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Ischaemia/reperfusion selectively attenuates coronary vasodilatation to an adenosine A_2- but not to an A_1-agonist in the dog

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1 The effects of myocardial ischaemia/reperfusion were tested on the coronary vasorelaxant responses to agonists selective for the A_1 and A_2 adenosine receptor subtypes in the dog. The left anterior descending (LAD) coronary artery was occluded distal to the first diagonal branch. The occlusion was maintained for 1 h, followed by 1 h of reperfusion.

2 In the first series of experiments, LAD and circumflex arteries were excised and contracted with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). Ischaemia/reperfusion did not significantly alter the vasorelaxation produced by either sodium nitroprusside (endothelium-independent) or acetylcholine (endothelium-dependent). The A_1 selective agonist, cyclopentyladenosine (CPA), produced coronary vasorelaxation in both normally perfused vessels and vessels subjected to ischaemia/reperfusion. In contrast, the relaxation produced by the A_2-selective agonist N6-{2-(3,5-dimethoxyphenyl)-2-(2-methylphenyl) ethyl} adenosine (DPMA) was significantly attenuated by ischaemia/reperfusion (14 fold shift in EC_{50}).

3 In the second series of experiments, coronary blood flow was increased by administration of the A_1 and A_2 agonists before and after ischaemia/reperfusion of the LAD in anaesthetized dogs. Both compounds dose-dependently increased coronary blood flow. The slopes of the dose-response functions to CPA or DPMA were not significantly altered in the normally perfused circumflex vascular bed. Similarly, the CPA dose-response function in the LAD was unaltered by ischaemia/reperfusion. However, the slope of the coronary vasodilator response to the A_2 agonist was significantly reduced following ischaemia/reperfusion of the LAD.

4 We conclude that ischaemia/reperfusion reduces responsiveness to an adenosine A_2 receptor subtype agonist, but not an A_1 receptor subtype agonist. These data confirm the independent nature of A_1- and A_2-mediated coronary vasodilatation.

Keywords: Cyclopentyladenosine; DPMA; endothelium; ischaemia; reperfusion; adenosine receptors; coronary blood flow; haemodynamics; vascular resistance

Introduction

Adenosine has been proposed as an important metabolic regulator of coronary blood flow (Berne, 1963). Purinoceptors have been classified as P_1 or P_2 receptors. Adenosine exhibits an almost complete selectivity for the P_1 receptor which can be further differentiated into A_1, A_2, and A_3 receptor subtypes (Van Calker *et al.*, 1979; Londos *et al.*, 1983; Zhou *et al.*, 1993). Selective agonists for A_1 and A_2 receptor sub-types have revealed that A_1 receptors mediate negative chronotropic and renal vasoconstrictor effects, while A_2 agonists primarily dilate the mesenteric and cerebral vascular beds (for review see Olsson & Pearson, 1990). We have been exploring the coronary vasorelaxant/vasodilator effects of an A_1 selective agonist, cyclopentyladenosine (CPA) and an A_2 selective agonist, N6-{2-(3,5-dimethoxyphenyl)-2- (2-methylphenyl)ethyl} adenosine (DPMA) (Cox *et al.*, 1991; Merkel *et al.*, 1992).

In addition to producing myocardial infarction, occlusion and reperfusion of a coronary artery produces changes in the remaining viable myoctyes, including depression of contractility, electrophysiological changes, and alteration of metabolic processes (Heyndrickx *et al.*, 1975; Jennings *et al.*, 1985; Kloner *et al.*, 1983; Bolli *et al.*, 1988). In clinical situations, this reperfusion injury can occur even when the ischaemic insult may not be so severe as to produce myocardial infarction, such as during percutaneous transluminal angioplasty (Cowley *et al.*, 1984). Recent evidence has suggested that a further effect of ischaemia/reperfusion is to alter reactivity in coronary vascular smooth muscle (Bolli *et al.*, 1990; Pearson *et al.*, 1990; Quillen *et al.*, 1990).

Adenosine and adenosine agonists have been shown to limit infarct size in several models of ischaemia/reperfusion (Lasley *et al.*, 1990; Norton *et al.*, 1992; Toombs *et al.*, 1992; Thornton *et al.*, 1992). In addition, ischaemia has been shown to inhibit the binding of a mixed adenosine agonist to an adenosine receptor identified as the A_2 receptor subtype in rat isolated working hearts subjected to global ischaemia (Zucchi *et al.*, 1992). Further, A_1 and A_2 receptors have been shown to desensitize by independent mechanisms in a vascular smooth muscle cell line (Ramkumar *et al.*, 1991). The present study was designed to define whether physiological stimuli (coronary occlusion followed by reperfusion) applied *in vivo* could produce a functional impairment of A_1- or A_2-mediated vasorelaxation. These experiments were conducted both on isolated coronary vessels and entirely *in vivo*. Further, we defined whether the vasorelaxant responses to endothelium-independent (sodium nitroprusside) or endothelium-dependent (acetylcholine, ACh) agents were altered by the 1 h ischaemia/1 h reperfusion protocol.

Methods

All experiments were conducted in accordance with a protocol approved by the Rhône-Poulenc Rorer Animal Care and Use Committee and conformed to the NIH Guide for the Care and Use of Laboratory Animals. Purpose-bred mongrel dogs (13.0–14.3 kg) were anaesthetized with sodium pentobarbitone (35 mg kg^{-1}, Abbott Laboratories, North Chicago, IL, U.S.A.). Animals were intubated and ventilated with room air. Body temperature was monitored and main-

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tained throughout the experiment. Femoral arterial and venous catheters were implanted and 0.9% saline (0.5 ml min^{-1}, i.v.) was infused throughout the experiment. Electrocardiogram electrodes were placed on the four extremities, with a fifth electrode placed on the ventral midline opposite the apex of the heart. All haemodynamic and electrocardiographic parameters were displayed on a Grass Model 7 polygraph. The chest was opened at the fifth intercostal space and a pericardial cradle established. The LAD coronary artery was isolated, and a micrometer- or screw-driven occluder was placed distal to the first diagonal branch. Generation or release of occlusions took place incrementally over a 10 min period. Ischaemia was maintained for 1 h, followed by a 1 h reperfusion period. The five lead electrocardiogram was analyzed after 5, 15, 30, and 60 min of ischaemia or reperfusion. No pharmacological interventions were used to minimize arrhythmias.

In vitro

At the end of the reperfusion period the animal was exsanguinated and the heart was removed immediately and placed in warm, oxygenated (37°C; 95% O_2, 5% CO_2) Krebs Henseleit buffer of the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25.0 and glucose 10.0 (pH 7.4). Starting approximatly 10 mm distal to the occlusion site, the LAD artery was carefully dissected out, cleaned of fat, blood and adhering tissue and cut into rings 2–3 mm wide. The left circumflex coronary artery was prepared similarly and used as control. Previous studies have shown no difference in vascular reactivity between these two arteries (Suba & Roth, 1987; Van Benthuysen *et al.*, 1987; Pearson *et al.*, 1990). Rings were transferred to water-jacketed tissue baths (10 ml) containing Krebs buffer and were mounted on L-shaped wire hooks between a stainless steel rod and a force transducer (Grass, FT-03) and changes in isometric tension were displayed on a Grass Model 7 Polygraph. Data were electronically captured using a Po-Ne-Mah acquisition system. The optimal lengths (L_o) for rings from the LAD and the circumflex were determined to be 2 g using 80 mM KCl (Ku, 1982). During the equilibration period, buffer was changed every 5 min for the first 15 min, and at 15 min intervals thereafter. Subsequently, tissues were stimulated twice with 36 mM KCl and then with 10 μM prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). After isometric tension had reached a steady state, cumulative doses of vasorelaxants were added to the baths. Subsequent concentrations were added only after tension had reached a new steady state. Each ring was exposed to one vasorelaxant only. Tension was expressed as percentage of maximal $PGF_{2\alpha}$-induced tension.

In vivo

In a second series of experiments, animals were instrumented as described above. In addition, the circumflex and LAD coronary arteries were instrumented with electromagnetic flow probes (Gould Statham Model 2202). A Millar pressure tip transducer was inserted into the left ventricle. Finally, pairs of sonomicrometry crystals (Triton Technology) were implanted in both the normally perfused and ischaemic myocardium. Animals with segment shortening of less than 5.0% during the control period were not included in the analysis. In addition, one animal did not become ischaemic despite complete occlusion of the LAD, and was also not included in the group data (individual data for this animal are provided in results section). Data were displayed on a Grass Model 7 polygraph and captured using a Po-Ne-Mah HD-4 computerized acquisition system.

The protocol involved intravenous administration of 3, 10, and 30 μg kg^{-1} of CPA, followed by 3, 10, and 30 μg kg^{-1} of DPMA. Higher doses were not tested due to the risk of adversely affecting the data by lengthening the protocol and/or downregulating the receptors with exogenous agonist. Intravenous (versus intracoronary) administration ensured that plasma levels of drug were equivalent in all branches of the circumflex and LAD vascular bed. All parameters were allowed to return to, or very near, baseline prior to administering the subsequent dose of adenosine agonist. All values illustrate the response at the time of peak change in coronary blood flow. The entire dosing protocol (for both CPA and DPMA) averaged 48.5 ± 4.5 min for the six animals. The LAD coronary artery was completely occluded over a 10 min period. The occlusion was maintained over 1 h then released over a 10 min period. The region was reperfused for 1 h and CPA and DPMA were again administered as described above.

An additional four animals were instrumented and drugs administered as described above except that the occluder was not tightened (sham ischaemia).

Drugs

Sodium nitroprusside, ACh, and prostaglandin $F_{2\alpha}$ were purchased from Sigma (St. Louis, MO, U.S.A.). Cyclopentyladenosine was produced by Research Biochemicals Inc. (Natick, MA, U.S.A.). CGS15943 (9-chloro-2-(2-furyl)[1,2,4] triazolo [1,5-c] quinazolin-5-amine) was a gift from Ciba Geigy (Summit, NJ, U.S.A.). DPMA [N6-{2-(3,5-dimethoxyphenyl)-2-(2-methylphenyl)ethyl} adenosine] was synthesized by Dr A. Spada and N. Setzer of the Department of Medicinal Chemistry, Rhône-Poulenc Rorer. For the *in vitro* studies, adenosine agonists were dissolved in dimethyl sulphoxide (DMSO, Sigma, St. Louis MO, U.S.A.) and serially diluted with Krebs buffer. Final DMSO concentration in the bath did not exceed 0.1%. For the *in vivo* studies, adenosine agonists and antagonists were initially dissolved in DMSO (Fisher Scientific, Fairlawn, NJ, U.S.A.). The final solution was 5% DMSO, 47.5% polyethylene glycol 200 (J.T. Baker, Inc. Phillipsberg, NJ, U.S.A.), and 47.5% normal (0.9%) saline (Abbot Laboratories). Solutions were then serially diluted with 0.9% saline.

Statistical analysis

In vitro EC_{50} values were determined from individual dose-response curves with a computerized curve fitting programme (RS/1, BBN Software Products Corp. Cambridge, MA, U.S.A.). Negative logarithmic transformations were conducted on the control and ischaemia/reperfusion EC_{50} values (− log M). These − log EC_{50} values were then compared by Student's two-tailed paired *t* test. The full dose-response functions were analyzed by two-way analysis of variance. The responses to individual doses with and without ischaemia/reperfusion were compared by Student's two-tailed *t* test.

In vivo Changes in baseline haemodynamics (Table 1) were compared by a repeated measures analysis of variance (followed by a Newman Keul's *post-hoc* test). Drug responses either before or after ischaemia/reperfusion were compared to vehicle also using a repeated measures analysis of variance. Slope analysis was conducted using the slopes of individual dose-response functions. Statistical analysis of the data using CPA demonstrated that the three doses used were on the linear aspect of the dose-response function under all four conditions ($R^2 = 0.986 \pm 0.005$, $n = 4$ (LAD and circumflex; before and after ischaemia/reperfusion)). Similarly, statistical analysis of the data obtained with DPMA also confirmed that these three doses were on the linear aspect of the dose-response function under all four conditions ($R^2 = 0.911 \pm 0.028$; $n = 4$ (LAD and circumflex, before and after ischaemia/reperfusion)). Student's paired *t* test was used for specific comparisons where appropriate. Values are means ± the standard error of the mean (s.e.mean). Significance was set at $P \leqslant 0.05$ for all tests.

Results

Arterial pressure prior to ischaemia was 119 ± 3 mmHg and heart rate was 171 ± 6 beats min^{-1} ($n = 7$). After ischaemia/reperfusion, neither arterial pressure (113 ± 6 mmHg) nor heart rate (161 ± 12 beats min^{-1}) were significantly altered. All seven hearts exhibited an elevated or depressed S-T segment and cyanosis. In five of seven dogs, heart rates were highly irregular during the reperfusion period. During the reperfusion period, three animals developed ventricular tachycardia, followed by ventricular fibrillation. Animals regained a largely sinus rhythm after defibrillation. During ventricular fibrillation, blood pressure was reduced to 20 ± 4 mmHg for 1.6 ± 0.3 min ($n = 3$). Reactivity of the isolated arteries from these animals was not markedly different. For example, the $-\log EC_{50}$ for vasorelaxation to ACh ($n = 6$ total) was 6.27 ± 0.19 ($n = 3$) in arteries from non-defibrillated hearts while the $-\log EC_{50}$ was 6.44 ± 0.26 ($n = 3$) in arteries from defibrillated hearts.

Vasorelaxation in vitro

$PGF_{2\alpha}$-contracted dog coronary arterial rings were relaxed by both the endothelium-independent dilator sodium nitroprusside and the endothelium-dependent agent ACh. For sodium nitroprusside (Figure 1a), vasorelaxation was not significantly different in rings from control and reperfused arteries ($-\log EC_{50}$ 7.01 ± 0.28 versus 7.41 ± 0.18; $n = 5$). For ACh (Figure 1b), vasorelaxation again was not significantly different ($-\log EC_{50}$ 6.35 ± 0.15 versus 6.42 ± 0.25 μM; $n = 6$). While ischaemia/reperfusion appeared to attenuate the relaxation to ACh at higher doses, this trend was not statistically significant even at the highest dose ($P = 0.07$).

In addition, $PGF_{2\alpha}$-contracted dog coronary rings from control and reperfused arteries were relaxed dose-dependently by the adenosine A_1-receptor agonist, CPA and the A_2-receptor agonist, DPMA (Figure 1c,d). For CPA, the vasorelaxation in control and ischaemia/reperfusion arteries was not significantly different ($-\log EC_{50}$ 5.69 ± 0.36 versus 5.73 ± 0.25; $n = 6$). In contrast, DPMA-induced vasorelaxation was significantly attenuated after ischaemia/reperfusion, with a log unit increase in the concentration necessary for half-maximal vasorelaxation ($-\log EC_{50}$ 6.57 ± 0.16 versus 5.64 ± 0.28; $n = 6$, $P \leqslant 0.01$).

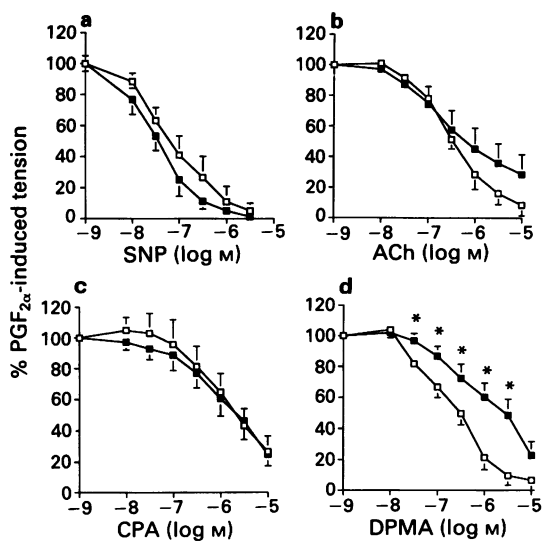

Figure 1 Concentration-response curves to the endothelium-independent vasorelaxant, sodium nitroprusside (a, $n = 5$), the endothelium-dependent vasorelaxant acetylcholine (b, $n = 6$), the A_1 selective agonist, cyclopentyladenosine (c, $n = 6$) and the A_2-selective agonist, DPMA (d, $n = 6$) in control (□) and ischaemia/reperfused (■) arteries. Control maximal isometric tension was 2.02 ± 0.44, 2.06 ± 0.82, 1.15 ± 0.46, and 1.76 ± 0.82 g for a,b,c and d, respectively. In vessels subjected to ischaemia/reperfusion, maximal isometric tension was 1.80 ± 0.54, 4.31 ± 0.98, 1.42 ± 0.52, and 1.99 ± 0.59 g for groups a,b,c and d, respectively. Note that only the vasorelaxation to the A_2-selective agonist is significantly affected by ischaemia/reperfusion.

Table 1 Effects of 1 h of ischaemia and 1 h of reperfusion on baseline haemodynamics

	Control	*Ischaemia*	*Reperfusion*
MAP (mmHg)	106 ± 6	107 ± 4	102 ± 4
HR (b.p.m.)	180 ± 12	193 ± 13	185 ± 13
dP/dt_{50} (mmHg s^{-1})	1757 ± 234	1882 ± 228	1751 ± 240
Circ BF (ml min^{-1})	23 ± 4	32 ± 2	25 ± 3
LAD BF (ml min^{-1})	15 ± 4	1 ± 2*	17 ± 5
SS Circ (%)	9.1 ± 1.2	8.6 ± 1.6	7.2 ± 1.1
SS LAD (%)	7.5 ± 0.7	− 3.6 ± 3.5*	− 2.1 ± 1.9*

Shown are mean arterial pressure (MAP), heart rate (HR), rate of change in left ventricular pressure (dP/dt_{50}), circumflex blood flow (Circ BF), left anterior descending arterial blood flow (LAD BF), and % segmental shortening (SS). Data are shown as mean ± standard error ($n = 6$). *indicates significantly different from control.

Maximal isometric tension

Maximal $PGF_{2\alpha}$-induced tension developed was significantly increased in rings from arteries subjected to ischaemia/reperfusion compared to controls ($P < 0.05$). Rings from control arteries developed 1.79 ± 0.64 g ($n = 7$) of tension; this value increased significantly after ischaemia/reperfusion to 2.57 ± 0.48 g, ($n = 7$). Isometric tension development to 36 mM KCl was not different between the two groups (4.55 ± 0.79 g control; 5.01 ± 0.70 g ischaemia/reperfusion, $n = 7$).

Vasodilatation in vivo

Table 1 illustrates the control values for mean arterial pressure, heart rate, $dP/dt50$ (rate of pressure rise at 50 mmHg), LAD blood flow, circumflex blood flow, and percent segmental shortening in both the circumflex and LAD vascular beds. In addition, Table 1 lists these parameters following 1 h of ischaemia, and again following 1 h of reperfusion. Segmental shortening in the myocardium perfused by the circumflex was not significantly affected by ischaemia/reperfusion of the LAD. In contrast, the myocardium perfused by the LAD was dysfunctional following the 1 h of ischaemia (Table 1). This region of the myocardium remained dysfunctional (as evidenced by the negative segmental shortening values) for the remainder of the protocol. This index of ischaemia could detect physiologically significant levels of collateral blood flow, as is discussed in detail in a subsequent paragraph.

The A_1 and A_2 agonists produced minimal effects on systemic haemodynamics at the point of maximal coronary vasodilatation. Table 2 lists changes in arterial pressure, heart rate, $dP/dt50$, and segmental shortening produced by vehicle, CPA and DPMA both before and after ischaemia/reperfusion. The A_1 agonist, CPA, did not significantly lower arterial pressure, either before or after ischaemia/reperfusion. The A_2 agonist, DPMA (at 30.0 μg kg^{-1}), lowered arterial pressure slightly compared to the response obtained with vehicle before but not after ischaemia/reperfusion. Heart rate was not significantly altered by either drug at the doses used. The value for $dP/dt50$ was modestly increased by the highest dose of CPA before ischaemia/reperfusion. DPMA did not alter $dP/dt50$ at any dose tested either before or after ischaemia/reperfusion. The highest dose of CPA did increase % segmental shortening in the circumflex region both before and after ischaemia/reperfusion. DPMA did not alter % segmental shortening in the circumflex field at any dose tested. In the LAD, CPA again increased % segmental

shortening at the highest dose tested, and DPMA again had no effect.

The A_1 agonist, CPA, produced a dose-dependent increase in blood flow in the normally perfused circumflex coronary artery before and after ischaemia/reperfusion of the LAD. Circumflex blood flow was significantly increased at 10.0 and 30.0 $\mu g\ kg^{-1}$ of CPA both before and after ischaemia/reperfusion (Figure 2). Ischaemia/reperfusion did significantly en-

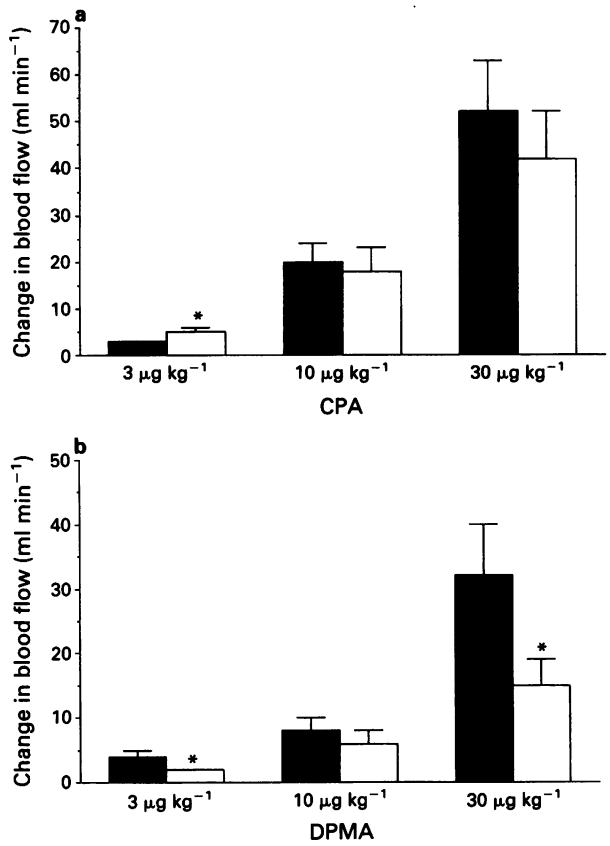

Figure 2 Effects of the A_1 agonist cyclopentyladenosine (a) and the A_2 agonist DPMA (b) on circumflex blood flow before (solid columns) and after (open columns) ischaemia/reperfusion of the left anterior descending (LAD) coronary artery. The adenosine agonists were administered intravenously at 3, 10, and 30 $\mu g\ kg^{-1}$ both before and after a 1 h ischaemia/1 h reperfusion protocol. Note that in the normally perfused circumflex coronary artery, the vasodilatation to cyclopentyladenosine is enhanced at the lowest dose and is unaltered at the two higher doses. In contrast, the vasodilator response to the A_2 agonist, DPMA, is significantly attenuated at the lowest and highest dose. $*P \leqslant 0.05$.

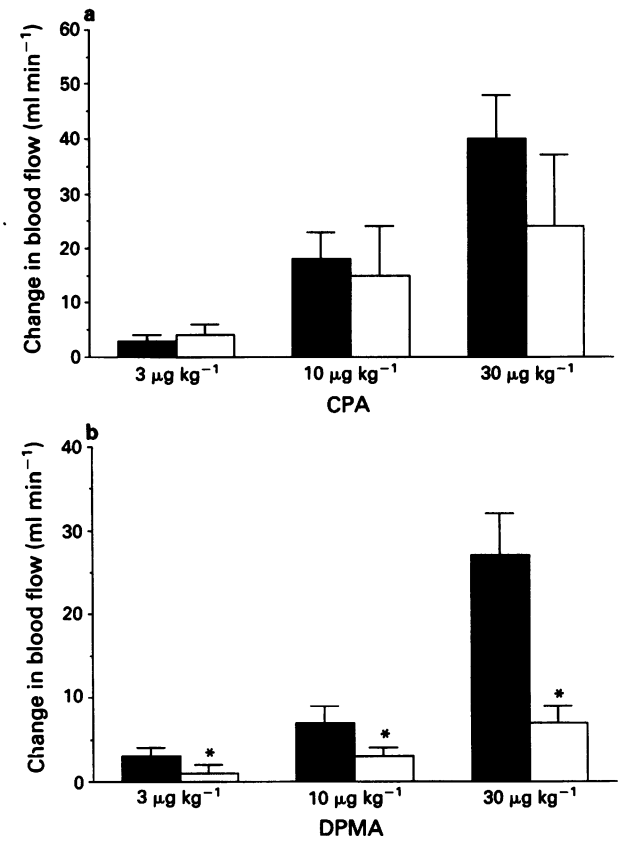

Figure 3 Effects of the A_1 agonist cyclopentyladenosine (a) and an A_2 agonist DPMA (b) on left anterior descending (LAD) coronary blood flow before (solid columns) and after (open columns) ischaemia/reperfusion. The adenosine agonists were administered intravenously at 3, 10, and 30 $\mu g\ kg^{-1}$ before and after a 1 h ischaemia/1 h reperfusion protocol. Note that the increase in blood flow produced by A_1 agonist tends to be reduced by ischaemia/reperfusion only at the highest dose while the response to the A_2 agonist is significantly attenuated at all doses. $*P \leqslant 0.05$.

Table 2 Change in haemodynamics and myocardial function produced by administration of vehicle compared with the A_1 agonist, CPA and the A_2 agonist, DPMA before and after ischaemia/reperfusion

		CPA			*DPMA*		
	Vehicle	*3 μg kg⁻¹*	*10 μg kg⁻*	*30 μg kg⁻¹*	*3 μg kg⁻¹*	*10 μg kg⁻¹*	*30 μg kg⁻¹*
MAP (mmHg)							
Pre	2 ± 1	1 ± 1	1 ± 2	− 2 ± 2	2 ± 1	0 ± 1	− 2 ± 1*
Post		− 1 ± 3	8 ± 4†	2 ± 3	2 ± 2	1 ± 3	− 2 ± 2
HR (b.p.m.)							
Pre	1 ± 1	− 2 ± 1	− 1 ± 1	− 9 ± 6	1 ± 3	5 ± 2	7 ± 2
Post		1 ± 3	0 ± 5	− 14 ± 6	0 ± 1	3 ± 1	0 ± 2†
dP/dt_{50} (mmHg s^{-1})							
Pre	11 ± 39	24 ± 30	56 ± 53	243 ± 58*	59 ± 24	361 ± 292	288 ± 28
Post		22 ± 46	144 ± 62	246 ± 204	10 ± 17	96 ± 47	58 ± 33†
SS Circ (%)							
Pre	− 0.7 ± 1.6	0.1 ± 0.2	0.2 ± 0.9	1.9 ± 1.1	0.1 ± 0.4	0.5 ± 0.4	1.2 ± 0.9
Post		1.0 ± 1.0	1.1 ± 1.4	3.8 ± 1.2	0.6 ± 0.4	0.4 ± 0.7	1.1 ± 0.4
SS LAD (%)							
Pre	− 0.3 ± 0.7	− 0.4 ± 0.4	− 0.1 ± 0.7	1.6 ± 0.4	0.4 ± 0.3	0.8 ± 0.3	0.9 ± 0.6
Post		3.2 ± 2.2	0.1 ± 0.2	1.0 ± 0.9	0.1 ± 0.3	0.3 ± 0.1	0.8 ± 0.3

Shown are mean arterial pressure (MAP), heart rate (HR), rate of change in left ventricular pressure (dP/dt_{50}), and percentage segmental shortening (% SS) in the Circumflex (Circ) and left anterior descending (LAD) coronary arteries. Significant difference from vehicle: $*P < 0.05$. Significant differences pre- versus post ischaemia/reperfusion: $\dagger P < 0.05$.

hance the increase, albeit modestly, to the lowest dose of CPA (Figure 2). Circumflex blood flow was significantly increased by the 30.0 $\mu g\,kg^{-1}$ dose of DPMA before ischaemia/reperfusion, and by 3.0, 10.0 and 30.0 $\mu g\,kg^{-1}$ after ischaemia/reperfusion (Table 1). The change in circumflex blood flow was attenuated by ischaemia/reperfusion of the LAD at both the lowest and the highest dose of DPMA (Figure 2).

CPA also increased coronary blood flow in the LAD before ischaemia/reperfusion, at the 10.0 and 30.0 $\mu g\,kg^{-1}$ doses, and after ischaemia/reperfusion at the 30.0 $\mu g\,kg^{-1}$ dose (Table 1). The changes in LAD blood flow were not significantly different before versus after ischaemia/reperfusion (Figure 3). The A_2 agonist, DPMA, also dose-dependently increased LAD blood flow before ischaemia/reperfusion (Table 1). However, the A_2 agonist did not significantly increase LAD blood flow following ischaemia/reperfusion, also shown in Table 1. Figure 3 illustrates the attenuated increases in LAD blood flow at all three doses of DPMA after ischaemia/reperfusion.

Slope analysis was conducted on the individual dose-response functions. Only the slope of the DPMA vasodilatation function in the LAD was altered by the ischaemia/reperfusion protocol.

Presence or absence of physiologically significant collateral blood flow was assessed functionally. In one animal, complete occlusion of the LAD did not produce myocardial dysfunction in the region monitored by the LAD sonomicrometry crystals (control = + 14.4%, ischaemia = + 9.4%, reperfusion = + 9.4%). As predicted, the A_2-mediated increase in LAD blood flow was not affected by the ischaemia/reperfusion protocol. For example, 30 $\mu g\,kg^{-1}$ of DPMA produced a 26 ml min^{-1} increase before ischaemia/reperfusion, and a 30 ml min^{-1} increase after ischaemia/reperfusion. In addition, DPMA increased LAD blood flow equally before and after the sham ischaemia/reperfusion protocol. For example, 10.0 $\mu g\,kg^{-1}$ of DPMA increased LAD blood flow by 13 ± 8 ml min^{-1} before sham ischaemia/reperfusion, and by 12 ± 6 ml min^{-1} after sham ischaemia/reperfusion ($n = 4$). These data further demonstrate the stability of DPMA in solution over the duration of the protocol.

Discussion and conclusions

In these experiments, we clearly demonstrate that both an adenosine A_1 and an adenosine A_2 receptor agonist can produce coronary vasorelaxation in the dog, *in vitro* as well as *in vivo*. Further, we found that ischaemia/reperfusion differentially attenuates coronary vasorelaxant responses to the A_2 receptor agonist while not affecting the vasorelaxant response to the A_1 receptor agonist. This differential effect is not due to endothelial damage, since the response to the endothelium-dependent vasorelaxant ACh is unaffected by ischaemia/reperfusion. Vascular smooth muscle also appears unaffected, since the response to the endothelium-independent vasorelaxant, sodium nitroprusside, is not altered.

Although our data indicate that maximal tension development to $PGF_{2\alpha}$ was significantly higher in rings from the ischaemic group compared to those from the control group, individual analysis revealed that only one of the four groups (CPA) displayed significantly higher vasoconstriction to $PGF_{2\alpha}$. The reason for this difference remains unclear since the rings from all four groups are derived from adjacent sections of the coronary arteries. However, analysis of the CPA data on an absolute gram (rather than a percentage) basis resulted in no significant difference in the dose-response curves. We therefore conclude that these intergroup differences have no influence on the interpretation of the results.

For the *in vitro* studies, it should be noted that the ischaemic vessels are taken 10 mm distal to the occluder. This calibre section of a conduit artery should not be subject to a pressure gradient allowing for collateral flow into the lumen of the vessel. This fact, coupled with the electrocardiographic data demonstrating S-T segment alterations and arrhythmias, clearly demonstrates that this region of the LAD was subjected to ischaemia. For the *in vivo* studies, we could clearly determine if collateral flow was present in physiologically significant amounts by examining the segmental shortening values in the ischaemic zone. As noted in the results section, one animal was excluded, based on insufficient reductions in segmental shortening following ischaemia/reperfusion. It should also be noted that the coronary vasodilatation produced by DPMA was not attenuated in this animal.

In the *in vivo* ischaemia/reperfusion studies, the protocol produces results which are commensurate with the increased complexity of the model. While the circumflex vascular bed was not subjected to ischaemia/reperfusion, the vasodilator responses to both the A_1 and A_2 agonist were altered at specific doses. The increase in coronary blood flow with CPA was enhanced at the lowest dose, and the vasodilator response to DPMA attenuated at the lowest and highest doses. However, slope analysis demonstrated no significant change in slope for either dose-response curve. We would hypothesize that the attenuated response to DPMA at the 3.0 and 30.0 $\mu g\,kg^{-1}$ doses is a response to the increased endogenous adenosine in the circumflex vascular bed. This adenosine may either originate from the ischaemic, dysfunctional LAD region, or be a result of the initial compensatory increase in myocardial work conducted by the circumflex region to maintain total cardiac function in spite of the dysfunctional myocardium in the LAD region. In support of this hypothesis, note the increase in baseline circumflex coronary blood flow (Table 1) following 1 h of ischaemia of the LAD (from 23 ± 4 to 32 ± 2 ml min^{-1}, $P < 0.05$, Student's paired t test).

Ischaemia/reperfusion of the LAD clearly attenuates the vasodilator response to the A_2 agonist, DPMA. The increase in coronary blood flow produced by DPMA is significantly reduced at all three doses. In addition, slope analysis revealed a significant reduction in slope for the three point dose-response function. In contrast, the increases in coronary blood flow produced by CPA were not significantly affected. It should be noted that a trend ($P = 0.06$) exists for the increase in coronary blood flow in response to the highest dose of CPA to be attenuated. We would hypothesize that CPA, at higher doses, may produce increases in coronary blood flow through activation of A_2 receptors, as well as A_1 receptors. However, this effect does not appear to occur at the lower two doses, and slope analysis reveals no change in the slope of the dose-response function.

In the present study, we have chosen to administer the A_1 and A_2 agonists intravenously, thus ensuring that plasma levels of drug were equivalent in all branches of the circumflex and LAD vascular beds. In addition, note that the doses used did not alter significantly mean arterial pressure or heart rate at the time of the peak increase in coronary blood flow. These data are in agreement with our previous results (Cox *et al.*, 1991) and that obtained with related A_1 and A_2 agonists (Gerencer *et al.*, 1992).

In previous studies, we have demonstrated that the vasorelaxant response of both the A_1 agonist, CPA and the A_2 agonist, DPMA can be completely blocked by the selective adenosine antagonist CGS15943 both *in vitro* in the dog (Cox *et al.*, 1991) and *in vitro* in the swine (Merkel *et al.*, 1992). CGS15943 is an antagonist selective for adenosine receptors but does not differentiate between A_1 and A_2 receptor subtypes (Jarvis *et al.*, 1991). These data are important since we have previously shown that coronary vasorelaxant responses to the A_1 agonist, CPA but not the A_2 agonist, DPMA, can be attenuated by the potassium channel blocker, glibenclamide in the swine (Merkel *et al.*, 1992). It should also be noted that low doses of CPA produce vasoconstriction in isolated coronary arteries from swine, but not from dog. The present study confirms the lack of A_1-mediated vasoconstriction in the dog, both *in vitro* and *in vivo*. Thus a species difference occurs with the constrictor, but not the dilato-

properties of CPA. Together, these data clearly demonstrate that the vasorelaxant actions of CPA are due to activation of a CGS15943-sensitive receptor. However, the glibenclamide and ischaemia/reperfusion data demonstrate that these vasorelaxant/vasodilator responses are not simply due to activation of A_2 receptors. The role of differing second messenger systems in separate species is not currently known.

Other investigators have examined the effect of ischaemia/reperfusion on the coronary vasorelaxant response to purinoceptor agonists. In one study, dog circumflex arteries were subjected to several levels of ischaemia/reperfusion. In this study, relaxation of conduit arteries in response to ACh, ADP, bradykinin, A23187, nitroglycerin and adenosine was not altered by a 1 h ischaemia/1 h reperfusion protocol. However, ischaemia/reperfusion did significantly blunt the EC_{50} to ACh and ADP in coronary microvessels (110–220 μm). However, adenosine was not tested in the microvessel model (Quillen *et al.*, 1990). In another study, the vasorelaxant effects of ADP on coronary arteries were blunted by a 1 h ischaemia/1 h reperfusion protocol (Pearson *et al.*, 1990). In the latter study, it would appear that the ischaemia may have been more severe, damaging the endothelium. In the study of Quillen *et al.* (1990), and consistent with our data, the endothelium is functional following our 1 h ischaemia/1 h reperfusion protocol. In the present study, there was a trend ($P = 0.07$) toward a blunted vasorelaxant response to ACh only at the higher doses. Despite the evidence for intact endothelium-dependent and independent vasorelaxation, the A_2 receptor agonist is functionally less active throughout the majority of the dose-response curve. This attenuated response occurred at doses where endothelium-dependent responses are clearly intact.

Thus, our data suggest that ischaemia/reperfusion selectively decreases A_2 receptor number, alters the coupling of A_2 receptors to its second messenger system, or alters the functioning of the second messenger system. While our data cannot differentiate between these possible mechanisms, we have clearly demonstrated a functional impairment of A_2, but not A_1, adenosine receptor-mediated coronary vasorelaxation. This effect may be in response to the elevated local adenosine concentrations which occur during ischaemia (Deussen *et al.*, 1988). This theory is supported by the findings of Ramkumar *et al.* (1991) who demonstrated that the downregulation of A_2 receptors by A_2 agonists occurred more rapidly ($t_{\frac{1}{2}} = 45$ min) than downregulation of A_1 receptors by A_1 agonists ($t_{\frac{1}{2}} = 8$ h) in a smooth muscle cell line (DDT MF-2). Therefore, one could speculate that ischaemia/reperfusion selectively decreases A_2 receptors, possibly by the accumulation of endogenous adenosine.

Our previous studies have shown that A_1-receptor-mediated vasorelaxation is linked to potassium channels (Merkel *et al.*, 1992). Additionally, potassium channel activators, such as aprikalim, have been shown to reduce ischaemia/reperfusion-mediated myocardial injury (Grover *et al.*, 1990; Auchampach *et al.*, 1991). Thus, by reducing adenosine 3′:5′-cyclic monophosphate (cyclic AMP)-(A_2) mediated vasodilatation in favor of an 'anti-ischaemic' potassium channel (A_1)-mediated vasodilatation, a greater mass of the myocardium may potentially be spared. These data support the development of selective adenosine agonists to treat ischaemia clinically followed by reperfusion.

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