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Inhibition by purines of the inotropic action of isoprenaline in rat atria

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1 The effects of a series of adenosine derivatives were examined on the catecholamine-stimulated electrically-driven rat left atrium *in vitro*.

2 All the purines tested reduced the positive inotropic action of isoprenaline, 0.1 μM, with the potency order: L-N^6-phenlylisopropyladenosine (L-PIA) > 5'-N-ethylcarboxamide adenosine (NECA) > D-PIA > 2-chloroadenosine > adenosine. Dipyridamole did not change the IC_{50} of adenosine. The adenosine deaminase inhibitor, 2'deoxycoformycin, produced a small but non-significant shift to the left of the adenosine concentration-response curve.

3 The cardiac depressant effects of these purines were reversed by theophylline and the IC_{50} values were unchanged in the presence of atropine or in atria taken from reserpine-treated rats.

4 It is concluded that the purine receptor mediating these effects should not be classified on the A_1/A_2 system. The relationship between functionally characterized purine receptors and those originally defined as modulating adenylate cyclase is discussed.

Introduction

Adenosine and related purines exert a number of potent effects on tissues of the body including the nervous system (Burnstock, 1981; Stone, 1981) kidney (Churchill, 1982), adipose tissue (Fredholm, 1978) the vascular system (Mustafa, 1980) and the heart. In the latter tissue adenosine has depressant actions, producing negative chronotropic and inotropic effects in various species (Drury & Szent-Györgyi, 1929; de Gubareff & Sleator, 1965; Schrader, Baumann & Gerlach, 1977; Szentmiklosi, Nemeth, Szegi, Papp & Szekeres, 1980; Brown, Burnstock, Cusack, Meghji & Moody, 1982; Evans, Schendon & Bristol, 1982; Collis, 1983), though it has recently been suggested that the mechanism of these effects may depend on the nature of the preparation. Thus the direct negative chronotropic action of adenosine on rat atria was not associated with any changes of cyclic adenosine 3'5'-monophosphate (AMP) levels, whereas the antagonism by adenosine of the positive inotropic effect of isoprenaline was associated with a reduction of isoprenaline-stimulated cyclic AMP production (Endoh, Maruyama & Taira, 1983).

The cardiac depressant actions of adenosine can be blocked by methylxanthines (Brown *et al.*, 1982; Collis, 1983) indicating that the receptor mediating these actions is of P_1 type in the classification proposed by Burnstock (1978). The P_1-receptor, however, appears to encompass two receptor populations, the A_1 (Van Calker, Muller & Hamprecht, 1979) or R_i site (Londos, Cooper & Wolff, 1980) at which purines cause an inhibition of adenylate cyclase and the A_2 or R_a site at which the cyclase is activated (Van Calker *et al.*, 1979; Londos *et al.*, 1980; Stone, 1981; 1982).

Recent attempts to determine which of these receptor populations is involved in the actions of adenosine on the heart have studied only the intrinsically generated tension of electrically driven guinea-pig atria (Collis, 1983). In view of the possibility that a different mechanism of action may be involved (Endoh *et al.*, 1983; see above) we have in the present study attempted to determine the type of purine receptor associated with the antagonism of the positive inotropic action of isoprenaline in the rat left atrium.

Methods

Rat isolated left atria were suspended in 25 ml organ baths, under 0.5 g tension and perfused with Krebs-Henseleit solution containing 1.25 mM calcium gassed with 95% O_2 and 5% CO_2 at 32°C. The solution was superfused continuously at a rate of 3 ml min^{-1}.

Electrical stimulation was delivered (1 Hz, 1 ms

duration, supra-maximal voltage) via parallel platinum electrodes. Tension was recorded isometrically on a Devices pen recorder.

After a 60 min equilibration period, control records of the inotropic response to isoprenaline 10^{-7} M were obtained; isoprenaline was administered directly into the organ bath in a volume of 0.1 ml. Adenosine, or one of its analogues was then added to the perfusing fluid for 40–60 min.

At the end of this time the response to isoprenaline was re-tested. Time-related alterations in the isoprenaline response were accounted for by using time matched controls and applying the correction factor technique of Hawthorn & Broadley (1982), to the results obtained from test preparations.

Where appropriate, reserpinized atria were obtained by treating rats with reserpine 10 mg kg^{-1} i.p. in a single dose 24 h before they were killed. The reserpine was suspended in one drop of Tween 80 and diluted with 0.9% saline to provide an injection volume of 1 ml kg^{-1}.

The antagonism of the depressant effects of purines by theophylline was studied by obtaining a constant (equilibrium) reduction of the isoprenaline responses using a perfused purine and then adding theophylline into the perfusing medium and determining the percentage increase of tension. For comparison the effects of theophylline were also examined on isoprenaline responses in the absence of purine agonists.

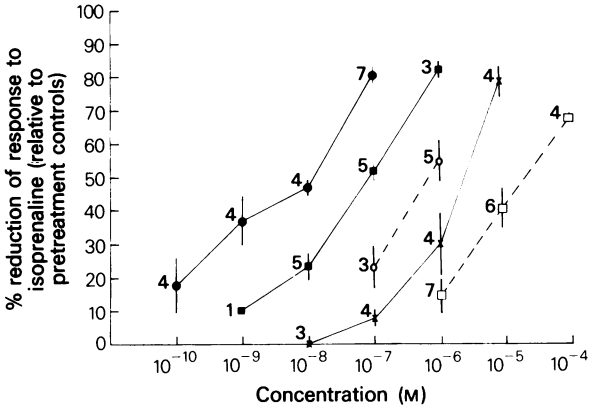

Figure 1 Concentration-response curves for the depression of inotropic responses to isoprenaline (0.1 μM) by L-N^6-phenylisopropyladenosine (L-PIA ●), 5′-N-ethylcarboxamide adenosine (NECA, ■), D-PIA (○), 2-chloroadenosine (X), adenosine (□). The points show the mean, and vertical bars the s.e.mean for the number of experiments indicated at each point.

Table 1 IC_{50} values for the inhibition of the positive inotropic effect of isoprenaline (10^{-7} M) by adenosine and derivatives

Compound	*IC_{50} (μM)*
L-PIA	0.019 ± 0.003 (4)*
NECA	0.081 ± 0.022 (3)
D-PIA	0.90 ± 0.29 (3)
2-Chloroadenosine	2.18 ± 0.031 (4)
Adenosine	27 ± 5.6 (4)

It was shown in separate experiments not included in Figure 1 that all the purines could produce complete inhibition of isoprenaline responses
* Mean ± s.e.mean (n)
L or D-PIA = L or D-N^6-phenylisopropyladenosine; NECA = 5′-N-ethylcarboxamide adenosine.

Materials

Adenosine hemisulphate, 2-chloroadenosine, theophylline, reserpine, tyramine HCl, atropine sulphate and (±)-isoprenaline HCl were purchased from Sigma Chemicals, and L-N^6-phenylisopropyladenosine (L-PIA) from Boehringer. The following were gifts of the manufacturers: 5′-N-ethylcarboxamide adenosine (Byk Gulden), D-PIA and dipyridamole (Boehringer), 2′-deoxycoformycin (Warner-Lambert).

Statistics

All statistical calculations of differences between points were performed using Student's t test.

Results

All the purines tested in this study depressed the contractile tension developed in response to isoprenaline, 10^{-7} M. The compounds included adenosine, 2-chloroadenosine, the D- and L-isomers of N^6-phenylisopropyladenosine (PIA), and 5′-N-ethylcarboxamide adenosine (NECA). Concentration-response curves for these agents are shown in Figure 1 and the IC_{50} values are summarised in Table 1. In particular it may be noted that L-PIA was approximately 56 times more potent than the D-isomer. (The interpolation of IC_{50} values from Figure 1 was validated in a separate series of tests showing that all the purines could cause complete inhibition of the responses to isoprenaline).

Perfusion with dipyridamole, 10 μM did not change the potency of any of the purines tested (including adenosine).

Table 2 Antagonism of the cardiac depressant effects of purines by theophylline

Purine	*Concentration*	*Effect of theophylline* (2×10^{-4} M) *Increase of tension with isoprenaline plus compound*	*Increase of tension with isoprenaline alone*
2-Chloroadenosine	2.4×10^{-6} M	96 ± 21.6 (4)*	18.5 ± 4.8 (4)
NECA	9×10^{-7} M	137 ± 41.6 (4)	15 ± 2.04 (4)
D-PIA	1.6×10^{-6} M	112 ± 34.7 (3)	12 ± 2.08 (3)
L-PIA	1.6×10^{-8} M	88 ± 28.3 (3)	11.3 ± 3.38 (3)
Control			
Carbachol	2×10^{-7} M	6.7 ± 4.03 (4)	

In order to take into account the positive inotropic effects of theophylline itself, the percentage increase by theophylline of the contractile tension in the presence of isoprenaline (10^{-7} M) and purine was compared with the effect on isoprenaline alone.

* Mean ± s.e.mean (n)

L-or D-PIA = L or D-N^6-phenylisopropyladenosine; NECA = 5'-N-ethylcarboxamide adenosine.

Effects of theophylline

Theophylline, 200 μM reversed the depressant effects of all the purines, producing an increase of developed tension of up to 250% of the purine depressed atrium, but only up to 31% increase of control isoprenaline stimulated tissue (Table 2). As an additional control it was shown that theophylline did not reverse the depressant effect of carbachol (0.2 μM) on the isoprenaline-stimulated atria (Table 2).

Atropine and reserpine

Atropine was not routinely included in the perfusion medium since there is evidence that purines may act on acetylcholine receptors (Ewald, 1976; Akasu, Hirai & Koketsu, 1981) and we did not wish to preclude the possibility that their cardiac depressant properties might be mediated through such sites. Several separate series of experiments were therefore carried out including atropine (1 μM) in the medium but the concentration-response curves and IC_{50} values obtained for purines were not significantly different from those shown (Figure 1 and Table 1).

The possibility was considered that part of the positive inotropic effect of isoprenaline might be mediated by the release of neuronal catecholamines, which in turn would be inhibited by purine acting on the nerve terminals (Hedqvist & Fredholm, 1979; Ribeiro, 1979; Stone, 1981). It was also considered that the antagonism of theophylline towards purine agonists might reflect, in part, a release of neuronal catecholamines (Berkowitz, Tarver & Spector, 1970; Waldeck, 1971). Six rats were therefore pretreated with reserpine, 10 mg kg^{-1} i.p. 24 h before they were killed. Atria from these animals showed no response to tyramine, 10 μM. Concentration-response curves were generated for L-PIA and NECA in these preparations but they showed no significant differences from non-reserpinized preparations.

Deoxycoformycin

Addition of the adenosine deaminase inhibitor 2-deoxycoformycin, 0.1 μM produced a slight shift to the left of the concentration-response curve for adenosine but not 2-chloroadenosine. The IC_{50} values for these compounds (18 ± 3.2 μM and 2.2 ± 0.14 μM; s.e.mean, $n = 4$) were not significantly different from control values (Table 1).

Discussion

In two respects the present results could be interpreted to indicate the existence of an A_1 receptor mediating the antagonism of isoprenaline stimulation of the heart. Firstly L-PIA was some 56 times more potent than D-PIA, a ratio characteristic of A_1 receptor activation (Smellie, Daly, Dunwiddie & Hoffer, 1979; Bruns, Daly & Snyder, 1980; Stone, 1981; 1982). There is much less stereospecificity in the activation of A_2 receptors (Smellie *et al.*, 1979; Bruns *et al.*, 1980).

Secondly there is only a five fold difference of potency between L-PIA and NECA, whereas differences an order of magnitude greater than this have been reported at A_2 receptors (Bruns, 1980; Bruns *et al.*, 1980).

However, the order of potency of all the compounds used in the present study was L-PIA > NECA > D-PIA > 2-chloroadenosine > adenosine whereas the order originally described by Londos *et al.* (1980) for the inhibition of adenylate cyclase by

the R_i receptor (considered equivalent to the A_1 receptor) was L-PIA > adenosine > NECA. The different position of adenosine in these schemes does not appear to reflect a difference in uptake or metabolism between the intact preparation used in the present experiments and the cultured or broken cell preparations of Van Calker *et al.* (1979) and Londos *et al.* (1980) as even 2-chloroadenosine proved to be weaker than L-PIA or NECA, and the responses to adenosine were not altered by dipyridamole or deoxycoformycin (Agarwal, 1982). The absence of any effect of dipyridamole is consistent with the report by Hopkins & Goldie (1971) that little adenosine uptake could be demonstrated in the rat heart, in contrast to guinea-pig. It cannot therefore be concluded that the receptor mediating the purine effects seen in the present experiments is identical to the R_i site of Londos *et al.* (1980) and the A_1 site of Van Calker *et al.* (1979).

In a study of the negative inotropic actions of purines on the guinea-pig atrium, Collis (1983) recently proposed the involvement of an A_1 receptor on the basis of the large difference of potency, over 100 fold, between the isomers of PIA, and the similar potencies of L-PIA and NECA. However, the order of potency NECA > L-PIA = 2-chloroadenosine > adenosine > D-PIA is very different from that used to define the inhibitory adenosine receptor (Londos *et al.*, 1980).

Similar problems have arisen in other studies where the pharmacology of purines has been examined in a functional system rather than a biochemical one. For example in the anococcygeus muscle Stone (1983) has reported the order PIA > NECA > 2-chloroadenosine > adenosine for the inhibition of neurally-evoked contractions. While this is not consistent with the involvement of an A_1 or A_2 receptor in this system it is the same order observed in the present study. In an investigation of the isolated atrium, trachea or ileum Brown *et al.* (1982) have also found that NECA was more potent that 2-chloroadenosine or adenosine in causing inhibition of contraction.

It may therefore be highly misleading to refer to purine receptors involved in a functional response as A_1 or A_2. The reason may be that the A_1/A_2 distinction only applies to the modulation of adenylate cyclase activity for which the nomenclature was devised. It seems clear that changes in the activity of adenylate cyclase are not necessarily involved in the effects of purines on the heart. Endoh *et al.* (1983) have concluded that whereas in the catecholamine-stimulated rat heart the depressant effect of adenosine is associated with a fall of cyclase activity (Schrader, *et al.*, 1977), the negative chronotropic action of adenosine is not associated with changes of cyclase function. In the guinea-pig heart Schutz & Tuisl (1981) have reported that although adenosine will reduce the cyclase activation produced by catecholamines neither L-PIA nor NECA even at 10 and 50 μM respectively were able to alter the basal or catecholamine-stimulated cyclase activity. Similarly adenylate cyclase is probably not involved in the inhibition of transmitter release (Kuroda, 1978; Reddington & Schubert, 1979; Smellie *et al.*, 1979; Dunwiddie & Hoffer, 1980).

On the other hand the action of purines both on the cardiac muscle cells and on nerve terminals probably involves an external receptor mediating a restriction of calcium influx (Schrader, Nees & Gerlach, 1977; Goto, Yatani & Tsuda, 1978; Ribeiro, 1979; Urthaler, Woods, James & Walker, 1981; Stone, 1981). One interpretation of these various results then may be that the purine receptors linked to calcium channels are pharmacologically distinct from those linked to adenylate cyclase. If this is indeed the case, it should prove possible to develop selective agonists and antagonists at the different sites.

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References

AGARWAL, R.P. (1982). Inhibitors of adenosine deaminase. *Pharmac. Ther.*, **17**, 399–429.

AKASU, T., HIRAI, K. & KOKETSU, K. (1981). Increase of acetylcholine receptor sensitivity by ATP. *Br. J. Pharmac.*, **74**, 505–507.

BERKOWITZ, B.A., TARVER, J.H. & SPECTOR, S. (1970). Release of norepinephrine in the CNS by theophylline and caffeine. *Eur. J. Pharmac.*, **10**, 64–71.

BROWN, C., BURNSTOCK, G., CUSACK, N.J., MEGHJI, P. & MOODY, C.J. (1982). Evidence for stereospecificity of the P_1-purinoceptor. *Br. J. Pharmac.*, **75**, 101–108.

BRUNS, R.F. (1980). Adenosine receptor activation in human fibroblasts: nucleoside agonists and antagonists. *Can. J. Physiol. Pharmac.*, **58**, 673–691.

BRUNS, R.F., DALY, J.W. & SNYDER, S.H. (1980). Adenosine receptors in brain membranes – binding of N^6-cyclohexyl-H^3-adenosine and 1, 3-diethyl-8-H^3-phenylxanthine. *Proc. natn. Acad. Sci. U.S.A.*, **77**, 5547–5551.

BURNSTOCK, G. (1978). A basis for distinguishing two types of purinergic receptors. In *Cell Membrane Receptors for Drugs and Hormones.* ed. Straub, R.W. & Bolis, L. pp. 107–118. New York: Raven Press.

BURNSTOCK, G. (1981). Neurotransmitters and trophic factors in the autonomic nervous system. *J. Physiol.*, **313**, 1–35.

CHURCHILL, P.C. (1982). Renal effect of 2-chloroadenosine and their antagonism by aminophylline

in anaesthetised rats. *J. Pharmac. exp. Ther.*, **222,** 319–323.

COLLIS, M.G. (1983). Evidence for an A_1 adenosine receptor in the guinea-pig atrium. *Br. J. Pharmac.*, **78,** 207–212.

DE GUBAREFF, T. & SLEATOR, W. (1965). Effects of caffeine on mammalian atrial muscle and its interaction with adenosine and calcium. *J. Pharmac. exp. Ther.*, **148,** 202–214.

DRURY, A.N. & SZENT-GYÖRGYI, A. (1929). The physiological activity of adenine compounds with special reference to their action upon the mammalian heart. *J. Physiol.*, **68,** 213–237.

DUNWIDDIE, T.V. & HOFFER, B.J. (1980). Adenine nucleotides and synaptic transmission in the *in vitro* hippocampus. *Br. J. Pharmac.*, **69,** 59–68.

ENDOH, M., MARUYAMA, M. & TAIRA, N. (1983). Adenosine-induced changes in rate of beating and cyclic nucleotide levels in rat atria: modification by islet-activating protein. In *Physiology and Pharmacology of Adenosine Derivatives.* ed. Daley, J.W., Kuroda, Y., Phillis, J.W., Shimizu H. & Ui, M., pp. 127–142. New York: Raven Press.

EVANS, D.B., SCHENDON, J.A. & BRISTOL, J.A. (1982). Adenosine receptors mediating cardiac depression. *Life Sci.*, **31,** 2425–2432.

EWALD, D.A. (1976). Potentiation of postjunctional cholinergic sensitivity of rat diaphragm muscle by high energy phosphate adenine nucleotides. *J. memb. biol.*, **29,** 47–65.

FREDHOLM, B.B. (1978). Local regulation of lipolysis in adipose tissue by fatty acids, prostaglandins and adenosine. *Med. Biol.*, **56,** 249–261.

GOTO, M., YATANI, A. & TSUDA, Y. (1978). Stabilizing effects of adenosine on the membrane currents and tension components of the bullfrog atrium. *Jap. J. Physiol.*, **28,** 611–625.

HAWTHORN, M.H. & BROADLEY, K.J. (1982). Evidence from use of neuronal uptake inhibition that β_1 adrenoceptors but not β_2 adrenoceptors are innervated. *J. Pharm. Pharmac.*, **34,** 664–666.

HEDQVIST, P. & FREDHOLM, B.B. (1979). Inhibitory effects of adenosine on adrenergic neuroreffector transmission in the rabbit heart. *Acta physiol. scand.*, **105,** 120–122.

HOPKINS, S.V. & GOLDIE, R.G. (1971). A species difference in the uptake of adenosine by heart. *Biochem. Pharmac.*, **20,** 3359–3365.

KURODA, Y. (1978). Physiological roles of adenosine derivatives which are released during neurotransmission in mammalian brain. *J. Physiol. (Paris)*, **74,** 436–470.

LONDOS, C., COOPER, D.M.F. & WOLFF, J. (1980). Subclasses of external adenosine receptors. *Proc. natn. Acad. Sci. U.S.A.*, **77,** 2551–2554.

MUSTAFA, S.J. (1980). Cellular and molecular mechanisms of coronary flow regulation by adenosine. *Mol. Cell. Biochem.*, **31,** 67–87.

REDDINGTON, M. & SCHUBERT, P. (1979). Parallel investigations of the effects of adenosine on evoked potentials and cyclic AMP accumulation in hippocampal slices of the rat. *Neurosci. Lett.*, **14,** 37–42.

RIBEIRO, J.A. (1979). Purinergic modulation of transmitter release. *J. theor. Biol.*, **80,** 259–270.

SCHRADER, J., BAUMANN, G. & GERLACH, E. (1977). Adenosine as inhibitor of myocardial effects of catecholamines. *Pflüg. Arch. ges. Physiol.*, **372,** 29–35.

SCHRADER, J., NEES, S. & GERLACH, E. (1977). Evidence for a cell surface adenosine receptor on coronary myocytes and atrial muscle cells. *Pflüg. Arch. ges Physiol.*, **69,** 251–257.

SCHUTZ, W. & TUISL, E. (1981). Evidence against adenylate cyclase coupled adenosine receptors in the guinea-pig heart. *Eur. J. Pharmac.*, **76,** 285–288.

SMELLIE, F.W., DALY, J.W., DUNWIDDIE, T.V. & HOFFER, B.J. (1979). The dextro and levorotatory isomers of N-phenylisopropyladenosine: Stereospecific effects on cyclic AMP formation and evoked synaptic responses in brain slices. *Life Sci.*, **25,** 1739–1748.

STONE, T.W. (1981). Physiological roles for adenosine and ATP in the nervous system. *Neuroscience*, **6,** 523–555.

STONE, T.W. (1982). Cell membrane receptor for purines, *Biosci. Rep.*, **2,** 77–90.

STONE, T.W. (1983). Purine receptors in the rat anococcygeus muscle. *J. Physiol.*, **335,** 591–608.

SZENTMIKLOSI, A.J., NEMETH, M., SZEGI, J. PAPP, J.G. & SZEKERES, L. (1980). Effect of adenosine on sinoatrial and ventricular automaticity of the guinea-pig. *Arch. Pharmac.*, **311,** 147–150.

URTHALER, F., WOODS, W.T., JAMES, T.N. & WALKER, A.A. (1981). Effects of adenosine on mechanical performance and electrical activity, in the canine heart. *J. Pharmac. exp. Ther.*, **216,** 254–260.

VAN CALKER, D., MULLER, M. & HAMPRECHT, B. (1979). Adenosine regulates, via two different types of receptors, the accumulation of cyclic AMP in cultured brain cells. *J. Neurochem.*, **33,** 999–1005.

WALDECK, B. (1971). Some effects of caffeine and aminophylline on the turnover of catecholamines in the brain. *J. Pharm. Pharmac.*, **23,** 824–8320.

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