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Characterization of adenosine receptors in guinea-pig isolated left atria

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1 The effects of purinergic stimulation on action potential, force of contraction, ^{86}Rb efflux and ^{45}Ca uptake were investigated in guinea-pig left atria.

2 Adenosine exerted a negative inotropic effect which was antagonized by adenosine deaminase but enhanced by dipyridamole.

3 The negative inotropic effect of adenosine was mimicked by 5′-(N-ethyl)-carboxamido-adenosine (NECA) and the isomers of N^6-(phenyl-isopropyl)-adenosine, **R**-PIA and **S**-PIA. NECA and **R**-PIA were about 1000 times more potent than adenosine, whereas **R**-PIA was about 100 times more potent than **S**-PIA.

4 The inotropic effects of adenosine (in the presence of dipyridamole), NECA, **R**-PIA and **S**-PIA were competitively antagonized either by theophylline (pA_2 about 4.5) or 8-phenyltheophylline (pA_2 about 6.3).

5 NECA and **R**-PIA shortened the action potential duration and increased the rate constant of the efflux of ^{86}Rb in a concentration-dependent manner with no differences in potency; the effects were competitively antagonized by 8-phenyltheophylline.

6 Barium ions reduced the efflux of ^{86}Rb under control conditions and antagonized the increase induced by NECA and **R**-PIA.

7 NECA and **R**-PIA significantly reduced ^{45}Ca uptake in beating preparations.

8 It is concluded that adenosine, NECA and **R**-PIA activate a common receptor population (P_1 or A_3) on the outside of the cell membrane of atrial heart muscle to increase the potassium conductance and to reduce the action potential and, thereby, calcium influx and force of contraction.

Introduction

The negative inotropic effect of adenosine in atrial heart muscle is accompanied by a decrease in the action potential duration (Johnson & McKinnon, 1956; Hollander & Webb, 1957; de Gubareff & Sleator, 1965). This effect of adenosine has been ascribed, mainly, to an increase in the potassium conductance of the myocardial cell membrane (Belardinelli & Isenberg, 1983; Jochem & Nawrath, 1983; Hutter & Rankin, 1984; Kurachi *et al.*, 1986). Characteristically, the myocardial effects of adenosine in multicellular atrial preparations are observed only at very high concentrations (in the millimolar range). This has raised the question as to whether or not the effects of adenosine are due to the stimulation of specific receptors or represent non-specific membrane effects.

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Two functionally and pharmacologically distinct adenosine receptors have been identified in the central nervous system, both being associated with membrane bound adenylate cyclase. One type of receptor mediates stimulation of activity; the other type mediates inhibition. Both types of receptor are located at the outer site of the membrane, seem to require an intact ribose moiety and have been termed A_1 and A_2 (van Calker *et al.*, 1979) or R_i and R_a (Londos *et al.*, 1980). In addition to these receptors, adenylate cyclases contain another site for adenosine action, termed the P-site, which mediates inhibition of activity (Londos & Wolff, 1977).

Burnstock & Meghji (1981) ascribed the effects of adenosine in guinea-pig atria to the stimulation of P_1-receptors. The effects of adenosine at P_1-receptors are blocked competitively by methylxanthines. Methylxanthines have also been shown to

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block the effects at the external R-site receptors but not those on the internal P-site (Londos & Wolff, 1977; Londos *et al.*, 1978; Schwabe, 1981; Daly, 1982).

Whereas the adenosine derivative (−)-N^6-(**R**-phenyl-isopropyl)-adenosine (**R**-PIA) has been shown to be more potent at A_1-receptors, 5′-(N-ethyl)-carboxamido-adenosine (NECA) is thought to be more potent at A_2-receptors (Londos *et al.*, 1980; Hüttemann *et al.*, 1984; Ukena *et al.*, 1984; 1987). Collis (1983) and Kurahashi & Paton (1986) interpreted the effects of adenosine in guinea-pig and rat atria, respectively, as mediated by A_1-receptors. Recently, Martens *et al.* (1987) described the existence of A_1-receptors in rat isolated ventricular myocytes. In radioligand binding studies, selective antagonists for A_1-receptors have been described (Lohse *et al.*, 1987; Stiles & Jacobson, 1987). Nevertheless, the distinction of A_1- and A_2-receptors in cardiac cells remains unclear because of the lack of, firstly, differences in the potency of **R**-PIA and NECA (Brückner *et al.*, 1985) and, secondly, the existence of selective antagonists in physiological experiments (Collis *et al.*, 1985; 1987).

In the present study, the functional effects of adenosine, **R**-PIA and NECA were studied quantitatively, in the absence and presence of inhibitors, in guinea-pig left atria. In addition to the effects on the force of contraction, the effects on action potential duration, ^{86}Rb efflux and ^{45}Ca uptake were studied. Preliminary accounts of this work have been published (Nawrath, 1986; Jahnel & Nawrath, 1986).

Methods

Preparations

Guinea-pigs of weight 250–400 g were killed by a blow to the head and bled from the carotid arteries. The hearts were quickly removed and transferred to a dissection chamber containing oxygenated warm Tyrode solution. Whole hearts were pinned down on Sylgard so that the left atria could be cut off. For flux studies and for the measurement of the force of contraction, the whole left atrial appendage was used. For electrophysiological recordings, left atria were opened and fine trabeculae were prepared by ligating both ends with a silk suture.

Solutions

Tyrode solution was prepared from stock solutions in distilled deionized water, and was of the following composition (in $mmol\,l^{-1}$): NaCl 136.9, KCl 5.4, $MgCl_2$ 1.05, NaH_2PO_4 0.42, $NaHCO_3$ 11.9, $CaCl_2$ 1.8, glucose 5.6. The solution was equilibrated with 95% O_2 and 5% CO_2 at 37°C (pH 7.4). For removal of extracellular Ca^{2+}, physiological salt solution (PSS) was prepared according to Leijten & van Breemen (1984) (composition in $mmol\,l^{-1}$: NaCl 140, KCl 4.6, EGTA 2.0, $MgCl_2$ 1.0, glucose 10.0, HEPES 5.0). The pH was adjusted at 0°C to 7.4 by addition of 0.1 $mol\,l^{-1}$ NaOH and the solution was bubbled with O_2.

Measurement of the action potential and the force of contraction

For the measurement of the action potential, atrial trabeculae were mounted horizontally in a 2 ml organ bath which was built into a perspex block that also contained a main reservoir of 100 ml Tyrode solution. Communication between both compartments was provided by connecting pores through which the fluids were driven by gas (95% O_2 and 5% CO_2). The preparations were fixed in the organ bath to keep the muscle length as constant as possible. One end of the preparation was positioned between two platinum electrodes and the other end connected to an inductive force displacement transducer via a stainless steel wire.

The preparations were electrically driven at 1 Hz by rectangular pulses of 0.1–1 ms duration at 10% above threshold intensity using a Grass stimulator (model S4) and isolation unit. Force of contraction (F_c) was recorded at the apex of the preload active tension curve via the inductive force displacement transducer in conjunction with a Hellige frequency carrier preamplifier.

The preparations were allowed to stabilize for at least 30 min. The effects of drugs were investigated by exposure to either single or to cumulatively increasing concentrations, achieved by adding drugs to the main Tyrode reservoir, and increasing the concentration after the establishment of a stable response.

The transmembrane potential was detected intracellularly by the use of 10–20 MΩ glass microelectrodes filled with KCl 3 $mol\,l^{-1}$. The signals were led off by means of a voltage follower with input capacity compensation (built by H. Ehrler, Homburg, Saar). Both transmembrane potential and tension were displayed on a cathode ray oscilloscope (Tektronix 5103N) and recorded on magnetic tape (Lyrec FM tape recorder, bandwidth 0–10 kHz). During the course of an experiment, all parameters could be observed on a digital scope (Nicolet Explorer I). For quantitative evaluation, all data were stored, amplified and edited by means of a transient recorder (Physical Data, 512A). The analogue output of the transient recorder was fed to an XY – pen recorder (MFE 815) for amplification. All evaluations were done from records read off the pen recordings.

For the measurement of the F_c only, the left atria were attached to a stainless steel hook built in a perspex rod and positioned next to two platinum electrodes. The preparations were then placed with the muscle holder in organ baths containing 5 ml Tyrode solution and electrically driven at 3 Hz. The muscles were connected via stainless steel wires to the transducer, the output of which was recorded on a Hellige pen recorder.

Measurement of ^{86}Rb efflux

Guinea-pig whole left atria were first exposed to about 10 MBq ^{86}Rb (specific activity: 79.2 GBq g^{-1}) for 90 min in Tyrode solution and then transferred to the test baths. The release of ^{86}Rb into nonradioactive Tyrode solution was then measured (a) for 30 min under control conditions and (b) for 15 min in the presence of a test substance. All preparations were kept at rest since changes in ^{86}Rb efflux are much more complicated in beating preparations, due to voltage- and time-dependent changes in various potassium conductances during the action potential. The bath solution was changed every 5 min and collected in scintillation vials for later determination of radioactivity. At the end of the experiment, the tissues were blotted, weighed and solubilized by the addition of 1 ml TS-1 (Zinsser, Frankfurt) and incubation at 65°C for three hours. Six ml of Minisolve (Zinsser, Frankfurt) was added to each sample in the counting vials. Radioactivity was determined by liquid scintillation counting in a Tricarb 3380 (Packard Instruments, Frankfurt).

Measurement of ^{45}Ca influx

The preparations were equilibrated and exposed for 30 min to control or test solutions. Thereafter, tissues were exposed to corresponding solutions containing 150 kBq ^{45}Ca (specific activity: 1.295 TBq g^{-1}) for 5 min. The experiments were carried out at a driving frequency of 3 Hz. Tissues were then washed three times for 5 min in ice-cold PSS containing 2 mmol l^{-1} EGTA gassed with O_2, blotted and dried at 60°C for three hours. Finally, the tissues were treated as described for the experiments with ^{86}Rb, and the ^{45}Ca content of each tissue was measured by liquid scintillation counting in a Tricarb 3380 (Packard Instruments, Frankfurt).

Chemicals

The following drugs were used (sources in parentheses): adenosine; adenosine deaminase (ADA): (−)-N^6-(**R**-phenyl-isopropyl)-adenosine (**R**-PIA); (+)-N^6-(S-phenyl-isopropyl)-adenosine (S-PIA) (Boehringer, Mannheim); 5′-(N-ethyl)-carboxamido-adenosine (NECA) (Byk Gulden, Konstanz); dipyridamole (Thomae, Biberach an der Riss); theophylline (Merck, Darmstadt); 8-phenyltheophylline (8-PT) (Sigma, München); barium chloride (Merck, Darmstadt); ^{45}Ca chloride and ^{86}Rb chloride (NEN, Dreieich). Tissue solubilizer TS-1; scintillation cocktail Minisolve (Zinsser, Frankfurt). All other chemicals were obtained from Merck, Darmstadt.

Evaluation of results and statistical analyses

Results are either demonstrated as original figures or expressed as means ± s.e.mean. EC_{50} values were determined by regression analysis and the two points on the steep portion of each individual concentration-response curve were taken into account. Confidence limits were calculated according to Documenta Geigy (1968). pA_2 values were evaluated according to Arunlakshana & Schild (1959). Peak levels of phasic contractions were evaluated and given as % of control values. Action potential recordings were analysed for duration (APD) at 20% and 90% of repolarization, APD_{20} and APD_{90}, respectively.

Intracellular potassium is kinetically homogeneous (Langer & Brady, 1966) and given the fact that Rb in tracer amounts passes through potassium channels, albeit to a smaller extent than potassium itself (Hille, 1973; Henquin *et al.*, 1979; Clay & Shlesinger, 1983; Plant, 1986), a single rate constant λ of ^{86}Rb efflux could be determined according to $\lambda = (\ln A_0 - \ln A)/t$ derived from $A = A_0 e^{-\lambda t}$. When appropriate, statistically significant differences were assessed by Student's *t* test or by analysis of variance (repeated measurements design according to Wallenstein *et al.*, 1980) followed by modified *t* statistics according to Dunnett (1964). Significant differences are marked by asterisks ($P < 0.01$).

Results

Adenosine produced a negative inotropic effect in guinea-pig left atria. The original tracing in Figure 1 shows a rapid decline of F_c upon the addition of adenosine 10 μmol l^{-1}, followed by a partial recovery of F_c to a new steady-state value. In the presence of adenosine deaminase (ADA) 1 u μmol^{-1}, the recovery of F_c was complete, whereas in the presence of dipyridamole 10 μmol l^{-1} the maximal effect of adenosine was strongly enhanced and the recovery phenomenon virtually abolished. This concentration of dipyridamole has been shown to inhibit the uptake of adenosine by about 80% in guinea-pig left atria (Hopkins, 1973; Nawrath *et al.*, 1985). The EC_{50} of the effects of adenosine in the steady-state was 3.9×10^{-5} mol l^{-1} (Table 1). The concentration-

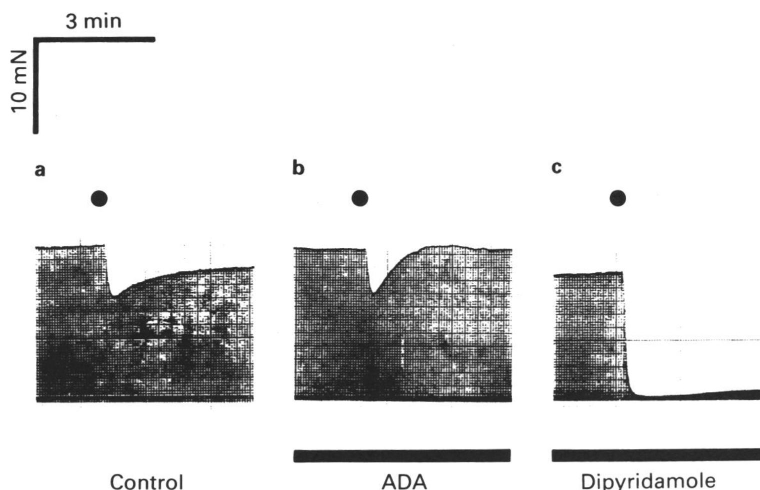

Figure 1 Effects of adenosine $10\,\mu mol\,l^{-1}$ (●) on force of contraction (F_c) in a guinea-pig left atrium (a) under control conditions, (b) in the presence of adenosine deaminase (ADA, $1\,u\,\mu mol^{-1}$ and (c) in the presence of dipyridamole $10\,\mu mol\,l^{-1}$. Original records from the same preparation which was exposed successively to all three conditions after wash periods of 15 min in drug-free Tyrode solution.

response relationships of adenosine were shifted to the left in the presence of dipyridamole $10\,\mu mol\,l^{-1}$ about 100 fold (not shown). Under these conditions, the EC_{50} of adenosine was reduced to $2.9 \times 10^{-7}\,mol\,l^{-1}$ (Table 1).

The fading of the effects of adenosine is probably related to the rapid uptake of adenosine and its deamination to inosine (Schrader *et al.*, 1972); inosine itself is completely ineffective (Nawrath *et al.*, 1985). It was therefore of interest to compare the effects of adenosine analogues which are not substrates for adenosine deaminase. NECA and **R**-PIA exerted negative inotropic effects at much lower concentrations. No fading of the effects of NECA and **R**-PIA as observed with adenosine, was seen (not shown). The EC_{50} of NECA was $3.8 \times 10^{-8}\,mol\,l^{-1}$ and the EC_{50}s of **R**-PIA and S-PIA were $3.3 \times 10^{-8}\,mol\,l^{-1}$ and $2.3 \times 10^{-6}\,mol\,l^{-1}$, respectively. The EC_{50} values, including 95% confidence limits, of all the agonists investigated are summarized in Table 1.

Table 1 Potencies of different adenosine agonists in guinea-pig left atria as measured by changes in force of contraction

	EC_{50} *(95% confidence limits)*	n
Adenosine	$3.9\ (3.3\text{–}4.8) \times 10^{-5}\,mol\,l^{-1}$	66
Adenosine + dipyridamole $10^{-5}\,mol\,l^{-1}$	$2.9\ (1.9\text{–}4.3) \times 10^{-7}\,mol\,l^{-1}$	16
R-PIA	$3.3\ (1.9\text{–}5.7) \times 10^{-8}\,mol\,l^{-1}$	12
S-PIA	$2.3\ (1.6\text{–}3.3) \times 10^{-6}\,mol\,l^{-1}$	8
NECA	$3.8\ (3.2\text{–}4.6) \times 10^{-8}\,mol\,l^{-1}$	12

We next investigated the effects of the adenosine agonists described so far in the presence of theophylline and/or 8-phenyltheophylline which have both been described as competitive antagonists for the effects of adenosine (Griffith *et al.*, 1981; Collis, 1983; Collis *et al.*, 1985). The evaluation of the interaction of adenosine agonists and antagonists on F_c in guinea-pig left atria was carried out according to Arunlakshana & Schild (1959). In our experiments, the interaction of adenosine and theophylline was described by a slope of 0.51 which is not compatible with a simple competitive interaction, since a 100 fold increase in the theophylline concentration produced only a 10 fold decrease in the inotropic response to adenosine.

It is possible that the removal of adenosine from the extracellular into the intracellular space may have strongly influenced the actual concentration of adenosine at the receptor sites. The experiments were therefore repeated in the presence of the uptake inhibitor dipyridamole $10\,\mu mol\,l^{-1}$. Under these conditions, the Schild plot yielded a slope of 1.04 and a pA_2 value of 4.48 was determined. The interaction of theophylline and **R**-PIA (without dipyridamole) was described by a slope of 1 and a pA_2 value of 4.65. In the presence of 8-phenyltheophylline, the inhibition of the effects of adenosine was almost 100 times greater than with theophylline. Furthermore, almost identical pA_2

Table 2 Evaluation of the interaction of adenosine agonists and antagonists on force of contraction in guinea-pig left atria

Agonist	*Antagonist*	*Slope*	pA_2 *(95% confidence limits)*	*n*
Adenosine	Theophylline	0.51	—	16
Adenosine + dipyridamole 10^{-5} mol l^{-1}	Theophylline	1.04	4.48 (4.19–5.16)	8
R-PIA	Theophylline	1.00	4.65 (4.49–4.86)	32
R-PIA	8-PT	0.99	6.43 (6.31–6.67)	25
S-PIA	8-PT	1.02	6.28 (5.99–7.03)	20
NECA	8-PT	1.04	6.25 (6.03–6.81)	12

The interaction of the agonists and antagonists was evaluated according to Arunlakshana & Schild (1959). 8-PT, 8-phenyltheophylline.

values of 6.43, 6.28 and 6.25 were found with **R**-PIA, S-PIA and NECA, respectively. The slopes and pA_2 values (including 95% confidence limits) of all Schild plots are summarized in Table 2.

The effects of NECA and **R**-PIA on action potential configuration, ^{86}Rb efflux and ^{45}Ca uptake were investigated, alone and in the presence of the antagonist 8-phenyltheophylline. Figure 2 shows that both substances, at maximally effective concentrations of 1 μmol l^{-1}, shorten the duration of the action potential to a similar extent. The effects of both NECA and **R**-PIA on APD_{20} and APD_{90} were concentration-dependent. The concentration-response relationships of both substances were shifted to the right about 10 fold in the presence of 8-phenyltheophylline 10 μmol l^{-1} (Figure 3). Figure 4 demonstrates that both NECA and **R**-PIA increased the efflux of ^{86}Rb. In this series of experiments, the atria were first exposed to ^{86}Rb and then washed repeatedly so that the efflux of the previously gained isotope could be measured. Under control conditions, a fairly stable rate constant of about 0.01 min^{-1} was calculated. After the addition of NECA 1 μmol l^{-1}, the rate constant was almost doubled within 15 min and returned virtually to control values after 30 min washout. Finally, **R**-PIA 1 μmol l^{-1} was added and, again, the rate constant of ^{86}Rb was significantly increased. The same experiments with NECA and **R**-PIA were also carried out in the presence of $BaCl_2$ 0.1 mmol l^{-1}. Ba ions seem to occupy potassium channels by electrostatic forces (Armstrong & Taylor, 1980; Armstrong *et al.*, 1982) and to reduce the conductance of potassium channels in various heart muscle preparations (Sperelakis *et al.*, 1967; Osterrieder *et al.*, 1982; Cohen *et al.*, 1983). Ba *per se* decreased the rate constant of ^{86}Rb

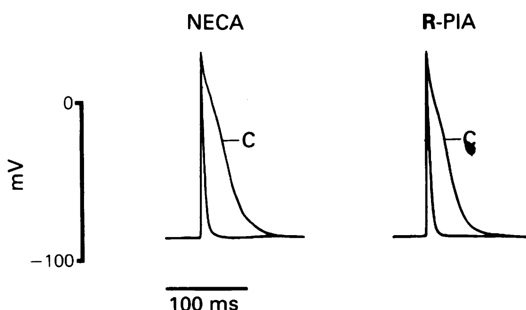

Figure 2 Effects of 5'-(N-ethyl)-carboxamido-adenosine (NECA) and (−)-N^6-(**R**-phenyl-isopropyl)-adenosine (**R**-PIA) (1 μmol l^{-1} each) on the configuration of the action potential in a guinea-pig left atrial trabecula. The same preparation was first exposed to NECA for 15 min, then washed for 45 min and finally exposed to **R**-PIA for 15 min. Original records under control conditions (C) and 15 min after the addition of the respective substance were graphically superimposed.

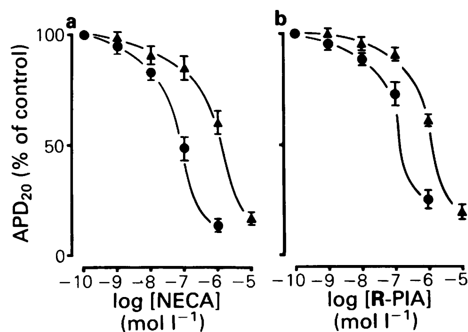

Figure 3 Effects of 5'-(N-ethyl)-carboxamido-adenosine (NECA) (a) and (−)-N^6-(**R**-phenyl-isopropyl)-adenosine (**R**-PIA) (b) on action potential duration at 20% repolarization (APD_{20}) in guinea-pig left atrial trabeculae. Cumulative concentration-response relationships under control conditions (●) and in the presence of 8-phenyltheophylline 10 μmol l^{-1} (▲). The effects of each concentration were observed for 15 min. Symbols represent means (n = 6 in each group) and vertical lines show s.e.mean.

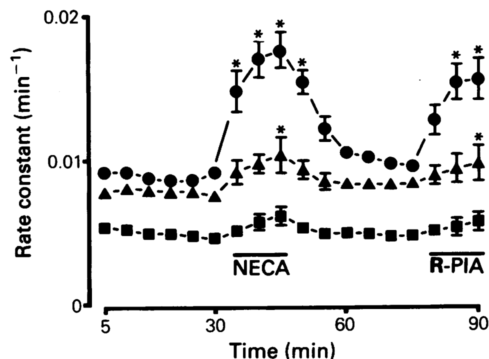

Figure 4 Time course of the effects of 5'-(N-ethyl)-carboxamido-adenosine (NECA) and (−)-N^6-(R-phenyl-isopropyl)-adenosine (R-PIA) (1 μmol l^{-1} each) on the rate constant of ^{86}Rb efflux in guinea-pig left atria under control conditions (●), in the presence of 8-phenyltheophylline 10 μmol l^{-1} (▲), and in the presence of $BaCl_2$ 0.1 mmol l^{-1} (■). Symbols represent means ($n = 6$ in each of the three groups); vertical lines show s.e.mean except when it is smaller than the size of the symbols. Significance level $P < 0.01$ is marked by an asterisk (control vs test values; analysis of variance).

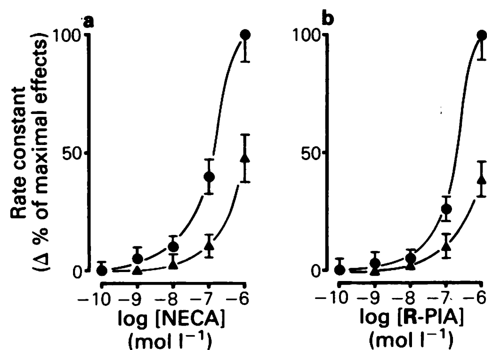

Figure 5 Effects of (a) 5'-(N-ethyl)-carboxamido-adenosine (NECA) and (b) (−)-N^6-(R-phenyl-isopropyl)-adenosine (R-PIA) on the rate constant of ^{86}Rb efflux under control conditions (●) and in the presence of 8-phenyltheophylline 10 μmol l^{-1} (▲). Cumulative concentration-response relationships are shown. Symbols represent means ($n = 6$ in each of the four groups); vertical lines show s.e.mean except when it is smaller than the size of the symbols used.

efflux by about 40%. In the presence of Ba, the effects of NECA and R-PIA on the efflux of ^{86}Rb were almost eliminated. 8-Phenyltheophylline 10 μmol l^{-1} also antagonized the increase in ^{86}Rb efflux by NECA or R-PIA, without significantly changing the control values.

The increase in ^{86}Rb efflux by NECA or R-PIA was concentration-dependent and the concentration-response relationships were shifted to the right by 8-phenyltheophylline (Figure 5).

The shortening of the action potential by adenosine analogues may be responsible for the negative inotropic effects of the substances by impairment of calcium influx during excitation (as already discussed by Grossman & Furchgott, 1964; Schrader *et al.*, 1975). Influx studies with ^{45}Ca generally reveal only relatively small changes (Grossman & Furchgott, 1964; Guthrie & Naylor, 1967; Meinertz *et al.*, 1973). Figure 6 demonstrates a small reduction of ^{45}Ca uptake (about 20%) in the presence of NECA or R-PIA (1 μmol l^{-1} each) which was statistically significant (comparison of control values versus all test values).

Discussion

We have confirmed and extended earlier results that adenosine has a negative inotropic effect in atrial heart muscle at relatively high concentrations. The effects of adenosine were antagonized in the presence of adenosine deaminase but enhanced in the presence of dipyridamole. This indicates that, firstly, receptors located outside on the cell surface probably mediate the inotropic effects and, secondly, an efficient uptake system for adenosine rapidly eliminates the effects of extracellularly applied adenosine. This may explain why maximal and stable effects of adenosine are established only at 1 mmol l^{-1}.

The effects of adenosine on F_c were equally well mimicked by NECA and R-PIA at about thousand times lower concentrations, R-PIA being about 100 times more potent than S-PIA. The observed large

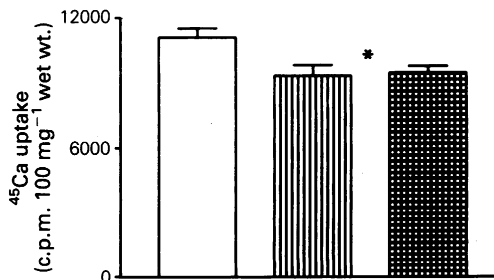

Figure 6 Effects of 5'-(N-ethyl)-carboxamido-adenosine (column with vertical lines) and (−)-N^6-(R-phenyl-isopropyl)-adenosine (cross-hatched column) (1 μmol l^{-1} each) on ^{45}Ca uptake (5 min) in beating (3 Hz) guinea-pig left atria; the open column shows the control value. Columns represent means (7 preparations in each group); vertical lines show s.e.mean. Significance level $P < 0.01$ is marked by an asterisk (control vs test values; analysis of variance).

difference in potency between R-PIA and S-PIA is in accord with an earlier study (Collis, 1983) and in agreement with the assumption that the inhibition of F_c by adenosine is mediated by an interaction with A_1-receptors. However, it would be surprising if R-PIA and NECA are equally effective at A_1-receptors. In addition, it is debatable whether or not any changes of the cyclic AMP content are responsible for the changes in F_c (Huang & Drummond, 1976; Anand-Srivastava & Cantin, 1983; Brückner *et al.*, 1985; Böhm *et al.*, 1988). Our results suggest that, in guinea-pig left atria, adenosine and both R-PIA and NECA stimulate a common receptor population which mediates the observed inotropic and electrophysiological changes. This hypothesis is in line with the physiological importance of specific adenosine receptors on the cell surface, but calls into question the significance of the receptor classification, A_1 and A_2 in atrial heart muscle. In the present study, two competitive inhibitors, theophylline and 8-phenyltheophylline, did not distinguish between the effects of adenosine, R-PIA, S-PIA or NECA. Other xanthine or non-xanthine derivatives, selective for A_1-receptors, have been described (Lohse *et al.*, 1987; Stiles & Jacobson, 1987; Daly *et al.*, 1988; Shamim *et al.*, 1988). Attempts to show selective inhibition of the effects of PIA and NECA on F_c in atrial heart muscle, however, have failed (Collis *et al.*, 1987). It is, therefore, possible that the inotropic effects of adenosine or its derivatives in the atrium are due to the activation of a receptor population which is unrelated to A_1- or A_2-receptors, and that these receptors may be better described as P_1 (Burnstock, 1972; 1978) or A_3 (Ribeiro *et al.*, 1986; Williams, 1987).

It has been suggested earlier that the effects of adenosine in the atrium may be ascribed to an increase in potassium conductance (Hartzell, 1979). This effect shortens the action potential and may, indirectly, diminish the entry of calcium during excitation. In the present study, it was shown that the effects of adenosine on action potential, potassium conductance and calcium entry are mimicked by NECA and R-PIA at micromolar concentrations. Again, no rank order of potency could be found for the effects of NECA and R-PIA which could serve as an indication for the separation of effects mediated by either A_1- or A_2-receptors.

An increase in the potassium conductance prevents the calcium slow inward current from running its normal time course (Ten Eick *et al.*, 1976). Therefore, from the present experiments, we cannot deduce whether NECA and R-PIA not only increase the potassium conductance but also decrease the calcium conductance of the myocardial cell membrane. Voltage clamp experiments are needed to provide this information.

The effects of purinergic stimulation in the atrium, including the effects on F_c, APD and potassium conductance (Nawrath *et al.*, 1985) are, therefore, virtually identical to those of cholinergic stimulation. It seems plausible from the results of many studies that the activation of either adenosine or acetylcholine receptors in atrial heart muscle produces an identical chain of cellular events via the same post receptor pathways. As such, adenosine may either help mediate the effects of cholinergic stimulation or serve as a reserve neurotransmitter in atrial heart muscle (Burnstock, 1986).

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