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Effects of Sarafotoxin S6c on Antidiuresis and Norepinephrine Overflow Induced by Stimulation of Renal Nerves in Anesthetized Dogs[1]

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ABSTRACT

We previously reported that endothelin (ET) may function as an inhibitory modulator of renal noradrenergic neurotransmission (Suzuki *et al., J. Cardiovasc. Pharmacol.* **19:** 905–910, 1992). In our study, we examined the effect of sarafotoxin S6c (S6c), a selective ET_B receptor agonist, on changes in renal function and norepinephrine overflow induced by renal nerve stimulation (RNS) in anesthetized dogs. RNS at a low frequency (0.5–2.0 Hz) caused significant decreases in urine flow, urinary excretion of sodium and fractional excretion of sodium and increased norepinephrine secretion rate, without affecting systemic and renal hemodynamics. RNS at a high frequency (2.5–5.0 Hz), which diminishes renal hemodynamics, produced more potent decreases in urine formation and increase in norepinephrine secretion rate than seen with low frequency RNS. When S6c (1 ng/kg/min) was infused intrarenally, there was a slight and transient increase in renal blood flow, and then this response was followed by a gradual reduction. S6c administration produced increase in the basal level of urine flow with no apparent effects on urinary excretion of sodium and fractional excretion of sodium. During S6c infusion, low frequency RNS-induced antidiuretic action and increase in norepinephrine secretion rate were markedly attenuated. Qualitatively, similar results were observed in the case of high frequency RNS. In addition, high frequency RNS-induced decreases in glomerular filtration rate and filtration fraction were significantly suppressed by S6c infusion. Taken together with our previous findings, it seems likely that ET plays an important role as an inhibitory modulator of renal noradrenergic neurotransmission, through ET_B receptor mechanisms.

ET is a family of potent vasoconstrictor peptides consisting of 21 amino acid residues, that was first isolated and characterized from the supernatant of cultured porcine aortic endothelial cells (Yanagisawa *et al*., 1988). Three distinct members of this family, namely ET-1, ET-2 and ET-3, were identified when a genomic DNA library was screened (Inoue *et al*, 1989). Furthermore, the amino acid sequence of a snake venom toxin, sarafotoxin, was shown to be highly homologous to ET (Kloog *et al*., 1989). Various biological actions of ET are mediated through specific cell surface receptors on target tissues. To date, two different ET receptor subtypes have been identified and cloned. The ET_A receptor is selective for ET-1, whereas the ET_B receptor binds with equal affinity to all three isoforms of ET and sarafotoxin peptides (Ambar *et al*., 1989; Arai *et al*., 1990; Sakurai *et al*., 1990). Of the sarafotoxins, S6c can bind selectively to the ET_B receptor, with high affinity (Williams *et al*., 1991). These peptides and receptors are widely distributed in both vascular and nonvascular tissues, including arteries, brain and kidney, mediating a large number of physiological responses (Rubanyi and Polokoff., 1994).

ET may function as a neuropeptide or a neuromodulator. Several investigators reported that ET exist in neuronal tissues (Shinmi *et al*., 1989, a and b; Matsumoto *et al*., 1989). ET-1 has also been shown to inhibit the transmural nerve stimulation-induced release of [^{3}H]NE and to enhance vasopressor responses to exogenous NE in guinea pig femoral artery (Wiklund *et al*., 1988), pulmonary artery (Wiklund *et al*., 1989a) and rat mesenteric artery (Tabuchi *et al*., 1989, a and b). In addition, the release of [^{3}H]acetylcholine induced by the transmural nerve stimulation in guinea pig ileum was suppressed by ET-1 (Wiklund *et al*., 1989b). These findings strongly suggest that ET-1 modulates the adrenergic and

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ABBREVIATIONS: S6c, sarafotoxin S6c; ET, endothelin; ET-1, endothelin-1; ET-2, endothelin-2; ET-3, endothelin-3; RNS, renal nerve stimulation; NE, norepinephrine; NESR, norepinephrine secretion rate; MAP, mean arterial blood pressure; HR, heart rate; RBF, renal blood flow; RVR, renal vascular resistance; GFR, glomerular filtration rate; FF, filtration fraction; UF, urine flow; $U_{Na}V$, urinary excretion of sodium; FE_{Na}, fractional excretion of sodium; NO, nitric oxide; PE, prostaglandin; AVP, arginine vasopressin; IMCD, inner medullary collecting duct.

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cholinergic neurotransmission through pre- and postsynaptic actions. We have also found that intrarenal arterial infusion of ET-1 inhibits NE overflow induced by electrical stimulation of renal nerves in anesthetized dogs (Suzuki *et al.*, 1992).

To investigate the ET receptor subtype participating in inhibitory effects on renal noradrenergic neurotransmission, we examined the effects of intrarenal arterial infusion of S6c, a selective ET_B receptor agonist, on renal actions and NE overflow induced by stimulation of renal nerves in anesthetized dogs.

Materials and Methods

Animal preparation. Experiments were done on 15 adult mongrel dogs of both sexes weighing 10 to 16 kg. The dogs were anesthetized with sodium pentobarbital (30 mg/kg i.v.) and given supplemental doses as required. The animals were placed on a heated surgical table that maintained the rectal temperature between 37 and 38°C. After tracheal intubation, respiration was supported by artificial ventilation with room air, using a Harvard respirator. Polyethylene catheters were placed in the right brachial artery and vein for arterial blood sampling and for the infusion of saline containing 0.45% inulin, respectively. Another catheter was also inserted into the abdominal aorta via the right femoral artery, to about the level of the renal arteries, and MAP and HR were monitored continuously with a pressure transducer (Nihon Koden, Tokyo, Japan AP601G). The left kidney was exposed through a retroperitoneal flank incision and the renal artery and vein were isolated from surrounding tissues. All visible renal nerve bundles along the renal artery were isolated, ligated and cut. For RNS, the distal cut portion was placed on bipolar platinum electrodes connected to an electric stimulator (Nihon Koden, SEN-7103). A flow probe (2.0–4.0 mm in a diameter, Nihon Koden) was attached around the left renal artery and the left RBF was measured continuously, using an electromagnetic blood flowmeter (Nihon Koden, MFV-2100). A curved 23-gauge needle was inserted into the left renal artery proximal to the flow probe for intrarenal arterial infusion of saline or drug solution at a rate of 0.48 ml/min. Another curved 18-gauge needle was inserted into the left renal vein for venous blood sampling. Finally, the left ureter was cannulated for urine collection. After completion of the surgery, a priming dose of inulin (20 mg/kg) was given, followed by a sustaining infusion of 0.9% saline containing inulin for the measurement of GFR, at a rate of 2.0 ml/min. MAP, HR and RBF were recorded continuously on a polygraph (Nihon Koden, RM6000G). Dogs were allowed 2 hr for equilibration before experimental treatments were begun.

Experimental protocol. Four RNS experiments were performed on each of 11 dogs. Each experiment consisted of a 10-min control period and a 10-min RNS period. Blood samples (3.0 ml) were taken at 5 min in the control period, 1 and 9 min in the RNS period from the right brachial artery and left renal vein. After measuring the systemic arterial hematocrit by the microcapillary method, plasma was separated immediately by centrifugation. Urine samples were collected during the latter 5 min in each period.

After a 2-hr stabilization, we started the first RNS experiment in which the renal nerves were stimulated at a low frequency (0.5–2.0 Hz; duration, 1.0 msec; and supramaximal voltage, 10–25 V) during the RNS period. The second RNS experiment was done after a 30-min interval for equilibration. In this experiment, RNS was applied at a high frequency (2.5–5.0 Hz). During these RNS experiments, saline was infused into the renal artery. About 60 min after termination of the second experiment, intrarenal arterial infusion of S6c (1 ng/kg/min) was started. After 15 min, two RNS experiments (the third and fourth) were repeated, in the same manner as described above. To estimate reproducibility of the renal actions induced by repeated RNS, separate experiments were done using saline instead of S6c during the third and fourth experiments. To observe the effects of S6c itself on renal functions, we did additional experiments using four dogs, in which the same experimental protocol without RNS was carried out.

Systemic and renal hemodynamic parameters (MAP, HR, RBF, RVR, GFR and FF) were determined at the midpoint in the control period and at 9 min after the start of RNS in the RNS period, respectively. Parameters for urine formation were determined during the last 5 min in each period.

Analytical procedures. The GFR was estimated from the inulin clearance. Urine and plasma inulin levels were measured spectrofluorometrically (Hitachi, 650–60) according to the method of Vurek and Pegram (1966). Urine and plasma sodium concentrations were determined, using a flame photometer (Hitachi, 205D). The plasma NE concentration was measured by high-performance liquid chromatography with an amperometric detector (Eikom, Kyoto, Japan, ECD-100), as previously described (Hayashi *et al.*, 1991). The NESR was calculated by:

$$\text{NESR (pg/g/min)} = (NE_V - NE_A)\,\text{RPF}$$

where RPF is renal plasma flow (ml/g/min), NE_V is renal venous plasma NE concentration (pg/ml) and NE_A is renal arterial plasma NE concentration (pg/ml). The RPF was calculated from RBF and a hematocrit measurement.

Drugs. S6c was purchased from Peptide Institute, Inc. (Osaka, Japan) and was dissolved in saline solution containing 0.1% heat-inactivating bovine serum albumin. Other chemicals were obtained from Nacalai Tesque, Inc. (Kyoto, Japan) and Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Statistical analysis. All values were expressed as mean ± S.E. Statistical significance of differences in values between control period and RNS period during saline or drug infusion was evaluated by paired Student's *t* test. RNS-induced changes during saline or drug infusion and changes in basal renal function induced by drug infusion were assessed by analysis of variance followed by a Bonferroni's multiple comparison test. For all comparisons, differences were considered significant at $P < .05$ and $P < .01$.

Results

Effects of S6c on RNS-induced renal actions. As shown in table 1 and figure 1, low frequency RNS significantly decreased UF, $U_{Na}V$ and FE_{Na}, by about 40, 35 and 30% from control values of 15.2 ± 3.5 μl/g/min, 4.02 ± 0.93 μEq/g/min and 2.4 ± 0.5%, respectively, without affecting systemic and renal hemodynamics. High frequency RNS produced more potent decreases in urine formation than seen with low frequency RNS; UF, $U_{Na}V$ and FE_{Na} decreased by about 60, 65 and 45% from control values of 15.5 ± 2.6 μl/g/min, 4.28 ± 0.63 μEq/g/min and 2.4 ± 0.3%, respectively. In addition, there were significant decreases in RBF, GFR and FF, and an increase in RVR. Intrarenal arterial infusion of S6c at the rate of 1 ng/kg/min resulted in a slight and transient increase in RBF at 1 to 2 min after start of the infusion, without any change in systemic hemodynamics, then RBF gradually decreased to below the basal level. S6c administration apparently did not alter $U_{Na}V$ and FE_{Na}, but did significantly increase the basal level of UF about 3-fold during saline infusion. In the presence of S6c, the low frequency RNS-induced reductions in urine formation were significantly attenuated, although the absolute change in UF induced by RNS was almost same as that during saline infusion. Observed decreases in UF, $U_{Na}V$ and FE_{Na} were about 15, 25 and 20% from control values of 45.3 ± 10.7 μl/g/min, 3.90 ± 0.82 μEq/g/min and 2.4 ± 0.5%, respectively. Similar suppressive effects of S6c on the RNS-induced an-

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TABLE 1
Effects of S6c on RNS-induced changes in systemic and renal hemodynamics

	MAP (mm Hg)	HR (beats/min)	RBF (ml/g/min)	RVR (mm Hg/ml/g/min)	GFR (ml/g/min)	FF (%)
Saline infusion						
Control	144.4 ± 5.1	174.3 ± 8.0	5.0 ± 0.5	30.9 ± 3.2	0.98 ± 0.07	34.3 ± 2.9
RNS (low frequency)	143.1 ± 5.0	175.3 ± 8.1	4.9 ± 0.5	30.8 ± 3.2	0.95 ± 0.06	33.6 ± 2.8
% change	−0.9 ± 0.6	0.5 ± 0.4	−0.7 ± 0.5	−0.2 ± 0.4	−2.4 ± 1.4	−1.9 ± 1.2
Control	142.4 ± 4.9	175.3 ± 8.5	4.9 ± 0.5	31.1 ± 3.6	1.03 ± 0.08	37.0 ± 4.2
RNS (high frequency)	142.4 ± 5.6	174.1 ± 8.8	4.3 ± 0.4[a]	35.4 ± 3.4[a]	0.65 ± 0.10[b]	26.7 ± 4.4[b]
% change	−0.1 ± 0.7	−0.7 ± 0.9	−12.7 ± 3.2[c]	15.3 ± 4.4[c]	−38.4 ± 6.4[c]	−29.5 ± 6.5[c]
S6c infusion						
Control	135.4 ± 5.8	175.0 ± 8.4	4.8 ± 0.6	31.5 ± 5.1	0.96 ± 0.08	35.9 ± 4.5
RNS (low frequency)	135.0 ± 5.8	176.7 ± 8.2	4.6 ± 0.6[a]	32.7 ± 5.0[b]	0.91 ± 0.07	35.5 ± 4.4
% change	−0.3 ± 0.3	1.0 ± 0.8	−4.6 ± 1.3	4.4 ± 1.4	−5.1 ± 2.6	−1.1 ± 1.9
Control	131.6 ± 6.8	176.9 ± 8.1	4.8 ± 0.7	31.4 ± 5.3	0.99 ± 0.07	36.7 ± 4.3
RNS (high frequency)	131.0 ± 7.4	175.7 ± 7.8	4.4 ± 0.6[a]	33.1 ± 5.3[a]	0.84 ± 0.08[b]	33.9 ± 3.6[a]
% change	−0.5 ± 1.1	−0.6 ± 0.3	−6.5 ± 2.1	6.8 ± 2.9	−16.3 ± 3.6[d]	−9.2 ± 3.6[d]

Each value represents the mean ± S.E. of seven dogs. Effects of RNS in each experimental condition were examined by Student's paired *t* test: [a] $P < .05$, [b] $P < .01$. RNS-induced changes were examined by Bonferroni's multiple comparison test: [c] $P < .01$ vs. low frequency RNS during saline infusion and [d] $P < .01$ vs. high frequency RNS during saline infusion.

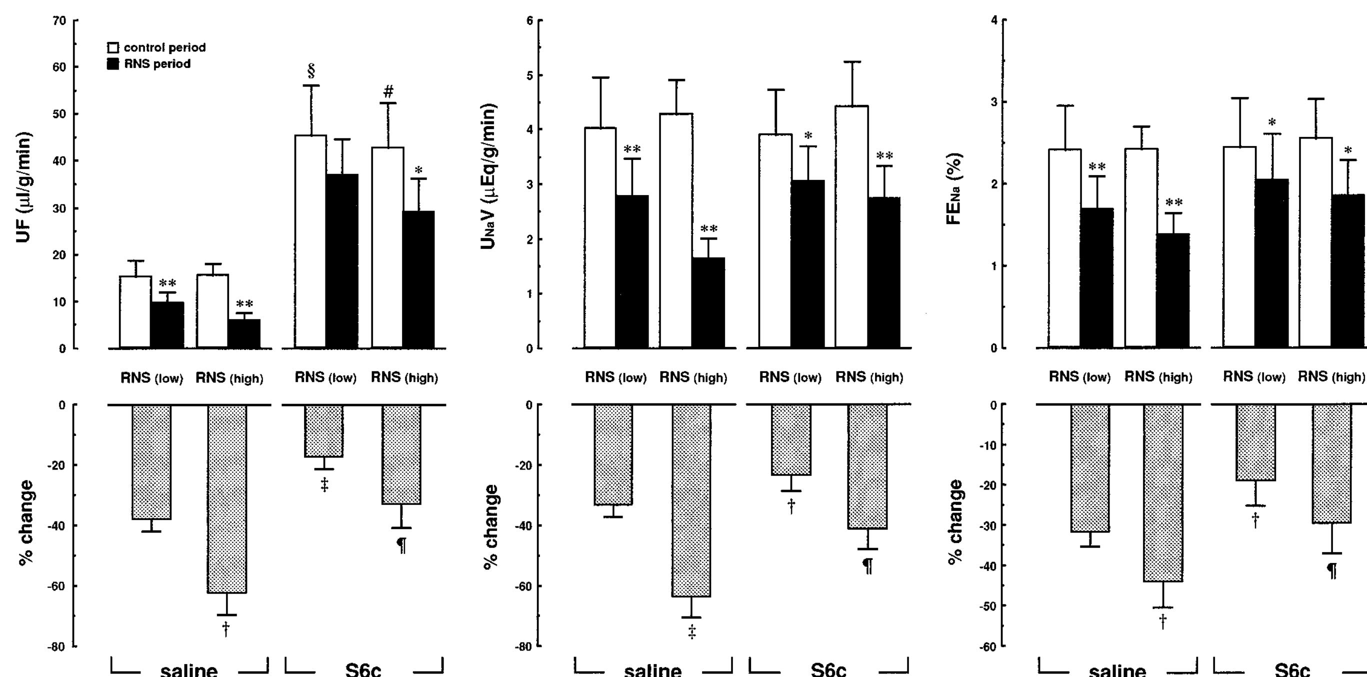

Fig. 1. Effects of S6c on RNS-induced changes in urine formation. Each value represents the mean ± S.E. of seven dogs. Effects of RNS in each experimental condition were examined by Student's paired *t* test: * $P < .05$, ** $P < .01$. RNS-induced changes and changes in basal urine formation induced by S6c infusion were examined by Bonferroni's multiple comparison test: † $P < .05$, ‡ $P < .01$ *vs.* low frequency RNS-induced change and ¶ $P < .01$ *vs.* high frequency RNS-induced change during saline infusion. § $P < .01$ *vs.* control value in low frequency RNS experiment and # $P < .01$ *vs.* control value in high frequency RNS experiment during saline infusion.

tidiuresis and antinatriuresis were observed in the case of high frequency RNS. In addition, high frequency RNS-induced decreases in GFR and FF were significantly attenuated by S6c infusion. In the low frequency RNS period during S6c infusion, a slight but significant decrease in RBF (4.6 ± 1.3% decrease) and an increase in RVR (4.4 ± 1.4% increase) were observed.

Assessment of reproducibility of repeated RNS-induced renal actions. Changes in renal hemodynamics and urine formation in response to repeated RNS were evaluated. The low (the first and third experiments) and the high (the second and fourth experiments) frequency RNS-induced renal actions were reproducible, respectively (data not shown).

Effects of S6c on RNS-induced increases in NESR. NESR significantly increased from a control value of −81 ± 108 to 497 ± 118 and 494 ± 113 pg/g/min at 1 and 9 min after low frequency RNS started, respectively. In the case of high frequency RNS, the NESR increased markedly from a control value of −100 ± 61 to 1057 ± 172 and 1066 ± 190 pg/g/min at 1 and 9 min after the start of RNS, respectively. In the following results, RNS-induced increases in NESR from the control are indicated as △NESR, to clarify changes in NESR induced by the RNS. Intrarenal arterial infusion of S6c significantly decreased △NESR during low frequency RNS (from 578 ± 70 and 575 ± 53 to 286 ± 63 and 291 ± 68 pg/g/min at 1 and 9 min after the start of RNS, respectively) (fig. 2). Similar inhibitory effects of S6c on RNS-induced NE

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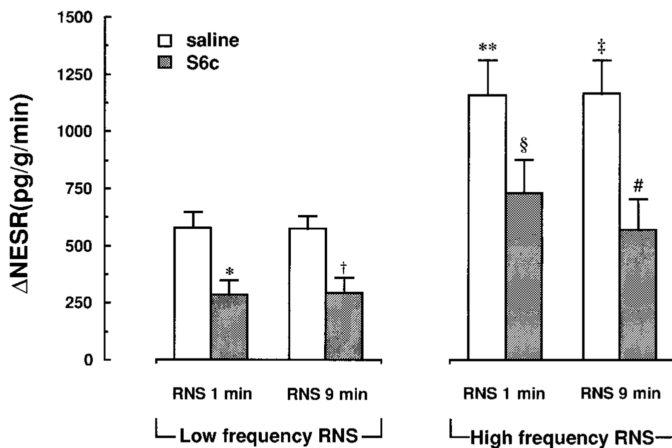

Fig. 2. Effects of S6c on △NESR with low and high frequency RNS. Each value represents the mean ± S.E. of seven dogs. RNS-induced changes during saline or S6c infusion were examined by Bonferroni's multiple comparison test: * $P < .05$, ** $P < .01$ *vs.* △NESR at 1 min after the start of low frequency RNS and † $P < .05$, ‡ $P < .01$ *vs.* △NESR at 9 min after the start of low frequency RNS during saline infusion. § $P < .01$, *vs.* △NESR at 1 min after the start of high frequency RNS and # $P < .01$, *vs.* △NESR at 9 min after the start of high frequency RNS during saline infusion.

release were observed at a high frequency RNS (from 1158 ± 156 and 1166 ± 148 to 732 ± 147 and 571 ± 134 pg/g/min at 1 and 9 min after the start of RNS, respectively).

Assessment of reproducibility of repeated RNS-induced increases in NESR. As shown in figure 3, in the first and third RNS experiments, low frequency RNS-induced increases in NESR were to the same extent (△NESR were 590 ± 151 and 807 ± 141 pg/g/min in the first experiment; 621 ± 101 and 783 ± 171 pg/g/min in the third experiment at 1 and 9 min after the start of RNS, respectively). Similar results were observed between second and fourth experiments at a high frequency RNS. (△NESR were 1381 ± 206 and 1422 ± 149 pg/g/min in the second experiment; 1361 ± 137 and 1364 ± 74 pg/g/min in the fourth experiment at 1 and 9 min after the start of RNS, respectively). Thus, RNS-induced increases in NESR were reproducible.

Assessment of the effects of S6c on renal functions. Table 2 shows the results of experiments done without RNS to evaluate the effects of S6c per se on renal function. In the first and second experiments, saline was infused, with no appreciable changes in systemic and renal hemodynamics,

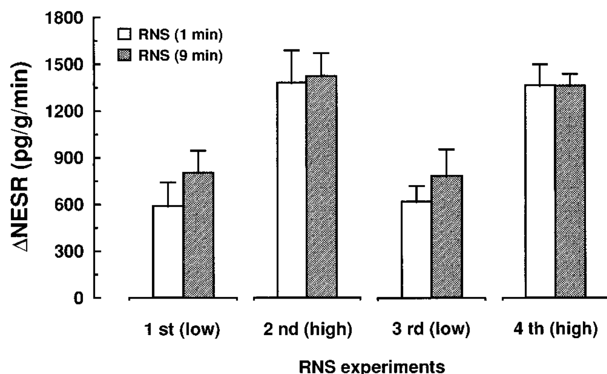

Fig. 3. Reproducibility of △NESR with repeated RNS. Experiments were done without infusion of S6c. Each value represents the mean ± S.E. of four dogs.

and urine formation. However, when S6c (1 ng/kg/min) was infused intrarenally, there was a transient increase in RBF, a response followed by a gradual reduction below basal values. In addition, S6c significantly increased the basal levels of UF, with no alteration in the excretion of sodium, a response sustained during the experimental periods.

Discussion

Our results clearly demonstrate that increase in the response of NE release from renal sympathetic nerve endings and changes in renal function induced by electrical stimulation of renal nerves was markedly suppressed by selective activation of the ET_B receptor by intrarenal administration of S6c. These findings strongly suggest that ETs play an important role as an inhibitory modulator of renal noradrenergic neurotransmission, through ET_B receptor mechanisms.

Intrarenal arterial infusion of ET-1 (1 ng/kg/min) inhibited the increased response of NESR to the RNS, whereas this peptide did not affect renal vasoconstriction and decreased urine formation, in response to the RNS (Suzuki *et al.*, 1992). By way of explanation, we suggested that presynaptic inhibition of ET-1 is neutralized by the peptide-induced potentiation of the responsiveness of α-adrenergic receptor to NE on postjunctional sites (Suzuki *et al.*, 1992). Other investigators noted that ET-1 enhanced the contractile responses to exogenous NE in isolated vascular vessels (Wiklund *et al.*, 1989a; Wong-Dusting *et al.*, 1990; Tabuchi *et al.*, 1990). However, ET-1 was seen to suppress the release of NE from nerve endings (Tabuchi *et al.*, 1989b; Wiklund *et al.*, 1988; Wiklund *et al.*, 1989a). In the case of intrarenal administration of ET-3 (2 ng/kg/min), we observed that RNS-induced changes in renal function were markedly suppressed by this peptide, in addition to the inhibitory effects of NE overflow induced by RNS (Matsumura *et al.*, 1996). Taken together with findings in our study using S6c and binding affinities of ET isopeptides to ET receptors (ET-1 $\gg$ ET-3, S6c, for ET_A receptors; ET-1 = ET-3 = S6c, for ET_B receptors) (Rubanyi and Polokoff, 1994), it is reasonable to consider that ET inhibit renal noradrenergic neurotransmission via ET_B receptors at the presynaptic site and that ET-1 can enhance NE-induced renal actions via ET_A receptors, probably at postsynaptic sites.

With respect to the inhibitory action on NESR via ET_B receptors, precise mechanisms and localization of receptors are unclear. It has been reported that ET-1 stimulates the release of PG in guinea pig and rat lungs (De Nucci *et al.*, 1988), rat mesenteric arteris (Rakugi *et al.*, 1989; Tabuchi *et al.*, 1989b) and also in the dog kidney (Miura *et al.*, 1989). PGE_2 is known to inhibit the release of NE by a prejunctional mechanism (Frame and Hedqvist, 1975). However, Tabuchi and colleagues (1989b) showed that the inhibition of PGE_2 production does not affect the presynaptic effect of ET-1. Furthermore, Wiklund *et al.*, (1990) reported that forskolin does not antagonize the prejunctional inhibitory effect of ET-1 in the rat vas deferens. Taken together, ET-1 induced inhibition of NE release does not appear to be directly linked to cyclic-AMP mechanisms.

It is well acknowledged that ET enhance the release of endothelium-derived NO as well as PGs (De Nucci *et al.*, 1988; Warner *et al.*, 1989). Recent reports suggested that endogenous NO may function as a neurotransmitter or a

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TABLE 2
Effects of S6c on systemic and renal hemodynamics, and urine formation

	MAP (mm Hg)	HR (beats/min)	RBF (ml/g/min)	RVR (mm Hg/ml/g/min)	GFR (ml/g/min)	FF (%)	UF (μl/g/min)	$U_{Na}V$ (μEq/g/min)	FE_{Na} (%)
Saline infusion									
First									
Control	136.8 ± 8.6	148.8 ± 14.5	3.8 ± 0.7	40.5 ± 9.3	0.86 ± 0.08	42.4 ± 11.2	12.7 ± 2.9	3.61 ± 0.58	2.8 ± 0.4
Experiment (without RNS)	135.5 ± 8.6	150.0 ± 13.6	3.8 ± 0.7	40.0 ± 8.9	0.86 ± 0.01	41.2 ± 8.2	12.6 ± 2.7	3.67 ± 0.52	2.9 ± 0.4
Second									
Control	135.0 ± 8.4	148.0 ± 14.9	3.8 ± 0.7	39.3 ± 8.1	0.84 ± 0.03	38.6 ± 7.2	13.5 ± 3.9	3.85 ± 0.90	3.0 ± 0.6
Experiment (without RNS)	133.5 ± 7.9	149.0 ± 14.6	3.9 ± 0.7	38.1 ± 7.7	0.86 ± 0.04	38.4 ± 6.1	14.5 ± 3.6	3.99 ± 0.82	3.1 ± 0.6
S6c infusion									
Third									
Control	133.8 ± 6.6	151.3 ± 9.3	3.6 ± 0.6	39.8 ± 6.2	0.83 ± 0.07	38.3 ± 5.0	40.4 ± 8.4[c]	4.68 ± 0.56	3.8 ± 0.4
Experiment (without RNS)	133.8 ± 7.7	152.8 ± 9.4	3.4 ± 0.6[b]	42.8 ± 6.8[a]	0.83 ± 0.05	41.4 ± 5.7	43.7 ± 10.6	4.66 ± 0.63	3.7 ± 0.4
Fourth									
Control	137.0 ± 10.5	157.0 ± 12.3	3.7 ± 0.6	40.6 ± 7.3	0.88 ± 0.06	40.1 ± 5.7	40.9 ± 9.2[d]	4.85 ± 1.02	3.7 ± 0.7
Experiment (without RNS)	137.8 ± 10.7	159.8 ± 13.2	3.4 ± 0.5[a]	43.6 ± 7.8[a]	0.83 ± 0.08	40.9 ± 5.4	41.4 ± 9.0	4.76 ± 0.94	4.0 ± 0.7

Each value represents the mean ± S.E. of four dogs. Effects of S6c in 3rd and 4th experimental condition were examined by Student's *t* test: [a] $P < .05$, [b] $P < .01$, compared with each control value. Effects of S6c on basal renal function were examined by Bonferroni's multiple comparison test: [c] $P < .01$ vs. control value in first experiment and [d] $P < .01$ vs. control value in second experiment during saline infusion.

neuromodulator in various tissues, because NO synthase inhibitors enhanced the transmural nerve stimulation-induced vasoconstriction in isolated vascular vessels (Bucher *et al.*, 1992; Shinozuka *et al.*, 1992; Toda and Okamura, 1992), and because the release of NE from adrenergic nerves of vascular vessels was inhibited when the endothelium was intact (Cohen and Weisbrod, 1988; Greenberg *et al.*, 1989). We found that intrarenal arterial infusion of N^G-nitro-L-arginine, an NO synthase inhibitor, enhanced the increased response of NE overflow and changes in renal function induced by RNS in anesthetized dogs (Egi *et al.*, 1994), whereas exogenously applied NO donor inhibited the RNS-induced renal actions and NE release (Maekawa *et al.*, 1996). Therefore, the inhibitory effect of ET-1 on renal presynaptic neurotransmission may be due to peptide-induced NO production.

In the RNS period at a low frequency, we observed an inconsistent result, *i.e.*, administration of S6c induced a slight but significant decrease in RBF and increase in RVR, whereas low and high frequency RNS-induced reduction in urine formation and high frequency RNS-induced renal vasoconstriction were significantly attenuated by this peptide. These contradictory findings may be related to renal vasoconstrictor effects of S6c. In fact, a similar renal vasoconstriction was observed in the additional study in which effects of S6c on renal hemodynamics and urine formation were examined without RNS (table 3). Other evidence suggests that ET_B receptors also exist on smooth muscle cells and mediate vasoconstriction (Hay, 1992; Harrison *et al.*, 1992; Teelink *et al.*, 1994), in addition to the general concept that they are located on vascular endothelial cells and mediate vasodilation via the release of prostacyclin and NO (De Nucci *et al.*, 1988; Warner *et al.*, 1989). Several studies demonstrated that stimulation of the ET_B receptor by the administration of S6c produced renal vasoconstriction in rats (Clozel *et al.*, 1992; Cristol *et al.*, 1993; Gellai *et al.*, 1994) and dogs (Clavell *et al.*, 1995). We also noted that intrarenal infusion of S6c in a higher dose (5 ng/kg/min) elicited a grater extent of renal vasoconstriction (with initial renal vasodilation) than seen in our study (unpublished observation).

In addition to renal vasoconstrictor effects, intrarenal administration of S6c produced significant increases in UF, with no apparent effects on $U_{Na}V$ and FE_{Na}. Several studies demonstrated that ET-1 inhibits AVP-induced water permeability in the rat IMCD (Oishi *et al.*, 1991; Nadler *et al.*, 1992) and AVP-stimulated cyclic-AMP accumulation (Tomita *et al.*, 1990). In the kidney, ET_B receptor mRNA is distributed mainly in the IMCD, a determination using a reverse transcription and polymerase chain reaction assay of rat nephron segments (Terada *et al.*, 1992). Furthermore, Edwards *et al.*, (1993) reported that AVP-induced increases in osmotic water permeability and cyclic-AMP accumulation in isolated rat IMCD were inhibited by S6c and that BQ-123, a selective ET_A receptor antagonist, had no effect on ET-1-induced inhibition of hydro-osmotic response to AVP. These results suggest that ET-1 has an antagonistic effect on antidiuretic effects of AVP in IMCD, and that these effects are mediated via activation of the ET_B receptor subtype. Thus, our results seem to be related to the inhibition of AVP-induced water reabsorption by administration of S6c.

In summary, the intrarenal administration of S6c inhibited the RNS-induced increased response of NE release from renal noradrenergic nerve endings and suppressed renal vasoconstriction and antidiuretic response to RNS. These findings provide evidence for a role of the ET_B receptor subtype in the kidney, *i.e.*, ETs play an important role as an inhibitory modulator of renal noradrenergic neurotransmission through ET_B receptor mechanisms.

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