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Effects of bosentan (Ro 47-0203), an ET_A-, ET_B-receptor antagonist, on regional haemodynamic responses to endothelins in conscious rats

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1 Regional haemodynamic responses to endothelin (ET)-1, -2 and -3 and big ET-1 (all at 500 pmol kg^{-1}) were assessed in the same conscious Long Evans rats ($n = 8$) in the absence or presence of the mixed ET_A-, ET_B-receptor antagonist, Ro 47-0203 (bosentan; 30 mg kg^{-1}).

2 Bosentan blocked the initial depressor, tachycardic and hindquarters hyperaemic vasodilator effects of ET-1, -2 and -3, and substantially curtailed the primary renal and secondary hindquarters vasoconstrictor responses. Bosentan did not inhibit the initial mesenteric vasoconstrictor action of ET-1, but reduced the duration of the later mesenteric vasoconstriction. In contrast, bosentan delayed the rate of onset, and reduced the duration, of the mesenteric vasoconstrictor actions of ET-2 and ET-3. The most likely explanation of this finding is that ET-1, but not ET-2 or ET-3, triggered a covert mesenteric vasodilator mechanism which was antagonized by bosentan.

3 Bosentan blocked all the effects of big ET-1, and, in a separate group of rats ($n = 7$), blocked all the haemodynamic effects of a lower dose of ET-1 (50 pmol kg^{-1}), with the exception of a slight mesenteric vasoconstriction.

4 The most straightforward explanation of the results is that the major haemodynamic effects of ET-1, -2 and -3, and all the effects of big ET-1, are mediated through ET_A- and/or ET_B-receptors that are effectively antagonized by bosentan.

Keywords: ET_A-receptors; ET_B-receptors; endothelins; big endothelin-1; bosentan

Introduction

Recent findings indicate that, while exogenous endothelin (ET)-1, and its analogues elicit initial depressor and vasodilator effects by activating ET_B-receptors, the subsequent pressor and vasoconstrictor actions of these peptides may involve both ET_A- and ET_B-receptors (Hiley *et al.*, 1989; Randall, 1991; Bigaud & Pelton, 1992; Clozel *et al.*, 1992; Gardiner *et al.*, 1992b; Moreland *et al.*, 1992; Cristol *et al.*, 1993; McMurdo *et al.*, 1993; Warner *et al.*, 1993).

These obervations raise the possibility that selective ET_A-receptor antagonists would be less useful than non-selective, ET_A-, ET_B-receptor antagonists in pathophysiological conditions in which impairment of regional blood flow was due to endogenous endothelin(s) (see Battistini *et al.*, 1993, for review). Recently, Clozel *et al.* (1993) demonstrated the ability of the non-selective ET_A-, ET_B-receptor antagonist, Ro 46-2005 (4-tert-butyl-*N*- [6-(2-hydroxy-ethoxy)-5-(3-methoxy-phenoxy)-4-pyrimidinyl]-benzenesulphonamide), to inhibit the decrease in renal blood flow following renal ischaemia, and also the cerebral vasospasm following subarachnoid haemorrhage in anaesthetized rats. In those models, Ro 46-2005 was without effect on systemic arterial blood pressure. However, Ro 46-2005 at a dose of 100 mg kg^{-1} caused a prolonged fall (30–40 mmHg) in mean arterial blood pressure in conscious, frusemide-treated, squirrel monkeys. Collectively, these results indicate that endogenous ETs may contribute to vasospasm in pathological conditions, and to the maintenance of mean arterial blood pressure in sodium depletion. However, at present, no data are available regarding the ability of non-selective ET_A-, ET_B-receptor antagonists to influence the regional haemodynamic actions of ETs.

Therefore, in the present work, we assessed regional haemodynamic responses to ET-1, ET-2 and ET-3, and big ET-1 in the absence and presence of the non-peptide, non-selective, ET_A-, and ET_B-receptor antagonist, bosentan (i.e., Ro 47-0203; 4-tert-butyl-*N*-[6-(2-hydroxy)-ethoxy)-5-(2-methoxy-phenoxy)-2, 2'-bipyrimidin-4yl] -benzenesulphonamide) (Clozel *et al.*, 1994).

Methods

Male Long Evans rats (350–450 g), bred in the Biomedical Services Unit, Queen's Medical Centre, Nottingham, were chronically instrumented with pulsed Doppler flow probes (renal, mesenteric and hindquarters) and intravascular catheters as described in detail previously (Gardiner *et al.*, 1991; 1992a). All surgery was carried out under sodium methohexitone anaesthesia (Brietal, Lilly; 40–60 mg kg^{-1}, i.p., supplemented as required). Experiments were not begun until at least 24 h after the last surgical intervention.

One group of animals ($n = 8$) was randomized to receive i.v. bolus doses of ET-1, ET-2, ET-3 and big ET-1 (all at 500 pmol kg^{-1}) on separate experimental days, before and 5 min after i.v. bolus injection of bosentan (30 mg kg^{-1}). The peptide injections on any experimental day were separated by at least 7 h; in pilot experiments we determined that repeated peptide injections at this interval evoked reproducible responses. The dose of the peptides was based on preliminary experiments showing that it evoked reproducible responses with respect to all components (see Results); the dose of bosentan was based on previous studies (Clozel *et al.*, 1994). Since bosentan did not abolish all the effects of the peptides (see Results), in a second group of animals ($n = 7$), responses to ET-1 at a 10 fold lower dose, i.e., 50 pmol kg^{-1}, were assessed before and 5 min after i.v. bolus injection of bosentan.

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Data analysis

During an experimental run, continuous recordings were made of instantaneous heart rate, and mean and phasic arterial blood pressure and Doppler shift signals. Vascular conductance was calculated (mean Doppler shift divided by mean arterial blood pressure), and changes in all variables relative to baseline were used to assess responses to peptides. For ET-1, ET-2 and ET-3 at 500 pmol kg^{-1}, measurements were made at 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 10, 15, 20, 25, 30 and 60 min after injection. For big ET-1, measurements were made 1, 2, 3, 4, 5, 10, 15, 20, 25, 30 and 60 min after

Table 1 Resting cardiovascular variables in the two groups of conscious, Long Evans rats studied

	Group 1	*Group 2*
Heart rate (beats min^{-1})	324 ± 10	301 ± 4
Mean arterial blood pressure (mmHg)	96 ± 3	100 ± 1
Renal Doppler shift (kHz)	6.8 ± 0.8	5.6 ± 0.6
Mesenteric Doppler shift (kHz)	6.6 ± 0.5	6.2 ± 0.5
Hindquarters Doppler shift (kHz)	4.0 ± 0.2	3.5 ± 0.3
Renal vascular conductance ([kHz $mmHg^{-1}$]10^3)	72 ± 10	56 ± 6
Mesenteric vascular conductance ([kHz $mmHg^{-1}$]10^3)	70 ± 6	62 ± 4
Hindquarters vascular conductance ([kHz $mmHg^{-1}$]10^3)	42 ± 3	35 ± 3

Group 1 ($n = 8$) were those that received ET-1, ET-2, ET-3 and big ET-1 at 500 pmol kg^{-1}. Group 2 ($n = 7$) were given ET-1 at 50 pmol kg^{-1} only. Values are mean ± s.e.mean.

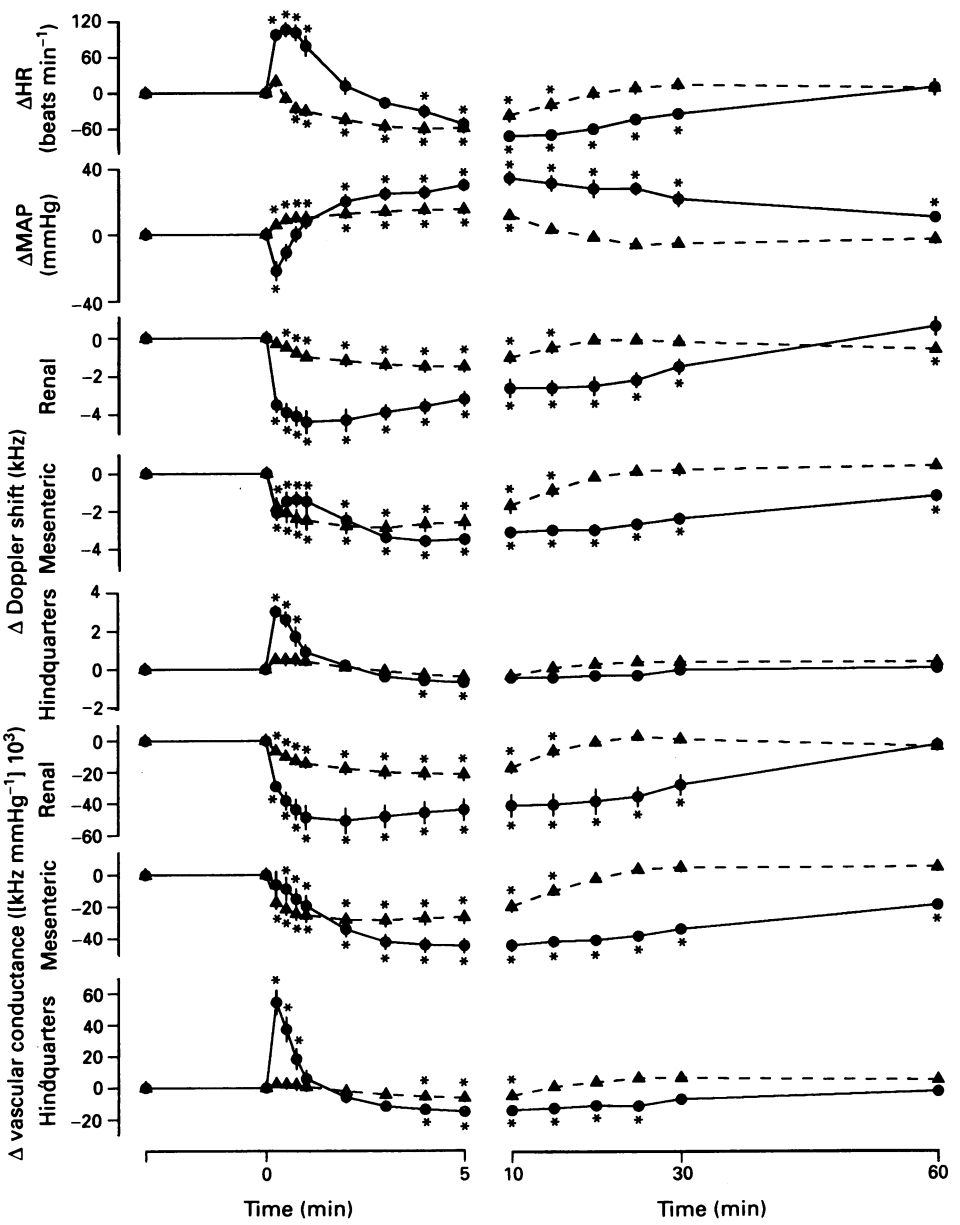

Figure 1 Cardiovascular responses to endothelin-1 (ET-1, 500 pmol kg^{-1}) in the absence (●) or presence (▲) of bosentan (30 mg kg^{-1}) in the same conscious, Long Evans rats ($n = 8$). Values are mean ± s.e.mean; $*P < 0.05$ versus baseline. Statistics for the differences between the responses in the absence and presence of bosentan are given in the text and Table 2.

injection. Responses to peptides were assessed by applying Friedman's test to the changes relative to the preinjection baseline; responses in the absence and presence of bosentan were compared by applying Wilcoxon's ranks sums test to integrated responses (areas under or over curves AUC_{0-60} min, AOC_{0-60} min, respectively). The same procedures were followed with the lower dose of ET-1 (50 pmol kg^{-1}), except that measurements were made only up to 10 min post-injection, since most variables were back to baseline levels by then. A P value <0.05 was taken as significant.

Peptides and drugs

ET-1, ET-2, ET-3 and big ET-1 were obtained from the Peptide Institute (Osaka, Japan), through their UK agents (Scientific Research Associates). Peptides were dissolved in saline containing 1% bovine serum albumin (Sigma, U.K.), and injected in a volume of 0.1 ml, flushed in with 0.1 ml saline. Bosentan (sodium salt) was synthesized at F. Hoffman – LaRoche Ltd., Basel Switzerland; it was dissolved in sterile water (60 mg ml^{-1}) and injected in a volume of 0.2 ml, flushed in with 0.1 ml saline.

Results

Resting cardiovascular variables in the two groups of rats studied are shown in Table 1.

Responses to ET-1 in the absence and presence of bosentan

High dose ET-1 ET-1 (500 pmol kg^{-1}) caused an initial fall in mean arterial blood pressure, and a tachycardia, accompanied by reductions in renal and mesenteric flows and conductances, but an increase in hindquarters flow and conductance (Figure 1, Table 2). Thereafter, there were pressor

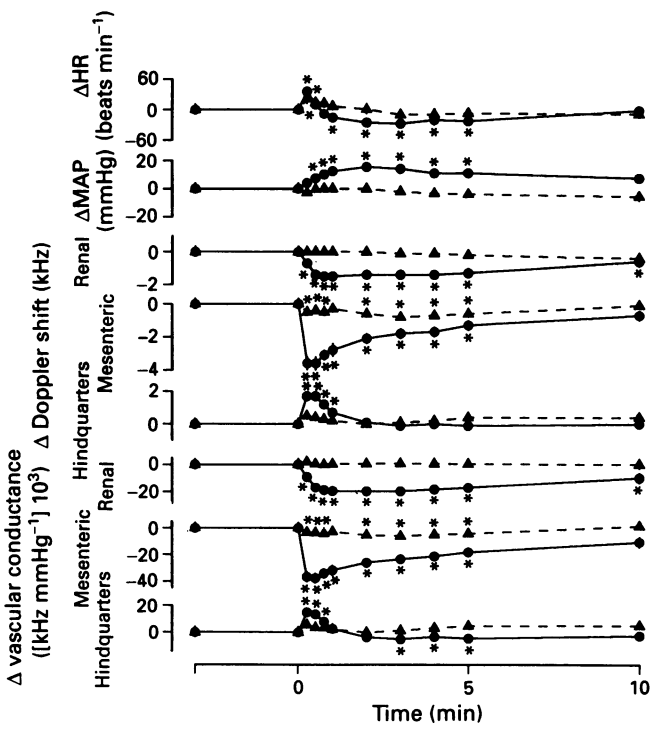

Figure 2 Cardiovascular responses to endothelin-1 (ET-1, 50 pmol kg^{-1}) in the absence (●) or presence (▲) of bosentan (30 mg kg^{-1}) in the same conscious, Long Evans rats ($n=7$). Values are mean ± s.e.mean; *$P<0.05$ versus baseline. Statistics for the differences between the responses in the absence and presence of bosentan are given in the text and Table 3.

Table 2 Integrated (AUC or AOC) cardiovascular responses to ET-1, ET-2, ET-3 or big ET-1 in the absence or presence of bosentan, respectively, in the same conscious, Long Evans rats ($n=8$)

	ET-1		*ET-2*		*ET-3*		*Big ET-1*	
	− *bosentan*	+ *bosentan*	− *bosentan*	+ *bosentan*	− *bosentan*	+ *bosentan*	− *bosentan*	+ *bosentan*
Heart rate (AUC, beats)	138 ± 23	7 ± 2†	30 ± 8*	14 ± 6	68 ± 17*	4 ± 4†	-	-
Heart rate (AOC, beats)	2529 ± 244	1117 ± 238†	1785 ± 227*	677 ± 188†	1387 ± 209*	833 ± 155†	3011 ± 549	473 ± 153†
Mean BP (AOC, mmHg min)	14 ± 4	0†	5 ± 2*	1 ± 1	6 ± 2	0†	-	-
Mean BP (AUC, mmHg min)	1212 ± 194	172 ± 32†	521 ± 113*	61 ± 23†	747 ± 90*	298 ± 41†	1121 ± 57	22 ± 19†
Renal conductance (AOC, [kHz mmHg^{-1}]10^3 min)	1742 ± 338	422 ± 85†	878 ± 136*	259 ± 101†	984 ± 88*	403 ± 114†	1068 ± 88*	330 ± 103†
Mesenteric conductance (AOC, [kHz mmHg^{-1}]10^3 min)	1962 ± 168	362 ± 65†	1161 ± 120*	308 ± 78†	1959 ± 251	498 ± 92†	1645 ± 215	388 ± 71†
Hindquarters conductance (AUC, [kHz mmHg^{-1}]10^3 min)	35 ± 8	7 ± 3†	15 ± 5*	8 ± 2	11 ± 2*	14 ± 3	-	-
Hindquarters conductance (AOC, [kHz mmHg^{-1}]10^3 min)	491 ± 109	75 ± 29†	375 ± 84*	32 ± 19†	475 ± 98	96 ± 78†	1158 ± 132*	40 ± 21†

Values are mean ± s.e.mean; †$P<0.05$ versus corresponding responses in the absence of bosentan; *$P<0.05$ versus corresponding responses to ET-1. The heart rate, mean arterial blood pressure and hindquarters vascular conductance changes evoked by ET-1, -2 and -3 were biphasic.

and bradycardic effects associated with reductions in renal, mesenteric and hindquarters flows and vascular conductances (Figure 1, Table 2).

When bosentan was injected, the majority of animals showed an alerting response and motor activity (such effects were not seen with vehicle injection). The motor activity was accompanied by a transient (less than 5 min) rise in heart rate and mean arterial blood pressure, but no reductions in regional blood flows. Hence, the pressor effect of bosentan was probably due to an activity-induced increase in cardiac output, and clearly was not an agonistic effect of the drug at ET-receptors, consisent with the findings of Clozel *et al.* (1994).

In the presence of bosentan, the initial depressor and tachycardic, and the hindquarters hyperaemic vasodilator effects of ET-1, were abolished (Figure 1, Table 2). Although the early (within 1 min) renal vasoconstrictor effect of ET-1 was attenuated (+ bosentan, Δ maximum vascular conductance = -15 ± 3 kHz mmHg$^{-1}10^3$;-bosentan, Δ maximum = -49 ± 8 kHz mmHg$^{-1}10^3$), the mesenteric vasoconstrictor response was not (Figure 1); however, the subsequent renal, mesenteric and hindquarters vasoconstrictor effects of ET-1, together with its pressor and bradycardic actions were all attenuated by bosentan (Figure 1, Table 2). Nevertheless, in the presence of bosentan, ET-1 still exerted significant cardiovascular effects (Figure 1).

Low dose ET-1 In the absence of bosentan, the 50 pmol kg^{-1} dose of ET-1 did not cause an initial depressor effect, and there was only a slight tachycardia (Figure 2). However, there were early increases in hindquarters flow and vascular conductance, in association with reductions in renal and mesenteric flows and vascular conductances (Figure 2, Table 3). Subsequently, there was a rise in mean arterial blood pressure and a bradycardia, together with a slight hindquarters vasoconstriction, but this was not accompanied by a reduction in flow (Figure 2, Table 3).

In the presence of bosentan, the pressor, bradycardic and renal and hindquarters haemodynamic effects of ET-1 were abolished. However, there were still significant, albeit slight, reductions in mesenteric flow and vascular conductance (Figure 2, Table 3).

Responses to ET-2 in the absence and presence of bosentan

Although the pattern of haemodynamic changes evoked by ET-2 (Figure 3) was similar to that of ET-1 (Figure 1), the initial depressor, tachycardic and hindquarters hyperaemic vasodilator effects of ET-2 were significantly less than those of ET-1 (Table 2; compare Figures 1 and 3), as was the initial renal vasoconstrictor effect (ET-2, Δ vascular conductance at 15 s = -9 ± 9 kHz mmHg$^{-1}10^3$; ET-1 = -29 ± 2 kHz mmHg$^{-1}10^3$). However, the initial mesenteric vasoconstrictor effect of ET-2 (Δ vascular conductance at 15 s = -45 ± 5 kHz mmHg$^{-1}10^3$) was significantly greater than that of ET-1 (-6 ± 9 kHz mmHg$^{-1}10^3$) (compare Figures 1 and 3). The subsequent pressor, bradycardic, and renal and mesenteric vasoconstrictor effects of ET-2 were less sustained than those of ET-1, and hence the integrated responses were smaller (Table 2).

In the presence of bosentan, the initial depressor effect of ET-2 was abolished, and there was no significant tachycardia, but since the responses in the absence of bosentan were small, there was no statistically significant effect of bosentan (Table 2). Likewise, the attentuation by bosentan of the initial hyperaemic hindquarters vasodilator response to ET-2 did not reach significance (Figure 3, Table 2). However, the early renal vasoconstriction was abolished, and the initial mesenteric vasoconstriction was attenuated (+ bosentan, Δ vascular conductance at 15 s = -14 ± 4 kHz mmHg$^{-1}10^3$) (Figure 3), and bosentan also reduced the durations of the renal and mesenteric vasoconstriction, and abolished the delayed hindquarters vasoconstriction (Figure 3, Table 2).

Responses to ET-3 in the absence and presence of bosentan

The profile of haemodynamic effect of ET-3 (Figure 4) was similar to that of ET-2 and ET-1, but compared to the latter, the initial tachycardia and hindquarters vasodilator actions of ET-3 were less (Table 2). Moreover, there was a significant difference between the early change in renal vascular conductance evoked by ET-3 (Δ vascular conductance at 15 s = 7 ± 4 kHz mmHg$^{-1}10^3$) and that seen with ET-1 (above), whereas the early mesenteric vasoconstrictor effect of ET-3 (Δ vascular conductance at 15 s = -41 ± 9 kHz mmHg^{-1} 10^3) was greater than that of ET-1 (above) (compare Figures 4 and 1). The durations of the pressor, bradycardic, renal vasoconstrictor effects, but not the mesenteric vasoconstrictor effect of ET-3, were less than those of ET-1 (Figure 4, Table 2).

Bosentan abolished the initial depressor and tachycardic effects of ET-3 (Figure 4, Table 2) and attenuated the early renal and mesenteric vasoconstrictions (renal, Δ vascular conductance at 1 min, + bosentan Δ = -10 ± 2 kHz mmHg^{-1} 10^3; − bosentan = -46 ± 5 kHz mmHg^{-1} 10^3; mesenteric, Δ vascular conductance at 15 s, + bosentan = -19 ± 3 kHz mmHg$^{-1}10^3$; − bosentan = -41 ± 9 kHz mmHg$^{-1}10^3$ (Figure 4, Table 2). Bosentan attenuated the subsequent pressor and bradycardic effects of ET-3, reduced the duration of the renal and mesenteric vasoconstrictor responses, and abolished the delayed hindquarters vasoconstrictor action of ET-3 (Figure 4, Table 2).

Response to big ET-1 in the absence and presence of bosentan

Big ET-1 evoked a slowly-developing pressor and bradycardic effect, accompanied by renal, mesenteric and hindquarters vasoconstrictions (Figure 5, Table 2). Big ET-1 thus differed from ET-1 in the absence of an initial depressor, tachycardic

Table 3 Integrated (AUC or AOC) cardiovascular responses to ET-1 (50 pmol kg^{-1}) in the absence or presence of bosentan in the same conscious, Long Evans rats ($n = 7$)

	ET-1	
	− bosentan	*+ bosentan*
Heart rate (AUC, beats)	14 ± 3	13 ± 5
Heart rate (AOC, beats)	191 ± 41	109 ± 45†
Mean BP (AUC, mmHg min)	110 ± 19	10 ± 5†
Renal conductance (AOC, [kHz mmHg^{-1}]10^3 min)	157 ± 32	16 ± 8†
Mesenteric conductance (AOC, [kHz mmHg^{-1}]10^3 min)	205 ± 34	41 ± 7†
Hindquarters conductance (AUC, [kHz mmHg^{-1}]10^3 min)	10 ± 2	4 ± 1
Hindquarters conductance (AOC, [kHz mmHg^{-1}]10^3 min)	38 ± 12	4 ± 2†

Values are mean ± s.e.mean; †$P < 0.05$ versus corresponding response in the absence of bosentan. The heart rate and hindquarters vascular conductance responses were biphasic.

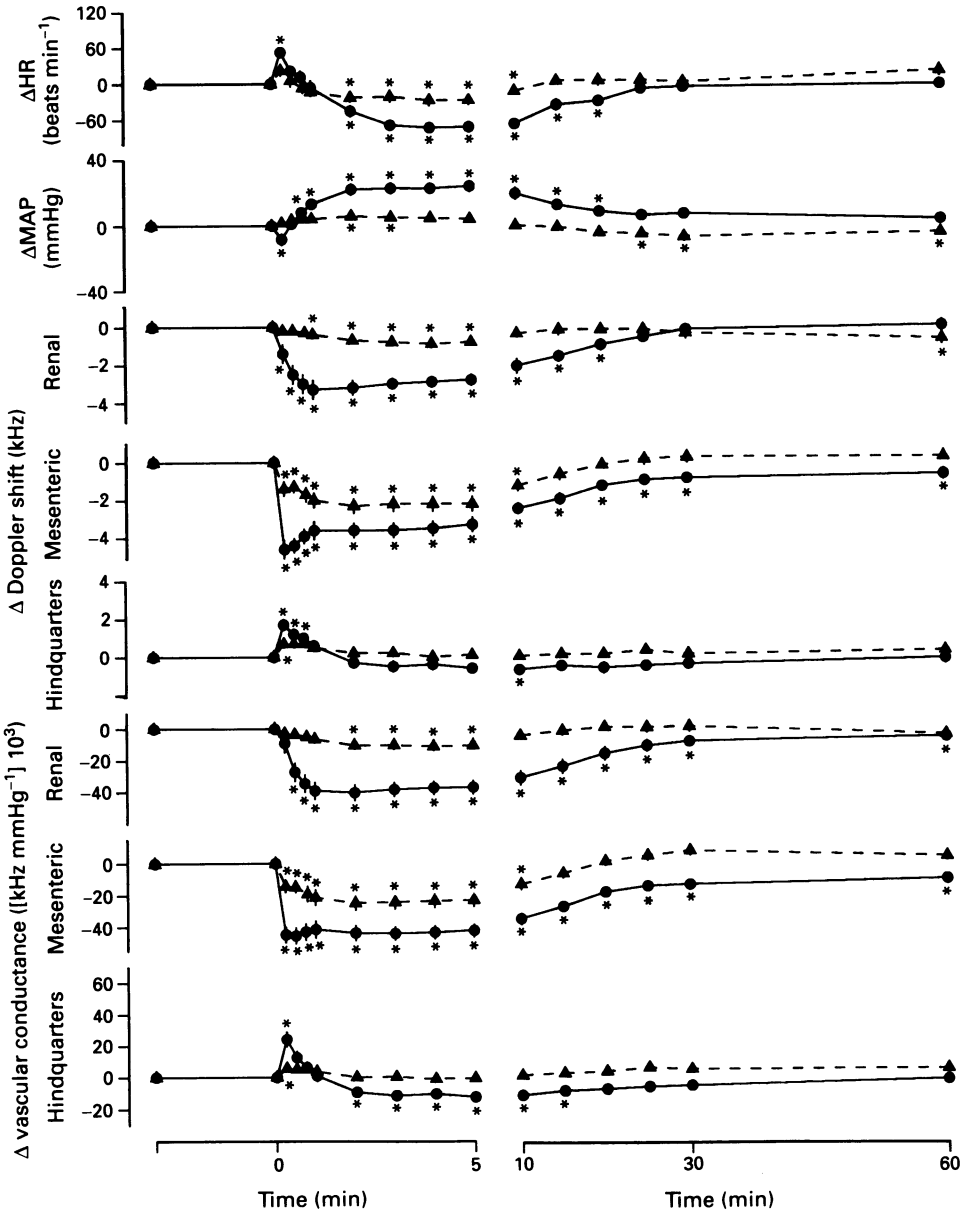

Figure 3 Cardiovascular responses to endothelin-2 (ET-2, 500 pmol kg^{-1}) in the absence (●) or presence (▲) of bosentan (30 mg kg^{-1}) in the same conscious, Long Evans rats ($n = 8$). Values are mean ± s.e.mean; *$P < 0.05$ versus baseline. Statistics for the differences between the responses in the absence and presence of bosentan are given in the text and Table 2.

and hindquarters vasodilator phase, although at a higher dose (10 nmol kg^{-1}), big ET-1 has such effects (Gardiner *et al.*, 1991). The integrated pressor and bradycardic, and mesenteric vasoconstrictor effects of big ET-1 were not different from those of ET-1, but the latter had greater renal vasoconstrictor and lesser hindquarters vasoconstrictor actions than did big ET-1 (Figure 5, Table 2).

Bosentan abolished the pressor, and renal, and hindquarters vasoconstrictor effects of big ET-1 (Figure 5, Table 2). In the presence of bosentan there were slight, delayed falls in mean arterial blood pressure and mesenteric flow and vascular conductance following administration of big ET-1 (Figure 5, Table 2).

Discussion

The major objectives of the present work were to assess the effectiveness of bosentan as an antagonist of the regional haemodynamic actions of ET-1, -2 and -3 and big ET-1 in conscious rats and, in so doing, to determine if there were residual responses to the peptides in the presence of bosentan, consistent with involvement of non-ET_A-, non-ET_B-receptors.

Effects of bosentan on responses to ET-1

At a dose of 500 pmol kg^{-1}, ET-1 elicited a marked initial hypotension, tachycardia and increases in hindquarters flow and vascular conductance; all these effects were blocked by bosentan. Furthermore, in the same experimental model, we have found that the ET_A-receptor antagonist, FR 139317, has no influence on these initial responses to ET-1 (Bennett *et al.*, 1993). Thus, these results, together, are consistent with an exclusive mediation of the hypotensive and hindquarters hyperaemic vasodilator effects of ET-1 by ET_B-receptors; it is likely that the tachycardia was a reflex response to the hypotension (Gardiner *et al.*, 1990a).

Although bosentan clearly attenuated the early renal vasoconstrictor effect of ET-1 at a dose of 500 pmol kg^{-1}, it tended to enhance the initial vasoconstrictor response in the mesenteric vascular bed. However, examination of the

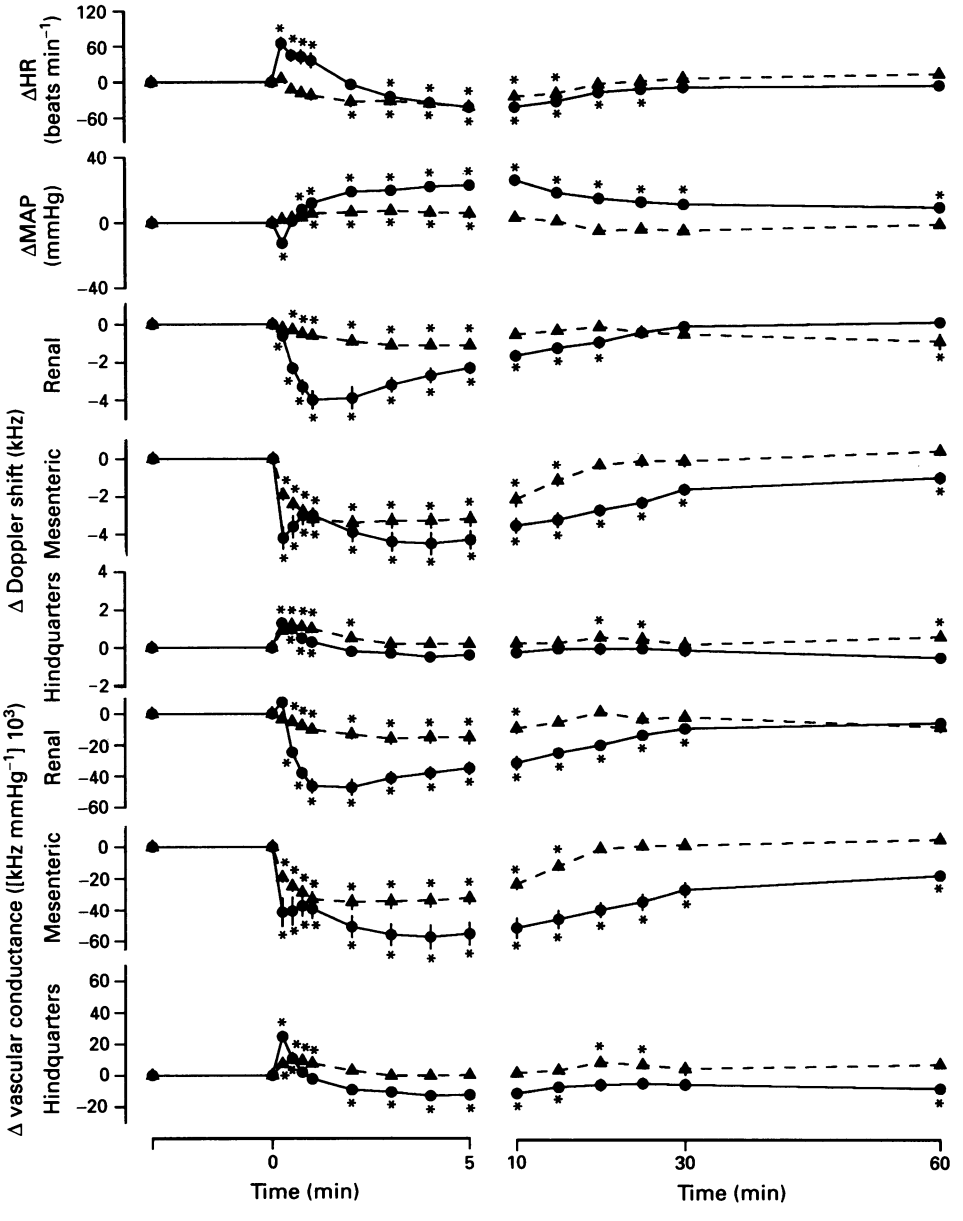

Figure 4 Cardiovascular responses to endothelin-3 (ET-3, 500 pmol kg^{-1}) in the absence (●) or presence (▲) of bosentan (30 mg kg^{-1}) in the same conscious, Long Evans rats ($n = 8$). Values are mean ± s.e.mean; *$P < 0.05$ versus baseline. Statistics for the differences between the responses in the absence and presence of bosentan are given in the text and Table 2.

changes in the arterial blood pressure and mesenteric blood flow showed that this was due to bosentan blocking a covert vasodilator component in this vascular bed that was acting to oppose the vasoconstrictor effect of ET-1. This interpretation is consistent with the finding that ET-1 at a dose of 50 pmol kg^{-1} had a rapid onset, mesenteric vasoconstrictor effect, that was clearly inhibited by bosentan. These observations indicate that, at a dose of 50 pmol kg^{-1}, ET-1 had a preferential mesenteric vasoconstrictor action, while at a dose of 500 pmol kg^{-1} addition mesenteric vasodilator mechanisms were activated by ET-1. It is feasible that the different pattern of haemodynamic changes in mesenteric and hindquarters vascular beds in response to ET-1, and the effect of bosentan thereupon, reflected different distributions of ET_A- and ET_B-receptors in the two vascular beds.

It is difficult to argue that bosentan more effectively blocked ET_B- than ET_A-receptors, if the latter predominate and, when activated, produce effects that oppose those due to ET_B-receptor stimulation. By the same token, it is not possible, straightforwardly, to quantitate the efffectiveness of bosentan in blocking the pressor and vasoconstrictor effects of ET-1, since, in the absence of bosentan, these actions were functionally antagonized by the depressor and vasodilator responses to ET-1 (Clozel *et al.*, 1994). Nonetheless, it was clear that bosentan markedly attenuated the pressor, and renal, mesenteric and hindquarters vasoconstrictor effects of ET-1 at a dose of 500 pmol kg^{-1}. Because, in the latter vascular bed, there was no reduction of flow in the presence of bosentan, it is possible that the vasoconstriction was an autoregulatory response to the rise in the perfusion pressure.

Although bosentan clearly shortened the duration of the pressor and vasoconstrictor effects, and diminished the maximum responses to ET-1 at a dose of 500 pmol kg^{-1}, significant responses remained. It is feasible that these were due to the involvement of receptors other than those of the ET_A- or ET_B-receptor subtype (see Huggins *et al.*, 1993, for review). However, since bosentan is a competitive antagonist (Clozel *et al.*, 1994) it is possible that the dose of bosentan was insufficient to inhibit fully the haemodynamic effects of this high dose of ET-1. Therefore, we assessed the effectiveness of bosentan against a lower dose of ET-1 (50 pmol kg^{-1}). Under these conditions, bosentan blocked all the

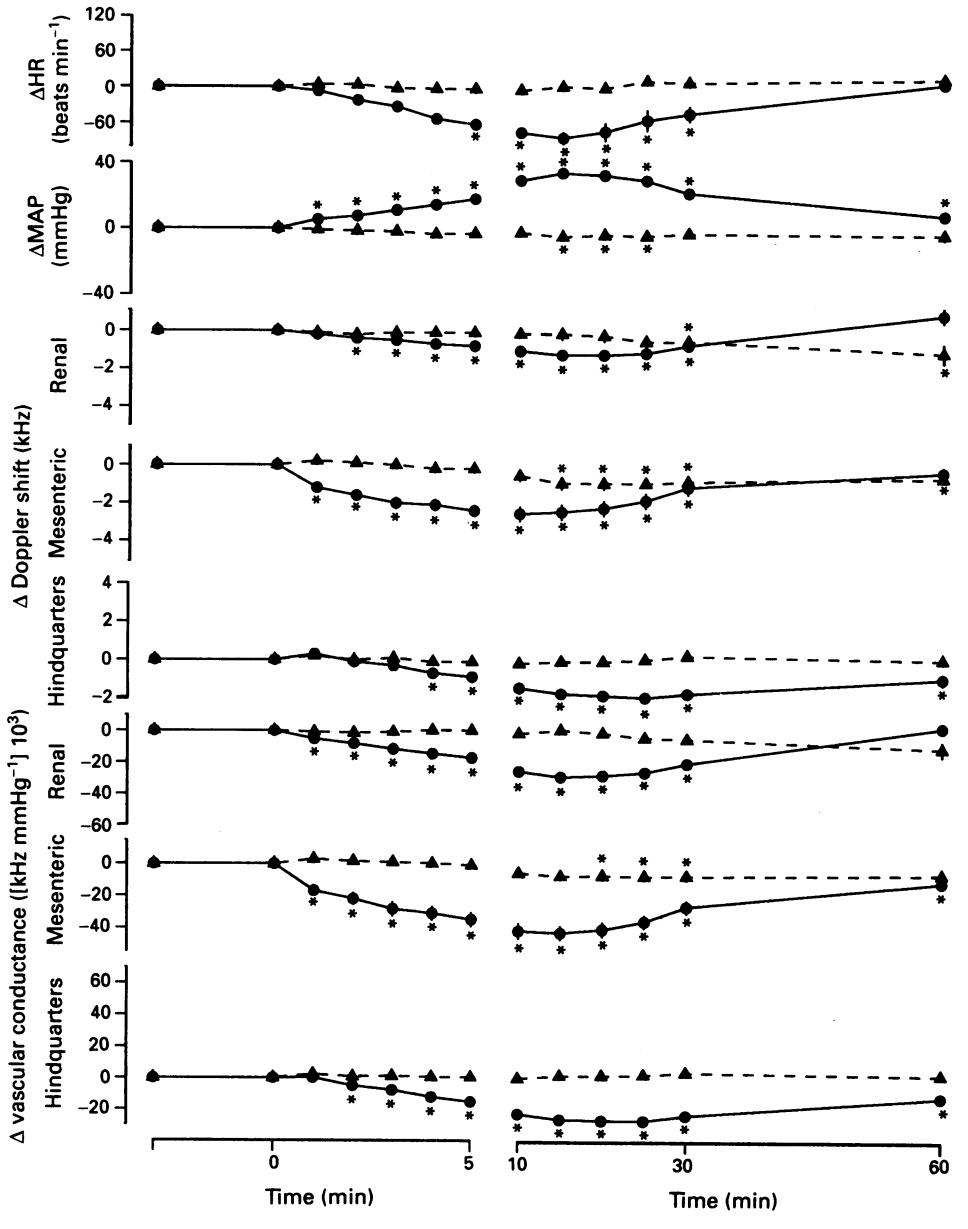

Figure 5 Cardiovascular responses to big endothelin-1 (big ET-1, 500 pmol kg^{-1}) in the absence (●) or presence (▲) of bosentan (30 mg kg^{-1}) in the same conscious, Long Evans rats ($n = 8$). Values are mean ± s.e.mean; $^*P < 0.05$ versus baseline. Statistics for the differences between the responses in the absence and presence of bosentan are given in the text and Table 2.

effects of ET-1, except for small reductions in mesenteric flow and vascular conductance. Thus, it seems likely that the majority of the effects of ET-1 were mediated through ET_A- and/or ET_B-receptors. However, we cannot dismiss an action of bosentan at other subtypes of ET receptors, in addition to ET_A- or ET_B-receptors. As mentioned above, it is feasible that, particularly at a dose of 500 pmol kg^{-1}, ET-1 activated other ET-receptor subtypes in addition to ET_A- and ET_B-receptors. Such a possibility is consistent with the finding that the maximum bradycardic effect of ET-1 was less inhibited by bosentan than was its maximum pressor effect (see Tables 2 and 3). Since the chronotropic actions of ET-1 are blocked by atropine and atenolol (Gardiner *et al.*, 1990a), it is feasible that any putative, non-ET_A-, non-ET_B-receptor-mediated influence of ET-1 on heart rate might be mediated through modulating cardiac autonomic control, possibly by an effect on baroreflex sensitivity.

Effects of bosentan on responses to ET-2 or ET-3

ET-2 and ET-3 had smaller initial depressor and hindquarters vasodilator effects than ET-1. Furthermore, they had more rapid onset vasoconstrictor actions in the mesenteric vascular bed than did ET-1, although this was not the case in the renal vascular bed. Thus, with the exception of the latter, we here, and previously (Gardiner *et al.*, 1990b, c,d), have not found *in vivo* functional evidence for more effective activation of vasodilator mechanisms, by ET- 3 or ET-2, than by ET-1.

In the majority of instances there was no evidence indicating that bosentan was less effective at inhibiting the actions of ET-2 or ET-3 than those of ET-1. Hence, our results are generally consistent with the proposal that the majority of the effects of ET-1, -2 and -3 are mediated through ET_A- and/or ET_B-receptors. The one apparent exception was the hindquarters hyperaemic vasodilator responses to ET-2 and ET-3 which, unlike those to ET-1, were not completely blocked by bosentan. However, these responses were so small their functional significance is doubtful.

Effects of bosentan on responses to big ET-1

All the effects of big ET-1 over the first 10 min after its administration were blocked by bosentan. Subsequently, even

in the presence of bosentan, there were slight reductions in mean arterial blood pressure and renal and mesenteric flows, and mesenteric vascular conductance. Considering the time course of these changes, it is likely they were due to the waning of the initial effects of bosentan (see Results), rather than to residual actions of big ET-1 in the presence of bosentan. Since bosentan so effectively blocked all the actions of big ET-1, it seems likely that the latter exerts its effects through ET_A- and/or ET_B-receptors. While this probably occurs after the local conversion of big ET-1 to ET-1 (Gardiner *et al.*, 1991), it has been suggested that unprocessed big ET-1 may also exert haemodynamic effects (Douglas & Hiley, 1991). If this is the case, then these actions appear to be mediated through ET_A- and/or ET_B-receptors, or possibly through another ET-receptor subtype that is antagonized by bosentan. Although it is theoretically possible that bosentan could have inhibited the haemodynamic effects of big ET-1 by preventing the generation of ET-1, Clozel *et al.* (1994) have reported that bosentan does not inhibit endothelin converting enzyme.

In conclusion, bosentan is an effective antagonist of the *in vivo* haemodynamic effects of ET-1, -2 and -3, and big ET-1 in conscious rats. Since previous studies have shown bosentan has no effect on responses to many substances, including catecholamines, prostanoids, angiotensin and vasopressin (Clozel *et al.*, 1994), this compound will be a powerful tool for discerning the putative role of endothelins in cardiovascular pathophysiology.

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