I., Alderley Park: N^6-cyclohexyladenosine, 5′-N-cyclopropylcarboxamide adenosine, 5′-N-ethylcarboxamide adenosine, D-N^6-phenylisopropyladenosine and 9-β-D-xylofuranosyl adenosine. All compounds were made up in aqueous solution.

Analysis of results

The negative inotropic response evoked by the agonists used in this study is expressed as a percentage of the maximal response evoked by 2-chloroadenosine (100 μM, response = 84.6 ± 0.8% decrease in force of contraction, $n = 57$). The slopes of the dose-response curves were calculated by regression analysis over the linear region (20–80%).

Data are expressed as mean ± s.e.mean. Significant differences between means ($P < 0.05$) were assessed by unpaired t tests and by two way analysis of variance.

Table 2 The effect of dipyridamole (10 μM) on the response of the guinea-pig atrium to adenosine, its analogues and acetylcholine

	Control			*Dipyridamole (100 μM)*		
Agonist	*pD_2*	*Max. response* **	n	*pD_2*	*Max response* **	n
Adenosine	5.40 ± 0.13	97.6 ± 2.0	7	6.95 ± 0.19*	102.1 ± 2.2	6
2-chloroadenosine	6.52 ± 0.06	100 (82.6 ± 1.8)$^\delta$	7	6.56 ± 0.07	100 (80.1 ± 2.9)$^\delta$	7
NECA	7.23 ± 0.08	102 ± 2.3	8	6.89 ± 0.16	95.1 ± 3.4	10
NCPCA	7.28 ± 0.09	101.0 ± 2.1	6	7.04 ± 0.08	101.0 ± 2.3	7
L-PIA	6.62 ± 0.06	95.1 ± 3.2	9	6.52 ± 0.24	84.2 ± 6.4	6
D-PIA	4.46 ± 0.11	†	6	3.96 ± 0.25	†	7
D-PIA (Atenolol 1 μM)	4.67 ± 0.10	†	6	4.33 ± 0.22	†	6
CHA	6.98 ± 0.09	104.3 ± 3.0	6	6.83 ± 0.15	102.6 ± 5.6	6
Acetylcholine	7.42 ± 0.08	105.8 ± 3.7	5	7.44 ± 0.16	102.7 ± 6.1	5

Values are expressed as mean ± s.e.mean of n determinations. For abbreviations see text.
†Maximal response not achieved due to poor solubility of D-PIA.
*Significantly different from control ($P < 0.001$).
$^\delta$% decrease in force of contraction.
**As % of 2-chloroadenosine (100 μM).

Results

Potency of agonists

Adenosine and its analogues decreased the force of contraction of the atrium in a concentration-dependent manner. The order of potency was NCPCA = NECA > CHA > L-PIA = 2-chloroadenosine > adenosine > D-PIA (Table 1). The slopes of the concentration-response curves and the maximal responses for these agonists were not significantly different; however, a maximal response to D-PIA could not be obtained due to the limited solubility of the compound. L-PIA was 144 times more potent than D-PIA. The P-site agonist, 9-β-D xylofuranosyl adenosine (1 mM) evoked a small direct decrease (17.3 ± 7.0%, $n = 4$) in the force of atrial contraction.

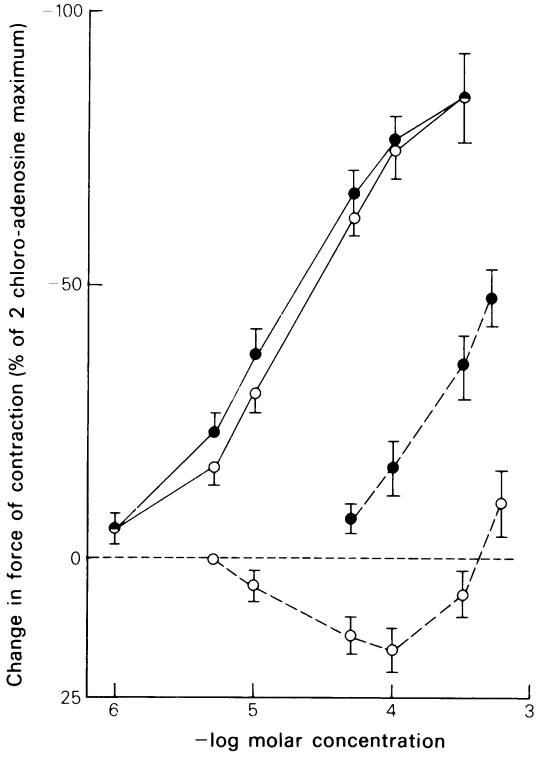

Figure 1 The effect of theophylline (100 μM) on responses of the guinea-pig atrium to D-N^6-phenylisopropyl adenosine (D-PIA) in presence and absence of atenolol (1 μM). D-PIA control (O——O); D-PIA plus atenolol (●——●); D-PIA plus theophylline (O--O); D-PIA plus theophylline plus atenolol (●--●).
Each point represents the mean of 6 experiments; vertical lines show s.e.mean.

Effect of dipyridamole

The purine transport inhibitor, dipyridamole (10 μM), caused a sustained reduction in the force of contraction of the atrium (−36.0 ± 5.2%, $P < 0.001$, $n = 8$). Submaximal responses evoked by adenosine were potentiated by dipyridamole, and this resulted in a significant increase in the pD_2 value for this agonist, equivalent to a 35 fold increase in sensitivity (Table 2). Dipyridamole had no significant effect on the pD_2 values of the other agonists used (Table 2).

Effect of theophylline

Submaximal responses evoked by L-PIA, CHA, NCPCA, NECA, 2-chloroadenosine and by adenosine (in presence of dipyridamole, 10 μM) were significantly attenuated by theophylline (100 μM). Responses evoked by acetylcholine were not altered (Table 1). The dose-response curves to adenosine and its analogues were shifted to the right in a parallel manner, with no change in maximal responses (Table 1).

Theophylline (100 μM) converted negative inotropic responses evoked by D-PIA (10–100 μM) into positive inotropic effects (Figure 1). These increases in the force of contraction were abolished by atenolol (1 μM, Figure 1). Atenolol (1 μM) had a small but non-significant effect on responses evoked by D-PIA in control conditions (Figure 1) but had no effect on responses evoked by the other purines in the presence of theophylline. There was no significant difference ($P = 0.16$, two way analysis of variance) between the sensitivity of adenosine and its analogues to the theophylline blockade (D-PIA evaluated in presence of atenolol).

Discussion

The cell surface adenosine receptor of the guinea-pig atrium appears to be of the A_1 sub-type. This conclusion is based on two lines of evidence. Firstly, the diastereo-isomers of PIA exhibited a large difference in potency as negative inotropes. A similar stereospecificity has been reported for A_1-receptors in brain tissue and at presynaptic sites in the rat vas deferens and guinea-pig ileum (Bruns *et al.*, 1980; Paton, 1981). By contrast, there is little difference in potency between L- and D-PIA at A_2-receptors (Bruns *et al.*, 1980; Brown & Collis, 1982). Secondly, there was little difference in potency between the 5′-substituted compounds (NECA, NCPCA) and the N^6 substituted analogues L-PIA and CHA. This observation is typical of drugs that interact with A_1-receptors; whereas at A_2-receptors, the 5′ substituted analogues are 20 to 50 times more potent than

CHA and L-PIA (Bruns, 1980; Bruns *et al.*, 1980; Brown & Collis, 1982).

The apparent order of potency of adenosine and its analogues could be influenced by tissue uptake and by deamination. In isolated tissues bathed in physiological saline solutions, transport into the tissue appears to be the major mechanism for the removal of extracellular purines. Inhibitors of adenosine transport such as dipyridamole potentiate responses evoked by adenosine in isolated tissues, whereas blockade of adenosine deaminase has little effect (Davies, Baird-Lambert & Jamieson, 1982). Since dipyridamole had no significant effect on responses evoked by the 5′ and N^6 substituted analogues, it is likely that their transport into the atrial myocyte is minimal. A similar conclusion was reached in a previous study of the guinea-pig trachea (Brown & Collis, 1982). However, an intracellular action of 2-chloroadenosine, possibly at the P-site, was revealed in the latter study since dipyridamole depressed the maximal response to this agonist and as responses were not significantly attenuated by theophylline. 2-Chloroadenosine does not appear to have an intracellular action in the atrium because dipyridamole had no effect on the maximal response and as theophylline blocked submaximal responses evoked by this agonist. The small negative inotropic response evoked by a high concentration of 9-β-D xylofuranosyl adenosine indicates that if the atrium does possess an intracellular P-site, activation of this site can only have a minor role in negative inotropic responses evoked by purines.

The order of agonist potency can be used to characterize receptor types if the agonists interact solely with the same receptor site. The similar magnitude and parallel displacement of concentration-response curves of the derivatives in the presence of theophylline indicate that all the purines interact with an adenosine (P_1)-receptor. Confirmation that the agonists act solely at an A_1-adenosine receptor must await the development of selective A_1-receptor antagonists.

Theophylline revealed a positive inotropic effect of D-PIA, which was susceptible to β-adrenoceptor blockade. D-PIA may therefore stimulate directly the β-adrenoceptor, or release endogenous catecholamines via a mechanism that is independent of adenosine receptors. The latter possibility appears more likely as contractile effects of D-PIA have also been observed in vascular preparations in which β-adrenoceptor stimulation would evoke relaxation (Brown & Collis, unpublished). D-PIA (D-N^6-phenylisopropyladenosine) has structural similarities to D-amphetamine (D-β-phenylisopropylamine). Consequently, the adenosine analogue may act in a similar manner to that of D-amphetamine causing the release of endogenous catecholamines and inhibiting neuronal uptake. There was no evidence for an interaction of the other purines with endogenous catecholamines as atenolol did not affect the responses evoked by them in the presence of theophylline.

Atenolol had a greater effect upon responses evoked by D-PIA in the presence than in the absence of theophylline. This paradox may be due to the functional antagonism that occurs between catecholamines and purine receptor agonists in the heart (Rockoff & Dobson, 1980). Thus, in control conditions, the inotropic effects of the catecholamines released by D-PIA would be antagonized by the simultaneous activation of adenosine receptors. Adenosine receptor blockade by theophylline would attenuate this functional antgonism between D-PIA and the released catecholamines. Consequently, the positive inotropic effects of the catecholamines would be enhanced in the presence of theophylline.

In conclusion, the guinea-pig atrium appears to possess an adenosine A_1-receptor that mediates a decrease in the force of contraction. Recent studies of the negative chronotropic effect of adenosine analogues in the rat atrium have also led to the identification of an A_1-receptor (Kurahashi & Paton, 1981). The A_1/A_2-adenosine receptor hypothesis was originally proposed on the basis of ligand binding and cyclic nucleotide studies. The present study, together with a previous paper on the guinea-pig trachea (Brown & Collis, 1982) demonstrate that A_1- and A_2-receptors can be identified in more intact systems by examining the relative potencies of agonists. It must be stressed, however, that the development of selective antagonists for these two putative receptors is necessary to confirm their separate existence. Such selective antagonists may also help to elucidate the role of adenosine in physiological and pathophysiological states.

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