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Reduction by prostaglandin E_1 or prostaglandin E_0 of myocardial infarct size in the rabbit by activation of ATP-sensitive potassium channels

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1 This study examined whether pretreatment of rabbits with infusions of prostaglandin E_1 (PGE_1) or prostaglandin E_0 (PGE_0) (which were terminated prior to the onset of ischaemia) reduce myocardial infarct size arising from coronary artery occlusion (60 min) and reperfusion (120 min). In addition, we investigated whether the observed cardioprotective effects of these two prostaglandins were due to the activation of ATP-sensitive potassium (K_{ATP}) channels.

2 In the anaesthetized rabbit, infarct size (expressed as a percentage of the area at risk) after 60 min of coronary artery occlusion followed by 2 h of reperfusion was $59 \pm 4\%$ ($n=10$). PGE_1 or PGE_0 treatment ($1.0\ \mu g\ kg^{-1}\ min^{-1}$), administered as 1 h pretreatments ($0.05\ ml\ min^{-1}$, i.v.), significantly reduced infarct size to $44 \pm 6\%$ ($n=6$) or $42 \pm 1\%$ ($n=6$), respectively. PGE_1 or PGE_0 pretreatment resulted in a significant reduction in mean arterial blood pressure, which returned to baseline within 15 min of discontinuation of the infusion (i.e. prior to LAL ligation).

3 The reduction in infarct size afforded by PGE_1 was abolished by pretreatment of rabbits with the K_{ATP} channel blockers, glibenclamide ($60 \pm 4\%$; $n=8$) or 5-hydroxydecanoate ($58 \pm 6\%$; $n=6$). Similarly, glibenclamide also largely attenuated the reduction in infarct size afforded by PGE_0 ($52 \pm 3\%$; $n=8$).

4 We propose that a 1 h pretreatment of PGE_1 or PGE_0 reduces infarct size by activating protein kinase C resulting in the opening of K_{ATP} channels.

Keywords: Prostaglandin E_1; prostaglandin E_0; glibenclamide; ATP-sensitive potassium channel; myocardial infarction; protein kinase C; ischaemic preconditioning

Introduction

Prostacyclin (PGI_2) and stable PGI_2-analogues, such as iloprost, exert potent anti-ischaemic effects in models of acute myocardial ischaemia and reperfusion in various species including the rabbit (Lefer *et al.*, 1978; Schrör *et al.*, 1981; Chiariello *et al.*, 1988). Similarly, prostaglandin E_1 (PGE_1) exerts beneficial effects on haemodynamic, biochemical, electrocardiographic and functional indices of ischaemia and reperfusion-related injury of the myocardium (Hutton *et al.*, 1973; Takano *et al.*, 1977; Riemersma *et al.*, 1977; Jugdutt *et al.*, 1981; Schrör *et al.*, 1988a; Simpson *et al.*, 1988). However, an improvement in biochemical indicators of ischaemic tissue injury, such as the loss of cytosolic marker enzymes from the ischaemic myocardium, is not necessarily associated with a reduction in infarct size (Thiemermann *et al.*, 1989).

The cardioprotective effects of PGE_1 have been attributed to systemic vasodilatation (resulting in a reduction in oxygen demand), coronary vasodilatation (resulting in an increase in coronary blood flow and, hence, oxygen supply), inhibition of platelet aggregation and in particular, inhibition of neutrophil activation (Hutton *et al.*, 1973; Jugdutt *et al.*, 1981; Schrör *et al.*, 1988a; Simpson *et al.*, 1988). However, cardioprotective effects of vasodilator prostaglandins also occur in isolated hearts perfused at constant flow with buffer solutions and subjected to global ischaemia and reperfusion (Araki & Lefer, 1980). This suggests, therefore, that vasodilatation and inhibition of platelet and neutrophil function are not a prerequisite for the cardioprotective effects of vasodilator prostaglandins. Thus, it has been proposed that the anti-ischaemic effects of these eicosanoids in isolated cells and tissues are due to a 'cytoprotective' or 'membrane stabilizing' effect, the mechanism of which is unknown (Schrör *et al.*, 1988b).

Ischaemic preconditioning which is defined as 'the protective adaptive mechanism produced by short periods of ischaemic stress resulting in a marked, albeit temporary, resistance of the myocardium to a subsequent more prolonged period of that same stress' (Murry *et al.*, 1986), is thought to be mediated by the translocation of inactive protein kinase C (PKC) from the cytosol to the membrane where it can be activated. This hypothesis is based on the findings demonstrating that (i) preconditioning is prevented by inhibitors of PKC, such as staurosporine, and (ii) preconditioning can be mimicked with activators of PKC, such as phorbol myristate acetate and oleyl acetyl glycerol (Ytrehus *et al.*, 1994). It is suggested that the activated PKC phosphorylates a membrane protein that may be linked to the ATP-sensitive potassium (K_{ATP}) channel, thus opening this channel. Indeed, inhibition of K_{ATP} channels with glibenclamide abolishes the cardioprotective effects of ischaemic preconditioning (Vegh *et al.*, 1993).

Interestingly, PGE_1 also activates PKC via EP_1 and EP_3 (subgroup A and D) receptors, and hence the IP_3 /DAG pathway (Hohlfeld, 1995). Thus, the vasodilator effects of PGE_1 in the coronary circulation of the rat are, at least in part, due to activation of K_{ATP} channels (Ney & Feelisch, 1995). Similarly, the coronary vasodilator effects of prostacyclin, prostaglandin E_2 and prostaglandin D_2 in the rat heart (Bouchard *et al.*, 1994), and of prostacyclin and iloprost in the rabbit heart (Jackson *et al.*, 1993) are (in part) due to activation of K_{ATP} channels, as they are attenuated by glibenclamide.

Here we investigate whether pretreatment ('pharmacological preconditioning') with PGE_1 (i) reduces infarct size in a rabbit model of acute myocardial ischaemia (60 min) and reperfusion (120 min), and (ii) whether any potential cardioprotective effect of PGE_1 is due to the activation of K_{ATP} channels. As PGE_1 is almost totally metabolized in one passage through the pulmonary circulation (Ferreira & Vane, 1967), it has been argued that any long-term cardioprotective effects seen with PGE_1 could be attributed to its more stable,

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active metabolite 13,14-dihydro-prostaglandin E_1 (PGE_0). We have therefore, also investigated the effects of PGE_0 on infarct size in the same model.

Methods

This study was carried out on 61 male rabbits (New Zealand White rabbits, Foxfield, U.K.) weighing 2.5 to 3.0 kg receiving a standard diet and water *ad libitum*.

Surgery and instrumentation

Ten minutes before surgery, animals were premedicated with Hypnorm i.m. (containing 0.315 mg ml^{-1} fentanyl citrate and 10 mg ml^{-1} fluanisone; Janssen Pharmaceutical Ltd.) at 0.1 ml kg^{-1}. General anaesthesia was then induced with sodium pentobarbitone (30 mg kg^{-1}, i.v. injected into the left marginal ear vein; Sagatal, May & Baker) and maintained with supplementary doses of sodium pentobarbitone as required. Lignocaine (Xylocaine 2%, Astra Pharmaceuticals) was also used for local anaesthesia. The rabbits were tracheotomized, intubated and ventilated with room air from a Harvard ventilator at a rate of 36–40 strokes min^{-1} and a tidal volume of 18–20 ml. Body temperature was maintained at 38 ± 1°C by means of a rectal probe thermometer attached to a homeothermic blanket control unit (Harvard Apparatus Ltd.). The left femoral artery was cannulated and connected to a pressure transducer (Spectramed P23XL) to monitor mean arterial blood pressure. Whilst monitoring pressure, another catheter was placed in the left ventricle, via the right common carotid artery, for measurement of left ventricular systolic pressure (LVSP) and administration of drugs. The left femoral vein was cannulated for the administration of drugs.

Myocardial ischaemia and reperfusion

The method of coronary artery occlusion and reperfusion in the anaesthetized rabbit was performed as previously described (Thiemermann *et al.*, 1989; McMurdo *et al.*, 1994). Briefly, rabbits were anaesthetized and instrumented as described above for haemodynamic recordings. Subsequently, a 2–3 cm left intercostal thoracotomy (4th intercostal space) was performed and the heart was suspended in a temporary pericardial cradle. A snare occluder was placed around the first anterolateral branch of the left coronary artery (LAL) (Maxwell *et al.*, 1987) 1 cm distal from its origin. In contrast to other species, the rabbit LAL supplies most of the left ventricle and apex of the left ventricular myocardium (Flores *et al.*, 1984). Care was taken not to include any veins draining blood from this area. After completion of the surgical procedure the animals were allowed to stabilize for 30 min before LAL ligation.

The coronary artery was occluded at time 0 by tightening of the occluder. This was associated with the typical electrocardiographic (ST-segment elevation and increase in R-wave amplitude) and haemodynamic (fall in LVSP) changes of myocardial ischaemia. After 60 min of acute myocardial ischaemia, the occluder was re-opened to allow a 2 h reperfusion, which was confirmed by the appearance of an 'epicardial blush'.

Haemodynamic measurements and electrocardiogram

Haemodynamic parameters, including mean arterial pressure (MAP), heart rate (HR), systolic and diastolic pressure (PA_d) and left ventricular systolic pressure (LVSP) were continuously recorded on a 4-channel Grass 7D polygraph recorder (Quincy, Mass., U.S.A.). However, detailed data analysis was only performed at −85 min (baseline), −75 min (after drug treatment), −15 min (just prior to end of PGE_1, PGE_0 or vehicle infusion), 0 min (just prior to LAL occlusion), 15, 30, 45, 60 min (occlusion period) and every hour during the subsequent reperfusion period (120, 180 min). Lead II electrocardiograms (ECGs) were recorded from sub-dermal platinum electrodes on a 7P4H Grass ECG-amplifier attached to Grass 4-channel recorder (Grass, Mass., U.S.A.). The heart rate was automatically calculated from left ventricular systolic pulse curves by means of a Grass 7P4H tachograph. The MAP was calculated as $(LVSP - PA_d) \times 0.33 + PA_d$. The pressure rate index, a relative indicator of myocardial oxygen consumption (Baller *et al.*, 1981) was calculated as the product of MAP and HR, and expressed in mmHg min^{-1}.

Measurements of area at risk and infarct size

After the 2 h reperfusion period, the LAL was reoccluded and Evans blue dye solution (4 ml of 2% w/v) injected into the left ventricle to distinguish between perfused and non-perfused (myocardium at risk) sections of the heart. The Evans blue solution stains the perfused myocardium, while the occluded vascular bed remains uncoloured. The dose of Evans blue dye used in this study is well within the range reported for nearly exclusive binding to plasma albumin (or other proteins) in the rabbit (Lindner & Heinle, 1982). The rabbits were killed with an overdose of anaesthetic. The heart was excised and sectioned into 4–5 mm thick slices. After removing the right ventricular wall, the area at risk and non-ischaemic myocardium were separated by following the line of demarcation between blue stained and unstained (pink/red) tissue. To distinguish between ischaemic and infarcted tissue, the area at risk was cut into small pieces and incubated (20 min at 37°C) with *p*-nitro-blue tetrazolium (NBT, 0.5 mg ml^{-1}; Sigma, Poole, Dorset). In the presence of intact dehydrogenase enzyme systems (normal myocardium), NBT forms a dark blue formazan, whilst areas of necrosis lack dehydrogenase activity and therefore do not stain (Nachlas & Shnitka, 1963).

Drug regimens

PGE_1, PGE_0 (1.0 μg kg^{-1} min^{-1}) or their vehicle-control (5% methanol in 0.9% NaCl for PGE_1 or 5% methyl acetate in 0.9% NaCl for PGE_0) were infused intravenously for 1 h at a rate of 0.05 ml min^{-1}, starting 75 min prior to LAL occlusion. Fifteen min before LAL occlusion, the infusion was switched off and the MAP was allowed to return to within 10% of its baseline value. Glibenclamide, a blocker of ATP-sensitive potassium (K_{ATP}) channels (Gross *et al.*, 1990), was administered as a bolus injection (2 ml volume) intravenously (i.v.) 10 min before infusion of PGE_1, PGE_0 or vehicle. Sodium 5-hydroxydecanoate (5-HD), an ischaemia-selective inhibitor of (K_{ATP}) channels (McCullough, *et al.*, 1991), was administered as a bolus injection (2 ml volume) into the left ventricle 10 min before infusion of PGE_1 or vehicle. As the different vehicle groups (methanol and methyl acetate) had no significant effect on any of the haemodynamic parameters or on infarct size, the data of these groups were pooled to form one vehicle-control group.

Thus, eight experimental groups were studied:
Group 1: vehicles (2 ml 20% dimethyl sulphoxide, DMSO, for glibenclamide, i.v. bolus, and 5% methanol, $n=5$, or methyl acetate, $n=5$, in saline for PGE_1/PGE_0, i.v. infusion) administered 85 min and 75 min respectively prior to LAL occlusion ($n=10$). Group II: Vehicle for glibenclamide (20% DMSO, 2 ml) followed 10 min later by a 1 h infusion of PGE_1 (1.0 μg kg^{-1} min^{-1}) starting 75 min prior to LAL occlusion ($n=6$). Group III: Glibenclamide (0.3 mg kg^{-1}, 2 ml) followed 10 min later by a 1 h infusion of PGE_1 (1.0 μg kg^{-1} min^{-1}) starting 75 min prior to LAL occlusion ($n=8$). Group IV: 5-Hydroxydecanoate (5 mg kg^{-1}, 2 ml) followed 10 min later by a 1 h infusion of vehicle for PGE_1 (5% methanol) starting 75 min before LAL occlusion ($n=8$). Group V: 5-Hydroxydecanoate (5 mg kg^{-1}, 2 ml) followed 10 min later by a 1 h infusion of PGE_1 (1.0 μg kg^{-1} min^{-1}) starting 75 min prior to LAL occlusion ($n=6$). Group VI: Vehicle for glibenclamide (20% DMSO, 2 ml) followed 10 min later by a 1 h infusion of

PGE_0 (1.0 μg kg^{-1} min^{-1}) starting 75 min prior to LAL occlusion ($n=6$). Group VII: Glibenclamide (0.3 mg kg^{-1}, 2 ml) followed 10 min later by a 1 h infusion of PGE_0 (1.0 μg kg^{-1} min^{-1}) starting 75 min before LAL occlusion ($n=8$). Group VIII: Glibenclamide (0.3 mg kg^{-1}, 2 ml) followed 10 min later by a 1 h infusion of vehicle for PGE_1 /PGE_0 (5% methanol or methyl acetate in saline respectively) starting 75 min before LAL occlusion ($n=6$).

Materials

Hypnorm was purchased from Janssen Pharmaceutical Co., (Oxford, U.K.), sodium pentobarbitone (Sagatal) from May and Baker (Dagenham, U.K.), lignocaine (Xylocaine) from Astra Pharmaceuticals (Kings Langley, U.K.) and heparin from Evans Med., (Middlesex, U.K.). Glibenclamide, Evans blue dye and NBT were obtained from Sigma Chemical Co., (Poole, U.K.). Glibenclamide was dissolved in 100% dimethyl sulphoxide (DMSO). After it was dissolved, the mixture was diluted (final volume 2 ml) with 20% DMSO. Prostaglandin E_1 and prostaglandin E_0 were obtained from Cayman Chemical Co. (Ann Arbor, U.S.A.). PGE_1 and PGE_0 were dissolved in methanol and methyl acetate respectively. Aliquots of PGE_1 and PGE_0 were stored frozen (−20°C) until use when they were diluted in 0.9% w/v saline. Sodium 5-Hydroxydecanoate was generously supplied by Dr Icilio Cavero from Rhône-Poulenc Rorer, France, and was freshly dissolved in 0.9% w/v saline each day.

Statistical comparisons

All values in the text, figures and tables are expressed as the means ± s.e.mean of n observations. Statistical analysis was performed by one-way analysis of variance (ANOVA) and end point determinations were analysed by Student's unpaired t test. A P value of less than 0.05 was considered statistically significant.

Results

Myocardial ischaemia and reperfusion

Of the 61 rabbits which underwent LAL occlusion, 1 (receiving vehicle) died within the experimental period due to ventricular fibrillation within 10–20 min of the ischaemic period. The area at risk of 2 rabbits (1 rabbit being treated with DMSO plus PGE_1 and 1 rabbit being treated with glibenclamide plus PGE_0) was below the cut off point of 20% of the left ventricle. The data obtained from these three rabbits were excluded from data analysis.

Haemodynamic data

Table 1 shows values for MAP, HR and pressure-rate index (PRI), an indicator of myocardial oxygen consumption (Baller *et al.*, 1981). Baseline haemodynamic data (−85 min) were

Table 1 Mean arterial pressure (MAP, mmHg), heart rate (HR, beats min^{-1}) and pressure rate index (PRI, mmHg $min^{-1} \times 10^3$) in rabbits subjected to 1 h coronary artery occlusion and 2 h reperfusion

Treatment		*−85 min*	*−75 min*	*−15 min*	*0 min*	*30 min*	*60 min*	*180 min*
20% DMSO +	MAP	63 ± 2	69 ± 3	66 ± 2	61 ± 3	61 ± 3	59 ± 3	56 ± 3
methanol/methyl	HR	226 ± 8	220 ± 6	226 ± 4	224 ± 4	221 ± 4	224 ± 5	221 ± 6
acetate								
$n=10$	PRI	14 ± 1	15 ± 1	15 ± 1	14 ± 1	13 ± 1	13 ± 1	12 ± 1
20% DMSO +	MAP	58 ± 2	67 ± 2	49 ± 1*	63 ± 2	57 ± 3	55 ± 2	54 ± 2
PGE_1	HR	220 ± 12	208 ± 12	229 ± 9	226 ± 10	212 ± 78	222 ± 6	219 ± 7
(1.0 μg kg^{-1} min^{-1})								
$n+6$	PRI	13 ± 1	14 ± 1	11 ± 0.5	14 ± 0.5	12 ± 1	12 ± 1	12 ± 1
Glibenclamide +	MAP	60 ± 2	71 ± 4	50 ± 3*	65 ± 3	56 ± 3	53 ± 3	54 ± 3
PGE_1	HR	234 ± 9	223 ± 8	233 ± 7	234 ± 8	219 ± 8	228 ± 7	229 ± 11
(1.0 μg kg^{-1} min^{-1})								
$n=8$	PRI	14 ± 1	16 ± 1	12 ± 1	15 ± 1	12 ± 1	12 ± 1	12 ± 1
5-HD +	MAP	66 ± 3	67 ± 3	66 ± 3	66 ± 3	64 ± 3	63 ± 3	64 ± 3
methanol	HR	227 ± 5	228 ± 6	228 ± 7	229 ± 6	239 ± 6*	249 ± 7*	252 ± 6*
vehicle								
$n=8$	PRI	15 ± 1	15 ± 1	15 ± 1	15 ± 1	15 ± 1	16 ± 1	16 ± 1*
5-HD +	MAP	70 ± 3	71 ± 3	58 ± 3*	71 ± 3	63 ± 2	62 ± 1	62 ± 3
PGE_1	HR	218 ± 3	217 ± 4	234 ± 5	231 ± 6	224 ± 8	230 ± 7	224 ± 5
(1.0 μg kg^{-1} min^{-1})								
$n=6$	PRI	16 ± 1	15 ± 1	13 ± 1	16 ± 1	14 ± 1	14 ± 1	14 ± 1
20% DMSO +	MAP	59 ± 2	67 ± 2	48 ± 2*	56 ± 2	52 ± 3	52 ± 2	54 ± 2
PGE_0	HR	225 ± 7	215 ± 8	250 ± 8*	244 ± 9	227 ± 15	233 ± 11	228 ± 7
(1.0 μg kg^{-1} min^{-1})								
$n=6$	PRI	13 ± 1	14 ± 1	11 ± 1*	14 ± 1	12 ± 1	12 ± 1	12 ± 0.5
Glibenclamide +	MAP	58 ± 2	70 ± 2	53 ± 4*	64 ± 2	59 ± 3	56 ± 2	55 ± 2
PGE_0	HR	230 ± 7	221 ± 7	244 ± 6	239 ± 7	229 ± 7	232 ± 6	229 ± 6
(1.0 μg kg^{-1} min^{-1})								
$n=8$	PRI	13 ± 1	15 ± 1	13 ± 1	15 ± 1	14 ± 1	13 ± 1	12 ± 0.5
Glibenclamide +	MAP	58 ± 4	65 ± 5	61 ± 5	61 ± 7	57 ± 7	57 ± 7	57 ± 5
methanol/methyl	HR	215 ± 7	205 ± 4	207 ± 3	207 ± 4	206 ± 5	217 ± 4	222 ± 6
acetate								
$n=4$	PRI	12 ± 1	13 ± 1	13 ± 1	13 ± 2	12 ± 2	12 ± 1	13 ± 1

Values are given as mean ± s.e.mean of n observations. The respective n-number for each group is provided in the left hand column. *$P < 0.05$ when compared to vehicle control.

similar in all groups investigated ($P>0.05$, see Table 1).

The bolus injection of glibenclamide (0.3 mg kg^{-1} i.v.) did not produce a significant effect on MAP when compared to the vehicle-control group, but did cause a transient increase in PRI in the glibenclamide plus PGE_1 or PGE_0-treated groups. In the groups pretreated with vehicle for glibenclamide (20% DMSO), infusion of either PGE_1 or PGE_0 resulted in a significant fall in MAP when compared with the vehicle control group ($P<0.05$). Within 15 min of termination of the PGE_1 or PGE_0 infusion (prior to LAL occlusion), however, the MAP returned to within 10% of the baseline value. Injection of 5-HD had no effect on any of the haemodynamic parameters measured nor did 5-HD alter the haemodynamic effects afforded by the subsequent infusion of PGE_1.

Area at risk and infarct size

The area of the left ventricle subjected to ischaemia that constituted the area at risk was similar in all groups ($P>0.05$, see Table 2).

In rabbits treated with vehicle alone, ischaemia (60 min) followed by reperfusion (2 h) resulted in an infarct size of $59\pm4\%$ ($n=10$) of the area at risk (Figure 1). Pretreatment of rabbits with an infusion of PGE_1 (1.0 $\mu g\ kg^{-1}\ min^{-1}$) resulted in a significant reduction in infarct size ($P<0.05$, $n=6$; Figure 1). This reduction in infarct size was abolished by pretreatment of rabbits with either glibenclamide (0.3 mg kg^{-1}, $n=8$) or 5-HD (5 mg kg^{-1}, $n=6$) (Figure 1), both blockers of K_{ATP} channels (Gross & Auchampach, 1990; McCullough *et al.*, 1991). When administered alone, neither glibenclamide ($54\pm10\%$; $n=8$, $P>0.05$) nor 5-HD ($55\pm7\%$; $n=8$, $P>0.05$) had any effect on myocardial infarct size when compared to vehicle control. Pretreatment with an infusion of PGE_0 (1.0 $\mu g\ kg^{-1}\ min^{-1}$) also resulted in a significant reduction in infarct size ($P<0.05$, $n=6$; Figure 1). This cardioprotective effect of PGE_0 was also abolished by pretreatment of rabbits with glibenclamide ($P<0.05$, $n=8$, Figure 1).

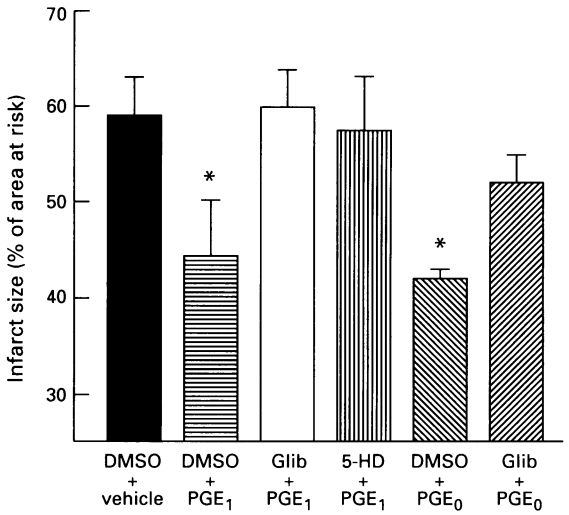

Figure 1 Infarct size expressed as a percentage of the area at risk. Rabbits received either vehicle (20% DMSO + 5% methanol or methyl acetate, $n=6$), vehicle for glibenclamide (20% DMSO) plus PGE_1 (1.0 $\mu g\ kg^{-1}\ min^{-1}$; DMSO + PGE_1, $n=6$), glibenclamide (0.3 mg kg^{-1} plus PGE_1 (1.0 $\mu g\ kg^{-1}\ min^{-1}$; Glib + PGE_1, $n=8$), 5-hydroxydecanoate (5 mg kg^{-1}) plus PGE_1 (1.0 $\mu g\ kg^{-1}\ min^{-1}$; 5-HD + PGE_1, $n=6$), vehicle for glibenclamide (20% DMSO) plus PGE_0 (1.0 $\mu g\ kg^{-1}\ min^{-1}$; DMSO + PGE_0, $n=6$), glibenclamide (0.3 mg kg^{-1}) plus PGE_0 (1.0 $\mu g\ kg^{-1}\ min^{-1}$; Glib + PGE_0, $n=8$). Results are expressed as mean $\pm$ s.e.mean of n observations. *$P<0.05$ when compared to vehicle control. Note that PGE_1 or PGE_0 (1.0 $\mu g\ kg^{-1}\ min^{-1}$) caused a significant reduction in infarct size ($P<0.05$ when compared to vehicle) which was abolished by pretreatment of the animals with glibenclamide, and (in the case of PGE_1) also by 5-hydroxydecanoate, both inhibitors of ATP-sensitive potassium channels.

Discussion

This study demonstrates that pretreatment of rabbits with infusions of either PGE_1 or PGE_0, which is discontinued 15 min prior to ligation of a coronary artery, substantially reduces myocardial infarct size arising from a subsequent period of myocardial ischaemia (60 min) followed by reperfusion (120 min).

What, then, is the mechanism by which pretreatment of animals with PGE_1 or PGE_0 causes this reduction in infarct size? We clearly demonstrate that the cardioprotective effects of PGE_1 was abolished by pretreatment of rabbits with glibenclamide or 5-hydroxydecanoate, two structurally different inhibitors of ATP-sensitive potassium (K_{ATP}) channels (McCullough *et al.*, 1991; Auchampach *et al.*, 1992). Similarly, glibenclamide also largely attenuated the cardioprotective effects of PGE_0. As (i) glibenclamide inhibits the induction of a calcium-independent isoform of nitric oxide synthase, an effect which is independent of the inhibition of K_{ATP} channels (Wu *et al.*, 1995); and (ii) glibenclamide produces systemic effects such as hypoglycaemia, it is possible that glibenclamide attenuates the cardioprotective effects of PGE_1 by a mechanism not related to the inhibition of K_{ATP} channels. To ensure that the inhibition by glibenclamide of the cardioprotective effects of PGE_1 is not due to a non-specific effect of this sulphonylurea, we used 5-HD, a novel and specific K_{ATP} channel antagonist. 5-HD has the further advantage of blocking the K_{ATP} channel only during ischaemia by competing with the ATP binding site, and unlike glibenclamide, does not affect pancreatic K_{ATP} channels (McCullough *et al.*, 1991; Natsuto *et al.*, 1992). Our findings that (i) both glibenclamide and 5-HD abolish the cardioprotective effects of PGE_1 in the anaesthetized rabbit,

Table 2 Area at risk (expressed as a % of left ventricle) in rabbits subjected to coronary artery (LAL) occlusion (60 min) and reperfusion (2 h)

Group	*Treatment*	*Area at risk* (% of left ventricle)	n
(I)	20% DMSO + 5% methanol or 5% methyl acetate	45 ± 3	10
(II)	20% DMSO + PGE_1 (1.0 $\mu g\ kg^{-1}\ min^{-1}$)	49 ± 3	6
(III)	Glibenclamide (0.3 mg kg^{-1}) + PGE_1 (1.0 $\mu g\ kg^{-1}\ min^{-1}$)	48 ± 3	8
(IV)	5-Hydroxydecanoate (5 mg kg^{-1}) + 5% methanol	43 ± 2	8
(V)	5-Hydroxydecanoate (5 mg kg^{-1}) + PGE_1 (1.0 $\mu g\ kg^{-1}\ min^{-1}$)	42 ± 5	6
(VI)	20% DMSO + PGE_0 (1.0 $\mu g\ kg^{-1}\ min$)$^{-1}$	43 ± 3	6
(VII)	Glibenclamide (0.3 mg kg^{-1}) + PGE_0 (1.0 $\mu g\ kg^{-1}\ min^{-1}$)	47 ± 3	8
(VIII)	Glibenclamide (0.3 mg kg^{-1}) + 5% methanol or 5% methyl acetate	50 ± 2	6

Values are given as mean $\pm$ s.e.mean of n observations. *Groups (II), (III), (VII) and (VIII) are significantly different ($P<0.05$) from the vehicle control group (I).

and (ii) that neither glibenclamide nor 5-HD (when administered alone) affected infarct size, demonstrates that the reduction in infarct size brought about by PGE_1 is due to the activation of K_{ATP} channels.

There is now good evidence that the potent cardioprotective effects caused by 'ischaemic preconditioning' of the myocardium are also due to activation of K_{ATP} channels, as (i) the cardioprotective effects of ischaemic preconditioning are abolished by the K_{ATP} channel inhibitors, glibenclamide or 5-HD (Auchampach *et al.*, 1992; Toombs *et al.*, 1993; Walsh *et al.*, 1994), and (ii) intracoronary administration of K_{ATP} channel openers (aprikalim, nicorandil, cromakalim, pinacidil), at doses which do not cause a significant fall in blood pressure, produce a marked reduction in infarct size (Grover *et al.*, 1990; Auchampach *et al.*, 1991; Gross *et al.*, 1992), which is of a similar magnitude to that seen with ischaemic preconditioning. Indeed, it has been proposed that the cardioprotective effects of ischaemic preconditioning are secondary to the release of endogenous adenosine, which in turn, activates the A_1 adenosine receptor (Liu *et al.*, 1991; Thornton *et al.*, 1992). A_1 receptor activation ultimately leads to the long-lasting opening of K_{ATP} channels by (i) stimulation of G proteins (e.g. G_0), and/or (ii) phosphorylation of the channel by protein kinase C (PKC). Opening of these channels leads to an increased K^+ efflux, a shortening of the cardiac action potential and, hence, membrane hyperpolarization. This K_{ATP} channel-induced membrane hyperpolarization prevents the opening of voltage-dependent (L-type) calcium channels which results in a reduced Ca^{2+} entry and reduced contractile energy consumption. The opening of K_{ATP} channels may also cause a decrease in ATP depletion, glycogen breakdown, and anaerobic glycolysis, thus preserving energy substrate (Grover *et al.*, 1989; 1992). Pronounced cardioprotective effects by ischaemic preconditioning have been demonstrated in models of myocardial ischaemia and reperfusion in the rat (Li & Kloner, 1992), rabbit (Cohen *et al.*, 1991), dog (Li *et al.*, 1990) and pig (Scott *et al.*, 1990).

Our hypothesis that the pronounced cardioprotective effects of PGE_1 or PGE_0 demonstrated in this study are due to activation of PKC resulting in the opening of K_{ATP} channels is based on the observations that (i) EP_3 receptors (of which there are four subgroups A-D) are present on bovine and porcine myocardial sarcolemma (Lopaschuk *et al.*, 1989; Hohlfeld, 1995), (ii) PGE_1 or PGE_0 activates PKC (via the activation of EP_1 and EP_3 (subgroups A and D) receptors and, hence, the IP_3 /DAG signal transduction pathway) (Hohlfeld, 1995), and (iii) the cardioprotective effects of PGE_1 or PGE_0 are largely attenuated by glibenclamide and (in the case of PGE_1) also by 5-HD (this study).

One could also argue that the cardioprotective effects of PGE_1 are due to inhibition of platelet and particularly neutrophil function, as well as increases in coronary blood flow. This is, however, unlikely as (i) these effects are mediated by transient increases in intracellular cyclic AMP, and not by PKC/K_{ATP} channels, (ii) the fall in MAP observed during PGE_1 infusion returned to baseline within 10 min of discontinuation of the infusion (i.e. prior to LAL ligation). This was not surprising, as PGE_1 is largely metabolized by a single passage through the pulmonary circulation (Ferreira & Vane, 1967).

Our finding here that the cardioprotective effects of PGE_1 or PGE_0 are due to activation of K_{ATP} channels and, hence are similar to the cardioprotective effects elicited by ischaemic preconditioning, raises the question whether the anti-ischaemic effects of PGE_1 observed in patients with peripheral arterial occlusive disease (PAOD) are also due to activation of K_{ATP} channels. Indeed, ischaemic preconditioning also protects against a subsequent more prolonged ischaemic insult in skeletal muscle, by a mechanism that is thought to involve PKC and the activation if K_{ATP} channels (Forrest *et al.*, 1992; Pang *et al.*, 1993; 1995).

In conclusion, this study demonstrates that pretreatment of rabbits with PGE_1 or PGE_0 caused a pronounced reduction in myocardial infarct size arising from regional ischaemia (60 min) and reperfusion (120 min). The cardioprotective effects of PGE_1 or PGE_0 are due to activation of K_{ATP} channels, as they were attenuated by glibenclamide. Thus, we propose that PGE_1 or PGE_0 exert potent cardioprotective effects by activation of PKC (via activation of EP_1 or more likely EP_3 receptors) resulting in the opening of K_{ATP} channels.

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References

ARAKI, H. & LEFER, A.M. (1980). Role of prostacyclin in the preservation of ischaemic myocardial tissue in the perfused cat heart. *Circ. Res.*, **47** 757–763.

AUCHAMPACH, J.A., GROVER, G.J. & GROSS G.J. (1992). Blockade of ischaemic preconditioning in dogs by the novel ATP dependent potassium channel antagonist sodium 5-hydroxydecanoate. *Cardiovasc. Res.*, **26,** 1054–1062.

AUCHAMPACH, J.A., MARUYAMA, M., CAVERO, I. & GROSS, G.J. (1991). The new K^+ channel opener aprikalim (RP 52891) reduces experimental infarct size in dogs in the absence of hemodynamic changes. *J. Pharmacol. Exp. Ther.*, **259,** 961–967.

BALLER, D., BRETSCHNEIDER, H.J. & HELLIGE, G. (1981). A critical look at currently used indirect indices of myocardial oxygen consumption. *Basic Res. Cardiol.*, **76,** 163–181.

BOUCHARD, J-F., DUMONT, E. & LAMONTAGNE, D. (1994). Evidence that prostaglandins I_2, E_2, and D_2 may activate ATP-sensitive potassium channels in the isolated rat heart. *Cardiovasc. Res.*, **28,** 901–905.

CHIARIELLO, M., GOLINO, P., CAPPELLI-BIGAZZI, M., AMBROSIO, G., TRITTO, I. & SALVATORE, M. (1988). Reduction in infarct size by the prostacyclin analogue iloprost (ZK 36374) after experimental coronary artery occlusion-reperfusion. *Am. Heart J.*, **115,** 449–504.

COHEN, M.V., LIU, G.S. & DOWNEY, J.M. (1991). Preconditioning causes improved wall motion as well as smaller infarcts after transient coronary occlusion in the rabbit. *Circulation*, **84,** 341–349.

FERREIRA, S.H. & VANE J.R. 1967). Prostaglandins: Their disappearance from and release into the circulation. *Nature*, **216,** 868–873.

FLORES, N.A., DAVIES, R.L.I., PENNY, W.J. & SHERIDAN, D.S. (1984). Coronary microangiography in the guinea pig, rabbit and ferret. *Int. J. Cardiol.*, **6,** 459 471.

FORREST, C.R., PANG, C.Y., BOYD, B., ZHONG, A. & MOUNSEY, R. (1992). The first report on acute ischemic preconditioning of muscle flaps. In *Proc. Plastic Surgery Res. Council, 37th Annual Meeting*. Toronto, 1992. pp. 81–85.

GROSS, G.J., AUCHAMPACH, J.A., MARUYAMA, M., WARLTIER, D.C. & PIEPER, G.M. (1992). Cardioprotective effects of nicorandil. *J. Cardiovasc. Pharmacol.*, **20,** 522–528.

GROSS, G.J., AUCHAMPACH, J.A. & MASAHIKO, M. (1990). Blockade of ATP-sensitive potassium channels exacerbates myocardial stunning in dogs. *Circulation*, **82,** (suppl. III), 459–465.

GROVER, G.J., DWONCZYK, S., PARHAM, C.S. & SLEPH, P.G. (1990). The protective effects of cromakalim and pinacidil on reperfusion function and infarct size in isolated rat hearts and anaesthetized dogs. *Cardiovasc. Drugs Ther.*, **15,** 465–474.

GROVER, G.J., MCCULLOUGH, J.R., HENRY, D.E., CONDOR, M.L. & SLEPH, P.G. (1989). The anti-ischaemic effects of the potassium channel activators pinacidil and cromakalim and the reversal of these effects with the potassium channel blocker glyburide. *J. Pharmacol. Exp. Ther.*, **251,** 98–110.

GROVER, G.J., SLEPH, P.G. & DWONCZYK, S. (1992). Role of myocardial ATP-sensitive potassium channels in mediating preconditioning in the dog heart and their possible interaction with adenosine A_1 receptors. *Circulation*, **86,** 1310–1316.

HOHLFELD, T. (1995). Regulation of prostaglandin E_1 receptors in myocardial ischaemia. In *Mediators in the Cardiovascular System: Regional Ischaemia.* ed. Schrör, K. & Pace-Asciak, C.R. *Agents and Actions*, (suppl. 45) pp. 93–100. Basel: Birkhäuser Verlag.

HUTTON, I., PARRATT, J.R. & LAWRIE, T.D.V. (1973). Cardiovascular effects of prostaglandin E_1 in experimental myocardial infarction. *Cardiovas. Res.*, **7,** 149–155.

JACKSON, W.F., KÖNIG, A., DAMBACHER, T. & BUSSE, R. (1993). Prostacyclin-induced vasodilation in rabbit heart is mediated by ATP-sensitive potassium channels. *Am. J. Physiol.*, **264,** H238–H243.

JUGDUTT, B.I., HUTCHINS, G.M., BULKLEY, B.H. & BECKER, L.C. (1981). Dissimilar effects of prostacyclin, prostaglandin E_1, and prostaglandin E_2 on myocardial infarct size after coronary occlusion in conscious dogs. *Circ. Res.*, **49,** 685–700.

LEFER, A.M., OGLETREE, M.L., SMITH, J.B., SILVER, M.J., NICOLAOU, K.C., BARNETTE, W.E. & GASIC, G.P. (1978). Prostacyclin: A potentially valuable agent for preserving myocardial tissue in acute myocardial ischaemia. *Science*, **200,** 52–54.

LI, G.C., VASQUEZ, J.A., GALLAGHER, K.P. & LUCCHESI, B.R. (1990). Myocardial protection with preconditioning. *Circulation*, **82,** 609–619.

LI, Y. & KLONER, R.A. (1992). Cardioprotective effects of ischaemic preconditioning are not mediated by prostanoids. *Cardiovasc. Res.*, **26,** 226–251.

LINDNER, V. & HEINLE, H. (1982). Binding properties of circulating Evans blue in rabbits as determined by disc electrophoresis. *Atherosclerosis*, **43,** 417–422.

LIU, G.S., THORNTON, J., VAN WINKLE, D.M., STANLEY, A.W.H., OLSSON, R.A. & DOWNEY, J.M. (1991). Protection against infarction afforded by preconditioning is mediated by A_1-adenosine receptors in the rabbit heart. *Circulation*, **84,** 350–356.

LOPASCHUK, G.D., MICHALAK, M., WANDLER, E.L., LERNER, R.W., PISCIONE, T.D., COCEANI, F. & OLLEY, P.M. (1989). Prostaglandin E receptors in cardiac sarcolemma: Identification and coupling of adenylate cyclase. *Circ. Res.*, **65,** 538–545.

MAXWELL, M.P., HEARSE, D.J. & YELLON, D.J. (1987). Species variation in the coronary collateral circulation during regional myocardial ischaemia: a critical determinant of the rate of evolution and extent of myocardial infarction. *Cardiovasc. Res.*, **21,** 737–746.

MCCULLOUGH, J.R., NORMANDIN, D.E., CONDER, M.L., SLEPH, P.G., DZWONCZYK, S. & GROVER G.J. (1991). Specific block of the anti-ischaemic actions of cromakalim by sodium 5-hydroxydecanoate. *Circ. Res.*, **69,** 949–958.

MCMURDO, L., THIEMERMANN, C. & VANE, J.R. (1994). The effects of the endothelin ET_A receptor antagonist, FR 139317, on infarct size in a rabbit model of acute myocardial ischaemia and reperfusion. *Br. J. Pharmacol.*, **112,** 75–80.

MURRY, C.E., JENNINGS, R.B. & REIMER, K.A. (1986). Preconditioning with ischaemia: a delay of lethal cell injury in ischaemic myocardium. *Circulation*, **74,** 1124–1136.

NACHLAS, M.M. & SHNITKA, T.K. (1963). Macroscopic identification of early myocardial infarct by alterations in dehydrogenase activity. *Am. J. Pathol.*, **43,** 379–405.

NATSUTO, T., TANAKA, I., TAKANO, M. & NORMA, A. (1992). Blockade of the ATP-sensitive K^+ channel by 5-hydroxydecanoate in guinea-pig ventricular myocytes. *J. Pharmacol. Exp. Ther.*, **260,** 702–708.

NEY, P. & FEELISCH, M. (1995). Vasodilator effects of PGE_1 in the coronary and systemic circulation of the rat are mediated by ATP-sensitive potassium (K^+) channels. In *Mediators in the Cardiovascular System: Regional Ischaemia.* ed. Schrör, K. & Pace-Asciak, C.R. *Agents and Actions*, (suppl. 45) pp. 71–76. Basel: Birkhäuser Verlag.

PANG, C.Y., FORREST, C.R. & MOUNSEY, R. (1993). Pharmacologic intervention in ischaemia-induced reperfusion injury in the skeletal muscle. *Microsurgery*, **14,** 176–182.

PANG, C.Y., YANG, R.Z., ZHONG, A., XU, N., BOYD, B. & FORREST, C.R. (1995). Acute ischaemic preconditioning protects against skeletal muscle infarction in the pig. *Cardiovasc. Res.*, **29,** 782–788.

RIEMERSMA, R.A., TALBOT, R.C., UNGER, A., MJOS, O.D. & OLIVER, M.F. (1977). Effects of prostaglandin E_1 on ST-segment elevation and regional myocardial blood flow during experimental myocardial ischaemia in dogs. *Eur. J. Clin. Invest.*, **7,** 515–521.

SCHRÖR, K., ADDICKS, K., DARIUS, H., OHLENDORF, R. & RÖSEN, P. (1981). PGI_2 inhibits ischaemia-induced platelet activation and prevents myocardial damage by inhibition of catecholamine release from adrenergic nerve terminals. Evidence for cAMP as common denominator. *Thromb. Res.*, **21,** 175–180.

SCHRÖR, K., SMITH III, E.F. & LEFER, A.M. (1988a). The cat as an in vivo model for myocardial ischemia and infarction. *Prog. Pharmacol.*, **6/4,** 31–91.

SCHRÖR, K., THIEMERMANN, C. & NEY, P. (1988b). Protection of the ischaemic myocardium from reperfusion injury by prostaglandin E_1 inhibition of ischaemia-induced neutrophil activation. *Naunyn-Schmied. Arch. Pharmacol.*, **338,** 268–274.

SCOTT, R.J., ROHMANN, S., BRAUN, E.R. & SCHAPER, W. (1990). Ischaemic preconditioning reduces infarct size in swine myocardium. *Circ. Res.*, **66,** 1133–1142.

SIMPSON, P.J., MICKELSON, J., FANTONE, J.C., GALLAGHER, K.P. & LUCCHESI, B.R. (1988). Reduction of experimental canine myocardial infarct size with prostaglandin E_1: Inhibition of neutrophil migration and activation. *J. Pharmacol. Exp. Ther.*, **244,** 619–624.

TAKANO, T., VYDEN, J.K., ROSE, H.B., CORDAY, E. & SWAN, H.J.C. (1977). Beneficial effects of PGE_1 in acute myocardial infarction. *Am. J. Cardiol.*, **39,** 297.

THIEMERMANN, C., THOMAS, R.G. & VANE, J.R. (1989). Defibrotide reduces infarct size in a rabbit model of experimental myocardial ischaemia and reperfusion. *Br. J. Pharmacol.*, **97,** 401–408.

THORNTON, J.D., LIU, G.S., OLSSON, R.A. & DOWNEY, J.M. (1992). Intravenous pretreatment with a A_1-selective adenosine analogue protects the heart against infarction. *Circulation*, **85,** 659–665.

TOOMBS, C.F., MOORE, T.L. & SHEBUSKI, R.J. (1993). Limitation of infarct size in the rabbit by ischaemic preconditioning is reversible with glibenclamide. *Cardiovasc. Res.*, **27,** 617–622.

VEGH, A., PAPP, J.G., SZEKERES, L. & PARRATT, J.R. (1993). Are ATP-sensitive potassium channels involved in the pronounced antiarrhythmic effects of preconditioning? *Cardiovasc. Res.*, **27,** 638–643.

WALSH, R.S., TSUCHIDA, A., DALY, J.J.F., THORNTON, J.D., COHEN, M.V. & DOWNEY, J.M. (1994). Ketamine-xylazine anaesthesia permits a K_{ATP} channel antagonist to attenuate preconditioning in rabbit myocardium. *Cardiovasc. Res.*, **28,** 1337–1341.

WU, C.-C., THIEMERMANN, C. & VANE, J.R. (1995). Glibenclamide-induced inhibition of the expression of inducible nitric oxide synthase in the cultured macrophages and in the anaesthetized rat. *Br. J. Pharmacol.*, **114,** 1273–1281.

YTREHUS, K., LIU, Y. & DOWNEY, J.M. (1994). Preconditioning protects ischaemic rabbit heart by protein kinase C activation. *Am. J. Physiol.*, **266,** H1145–H1152.

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