ELSEVIER

European Journal of Pharmacology 359 (1998) 177–184

ejp

Renal effects of endothelin in anesthetized rabbits

Naoto Ono, Toshiyuki Matsui, Makoto Yoshida, Mizue Suzuki-Kusaba, Hiroaki Hisa *, Susumu Satoh

Department of pharmacology, Pharmaceutical Institute, Tohoku University, Aobayama, Sendai 980-8578, Japan

Received 29 July 1998; revised 24 August 1998; accepted 28 August 1998

Abstract

Intrarenal arterial infusion of endothelin-1 (1, 3 and 10 ng/kg per min) reduced renal blood flow, urine flow rate and urinary Na^+ excretion without affecting fractional Na^+ excretion in anesthetized rabbits. An endothelin ET_A receptor antagonist (*R*)2-[(*R*)-2-[(*S*)-2-[[1-(hexahydro-1*H*-azepinyl)]carbonyl]amino-4-methyl-pentanoyl]amino-3-[3-(1-methyl-1*H*-indolyl)]propionyl]amino-3-(2-pyridyl)propionic acid (FR139317, 1 μg/kg per min) attenuated the endothelin-1 (1 ng/kg per min)-induced renal responses. An endothelin ET_B receptor antagonist *N-cis* 2,6-dimetylpiperidinocarbonyl-L-γ-metylleucyl-D-1-methoxycarbonyltryptophanyl-D-norleucine (BQ-788, 1 μg/kg per min) potentiated the endothelin-1-induced changes in renal blood flow, urine flow rate and urinary Na^+ excretion. A nitric oxide (NO) synthase inhibitor N^{ω}-nitro-L-arginine methyl ester (L-NAME, 50 μg/kg per min) also potentiated the endothelin-1-induced reductions in urine flow rate and urinary Na^+ excretion but not the reduction in renal blood flow. Endothelin-1 reduced fractional Na^+ excretion in the presence of BQ-788 or L-NAME. A spontaneous NO donor 1-hydroxy-2-oxo-3-(*N*-methyl-3-aminopropyl)-3-methyl-1-triazene (30 ng/kg per min) slightly attenuated the antinatriuresis but not the vasoconstriction induced by endothelin-1. These results suggest that in the rabbit kidney in vivo endothelin ET_A receptors mediate endothelin-1-evoked vasoconstriction and tubular Na^+ reabsorption, that the concomitant stimulation of endothelin ET_B receptors by endothelin-1 counteracts both the ET_A receptor-mediated vascular and tubular actions, and that the tubular action, but not the vascular action, of endothelin-1 is also susceptible to changes in renal NO level.

Keywords: Endothelin-1; Nitric oxide (NO); Endothelin ET_A receptor; Endothelin ET_B receptor; Kidney; (Rabbit)

1. Introduction

Endothelin, a 21-amino acid peptide that is released from vascular endothelial cells, causes transient hypotension followed by potent and continuous hypertension (Yanagisawa et al., 1988). It has been reported that endothelin reduces renal blood flow and glomerular filtration rate (Kon and Badr, 1991). The renal medulla contains the highest concentration of endothelin in the body (Kitamura et al., 1989). The production of endothelin is confined to endothelial cells in the renal microvessels (MacCumber et al., 1989) and glomeruli (Marsden et al., 1991) and in the renal tubular epithelial cells (Shichiri et al., 1989). Therefore, endothelin is regarded as a modulator of renal functions.

Endothelin receptors have two main subtypes, namely, ET_A and ET_B. However, the renal effects of endothelin and the participation of endothelin ET_A and ET_B receptors in them are still controversial. Endothelin is reported to induce antinatriuresis (Goetz et al., 1988; Miller et al., 1989) or natriuresis (King et al., 1989; Munger et al., 1991). Endothelin induces renal vasoconstriction via endothelin ET_A receptors (Clavell et al., 1995) or ET_B receptors (Matsuura et al., 1996), or vasodilation via endothelin ET_B receptors (Yukimura et al., 1994).

Nitric oxide (NO) has been suggested to contribute to the maintenance of renal circulation and urine formation and to modulate the neural and humoral control of the renal functions. NO synthase inhibitors such as *N*-monomethyl-L-arginine (L-NMMA), N^G-nitro-L-arginine (L-NA) and N^{ω}-nitro-L-arginine methyl ester (L-NAME) induce renal vasoconstriction and antinatriuresis (Tolins et al., 1990; Majid et al., 1993) and facilitate norepinephrine- and angiotensin II-induced renal vasoconstriction and antinatriuresis (Adachi et al., 1996; Ono et al., 1998) in experimental animals. Our recent studies have also shown that a spontaneous NO donor 1-hydroxy-2-oxo-3-(*N*-methyl-3-

* Corresponding author. Telefax: +81-22-217-6835; Fax: +81-22-217-6835; E-mail: hhisa@mail.pharm.tohoku.ac.jp

0014-2999/98/$ - see front matter
PII: S0014-2999(98)00647-5

Table 1
Systemic and renal effects of endothelin-1 in Group 1

	Basal	Endothelin-1					
		1 ng/kg per min		3 ng/kg per min		10 ng/kg per min	
		10–20	20–30 min	10–20	20–30 min	10–20	20–30 min
MAP (mmHg)	80 ± 2	80 ± 2	79 ± 3	77 ± 3	77 ± 4	77 ± 2	77 ± 3
HR (bpm)	239 ± 11	237 ± 12	232 ± 11	230 ± 12	225 ± 12[a]	224 ± 14[b]	220 ± 17[b]
RBF (ml/min)	42 ± 5	40 ± 4	29 ± 4[b]	27 ± 3[b]	18 ± 4[b]	14 ± 3[b]	12 ± 2[b]
GFR (ml/min)	5.6 ± 1.0	5.7 ± 1.4	4.3 ± 0.6	3.8 ± 0.9[a]	2.8 ± 0.9[b]	2.2 ± 0.9[b]	1.7 ± 0.8[b]
FF (%)	23 ± 2	23 ± 3	26 ± 3	24 ± 3	25 ± 6	28 ± 11	25 ± 11
UV (ml/min)	0.29 ± 0.06	0.26 ± 0.06	0.22 ± 0.07	0.18 ± 0.06	0.11 ± 0.04[b]	0.08 ± 0.03[b]	0.04 ± 0.03[b]
UNaV (μEq/min)	19.2 ± 4.1	17.6 ± 4.2	13.7 ± 3.9[a]	10.2 ± 3.0[b]	7.9 ± 2.6[b]	4.8 ± 1.4[b]	2.6 ± 1.5[b]
FENa (%)	2.8 ± 0.7	2.6 ± 0.7	2.5 ± 0.9	2.1 ± 0.7	2.9 ± 1.0	3.3 ± 1.1	3.6 ± 2.0

Values are means ± S.E.
MAP, mean arterial pressure; HR, heart rate; RBF, renal blood flow; GFR, glomerular filtration rate; FF, filtration fraction; UV, urine flow rate; UNaV, urinary Na^+ excretion; FENa, fractional Na^+ excretion; Endothelin-1 was infused into the renal artery. $n = 6$.
[a] $P < 0.05$, [b] $P < 0.01$ compared with the corresponding basal value.

aminopropyl)-3-methyl-1-triazene (NOC 7) suppresses angiotensin II- and norepinephrine-induced renal responses in anesthetized rabbits (Adachi et al., 1997; Ono et al., 1998). Since endothelin-1 and endothelin-3 have been suggested to enhance the production of nitric oxide through stimulation of endothelin ET_B receptors located on endothelial cells (Hirata et al., 1995), NO may modify the physiological and pharmacological actions of endothelin in the kidney.

In the present study, to clarify whether endothelin ET_A or ET_B receptors participate in, and whether NO interacts with, the renal actions of endothelin, we examined effects of an endothelin ET_A receptor antagonist (*R*)2-[(*R*)-2-[(*S*)-2-[[1-(hexahydro-1*H*-azepinyl)]carbonyl]amino-4-methylpentanoyl]amino-3-[3-(1-methyl-1*H*-indolyl)]propionyl]-amino-3-(2-pyridyl)propionic acid (FR139317), an endothelin ET_B receptor antagonist *N-cis* 2,6-dimetylpiperiinocarbonyl-L-γ-metylleucyl-D-1-methoxycarbonyltryptohanyl-D-norleucine (BQ-788), a NO synthase inhibitor L-NAME and a NO donor NOC 7 on endothelin-1-induced changes in renal functions in anesthetized rabbits.

2. Materials and methods

2.1. Preparation

Male Japanese white rabbits (2.5–3.5 kg) were anesthetized with sodium pentobarbital (40 mg/kg) injected through a marginal ear vein. The trachea was cannulated and then artificially ventilated with room air (stroke volume 50 ml, 25 rpm). A double-lumen catheter was inserted into the right femoral vein for drug administration. Anesthesia was maintained by continuous infusion of pentobarbital (2–4 mg/kg per h, i.v.) throughout the experiments. Inulin, dissolved in plasma extender solution (consisting of NaCl, 0.5 g; KCl, 0.03 g; $CaCl_2$, 0.02 g; glucose, 1.5 g; sodium lactate, 0.224 g in 100 ml), was given i.v. at a priming dose of 50 mg/kg and at a maintenance dose of 1 mg/kg per min (0.1 ml/kg per min). The right femoral artery was cannulated for collection of arterial blood samples and measurement of arterial blood pressure with a pressure transducer (model TP-200T, Nihon Kohden, Tokyo, Japan). The left kidney was exposed by a retroperitoneal flank incision, and the animal was suspended by clamping one of its lumbar spinous processes to facilitate manipulation. A catheter for urine collection was inserted into the ureter. All visible renal nerves were dissected away from the renal vessels and cut

Table 2
Systemic hemodynamics during the experiments in Groups 2–6

	Blank	Basal	Endothelin-1		
			20–30 min	30–40 min	40–50 min
Group 2 ($n = 12$)		Vehicle			
MAP (mmHg)	81 ± 3	83 ± 2	84 ± 3	83 ± 3	83 ± 3
HR (bpm)	215 ± 7	218 ± 7	216 ± 6	215 ± 8	214 ± 8[b]
Group 3 ($n = 6$)		FR139317			
MAP (mmHg)	81 ± 1	77 ± 1	78 ± 1	78 ± 2	79 ± 2
HR (bpm)	230 ± 11	223 ± 12	219 ± 13	216 ± 14[b]	216 ± 15[b]
Group 4 ($n = 6$)		BQ-788			
MAP (mmHg)	72 ± 6	70 ± 6	69 ± 6	71 ± 7	70 ± 7
HR (bpm)	191 ± 14	188 ± 14	181 ± 14[b]	179 ± 14[b]	177 ± 14[b]
Group 5 ($n = 8$)		L-NAME			
MAP (mmHg)	83 ± 2	91 ± 2[a]	94 ± 3[b]	95 ± 2[b]	96 ± 3[b]
HR (bpm)	237 ± 4	228 ± 5[a]	217 ± 6[b]	214 ± 6[b]	212 ± 6[b]
Group 6 ($n = 6$)		NOC 7			
MAP (mmHg)	84 ± 3	80 ± 2	81 ± 3	81 ± 3	82 ± 3
HR (bpm)	220 ± 11	217 ± 13	214 ± 15	212 ± 15	211 ± 15[b]

Values (means ± S.E.) were obtained in the absence of drug ('Blank'), before endothelin-1 infusion ('Basal') and during endothelin infusion.
MAP, mean arterial pressure; HR, heart rate. Endothelin-1 (1 ng/kg per min), FR139317 (1 μg/kg per min), BQ-788 (1 μg/kg per min), L-NAME (50 μg/kg per min) and NOC 7 (30 ng/kg per min) were infused into the renal artery.
[a] $P < 0.05$; [b] $P < 0.01$ compared with the corresponding basal value.

after ligation to exclude possible effects of drugs on the neural control of renal function. An electromagnetic flow probe (1.5 mm in diameter, Nihon Kohden) was attached to the renal artery to measure renal blood flow with a square-wave flowmeter (model MF-27, Nihon Kohden). A curved 30-gauge needle connected to PE 10 tubing with four side branches (for single or combined intrarenal arterial infusion of drugs) was placed in the renal artery for drug infusion. Heparinized 0.9% saline (250 units/ml) was continuously infused via this catheter (1.0 ml/h) throughout the experiments. Blood pressure, heart rate and renal blood flow were recorded with a polygraph system (model PM-6000, Nihon Kohden). After completion of surgery, more than 90 min was allowed for stabilization with continuous monitoring of urine flow and hemodynamics. The animals were divided into six groups.

2.2. Experimental protocols

2.2.1. Group

Urine was collected over a 10-min period and 0.6 ml of arterial blood was withdrawn at the midpoint of urine collection ($n = 6$). Endothelin-1 was then infused into the renal artery at increasing doses of 1, 3 and 10 ng/kg per

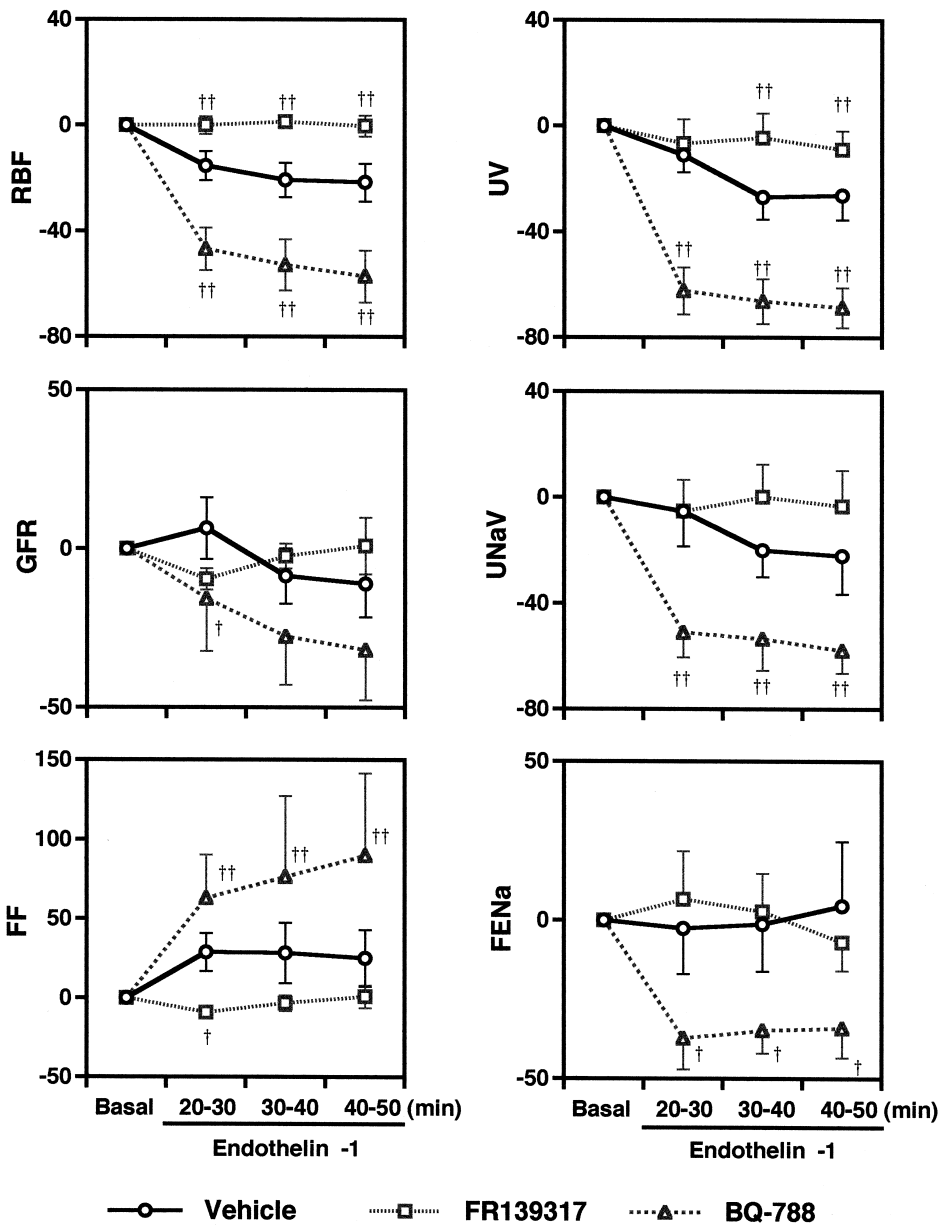

Fig. 1. Effects of vehicle (Group 2, $n = 12$), FR139317 (1 μg/kg per min, Group 3, $n = 6$) or BQ-788 (1 μg/kg per min, Group 4, $n = 6$) on endothelin-1 (1 ng/kg per min)-induced renal responses. Values (means ± S.E.) represent percent changes from the basal levels. RBF, renal blood flow; GFR, glomerular filtration rate; FF, filtration fraction; UV, urine flow rate; UNaV, urinary Na^+ excretion; FENa, fractional Na^+ excretion. †$P < 0.05$, ††$P < 0.01$ compared with the corresponding vehicle control value (Group 2).

min for 30 min each. Urine and blood samples were collected during 10–20 min and 20–30 min of endothelin-1 infusion at each dose.

2.2.2. Groups 2–6

Urine was collected over a 10-min period and 0.6 ml of arterial blood was withdrawn at the midpoint of urine collection. Then intrarenal arterial infusion of vehicle (0.9% saline, Group 2, $n = 12$), FR139317 (1 μg/kg per min, Group 3, $n = 6$), BQ-788 (1 μg/kg per min, Group 4, $n = 6$), L-NAME (50 μg/kg per min, Group 5, $n = 8$) or NOC 7 (30 ng/kg per min, Group 6, $n = 6$) was started. Thirty minutes after the start of drug infusion, urine and blood samples were collected again, and then endothelin-1 was infused into the renal artery at 1 ng/kg/min during simultaneous infusion of the drug. Urine and blood samples were collected during 20–30 min, 30–40 min and 40–50 min of endothelin-1 infusion.

2.3. Measurements

Blood samples were transferred to ice-chilled tubes containing ammonium EDTA (6 mg/ml blood) and then centrifuged to obtain plasma samples. Glomerular filtration

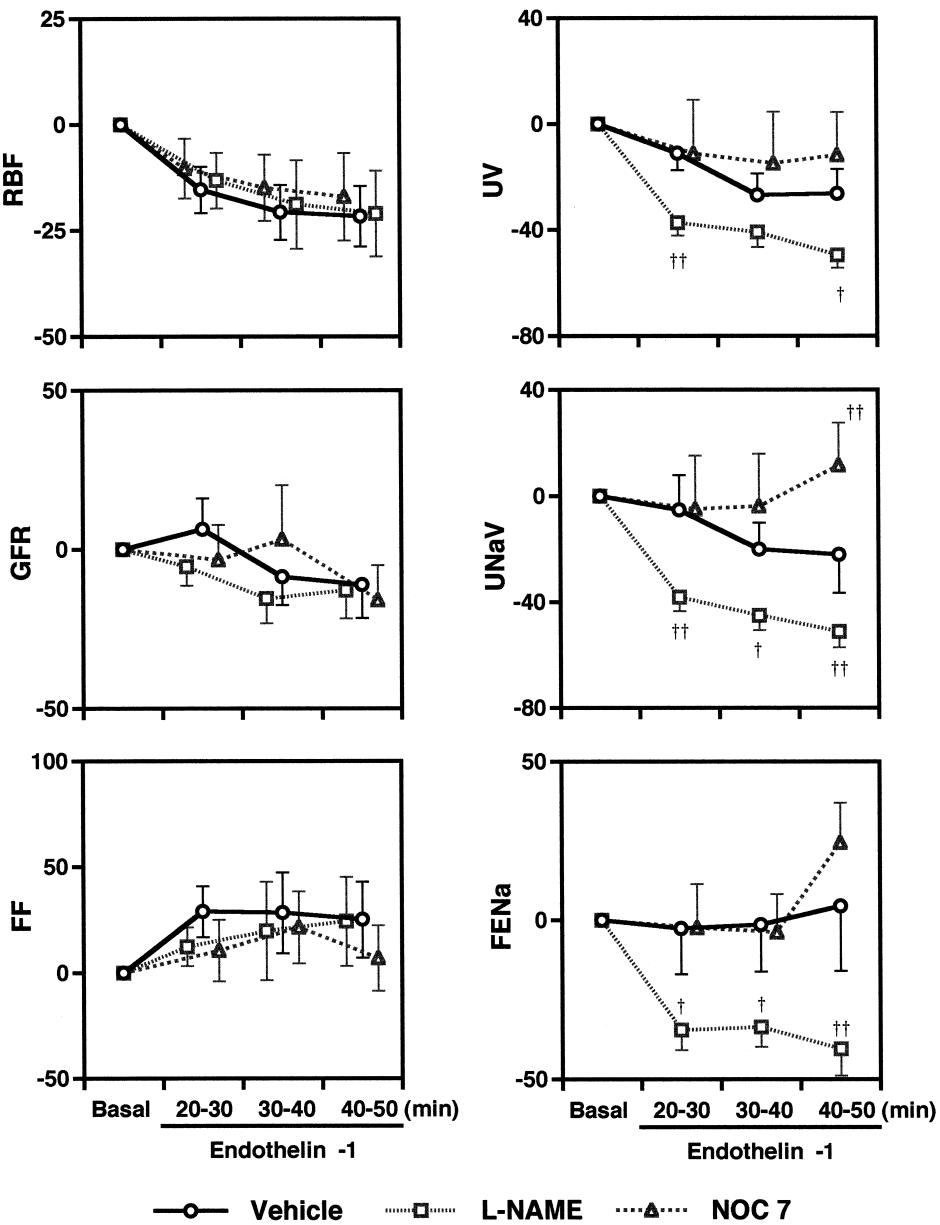

Fig. 2. Effects of L-NAME (50 μg/kg per min, Group 5, $n = 8$) or NOC 7 (30 ng/kg per min, Group 6, $n = 6$) on endothelin-1 (1 ng/kg per min)-induced renal responses. Values (means ± S.E.) represent percent changes from the basal levels. RBF, renal blood flow; GFR, glomerular filtration rate; FF, filtration fraction; UV, urine flow rate; UNaV, urinary Na^+ excretion; FENa, fractional Na^+ excretion. †$P < 0.05$, ††$P < 0.01$ compared with the corresponding vehicle control value (Group 2).

rate was determined as inulin clearance. Inulin concentration in plasma and urine was measured by the anthrone method. Na^+ and K^+ were measured by flame photometry (model 775A, Hitachi).

2.4. Drugs

Endothelin-1 (Peptide Institute, Osaka, Japan), L-NAME (Sigma Chemical, St. Louis, MO, USA) and FR139317 (Fujisawa Pharmaceutical, Tsukuba, Japan) were dissolved in 0.9% saline. BQ-788 (Banyu Pharmaceutical, Tsukuba, Japan) and NOC 7 (Dojindo Laboratories, Kumamoto, Japan) were dissolved in small amount of dimethylsulfoxide and 0.1 M NaOH, respectively, and diluted with 0.9% saline (the final concentration of dimethylsulfoxide in the drug solution was less than 1%). In preliminary experiments, we had confirmed that NaOH or dimethylsulfoxide within the same concentration range as used in this study did not affect the renal parameters.

2.5. Statistics

All values are expressed as means ± S.E. Effects of endothelin-1 were analyzed by analysis of variance (ANOVA) for single-factor repeated measures. When ANOVA showed a statistical difference, the values in the basal period and in the endothelin-1 infusion period were compared by using Dunnett's test. Effects of drugs on the percent changes induced by endothelin-1 in the vehicle group (Group 2) and the drug infusion group (Groups 3–6) were analyzed by three-factor ANOVA. When ANOVA showed a statistical difference, the values for each endothelin-1 infusion period in the vehicle group and in the drug infusion group were compared by using Dunnett's test. Student's paired *t*-test was used to compare values before drug infusion (blank) and during FR139317, BQ-788, L-NAME or NOC 7 infusion before the start of endothelin infusion (basal). Differences at $P < 0.05$ were considered to be statistically significant.

3. Results

Intrarenal arterial infusion of endothelin-1 (1, 3 and 10 ng/kg/min) gradually reduced renal blood flow, glomerular filtration rate, urine flow rate and urinary Na^+ excretion in a dose-dependent manner while causing little change in filtration fraction or fractional Na^+ excretion (Group 1, Table 1). Endothelin-1 did not affect the mean arterial pressure in either experimental group (Tables 1 and 2).

In non-treated rabbits (Group 2), intrarenal arterial infusion of endothelin-1 at 1 ng/kg per min gradually reduced renal blood flow, glomerular filtration rate, urine flow rate and urinary Na^+ excretion by about 25–30% while causing little change in fractional Na^+ excretion (Fig. 1). The endothelin-1-induced renal responses in Groups 2–6 are shown in Figs. 1 and 2 as percent changes from the basal values shown in Table 3.

Intrarenal arterial infusion of FR139317 (1 μg/kg per min) or BQ-788 (1 μg/kg per min) did not affect systemic or renal hemodynamics or urinary parameters (Tables 2 and 3). FR139317 suppressed the endothelin-1-induced changes in renal blood flow, urine flow rate and urinary Na^+ excretion (Group 3, Fig. 1). BQ-788 potentiated the endothelin-1-induced reductions in renal blood flow, glomerular filtration rate, urine flow rate and urinary Na^+ excretion (Group 4, Fig. 1). During BQ-788 infusion, endothelin-1 significantly increased filtration fraction and reduced fractional Na^+ excretion (Group 4, Fig. 1).

Table 3
Effects of vehicle, FR139317, BQ-788, L-NAME and NOC 7 on renal functions in Groups 2–6

	Blank	Basal
Group 2 ($n = 12$)		Vehicle
RBF (ml/min)	33 ± 4	33 ± 3
GFR (ml/min)	3.8 ± 0.2	3.7 ± 0.2
FF (%)	24 ± 4	23 ± 4
UV (ml/min)	0.45 ± 0.06	0.41 ± 0.04
UNaV (μEq/min)	36.7 ± 7.0	33.7 ± 4.2
FENa (%)	7.9 ± 1.5	7.3 ± 1.1
Group 3 ($n = 6$)		FR139317
RBF (ml/min)	30 ± 4	30 ± 4
GFR (ml/min)	4.1 ± 0.5	4.5 ± 0.5
FF (%)	27 ± 7	27 ± 5
UV (ml/min)	0.30 ± 0.08	0.32 ± 0.05
UNaV (μEq/min)	18.1 ± 3.6	18.1 ± 2.3
FENa (%)	3.6 ± 0.7	3.6 ± 0.5
Group 4 ($n = 6$)		BQ-788
RBF (ml/min)	23 ± 3	20 ± 4
GFR (ml/min)	2.9 ± 0.5	2.4 ± 0.5
FF (%)	24 ± 4	23 ± 4
UV (ml/min)	0.33 ± 0.04	0.24 ± 0.04
UNaV (μEq/min)	22.8 ± 2.6	16.0 ± 2.8
FENa (%)	6.3 ± 1.1	5.8 ± 1.4
Group 5 ($n = 6$)		L-NAME
RBF (ml/min)	36 ± 3	24 ± 4[b]
GFR (ml/min)	4.2 ± 0.6	3.9 ± 0.4
FF (%)	22 ± 4	40 ± 9[a]
UV (ml/min)	0.37 ± 0.03	0.24 ± 0.03[b]
UNaV (μEq/min)	25.1 ± 4.7	15.7 ± 3.3[b]
FENa (%)	5.2 ± 1.4	3.5 ± 1.1[b]
Group 6 ($n = 6$)		NOC 7
RBF (ml/min)	32 ± 3	33 ± 3
GFR (ml/min)	4.4 ± 0.7	4.7 ± 0.5
FF (%)	25 ± 3	26 ± 3
UV (ml/min)	0.28 ± 0.05	0.28 ± 0.06
UNaV (μEq/min)	19.9 ± 2.4	19.6 ± 4.0
FENa (%)	3.6 ± 0.6	3.1 ± 0.5

Values (means ± S.E.) were obtained in the absence of drug ('Blank') and before endothelin-1 infusion ('Basal'). FR139317 (1 μg/kg per min), BQ-788 (1 μg/kg per min), L-NAME (50 μg/kg per min) and NOC 7 (30 ng/kg per min) were infused into the renal artery. Abbreviations are as in Table 1.
[a] $P < 0.05$; [b] $P < 0.01$ compared with the corresponding blank value.

Intrarenal arterial infusion of L-NAME (50 μg/kg per min) reduced renal blood flow, urine flow rate, urinary Na^+ excretion and fractional Na^+ excretion (Group 5, Table 3). L-NAME potentiated the endothelin-1-induced changes in urine flow rate and urinary Na^+ excretion without affecting the change in renal blood flow or glomerular filtration rate (Group 5, Fig. 2). During L-NAME infusion, endothelin-1 significantly reduced fractional Na^+ excretion (Group 5).

Intrarenal arterial infusion of NOC 7 (30 ng/kg per min, Group 6) did not affect the basal values (Table 3) or the endothelin-1-induced decrease in renal blood flow (Fig. 2), but it tended to attenuate the endothelin-1-induced reduction in urine flow rate and suppressed the reduction in urinary Na^+ excretion (Fig. 2).

In additional experiments ($n = 4$), we examined the effects of simultaneous administration of BQ-788 (1 μg/kg per min) and FR139317 (1 μg/kg per min) on the endothelin-1-induced renal responses ($n = 4$). Endothelin-1 (1 ng/kg per min) failed to affect the renal parameters in the presence of these two antagonists. The values before and during endothelin-1 infusion (40–50 min of infusion) were as follows: renal blood flow, 25 ± 3 and 25 ± 3 ml/min; glomerular filtration rate, 2.0 ± 0.4 and 1.9 ± 0.2 ml/min; urine flow rate, 0.20 ± 0.05 and 0.22 ± 0.07 ml/min; urinary Na^+ excretion, 19.0 ± 4.2 and 20.8 ± 6.2 μEq/min; and fractional Na^+ excretion, $8.8 \pm 2.6\%$ and $9.7 \pm 3.4\%$, respectively.

4. Discussion

The present study was performed to elucidate the roles of endothelin ET_A and ET_B receptors and the NO system in the renal actions of endothelin. The effects of FR139317 (endothelin ET_A receptor antagonist), BQ-788 (endothelin ET_B receptor antagonist), L-NAME (NO synthase inhibitor) and NOC 7 (NO donor) on endothelin-1-induced changes in renal hemodynamics and urine formation were evaluated in anesthetized rabbits.

In the first series of experiments (Group 1), the dose–response relationship of endothelin-1 was examined to clarify its renal actions. Intrarenal arterial infusion of endothelin-1 (1, 3 and 10 ng/kg per min) reduced renal blood flow, glomerular filtration rate, urine flow rate and urinary Na^+ excretion in a dose-dependent manner. However, no statistically significant change in fractional Na^+ excretion was observed with any dose of endothelin-1, implying that endothelin-1 does not affect renal tubular Na^+ reabsorption. Although endothelin-1 is reported to reduce both glomerular filtration rate and fractional Na^+ excretion in anesthetized dogs (Takagi et al., 1993) and rats (Matsumura et al., 1989), our present results suggest that endothelin-1, like angiotensin II (Adachi et al., 1996, 1997), induces antinatriuresis predominantly through renal vasoconstriction and hypofiltration in anesthetized rabbits.

In the following series of experiments (Groups 2 to 6), endothelin was infused at a dose of 1 ng/kg per min, a dose which had been found in Group 1 experiments to reduce urine flow rate and urinary Na^+ excretion to almost the same extent (by about 25–30%) as the reduction obtained by infusion of angiotensin II or norepinephrine in our previous studies (Adachi et al., 1996, 1997; Ono et al., 1998). Infusion of endothelin at this dose alone in non-treated rabbits elicited moderate renal responses (Group 2).

It has been reported that an endothelin ET_A receptor antagonist BQ-123 does not suppress the endothelin-1-induced reduction in renal blood flow in anesthetized rats (Cristol et al., 1993). Recent studies, however, have shown that BQ-123 prevents vasoconstrictor responses to endothelin-1 in perfused rabbit kidneys (D'Orléans-Juste et al., 1995) and in anesthetized dogs (Clavell et al., 1995). In our study, FR139317 abolished the endothelin-1-induced reductions in renal blood flow, glomerular filtration rate, urine flow rate and urinary Na^+ excretion (Group 3). These results suggest that in anesthetized rabbits endothelin-1-induced renal vasoconstriction and hypofiltration are mediated by endothelin ET_A receptors.

BQ-788 has been reported to enhance the endothelin-1-induced reduction in renal blood flow in anesthetized rats (Matsuura et al., 1996) and the elevation of perfusion pressure in isolated rabbit kidneys (D'Orléans-Juste et al., 1995). In our present study, BQ-788 potentiated the endothelin-1-induced reductions in renal blood flow, glomerular filtration rate, urine flow rate and urinary Na^+ excretion (Group 4). Furthermore, endothelin-1 reduced fractional Na^+ excretion during BQ-788 infusion. It should be noted that infusion of endothelin-1 alone at the higher dose (3 ng/kg per min, Group 1) failed to affect fractional Na^+ excretion, although it reduced the values of other renal parameters to almost the same extent as observed in these experiments (Group 4). Therefore the endothelin-1-induced reduction in fractional Na^+ excretion in the presence of BQ-788 does not depend on changes in renal hemodynamics or glomerular filtration. We also observed that the facilitatory effects of BQ-788 on the endothelin-1-induced renal responses were abolished by simultaneous infusion of FR139317. Our present results suggest that endothelin-1-induced renal vasoconstriction and hypofiltration, both of which are mediated by endothelin ET_A receptors, are attenuated by concomitant stimulation of endothelin ET_B receptors, and that endothelin-1 can facilitate tubular Na^+ reabsorption through stimulation of endothelin ET_A receptors, but the renal tubular action is counteracted by endothelin ET_B receptor-mediated mechanisms.

It has been reported that pretreatment with a NO synthase inhibitor L-NMMA enhances the reduction in renal blood flow observed during intravenous infusion of endothelin-1 in anesthetized dogs (Lerman et al., 1992), and that a soluble guanylate cyclase inhibitor methylene blue augments the endothelin-induced elevation of perfusion pressure in isolated rat kidneys (Hirata et al., 1991). Thus

NO seems to suppress endothelin-induced vasoconstriction in the kidney. However, it has also been reported that a NO synthase inhibitor L-NNA does not enhance the endothelin-1-induced reduction in renal blood flow in conscious dogs (Fitzgerald et al., 1995). In the present study, the endothelin-1-induced reduction in renal blood flow was not enhanced by L-NAME treatment (Group 5). Neither did NOC 7, which can effectively suppress angiotensin II-induced renal vasoconstriction (Adachi et al., 1997), affect the endothelin-1-induced renal vasoconstriction (Group 6). NO may therefore not modify the renal vascular response to endothelin-1 in anesthetized rabbits.

However, L-NAME potentiated and NOC 7 tended to suppress the reductions in urine flow rate and urinary Na^+ excretion induced by endothelin-1 (Groups 5 and 6). Endothelin-1 reduced fractional Na^+ excretion in the presence of L-NAME (Group 5). These results suggest that NO counteracts the endothelin-1-induced antinatriuresis through suppression of tubular Na^+ reabsorption. In the rat proximal straight tubules or in the rabbit inner medullary collecting duct cells, endothelin prevents tubular Na^+ reabsorption by inhibition of Na^+, K^+-ATPase activity (Zeidel et al., 1989; Garvin and Sanders, 1991). In the cultured porcine kidney epithelial cell line ($LLC\text{-}PK_1$ cells), endothelin-1 or endothelin-3 elevates cGMP levels via formation of an endothelium-derived relaxing factor-like substance (Ishii et al., 1991), and stimulation of endothelin ET_B receptors enhances cGMP production (Ozaki et al., 1994). Cyclic GMP has been suggested to inhibit amiloride-sensitive Na^+ channels (Cantiello and Ausiello, 1986; Mohrmann et al., 1987) or Na^+, K^+-ATPase activity (Aperia et al., 1994; Scavone et al., 1995). In preliminary experiments, we observed that an endothelin ET_B receptor agonist sarafotoxin S6c elevated fractional Na^+ excretion in anesthetized rabbits, an effect which was attenuated by L-NAME pretreatment. It is therefore possible that NO participates in the endothelin ET_B receptor-mediated suppression of tubular Na^+ reabsorption during endothelin-1 infusion.

In conclusion, the present study suggests that in the rabbit kidney in vivo (1) endothelin-1 causes hypoperfusion and hypofiltration through stimulation of endothelin ET_A receptors, (2) stimulation of endothelin ET_A receptors can evoke tubular Na^+ reabsorption, but the tubular action is masked and the vascular action is blunted by simultaneous stimulation of endothelin ET_B receptors and (3) the renal NO system counteracts the endothelin ET_A receptor-mediated tubular action but not the vascular action of endothelin-1.

Acknowledgements

This work was supported by The Research Foundation for Pharmaceutical Sciences, Japan (to H. H.). BQ-788 was generously provided by Banyu Pharmaceutical, Tsukuba, Japan and FR139317 was generously provided by Fujisawa Pharmaceutical, Tsukuba, Japan.

References

Adachi, Y., Hashimoto, K., Hisa, H., Yoshida, M., Suzuki-Kusaba, M., Satoh, S., 1996. Angiotensin II-induced renal responses in anesthetized rabbits: effects of N^{ω}-nitro-L-arginine methyl ester and losartan. Eur. J. Pharmacol. 308, 165–171.

Adachi, Y., Hashimoto, K., Ono, N., Yoshida, M., Suzuki-Kusaba, M., Hisa, H., Satoh, S., 1997. Renal effects of a nitric oxide donor, NOC 7, in anesthetized rabbits. Eur. J. Pharmacol. 324, 223–226.

Aperia, A., Holtback, U., Syren, M.L., Svensson, L.B., Fryckstedt, J., Greengard, P., 1994. Activation/deactivation of renal Na^+, K^+-ATPase: a final common pathway for regulation of natriuresis. FASEB J. 8, 436–439.

Cantiello, H.F., Ausiello, D.A., 1986. Atrial natriuretic factor and cGMP inhibit amiloride-sensitive Na^+ transport in the cultured renal epithelial cell line, LLC-PK1. Biochem. Biophys. Res. Commun. 134, 852–860.

Clavell, A.L., Stingo, A.J., Margulies, K.B., Brandt, R.R., Burnett Jr., J.C., 1995. Role of endothelin receptor subtypes in the in vivo regulation of renal function. Am. J. Physiol. 268, F455–F460.

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, 108, 776–779.

D'Orléans-Juste, P., Yano, M., Maurice, M.C., Gratton, J.P., 1995. Hyperresponsiveness to ET-1 after treatment with a mixture of ET_A and ET_B receptor antagonists in the rabbit in vivo and in vitro. J. Cardiovasc. Pharmacol. 26 (Suppl. 3), S369–S372.

Fitzgerald, S.M., Evans, R.G., Christy, I.J., Anderson, W.P., 1995. Nitric oxide synthase blockade and renal vascular responses to norepinephrine and endothelin-1 in conscious dogs. J. Cardiovasc. Pharmacol. 25, 979–985.

Garvin, J., Sanders, K., 1991. Endothelin inhibits fluid and bicarbonate transport in part by reducing Na^+/K^+ ATPase activity in the rat proximal straight tubule. J. Am. Soc. Nephrol. 2, 976–982.

Goetz, K.L., Wang, B.C., Madwed, J.B., 1988. Cardiovascular, renal, and endocrine responses to intravenous endothelin in conscious dogs. Am. J. Physiol. 255, R1064–R1068.

Hirata, Y., Matsuoka, H., Kimura, K., Sugimoto, T., Hayakawa, H., Suzuki, E., Sugimoto, T., 1991. Role of endothelium-derived relaxing factor in endothelin-induced renal vasoconstriction. J. Cardiovasc. Pharmacol. 17 (Suppl. 7), S169–S171.

Hirata, Y., Hayakawa, H., Suzuki, E., Kimura, K., Kikuchi, K., Nagano, T., Hirobe, M., Omata, M., 1995. Direct measurements of endothelium-derived nitric oxide release by stimulation of endothelin receptors in rat kidney and its alteration in salt-induced hypertension. Circulation 91, 1229–1235.

Ishii, K., Warner, T.D., Sheng, H., Murad, F., 1991. Endothelin increases cyclic GMP levels in LLC-PK1 Porcine kidney epithelial cells via formation of an endothelium-derived relaxing factor-like substance. J. Pharmacol. Exp. Ther. 259, 1102–1108.

King, A.J., Brenner, B.M., Anderson, S., 1989. Endothelin: a potent renal and systemic vasoconstrictor peptide. Am. J. Physiol. 256, F1051–F1058.

Kitamura, K., Tanaka, T., Kato, J., Ogawa, T., Eto, T., Tanaka, K., 1989. Immunoreactive endothelin in rat kidney inner medulla: marked decrease in spontaneously hypertensive rats. Biochem. Biophys. Res. Commun. 162, 38–44.

Kon, V., Badr, K.F., 1991. Biological actions and pathophysiologic significance of endothelin in the kidney. Kidney Int. 40, 1–12.

Lerman, A., Sandok, E.K., Hildebrand Jr., F.R., Burnett Jr., J.C., 1992. Inhibition of endothelium-derived relaxing factor enhances endothelin-mediated vasoconstriction, 85, 1894–1898.

MacCumber, M.W., Ross, C.A., Glaser, B.M., Snyder, S.H., 1989. Endothelin: visualization of mRNAs by in situ hybridization provides evidence for local action. Proc. Natl. Acad. Sci. USA 86, 7285–7289.

Majid, D.S.A., Williams, A., Navar, L.G., 1993. Inhibition of nitric oxide synthesis attenuates pressure-induced natriuretic responses in anesthetized dogs. Am. J. Physiol. 264, F79–F87.

Marsden, P.A., Dorfman, D.M., Collins, T., Brenner, B.M., Orkin, S.H., Ballermann, B.J., 1991. Regulated expression of endothelin-1 in glomerular capillary endothelial cells. Am. J. Physiol. 261, F117–F125.

Matsumura, Y., Hisaki, K., Ohyama, T., Hayashi, K., Morimoto, S., 1989. Effects of endothelin on renal function and renin secretion in anesthetized rats. Eur. J. Pharmacol. 166, 577–580.

Matsuura, T., Yukimura, T., Kim, S., Miura, K., Iwao, H., 1996. 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. Jpn. J. Pharmacol. 71, 213–222.

Miller, W.L., Redfield, M.M., Burnett Jr., J.C., 1989. Integrated cardiac, renal, and endocrine actions of endothelin. J. Clin. Invest. 83, 317–320.

Mohrmann, M., Cantiello, H.F., Ausiello, D.A., 1987. Inhibition of epithelial Na^+ transport by atriopeptin, protein kinase C, and pertussis toxin. Am. J. Physiol. 253, F372–F376.

Munger, K.A., Sugiura, M., Takahashi, K., Inagami, T., Badr, K.F., 1991. A role for atrial natriuretic peptide in endothelin-induced natriuresis. J. Am. Soc. Nephrol. 1, 1278–1283.

Ono, N., Adachi, Y., Hashimoto, K., Yoshida, M., Suzuki-Kusaba, M., Hisa, H., Satoh, S., 1998. A nitric oxide donor NOC 7 suppresses renal responses induced by norepinephrine and angiotensin II in the NO-depleted denevated rabbit kidney. Eur. J. Pharmacol. 342, 285–289.

Ozaki, S., Ihara, M., Saeki, T., Fukami, T., Ishikawa, K., Yano, M., 1994. Endothelin ET_B receptors couple to two distinct signalling pathways in porcine kidney epithelial LLC-PK1 cells. J. Pharmacol. Exp. Ther. 270, 1035–1040.

Scavone, C., Scanlon, C., McKee, M., Nathanson, J.A., 1995. Atrial natriuretic peptide modulates sodium and potassium-activated adenosine triphosphatase through a mechanism involving cyclic GMP and cyclic GMP-dependent protein kinase. J. Pharmacol. Exp. Ther. 272, 1036–1043.

Shichiri, M., Hirata, Y., Emori, T., Ohta, K., Nakajima, T., Sato, K., Sato, A., Marumo, F., 1989. Secretion of endothelin and related peptides from renal epithelial cell lines. FEBS Lett. 253, 203–206.

Takagi, H., Takahara, A., Tomura, Y., Yamagata, T., Hisa, H., Satoh, S., 1993. Effects of nifedipine, 8-(*N*, *N*-diethylamino) octyl-3, 4, 5-trimethoxybenzoate hydrochloride and atrial natriuretic peptide on endothelin-induced antinatriures in dogs. J. Pharmacol. Exp. Ther. 267, 456–461.

Tolins, J.P., Palmer, R.M.J., Moncada, S., Raij, L., 1990. Role of endothelium-derived relaxing factor in regulation of renal hemodynamic responses. Am. J. Physiol. 258, H655–H662.

Yanagisawa, M., Kurihara, H., Kimura, S., Tomobe, Y., Kobayashi, M., Mitsui, Y., Yazaki, Y., Goto, K., Masaki, T., 1988. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 332, 411–415.

Yukimura, T., Yamashita, Y., Miura, K., Kim, S., Iwao, H., Takai, M., Okada, T., 1994. Renal vasodilating and diuretic actions of a selective endothelin ET_B receptor agonist, IRL1620. Eur. J. Pharmacol. 264, 399–405.

Zeidel, M.L., Brady, H.R., Kone, B.C., Gullans, S.R., Brenner, B.M., 1989. Endothelin, a peptide inhibitor of Na^+–K^+-ATPase in intact renal tubular epithelial cells. Am. J. Physiol. 257, C1101–C1107.