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Effects of FK409, a nitric oxide donor, on renal responses to renal nerve stimulation in anesthetized dogs

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Abstract

We examined the effects of (±)-(*E*)-4-ethyl-2-[(*E*)-hydroxyimino]-5-nitro-3-hexenamide (FK409), a nitric oxide (NO) donor, on renal actions and norepinephrine overflow induced by renal nerve stimulation in anesthetized dogs, with or without N^G-nitro-L-arginine (NOARG), a NO synthase inhibitor. Renal nerve stimulation at a low frequency (0.5–2.0 Hz) produced significant decreases in urine flow and urinary excretion of Na^+ and increases in norepinephrine secretion rate. Renal nerve stimulation at a high frequency (2.5–5.0 Hz) which diminishes renal hemodynamics, elicited more marked decreases in urine formation and increases in norepinephrine secretion rate. Intrarenal arterial infusion of FK409 (0.25 μg/kg/min) failed to alter renal actions and increases in norepinephrine secretion rate in response to both low- and high frequency renal nerve stimulation. When NOARG (40 μg/kg/min) was administrated intrarenally, low-frequency renal nerve stimulation caused a potent antidiuresis and renal vasoconstriction. The renal nerve stimulation-induced increase in norepinephrine secretion rate was markedly enhanced by NOARG infusion. Simultaneous infusion of FK409 markedly attenuated the NOARG-induced enhancement of renal actions and increases in norepinephrine secretion rate, in response to low-frequency renal nerve stimulation. These results suggest that exogenous NO suppresses the renal nerve stimulation-induced norepinephrine overflow and renal actions in NO-depleted conditions. We also propose that endogenous NO functions tonically as an inhibitory modulator of renal noradrenergic neurotransmission. © 1998 Elsevier Science B.V.

Keywords: Nitric oxide (NO); FK409; N^G-nitro-L-arginine; Renal nerve stimulation; Norepinephrine overflow; Renal function

1. Introduction

Nitric oxide (NO) is synthesized from the amino acid L-arginine by a NO synthase (Moncada et al., 1991a) and elicits smooth muscle relaxations by activation of soluble guanylate cyclase, which is followed by increases in cyclic guanosine monophosphate (cGMP) formation in vascular smooth muscle cells (Ignarro, 1990; Moncada et al., 1991a). NO synthase is competitively inhibited by L-arginine derivatives such as N^G-nitro-L-arginine (NOARG) and N^G-nitro-L-arginine methyl ester. NO synthase inhibitors have been used widely to determine the role of endogenous NO.

Several studies have shown that NO may function as a neurotransmitter or a neuromodulator. NO synthase inhibitors enhanced electrically stimulated vasoconstriction but failed to increase norepinephrine release in the rat tail artery (Bucher et al., 1992), the rabbit pulmonary artery (Shinozuka et al., 1992) and the monkey mesenteric artery (Toda and Okamura, 1992). Other investigators demonstrated that the intact endothelium inhibits the release of the adrenergic neurotransmitter in the rabbit carotid artery (Cohen and Weisbrod, 1988) and canine pulmonary arteries and veins (Greenberg et al., 1989). In addition, NO inhibits norepinephrine release via a prejunctional mechanism in the rat tail artery (Vo et al., 1991). It was also reported that NO of predominantly neuronal origin exerts an inhibitory effect over the evoked norepinephrine release from sympathetic nerves in the rat heart (Schwarz et al., 1996). In a previous study, we noted that intrarenal arterial infusion of NOARG enhances norepinephrine overflow induced by electrical stimulation of renal nerves in anesthetized dogs (Egi et al., 1994). In addition, exogenous norepinephrine-induced renal vasoconstriction and antidiuresis were augmented by the intrarenal administration of

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NOARG (Matsumura et al., 1995). These findings suggest that NO modulates noradrenergic neurotransmission via pre- and postjunctional mechanisms.

In the present study, we used a spontaneous NO donor, (±)-(*E*)-4-ethyl-2-[(*E*)-hydroxyimino]-5-nitro-3-hexenamide (FK409). The compound produces a potent vasorelaxation in the isolated dog coronary artery (Yamada et al., 1991) and rat aorta (Isono et al., 1993). The spontaneous NO release following decomposition of the compound, can account for the above biological actions. (Kita et al., 1994). Therefore, we examined the effects of FK409 to evaluate the direct effect of NO on renal actions and norepinephrine overflow in response to renal nerve stimulation in anesthetized dogs.

2. Materials and methods

2.1. Surgical procedures

Experiments were performed on adult mongrel dogs of both sexes weighing 10–18 kg. The dogs were anesthetized with sodium pentobarbital (30 mg/kg i.v.) and given maintenance doses as needed. 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 infusion of saline containing 0.45% inulin, respectively. Mean arterial blood pressure and heart rate were monitored with a pressure transducer (Nihon Koden, Tokyo, AP601G) connected to a catheter placed in the abdominal aorta via the right femoral artery. The left kidney was exposed retroperitoneally through a flank incision and the renal artery was isolated from surrounding tissue. All visible nerve fibers along the renal artery were isolated, ligated and cut. For renal nerve stimulation, the distal cut portion was placed on bipolar platinum electrodes connected to an electric stimulator (Nihon Koden, SEN-7103). An electromagnetic flow prove (2.0–3.5 mm in a diameter, Nihon Koden) connected to a square-wave flowmeter (Nihon Koden, MFV-2100) was attached at the left renal artery to measure renal blood flow continuously. A curved 23-gauge needle connected to polyethylene tubing was inserted into the left renal artery proximal to the flow prove for infusion of drug solution or saline at a rate of 0.48 ml/min. Another curved 18-gauge needle connected to polyethylene catheters was inserted into the left renal vein for venous blood sampling. Finally, the left ureter was cannulated for urine collection. After completion of the surgical procedures, a priming dose of inulin (20 mg/kg) was given, followed by a sustaining infusion of 0.9% saline containing 0.45% inulin for the measurement of glomerular filtration rate, at a rate of 2.0 ml/min. Mean arterial blood pressure, heart rate and renal blood flow were recorded continuously on a polygraph (Nihon Koden, RM6000G). Approximately 90–120 min were allowed for stabilization.

2.2. Experimental protocol

2.2.1. Experiment A

After stabilization, we performed four renal nerve stimulation experiments in each of 7 dogs. Each experiment consisted of a 10 min control period and a 10 min renal nerve stimulation period. Blood samples (3.0 ml) were taken at 5 min in the control period, 1 and 9 min in the renal nerve stimulation period from the right brachial artery and left renal vein, respectively. 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.

The first stimulation experiments was performed at low frequency (0.5–2.0 Hz; duration, 1.0 ms; and supramaximal voltage, 10–25 V) during the renal nerve stimulation period. The second renal nerve stimulation experiment was started after a 30 min equilibration interval. In this experiment, renal nerves were stimulated at a high frequency (2.5–5.0 Hz). These renal nerve stimulation experiments were performed under conditions of intrarenal arterial infusion of saline at a rate of 0.48 ml/min. Approximately 60 min after termination of the second experiment, intrarenal arterial infusion of FK409 (0.25 μg/kg/min) was started. Ten minutes after the drug infusion, renal nerve stimulation experiments of both low and high frequency were repeated during the infusion of FK409, in the same manner as described above. FK409 was infused at a dose which did not significantly affect systemic and renal hemodynamics.

2.2.2. Experiment B

In separate experiments, we evaluated the effect of FK409 on renal nerve stimulation-induced renal actions, with or without blockade of endogenous NO generation by NOARG, a NO synthase inhibitor. Each renal nerve stimulation experiment was performed in the same manner as experiment A. One group of animals ($n = 7$) was used to examine the effects of intrarenal arterial infusion of FK409 on renal nerve stimulation-induced renal actions, in the absence of NOARG. Two renal nerve stimulation experiments were performed on each dog. After stabilization, we started the first renal nerve stimulation experiments at a low frequency during the renal nerve stimulation period. During the first renal nerve stimulation experiment, saline was infused into the renal artery. About 30 min after termination of the first renal nerve stimulation experiment, intrarenal arterial infusion of FK409 (0.25 μg/kg/min) was started. After 10 min, the second renal nerve stimulation experiments were performed under conditions of drug infusion, in the same manner as described above. Another

group of dogs ($n = 7$) was used to examine the effect of FK409 on renal nerve stimulation-induced renal actions, in the presence of NOARG. We performed two renal nerve stimulation experiments in each dog. After stabilization, intrarenal arterial infusion of NOARG (40 μg/kg/min) was started and 30 min later the first renal nerve stimulation experiments at a low frequency was done. The second renal nerve stimulation experiment was performed during the simultaneous infusion of FK409 with NOARG. The dose of NOARG was determined by examining an inhibitory effect on acetylcholine-induced renal vasodilation.

2.3. Analytical procedures

Glomerular filtration rate was estimated from 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). NO metabolites (NO_x) in urine was measured using an autoanalyser (Tokyo Kasei Kogyo, Tokyo, TCl-NOX 1000). Urine was diluted with carrier solution (0.07% ethylenediamine tetra acetic acid and in 0.3% NH_4Cl) and passed through a cadmium reduction column to reduce from NO_3^- to NO_2^-, which reacts with Griess reagent (1% sulfanilamide and 0.1% *N*-1-naphthylethylenediamine dihydrochloride in 5% HCl). The absorbance at 540 nm was measured using a flow-through visible spectrophotometer (Tokyo Kasei Kogyo, S-3250). NO_2^- was used as standard. The plasma norepinephrine concentration was measured by high-performance liquid chromatography with an amperometric detector (Eikom, Kyoto, EC-100), as previously reported (Hayashi et al., 1991). The norepinephrine secretion rate (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 norepinephrine concentration (pg/ml), and NE_A is renal arterial plasma norepinephrine concentration (pg/ml).

2.4. Drugs

FK409 was a kind gift from Fujisawa Pharmaceutical (Osaka). NOARG was purchased from Peptide Institute (Osaka). All drugs were dissolved in saline solution. Other chemicals were obtained from Nacalai Tesque, (Kyoto) and Wako Pure Chemical Industries (Osaka).

2.5. Statistical analysis

Data are expressed as mean ± S.E.M. For statistical analysis, we used paired Student's *t*-test for comparison of values between the control period and renal nerve stimulation period during saline or drug infusion. Renal nerve stimulation- or drug-induced changes were assessed by analysis of variance (ANOVA) followed by a Bonferroni's multiple comparison test. For all comparisons, differences were considered significant at $P < 0.05$ and $P < 0.01$.

3. Results

3.1. Effects of FK409 on renal actions induced by renal nerve stimulation (experiment A)

As shown in Table 1, low-frequency renal nerve stimulation (0.5–2.0 Hz) significantly decreased urine flow, urinary excretion of Na^+ and fractional excretion of Na^+, (urine flow, urinary excretion of Na^+ and fractional excretion of Na^+ decreased by about 50, 50 and 45%, respectively) without affecting systemic and renal hemodynamics. High frequency renal nerve stimulation (2.5–5.0 Hz) produced more potent decreases in urine formation than seen with low frequency renal nerve stimulation (urine flow, urinary excretion of Na^+ and fractional excretion of Na^+ decreased by about 80, 80 and 50%, respectively). In addition, there were significant decreases in renal blood flow, glomerular filtration rate and filtration fraction, and increase in renal vascular resistance. Intrarenal arterial infusion of FK409 (0.25 μg/kg/min) tended to increase urine flow, urinary excretion of Na^+ and fractional excretion of Na^+ but with no statistical significance. In the presence of FK409, low frequency renal nerve stimulation reduced urine formation, the extent of these changes being similar to those seen in the absence of FK409. The high frequency renal nerve stimulation-induced potent antidiuresis and antinatriuresis was also observed in the case of FK409 infusion. In addition, high frequency renal nerve stimulation-induced decreases in renal blood flow, glomerular filtration rate and filtration fraction and the increases in renal vascular resistance were not affected by infusion of the drug.

3.2. Effects of FK409 on renal nerve stimulation-induced increase in norepinephrine secretion rate (experiment A)

The low-frequency renal nerve stimulation significantly increased norepinephrine secretion rate from a control value of -110 ± 65 to 418 ± 126 and 551 ± 125 pg/g/min at 1 and 9 min after starting renal nerve stimulation. In case of high-frequency renal nerve stimulation, the norepinephrine secretion rate increased markedly from a control value of -214 ± 115 to 1506 ± 220 and 1635 ± 357 pg/g/min at 1 and 9 min after the start of renal nerve stimulation, respectively. In the following results, renal nerve stimulation-induced increases in norepinephrine secretion rate from the control are indicated as ΔNESR, to clarify changes in norepinephrine secretion rate induced by renal nerve stimulation. As shown in Fig. 1, FK409 infusion did not affect ΔNESR during low

Table 1
Effects of FK409 on RNS-induced changes in systemic hemodynamics and renal functions in anesthetized dogs

	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									
Control	132.0±4.9	153.3±16.1	4.3±0.5	32.6±3.6	0.88±0.10	34.1±1.9	16.3±2.6	4.60±0.86	3.8±0.8
RNS (low)	133.4±4.6	153.4±16.4	4.3±0.5	33.6±4.1	0.83±0.11	32.4±2.3	7.5±1.5[b]	2.21±0.48[b]	1.9±0.5[b]
% change	1.1±0.6	−0.0±0.9	−2.7±0.8	4.0±1.1	−5.4±5.6	−4.5±5.5	−51.9±7.3	−51.6±5.1	−45.1±6.2
Control	127.4±5.2	160.1±23.5	4.4±0.5	31.3±4.2	0.89±0.10	32.8±1.9	16.3±3.1	4.49±0.71	3.6±0.6
RNS (high)	128.6±5.8	158.7±22.6	3.2±0.5[b]	50.6±10.9[a]	0.37±0.09[b]	18.7±3.2[b]	2.5±0.6[b]	0.73±0.20[b]	1.5±0.3[b]
% change	0.9±1.8	−0.7±0.7	−30.3±6.6[d]	55.3±19.8[c]	−60.5±6.8[d]	−44.6±7.3[d]	−80.6±4.7[c]	−82.8±3.6[d]	−51.9±12.1
FK409 infusion									
control	121.1±8.2	167.1±20.3	4.6±0.4	28.6±3.9	0.92±0.10	32.7±2.3	22.1±4.6	5.91±0.85	4.5±0.7
RNS (low)	121.6±7.6	167.7±20.7	4.5±0.4	29.1±3.9	0.90±0.09	32.6±2.1	11.7±1.7[a]	3.74±0.62[b]	2.9±0.5[b]
% change	0.6±1.0	−0.1±0.9	−1.3±0.7	1.9±1.7	0.3±2.4	0.0±2.3	−36.4±10.5	−36.1±6.3	−35.8±5.5
Control	116.9±9.0	164.9±20.3	4.6±0.4	27.4±3.9	0.85±0.09	29.7±2.3	18.8±2.5	5.28±0.74	4.2±0.5
RNS (high)	119.0±9.3	165.9±20.1	3.3±0.5[b]	43.5±9.0[a]	0.36±0.09[b]	18.1±4.1[b]	3.8±0.9[b]	1.01±0.22[b]	2.4±0.6[a]
% change	1.8±1.0	0.8±0.8	−29.9±6.9[f]	57.7±22.1[e]	−59.9±7.4[f]	−42.0±10.8[f]	−77.9±6.2[f]	−79.9±5.3[f]	−41.9±15.2

Each value represents the mean±S.E.M. of seven dogs. Effects of RNS in each experimental condition were examined by paired Student's *t*-test: [a]$P < 0.05$, [b]$P < 0.01$.
RNS-induced changes were assessed by analysis of variance (ANOVA) followed by a Bonferroni's multiple comparison test: [c]$P < 0.05$, [d]$P < 0.01$ versus low frequency RNS during saline infusion and [e]$P < 0.05$, [f]$P < 0.01$ versus low frequency RNS during FK409 infusion.

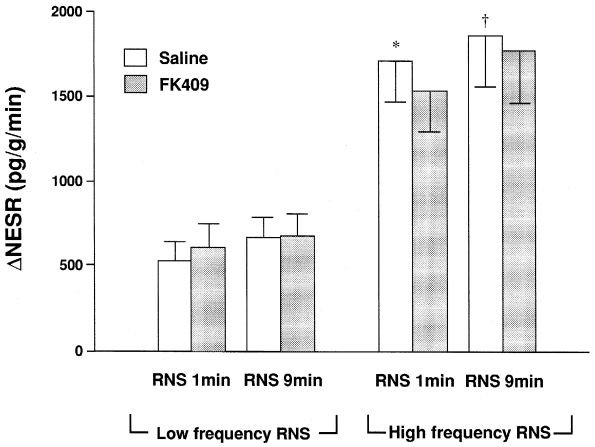

Fig. 1. Effects of FK409 on renal nerve stimulation-induced changes in ΔNESR. Each value represents the mean ± S.E.M. of seven dogs. Renal nerve stimulation-induced changes during saline or FK409 infusion were assessed by analysis of variance (ANOVA) followed by a Bonferroni's multiple comparison test: $^{*}P < 0.01$, versus ΔNESR at 1 min after the start of low frequency renal nerve stimulation and $^{\dagger}P < 0.01$, versus ΔNESR at 9 min after the start of low frequency renal nerve stimulation during saline infusion.

frequency renal nerve stimulation (from 528 ± 100 and 661 ± 115 to 599 ± 134 and 677 ± 120 pg/g/min at 1 and 9 min after the start of low-frequency renal nerve stimulation, respectively). Similar, high frequency renal nerve stimulation-induced norepinephrine release was not affected by the FK409 infusion (from 1719 ± 213 and 1849 ± 369 to 1536 ± 301 and 1764 ± 382 pg/g/min at 1 and 9 min after the start of high-frequency renal nerve stimulation, respectively).

3.3. Effects of FK409 on renal actions induced by low-frequency renal nerve stimulation with or without NOARG (experiment B)

Intrarenal arterial infusion of FK409 did not affect basal and low frequency renal nerve stimulation-induced changes in renal hemodynamics, as observed in experiment A. In addition, basal and renal nerve stimulation-induced changes in urine formation were not affected by the FK409 infusion (Figs. 2 and 3). When NOARG (40 μg/kg/min) was administrated intrarenally, the basal level of renal blood flow was decreased and that of renal vascular resistance was increased. In the presence of NOARG, low frequency renal nerve stimulation significantly decreased renal blood flow and glomerular filtration rate by about 20 and 40% from control values of 3.0 ± 0.3 ml/g/min and 0.82 ± 0.06 ml/g/min, respectively, and increased renal vascular resistance by about 25% from the control value of 51.7 ± 5.3 mm Hg/ml/g/min (Fig. 2). As shown in Fig. 3, the drugs also elicited decreases in urine flow, urinary excretion of Na^{+} and fractional excretion of Na^{+}. The renal

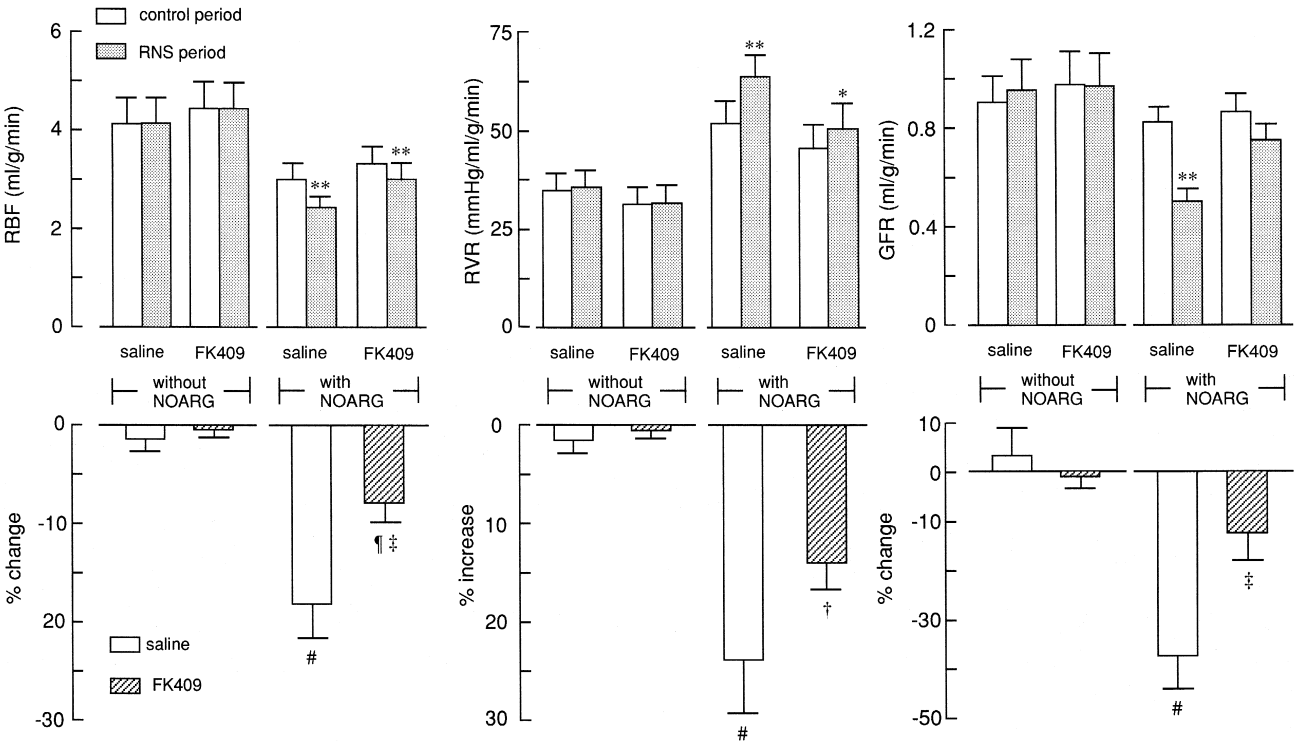

Fig. 2. Effects of FK409 on low frequency renal nerve stimulation-induced changes in renal hemodynamic. Each value represents the mean ± S.E.M. of seven dogs. Effects of renal nerve stimulation in each experimental condition were examined by Student's *t*-test: $^{*}P < 0.05$, $^{**}P < 0.01$. Renal nerve stimulation- or drug-induced changes were assessed by analysis of variance (ANOVA) followed by a Bonferroni's multiple comparison test: $^{\#}P < 0.01$, versus renal nerve stimulation-induced change during saline infusion without NOARG. $^{\P}P < 0.05$, versus renal nerve stimulation-induced change during FK409 infusion without NOARG. $^{\dagger}P < 0.05$, $^{\ddagger}P < 0.01$ versus renal nerve stimulation-induced change during saline infusion with NOARG.

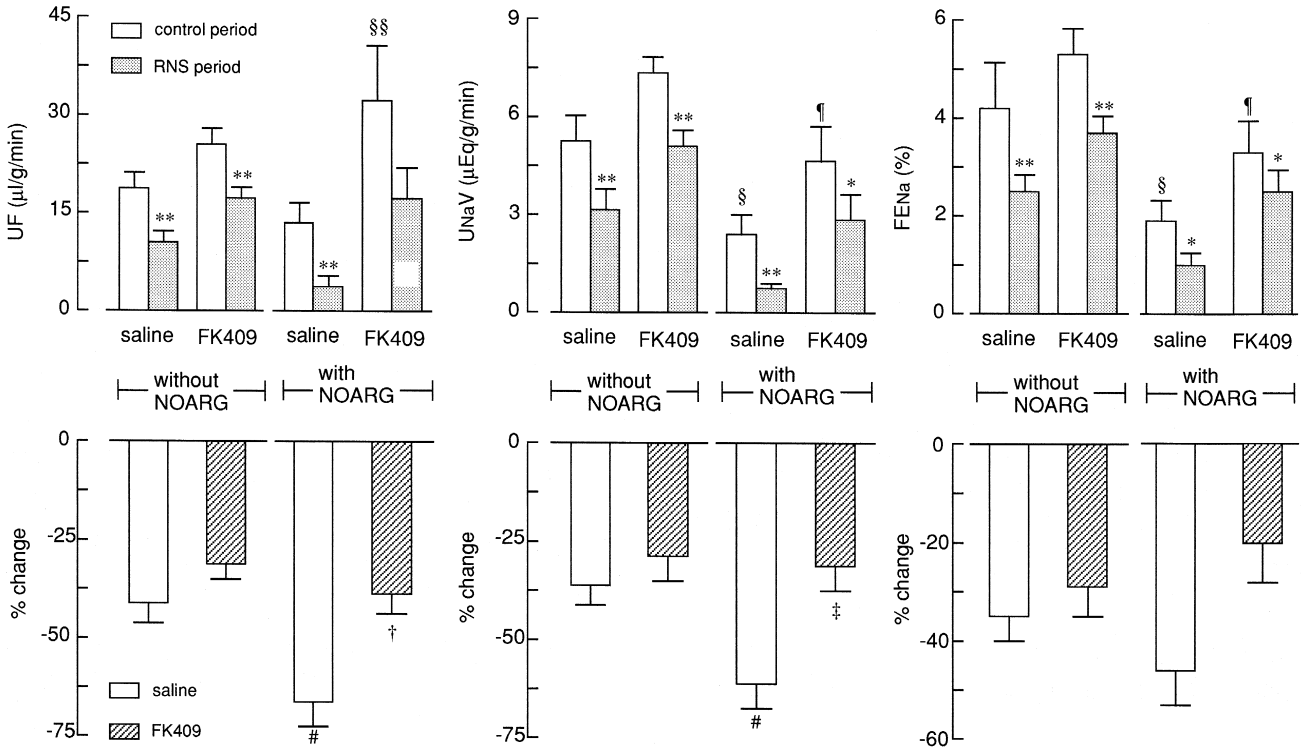

Fig. 3. Effects of FK409 on renal nerve stimulation-induced changes in urine formation. Each value represents the mean ± S.E.M. of seven dogs. Effects of renal nerve stimulation in each experimental condition were examined by Student's *t*-test: $^{*}P < 0.05$, $^{**}P < 0.01$. Renal nerve stimulation- or drug-induced changes were assessed by analysis of variance (ANOVA) followed by a Bonferroni's multiple comparison test: $^{\#}P < 0.05$, versus renal nerve stimulation-induced change during saline infusion without NOARG. $^{\dagger}P < 0.05$, $^{\ddagger}P < 0.01$ versus renal nerve stimulation-induced change during saline infusion with NOARG. $^{\S}P < 0.01$ versus control value during saline infusion without NOARG. $^{\P}P < 0.05$ versus control value during FK409 infusion without NOARG. $^{\S\S}P < 0.01$ versus control value during saline infusion with NOARG.

nerve stimulation-induced potent reduction in urine formation was also observed during NOARG infusion (urine flow, from 13.8 ± 3.0 to 3.8 ± 0.6 μl/g/min; urinary excretion of Na^+, from 2.39 ± 0.58 to 0.70 ± 0.15 μEq/g/min; fractional excretion of Na^+, from 1.9 ± 0.4 to 1.0 ± 0.2%). When FK409 was infused into the renal artery in the presence of NOARG, the renal nerve stimulation-induced renal vasoconstriction observed with NOARG alone was suppressed markedly (Fig. 2). In the presence of NOARG, FK409 increased the basal levels of urine flow, urinary excretion of Na^+ and fractional excretion of Na^+ (with a statistical significance only in urine flow), in contrast to FK409 alone. In addition, the NOARG-induced enhancement of renal nerve stimulation-induced reduction in urine formation was abolished by the simultaneous infusion of FK409 (Fig. 3).

3.4. Effects of FK409 on renal nerve stimulation-induced norepinephrine secretion rate with or without NOARG (experiment B)

Norepinephrine secretion rate increased from a control value of -99 ± 72 to 498 ± 125 and 600 ± 143 pg/g/min at 1 and 9 min after the initiation of low-frequency renal

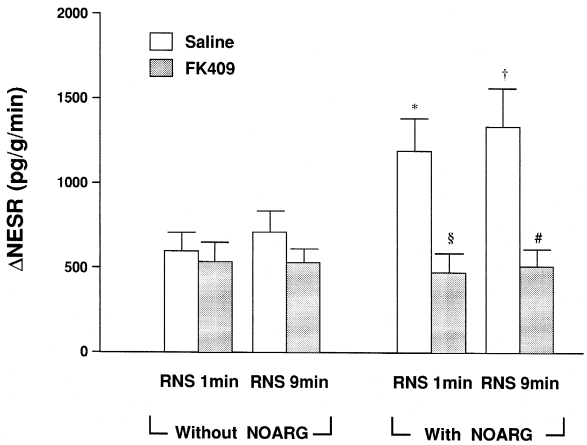

Fig. 4. Effects of FK409 on low frequency renal nerve stimulation-induced changes in ΔNESR with or without NOARG. Each value represents the mean ± S.E.M. of seven dogs. Effects of renal nerve stimulation in each experimental condition were assessed by analysis of variance (ANOVA) followed by a Bonferroni's multiple comparison test: $^{*}P < 0.05$, versus ΔNESR at 1 min after the start of renal nerve stimulation without NOARG. $^{\dagger}P < 0.05$, versus ΔNESR at 9 min after the start of renal nerve stimulation without NOARG. $^{\S}P < 0.01$, versus ΔNESR at 1 min after the start of renal nerve stimulation during NOARG infusion. $^{\#}P < 0.05$ versus ΔNESR at 9 min after the start of renal nerve stimulation during NOARG infusion.

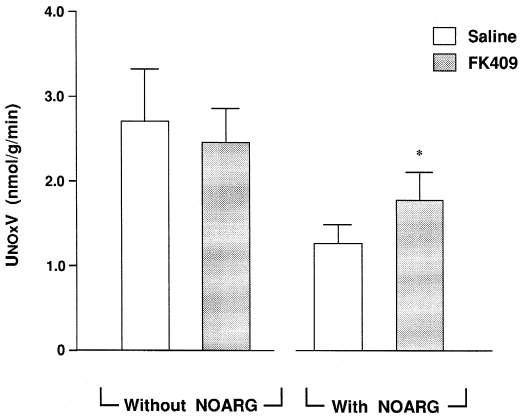

Fig. 5. Effects of FK409 on urinary excretion of NO metabolites (UNOXV) with or without NOARG. Each value represents the mean ± S.E.M. of seven dogs. $^{*}P < 0.05$, versus control value during saline infusion with NOARG.

nerve stimulation, respectively. As shown in Fig. 4, FK409 infusion failed to change ΔNESR during low-frequency renal nerve stimulation (from 597 ± 96 and 699 ± 123 to 532 ± 111 and 526 ± 79 pg/g/min at 1 and 9 min after the start of low-frequency renal nerve stimulation, respectively) as observed in experiment A. When NOARG was administrated intrarenally, the basal level of norepinephrine secretion rate was not affected by the drug infusion (−69 ± 115 versus −99 ± 72 pg/g/min). During NOARG infusion, there were marked increases in ΔNESR during low-frequency renal nerve stimulation, compared with saline infusion. Simultaneous infusion of FK409 markedly suppressed NOARG-induced accelerating effects on ΔNESR (from 1182 ± 189 and 1328 ± 236 to 467 ± 113 and 506 ± 85 pg/g/min at 1 and 9 min after the start of low-frequency renal nerve stimulation, respectively).

3.5. Effects of FK409 on urinary excretion of NO metabolites, with or without NOARG (experiment B)

As shown in Fig. 5, intrarenal arterial infusion of FK409 did not affect the basal level of urinary excretion of NO metabolites (UNO_xV) (from 2.7 ± 0.6 to 2.4 ± 0.4 nmol/g/min). When NOARG was administrated intrarenally, the basal levels of UNO_xV decreased to about 50% of those seen with saline infusion. Simultaneous infusion of FK409 significantly increased the UNO_xV about 40% (from 1.2 ± 0.2 to 1.7 ± 0.3 nmol/g/min).

4. Discussion

In the present study, the intrarenal arterial infusion of FK409, which releases NO spontaneously, was performed to evaluate the direct effect of exogenous NO on renal actions and norepinephrine overflow in response to renal nerve stimulation. Renal nerve stimulation at a low frequency produced significant decreases in urine flow, urinary excretion of Na^+ and fractional excretion of Na^+ with no effect on systemic and renal hemodynamics. High frequency renal nerve stimulation elicited decreases in renal blood flow, glomerular filtration rate and filtration fraction, in addition to more marked antidiuresis and antinatriuresis. We determined norepinephrine secretion rate during the renal nerve stimulation as an index of norepinephrine release from renal noradrenergic nerve endings. Renal nerve stimulation produced frequency-dependent increases of norepinephrine secretion rate. Intrarenal arterial infusion of FK409 did not affect renal hemodynamic and excretory responses to renal nerve stimulation. Furthermore, there were no differences in the increased responses of norepinephrine secretion rate to the renal nerve stimulation, between with or without FK409 infusion. In the present study, a relatively low dose (0.25 μg/kg/min) of the agent, which does not significantly affect systemic and renal hemodynamics, was given. Thus, our results indicate that FK409 at this dose does not modulate renal noradrenergic neurotransmission, under normal conditions.

Several studies demonstrated that endogenous NO may function as a neurotransmitter and/or neuromodulator in various organs, since the presence of an intact endothelium inhibits electrical nerve stimulation induced-norepinephrine release from adrenergic nerves (Cohen and Weisbrod, 1988; Greenberg et al., 1989). In addition, NO synthase inhibitors enhanced norepinephrine release via a prejunctional mechanism in the rat tail artery (Vo et al., 1991) and the rat heart (Schwarz et al., 1996). We previously noted that changes in renal function and norepinephrine overflow induced by renal nerve stimulation were enhanced by NOARG, a NO synthase inhibitor (Egi et al., 1994; Maekawa et al., 1996; Matsuo et al., in press). In the present study, low frequency renal nerve stimulation during NOARG infusion elicited marked reductions in urine flow, urinary excretion of Na^+ and fractional excretion of Na^+. In addition, there were significant decreases in renal blood flow, glomerular filtration rate and filtration fraction and increases in renal vascular resistance. Augmention of NOARG on renal hemodynamic and excretory responses to renal nerve stimulation were accompanied by increases in norepinephrine secretion rate. Thus, endogenous NO probably has a role as inhibitory modulator of renal noradrenergic neurotransmission, at prejunctional sites.

In this study, we examined effects of FK409 on the renal nerve stimulation-induced renal actions, also in the presence of NOARG, since we previously noted that renal hemodynamic and excretory responses to FK409 (the intrarenal arterial infusion at 10 μg/kg/min) in anesthetized rats were markedly enhanced in the presence of NOARG (Urabe et al., 1997). Simultaneous infusion of FK409 abolished the NOARG-induced enhancement of decreases in urine formation and renal vasoconstriction in response to low-frequency renal nerve stimulation. Fur-

thermore, augmentation of the renal nerve stimulation-induced increase in norepinephrine secretion rate by NOARG was also abolished by intrarenal administration of FK409. Maekawa et al. (1996) noted that Na^+ nitroprusside markedly attenuated the renal nerve stimulation-induced renal actions and norepinephrine overflow, in the presence of NO synthase inhibitor. Taken together, inhibitory effects of exogenous NO on renal noradrenergic neurotransmission appears to be augmented under NO-depleted conditions.

Adenosine may play an important role in the kidney physiology and pathophysiology. Adenosine has been shown to function as an inhibitory neuromodulator in the rabbit kidney (Hedqvist and Fredholm, 1976). Intrarenal arterial infusion of adenosine in vivo elicits biphasic responses, a transient renal vasoconstriction followed by sustained vasodilation in dogs (Spielman and Arend, 1991) and diuresis and natriuresis in rats (Miyamoto et al., 1988). It has been also reported that adenosine concentration increases with renal ischemia (Miller et al., 1978). Therefore, adenosine serving as neuromodulator in the kidney is likely to be of (patho-)physiological significance. Okumura et al. (1992) reported that the magnitude and duration of renal vasoconstriction produced by adenosine were enhanced by NO synthase inhibitor, indicated that NO plays a role in the renal vascular action of adenosine. Thus, NO may mediate the neuromodulating effects of adenosine.

NO synthase inhibitors diminishes urine formation without affecting glomerular filtration rate (Lahera et al., 1991; Majid et al., 1993; Egi et al., 1994). In the present study, the intrarenal arterial infusion of NOARG decreased the basal levels of renal excretory parameters. In addition, several studies have shown that NO inhibits Na^+ reabsorption, via generation of cyclic GMP in rabbit proximal tubules (Roczniak and Burns, 1996) and mouse cortical collecting ducts (Stoos et al., 1992). These results indicate that NO may function as a regulatory factor in tubular reabsorption of Na^+ and water. In our study, FK409 infusion tended to slightly increase urine formation in the absence of NOARG. On the other hand, when FK409 was infused simultaneously with NOARG, the basal level of urine flow was significantly increased and was accompanied by an increase in UNOXV. Urabe et al. (1997) noted that intrarenal arterial infusion of FK409 to normal rats at 10 $\mu g/kg/min$, a dose which produces a significant decrease in blood pressure, caused renal vasodilation and diuretic effects. These effects were accompanied by increased levels of UNOXV. They also found that renal effects of FK409 were extremely potent in chronic NOARG-treated hypertensive rats, compared with events in normal rats. These results are qualitatively consistent with reports of Moncada et al. (1991b), who demonstrated that treatment with NO synthase inhibitors enhanced vascular responses to glyceryl trinitrate and Na^+ nitroprusside, in vivo and in vitro. They suggested that the removal of basal NO production in the cardiovascular system leads to a specific supersensitivity to nitrovasodilators and that the phenomena occur at the level of guanylate cyclase. Thus, it seems likely that diminution of basal NO production by treatment with NOARG augments the sensitivities of renal vasculature and renal tubules to FK409.

In conclusion, our results suggest that exogenous NO suppresses the renal nerve stimulation-induced norepinephrine overflow and renal actions in NO-depleted conditions. We also propose that endogenous NO functions tonically as an inhibitory modulator of renal noradrenergic neurotransmission.

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