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Renal hemodynamic and excretory responses in anesthetized rats to FK409, a novel nitric oxide donor

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Abstract

Renal hemodynamic and excretory responses to (±)-(*E*)-4-ethyl-2-[(*E*)-hydroxyimino]-5-nitro-3-hexenamide (FK409), a novel nitric oxide (NO) donor, were examined using anesthetized rats. When FK409 was infused into the renal artery of normal rats at 10 μg/kg per min, a moderate renal vasodilating effect was observed with a decrease in mean arterial blood pressure. Urine flow, urinary excretion of sodium and fractional excretion of sodium significantly increased by about 85%, 110% and 75%, respectively, compared with each control value. Simultaneously, urinary excretion of NO metabolites (UNO_xV) was markedly increased with the administration of FK409. In hypertensive rats treated with N^G-nitro-L-arginine (NOARG), the NO synthase inhibitor, FK409 produced a potent renal vasodilation, although the hypotensive effect of the agent was comparable to that seen in normal rats. In addition, glomerular filtration rate was significantly elevated by the agent. There were marked increases in the excretory responses, i.e., levels of urine flow, urinary excretion of sodium and fractional excretion of sodium were increased to about 3-, 6- and 5-fold of each control value, respectively. The extent of increment of UNO_xV was similar to that seen in normal rats. These results clearly indicate that FK409 causes renal vasodilation and diuresis, via NO formation. Renal hemodynamic and excretory responses to the agent are sensitive in NO-depleted conditions. FK409 and related compounds may be useful for the treatment of renal diseases, in cases where the basal NO formation is impaired.

Keywords: FK409; Nitric oxide (NO); N^G-Nitro-L-arginine; Renal function; Urine formation

1. Introduction

In vascular endothelium, nitric oxide (NO) is synthesized from the amino acid L-arginine by an enzyme, the NO synthase (Moncada et al., 1991a). This NO accounts for the biological actions of endothelium-derived relaxing factor, and acts via the stimulation of soluble guanylate cyclase in vascular smooth muscle cells (Ignarro, 1990; Moncada et al., 1991a). Inhibition of NO synthesis by N^G-nitro-L-arginine (NOARG) and other arginine analogs induces a hypertensive response and decreases local blood flow in laboratory animals (Gardiner et al., 1990; Lahera et al., 1991). These observations indicate that the synthesis and release of NO at the basal level tonically contribute to the regulation of vascular tone in the cardiovascular system. In the kidney, intrarenal arterial infusion of NO synthase inhibitors causes potent renal vasoconstriction and antidiuresis (Egi et al., 1994; Baylis et al., 1990). The NO synthase inhibitor NOARG has been reported to impair pressure-induced natriuresis and renal autoregulation, in anesthetized dogs (Salom et al., 1992; Majid et al., 1993b), thereby suggesting that endogenous NO plays an important role in the regulation of renal vascular tone and renal tubular reabsorption of sodium and/or water. Much of the evidence with respect to the functional role of NO in the kidney has been derived from experiments using NO synthase inhibitors.

FK409, (±)-(*E*)-4-ethyl-2-[(*E*)-hydroxyimino]-5-nitro-3-hexenamide, is a structurally unique vasodilator discovered from the fermentation product of *Streptomyces griseosporeus* (Hino et al., 1989). Kita et al. (1994a) reported that biological actions of FK409 can be accounted for by spontaneous NO release following decomposition of the compound. FK409 produces a potent vasorelaxation in the isolated dog coronary artery (Yamada et al., 1991) and rat aorta (Isono et al., 1993). Furthermore, it has been reported that antiplatelet effects (Kita et al., 1994b) and antianginal effects (Kita et al., 1994c) of FK409 are more potent than those of organic nitrates such as isosorbide dinitrate, these effects being based on the potential of

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spontaneous NO generation. In light of these characteristics of FK409, it seemed important to evaluate renal effects of this compound.

We examined renal hemodynamic and excretory responses in case of intrarenal arterial infusions of a novel NO donor, FK409, into anesthetized rats. In addition, renal effects of FK409 in normal rats were compared with those in NOARG-treated hypertensive rats in which endogenous NO production is impaired.

2. Materials and methods

2.1. NOARG treatment

Experiments were done on male Sprague-Dawley rats, weighing 300–330 g. For NO synthase inhibition, these rats were given drinking water containing NOARG at a concentration of 2.74 mM for 7 days, as described previously (Fujita et al., 1995). Control animals were provided plain tap water throughout the study.

2.2. Surgical procedure and experimental protocol

The rats were anesthetized with an intraperitoneal injection of sodium thiobutabarbital (Inactin, 100 mg/kg) and placed on a heated surgical tray that maintained the rectal temperature between 37 and 38°C. After tracheotomy, cannulas were placed in the right femoral vein for infusion of physiological saline containing 1% inulin for estimation of glomerular filtration rate and in the right femoral artery for measurements of mean arterial blood pressure and heart rate, using a pressure transducer (Nihon Koden, AP601G, Tokyo, Japan). The left carotid artery was also cannulated for blood sampling. After making an abdominal midline incision, the left kidney was exposed, and the renal artery was carefully stripped of connective tissues, followed by the application of 5% phenol in 70% ethanol to exclude the influence of renal sympathetic nerves. An electromagnetic flow probe (1.0 mm in diameter, Nihon Koden) connected to a square-wave flowmeter (Nihon Koden, MFV-2100) was positioned on the left renal artery to measure renal blood flow. A curved 30-gauge needle connected to polyethylene tubing was inserted into the left renal artery proximal to the flow probe for infusion of drug solution or saline, at a rate of 0.04 ml/min. Finally, the left ureter was cannulated for collection of timed urine samples. Following termination of surgical procedures, about 2 ml of inulin solution was slowly infused to supplement the loss of body fluid and as a priming dose of inulin (about 70 mg/kg), followed by a sustaining infusion of the solution at a rate of 0.03 ml/min. Mean arterial blood pressure, heart rate and renal blood flow were recorded continuously on a polygraph (Nihon Koden, RM6000G), throughout all the experiments. A 60–90 min period was allowed for stabilization of blood pressure, renal blood flow and urine flow.

After the equilibration period, urine samples were collected during two 15-min control clearance periods. Following the control periods, FK409 (3, 10 μg/kg per min) was infused into the renal artery for 20 min. No urine was collected during the first 5 min after start of the infusion, to allow for the dead space in the collection system. Urine samples were then collected during 15-min clearance periods (experimental period). Blood samples (0.25 ml) were obtained at the midpoint of the control periods and at the end of the experimental period. The blood loss was replaced by giving an equal volume of blood from donor rats. After measuring the systemic arterial hematocrit by the microcapillary method, plasma was immediately separated by centrifugation. Results in the second control period served as the control values of renal hemodynamic and excretory responses.

Hemodynamic parameters such as mean arterial blood pressure, heart rate, renal blood flow and renal vascular resistance were determined at the midpoint in each period, i.e., at 12.5 min after the drug infusion, in the experimental period, because hemodynamic effects of FK409 occurred gradually after the infusion, and reached a plateau at about 10 min.

In some rats, time-control experiments were done, and here, physiological saline instead of FK409 was infused. Levels of blood pressure, renal hemodynamics and urine formation were constant throughout all clearance periods.

2.3. Analytical procedure

Urine samples were collected in preweighed tubes and urine volumes were determined gravimetrically. Glomerular filtration rate was estimated from inulin clearance. Urine and plasma inulin levels were measured by spectrofluorometry (Hitachi, 650-60), according to the method of Vurek and Pegram (1966). Urinary sodium concentration was determined, using a flame photometer. The level of NO metabolites (NO_x) in urine was measured using an autoanalyser (Tokyo Kasei Kogyo, TCI-NOX 1000, Tokyo, Japan). Briefly, urine samples were diluted with de-ionized water (1:50), the sample was mixed with carrier solution (0.07% ethylenediaminetetraacetic acid and 0.3% NH_4Cl) and passed through the cadmium reduction column to reduce from NO_3^- to NO_2^-, which reacts with Griess reagent (1% sulfonamide, 0.1% *N*-1-naphthylethylenediamine dihydrochloride, 5% HCl). Absorbance at 540 nm was measured using a flow-through visible spectrophotometer (Tokyo Kasei Kogyo, S-3250). NO_2^- was used as standard.

Renal vascular resistance (RVR, mmHg/ml per g per min) was calculated from the formula RVR = MAP/RBF, where MAP is mean arterial blood pressure (mmHg), RBF is renal blood flow (ml/g per min). Filtration fraction (FF, %) was calculated from the formula FF = GFR/[RBF × (1

$-$ Hct)] $\times$ 100, where GFR is glomerular filtration rate (ml/g per min), Hct is hematocrit. Fractional excretion of sodium (FE_{Na}, %) was calculated from the formula $FE_{Na} = U_{Na}V/(P_{Na} \times GFR) \times 100$, where $U_{Na}V$ is urinary excretion of sodium (μEq/g per min), P_{Na} is the plasma sodium concentration (mEq/l).

2.4. Drugs

FK409, a kind gift from Fujisawa Pharmaceutical (Osaka, Japan), was dissolved in saline (0.9%) just prior to administration. Other drugs were purchased from Nacalai Tesque (Kyoto, Japan).

2.5. Statistical analysis

Data are expressed as mean $\pm$ S.E.M. For statistical analysis, we used the paired Student's *t*-test for comparison of values between the control and experimental periods, in the same group. For comparison of values between normal and NOARG-treated groups, the unpaired Student's *t*-test was used. Differences were considered significant at $P < 0.05$.

3. Results

3.1. Effects of intrarenal arterial infusion of FK409 on renal hemodynamic and excretory responses in normal rats

When the intrarenal arterial infusion of FK409 was given to normal rats at a dose of 3 μg/kg per min, there was a slight decrease in mean arterial blood pressure (5–10 mmHg). Although a slight increase in renal blood flow (4–8% compared with the control) was also observed, glomerular filtration rate and urine formation remained unchanged during the drug infusion.

Changes in systemic and renal hemodynamics in normal rats in response to FK409 at a dose of 10 μg/kg per min are summarized in Table 1. Mean arterial blood pressure significantly decreased during the infusion of FK409. Simultaneously, significant increase in renal blood flow and decrease in renal vascular resistance were observed by the treatment with FK409. Glomerular filtration rate remained at the basal level, with a tendency to decrease in the filtration fraction. As shown in Fig. 1, urine flow, urinary excretion of sodium and fractional excretion of sodium increased by about 85%, 110% and 75% from each control value of 9.92 ± 1.87 μl/g per min, 1.90 ± 0.56 μEq/g per min and 0.90 ± 0.34%, respectively. Urinary excretion of NO_x (UNO_xV) was also increased with the administration of FK409 (Table 2).

3.2. Effects of intrarenal arterial infusion of FK409 on renal hemodynamic and excretory responses in NOARG-treated hypertensive rats

Animals given NOARG for 7 days showed evidence of hypertension and potent renal vasoconstriction. As shown in Table 1, basal values of mean arterial blood pressure and renal vascular resistance were significantly increased, and that of renal blood flow was markedly decreased, compared with values for normal rats. In addition, basal levels of urine formation tended to decrease (Fig. 1). When FK409 was administered to NOARG-treated rats, about 20% decrease in mean arterial blood pressure was observed. Heart rate slightly but significantly increased. The administration of FK409 to the NOARG-treated rats produced a potent renal vasodilation. Observed changes in renal blood flow and renal vascular resistance were significantly greater than those seen with normal rats. A signifi-

Table 1
Effects of intrarenal artery infusion FK409 on systemic and renal hemodynamics in normal rats and NOARG-treated hypertensive rats

	MAP (mmHg)	HR (beats/min)	RBF (ml/g per min)	RVR (mmHg/ml per g per min)	GFR (ml/g per min)	FF (%)
Normal rats						
Control	131 ± 2	380 ± 13	8.92 ± 0.86	16.0 ± 2.0	1.81 ± 0.23	38.3 ± 3.6
FK409	113 ± 3 [b]	390 ± 11	10.20 ± 1.01 [b]	12.0 ± 1.5 [b]	1.87 ± 0.23	33.6 ± 2.4
% Change	-14.3 ± 2.2	3.1 ± 2.3	14.7 ± 3.7	-24.7 ± 2.5	4.9 ± 2.5	10.4 ± 3.9
NOARG-treated hypertensive rats						
Control	166 ± 3 [d]	393 ± 9	6.44 ± 0.53 [c]	26.7 ± 2.0 [d]	1.22 ± 0.12	38.6 ± 3.0
FK409	135 ± 3 [b]	420 ± 5 [a]	8.68 ± 0.57 [b]	15.9 ± 1.1 [b]	1.55 ± 0.09 [a]	34.6 ± 2.4
% Change	-19.4 ± 2.6	7.3 ± 2.7	36.1 ± 3.4 [d]	-40.2 ± 1.6 [d]	31.6 ± 10.1 [d]	-7.2 ± 7.4

Each value represents the mean $\pm$ S.E.M. (normal, $n = 7$; NOARG, $n = 8$). [a] $P < 0.05$; [b] $P < 0.01$, compared with each control value. [c] $P < 0.05$; [d] $P < 0.01$, compared with the value of normal rats. MAP, mean arterial blood pressure; HR, heart rate; RBF, renal blood flow; RVR, renal vascular resistance; GFR, glomerular filtration rate; FF, filtration fraction.

Table 2
Effects of the intrarenal arterial infusion of FK409 on urinary excretion of NO metabolites (UNO_xV) in normal rats and NOARG-treated hypertensive rats

	UNO_xV (nmol/g per min)	
	Normal rats	NOARG-treated hypertensive rats
Control	4.17 ± 0.49	1.27 ± 0.14 [b]
FK409	6.61 ± 0.62 [a]	3.14 ± 0.23 [a]

Each value represent the mean ± S.E.M. (normal, $n = 7$; NOARG, $n = 8$). [a] $P < 0.01$, compared with each control value. [b] $P < 0.01$, compared with the value of normal rats.

cant increase in glomerular filtration rate was also evident when FK409 was infused (Table 1). As shown in Fig. 1, the administration of FK409 produced potent diuretic and natriuretic effects. Urine flow, urinary excretion of sodium and fractional excretion of sodium increased to about 4-fold, 7-fold and 5-fold over each control value of 6.85 ± 0.61 μl/g per min, 0.71 ± 0.11 μEq/g per min and 0.42 ± 0.08%, respectively (Fig. 1). Although the basal level of UNO_xV in NOARG-treated rats was much lower than that in normal rats, absolute changes in UNO_xV in response to FK409 did not differ between the two groups (Table 2).

4. Discussion

Accumulating evidence suggests that endogenous NO has a major role in regulating blood pressure, by controlling vascular tone (Moncada et al., 1991a). Recent studies have also indicated the physiological importance of NO in the regulation of renal hemodynamics and function, as based on findings indicating that intrarenal arterial infusion of NO synthase inhibitors produces renal vasoconstriction (Egi et al., 1994; Lahera et al., 1991; Majid et al., 1993a; Tolins et al., 1990). These agents are also reported to impair the autoregulation of renal blood flow in anesthetized dogs (Kiyomoto et al., 1992). In addition, several studies on anesthetized animals have demonstrated that the administration of NO synthase inhibitors diminishes urine formation (Lahera et al., 1991; Majid et al., 1993b; Tolins et al., 1990). These agents inhibit the pressure-induced natriuretic response in anesthetized dogs (Salom et al., 1992; Majid et al., 1993b). Alberola et al. (1992) noted that a NO synthase inhibitor given in a dose that does not affect baseline renal functions, increases proximal lithium reabsorption during saline volume expansion in anesthetized dogs. Thus, endogenous NO appears to tonically participate in the regulation of renal function, at renal vascular and at tubular levels.

Most of findings described above are derived from studies using NO synthase inhibitors such as NOARG and N^G-nitro-L-arginine methyl ester (L-NAME). In the present study, a novel NO donor, FK409, which spontaneously releases NO following decomposition of the compound (Kita et al., 1994a), was intrarenally administered to evaluate the direct effect of NO on renal hemodynamics and urine formation in anesthetized rats. Intrarenal arterial infusion of FK409 to normal rats produced a renal vasodi-

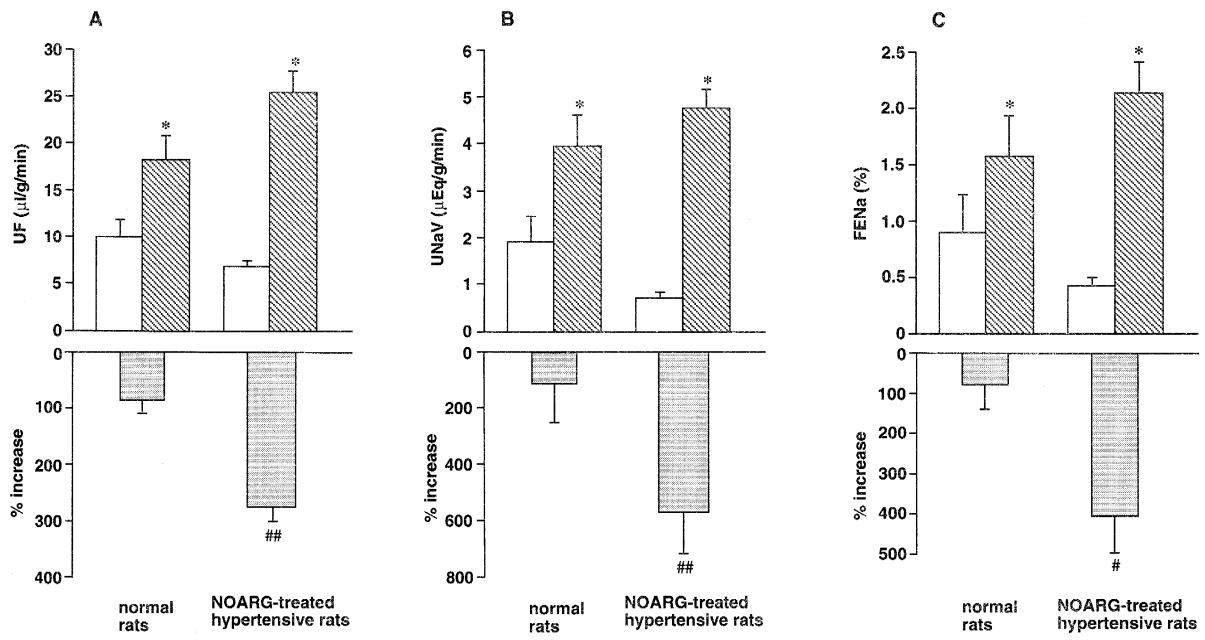

Fig. 1. Effects of intrarenal arterial infusion of FK409 on urine flow (UF, A), urinary excretion of sodium ($U_{Na}V$, B) and fractional excretion of sodium (FE_{Na}, C) in normal rats and NOARG-treated hypertensive rats. Each value represents the mean ± S.E.M. * $P < 0.01$, compared with each control value. # $P < 0.05$, ## $P < 0.01$, compared with the value of normal rats. Open column, control; hatched column, FK409 infusion; shaded column, percent increase from control.

lation. The agent elicited significant diuretic and natriuretic responses, without affecting glomerular filtration rate. In addition, the FK409-induced renal actions were accompanied by an increase in UNO_xV. These results using a novel NO donor strongly support the functional role of NO as a regulatory factor in renal vascular tonus and tubular reabsorption.

In normal rats, the intrarenal arterial infusion of FK409 significantly increased renal blood flow, with no alternation in glomerular filtration rate, thereby suggesting that renal vasodilation occurred predominantly in efferent arterioles during infusion of the compound. On the other hand, we and others have found that intrarenal arterial infusion of NOARG reduced renal blood flow without affecting glomerular filtration rate, as accompanied by an increase in filtration fraction (Baylis et al., 1993; Egi et al., 1994, 1995). Taken together, it seems that tonic release of endogenous NO probably occurs preferentially in the efferent arterioles to act as a renal vasodilator and that postglomerular sites are relatively sensitive to exogenously applied NO.

In the present study, the renal effects of FK409 were also investigated using NOARG-treated hypertensive rats in which endogenous NO production is impaired. The rats treated with NOARG for 7 days showed hypertension and potent renal vasoconstriction, as described previously (Bank et al., 1994; Dananberg et al., 1993; Fujita et al., 1995). In addition, there was a marked decrease in UNO_xV and a tendency toward a decrease in basal urine formation. Intrarenal arterial infusion of FK409 in a dose of 10 μg/kg per min to NOARG-treated rats markedly increased renal blood flow and decreased renal vascular resistance. In contrast to the case of normal rats, the agent caused significant increases in glomerular filtration rate. FK409 also caused remarkable diuretic and natriuretic actions in these rats. These renal actions, in particular excretory responses, were extremely potent compared with cases seen with normal rats, although the amount of increment of UNO_xV was comparable to that in normal rats. Our results are qualitatively in agreement with findings of Moncada et al. (1991b), who demonstrated that treatment with NO synthase inhibitors enhanced vascular responses to glyceryl trinitrate and sodium nitroprusside, in vivo and in vitro. They suggested that 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 (Moncada et al., 1991b). 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. The possible involvement of NOARG-induced hypertension per se can be excluded in the above augmentation. When the same dose of FK409 was administered into the renal artery of deoxycorticosterone acetate (DOCA)-salt-induced hypertensive rats, renal vasodilatory and excretory responses to the compound were slightly less potent, compared with those seen in normal rats (unpublished observation).

Several studies have indicated that acute or chronic inhibition of NO synthesis enhances renal vasoconstrictor responses to vasoactive substances (Hajj-Ali et al., 1994; Ito et al., 1991; Matsumura et al., 1995; Yoshida et al., 1996). Pollock et al. (1993) noted that hypertension caused by chronic inhibition of NO synthase was prevented by pretreatment with an angiotensin II receptor antagonist. Thus, long-term inhibition of NO synthase may induce changes in vascular sensitivity to constrictor agents. Based on the view that the interaction between NO and several vasoconstrictor substances is an important regulatory factor of renal vascular tonus (Lücher et al., 1991), we cannot rule out the possibility that an increased basal tone of renal vasculature by enhancement of responses to some vasoconstrictor substance may amplify the vasodilatory activity of FK409, in NOARG-treated rats.

In some disease states, including hypertension and hyperlipidemia, endothelium-derived vasorelaxation is attenuated (Hayakawa et al., 1993; Girerd et al., 1990). Such impaired vasorelaxation has been mostly attributed to an attenuated endothelium-derived NO formation. Regardless of the mechanisms, our results in NOARG-treated rats suggest that FK409 has merit as a pharmacological tool to ameliorate the diminished renal function, under pathological conditions in which endogenous NO production is impaired.

In conclusion, our results clearly indicate that FK409 causes renal vasodilation and diuresis, probably via NO formation. Renal hemodynamic and excretory responses to the agent are sensitive, under NO-depleted conditions. FK409 and related compounds may also be useful in treatment of subjects with renal diseases, in which basal NO formation is impaired.

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