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Effects of the ET_A/ET_B receptor antagonist, bosentan on endothelin-1-induced myocardial ischaemia and oedema in the rat

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1 The purposes of this study were to assess the role of ET_B receptors in mediating endothelin-1 (ET-1)-induced myocardial ischaemia and oedema in rats and to study the inhibitory action of the novel non-peptide ET_A/ET_B receptor antagonist, bosentan on these actions of ET-1.

2 Intravenous bolus injection of ET-1 (1 nmol kg^{-1}) into anaesthetized rats produced marked ST segment elevation of the electrocardiogram without causing arrhythmias. ST segment elevation developed within 30–50 s and persisted for at least 30 min following injection of the peptide.

3 Pretreatment of the animals with bosentan (10 mg kg^{-1}, i.v.) inhibited on average by 96% the ST segment elevation elicited by ET-1 (1 nmol kg^{-1}) compared to the 82% inhibition observed with the ET_A receptor-selective antagonist, FR 139317 (2.5 mg kg^{-1}, i.v.).

4 Bolus injection of ET-1 (1 nmol kg^{-1}, i.v.) to conscious chronically catheterized rats evoked a transient depressor response followed by a prolonged pressor effect. Corresponding to changes in blood pressure, a transient tachycardia and a sustained bradycardia were observed. ET-1 (1 nmol kg^{-1}) enhanced albumin extravasation by 119 and 93% in the left ventricle and right atrium, respectively, as measured by the local extravascular accumulation of Evans blue dye.

5 Pretreatment of the animals with bosentan (10 mg kg^{-1}) inhibited by 71 and 90% the depressor and pressor actions of ET-1 (1 nmol kg^{-1}) and the accompanying tachycardia and bradycardia, respectively. FR 139317 (2.5 mg kg^{-1}) attenuated the pressor response to ET-1 and accompanying bradycardia by 75%, without affecting the depressor action and accompanying tachycardia. ET-1-induced albumin extravasation was completely inhibited by bosentan (10 mg kg^{-1}) both in the left ventricle and right atrium, compared to the 86% inhibition observed with FR 139317 (2.5 mg kg^{-1}).

6 Like ET-1, the ET_B receptor-selective agonist, IRL 1620 (0.3 and 1 nmol kg^{-1}, i.v.) also produced dose-dependent ST segment elevation in anaesthetized rats and enhanced albumin extravasation (up to 141% of control) in the left ventricle and right atrium, respectively, in conscious rats. These effects of IRL 1620 were completely prevented by bosentan (10 mg kg^{-1}).

7 These results indicate that ET_B receptors, albeit to a lesser extent than ET_A receptors, are also involved in mediating ET-1-induced myocardial ischaemia and oedema in the rat, and suggest the therapeutic potential for bosentan in the treatment of ischaemic myocardial diseases.

Keywords: Endothelin; ET_A and ET_B receptors; bosentan; IRL 1620; myocardial ischaemia; vascular permeability; rat heart

Introduction

Several lines of evidence suggest a role for endothelin-1 (ET-1) in the pathogenesis of myocardial ischaemia. Elevated plasma ET-1 levels have been reported in the coronary circulation under ischaemic and hypoxic conditions both in patients and laboratory animals (for recent review see Rubanyi & Polokoff, 1994). ET-1 is a potent constrictor of coronary arteries *in vivo* and *in vitro* and is thought to have direct myocardial effects such as positive inotropy (see Rubanyi & Polokoff, 1994). Furthermore, intracoronary or intravenous administration of ET-1 evokes ST segment elevations of the electrocardiogram, similar to the clinical phenomenon of Prinzmetal angina (Harada *et al.*, 1993; Filep *et al.*, 1994b) and enhances myocardial oedema formation in the rat (Filep *et al.*, 1992; 1994b). In addition, anti-ET-1 antibodies were found to protect ischaemic rat hearts (Watanabe *et al.*, 1991).

The ET receptor subtypes, ET_A (which is highly selective for ET-1) and ET_B (non-isopeptide selective) are expressed in cardiac tissues (Arai *et al.*, 1990; Sakurai *et al.*, 1990; Molenaar *et al.*, 1993; Davenport *et al.*, 1995) and the existence of a third, non ET_A/ET_B receptor subtype in the pig coronary circulation has also been reported (Harrison *et al.*, 1992). Numerous *in vitro* studies and experiments using isolated perfused hearts suggested that all of these three receptor subtypes may mediate the vasoconstrictor action of ET-1 (see Rubanyi & Polokoff, 1994). Our previous findings that the ET_A receptor-selective antagonists, BQ-123 and FR 139317 effectively, though not completely, attenuated ET-1-induced ischaemia and oedema in the rat coronary circulation (Filep *et al.*, 1992; 1994b) indicated a role for ET receptor subtypes other than ET_A in these events. The aim of the present experiments was to assess the involvement of ET_B receptors by comparing the inhibitory effects of the novel ET_A/ET_B receptor antagonist, bosentan (Clozel *et al.*, 1994) to those of the ET_A receptor-selective antagonist, FR 139317 (Aramori *et al.*, 1993; Sogabe *et al.*, 1993) on myocardial ischaemia and oedema elicited by ET-1 and by examining the cardiac effects of IRL 1620, an ET_B receptor-selective agonist (Takai *et al.*, 1992).

Methods

Vascular permeability measurements

The experiments were performed on conscious, chronically catheterized male Wistar rats weighing 200–240 g. The animals were housed in individual metabolic cages and catheters were

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implanted into the abdominal aorta and vena cava as described previously (Filep & Fejes-Tóth, 1986). During the experiments the animals could move freely and had free access to food and water. Mean arterial blood pressure (MABP) and heart rate were monitored continuously by a blood pressure analyzer (Micro-Med, Louisville, KY, U.S.A.) using a COBE CDX III pressure transducer.

On the day of the experiment, following an equilibration period of 1 h, basal cardiovascular parameters were measured for 20 min before drug administration. To measure protein extravasation, Evans blue dye (20 mg kg^{-1}) which binds to plasma albumin, was injected i.v. together with ET-1 (1 nmol kg^{-1}). Previous experiments showed that at this dose, ET-1 markedly enhances albumin extravasation in the rat coronary circulation (Filep *et al.*, 1992). The animals were pretreated with bosentan (10 mg kg^{-1}, i.v.) or FR 139317 (2.5 mg kg^{-1}, i.v.) for 10 and 5 min, respectively, before injection of ET-1. Our previous studies have demonstrated that bosentan and FR 139317 at the doses employed caused maximum inhibition of the depressor and/or pressor actions of ET-1 in the conscious rat (Filep *et al.*, 1994a). An additional group of rats were given the selective ET_B receptor agonist, IRL 1620 (0.3 or 1 nmol kg^{-1}, i.v.) in the absence and presence of bosentan (10 mg kg^{-1}). Ten min after injection of ET-1 or IRL 1620, the animals were anaesthetized with sodium pentobarbitone (50 mg kg^{-1}, i.v.) and the heart was perfused with 40 ml 0.9% NaCl through a catheter inserted in the abdominal aorta. Portions of the anterior wall of the left ventricle and right atrium were then excised and tissue Evans blue dye content was measured by spectrophotometry following extraction by formamide as described previously (Filep *et al.*, 1992).

Electrocardiogram measurements

Male Wistar rats (200–240 g) were anaesthetized with sodium pentobarbitone (50 mg kg^{-1}, i.p.). Catheters were inserted into the left femoral artery and vein and electrodes were placed on the left and right forlegs and left hindleg. The change in ST segment of the lead II electrocardiogram (ECG) was used to monitor coronary ischaemia. Following control cardiovascular and ECG measurements, the animals were pretreated with bosentan (10 mg kg^{-1}, i.v.), FR 139317 (2.5 mg kg^{-1}, i.v.) or their vehicle before injection of ET-1 or IRL 1620 (0.3 or 1 nmol kg^{-1}) as described above. The animals were monitored for 30 min following the injection of ET-1. Each animal received only one injection of ET-1 or IRL 1620 and one type of pretreatment. Lead II ECG was recorded with a Siemens Sirecust 341 electrocardiograph (Germany).

All procedures were in accordance with the Guidelines of the Canadian Council of Animal Care and were approved by the local Animal Care Committee.

Drugs and chemicals

ET-1 and IRL 1620 (Suc-[Glu^9, $Ala^{11,15}$]endothelin-1(18–21)) were synthesized in our laboratories by solid-phase methodology. The purity of the preparations were greater than 97% as measured by high performance liquid chromatography. ET-1 and IRL 1620 were dissolved in distilled water and stored at –20°C. On the day of the experiments an aliquot was removed and diluted further in 0.9% NaCl. Bosentan (Ro 47-0203, 4-*tert*-butyl-N-[6-(2 hydroxy-ethoxy)-5-(2-methoxy-phenoxy)-2,2'bipyrimidin-4-yl]-benzene-sulphonamide, Hoffman-La Roche Ltd., Basel, Switzerland) and FR 139317 ((**R**)2-[(**R**)-2-[(**S**)-2-[[1-(hexahydro-1*H*-azepinyl)]-carbonyl]amino-4-methyl-pentanoyl]amino-3-(2-pyridyl)propionic acid, Fujisawa Pharmaceutical Co., Osaka, Japan) were dissolved in distilled water containing 300 mM glucose and were freshly prepared each day.

Statistical analysis

Results are expressed as means ± s.e.mean. Statistical analysis of the data was performed by one-way analysis of variance using ranks (Kruskal-Wallis test) followed by Dunn's multiple contrast hypothesis test when various treatments were compared to the same control group, or by the Mann-Whitney U test for unpaired observation. A level of $P<0.05$ was considered significant for all tests.

Results

Baseline values for MABP and heart rate were significantly lower in conscious (108 ± 1 mmHg and 331 ± 4 beats min^{-1}, respectively, $n=43$) than in anaesthetized rats (126 ± 1 mmHg and 431 ± 5 beats min^{-1}, respectively, $n=44$, $P<0.001$).

Effects of bosentan and FR 139317 on ET-1 and IRL 1620-induced changes in blood pressure and heart rate

As well established, ET-1 (1 nmol kg^{-1}) produced a sustained pressor effect preceded by a transient depressor action in both conscious and anaesthetized animals (Table 1). However, the peak depressor response to ET-1 was more pronounced in anaesthetized than in conscious rats, whereas the peak pressor

Table 1 Effects of bosentan and FR 139317 on endothelin-1 (ET-1) and IRL 1620-induced maximum changes in mean arterial blood pressure (MABP) and accompanying changes in heart rate in conscious and anaesthetized rats

		Basal		*Maximum depressor response*		*Maximum pressor response*	
		MABP (mmHg)	*Heart rate* (beats min^{-1})	*ΔMABP* (mmHg)	*ΔHeart rate* (beats min^{-1})	*ΔMABP* (mmHg)	*ΔHeart rate* (beats min^{-1})
Conscious rats							
ET-1	6	110 ± 4	338 ± 12	–17 ± 2	2 ± 5	44 ± 3	–40 ± 11
Bosentan + ET-1	4	107 ± 3	353 ± 15	–5 ± 1*	2 ± 4	5 ± 1**	–5 ± 2**
FR 139317 + ET-1	4	109 ± 4	330 ± 11	–18 ± 3	2 ± 4	10 ± 1*	–14 ± 5*
IRL 1620	4	111 ± 3	330 ± 16	–11 ± 2	3 ± 2	17 ± 3	–19 ± 7
Bosentan + IRL 1620	4	111 ± 2	317 ± 8	–2 ± 1#	2 ± 2	2 ± 1##	–3 ± 2#
FR 139317 + IRL 1620	4	110 ± 3	346 ± 16	–9 ± 1	3 ± 3	20 ± 1	–20 ± 3
Anaesthetized rats							
ET-1	6	125 ± 2	439 ± 12	–31 ± 3	8 ± 1	28 ± 2	–22 ± 5
Bosentan + ET-1	4	124 ± 5	411 ± 28	–8 ± 3*	4 ± 2	3 ± 1*	–1 ± 2*
FR 139317 + ET-1	4	124 ± 3	441 ± 18	–30 ± 3	10 ± 2	7 ± 2*	–8 ± 2*
IRL 1620	4	127 ± 3	434 ± 15	–18 ± 3	6 ± 2	7 ± 2	–6 ± 2
Bosentan + IRL 1620	4	127 ± 2	415 ± 9	–1 ± 1#	1 ± 1	1 ± 1#	0 ± 2
FR 139317 + IRL 1620	4	126 ± 3	435 ± 22	–19 ± 2	5 ± 2	8 ± 3	–4 ± 2

Following control measurements, the animals were pretreated with bosentan (10 mg kg^{-1}, i.v.) or FR 139317 (2.5 mg kg^{-1}, i.v.) for 10 min before injection of ET-1 (1 nmol kg^{-1}, i.v.) or IRL 1620 (1 nmol kg^{-1}, i.v.). The values are means ± s.e.mean for n experiments. *$P<0.05$, **$P<0.01$ (compared to ET-1), #$P<0.05$, ##$P<0.01$ (compared to IRL 1620).

response to ET-1 was significantly greater in conscious than in anaesthetized animals (Table 1). Similar changes were observed with IRL 1620 (1 nmol kg^{-1}) (Table 1). Corresponding to the changes in MABP, the depressor response was accompanied by a transient tachycardia, whereas prolonged increases in MABP were associated with marked decreases in heart rate (Table 1). Neither bosentan (10 mg kg^{-1}) nor FR 139317 (2.5 mg kg^{-1}) by itself affected significantly MABP and heart rate. Bosentan inhibited on average by 71 and 74% the depressor response to 1 nmol kg^{-1} ET-1 in conscious and anaesthetized rats, respectively, and attenuated by about 90% the pressor response and concomitant bradycardia both in conscious and anaesthetized animals. FR 139317 did not modify the depressor action of ET-1, whereas it attenuated by 75–77% ET-1-induced increase in MABP and concomitant bradycardia (Table 1). Bosentan, but not FR 139317, attenuated by about 90–95% IRL 1620 (1 nmol kg^{-1})-induced changes in MABP and heart rate (Table 1).

Effects of bosentan and FR 139317 on ET-1-induced albumin extravasation

In agreement with our previous observations, in the conscious rat, ET-1 at 1 nmol kg^{-1} increased albumin accumulation in the left ventricle and right atrium on average by 119 and 93%, respectively (Figure 1). Pretreatment of the animals with FR 139317 (2.5 mg kg^{-1}) reduced ET-1-induced albumin extravasation in these tissues by 86% (Figure 1), whereas no increases in tissue Evans blue content could be detected in bosentan (10 mg kg^{-1})-treated animals in response to ET-1 (Figure 1). None of the antagonists alone affected the tissue content of Evans blue dye (data not shown).

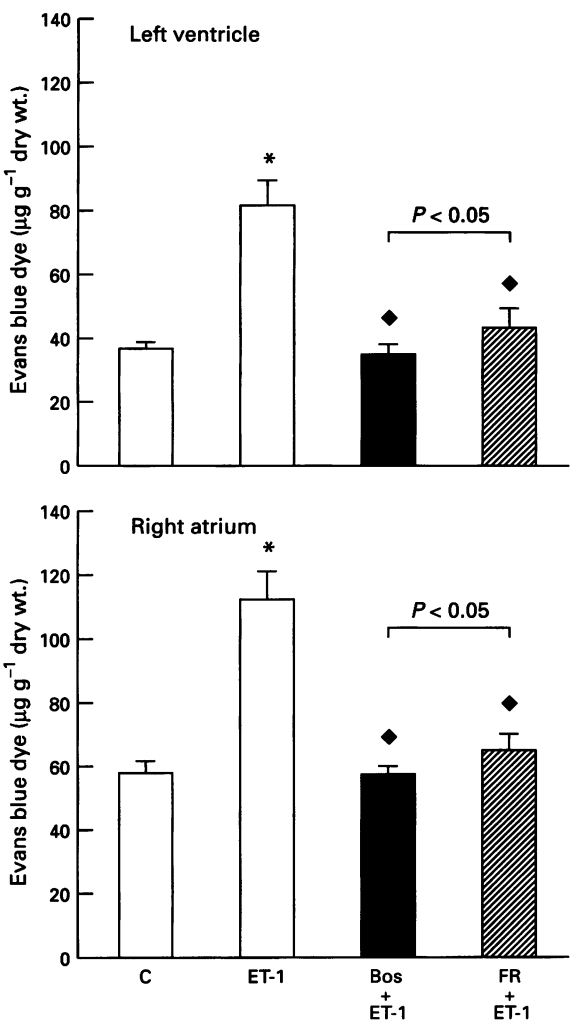

Figure 1 Effects of bosentan and FR 139317 on endothelin-1 (ET-1)-induced albumin extravasation in the coronary circulation of conscious rats. The animals were pretreated with vehicle (C, control), bosentan (Bos, 10 mg kg^{-1}, i.v.) or FR 139317 (FR, 2.5 mg kg^{-1}, i.v.) for 10 min before i.v. bolus injection of ET-1 (1 nmol kg^{-1}) or their vehicle (control, C) plus Evans blue dye (20 mg kg^{-1}). Ten minutes later, the rats were anaesthetized and the heart was perfused with 0.9% NaCl. The permeability measurements were made 15 min after the injection of the dye. Values are mean with s.e.mean, $n=6$ for control and ET-1, and $n=4$ for all other treatments. $^*P<0.05$ (compared to control by Dunn's multiple contrast hypothesis test); ◆$P<0.05$ (compared to ET-1).

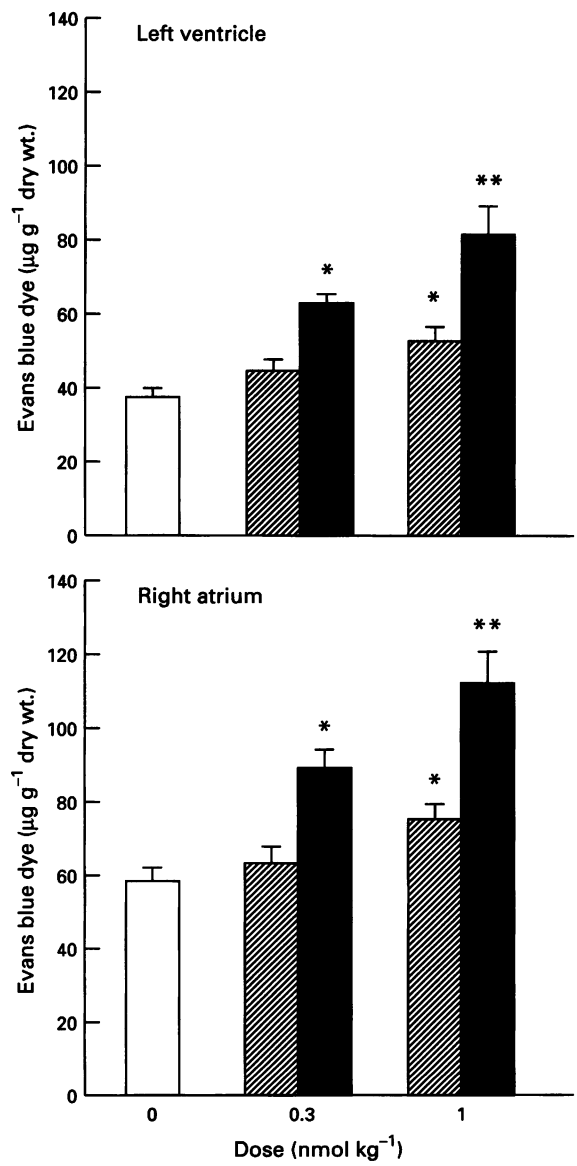

Figure 2 Comparison of the effects of IRL 1620 and endothelin-1 (ET-1) on albumin extravasation in the coronary circulation of conscious rats. The animals received a bolus i.v. injection of vehicle (open column), IRL 1620 (hatched columns) or ET-1 (solid columns) plus Evans blue dye (20 mg kg^{-1}). Ten min later, the rats were anaesthetized and the heart was perfused with 0.9% NaCl. The permeability measurements were made 15 min after the injection of the dye. Values are means with s.e.mean, $n=4$ for IRL 1620, 0.3 and 1 nmol kg^{-1}, $n=5$ for ET-1, 0.3 nmol kg^{-1} and $n=6$ for all other treatments. $^*P<0.05$; $^{**}P<0.01$ (compared to vehicle).

Effects of bosentan on IRL 1620-induced protein extravasation

Bolus i.v. injection of IRL 1620 increased tissue albumin accumulation in a dose-dependent manner, although on a molar basis, it appeared to be about 4 times less potent than ET-1 (Figure 2). For instance, at 1 nmol kg^{-1}, IRL 1620 evoked on average 29 and 41% increases in albumin extravasation in the right atrium and left ventricle, respectively (Figure 2). These actions of IRL 1620 were completely prevented by bosentan (10 mg kg^{-1}) in both vascular beds studied. Evans blue dye content was 58 ± 4 μg g^{-1} dry tissue weight ($n=6$) and 59 ± 3 μg g^{-1} dry tissue weight ($n=4$) in the right atrium of control and bosentan plus IRL 1620-treated animals, respectively ($P>0.1$), and 37 ± 2 μg g^{-1} dry tissue weight and 36 ± 3 μg g^{-1} dry tissue weight in the left ventricle of control and bosentan plus IRL 1620-treated animals, respectively ($P>0.1$).

Effects of bosentan and FR 139317 on ET-1-induced ST segment elevation

Bolus i.v. injections of ET-1 (1 nmol kg^{-1}) to anaesthetized rats produced ST segment elevation within 30–50 s. No complete recovery to control levels was observed within 30 min following injection of ET-1. At the dose employed, ET-1 did not produce arrhythmias. Bosentan (10 mg kg^{-1}) attenuated the ST segment elevation evoked by ET-1 (1 nmol kg^{-1}) by 96%, compared to the 82% inhibition observed in animals pretreated with FR 139317 (2.5 mg kg^{-1}) ($P<0.05$) (Figure 3). None of the ET receptor antagonists alone caused significant changes in ST segment (data not shown).

Effects of bosentan on IRL 1620-induced ST segment elevation

Bolus i.v. injections of the ET_B receptor-selective agonist, IRL 1620 also evoked ST segment elevation in a dose-dependent fashion (Figure 4). ST segment elevations were observed within 50–90 s and persisted for 10–15 min. On a molar basis, IRL 1620 appeared to be about 3.6 times less potent than ET-1 (Figure 4). IRL 1620 (1 nmol kg^{-1})-induced ST segment elevation was almost completely prevented by bosentan (10 mg kg^{-1}) (0.003 ± 0.001 mV, $n=4$, $P>0.05$ compared to vehicle).

Discussion

The present results showed that in addition to ET_A receptors, ET_B receptors are also involved in mediating ET-1-induced ST segment elevation and myocardial oedema in the rat.

Since the ECG measurements were performed in anaesthetized animals, we compared the effects of bosentan and FR 139317 on the haemodynamic effects of ET-1 in conscious and anaesthetized rats. Despite the differences in the magnitude of ET-1-induced changes in MABP and heart rate in anaesthetized and conscious rats, these antagonists produced similar inhibition. In confirmation of earlier observations, the present study also shows that both ET_A and ET_B receptors mediate the pressor action of ET-1 in both conscious (Ihara *et al.*, 1992; Filep *et al.*, 1992; 1994a; Webb *et al.*, 1992; Sogabe *et al.*, 1993) and anaesthetized rats (Williams *et al.*, 1991; Clozel *et al.*, 1992; McMurdo *et al.*, 1993). This conclusion is supported by the findings that the maximum inhibition of the ET-1 pressor effect that could be achieved with bosentan was significantly greater than that observed with FR 139317, and that the ET_B receptor-selective agonist IRL 1620 can also evoke pressor responses. However, a third, non-ET_A, non-ET_B receptor may also be involved in the generation of the pressor response as ET-1 produced a small, 2–9 and 1–5 mmHg increases in MABP in conscious and anaesthetized animals pretreated with bosentan. Whether this receptor is similar to the ET-3 selective (termed ET_C) receptor cloned from *Xenopus* melanophores (Karne *et al.*, 1993) or the receptor described in the pig coronary circulation (Harrison *et al.*, 1992) remains to be investigated. The transient depressor action of ET-1 was unaffected by FR 139317, whereas it was markedly inhibited by bosentan. The observations that the maximal inhibition

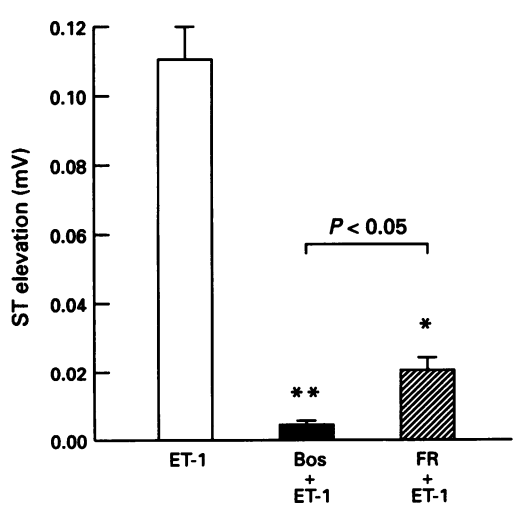

Figure 3 Effects of bosentan and FR 139317 on ST segment elevation elicited by i.v. administration of endothelin-1 (ET-1 1 nmol kg^{-1}) in anaesthetized rats. The animals were pretreated with vehicle, bosentan (Bos, 10 mg kg^{-1}) or FR 139317 (FR, 2.5 mg kg^{-1}) for 10 min before injection of ET-1 (1 nmol kg^{-1}). Values are means with s.e.mean, $n=6$ for ET-1, $n=4$ for all other treatments. $^*P<0.05$; $^*P<0.01$ (compared to ET-1 by Dunn's multiple contrast hypothesis test).

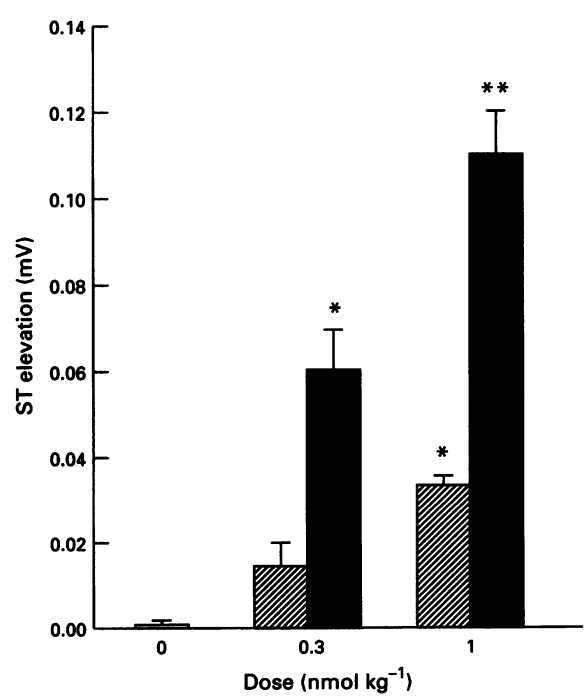

Figure 4 ST segment elevations elicited by intravenous administration of IRL 1620 (hatched columns) or endothelin-1 (ET-1, solid columns) in anaesthetized rats. Values are means with s.e.mean, $n=3$ for IRL 1620, 0.3 nmol kg^{-1}, $n=6$ for ET-1, 1 nmol kg^{-1} and $n=4$ for all other treatments. $^*P<0.05$, $^{**}P<0.01$ (compared to vehicle by Dunn's multiple contrast hypothesis test).

that can be achieved with bosentan did not exceed 80%, and the maximum decrease in MABP that can be achieved with IRL 1620 was about 50% of the apparent peak response to ET-1 (Filep *et al.*, 1994a) would suggest that a component of the ET-1 depressor response is either not mediated through ET_B receptors or is mediated via a subtype of ET_B receptor, which cannot be blocked with bosentan. However, this latter explanation is unlikely as bosentan antagonizes both known ET_B receptor subtypes (i.e. which mediate vasoconstriction and endothelium-dependent relaxation) with similar K_i values (Clozel *et al.*, 1994).

The depressor and pressor actions of ET-1 and IRL 1620 were accompanied by a transient increase and prolonged decrease in heart rate. Attenuation of the ET-1 depressor or pressor responses by endothelin receptor antagonists resulted in amelioration of the accompanying tachycardia or bradycardia, respectively, in both anaesthetized and conscious rats. These findings would suggest that changes in heart rate were secondary to changes in MABP.

Previous studies demonstrated that both intracoronary and intravenous administration of ET-1 elevates coronary resistance and induces ST segment elevations in rats, whereas arrhythmias could only be observed following intracoronary administration of the peptide (Harada *et al.*, 1993; Filep *et al.*, 1994b). It should be noted that following i.v. injection of 1 nmol kg^{-1} ET-1, the peak plasma concentration of the peptide in the coronary circulation might be about 50 fold lower than those levels that might have been achieved by intracoronary injection of 7 nmol kg^{-1} ET-1 in rats (Harada *et al.*, 1993). However, the peak plasma levels of ET-1 following i.v. administration of the peptide are still at least two orders of magnitude higher than those detected in various myocardial ischaemia models (see Rubanyi & Polokoff, 1994). It is possible that plasma ET-1 levels do not reflect local production or concentration of the peptide. The pro-ischaemic and arrhythmogenic actions of ET-1 are thought be be mediated via different mechanisms. Indeed, the ST segment elevation is possibly due to myocardial ischaemia related to coronary vasoconstriction, whereas a direct action of ET-1 on the myocardium has been implicated in inducing arrhythmias (Harada *et al.*, 1993). Therefore, the absence of arrhythmias in our experiments would indicate that ET-1 and IRL 1620 acted primarily on the coronary vascular smooth muscle rather than on the myocardium. We have previously reported that ET_A receptor blockade markedly, though not completely, protected the heart from ST segment elevation. The present results provide two lines of evidence that ET_B receptors are also involved in mediating the pro-ischaemic action of ET-1. Firstly, the non-selective ET_A/ET_B receptor antagonist, bosentan, completely prevented the ST segment elevation evoked by ET-1, compared to the 82% inhibition observed with FR 139317. Secondly, the ET_B receptor-selective agonist, IRL 1620, also induced ST segment elevations, albeit, on a molar basis, it appeared to be less potent than ET-1.

In addition to evoking ST segment elevation, ET-1 can also promote albumin extravasation in the coronary vascular bed of conscious rats (Filep *et al.*, 1992; 1994b). These studies have demonstrated that the permeability enhancing action of ET-1 is mediated predominantly via ET_A receptors both in the left ventricle and right atrium. In the present experiments, bosentan was a more potent inhibitor of the permeability effect of ET-1 than FR 139317, indicating the involvement of ET_B receptors. Additional evidence supporting a role for ET_B receptors is derived from the experiments with IRL 1620. IRL 1620 also produced significant increases in protein extravasation in both vascular beds studied, albeit, as with inducing ST segment elevation, it was less potent than ET-1.

Although in the present study parallel changes were observed in MABP and albumin extravasation, previous results from our laboratories have demonstrated that an increase in albumin extravasation elicited by ET-1 is not simply a consequence of changes in MABP (Filep *et al.*, 1994a), but, as with other mediators, it can primarily be attributed to formation of interendothelial cell gaps exclusively in the venules (Grega *et al.*, 1986). ET-1 may induce gap formation directly and/or through release of secondary mediators, such as thromboxane A_2 or platelet-activating factor (Filep *et al.*, 1994b). A direct gap forming effect of endothelin-1 is most likely mediated through activation of ET_B receptors, since it appears that the ET_B type of endothelin receptor is predominantly expressed on endothelial cells derived from peripheral large vessels (Takayanagi *et al.*, 1991). The findings that IRL 1620 is capable of enhancing albumin extravasation in the coronary (present study) and other vascular beds (Filep *et al.*, 1994a) lend further support to this notion. However, IRL 1620 did not mimic completely the permeability enhancing effect of ET-1, indicating that ET-1-induced albumin extravasation is only partially mediated by activation of ET_B receptors. Recent studies demonstrated the presence of ET_A receptors on endothelial cells prepared from rat and human brain microvessels (Vigne *et al.*, 1990; Stanimirovic *et al.*, 1994). These observations raise the possibility that stimulation of endothelial ET_A receptors might also lead directly to gap formation. However, it is not known at present whether coronary microvascular endothelial cells possess ET_A receptors. Alternatively, ET-1 may induce gap formation through release of thromboxane A_2 and platelet-activating factor via the activation of ET_A receptors as indicated by the similar degree of inhibition of ET-1-induced albumin extravasation in the rat coronary circulation following ET_A, thromboxane A_2 or platelet-activating factor receptor blockade (Filep *et al.*, 1994b). An increase in systemic blood pressure and consequently in capillary hydrostatic pressure would facilitate albumin extravasation only when gaps are formed (Grega *et al.*, 1986). Activation of ET_B receptors may lead to elevation of capillary hydrostatic pressure since ET-1 is a more potent constrictor of venous then arterial vessels (Yang *et al.*, 1989; Warner, 1990) and ET_A-like contractile receptors predominate on arterial and ET_B-like contractile receptors on venous smooth muscle (Moreland *et al.*, 1992). Thus, attenuation of ET-1-induced coronary vasoconstriction by bosentan or to a lesser extent by FR 139317 would decrease hydrostatic pressure in the coronary vascular bed, which, in turn, could contribute to the decrease in albumin extravasation.

In conclusion, the present study has demonstrated that in addition to ET_A receptors, ET_B receptors are also involved in mediating the pro-ischaemic and microvascular permeability enhancing effects of endothelin-1 in the rat coronary circulation. These findings also suggest that a non-selective ET_A/ET_B receptor antagonist, such as bosentan may have a greater therapeutic potential than ET_A receptor-selective antagonists in the treatment of acute ischaemic heart diseases.

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