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Endothelin-1-induced reduction of myocardial infarct size by activation of ATP-sensitive potassium channels in a rabbit model of myocardial ischaemia and reperfusion

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1 This study examined whether endothelin-1 (ET-1) reduces infarct size in a rabbit model of acute coronary artery occlusion (60 min) and reperfusion (120 min). In addition, we investigated whether the observed cardioprotective effect of ET-1 was due to the activation of ATP-sensitive potassium (K_{ATP}) channels by using two selective antagonists, glibenclamide and sodium 5-hydroxydecanoate (5-HD).

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 $55 \pm 4\%$ ($n=11$). ET-1 (0.3 nmol kg^{-1}), administered as a bolus injection into the left ventricle, had no effect on infarct size ($62 \pm 2\%$, $n=4$). A lower dose of ET-1 (0.03 nmol kg^{-1}) resulted in a significant reduction in infarct size (infarct size $43 \pm 3\%$; $P<0.05$, $n=16$). The higher dose (0.3 nmol kg^{-1}), but not the lower dose of ET-1 caused a significant rise in blood pressure, pressure rate index and hence, myocardial oxygen consumption.

3 The reduction in infarct size afforded by ET-1 (0.03 nmol kg^{-1}) was abolished by pretreatment of rabbits with the K_{ATP} channel inhibitors, glibenclamide (0.3 mg kg^{-1}) and 5-HD (5 mg kg^{-1}), (infarct size 59 ± 3 and $63 \pm 4\%$ respectively; $n=4-9$).

4 We propose that ET-1 reduces infarct size by opening K_{ATP} channels.

Keywords: Endothelin-1; 5-hydroxydecanoate; glibenclamide; ATP-sensitive potassium channel; myocardial infarction; ischaemic preconditioning

Introduction

Endothelin-1 (ET-1) is a potent coronary vasoconstrictor in numerous species including man (see Rubanyi & Polokoff, 1994). Enhanced plasma levels of ET-1 in man are associated with a variety of cardiovascular disorders including acute myocardial infarction, angina pectoris, coronary artery vasospasm and congestive heart failure (see Hasdai *et al.*, 1994). Ischaemia/reperfusion of the heart enhances the coronary vasoconstriction elicited by ET-1 (see Rubanyi & Polokoff, 1994). Thus, increases in circulating ET-1 following acute myocardial ischaemia could lead to excessive coronary vasoconstriction and subsequently to an increase in infarct size. It is, however, unclear whether elevated plasma levels of ET-1 are merely a surrogate marker of myocardial ischaemia or whether endogenous ET-1 contributes to the underlying pathophysiology of ischaemic injury of the myocardium (e.g. by extension of infarct size).

Studies into the role of endogenous ET-1 in the extension of ischaemic injury of the myocardium have led to controversial results. For instance, monoclonal antibodies directed against ET-1 reduce myocardial infarct size in anaesthetized rats (Watanabe *et al.*, 1991) or rabbits (Kusumoto *et al.*, 1993) suggesting that ET-1 contributes to the extension of infarct size. The non-selective ET_A and ET_B receptor antagonist, TAK-044 (Kikuchi *et al.*, 1994) reduced infarct size in a rat model of myocardial ischaemia and reperfusion (Watanabe *et al.*, 1995). Intracoronary infusion of the ET_A receptor antagonist, BQ-123 (Ihara *et al.*, 1992) also led to a 40% reduction in infarct size in the dog (Grover *et al.*, 1993). However, there are also several well-designed studies which do not show a reduction in experimental infarct size with ET_A receptor antagonists. For instance, intravenous infusion of BQ-123 did not reduce infarct size in a canine model of ischaemia and reperfusion (Krause *et al.*, 1994). In the anaesthetized rabbit, FR 139317 (Sogabe *et al.*, 1993) also failed to reduce infarct size arising from ischaemia and reperfusion (McMurdo *et al.*, 1994). Interestingly, intracoronary infusion of FR 139317 actually increased infarct size in the dog, suggesting that endogenous ET-1 may have cardioprotective effects (Velasco *et al.*, 1993).

Two endothelin receptors have been cloned and expressed, namely ET_A (Arai *et al.*, 1990) and ET_B (Sakurai *et al.*, 1990). The vasoconstrictor effects of ET-1 are mainly mediated by activation of ET_A receptors (Bigaud & Pelton, 1992) although ET_B receptors (located on the vascular smooth muscle) also mediate vasoconstriction in some vascular beds (Douglas *et al.*, 1992; Moreland *et al.*, 1992; Cristol *et al.*, 1993). Activation of protein kinase C (PKC) may play a role in the contraction of vascular smooth muscle elicited by ET-1 (see Rubanyi & Polokoff, 1994), and the selective PKC inhibitor, staurosporine, significantly attenuated ET-1 induced coronary vasoconstriction in anaesthetized pigs (Egashira *et al.*, 1990).

Ischaemic preconditioning, which can be 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; Parratt, 1994), is thought to be mediated by the translocation of inactive PKC from the cytosol to the membrane where it can be activated. Activated PKC phosphorylates a membrane protein that may be linked to the ATP-sensitive potassium (K^+) channel, thus opening this channel. Indeed, inhibition of ATP-sensitive potassium channels with glibenclamide or the ischaemia-sensitive inhibitor sodium 5-hydroxydecanoate abolishes the cardioprotective effects of ischaemic preconditioning (Auchampach *et al.*, 1992; Vegh *et al.*, 1993).

As ET-1 also activates PKC (see above), the aims of the present study were to elucidate (i) whether pretreatment with ET-1 reduces infarct size in a rabbit model of acute myocardial ischaemia and reperfusion, and (ii) whether any potential cardioprotective effect of ET-1 is due to the activation of ATP-sensitive K^+ channels.

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Methods

Animals

The study was carried out on 62 male rabbits (New Zealand White rabbits, Foxfield, U.K.) weighing 2.5 to 3.0 kg and receiving a standard diet and water *ad libitum*.

Surgery and instrumentation

Ten minutes before surgery, all 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 $38 \pm 1°C$ 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.

Myocardial ischaemia and reperfusion

The method of coronary artery occlusion and reperfusion in the anaesthetized rabbit was as previously described (Thiemermann *et al.*, 1989b; 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 intercosal space) was performed and the heart was suspended in a temporary pericardial cradle. A snare occluder was placed around the first antero-lateral 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 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 left ventricular systolic pressure; LVSP) changes of myocardial ischaemia. After 60 min of acute myocardial ischaemia, the occluder was reopened 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 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 −10 min (baseline; immediately prior to drug treatment), 0 min (time of occlusion; 10 min after drug treatment), 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 $(LVSP\text{-}PA_d) \times 0.33 + PA_d$. The pressure rate index (PRI), a relative indicator of myocardial oxygen consumption (Baller *et al.*, 1981) was calculated as the product of MAP and HR, and expressed in mmHg $min^{-1} \times 10^3$.

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 (Linder & 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

ET-1 (0.03 or 0.3 nmol kg^{-1}) or its vehicle-control (0.1% bovine serum albumin, BSA, in 0.9% NaCl) were administered 10 min prior to LAL occlusion as a bolus injection (2 ml volume) into the left ventricle. Sodium 5-hydroxydecanoate (5-HD; 5 mg kg^{-1}), an ischaemia-selective inhibitor of ATP-sensitive potassium channels (McCullough *et al.*, 1991), was administered as a bolus injection (2 ml volume) into the left ventricle immediately before injection of either ET-1 (0.03 nmol kg^{-1} only) or vehicle. Glibenclamide, a blocker of ATP-sensitive potassium channels (Gross & Auchampach, 1992), was administered as a bolus injection (2 ml volume) intravenously (i.v.) 10 min before injection of ET-1 (0.03 nmol kg^{-1} only) or vehicle into the left ventricle. Thus, seven experimental groups were studied:

Group I: Vehicles (2 ml 0.9% NaCl for 5-HD and 0.1% BSA in saline for ET-1) administered 10 min prior to LAL occlusion ($n=11$). Group II: Vehicle for 5-HD (0.9% saline, 2 ml) followed by an injection of ET-1 (0.03 nmol kg^{-1}) administered 10 min prior to LAL occlusion ($n=16$). Group III: Vehicle for 5-HD (0.9% saline, 2 ml) followed by an injection of ET-1 (0.3 nmol kg^{-1}) administered 10 min prior to LAL occlusion ($n=4$). Group IV: Injection of 5-HD (2 ml, 5 mg kg^{-1}) followed by an injection of ET-1 (0.03 nmol kg^{-1}) administered 10 min prior to LAL occlusion ($n=9$). Group V: Injection of 5-HD (5 mg kg^{-1}) followed by vehicle for ET-1 (0.1% BSA in 0.9% NaCl, 2 ml) administered 10 min prior to LAL occlusion ($n=10$). Group VI: Injection of glibenclamide (0.3 mg kg^{-1}) followed 10 min later by an injection of ET-1 (0.03 nmol kg^{-1}) administered 10 min prior to LAL occlusion ($n=4$). Group VII: Injection of glibenclamide (0.3 mg kg^{-1}) followed 10 min later by an injection of vehicle for ET-1 (0.1% BSA in 0.9% NaCl, 2 ml) administered 10 min prior to LAL occlusion ($n=5$).

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 a vehicle (300 μl) containing equal parts of 1 M NaOH, ethanol, and polyethylene glycol. After it was dissolved, the mixture was diluted (final volume 2 ml) with 0.9% saline. This vehicle mix has previously been shown to have no effect on infarct size (Thornton *et al.*, 1993). Endothelin-1 (human) was supplied by Peptide Institute Inc. (Osaka, Japan) and was reconstituted in 0.1% acetic acid. 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. Aliquots of ET-1 were stored frozen (−20°C) until use when they were diluted in 0.9% w/v saline containing 0.1% w/v bovine serum albumin.

Statistical comparison

All values in the text, figures and tables are expressed as the mean ± s.e.mean of n observations. Statistical analysis was performed by one-way analysis of variance (ANOVA) and end point determinations were analysed by an unpaired Students t test. A P value of less than 0.05 was considered statistically significant.

Results

Myocardial ischaemia and reperfusion

Of the 62 rabbits which underwent LAL occlusion, 2 died within the experimental period due to ventricular fibrillation within 10–20 min of the ischaemic period (1 rabbit receiving vehicle and 1 rabbit receiving ET-1 at 0.3 nmol kg^{-1}). The area at risk of 1 rabbit (treated with 5-HD, 5 mg kg^{-1} + 0.1% BSA) 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). Baseline haemodynamic data (−10 min) were similar in all groups investigated ($P>0.05$, see Table 1).

When compared to the control group, injection of high dose ET-1 (0.3 nmol kg^{-1}) caused, within 5 min, a large (approximately 36 mmHg) increase in MAP. The MAP for this group was still raised at 15 min (i.e. 15 min after the onset of LAL occlusion) when compared to vehicle control (74 ± 1 mmHg and 60 ± 2 mmHg respectively; $P<0.05$), but had returned to baseline levels by 30 min (Table 1). This rise in MAP, was associated with a significant, but transient, rise in PRI. Surprisingly, rabbits treated with 5-HD + 0.1% BSA showed an increase in HR at 60, 120 and 180 min when compared to vehicle control ($P<0.05$). This change in HR was accompanied by an increase in PRI when compared to vehicle control ($P<0.05$ at 180 min).

Area at risk and infarct size

The area of the left ventricle at risk was similar in all experimental groups (approximately 45%). (Table 2).

In rabbits treated with vehicle alone, ischaemia (60 min) followed by reperfusion (2 h) resulted in an infarct size of 55 ± 4% of the area at risk (Figure 1). Administration of ET-1 at 0.3 nmol kg^{-1} (high dose) had no significant effect on infarct size (62 ± 2%, $n=4$) when compared to vehicle control. Treatment with ET-1 at 0.03 nmol kg^{-1} (low dose) resulted in a significant (22%) reduction in infarct size ($P<0.05$, $n=16$; Figure 1). This reduction in infarct size was abolished by pretreatment of rabbits with 5-HD (5 mg kg^{-1}, $n=9$) or glibenclamide (0.3 mg kg^{-1}, $n=4$), both blockers of ATP-sensitive K^+ channels (Gross & Auchampach, 1992). When administered alone, neither 5-HD (52 ± 6%, $n=10$) nor glibenclamide (61 ± 8%, $n=5$) had any effect on myocardial infarct size ($P>0.05$, when compared to vehicle control).

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

Treatment		−10 min	0 min	30 min	60 min	120 min	180 min
Saline + 0.1%	MAP	61 ± 2	63 ± 2	58 ± 2	57 ± 2	54 ± 2	54 ± 2
BSA	HR	218 ± 8	219 ± 7	221 ± 7	226 ± 6	221 ± 6	219 ± 6
$n=11$	PRI	13 ± 1	14 ± 1	13 ± 1	13 ± 1	12 ± 1	12 ± 1
Saline + ET-1	MAP	62 ± 2	67 ± 2	62 ± 2	62 ± 3	60 ± 2	62 ± 3
(0.03 nmol kg^{-1})	HR	218 ± 7	211 ± 7	215 ± 6	223 ± 5	214 ± 5	215 ± 6
$n=16$	PRI	14 ± 1	14 ± 1	13 ± 1	13 ± 1	13 ± 1	14 ± 1
Saline + ET-1	MAP	60 ± 3	95 ± 6*	65 ± 4	59 ± 5	53 ± 5	56 ± 5
(0.3 nmol kg^{-1})	HR	226 ± 12	217 ± 13	224 ± 14	228 ± 9	215 ± 8	215 ± 10
$n=4$	PRI	14 ± 1	21 ± 2	15 ± 1	13 ± 1	11 ± 1	12 ± 1
5-HD + ET-1	MAP	62 ± 2	66 ± 2	59 ± 4	59 ± 3	55 ± 3	54 ± 4
(0.03 nmol kg^{-1})	HR	221 ± 10	215 ± 10	208 ± 14	213 ± 14	223 ± 8	226 ± 7
$n=9$	PRI	14 ± 1	14 ± 1	13 ± 1	13 ± 1	12 ± 1	12 ± 1
5-HD + 0.1%	MAP	66 ± 2	66 ± 3	63 ± 3	61 ± 3	60 ± 3	61 ± 3
BSA	HR	227 ± 4	227 ± 5	237 ± 5	248 ± 5*	243 ± 4*	252 ± 5*
$n=10$	PRI	15 ± 1	15 ± 1	15 ± 1	15 ± 1	14 ± 1	15 ± 1*
Glibenclamide	MAP	58 ± 3	63 ± 4	58 ± 4	58 ± 3	60 ± 5	58 ± 4
+ ET-1 (0.03)	HR	232 ± 17	227 ± 18	223 ± 16	218 ± 10	220 ± 8	219 ± 7
$n=4$	PRI	14 ± 1	14 ± 2	13 ± 2	13 ± 1	13 ± 1	13 ± 0
Glibenclamide	MAP	58 ± 4	61 ± 5	57 ± 7	57 ± 7	58 ± 7	57 ± 5
+ 0.1% BSA	HR	215 ± 7	207 ± 3	206 ± 5	217 ± 4	225 ± 5	222 ± 6
$n=5$	PRI	12 ± 1	13 ± 1	12 ± 2	12 ± 1	13 ± 1	13 ± 1

Rabbits received either vehicle (saline + 0.1% BSA, $n=11$), saline + ET-1 at 0.03 nmol kg^{-1} ($n=16$), saline + ET-1 at 0.3 nmol kg^{-1} ($n=4$), sodium 5-hydroxydecanoate (5-HD) at 5 mg kg^{-1} + ET-1 at 0.03 nmol kg^{-1} ($n=9$), 5-HD at 5 mg kg^{-1} + 0.1% BSA ($n=10$), glibenclamide at 0.03 mg kg^{-1} + ET-1 at 0.03 nmol kg^{-1} ($n=4$) or glibenclamide at 0.03 mg kg^{-1} + 0.1% BSA ($n=10$). 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. Note that −10 min represents baseline (immediately prior to drug treatment) and 0 min represents the onset of LAL occlusion (10 min after drug treatment).

Table 2 Area at risk (expressed as a percentage 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)	Saline + 0.1% BSA	44.7 ± 2.1	11
(II)	Saline + ET-1 (0.03 nmol kg^{-1})	43.7 ± 2.5	16
(III)	Saline + ET-1 (0.3 nmol kg^{-1})	41.3 ± 5.4	4
(IV)	5-HD + ET-1 (0.03 nmol kg^{-1})	50.9 ± 1.9	9
(V)	5-HD + 0.1% BSA	42.9 ± 2.8	10
(VI)	Glibenclamide + ET-1 (0.03 nmol kg^{-1})	48.9 ± 2.6	5
(VII)	Glibencalmide + 0.1% BSA	40.7 ± 6.0	4

Rabbits received either vehicle (saline + 0.1% BSA, $n = 11$), saline + ET-1 at 0.03 nmol kg^{-1} ($n = 16$), saline + ET-1 at 0.3 nmol kg^{-1} ($n = 4$), sodium 5-hydroxydecanoate (5-HD) at 5 mg kg^{-1} + ET-1 at 0.03 nmol kg^{-1} ($n = 9$), 5-HD at 5 mg kg^{-1} + 0.1% BSA ($n = 10$), glibenclamide at 0.03 mg kg^{-1} + ET-1 at 0.03 nmol kg^{-1} ($n = 4$) or glibenclamide at 0.03 mg kg^{-1} + 0.1% BSA ($n = 10$). Values are given as mean ± s.e.mean of n observations. *$P < 0.05$ when compared to vehicle control.

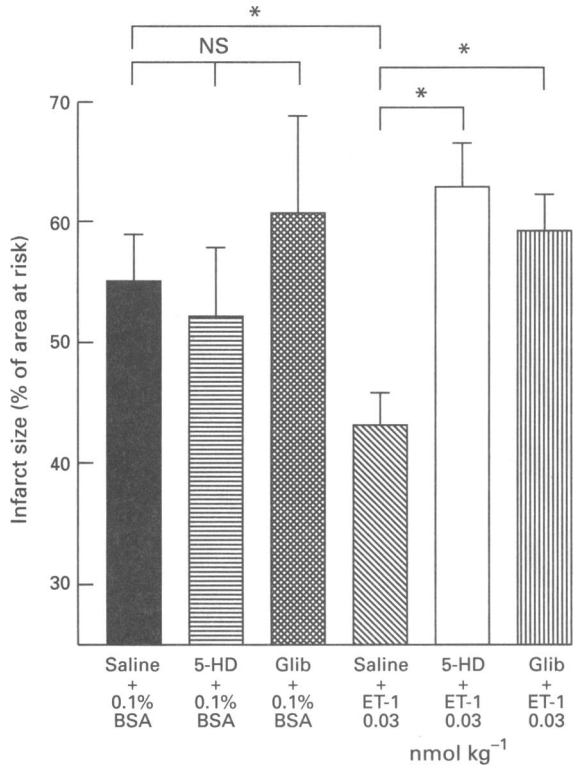

Figure 1 Infarct size expressed as a percentage of the area at risk. Rabbits received either vehicle (Saline + 0.1% BSA, $n = 11$), saline plus endothelin-1 at 0.03 nmol kg^{-1} (Saline + ET-1, $n = 16$), sodium 5-hydroxydecanoate at 5 mg kg^{-1} plus endothelin-1 at 0.03 nmol kg^{-1} (5-HD + ET-1, $n = 9$) or glibenclamide at 0.3 mg kg^{-1} plus endothelin-1 at 0.03 nmol kg^{-1} (Glib + ET-1, $n = 4$). Results are expressed as mean ± s.e.mean of n observations. *$P < 0.05$ when compared to vehicle control. Note that ET-1 (0.03 nmol kg^{-1}) caused a significant reduction in infarct size ($P < 0.05$ when compared to vehicle) which was abolished by pretreatment of the animals with 5-HD, an inhibitor of ATP-sensitive potassium channels.

Discussion

Here we demonstrate that pretreatment of rabbits with a low dose of endothelin-1 (0.03 nmol kg^{-1}), which did not cause a significant increase in blood pressure or pressure-rate index, caused a significant reduction in infarct size in a rabbit model of myocardial ischaemia (60 min) and reperfusion (2 h). In contrast, a higher dose of endothelin-1 (0.3 nmol kg^{-1}), which caused a significant rise in blood pressure, pressure rate index and, hence, myocardial oxygen consumption (Baller *et al.*, 1981), had no protective effect on infarct size in this model of ischaemia and reperfusion.

What therefore, was the mechanism by which ET-1 caused this reduction in infarct size? Clearly, the cardioprotective effect afforded by ET-1 was abolished by pretreatment of rabbits with glibenclamide and 5-HD, two structurally different inhibitors of ATP-sensitive potassium (K_{ATP}) channels (Auchampach *et al.*, 1992). Glibenclamide, a sulphonylurea K_{ATP} channel blocker, produces systemic metabolic effects such as markedly reducing blood glucose levels (Auchampach *et al.*, 1992). It is, therefore, possible that glibenclamide attenuates the cardioprotective effects of ET-1 by a mechanism not related to the inhibition of K_{ATP} channels. Indeed, we have previously reported that glibenclamide (at doses lower than the ones used in this study) inhibits the induction of a calcium-independent isoform of nitric oxide synthase (iNOS); an effect which is also independent of the inhibition of K_{ATP} channels (Wu *et al.*, 1995). To ensure that the inhibition by glibenclamide of the cardioprotective effects of ET-1 was 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 ET-1 in the anaesthetized rabbit, and (ii) that neither glibenclamide nor 5-HD (when administered alone) affected infarct size, demonstrate that the reduction in infarct size brought about by ET-1 was due to the activation of K_{ATP} channels.

There is now good evidence that activation of K_{ATP} channels importantly contributes to the reductions in infarct size caused by ischaemic preconditioning in numerous animal species including the rabbit (Auchampach *et al.*, 1992; Toombs *et al.*, 1993; Walsh *et al.*, 1994). This cardioprotection is abolished by glibenclamide (Toombs *et al.*, 1993). Indeed, it has been proposed (Parratt, 1994) that the activation of K_{ATP} channels afforded by ischaemic preconditioning is secondary to the activation of protein kinase C (PKC) by endogenous mediators such as adenosine (Ytrehus *et al.*, 1994). As (i) ET-1 activates PKC (see Rubanyi & Polokoff, 1994) and (ii) the coronary vasoconstrictor effects caused by ET-1 in the anaesthetized pig are attenuated by the PKC inhibitor, staurosporine (Egashira *et al.*, 1990), we propose that the reduction in infarct size caused by ET-1 in the anaesthetized rabbit is due to activation of PKC, which in turn results in the phosphorylation and opening of K_{ATP} channels. The concept that a potent coronary vasoconstrictor, such as ET-1 (at least at doses which do not cause a significant increase in after load and, hence, myocardial oxygen consumption) can reduce infarct size is not entirely surprising, as intracoronary infusion of noradrenaline also causes a reduction in infarct size by mimicking ischaemic preconditioning (Bankwala *et al.*, 1993; Tsuchida *et al.*, 1994). As α_1-adrenoceptors couple directly with phospholipase C (the activator pathway for PKC) this cardioprotection is also likely to be mediated by PKC and K_{ATP} channels.

What then is the mechanism by which exogenous ET-1 causes the opening of K_{ATP} channels? In the anaesthetized rabbit, exogenous ET-1 causes the release of endogenous prostacyclin into the circulation (Thiemermann *et al.*, 1989a; Lidbury *et al.*, 1990). As prostacyclin reduces infarct size in various animal species including the rabbit (Chiariello *et al.*, 1988), and the vasodilator effects of prostacyclin are, at least in part, due to activation of K_{ATP} channels (Jackson *et al.*, 1993), one could argue that the cardioprotective effects elicited by ET-1 in the anaesthetized rabbit are mediated by prostacyclin. However, this is unlikely as there is good evidence that the cardioprotective effects of the stable prostacyclin-analogue, iloprost, in a rabbit model of global myocardial ischaemia and reperfusion (*in vitro*) are not attenuated by glibenclamide (1 μM) (Vesper & Schrör, 1994). Alternatively, activation of G_i proteins results in the opening of K_{ATP} channels and may contribute to the cardioprotective effects of ischaemic preconditioning (Kirsch *et al.*, 1990; Thornton *et al.*, 1993). As ET-1 causes the activation of G_i protein in numerous tissues, including rat myocytes (see Rubanyi & Polokoff, 1994) it is possible that the opening of K_{ATP} channels by ET-1 is secondary to the activation of G_i protein, an effect which may or may not involve the activation of PKC.

In conclusion, this study demonstrates that a low dose of exogenous ET-1, which does not cause a significant increase in afterload and myocardial oxygen consumption, reduces infarct size in a rabbit model of acute myocardial ischaemia and reperfusion. This cardioprotective effect of exogenous ET-1 was abolished by the K_{ATP} channel inhibitors glibenclamide and 5-HD. We propose that exogenous ET-1, like ischaemic preconditioning, opens ATP-sensitive potassium channels, which in turn protect myocytes against the injury arising from ischaemia and reperfusion. The question as to (i) which ET-receptor subtype mediates the cardioprotective effects of ET-1, and (ii) whether endogenous ET-1 contributes to the cardioprotective effects of ischaemic preconditioning, warrant further investigation using selective antagonists of the ET_A and/or ET_B receptors.

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