0022-3565/93/2671-0456$03.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 267, No. 1
Printed in U.S.A.

Effects of Nifedipine, 8-(N,N-Diethylamino)Octyl-3,4,5-Trimethoxybenzoate Hydrochloride and Atrial Natriuretic Peptide on Endothelin-Induced Antinatriuresis in Dogs

HIROMITSU TAKAGI, AKIRA TAKAHARA, YUICHI TOMURA, TSUYOSHI YAMAGATA, HIROAKI HISA and SUSUMU SATOH[1]

Department of Pharmacology, Pharmaceutical Institute, Tohoku University, Sendai, Japan

Accepted for publication June 28, 1993

ABSTRACT

Nifedipine, 8-(N,N-diethylamino)octyl-3,4,5-trimethoxybenzoate hydrochloride (TMB-8) or atrial natriuretic peptide (ANP) was infused into the renal artery before and during intrarenal arterial infusion of endothelin-1 (ET) in anesthetized dogs. Before ET infusion, nifedipine (0.1 $\mu g\ kg^{-1}\ min^{-1}$), TMB-8 (75 $\mu g\ kg^{-1}\ min^{-1}$) or ANP (10 $ng\ kg^{-1}\ min^{-1}$) increased the urine flow rate, urinary sodium excretion and fractional sodium excretion with little change in renal blood flow or glomerular filtration rate. ET (2 $ng\ kg^{-1}\ min^{-1}$) reduced the basal renal blood flow, glomerular filtration rate, urine flow rate, urinary sodium excretion and fractional sodium excretion. Both nifedipine and TMB-8 induced natriuresis during ET infusion; but only TMB-8 completely reversed the ET-induced reduction in fractional sodium excretion and partially antagonized the reductions in urine flow rate and urinary sodium excretion. ANP did not induce substantial urinary responses during ET infusion. Neither nifedipine, TMB-8 nor ANP reversed the ET-induced decreases in renal blood flow and glomerular filtration rate. The present study suggests that in the dog kidney 1) the ET-induced antinatriuresis is caused in part by enhancement of tubular sodium reabsorption, 2) the tubular action of ET depends on TMB-8-sensitive calcium movements but not calcium influx through dihydropyridine-sensitive channels and 3) ANP cannot counteract the ET-induced antinatriuresis.

ET, a potent and long-lasting vasoconstrictor peptide (Yanagisawa *et al.*, 1988), is well known to affect renal hemodynamics and urinary sodium and water excretion. ET induces potent renal vasoconstriction and reduces the GFR (Kon and Badr, 1991). Although ET can inhibit renal tubular sodium reabsorption (Perico *et al.*, 1990), which may be related in part to inhibition of Na^+-K^+-ATPase activity in renal tubular cells (Zeidel *et al.*, 1989), many reports have shown that ET reduces the UNaV in rats (Cao and Banks, 1990; Hirata *et al.*, 1989; Matsumura *et al.*, 1989) and in dogs (Cao and Banks, 1990; Goetz *et al.*, 1988; Miller *et al.*, 1989). The antinatriuresis induced by ET may result from a substantial decrease in GFR, which would overcome the tubular action of ET. However, ET also reduces the FENa in some studies (Matsumura *et al.*, 1989; Miller *et al.*, 1989). The ET-induced antinatriuresis therefore seems to involve a component that is independent of the change in GFR.

ET raises the free calcium concentration in vascular smooth muscle cells (Marsden *et al.*, 1989). Some reports suggest that the influx of extracellular calcium through voltage-dependent channels participates in the vascular effect of ET *in vitro* (Yanagisawa *et al.*, 1988) and *in vivo* (Madeddu *et al.*, 1990). ET also elevates the free calcium level in mesangium cells (Simonson and Dunn, 1991) and in collecting duct cells (Naruse *et al.*, 1991). Thus, the increase in intracellular calcium may mediate the renal responses induced by ET.

Receptor binding studies have shown that the localization of ET receptors in the kidney (Jones *et al.*, 1989; Kohzuki *et al.*, 1989) overlaps the binding sites of ANP (Chai *et al.*, 1986). By contrast with ET, ANP increases UNaV and urinary water excretion, which may be the result of renal vasodilation and/or inhibition of tubular sodium reabsorption (Needleman *et al.*, 1985). ANP is also reported to reverse the contractile effect of ET on an isolated vasculature (Opgenorth and Novosad, 1990). These findings suggest some interactions between renal actions of ET and ANP.

Considering these data, calcium entry blockers and ANP would be expected to antagonize the ET-induced antinatriuresis. Katoh *et al.* (1990) reported that simultaneous infusion of ET with nicardipine or ANP into the rat kidney caused

Received for publication April 15, 1993.
[1] This work was supported in part by Grant-in-Aid for Scientific research (The Ministry of Education, Japan) no. 03670089 and by the Sapporo Bioscience Foundation (Tokyo, Japan).

ABBREVIATIONS: ANP, atrial natriuretic peptide; ET, endothelin; FENa, fractional sodium excretion; GFR, glomerular filtration rate; RBF, renal blood flow; UV, urine flow rate; UNaV, urinary sodium excretion; TMB-8, 8-(N,N-diethylamino)octyl-3,4,5-trimethoxybenzoate hydrochloride.

smaller renal responses than ET infusion alone. However, some studies have provided evidence against the inhibitory effects of calcium entry blockers or ANP on the ET-induced renal responses (Cao and Banks, 1990; Ota *et al.*, 1992). This issue is still controversial.

Recent reports from our laboratory demonstrated that TMB-8, a putative inhibitor of calcium release from intracellular stores (Chiou and Malagodi, 1975), induced natriuresis (Takahara *et al.*, 1991) and suppressed renal nerve stimulation-induced antinatriuresis in dogs (Ogasawara *et al.*, 1993). The mechanisms of renal sodium handling may involve pathways that depend on the TMB-8-sensitive calcium movements. It is possible that the ET-induced antinatriuresis is also susceptible to TMB-8.

In the present study, we examined whether the ET-induced antinatriuresis could be counteracted with a calcium entry blocker, a calcium release inhibitor or ANP in anesthetized dogs. The effects of nifedipine, TMB-8 and ANP on urinary parameters were compared before and during intrarenal arterial infusion of ET.

Methods

Animal preparation. Mongrel dogs of either sex weighing 10 to 25 kg were anesthetized with sodium pentobarbital (30 mg/kg i.v.) and then artificially ventilated with room air. Decamethonium bromide (0.25 mg kg^{-1} min^{-1} i.v.) was given to prevent spontaneous active respiratory movement. Anesthesia was maintained by a continuous i.v. infusion of pentobarbital sodium (5 mg kg^{-1} hr^{-1}) throughout the experiments. Inulin, dissolved in 0.45% NaCl and 2.5% dextrose, was given i.v. at a priming dose of 50 mg/kg and at a maintenance dose of 1 mg kg^{-1} min^{-1}. The right brachial artery was cannulated for collection of blood samples and measurement of systemic blood pressure with a pressure transducer (model TP-200T, Nihon Kohden, Tokyo, Japan). The right and left kidneys were exposed by retroperitoneal flank incisions. Catheters for urine collection were inserted into both the right and left ureters. All visible renal nerves were dissected away from the renal vessels and cut after ligation. Electromagnetic flow probes (2.5–3.5 mm in diameter, Nihon Kohden) were attached to the renal arteries to measure the RBF with a square-wave flowmeter (model MF-27, Nihon Kohden). Curved 25-gauge needles connected to polyethylene tubes were inserted into the left renal artery for drug infusion. The heart rate was monitored by electrocardiography. The mean arterial pressure, heart rate and RBF were recorded with a polygraph system (Nihon Kohden). After completion of surgery, 60 to 90 min were allowed

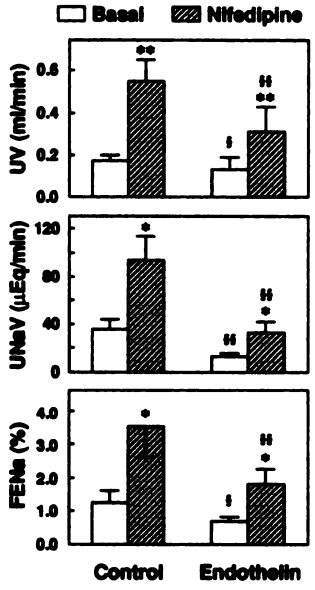

Fig. 1. Effects of nifedipine (0.1 μg kg^{-1} min^{-1}) on UV, UNaV and FENa before and during ET infusion (2 ng kg^{-1} min^{-1}, group 1). The values are the means ± S.E. $n = 6$. *P < .05, **P < .01 compared with basal values. §P < .05, §§P < .01 compared with values at corresponding sampling points before ET infusion (Control).

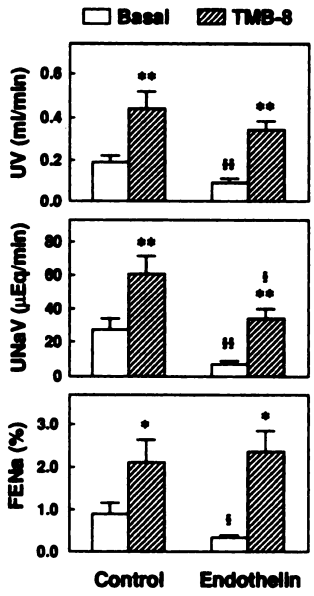

Fig. 2. Effects of TMB-8 (75 μg kg^{-1} min^{-1}) on UV, UNaV and FENa before and during ET infusion (2 ng kg^{-1} min^{-1}, group 2). The values are the means ± S.E. $n = 6$. *P < .05, **P < .01 compared with basal values. §P < .05, §§P < .01 compared with values at corresponding sampling points before ET infusion (Control).

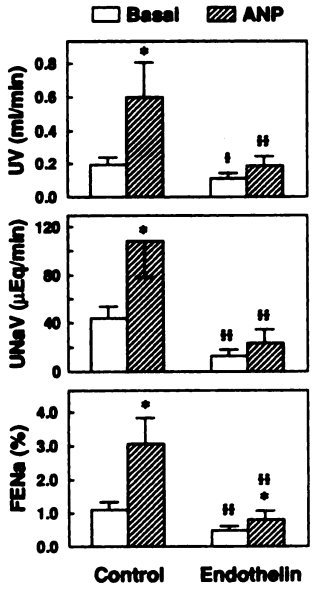

Fig. 3. Effects of ANP (10 ng kg^{-1} min^{-1}) on UV, UNaV and FENa before and during ET infusion (2 ng kg^{-1} min^{-1}, group 3). The values are the means ± S.E. $n = 6$. *P < .05 compared with basal values. §P < .05, §§P < .01 compared with values at corresponding sampling points before ET infusion (Control).

TABLE 1

Effects of nifedipine, TMB-8 and ANP on hemodynamics before and during infusion of ET

The values are the means ± S.E. Nifedipine (0.1 μg $kg^{-1}min^{-1}$, group 1, $n = 6$), TMB-8 (75 μg $kg^{-1}min^{-1}$, group 2, $n = 6$) or ANP (10 ng $kg^{-1}min^{-1}$, group 3, $n = 6$) was infused into the renal artery before and during intrarenal arterial infusion of endothelin (2 ng $kg^{-1}min^{-1}$).

	Control		ET	
	Basal	Nifedipine	Basal	Nifedipine
MAP (mm Hg)	115 ± 7	114 ± 8	115 ± 8	113 ± 9
RBF (ml/min)	146 ± 13	164 ± 16	88 ± 12*	93 ± 15*
GFR (ml/min)	19 ± 1	18 ± 1	12 ± 1*	13 ± 1*
	Basal	TMB-8	Basal	TMB-8
MAP (mm Hg)	136 ± 6	137 ± 6	133 ± 6	132 ± 6
RBF (ml/min)	135 ± 13	133 ± 13	83 ± 8*	80 ± 7*
GFR (ml/min)	23 ± 3	23 ± 3	13 ± 3*	13 ± 3*
	Basal	ANP	Basal	ANP
MAP (mm Hg)	125 ± 6	124 ± 7	123 ± 6	122 ± 5
RBF (ml/min)	196 ± 22	183 ± 25	109 ± 17*	104 ± 18*
GFR (ml/min)	26 ± 3	24 ± 3	15 ± 3*	17 ± 4*

*$P < .01$

for stabilization with continuous monitoring of the UV and hemodynamics.

Experimental protocol. The experiments were started after RBF and UV had reached constant levels for at least 30 min. The urine was collected over a 10-min period and arterial blood was withdrawn at the midpoint of each urine collection period. After sampling for basal values, nifedipine (0.1 μg kg^{-1} min^{-1}, group 1, $n = 6$), TMB-8 (75 μg kg^{-1} min^{-1}, group 2, $n = 6$) or ANP (10 ng kg^{-1} min^{-1}, group 3, $n = 6$) was infused into the renal artery at a flow rate of 0.1 ml/min using a motor-driven syringe pump (model 11, Harvard Apparatus, South Natick, MA). Beginning at 5 min after the start of drug infusion, urine was collected again and the drug infusion was stopped. About 30 min after the end of the drug infusion, a urine sample was obtained and ET was then infused intrarenally at a rate of 2 ng kg^{-1} min^{-1} (0.1 ml/min). Twenty-five minutes after the start of the ET infusion, urine sampling and the infusion of nifedipine, TMB-8 or ANP were performed during simultaneous infusion of ET similarly as in the period before ET infusion (control period).

The reproducibility of changes in UV, UNaV and FENa to consecutive infusions of nifedipine (group 4, $n = 5$), TMB-8 (group 5, $n = 5$) or ANP (group 6, $n = 7$) was also examined in the absence of ET.

The stability of the renal parameters during ET infusion (2 ng kg^{-1} min^{-1}) was examined in an additional five dogs (group 7). Urine was collected before the ET infusion and at 25 to 35 min and 40 to 50 min during the ET infusion. Plasma and urinary osmolalities were also measured.

Measurements. Blood samples were transferred into chilled tubes containing diammonium EDTA (2 mg/ml of blood) and then centrifuged to obtain plasma samples. The GFR was determined by inulin clearance. The sodium concentration, inulin concentration and osmolality in the plasma and urine were determined by flame photometry, the anthrone method and the freezing point depression method, respectively.

Data analysis. The values are expressed as the means ± S.E. The data were transformed to logarithms before the application of the statistical procedures when necessary. The values between the two experimental periods (control *vs.* ET or vehicle infusion periods) in groups 1 to 6 were compared by analysis of variance for multifactor repeated measures and simple main effects. The basal values and the values at each sampling point during ET infusion in group 7 were compared by analysis of variance for single-factor repeated measures and Dunnett's test. Ratios of UV, UNaV and FENa were calculated by dividing the values of the urinary parameters in the ET infusion period by the values at corresponding sampling points (before and during the infusion of nifedipine, TMB-8 or ANP) in the control period in groups 1 to 3 (ET/control ratios). The ratios (basal *vs.* drug infusion) were compared by Student's paired *t* test. Differences at a P value $< .05$ were considered to be statistically significant in all statistical procedures.

Drugs. ET (Peptide Institute Inc., Osaka, Japan) and ANP (Human 1-28, Peptide Institute) were dissolved in distilled water and diluted with 0.9% saline. TMB-8 (Sigma Chemical Co., St. Louis, MO) was dissolved in 0.9% saline. Nifedipine (Sigma) was dissolved in a small amount of ethanol and diluted with 0.9% saline (the final concentration of ethanol was less than 1%).

Results

During the control period, infusion of nifedipine, TMB-8 or ANP (groups 1–3, respectively) increased UV, UNaV and FENa (figs. 1-3) with few changes in RBF or GFR (table 1). ANP tended to reduce RBF but the change was not statistically significant. The urinary parameters returned to the basal levels about 30 min after stopping the drug infusion (data not shown).

The infusion of ET gradually reduced the RBF. Because RBF stabilized about 20 min after the start of ET infusion, urine collection for basal values was started at 25 min of the ET infusion. ET significantly reduced the RBF and GFR (table 1) and the UV, UNaV and FENa (figs. 1–3). One animal of six in group 1 experiments had a slight increase in UV during ET

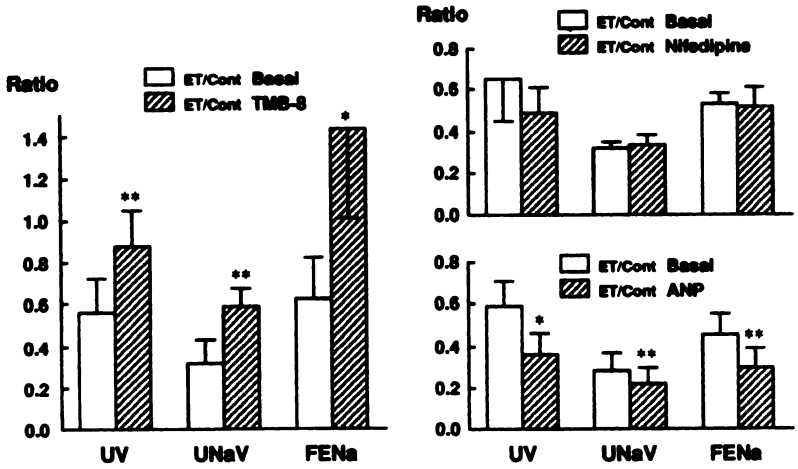

Fig. 4. Ratios of UV, UNaV and FENa in the ET infusion period (ET) to those at corresponding sampling points [before and during infusion of nifedipine (group 1, $n = 6$), TMB-8 (group 2, $n = 6$) or ANP (group 3, $n = 6$)] in the control period (Cont). The values are the means ± S.E. *$P < .05$, **$P < .01$ compared with the ratios of basal values (the values before the infusion of TMB-8 or ANP).

TABLE 2

The reproducibility of the urinary responses to nifedipine, TMB-8 and ANP

The values are the means ± S.E. Nifedipine (0.1 μg $kg^{-1}min^{-1}$, group 4, $n = 5$), TMB-8 (75 μg $kg^{-1}min^{-1}$, group 5, $n = 5$) or ANP (10 ng $kg^{-1}min^{-1}$, group 6, $n = 7$) was infused into the renal artery before and during intrarenal arterial infusion of vehicle (0.9% saline).

	Control		Vehicle	
	Basal	Nifedipine	Basal	Nifedipine
UV (ml/min)	0.15 ± 0.02	0.47 ± 0.06*	0.16 ± 0.03	0.57 ± 0.10*
UNaV (μEq/min)	24 ± 3	66 ± 4*	23 ± 4	66 ± 5*
FENa (%)	0.78 ± 0.10	2.21 ± 0.36*	0.74 ± 0.11	2.73 ± 0.40*
	Basal	TMB-8	Basal	TMB-8
UV (ml/min)	0.10 ± 0.01	0.22 ± 0.03*	0.11 ± 0.02	0.22 ± 0.05*
UNaV (μEq/min)	14 ± 4	34 ± 8*	16 ± 3	33 ± 6*
FENa (%)	0.38 ± 0.08	1.00 ± 0.33*	0.53 ± 0.12	0.99 ± 0.35*
	Basal	ANP	Basal	ANP
UV (ml/min)	0.25 ± 0.07	0.73 ± 0.17*	0.23 ± 0.08	0.70 ± 0.16*
UNaV (μEq/min)	42 ± 9	113 ± 24*	34 ± 7	109 ± 22*
FENa (%)	1.13 ± 0.25	3.29 ± 0.69*	0.91 ± 0.17	3.17 ± 0.62*

* $P < .01$ compared with each basal value.

TABLE 3

Effects of ET alone on hemodynamics and urinary excretion

The values are the means ± S.E. ($n = 5$, group 7). ET (2 ng $kg^{-1}min^{-1}$), was infused into the renal artery.

	Basal	ET	
		25–35 min	40–50 min
MAP (mm Hg)	135 ± 7	133 ± 7	133 ± 7
RBF (ml/min)	141 ± 20	105 ± 12**	100 ± 12**
GFR (ml/min)	21 ± 3	13 ± 2**	13 ± 2**
UV (ml/min)	0.27 ± 0.01	0.21 ± 0.03*	0.21 ± 0.04*
UNaV (μEq/min)	41 ± 10	16 ± 4**	16 ± 5**
FENa (%)	1.29 ± 0.17	0.89 ± 0.19*	0.88 ± 0.23*
UOsm (mOsm/kg)	549 ± 120	352 ± 51*	306 ± 42*
COsm (ml/min)	0.53 ± 0.10	0.27 ± 0.05**	0.25 ± 0.05**
TCH_2O (ml/min)	0.26 ± 0.11	0.06 ± 0.04*	0.04 ± 0.04*

* $P < .05$, ** $P < .01$ compared with each basal value.

infusion despite decreases in UNaV and FENa. The difference in the mean values of basal UV (fig. 1, control *vs.* ET infusion) was therefore smaller than those observed in other experimental groups.

Nifedipine increased the UV, UNaV and FENa during ET infusion but the values were lower than those obtained with nifedipine during the control period (fig. 1). TMB-8 also caused natriuresis during ET infusion (fig. 2). By contrast with nifedipine, TMB-8 elevated FENa almost to the same extent as during the control period. ANP did not induce a substantial increase in UV, UNaV or FENa although the change in FENa was statistically significant (fig. 3). Neither nifedipine, TMB-8 nor ANP counteracted the ET-induced decreases in RBF and GFR (table 1).

Neither nifedipine, TMB-8, ANP or ET caused statistically significant changes in the mean arterial pressure (table 1). The renal hemodynamics and urinary excretion in the contralateral noninfused kidneys were stable throughout the experiments (data not shown).

Figure 4 shows ET/control ratios of UV, UNaV and FENa in groups 1 to 3. The ET/control ratios during TMB-8 infusion were significantly higher than the ratios before TMB-8 infusion (presented as "Basal" in the figure), showing that the decreases in UV, UNaV and FENa during the infusion of ET plus TMB-8 were smaller than the decreases during the infusion of ET alone. However, ANP significantly lowered the ratios. Nifedipine did not affect the ratios.

Table 2 shows the reproducibility of the changes in UV, UNaV and FENa induced by consecutive infusions of nifedipine (group 4), TMB-8 (group 5) or ANP (group 6) in the absence of ET. The first and the second infusion of each drug increased these urinary parameters to the same extent.

The changes in renal hemodynamics and urinary excretion were stable in two sampling periods during the infusion of ET alone (group 7, table 3). Urinary osmolality, osmotic clearance and free water reabsorption were also reduced by ET infusion.

Discussion

In the present study, changes in UNaV and water excretion in response to nifedipine, TMB-8 and ANP were compared before and during infusion of ET. All drugs were infused into the renal artery at doses that did not affect systemic hemodynamics.

During the control period, nifedipine, TMB-8 and ANP increased UV and UNaV with little change in RBF and GFR. Accordingly, FENa increased in each experimental group. These drugs may suppress tubular sodium reabsorption to induce natriuresis, as shown in previous reports from our laboratory (Imagawa *et al.*, 1986a; Hisa *et al.*, 1989; Takahara *et al.*, 1991).

ET infusion reduced RBF, GFR, UV and UNaV. Because the change in UNaV was greater than the change in GFR, ET infusion also reduced the calculated FENa. Thus, the ET-induced antinatriuresis may not be explained only by the reduction in GFR. ET elevates FENa in the isolated perfused rat kidney (Perico *et al.*, 1990). However, the elevation of FENa has not been observed in many studies with *in vivo* kidneys (Katoh *et al.*, 1990; Matsumura *et al.*, 1989; Miller *et al.*, 1989; Stacy *et al.*, 1990). Consistent with our present observation, Matsumura *et al.* (1989) reported that intrarenal arterial infusion of ET reduced FENa in rats. ET therefore could enhance renal tubular sodium reabsorption in some experimental conditions *in vivo*. The ET-induced antinatriuresis observed in the present study may be related both to the decreased glomerular filtration and the increased tubular sodium reabsorption.

ET also reduced both urinary osmolality and free water reabsorption (group 7, table 3), suggesting that ET suppresses reabsorption of solute-free water, probably at the distal tubules or the collecting ducts. The ET-induced decrease in UV (by 20–40%) tended to be smaller than the decrease in UNaV (by about 60%, groups 1–3 and 7), which might be the result of the concomitant increase in free water excretion.

There have been controversial results concerning whether the ET-induced renal responses could be counteracted with calcium antagonists and ANP. Simultaneous infusion of nicardipine or ANP suppressed the ET-induced renal vasoconstriction and antinatriuresis in normotensive rats (Katoh *et al.*,

1990). Additional infusion of ANP reversed the changes in renal hemodynamics caused by ET, resulting in pronounced natriuresis in spontaneously hypertensive rats (Hirata *et al.*, 1989). However, pretreatment with verapamil (Cao and Banks, 1990) or ANP (Ota *et al.*, 1992) cannot inhibit renal vasoconstriction and antinatriuresis induced by ET in anesthetized rats and dogs. Opgenorth and Novosad (1990) demonstrated that ANP relaxed the rabbit aorta rings precontracted with ET but ANP pretreatment did not prevent the ET-induced contraction. The ET-induced renal responses also seem to be resistant to drug pretreatment. Calcium antagonists and ANP therefore might counteract the ET-induced renal responses if they are administered in the presence of ET.

In our study, the addition of nifedipine during the ET infusion period significantly increased UV, UNaV and FENa nearly to or more than the levels of basal values in the control period (fig. 1). In this regard, nifedipine could reverse the ET-induced decreases in urinary sodium and water excretion. However, the values were still lower than the levels observed during nifedipine infusion in the control period. Nifedipine therefore does not seem to antagonize the ET-induced urinary responses. The ET/control ratios of the urinary parameters (the values during the ET infusion period divided by the values during the control period, fig. 4) remained unaffected during nifedipine infusion. Thus, there may be little or no interaction between the opposite urinary responses induced by ET and by nifedipine. The present results suggest that the calcium influx through dihydropyridine-sensitive channels does not participate in the ET-induced antinatriuresis in the dog kidney.

Because ANP did not induce substantial increases in the urinary parameters during ET infusion (fig. 3), ANP may not counteract the ET-induced antinatriuresis. ANP significantly lowered the ET/control ratios of UV, UNaV and FENa (fig. 4), which would imply that the ET-induced antinatriuresis can overcome the ANP-induced natriuresis. ET may inhibit the natriuretic action of ANP, as suggested by Zimmerman *et al.* (1990). In the *in vivo* rat kidney, Sano *et al.* (1992) showed that HS-142-1, an antagonist for guanylate cyclase-linked ANP receptors, inhibits ANP-induced increases in urinary sodium and cyclic GMP excretion rates. The natriuretic action of ANP and its vasodilatory action (Winquist, 1986) may depend on the cyclic GMP production. Because ET inhibits ANP-stimulated cyclic GMP production in vascular smooth muscle (Jaiswal, 1992), ET may inhibit cyclic GMP production at renal tubular sites and, thereby, interfere with the natriuretic action of ANP. Alternatively, the ET-induced renal hypoperfusion itself may be responsible for the blunted natriuretic response to ANP.

The addition of TMB-8 during the ET infusion period significantly increased FENa to the same level as observed during TMB-8 infusion in the control period (fig. 2), indicating that TMB-8 completely antagonized the ET-enhanced tubular sodium reabsorption. Although TMB-8 did not completely reverse the ET-induced decrease in UNaV, TMB-8 significantly elevated the ET/control ratios of UV, UNaV and FENa (fig. 4). Thus, the concomitant infusion of ET with TMB-8 caused smaller urinary responses than did ET infusion alone. The TMB-8-induced natriuresis can counteract the ET-induced antinatriuresis in the dog kidney. A recent study from our laboratory has suggested existence of a TMB-8-sensitive pathway in the neural control mechanism of tubular sodium reabsorption (Ogasawara *et al.*, 1993). It is unlikely, however, that ET evokes neurotransmitter release from renal nerve endings to enhance tubular sodium reabsorption because tonic renal nerve activity was almost eliminated by surgical denervation. We could postulate that ET directly activates the renal tubular mechanisms of sodium reabsorption by stimulating calcium release from TMB-8-sensitive intracellular stores.

ET has been suggested to produce biphasic elevation of the free calcium concentration in vascular smooth muscle cells (Marsden *et al.*, 1989) and mesangial cells (Simonson and Dunn, 1991), a steep and transient elevation accompanied by phosphoinositol breakdown and a sustained elevation resulting from extracellular calcium influx. Because we administered TMB-8 after ET had already caused antinatriuresis, TMB-8 may have affected the sustained phase of elevation of intracellular calcium. It is unclear whether the enhanced tubular sodium reabsorption during ET infusion is maintained by intracellular calcium release. Therefore, we cannot rule out the possibility that the influx of extracellular calcium through pathways that are also sensitive to TMB-8 but not to dihydropyridine calcium antagonists plays an essential role in the tubular action of ET.

Nifedipine or ANP did not affect the decreased RBF and GFR during ET infusion (table 1). TMB-8 did not counteract the ET-induced renal vasoconstriction despite complete inhibition of the renal tubular action of ET. This may be responsible for the incomplete reversal of UNaV with TMB-8 during ET infusion. Studies in anesthetized dogs showed that a decrease in RBF induced by an intrarenal bolus injection of angiotensin II was attenuated during infusion of nifedipine at a rate of 0.3 to 1.0 μg/min (Imagawa *et al.*, 1986b); TMB-8, at 30 and 100 $\mu g\ kg^{-1}\ min^{-1}$ (Takahara *et al.*, 1990); or ANP, at 10 and 50 $ng\ kg^{-1}\ min^{-1}$ (Hisa *et al.*, 1992). The drug doses applied in the present study therefore seem to be sufficient to act on the renal vasculature, although the experimental protocols were different from those applied in the previous studies. The ET-induced vasoconstriction in the dog kidney may not be susceptible to the calcium antagonists and ANP. However, these drugs were infused at doses that did not increase basal RBF during the control period, although they caused significant natriuresis. Experiments with higher doses of these drugs would be required to elucidate the mechanisms of the ET-induced renal vasoconstriction.

In summary, the present study showed that ET decreases absolute sodium excretion and FENa, which can be counteracted with TMB-8 but not with nifedipine in the dog kidney, and that the ET-induced antinatriuresis overcomes the natriuretic action of ANP. ET may elicit TMB-8-sensitive calcium movement, probably calcium release from intracellular stores, to enhance tubular sodium reabsorption.

References

CAO, L. AND BANKS, R. O.: Cardiovascular and renal actions of endothelin: Effects of calcium-channel blockers. Am. J. Physiol. **258:** F254–F258, 1990.

CHAI, S. Y., SEXTON, P. M., ALLEN, A. M., FIGDOR, R. AND MENDELSOHN, F. A. O.: *In vitro* autoradiographic localization of ANP receptors in rat kidney and adrenal gland. Am. J. Physiol. **250:** F753–F757, 1986.

CHIOU, C. Y. AND MALAGODI, M. H.: Studies on the mechanism of action of a new Ca^{2+} antagonist, 8-(N,N-diethylamino)octyl-3,4,5-trimethoxybenzoate hydrochloride in smooth and skeletal muscles. Br. J. Pharmacol. **53:** 279–285, 1975.

GOETZ, K. L., WANG, B. C., MADWED, J. B., ZHU, J. L. AND LEADLEY, R. J. JR.: Cardiovascular, renal, and endocrine responses to intravenous endothelin in conscious dogs. Am. J. Physiol. **255:** R1064–R1068, 1988.

HIRATA, Y., MATSUOKA, H., KIMURA, K., FUKUI, K., HAYAKAWA, H., SUZUKI, E., SUGIMOTO, T., SUGIMOTO, T., YANAGISAWA, M. AND MASAKI, T.: Renal vasoconstriction by the endothelial cell-derived peptide endothelin in spontaneously hypertensive rats. Circ. Res. **65:** 1370–1379, 1989.

HISA, H., TOMURA, Y. AND SATOH, S.: Atrial natriuretic factor suppresses neural stimulation of renin release in dogs. Am. J. Physiol. **257:** E332–E335, 1989.

HISA, H., TOMURA, Y., YAMAGATA, T. AND SATOH, S.: Atrial natriuretic peptide suppresses renal vasoconstriction induced by angiotensin II and norepinephrine in dogs. Eur. J. Pharmacol. **222:** 265–271, 1992.

IMAGAWA, J., KUROSAWA, H. AND SATOH, S.: Effects of nifedipine on renin release and renal function in anesthetized dogs. J. Cardiovasc. Pharmacol. **8:** 636–640, 1986a.

IMAGAWA, J., KUSABA-SUZUKI, M. AND SATOH, S.: Preferential inhibitory effect of nifedipine on angiotensin II-induced renal vasoconstriction. Hypertension **8:** 897–903, 1986b.

JAISWAL, R. K.: Endothelin inhibits the atrial natriuretic factor stimulated cGMP production by activating the protein kinase C in rat aortic smooth muscle cells. Biochem. Biophys. Res. Commun. **182:** 395–402, 1992.

JONES, C. R., HILEY, C. R., PELTON, J. T. AND MILLER, R. C.: Autoradiographic localisation of endothelin binding sites in kidney. Eur. J. Pharmacol. **163:** 379–382, 1989.

KATOH, T., CHANG, H., UCHIDA, S., OKUDA, T. AND KUROKAWA, K.: Direct effects of endothelin in the rat kidney. Am. J. Physiol. **258:** F397–F402, 1990.

KOHZUKI, M., JOHNSTON, C. I., CHAI, S. Y., CASLEY, D. J. AND MENDELSOHN, F. A. O.: Localization of endothelin receptors in rat kidney. Eur. J. Pharmacol. **160:** 193–194, 1989.

KON, V. AND BADR, K. F.: Biological actions and pathophysiologic significance of endothelin in the kidney. Kidney Int. **40:** 1–12, 1991.

MADEDDU, P., YANG, X.-P., ANANIA, V., TROFFA, C., PAZZOLA, A. SORO, A., MANUNTA, P., TONOLO, G., DEMONTIS, M. P., VARONI, M. V., MELIS, M. G. AND GLORIOSO, N.: Efficacy of nifedipine to prevent systemic and renal vasoconstrictor effects of endothelin. Am. J. Physiol. **259:** F304–F311, 1990.

MARSDEN, P. A., DANTHULURI, N. R., BRENNER, B. M.,. BALLERMANN, B. J. AND BROCK, T. A.: Endothelin action on vascular smooth muscle involves inositol trisphosphate and calcium mobilization. Biochem. Biophys. Res. Commun. **158:** 86–93, 1989.

MATSUMURA, Y., HISAKI, K., OHYAMA, T., HAYASHI, K. AND MORIMOTO, S.: Effects of endothelin on renal function and renin secretion in anesthetized rats. Eur. J. Pharmacol. **166:** 577–580, 1989.

MILLER, W. L., REDFIELD, M. M. AND BURNETT, J. C., JR.: Integrated cardiac, renal, and endocrine actions of endothelin. J. Clin. Invest. **83:** 317–320, 1989.

NARUSE, M., UCHIDA, S., OGATA, E. AND KUROKAWA, K.: Endothelin 1 increases cell calcium in mouse collecting tubule cells. Am. J. Physiol. **261:** F720–F725, 1991.

NEEDLEMAN, P., ADAMS, S. P., COLE, B. R., CURRIE, M. G., GELLER, D. M., MICHENER, M. L., SAPER, C. B., SCHWARTZ, D. AND STANDAERT, D. G.: Atriopeptins as cardiac hormones. Hypertension **7:** 469–482, 1985.

OGASAWARA, A., HISA, H. AND SATOH, S.: An intracellular calcium release inhibitor TMB-8 suppresses renal nerve stimulation-induced antinatriuresis in dogs. J. Pharmacol. Exp. Ther. **264:** 117–121, 1993.

OPGENORTH, T. J. AND. NOVOSAD, E. I.: Atrial natriuretic factor and endothelin interactions in control of vascular tone. Eur. J. Pharmacol. **191:** 351–357, 1990.

OTA, K., KIMURA, T., SHOJI, M., INOUE, M., SATO, K., OHTA, M., YAMAMOTO, T., TSUNODA, K., ABE, K. AND YOSHINAGA, K.: Interaction of ANP with endothelin on cardiovascular, renal, and endocrine function. Am. J. Physiol. **262:** E135–E141, 1992.

PERICO, N., DADAN, J., GABANELLI, M. AND REMUZZI, G.: Cyclooxygenase products and atrial natriuretic peptide modulate renal response to endothelin. J. Pharmacol. Exp. Ther. **252:** 1213–1220, 1990.

SANO, T., MORISHITA, Y., MATSUDA, Y. AND YAMADA, K.: Pharmacological profile of HS-142-1, a novel nonpeptide atrial natriuretic peptide antagonist of microbial origin. I. Selective inhibition of the actions of natriuretic peptides in anesthetized rats. J. Pharmacol. Exp. Ther. **260:** 825–831, 1992.

SIMONSON, M. S., AND DUNN, M. J.: Endothelins: A family of regulatory peptides. Hypertension **17:** 856–863, 1991.

STACY, D. L., SCOTT, J. W. AND GRANGER, J. P.: Control of renal function during intrarenal infusion of endothelin. Am. J. Physiol. **258:** F1232–F1236, 1990.

TAKAHARA, A., KUSABA-SUZUKI, M., HISA, H. AND SATOH, S.: Effects of a novel Ca^{2+} entry blocker, CD-349, and TMB-8 on renal vasoconstriction induced by angiotensin II and vasopressin in dogs. J. Cardiovasc. Pharmacol. **16:** 966–970, 1990.

TAKAHARA, A., OGASAWARA, A., SUZUKI-KUSABA, M., HISA, H. AND SATOH, S: Natriuretic effect of TMB-8 in anaesthetized dogs. Clin. Exp. Pharmacol. Physiol. **18:** 255–258, 1991.

WINQUIST, R. J.: Possible mechanisms underlying the vasorelaxant response to atrial natriuretic factor. Fed. Proc. **45:** 2371–2375, 1986.

YANAGISAWA, M., KURIHARA, H., KIMURA, S., TOMOBE, Y., KOBAYASHI, M., MITSUI, Y., YAZAKI, Y., GOTO, K. AND MASAKI, T.: A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature (Lond.) **332:** 411–415, 1988.

ZEIDEL, M. L., BRADY, H. R., KONE B. C., GULLANS, S. R. AND BRENNER, B. M.: Endothelin, a peptide inhibitor of Na^+-K^+-ATPase in intact renal tubular epithelial cells. Am. J. Physiol. **257:** C1101–1107, 1989.

ZIMMERMAN, R. S., MARTINEZ, A. J., MACPHEE, A. A. AND BARBEE, R. W.: Atrial natriuretic factor blocks the pressor action of endothelin. J. Cardiovasc. Pharmacol. **16:** 865–870, 1990.

Send reprint requests to: H. Hisa, Ph.D., Department of Pharmacology, Pharmaceutical Institute, Tohoku University, Aobayama, Sendai 980, Japan.