

Selective Blockade of Endothelin Receptor Subtypes on Systemic and Renal Vascular Responses to Endothelin-1 and IRL1620, a Selective Endothelin ET_B-Receptor Agonist, in Anesthetized Rats

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ABSTRACT—By using BQ-788 as a selective endothelin ET_B-receptor antagonist and FR139317 as a selective endothelin ET_A-receptor antagonist, we have characterized the receptor subtypes mediating the systemic and renal vascular effects of endothelin-1 and IRL1620, a selective endothelin ET_B-receptor agonist (succinyl-[Glu⁹,Ala^{11,5}]-endothelin-1(8–21)), in anesthetized rats. Bolus intravenous injection of endothelin-1 (0.5 nmol/kg) and IRL1620 (1.65 nmol/kg) produced a transient fall in systemic blood pressure followed by a sustained increase. The initial fall in blood pressure observed after endothelin-1 and IRL1620 administration was completely blocked by BQ-788 (0.5 μmol/kg, i.v.), whereas the pressor response was blocked by FR139317 (0.8 μmol/kg, i.v.). Renal blood flow was decreased and calculated renal vascular resistance was dramatically increased by endothelin-1 and IRL1620. The reduction of renal blood flow by endothelin-1 was significantly suppressed by FR139317 but potentiated by BQ-788. Both BQ-788 and FR139317 partially blocked the renal vasoconstriction by IRL1620. Pretreatment by BQ-788 itself decreased renal blood flow by 14.1%. These results indicate that the systemic depressor responses induced by endothelin-1 and IRL1620 are mediated through the endothelin ET_B-receptor, and the pressor responses are mediated through the endothelin ET_A-receptor. In the renal vasculature of anesthetized rats, it is suggested that vasoconstriction is mediated through both endothelin ET_A- and ET_B-receptors and that endothelin ET_B-receptors may be also involved in vasodilating responses to endothelin peptides.

Keywords: Endothelin-1, Systemic blood pressure, Renal blood flow, BQ-788, FR139317

Endothelin-1 is a 21-amino acid vasoconstrictor peptide isolated from cultured endothelial cells (1). Endothelin-1 caused an increase in renal vascular resistance and a decrease in renal blood flow (2–6). The circulating plasma level of endothelin-1 was increased both clinically and in animal models of renal failure, and expression of endothelin-1 mRNA in the kidney of the rat was increased after ischemia-reperfusion or after cyclosporin administration (7, 8). Selective antibodies or antagonists for endothelin-1 protected the kidney against ischemia-reperfusion injury and the nephrotoxic effects of cyclosporin (6, 9, 10), thereby suggesting a pathophysiological role of the peptide in the kidney.

To date, two endothelin receptor subtypes, endothelin ET_A- and ET_B-receptors have been cloned and characterized (11, 12). Initially, it was thought that the endothelin ET_A-receptor was located on vascular smooth muscle to mediate vasoconstriction and the endothelin ET_B-recep-

tor on the endothelium where it mediated vasodilation through release of nitric oxide and prostaglandins (13–17).

In the kidney, however, the suggestion that non-endothelin ET_A-receptors mediate vasoconstriction first came from *in vitro* studies in which selective antagonists of the endothelin ET_A-receptor failed to completely inhibit endothelin-1 induced constrictions (18). Subsequently, it has been shown that the renal vasoconstriction in the rat is not dependent on endothelin ET_A-receptor activation (19–21), but may be due to endothelin ET_B-receptor activation (22–24) or to a novel endothelin receptor to be characterized (21, 25). Recently, Wellings et al. (26) showed that a non-selective mixed endothelin ET_A/ET_B-receptor antagonist completely blocked the vasoconstriction induced by endothelin-1, while the selective endothelin ET_A-receptor antagonists partially blocked the vasoconstriction by endothelin-1 in isolated per-

fused rat kidney. They concluded that endothelin-1-induced renal vasoconstriction is mediated through endothelin ET_A- and ET_B-like receptors.

The use of a selective endothelin ET_B-receptor antagonist is now essential to discriminate clearly between systemic and renal vascular effects of endothelin peptides, mediated via distinct receptor populations. Ishikawa et al. (27) have recently reported that a novel compound, BQ-788, competitively antagonized the vasoconstriction induced by a selective endothelin ET_B-receptor agonist, BQ-3020, in the rabbit pulmonary artery.

The present experiments were conducted to elucidate the role of endothelin receptor subtypes in the systemic and renal vascular responses to endothelin peptides. We examined the effects of endothelin-1 and a selective agonist for the endothelin ET_B-receptor on blood pressure and renal blood flow in anesthetized rats treated with BQ-788 as a selective endothelin ET_B-receptor antagonist or FR139317 as a selective endothelin ET_A-receptor antagonist.

MATERIALS AND METHODS

Animal preparations and experimental design

Experiments were performed on male Sprague-Dawley rats weighing 280–350 g (Charles River, Osaka). The rats were anesthetized with thiopental sodium (100 mg/kg, i.p.) and given additional doses as required. Respiration was maintained by spontaneous breathing through an endotracheal tube (PE240; Nippon Becton Dickinson, Tokyo). A polyethylene catheter (SP31; Natsume Seisakusyo, Tokyo) was placed in the right femoral artery for direct arterial pressure monitoring by a pressure transducer (P23ID; Nihon Kohden, Tokyo), 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 drugs.

The left renal artery was exposed through a retroperitoneal flank incision as reported previously (28). A flow probe was placed around the left renal artery, and renal blood flow was measured continuously using an electromagnetic blood flowmeter (MVF 2100, Nihon Kohden). The corresponding % changes in renal vascular resistance were calculated by dividing blood pressure by renal blood flow. The integrated responses of blood pressure and renal blood flow to endothelin peptides were evaluated with the area under or over the response curves, during 10 min after the i.v. injection. They were determined by a Macintosh LC III using a public domain NIH Image program.

The rats were continuously infused with physiological saline at a rate of 0.9 ml/hr per 100 grams body weight

via a catheter placed in the femoral vein, throughout the experiment. After an equilibrium period of 60–90 min, the rats were divided into 3 groups and given either BQ-788 (0.5 μ mol/kg) (n=21), FR139317 (0.8 μ mol/kg) (n=18) or vehicle (0.1 ml of 0.9% saline containing 0.1% bovine serum albumin) (n=22) intravenously over 10 min. Five minutes later, each group of rats was given either endothelin-1 (0.1 or 0.5 nmol/kg) (n=4–8) or IRL1620 (0.33 or 1.65 nmol/kg) (n=4–7), all as i.v. bolus injections (50 μ l, 10 sec).

Chemicals

Endothelin-1 was purchased from Peptide Institute, Inc. (Minoo). IRL1620, succinyl-[Glu⁹,Ala^{11,5}]-endothelin-1(8–21) (29), was synthesized at Ciba-Geigy (Takarazuka). 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 (30), was a gift from Fujisawa Pharmaceutical Co. (Osaka). BQ-788, *N*-cis-2,6-dimethylpiperidino-carbonyl-L- γ -methylleucyl-D-1-methoxycarbonyltryptophanyl-D-norleucine (27), was obtained from Banyu Pharmaceuticals (Tsukuba). Other chemicals were purchased from Ishizu Chemical Co. (Osaka).

Statistical analyses

All data are shown as means \pm S.E.M. Experimental data were subjected to an analysis of variance with the Duncan new multiple range test. A P value smaller than 0.05 was considered to be statistically significant.

RESULTS

Effects of endothelin-1 and IRL1620 on blood pressure

The blood pressure decreased transiently from the baseline values of 110.5 \pm 2.0 and 111.2 \pm 1.6 mmHg to minimum values of 84.5 \pm 1.9 and 68.1 \pm 1.3 mmHg within a minute following i.v. administration of endothelin-1 at doses of 0.1 and 0.5 nmol/kg, respectively (Table 1). The blood pressure reverted to the preinjection value within 2–3 min and then showed a sustained and dose-dependent increase; a maximum value was attained within 7–10 min after the injection (Figs. 1 and 2). Bolus i.v. injection of IRL1620 also produced a transient fall in blood pressure, followed by a significant pressor effect at a higher dose of IRL1620 (1.65 nmol/kg) (Figs. 1 and 3, Table 1).

Effects of BQ-788 and FR139317 on blood pressure responses to endothelin-1 and IRL1620

The initial fall in blood pressure after i.v. administration of endothelin-1 or IRL1620 was completely abolished by BQ-788, but was not affected by FR139317,

Table 1. Effects of endothelin-1 and IRL1620 on blood pressure and renal blood flow in anesthetized rats

	Dose	n	BP (mmHg)			RBF (ml/min/g)	
			baseline	depressor	pressor	baseline	decrease
ET-1	0.1 nmol/kg	4	110.5±2.0	-26.0±1.7 ^a	5.3±1.4 ^a	7.2±0.7	-1.1±0.2 ^a
	0.5 nmol/kg	7	111.2±1.6	-43.1±2.2 ^a	22.6±2.7 ^a	6.2±0.2	-5.6±0.3 ^a
IRL1620	0.33 nmol/kg	4	110.8±6.3	-22.5±4.2 ^a	8.3±2.3	7.4±0.4	-1.1±0.2 ^a
	1.65 nmol/kg	7	109.9±2.0	-33.1±1.6 ^a	14.6±1.5 ^a	6.4±0.2	-4.7±0.4 ^a

ET-1, Endothelin-1; BP, blood pressure; RBF, renal blood flow; ml/min/g, ml/min per gram kidney weight. Data are means±S.E.M. Changes in blood pressure and renal blood flow from the baseline values are shown. ^aP<0.05, compared with each baseline value.

when estimated either by peak values (Figs. 2 and 3) or the area over the response curve (Tables 2 and 3). The depressor responses to the higher dose of endothelin-1 in the presence of BQ-788 (area over the response curve=0.3±0.1 mmHg·min) were remarkably smaller than those seen in the naive rats (39.0±5.8 mmHg·min) or in the rats treated with FR139317 (39.5±6.0 mmHg·min) (Table 2).

The endothelin ET_A-receptor antagonist FR139317 significantly suppressed the systemic pressor effects of endothelin-1 when estimated by the peak values (Figs. 2 and 4) or the area under the response curve (Table 2). The pressor effects by the higher dose of endothelin-1 (area under the response curve=128.2±33.9 mmHg·min) were significantly decreased in the presence of FR139317 (33.3±5.1 mmHg·min) (Table 2). In contrast, the treatment with BQ-788 significantly enhanced the pressor responses to endothelin-1 at the doses used in the present

experiment, when estimated by the peak values (Figs. 2 and 4) or the area under the response curve (Table 2).

The IRL1620-induced initial fall in blood pressure was abolished by BQ-788 but not by FR139317. The sustained pressor response was observed when a higher dose of IRL1620 was given; This was significantly suppressed by FR139317 (Fig. 3, Table 3).

Renal vascular effects of endothelin-1 and IRL1620

Endothelin-1 produced a dose-related reduction in renal blood flow by 14±2% and 90±3% at the doses of 0.1 and 0.5 nmol/kg, respectively (Table 1). The renal blood flow reached a minimum within a minute, respectively (Figs. 1 and 2). The area over the response curve values of endothelin-1-induced reduction in renal blood flow at doses of 0.1 and 0.5 nmol/kg were 10.3±2.4 and 37.3±3.8 ml per gram kidney weight (ml/g), respectively (Table 2). IRL1620 also caused a dose-related reduction in renal blood flow (Tables 1 and 3). At the higher doses of endothelin-1 and IRL1620, the renal blood flow did not revert to the pre-injection value and remained at a significant lower value at 25 min after injection (Fig. 1). The calculated renal vascular resistance was remarkably increased by endothelin-1 (Fig. 2) and IRL1620 (Fig. 3).

Effects of BQ-788 and FR139317 on renal vasoconstrictor responses to endothelin-1 and IRL1620

The remarkable reduction of renal blood flow by 90.7±2.9% (area over the response curve=37.3±3.8 ml/g) following a higher dose of endothelin-1 (0.5 nmol/kg) was suppressed by FR139317, but the increase in renal vascular resistance by endothelin-1 (0.5 nmol/kg) was enhanced by pretreatment of BQ-788 (Fig. 2, Table 2). Furthermore, the reduction of renal blood flow by a lower dose of endothelin-1 (0.1 nmol/kg) was apparently enhanced in rats treated with BQ-788 when estimated by the peak value (Fig. 5). The area over the response curve of renal blood flow by endothelin-1 was increased twofold from 10.3±2.4 to 20.2±3.2 ml/g in the presence of BQ-788 (Table 2). The increase in renal vascular resistance by

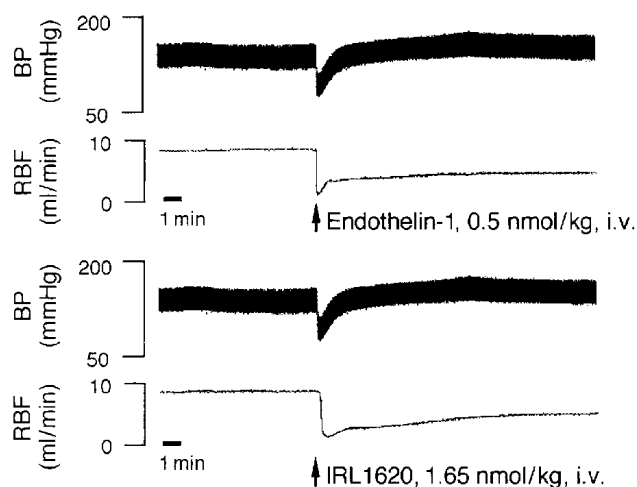


Fig. 1. Representative recordings of the change of blood pressure and renal blood flow in response to endothelin-1 (0.5 nmol/kg) and IRL1620 (1.65 nmol/kg). Endothelin-1 or IRL1620 were administered as i.v. bolus injections. BP, blood pressure; RBF, renal blood flow.

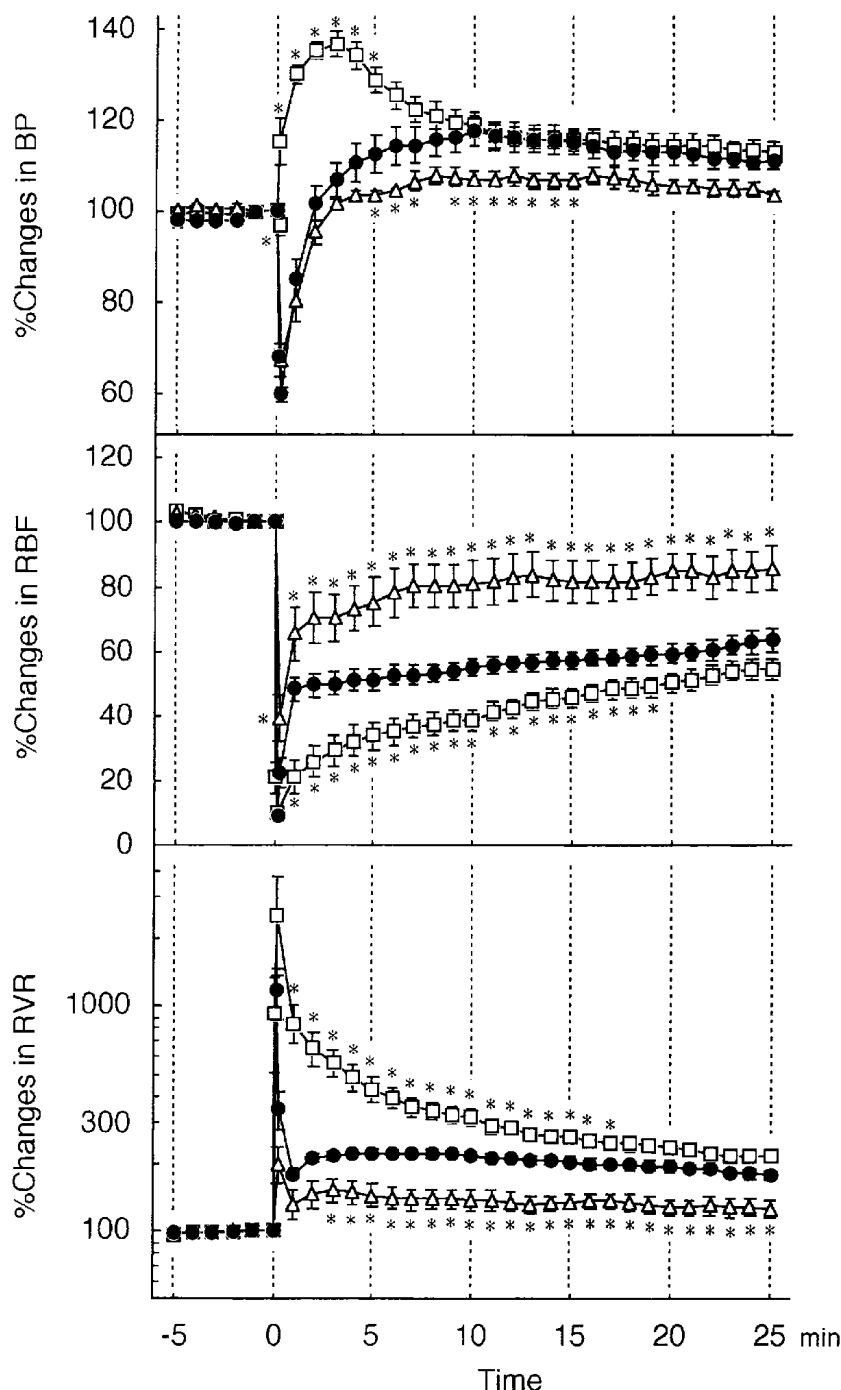


Fig. 2. Effects of BQ-788 ($0.5 \mu\text{mol/kg}$) and FR139317 ($0.8 \mu\text{mol/kg}$) on endothelin-1-induced changes in blood pressure (BP), renal blood flow (RBF) and renal vascular resistance (RVR) in anesthetized rats. Time course effects on BP, RBF and RVR of endothelin-1 (0.5 nmol/kg , i.v., given at time = 0) in rats treated with saline containing 0.1% bovine serum albumin (0.1 ml) (●, $n=7$), treated with BQ-788 (□, $n=8$), or treated with FR139317 (△, $n=5$). Data are expressed as means \pm S.E.M. (vertical bars). * $P < 0.05$, compared with the vehicle-treated control group.

endothelin-1 was enhanced by BQ-788 but attenuated by FR139317 (Fig. 2).

When estimated by the peak value (Fig. 5) or the area over the response curve (Table 3), the reduction of renal

blood flow by a lower dose of IRL1620 was not affected by BQ-788 or FR139317. However, the reduction of renal blood flow following a higher dose of IRL1620 was partially but significantly suppressed by BQ-788 (Fig. 3). The

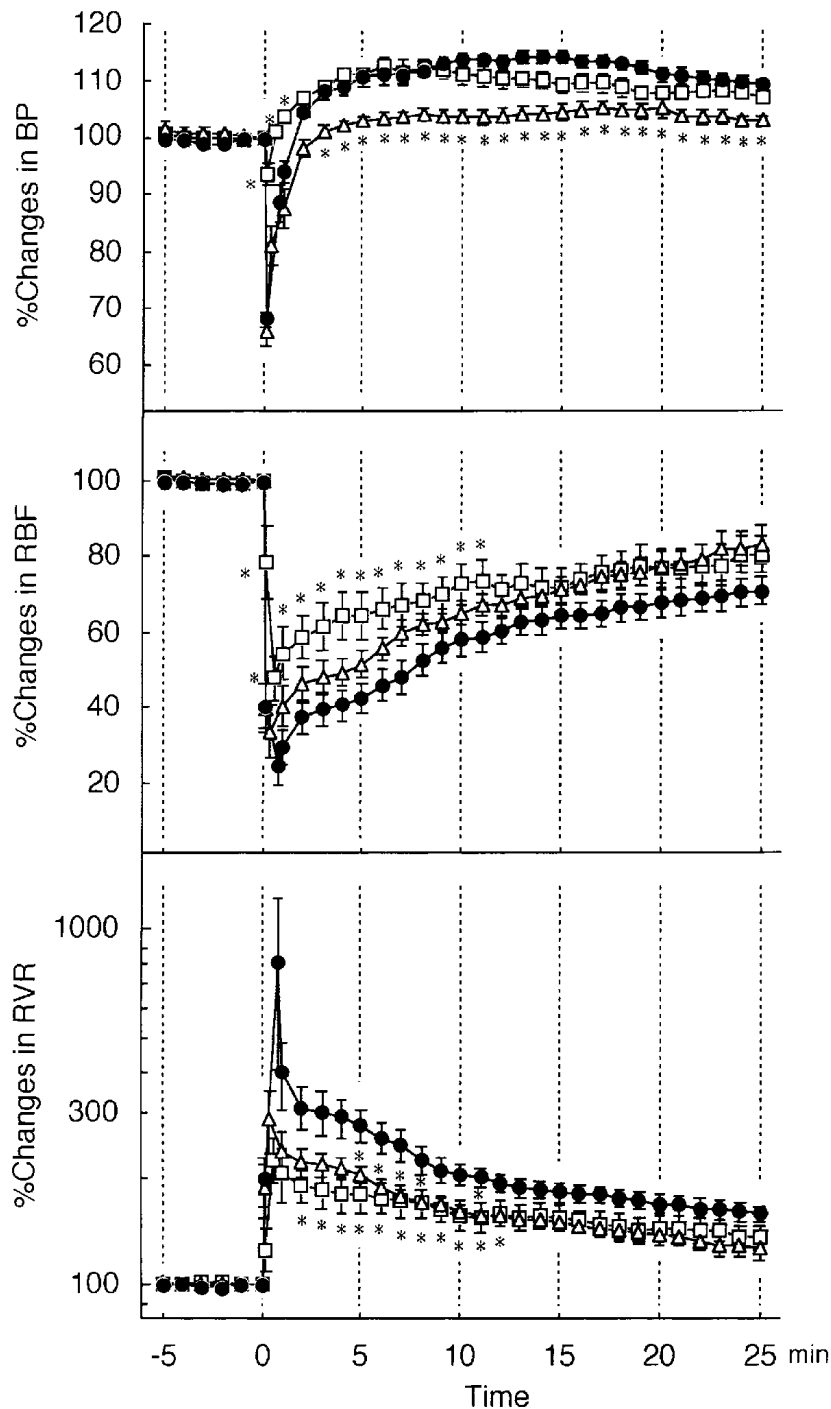


Fig. 3. Effects of BQ-788 ($0.5 \mu\text{mol/kg}$) and FR139317 ($0.8 \mu\text{mol/kg}$) on IRL1620-induced changes in blood pressure (BP), renal blood flow (RBF) and renal vascular resistance (RVR) in anesthetized rats. Time course effects on BP, RBF and RVR of IRL1620 (1.65 nmol/kg , i.v., given at time=0) in rats treated with saline containing 0.1% bovine serum albumin (0.1 ml) (\bullet , $n=7$), treated with BQ-788 (\square , $n=4$), or treated with FR139317 (\triangle , $n=5$). Data are expressed as means \pm S.E.M. (vertical bars). * $P < 0.05$, compared with the vehicle-treated control group.

area over the response curve of renal blood flow and increased renal vascular resistance by a higher dose of IRL1620 was significantly attenuated in the presence of BQ-788 (Table 3). The increase in renal vascular

resistance was also significantly suppressed by FR139317, although the area over the response curve of renal blood flow was not affected (Fig. 3, Table 3).

Table 2. The integrated systemic and renal responses to endothelin-1 estimated by the area under or over the response curves in rats treated with and without BQ-788 or FR139317

	Endothelin-1 (0.1 nmol/kg)			Endothelin-1 (0.5 nmol/kg)		
	without (n=4)	with BQ-788 (n=5)	with FR139317 (n=4)	without (n=7)	with BQ-788 (n=8)	with FR139317 (n=5)
Decrease in BP						
AOC (mmHg·min)	7.7±1.8	0.1±0.0	21.3±6.5 ^a	39.0±5.8	0.3±0.1 ^a	39.5±6.0
Increase in BP						
AUC (mmHg·min)	20.7±5.1	90.4±22.0 ^a	3.5±2.7	128.2±33.9	311.4±15.2 ^a	33.3±5.1 ^a
Reduction in RBF						
AOC (ml/g)	10.3±2.4	20.2±3.2 ^a	1.2±0.4 ^a	37.3±3.8	42.1±2.5	14.6±4.4 ^a

BP, blood pressure; RBF, renal blood flow; AOC, the area over the response curve; AUC, the area under the response curve; ml/g, ml per gram kidney weight. Data are expressed as means±S.E.M. ^aP<0.05, when compared with the value obtained from each control rat without treatment.

Table 3. The integrated systemic and renal responses to IRL1620 estimated by the area under or over the curves in rats treated with and without BQ-788 or FR139317

	IRL1620 (0.33 nmol/kg)			IRL1620 (1.65 nmol/kg)		
	without (n=4)	with BQ-788 (n=4)	with FR139317 (n=4)	without (n=7)	with BQ-788 (n=4)	with FR139317 (n=5)
Decrease in BP						
AOC (mmHg·min)	11.5±4.9	0.1±0.0 ^a	11.5±3.6	21.8±2.9	0.6±0.3 ^a	32.3±4.6
Increase in BP						
AUC (mmHg·min)	45.6±15.6	81.7±16.4	25.4±9.7	90.2±11.4	108.1±10.4	23.6±7.0 ^a
Reduction in RBF						
AOC (ml/g)	7.4±2.8	4.1±2.1	6.9±0.8	43.0±5.9	20.5±3.0 ^a	32.2±1.7

BP, blood pressure; RBF, renal blood flow; AOC, the area over the response curve; AUC, the area under the response curve; ml/g, ml per gram kidney weight. Data are expressed as means±S.E.M. ^aP<0.05, when compared with the value obtained from each control rat without treatment.

Effect of BQ-788 and FR139317 on blood pressure and renal blood flow

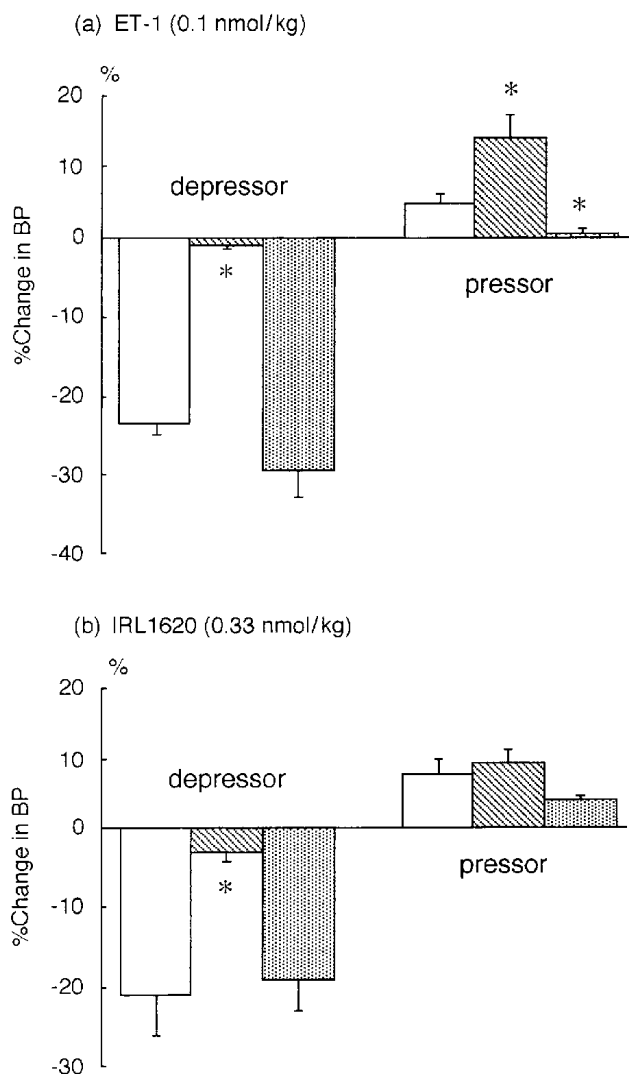
As shown in Fig. 6, intravenous administration of BQ-788 exerted a very small but significant increase in blood pressure by $4.0 \pm 0.6\%$, and FR139317 induced a fall in blood pressure by $3.1 \pm 0.5\%$. BQ-788 significantly reduced renal blood flow by $14.1 \pm 1.2\%$, but FR139317 did not.

DISCUSSION

Intravenous injection of endothelin-1 at a dose of 0.5 nmol/kg into anesthetized rats produced an initial transient depressor response followed by a sustained pressor response, as reported by other investigators (20, 24, 31). These changes in blood pressure were mediated via at least two different endothelin receptors. Intravenous injections of endothelin ET_B-receptor agonists, endothelin-

3 and sarafotoxin 6c, into anesthetized rats produced initial transient depressor responses (20, 32). We also demonstrated the fall in blood pressure observed after i.v. administration of the specific endothelin ET_B-receptor agonist IRL1620 at doses of 0.33 and 1.65 nmol/kg. The depressor responses of endothelin ET_B-receptor stimulation by sarafotoxin 6c and [Ala^{1,3,11,15}]endothelin-1 (19), IRL1620 and endothelin-1 in the presence of the endothelin ET_A-receptor antagonist in the present experiment have suggested that the depressor activity of endothelin-1 may be endothelin ET_B-receptor-mediated. Furthermore, Wellings et al. (26) showed that a non-selective endothelin receptor antagonist, PD145065, completely prevented any blood pressure responses.

More recently, Ishikawa et al. (27) have developed a potent and selective endothelin ET_B-receptor antagonist BQ-788, which competitively antagonized the vasoconstriction induced by a selective endothelin ET_B-receptor



agonist BQ-3020 in the rabbit pulmonary artery with a pA_2 value of 8.4. Binding assays have shown that BQ-788 has a 1000-fold higher affinity in endothelin ET_B -receptor-containing membrane preparations than ET_A -receptor-containing ones. In the present experiment in anesthetized rats, the depressor responses to endothelin-1 and IRL1620 were completely blocked by BQ-788, but unaffected by the endothelin ET_A -receptor antagonist FR139317, which are consistent with the findings by Ishikawa et al. (27), demonstrating that the depressor responses to endothelin peptides are mediated through the endothelin ET_B -receptor subtype.

A number of studies have demonstrated that the rise in blood pressure induced by endothelin-1 were blocked by BQ-123, the endothelin ET_A -receptor antagonist (19, 20, 24). In the present experiment, the pressor response to endothelin-1 was completely abolished by FR139317, and in contrast, it was enhanced by BQ-788. These results and others clearly indicated that the sustained pressor response to endothelin-1 is mediated by endothelin ET_A -receptor in rats.

The higher dose of IRL1620 exerted a small but significant increase in blood pressure following a transient depressor response. The same results were reported by

Fig. 4. Responses of blood pressure (BP) to endothelin-1 (0.1 nmol/kg) (a) or IRL1620 (0.33 nmol/kg) (b) in rats treated with and without BQ-788 or FR139317. Open columns, endothelin-1 or IRL1620 alone; hatched columns, with BQ-788; stippled columns, with FR139317. Each column is expressed as the mean \pm S.E.M. (vertical bar) of % changes from the value before the peptide injection ($n=4-5$). * $P < 0.05$, compared with the value obtained from each control rat without treatment.

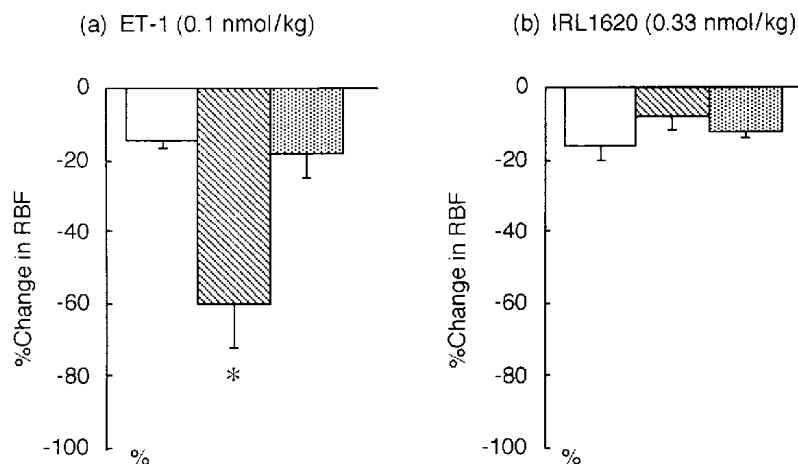


Fig. 5. Responses of renal blood flow (RBF) to endothelin-1 (0.1 nmol/kg) (a) or IRL1620 (0.33 nmol/kg) (b) in rats treated with and without BQ-788 or FR139317. Open columns, endothelin-1 or IRL1620 alone; hatched columns, with BQ-788; stippled columns, with FR139317. Each column is expressed as the mean \pm S.E.M. (vertical bar) of % reduction from the value before the peptide injection ($n=4-5$). * $P < 0.05$, compared with the value obtained from each control rat without treatment.

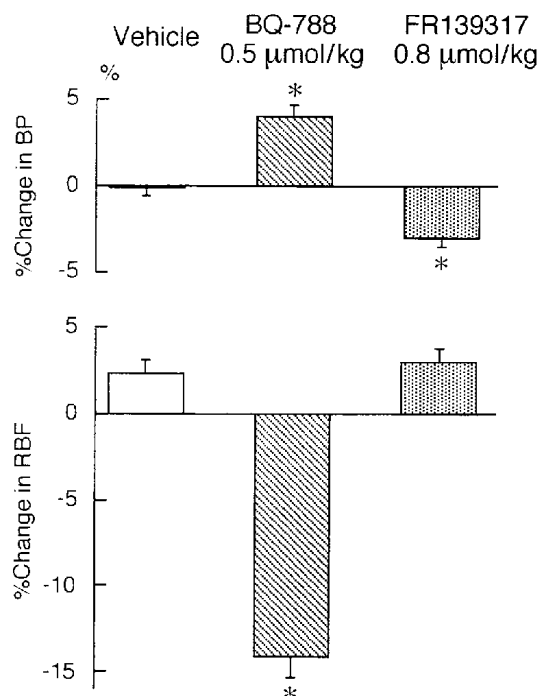


Fig. 6. Effects on blood pressure (BP) and renal blood flow (RBF) of i.v. administration of vehicle (0.1 ml of 0.9% saline containing 0.1% bovine serum albumin, $n=22$, open columns), BQ-788 (0.5 μmol/kg, $n=21$, hatched columns) and FR139317 (0.8 μmol/kg, $n=18$, stippled columns). Data are expressed as means ± S.E.M. (vertical bars) of % changes from baseline values. * $P < 0.05$, compared with the vehicle group.

Gardiner et al. (33) who showed that the endothelin ET_B -receptor agonist BQ-3020 had dose-dependent pressor effects in conscious rats. The findings that the pressor effect of sarafotoxin 6c was antagonized by BQ-123 (34) and that of BQ-3020 by FR139317 (33) may suggest either the presence of another receptor subtype or that there may be an interaction between the endothelin ET_B -receptor agonists and endothelin ET_A -receptors or between endothelin ET_A -receptor antagonists and endothelin ET_B -receptors. In our experiment, the pressor effect of a higher dose of IRL1620 was antagonized by the endothelin ET_A -receptor antagonist FR139317 but not by the endothelin ET_B -receptor antagonist BQ-788. Hence, our observation together with those of others suggested that IRL1620 at the higher dose could induce a relatively weak endothelin ET_A -mediated vasoconstriction in rats.

The renal blood flow was remarkably reduced following i.v. injections of both endothelin-1 and IRL1620 in the present experiment. The suggestion that non-endothelin ET_A -receptors mediate renal vasoconstriction came from in vitro studies in which selective antagonists of the endothelin ET_A -receptor failed to inhibit completely endothelin induced vasoconstriction in the kidney (18, 22,

24). Furthermore, it has been shown that the endothelin-1-induced renal vasoconstriction in the rat was largely independent of endothelin ET_A -receptor activation (19–21), but rather due to activation of endothelin ET_B -receptors (18, 22, 23) or to a yet to be characterized novel endothelin receptor (21, 25). A number of studies have demonstrated that endothelin ET_B -receptor agonists (e.g., sarafotoxin 6c, [Ala^{1,3,11,15}]endothelin-1 and endothelin-3) induced renal vasoconstriction, and these responses were not inhibited by BQ-123 (19, 20). Similarly, endothelin ET_B -receptor mediated vasoconstriction was reported in rat mesenteric artery (19, 23, 33), swine pulmonary vein (35) and rabbit saphenous vein (35, 36). A non-selective receptor (endothelin ET_A and ET_B) antagonist, PD145065, blocked the reduction in renal blood flow induced by sarafotoxin 6c (26). Our results show that the selective endothelin ET_B -receptor agonist IRL1620 also produced renal vasoconstriction, which was partially blocked by BQ-788, thereby indicating that the stimulation of endothelin ET_B -receptor caused renal vasoconstriction in rats.

On the contrary, Gardiner et al. (33) showed that renal vasoconstrictor responses to endothelin-1 and BQ-3020 were attenuated by FR139317 in rats. We also demonstrated that FR139317 partially and significantly attenuated renal vasoconstriction by a non-selective receptor agonist, endothelin-1. Thus, it was suggested that the endothelin ET_A -receptor as well as endothelin ET_B -receptor may be involved, at least in part, in the renal vasoconstriction of endothelin-1, the endothelin ET_B -receptor having a predominant role.

Reduction of renal blood flow by the endothelin ET_B -receptor stimulation by IRL1620 was significantly suppressed in rats treated with BQ-788. However, unexpectedly, BQ-788 enhanced endothelin-1-induced reduction of renal blood flow in the present experiment. It might be feasible that the endothelin ET_B -receptor stimulation in the kidney would exert a vasodilating action in addition to vasoconstriction for the following reasons: First, at the dose of BQ-788 used in the present experiment, the decrease in blood pressure (systemic vasodilation) induced by endothelin-1 and IRL1620 was completely abolished. Second, i.v. administration of BQ-788 alone reduced renal blood flow by $14.1 \pm 1.2\%$ with a minimum change in blood pressure, suggesting that the endothelin ET_B -receptor stimulation by endogenous endothelin peptides may possibly exert renal vasodilation. Moreover, the endothelin ET_B -receptor located on the endothelial cells was reported to mediate vasodilation through enhanced production and release of prostaglandins (37) and/or nitric oxide (14). The renal production of vasodilating substances such as nitric oxide and prostaglandins were reported to be enhanced in anesthetized

dogs administered endothelin peptides (15) or the endothelin ET_B-receptor agonist IRL1620 (16).

In summary, the systemic depressor responses induced by endothelin-1 and IRL1620 are mediated by endothelin ET_B-receptors, and the pressor responses are induced by endothelin ET_A-receptors. In the kidney, it is suggested that both endothelin ET_B- and ET_A-receptors have an important role in the regulation of renal hemodynamics.

It is of note that BQ-788 completely blocked vaso-depressor action of either endothelin-1 and IRL1620, whereas it only partially attenuated the renal vasoconstriction elicited by endothelin ET_B-receptor agonist. This may indicate that BQ-788 has higher affinity to the endothelin ET_{B1}-receptor subtype mediating vasodilation located on the endothelium, which was described previously (25, 38), than to the endothelin ET_{B2}-receptor subtype mediating renal vasoconstriction located on vascular smooth muscle.

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