Br. J. Pharmacol. (1988), **93**, 729–738

Adenosine inhibits the positive inotropic effect of 3-isobutyl-1-methylxanthine in papillary muscles without effect on cyclic AMP or cyclic GMP

[1] Michael Böhm, Reinhard Brückner, Joachim Neumann, Monika Nose, [2] Wilhelm Schmitz & Hasso Scholz

Abteilung Allgemeine Pharmakologie, Universitäts-Krankenhaus Eppendorf, Universität Hamburg, Martinistrasse 52, D-2000 Hamburg 20, F.R.G.

1 Adenosine and the adenosine receptor agonist (−)-N^6-phenylisopropyladenosine (PIA) produced a small positive and negative inotropic effect, respectively, in isolated electrically driven papillary muscles of guinea-pigs.

2 Adenosine ($100\,\mu mol\,l^{-1}$) had no effect on cyclic AMP or cyclic GMP content. PIA ($100\,\mu mol\,l^{-1}$) slightly increased cyclic AMP.

3 In the presence of 3-isobutyl-1-methylxanthine (IBMX; $60\,\mu mol\,l^{-1}$), which increased force of contraction 2 fold, adenosine and PIA exerted strong negative inotropic effects. PIA was more potent than adenosine (mean IC_{25} 2.1 and $168\,\mu mol\,l^{-1}$, respectively).

4 In contrast, the nucleosides did not affect the increase in force of contraction produced by elevating extracellular Ca^{2+} concentration.

5 The IBMX-antagonistic effects of adenosine and PIA were not accompanied by modification of the IBMX-induced increase in cyclic AMP and cyclic GMP.

6 The effects of adenosine and PIA on force of contraction were accompanied by a partial reversal of the IBMX-induced increase in the maximal rate of depolarization of slow action potentials.

7 It is concluded that adenosine and PIA are able to attenuate the positive inotropic effect of a phosphodiesterase inhibitor. This effect is unlikely to be due to a reduction of the IBMX-induced increase in cyclic AMP content. It is conceivably due to an inhibition of the stimulant action of cyclic AMP on slow Ca^{2+} channels leading to the reduction of the slow inward current which in turn reduces force of contraction.

Introduction

Adenosine has been shown to antagonize the positive inotropic effects of β-sympathomimetic agents, e.g. of isoprenaline in various heart preparations (Schrader *et al.*, 1977; Dobson, 1978; 1983; Hughes & Stone, 1983; Böhm *et al.*, 1984; 1985a, b, c). Since increased amounts of adenosine are released from the heart during isoprenaline stimulation it has been suggested that adenosine may play a physiological role as a negative feed-back regulator protecting the heart against excessive sympathetic stimulation (Schrader *et al.*, 1977; Dobson, 1978). The mechanism of the interaction between adenosine and isoprenaline is still a matter of debate. Some workers have attributed it to an inhibition of the isoprenaline-stimulated adenylate cyclase activity with a subsequent reduction of the myocardial cyclic AMP content (Schrader *et al.*, 1977; Dobson, 1978). However, an additive stimulatory effect of adenosine on the cyclic AMP content (Huang & Drummond, 1978) as well as no effect of adenosine on cyclic AMP levels (Schmitz *et al.*, 1981; Böhm *et al.*, 1984) have also been reported. In addition, adenosine has as yet been reported to elicit negative inotropic effects in ventricular preparations only if force of contraction has been increased by agents that stimulate adenylate cyclase activity and hence increase myocardial cyclic AMP content (Baumann *et al.*,

[1] Present address: Medizinische Klinik I, Universität München, Marchioninistrasse 15, D-8000 München 70, F.R.G.

[2] Author for correspondence.

1981). For instance the effects of the adenylate cyclase stimulating agents dopamine and histamine, like the effects of isoprenaline, are also antagonized, while the Ca^{2+}-induced positive inotropic effect is not affected by adenosine (Dobson *et al.*, 1980; Baumann *et al.*, 1981; Böhm *et al.*, 1984). Therefore, it has been proposed that adenosine may exert its negative inotropic effect by inhibiting a late step in the series of events by which hormones induce activation of adenylate cyclase (Baumann *et al.*, 1981).

The present experiments were performed to investigate whether or not a positive inotropic effect evoked by phosphodiesterase inhibition (and not by adenylate cyclase stimulation) can also be antagonized by adenosine. Therefore, we investigated the effects of adenosine on the positive inotropic and cyclic AMP elevating effects of the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX) in isolated electrically driven papillary muscles from guinea-pig hearts. Cyclic GMP content was also measured because negative inotropic effects have been attributed to increased cyclic GMP levels (see Goldberg *et al.*, 1975; Nawrath *et al.*, 1981). Furthermore, the effects of the drugs on normal and slow action potentials were also investigated. In order to characterize further the cardiac effects of adenosine the adenosine derivative (−)-N^6-phenylisopropyladenosine (PIA) which is an agonist at cardiac adenosine receptors located at the external surface of the cell membrane (Hosey *et al.*, 1984; Lohse *et al.*, 1985; Böhm *et al.*, 1985a; Brückner *et al.*, 1985a; Schmitz *et al.*, 1985) was also studied. Parts of the results have been reported in a preliminary communication (Neumann *et al.*, 1984).

Methods

Contractile force and action potential measurements

These experiments were performed on electrically driven papillary muscles isolated from the right ventricles of guinea-pig hearts. In order to exclude any interference from endogenous catecholamines the animals were pretreated with reserpine (5 mg kg^{-1}, i.p., 16–18 h before they were killed). The animals (either sex; 250–360 g) were killed by a blow on the head and bled from the carotid arteries. Their hearts were quickly excised and the papillary muscles (diameter < 1.0 mm, length 3–6 mm) were dissected from the hearts in aerated bathing solution (composition see below) at room temperature. The preparations were attached to a bipolar platinum stimulating electrode and suspended individually in glass tissue chambers for recording isometric contractions (for further details see Böhm *et al.*, 1984). The bathing solution (10 ml) was a modified Tyrode solution containing (mmol l^{-1}): NaCl 119.8, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1.05, NaH_2PO_4 0.42, $NaHCO_3$ 22.6, Na_2EDTA 0.05, ascorbic acid 0.28, glucose 5.0. It was continuously gassed with 95% O_2 : 5% CO_2 and maintained at 35°C; the pH was 7.4. The force of contraction was measured with an inductive force transducer (W. Fleck, Mainz, FRG) attached to a Hellige Helco Scriptor recorder. Each muscle was stretched to the length at which force of contraction was maximal. The resting force (approximately 5 mN) was kept constant throughout the experiment. The preparations were electrically paced at 1 Hz with rectangular pulses of 5 ms duration (Grass stimulator SD 9); the voltage was about 20% greater than threshold.

All preparations were allowed to equilibrate in drug-free bathing solution until complete mechanical stabilization had been achieved. All drugs were freshly dissolved in pre-warmed and pre-aerated bathing solution. Concentration-dependent mechanical effects of adenosine or PIA in the presence of IBMX were obtained in the following manner. First the muscle preparations were exposed to IBMX for 15 min. Maintaining the concentration of IBMX constant, adenosine or PIA were added cumulatively. The time of exposure to each drug concentration was 5 min. The time points were chosen so that the force of contraction had reached equilibrium at each concentration.

For recording transmembrane potentials, guinea-pig papillary muscles were mounted horizontally in a 2 ml chamber continuously perfused with pre-warmed and pre-aerated bathing solution. The resting and evoked action potentials were measured in a conventional way (Brückner *et al.*, 1980) between an intracellular glass microelectrode filled with 3 mol l^{-1} KCl and an extracellular Ag-AgCl electrode. For recording slow action potentials the potassium concentration was increased to 22 mmol l^{-1} without isotonic compensation in order to inactivate the fast sodium channels. The force of contraction, was simultaneously monitored, and the transmembrane potentials (displayed on a Tektronix model 5103 N oscilloscope) were recorded with a high speed camera (Grass C-4).

Determination of cyclic nucleotide levels

Papillary muscles were prepared and mounted individually in the organ bath as described for the contraction experiments. At the end of the incubation time with IBMX, adenosine, PIA or drug-free solution they were quickly frozen in liquid nitrogen (for details see Dönges *et al.*, 1977). Cyclic AMP and cyclic GMP were measured by radioimmunoassay according to the method of Harper & Brooker (1975) as described by Böhm *et al.* (1984). Recoveries,

run with each experiment, were not altered by the substances under investigation; the average recovery of 5 pmol non-radioactive cyclic AMP and cyclic GMP added to 500 µl of trichloroacetic acid homogenate was 108 ± 5.5% ($n = 40$) and 65.2 ± 5.2% ($n = 97$), respectively. The values shown were corrected for recoveries. All measurable material was destroyed by treatment with phosphodiesterase.

Drugs and solvents

Drugs used were adenosine, (−)-N^6-phenylisopropyladenosine (PIA) (both from Boehringer Mannheim, FRG), 3-isobutyl-1-methyl-xanthine (IBMX; EGA-Chemie, Steinheim/Albuch, FRG), atropine sulphate (Merck, Darmstadt, FRG), cyclic adenosine 3',5'-monophosphate, cyclic guanosine 3',5'-monophosphate (both from Boehringer Mannheim, FRG), adenosine-5'-triphosphate (Serva, Heidelberg, FRG), 2'-0-succinyl adenosine 3',5'-monophosphate tyrosine methyl ester and 2'-0-succinyl guanosine 3',5'-monophosphate tyrosine methyl ester (both from Sigma Chem. Co., München, FRG) were iodinated using $Na^{125}I$ (Amersham-Buchler, Braunschweig, FRG) as described by Struck *et al.* (1977). All other chemicals were of analytical or best commercial grade available. Deionized and twice-distilled water was used throughout.

Statistical methods

Values presented are means ± s.e. mean. Statistical significance was estimated by Student's t test for paired or unpaired observations. A P value of less than 0.05 was considered significant. Concentrations of drugs which produced 25% inhibition (IC_{25} values) were determined graphically in each experiment.

Results

Effects of adenosine and PIA on force of contraction and cyclic nucleotide content

Adenosine alone had a small positive inotropic effect at high concentrations (100–1000 µmol l^{-1}; Figure 1a). In contrast, PIA had a small negative inotropic effect amounting to less than 10% at the high concentration of 100 µmol l^{-1} (Figure 1b). The limited solubility prevented the testing of higher concentrations than 300 µmol l^{-1}. The inotropic effects of adenosine and PIA were not affected by 10 µmol l^{-1} atropine (data not shown).

In the presence of IBMX (60 µmol l^{-1}) the effects of adenosine and PIA on force of contraction were quite different from those of adenosine (Figure 1a) and PIA applied alone (Figure 1b). IBMX itself increased force of contraction by about 190%. Additionally administered adenosine had a concentration-dependent (10–1000 µmol l^{-1}) negative inotropic effect, reducing force of contraction by about 50% of the IBMX value at the highest concentration tested. The negative inotropic effect of PIA started at 0.1 µmol l^{-1} and reduced force

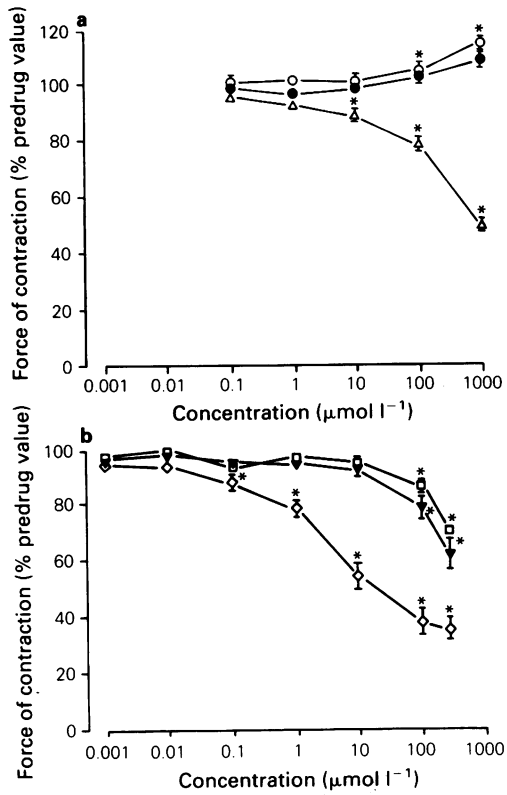

Figure 1 Concentration-response curves for the effects of adenosine (a) and (−)-N^6-phenylisopropyladenosine (PIA; b) on force of contraction in isolated electrically driven papillary muscles from guinea-pigs. Ordinates: Force of contraction in % predrug value. Abscissae: drug concentration in µmol l^{-1}. (a) Effects of adenosine alone (○, $n = 6$), adenosine in the presence of 60 µmol l^{-1} IBMX (△, $n = 7$) and adenosine in the presence of 3.6 mmol l^{-1} Ca^{2+} (●, $n = 11$). (b) Effects of PIA alone (□, $n = 8$), PIA in the presence of 60 µmol l^{-1} IBMX (◇, $n = 7$), and PIA in the presence of 3.6 mmol l^{-1} Ca^{2+} (▼, $n = 8$). The predrug value of force of contraction before any drug addition was 1.1 ± 0.2 mN ($n = 47$). IBMX increased force of contraction to 2.5 ± 0.3 mN ($n = 14$) and 3.6 mmol l^{-1} Ca^{2+} to 2.9 ± 0.5 mN ($n = 19$). The normal Ca^{2+} concentration was 1.8 mmol l^{-1}. Asterisks denote significant differences from predrug values.

of contration maximally to about 50% at $100\,\mu mol\,l^{-1}$. The IC_{25} values for adenosine and PIA in the presence of IBMX were 168 ± 25.5 ($n = 7$) and $2.1 \pm 0.7\,\mu mol\,l^{-1}$ ($n = 7$), respectively. The effects of both substances were not affected by $10\,\mu mol\,l^{-1}$ atropine (data not shown) and hence were not due to a stimulation of muscarinic cholinoceptors.

The positive inotropic effect of an increased extracellular Ca^{2+} concentration is known to occur without changes in cyclic AMP content (Watanabe & Besch, 1974; Dönges *et al.*, 1977). Therefore, the influence of adenosine and PIA on the positive inotropic effect of Ca^{2+} was investigated to find out whether the adenosine- and PIA-induced decrease in force of contraction could only be observed after the cyclic AMP content had been increased. Doubling the Ca^{2+} concentration from 1.8 to $3.6\,mmol\,l^{-1}$ increased force of contraction to about 280%. Additionally applied adenosine (Figure 1a) or PIA (Figure 1b) had the same effect as adenosine and PIA alone, i.e. adenosine had a small positive inotropic and PIA only a marginal negative inotropic effect at high concentrations. Thus, the positive inotropic effect of Ca^{2+}, in contrast to that of IBMX, was not antagonized by adenosine or PIA.

In the following experiments we investigated whether or not changes in cyclic nucleotide content are involved in the mechanical effects of adenosine and PIA. In these experiments cyclic AMP and cyclic GMP content and force of contraction were determined in the same muscle preparations. Figure 2 (left panel) shows that the small positive inotropic effect of adenosine ($100\,\mu mol\,l^{-1}$) was not accompanied by a significant change in cyclic AMP or cyclic GMP content either at 1 min or at 5 min. PIA ($100\,\mu mol\,l^{-1}$) slightly increased the cyclic AMP content after 5 min (Figure 2, right panel). Cyclic GMP content was not significantly altered.

Figure 3 shows the results obtained in the presence of IBMX ($60\,\mu mol\,l^{-1}$). It is evident that the positive inotropic effect of IBMX 15 min (b), 16 min

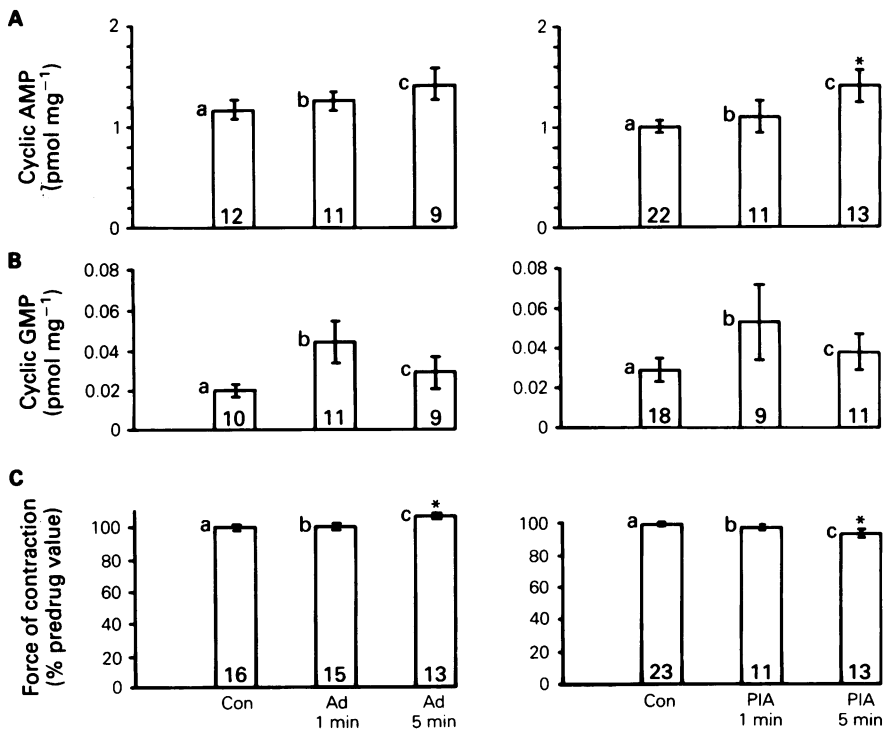

Figure 2 Effects of adenosine ($100\,\mu mol\,l^{-1}$; left panel) and (−)-N^6-phenylisopropyladenosine (PIA; $100\,\mu mol\,l^{-1}$; right panel) on cyclic AMP content (A), cyclic GMP content (B) and force of contraction (C) in electrically driven papillary muscles from guinea pigs. (a) Control in drug-free bathing solution; (b and c) after 1 and 5 min incubation time with adenosine and PIA, respectively. The numbers in the columns denote the numbers of experiments. Predrug values of force of contraction were 1.1 ± 0.1 mN ($n = 91$). Asterisks denote significant differences from control.

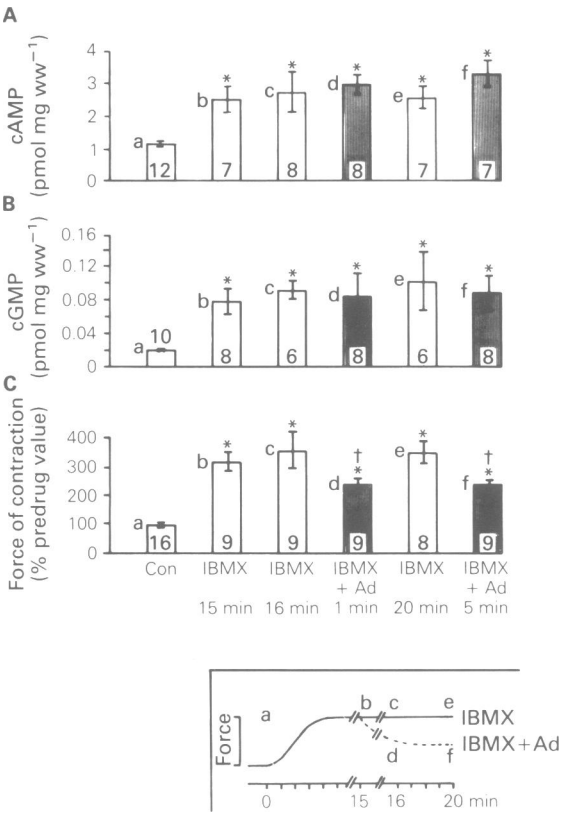

Figure 3 Cyclic AMP content (A), cyclic GMP content (B) and force of contraction (C) of electrically driven papillary muscles from guinea-pigs in drug-free bathing solution as control (Con) (a; see also schematic inset) and 15 min (b), 16 min (c) and 20 min (e) after addition of 60 μmol l^{-1} 3-isobutyl-1-methylxanthine (IBMX). Fifteen min after incubation with IBMX, adenosine (Ad; 100 μmol l^{-1}) was applied additionally for 1 min (d) or 5 min (f). The numbers in the columns denote the numbers of experiments. The predrug value of force of contraction was 1.4 ± 0.1 mN (n = 60). *P < 0.05 vs. (a); †P < 0.05 (d) vs. (c); (f) vs. (e).

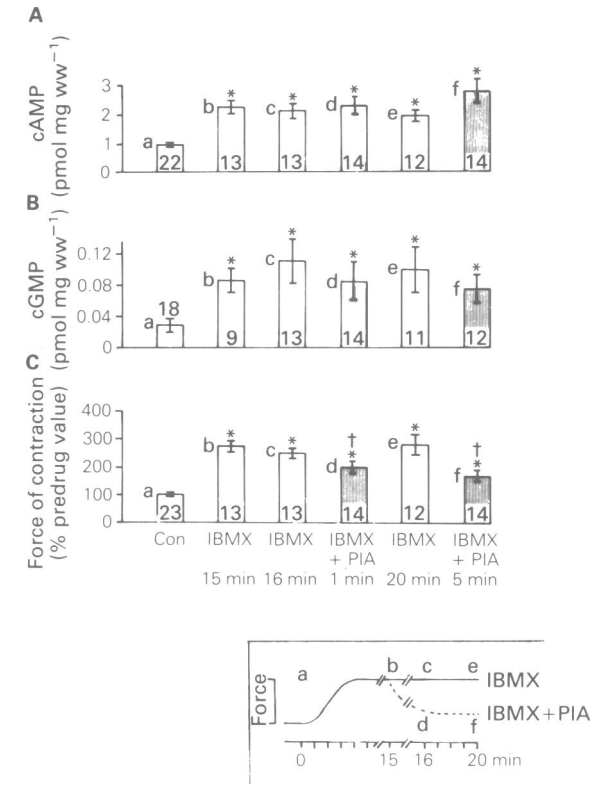

Figure 4 Cyclic AMP content (A), cyclic GMP content (B) and force of contraction (C) of electrically driven guinea-pig papillary muscles in drug-free bathing solution as control (Con) (a; see also schematic inset) and 15 min (b), 16 min (c) and 20 min (e) after addition of 60 μmol l^{-1} 3-isobutyl-1-methylxanthine (IBMX). Fifteen min after incubation with IBMX, (−)-N^6-phenylisopropyladenosine (PIA; 60 μmol l^{-1}) was applied additionally for 1 min (d) or 5 min (f). The numbers in the columns denote the numbers of experiments. The predrug value of force of contraction was 1.0 ± 0.1 mN (n = 89). *P < 0.05 vs. (a), †P < 0.05 (d) vs. (c); (f) vs. (e).

(c) and 20 min (e) after drug addition was accompanied by a doubling of the cyclic AMP content and by an about 3 fold increase in cyclic GMP content. Adenosine (100 μmol l^{-1}), additionally applied for 1 min (d) or 5 min (f), partially antagonized the positive inotropic effect of IBMX. However, the cyclic AMP and cyclic GMP content of the papillary muscles remained elevated, i.e. the cyclic nucleotide contents in the presence of adenosine did not differ from the corresponding values obtained with IBMX alone (d vs. c and f vs. e). Similar results were obtained with the adenosine receptor agonist PIA (Figure 4). PIA also antagonized the IBMX-induced increase in force of contraction without affecting the IBMX-induced increase in cyclic AMP and cyclic GMP content.

Effects of adenosine and PIA on normal and slow action potentials

In order to find out whether the mechanical effects of adenosine and PIA can be attributed to corresponding electrophysiological changes, we studied the effects of adenosine and PIA alone and in the presence of IBMX on normal and slow action potentials. The data are summarized in Table 1. The small

Table 1 Effect of adenosine (Ad; 100 μmol l^{-1}) or (−)-N^6-phenylisopropyladenosine (PIA; 100 μmol l^{-1}), 3-isobutyl-1-methylxanthine (IBMX; 60 μmol l^{-1}) or Ca^{2+} (3.6 mmol l^{-1}) and Ad or PIA plus IBMX or Ca^{2+} on APD_{90} and APD_{20} (action potential duration measured at 90% and 20% repolarization) of normal action potentials and on dV/dt_{max} (maximal rate of depolarization) of slow action potentials

	Normal action potential		*Slow action potential*
	APD_{90} (ms)	APD_{20} (ms)	dV/dt_{max} ($V s^{-1}$)
Control	223.0 ± 31.9 (4)	118.3 ± 6.5 (4)	4.6 ± 0.5 (4)
Ad	221.0 ± 28.7 (4)	117.1 ± 5.6 (4)	4.7 ± 0.6 (4)
Control	230.5 ± 5.4 (6)	115.8 ± 6.3 (6)	3.9 ± 0.3 (4)
IBMX	207.2 ± 4.1 (6)[a]	121.8 ± 7.8 (6)	11.0 ± 0.5 (4)[a]
IBMX + Ad	211.5 ± 4.0 (6)	120.7 ± 8.4 (6)	7.1 ± 0.5 (4)[a, b]
Control	249.0 ± 10.8 (4)	131.3 ± 9.6 (4)	4.3 ± 0.5 (4)
PIA	251.5 ± 16.5 (4)	131.2 ± 11.3 (4)	4.2 ± 0.6 (4)
Control	223.9 ± 9.0 (5)	119.3 ± 6.6 (5)	3.2 ± 0.3 (4)
IBMX	199.9 ± 9.9 (5)[a]	117.4 ± 3.5 (5)	9.4 ± 1.1 (4)[a]
IBMX + PIA	206.4 ± 9.5 (5)[a]	112.9 ± 4.5 (5)	5.1 ± 0.6 (4)[a, b]
Control	—	—	3.6 ± 0.3 (6)
Ca^{2+}	—	—	5.8 ± 0.3 (6)[a]
Ca^{2+} + PIA	—	—	5.7 ± 0.4 (6)[a]

Action potentials were measured before (Control) and 15 min after addition of IBMX or Ca^{2+} and 5 min after addition of Ad or PIA in the continued presence of IBMX. The numbers in parentheses denote the numbers of experiments.

[a] $P < 0.05$ vs. Control; [b] $P < 0.05$ vs. IBMX

— not determined

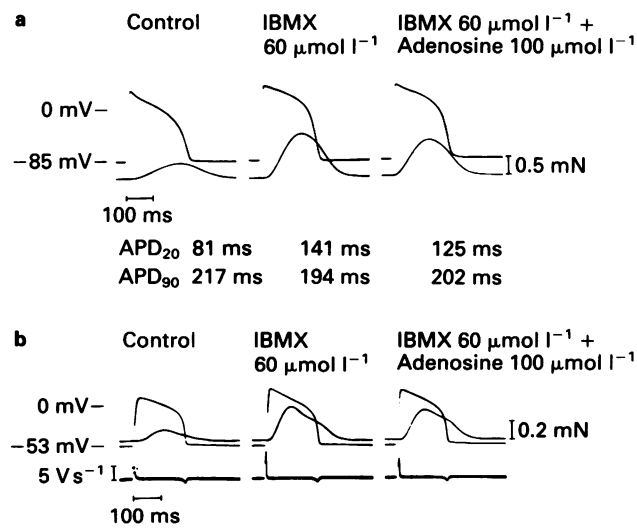

Figure 5 Effect of 3-isobutyl-1-methylxanthine (IBMX) and IBMX plus adenosine on normal (a) and slow action potentials (b) in electrically driven (1 Hz) papillary muscles from guinea-pigs. Force of contraction was measured simultaneously. (a) Normal action potentials (mV; upper traces) and force of contraction (mN; lower traces). (b) Slow action potentials (mV) and force of contraction (mN; upper traces); maximal rate of depolarization ($V s^{-1}$, lower traces). Slow action potentials were elicited after raising the potassium concentration from 5.4 to 22 mmol l^{-1}. Recordings were performed before (control), 15 min after addition of IBMX and 5 min after addition of adenosine in the continued presence of IBMX.

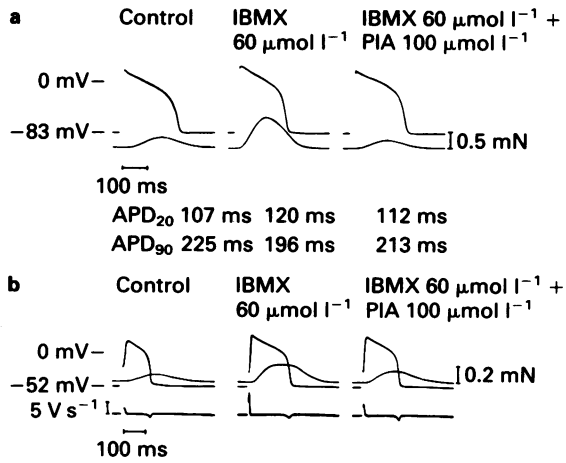

Figure 6 Effects of 3-isobutyl-1-methylxanthine (IBMX) and IBMX plus (−)-N^6-phenylisopropyladenosine (PIA) on normal (a) and slow action potential (b) in electrically driven (1 Hz) papillary muscles from guinea-pigs. Force of contraction was measured simultaneously. (a) Normal action potentials (mV; upper traces) and force of contraction (mN; lower traces). (b) Slow action potentials (mV) and force of contraction (mN; upper traces); maximal rate of depolarization ($V s^{-1}$; lower traces). Slow action potentials were elicited after raising the potassium concentration from 5.4 to 22 mmol l^{-1}. Recordings were performed before (Control), 15 min after addition of IBMX and 5 min after the addition of adenosine or PIA in the continued presence of IBMX.

positive inotropic effect of adenosine alone (100 μmol l^{-1}) was not accompanied by detectable changes in the shape of the normal action potential or of the slow action potential. PIA (100 μmol l^{-1}) also failed to produce significant changes in the shape of the normal and slow action potential.

The IBMX-induced increase in force of contraction was accompanied by a shortening of the normal action potential at the end of the repolarization phase (APD_{90}; Figure 5a and 6a). The decrease in force of contraction 5 min after the additional application of adenosine (100 μmol l^{-1}) was not accompanied by significant changes of the IBMX-induced electrophysiological changes (Figure 5a).

IBMX (60 μmol l^{-1}) increased the maximal rate of depolarization (dV/dt_{max}) of slow action potentials (Figure 5b) evoked in potassium depolarized papillary muscles. In this case adenosine (100 μmol l^{-1}) partially reversed not only the mechanical but also the electrophysiological effects of IBMX. Similar results were obtained with PIA (100 μmol l^{-1}; Figure 6). In contrast, the increase in dV/dt_{max} induced by increasing the Ca^{2+} concentration from 1.8 to 3.6 mmol l^{-1} was not affected by PIA (Table 1).

Discussion

In isolated electrically driven guinea-pig papillary muscles, adenosine produced a small positive inotropic effect while the adenosine receptor agonist PIA produced a small negative inotropic effect at high concentrations (100 μmol l^{-1}). As all the animals had been pretreated with reserpine the positive inotropic effect of adenosine cannot be explained by a release of endogenous catecholamines. Since propranolol (1 μmol l^{-1}) and phentolamine (5 μmol l^{-1}) did not influence the positive inotropic effect of adenosine (data not shown) it is also unlikely to be due to a stimulation of β- or α-adrenoceptors. Furthermore, changes in cyclic AMP or cyclic GMP content are not involved because the content of the cyclic nucleotides remain unchanged. Burnstock & Meghji (1981) did not observe an effect of adenosine in guinea-pig ventricles but in contrast to this study they used ventricular strips and a higher rate of stimulation (2.5 Hz) which could account for the divergent results (see Dobson *et al.*, 1980). Furthermore, in rat ventricle they describe a slightly enhanced contractile force in the presence of adenosine (0.3 mmol l^{-1}), although this effect was not significant (Burnstock & Meghji, 1983). In the present study the small negative inotropic effect of PIA alone was not influenced by atropine and hence was not due to stimulation of muscarinic cholinoceptors. The small increase in cyclic AMP after PIA can probably be explained by an inhibition of phosphodiesterase activity described for high concentrations of PIA (Meyer *et al.*, 1984). Since neither adenosine nor PIA produced a detectable change in the shape of the normal and slow action potential the mechanism(s) of the small direct inotropic effects of these drugs remain to be elucidated.

Quite different results were obtained in the presence of IBMX, which is known to be a potent inhibitor of cyclic AMP and cyclic GMP phosphodiesterase activity in guinea-pig ventricular preparations (Berger *et al.*, 1985). In the presence of IBMX, adenosine exerted a negative inotropic effect, i.e., it partially reversed the positive inotropic effect of IBMX. PIA had a similar negative inotropic effect but its potency was greater than that of adenosine. The lower potency of adenosine is probably not the result of rapid breakdown of adenosine by endogenous adenosine deaminase, because treatment with the adenosine deaminase inhibitor deoxycoformycin does not increase the potency of adenosine (unpublished experiments). Adenosine and PIA also reduced the positive inotropic effect of the non-xanthine phosphodiesterase inhibitor amrinone (Böhm *et al.*, 1985b; Schmitz *et al.*, 1985). Judged from IC_{25} values adenosine and PIA were 2 and 10 times more potent, respectively in the presence of

amrinone than in the presence of IBMX. This may indicate that IBMX also exerts a small adenosine-antagonistic effect.

The present data and the results of other studies suggest that adenosine and PIA antagonize not only the positive inotropic effect of substances stimulating adenylate cyclase activity and hence enhancing the formation of cyclic AMP (see Baumann *et al.*, 1981; Dobson, 1983; Böhm *et al.*, 1984), but also of substances which inhibit cyclic AMP breakdown. An increased cyclic AMP content seems to be a prerequisite for demonstrating the negative inotropic effects of adenosine and its derivative in ventricular heart preparations. For example, the increase in force of contraction due to an increase in extracellular Ca^{2+} concentration was not antagonized by adenosine or PIA. Similarly the cyclic AMP-independent positive inotropic effects of the so-called Ca^{2+} agonist Bay K 8644 (Böhm *et al.*, 1985b), the cardiac glycoside dihydro-ouabain (Böhm *et al.*, 1985a; Schmitz *et al.*, 1985) and the α-adrenoceptor agonist phenylephrine (Endoh & Yamashita, 1980) are not reduced by adenosine.

It is noteworthy that the negative inotropic effect of adenosine was mimicked by PIA. PIA is an adenosine derivative known to act selectively at adenosine receptors located at the external surface of the cell membrane (see Londos *et al.*, 1980). Thus, it is likely that the negative inotropic effects of adenosine and PIA involve activation of cardiac adenosine receptors. The existence of these receptors has been shown by radioligand binding as well as functional studies (Hughes & Stone, 1983; Hosey *et al.*, 1984; Lohse *et al.*, 1985; Brückner *et al.*, 1985a; Böhm *et al.*, 1985a).

Since adenosine and PIA inhibit the positive inotropic effect only of substances which increase myocardial cyclic AMP levels, either by stimulation of adenylate cyclase activity (see Baumann *et al.*, 1981) or by inhibition of phosphodiesterase activity (this paper), one would expect the mechanical effects of these agents to be accompanied by changes in cyclic AMP content. In agreement with previous work (Biegon *et al.*, 1980; Brown *et al.*, 1980; Brückner *et al.*, 1985b) the positive inotropic effect of IBMX was accompanied by a marked increase in cyclic AMP- and cyclic GMP-content. However, the negative inotropic effects of additionally applied adenosine or PIA were not accompanied by changes of the IBMX-induced increase in cyclic AMP and cyclic GMP content and hence cannot be attributed to a decrease in cyclic AMP or an increase in cyclic GMP. Concerning the effect of adenosine on the isoprenaline-induced increase of the cyclic AMP content conflicting results have been obtained. Huang & Drummond (1978) found a further increase in cyclic AMP content; their experiments were performed on guinea-pig ventricular slice preparations which did not contract, whereas in the present study cyclic AMP content and force of contraction were measured in the same intact contracting preparations. On the other hand Schrader *et al.* (1977) found a decrease of the isoprenaline-induced elevation of the cyclic AMP content in isolated spontaneously beating guinea-pig hearts (Langendorff technique). Furthermore, no change of the isoprenaline elevated cyclic AMP content in intact contracting papillary muscles from guinea-pigs has been reported (Schmitz *et al.*, 1982; Böhm *et al.*, 1984). Although the reason for the discrepancies concerning the effects of adenosine on cyclic AMP content remain obscure, the different preparations used may play a role. However, it has been shown that the adenosine receptor agonists PIA and 5'-N-ethylcarboxamideadenosine (NECA) do not inhibit (Schütz & Tuisl, 1981; Böhm *et al.*, 1985a) or only slightly inhibit (LaMonica *et al.*, 1985; Linden *et al.*, 1985) adenylate cyclase activity in cardiac particulate membrane preparations suggesting that the cardiac adenosine receptor is not coupled to adenylate cyclase. This view is supported by the fact that PIA and NECA are equipotent in reducing force of contraction (Brückner *et al.*, 1985a; Böhm *et al.*, 1985a). In contrast, in tissues in which adenosine receptors mediate inhibition of adenylate cyclase the potency ranking PIA > NECA is characteristic (see Londos *et al.*, 1980).

It should be noted that PIA is more potent than adenosine at reducing the force of contraction than the parent compound adenosine. This may be due to the fact that these analogues are poor substrates for the adenosine transporter as indicated by the failure of transport inhibitors to potentiate their effects (Böhm *et al.*, 1985a). In addition, PIA is not degraded to metabolites by adenosine deaminase which converts adenosine to inotropically inactive inosine. From previous studies (Böhm *et al.*, 1984) and the present work it can be concluded that the reduction in force of contraction produced by adenosine and PIA is not due to corresponding changes in cyclic nucleotide content. In contrast, the effects on force of contraction may be explained by corresponding electrophysiological effects. The negative inotropic effects of adenosine and PIA were accompanied by a partial reversal of the IBMX-induced increase of dV/dt_{max} of the slow action potential. Since dV/dt_{max} of slow action potentials can be taken as a measure of the slow Ca^{2+} inward current (Cranefield, 1975; Carmeliet, 1980) it is concluded that adenosine and PIA reduced the IBMX-induced increase in slow inward current. The reduced Ca^{2+} inward current during excitation in turn leads to a decrease in force of contraction. Similarly it has been shown that adenosine reduces the isoprenaline-

induced increase in slow inward current in bovine and guinea-pig ventricular myocytes (Isenberg & Belardinelli, 1984) as well as in guinea-pig papillary muscles (Schmitz *et al.*, 1985).

The lack of effect of PIA on the Ca^{2+}-induced increase in force of contraction as well as the increase in dV/dt_{max} of the slow action potential support the conclusion that the negative inotropic effects of adenosine and PIA in the presence of IBMX are indeed due to a decrease in slow Ca^{2+} inward current.

Although the IBMX-antagonistic effects of adenosine and PIA occurred without a reduction of the cyclic AMP content, the negative inotropic effects of these substances in ventricular heart preparations can be evoked only in the presence of positive inotropic agents which elevate cyclic AMP (Endoh & Yamashita, 1980; Baumann *et al.*, 1981; Dobson, 1983; Böhm *et al.*, 1984; 1985b). Thus, the IBMX-antagonistic effects of adenosine and PIA may be explained by an inhibition of a step beyond cyclic AMP accumulation. For instance, the nucleosides may interact with the phosphorylation of the sarcolemmal Ca^{2+} channel and thereby reduce the Ca^{2+} influx during excitation.

In summary, adenosine and the adenosine receptor agonist PIA are able to antagonize the positive inotropic effect of the phosphodiesterase inhibitor IBMX. This negative inotropic effect is not due to changes in the cyclic AMP or cyclic GMP content. It may, however, be due to an inhibition of the action of cyclic AMP which leads to the observed reduction of the IBMX-induced increase in slow inward current.

This work was supported by the Deutsche Forschungsgemeinschaft.

References

BAUMANN, G., SCHRADER, J. & GERLACH, E. (1981). Inhibitory action of adenosine on histamine- and dopamine-stimulated cardiac contractility and adenylate cyclase in guinea-pigs. *Circ. Res.*, **48**, 259–266.

BERGER, C., MEYER, W., SCHOLZ, H. & STARBATTY, J. (1985). Effects of the benzimidazole derivatives pimobendane and 2-(4-hydroxyphenyl)-5-(5-methyl-3-oxo-4,5-dihydro-2H-6-pyridazinyl) benzimidazole HCl on phosphodiesterase activity and force of contraction in guinea-pig hearts. *Arzneim. Forsch.*, **35**, 11, 1668–1673.

BIEGON, R.L., EPSTEIN, P.M. & PAPPANO, A.J. (1980). Muscarinic antagonism of the effects of a phosphodiesterase inhibitor (methylisobutylxanthine) in embryonic chick ventricle. *J. Pharmacol. Exp. Therap.*, **215**, 348–356.

BÖHM, M., BRÜCKNER, R., HACKBARTH, I., HAUBITZ, B., LINHART, R., MEYER, W., SCHMIDT, B., SCHMITZ, W. & SCHOLZ, H. (1984). Adenosine inhibition of catecholamine-induced increase in force of contraction in guinea-pig atrial and ventricular heart preparations. Evidence against a cyclic AMP- and cyclic GMP-dependent effect. *J. Pharmacol. Exp. Therap.*, **230**, 483–492.

BÖHM, M., BRÜCKNER, R., MEYER, W., NOSE, M., SCHMITZ, W., STARBATTY, J. & SCHOLZ, H. (1985a). Evidence for adenosine receptor-mediated isoprenaline-antagonistic effects of the adenosine analogs PIA and NECA on force of contraction in guinea-pig atrial and ventricular cardiac preparations. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **331**, 131–139.

BÖHM, M., BURMANN, H., MEYER, W., NOSE, M., SCHMITZ, W. & SCHOLZ, H. (1985b). Positive inotropic effect of Bay K 8644: cAMP-independence and lack of inhibitory effect of adenosine. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **329**, 447–450.

BÖHM, M., MEYER, W., MÜGGE, A., SCHMITZ, W. & SCHOLZ, H. (1985c). Functional evidence for the existence of adenosine receptors in the human heart. *Eur. J. Pharmacol.*, **116**, 323–326.

BRÜCKNER, R., SCHMITZ, W., SCHOLZ, H., SCHUMACHER, A., ERDMANN, E., KRAWIETZ, W. & WERDAN, K. (1980). Negative inotropic effect of vanadate in ventricular myocardium in the presence of 3-isobutyl-1-methylxanthine or isoprenaline. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **315**, 147–153.

BRÜCKNER, R., FENNER, A., MEYER, W., NOBIS, T.-M., SCHMITZ, W. & SCHOLZ, H. (1985a). Cardiac effects of adenosine and adenosine analogs in guinea-pig atrial and ventricular preparations: Evidence against a role of cyclic AMP and cyclic GMP. *J. Pharmacol. Exp. Therap.*, **234**, 766–774.

BRÜCKNER, R., GRAMANN, S., NOSE, M., SCHMITZ, W. & SCHOLZ, H. (1985b). Isoprenaline-like effects of the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine on mechanical, biochemical and electrophysiological parameters in the mammalian heart. *Experientia*, **41**, 732–734.

BROWN, B.S., POLSON, J.B., KRZANOWSKI, J.J. & WIGGINS, J.R. (1980). Influence of isoproterenol and methylisobutylxanthine on the contractile and cyclic nucleotide effects of methacholine in isolated rat atria. *J. Pharmacol. Exp. Therap.*, **212**, 325–332.

BURNSTOCK, G. & MEGHJI, P. (1981). Distribution of P_1- and P_2-purinoceptors in the guinea-pig and frog heart. *Br. J. Pharmacol.*, **73**, 879–885.

BURNSTOCK, G. & MEGHJI, P. (1983). The effect of adenyl compounds on the rat heart. *Br. J. Pharmacol.*, **79**, 211–218.

CARMELIET, E. (1980). The slow inward current: non-voltage-clamp studies. In *The Slow Inward Current and Cardiac Arrhythmias.* ed. Zipes, D.P., Bailey, J.C. & Elharrar, V. pp. 97–110. The Hague, Boston, London: Martinus Nijhoff Publishers.

CRANEFIELD, P.F. (1975) *The Conduction of the Cardiac Impulse.* pp. 75–115. Mount Kisko New York: Futura Publishing Company.

DOBSON, J.G., JR., SCHWAB, G.E., ROSS, J., JR. & MAYER, S.E. (1980). Comparison of the biochemical composition of four preparations of contracting cardiac muscle. *Am. J. Physiol.*, **227**, 1452–1457.

DOBSON, J.G., JR. (1978). Reduction by adenosine of the isoproterenol-induced increase in cyclic adenosine 3',5'-mono-phosphate formation and glycogen phosphorylase activity in rat heart muscle. *Circ. Res.*, **43**, 785–792.

DOBSON, J.G., JR. (1983). Mechanism of adenosine inhibition of catecholamine-induced responses in heart. *Circ. Res.*, **52**, 151–160.

DÖNGES, C., HEITMANN, M., JUNGBLUTH, H., MEINERTZ, T., SCHMELZLE, B. & SCHOLZ, H. (1977). Effectiveness of theophylline to increase cyclic AMP levels and force of contraction in electrically paced guinea-pig auricles. Comparison with isoprenaline, calcium and ouabain. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **301**, 87–97.

ENDOH, M. & YAMASHITA, S. (1980). Adenosine antagonizes the positive inotropic action mediated via β-, but not α-adrenoceptors in the rabbit papillary muscle. *Eur. J. Pharmacol.*, **65**, 445–448.

GOLDBERG, U.D., HADDOX, M.K., NICOL, S.E., GLASS, D.B., SANFORD, C.H., KUEHL, F.A., JR. & ESTENSEN, R. (1975). Biologic regulation through opposing influences of cyclic GMP and cyclic AMP: the Yin Yang hypothesis. *Adv. Cyclic Nucl. Res.*, **5**, 307–330.

HARPER, G.F. & BROOKER, G. (1975). Femtomol sensitive radio-immunoassay for cyclic AMP and cyclic GMP after 2'-0-acetylation by acetic anhydride in aqueous solution. *J. Cyclic Nucl. Res.*, **1**, 207–218.

HOSEY, M.M., MCMAHON, K.K. & GREEN, R.D. (1984). Inhibitory adenosine receptors in the heart: Characterization by ligand binding studies and effects on β-adrenergic receptor stimulated adenylate cyclase and membrane protein phosphorylation. *J. Mol. Cell. Cardiol.*, **16**, 931–942.

HUANG, M. & DRUMMOND, G.I. (1978). Interaction between adenosine and catecholamines on cyclic AMP accumulation in guinea pig ventricular myocardium. *Biochem. Pharmacol.*, **27**, 187–191.

HUGHES, P.R. & STONE, T.W. (1983). Inhibition by purines of the inotropic action of isoprenaline in rat atria. *Br. J. Pharmacol.*, **80**, 149–153.

ISENBERG, G. & BELARDINELLI, L. (1984). Ionic basis for the antagonism between adenosine and isoproterenol on isolated ventricular myocytes. *Circ. Res.*, **55**, 309–325.

LAMONICA, D.A., FROHLOFF, N. & DOBSON, J.G., JR. (1985). Adenosine inhibition of catecholamine-stimulated cardiac membrane adenylate cyclase. *Am. J. Physiol.*, **248**, H737–744.

LINDEN, J., HOLLEN, C.E. & PATEL, A. (1985). The mechanism by which adenosine and cholinergic agents reduce contractility in rat myocardium. Correlation with cyclic adenosine monophosphate and receptor densities. *Circ. Res.*, **56**, 728–735.

LOHSE, M.J., UKENA, D. & SCHWABE, U. (1985). Demonstration of R_i-type adenosine receptors in bovine myocardium by radio-ligand binding. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **328**, 310–316.

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

MEYER, W., NOSE, M., SCHMITZ, W. & SCHOLZ, H. (1984). Adenosine and adenosine analogs inhibit phosphodiesterase activity in the heart. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **328**, 207–209.

NAWRATH, H., GRUSCHWITZ, P. & ZONG, X. (1981). A negative inotropic effect of acetylcholine in the presence of several phosphodiesterase inhibitors. *Experientia*, **37**, 755–756.

NEUMANN, J., NOSE, M. & SCHMITZ, W. (1984). Effects of adenosine and (−)-N^6-phenylisopropyladenosine on force of contraction and the cAMP and cGMP content in guinea-pig papillary muscles in the presence of isobutylmethylxanthine. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **325**, R 46.

SCHMITZ, W., HACKBARTH, I., HAUBITZ, B., LINHART, R., MEYER, W. & SCHOLZ, H. (1981). Effect of adenosine and isoprenaline on force of contraction and on the c-AMP system in guinea-pig atria. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **316**, R 34.

SCHMITZ, W., BÖHM, M., HACKBARTH, I. & SCHMIDT, B. (1982). Effects adenosine on force of contraction and the cAMP and cGMP content in guinea-pig hearts in the presence of isoprenaline. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **319**, R 38.

SCHMITZ, W., BÖHM, M., BRÜCKNER, R., BURMANN, H., MEYER, W., NEUMANN, J., NOSE, M. & SCHOLZ, H. (1985). Relationship between the effects of cAMP-increasing agents and adenosine in the mammalian heart. In *Adenosine: Receptors and Modulation of Cell Function.* ed. Stefanovich, V., Rudolphi, K. & Schubert, P. pp. 295–305. Oxford: IRL Press.

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

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

STRUCK, C.J., AHNERT, G., GLOSSMANN, H. & SCHAEG, W. (1977). Solid phase radioimmunoassay for cyclic AMP using staphylococcal protein as antibody adsorbent. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **298**, 67–73.

WATANABE, A.M. & BESCH, H.R. (1974). Cyclic adenosine mono-phosphate modulation of slow calcium influx channels in guinea-pig hearts. *Circ. Res.*, **35**, 316–324.

(Received June 15, 1987
Revised October 5, 1987
Accepted October 23, 1987)