British Journal of Pharmacology (1997) 122, 993–998

Subtypes of endothelin receptors that mediate venous effects of endothelin-1 in anaesthetized rats

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1 The subtypes of endothelin receptors that mediate the effects of endothelin-1 (ET-1) on mean arterial pressure (MAP), heart rate (HR), mean circulatory filling pressure (MCFP), arterial resistance (R_A), cardiac output (CO) and venous resistance (R_V) were characterized in 9 groups of pentobarbitone-anaesthetized rats via the injection of ET-1 in the absence and presence of bosentan (Ro 47-0203, ET_A- and ET_B-receptor antagonist), PD 142893 (ET_A- and ET_B-receptor antagonist) or FR 139317 (ET_A-receptor antagonist), as well as injection of the ET_B-receptor agonist, IRL 1620.

2 Cumulative i.v. bolus injections of ET-1 or IRL 1620 (0.5, 1 and 2 nmol kg^{-1}) dose-dependently increased MAP (ET: by 22, 34 and 44; IRL: 8, 17 and 28 mmHg), R_A (ET: 62, 108 and 162; IRL: 51, 63 and 86% over baseline), R_V (ET: 70, 132 and 179; IRL: 81, 89 and 98% over baseline) and MCFP (ET: 1.1, 1.8 and 1.9; IRL: 0.9, 1.0 and 1.2 mmHg) and reduced CO (ET: −18, −35 and −44; IRL: −24; −26; −25% below baseline). Equimolar doses of ET-1 and IRL 1620 caused similar initial transient depressor responses. Saline did not modify any haemodynamic variables in the time-control group.

3 Bosentan (10 mg kg^{-1}, i.v.) inhibited ET-induced increases in MAP, R_V, R_A and MCFP and decrease in CO. PD 142893 (22 mg kg^{-1}, i.v.) abolished ET-induced changes on MAP, R_V, R_A and CO, but did not alter effects on MCFP. Bosentan alone did not cause haemodynamic changes, but PD 142893 alone elevated MCFP (0.9 ± 0.3 mmHg at 1 h after injection) and did not alter other variables. Both antagonists abolished the initial depressor effects of ET-1.

4 FR 139317 (1 mg kg^{-1}, i.v.) partially inhibited the increases in MAP, R_V, R_A and MCFP and decreases in CO elicited by ET-1, but did not alter the transient depressor response of ET-1.

5 The results show that both ET_A- and ET_B-receptors mediate the arterial and venous constrictor effects of ET-1. Bosentan is more efficacious than PD 142893 in inhibiting the venous effects of ET-1.

Keywords: Endothelin-1 (ET-1); mean circulatory filling pressure (MCFP); cardiac output; venous resistance; body venous tone; endothelin receptor antagonist; PD 142893; bosentan (Ro 47-0203); FR 139317; ET_B-receptor agonist; IRL 1620

Introduction

Endothelin-1 (ET-1) is well-known to cause a transient depressor followed by a long-lasting pressor response via elevation of systemic arterial resistance (Clarke *et al*., 1989; Lerman *et al*., 1991). Whereas the initial depressor response to ET-1 is due to arterial dilatation via the activation of endothelial ET_B-receptors and subsequent release of nitric oxide and/or prostacyclin (Filep *et al*., 1991; 1994; Fujitani *et al*., 1993; Hirata *et al*., 1993; Karaki *et al*., 1993), the prolonged arterial constriction is probably mediated by the activation of smooth muscle ET_A- as well as ET_B-receptors (Clozel *et al*., 1992; Sumner *et al*., 1992; Batra *et al*., 1993; Shetty *et al*., 1993; Warner *et al*., 1993; Filep *et al*., 1994; Gardiner *et al*., 1994; Beyer *et al*., 1995; Sharifi & Schiffrin, 1996).

In vitro studies show that ET-1 constricts veins via the activation of ET_A- and/or ET_B-receptors. Both ET_A- and ET_B-receptors mediate contractile response to ET-1 in human isolated internal mammary (Seo *et al*., 1994) and saphenous (White *et al*., 1994) veins. Human renal (Maguire *et al*., 1994), coronary (Opgaard *et al*., 1994) and omental (Riezebos *et al*., 1994) veins and rat mesenteric veins (D'Orléans-Juste *et al*., 1993) contract via the activation of primarily ET_A-receptors. In contrast, endothelins constrict via the activation of primarily ET_B-receptors in rabbit jugular (Sumner *et al*., 1992) and saphenous (Moreland *et al*., 1992; 1994; Gray *et al*., 1994; Douglas *et al*., 1995) veins as well as in canine saphenous (Moreland *et al*., 1994) and femoral (Miller & Michener, 1995) veins. *In situ* studies in cat perfused, sympathectomized gastrocnemius muscle preparations (Ekelund *et al*., 1993), human forearm veins (Haynes *et al*., 1995b) and dorsal hand veins (Strachan *et al*., 1995) show that ET-1 venoconstricts via the activation of both ET_A- and/or ET_B-receptors.

We showed that i.v. injection of high doses of ET-1 into conscious rats caused small increases in mean circulatory filling pressure (MCFP) (Waite & Pang, 1990; 1992). MCFP is the systemic pressure obtained after circulatory arrest and immediate equilibration of arterial and venous pressures and is an index of the driving force of venous return (Rothe, 1993; Pang, 1994). In pentobarbitone-anaesthetized rats, i.v. injections of ET-1 slightly increased MCFP, but markedly elevated venous resistance (Palacios *et al*., 1997), which is the ratio of pressure gradient and flow between upstream and downstream veins and is a significant factor controlling cardiac output due to low pressure in the venous system (Rothe, 1993; Pang, 1994).

The present study characterizes the subtypes of endothelin-receptors that mediate the venoconstrictor effects of ET-1 in pentobarbitone-anaesthetized rats via the use of mixed ET_A- and ET_B-receptor antagonists, namely, bosentan (Ro 47-0203, 4-tert-butyl-N-[6-(2-hydroxy-ethoxy)-5-(2-methoxy-phenoxy)-2,2′-bipyrimidin-4-yl]-benzenesulfon-amide) (Clozel *et al*., 1994) and PD 142893 (Ac-[3,3-D-diphenylalanine]-L-Leu-L-Asp-L-Ile-L-Trp) (Doherty *et al*., 1993), the ET_A-selective receptor antagonist, FR 139317 ((**R**)2-[(**R**)-2-[(**S**)-2-[[1-(hexahydro-1H-azepinyl)]carbonyl]amino-4-methylpentanoyl]amino-3-[3-(1-methyl-1H-indoyl)]propionyl]amino-3-(2-pyridyl)-propionic acid) (Sogabe *et al*., 1993), and the selective ET_B-receptor agonist, IRL 1620 (Suc-[Glu^9, $Ala^{11,15}$]endothelin-1 (18-21)) (Takai *et al*., 1992).

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Methods

Animal preparation

Male Sprague-Dawley rats weighing 420–500 g were anaesthetized with pentobarbitone (60 mg kg^{-1}, i.p.). Body temperature was maintained at 36–37°C via a rectal thermometer and a heat lamp connected to a Thermistemp Temperature Controller (Model 71; Yellow Springs Instrument Co. Inc., OH, U.S.A.). A polyethylene catheter (PE50) filled with heparin-treated saline (0.9% NaCl, 25 iu ml^{-1}) was inserted into the left iliac artery for continuous measurement of mean arterial pressure (MAP) by a pressure transducer (P23DB, Gould Statham, CA, U.S.A.) and the recording was displayed on a Grass polygraph recorder (Model RPS, 7C8). The heart rate (HR) was determined electronically from the systemic blood pressure trace by a cardiotachograph (Grass, Model 7P4G). PE50 cannulae were also implanted into the right iliac vein for the administration of vehicle or drugs, and the inferior vena cava via the left iliac vein for monitoring of central venous pressure (CVP) by another pressure transducer (P23DB, Gould Statham). A saline-filled, balloon-tipped catheter was inserted into the right atrium through the right external jugular vein. The proper location of the balloon was tested by transient inflation of the balloon, which when correctly placed, stopped the circulation within 5 s of inflation. This led to a simultaneous decrease in MAP to 20–25 mmHg and an increase in CVP to a plateau value. Additional cannulae were inserted into the left ventricle via the right carotid artery, for the injection of radioactively-labelled microspheres for the measurement of cardiac output (CO), and into the right iliac artery for the withdrawal of a reference blood sample, as described in Wang *et al.* (1995) and Waite *et al.* (1995). The position of the ventricular catheter was verified by the appearance of the ventricular pulse pressure.

The method for determining MCFP has been described in detail elsewhere (see Tabrizchi & Pang, 1992; Wang *et al.*, 1995). Briefly, steady-state readings of MAP and CVP were obtained 4–5 s after inflation of the atrial balloon. To avoid the need to equilibrate arterial and venous pressures during circulatory arrest, the arterial pressure contributed by the small amount of trapped arterial blood was corrected by the following formula: MCFP = VPP + 1/60 (FAP − VPP), where FAP and VPP represent, respectively, the final arterial pressure and venous plateau pressure obtained within 5 s of circulatory arrest, and 1/60 represents the ratio of arterial to venous compliance.

Microsphere technique

A well-stirred suspension (150 μl) containing 20,000–25,000 microspheres (15 μm diam.; DuPont Canada, Ontario, Canada) labelled with ^{57}Co were injected and flushed over 10 s into the left ventricle at the end of a 30 min stabilization period (baseline measurements) and at 10 min after i.v. injection of PD 142893, bosentan or FR 139317 as well as at 13 min after i.v. bolus injections of endothelin, IRL 1620 or the vehicle. Blood was withdrawn for 1 min at 0.35 ml min^{-1} from the right iliac arterial cannula into a heparin-treated syringe (0.9% NaCl, 50 iu ml^{-1}) starting at 10 s before the injection of each set of microspheres with a Harvard infusion/withdrawal pump. The radioactivity contained in the blood samples, syringes used for injection of microspheres and collection of blood, and test tubes used for holding the radiolabelled samples was counted by means of a 1185 Series Dual Channel Automatic Gamma Counter (Nuclear-Chicago, IL, U.S.A.) with a 3 inch NaI crystal at energy settings of 80–160 keV. The withdrawn blood was slowly injected back into the rats immediately after the counting of radioactivity.

Experimental protocol

Rats were randomly divided into 9 groups ($n = 6$ each). After 30 min of equilibration, baseline readings of MAP, HR and CO followed by MCFP were taken. Afterwards, cumulative doses of ET-1 (0.5, 1 and 2 nmol kg^{-1}) or equal volumes of saline (0.2, 0.2 and 0.4 ml kg^{-1}) were injected as an i.v. bolus, into two groups at dose-intervals of 15 min. Haemodynamic measurements were taken at 13 min after injection, at the plateau phase of the response to ET-1. Two other groups received bosentan (10 mg kg^{-1}, 0.4 ml kg^{-1}, injected i.v. over 2 min) followed 12 min later by injection of cumulative doses of either ET-1 or saline. Another two groups received PD 142893 (22 mg kg^{-1}, 0.4 ml kg^{-1}, i.v., injected over 1 min) and two other groups received FR 139317 (1 mg kg^{-1}, 0.4 ml kg^{-1}, i.v., injected over 1 min); dose-response curves to ET-1 or saline were also constructed as described for bosentan. A final group received injections of cumulative doses of IRL 1620 (0.5, 1 and 2 nmol kg^{-1}, i.v. bolus) at 15 min intervals. In 3 rats, FR 139317 (1 mg kg^{-1}, i.v.) was given 12 min before the IRL 1620 curve was constructed to evaluate its selectivity for ET_A-receptors.

Drugs

The following drugs were used: ET-1 (human/porcine, Peninsula Lab., Inc., Belmont, CA, U.S.A.), PD 142893 and FR 139317 (Parke-Davis Pharmaceutical Research Div., Ann Arbor, MI, U.S.A.), bosentan (F. Hoffmann-La Roche Ltd, Preclinical Research Dept., Basel, Switzerland), and IRL 1620 (Peptide Institute Inc., Osaka, Japan). All drugs were dissolved in saline (0.9% NaCl) except for bosentan that was dissolved in distilled water. Aliquots of stocks ET-1 and IRL 1620 were stored at −20°C until use. PD 142893, bosentan and FR 139317 were freshly prepared before use.

Calculations and statistical analysis

CO (ml min^{-1}), arterial resistance (R_A, mmHg min ml^{-1}) and venous resistance (R_V, mmHg min ml^{-1}) were calculated according to the formulae:

$$CO = \frac{\text{rate of withdrawal of blood} \times \text{total injected c.p.m.}}{\text{c.p.m. in withdrawn blood}}$$

$$R_A = \frac{MAP}{CO}$$

$$R_V = \frac{MCFP - CVP}{CO}$$

Due to a technical difficulty in monitoring right atrial pressure in small animals, CVP rather than right atrial pressure was used to calculate pressure gradient to venous return (MCFP − right atrial pressure), as mean CVP is nearly identical to mean right atrial pressure (Rothe, 1993).

All results are expressed as mean ± s.e.mean and analysed by analysis of variance followed by Duncan's multiple range test, with $P < 0.05$ selected as the criterion for statistical significance.

Results

There was no significant difference in the baseline values of MAP (ranged from 99 ± 2 to 102 ± 6 mmHg), HR (340 ± 10 to 368 ± 10 beats min^{-1}), R_V (0.040 ± 0.003 to 0.046 ± 0.004 mmHg min ml^{-1}), R_A (1.04 ± 0.05 to 1.10 ± 0.04 mmHg min ml^{-1}), CO (94 ± 4 to 98 ± 8 ml min^{-1}) and MCFP (4.8 ± 0.2 to 5.3 ± 0.3 mmHg) between the nine groups of pentobarbitone-anaesthetized rats.

The vehicle did not significantly alter any variables in the time-control group (Figure 1). Injection of each dose of ET-1 (0.5, 1 and 2 nmol kg^{-1}) evoked an initial transient (≈45 s duration) fall in MAP (−28 ± 5, −21 ± 2 and −18 ± 4 mmHg, respectively). Thereafter, there was a prolonged, dose-dependent rise in MAP that reached a plateau within 7–10 min after

injection (Figure 1a). At the plateau phase of the pressor response to ET-1, HR and CO were decreased, while R_V, R_A and MCFP were increased (Figure 1b,c,d,e,f).

The transient depressor response elicited by each dose of ET-1 was abolished by bosentan and PD 142893 but unaffected by FR 139317. Decreases in MAP by ET-1 (0.5, 1 and 2 nmol kg^{-1}) in FR 139317-treated rats were -22 ± 3, -17 ± 3 and -13 ± 2 mmHg; respectively, and these readings were not significantly different from the corresponding readings in rats injected with ET-1.

I.v. injection of each dose of IRL 1620 (0.5 and 1 nmol kg^{-1}) also elicited a biphasic MAP response, with a transient fall (-20 ± 2, -21 ± 3 and -20 ± 2 mmHg, respectively) followed by a dose-dependent sustained increase in MAP (Figure 1a). IRL 1620 also reduced HR and CO and increased R_V, R_A and MCFP (Figure 1b,c,d,e,f).

The IRL 1620-induced increases in MAP (0.5 and 1 nmol kg^{-1}), R_A (1 and 2 nmol kg^{-1}) and R_V (2 nmol kg^{-1}), and the reduction in CO (2 nmol kg^{-1}) were significantly less than those of ET-1. In these rats, a higher dose (4 nmol kg^{-1}) of IRL 1620 caused either similar effects to those elicited by the 2 nmol kg^{-1} dose or was less effective (results not shown).

In the presence of bosentan (10 mg kg^{-1}), the vehicle did not cause any haemodynamic effects. Bosentan abolished the effects of ET-1 on R_V and MCFP (effects insignificantly different from corresponding readings in the time-controls) and markedly inhibited the effects of ET-1 on MAP, R_A, HR and CO (Figure 2). Pretreatment with bosentan (20 mg kg^{-1}, $n=3$) did not cause greater inhibition of the haemodynamic effects of ET-1 (results not shown).

In PD 142893-pretreated time-control rats, saline did not alter MAP, HR, R_V R_A or CO but increased MCFP at the last reading ($P<0.05$ from the pre-PD 142893 baseline as well as the vehicle time-control values in Figure 1). These results suggest that PD 142893 has a partial agonistic action on MCFP. In the presence of PD 142893, the effects of ET-1 on MAP, R_V, R_A and CO were abolished, but the effects on MCFP and HR were unaffected (Figure 3).

In rats pretreated with FR 139317, injection of saline did not cause any significant haemodynamic changes (Figure 4). FR 139317 partially inhibited the effects of ET-1 on MAP, HR, R_V, R_A, CO and MCFP; there was a tendency for a greater inhibition with lower doses than with higher doses of ET-1. Higher doses of FR 139317 (5 and 10 mg kg^{-1}, $n=3$ each) did not cause further inhibition of the effects of ET-1 on MAP, HR, R_V, R_A, CO and MCFP (results not shown). Pretreatment with FR 139317 (1 mg kg^{-1}, i.v., $n=3$) did not inhibit IRL 1620-induced changes in MAP, HR, R_V, R_A CO and MCFP (results not shown) indicating its selectivity for ET_A-receptors.

Discussion

Similar to results from a previous study (Palacios *et al*., 1997), an i.v. bolus injection of ET-1 in pentobarbitone-anaesthetized rats caused an initial transient decrease (<1 min) followed by a prolonged increase in MAP. At 13 min after injection, ET-1 significantly increased MAP, MCFP, R_A and R_V, and reduced CO as well as HR. The maximum increase in MCFP (2.0 mmHg) induced by ET-1 was less than those elicited by noradrenaline (3.3 mmHg), angiotensin II (4.8 mmHg) and the α_2-adrenoceptor agonist BHT 920 (3.0 mmHg) (Pang & Tabrizchi, 1986). The rise in MAP elicited by ET-1 was due to

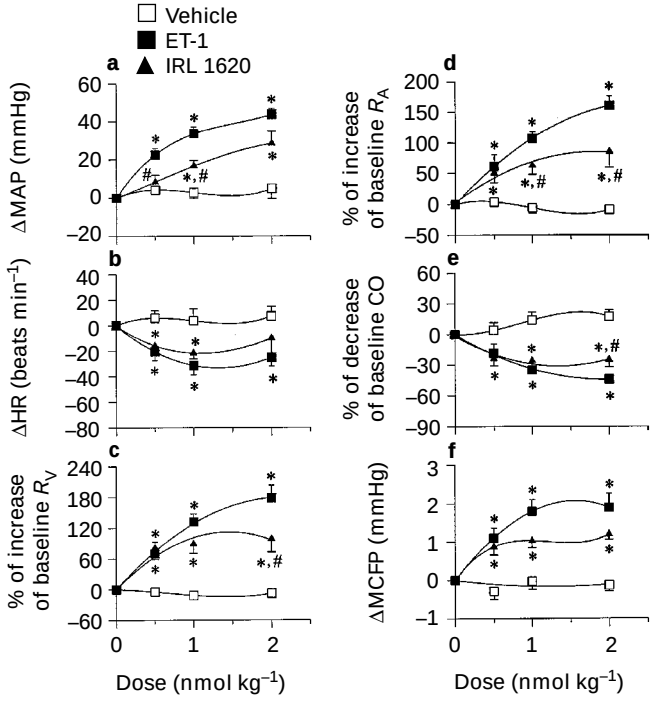

Figure 1 Changes induced by cumulative i.v. bolus injections of endothelin-1 (ET-1), IRL 1620 or equivalent volumes of vehicle (0.9% NaCl) on mean arterial pressure (MAP, a), heart rate (HR, b), venous resistance (R_V, c), arterial resistance (R_A, d), cardiac output (CO, e) and mean circulatory filling pressure (MCFP, f) in three groups of pentobarbitone-anaesthetized rats ($n=6$ each). The measurements were obtained 13 min after the injection of ET-1, IRL 1620 or vehicle. Points show the mean and vertical lines indicate s.e.mean. *Significantly different ($P<0.05$) from the corresponding values in the vehicle group. #Significantly different ($P<0.05$) from the corresponding values in ET-1-treated group.

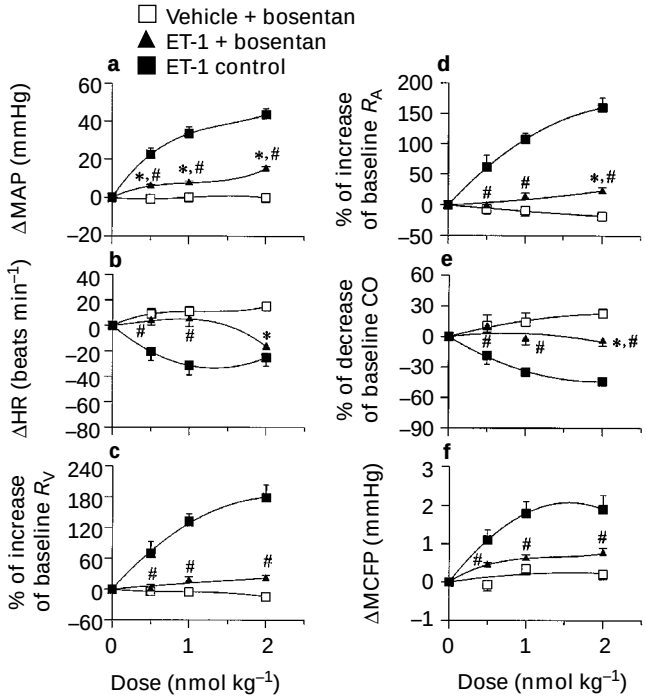

Figure 2 Changes induced by cumulative i.v. bolus injections of endothelin-1 (ET-1) or equivalent volumes of vehicle (0.9% NaCl) on mean arterial pressure (MAP, a), heart rate (HR, b), venous resistance (R_V, c), arterial resistance (R_A, d), cardiac output (CO, e) and mean circulatory filling pressure (MCFP, f) in two groups of pentobarbitone-anaesthetized rats ($n=6$ each) pretreated with bosentan (10 mg kg^{-1}, i.v.) 12 min before the start of the dose-response curves. The control ET-1-treated group is also plotted (control). The measurements were obtained 13 min after the injection of ET-1 or vehicle. Points show the mean and vertical lines indicate s.e.mean. *Significantly different ($P<0.05$) from the corresponding values in the vehicle group. #Significantly different ($P<0.05$) from the corresponding values in the control ET-1-treated group.

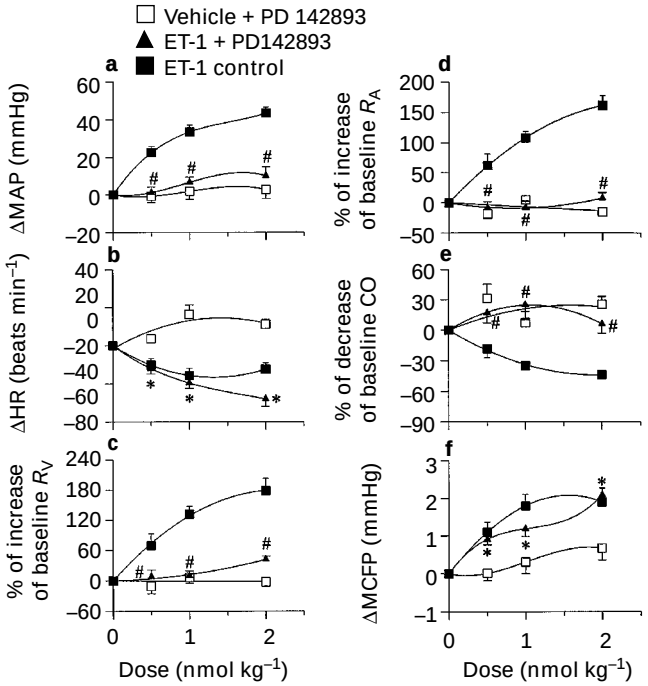

Figure 3 Changes induced by cumulative i.v. bolus injections of endothelin-1 (ET-1) or equivalent volumes of vehicle (0.9% NaCl) on mean arterial pressure (MAP, a), heart rate (HR, b), venous resistance (R_V, c), arterial resistance (R_A, d), cardiac output (CO, e) and mean circulatory filling pressure (MCFP, f) in two groups of pentobarbitone-anaesthetized rats ($n=6$ each) pretreated with PD 142893 (22 mg kg^{-1}, i.v.) 12 min before the start of the dose-response curves. The control ET-1-treated group is also plotted. The measurements were obtained 13 min after the injection of ET-1 or vehicle. Points show the mean and vertical lines indicate s.e.mean. *Significantly different ($P<0.05$) from the corresponding values in the vehicle group. #Significantly different ($P<0.05$) from the corresponding values in the control ET-1-treated group.

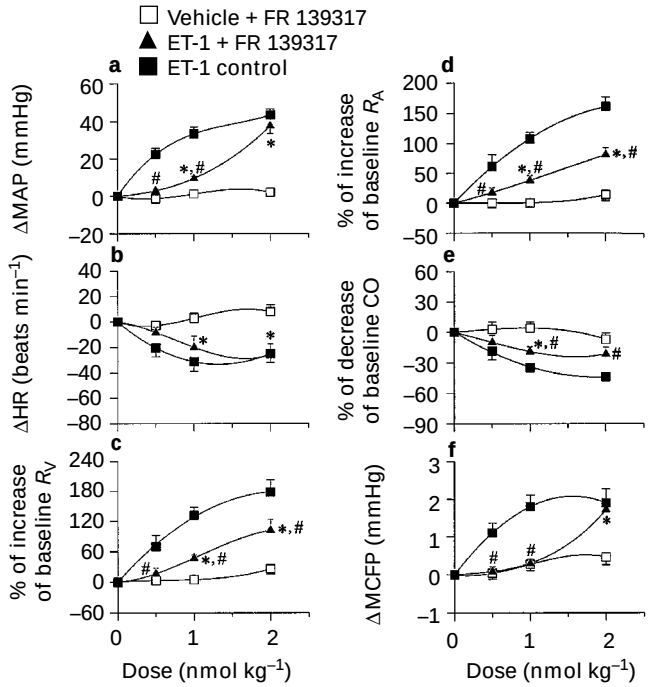

Figure 4 Changes induced by cumulative i.v. bolus injections of endothelin-1 (ET-1) or equivalent volumes of vehicle (0.9% NaCl) on mean arterial pressure (MAP, a), heart rate (HR, b), venous resistance (R_V, c), arterial resistance (R_A, d), cardiac output (CO, e) and mean circulatory filling pressure (MCFP, f) in two groups of pentobarbitone-anaesthetized rats ($n=6$ each) pretreated with FR 139317 (1 mg kg^{-1}, i.v.) 12 min before the start of the dose-response curves. The control ET-1-treated group is also plotted. The measurements were obtained 13 min after the injection of ET-1 or vehicle. Points show the mean and vertical lines indicate s.e.mean. *Significantly different ($P<0.05$) from the corresponding values in the vehicle group. #Significantly different ($P<0.05$) from the corresponding values in the control ET-1-treated group.

increases in systemic arterial resistance, as CO was decreased. The fall in CO elicited by ET-1 (0.5, 1 and 2 nmol kg^{-1}) was the result of increases in arteriolar (+62, +108 and +162% over baseline, respectively) and venous (+70, +132 and +179%) resistances and fall in HR.

The ET_B-receptor agonist IRL 1620, similar to ET-1, caused a biphasic MAP response. The initial depressor responses were similar in magnitude to those of ET-1 and were not blocked by FR139317 (results not shown). The activation of ET_B-receptors has been shown to mediate the release of nitric oxide and/or prostacyclin (see Introduction). At 13 min after injection, IRL 1620 (0.5, 1 and 2 nmol kg^{-1}) decreased CO and HR and increased MAP, MCFP, R_A (+51, +63, +86% over baseline, respectively) and R_V (+81, +89, +98% over baseline). FR 139317 did not inhibit haemodynamic responses to IRL 1620 (results not shown). The pressor response to IRL 1620 was due to the constriction of arterioles since R_A was increased and CO was decreased. Reduced CO elicited by IRL 1620 was due to a reduction in HR and constrictions of both arterial and venous resistance vessels. IRL 1620, like ET-1, has similar efficacy in constricting resistance arteries and veins. The exact site whereby IRL 1620 raised venous resistance is unknown. An important location of venous resistance is the hepatic bed where a pressure gradient of 4 to 15 mmHg exists between the portal vein and the inferior vena cava at the exit junction of the hepatic vein (Tabrizchi *et al.*, 1993; Pang, 1994). An increase in venous resistance would be expected to reduce venous return and therefore cardiac output due to the accumulation of blood in the venules of the splanchnic bed.

Maximum MAP, R_A, R_V and CO responses to IRL 1620 were significant less than those to ET-1, suggesting the overlapping roles of ET_A- and ET_B-receptors in the mediation of these responses. Maximum responses to IRL 1620 were achieved since haemodynamic responses to a higher dose (4 nmol kg^{-1}) were either similar or less than those to 2 nmol kg^{-1}. In accordance with our findings, IRL 1620 caused less systemic vasoconstriction in anaesthetized rats (Beyer *et al.*, 1995) and a smaller increase in MAP in conscious rats (Filep *et al.*, 1994) than ET-1 did. Sarafotoxin S6c also caused a smaller pressor response in pithed rats (Flynn *et al.*, 1995) and less constriction *in situ* in human forearm resistance arteries (Haynes *et al.*, 1995a) and dorsal hand veins (Haynes *et al.*, 1995b; Strachan *et al.*, 1995) than did ET-1. To our knowledge, this is the first study to quantify the constrictor roles of both ET_A- and ET_B-receptors in the venous system *in vivo*.

Pretreatment with bosentan (10 mg kg^{-1}) inhibited completely the effects of all doses of ET-1 on R_V and MCFP but incompletely (though markedly) inhibited the effects of ET-1 on MAP, HR, R_A and CO. We are convinced that maximum inhibition of ET-1 effects was obtained since bosentan (20 mg kg^{-1}; $n=3$) caused similar inhibition of ET-1 responses in preliminary studies as did 10 mg kg^{-1}. Furthermore, Filep *et al.* (1994) showed that bosentan, at 10 and 20 mg kg^{-1}, caused similar (87%) inhibition of the pressor effect of ET-1 (1 nmol kg^{-1}) in conscious rats. In the present study, bosentan alone (10 mg kg^{-1}) did not cause any haemodynamic changes suggesting that it has negligible partial agonistic activity.

Doherty *et al.* (1993) showed that PD 142893 at 11 mg kg^{-1} inhibited the depressor but not the pressor response to ET-1 in anaesthetized, ganglionic-blocked rats. In our preliminary studies ($n=5$), PD 142893 11 mg kg^{-1} also inhibited the transient depressor but not the pressor effects of ET-1. This dose of PD 142893 also did not attenuate the effects of ET-1 on R_V, R_A and CO. However, PD 142893 at 22 mg kg^{-1} (i.v., bolus) abolished the transient depressor effects of ET-1 (results

not shown) as well as the sustained effects of ET-1 on MAP, R_V, R_A and CO, but did not inhibit the effects of ET-1 on HR and MCFP. PD 142893 alone did not alter MAP, R_V, R_A, CO or HR, but slightly increased MCFP with time suggesting that it has a slight partial agonistic action on MCFP; the receptors involved in the rise of MCFP remain unclear.

The selective ET_A-receptor antagonist, FR 139317 (1 mg kg^{-1}) partially inhibited the effects of ET-1 on MAP, HR, R_V, R_A, CO and MCFP. Incomplete inhibition of all haemodynamic effects of ET-1 by FR 139317 was not due to a short duration of action of the compound or an insufficient dose, since higher doses of FR 139317 (5 and 10 mg kg^{-1}) did not further suppress the effects of ET-1 in preliminary studies. Moreover, after the completion of the dose-response curve to saline in time-control rats pretreated with FR 139317 (1 mg kg^{-1}), the effects of ET-1 (0.5 and 1 nmol kg^{-1}) were still similarly (results not shown) inhibited as in FR 139317-pretreated rats given only ET-1. FR 139317 (1 mg kg^{-1}) neither inhibited the depressor response to ET-1 nor haemodynamic changes elicited by IRL 1620 (0.5, 1 and 2 nmol kg^{-1}, i.v. bolus) indicating its selectivity for ET_A-receptors. Our results show that the haemodynamic effects elicited by ET-1 are partially mediated via the activation of ET_A-receptors. Incomplete inhibition of the pressor effects of ET-1 by FR 139317 (Bird & Waldron, 1993; Filep *et al*., 1994; Gardiner *et al*., 1994) or the ET_A-receptor antagonist BQ 123 (McMurdo *et al*., 1993) has been described previously.

To summarize, our results on the efficacy of IRL 1620 at elevating R_A, R_V and MCFP show that ET_B-receptors mediate both arterial and venous tone. The ability of FR139317 to suppress partially the effects of ET-1 (but not those of IRL 1620) on R_A, R_V and MCFP suggest that ET_A-receptors are also involved. While bosentan and PD 142893 almost completely inhibited the effects of ET-1 on R_A and R_V, bosentan but not PD 142893 inhibited the effects of ET-1 on MCFP. It has been suggested that contractile responses to ET-1 may involve the activation of a third subtype of receptor that is neither ET_A nor ET_B (Harrison *et al*., 1992; Filep *et al*., 1994). Whether or not additional receptors are involved is unclear and has to await the development of selective antagonists for the yet unknown receptors.

Our results suggest that ET-1 increases R_A, R_V and MCFP via the activation of both ET_A- and ET_B-receptors. ET-1 is unlikely to play a significant role in the regulation of vascular tone during normal conditions since plasma levels of endothelins are low. Circulating levels of ET-1 are markedly increased in pathophysiological conditions such as renal failure, heart failure and various states of shock (cardiogenic, haemorrhage, septic) (see reviews by Tamirisa *et al*., 1995; Gray & Webb, 1996; Noll *et al*., 1996). Under such conditions, ET-1 may play a significant constrictor role in both the arterial as well as the venous system *in vivo*.

This work was supported by Heart & Stroke Foundation of B.C. & Yukon. B.P. is the recipient of a postdoctoral fellowship from the Ramón Areces Foundation in Spain. We thank Dr A.M. Doherty (Parke-Davis, U.S.A.) for the generous supply of PD 142893 and FR 139317, Dr M. Clozel (Hoffmann-La Roche Ltd, Switzerland) for bosentan and Dr Y. Kishida (Peptide Institute Inc., Japan) for IRL 1620.

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(Received July 18, 1997
Accepted August 6, 1997)