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Renal vascular effects of the selective endothelin receptor antagonists in anaesthetized rats

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1 Endothelin (ET) is a potent vasoconstrictor peptide which has been shown to have an important role in the regulation of systemic and renal haemodynamics. In order to elucidate the role of endogenous ET in the kidney, we examined the effects of ET receptor antagonists on systemic and renal vasculature in normotensive anaesthetized rats.

2 Intravenous injection of a selective ET_A receptor antagonist, FR139317 (0.5 μmol kg^{-1}, for 20 min) induced a very small fall in blood pressure. Similarly, a non-selective ET_A/ET_B receptor antagonist, TAK-044 (12.5 μmol kg^{-1}, for 20 min) slightly decreased blood pressure. A selective ET_B receptor antagonist, BQ-788 (0.5 μmol kg^{-1}, for 20 min) had no effect on blood pressure.

3 FR139317 and TAK-044 did not affect renal blood flow or calculated renal vascular resistance. In contrast, BQ-788 significantly reduced renal blood flow by $18.2 \pm 2.4\%$ and increased renal vascular resistance. Furthermore, the renal vascular action of BQ-788 was not observed when combined with FR139317.

4 Pretreatment with a nitric oxide (NO) synthase inhibitor N^{ω}-nitro-L-arginine methyl ester (L-NAME, 37 μmol kg^{-1}, i.v.) and a cyclo-oxygenase inhibitor ibuprofen (44 μmol kg^{-1}, i.v.) completely abolished the BQ-788-mediated renal vasoconstriction.

5 These results indicate that activation of ET_B receptors by endogenous ET acts as a physiological brake for the ET_A-mediated renal vasoconstriction; this effect appears to be mediated by stimulation of NO and/or vasodilator prostaglandin(s) release.

Keywords: Endothelin; ET receptor antagonists; FR139317; BQ-788; TAK-044; N^{ω}-nitro-L-arginine methyl ester; ibuprofen

Introduction

Endothelin (ET) is a vasoconstrictor peptide isolated from cultured endothelial cells (Yanagisawa *et al*., 1988). Accumulating evidence suggests pathophysiological roles for ET in experimental animals (Vane, 1994; Tamirisa *et al*., 1995). To date, two ET receptor subtypes, ET_A and ET_B, have been cloned and characterized (Arai *et al*., 1990; Sakurai *et al*., 1990). The ET_A receptor is located on vascular smooth muscle cells where it mediates vasoconstriction. Although the ET_B receptor has been thought to be limited to vascular endothelial cells where it mediates vasodilatation through release of nitric oxide (NO) and prostaglandin(s) (de-Nucci *et al*., 1988; Warner *et al*., 1989; Miura *et al*., 1991; Yamashita *et al*., 1991; D'Orleans-Juste *et al*., 1994; Yukimura *et al*., 1994), recent evidence suggests that the ET_B receptors are also expressed in vascular smooth muscle cells and mediate vasoconstriction in certain vascular beds (Fukuroda *et al*., 1992; Ihara *et al*., 1992; Warner *et al*., 1993).

Several ET antagonists have been synthesized to evaluate the physiological or pathophysiological roles of endogenous ET and its receptor subtypes. FR139317 is a selective ET_A receptor antagonist that inhibits ET-1-induced vasoconstriction *in vitro* and *in vivo* (Sogabe *et al*., 1993). In contrast, BQ-788 is an ET_B-selective antagonist that competitively antagonizes the vasoconstriction induced by a selective ET_B receptor agonist in the rabbit pulmonary artery, with pA_2 value of 8.4 (Ishikawa *et al*., 1994). Binding assays have shown that BQ-788 has a 1000 fold higher affinity in ET_B than in ET_A receptor-containing membrane preparations. Consistent with this, we have shown that in anaesthetized rats, the depressor responses to ET-1 and the ET_B receptor agonist IRL1620 were completely blocked by BQ-788, but unaffected by the ET_A receptor antagonist, FR139317 (Matsuura *et al*., 1996). TAK-044 is a mixed ET_A/ET_B receptor antagonist and has high affinity for both ET_A and ET_B receptors (Ikeda *et al*., 1994). It antagonized contractile responses to ET-1 in porcine coronary arteries *in vitro* (Watanabe *et al*., 1995). Furthermore, TAK-044 dose- dependently blocks the depressor and pressor responses to bolus doses of ET-1 and sarafotoxin S6c in rats (Ikeda *et al*., 1994; Watanabe *et al*., 1995).

The physiological relevance of the endogenous generation of ET in controlling renal haemodynamics remains to be elucidated. If basal generation of ET contributes to renal vascular tone, then drugs that inhibit the generation or actions of ET would affect basal renal haemodynamics. Some studies have shown no apparent effect of anti-ET treatment on renal blood flow in normotensive animals (Gardiner *et al*., 1991; Pollock & Opgenorth, 1993; Gellai *et al*., 1994). Whereas other studies have demonstrated that several ET receptor antagonists increased renal blood flow or decreased renal vascular resistance only in hypertensive animals (Fujihara *et al*., 1995; Doucet *et al*., 1996; Hocher *et al*., 1996). However, these studies only used either ET_A receptor antagonists, such as BQ-123 and FR139317, or mixed ET_A/ET_B receptor antagonists, such as bosentan. Thus, a selective ET_B receptor antagonist is now essential to clarify the physiological role of endogenous ET.

Therefore, we examined the systemic and renal vascular effects of systemic administration of the selective ET receptor (ET_A and ET_B) antagonists as well as the non-selective ET_A/ET_B receptor antagonist in anaesthetized rats. Furthermore, in order to elucidate the possible interaction between the ET_A and ET_B receptors, we also examined combined administration of BQ-788 with FR139317. In addition, we investigated whether the renal vascular effects of BQ-788 are related to ET_B-mediated NO and/or vasodilator prostaglandin(s) production.

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Methods

Animal preparations and experimental design

Experiments were performed on male Sprague-Dawley rats weighing 280–330 g (Charles River Inc, Japan). The rats were anaesthetized with thiopentone sodium (0.38 mmol kg^{-1}, i.p.) and given additional doses to maintain surgical anaesthesia. The trachea was cannulated (PE240; Nippon Becton Dickinson) to facilitate respiration. Body temperature was maintained at 37.0–38.0°C by a homeothermic blanket (ATB-1100; Nihon Kohden). A polyethylene catheter (SP31; Natsume Seisakusyo) was placed in the right femoral artery for monitoring direct arterial pressure by a pressure transducer (P23ID; Nihon Kohden), and blood pressure was recorded on a polygraph (RM6100; Nihon Kohden). The right femoral vein was cannulated (PE50; Nippon Becton Dickinson) for the i.v. infusion of physiological saline or drug solution at a rate of 0.9 ml h^{-1} 100 g^{-1} body weight.

The left kidney was exposed through a retroperitoneal frank incision and the left renal artery painted with 10% phenol in 70% ethanol to facilitate observation of the direct effects of the peptide on the renal vasculature, as shown previously (Lu *et al*., 1994). A flow probe was placed around the left renal artery and renal blood flow was measured continuously by an electromagnetic flowmeter (MVF 2100; Nihon Kohden). Renal vascular resistance was calculated as mean blood pressure divided by renal blood flow.

After the completion of surgery, the rats were left for 60 to 90 min to allow stabilization of systemic blood pressure and renal blood flow. The rats were randomly assigned to one of the following protocols.

Protocol 1: systemic and renal vascular effects of the ET receptor antagonists

After a stabilizing period, ET receptor antagonists (FR139317; 0.5 μmol kg^{-1}, $n=6$, BQ-788; 0.5 μmol kg^{-1}, $n=7$, TAK-044; 12.5 μmol kg^{-1}, $n=6$) or vehicle (0.9% saline; $n=5$) were intravenously infused for 20 min. After the drug or vehicle administration we observed blood pressure and renal blood flow for an additional 60 min.

In addition, FR139317 and BQ-788 were co-administered (0.5 μmol kg^{-1}, i.v., for 20 min each, $n=6$) to examine a possible interaction between ET_A and ET_B receptors in the regulation of renal haemodynamics.

Protocol 2

We carried out another set of experiments to test whether the renal effects of BQ-788 are related to ET_B-mediated NO and/or vasodilator prostaglandin(s) production. Eight rats prepared as above were given an intravenous bolus injection of a NO synthase inhibitor N^{ω}-nitro-L-arginine methyl ester (L-NAME, 37 μmol kg^{-1}) and a cyclo-oxygenase inhibitor ibuprofen (44 μmol kg^{-1}). Thirty minutes later, the haemodynamic effects of BQ-788 (0.5 μmol kg^{-1}, for 20 min) or BQ-788 vehicle (0.9% saline containing 0.1% HCO 60) were studied.

Chemicals

FR139317 ((**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) was a gift from Fujisawa Pharmaceutical Co. (Osaka). BQ-788 (N-*cis*-2,6-dimethylpiperidinocarbonyl-L-γ-methylleucyl-D-1-methoxycarbonyltryptophanyl-D-norleucine) was donated from Banyu Pharmaceuticals (Tsukuba). TAK-044 (cyclo [D-α-aspartyl-3-[(4-phenylpiperazin-1-yl)carbonyl]-L-alanyl-L-α-aspartyl-D-2-(2-thienyl)glycyl-L-leucyl-D-tryptophyl] disodium salt) was gifted from Takeda Chemical Industries, Ltd. (Osaka). Other chemicals were purchased from Ishizu Chemical Co. (Osaka).

Statistical analysis

Results presented in the paper are shown as mean ± s.e.mean. Data were analysed by either one-way analysis of variance (ANOVA) or a complete randomized block ANOVA where appropriate. Following ANOVA, individual statistical differences were determined by Duncan's multiple range comparison test. A P value less than 0.05 was considered to be statistically significant.

Results

Effects of ET receptor antagonists on blood pressure and renal vascular tone

Before the intravenous administration of drugs, there were no statistical differences in the basal haemodynamic parameters in each group (Table 1).

Infusion of FR139317 resulted in a very small decrease in blood pressure, by 4.7 ± 1.5% at 20 min (Figure 1). Similarly, the non-selective ET_A/ET_B receptor antagonist, TAK-044, induced a slight fall in blood pressure (Figure 2), whereas, infusion of BQ-788 did not affect blood pressure (Figures 1 and 2).

Renal blood flow remained unchanged by FR139317 administration. Similarly, TAK-044 did not change renal blood flow. The calculated renal vascular resistance was not affected by either FR139317 or TAK-044 (Figures 1 and 2). In contrast, renal blood flow started to decline with the initiation of BQ-788 infusion and reached a plateau during the infusion period. Renal blood flow decreased by 18.2 ± 2.4% at the end of the administration of BQ-788. After the cessation of infusion, renal blood flow gradually reverted toward the control level. The renal vascular resistance was markedly increased in BQ-788 infused rats (Figures 1 and 2).

However, combined administration of FR139317 with BQ-788 abolished the renal vasoconstriction caused by BQ-788

Table 1 Basal haemodynamic parameters obtained before intravenous administration of drugs

	Vehicle ($n=5$)	*FR139317* ($n=6$)	*BQ-788* ($n=7$)	*TAK-044* ($n=6$)	*FR139317 + BQ-788* ($n=6$)
Mean blood pressure (mmHg)	109.8 ± 1.2	112.8 ± 1.0	113.6 ± 2.9	114.0 ± 2.5	111.7 ± 1.7
Renal blood flow (ml min^{-1} g^{-1} kidney wt)	7.2 ± 0.5	7.2 ± 0.5	7.0 ± 0.4	7.3 ± 0.3	7.1 ± 0.4
Renal vascular resistance (mmHg min g ml^{-1})	15.5 ± 1.1	16.0 ± 0.9	16.5 ± 1.0	15.8 ± 0.6	15.8 ± 0.6

Basal haemodynamic parameters did not differ between those rats treated with drugs compared to the vehicle group treated with 0.9% saline. Values are expressed as mean ± s.e.mean.

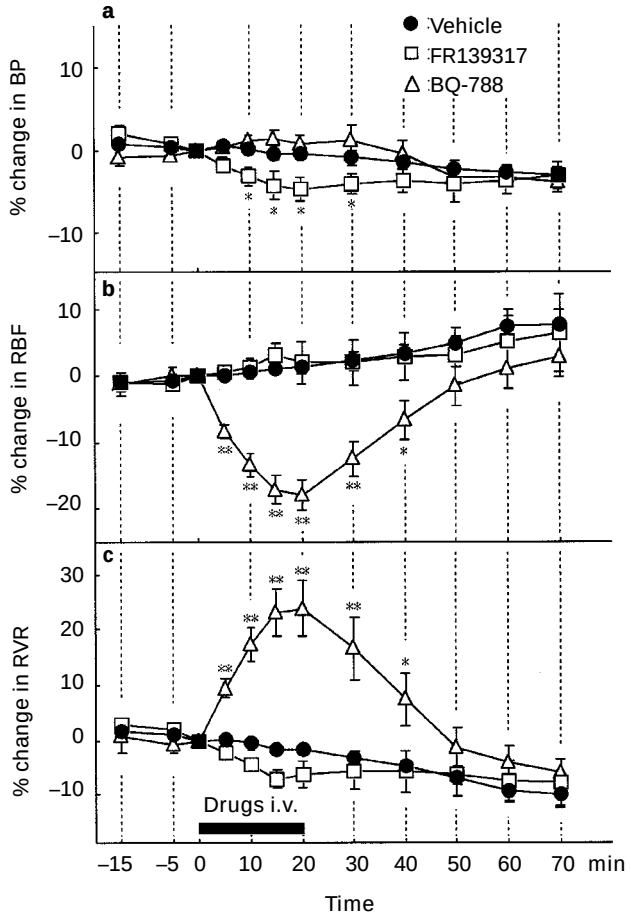

Figure 1 Effects of BQ-788 and FR139317 on (a) blood pressure, (b) renal blood flow and (c) renal vascular resistance in anaesthetized rats. Data are shown as mean and vertical lines indicate s.e.mean. Drugs were infused intravenously at a rate of 0.5 μmol kg^{-1} from time 0 to 20. (a) BP, mean blood pressure; (b) RBF, renal blood flow; (c) RVR, renal vascular resistance. *$P<0.05$ and **$P<0.01$ compared to the vehicle-treated (0.9% saline) control group. The solid horizontal bar indicates the period of i.v. infusion of the drugs.

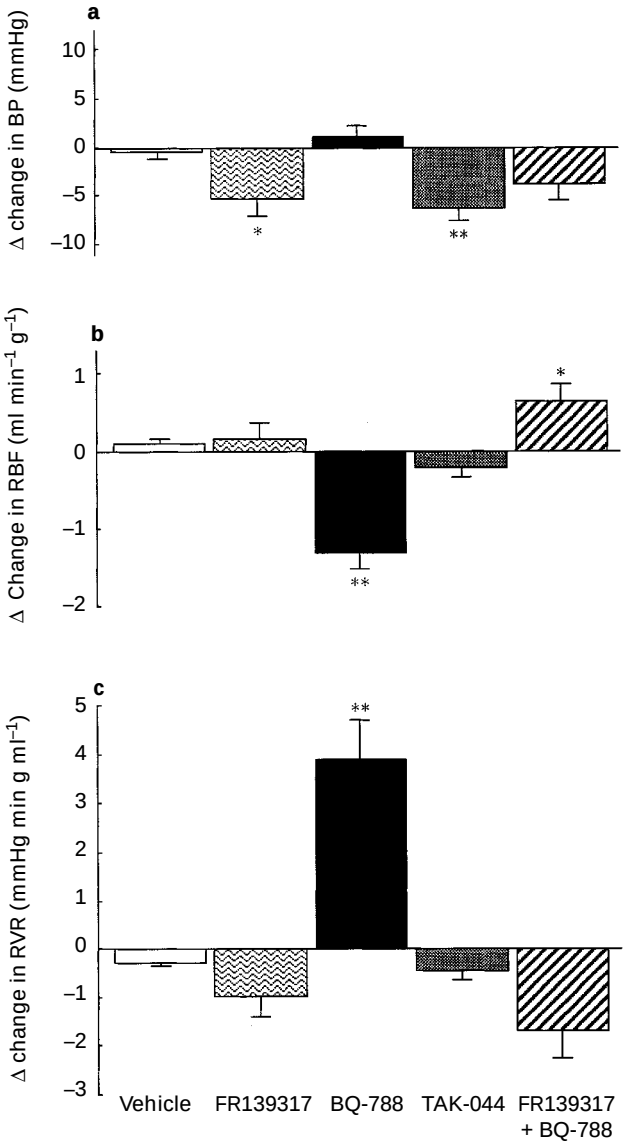

Figure 2 Effects of FR139317, BQ-788, TAK-044 and a combination of FR139317 and BQ-788 on (a) blood pressure, (b) renal blood flow and (c) renal vascular resistance in anaesthetized rats. Data are shown as mean ± s.e.mean. BP, mean blood pressure; RBF, renal blood flow; RVR, renal vascular resistance. *$P<0.05$ and **$P<0.01$ compared to the vehicle-treated (0.9% saline) control group.

alone (Figure 2). In fact, renal blood flow was increased, but calculated renal vascular resistance remained unchanged, by this co-administration.

Effects of L-NAME and ibuprofen on the BQ-788-induced renal vasoconstriction

Administration of L-NAME and ibuprofen elicited a sustained increase in blood pressure and remained elevated. Blood pressure increased from 109.1 ± 0.5 to 135.6 ± 2.0 mmHg 30 min after the administration of these blockers ($P<0.01$) (Figure 3). Similarly, L-NAME and ibuprofen caused a renal vasoconstriction characterized by a slow onset and a long duration. Thirty minutes after the injection, the renal blood flow was decreased from 6.8 ± 0.1 to 5.2 ± 0.2 min min^{-1} g^{-1} kidney wt ($P<0.01$), and calculated renal vascular resistance was increased from 16.2 ± 0.3 to 26.5 ± 1.6 mmHg ml g ml^{-1} ($P<0.01$). The renal blood flow reached a plateau and remained decreased. After the treatment with L-NAME and ibuprofen, neither BQ-788 nor vehicle affected blood pressure, renal blood flow or renal vascular resistance (Figure 3).

Discussion

In this paper, we studied the role of endogenous ET in rat renal vasculature, especially any effects mediated via the ET_B receptor. Our experiments, by use of various antagonists of the ET receptors, demonstrated that endogenous ET regulates renal haemodynamics and this seems to be mediated by a unique interaction between the ET_A and ET_B receptors.

Our data showed that blockade of the ET_B receptor by BQ-788 resulted in renal vasoconstriction in anaesthetized rats. Although BQ-788 was dissolved in 0.9% saline containing 0.1% polyoxyethylene-hydrogenated Castor Oil 60 (HCO 60) solution, preliminary experiments confirmed that vehicle for BQ-788 had no effect on blood pressure or renal blood flow (data not shown). In contrast, the ET_A receptor blockade by FR139317 had no effect on renal haemodynamics. These data imply that endogenous ET peptides physiologically dilate renal vessels via the ET_B receptor, whereas the ET_A receptor remains inactive and does not appear to participate in the regulation of renal blood flow. However, this notion is unlikely because TAK-044, which blocks both ET_A and ET_B receptors, did not affect basal renal haemodynamics. If endogenous ET dilated renal vessels, the mixed-receptor antagonist, TAK-044 should have elicited renal vasoconstriction. Furthermore, the renal

vasoconstrictor action of BQ-788 was abolished when the ET_A receptor was simultaneously blocked by FR139317. Since FR139317 had no effects on the vasoconstrictor action of high potassium, noradrenaline or histamine (Cardell *et al*., 1993), it is unlikely that FR139317 blocked the BQ-788-induced renal vasoconstriction in a non-specific manner. These results strongly suggest that the renal response to ET_B receptor inhibition reflects a unique interaction between the ET_A and ET_B receptor in the kidney; the renal ET_B receptor may act as a physiological brake on ET_A receptor-mediated renal vasoconstriction rather than having a direct vasodilator action. As a result, selective inhibition of the ET_B receptor in anaesthetized rats would unmask the ET_A receptor-mediated renal vasoconstriction.

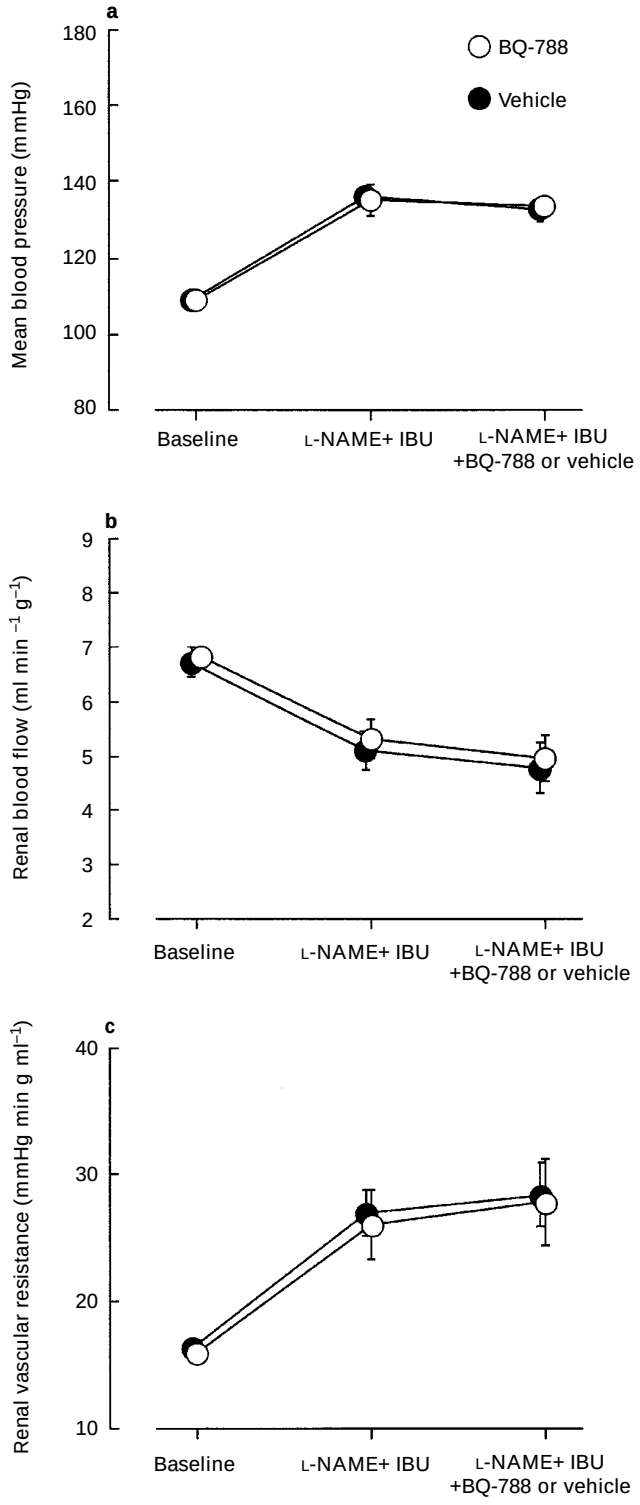

Figure 3 Effects of BQ-788 and BQ-788-vehicle on (a) blood pressure, (b) renal blood flow and (c) renal vascular resistance during blockade of nitric oxide (NO) and prostaglandin(s) synthesis. Data are shown as mean and vertical lines indicate s.e.mean. N^{ω}-nitro-L-arginine methyl ester (L-NAME) (37 μmol kg^{-1}) and ibuprofen (IBU) (44 μmol kg^{-1}) were administered as an intravenous bolus injection. Thirty minutes later, BQ-788 or BQ-788-vehicle (0.9% saline containing 0.1% HCO 60) was infused for 20 min. Changes in both cases were not significant ($P>0.05$) but parallel.

Since the ET_B receptor on the endothelium stimulates the release of NO and/or prostaglandin(s) (de-Nucci *et al*., 1988; Warner *et al*., 1989; Miura *et al*., 1991; Yamashita *et al*., 1991; D'Orleans-Juste *et al*., 1994; Yukimura *et al*., 1994), such mechanisms may be operating in the renal action of BQ-788 seen in the present experiments. This hypothesis was tested by using inhibitors of NO synthase and cyclo-oxygenase. Pretreatment with L-NAME and ibuprofen completely abolished the renal vasoconstrictor action of BQ-788. As systemic blood pressure and renal vascular resistance dramatically increase after treatments with these inhibitors, one may speculate that the alterations in the haemodynamics by these inhibitors may abolish the renal action mediated by BQ-788 in a non-specific manner. However, this notion is unlikely since blockade of NO or prostaglandin(s) synthase *in vivo* enhances the effects of different vasoconstrictors, such as angiotensin II (AII) or noradrenaline (Aiken & Vane, 1973; Parekh *et al*., 1996). Thus, activation of the ET_B receptor by endogenous ET peptide(s) may buffer the ET_A receptor-mediated renal vasoconstrictor potential via NO and/or prostaglandin(s). In the present experiments, relative contributions of NO and prostaglandin(s) to the renal haemodynamic action of BQ-788 were not tested. Nevertheless, we have evidence that both prostaglandins and NO are involved in the renal vasodilating action of ET-3 (Yamashita *et al*., 1991).

Interactions between vasoconstrictors and prostaglandin/NO have been described previously. Chatziantoniou *et al*. (1993) showed that intrarenal administration of AII produced renal vasoconstriction that was effectively buffered by vasodilator prostaglandin(s) at a dose that did not affect baseline blood pressure or renal blood flow. They proposed that the vasodilator mechanisms activated by prostaglandin(s) interact with the vasoconstrictor mechanism(s) of AII at a common signal(s). In another study, endotoxin administration enhanced NO production that mediated the vascular hyporeactivity to noradrenaline (Szabo *et al*., 1993). These examples support our hypothesis that the ET_B receptor acts as a physiological brake against the ET_A-mediated renal vasoconstriction through the release of NO and/or prostaglandin(s).

Recently, Gellai *et al*. (1996) showed that intravenous administration of RES-701-1, a selective ET_B antagonist, elicited systemic hypertension and renal vasoconstriction in conscious rats. Although the ET_A antagonist, BQ-123 and mixed ET receptor antagonist, SB-209670 did not have any effects on systemic and renal haemodynamics, SB-209670 but not BQ-123 completely abolished the systemic and renal action of RES-701-1. They concluded that the predominant role of endogenous ET is vasodilatation and the ET_A receptor plays a negligible role in the control of vascular tone in rats. However, it should be pointed out that if their conclusion were correct, the mixed ET antagonist should have resulted in systemic and renal vasoconstriction. Their data are in part consistent with our findings in that the ET_B antagonist, but neither the ET_A nor the mixed ET receptor antagonist, elicited renal vasoconstriction. However, the effects of the ET_A antagonist on the renal vasoconstrictor action of ET_B receptor blockade were different. Furthermore, the vasoconstrictor action of the ET_B receptor antagonist was apparent in both systemic and renal vasculature in their study, whereas it was only observed in the kidney in our experiments. These differences may arise from different experimental conditions; Gellai *et al*. (1996) used conscious rats whereas we used anaesthetized rats. Alternatively, it may be attributable to differences in the receptor subtype selectivity of ET_B receptor antagonists; ET_{B1} receptors

but not ET_{B2} receptors are sensitive to RES-701-1 whereas both ET_B receptor subtypes can be blocked by BQ-788 (Karaki *et al*., 1994). Furthermore, specific binding of ET-1 that is not displaced by ligands to ET_A and ET_B receptors exists in the kidney, making interpretation of their results and ours more complicated (Yukimura *et al*., 1996).

We showed here that systemic administration of the ET_A receptor antagonist, FR139317 and the non-selective ET_A/ET_B receptor antagonist, TAK-044 caused only slight hypotension by approximately 5.0%. In addition, BQ-788 alone and combined administration of BQ-788 and FR139317 did not elicit any systemic haemodynamic changes. Some investigators have also shown that anti-ET treatment does not affect blood pressure in normotensive animals (Gardiner *et al*., 1991; 1994b; Douglas *et al*., 1992; Nishikibe *et al*., 1993; Sogabe *et al*., 1993; Filep *et al*., 1994). These data suggest that endogenous ET plays a minor role in the control of systemic blood pressure in the rats.

The doses of FR139317, BQ-788 and TAK-044 that we used in this study should be sufficient to block effectively ET_A, ET_B and both receptors, respectively. We and other investigators have previously shown that this dose of FR139317 antagonizes arterial hypertension induced by ET-1, but does not alter the early ET_B-mediated vasodepressor response, whereas BQ-788 at a dose of 0.5 μmol kg^{-1} completely abolished the latter alone (Gardiner *et al*., 1994a; Matsuura *et al*., 1996). Indeed, Gardiner *et al*. (1994a) have shown that higher doses of FR139317 do not cause any greater inhibition of responses to ET-1 than a dose of 0.5 μmol kg^{-1}. TAK-044 completely blocked the ET-1-induced depressor and pressor responses at a dose of 10 mg kg^{-1} (=10.3 μmol kg^{-1}) (Ikeda *et al*., 1994; Watanabe *et al*., 1995).

In conclusion, our results indicate that the ET_B receptor acts as a physiological brake for the potent ET_A-mediated renal vasoconstriction. This ET_B-mediated action of renal haemodynamics is dependent on the stimulation of NO and/or vasodilator prostaglandin(s) release. This kind of interaction between the two ET receptors should always be considered when the effects of selective receptor antagonists are analysed.

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