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The effects of the endothelin ET_A receptor antagonist, FR 139317, on infarct size in a rabbit model of acute myocardial ischaemia and reperfusion

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1 The effects were investigated of the ET_A receptor antagonist, FR 139317, on endothelin-1 (ET-1)-induced coronary vasoconstriction in the isolated perfused heart of the rabbit. In addition, this study examined whether FR 139317 reduced infarct size in a rabbit model of coronary artery occlusion and reperfusion.

2 In the rabbit isolated perfused heart, ET-1 (1–100 pmol) elicited a dose-dependent increase in coronary perfusion pressure (CPP). For example, 30 pmol ET-1 caused CPP to rise by 22 ± 8 mmHg and 100 pmol ET-1 by 47 ± 10 mmHg ($n = 8$). Infusion of FR 139317 (1 μM) significantly attenuated the increase in CPP caused by ET-1 (30 pmol: 3 ± 1 mmHg, 100 pmol: 8 ± 2 mmHg; $n = 8$).

3 In the anaesthetized rabbit, infarct size (expressed as a percentage of the area at risk) after 45 or 60 min of coronary artery occlusion followed by 2 h of reperfusion was 47 ± 6% ($n = 6$) and 55 ± 7% ($n = 5$), respectively. A continuous infusion of FR 139317 (0.2 mg kg^{-1} min^{-1} preceded by a loading dose of 1.0 mg kg^{-1}, i.v.; $n = 5–6$) had no effect on the extent of the myocardial infarct size (45 min: 47 ± 6%; 60 min: 49 ± 7%). Even a three-times higher dose (0.6 mg kg^{-1} min^{-1} preceded by a loading dose of 3 mg kg^{-1}, i.v.; $n = 4$) of FR 139317 had no effect on myocardial infarct size (48 ± 5%) after 45 min occlusion of the antero-lateral branch of the left coronary artery (LAL) and 2 h reperfusion.

4 In a separate group of experiments, the LAL was occluded for 60 min and subsequently reperfused for 6 h. FR 139317 (0.6 mg kg^{-1} min^{-1} preceded by a loading dose of 3 mg kg^{-1}, i.v.; $n = 4$) had no significant effect on infarct size even in this long reperfusion model (control: 48 ± 3%, FR 139317: 61 ± 6%).

5 Thus, the vasoconstrictor effects elicited by ET-1 in the coronary vasculature of the rabbit are primarily mediated via the ET_A receptor, for they were inhibited by the ET_A receptor antagonist, FR 139317. However, an enhanced formation of endogenous ET-1 does not play a major role in ischaemia/reperfusion injury of the rabbit heart, for FR 139317 had no effect on infarct size.

Keywords: Endothelin-1; endothelin ET_A receptor; FR 139317; myocardial infarction

Introduction

Endothelin-1 (ET-1) is a potent coronary vasoconstrictor in a variety of species including rat (Baydoun *et al.*, 1989; Neubauer *et al.*, 1990a), rabbit (Hirata *et al.*, 1990), pig (Ezra *et al.*, 1989), dog (Clozel & Clozel, 1989; Larkin *et al.*, 1989) and man (Chester *et al.*, 1989; Clarke *et al.*, 1989). Moreover, ischaemia/reperfusion of the isolated perfused heart of the rat enhances the coronary vasoconstriction elicited by ET-1 (Neubauer *et al.*, 1990b; 1991; Stewart & Baffour, 1990; Brunner *et al.*, 1992; Grover *et al.*, 1992; Watts *et al.*, 1992; McMurdo *et al.*, 1993b). Enhanced plasma levels of ET-1 in man are associated with a variety of cardiovascular disorders including acute myocardial infarction (Miyauchi *et al.*, 1989; Salminen *et al.*, 1989), angina pectoris (Nakao *et al.*, 1989), coronary artery vasospasm (Matsuyama *et al.*, 1990) and congestive heart failure (Grenier *et al.*, 1990). The hypothesis that endogenous ET-1 plays a role in the extension of ischaemia/reperfusion injury of the heart is supported by the findings that (i) intravenous administration of a monoclonal antibody against ET-1 reduces infarct size in a model of coronary artery occlusion and reperfusion in the anaesthetized rat (Watanabe *et al.*, 1991) or anaesthetized rabbit (Kusumoto *et al.*, 1993) and (ii) infusion of the endothelin-converting enzyme inhibitor, phosphoramidon, results in a substantial reduction in infarct size in the anaesthetized rat (Grover *et al.*, 1992).

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 due to activation of the ET_A receptor although ET_B receptors also mediate some vasoconstriction (Douglas *et al.*, 1992; Bigaud & Pelton, 1992; Moreland *et al.*, 1992). The discovery of distinct endothelin receptors has prompted the development of selective endothelin receptor antagonists, such as FR 139317 (Sogabe *et al.*, 1992). FR 139317 is a linear tripeptide, which binds selectively to ET_A receptors and inhibits ET-1 binding to porcine and human aortic microsomes, antagonizes ET-1-induced contractions of rabbit isolated aorta and attenuates the pressor response to ET-1 in conscious rats (Sogabe *et al.*, 1992; 1993) and anaesthetized rabbits (McMurdo *et al.*, 1993a).

Here, we determine which endothelin receptor-subtype mediates ET-1-induced coronary vasoconstriction in the isolated perfused heart of the rabbit and go on to investigate whether the ET_A receptor antagonist, FR 139317, reduces infarct size in a rabbit model of coronary artery occlusion and reperfusion.

Methods

Rabbit isolated perfused hearts

Male New Zealand White rabbits (2–3 kg) were premedicated with Hypnorm (0.1 ml kg^{-1}, i.m.; containing fentanyl citrate at 0.315 mg ml^{-1} and fluanisone at 10 mg ml^{-1}). General anaesthesia was then induced with

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sodium pentobarbitone (30 mg kg^{-1}, i.v.) and anaesthesia was maintained with supplementary doses of sodium pentobarbitone as required. The trachea was cannulated and the rabbits were ventilated with room air from a Harvard ventilator at a rate of 36–40 strokes per min and a tidal volume of 18–20 ml. Heparin (100 iu kg^{-1}) was given into the marginal ear vein, the thorax was opened and the heart was rapidly excised and immersed in ice cold saline. The aorta was dissected free and cannulated with a glass cannula and the heart was transferred to the perfusion apparatus. The coronary circulation was perfused at a constant flow (25 ml min^{-1}) with Krebs solution containing (in mM): NaCl 118, $NaHCO_3$ 25, KCl 3, $MgSO_4$ 1.2, NaH_2PO_4 1.2, glucose 11 and $CaCl_2$ 1.4. The buffer was bubbled with 95% O_2, 5% CO_2 and kept at 37°C. Air temperature around the hearts was maintained by means of a heated (37°C) water-jacket. In this constant flow system, changes in coronary perfusion pressure (CPP) reflect changes in coronary vascular resistance. CPP was recorded with a Transamerica type 4-422-0001 pressure transducer. In addition, developed tension (DT) and heart rate (HR) were measured by means of a hook attached to the apex of the heart which was connected by a thread to an isometric transducer (Grass Instruments, model FT03). The output of each transducer was displayed on a polygraph recorder (Grass Instruments, model 7D). Drugs were administered via an injection port 3 cm distal to the aortic cannula. The hearts were subjected to a resting tension of 2 g and allowed to equilibrate for 20 min in order to establish stable baseline parameters.

ET-1 (1, 3, 10, 30 or 100 pmol) bolus injections were given 10 min after the start of a continuous infusion of vehicle (20% dimethylsulphoxide; DMSO) or FR 139317 (1 μM).

Myocardial ischaemia and reperfusion in the anaesthetized rabbit

Surgical procedure Rabbits were anaesthetized and ventilated as described above. Body temperature was monitored and maintained at 38 ± 1°C by means of a rectal probe thermometer attached to a homeothermic blanket control unit (Harvard, Edenbridge, Kent). The left femoral vein was cannulated for drug administration. The left femoral artery was cannulated for the measurement of blood pressure from which mean arterial pressure (MAP) and HR were derived. Haemodynamic parameters were continuously recorded on a polygraph recorder (Grass Instruments, 7D). Lead II electrocardiograms (ECGs) were recorded from subdermal platinum electrodes. ST-segment changes were calculated as absolute differences with respect to the J-point of the QRS complex (of at least 10 cardiac cycles) and expressed as Δ mV. Another catheter was placed in the left ventricle via the right common carotid artery for injection of Evans blue dye solution. A 2–3 cm left intercostal thoracotomy (4th intercostal space) was performed to expose the heart and a snare occluder was placed around the first antero-lateral branch of the left coronary artery (LAL) 1 cm distal from its origin. In contrast to other species, the rabbit LAL supplies most of the left ventricular myocardium including most of the septum and apex (Flores *et al.*, 1984). Care was taken not to include veins draining blood from this area whenever possible.

Measurement of infarct size After the reperfusion period, the LAL was re-occluded and Evans blue dye solution (4 ml of 2% w/v) was 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). After injection of Evans blue dye, 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 tissue. To distinguish between ischaemic and infarcted tissue, the area at risk was chopped and incubated in *p*-nitroblue tetrazolium (NBT, 0.5 mg ml^{-1} for 20 min) at 37°C. In the presence of intact dehydrogenase enzyme systems (normal myocardium). NBT forms blue coloured precipitates, while areas of necrosis lack dehydrogenase activity and, therefore, fail to stain (Nachlas & Shnitka, 1963). Tissue was divided into stained (blue) or unstained (red) groups. All tissues from the left ventricle were weighed. Area at risk was expressed as a percentage of the left ventricle, and the infarct size was calculated as a percentage of the area at risk.

Experimental design All rabbits were allowed to stabilize for 30 min before being assigned to one of eight groups: (1) 30 min LAL occlusion and 2 h reperfusion treated with vehicle ($n = 4$); (2) 45 min LAL occlusion and 2 h reperfusion treated with vehicle ($n = 5$); (3) 45 min LAL occlusion and 2 h reperfusion treated with a low dose of FR 139317 (0.2 mg kg^{-1} min^{-1} preceded by a loading dose of 1.0 mg kg^{-1}, i.v., bolus; $n = 5$); (4) 45 min LAL occlusion and 2 h reperfusion treated with a high dose of FR 139317 (0.6 mg kg^{-1} min^{-1} preceded by a loading dose of 3.0 mg kg^{-1}, i.v., bolus, $n = 4$); (5) 60 min LAL occlusion and 2 h reperfusion treated with vehicle ($n = 5$); (6) 60 min LAL occlusion and 2 h reperfusion treated with a low dose of FR 139317 (0.2 mg kg^{-1} min^{-1} preceded by a loading dose of 1.0 mg kg^{-1}, i.v., bolus, $n = 6$); (7) 60 min LAL occlusion and 6 h reperfusion treated with vehicle ($n = 5$); (8) 60 min LAL occlusion and 6 h reperfusion treated with a high dose of FR 139317 (0.6 mg kg^{-1} min^{-1} preceded by a loading dose of 3.0 mg kg^{-1}, i.v., bolus, $n = 4$).

Vehicle or FR 139317 infusion was started 10 min prior to LAL occlusion and continued throughout the reperfusion period.

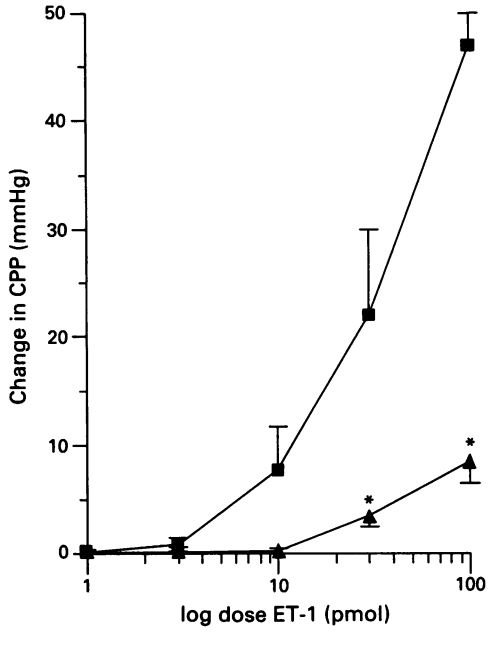

Figure 1 Changes in coronary perfusion pressure (CPP) induced by endothelin-1 (ET-1, 1-100 pmol, bolus injection) in rabbit isolated hearts treated with either vehicle (■, $n = 8$) or with FR 139317 (▲, 1 μM continuous infusion, $n = 8$). Data are mean ± s.e.mean of n observations. $^*P < 0.05$ when compared to vehicle control.

Materials

Hypnorm was purchased from Janssen Pharmaceutical Co., (Oxford, UK), sodium pentobarbitone (Sagatal) from May & Baker (Dagenham, UK) and heparin from Evans Med., (Middlesex, UK). The Krebs buffer salts, DMSO, bovine serum albumin, Evans blue dye and NBT were obtained from Sigma Chem. Co., (Poole, UK). ET-1 was supplied by Scientific Marketing Associates (London, UK) and was reconstituted in 0.1% acetic acid and diluted in 0.9% w/v saline containing 1% w/v bovine serum albumin and 0.06% sodium bicarbonate. FR 139317 (**R**)-2[(**R**)-2-[(S)-2-1-(hexahydro-1H-azepinyl)]carbonyl]amino-4-methylpentanoyl] amino-3[3-(1-methyl-1H-indolyl]proprionyl] amino-3-(2-pyridyl) proprionic acid was provided by Dr Annette M. Doherty of the Medicinal Chemistry Department at Parke-Davis Pharmaceutical Research Division of Warner-Lambert Co. (Ann Arbor, U.S.A.). FR 139317 was dissolved in 20% DMSO or in saline (6 h reperfusion experiments). Aliquots of ET-1 and FR 139317 were stored frozen (− 20°C) until use.

Statistical comparisons

All values in the figures, tables and text are expressed as mean ± s.e.mean of n observations. Statistical evaluation of the data was by Student's t test for unpaired determinations or by ANOVA. A P value of less than 0.05 was considered significant.

Results

Effects of FR 139137 on the coronary vasoconstriction elicited by ET-1 in the isolated perfused heart of the rabbit

After the 20 min equilibration period, the mean resting CPP was 29 ± 1 mmHg, the DT was 10 ± 1 g and HR was 158 ± 4 beats min^{-1} ($n = 16$). Infusion of vehicle (20% DMSO) or FR 139317 (1 μM) had no effect on these parameters.

ET-1 induced a dose-dependent increase in CPP. For example, 30 pmol ET-1 caused CPP to rise by 22 ± 8 mmHg and 100 pmol ET-1 by 47 ± 10 mmHg ($n = 8$), respectively (Figure 1). The coronary vasoconstriction elicited by ET-1 was significantly attenuated in the presence of a continuous infusion of FR 139317 (1 μM). For example, 30 pmol and 100 pmol ET-1 elicited rises in CPP of only 3 ± 1 mmHg and 8 ± 2 mmHg, respectively ($n = 8$, Figure 1). ET-1 had no effect on HR or DT in the absence or presence of FR 139317 (data not shown).

Effects of FR 139317 on infarct size in a rabbit model of acute myocardial ischaemia and reperfusion

Of the 48 rabbits which underwent LAL occlusion, 10 died within the experimental period due to ventricular fibrillation or cardiac failure and these were excluded from the study. Nine of these died within 5–20 min of the ischaemic period (7 rabbits receiving vehicle and 2 rabbits receiving FR 139317 at 0.2 mg kg^{-1} min^{-1} preceded by a loading dose of 1.0 mg kg^{-1}, i.v.). One rabbit died during reperfusion (receiving FR 139317 at 0.6 mg kg^{-1} min^{-1} preceded by a loading dose of 3 mg kg^{-1}, i.v.).

Haemodynamic data mean resting values for MAP was 61 ± 2 mmHg and for HR was 237 ± 4 beats min^{-1} ($n = 38$). Infusion of FR 139317 at the highest dose used (0.6 mg kg^{-1} min^{-1} preceded by a loading dose of 3 mg kg^{-1}, i.v.) elicited a transient rise (16 ± 4 mmHg) in blood pressure which returned to control levels within 20 min. LAL occlusion (45 or 60 min) and reperfusion (2 or 6 h) had no significant effect on MAP or HR in any of the groups studied (Table 1).

Area at risk and infarct size: The area of the left ventricle at risk (ischaemic myocardium) was similar in all groups studied (Table 2). In hearts subjected to 30 min LAL occlusion plus 2 h of reperfusion, infarct size (expressed as a percentage of area at risk) was 10 ± 1% ($n = 4$). Due to the small infarct size obtained, this model was not used to investigate the effects of FR 139317.

Infarct size after 45 or 60 min of LAL occlusion followed by 2 h of reperfusion in vehicle-treated rabbits was 47 ± 6% ($n = 6$) and 55 ± 7% ($n = 5$), respectively (Table 2). A continuous infusion of FR 139317 (0.2 mg kg^{-1} min^{-1} preceded by a loading dose of 1.0 mg kg^{-1}, i.v.; $n = 5–6$) had no effect on the extent of the myocardial infarct (45 min: 47 ± 6%; 60 min; 49 ± 7%, Table 2). Even a three-times higher dose (0.6 mg kg^{-1} min^{-1} preceded by a loading dose of 3 mg kg^{-1}, i.v.; $n = 4$) of FR 139317 had no effect on myocardial infarct size (48 ± 5%) after 45 min LAL occlusion and 2 h reperfusion (Table 2).

When the LAL was occluded for 60 min and subsequently reperfused for 6 h, infarct size in vehicle-treated animals was 48 ± 3% ($n = 5$). FR 139317 (0.6 mg kg^{-1} min^{-1} preceded by

Table 1 Mean arterial pressure (MAP; mmHg) and heart rate (HR; beats min^{-1}) in rabbits subjected to: (1) 30 min coronary artery (LAL) occlusion and 2 h reperfusion; (2), (3), (4) 45 min LAL occlusion and 2 h reperfusion; (5), (6) 60 min LAL occlusion and 2 h reperfusion; or (7), (8) 60 min LAL occlusion and 6 h reperfusion. Rabbits received either vehicle, FR 139317 at 0.2 mg kg^{-1} min^{-1} or FR 139317 at 0.6 mg kg^{-1} min^{-1}.

Group	*Treatment* (mg kg^{-1} min^{-1})		*Basal*	*End of occlusion*	*End of reperfusion*
(1)	Vehicle	MAP	61 ± 5	57 ± 5	63 ± 6
		HR	247 ± 14	240 ± 14	235 ± 12
(2)	Vehicle	MAP	60 ± 3	60 ± 3	58 ± 5
		HR	245 ± 12	229 ± 9	239 ± 8
(3)	FR 139317 (0.2)	MAP	62 ± 3	59 ± 2	53 ± 4
		HR	244 ± 8	232 ± 8	244 ± 6
(4)	FR 139317 (0.6)	MAP	58 ± 6	60 ± 4	64 ± 7
		HR	263 ± 24	248 ± 22	267 ± 11
(5)	Vehicle	MAP	55 ± 5	55 ± 4	53 ± 5
		HR	215 ± 7	213 ± 5	209 ± 2
(6)	FR 139317 (0.2)	MAP	68 ± 6	64 ± 5	61 ± 5
		HR	227 ± 6	236 ± 7	253 ± 6
(7)	Vehicle	MAP	64 ± 2	70 ± 2	72 ± 4
		HR	230 ± 6	219 ± 9	213 ± 10
(8)	FR 139317 (0.6)	MAP	76 ± 5	73 ± 6	71 ± 8
		HR	235 ± 11	226 ± 13	232 ± 11

Values are given as mean ± s.e.mean of 4–6 observations for each group.

Table 2 Area at risk (expressed as a percentage of left ventricle) and infarct size (expressed as a percentage of area at risk) in rabbits subjected to coronary artery (LAL) occlusion (30, 45 or 60 min) and reperfusion (2 or 6 h)

Group	Treatment ($mg\ kg^{-1}\ min^{-1}$)	Occlusion (min)	Reperfusion (h)	Area at risk (%)	Infarct (%)	n
(1)	Vehicle	30	2	30 ± 4	10 ± 1	4
(2)	Vehicle	45	2	42 ± 4	47 ± 6	5
(3)	FR 139317 (0.2)	45	2	44 ± 2	47 ± 6	5
(4)	FR 139317 (0.6)	45	2	46 ± 8	48 ± 5	4
(5)	Vehicle	60	2	34 ± 3	55 ± 7	5
(6)	FR 139317 (0.2)	60	2	33 ± 3	49 ± 7	6
(7)	Vehicle	60	6	35 ± 4	48 ± 3	5
(8)	FR 139317 (0.6)	60	6	39 ± 6	61 ± 6	4

Rabbits received either vehicle, FR 139317 at $0.2\ mg\ kg^{-1}\ min^{-1}$ preceded by a loading dose of $1.0\ mg\ kg^{-1}$ or FR 139317 at $0.6\ mg\ kg^{-1}\ min^{-1}$ preceded by a loading dose of $3.0\ mg\ kg^{-1}$. Values are given as mean ± s.e.mean of n observations.

Table 3 Peak elevation in ST-segment in rabbits subjected to: (1) 30 min coronary artery (LAL) occlusion and 2 h reperfusion; (2), (3), (4) 45 min LAL occlusion and 2 h reperfusion; (5), (6) 60 min LAL occlusion and 2 h reperfusion; or (7), (8) 60 min LAL occlusion and 6 h reperfusion

Group	Treatment ($mg\ kg^{-1}\ min^{-1}$)	ST elevation (mV)
(1)	Vehicle	0.28 ± 0.01
(2)	Vehicle	0.29 ± 0.03
(3)	FR 139317 (0.2)	0.24 ± 0.07
(4)	FR 139317 (0.6)	0.26 ± 0.02
(5)	Vehicle	0.24 ± 0.02
(6)	FR 139317 (0.2)	0.26 ± 0.02
(7)	Vehicle	0.36 ± 0.02
(8)	FR 139317 (0.6)	0.28 ± 0.01

Rabbits received either vehicle, FR 139317 at $0.2\ mg\ kg^{-1}\ min^{-1}$ preceded by a loading dose of $1.0\ mg\ kg^{-1}$ or FR 139317 at $0.6\ mg\ kg^{-1}\ min^{-1}$ preceded by a loading dose of $3.0\ mg\ kg^{-1}$. Values are given as mean ± s.e.mean of 4–6 observations for each group.

a loading dose of $3\ mg\ kg^{-1}$, i.v.; $n = 4$) had no significant effect on infarct size even in this longer reperfusion model (61 ± 6%, Table 2).

Electrocardiogram changes: basal values for the ST-segment in the lead II ECG ranged from 0.004 ± 0.01 mV to 0.03 ± 0.01 mV. In different vehicle-treated control groups, LAL occlusion elicited a peak rise in the ST-segment ranging from 0.24 mV ± 0.07 mV to 0.36 ± 0.02 mV which remained elevated throughout the occlusion period (Table 3). Upon reperfusion, the ST-segment gradually returned to almost basal levels, with end values of 0.01 ± 0.01 mV to 0.05 ± 0.02 mV. FR 139317 ($0.2\ mg\ kg^{-1}\ min^{-1}$ preceded by a loading dose of $1.0\ mg\ kg^{-1}$ or $0.6\ mg\ kg^{-1}\ min^{-1}$ preceded by a loading dose of $3\ mg\ kg^{-1}$, i.v.) had no effect on the ischaemia-induced ST-segment elevation in any of the models studied (Table 3).

Discussion

Here we demonstrate that the ET_A receptor antagonist, FR 139317, attenuates the coronary vasoconstrictor effects elicited by ET-1 in the isolated perfused heart of the rabbit. However, FR 139317, in doses which attenuate by 83–89% the ET_A-mediated pressor effects of exogenous ET-1 ($1\ nmol\ kg^{-1}$) in the anaesthetized rabbit (McMurdo *et al.*, 1993a), has no effect on infarct size and ST-segment elevation in three different models of myocardial ischaemia and reperfusion in the rabbit. This is in contrast to previous studies which suggested that an enhanced formation of endogenous ET-1 contributes to ischaemia/reperfusion injury of the heart. Thus, an antibody to ET-1 (Watanabe *et al.*, 1991) or the endothelin-converting enzyme inhibitor, phosphoramidon (Grover *et al.*, 1992) reduce infarct size in models of coronary artery occlusion and reperfusion in the anaesthetized rat. Furthermore, pretreatment of rabbits with an antibody to ET-1 reduced infarct size caused by 30 min occlusion of the first branch of the left circumflex coronary artery followed by 24 h of reperfusion from 61 ± 5% (control) to 37 ± 5% (treatment, $n = 5$). In this study, the areas at risk in control and treated animals were 43 ± 5% and 44 ± 3%, respectively (Kusumoto *et al.*, 1993).

Why then, does the ET_A receptor antagonist, FR 139317, not reduce infarct size in this study? Clearly, FR 139317 ($0.2\ mg\ kg^{-1}\ min^{-1}$ preceded by a loading dose of $1.0\ mg\ kg^{-1}$) had no effect on infarct size caused by 60 min of LAL occlusion and 2 h of reperfusion. Considering that collateral blood supply to the ischaemic myocardium after complete coronary artery occlusion is minimal in the rabbit heart (Maxwell *et al.*, 1987), one could argue that the degree of the ischaemic insult caused by 60 min of LAL occlusion was too severe to allow therapeutic intervention. However, drugs such as the prostacyclin analogue, iloprost (Chiariello *et al.*, 1988), the fibrinolytic compound, defibrotide (Thiemermann *et al.*, 1989) and the anti-platelet agent, cloricromene (Lidbury *et al.*, 1993) elicit a substantial reduction in infarct size in this model. Moreover, FR 139317 ($0.2\ mg\ kg^{-1}\ min^{-1}$ preceded by a loading dose of $1.0\ mg\ kg^{-1}$) had no effect on infarct size even when the duration of coronary artery occlusion was reduced to 45 min. Investigation of the effects of FR 139317 on the ischaemic injury caused by shorter periods of LAL occlusion was not feasible, for 30 min of LAL occlusion caused only a very small infarction (10 ± 1% of area at risk). One could also argue that the dose of FR 139317 used in this study was too low to antagonize the effects of endogenous ET-1 in the coronary vasculature of the rabbit. However, this is unlikely, for even a three times higher dose of FR 139317 ($0.6\ mg\ kg^{-1}\ min^{-1}$ preceded by a loading dose of $3.0\ mg\ kg^{-1}$) did not reduce the infarct size caused by 45 min of LAL occlusion followed by 2 h of reperfusion. Assuming a blood volume of $60–70\ ml\ kg^{-1}$ the dose of FR 139317 used should result in plasma levels of more than 100 μM at 10 min after the start of the continuous infusion of FR 139317. Clearly this concentration of the antagonist is sufficient to block the vasoconstrictor effects caused by ET-1 in the coronary vasculature of the rabbit (this study).

In anaesthetized rabbits subjected to coronary artery occlusion (30 min) and reperfusion (24 h), plasma levels of ET-1 are maximally elevated (approximately 2.5 fold) at 3 h after reperfusion (Kusumoto *et al.*, 1993). Thus, ET-1 may play a

pathophysiological role after longer periods of reperfusion and hence, 2 h of reperfusion may not have been long enough to see a potential beneficial action of FR 139317. Therefore, we also investigated the effects of FR 139317 ($0.6\ mg\ kg^{-1}\ min^{-1}$ preceded by a loading dose of $3.0\ mg\ kg^{-1}$) in rabbits in which the LAL was occluded for 60 min and subsequently reperfused for 6 h. However, even when the ischaemic insult was followed by this prolonged reperfusion period, FR 139317 did not reduce infarct size. This suggests that if endogenous ET-1 does play a pathophysiological role in the extension of myocardial ischaemia/reperfusion injury, then it does so between 6 and 24 h of reperfusion.

Interestingly, 9 out of 48 rabbits died within 5–20 min of myocardial ischaemia due to ventricular fibrillation. Of these 9 animals, 7 were treated with vehicle and 2 were treated with FR 139317. This result indicates that inhibition of ET_A receptors with FR 139317 reduces the incidence of ventricular fibrillation and, hence, further studies are necessary to elucidate the role of ET-1 in the pathogenesis of ischaemia-induced arrhythmias.

Ischaemia/reperfusion of the rat heart causes an increase in ET-1 receptor binding sites (Liu *et al.*, 1990). We have shown here that under non-ischaemic conditions, ET_A receptors mediate the rise in CPP elicited by ET-1 in the isolated perfused heart of the rabbit. However, it is unknown which endothelin receptor subtype is upregulated following ischaemia-reperfusion injury of the heart. If other (non-ET_A) receptors are increased, then this would explain why FR 139317 is ineffective in reducing myocardial infarct size. It would be interesting to examine the effects of an ET_B or non-selective receptor antagonist in this model of coronary artery occlusion and reperfusion.

Thus, we demonstrate here that endogenous ET-1 does not play a major role in the extension of infarct size in these models of LAL occlusion (45 or 60 min) and reperfusion (2 or 6 h) in the anaesthetized rabbit.

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