British Journal of Pharmacology (1999) 128, 385–395
http://www.stockton-press.co.uk/bjp

The effect of body temperature on myocardial protection conferred by ischaemic preconditioning or the selective adenosine A_1 receptor agonist GR79236, in an anaesthetized rabbit model of myocardial ischaemia and reperfusion

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1 The cardioprotective effect of N-[(1S, trans)-2-hydroxycyclopentyl]adenosine (GR79236), an adenosine A_1 receptor agonist, was compared with that produced by ischaemic preconditioning in an anaesthetized rabbit model of myocardial ischaemia and reperfusion. In addition, we examined the effect of different body core temperatures on GR79236- or ischaemic preconditioning-induced cardioprotection when administered prior to ischaemia, and on cardioprotection induced by GR79236 administered 10 min prior to the onset of reperfusion.

2 When rabbits were subjected to 30 min occlusion of the left coronary artery, followed by 2 h reperfusion, GR79236 (3×10^{-8} mol kg^{-1} i.v. (10.5 μg kg^{-1} i.v.)) or ischaemic preconditioning (5 min ischaemia followed by 5 min reperfusion), administered or applied 10 min prior to the occlusion, significantly limited the development of infarction. The cardioprotective effect of ischaemic preconditioning was significantly greater than that seen after administration of GR79236. Pre-treatment with the selective adenosine A_1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, 3.3×10^{-6} mol kg^{-1} (1 mg kg^{-1} i.v.)), prevented the cardioprotective effect of GR79236, but not that of ischaemic preconditioning.

3 Maintaining body core temperature at 38.5°C rather than at 37.0°C did not influence infarct size in control groups of rabbits, but reduced the cardioprotective effect of GR79236 when administered 10 min prior to occlusion *or* 10 min prior to the onset of reperfusion. The cardioprotective effect of ischaemic preconditioning was not temperature-dependent.

4 In conclusion, myocardial protection conferred by GR79236 in anaesthetized rabbits is mediated *via* adenosine A_1 receptors. Myocardial protection can be conferred when GR79236 is administered before the onset of ischaemia *or* reperfusion, and is reduced when body core temperature is maintained at 38.5°C rather than at 37.0°C. In contrast, myocardial protection conferred by ischaemic preconditioning is not reduced by adenosine A_1 receptor blockade, or by maintaining body core temperature at 38.5°C rather than at 37.0°C. These findings point to distinct differences in the mechanisms of induction of myocardial protection by adenosine A_1 receptor agonist and ischaemic preconditioning. They also highlight the need for careful control of body core temperature when investigating the phenomenon of cardioprotection.

Keywords: Myocardial protection; ischaemic preconditioning; GR79236; adenosine A_1 receptor agonist

Abbreviations: DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; GR79236 (N-[(1S, trans)-2-hydroxycyclopentyl]adenosine; HR, heart rate; IPC, ischaemic preconditioning; R-PIA, R(−)N^6-2-phenylisopropyl adenosine; TTC, triphenyltetrazolium chloride

Introduction

Since its first description by Murry *et al*., 1986, there has been great effort to elucidate the mechanism by which ischaemic preconditioning confers its cardioprotective effect. The phenomenon of myocardial ischaemic preconditioning, whereby a brief period of ischaemia, followed by reperfusion, attenuates the myocardial ischaemia-reperfusion injury associated with a more sustained period of ischaemia, is a powerful protective mechanism which has been demonstrated in all animal models yet studied. Ischaemic preconditioning induces two phases of myocardial protection. The initial, acute, phase of protection described by Murry *et al*. (1986) lasts 1–2 h (Murry *et al*., 1991; van Winkle *et al*., 1991; Li *et al*., 1992; Miura *et al*., 1992). This is followed by a delayed phase, or second window, of protection (Marber *et al*., 1993; Kuzuya *et al*., 1993), that develops 12–24 h after ischaemic preconditioning, and lasts for at least a further 48 h (Baxter *et al*., 1997).

Early studies showed that the protective properties of the acute phase of ischaemic preconditioning can be prevented by pre-treatment with non-selective adenosine receptor antagonists (Liu *et al*., 1991; Downey *et al*., 1993). Pre-treatment with adenosine or selective A_1, but not A_2, receptor agonists, mimics the infarction-limiting effect of ischaemic preconditioning (Downey *et al*., 1993). Taken together, these data suggest a role for adenosine A_1 receptors in the process of ischaemic preconditioning, at least in the rabbit, with evidence for a similar role in the dog and pig (Grover *et al*., 1992; Martin *et al*., 1993; Auchampach & Gross, 1993; van Winkle *et al*., 1992). Adenosine receptors have also been implicated in

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triggering the delayed phase of myocardial protection (Baxter *et al*., 1994).

N-[(1S, trans)-2-hydroxycyclopentyl]adenosine (GR79236) is a potent and selective agonist at adenosine A_1 receptors (Gurden *et al*., 1993), and induces both the classic, acute protection, and the delayed phase of protection, in a rabbit model of myocardial ischaemia and reperfusion (Travers *et al*., 1998). GR79236 also reduces infarct size in a pig model of myocardial ischaemia and reperfusion (Louttit *et al*., 1999). Although GR79236 induces cardioprotection in the rabbit model, we have noted, at times, that the extent of protection seen can be variable, particularly during periods of high ambient temperature. Interestingly, McClanahan *et al*. (1994) have shown that pentostatin, an inhibitor of adenosine deaminase, reduced infarct size in pigs only when combined with mild hypothermia, suggesting that the cardioprotective effect of adenosine can be influenced by temperature. We therefore investigated whether variations in body core temperature could be responsible for the variation in cardioprotective activity sometimes seen with GR79236 in anaesthetized rabbits. In addition, we investigated whether myocardial protection induced by ischaemic preconditioning, the mechanism which GR79236 is assumed to mimic, is similarly influenced by body core temperature.

The cardioprotective effects of adenosine A_1 receptor agonists administered prior to ischaemia are well established. However, the evidence to suggest that they are beneficial when administered *during* ischaemia, just prior to reperfusion, is controversial. Using an anaesthetized rabbit model, Thornton *et al*. (1992) were not able to show protection when a 5 min infusion of N^6-(phenyl-2R-isopropyl)-adenosine (R-PIA) was administered, commencing 2 min prior to reperfusion. However, using a different A_1 receptor agonist, cyclopentyladenosine, infused for 65 min, starting 5 min prior to reperfusion in the anaesthetized rabbit, Norton *et al*. (1992) *did* show a reduction in infarct size. In addition, we have recently shown that GR79236 reduces infarct size when administered 10 min prior to the onset of reperfusion, in an anaesthetized pig model of myocardial ischaemic and reperfusion (Louttit *et al*., 1999). We have therefore investigated the effect of GR79236, administered 10 min prior to the onset of reperfusion, on the development of infarction in the rabbit model, and determined whether any protective activity seen is influenced by temperature.

Methods

Surgical procedures

This research complied with national legislation and with Company policy on the Care and Use of Animals and with related codes of practice.

Male New Zealand White rabbits (2.4–3.2 kg, Charles River Ltd.) were anaesthetized to effect with pentobarbitone sodium (approximately 45 mg kg^{-1} i.v.). A tracheotomy was performed and the animal artificially ventilated with room air (ventilation volume 3.5 ml kg^{-1}; ventilation rate of 60 inspirations min^{-1}) supplemented with oxygen, to maintain blood pH and blood gas tensions close to those seen in conscious rabbits (unpublished observations; pH 7.34; PCO_2 36.4; PO_2 91.6; $n=44$). A femoral artery was cannulated to allow measurement of arterial pressure from which heart rate was derived, and for the withdrawal of arterial blood for the analysis of blood pH and blood gas tensions. A catheter was placed in the left jugular vein for the administration of anaesthetic, as required, and drugs. The heart was exposed *via* a left lateral thoracotomy in the 5th intercostal space, and a suture passed under a branch of the left coronary artery to form a snare so that a reversible occlusion could be applied. The thoracotomy was covered, and remained so, with the exception of the time when the snare around the coronary artery was being tightened or released, to minimise heat loss. Rabbit body core temperature was monitored using a thermistor (Digitron, RS Components; calibrated measurement uncertainty ± 0.3°C) placed in the colon, and carefully maintained (± 0.3°C) at either 37.0°C or 38.5°C by manually adjusting the heat setting of a homeothermic blanket control unit.

Experimental design

After a stabilization period of approximately 30 min, an intravenous bolus dose of heparin (250 iu kg^{-1} i.v.) was administered to prevent thrombus formation during the experimental protocol. Myocardial ischaemia was induced by tightening the snare around the coronary artery for a period of 30 min. Blood flow was restored by release of the snare, and the myocardium reperfused for 2 h. Twenty minutes before the end of the reperfusion period, a second bolus dose of heparin (500 iu kg^{-1} i.v.) was administered.

Three series of experiments were performed; the experimental protocols are shown in Figure 1. In the first group of experiments (Series 1), the effect of the adenosine A_1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, 3.3×10^{-6} mol kg^{-1} i.v. (1 mg kg^{-1} i.v.)), or its vehicle (1 ml kg^{-1} i.v.), on the cardioprotective effect of GR79236 (3×10^{-8} mol kg^{-1} i.v. (10.5 μg kg^{-1} i.v.)) or ischaemic preconditioning (5 min ischaemia followed by 5 min reperfusion), was investigated in rabbits in which body core temperature was maintained at 37.0 ± 0.3°C. GR79236 or its vehicle (saline; 1 ml i.v.) was administered, or ischaemic preconditioning applied, 10 min prior to coronary artery occlusion. DPCPX or its vehicle was administered 10 min prior to the administration of GR79236, saline, or the onset of ischaemic preconditioning. In a subsequent group of experiments (Series 2), the effect of body core temperature on GR79236- or ischaemic preconditioning-induced cardioprotec-

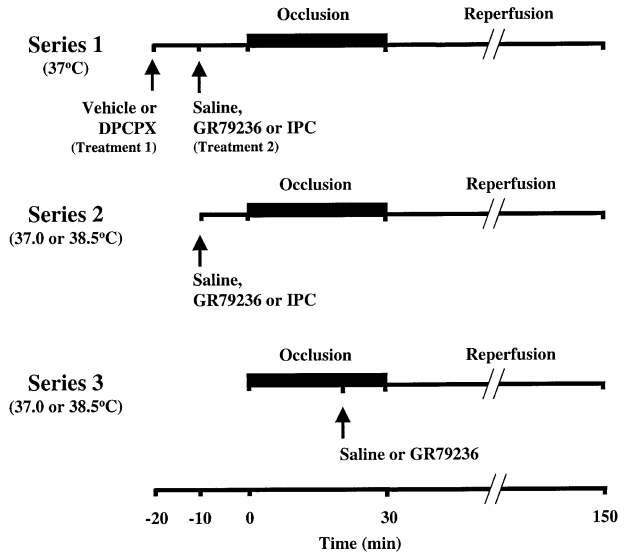

Figure 1 Experimental protocols for Series 1, 2 and 3 experiments. Ischaemic preconditioning (IPC) is 5 min of ischaemia followed by 5 min reperfusion.

tion was examined. Rabbits were maintained ($\pm 0.3^{\circ}C$) at either 37.0 or 38.5°C, and GR79236 or saline, or ischaemic preconditioning, applied 10 min prior to occlusion. Finally, in the third series of experiments (Series 3), GR79236 or saline was administered 10 min prior to the onset of reperfusion in rabbits maintained ($\pm 0.3^{\circ}C$) at either 37.0 or 38.5°C.

All experiments were recorded and analysed using a Modular Instruments Inc. data acquisition system and the Bioreport haemodynamics software package. Drug-induced change in mean arterial pressure (MAP) or heart rate (HR) was expressed as per cent change from the pre-dose value.

At the end of the experimental protocol, hearts were removed and the aorta perfused retrogradely with heparinized saline to flush the coronary vasculature of residual blood. The coronary artery was re-occluded and the coronary vasculature perfused with a suspension of fluorescent Zn/Cd particles (1–10 μm diameter). After the hearts had been frozen, they were cut into 2 mm thick slices parallel with the atrioventricular groove, and the right ventricle discarded. The slices were allowed to thaw and then incubated in a 1% solution of triphenyltetrazolium chloride (TTC) in phosphate buffered saline at 37.0°C for 10 min.

Infarcted myocardium remained unstained by the TTC. Any area of the heart not containing fluorescent particles was defined as the risk zone for infarction. The areas of infarcted tissue, risk zone and total ventricular area was measured using digital planimetry. Infarct size was expressed as a percentage of the risk zone, and the risk zone as a percentage of the total left ventricular area.

Drugs and reagents

GR79236 was synthesized in Medicinal Chemistry, Glaxo Research and Development, dissolved in sterile saline, and administered over a period of 1 min. DPCPX was obtained from Research Biochemicals International (RBI, Sigma-Aldrich, Poole, Dorset), dissolved in 200 μl DMSO and 200 μl 1 M NaOH, and made up to volume using sterile saline (total volume of DMSO and 1 M NaOH used amounted to 6% v v^{-1}). One ml kg^{-1} i.v. of the DPCPX solution was administered over a period of 2 min. Sodium pentobarbitone (Sagatal) was obtained from Rhone-Merieux (Harlow, Essex), heparin from Leo Pharmaceuticals (Princess Risborough, Bucks). Triphenyltetrazolium chloride and phosphate buffered saline was obtained from Sigma-Aldrich (Poole, Dorset), and zinc-cadmium sulphide fluorescent particles were obtained from Duke Scientific (Christison Ltd., Gateshead, Tyne and Wear), and suspended in saline immediately prior to use.

Statistical analysis

Values shown are arithmetic mean ± s.e.mean where n represents the number of observations. The data were analysed using analysis of variance. In the analysis of MAP and HR the data were adjusted to account for the basal measurements. A P value of <0.05 was considered to indicate a statistically significant difference.

Results

Series 1 experiments – effect of DPCPX on cardioprotection conferred by GR79236 or ischaemic preconditioning

Haemodynamics Mean arterial pressure (MAP) and heart rate (HR) values at the beginning of the experimental protocol, and at time points throughout the experiment, are shown in Table 1. Changes in MAP or HR in response to the different treatments are shown in Figure 2.

There were no statistically significant differences in basal MAP or HR values between the groups. Intravenous administration of the vehicle for DPCPX, or the vehicle (saline) for GR79236, had little effect on MAP or HR. In rabbits pre-treated with the vehicle for DPCPX, GR79236 reduced MAP and HR by approximately 15%. The effect on MAP was transient and had returned to basal values just prior to occlusion. However, HR remained significantly ($P<0.01$) lower, just prior to occlusion, than in all other groups (Table 1). Haemodynamic variations caused by the occlusion-reperfusion protocol prevented us making an assessment of the duration of action of GR79236. However, in previous studies, we have found that GR79236-induced bradycardia lasts for approximately 30 min (unpublished observation).

Table 1 Haemodynamic values for Series 1 experiments

		Mean arterial pressure (MAP; mmHg)					*Heart rate (HR; beats min^{-1})*				
Treatments 1/2	n	*Prior to treatment*	*Prior to occlusion*	*Prior to reperfusion*	*+1 h into reperfusion*	*+2 h into reperfusion*	*Prior to treatment*	*Prior to occlusion*	*Prior to reperfusion*	*+1 h into reperfusion*	*+2 h into reperfusion*
Vehicle/Saline	7	81.1	83.9	75.8	72.9	75.2	246.2	252.2	211.6	242.5	246.5
		(4.6)	(4.7)	(4.1)	(3.5)	(3.6)	(10.0)	(10.1)	(8.8)	(7.4)	(7.5)
Vehicle/GR	6	85.4	79.5	72.2	67.7	75.0	239.7	216.7#	224.1	240.7	239.4
		(3.0)	(4.8)	(4.1)	(4.8)	(4.6)	(4.1)	(5.1)	(6.3)	(6.5)	(12.1)
Vehicle/IPC	6	87.9	84.5	80.4	76.3	74.2	241.9	244.7	240.7	248.9	247.4
		(2.0)	(1.8)	(1.9)	(3.0)	(1.9)	(5.0)	(5.8)	(8.9)	(8.4)	(5.6)
DPCPX/Saline	6	85.0	92.1*	78.2	66.6	61.1**	225.1	238.8	227.2*	243.6*	253.1*
		(2.5)	(2.4)	(1.8)	(6.9)	(6.3)	(9.4)	(10.0)	(6.6)	(13.1)	(13.7)
DPCPX/GR	6	80.8	89.1*	81.1	68.1	64.4**	252.4	270.8	257.2*	273.3*	274.2*
		(2.7)	(2.9)	(2.0)	(3.9)	(4.9)	(3.9)	(4.0)	(5.2)	(4.0)	(3.2)
DPCPX/IPC	6	86.9	86.0*	80.2	73.2	65.9**	237.2	239.0	248.1*	263.8*	261.3*
		(2.3)	(4.9)	(4.8)	(6.0)	(5.5)	(7.6)	(16.6)	(15.3)	(14.6)	(14.6)

Data are expressed as mean ± s.e.mean of n observations. Basal readings were taken just prior to treatment 1. Treatment 1 (vehicle for DPCPX, or DPCPX (3.3×10^{-6} mol kg^{-1} i.v.)), was administered 10 min prior to treatment 2 (saline (1 ml i.v.), GR79236 (GR; 3×10^{-8} mol kg^{-1} i.v.) or ischaemic preconditioning (IPC; 1×5 min ischaemia followed by 5 min reperfusion)). *MAP prior to occlusion and HR prior to the onset of reperfusion, and 1 and 2 h into reperfusion, in DPCPX pre-treated groups was significantly ($P<0.01$) higher than in vehicle pre-treated groups. **MAP in DPCPX pre-treated groups 2 h into reperfusion) was significantly ($P<0.01$) lower than in vehicle pre-treated groups. #HR prior to occlusion after pre-treatment with vehicle for DPCPX followed by GR79236, was significantly ($P<0.01$) lower compared to all other groups at this time point.

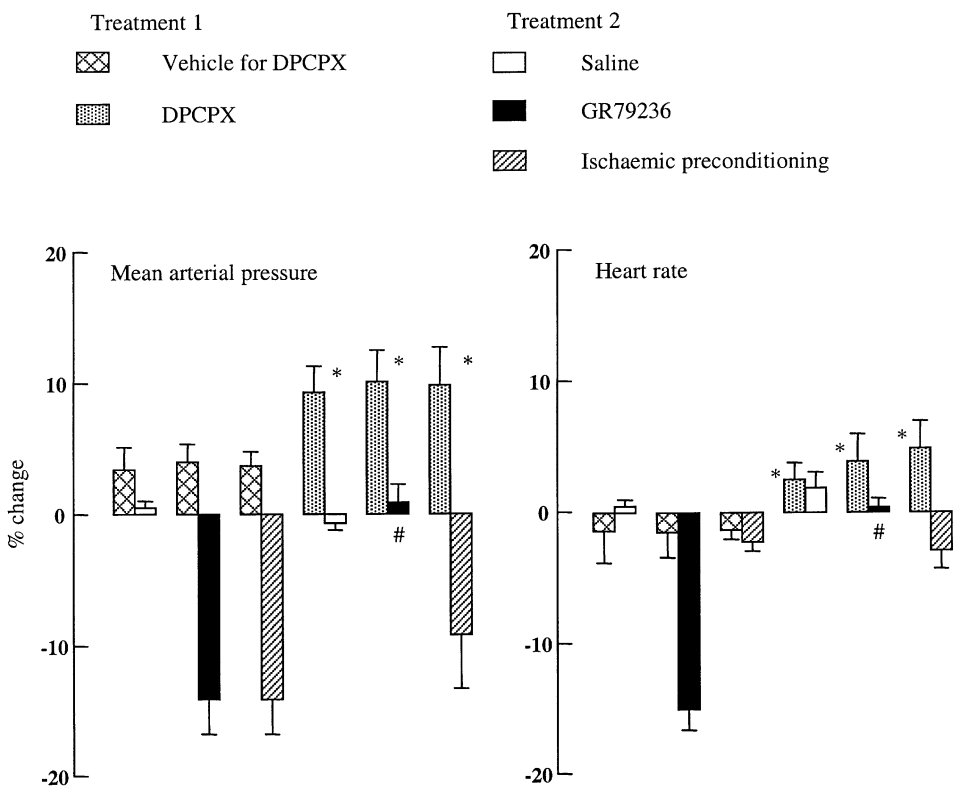

Figure 2 Effect of DPCPX (3.3×10^{-6} mol kg^{-1} i.v.), or its vehicle (1 ml kg^{-1} i.v.), on mean arterial pressure (MAP) and heart rate (HR), and on peak changes in MAP and HR produced following administration of saline (1 ml i.v.), GR79236 (3×10^{-8} mol kg^{-1} i.v.), or application of ischaemic preconditioning (5 min ischaemia followed by 5 min reperfusion). In each pair of histobars, the first bar shows the effect of treatment 1, and the second bar shows the effect of treatment 2. Data are expressed as mean percentage peak change in MAP and HR $\pm$ s.e.mean ($n = 6-7$). *Pre-treatment with DPCPX produced a significant ($P < 0.01$) increase in MAP and HR compared to pre-treatment with vehicle. #GR79236-induced peak reductions in MAP and HR after pre-treatment with DPCPX are significantly ($P < 0.01$) smaller than after pre-treatment with vehicle.

Table 2 Risk zone size in Series 1, Series 2 and Series 3 experiments

Series 1				*Series 2 (prior to occlusion)*			*Series 3 (prior to reperfusion)*		
Treatment 1	*Treatment 2*	n	*Risk zone (%)*	*Treatment*	n	*Risk zone (%)*	*Treatment*	n	*Risk zone (%)*
Vehicle	Saline	7	28.5 $\pm$ 1.6	Saline 37.0°C	6	35.0 $\pm$ 3.3	Saline 37.0°C	6	32.9 $\pm$ 4.7
Vehicle	GR79236	6	26.9 $\pm$ 1.3	GR79236 37.0°C	6	34.0 $\pm$ 4.0	GR79236 37.0°C	6	30.0 $\pm$ 3.6
Vehicle	IPC	6	26.5 $\pm$ 2.5	IPC 37.0°C	6	31.9 $\pm$ 2.7	–		–
DPCPX	Saline	6	31.2 $\pm$ 3.0	Saline 38.5°C	6	31.6 $\pm$ 4.1	Saline 38.5°C	6	32.1 $\pm$ 2.8
DPCPX	GR79236	6	28.4 $\pm$ 2.3	GR79236 38.5°C	6	24.5 $\pm$ 3.7	GR79236 38.5°C	6	26.5 $\pm$ 2.5
DPCPX	IPC	6	27.5 $\pm$ 3.0	IPC 38.5°C	6	30.3 $\pm$ 3.8	–		–

The risk zone is expressed as a percentage of the left ventricle. Data are expressed as mean $\pm$ s.e.mean of n observations. There were no statistically significant differences between the groups.

Also in rabbits pre-treated with vehicle for DPCPX, ischaemic preconditioning markedly reduced MAP in most instances. However, this effect was transient, and MAP had returned to basal levels just prior to occlusion. HR was little changed by the ischaemic preconditioning protocol. When compared with the effects of its vehicle, DPCPX caused a significant ($P < 0.01$) increase in MAP and HR, and HR remained significantly ($P < 0.01$) elevated prior to occlusion. However, there were no significant differences in MAP or HR between the groups of saline- or GR79236-treated, or ischaemically preconditioned groups of rabbits (Table 1). Pre-treatment with DPCPX prevented GR79236-induced reductions in MAP and HR, but did not influence haemodynamic changes observed after treatment with saline or during ischaemic preconditioning (Figure 2).

In rabbits pre-treated with the vehicle for DPCPX, MAP tended to decrease, and HR tended to increase, over the course of the experiments, but these effects did not reach statistical significance. In rabbits pre-treated with DPCPX, similar trends were observed, but MAP was significantly ($P < 0.01$) lower 2 h into repefusion, and HR significantly ($P < 0.01$) higher just prior to the onset of reperfusion, and 1 and 2 h into reperfusion, compared to those pre-treated with vehicle for DPCPX.

Infarct size Left coronary artery occlusion rendered approximately 28% of the left ventricle at risk of infarction. There were no significant differences in risk zone size between the groups (Table 2). DPCPX had little effect on infarct size in its own right. GR79236 produced a significant

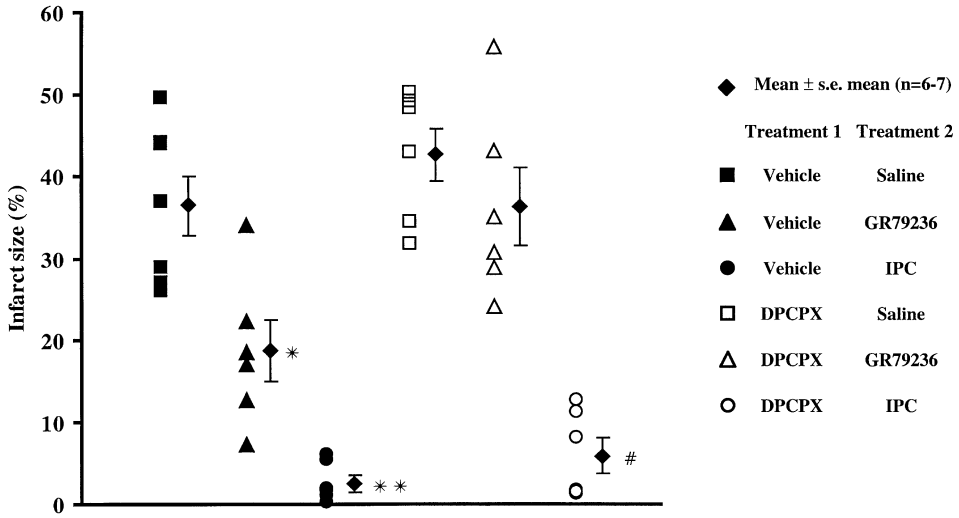

Figure 3 Effect of pre-treatment with DPCPX (3.3×10^{-6} mol kg^{-1} i.v.) or its vehicle (1 ml kg^{-1} i.v.) on the development of infarction after treatment with GR79236 (3×10^{-8} mol kg^{-1} i.v.) or ischaemic preconditioning (IPC; 5 min ischaemia, followed by 5 min reperfusion). The filled diamonds show mean infarct size expressed as a percentage of the risk zone, the error bars represent s.e.mean. All other symbols show infarct size for each individual rabbit. *In the presence of the vehicle for DPCPX, GR79236 evoked a significant (<0.01) limitation in the development of infarction compared to saline. **In the presence of vehicle for DPCPX, IPC evoked a significant ($P<0.01$) limitation in the development of infarction compared to GR79236. #In the presence of DPCPX, IPC evoked a significant ($P<0.01$) limitation in the development of infarction compared to GR79236 and saline.

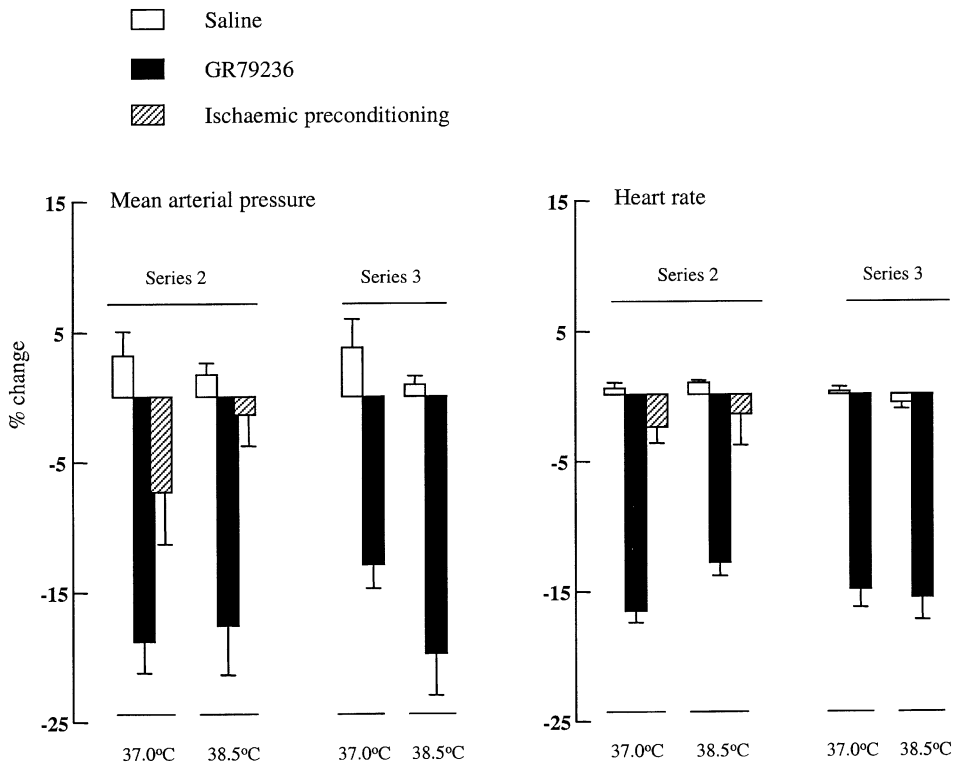

Figure 4 Haemodynamic effect of saline (1 ml i.v.) GR79236 (3×10^{-8} mol kg^{-1} i.v.) or ischaemic preconditioning (5 min ischaemia followed by 5 min reperfusion) administered prior to occlusion, and of saline (1 ml i.v.) or GR79236 (3×10^{-8} mol kg^{-1} i.v.) administered 10 min prior to the onset of reperfusion, in rabbits whose body core temperature was maintained at 37.0°C or at 38.5°C. Data are expressed as mean percentage peak change in MAP or HR ± s.e.mean ($n=6$).

($P<0.01$) limitation of the development of infarction, as did ischaemic preconditioning. The limitation in the development of infarction by ischaemic preconditioning was significantly ($P<0.01$) greater than that seen after administration of GR79236. Myocardial protection induced by GR79236, but not that by ischaemic preconditioning, was prevented by pre-treatment with DPCPX. These results are shown in Figure 3.

Series 2 experiments – influence of body core temperature on cardioprotection induced by GR79236 administered prior to occlusion, or by ischaemic preconditioning

Haemodynamics MAP and HR values at the beginning of the experimental protocol, and at time points throughout the duration of the experiment, are shown in Table 3. Changes in

Table 3 Haemodynamic values for Series 2 experiments

		Mean arterial pressure (MAP; mmHg)					*Heart rate (HR; beats min^{-1})*				
Group	n	*Prior to treatment*	*Prior to occlusion*	*Prior to reperfusion*	*+1 h into reperfusion*	*+2 h into reperfusion*	*Prior to treatment*	*Prior to occlusion*	*Prior to reperfusion*	*+1 h into reperfusion*	*+2 h into reperfusion*
Saline	6	81.0	84.7	79.5	76.4	72.8	240.1	242.3	234.2	254.8	256.9
37.0°C		(2.7)	(3.8)	(2.8)	(4.1)	(4.3)	(7.4)	(8.3)	(8.1)	(9.0)	(7.1)
GR79236	6	80.6	73.9*	71.0	78.8	81.0	250.6	216.6##	233.5	261.2	254.8
37.0°C		(2.7)	(3.0)	(2.5)	(2.3)	(3.2)	(9.6)	(5.8)	(5.2)	(8.7)	(8.6)
IPC	6	86.9	82.4*	81.3	79.4	71.5	245.7	241.1	243.0	261.9	258.5
37.0°C		(2.9)	(4.3)	(3.6)	(3.2)	(4.9)	(10.0)	(12.0)	(10.4)	(8.3)	(8.2)
Saline	6	79.3	81.3	70.2	69.1#	64.6#	265.7**	271.8	247.2	266.3	268.7
38.5°C		(2.2)	(2.4)	(4.5)	(2.3)	(2.0)	(10.2)	(12.4)	(13.9)	(10.4)	(9.5)
GR79236	6	78.3)	71.2*	71.0	67.8#	64.3#	281.7**	256.5##	273.0	290.7	291.5
38.5°C		(1.2)	(2.3)	(3.1)	(3.9)	(3.9)	(9.2)	(9.2)	(14.0)	(10.8)	(9.1)
IPC	6	80.3	77.3*	75.9	68.9#	62.8#	265.4**	263.9	263.4	270.9	267.8
38.5°C		(5.2)	(5.8)	(3.9)	(5.8)	(4.5)	(11.6)	(12.2)	(9.3)	(12.6)	(11.8)

Data are expressed as mean ± s.e.mean of *n* observations. Basal readings were taken just prior to drug administration. Saline or GR79236 (3×10^{-8} mol kg^{-1} i.v.) was administered, or ischaemic preconditioning (IPC) applied, 10 min prior to occlusion, in rabbits whose body core temperature was maintained at 37°C or at 38.5°C. *MAP in the GR79236- and IPC-treated groups was significantly lower ($P<0.01$) than in the saline-treated groups across both temperatures. **Basal HR at 38.5°C was significantly ($P<0.05$) higher than basal HR at 37.0°C. #MAP at 38.5°C was significantly ($P<0.01$) lower 1 and 2 h into reperfusion than at 37.0°C for the corresponding time points. ##HR in the GR79236-treated groups was significantly ($P<0.01$) lower prior to occlusion than saline or IPC for the corresponding time points.

Table 4 Haemodynamic values for Series 3 experiments

		Mean arterial pressure (MAP; mmHg)					*Heart rate (HR; beats min^{-1})*				
Group	n	*Prior to occlusion*	*Prior to treatment*	*Prior to reperfusion*	*+1 h into reperfusion*	*+2 h into reperfusion*	*Prior to occlusion*	*Prior to treatment*	*Prior to reperfusion*	*+1 h into reperfusion*	*+2 h into reperfusion*
Saline	6	79.0	73.0	75.6	72.0	70.3	240.6	229.6	231.5	254.0	252.3
37.0°C		(3.2)	(3.5)	(2.7)	(4.0)	(6.1)	(10.2)	(8.6)	(8.0)	(8.8)	(8.5)
GR79236	6	71.8	72.4	64.2	71.5	68.5	243.0	231.1	201.4**	239.5	249.2
37.0°C		(3.7)	(4.8)	(4.7)	(2.6)	(2.5)	(11.9)	(8.6)	(7.3)	(9.7)	(10.9)
Saline	6	82.1	84.0	84.2	78.8	76.2	283.4*	273.5	271.7	280.2	288.6
38.5°C		(4.2)	(3.1)	(3.0)	(3.8)	(3.5)	(9.5)	(12.7)	(12.7)	(7.5)	(9.8)
GR79236	6	77.0	80.1	65.9	70.1	72.0	262.2*	258.4	222.0**	261.8	266.9
38.5°C		(4.0)	(2.5)	(4.7)	(4.1)	(4.5)	(12.6)	(7.2)	(5.2)	(11.5)	(11.7)

Data are expressed as mean ± s.e.mean of *n* observations. Basal readings were taken just prior to occlusion. Saline or GR79236 (3×10^{-8} mol kg^{-1} i.v.) was administered 10 min prior to occlusion in rabbits whose body core temperature was maintained at 37.0°C or at 38.5°C. *Basal HR at 38.5°C was significantly ($P<0.05$) higher than basal HR at 37.0°C. **HR in GR79236-treated groups was significantly ($P<0.01$) lower than in saline-treated groups at the corresponding time point.

MAP or HR in response to the different treatments are shown in Figure 4.

There were no significant differences in basal MAP values between the groups. Basal HR was significantly ($P<0.05$) higher in the groups of rabbits in which body temperature was maintained at 38.5°C compared to those kept at 37.0°C (Table 3). Intravenous administration of GR79236 reduced MAP and HR, whereas ischaemic preconditioning produced transient reductions in MAP, but had little (<10%) effect on HR (Figure 4). These effects on MAP and HR were not significantly influenced by body temperature. MAP was still significantly ($P<0.01$) lower just prior to occlusion after treatment with GR79236 or after ischaemic preconditioning than in vehicle-treated groups of rabbits, and HR was significantly ($P<0.01$) lower in GR79236-treated groups of rabbits than in those that received vehicle or ischaemic preconditioning (Table 3). MAP tended to decline over the course of the experiment in all groups, and was significantly ($P<0.01$) lower 1 and 2 h into reperfusion in rabbits where body temperature was maintained at 38.5°C, compared with that seen in those maintained at 37.0°C.

Infarct size Occlusion of the left coronary artery for 30 min rendered approximately 30% of the left ventricle at risk of infarction. There were no significant differences in risk zone size between the groups (Table 2). Infarct size was not significantly different in saline-treated rabbits where body temperature was maintained at 37.0 or at 38.5°C. When body temperature was maintained at 37.0°C, GR79236 significantly ($P<0.05$) limited the development of infarction. However, when body temperature was maintained at 38.5°C, infarct size was not significantly different from that in the saline-treated control groups of rabbits at this temperature (Figure 5). In contrast to the cardioprotective effect of GR79236, limitation of infarct development conferred by ischaemic preconditioning was unaffected by temperature. Ischaemic preconditioning-induced protection was significantly ($P<0.05$) greater than that induced by GR79236 at both temperatures tested (Figure 5).

Series 3 experiments – cardioprotective effect of GR79236 administered prior to reperfusion and the influence of temperature

Haemodynamics MAP and HR values at the beginning of the experimental protocol, and at time points throughout the duration of the experiment, are shown in Table 4. Changes in MAP or HR in response to the different treatments are shown in Figure 4.

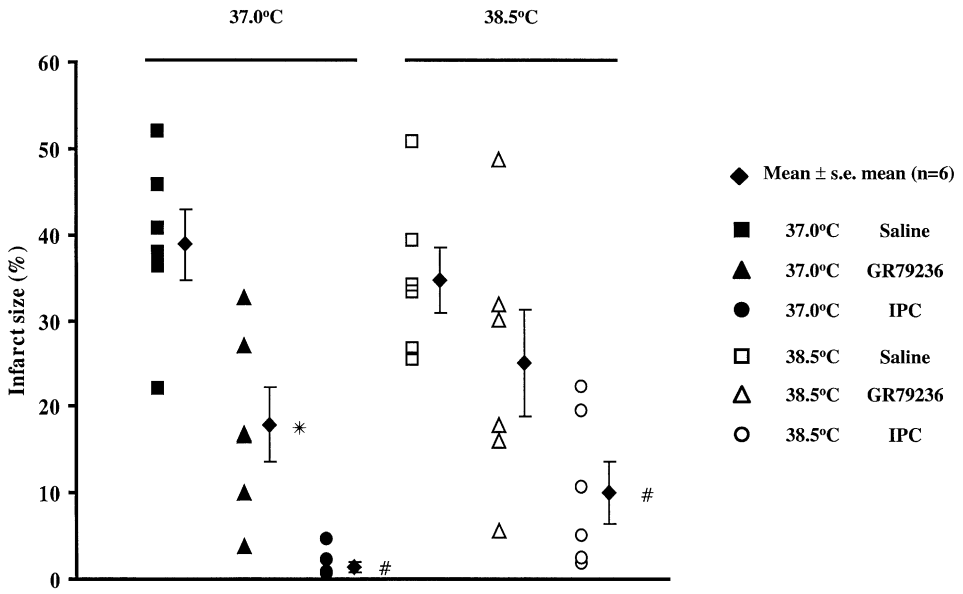

Figure 5 Effect of saline (1 ml i.v.), GR79236 (3×10^{-8} mol kg^{-1} i.v.), or ischaemic preconditioning (IPC; 5 min ischaemia followed by 5 min reperfusion) on the development of infarction when administered or applied 10 min prior to occlusion, and the influence of maintaining body core temperature at 37.0°C or at 38.5°C. The filled diamonds show mean infarct size expressed as a percentage of the risk zone, the error bars represent s.e.mean. All other symbols show infarct size for each individual rabbit. *At 37°C GR79236 evoked a significant ($P<0.05$) limitation of the development of infarction compared to saline. #IPC evoked a significant ($P<0.05$) limitation of the development of infarction compared to GR79236 at the corresponding temperature.

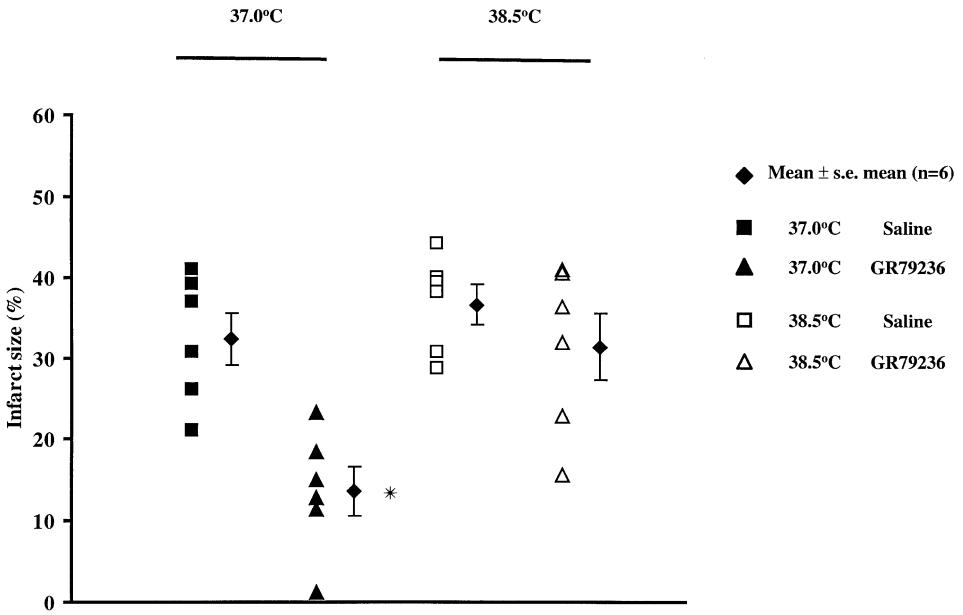

Figure 6 Effect of saline (1 ml i.v.) or GR79236 (3×10^{-8} mol kg^{-1} i.v.) on the development of infarction, administered 10 min prior to the onset of reperfusion, and the influence of maintaining body core temperature at 37.0°C or at 38.5°C. The filled diamonds show mean infarct size, the error bars represent s.e.mean. All other symbols show infarct size for each individual rabbit. *At 37.0°C GR79236 evoked a significant ($P<0.01$) limitation in the development of infarction compared to all other treatments.

There were no significant differences in basal MAP values between the groups. Basal HR values were significantly ($P<0.05$) higher when body temperature was maintained at 38.5 rather than at 37.0°C (Table 4). Intravenous administration of GR79236 reduced MAP and HR, but these changes were not significantly influenced by body temperature. The reduction in MAP caused by GR79236 returned to basal levels just prior to reperfusion. However, HR remained significantly ($P<0.01$) lower just prior to reperfusion, than that seen in saline-treated groups of rabbits.

Infarct size Occlusion of the left coronary artery for 30 min rendered approximately 30% of the left ventricle at risk of infarction. There were no significant differences in risk zone size between the groups (Table 2). In groups of rabbits treated with saline 10 min prior to the onset of reperfusion, development of infarction was similar whether body temperature was maintained at either 37.0 or 38.5°C. When body temperature was maintained at 37.0°C, GR79236 significantly ($P<0.01$) limited infarct development, but this effect was lost when body temperature was maintained at 38.5°C (Figure 6).

Discussion

Cardioprotective effect of GR79236 – comparison with ischaemic preconditioning

In keeping with the current literature describing myocardial protection induced by adenosine A_1 receptor agonists (see Introduction), we have shown that GR79236 (Gurden *et al*., 1993), an adenosine A_1 receptor agonist, has cardioprotective properties in an anaesthetized rabbit model of myocardial ischaemia and reperfusion (Travers *et al*., 1998). In the present study, when administered 10 min prior to occlusion, GR79236 (3×10^{-8} mol kg^{-1} i.v.) significantly limited the development of infarction, compared to the vehicle-treated control group. Similarly, and as described in the literature, ischaemic preconditioning (one 5 min episode of ischaemia followed by 5 min reperfusion), also markedly limited the development of infarction in this model. This reduction in the development of infarction with ischaemic preconditioning was significantly greater than that conferred by GR79236, suggesting that adenosine A_1 receptor activation might not be the only pathway through which ischaemic preconditioning can confer myocardial protection in this model. The lesser degree of protection conferred by GR79236 is unlikely to reflect the dose used; previous (unpublished) experiments using a range of doses of GR79236 revealed that the cardioprotective effect of 1 or 3×10^{-8} mol kg^{-1} i.v. are approximately equal. Thus, the maximum protective effect of A_1 receptor stimulation seems to be less than that of ischaemic preconditioning. In keeping with activation of adenosine A_1 receptors, GR79236 reduced heart rate (HR), and this was associated with a fall in mean arterial pressure (MAP). The effect on HR had not fully recovered to baseline values at the time of occlusion but, in previous (unpublished) studies, we have found that the haemodynamic effects of a single bolus dose of GR79236 (3×10^{-8} mol kg^{-1} i.v.) in the anaesthetized rabbit last approximately 30 min. It seems unlikely that these haemodynamic events are responsible for the protective properties of GR79236. Changes in MAP or HR (Ytrehus *et al*., 1994), or rate pressure product (Chien *et al*., 1994), were found not to significantly influence infarct size in the rabbit. In addition, the adenosine A_1 receptor agonist R-PIA, significantly limited the development of infarction even when the bradycardia and severe hypotension that it normally produced were prevented by pacing (Tsuchida *et al*., 1992). In contrast, the adenosine A_2 receptor agonist, 2-[4-(2-Carboxyethyl)phenethyl-amino]-5′-*N*-ethylcarboxamido adenosine (CGS 21680) elicited similar reductions in blood pressure to R-PIA, but failed to limit infarct development in the anaesthetized rabbit (Thornton *et al*., 1992). Furthermore, GR79236-induced cardioprotection occurs independently of changes in HR and MAP (Louttit *et al*., 1999), albeit in the pig, and GR79236-induced myocardial protection in the rabbit is similar, and maximal, for doses of 1 or 3×10^{-8} mol kg^{-1} i.v. (unpublished observations), whereas the higher dose of GR79236 produces a more marked bradycardia. Thus, the protection conferred occurs in the face of differing degrees of change in MAP and HR. For the same reasons, and since there was little difference between control infarct size at 37.0 or at 38.5°C, the differences seen in *basal* HR (Tables 3 & 4) are also unlikely to account for the cardioprotective properties of this drug.

Involvement of adenosine A_1 receptors in myocardial protective mechanisms

Whilst having little effect on infarct size in its own right, the adenosine A_1 receptor antagonist DPCPX (3.3×10^{-6} mol kg^{-1} i.v.) abolished GR79236-induced cardioprotective and haemodynamic effects, thus confirming a role for adenosine A_1 receptors in the mechanism of action of GR79236. The protective effect of ischaemic preconditioning can be prevented with the non-selective adenosine receptor agonist 8-(*p*-sulphophenyl)theophylline (8-SPT) in anaesthetized rabbits (Liu *et al*., 1991; Downey *et al*., 1993; and our unpublished observations). However, in the present study, DPCPX did not block the cardioprotective effect of ischaemic preconditioning. This might seem surprising because DPCPX *does* block cardioprotection evoked by ischaemic preconditioning in the pig (Schwarz *et al*., 1991; Louttit *et al*., 1999), ferret (Gomoll, 1996) and dog (Auchampach & Gross, 1993). The present results suggest that in the rabbit heart, ischaemic preconditioning appears to be able to confer protection *via* an adenosine A_1 receptor-independent route. Indeed, Downey *et al*. (1993) have reported that DPCPX does not block protection conferred by ischaemic preconditioning in the rabbit heart *in situ* (although no data were presented to support this observation); similar findings have been made in rabbit isolated hearts (Liu *et al*., 1994; Lasley and Mentzer, 1995), and cardiac myocytes preconditioned by simulated ischaemia (Armstrong & Ganote, 1994). In contrast, Rice *et al*. (1996) and Wang *et al*. (1997) reported that DPCPX reduced, but did not abolish, cardiomyocyte protection afforded by simulated ischaemic preconditioning. Taken together, these findings suggest that stimulation of adenosine A_1 receptors does not play a predominant role in the protection conferred by ischaemic preconditioning in the rabbit heart. Thus, the pathway through which ischaemic preconditioning is mediated might be different depending on the species studied. Recently, a novel adenosine A_1-like receptor, insensitive to DPCPX, and termed the A_3 receptor, has been cloned and characterized (Zhou *et al*., 1992). A definitive role for this receptor as a possible route through which ischaemic preconditioning might be mediated has been precluded by a lack of selective agonists and antagonists. However, cautious interpretation of experiments using the pharmacological tools available, has shown that protection can be induced in rabbit isolated cardiomyocytes (Armstrong & Ganote, 1994), rabbit isolated hearts (Tracy *et al*., 1997) and rabbit heart *in situ* (Smith *et al*., 1996), *via* an adenosine receptor with properties consistent with those of the adenosine A_3 receptor. It is even conceivable that a considerable degree of redundancy exists in the mechanism(s) through which ischaemic preconditioning confers protection in the rabbit heart. Thus, stimulation of either adenosine A_1 or A_3 receptors might maximally protect the myocytes, so that even after blockade of a single population (e.g. A_1 receptors), the overall extent of the protection remains undiminished.

Effect of temperature on myocardial protective mechanisms

McClanahan *et al*. (1994) showed in anaesthetized pigs, that pentostatin, an agent that would be expected to increase the concentration, and prolong the presence, of endogenous adenosine in the myocardium, reduced infarct size when body temperature was maintained at 35.0°C. However, when body temperature was maintained at 37.0°C, the protective effect of pentostatin was lost. Thus, in their studies, temperature alone was shown to influence the effect of an agent that confers cardioprotection, presumably indirectly *via* adenosine receptors, and this effect occurred over a temperature range that did not influence infarct size in vehicle-treated pigs. Previously, we have found that the cardioprotective effect of GR79236 in rabbits can be variable, particularly under conditions of high

ambient temperature. Those experiments were carried out using a homeothermic blanket control system, with the thermistor placed in the oesophagus, to control body temperature. However, when we measured rabbit body core temperature using a thermometer placed in the colon, *via* the rectum, we found that it was approximately 1–1.5°C greater than that measured simultaneously from the oesophageal probe, and that this difference could vary on a day-to-day basis. We cannot account for this observation, but it is possible that the air moving in and out of the trachea produces a cooling effect on the thermistor in the oesophagus, resulting in greater heat input from the blanket. We therefore carried out studies to investigate whether differences in body core temperature could influence the cardioprotective effect of GR79236 because, in the light of the experiments carried out by McClanahan *et al.* (1994), small variations in body temperature within an experimental group might explain the variable results we obtained with GR79236. Subsequently, we used a calibrated Digitron temperature probe placed in the colon, *via* the rectum, and adjusted the homeothermic blanket control unit manually, so that rabbit body core temperature was maintained within ± 0.3°C of the desired temperature.

In vehicle-treated control groups of rabbits, infarct sizes were similar irrespective of whether body temperature was maintained at 37.0 or at 38.5°C (Figure 5). GR79236 administered 10 min prior to occlusion significantly limited the development of infarction compared to control groups of rabbits maintained at 37.0°C, but when the drug was administered to rabbits maintained at 38.5°C, the cardioprotective effect of GR79236 became less consistent, and mean infarct size was not significantly different from that seen in the control group at this temperature. Ischaemic preconditioning was also markedly protective, but in contrast to the cardioprotective effect of GR79236, was not significantly influenced by temperature. However, the limitation of infarct development in ischaemically preconditioned rabbits was less consistent when the experiment was carried out at 38.5°C (Figure 5), so perhaps a further increase in body temperature might render ischaemic preconditioning less effective. This observation may lend support to the concept that more than one receptor mediates protection conferred by ischaemic preconditioning in the rabbit, but with only one of the receptors (A_1) being influenced by temperature. Indeed, Stojanov & Proctor (1990) and Broadley *et al.* (1985), have shown that temperature-induced variability in tissue responsiveness to adenosine analogues is linked to activation of adenosine A_1, but not A_2, receptors. Thus, it is possible that coupling of GR79236 to the adenosine A_1 receptor, and/or subsequent signal transmission, is altered with an elevation of body temperature. We therefore compared the magnitude of GR79236-induced haemodynamic changes at 37.0°C with those seen at 38.5°C, and found GR79236-induced changes in MAP or HR were not different at the two temperatures (Figure 4). Similarly, in studies in which GR79236 was administered prior to reperfusion (Series 3), there were no significant differences between reductions in MAP and HR at the two temperatures (Figure 4). Thus, in the present study, we found no convincing evidence to support the concept that signal transduction *via* adenosine A_1 receptors is modulated by temperature.

Whether or not adenosine A_1 receptor agonists can protect the myocardium when administered *during* (rather than before) ischaemia, is controversial (see Introduction). When GR79236 was administered 10 min prior to the onset of reperfusion in rabbits in which body temperature was maintained at 37.0°C, the development of infarction was limited by a similar extent to that seen when GR79236 was administered prior to coronary artery occlusion at this temperature (Figure 6). This suggests that most of the damage to the myocardium occurs during the last 10 min of ischaemia, and/or during reperfusion. When GR79236 was administered 10 min prior to the onset of reperfusion in rabbits where body temperature was maintained at 38.5°C, the cardioprotective effect of GR79236 was lost (Figure 6). Thus, treatment with an adenosine A_1 receptor agonist prior to ischaemia, or just prior to reperfusion, is equally protective and, in both cases, the protective effect is less when body temperature is maintained at 38.5°C rather than at 37.0°C. This might explain why Baxter *et al.* (personal communication), were unable to limit the development of infarction by administration of GR79236 just prior to the onset of reperfusion, since in their studies, body core temperature was maintained between 38.0 and 39.0°C.

There is an increasing amount of evidence in the literature to show that development of infarction is markedly temperature-sensitive. Chien *et al.* (1994) measured infarct size in 18 rabbits in which body temperature was maintained at a given level within a range of 35–42°C. The relationship between infarct size and temperature was such that for every 1°C increase in body temperature, there was a 10% increase in infarct size. Hale & Kloner (1997) made a similar observation in rabbit hearts, by inducing regional hypothermia of the risk zone. In the anaesthetized pig, over a temperature range of 35–39°C, there is a 20% increase in infarct size with each 1°C increase in body temperature (Dunker *et al.*, 1996). It might not be surprising that we did not see a difference in infarct size in control rabbits maintained at 37.0 or at 38.5°C, because of comparatively smaller difference between these temperatures and those evaluated by other authors, and the relatively small numbers of animals used. Nevertheless, it is established that if the severity of an ischaemic insult is increased by prolonging the period of the sustained occlusion, the protection conferred by ischaemic preconditioning is lost (van den Doel *et al.*, 1998). Thus, there becomes a time during the infarction-inducing process beyond which ischaemic preconditioning is unable to protect the myocardium from ischaemia and reperfusion-induced injury. It is therefore tempting to hypothesise that an increase in body temperature may impair cardioprotective mechanisms, and thus make it more difficult for GR79236 to confer myocardial protection. What these mechanisms might be remains unknown. However, glucose utilization and lactate production in non-ischaemic or ischaemic hearts has been shown to be increased at higher temperatures suggesting a greater metabolic demand (Ichihara *et al.*, 1981). The damage induced when oxygen or calcium was re-introduced after anoxic or calcium-free perfusion (oxygen and calcium paradoxes, respectively), was highly temperature-dependent in rat isolated hearts (Hearse *et al.*, 1978). In both of these paradoxes, cellular damage increased sharply over a temperature range of 33–36°C. Thus, increasing the temperature at which these investigations are carried out, promotes processes that might be associated with ischaemia reperfusion-induced damage, and this may make it more difficult to protect against ischaemia and reperfusion injury.

In conclusion, the present study has shown that the adenosine A_1 receptor agonist GR79236 reduces infarct size in an anaesthetized rabbit model of myocardial ischaemia and reperfusion, an effect mediated by adenosine A_1 receptors. The protective effect of ischaemic preconditioning in the rabbit seems not, however, to be attributable to activation of adenosine A_1 receptors. The body temperature at which experiments are carried out can markedly influence the extent of protection afforded by GR79236, but not that of ischaemic

preconditioning, over a temperature range that does not significantly influence infarct size in its own right. Thus, even small differences in body temperature might be expected to induce variability within an experimental group and significantly influence the outcome of the study. For this reason, it is essential when carrying out studies of this nature, to maintain body core temperature within a very strictly predetermined range. In addition, our results confirm that administration of an adenosine A_1 receptor agonist can reduce infarct size when administered just prior to the onset of reperfusion, suggesting that the damage against which GR79236 protects occurs mainly during reperfusion.

We would like to thank Mark Lennon for his considerable efforts, and rapid responses, to our requests for statistical analyses and advice.

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(Received December 7, 1998
Revised June 17, 1999
Accepted June 24, 1999)