Original Article

Role of Nitric Oxide in Regional Blood Flow in Angiotensin II-Induced Hypertensive Rats

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The present study was designed to evaluate the contribution of nitric oxide (NO) to regional hemodynamics during the early phase of angiotensin II (Ang II)-induced hypertension. The responses of regional blood flow to chronic NO synthase inhibition with *N*^G-nitro-L-arginine methyl ester (L-NAME) were assessed using radioactive microspheres in conscious Ang II-infused hypertensive rats. Ang II-infused rats (270 ng/kg/min, subcutaneously for 12 days; *n*=11) showed higher mean arterial pressure (MAP: 153 ± 4 mmHg) and total peripheral resistance (TPR: 1.61 ± 0.06 mmHg/min/ml), and lower cardiac output (CO: 102 ± 3 ml/min) than vehicle-infused normotensive rats (115 ± 2 mmHg, 0.96 ± 0.05 mmHg/min/ml and 130 ± 7 ml/min, *n*=11, respectively). The blood flow rates in the brain, spleen, large intestine and skin were significantly reduced in Ang II-infused rats compared with vehicle-infused rats, while those in the lung, heart, liver, kidney, adrenal gland, small intestine, and skeletal muscle were similar. Treating Ang II-infused rats with L-NAME (75 mg/l in drinking water for 10 days, *n*=11) resulted in higher MAP (166 ± 6 mmHg) and TPR (1.89 ± 0.18 mmHg/min/ml) and lower CO (87 ± 7 ml/min) than untreated Ang II-infused rats. L-NAME-treated Ang II-infused rats showed widespread increases in regional vascular resistance and reduced blood flow rates in the kidney (3.81 ± 0.27 ml/min/g) and skeletal muscle (0.20 ± 0.03 ml/min/g) compared with untreated Ang II-infused rats (6.88 ± 0.27 and 0.33 ± 0.04 ml/min/g, respectively). However, there were no significant differences in the flow rates of other organs investigated between these animals. An NO donor, ($\pm$)-(E)-4-ethyl-2-[(E)-hydroxyimino]-5-nitro-3-hexenamide (FK409; $30 \mu\text{g/kg/min}$, i.v.), significantly decreased MAP (110 ± 6 mmHg) and TPR (1.23 ± 0.18 mmHg/min/ml) without significant changes in CO (89 ± 9 ml/min) in L-NAME-treated Ang II-infused rats. Furthermore, FK409 partially reversed blood flow rates in the kidney (4.72 ± 0.40 ml/min/g) and skeletal muscle (0.25 ± 0.02 ml/min/g) in these animals. These results suggest that NO counteracts, at least in part, the vasoconstrictor effects of elevated Ang II levels in renal and skeletal muscle vascular beds, and is an important modulator in the regulation of blood flow to these organs during the development of Ang II-induced hypertension. (Hypertens Res 2001; 24: 421–427)

Key Words: angiotensin II, nitric oxide, hypertension, regional blood flow, ($\pm$)-(E)-4-ethyl-2-[(E)-hydroxyimino]-5-nitro-3-hexenamide (FK409)

Introduction

Angiotensin II (Ang II) exerts a powerful hypertensinogenic

influence when its level of activity is not appropriate for existing physiological status (1–3). Chronic administration of suppressor doses of Ang II infusions into uninephrectomized rats leads to a slowly developing hypertension which resem-

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bles two-kidney, one-clip (2K1C) Goldblatt renovascular hypertension. In these models of animals, plasma and tissue Ang II levels are increased (1–11) and renal vascular reactivity to Ang II is enhanced (6, 8). Nevertheless, in the kidneys of chronic Ang II-infused hypertensive rats, basal renal function and microcirculatory status are either not reduced or not reduced as much as would be expected from the elevated intrarenal Ang II levels (4, 9, 12). Many studies have suggested that during the development of Ang II-induced hypertension, renal vasoconstriction elicited by elevated Ang II levels is partially counteracted by enhanced activity of nitric oxide (NO) (1, 4, 5, 7, 9, 12). Deng *et al.* (12) reported that urinary excretion of nitrates and nitrites is maintained in Ang II-infused hypertensive rats. Using a blood-perfused juxtamedullary nephron preparation, Ichihara *et al.* (7) demonstrated that NO inhibition enhances afferent arteriolar reactivity to Ang II in this animal model. Studies at the whole kidney level showed that NO synthesis inhibition-induced reduction in renal blood flow is significantly greater in chronically Ang II-infused rats than in normotensive rats (4). Recently, studies using Western blot analysis have demonstrated a significant elevation in endothelial NO synthase levels in the renal cortex of chronically Ang II-infused hypertensive rats (5).

Similar regulatory interactions between NO and Ang II have been demonstrated in other vascular beds (13, 14). Gruetter *et al.* (13) reported that hemoglobin or methylene blue enhances Ang II-induced contraction in isolated rat aorta and bovine coronary arteries. Studies using the *in vivo* microsphere method have shown that blocking Ang II AT₁ receptors by losartan attenuates the visceral hemodynamic responses to acute inhibition of NO synthase with *N*^G-nitro-L-arginine methyl ester (L-NAME) in normotensive rats (14). Thus, these observations suggest that NO contributes to maintaining regional hemodynamics in several organs, not only as a potent local vasodilator but also by virtue of its interaction with local vascular effects of Ang II.

In Ang II-dependent hypertension, increased local NO activity could be caused by the progressive increases in arterial pressure causing a greater endothelial shear stress, which is a potent stimulus for endothelial-derived NO synthesis and release (5, 9, 15), or could be due to direct actions mediated by sustained stimulation of Ang II receptors (9, 16, 17). Consequently, increased NO formation may partially counteract the chronic vasoconstrictor influence of elevated Ang II levels to allow maintenance of blood flow in several vascular beds. Therefore, the present study was designed to evaluate the influence of NO on regional hemodynamics during the development of Ang II-induced hypertension. Accordingly, the effects of chronic inhibition of NO synthase with L-NAME on systemic and regional hemodynamics, as measured using a radioactive microsphere method (10, 18), were examined in Ang II-infused hypertensive rats. We also examined the effects of an NO donor, (±)-(E)-4-ethyl-2-[(E)-hydroxyimino]-5-nitro-3-hexenamide (FK409) (19), on sys-

temic and regional hemodynamics in L-NAME-treated Ang II-infused hypertensive rats.

Materials and Methods

Animal Preparation

Male Sprague-Dawley rats were housed in separate cages and maintained in a temperature-controlled room under a 12-h light/dark cycle. Throughout the experiments, animals had free access to standard rat chow and prepared water. All surgical and experimental procedures were performed according to the guidelines for the care and use of animals as established by Kagawa Medical University.

Rats weighing 280 to 305 g at the beginning of the experiments were randomly divided into three groups: a group of rats receiving vehicle (5% acetic acid) infusion (vehicle rats, *n* = 11); a group receiving Ang II infusion (Ang II rats, *n* = 11); and a group receiving Ang II infusion and L-NAME treatment (Sigma Chemical Co., St. Louis, USA) (Ang II/L-NAME rats, *n* = 8). Ang II (Sigma Chemical Co.) was infused for 12 days at a rate of 270 ng/kg/min *via* an osmotic minipump (model 2002; Alza Co., Palo Alto, USA) implanted subcutaneously at the dorsum of the neck under sodium pentobarbital anesthesia (50 mg/kg, *i.p.*), as previously reported (4–8, 10). For Ang II/L-NAME rats, L-NAME administration (75 mg/l in drinking water) was started from 2 days after the implantation of an osmotic minipump. The dose of L-NAME was chosen based on the results of previous *in vivo* microsphere studies (20). L-NAME solution was replaced every 24 h. Vehicle rats and Ang II rats received only tap water. Mean blood pressure (MBP) was measured in conscious rats by tail-cuff plethysmography to monitor the progression of hypertension.

Measurements of Systemic and Regional Hemodynamics

The following surgical procedures were performed 11 days after the implantation of an osmotic minipump. Under sodium pentobarbital anesthesia (50 mg/kg, *i.p.*), polyethylene catheters (PE-50) were placed in the femoral artery for the sampling of reference blood and direct measurement of mean arterial pressure (MAP). Another catheter was also placed in the left ventricle *via* the right carotid artery for the injection of microspheres. These catheters were filled with heparinized saline (100 IU/ml) and exteriorized through a cutaneous tunnel at the back of the neck after confirmation of their tip locations by pressure tracings. Animals were allowed to recover for 24 h before initiation of the experimental procedures. The catheterized rats were placed in a small plastic chamber. The femoral arterial catheter was connected to the pressure transducer, and MAP was continuously recorded on a multichannel polygraph (Nihondenki-Sanei, Tokyo, Japan). Radioactive microspheres were used to meas-

Table 1. Systemic Hemodynamics at the Time of Injection of Microspheres

	Vehicle-infused rats	Ang II-infused rats	Ang II-infused rats treated with L-NAME
Number of rats	11	11	8
Body weight (g)	338±6	318±7*	300±5*,†
Mean arterial pressure (mmHg)	115±2	153±4**	166±6**,†
Cardiac output (ml/min)	130±7	102±3*	87±7**,†
Total peripheral resistance (mmHg/min/ml)	0.96±0.05	1.61±0.06**	1.89±0.18**,†

All values are means±SE. * $p < 0.05$, ** $p < 0.01$ vs. control rats. † $p < 0.05$, vs. Ang II-infused rats. Ang II, angiotensin II; L-NAME, *N*^G-nitro-L-arginine methyl ester.

ure the cardiac output (CO) and regional blood flow, as previously reported (10, 18). Briefly, radionuclide-labelled microspheres (^{141}Ce ; New England Nuclear, Boston, USA) $15 \pm 3 \mu\text{m}$ in diameter, were used. Following a 60-min stabilization period during which rats adjusted to the chamber, 0.25 ml of saline solution containing 75,000 microspheres was injected into the left ventricle. The injection was performed over a 15-s period. Arterial blood samples for reference blood were obtained using a withdrawal pump at a rate of 0.55 ml/min starting immediately before the injection of the microspheres and ending 60 s later. In Ang II/L-NAME rats, systemic and regional hemodynamic responses to FK409 (Fujisawa Pharmaceutical Co., Tokyo, Japan) were also examined. Fifteen minutes after the first injection of microspheres (^{141}Ce), FK409 was infused intravenously at a rate of $30 \mu\text{g/kg/min}$ for 10 min from a catheter placed in the femoral vein. Subsequently, a second injection of microspheres (^{85}Sr ; New England Nuclear) was performed as described above. Preliminary experiments showed that FK409 at a rate of $30 \mu\text{g/kg/min}$ decreased MAP with a maximum reduction at 10 min after the start of infusion.

After termination of the injection of microspheres, the animals were euthanized *via* an excess dose of sodium pentobarbital. The brain, lungs, heart, liver, spleen, kidneys, adrenal glands, intestines (small and large), hindlimb skeletal muscle and skin were then removed and weighed. Due to the relatively low blood flow to the skeletal muscle and skin, at least 5 g of these tissues were removed. Skeletal muscle and skin samples contained 300–400 microspheres from each microsphere injection and all other tissues contained over 500 microspheres. The activities of each batch of microspheres in stock solution, reference blood and tissue samples were analyzed using a gamma scintillation counter.

The CO was calculated as previously reported (10, 18). Briefly, $\text{CO (ml/min)} = [\text{injected isotope counts (cpm)} / \text{reference blood count (cpm)}] \times 0.65 \text{ (ml/min)}$. The fraction of CO to each organ was calculated from the ratio of radioactivity of each organ to total injected radioactivity. Total injected radioactivity was obtained by subtracting the residual radioactivity from the radioactivity before injection. Absolute organ flow was calculated as follows: organ blood

flow (ml/min) = [organ isotope counts (cpm) / reference blood counts (cpm)] $\times 0.65 \text{ (ml/min)}$. The organ blood flow is expressed as ml/g of organ/min.

Statistical Analysis

All values are expressed as the means±SE. For each variable, simultaneous multiple comparisons of group means were made with the use of analysis of variance and Fisher's PLSD test. Statistical comparisons of the differences in the responses were performed using analysis of variance followed by Newman-keuls test. Values of $p < 0.05$ were considered to indicate statistical significance.

Results

Systemic Hemodynamics

At the start of the study, body weights were similar among the three groups (vehicle rats, $305 \pm 8 \text{ g}$; Ang II rats, $303 \pm 5 \text{ g}$; Ang II/L-NAME rats, $293 \pm 6 \text{ g}$). MBP values measured by tail-cuff plethysmography at the initiation of the study were also similar among the three groups (data not shown). On days 5 and 10 of Ang II or vehicle infusion, MBP as measured by tail-cuff plethysmography was significantly elevated to 146 ± 6 and $168 \pm 11 \text{ mmHg}$ in Ang II rats compared with vehicle rats (104 ± 4 and $108 \pm 4 \text{ mmHg}$, $p < 0.05$, respectively). Ang II/L-NAME rats on days 5 ($174 \pm 10 \text{ mmHg}$, $p < 0.05$) and 10 ($189 \pm 11 \text{ mmHg}$, $p < 0.05$) of Ang II infusion showed significantly higher MBPs than untreated Ang II rats ($p < 0.05$, respectively).

The systemic parameters for the three groups of rats used in the microsphere experiments are presented in Table 1. Consistent with a previous report (21), average body weight in Ang II rats was lower than that in vehicle rats ($p < 0.05$). Furthermore, Ang II/L-NAME rats showed lower body weight than Ang II rats ($p < 0.05$). Direct measurement of MAP *via* the femoral artery gave results similar to those for MBP measured using tail-cuff plethysmography. Ang II infusion for 12 days significantly increased MAP ($p < 0.01$) and TPR ($p < 0.01$), but reduced CO ($p < 0.05$). Ang II/L-

Fig. 1. Regional blood flow in vehicle-infused normotensive rats (vehicle rats, $n=11$), angiotensin II (Ang II)-infused hypertensive rats (Ang II rats, $n=11$), and Ang II-infused hypertensive rats treated with N^G -nitro-L-arginine methyl ester (Ang II/L-NAME rats, $n=8$). * $p<0.05$ vs. vehicle-infused rats. † $p<0.05$, †† $p<0.01$ vs. Ang II rats.

Fig. 2. Regional vascular resistance in vehicle-infused normotensive rats (vehicle rats, $n=11$), angiotensin II (Ang II)-infused hypertensive rats (Ang II rats, $n=11$), and Ang II-infused hypertensive rats treated with N^G -nitro-L-arginine methyl ester (Ang II/L-NAME rats, $n=8$). * $p<0.05$ vs. vehicle-infused rats. † $p<0.05$, †† $p<0.01$ vs. Ang II rats.

Table 2. The Percent Distribution of Cardiac Output to Each Organ in Vehicle-Infused Rats, Angiotensin II (Ang II)-Infused Rats, and Ang II-Infused Rats Treated with N^G -Nitro-L-Arginine Methyl Ester (L-NAME)

	Vehicle-infused rats	Ang II-infused rats	Ang II-infused rats treated with L-NAME
Brain	1.54±0.11	1.08±0.12*	1.17±0.12*
Lung	0.91±0.32	1.00±0.17	0.74±0.10
Heart	5.55±0.40	6.67±0.67	7.43±0.68
Liver	15.2±1.1	15.1±1.2	17.1±1.3
Spleen	1.36±0.12	0.58±0.07**	0.59±0.11**
Kidney	15.7±0.6	15.4±0.8	9.35±0.34**††
Adrenal gland	0.21±0.02	0.23±0.02	0.27±0.04
Small intestine	5.49±0.37	5.90±0.71	6.24±0.79
Large intestine	3.34±0.24	3.13±0.24*	2.95±0.30
Skeletal Muscle	0.65±0.09	0.69±0.07	0.56±0.04*†
Skin	0.61±0.08	0.35±0.05*	0.45±0.06*

All values are means±SE in %. * $p<0.05$, ** $p<0.01$ vs. control rats. † $p<0.05$, †† $p<0.01$ vs. Ang II-infused rats.

NAME rats showed further elevated MAP ($p<0.05$) and TPR ($p<0.05$) levels, and reduced CO ($p<0.05$) compared with untreated Ang II rats.

Regional Hemodynamics

In agreement with previous studies (10), the blood flow rates in the brain, spleen, large intestine and skin were significantly reduced in Ang II rats compared with vehicle rats ($p<0.05$, respectively, Fig. 1). In contrast, there were no statistically significant changes in the flow of the lung, heart, liver, kidney, adrenal gland, small intestine or skeletal muscle between vehicle and Ang II rats (Fig. 1). Ang II rats showed widespread increases in organ vascular resistance including the

brain, heart, liver, spleen, kidney, adrenal gland, intestine (small and large), skeletal muscle and skin ($p<0.05$, respectively) compared with vehicle-infused rats, whereas there were no statistically significant changes in vascular resistance of the lung (Fig. 2). Ang II/L-NAME rats showed markedly reduced blood flow rates in the kidney ($3.81±0.27$ mL/min/g, $p<0.01$) and skeletal muscle ($0.20±0.03$ mL/min/g, $p<0.05$) compared with untreated Ang II rats ($6.88±0.27$ and $0.33±0.04$ mL/min/g, respectively); however, there were no significant differences in the flow rates of other organs between these animals (Fig. 1). Vascular resistance of the kidney ($45.4±4.5$ mmHg/mL/min/g) and skeletal muscle ($1,324±131$ mmHg/mL/min/g) in Ang II/L-NAME rats were significantly elevated compared with those in untreated Ang II rats

Fig. 3. A: Effects of FK409 (30 µg/kg/min, i.v.) on regional blood flow in angiotensin II (Ang II)-infused hypertensive rats treated with N^G-nitro-L-arginine methyl ester (AngII/L-NAME rats, n=8). Data are expressed as percent changes of the control values. *p<0.05 vs. controls. B: Effects of FK409 (30 µg/kg/min, i.v.) on regional vascular resistance in Ang II/L-NAME rats (n=8). Data expressed as percent changes of the control values. *p<0.05 vs. controls.

(24.5 ± 1.4 and 892 ± 108 mmHg/ml/min/g, $p < 0.01$ and $p < 0.05$, respectively) (Fig. 2). The average percent distributions of CO to each organ are presented in Table 2. The percent distribution of CO to the brain ($p < 0.05$), spleen ($p < 0.01$) and skin ($p < 0.05$) were significantly reduced in Ang II rats compared with vehicle rats. In contrast, there were no significant differences in the percent distribution of CO to the lung, heart, liver, kidney, adrenal gland, intestine (small and large) or skeletal muscle between vehicle and Ang II rats. L-NAME treatment did not alter the percent distribution of CO to the brain, lung, heart, liver, spleen, adrenal gland, intestine (small and large) or skin, but significantly decreased the percent distribution to the kidney and skeletal muscle ($p < 0.01$ and $p < 0.05$, respectively) in Ang II rats (Table 2).

Effects of FK409 on the Systemic and Regional Hemodynamics in AngII/L-NAME Rats

Intravenous infusion of FK409 at a rate of 30 µg/kg/min significantly decreased MAP by 34 ± 3% from 166 ± 6 to 110 ± 6

mmHg and TPR by 36 ± 5% from 1.89 ± 0.17 to 1.23 ± 0.18 mmHg/min/g without significant changes in CO (89 ± 9 ml/min) in Ang II/L-NAME rats. Figure 3 shows the effects of FK409 on regional hemodynamics in Ang II/L-NAME rats. During FK 409 infusion, the blood flow rates in the brain, heart, liver, kidney, adrenal gland, intestine (small and large), skeletal muscle, and skin were significantly increased, whereas the blood flow in the lung was not changed and flow in the spleen was significantly decreased by 42 ± 8% (Fig. 3A). On the basis of group comparisons, however, the blood flow rates in the spleen, kidney and skeletal muscle in FK409-treated Ang II/L-NAME rats (0.70 ± 0.12, 4.72 ± 0.40, and 0.25 ± 0.02 ml/min/g, respectively) were significantly lower than those in untreated Ang II rats (1.42 ± 0.15, 6.88 ± 0.27, and 0.33 ± 0.04 ml/min/g, respectively). In contrast, the blood flow rates in the brain and hearts in FK409-treated Ang II/L-NAME rats (1.06 ± 0.05 and 8.48 ± 0.26 ml/min/g, respectively) were significantly higher than those in untreated Ang II rats (0.81 ± 0.04 and 6.95 ± 0.31 ml/min/g, respectively). During FK409 infusion, vascular resistances were significantly decreased in every organ and tissue studied except the spleen, in which the resistance was significantly increased by 24 ± 8% in Ang II/L-NAME rats. The magnitude of the reduction in the regional resistance was not uniform among the organs and tissues studied, but ranged from a maximum of 54% in the skin to 26% in the lung (Fig. 3B).

Discussion

Chronic infusion of subpressor doses of Ang II over the course of 12 days elicited a degree of hypertension similar to that reported previously (4–8, 10). As compared with vehicle-infused rats, Ang II-infused hypertensive rats showed a marked elevation of TPR and a reduction in CO. In agreement with previous studies (10), the blood flow rates in the brain, spleen, large intestine and skin of Ang II-infused hypertensive rats were significantly reduced compared with those in vehicle-infused normotensive animals. In contrast, the flow rates of the lung, heart, liver, kidney, adrenal gland, small intestine, and skeletal muscle were maintained. These results indicate that the regional hemodynamic responses to chronically elevated Ang II levels were not uniform among the organs studied. While the mechanisms responsible for the heterogeneity of regional hemodynamics in Ang II-infused hypertensive rats are multifarious as described previously (10), at least one important mechanism of these differences may be regional heterogeneity of the formation (or sensitivity) of local NO.

In the present study, Ang II/L-NAME rats showed markedly reduced CO as well as markedly reduced blood flow rates in the kidney and skeletal muscle compared with Ang II rats, whereas there were no significant differences in the flow rates of other organs and tissues between these animals. Thus, the finding that not all regional flows decreased commensurately with the fall in CO provides evidence for a

differential modulatory influence of NO on regional blood flow during the development of Ang II-induced hypertension. Possible explanations for these regional variations in response to chronic L-NAME administration include regional heterogeneity of NO synthase activity (22, 23), variability in the pharmacokinetics of L-NAME (24), differing characteristics of shear stress stimuli (25), and variations in the Ang II-induced NO release (14). Sigmon and Beierwaltes (14) found that losartan caused the greatest blunting of L-NAME-induced response in renal circulation among the organs they investigated. They also observed that renal hemodynamic responses to L-NAME were greatly diminished in conscious losartan-treated animals (14). Based on these findings, they hypothesized that in anesthetized animals, the increased interaction between Ang II and NO in renal hemodynamics is related to anesthetic-induced increase in the circulating Ang II levels. In support of this hypothesis, earlier studies performed in conscious rats with chronically elevated Ang II by dietary sodium restriction demonstrated that the interaction between Ang II and NO was significantly enhanced and influenced the renal hemodynamics in these animals (26). Similarly, other studies have shown that the reduction in renal blood flow induced by NO synthase inhibition was significantly greater in Ang II-infused rats (4) or transgenic hypertensive rat strain TGR(mRen2) rats (27) than in normotensive animals. In the present study, we found that the kidney responded to chronic inhibition of NO synthase with the greatest decrease in blood flow among all organs and tissues tested in Ang II rats. Collectively, these data are consistent with the hypothesis that NO activity counteracts the vasoconstrictor influence of elevated circulating Ang II levels and thus helps maintain renal blood flow during the development of Ang II-induced hypertension.

The contribution of NO to Ang II-dependent influence on skeletal muscle hemodynamics is less clear. Studies performed on heart failure rats has shown that L-NAME-induced reductions in hindlimb skeletal muscle vascular conductance were abolished by losartan (28), indicating the presence of regulatory interactions between NO and Ang II in the skeletal muscle vasculature. In studies using normotensive conscious Brattleboro rats under resting conditions, the hindquarters skeletal muscle was found to respond to oral ingestion of L-NAME with the greatest decrease in blood flow of all the organs examined (29). It should be noted, however, that the effects of NO synthase inhibitor on skeletal muscle vascular tone are not uniform among several regions (30). Furthermore, in the above-mentioned study, rats drinking L-NAME showed more marked skeletal muscle vasoconstriction than those drinking *N*^G-monomethyl-L-arginine, despite the fact that both drugs induced a similar degree of hypertension (29). Further studies will be needed to determine the contribution of NO in regulating skeletal muscle circulation in Ang II-induced hypertension.

In the present study, FK409 decreased MAP and TPR in

Ang II/L-NAME rats, such that these levels were significantly lower than those in untreated Ang II-infused rats. We anticipated that L-NAME-induced reductions in renal and skeletal muscle blood flow would be completely reversed by FK409; however, treating Ang II/L-NAME rats with FK409 significantly increased but failed to reverse these blood flow rates to the values observed in untreated Ang II rats. These observations are in accordance with those of Majid *et al.* (31), who reported that *N*^G-nitro-L-arginine induced reductions in renal blood flow were not fully reversed by acute administration of the NO donor, *S*-nitroso-*n*-acetylpenicillamine. Other studies have shown that chronic L-NAME hypertension was associated with a loss of renal autoregulatory adjustments in afferent arteriolar diameter (32). Furthermore, FK409-induced reduction in MAP has been shown to cause a reflex increase in sympathetic tone leading to vasoconstriction in several organs and tissues (33). Therefore, the possibility exists that, during FK409 infusion, reductions in arterial perfusion may interfere with NO-dependent renal and skeletal muscle vasodilation. In support of this possibility, we here observed that FK409 significantly increased heart rate (data not shown) and decreased the blood flow rate in the spleen, which organ is known to be highly sensitive to the changes in sympathetic tone (33, 34). Another possibility is that chronic treatment with L-NAME causes severe vascular injury in the kidney as well as in skeletal muscle, as suggested by other investigators (35, 36).

In summary, chronic NO synthase inhibition by L-NAME reduced the blood flow rates in the kidney and skeletal muscle in Ang II-induced hypertensive rats, and these effects were partially reversed by the administration of an NO donor, FK409. These results support the hypothesis that local NO serves as an important endogenous vasodilator system that maintains renal and skeletal muscle hemodynamics during the development of Ang II-induced hypertension.

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References

1. Navar LG, Inscho EW, Majid SA, Imig JD, Harrison-Bernard LM, Mitchell KD: Paracrine regulation of the renal microcirculation. *Physiol Rev* 1996; **76**: 425–536.
2. Navar LG, Harrison-Bernard LM, Imig JD, Wang CT, Cervenka L, Mitchell KD: Intrarenal angiotensin II generation and renal effects of AT₁ receptor blockade. *J Am Soc Nephrol* 1999; **10** (Suppl 12): S266–S272.
3. Navar LG, Harrison-Bernard LM: Intrarenal angiotensin II augmentation in angiotensin II dependent hypertension. *Hypertens Res* 2000; **23**: 291–301.
4. Chin SY, Wang CT, Majid DS, Navar LG: Renoprotective effects of nitric oxide in angiotensin II-induced hyperten-

- sion in the rat. *Am J Physiol* 1998; **274**: F876–F882.
5. Chin SY, Pandey KN, Shi SJ, Kobori H, Moreno C, Navar LG: Increased activity and expression of Ca(2+)-dependent NOS in renal cortex of ANG II-infused hypertensive rats. *Am J Physiol* 1999; **277**: F797–F804.
6. Ichihara A, Inscho EW, Imig JD, Michel RE, Navar LG: Role of renal nerves in afferent arteriolar reactivity in angiotensin-induced hypertension. *Hypertension* 1997; **29**: 442–449.
7. Ichihara A, Imig JD, Inscho EW, Navar LG: Interactive nitric oxide-angiotensin II influences on renal microcirculation in angiotensin II-induced hypertension. *Hypertension* 1998; **31**: 1255–1260.
8. Imig JD: Afferent arteriolar reactivity to angiotensin II is enhanced during the early phase of angiotensin II hypertension. *Am J Hypertens* 2000; **13**: 810–818.
9. Navar LG, Ichihara A, Chin SY, Imig JD: Nitric oxide-angiotensin II interactions in angiotensin II-dependent hypertension. *Acta Physiol Scand* 2000; **168**: 139–147.
10. Nishiyama A, Fukui T, Fujisawa Y, et al: Systemic and regional hemodynamic responses to Tempol in angiotensin II infused hypertensive rats. *Hypertension* 2001; **37**: 77–83.
11. Zou LX, Hymel A, Imig JD, Navar LG: Renal accumulation of circulating angiotensin II in angiotensin II-infused rats. *Hypertension* 1996; **27**: 658–662.
12. Deng X, Welch WJ, Wilcox CS: Role of nitric oxide in short-term and prolonged effects of angiotensin II on renal hemodynamics. *Hypertension* 1996; **27**: 1173–1179.
13. Gruetter CA, Ryan ET, Lemke SM, Bailly DA, Fox MK, Schoepp DD: Endothelium-dependent modulation of angiotensin II-induced contraction in blood vessels. *Eur J Pharmacol* 1988; **146**: 85–95.
14. Sigmon DH, Beierwaltes WH: Angiotensin II: nitric oxide interaction and the distribution of blood flow. *Am J Physiol* 1993; **265**: R1276–R1283.
15. Ballermann BJ, Dardik A, Eng E, Liu A: Shear stress and the endothelium. *Kidney Int Suppl* 1998; **67**: S100–S108.
16. Siragy HM, Carey RM: The subtype 2 (AT₂) angiotensin receptor mediates renal production of nitric oxide in conscious rats. *J Clin Invest* 1997; **100**: 264–269.
17. Thorup C, Kornfeld M, Goligorsky MS, Moore LC: AT₁ receptor inhibition blunts angiotensin II-stimulated nitric oxide release in renal arteries. *J Am Soc Nephrol* 1999; **10** (Suppl 11): S220–S224.
18. He H, Bessho H, Fujisawa Y, et al: Effects of a synthetic rat adrenomedullin on regional hemodynamics in rats. *Eur J Pharmacol* 1995; **273**: 209–214.
19. Kita Y, Hirasawa Y, Maeda K, Nishio M, Yoshida K: Spontaneous nitric oxide release accounts for the potent pharmacological actions of FK409. *Eur J Pharmacol* 1994; **257**: 123–130.
20. Huang M, Manning RD, Jr, LeBlanc MH, Hester RL: Overall hemodynamic studies after the chronic inhibition of endothelial-derived nitric oxide in rats. *Am J Hypertens* 1995; **8**: 358–364.
21. Cassis LA, Marshall DE, Fetting MJ, Rosenbluth B, Loder RA: Mechanisms contributing to angiotensin II regulation of body weight. *Am J Physiol* 1998; **274**: E867–E876.
22. Forstermann U, Boissel JP, Kleinert H: Expressional control of the 'constitutive' isoforms of nitric oxide synthase (NOS I and NOS III). *FASEB J* 1998; **12**: 773–790.
23. Norris PJ, Charles IG, Scorer CA, Emson PC: Studies on the localization and expression of nitric oxide synthase using histochemical techniques. *Histochem J* 1995; **27**: 745–756.
24. Greenblatt EP, Loeb AL, Longnecker DE: Marked regional heterogeneity in the magnitude of EDRF/NO-mediated vascular tone in awake rats. *J Cardiovasc Pharmacol* 1993; **21**: 235–240.
25. Hutcheson IR, Griffith TM: Release of endothelium-derived relaxing factor is modulated both by frequency and amplitude of pulsatile flow. *Am J Physiol* 1991; **261**: H257–H262.
26. Sigmon DH, Newman JM, Beierwaltes WH: Angiotensin II: endothelium-derived nitric oxide interaction in conscious rats. *J Am Soc Nephrol* 1994; **4**: 1675–1682.
27. Dedeoglu IO, Springate JE: Effect of nitric oxide inhibition on blood pressure and renal function in TGR(mRen2)27 rats. *Pediatr Res* 1998; **43**: 203–208.
28. Symons JD, Stebbins CL, Musch TI: Interactions between angiotensin II and nitric oxide during exercise in normal and heart failure rats. *J Appl Physiol* 1999; **87**: 574–581.
29. Gardiner SM, Compton AM, Bennett T, Palmer RM, Moncada S: Regional haemodynamic changes during oral ingestion of N^G-monomethyl-L-arginine or N^G-nitro-L-arginine methyl ester in conscious Brattleboro rats. *Br J Pharmacol* 1990; **101**: 10–12.
30. Shen W, Lundborg M, Wang J, et al: Role of EDRF in the regulation of regional blood flow and vascular resistance at rest and during exercise in conscious dogs. *J Appl Physiol* 1994; **77**: 165–172.
31. Majid DS, Omoro SA, Chin SY, Navar LG: Intrarenal nitric oxide activity and pressure natriuresis in anesthetized dogs. *Hypertension* 1998; **32**: 266–272.
32. Bouriquet N, Casellas D: Chronic L-NAME hypertension in rats and autoregulation of juxtamedullary preglomerular vessels. *Am J Physiol* 1995; **269**: F190–F197.
33. Diepstra G, Gonyea W, Mitchell JH: Distribution of cardiac output during static exercise in the conscious cat. *J Appl Physiol* 1982; **52**: 642–646.
34. Tkachenko BI, Medvedeva NJ, Pozdnjakov PK: The character of the capacitance vessel responses in the spleen and intestine under electrical stimulation of the sympathetic fibres. *Experientia* 1974; **30**: 1413–1414.
35. Anderson JE: A role for nitric oxide in muscle repair: nitric oxide-mediated activation of muscle satellite cells. *Mol Biol Cell* 2000; **11**: 1859–1874.
36. Watanabe S, Ono H, Ishimitsu T, Matsuoka H, Ono Y, Fujimori T: Calcium antagonist inhibits glomerular cell apoptosis and injuries of L-NAME exacerbated nephrosclerosis in SHR. *Hypertens Res* 2000; **23**: 683–691.