Cardiovascular selectivity of adenosine receptor agonists in anaesthetized dogs

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1 In order to determine the relevance of adenosine (Ado) receptor classification obtained from *in vitro* methods to the cardiovascular actions of Ado agonists *in vivo*, the cardiovascular effects of adenosine 5'-monophosphate (AMP), N⁶-cyclohexyladenosine (CHA, 400 fold A₁-selective), 5'-N-ethylcarboxamidoadenosine (NECA, A₁ ≈ A₂) and 2-phenylaminoadenosine (PAA, 5 fold A₂-selective) were compared in open-chest, fentanyl-pentobarbitone anaesthetized dogs.

2 Graded doses of CHA (10 to 1000 µg kg⁻¹), NECA (0.5 to 100 µg kg⁻¹) or PAA (0.1 to 20 µg kg⁻¹) were administered intravenously and changes in haemodynamics and myocardial contractility were assessed 10 min following each dose. The effects of graded infusions of AMP (200 to 1000 µg kg⁻¹ min⁻¹) were also evaluated.

3 AMP and each of the Ado analogues (NECA > PAA > CHA) increased the systemic vascular conductance index (SVCI) in a dose-dependent manner and reduced mean arterial pressure (MAP). At doses causing similar increases in SVCI, these agonists caused (i) similar reflex increases in heart rate (HR) and cardiac index (CI) and decreases in AV conduction interval (AV_i) and (ii) similar increases in coronary vascular conductance (CVC).

4 After cardiac autonomic blockade with atropine (0.2 mg kg⁻¹) and propranolol (1 mg kg⁻¹), AMP, CHA and PAA still increased SVCI and CVC and decreased MAP. CHA and PAA had no marked effects on HR, CI or AV_i. As in the absence of cardiac autonomic blockade, equieffective vasodilator doses of CHA and PAA had identical effects on CVC, CI and AV_i.

5 Myocardial contractility, as assessed by E_{max} measurements, was stimulated by AMP in control animals. Following cardiac autonomic blockade, PAA increased contractility while AMP and CHA had no significant effects.

6 Despite marked differences in receptor selectivity *in vitro*, no marked differences between the actions of these A₁- and A₂-selective Ado receptor agonists on the cardiovascular system *in vivo* were apparent. Difficulties therefore exist in the application of *in vitro* Ado receptor selectivity data to the prediction of the cardiovascular effects of Ado agonists *in vivo*.

Keywords: Adenosine receptors; haemodynamics; myocardial contractility; AV nodal conduction; adenosine 5'-monophosphate; N⁶-cyclohexyladenosine; 5'-N-ethylcarboxamidoadenosine; 2-phenylaminoadenosine

Introduction

Adenosine (Ado) is an endogenous nucleoside that participates in a multitude of biochemical and physiological processes throughout the body. In the cardiovascular system, Ado affects both vascular conductance (see review by Collis, 1989) and cardiac function (see review by Belardinelli *et al.*, 1989). It is used clinically in the diagnosis and management of some forms of cardiac dysrhythmias (DiMarco *et al.*, 1983) and for the induction of deliberate hypotension during anaesthesia (Sollevi *et al.*, 1984). Ado effects are mediated by two main subtypes of cell-surface receptors classified as A₁ or A₂ based on the relative pharmacological potencies of a series of reference Ado agonists (Linden, 1991). Based mostly on studies *in vitro*, A₁-selective agonists are considered to generate negative inotropic, chronotropic and dromotropic responses (Evans *et al.*, 1982; DiMarco *et al.*, 1983; Romano *et al.*, 1991) that would tend to reduce the oxygen demand of the myocardium. A₂-mediated responses include coronary vasodilatation (Berne, 1980; King *et al.*, 1990) and inhibition of platelet aggregation (Quattrin *et al.*, 1988) and neutrophil function (Cronstein *et al.*, 1985) and would therefore tend to preserve or increase myocardial oxygen supply. Ado-induced changes in myocardial efficiency (Headrick & Willis, 1989) and energy substrate utilization (Finegan *et al.*, 1992) have also been demonstrated.

The marked systemic vasodilatation resulting from Ado receptor stimulation may be of therapeutic benefit in the

management of acute, low output cardiac failure. We have shown previously (Finegan *et al.*, 1990), in normal anaesthetized dogs, that afterload reduction due to Ado receptor-mediated vasodilatation, increases cardiac output more than equieffective afterload reducing doses of sodium nitroprusside (SNP). A similar result was also observed in a canine model of acute left ventricular dysfunction (Finegan & Clanachan, 1991).

Potential advantages of Ado receptor agonists in this role include inhibition of atrial and ventricular automaticity (Szentmiklosi *et al.*, 1980; Wainwright & Parratt, 1988), anti-renin activity (Lagerkranser *et al.*, 1989) and protection of cardiac muscle from ischaemic damage (Olafsson *et al.*, 1987; Babbitt *et al.*, 1989; Pitarys *et al.*, 1991) in addition to their potent vascular smooth muscle relaxant properties (Collis, 1989).

It is not clear what balance of A₁ to A₂ activity would be ideal. A₂ receptor stimulation is required for vasodilatation and afterload reduction while A₁ stimulation, although apparently responsible for cardioprotection (Liu *et al.*, 1991) and attenuating reflex tachycardia, may, if excessive, cause depression of myocardial contractility or blockade of atrio-ventricular (AV) conduction.

Considerable data on the selectivity of Ado agonists in CNS tissues have been derived from receptor binding assays *in vitro*, but the relevance of these classification systems to the cardiovascular effects of Ado agonists *in vivo* is unclear. Indeed, some reports have indicated that Ado receptors involved in canine sino-atrial (SA) nodal function *in vivo*

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have an unusual order of agonist potency that does not conform to the conventional A_1 receptor (Belloni *et al.*, 1989a) identified from tissue and binding studies *in vitro*.

This study was designed to examine in detail the effects of selective Ado receptor agonists on haemodynamics and myocardial contractility *in vivo* and to determine if their classification based on *in vitro* methods is applicable to the prediction of their cardiovascular properties *in vivo*.

Methods

This study was approved by the Health Sciences Animal Care Committee of the University of Alberta. Experiments were performed on healthy mongrel dogs ($n = 28$) of either sex weighing between 24 and 28 kg (mean 26.2 kg). Animals were anaesthetized with pentobarbitone (30 mg kg^{-1}) and ventilated with an O_2 -enriched air mixture to maintain arterial O_2 tension greater than 120 mmHg. Anaesthesia was maintained by a constant infusion ($4 \text{ ml kg}^{-1} \text{ h}^{-1}$) of fentanyl ($20 \text{ } \mu\text{g kg}^{-1} \text{ h}^{-1}$), pentobarbitone ($3 \text{ mg kg}^{-1} \text{ h}^{-1}$) and pancuronium ($60 \text{ } \mu\text{g kg}^{-1} \text{ h}^{-1}$) in 0.9% NaCl following loading doses of fentanyl ($40 \text{ } \mu\text{g kg}^{-1}$) and pancuronium ($80 \text{ } \mu\text{g kg}^{-1}$).

The right external jugular vein was cannulated for passage of a pulmonary artery thermodilution catheter (Swan-Ganz) for determination of cardiac output (average of 3 individual measurements), pulmonary artery pressure (PAP) and central venous pressure (CVP) and for fluid loading (10 ml kg^{-1} of 6% Dextran 70 in 0.9% NaCl) and anaesthetic infusion. A triple lumen catheter was inserted in the left external jugular vein for infusion of test drugs. A catheter was placed in the aortic arch via the left femoral artery to measure aortic pressure and allow blood sampling at regular intervals for determination of blood gasses, pH and electrolytes. Normal CO_2 tension was maintained by continuous monitoring of end-tidal CO_2 . Acidosis and hypokalaemia were corrected, when required, by infusion of sodium bicarbonate and potassium chloride, respectively. The right femoral artery was cannulated for insertion of a precalibrated, high fidelity (Millar) pressure transducer tipped catheter into the left ventricle (LV) for measurement of LV pressure.

Following a left lateral thoracotomy, two piezoelectric crystals (5 MHz, 1.5–2.0 mm diameter) were inserted into the LV subendocardium along its long axis to allow continuous measurement of myocardial segment length (SL) and an electromagnetic flow probe was positioned on the left anterior descending coronary artery (LAD) proximal to the first diagonal branch for the continuous measurement of LAD blood flow. Positive end-expiratory pressure of 5 cm H_2O was applied to the airway to prevent atelectasis.

Data Collection

Heart rate (HR), ECG, systolic, diastolic and mean (MAP) arterial pressure, LAD blood flow, LV pressure, and SL were continuously recorded. For the assessment of AV nodal conduction intervals (AV_i), stimulus artifact-R intervals were averaged from 5 consecutive cardiac cycles during 5 s periods of atrial pacing at 2.5 Hz (a bipolar pacing electrode was placed in the right atrium via the left femoral vein). Myocardial contractility was assessed by E_{max} , which was determined from the end-systolic pressure segment length (ESPSL) relationship (Nozawa *et al.*, 1988). E_{max} was determined at appropriate times of the protocol when cardiac preload was altered by inflation of a 40 ml 22F balloon catheter that was placed in the inferior vena cava. Inflation was maintained for only 4 to 6 cardiac cycles to minimize potential baroreceptor-mediated changes in the inotropic state of the LV. Stroke index (SI), cardiac index (CI), coronary vascular conductance (CVC) and pulmonary (PVC) and systemic vascular conductance (SVCI) indices were calculated from standard formulae (Keefer & Barash, 1985).

Drug administration protocol

Separate groups of dogs were used to evaluate the cardiovascular effects of N⁶-cyclohexyladenosine (CHA), 2-phenyl-aminoadenosine (PAA) and 5'-N-ethylcarboxamido-adenosine (NECA). Each animal within a drug treatment group received all doses of a particular agonist. The effects of adenosine 5'-monophosphate (AMP) were also studied, but because of its extremely short plasma half-life, it was administered as continuous infusions of 200, 400 and $1000 \text{ } \mu\text{g kg}^{-1} \text{ min}^{-1}$ for 10 min per dose level.

Initial baseline values were recorded 30 min following completion of instrumentation and repeated every 10 min for 30 min to assess the stability of the experimental preparation. Haemodynamic variables were then recorded in the steady state after 10 min of infusion of AMP or 10 min after the slow (1 min) intravenous administration of each dose level of each Ado analogue.

Cardiac autonomic blockade

In separate groups of animals, the effects of AMP, CHA and PAA were determined following cardiac autonomic blockade. After baseline measurements, propranolol (1 mg kg^{-1}) and atropine (0.2 mg kg^{-1}) were administered intravenously and the blockade was maintained by constant infusion of propranolol ($0.5 \text{ mg kg}^{-1} \text{ h}^{-1}$) and atropine ($0.05 \text{ mg kg}^{-1} \text{ h}^{-1}$). Completeness of blockade was confirmed by the absence of a cardiac response to isoprenaline and acetylcholine. AMP, CHA and PAA were then administered as described above.

Statistical analysis

Cardiovascular data in the text are means $\pm$ s.e. and drug dosages are expressed as geometric means with 95% confidence limits. Statistical analysis was performed by means of analysis of variance for repeated measures and between group comparisons were made by the Mann-Whitney U test. Differences were judged to be statistically significant when $P < 0.05$.

Drugs

AMP and atropine were dissolved in 0.9% saline. Stock solutions of propranolol, PAA and NECA were prepared with saline containing 10% dimethylsulphoxide (DMSO). CHA was dissolved initially in 95% ethanol and then diluted in saline. Ado analogues were purchased from Research Biochemicals, Inc. and all other agents were obtained from Sigma Chemical Co.

Results

Baseline cardiovascular parameters in control animals are shown in Table 1. It should be noted that the addition of fentanyl to the anaesthetic regimen resulted in values for HR that approximated those of normal conscious animals ($90 \text{ beats min}^{-1}$) and are unlike those reported during anaesthesia with pentobarbitone alone ($150 \text{ beats min}^{-1}$).

Haemodynamic effects of AMP in control animals

Graded infusions of AMP (Table 1) produced rapid, step-wise reductions in MAP that were due to systemic vasodilatation as evidenced by marked increases in SVCI. HR and CI increased; the greater proportional increase in CI indicated that SI also increased. As expected, AMP elevated coronary blood flow by up to 4 fold. CVC, a parameter that is independent of mean arterial pressure, and hence coronary perfusion pressure, was also significantly increased by up to 5.5 fold. Despite the increase in CI, there was only a slight, but significant increase in PAP due to a concomitant increase

Table 1 Cardiovascular effects of intravenous infusions of AMP in fentanyl-pentobarbitone anaesthetized dogs

	Baseline	200	AMP infusion rate ($\mu\text{g kg}^{-1} \text{min}^{-1}$) 400	1000	P
MAP	112 $\pm$ 3	102 $\pm$ 2	98 $\pm$ 2	90 $\pm$ 3	<0.0001
SVCI	360 $\pm$ 20	560 $\pm$ 50	720 $\pm$ 50	940 $\pm$ 60	<0.0001
HR	89 $\pm$ 3	111 $\pm$ 5	143 $\pm$ 9	163 $\pm$ 8	<0.0001
CI	3.0 $\pm$ 0.1	4.2 $\pm$ 0.3	5.2 $\pm$ 0.3	6.2 $\pm$ 0.3	<0.0001
SI	34 $\pm$ 1	38 $\pm$ 2	37 $\pm$ 2	39 $\pm$ 2	<0.05
CBF	35 $\pm$ 2	58 $\pm$ 17	115 $\pm$ 17	146 $\pm$ 14	<0.0001
CVC	5.0 $\pm$ 0.5	9.2 $\pm$ 2.8	19.3 $\pm$ 3.1	27.5 $\pm$ 3.2	<0.0001
LVEDP	10 $\pm$ 1	9 $\pm$ 1	8 $\pm$ 1	7 $\pm$ 1	<0.05
CVP	5.6 $\pm$ 0.3	5.3 $\pm$ 0.3	5.3 $\pm$ 0.3	5.3 $\pm$ 0.3	<0.05
PAP	17 $\pm$ 1	19 $\pm$ 1	20 $\pm$ 1	22 $\pm$ 1	<0.0001
PRVI	220 $\pm$ 20	200 $\pm$ 20	190 $\pm$ 10	170 $\pm$ 10	<0.0001
LVS WI	48.1 $\pm$ 2.9	48.3 $\pm$ 2.5	44.8 $\pm$ 2.6	42.0 $\pm$ 1.5	<0.01
RVS WI	5.2 $\pm$ 0.2	6.8 $\pm$ 0.4	7.6 $\pm$ 0.5	8.5 $\pm$ 0.5	<0.0001
RPP	11.7 $\pm$ 0.3	13.8 $\pm$ 0.6	17.5 $\pm$ 1.0	19.1 $\pm$ 1.1	<0.0001
AV _i	151 $\pm$ 7	121 $\pm$ 7	108 $\pm$ 8	101 $\pm$ 7	<0.0001

Values are means $\pm$ s.e., $n = 15$. Data are shown for baseline conditions and during three graded infusions of AMP on MAP, mean arterial pressure (mmHg); SVCI, systemic vascular conductance index ($\text{dyne s cm}^{-5} \text{m}^2$)⁻¹; HR, heart rate (beats min^{-1}); CI, cardiac index ($\text{l min}^{-1} \text{m}^{-2}$); SI, stroke index (ml m^{-2}); CBF, coronary blood flow (ml min^{-1}); CVC, coronary vascular conductance ($\text{dyne s cm}^{-5} \text{m}^2$)⁻¹; LVEDP, left ventricular end diastolic pressure (mmHg); CVP, central venous pressure (mmHg); PAP, mean pulmonary artery pressure (mmHg); PVRI, pulmonary vascular resistance index ($\text{dyne s cm}^{-5} \text{m}^2$); LVS WI, left ventricular stroke work index ($\text{g-m m}^{-2} \text{beat}^{-1}$); RVS WI, right ventricular stroke work index ($\text{g-m m}^{-2} \text{beat}^{-1}$); RPP, rate pressure product (mmHg beats min^{-1}); AV_i, atrio-ventricular nodal conduction (PR) interval (ms). *P* values were determined by analysis of variance for repeated measures.

in PVC. CVP was unchanged. Calculation of indices of myocardial work (Table 1) showed that rate-pressure product (RPP) and right ventricular stroke work (RVS WI) were elevated by AMP whereas left ventricular stroke work (LVS WI) was reduced by AMP.

Haemodynamic effects of AMP after cardiac autonomic blockade

Relative to baseline values (Table 1), cardiac autonomic blockade with propranolol and atropine significantly increased HR (to 154 ± 9 , $P < 0.001$) and altered the other cardiovascular parameters that are HR-dependent; namely, it increased CI (to 3.6 ± 0.4 , $P < 0.01$) and RPP (to 21.5 ± 1.3 , $P < 0.001$) and depressed AV_i (to 115 ± 7 , $P < 0.01$) and SI (to 23 ± 3 , $P < 0.0001$). There were no alterations in the other baseline values. The graded doses of AMP, administered during cardiac autonomic blockade, reduced MAP ($P < 0.0001$) and increased SVCI ($P < 0.0001$). Although HR was slightly depressed ($P < 0.05$) by AMP, CI was increased ($P < 0.001$) due to a significant increase in SI of about 60% ($P < 0.0001$). While coronary blood flow was elevated ($P < 0.05$) by up to about 2 fold, CVC was increased ($P < 0.0001$) by up to 4.5 fold to a value similar to that in unblocked dogs. Myocardial work, as assessed by RPP was reduced ($P < 0.001$) by AMP, while RVS WI was increased ($P < 0.001$) and LVS WI remained unchanged ($P < 0.05$).

Haemodynamic effects of Ado analogues in control animals

Each of the Ado analogues, CHA, PAA and NECA decreased MAP (Figure 1). As an index of their relative hypotensive potency, doses that caused a 20 mmHg decrease in MAP (ED₋₂₀) were calculated. Geometric mean (with 95% confidence limits) were as follows (nmol kg^{-1}); CHA, 270 (90 to 810), NECA, 7.7 (1.9 to 31); PAA, 6.6 (1.3 to 33) indicating an order of hypotensive effectiveness of PAA = NECA $35 \times >$ CHA. All agonists increased SVCI (Figure 1); mean ED₊₃₀₀ doses were CHA, 630 (380 to 1030), NECA, 7.1 (4.1 to 12) and PAA, 22 (11 to 42) which indicates an order of effectiveness as systemic vasodilators of

NECA $3.1 \times >$ PAA $29 \times >$ CHA. HR (Figure 2) was elevated by each of the agonists with an order of potency that was similar to that for the changes in MAP; ED₊₅₀ doses (nmol kg^{-1}) were CHA, 770 (545 to 1080), NECA, 5.1 (2.3 to 11) and PAA 11 (6.2 to 20). CI (Figure 2) was also increased but SI was unchanged.

Coronary blood flow was increased by each of the Ado analogues (Figure 3) due to increases in CVC. Doses (nmol kg^{-1}) causing increases in CVC of 10 units (ED₊₁₀) were as follows; CHA 290 (160 to 540), NECA 4.2 (2.7 to 6.4), PAA 18 (7.6 to 42) yielding an order of effectiveness of NECA $4 \times >$ PAA $22 \times >$ CHA. NECA had a marked biphasic effect on coronary blood flow with higher doses tending to cause smaller alterations. This was probably due to marked reductions in MAP, and hence coronary perfusion pressure, with the higher doses; no biphasic responses were observed in the changes in CVC (Figure 3). CHA, NECA and PAA also produced dose-dependent increases in AV nodal excitation, as indicated by reductions in AV_i (Figure 4).

Haemodynamic effects of Ado analogues after cardiac autonomic blockade

After cardiac autonomic blockade, the Ado analogues, CHA and PAA still caused marked decreases in MAP. ED₋₂₀ values (nmol kg^{-1}) for CHA and PAA were 79 (46 to 140) and 2.4 (0.5 to 10), respectively. The hypotensive potency ratio of PAA and CHA (33 fold) was similar to that in the unblocked condition (Figure 1). Also, their potency ratio (21 fold) for increases in SVCI (ED₊₃₀₀ values for CHA and PAA were 500 (300 to 820) and 23 (12 to 46) nmol kg^{-1} , respectively) was similar to that in the unblocked condition (Figure 1). The increases in HR and CI, that accompanied drug-induced hypotension in control animals, were absent following cardiac autonomic blockade (Figure 2). At the highest dose of CHA studied, maximal increases in SVCI were demonstrable that were accompanied by only small decreases in HR. Both CHA and PAA increased coronary blood flow (Figure 3) and the changes in CVC (ED₊₁₀ values of 234 (208 to 263) and 5.7 (4.3 to 7.5) nmol kg^{-1} , respectively) were similar to those in control animals (Figure 3). No

changes in AV_i were demonstrable with the doses of CHA or PAA used in this study (Figure 4).

Comparison of the effects of Ado analogues at equi-effective vasodilator doses

As a further probe of the selectivity of these Ado analogues *in vivo*, changes in HR and AV_i (presumed A_1 -mediated events) were compared at equieffective vasodilator (a

presumed A_2 -mediated event) dosages (Figure 5). Reflex tachycardia, that was observed with each of the analogues in control animals, was greatest with PAA. After cardiac autonomic blockade, no consistent alterations in HR occurred. No differences between the effects of CHA and PAA on AV_i were seen; with both agents (reflex) reductions in AV_i were observed in control animals, but following cardiac autonomic blockade AV_i remained constant.

The relative selectivity between effects on the coronary and

Figure 1 Dose-response relationships for the effects of adenosine (Ado) receptor agonists on mean arterial pressure (mmHg, a,b) and systemic vascular conductance index (conductance units, CU, $\text{dyne s cm}^{-5} \text{m}^{-2}$)⁻¹, c,d) in control animals (a,c) or following cardiac autonomic blockade (b,d). Graded doses (ordinates; $\mu\text{g kg}^{-1}$, log scale) of N^6 -cyclohexyladenosine ($\blacksquare$), 2-phenylaminoadenosine ($\bullet$) and 5'-N-ethylcarboxamidoadenosine ($\blacklozenge$) were administered intravenously and responses recorded after 10 min. Values are mean changes from baseline $\pm$ s.e.(vertical bars), $n > 5$ observations.

Figure 2 Dose-response relationships for the effects of adenosine (Ado) receptor agonists on heart rate (beats, min^{-1} , a,b) and cardiac index ($\text{l min}^{-1} \text{m}^{-2}$, c,d) in control animals (a,c) or following cardiac autonomic blockade (b,d). Graded doses (ordinates; $\mu\text{g kg}^{-1}$, log scale) of N^6 -cyclohexyladenosine ($\blacksquare$), 2-phenylaminoadenosine ($\bullet$) and 5'-N-ethylcarboxamidoadenosine ($\blacklozenge$) were administered intravenously and responses recorded after 10 min. Values are mean changes from baseline $\pm$ s.e.(vertical bars), $n > 5$ observations.

systemic vasculature were assessed by plotting agonist-induced increases in CVC against increases in SVCI (Figure 6). The resulting relationships were identical for CHA, NECA and PAA in control animals and for CHA and PAA in animals following cardiac autonomic blockade. Consequently, preferential coronary, relative to systemic, vasodilator activity of either of these Ado receptor agonists was not apparent.

Figure 3 Dose-response relationships for the effects of adenosine (Ado) receptor agonists on coronary artery blood flow (ml min⁻¹, a,b) and coronary vascular conductance (conductance units, CU, (dyne s cm⁻⁵ m⁻²)⁻¹, c,d) in control animals (a,c) or following cardiac autonomic blockade (b,d). Graded doses (ordinates: μg kg⁻¹, log scale) of N⁶-cyclohexyladenosine (■), 2-phenylaminoadenosine (●) and 5'-N-ethylcarboxamidoadenosine (◆) were administered intravenously and responses recorded after 10 min. Values are mean changes from baseline ± s.e.(vertical bars), *n* > 4 observations.

Effects of AMP and Ado analogues on myocardial contractility (E_{max})

In order to assess the direct effects of AMP and the Ado receptor agonists on myocardial contractility, independent of changes in HR and cardiac loading, E_{max} values were estimated from LV pressure-dimension loops (Table 2) following equieffective vasodilator doses. AMP significantly in-

Figure 4 Dose-response relationships for the effects of adenosine (Ado) receptor agonists on LV stroke work index (g-m beat⁻¹, a,b) and AV_i (paced PR interval, ms, c,d) in control animals (a,c) or following cardiac autonomic blockade (b,d). Graded doses (ordinates: μg kg⁻¹, log scale) of N⁶-cyclohexyladenosine (■), 2-phenylaminoadenosine (●) and 5'-N-ethylcarboxamidoadenosine (◆) were administered intravenously and responses recorded after 10 min. Values are mean changes from baseline ± s.e.(vertical lines), *n* > 5 observations.

creased $E_{\max}$, but no significant changes were demonstrable with the low numbers of animals included in the CHA, NECA and PAA groups. Following cardiac autonomic blockade, PAA significantly increased this index of myocardial contractility whereas the other agents had no effect.

Discussion

We found that the predominant action of AMP and the three Ado receptor agonists (CHA, PAA, and NECA) was systemic and coronary vasodilatation. The reduction in MAP elicited reflex increases in HR, CI and AV nodal conduction velocity. Even after cardiac autonomic blockade with atropine and propranolol, and when reflex activation of the

heart was eliminated, still only minor differences between CHA and PAA were apparent. CHA is an effective vasodilator, but less potent than PAA or NECA, and each agonist elicited similar cardiac effects at equieffective vasodilator dosages. N^6 -[(R)-1-methyl-2-phenylethyl]adenosine (R-PIA) elicited responses that were similar to CHA ($n=3$, data not shown). Thus, despite marked selectivity of these agonists in *in vitro* assays, no obvious differences in their cardiovascular selectivity were demonstrable *in vivo*.

Figure 5 Relationships between drug-induced changes in heart rate (beats min^{-1} , a,b) and AV_i (paced PR interval, ms, c,d) and changes in systemic vascular conductance index (conductance units, CU, ($\text{dyne s cm}^{-5} \text{ m}^{-2}$) $^{-1}$) in control animals (a,c) or following cardiac autonomic blockade (b,d). Graded doses of N^6 -cyclohexyladenosine (■), 2-phenylaminoadenosine (●) and 5'-N-ethylcarboxamido-adenosine (◆) were administered intravenously and responses recorded after 10 min. Values are mean $\pm$ s.e., $n=5$ subjects.

Figure 6 Relationships between drug-induced changes in coronary vascular conductance and changes in systemic vascular conductance index (conductance units, CU, ($\text{dyne s cm}^{-5} \text{ m}^{-2}$) $^{-1}$) in control animals (a) or following cardiac autonomic blockade (b). Graded doses of N^6 -cyclohexyladenosine (■), 2-phenylaminoadenosine (●) and 5'-N-ethylcarboxamido-adenosine (◆) were administered intravenously and responses recorded after 10 min. Values are mean $\pm$ s.e., $n=5$ subjects.

Table 2 Effect of equieffective vasodilator doses of adenosine 5'-monophosphate (AMP) and adenosine (Ado) analogues on myocardial contractility ($E_{\max}$, mmHg mm^{-1}) in anaesthetized dogs in the absence (control) or presence of cardiac autonomic blockade

	Control	Blocked
Baseline	32 $\pm$ 3 (15)	45 $\pm$ 6 (8)
AMP	*50 $\pm$ 5 (15)	49 $\pm$ 8 (6)
CHA	49 $\pm$ 8 (2)	39 $\pm$ 6 (4)
NECA	39 $\pm$ 8 (3)	ND
PAA	65 $\pm$ 15 (3)	*95 $\pm$ 14 (4)

Values are shown for equieffective systemic vasodilating doses of AMP ($400 \mu\text{g kg}^{-1} \text{ min}^{-1}$), N^6 -cyclohexyladenosine (CHA, $200 \mu\text{g kg}^{-1}$), 5'-N-ethylcarboxamido-adenosine (NECA, $2 \mu\text{g kg}^{-1}$) and 2-phenylaminoadenosine (PAA, $10 \mu\text{g kg}^{-1}$) and are means $\pm$ s.e. (n observations). Significant differences from baseline are indicated by *. ND indicates not done.

Table 3 Relative potencies of N⁶-cyclohexyladenosine (CHA), 5'-N-ethylcarboxamidoadenosine (NECA) and 2-phenylamino-adenosine (PAA) *in vitro* and *in vivo*

	CHA	NECA	PAA
<i>In vitro</i> (nM) data from rat striatal membranes (Bruns <i>et al.</i> , 1986)			
A ₁ affinity	1.3 (1)	6.3 (0.21)	560 (0.002)
A ₂ affinity	510 (1)	10 (51)	120 (4.3)
A ₂ /A ₁ selectivity	390	1.6	0.21
<i>In vivo</i> (nmol kg ⁻¹)			
MAP (ED ₋₂₀)	270 (1)	Control 7.7 (35)	6.6 (41)
SVCI (ED ₊₃₀₀)	625 (1)	7.1 (88)	22 (28)
CVC (ED ₊₁₀)	293 (1)	4.2 (70)	18 (16)
<i>In vivo</i> (nmol kg ⁻¹) Cardiac Autonomic Blockade			
MAP (ED ₋₂₀)	80 (1)	—	2.4 (34)
SVCI (ED ₊₃₀₀)	500 (1)	—	23 (21)
CVC (ED ₊₁₀)	234 (1)	—	5.7 (41)

For *in vivo* data, doses of agonist (nmol kg⁻¹) that elicited decreases in MAP of 20 mmHg (ED₋₂₀) and increases in SVCI of 300 units (ED₊₃₀₀) and CVC of 10 units (ED₊₁₀) were determined from individual dose-response curves for each animal. Values in Table represent geometric means from 5 experiments. Values in parentheses indicate potency relative to CHA. For abbreviations, see footnote to Table 1.

Definitive classification of Ado receptors in cardiovascular tissues has not yet been accomplished due to the relatively small population of receptors in the various parts of the heart and the lack of high affinity and selective Ado receptor antagonists. Furthermore, determination of the functional effects of these receptor subtypes has been difficult due to the often contrasting results obtained with the use of different experimental preparations. Nevertheless, current attempts revolve around the use of the presently available selective A₁ and A₂ agonists. Studies using receptor binding methodology (e.g., Bruns *et al.*, 1986) have shown that many of the N⁶-substituted Ado analogues are A₁ selective, such as CHA (A₁/A₂ potency ratio: 390) which we used to determine the functional effects of A₁ receptor agonists. To study the effects of A₂ receptor activation, we used the A₂ selective compound, PAA (CV-1808, A₁/A₂ potency ratio: 0.2), which is of particular interest since there is some evidence that it possesses coronary selectivity (Kawazoe *et al.*, 1980). The potent, but non-selective 5'-substituted analogue NECA (Bruns *et al.*, 1986) was used to elucidate the cardiovascular effects arising from simultaneous A₁ and A₂ receptor activation and to compare its effects to those of its parent compound Ado. Ado was administered as the soluble prodrug, AMP, which is rapidly dephosphorylated to Ado by 5'-nucleotidase located on the plasma membrane of many cells, including vascular endothelial cells (Pelleg *et al.*, 1987; Coade & Pearson, 1989).

Numerous studies have shown Ado-induced reductions in both the rate and force of cardiac contractions (Evans *et al.*, 1982), depression of A-V nodal conduction velocity (DiMarco *et al.*, 1983) and inhibition of atrial and ventricular automaticity (Szentmiklosi *et al.*, 1980; Wainwright & Parratt, 1988). These effects are mediated by the A₁ subclass of Ado receptors and are easily demonstrable with A₁-selective agonists such as CHA (Haleen & Evans, 1985; Hamilton *et al.*, 1987; Oei *et al.*, 1988) and R-PIA (Caparrotta *et al.*, 1987; Jahnel & Nawrath, 1989; Shryock *et al.*, 1989). A₁-mediated responses, including negative chronotropic and dromotropic actions as well as cardioprotection, are also apparent in canine hearts, although some evidence suggests that dogs possess an unusual form of A₁ receptor. Consequently it was surprising that in this study, an effect of each of these agonists was an apparent cardiac excitation. This was mediated by baroreceptor reflex activation in response to drug-induced hypotension, for in the presence of cardiac autonomic blockade, cardiac excitation was absent.

Nevertheless, even under conditions of autonomic blockade, marked cardiac depression was not observed, even with the supposedly A₁ selective agonist, CHA, at doses that caused maximal systemic vasodilatation. This absence of A₁ receptor responses may be due to a lower A₁ receptor density and/or less efficient transduction mechanisms relative to A₂ receptor systems.

A-V nodal blockade can be easily demonstrated in dogs (Belhassen & Pelleg, 1984) and man (DiMarco *et al.*, 1983) with bolus dosing protocols (high cardiac and lower systemic concentrations of agonist). In this study, the slower drug administration and the measurement of responses under equilibrium conditions 10 min after drug administration, probably contributed to the observed lack of significant cardiac depression. The drug evaluation procedure used here, although subject to potential differences in the tissue partitioning of each agonist, is more appropriate than bolus dosing for the assessment of receptor selectivity among different cardiovascular effector systems.

A second factor that may have contributed to the observed lack of cardiac depression was the drug combination used for anaesthesia. Our animals were anaesthetized with fentanyl and pentobarbitone and had a basal HR of 89 beats min⁻¹, a value close to that in conscious dogs. Most of the *in vivo* studies that have demonstrated Ado receptor-mediated depression of cardiac rate and/or contractility have usually been in animals anaesthetized with pentobarbitone alone. In such canine preparations, basal HR is approximately 150 beats min⁻¹, indicating an autonomic imbalance (consisting of a relative sympathetic excitation) relative to the conscious state (Belloni *et al.*, 1989b). Indeed, in conscious man (Biagioni *et al.*, 1987) and rabbits (Chen & Clanachan, unpublished) intravenous Ado causes tachycardia. The involvement of an intact baroreceptor reflex response may even give the appearance of a selective Ado-induced tachycardia (Fuller *et al.*, 1987) because increased efferent sympathetic activity, that attempts to increase HR, systemic vascular resistance and cardiac output, may be able to compensate adequately for concomitant Ado-induced systemic vasodilatation and hypotension.

In this study, AMP-induced hypotension elicited reflex increases in myocardial contractility in normal anaesthetized animals. After autonomic blockade, E_{max} was unaltered by AMP and by CHA. PAA, however, appeared to stimulate myocardial contractility as it significantly increased E_{max}. The

mechanism for this positive inotropic action was not investigated in this study but may have been related to 'Greggs phenomenon', where increased coronary blood flow enhances LV contractile function (Goto *et al.*, 1991). If this were the case, CHA and AMP, that also caused similar increases in coronary blood flow, would also be expected to increase $E_{\max}$. The lack of any such increase in $E_{\max}$ with these agonists is consistent with a mild direct negative inotropic action that was compensated for by the Gregg effect. An alternate explanation for the PAA-induced increase in $E_{\max}$ is that it arose from stimulation of A_2 receptors that have been shown to be present on myocytes (Behnke *et al.*, 1990) and that may be capable of eliciting small positive inotropic responses (Bruckner *et al.*, 1985).

Comparison of the relative potencies of the agonists used in this study (Table 3) shows that, for A_2 -mediated responses *in vivo*, e.g., vasodilatation, NECA had a potency ratio to CHA (ranging from 38 fold for CVC to 88 fold for SVRI) that was similar to that observed in *in vitro* binding assays (approximately 51 fold). However, for PAA, significantly greater potency ratios relative to CHA were found *in vivo* (ranging from 13 to 29 for CVC and SVCI, respectively) than that reported previously based on *in vitro* assays (4 to 5). This apparent greater A_2 -selectivity *in vivo* may be related to additional activities of this substance such as nucleoside transport (NT) inhibition (Chiba & Watanabe, 1983). Other studies have demonstrated that PAA has an affinity for NT inhibitory sites in endothelial cells (K_i value of about 200 nM) that is comparable to its affinity for A_2 receptors (Clanachan, unpublished).

An equivalent comparison of relative potencies of these agonists for A_1 receptors *in vivo* and *in vitro* cannot be made

because no clear A_1 responses were demonstrable *in vivo*.

Some Ado agonists, e.g., PAA, have been reported to exert coronary-selective vasodilatation (Kawazoe *et al.*, 1980). Comparison of changes in CVC with changes in SVCI showed these relationships to be identical for PAA, CHA and NECA, either before, or after cardiac autonomic blockade. Thus, using experimental conditions that avoid pharmacokinetic conditions favouring cardiac and coronary responses, no coronary selectivity was observed.

Our research shows that both systemic and coronary vasodilatation without A-V blockade and direct depression of LV contractility ($E_{\max}$) is demonstrable when agonists are administered slowly and allowed to equilibrate among cardio-vascular effector tissues. These results are of importance in the design and evaluation of new Ado agonists for cardiovascular indications. Sufficient Ado receptor-mediated systemic vasodilatation and afterload reduction to reduce cardiac workload and O_2 consumption, in combination with direct A_1 receptor-mediated cardioprotection (Liu *et al.*, 1991), should be obtainable without adverse negative inotropic and dromotropic effects.

While classification of Ado receptor subtypes can be performed in isolated tissues or with binding affinities *in vitro*, functional selectivity of Ado agonists *in vivo* cannot be predicted easily because of several interfering mechanisms, including compensation by autonomic reflexes, relative density of receptors, drug inactivation rates and efficiency of transduction mechanisms.

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