Role of Endogenous Angiotensin II in Renal Hemodynamic and Excretory Responses to L-Arginine Infusion

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The purpose of this study was to investigate whether endogenous angiotensin II has a functional role in renal hemodynamic and excretory changes induced by L-arginine, a substrate for nitric oxide (NO), in anesthetized rats. During the intravenous infusion of L-arginine (50, 100, 200 $\mu\text{mol/kg} \cdot \text{min}$), there was no significant change in systemic or renal hemodynamics, but urine flow and urinary sodium excretion markedly increased in a dose-dependent manner. Simultaneously, L-arginine infusion produced an increase in urinary excretion of NO metabolites, NO_2^- and NO_3^- . Treatment with L-158809 {5,7-dimethyl-2-ethyl-3-[[2'-(1*H*-tetrazol-5-yl)[1,1']-biphenyl-4-yl]methyl]-3*H*-imidazo[4,5-*b*]pyridine} (0.3 mg/kg), a selective angiotensin II type I receptor antagonist, caused a reduction in mean arterial pressure, and a rise in renal blood flow and glomerular filtration rate, with no changes in excretory responses. In the presence of L-158809, L-arginine-induced diuretic and natriuretic actions were observed to the same extent as seen in the absence of L-158809. These data suggest that the infusion of L-arginine causes diuresis and natriuresis, possibly *via* the formation of nitric oxide in the kidney, and that endogenous angiotensin II is not involved in the L-arginine-induced renal actions.

Key words L-arginine; nitric oxide; angiotensin II; L-158809; renal function; urine formation

Nitric oxide (NO), known as an endothelium-derived relaxing factor, is formed from the terminal guanidino nitrogen atom of L-arginine by the action of NO synthase in the endothelial cell. Accumulating evidence suggests that endogenous NO plays an important role in the control of systemic and renal hemodynamics.¹⁾ This evidence is derived largely from studies using NO synthase inhibitors such as *N*^G-nitro-L-arginine methyl ester (NAME). Several investigators noted that these inhibitors led to a sustained rise in blood pressure, a rise in renal vascular resistance (RVR), and a reduction in renal perfusion flow and glomerular filtration rate (GFR) in anesthetized rats.²⁾ Urine flow (UF) and the urinary excretion of sodium (UNaV) are also affected by the administration of the NO synthase inhibitor, although these responses appear to be dependent on the dose used in the experiments. Lahera *et al.*³⁾ found that NAME at a dose which does not cause any significant change in blood pressure produces anti-diuresis and antinatriuresis. At larger doses, this inhibitor produces hypertension which overrides the initial antinatriuretic effect.

NO may interact with endogenous renal vasoconstrictors in regulating renal hemodynamics and urine formation. Indeed, endogenous NO was reported to counteract the effect of angiotensin II in renal hemodynamic and excretory responses. Intrarenal arterial infusion of NO synthase inhibitor markedly amplified the renal vasoconstriction and antinatriuresis induced by angiotensin II.^{4,5)} Other investigators reported that an angiotensin II blockade with losartan, an angiotensin II type I (AT_1) receptor antagonist, had little effect on renal hemodynamic and excretory responses to acute NO synthase blockade.⁶⁾ Conversely, De Nicola *et al.*⁷⁾ reported that several renal effects of *N*^G-monomethyl-L-arginine, a NO synthase inhibitor, in anesthetized rats were attenuated or prevented by pretreatment with

losartan. Thus, the interaction between angiotensin II and NO in renal hemodynamic and excretory responses has not been fully elucidated. In addition, although there is evidence that exogenously applied L-arginine produces renal vasodilation and diuresis by increasing NO production,^{8,9)} it is unknown whether endogenous angiotensin II influences renal hemodynamic and excretory responses to L-arginine.

In the present study, to evaluate whether endogenous angiotensin II modulates renal hemodynamic and excretory responses to L-arginine, we examined L-arginine-induced renal actions in the absence or presence of L-158809 {5,7-dimethyl-2-ethyl-3-[[2'-(1*H*-tetrazol-5-yl)-[1,1']-biphenyl-4-yl]methyl]-3*H*-imidazo[4,5-*b*]pyridine}, a selective AT_1 receptor antagonist,¹⁰⁾ using anesthetized rats.

MATERIALS AND METHODS

Surgical Procedure Experiments were performed using male Sprague-Dawley rats weighing 350–420 g. The rats were anesthetized with an intraperitoneal injection of sodium thiobarbital (Inactin, 100 mg/kg) and were placed on an operating table maintained at a temperature between 37 and 38 °C. After tracheotomy, cannulas were placed in the left jugular vein for the infusion (0.04 ml/min) of physiological saline containing 1% inulin to estimate GFR and in the carotid artery for blood sampling. Furthermore, the right femoral vein and artery were cannulated for the infusion of L-arginine or saline, at a rate of 0.04 ml/min, and for mean arterial pressure (MAP) and heart rate (HR) measurements with a pressure transducer (Nihon Koden, Tokyo, Japan, AD601G). After an abdominal midline incision was done, the left kidney was exposed and the renal artery was carefully stripped of connective tissues, followed by the use of 5% phenol

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for denervation. An electromagnetic probe was attached to the renal artery and renal blood flow (RBF) was measured using a square-wave flowmeter (Nihon Koden, MFV-200). A cannula was inserted into the left ureter for urine collection. At the end of the operation, 2 ml of saline containing inulin (70 mg/kg) was slowly injected for supplement of body fluid loss and priming dose of inulin, followed by the infusion of saline containing 1% inulin. MAP, HR, RBF were recorded continuously on a polygraph (Nihon Koden, RM 6000G). After completion of the surgical procedure the rats were allowed to stabilize MAP, RBF and UF for about 60 min.

Experimental Protocol The dose-response effects of L-arginine (50, 100, 200 $\mu\text{mol/kg}\cdot\text{min}$) on renal hemodynamic and excretory responses were studied in three groups of seven rats each. Urine was collected from the left ureter during two consecutive 20-min periods as a control. Following the control periods, the intravenous infusion of L-arginine was started. After 15-min stabilization period, urine was collected during two consecutive 20-min periods as experimental periods. Blood samples were obtained at the midpoint of each period. The blood loss was supplemented by an equal volume of blood from donor rats.

The effect of L-158809 on renal hemodynamic and excretory responses to L-arginine was studied in two groups of seven rats each. L-158809 (0.3 mg/kg) or vehicle were administered intravenously. After 30-min equilibration period, a 40-min urine collection was done before L-arginine administration as a control. L-Arginine was then infused into the femoral vein at a dose of 100 $\mu\text{mol/kg}\cdot\text{min}$ for 55 min (15-min stabilization period plus 40-min urine collection period). In a separate experiment, we verified that the dose of L-158809 used in this study inhibited exogenous angiotensin II (100 ng/kg)-induced pressor response by about 70% during a 4 h period (data not shown).

Analytical Procedures Urine samples were collected in pre-weighed tubes and urine volumes were determined gravimetrically. 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.¹¹⁾ The urinary sodium concentration was determined using a flame photometer. The urinary excretion of NO_2^- and NO_3^- (NO metabolites), the stable oxidation products of NO, was measured to evaluate NO production in the kidney.¹²⁾ NO metabolite levels in urine were measured by an autoanalyzer (Tokyo Kasei Kogyo, TCI-NOX 1000, Tokyo, Japan). Briefly, urine samples were diluted with de-ionized water (1:50). The sample was mixed with a carrier solution (0.07% ethylenediamine tetra acetic acid and 0.3% NH_4Cl) and applied to a copperized cadmium reduction column to reduce NO_3^- to NO_2^- , which reacts with Griess reagent (1% sulfonamide, 0.1% *N*-1-naphthylethylenediamine dihydrochloride, 5% HCl). The absorbance at 540 nm was measured by a flow-through visible spectrophotometer (Tokyo Kasei Kogyo, S-3250). NO_2^- was used as the standard.

Drugs L-158809 was a kind gift from Banyu Pharmaceutical Co., Ltd. L-158809 was dissolved in saturated

NaHCO_3 and diluted to 15% NaHCO_3 in saline. Sulfonamide and *N*-1-naphthylethylenediamine dihydrochloride were purchased from Tokyo Kasei Kogyo. Other chemicals were purchased from Nacalai Tesque, Inc. (Kyoto, Japan).

Statistical Analysis All values were expressed as means $\pm$ S.E.M. For statistical analysis, we used unpaired Student's *t* test for two-group comparison and one-way analysis of variance combined with Dunnett's multiple range test for multiple comparison. Differences were considered significant at $p < 0.05$.

RESULTS

Effects of L-Arginine on Systemic and Renal Hemodynamics The effects of L-arginine on systemic and renal hemodynamics are summarized in Table 1. L-Arginine at 50, 100, and 200 $\mu\text{mol/kg}\cdot\text{min}$ did not significantly alter the levels of MAP during the drug infusion. There was also no significant alternation in HR throughout the experimental period at 50 or 100 $\mu\text{mol/kg}\cdot\text{min}$ L-arginine. At the highest dose of L-arginine (200 $\mu\text{mol/kg}\cdot\text{min}$), there was a significant decrease in HR during the second experimental period. No significant changes in RBF or GFR were observed throughout the experiment.

Effects of L-Arginine on Excretory Responses Figure 1 illustrates the effect of L-arginine on UF. The control values in the first period were 8.78 ± 1.95 , 6.33 ± 1.09 , and 5.53 ± 1.09 $\mu\text{l/g}\cdot\text{min}$ in the group treated with 50, 100, and 200 $\mu\text{mol/kg}\cdot\text{min}$ L-arginine, respectively. The administration of L-arginine produced a diuretic action in a dose-dependent manner. In the second experimental period, UF was significantly increased to 14.98 ± 3.52 , 30.89 ± 2.92 , and 71.45 ± 8.32 $\mu\text{l/min}\cdot\text{g}$, respectively, by L-arginine infusion at 50, 100, and 200 $\mu\text{mol/kg}\cdot\text{min}$. These diuretic effects gradually disappeared after termination of the drug infusion (data not shown).

Figure 2 shows the effect of L-arginine on UNaV. L-Arginine (50 $\mu\text{mol/kg}\cdot\text{min}$) slightly increased UNaV, but

Table 1. Effects of L-Arginine on Systemic and Renal Hemodynamics in Anesthetized Rats

	C1	C2	E1	E2
L-Arginine (50 $\mu\text{mol/kg}\cdot\text{min}$)				
MAP	125 ± 7	121 ± 7	117 ± 8	113 ± 8
HR	411 ± 17	404 ± 16	394 ± 17	390 ± 19
RBF	5.74 ± 0.49	5.77 ± 0.40	5.99 ± 0.51	5.64 ± 0.36
GFR	1.61 ± 0.16	1.44 ± 0.16	1.62 ± 0.19	1.56 ± 0.17
L-Arginine (100 $\mu\text{mol/kg}\cdot\text{min}$)				
MAP	127 ± 3	119 ± 4	122 ± 5	117 ± 4
HR	403 ± 21	393 ± 21	384 ± 20	374 ± 20
RBF	4.68 ± 0.38	4.59 ± 0.46	5.32 ± 0.57	5.13 ± 0.56
GFR	1.22 ± 0.14	1.27 ± 0.17	1.40 ± 0.09	1.20 ± 0.15
L-Arginine (200 $\mu\text{mol/kg}\cdot\text{min}$)				
MAP	125 ± 3	128 ± 4	126 ± 4	126 ± 4
HR	405 ± 6	406 ± 8	390 ± 7	$375 \pm 8^{**}$
RBF	5.40 ± 0.47	5.32 ± 0.49	5.67 ± 0.52	5.19 ± 0.50
GFR	1.58 ± 0.11	1.61 ± 0.10	1.56 ± 0.12	1.43 ± 0.16

Each value represents the mean $\pm$ S.E.M. MAP, mean arterial pressure; HR, heart rate; RBF, renal blood flow; GFR, glomerular filtration rate; C1, the first control period; C2, the second control period; E1, the first experimental period; E2, the second experimental period; $^{**} p < 0.01$, compared with each value of C1.

this increment was not statistically significant compared with the control value. A significant increase in UNaV was evident during the infusion of 100 and 200 $\mu\text{mol/kg} \cdot \text{min}$ L-arginine. In the second experimental period, UNaV was increased to about 2.5- and 4-fold of each control value.

The administration of L-arginine resulted in an increase in the urinary excretion of NO metabolites (UNO_xV) (Fig.

Fig. 1. Effect of L-Arginine on Urine Flow (UF) in Anesthetized Rats

Each value represents the mean $\pm$ S.E.M. * $p < 0.05$; ** $p < 0.01$, compared with each value of C1. open column, 50 $\mu\text{mol/kg} \cdot \text{min}$; dotted column, 100 $\mu\text{mol/kg} \cdot \text{min}$; hatched column, 200 $\mu\text{mol/kg} \cdot \text{min}$.

Fig. 2. Effect of L-Arginine on Urinary Excretion of Sodium (UNaV) in Anesthetized Rats

Each value represents the mean $\pm$ S.E.M. * $p < 0.05$; ** $p < 0.01$, compared with each value of C1. open column, 50 $\mu\text{mol/kg} \cdot \text{min}$; dotted column, 100 $\mu\text{mol/kg} \cdot \text{min}$; hatched column, 200 $\mu\text{mol/kg} \cdot \text{min}$.

3). At 100 and 200 $\mu\text{mol/kg} \cdot \text{min}$ L-arginine, there were significant increases in UNO_xV during the experimental periods. In the second experimental period, UNO_xV increased to about 2.5- and 2.1-fold of each control value, respectively.

Effects of L-158809 on Renal Hemodynamic and Excretory Responses to L-Arginine Infusion To evaluate the possible involvement of endogenous angiotensin II in L-arginine-induced renal action, we examined the effect of L-arginine at a dose of 100 $\mu\text{mol/kg} \cdot \text{min}$ in the absence or presence of L-158809. As described above, L-arginine at this dose produced notable diuretic and natriuretic action with a significant increase in UNO_xV without affecting systemic and renal hemodynamics (Tables 2 and 3). When L-158809 was administered, there was a significant decrease in MAP (about 15%) and the decrease level was stable during the drug infusion. An approximate 30% increase in RBF was observed, and the calculated RVR was markedly decreased. GFR was also elevated by the drug infusion. In the presence of L-158809, the administration of L-arginine did not influence these hemodynamic parameters (Table 2). As shown in Table 3, L-158809 did not affect the basal levels of urine formation. When L-arginine was administered in the presence of L-158809, observed changes in UF, UNaV, the fractional excretion of sodium (FENa) and UNO_xV were similar to those seen in the absence of L-158809.

Fig. 3. Effect of L-Arginine on Urinary Excretion of NO Metabolites (UNO_xV) in Anesthetized Rats

Each value represents the mean $\pm$ S.E.M. * $p < 0.05$; ** $p < 0.01$, compared with each value of C1. open column, 50 $\mu\text{mol/kg} \cdot \text{min}$; dotted column, 100 $\mu\text{mol/kg} \cdot \text{min}$; hatched column, 200 $\mu\text{mol/kg} \cdot \text{min}$.

Table 2. Effects of L-158809 on L-Arginine (100 $\mu\text{mol/kg} \cdot \text{min}$)-Induced Changes in Systemic and Renal Hemodynamics in Anesthetized Rats

	MAP (mmHg)	HR (beats/min)	RBF (ml/g · min)	RVR (mmHg/ml/g · min)	GFR (ml/g · min)
Vehicle					
Control	123 $\pm$ 4	398 $\pm$ 20	4.64 $\pm$ 0.41	27.5 $\pm$ 1.8	1.25 $\pm$ 0.15
L-Arginine	120 $\pm$ 5	378 $\pm$ 20	5.23 $\pm$ 0.56	23.9 $\pm$ 1.8	1.30 $\pm$ 0.11
% change	-2.6 $\pm$ 2.9	-4.5 $\pm$ 2.4	12.2 $\pm$ 5.4	-12.9 $\pm$ 3.3	10.9 $\pm$ 12.1
L-158809					
Control	107 $\pm$ 6*	393 $\pm$ 8	5.98 $\pm$ 0.38*	17.9 $\pm$ 1.2*	1.79 $\pm$ 0.17*
L-Arginine	109 $\pm$ 8	389 $\pm$ 10	6.67 $\pm$ 0.38	16.6 $\pm$ 1.3	1.88 $\pm$ 0.17
% change	2.8 $\pm$ 1.5	-1.0 $\pm$ 1.5	11.8 $\pm$ 2.9	-7.5 $\pm$ 3.1	11.3 $\pm$ 9.1

Each value represents the mean $\pm$ S.E.M. * $p < 0.05$, compared with each control value of vehicle.

Table 3. Effect of L-158809 on L-Arginine (100 $\mu\text{mol/kg}\cdot\text{min}$)-Induced Changes in Excretory Response in Anesthetized Rats

	UF ($\mu\text{l/g}\cdot\text{min}$)	UNaV ($\mu\text{Eq/g}\cdot\text{min}$)	FENa (%)	UNO _x V ($\text{nmol/g}\cdot\text{min}$)
Vehicle				
Control	6.08 $\pm$ 1.03	1.18 $\pm$ 0.30	0.62 $\pm$ 0.15	2.49 $\pm$ 0.37
L-Arginine	24.63 $\pm$ 3.20**	2.40 $\pm$ 0.51*	1.40 $\pm$ 0.37*	5.48 $\pm$ 0.90**
% change	358.6 $\pm$ 58.7	198.3 $\pm$ 77.1	164.0 $\pm$ 50.8	120.6 $\pm$ 17.7
L-158809				
Control	7.08 $\pm$ 1.02	1.84 $\pm$ 0.63	0.70 $\pm$ 0.21	2.65 $\pm$ 0.47
L-Arginine	29.22 $\pm$ 6.43**	5.51 $\pm$ 1.89*	1.91 $\pm$ 0.53*	5.70 $\pm$ 0.72**
% change	322.4 $\pm$ 49.7	213.6 $\pm$ 41.1	189.6 $\pm$ 32.0	127.9 $\pm$ 19.7

Each value represents the mean $\pm$ S.E.M. UF, urine flow; UNaV, urinary excretion of sodium; FENa, fractional excretion of sodium; UNO_xV, urinary excretion of NO metabolites. * $p < 0.05$; ** $p < 0.01$, compared with each control value.

DISCUSSION

Several studies have demonstrated that the infusion of the NO precursor L-arginine to conscious and anesthetized rats causes diuresis and natriuresis.^{2,13} We also observed that the infusion of L-arginine (50 to 200 $\mu\text{mol/kg}\cdot\text{min}$) produced diuresis and natriuresis with no alternation in systemic and renal hemodynamics. Furthermore, the L-arginine-induced diuretic action was accompanied by increased UNO_xV. Thus, our findings support the concept that exogenously applied L-arginine causes diuresis and natriuresis, which is probably mediated by the increased formation of NO in the kidney.

Many studies have found that the intrarenal arterial infusion of NO synthase inhibitors caused renal vasoconstriction and antidiuresis.¹¹ These inhibitors impaired pressure-induced natriuresis in anesthetized dog.¹⁴ Thus, NO seems to play an important role in the regulation of renal function. Moreover, NO has been suggested to exert its influence on the renal vasculature and tubules by blunting the action of endogenous vasoconstrictor agents. To date, such an interaction has been demonstrated for angiotensin II,¹⁵ norepinephrine¹⁶ and endothelin-1.¹⁷ In regard to angiotensin II, Alberola *et al.*⁴ reported that the inhibition of NO synthase within the kidney markedly enhances the reduction in GFR and the rise in RVR induced by angiotensin II. Additionally, angiotensin II-induced reductions in FENa and the fractional excretion of lithium were also enhanced by NO synthase inhibition. However, this evidence was derived from studies using NO synthase inhibitors. To our knowledge, there have been no reports examining the possible involvement of endogenous angiotensin II in the renal hemodynamic and excretory responses to NO substrate. In the present study, the infusion of L-arginine at a rate of 100 $\mu\text{mol/kg}\cdot\text{min}$ caused diuresis without affecting renal hemodynamics. In the L-158809-treated group, L-arginine also produced diuresis and natriuresis without significant alterations in renal hemodynamics, and the observed changes in urine formation were to the same extent as those seen in the vehicle group. These findings suggest that endogenous angiotensin II does not play a functional role in L-arginine-induced diuretic and natriuretic actions.

Terada *et al.*¹⁸ reported that constitutive NO synthase was detected in the inner medullary collecting duct, inner medullary thin limb, glomerulus, vasa recta and arcuate

artery. Furthermore, it has been shown that NO inhibits the active transport of sodium and increases cyclic-GMP content in cultured mouse cortical duct cells.¹⁹ This would suggest that NO has direct renal tubular effects on sodium reabsorption *via* cyclic-GMP formation. We found that the administration of L-arginine increased UF, UNaV and FENa accompanied by an increase in UNO_xV. Thereby, it seems reasonable to consider that L-arginine-induced diuretic and natriuretic actions were exerted by the generation of NO from L-arginine at these sites. Terada *et al.*²⁰ found that using an assay of reverse transcription and polymerase chain reaction, localization of mRNA coding for the AT₁ receptor was present in these sites. De Nicora *et al.*⁷ reported that Dup753, an AT₁ receptor antagonist, prevented both glomerular and tubular actions induced by NO synthase inhibitor in the Munich-Wistar rat, thereby suggesting that NO is a physiological antagonist of angiotensin II at both glomerular and tubular sites. The reason for the discrepancy from our data is unknown. One possibility is that endogenous angiotensin II may interact with basal levels of NO in a different mode, compared with exogenously applied L-arginine-derived NO.

One problem arising from our study is related to the ability of various amino acids to produce moderate renal vasodilation and diuresis. Chen *et al.*¹³ reported that the intravenous infusion of mixed amino acids causes a rise in renal perfusion flow, UF and sodium excretion. However, in their report, these changes induced by mixed amino acids were much less potent than those seen with L-arginine. In addition, Cernadas *et al.*⁹ noted that the bolus administration of L-arginine to conscious rats caused potent diuresis and natriuresis associated with increased levels of urinary cyclic-GMP, which is implicated as the second messenger of NO. The diuretic action and increment of cyclic-GMP level induced by L-arginine was significantly inhibited by the NO synthase inhibitor. In our study, there was a positive correlation between urine volume and the urinary excretion of NO metabolites at 50 and 100 $\mu\text{mol/kg}\cdot\text{min}$ L-arginine. However, there was no such correlation when L-arginine at 200 $\mu\text{mol/kg}\cdot\text{min}$ was infused. Thereby, it seems likely that the potent diuretic action induced by 200 $\mu\text{mol/kg}\cdot\text{min}$ L-arginine was due not only to an L-arginine-NO pathway, but also to other effects of L-arginine.

In conclusion, the intravenous infusion of L-arginine

caused diuresis and natriuresis, accompanied by an increase in UNO_xV in anesthetized rats. In the presence of L-158809, L-arginine-induced diuretic and natriuretic actions were comparable to those seen in the absence of L-158809. These findings suggest that the diuresis and natriuresis induced by L-arginine are at least partly dependent on the generation of NO in the kidney. Furthermore, it is likely that endogenous angiotensin II does not interact with the exogenous L-arginine-induced diuretic and natriuretic actions.

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