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Sulprostone-induced reduction of myocardial infarct size in the rabbit by activation of ATP-sensitive potassium channels

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1 This study examined whether (i) a 1 h pretreatment with or (ii) a continuous infusion of sulprostone reduces myocardial infarct size arising from coronary artery occlusion (60 min) and reperfusion (120 min) in the anaesthetized rabbit. In addition, we investigated whether the observed cardioprotective effect of this selective agonist of prostanoid EP_1/EP_3 receptors were due to the activation of ATP-sensitive potassium (K_{ATP}) channels.

2 In anaesthetized rabbits pretreated with vehicle (5% ethanol in 0.9% saline; 0.05 ml min^{-1}, i.v.) infarct size (expressed as a percentage of the area at risk) after 60 min of coronary artery occlusion followed by 120 min of reperfusion was $59 \pm 4\%$ ($n=10$). Pretreatment of rabbits with sulprostone (1.0 $\mu g\ kg^{-1}\ min^{-1}$ for 1 h, discontinued immediately prior to coronary artery occlusion) did not reduce infarct size ($60 \pm 4\%$; $n=4$). In contrast, a continuous infusion of sulprostone (1.0 $\mu g\ kg^{-1}\ min^{-1}$) starting 10 min prior to the onset of LAL occlusion and continued throughout the experiment, significantly reduced infarct size ($41 \pm 5\%$, $n=6$) when compared to the respective vehicle-treated controls ($57 \pm 4\%$, $n=10$; $P<0.05$). Sulprostone (pretreatment or continuous infusion) had no effect on any of the haemodynamic parameters measured.

3 The reduction in infarct size afforded by continuous infusion of sulprostone was abolished by pretreatment of rabbits with the K_{ATP} channel blocker 5-hydroxydecanoate (5-HD 5 $\mu g\ kg^{-1}$; $63 \pm 4\%$; $n=6$). When administered alone, 5-HD had no effect on infarct size when compared to control (52 ± 6, $n=10$).

4 We propose that a continuous infusion of the selective EP_1/EP_3 prostanoid receptor agonist, sulprostone, reduces infarct size in the anaesthetized rabbit by a mechanism that involves the opening of K_{ATP} channels.

Keywords: Sulprostone; E-type prostaglandin receptors; sodium 5-hydroxydecanoate; ATP-sensitive potassium channel; myocardial infarction; protein kinase C; ischaemic preconditioning

Introduction

E-type prostaglandins (PGs), such as PGE_1, exert 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.*, 1988b; Simpson *et al.*, 1988; Hide *et al.*, 1994). 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, all of which are mediated by prostanoid EP_2 receptors (Kloeze, 1967; Hutton *et al.*, 1973; Jugdutt *et al.*, 1981; Schrör *et al.*, 1988b; 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 (EP_2-mediated effects) are not a pre-requisite for the cardioprotective effects of vasodilator prostaglandins. Thus, it has been proposed that the anti-ischaemic effects of these prostanoids in isolated cells and tissues are due to a 'cytoprotective' or 'membrane stabilizing' effect, the mechanism of which is unknown (see Schrör *et al.*, 1988a).

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 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 (see Parratt & Kane, 1994). Indeed, inhibition of K_{ATP} channels with glibenclamide or sodium 5-hydroxydecanoate (5-HD) abolishes the cardioprotective effects of ischaemic preconditioning (Vegh *et al.*, 1993; Hide & Thiemermann, 1996).

On the basis of their responses to various agonists and antagonists, E-type prostanoid receptors have been divided into four subtypes, EP_1, EP_2, EP_3 and EP_4 (Coleman *et al.*, 1990). The EP_2 and EP_4 prostanoid receptors are linked via a G-protein, G_s, to stimulation of adenylate cyclase and an increase in cyclic cAMP. In contrast, EP_1 and EP_3 are linked to multiple G-proteins (G_q and G_i/G_q respectively; see *Trends Pharmacol. Sci.*, 1995) and activation of these receptors results in the hydrolysis of phosphatidylinositol (PI) and, hence, PKC activation (Hohlfeld, 1995; Katoh *et al.*, 1995).

We have previously demonstrated that PGE_1 (1 $\mu g\ kg^{-1}\ min^{-1}$, 1 h pretreatment) reduces infarct size in a rabbit model of acute myocardial ischaemia (60 min) and reperfusion (120 min). This cardioprotection was reduced by inhibition of K_{ATP} channels with glibenclamide and 5-HD (Hide *et al.*, 1995) suggesting that the opening of these channels by PGE_1 contributes to the reduction in infarct size afforded by this prostanoid. As PGE_1 is a non-selective agonist of EP_1, EP_2 or EP_3

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receptors, the receptor which mediates the activation of K_{ATP} channels afforded by PGE_1 is unknown. Moreover, it is unclear whether activation of the EP_2 receptor, which mediates many of the anti-ischaemic effects of PGE_1 or prostacyclin (see above), is essential for cardioprotection.

Thus, this study investigates whether pretreatment ('pharmacological preconditioning') or a continuous infusion of sulprostone, a selective agonist of EP_1/EP_3 receptors (Coleman *et al.*, 1987; 1990), reduces infarct size in a rabbit model of acute myocardial ischaemia (60 min) and reperfusion (120 min). In a separate subsequent study, we have also investigated whether the observed cardioprotective effects of sulprostone are due to the activation of K_{ATP} channels.

Methods

Experimental exclusion criteria

This study was carried out on 51 male rabbits (New Zealand White rabbits, Foxfield, U.K.) weighing 2.5 to 3.0 kg receiving a standard diet and water *ad libitum*. The exclusion criteria for this series of experiments included (i) an area at risk of less than 20%, or more than 60% of the left ventricle, (ii) death of the rabbit within 10–20 min of LAL occlusion due to ventricular fibrillation or (iii) MAP of less than 25 mmHg (e.g. due to cardiac failure). Hence, 1 rabbit died before being attached to the ventilator (respiratory arrest) and of the 50 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 and 1 died of cardiac failure during the reperfusion period (receiving sulprostone as a continuous infusion). The area at risk of 2 rabbits (1 rabbit being treated with sulprostone as a continuous infusion and 1 rabbit being treated with 5-HD plus sulprostone) was above the cut off point of 60% of the left ventricle. The data obtained from these five rabbits were excluded from data analysis. The experimental protocol employed in this study is shown in Figure 1.

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) at 0.1 ml kg^{-1}. General anaesthesia was then induced with sodium pentobarbitone (20 mg kg^{-1}, i.v. injected into the left marginal ear vein; Sagatal) and maintained with supplementary doses of sodium pentobarbitone as required. Lignocaine (Xylocaine 2%) was also used for local anaesthesia. The rabbits were tracheotomised, intubated and ventilated with room air from a Harvard ventilator at a rate of 36–40 strokes per minute 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 (MAP). 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; Hide *et al.*, 1995). 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 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 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 (MI). 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'.

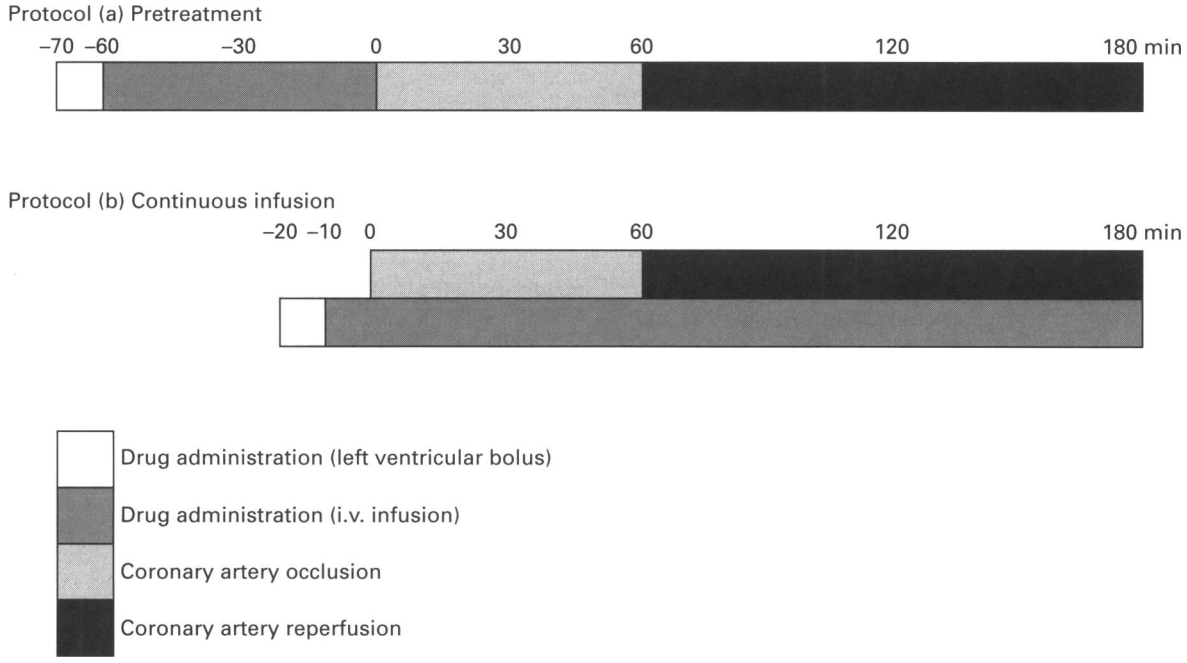

Figure 1 Schematic representation of the experimental protocols employed in this study.

Haemodynamic measurements and electrocardiogram Haemodynamic parameters, including 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 −70 min (baseline), −60 min (after 5-HD treatment), 0 min (just prior to end of sulprostone or vehicle infusion and before LAL occlusion), 15, 30, 45, 60 min (occlusion period) and every hour during the subsequent reperfusion period (120, 180 min) for animals pretreated with sulprostone. In animals treated with a continuous infusion of sulprostone, haemodynamic measurements were obtained at −20 min (baseline), −10 min (after 5-HD treatment and just prior to start of sulprostone infusion), 0 min (just prior to LAL occlusion) and at the same time points during occlusion and reperfusion as the pretreated group. 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.) to confirm successful LAL occlusion (rise in ST-segment and increase in R-wave amplitude) and reperfusion (fall in ST-segment elevation and increase in Q-wave amplitude). The heart rate was automatically calculated from left ventricular systolic pulse curves by means of a Grass 7P4H tachograph. 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 (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). Pieces were separated according to staining and weighed in order to determine the infarct size as a percentage of the area at risk.

Drug regimens Sulprostone (1.0 μg kg^{-1} min^{-1}) or its vehicle-control (5% ethanol in 0.9% NaCl) were infused intravenously (i) for 1 h at a rate of 0.05 ml min^{-1}, starting 60 min prior to LAL occlusion or (ii) as a continuous infusion (1 μg kg^{-1} min^{-1} at a rate of 0.05 ml min^{-1}) starting 10 min prior to LAL occlusion and continuing throughout the experiment. 5-HD (5 mg kg^{-1}), an ischaemia selective blocker of ATP-sensitive potassium (K_{ATP}) channels (McCullough *et al.*, 1991; Auchampach *et al.*, 1992; Hide *et al.*, 1995), was administered as a bolus injection (2 ml volume) into the left ventricle 10 min before infusion of sulprostone or vehicle.

Thus, six experimental groups were studied: (I) Control pretreatment: vehicle (5% ethanol in saline, 1 h i.v. infusion), commencing 60 min prior to LAL occlusion ($n=10$). (II) Sulprostone pretreatment: sulprostone (1.0 μg kg^{-1} min^{-1}, 1 h infusion) commencing 60 min prior to LAL occlusion ($n=4$). (III) Control, continuous infusion: vehicles (2 ml 0.9% w/v saline for 5-HD, left ventricular bolus and 5% ethanol in saline for sulprostone, continuous i.v. infusion) starting −20 min and −10 min respectively prior to LAL occlusion ($n=10$). (IV) Sulprostone, continuous infusion: vehicle for 5-HD (0.9% saline, 2 ml) followed 10 min later by an i.v. infusion of sulprostone (1.0 μg kg^{-1} min^{-1}) starting 10 min prior to LAL occlusion and continued throughout the remainder of the experiment ($n=6$). (V) 5-HD plus sulprostone: 5-HD (5 mg kg^{-1}, 2 ml) followed 10 min later by an i.v. infusion of sulprostone (1.0 μg kg^{-1} min^{-1}) starting 10 min prior to LAL occlusion and continued throughout the remainder of the experiment ($n=6$). (VI) 5-HD only: 5-HD (5 mg kg^{-1}, 2 ml) followed 10 min later by an i.v. infusion of 5% ethanol in 0.9% saline (at a rate of 0.05 ml min^{-1}) starting 10 min prior to LAL occlusion and continued throughout the remainder of the experiment ($n=10$).

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.). Evans blue dye and NBT were obtained from Sigma Chemical Co. (Poole, U.K.). Sulprostone was a gift from Schering AG, Germany, and was dissolved in ethanol and stored in aliquots at −20°C until required. The stock was diluted to the required concentration in 0.9% w/v saline each day. Sodium 5-hydroxydecanoate was obtained from Affiniti Research Products Ltd. (Exeter, U.K.) and was freshly dissolved in 0.9% w/v saline each day.

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 Student's unpaired *t* test. A *P* value of less than 0.05 was considered statistically significant.

Results

Myocardial ischaemia and reperfusion

Haemodynamic data Tables 1 and 2 show values for MAP, HR and pressure-rate index (PRI), an indicator of myocardial oxygen consumption (Baller *et al.*, 1981). Baseline haemodynamic data were similar in all groups investigated ($P>0.05$, see Table 1).

Sulprostone (1 μg kg^{-1} min^{-1}) administered either as a pretreatment or continuous infusion had no effect on MAP, HR or PRI. The bolus injection of 5-HD into the left ventricle (5 mg kg^{-1}) also had no effect on any of the haemodynamic parameters measured, nor did 5-HD alter the haemodynamic effects afforded by the subsequent infusion of sulprostone.

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 3).

In rabbits treated with vehicle alone (pretreatment), ischaemia (60 min) followed by reperfusion (2 h) resulted in an infarct size of 59 ± 4% ($n=10$) of the area at risk (Figure 2). Pretreatment of rabbits with an infusion of sulprostone (1.0 μg kg^{-1} min^{-1}) had no effect on infarct size when compared with vehicle ($n=4$; Figure 2). Treatment with a continuous infusion of vehicle resulted in an infarct size of 58 ± 4% ($n=10$). Administration of sulprostone as a continuous i.v. infusion starting 10 min prior to LAL occlusion and continued throughout the experiment significantly reduced myocardial infarct size when compared to control ($P<0.05$, $n=6$; Figure 2). This reduction in infarct size was abolished by pretreatment of rabbits with 5-HD (5 mg kg^{-1}, $n=6$) (Figure

Table 1 Mean arterial blood 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/2 h reperfusion and pretreated for 1 h with either vehicle or sulprostone

		Time				
Treatment	*Parameter*	*-60 min*	*0 min*	*30 min*	*60 min*	*180 min*
Control	MAP	63±2	63±3	61±3	59±3	56±3
pretreatment	HR	226±8	224±3	221±14	224±5	221±16
$n=10$	PRI	14±1	14±1	13±1	13±1	12±1
Sulprostone	MAP	62±6	70±10	66±8	60±9	66±6
pretreatment	HR	206±15	193±11	214±12	215±16	226±16
$n=4$	PRI	13±1	13±1	14±2	13±3	15±2

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.

Table 2 Mean arterial blood 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/2 h reperfusion and treated with a continuous infusion of sulprostone starting 10 min prior to LAL occlusion and continued until the end of the experiment (or vehicle)

		Time					
Treatment	*Parameter*	*-20 min*	*-10 min*	*0 min*	*30 min*	*60 min*	*180 min*
Control	MAP	62±3	61±2	62±3	58±2	56±3	54±3
continuous	HR	225±8	224±7	222±7	218±9	222±8	225±7
infusion, $n=10$	PRI	14±1	14±1	14±1	13±1	12±1	12±1
Sulprostone	MAP	62±3	64±2	67±3	64±2	64±2	62±2
continuous	HR	232±10	220±9	211±8	207±10	207±10	197±8
infusion, $n=6$	PRI	15±1	14±1	14±1	14±1	14±1	12±1
5-HD+	MAP	62±4	62±5	65±5	64±6	64±6	56±5
sulprostone	HR	233±2	230±10	213±12	208±12	208±12	195±12
$n=6$	PRI	15±1	14±1	14±1	13±1	13±1	11±1
5-HD	MAP	65±2	65±2	66±3	63±3	61±3	61±3
only	HR	227±4	227±4	227±5	237±5	248±5	252±5
$n=10$	PRI	15±1	15±1	15±1	15±1	14±1	15±1

5-HD was administered as a bolus in the left ventricle 10 min before onset of the sulprostone infusion. 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.

Table 3 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)	Control pretreatment	45±3	10
(II)	Sulprostone pretreatment	47±4	4
(III)	Control continuous infusion	43±3	10
(IV)	Sulprostone continuous infusion	40±5	6
(V)	5-HD+sulprostone	46±4	6
(VI)	5-HD only	43±3	10

Values are given as mean±s.e.mean of n observations.

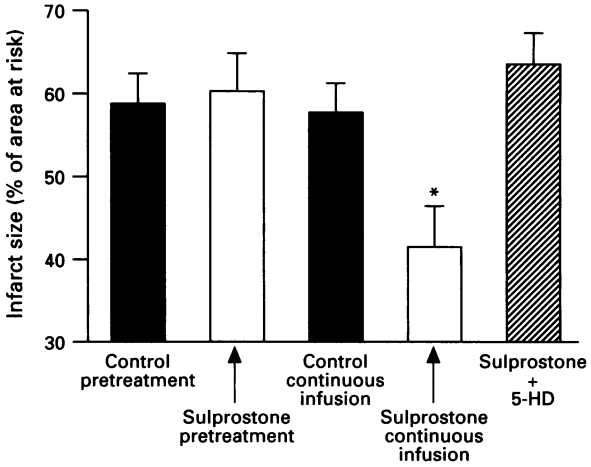

Figure 2 Infarct size (IS) expressed as a percentage of the area at risk. Columns are mean±s.e.mean. Sulprostone (continuous infusion; 1 $\mu g\,kg^{-1}\,min^{-1}$) reduced IS when compared to control and this cardioprotection was abolished by pretreatment with sodium 5-hydroxydecanoate (5-HD). *P-value<0.05 vs (i) control continuous infusion and (ii) 5-HD+sulprostone groups, $n=4-10$ per group.

2), a blocker of K_{ATP} channels. When administered alone, 5-HD (55±7%; $n=8$, $P>0.05$) had no effect on myocardial infarct size compared to vehicle control.

Discussion

This study demonstrates that a continuous infusion of the EP_1 and EP_3 prostanoid receptor agonist, sulprostone, reduces infarct size in a rabbit model of regional myocardial ischaemia (60 min) and reperfusion (120 min).

What then, is the mechanism by which sulprostone causes this reduction in infarct size? Clearly, a reduction in blood pressure and, hence, afterload does not contribute to the anti-ischaemic effects of sulprostone, as sulprostone did not exert any haemodynamic effects at the dose used in this study. However, a higher dose of sulprostone increased, rather than decreased, mean arterial blood pressure (unpublished observation). Similarly, higher doses of sulprostone cause vasoconstriction in the human isolated pulmonary artery, and this effect is due to activation of EP_3 receptors (Qian *et al.*, 1994). One could also argue that sulprostone, like prostacyclin or E-type prostaglandins, reduces infarct size by inhibiting the ac-

tivation of platelets and particularly polymorphonuclear neutrophils (PMN's). This is, however, extremely unlikely as these effects are due to activation of EP_2 receptors. Indeed, sulprostone potentiates, rather than inhibits, the aggregation of human platelets in response to ADP or PAF; and this pro-aggregatory effect is secondary to activation of EP_3 receptors and G_i resulting in inhibition of adenylate cyclase and increase in intracellular calcium (Ashby, 1988; Mathew & Jones, 1993). Similarly, sulprostone does not inhibit the activity of human (Wheeldon & Vardy, 1993; Talpain *et al.*, 1995) or rat PMN's (Wise & Jones, 1994), but may enhance PMN activity due to activation of EP_3 receptors (Armstrong, 1992; Wheeldon & Vardey, 1993).

The reduction in infarct size afforded by sulprostone was, however, abolished by pretreatment of rabbits with 5-HD, an ischaemia-selective inhibitor of K_{ATP} channels (McCullough *et al.*, 1991). Thus, we propose that the cardioprotective effects of sulprostone are due to the opening of K_{ATP} channels. What then is the mechanism by which sulprostone causes the activation of K_{ATP} channels? Activation of G_q by EP_1/EP_3 (subgroups A and D) ultimately results, via phospholipase C (PLC)-mediated phosphoinositol (PI) hydrolysis, in the activation of protein kinase C (PKC; Hohlfeld, 1995; Katoh *et al.*, 1995). The subsequent phosphorylation and opening of K_{ATP} channels by PKC leads to an increased potassium 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 calcium entry and reduced contractile energy consumption. The opening of K_{ATP} channels may also prevent ATP depletion, glycogen breakdown, and anaerobic glycolysis, thus preserving energy substrate (Grover *et al.*, 1989; 1992). In the case of EP_1 receptors, a potential phosphorylation site for PKC is located in the third intracellular loop and phosphorylation of this site may result in the uncoupling of EP_1 from its associated G-protein. PKC induces both short term and long term desensitization of EP_1 and is therefore an important feedback regulator of the signal transduction of EP_1 receptors (Katoh *et al.*, 1995).

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; Hide & Thiemermann, 1996) 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 (Auchampach *et al.*, 1991; Gross *et al.*, 1992; Grover *et al.*, 1990), 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 mediators such as adenosine (Liu *et al.*, 1991; Thornton *et al.*, 1992) which, via the stimulation of G protein-coupled (G_q/G_0) receptors and activation of PKC, ultimately leads to the long-lasting opening of K_{ATP} channels. Similarly, the reductions in infarct size caused by E-type prostaglandins, such as PGE_1, and by endothelin-1 are due to the activation of K_{ATP} channels, as the cardioprotective effects of these autacoids are attenuated by glibenclamide and 5-HD (Hide *et al.*, 1995a,b).

Surprisingly, we found that the pretreatment of rabbits with a 1 h infusion of sulprostone did not result in a significant reduction in infarct size. It is likely that the degree of activation of EP_1 or EP_3 receptors and, hence, the subsequent activation of PKC afforded by the pretreatment with sulprostone is smaller than the one achieved with a continuous infusion of sulprostone. Thus it is possible that pretreatment with sulprostone only leads to a degree of PKC activation which is below the threshold for a long-lasting opening of K_{ATP} channels. This hypothesis is supported by the finding that a specific degree of PKC activation ('Threshold Hypothesis') is required to initiate cardioprotection (Goto *et al.*, 1995).

In conclusion, this study demonstrates that sulprostone (when administered as a continuous infusion during ischaemia and reperfusion) causes a pronounced reduction in infarct size caused by regional myocardial ischaemia (60 min) and reperfusion (120 min) in the anaesthetized rabbit. The cardioprotective effect of sulprostone is due to opening of K_{ATP} channels. We propose that the opening of these channels by sulprostone is secondary to the stimulation of EP_1/EP_3 prostanoid receptors and subsequent activation of PKC. Further studies are, however, necessary to elucidate whether EP_1 receptors-like EP_3 receptors- are present on the sarcolemma of cardiac myocytes (Lopaschuk *et al.*, 1989; Hohlfeld, 1995). Our results imply that a significant degree of cardioprotection can be achieved by prostanoids which do not activate EP_2 receptors and, hence, exert haemodynamic (side) effects. The cardioprotective effects of prostacyclin (and its analogues) as well as E-type prostaglandins in isolated hearts perfused at constant flow with buffer solution have, in the absence of coronary vasodilatation and of blood-borne cells, been attributed to a 'cytoprotective' effect, the mechanism of which is unknown. Clearly, the activation of (i) EP_1 or EP_3 receptors, (ii) PKC and (iii) ultimately K_{ATP} channels (e.g. by sulprostone) represents a novel mechanism underlying the protective effects of certain prostanoids which is independent of haemodynamic effects or inhibition of the function of platelets and PMNs. We speculate that the activation of PKC and K_{ATP} channels may contribute to the 'cytoprotective' effects of prostaglandins.

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