Br. J. Pharmacol. (1994), 112, 477-486

Effects of an ET_1-receptor antagonist, FR139317, on regional haemodynamic responses to endothelin-1 and [$Ala^{11,15}$]Ac-endothelin-1 (6-21) in conscious rats

[1]S.M. Gardiner, P.A. Kemp, J.E. March, T. Bennett, *A.P. Davenport & †L. Edvinsson

Department of Physiology & Pharmacology, University of Nottingham Medical School, Queen's Medical Centre, Nottingham NG7 2UH; *Department of Clinical Pharmacology, University of Cambridge and †Department of Experimental Research, University of Lund, Sweden

1 In conscious, Long Evans rats, chronically instrumented for the measurement of regional haemodynamics, we compared responses to the putative, selective ET_B-receptor agonist, [$Ala^{1,3,11,15}$]endothelin-1 (ET-1), obtained from two sources (Microchemical Laboratory, Cambridge (MLC) and Neosystem Laboratory, France (NLF)). [$Ala^{1,3,11,15}$]ET-1 (0.15, 0.3, 1 and 10 nmol kg^{-1}) from MLC caused dose-dependent pressor effects, accompanied by reductions in renal and, particularly, mesenteric flows and vascular conductances; there was an initial hyperaemic vasodilatation in the hindquarters which was not dose-dependent, and only with the highest dose was there a subsequent vasoconstriction. There was no significant initial depressor response to [$Ala^{1,3,11,15}$]ET-1 from MLC at any dose. However, the peptide from NLF exerted dose-dependent depressor and hindquarters vasodilator actions, and subsequent pressor effects. The difference between the two peptide preparations remains unexplained, but it appears that the [$Ala^{1,3,11,15}$]ET-1 from MLC may activate ET_B-receptors mediating vasoconstriction, more effectively than those mediating vasodilatation.

2 We also assessed responses to the putative ET_B-receptor agonist, [$Ala^{11,15}$]Ac-ET-1 (6-21) (BQ-3020), and determined the effects of the selective ET_A-receptor antagonist, FR139317, on responses to ET-1 and BQ-3020 at doses matched for their initial depressor and subsequent pressor effects (ET-1, 0.5 nmol kg^{-1}, BQ-3020, 10 nmol kg^{-1}), and also at doses that did not affect mean arterial blood pressure, but which were matched for their renal vasoconstrictor effects (ET-1, 7.5 pmol kg^{-1}; BQ-3020, 0.15 nmol kg^{-1}).

3 BQ-3020 (0.15, 0.3 and 1 nmol kg^{-1}) had dose-dependent pressor effects accompanied by reductions in renal and, particularly, mesenteric flows and vascular conductances. BQ-3020 at a dose of 10 nmol kg^{-1} elicited an initial depressor response which coincided with an attenuated mesenteric vasoconstrictor effect, accompanying a marked hindquarters vasodilatation. Hence, it appears that BQ-3020 may activate both vasoconstrictor and vasodilator ET_B-receptors.

4 FR139317 (0.5 μmol kg^{-1}) caused attenuation of the pressor, and renal, mesenteric and hindquarters vasoconstrictor effects of ET-1 (0.5 nmol kg^{-1}) and of BQ-3020 (10 nmol kg^{-1}), but the reductions of the pressor and renal vasoconstrictor effects of ET-1 were greater than those of BQ-3020. However, in the presence of FR139317, significant pressor and renal, mesenteric and hindquarters vasoconstrictor responses to ET-1 and BQ-3020 still occurred, and the initial depressor and hindquarters vasodilator responses to both peptides were unchanged.

5 ET-1 at a dose of 7.5 pmol kg^{-1} and BQ-3020 at a dose of 0.15 nmol kg^{-1} had similar renal vasoconstrictor effects. However, the response to ET-1 was almost abolished by FR139317 whereas that to BQ-3020 was unaffected. Moreover, under these conditions, the mesenteric vasoconstrictor and hindquarters vasodilator responses to ET-1 and to BQ-3020 were not changed by FR139317.

6 Collectively, these results are consistent with the haemodynamic effects of ET-1 and BQ-3020 involving ET_A-receptors or ET_B-receptors, or ET_A- and ET_B-receptors, depending on the dose of agonist and the regional haemodynamic effect considered.

Keywords: ET_A-receptors; ET_B-receptors; FR139317; regional haemodynamics in Long Evans rats

Introduction

It has been suggested that endothelin (ET) receptors can be classified into three prototypes, namely, ET_A-receptors that exist on vascular smooth muscle and are responsible for vasoconstriction, ET_B-receptors that exist on endothelial cells and are responsible for vasodilatation, and ET_C-receptors that exist on neurones (Masaki *et al.*, 1992). However, already there is evidence that ET_B-receptors may mediate vasoconstriction (e.g. Hiley *et al.*, 1989; Randall, 1991; Bigaud & Pelton, 1992; Clozel *et al.*, 1992; Gardiner *et al.*, 1992b; Moreland *et al.*, 1992; Cristol *et al.*, 1992; Douglas *et al.*, 1992; McMurdo *et al.*, 1993).

Recently, it was reported that the renal and coeliac vasoconstrictor effects of ET-1 in anaesthetized rats were resistant to antagonism by the ET_A-receptor antagonist, BQ-123 (Bigaud & Pelton, 1992; Cristol *et al.*, 1993; Pollock & Opgenorth, 1993). This observation is consistent with these effects of ET-1 being mediated by ET_B-receptors. However, the picture is confused by the finding that the renal and coeliac, but not the mesenteric, vasoconstrictor effects of the ET_B-receptor agonist, [$Ala^{1,3,11,15}$]ET-1 (Hiley *et al.*, 1990; Saeki *et al.*, 1991), were blocked by BQ-123 (Bigaud & Pelton, 1992). In addition, Bigaud & Pelton (1992) found that the mesenteric vasoconstrictor effect of ET-1 was significantly inhibited by BQ-123, whereas Douglas *et al.* (1992) found it was not. In order to determine if these anomalous findings pertain only to the use of [$Ala^{1,3,11,15}$]ET-1 and BQ-123 in anaesthetized rats we have assessed, in conscious rats, regional haemodynamic responses to [$Ala^{1,3,11,15}$]

[1] Author for correspondence.

ET-1 and to [Ala^{11,15}]Ac-ET-1 (6-21) (BQ-3020) (Ihara *et al.*, 1992; Molenaar *et al.*, 1992), which is also a selective ET_B-receptor agonist. In addition, we analysed the effect of the ET_A-receptor antagonist, FR139317 (i.e., ((**R**) 2-[(**R**)-2-[(**S**)-2[[1-(hexahydro-1H-azepinyl)]-carbonyl] amino-4-methyl-pentanoyl] amino-3-[3-(1-methyl-1H-indolyl)]propionyl]amino-3-(2-pyridyl) propionic acid) (Sun *et al.*, 1992; Sogabe *et al.*, 1993; Cardell *et al.*, 1993) on regional haemodynamic responses to doses of ET-1 and BQ-3020, matched for their initial depressor and subsequent pressor effects, or for their renal vasoconstrictor effects. Parts of this work have been presented to the British Pharmacological Society (Gardiner *et al.*, 1992b; 1993; Bennett *et al.*, 1993).

Methods

Male, Long Evans rats (350–450 g) were studied. Animals were anaesthetized (sodium methohexitone, Brietal, Lilly, 40–60 mg kg^{-1}, i.p., supplemented as required) and had pulsed Doppler flow probes (Haywood *et al.*, 1981) fitted around the left renal and superior mesenteric arteries, and the distal abdominal aorta (to monitor flow to the hindquarters) (Gardiner *et al.*, 1991; 1992a). At least 7 days later, rats were anaesthetized (sodium methohexitone, 40 mg kg^{-1}, i.p.) and a catheter was inserted into the distal abdominal aorta (via the ventral caudal artery) and three catheters were inserted into the right jugular vein. Catheters ran subcutaneously to emerge at the back of the neck at the same point as the probe wires; the latter were soldered into a microconnector (Microtech Inc, Boothwyn, U.S.A.) that was clamped into a harness fitted to the rat. A flexible spring was connected to the harness, and the catheters were threaded through this for protection. The spring was supported by a counterbalanced lever that allowed the animals free movement in their individual home cages in which they were left for at least 24 h with free access to food and water before experiments were begun. The experiments performed were as follows:-

Protocol 1: regional haemodynamic responses to [Ala^{1,3,11,15}]ET-1

Increasing i.v. bolus doses of [Ala^{1,3,11,15}]ET-1 (0.15, 0.3, 1 and 10 nmol kg^{-1}) were administered to seven Long Evans rats. The doses were separated by intervals of at least 1 h to allow all variables to return to baseline before the next dose was administered. The peptide used in these experiments was synthesized in the microchemical laboratory in Cambridge (MLC) and the results showed no significant depressor responses at any dose (see Results) whereas Bigaud & Pelton (1992) reported marked depressor responses to [Ala^{1,3,11,15}]ET-1. Since the experiments of Bigaud & Pelton (1992) were carried out in pentobarbitone-anaesthetized Sprague-Dawley rats, we also investigated the effect of the [Ala^{1,3,11,15}]ET-1 obtained from MLC under identical conditions; it had no depressor effect.

The [Ala^{1,3,11,15}]ET-1 used by Bigaud & Pelton (1992) was obtained from Neosystem Laboratory, France (NLF). Therefore, in a second group of rats ($n = 6$) we assessed haemodynamic responses to this peptide at doses of 0.15, 0.3, 1 and 10 nmol kg^{-1}. While we were able to confirm this peptide had dose-dependent initial depressor effects (see Results) these were very transient (duration about 30 s) in contrast to the effects reported by Bigaud & Pelton (1992) which lasted for at least 3 min. In order to determine if the depressor effect of the [Ala^{1,3,11,15}]ET-1 from NLF was more prolonged in the experimental model studied by Bigaud & Pelton (1992) we also investigated its effects in pentobarbitone-anaesthetized Sprague-Dawley rats ($n = 2$).

Protocol 2: regional haemodynamic responses to BQ-3020

Increasing bolus doses of BQ-3020 (0.1, 0.3, 1 and 10 nmol kg^{-1}) were administered to another eight Long Evans rats, using the same dosing schedule as above.

Protocol 3: regional haemodynamic responses to ET-1 or BQ-3020 before and after FR139317

From the above experiments, we determined that the initial depressor and subsequent pressor effects of ET-1 at a dose of 0.5 nmol kg^{-1}, and BQ-3020 at a dose of 10 nmol kg^{-1}, were similar. Therefore, we then assessed (in the same animals, but on different days) regional haemodynamic responses to ET-1 or BQ-3020 at these doses before and 5 min after FR139317 at a dose of 0.5 μmol kg^{-1}. This dose was chosen because higher doses of FR139317 had pressor effects, and did not cause any greater inhibition of responses to ET-1 or BQ-3020 than did the dose of 0.5 μmol kg^{-1}.

Six of the animals that underwent this protocol came from the group of eight studied in protocol 2 (on another experimental day). An additional two animals were investigated to make $n = 8$ for the assessment of the effects of FR139317 on responses to ET-1 or BQ-3020. Four extra animals, together with some of those in this protocol (i.e. protocol 3) were also investigated on another day to determine the reproducibility of responses to ET-1 ($n = 8$) or BQ-3020 ($n = 7$) before and after saline administration (0.1 ml), to control for the effect of FR139317 vehicle.

From our initial findings (see Results) it appeared that some of the effects of the 10 nmol kg^{-1} dose of BQ-3020 were inhibited by FR139317. This raised the possibility that this relatively high dose of BQ-3020 was acting on ET_A-receptors. Therefore, in a separate group of eight rats we assessed responses to low doses of ET-1 (7.5 pmol kg^{-1}) and BQ-3020 (0.15 nmol kg^{-1}), before and after saline, and before and after FR139317 (0.5 μmol kg^{-1}) administration. The doses of ET-1 and BQ-3020 were matched for their renal vasoconstrictor effects.

Drugs and peptides

ET-1 was obtained from the Peptide Institute (Osaka, Japan) through their UK agents (Scientific Marketing Associates); FR139317 was a gift from Dr Jo Mori (Fujisawa Pharmaceutical Co., Osaka, Japan). [Ala^{1,3,11,15}]ET-1 and BQ-3020, obtained from MLC, were preparaed by solid phase t-Boc chemistry, purified by gel filtration, and the sequence confirmed by microsequence analysis; peptide concentration was determined by u.v. spectrophotometry. Peptide purity was confirmed by analytical high performance liquid chromatography using a Vydac C18 column. The gradient was 30% acetonitrile in water containing 0.1% trifluoroacetic acid at time 0 to 38% acetonitrile in water (0.1% trifluoroacetic acid) after 45 min. For each MLC peptide, a single peak was found using spectrophotometric detection at 206 nm.

All substances were dissolved in sterile isotonic saline (154 nmol l^{-1} NaCl) containing 1% bovine serum albumin (Sigma). Bolus injections were given in a volume of 100 μl and flushed in with 150 μl of isotonic saline.

Data analysis

Throughout an experimental protocol, continuous recordings were made of instantaneous heart rate, phasic and mean arterial blood pressures, and phasic and mean Doppler shift signals. Percentage changes in mean Doppler shift signals were taken as an index of flow changes, and the corresponding % changes in vascular conductances were calculated by dividing the mean Doppler shift signal by the mean arterial blood pressure (Gardiner *et al.*, 1991; 1992a).

Responses to [Ala^{1,3,11,15}]ET-1, BQ-3020, or ET-1 were

assessed from the values measured at 0.25, 0.5, 0.75, 1, 2, 3, 4 and 5 min; these responses were analysed by Friedman's test applied to the changes relative to the pretreatment baseline. Comparisons of the responses to [$Ala^{1,3,11,15}$]ET-1 or BQ-3020 under different conditions were made by the Mann-Whitney U test applied to integrated responses (i.e. areas under or over curves; AUC and AOC 0–5 min, respectively). The effects of FR139317 on responses to ET-1 or BQ-3020 were assessed by comparing integrated responses in the absence and presence of FR139317 by Wilcoxon's test. A value of $P<0.05$ was taken as significant.

Results

Regional haemodynamic responses to [$Ala^{1,3,11,15}$]ET-1

The injection of [$Ala^{1,3,11,15}$]ET-1 obtained from MLC caused dose-dependent pressor effects and bradycardia. There was an initial, variable tachycardia (Figures 1 and 2), but there was not a consistent, initial hypotensive response to [$Ala^{1,3,11,15}$]ET-1 at doses of 0.15, 0.3 or 1 nmol kg^{-1} (Figure 1). Even at a dose of 10 nmol kg^{-1}, the peptide did not have an early depressor effect in all animals, and in those animals in which such a response occurred it was slight, and so brief (Figure 2) that it had gone by the first post-injection measurement (i.e. 0.25 min; Figure 1). [$Ala^{1,3,11,15}$]ET-1 from MLC in doses as high as 30 nmol kg^{-1}, in pentobarbitone-anaesthetised Sprague-Dawley rats (i.e., the model studied by Bigaud & Pelton (1992)), caused no initial depressor response (data not shown).

The pressor effects of [$Ala^{1,3,11,15}$]ET-1 obtained from MLC were accompanied by dose-dependent reductions in renal and mesenteric flows and vascular conductances; the changes in the mesenteric vascular bed were greatest (Figures 1 and 2). In the hindquarters vascular bed there was an initial hyperaemia and vasodilatation, although the latter was not dose-dependent (Figures 1 and 2). Only with the highest dose of [$Ala^{1,3,11,15}$]ET-1 was there a subsequent hindquarters vasoconstriction (Figure 1).

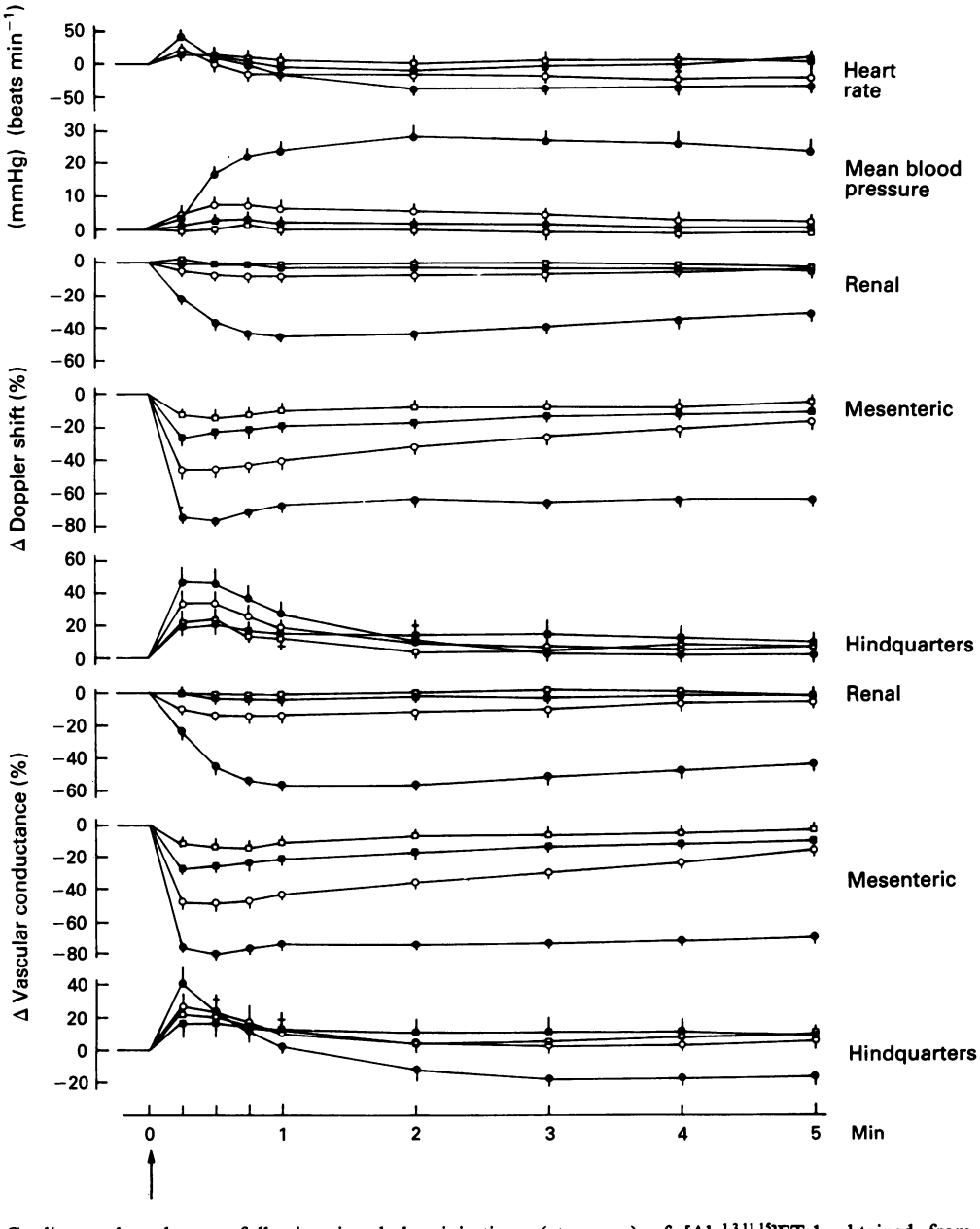

Figure 1 Cardiovascular changes following i.v. bolus injections (at arrow) of [$Ala^{1,3,11,15}$]ET-1 obtained from MLC (□, 0.15 nmol kg^{-1}; ■, 0.3 nmol kg^{-1}; ○, 1 nmol kg^{-1}; ●, 10 nmol kg^{-1}) in the same conscious Long Evans rats ($n = 7$). Values are mean with s.e.mean.

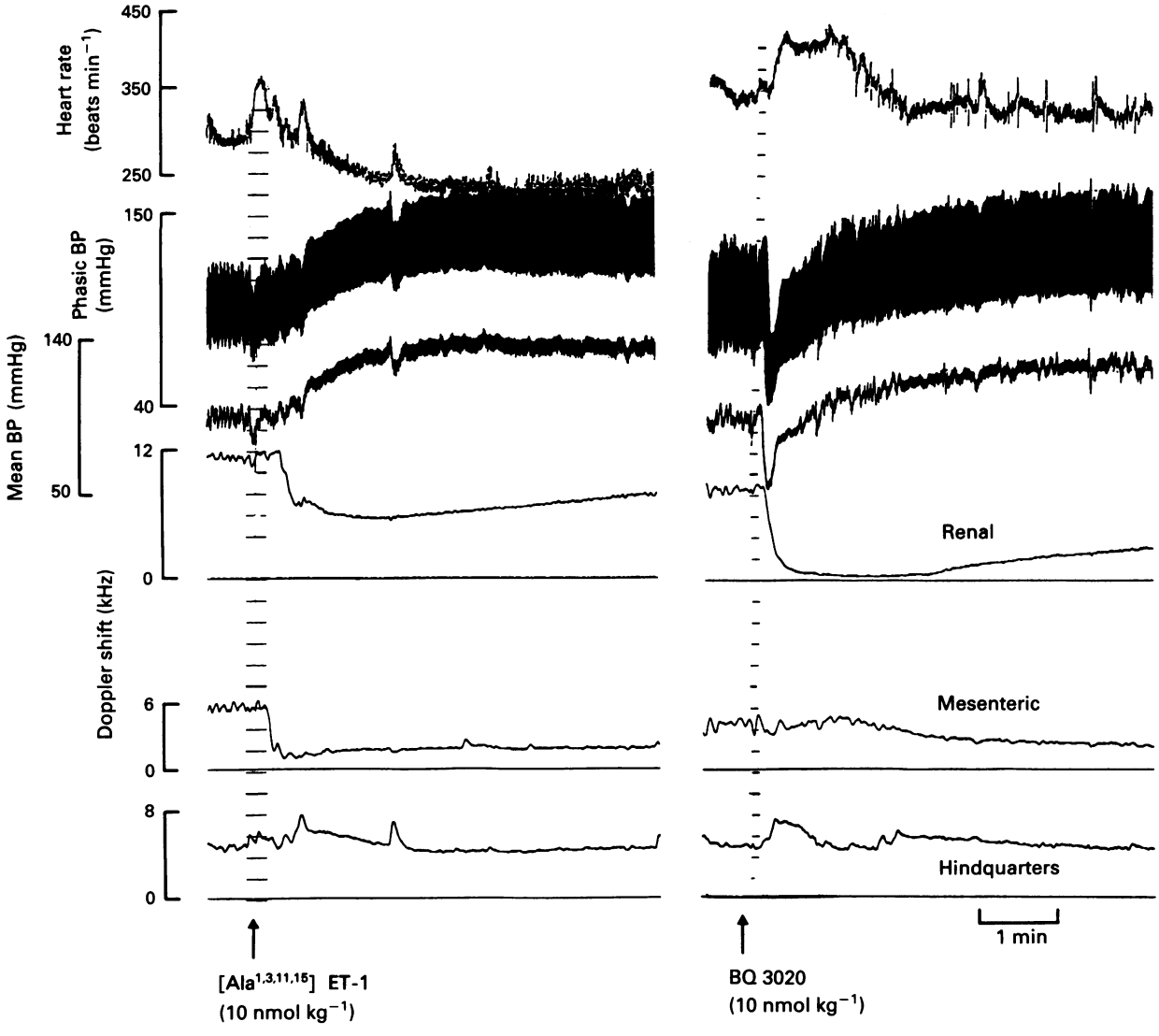

Figure 2 Cardiovascular changes following i.v. injection of [Ala^{1,3,11,15}]ET-1 or [Ala^{11,15}]Ac-ET-1 (6–21) (i.e., BQ-3020) (both from MLC) (10 nmol kg^{-1}) in conscious Long Evans rats.

[Ala^{1,3,11,15}]ET-1 obtained from NLF exerted initial, dose-dependent depressor effects accompanied by hindquarters hyperaemic vasodilatation, and reductions in renal and mesenteric flows and conductances (Figure 3). Thereafter, there was a variable pressor effect and hindquarters vasoconstriction (Figure 3). Although this pattern of effect was more similar to that observed by Bigaud & Pelton (1992) than that seen with [Ala^{1,3,11,15}]ET-1 obtained from MLC (see above), the depressor effect of the peptide from NLF was very transient (i.e., lasting about 30 s) in contrast to the findings of Bigaud & Pelton (1992) (i.e., a depressor effect lasting at least 3 min). The depressor effect of [Ala^{1,3,11,15}]ET-1 from NLF was no more prolonged in pentobarbitone-anaesthetized Sprague-Dawley rats (data not shown).

Regional haemodynamic responses to BQ-3020

BQ-3020 at doses of 0.15, 0.3 and 1 nmol kg^{-1} caused increasing pressor effects with no initial hypotensive response (Figure 4). However, at a dose of 10 nmol kg^{-1}, BQ-3020 had a substantial early depressor effect (Figures 2 and 4) which opposed its subsequent pressor action (Figures 2 and 4). The heart rate changes following BQ-3020, were not dose-dependent (Figures 2 and 4).

The pressor effects of BQ-3020 were accompanied by dose-dependent reductions in renal flow and vascular conductance (Figure 4). There were also dose-dependent reductions in mesenteric flow and vascular conductance with BQ-3020 at doses of 0.15, 0.3 and 1 nmol kg^{-1}, and over this dose-range the effects in the mesenteric vascular bed were greater than those in the kidney (Figure 4). However, with the 10 nmol kg^{-1} dose of BQ-3020, the reduction in mesenteric flow and vascular conductance was delayed (Figures 2 and 3), such that the integrated response was not different from that seen with BQ-3020 at a dose of 1 nmol kg^{-1}. Moreover, the mesenteric vascular response to the highest dose of BQ-3020 was less than that seen in the renal vascular bed (Figures 2 and 4).

In the hindquarters vascular bed, there was an initial, hyperaemic vasodilator response to BQ-3020; the effect on hindquarters vascular conductance was greatest with the highest dose of BQ-3020 (Figure 4). There was a subsequent hindquarters vasoconstriction, although the flow changes were variable (Figure 4).

Regional haemodynamic responses to ET-1 and BQ-3020 in the absence and presence of FR139317

ET-1 (0.5 nmol kg^{-1}) caused an initial hypotension and tachycardia followed by a pressor effect and bradycardia

(Figure 5, Table 1). There were reductions in renal and mesenteric flows and vascular conductances and an initial increase, and subsequent decrease, in hindquarters flow and vascular conductance (Figure 5, Table 1).

In the presence of FR139317 (0.5 μmol kg^{-1}) the pressor, and renal, mesenteric and hindquarters vasoconstrictor effects of ET-1 were significantly reduced (Figure 5, Table 1). However, the initial depressor and hindquarters vasodilator effects

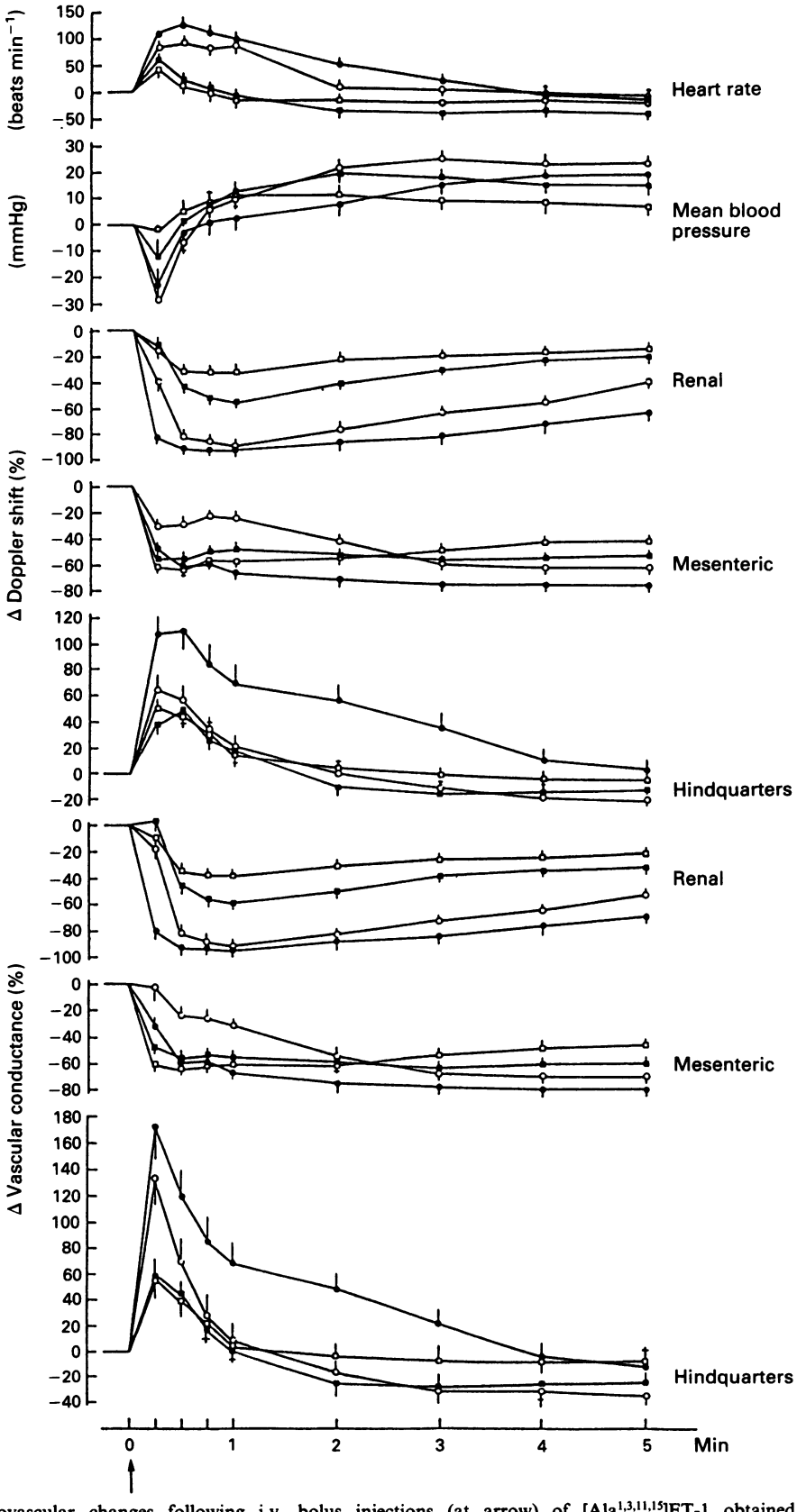

Figure 3 Cardiovascular changes following i.v. bolus injections (at arrow) of [$Ala^{1,3,11,15}$]ET-1 obtained from NLF (□, 0.15 nmol kg^{-1}; ■, 0.3 nmol kg^{-1}; ○, 1 nmol kg^{-1}; ●, 10 nmol kg^{-1}) in the same conscious Long Evans rats ($n = 7$). Values are mean with s.e.mean.

of ET-1 were not affected significantly by FR139317 although the duration of the former tended to be increased (Figure 5, Table 1).

FR139317 also attenuated the pressor and renal, mesenteric and hindquarters vasoconstrictor effects of BQ-3020 (10 nmol kg^{-1}) while leaving its depressor and hindquarters vasodilator action intact (Figure 6, Table 1). The inhibitory effects of FR139317 on the pressor and renal vasoconstrictor actions of BQ-3020 were significantly less than on those of ET-1, whereas the inhibition of the mesenteric vasoconstriction and of the hindquarters vasoconstriction were not significantly different from ET-1 and BQ-3020 (Table 1). In

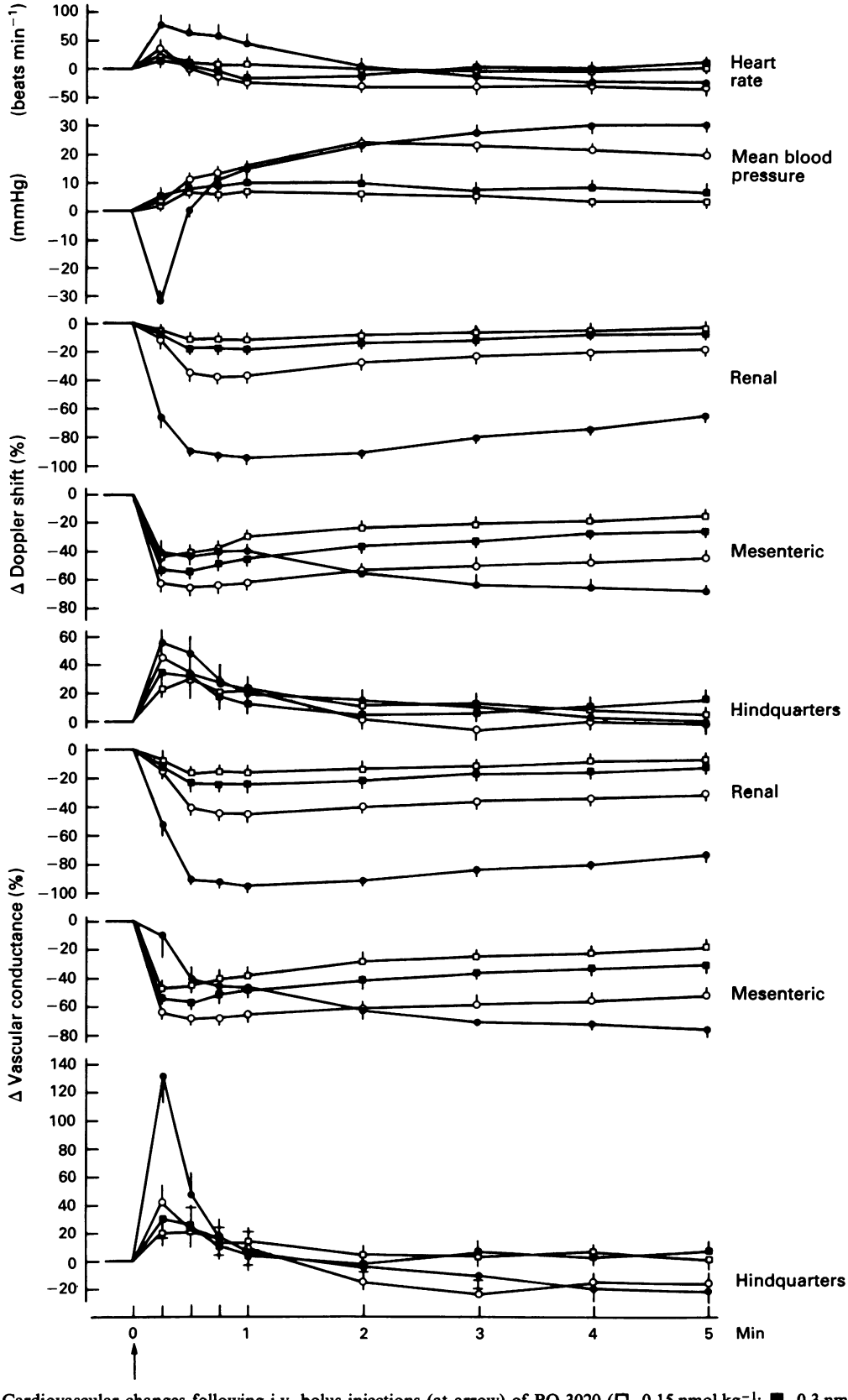

Figure 4 Cardiovascular changes following i.v. bolus injections (at arrow) of BQ-3020 (□, 0.15 nmol kg^{-1}; ■, 0.3 nmol kg^{-1}; ○, 1 nmol kg^{-1}; ●, 10 nmol kg^{-1}) in the same conscious Long Evans rats ($n = 8$). Values are mean with s.e.mean.

the presence of FR139317, both ET-1 and BQ-3020 still elicited significant haemodynamic effects (Figures 5 and 6, Table 1).

With repeated administration of ET-1 or BQ-3020 in the presence of saline, rather than FR139317, there were no significant changes in haemodynamic responses (data not shown).

As explained in Methods, we carried out an additional experiment to determine if FR139317 (0.5 μmol kg^{-1}) affected the haemodynamic responses to low doses of ET-1 or BQ-3020. ET-1 at a dose of 7.5 pmol kg^{-1} had no significant effect on mean arterial blood pressure, but it caused significant reductions in renal and mesenteric flows (AOC, -43 ± 9 and -50 ± 4% min, respectively) and vascular conductances (AOC, -44 ± 10 and -50 ± 5% min, respectively), and significant increases in hindquarters flow (AUC, 61 ± 10% min) and vascular conductance (65 ± 22% min). In the presence of FR139317, the ET-1-induced reductions in renal flow (AOC, -10 ± 4% min) and vascular conductance (AOC -9 ± 3% min) were significantly attenuated, but the reductions in mesenteric flow (AOC, -60 ± 5% min) and vascular conductance (AOC, -54 ± 7% min), and the increases in hindquarters flow (AUC, 47 ± 17% min) and vascular conductance (AUC, 55 ± 18% min) were not different from those seen in the absence of FR139317.

In this experiment, BQ-3020 at a dose of 0.15 nmol kg^{-1} had similar haemodynamic effects to those in the previous experiment (renal flow, AOC -43 ± 7; mesenteric flow,

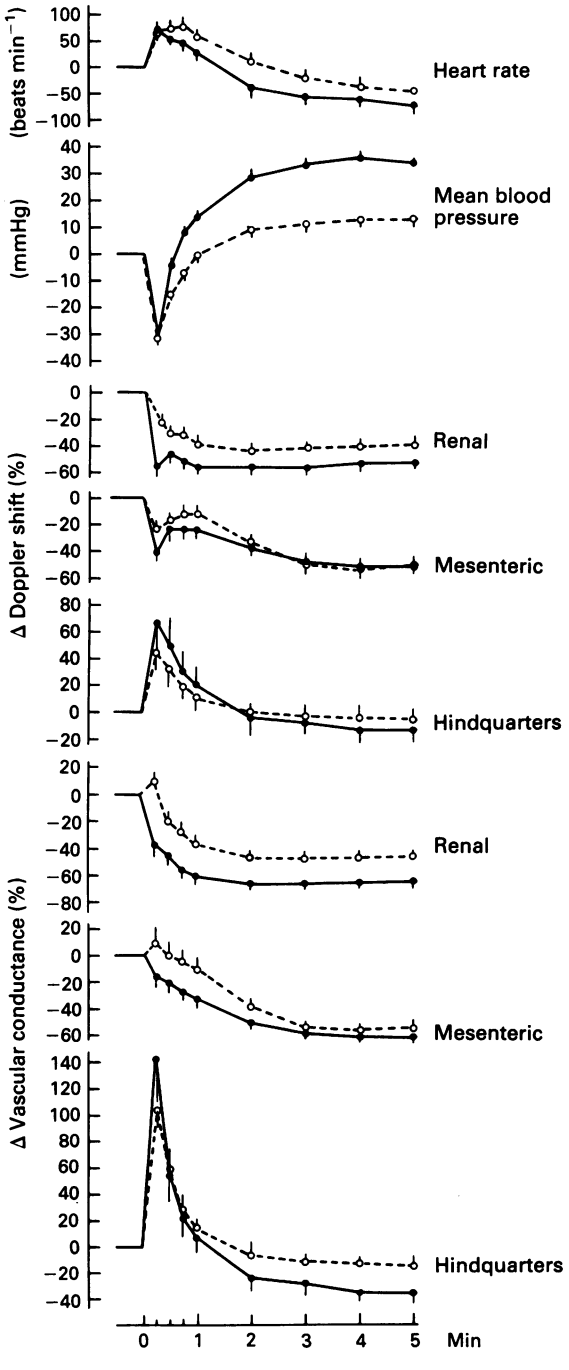

Figure 5 Cardiovascular changes in response to endothelin-1 (0.5 nmol kg^{-1}) in the absence (●) or presence (○) of FR139317 (0.5 μmol kg^{-1}) in the same conscious, Long Evans rats ($n = 8$). The statistics for the differences in the integrated responses are shown in Table 1. Values are mean with s.e.mean.

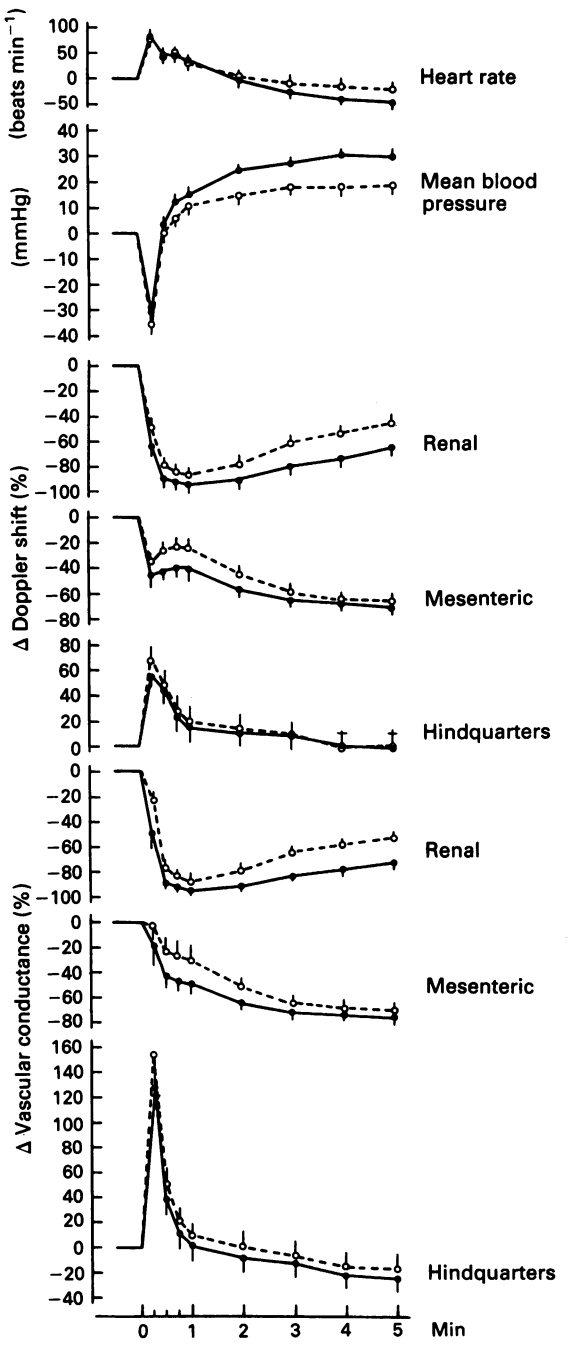

Figure 6 Cardiovascular changes in response to BQ-3020 (10 nmol kg^{-1}) in the absence (●) or presence (○) of FR139317 (0.5 μmol kg^{-1}) in the same conscious, Long Evans rats ($n = 8$). The statistics for the differences in the integrated responses are shown in Table 1. Values are mean with s.e.mean.

Table 1 Integrated cardiovascular responses (areas under or over curves; AUC, $AOC_{0-5\,min}$) following bolus injection of endothelin-1 (ET-1, 0.5 nmol kg^{-1}) or BQ-3020 (10 nmol kg^{-1}) in the absence and presence of FR139317 (0.5 μmol kg^{-1}) in the same conscious Long Evans rats

	ET-1	*ET-1 + FR139317*	*BQ-3020*	BQ3020 + *FR139317*
Heart rate (AUC; beats)	72 ± 17	107 ± 13	90 ± 15	101 ± 29
Heart rate (AOC; beats)	− 213 ± 52	− 101 ± 21	− 108 ± 33	− 66 ± 33
Mean blood pressure (AOC; mmHg min)	− 14 ± 1	− 18 ± 2	− 13 ± 1	− 15 ± 2
Mean blood pressure (AUC; mmHg min)	126 ± 7	40 ± 5*	113 ± 10	72 ± 9*
Renal flow (AOC; % min)	− 269 ± 16	− 190 ± 9*	− 405 ± 13	− 323 ± 7*
Mesenteric flow (AOC; % min)	− 202 ± 16	− 187 ± 13*	− 289 ± 23	− 244 ± 25*
Hindquarters flow (AUC; % min)	83 ± 39	54 ± 19	100 ± 49	97 ± 31
Hindquarters flow (AOC; % min)	− 65 ± 20	− 28 ± 11*	− 38 ± 16	− 28 ± 14
Renal conductance (AOC; % min)	− 305 ± 17	− 198 ± 9*	− 417 ± 12	− 355 ± 9*
Mesenteric conductance (AOC; % min)	− 245 ± 15	− 195 ± 12*	− 314 ± 22	− 259 ± 25*
Hindquarters conductance (AUC; % min)	76 ± 29	67 ± 16	81 ± 37	91 ± 27
Hindquarters conductance (AOC; % min)	− 120 ± 26	− 48 ± 14*	− 92 ± 23	− 57 ± 21*

$n = 8$
*$P < 0.05$ versus corresponding response in the absence of FR139317 (Wilcoxon's test).

AOC − 153 ± 20; hindquarters flow, AUC 56 ± 8; renal vascular conductance, AOC − 54 ± 8; mesenteric vascular conductance, AOC − 161 ± 21; hindquarters vascular conductance, AUC 49 ± 5% min). In the presence of FR139317, the responses to BQ-3020 were not changed significantly (renal flow, AOC − 41 ± 8; mesenteric flow, AOC − 146 ± 13; hindquarters flow, AUC 56 ± 13; renal vascular conductance, AOC − 53 ± 10; mesenteric vascular conductance, AOC − 157 ± 14; hindquarters vascular conductance, AUC 49 ± 12% min).

Discussion

Our objectives were: (1) to determine if the regional haemodynamic effects of [$Ala^{1,3,11,15}$]ET-1 in conscious rats were as described for anaesthetized rats; (2) to ascertain if BQ-3020 had effects similar to [$Ala^{1,3,11,15}$]ET-1; and (3) to delineate effects of FR139317 on responses to ET-1 and BQ-3020 in order to assess the possible involvement of ET_A- and ET_B-receptors, and to compare the results with those obtained by others using the ET_A-receptor antagonist, BQ-123, in anaesthetized rats.

Regional haemodynamic responses to [$Ala^{1,3,11,15}$]ET-1 and BQ-3020

Bigaud & Pelton (1992) recently described the regional haemodynamic effects of [$Ala^{1,3,11,15}$]ET-1 in pentobarbitone-anaesthetized Sprague-Dawley rats, acutely instrumented with pulsed Doppler flow probes. They found that [$Ala^{1,3,11,15}$]ET-1 obtained from NLF caused dose-dependent, early depressor, and subsequent pressor, effects accompanied by renal and mesenteric vasoconstriction; there was an early hindquarters vasodilatation, but no subsequent vasoconstriction, and no change in heart rate at any stage. Our results with [$Ala^{1,3,11,15}$]ET-1 obtained from NLF showed a similar pattern of change in regional haemodynamics. However, under all experimental conditions we found the depressor effect of the peptide was very transient (about 30 s) in contrast to the long duration (at least 3 min) of the effect described by Bigaud & Pelton (1992); we cannot explain this difference. The [$Ala^{1,3,11,15}$]ET-1 obtained from MLC was confirmed in structure and purity, i.e., no shorter analogues or contaminants were detectable. Thus, the finding that this peptide caused only modest hindquarters vasodilatation and was completely devoid of an initial depressor effect in doses up to 30 nmol kg^{-1} indicates it activated ET_B-receptors mediating vasoconstriction more effectively than ET_B-receptors mediating vasodilatation. This is consistent with the recent report from Gray & Clozel (1994) showing that the mixed, non-peptide endothelin antagonist, bosentan, distinguished between those ET_B-receptors on vascular smooth muscle and those on endothelial cells. However, the reason for the difference between the effects of [$Ala^{1,3,11,15}$]ET-1 from MLC and NLF is unknown.

Neither [$Ala^{1,3,11,15}$]ET-1 obtained from MLC or NLF, nor BQ-3020, showed any signs of preferential activation of vasodilator responses, except in the hindquarters vascular bed. The 3 nmol kg^{-1} dose of [$Ala^{1,3,11,15}$]ET-1 (from NLF) and the 10 nmol kg^{-1} dose of BQ-3020 caused less marked initial mesenteric vasoconstriction than was seen with lower doses, possibly due to recruitment of an opposing vasodilator mechanism (see below). However, such a phenomenon was not apparent with [$Ala^{1,3,11,15}$]ET-1 obtained from MLC. Nonetheless, collectively, the results are consistent with ET_B-receptors being responsible for renal, and mesenteric vasoconstrictor, and hindquarters vasodilator, responses to [$Ala^{1,3,11,15}$]ET-1 and BQ-3020, but these observations, particularly with higher doses of the peptides, do not exclude the involvement of ET_A-receptors, or other receptor sub-types.

Effects of FR139317 on responses to ET-1 and BQ-3020

Since, in our experiments, [$Ala^{1,3,11,15}$]ET-1 from MLC or NLF did not elicit a pattern of haemodynamic responses entirely like that of ET-1, we compared the effects of the ET_A-receptor antagonist, FR139317, on responses to ET-1

and BQ-3020, both at doses matched for their initial depressor and subsequent pressor effects, and at doses matched for their renal vasoconstrictor effects.

FR139317 (0.5 μmol kg^{-1}) abolished the renal vasoconstrictor effect of ET-1 (7.5 pmol kg^{-1}), but did not change its mesenteric vasoconstrictor or hindquarters vasodilator action. Moreover, the renal and mesenteric vasoconstrictor, and hindquarters vasodilator, effects of BQ-3020 (0.15 nmol kg^{-1}) were all uninfluenced by FR139317. These results are consistent with the renal vasoconstriction caused by low doses of ET-1 being mediated through ET_A-receptors, while ET_B-receptors (or other receptor subtypes) appear to be responsible for the mesenteric vasoconstrictor and hindquarters vasodilator action of ET-1, and for all the effects of BQ-3020 at a low dose.

Recent studies, in anaesthetized rats, have reported that the renal vasoconstrictor effects of ET-1 are not influenced by the ET_A-receptor antagonist, BQ-123 (Bigaud & Pelton, 1992; Cristol *et al.*, 1993; Pollock & Opgenorth, 1993). However, Bigaud & Pelton (1992) used a higher dose of ET-1 than we did, and hence these effects may have been harder to antagonize; this proposition is consistent with the finding that BQ-123 had some inhibitory effect on the renal vasoconstrictor response to ET-1 at a dose of 0.1 nmol kg^{-1} (Cristol *et al.*, 1993). Since Pollock & Opgenorth (1993) used infusions of ET-1, it is feasible that steady-state responses to the peptide are more dependent on ET_B-receptors. It is also possible that FR139317 is more effective than BQ-123 in blocking ET_A receptors, possibly because the physicochemical properties of a modified linear tripeptide (FR139317) may confer greater stability and/or bioavailability compared to BQ-123.

However, it could also be argued that FR139317 is less selective for ET_A-receptors than is BQ-123; but the lack of effect of FR139317 against the responses to BQ-3020 (0.15 nmol kg^{-1}) argues against this, at least as far as ET_B-receptors are concerned. By the same token, the failure of FR139317 to influence the mesenteric vasoconstrictor or hindquarters vasodilator effects of low doses of ET-1 or BQ-3020 is consistent with these actions being mediated by ET_B-receptors. It is notable that the hindquarters vasodilator effect of BQ-3020 was not different from that of ET-1 whereas its mesenteric vasoconstrictor effect was much more marked. This, together with the lack of dose-dependency of the hindquarters vasodilator effect of BQ-3020 indicates that mechanisms other than, or in addition to, activation of ET_B-receptors may be involved in the responses in the two vascular beds.

A different pattern of effects of FR139317 was seen when responses to higher doses of ET-1 (0.5 nmol kg^{-1}) and BQ-3020 (10 nmol kg^{-1}) were studied. FR139317 (0.5 μmol kg^{-1}) caused 66% inhibition of the pressor effects of ET-1 (0.5 nmol kg^{-1}) and 36% inhibition of the pressor effects of BQ-3020 (10 nmol kg^{-1}). Considering the results above, indicating that FR139317 (0.5 μmol kg^{-1}) does not inhibit ET_B-receptor-mediated events, it is likely that the inhibitory actions of FR139317 on the pressor effect of BQ-3020 was due to the high dose of the latter acting on ET_A-receptors, although we cannot exclude the possibility that both FR 139317 and BQ-3020 interact with non-ET_A- and non-ET_B-receptors.

The fact that FR139317 had a significantly greater inhibitory effect on the pressor action of ET-1 than on that of BQ-3020 is consistent with a predominance of ET_A-receptors in the former response, but, even with ET-1, a significant pressor response still occurred in the presence of FR139317. These results are consistent with a variable component of the pressor response to ET-1 and BQ-3020 being due to ET_B-receptors, depending on the agonist, and are consistent with the findings of Bigaud & Pelton (1992) and Cristol *et al.* (1993). As mentioned in Methods, a 10 fold higher dose of FR139317 had no greater inhibitory effect on responses to ET-1 or BQ-3020, so our results were probably not due to incomplete antagonism of ET_A-receptors.

FR139317 also caused greater inhibition of the renal vasoconstrictor effect of ET-1 (35%) than of BQ-3020 (19%), indicating differential involvement of ET_A- and ET_B-receptors. Our results, showing total loss of the renal vasoconstrictor effects of a low dose of ET-1, and a significant inhibition of the renal vasoconstrictor effect of a high dose of ET-1, are consistent with a predominance of ET_B-receptors in this latter event, compared to an exclusive involvement of ET_A-receptors in the former. The slight inhibitory effect of FR139317 on the renal vasoconstrictor response to BQ-3020 is consistent with the latter agonist influencing ET_A- as well as ET_B-receptors at high doses.

The small inhibition of the mesenteric vasoconstrictor effects of high doses of ET-1 and BQ-3020 by FR139317 indicates a slight involvement of ET_A-receptors. Since at low doses, these agonists caused mesenteric vasoconstriction that was unaffected by FR139317, then both results are consistent with a preponderance of ET_B-receptors in this action (see also Bigaud & Pelton, 1992). It is notable that at low doses, ET-1 appeared to act selectively on ET_A-receptors in the kidney and on ET_B-receptors in the mesenteric vascular bed, possibly because of differences in receptor populations.

Inhibition of the late hindquarters vasoconstrictor effects of ET-1 and BQ-3020 by FR139317 was not significantly different; assuming the effect of FR139317 on the response to BQ-3020 was due to the latter acting on ET_A-receptors, it appears about half the hindquarters vasoconstrictor effect of ET-1 and BQ-3020 was due to stimulation of ET_B-receptors. In contrast, the whole of the hindquarters vasodilator (and depressor) effect of ET-1 and BQ-3020 was uninfluenced by FR139317, consistent with this being entirely dependent on ET_B-receptors. But, as mentioned above, the hindquarters vasodilator response was not clearly dose-dependent, although, at the highest dose of BQ-3020, there was a marked increase in the rise in calculated hindquarters vascular conductance that it caused. However, this was due to the occurrence of an initial depressor effect coinciding with a relative attenuation of the mesenteric vasoconstriction. Thus, at higher doses it may be that BQ-3020 (and ET-1) activate additional (possibly non ET_A-, non ET_B-receptor-mediated) vasodilator mechanisms.

In summary, one explanation of our results is that, at a low dose (7.5 pmol kg^{-1}), ET-1 activates renal ET_A-receptors (vasoconstriction), mesenteric ET_B-receptors (vasoconstriction) and hindquarters ET_B-receptors (vasodilatation). At a higher dose (0.5 nmol kg^{-1}), ET-1 has a pressor effect (dependent on ET_A- $>$ ET_B-receptors), and vasoconstrictor effects in the renal (ET_B- $>$ ET_A-receptors), mesenteric (ET_B- $>>$ ET_A-receptors) and hindquarters (ET_A- $\geq$ ET_A-receptors) vascular beds, together with an initial depressor and hindquarters vasodilator effect (ET_B-receptors only).

BQ-3020 at a low dose (0.5 nmol kg^{-1}) causes renal and mesenteric vasoconstriction (ET_B-receptors), and hindquarters vasodilatation (ET_B-receptors). At a dose of 10 nmol kg^{-1}, BQ-3020 has a pressor effect (ET_B $>$ ET_A-receptors) and causes vasoconstriction in renal (ET_B- $>>$ ET_A-receptors), mesenteric (ET_B- $>>$ ET_A-receptors) and hindquarters (ET_B- $\geq$ ET_A-receptors) vascular beds preceded by an early depressor and hindquarters vasodilator effect (ET_B-receptors). However, it is feasible that some, or all, the effects here attributed to ET_B-receptors involve, to varying extents, subtypes of ET_B-receptors and/or ET-receptors that are not of the ET_A- or ET_B-receptor subtype.

References

BENNETT, T., GARDINER, S.M., KEMP, P.A., DAVENPORT, A.P. & EDVINSSON, L. (1993). Influence of an ET_A-receptor antagonist on regional haemodynamic responses to endothelin-1 (ET-1) and [$Ala^{11,15}$]Ac-ET-1(6-21) in conscious rats. *Br. J. Pharmacol.*, **109**, 57p.

BIGAUD, M. & PELTON, J.T. (1992). Discrimination between ET_A- and ET_B-receptor-mediated effects of endothelin-1 and [$Ala^{1,3,11,15}$] endothelin-1 by BQ-123 in the anaesthetized rat. *Br. J. Pharmacol.*, **107**, 912–918.

CARDELL, L.O., UDDMAN, R. & EDVINSSON, L. (1993). A novel ET_A-receptor antagonist, FR139317, inhibits endothelin-induced contractions of guinea-pig pulmonary arteries, but not trachea. *Br. J. Pharmacol.*, **108**, 448–452.

CLOZEL, M., GRAY, G.A., BREU, V., LÖFFLER, B.-M. & OSTERWALDER, R. (1992). The endothelin ET_B-receptor mediates both vasodilatation and vasoconstriction *in vivo*. *Biochem. Biophys. Res. Commun.*, **186**, 867–873.

CRISTOL, J.-P., WARNER, T.D., THIEMERMANN, C. & VANE, J.R. (1993). Mediation via different receptors of the vasoconstrictor effects of endothelins and sarafotoxins in the systemic circulation and renal vasculature of the anaesthetized rat. *Br. J. Pharmacol.*, **108**, 776–779.

DOUGLAS, S.A., ELLIOT, J.D. & OHLSTEIN, E.H. (1992). Regional vasodilataton to endothelin-1 is mediated by a non ET_A receptor subtype in the anaesthetized rat: effect of BQ-123 on systemic haemodynamic responses. *Eur. J. Pharmacol.*, **221**, 315–324.

GARDINER, S.M., COMPTON, A.M., KEMP, P.A. & BENNETT, T. (1991). The effects of phosphoramidon on the regional haemodynamic responses to human proendothelin [1-38] in conscious rats. *Br. J. Pharmacol.*, **103**, 2009–2015.

GARDINER, S.M., KEMP, P.A. & BENNETT, T. (1992a). Inhibition by phosphoramidon of the regional haemodynamic effects of proendothelin-1 and -3 in conscious rats. *Br. J. Pharmacol.*, **107**, 584–590.

GARDINER, S.M., KEMP, P.A., BENNETT, T. & DAVENPORT, A.P. (1992b). Regional haemodynamic response to [$Ala^{1,3,11,15}$]endothelin-1 in conscious rats. *Br. J. Pharmacol.*, **107**, Suppl. 415P.

GARDINER, S.M., KEMP, P.A., BENNETT, T. & DAVENPORT, A.P. (1993). Regional haemodynamic responses to the ET_B-receptor-selective agonist, BQ3020, in conscious rats. *Br. J. Pharmacol.*, **109**, Suppl. 131P.

GRAY, G.A. & CLOZEL, M. (1994). Three endothelin receptor subtypes suggested by the differential potency of bosentan, a novel endothelin receptor antagonist, in isolated tissues. *Br. J. Pharmacol.*, Suppl. (in press).

HAYWOOD, J.R., SHAFFER, R.A., FASTENOW, C., FINK, G.D. & BRODY, M.J. (1981). Regional blood flow measurement with pulsed Doppler flow meter in conscious rat. *Am. J. Physiol.*, **241**, H273–H278.

HILEY, C.R., DOUGLAS, S.A. & RANDALL, M.D. (1989). Pressor effects of endothelin-1 and some analogs in the perfused superior mesenteric arterial bed of the rat. *J. Cardiovasc. Pharmacol.*, **13**, (suppl. 5), S197–S199.

HILEY, C.R., JONES, C.R., PELTON, J.T. & MILLER, R.C. (1990). Binding of [^{125}I]-endothelin-1 to rat cerebellar homogenates and its interactions with some analogues. *Br. J. Pharmacol.*, **101**, 319–324.

IHARA, M., SAEKI, T., FUKURODA, T., KIMURA, S., OZAKI, S., PATEL, A.C. & YANO, M. (1992). A novel radioligand [^{125}I]BQ-3020 selective for endothelin (ET_B) receptors. *Life Sci.*, **51**, 47–52.

MASAKI, T., YANAGISAWA, M. & GOTO, K. (1992). Physiology and pharmacology of endothelins. *Med. Res. Rev.*, **12**, 391–421.

MOLENAAR, P., KUC, R.E. & DAVENPORT, A.P. (1992). Characterisation of two new ET_B selective radioligands [^{125}I]-BQ-3020 and [^{125}I]-[$Ala^{1,3,11,15}$]ET-1 in human heart. *Br. J. Pharmacol.*, **107**, 637–639.

MCMURDO, L., CORDER, R., THIEMERMANN, C. & VANE, J.R. (1993). Incomplete inhibition of the pressor effects of endothelin-1 and related peptides in the anaesthetized rat with BQ-123 provides evidence for more than one vasoconstrictor receptor. *Br. J. Pharmacol.*, **108**, 557–561.

MORELAND, S., MCMULLEN, D.M., DELANEY, C.L., LEE, V.G. & HUNT, J.T. (1992). Venous smooth muscle contains vasoconstrictor ET_B-like receptors. *Biochem. Biophys. Res. Commun.*, **184**, 100–106.

POLLOCK, D.M & OPGENORTH, T.J. (1993). Evidence for endothelin-induced renal vasoconstriction independent of ET_A-receptor activation. *Am. J. Physiol.*, **264**, R222–R226.

RANDALL, M.D. (1991). Vascular activities of the endothelins. *Pharmacol. Ther.*, **50**, 73–93.

SAEKI, T., IHARA, M., FUKURODA, T., YAMAGIWA, M. & YANO, M. (1991). [$Ala^{1,3,11,15}$]endothelin-1 analogs with ET_B agonistic activity. *Biochem. Biophys. Res. Commun.*, **179**, 286–292.

SOGABE, K., NIREI, H., SHOUBO, M., NOMOTO, A., AO, S., NOTSU, Y. & ONO, T. (1993). Pharmacological profile of FR139317, a novel, potent endothelin ET_A receptor antagonist. *J. Pharmacol. Exp. Ther.*, **264**, No. 3.

SUN, X., HEDNER, T., FONG, Q. & EDVINSSON, L. (1992). Inhibition of endothelin (ET-1) induced pressor responses by the endothelin (ET_A) receptor antagonist FR139317 in the pithed rat. *Blood Press.*, **1**, 108–112.

(Received April 23, 1993
Revised January 21, 1994
Accepted February 14, 1994)