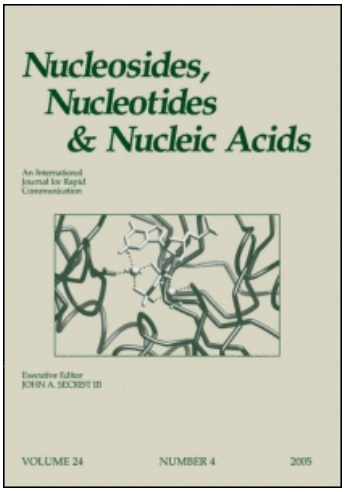

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NEGATIVE CHRONOTROPIC ACTION OF ADENOSINE IN RAT ATRIA: EVIDENCE FOR ACTION AT A_1 RECEPTORS

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ABSTRACT. Adenosine produces its negative chronotropic action in rat atria through activation of a P_1-purinoceptor of the A_1 sub-type.

INTRODUCTION

It has been known since 1929 that adenosine has actions on the heart involving the coronary vasculature, contractility and electrophysiological activity[1]. The cardiac actions of adenosine have been recently reviewed by Burnstock[2] and Lubbe[3]. Adenosine has negative chronotropic and inotropic actions on atrial muscle mediated through actions on discrete receptors. The classification of adenosine receptors has been reviewed recently by a number of workers [2,4,5].

In guinea-pig atria, adenosine has been shown to act on P_1-purinoceptors of the A_1 sub-type[6]. The aim of the present study was to examine the nature of the adenosine receptors responsible for the negative chronotropic action of adenosine in rat atria and to attempt to determine to which class they belong. A preliminary account of a portion of this work has been presented elsewhere[7].

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METHODS

Tissue Preparation

Male Wistar rats (250-350g) were killed by cervical dislocation and by exsanguination. The chest was opened, the heart rapidly removed and the atria then dissected from the remainder of the heart. The atria or the left atria alone were then mounted in a 5ml organ bath filled with a modified Krebs solution at 30°C.

Recording of rate or force of contractility

Atria were attached to a force-displacement transducer (Grass FT03C) and tachograph (Grass 7PIF), and isometric contractions and heart rate were recorded on a polygraph (Grass RPS7C8). Preparations were set up under 500mg tension for a 60 min equilibration period before starting the experiments. During this period, the medium was replaced several times and the tension readjusted to 500mg as required. After 60 min, atria were generally beating at a constant rate.

Adenosine and adenosine analogs were added to the bath cumulatively when the maximum inhibition of rate had been produced by the preceeding concentration of adenosine or analog. The % inhibition of the control rate of contraction produced 30% inhibition of the control rate of contraction (IC_{30}) was determined by linear regression using a Hewlett-Packard HP-85 computer for each separate preparation studied, and is expressed either as this concentration (with 95% confidence limits) or as the negative logarithm of this concentration.

Medium

The composition of the modified Krebs solution was (mM): NaCl, 116; KCl, 5.0; $CaCl_2$, 1.5; $MgCl_2$, 1.2; NaH_2PO_4, 1.2; $NaHCO_3$, 22.2; D-glucose, 11.2; Na_2EDTA, 0.04 in distilled, deionized water. The medium was maintained at 30°C, and equilibtrated with 95% O_2/5%CO.

Drugs

The drugs used in this study were obtained from the individuals or organizations shown, those indicated by an asterisk being generously donated: N-cyclopropyl-adenosine-5'-uronamide (NCPCA)* (Abbot Laboratories); L-adenosine* (Dr. N.J. Cusack); N^6-R-[1-phenyl-2-propyl]-

adenosine (R-PIA)* and N^6-S-[1-phenyl-2-propyl]adenosine (S-PIA)* (Dr. J.W. Daly; adenosine 5'-acetate*, N^6-(benzyl)adenosine*, cyclic-2',3'-thiocarbonate adenosine*, 2-fluoro-adenosine* and N^6-(2-hydroxylethyl)-adenosine* (National Cancer Institute) and eritadenine* (Dr. T. Trost). All other drugs and chemicals were obtained from Sigma Chemical Co.

Statistical Analysis of Data

Results were expressed as the mean ± standard error of the mean (S.E.M.) for changes in rate or force of contractions, and as geometric mean ± S.E.M. for IC_{30} values. The significance of differences was determined using Student's 't' test. Differences were considered significant when $p<0.05$ (two-tailed test).

RESULTS

Effect of adenosine on spontaneously beating atria and on atria accelerated by p-tyramine or (-)-noradrenaline

In spontaneously beating atria, the addition of adenosine (10^{-7} - 10^{-4}M) produced a concentration-dependent negative chronotropic action. At 10^{-4}M, adenosine caused arrhythmias or cardiac standstill in 3 of 9 preparations, and in the remaining preparations only reduced heart rate by 46%. Consequently, it was not possible to determine an IC_{50} value for adenosine in spontaneously beating atria. Instead the IC_{30} value for adenosine and analogs was determined in all subsequent studies, and was found to be 28.8μM for adenosine (Table 1).

Addition of p-tyramine (1-30μM) or of (-)-noradrenaline (0.01-1.0μM) increased the rate of contraction in a concentration-dependent manner (Table 1), the increased rate of contraction reaching a steady rate within 10 min and being maintained for a further 20 min. The addition of adenosine to such accelerated preparations during this period produced a concentration-dependent negative chronotropic response, and the IC_{30} for adenosine was determined by cumulative addition. It was found that the negative chronotropic action of adenosine was significantly increased in preparations whose rate had been accelerated by p-tyramine (1-10μM) or (-)-noradrenaline (0.03-1.0μM), the maximal increase in potency of

TABLE 1

Negative chronotropic action of adenosine in spontaneously beating rat atria. IC_{30} values were calculated from cumulative dose-response curves. The number of observations is shown in parentheses. *, $p<0.05$, compared to spontaneous preparation.

Experimental situation (M)	Heart rate before addition of adenosine (beats/min)	Adenosine, IC_{30} value (M) Mean (95% confidence limits)
Spontaneous	125 ± 5 (12)	$2.88\ (2.10\text{-}3.92)\times10^{-5}$
Presence of p-tyramine		
(1μM)	153 ± 7 (7)*	$1.09\ (0.85\text{-}1.39)\times10^{-5}$*
(3.8μM)	228 ± 9 (9)*	$2.86\ (2.29\text{-}3.56)\times10^{-6}$*
(10μM)	278 ± 9 (6)*	$1.39\ (1.21\text{-}1.61)\times10^{-5}$*
(30μM)	281 ± 6 (8)*	$3.11\ (2.75\text{-}3.52)\times10^{-5}$
Presence of (-)-noradrenaline		
(0.01μM)	146 ± 4 (8)*	$1.70\ (0.84\text{-}3.42)\times10^{-5}$
(0.03μM)	174 ± 7(13)*	$4.54\ (2.75\text{-}7.48)\times10^{-6}$*
(0.1 μM)	198 ± 6(12)*	$2.81\ (1.99\text{-}3.97)\times10^{-6}$*
(1.0 μM)	256 ± 6 (6)*	$5.84\ (5.32\text{-}6.43)\times10^{-6}$*

adenosine being about ten-fold in tissues continuously exposed to 3.8μM p-tyramine or 0.1μM (-)-noradrenaline (Table 1).

Effect of theophylline on action of adenosine

The addition of 10μM theophylline did not alter the rate of contraction in spontaneous preparations or in preparation accelerated by 3.8μM p-tyramine. However addition of 40μM and 100μM theophylline increased the rates of contraction of both spontaneous and accelerated preparations.

In subsequent studies, atria were exposed to 10μM theophylline prior to the determination of the IC_{30} concentration for adenosine. In atria

with an increased rate of contraction due to exposure to 3.8μM p-tyramine, 10μM theophylline significantly reduced the negative chronotropic action of adenosine being 1.0 (0.8-1.3) x 10^{-5}M and 2.9 (2.3-3.6) x 10^{-6}M in the presence and absence of theophylline respectively. In spontaneously beating preparation, 10μM theophylline did not significantly alter the negative chronotropic action of adenosine.

Structure-activity relations for the negative chronotropic action of adenosine in spontaneously beating atria and in atria accelerated by 3.8μM p-tyramine.

Structure-activity relations for the negative chronotropic actions of adenosine were examined using 24 adenosine analogs in both spontaneously beating preparations and in atria accelerated by the addition of 3.8μM p-tyramine (Tables 2 and 3).

Analogs with substitutions at the C2 of the purine moiety were considerably more potent than adenosine. An amino group at C6 of the purine moiety was essential for activity since analogs without such a group were inactive (e.g. 6-mercaptopurine riboside, 6-methoxypurine riboside, purine riboside, inosine), while disubstitution at the N^6 position (e.g. N^6-dimethyladenosine) resulted in a considerable loss of activity. The N^6 substituted compound R-PIA was an extremely potent analog, being well over 900-fold more potent than adenosine. The diastereomer, S-PIA was about fifty-fold less potent than R-PIA.

Substitutions at the 2' and/or 3' positions on the riboside moiety generally resulted in a reduction in or a loss of activity. By contrast, the N-cyclopropyl adenosine-5'-uronamide (NCPCA) was an extremely potent analog, being over 350-fold more potent than adensoine. L-adenosine was inactive.

DISCUSSION

The cellular electrophysiological actions of adenosine on cardiac tissue have been recently reviewed[8]. In the mammalian atrium, adenosine suppresses the inward calcium content and increases the outward potassium current. These result in a shortened action potential and hyperpolarization of the resting membrane potential.

The negative chronotropic action of adenosine was significantly greater in atria accelerated by either (-)-noradrenaline or p-tyramine than in spontaneously beating preparations. Similarly in guinea-pig atria veratramine had a greater negative chronotropic action in atria

TABLE 2

Structure-activity relations for negative chronotropic actions of spontaneously beating rat atria and in atria accelerated by 3.8μM p-tyramine. Mean ± S.E.M. of not less than 5 preparations. NA, analog failed to produce 30% inhibition rate at 100μM.

Analogue	-log IC_{30} value (M) Spontaneous	Tyramine Accelerated
Adenosine	4.46 ± 0.14	5.52 ± 0.09
Purine modified		
2-chloroadenosine	6.67 ± 0.24	7.32 ± 0.15
2-fluoroadensoine	6.46 ± 0.23	7.12 ± 0.24
N^6-(methyl)adenosine	4.75 ± 0.14	5.78 ± 0.12
N^6-(dimethyl)adenosine	NA	4.48 ± 0.27
N^6-(benzyl)adenosine	5.01 ± 0.22	6.05 ± 0.09
N^6-(2-hydroxyethyl)adenosine	6.71 ± 0.15	7.77 ± 0.11
N^6-R-PIA	8.11 ± 0.16	8.47 ± 0.66
N^6-S-PIA	6.45 ± 0.07	6.77 ± 0.19
Riboside modified		
2'-deoxyadenosine	NA	4.98 ± 0.24
2'-O-methyladenosine	NA	4.89 ± 0.06
3'-deoxyadenosine	4.87 ± 0.25	5.96 ± 0.23
cyclic-2',3'-thiocarbonate adenosine	NA	5.43 ± 0.09
adenosine 5'-acetate	5.10 ± 0.21	5.66 ± 0.12
NCPCA	7.58 ± 0.07	8.09 ± 0.03

accelerated by noradrenaline or histamine than in spontaneously beating atria[9]. These workers concluded that there was a physiological antagonism between veratramine and cardio-accelerator agents rather than a pharmacological antagonism. Adenosine also inhibited the cardio-accelerator action of histamine in guinea-pig atria[1].

In the present study, the negative chronotropic action of adenosine in rat atria was inhibited by theophylline, while for activity an amino group at position 6 on the purine moiety and hydroxyl groups at the 2'

TABLE 3

Adenosine analogs that failed to produce a negative chronotropic action at 100μM in spontaneously beating atria or in atria accelerated by 3.8μM p-tyramine.

Inactive analogs
Purine modified
6-mercaptopurine riboside
6-methoxypurine riboside
8-bromoadenosine
purine riboside
adenine
inosine
Riboside modified
L-adenosine
2',3'-isopropylideneadenosine
2',3'-diacetyladenosine
eritadenine

and 3' positions on the riboside moiety were required. These characteristics indicate that the adenosine receptor in rat atria, responsible for the reduction in heart rate, can be classified as a P_1 purinoceptor, as suggested previously[11], and that it possesses R site characteristics as proposed by Samet and Rutledge[12].

The question arises as to whether the receptors can be further classified as A_1 or A_2 in type. It is appreciated that not all investigators agree on how, or indeed whether, adenosine receptors should be classified as A_1 or A_2 on the basis of pharmacological studies only[5]. In general, such attempts have utilised the rank order of potency of N^6 substituted analogs such as R- and S-PIA and N^6-cyclohexyladenosine, the 2-substituted analog 2-chloroadenosine, and the adenosine-5'-uronamides

N-ethyladenosine-5'-uronamide (NECA) and NCPCA[13]. In the present study, the rank order of potency found was: R-PIA>NCPCA>2-chloroadenosine>S-PIA>adenosine, while R-PIA was more than 50-fold more potent then S-PIA. These features suggest that the receptors in rat atria responsible for the negative chronotropic response to adenosine may be classified as A_1 in type. The marked potency of the adenosine-5'-uronamides at putative A_1 receptors has also been observed in other tissues [6,13,14].

The adenosine receptors responsible for the negative chronotropic and inotropic actions of adenosine in guinea-pig atria have also been reported to belong to the A_1 and sub-type[6,15], as have those responsible for the negative inotropic action of adenosine in rat atria[16,17].

In isolated cardiac preparations, adenosine inhibits both the positive chronotropic and inotropic responses to catecholamines[18,20]. This effect has been reported by some workers to result predominantly or entirely from the inhibition of adenylate cyclase activity by adenosine[19,20], whereas others have concluded that this action is not related to changes in tissue content of either cyclic AMP or cyclic GMP[18]. Islet-activating protein isolated from Bordetella pertussis toxin attenuated the negative chronotropic action of adenosine in spontaneously beating rat atria and in atria accelerated by isoprenaline[21]. This protein has been found to block the effect of hormones and drugs that inhibit adenylate cyclase, apparently as a result of ADP ribosylation[22].

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