Long-term Administration of L-Arginine Improves Nitric Oxide Release From Kidney in Deoxycorticosterone Acetate–Salt Hypertensive Rats

Hiroshi Hayakawa, Yasunobu Hirata, Etsu Suzuki, Kenjiro Kimura, Kazuya Kikuchi, Tetsuo Nagano, Masaaki Hirobe, Masao Omata

Abstract To examine the effects of L-arginine (L-Arg) on endothelial function, we administered 0.5 g/L L-Arg in drinking water to deoxycorticosterone acetate (DOCA)-salt rats for 8 weeks and then measured nitric oxide (NO) release from isolated kidneys using a newly developed real-time chemiluminescence method. Renal pathology was also analyzed. Acetylcholine caused much smaller declines in renal perfusion pressure (10⁻⁷ mol/L acetylcholine: −24±2% [SEM] versus −50±2%, \(P<.001\)) and NO release in DOCA-salt rats (+3±1 versus +33±3 fmol/min per gram kidney weight, \(P<.001\)) compared with control rats. L-Arg did not influence the time course of systolic blood pressure elevation in DOCA-salt rats (211±5 versus 208±6 mm Hg, DOCA versus L-Arg/DOCA, \(P=NS\)). However, oral administration of L-Arg improved acetylcholine-induced declines in renal perfusion pressure (10⁻⁷ mol/L acetylcholine: L-Arg/DOCA, −33±3%, \(P<.01\) versus DOCA). This change was associated with an increase in NO release by acetylcholine (10⁻⁷ mol/L acetylcholine: L-Arg/DOCA, +10±1 mol/min per gram kidney weight, \(P<.05\) versus DOCA). However, morphological changes in renal vessels and glomeruli were similar between DOCA and L-Arg/DOCA rats. These results suggest that L-Arg administration partially reverses renal endothelial function with respect to vasorelaxation and NO release independent of blood pressure changes, indicating that hypertensive vessels seem to be depleted of L-Arg and/or have defects in the availability of L-Arg for NO synthesis.

Key Words • nitric oxide • endothelium • arginine • deoxycorticosterone • kidney

Vascular endothelial cells produce various vasoactive substances. One of them, endothelium-derived nitric oxide (EDNO), exhibits a potent antithrombotic and vasodilator action and therefore may participate in the regulation of vascular tone. In fact, in most disease conditions with arterial sclerosis, including hypertension and hyperlipidemia, endothelium-dependent vasorelaxation is attenuated. Such impaired vasorelaxation has been considered to be attributable mostly to attenuated EDNO release, with some exceptions. It has been demonstrated that EDNO is synthesized by nitric oxide (NO) synthase from a substrate of L-arginine (L-Arg). This implies that L-Arg administration may increase EDNO release and in turn improve the altered endothelium-dependent vasorelaxation. Intravenous infusion of L-Arg quickly lowered blood pressure (BP) in healthy humans. The addition of L-Arg to the incubation buffer improved agonist-induced endothelial-dependent vasorelaxation of arterial strips from animals with hyperlipidemia and those with heart failure. However, there have been only a few reports on the long-term effects of L-Arg administration. Chen and Sanders demonstrated the beneficial effect of L-Arg by showing that oral administration of L-Arg for 2 weeks abolished BP elevation in Dahl salt-sensitive rats. However, it has not been determined whether such effects are also observed in different types of hypertension.

We have recently developed a highly sensitive assay system for EDNO that can be directly applied to physiological solutions. We reported that this system was able to detect NO release from the rat isolated kidney and monitor NO release during endothelium-dependent vasodilation simultaneously with renal perfusion pressure (RPP). In the present study we applied this to isolated perfused kidneys from deoxycorticosterone acetate (DOCA)–salt hypertensive rats and examined the long-term effects of L-Arg administration on BP, vascular damage, and NO release.

Methods Six-week-old Wistar rats were uninephrectomized, implanted subcutaneously with a silicon pellet containing 200 mg DOCA per kilogram of body weight, and provided with 0.9% saline. Control rats were also uninephrectomized but given normal tap water. DOCA-salt and control rats were divided into two groups, one with and one without L-Arg administration. L-Arg at 0.5 g/L was added to 0.9% saline in DOCA-salt rats and to tap water in the control group. After 8 weeks of treatment, NO release from the four groups of isolated rat kidneys was compared.
Renal perfusion flow was set at 5 mL/min throughout the study. After an equilibrium period, vehicle, 10^{-8} mol/L acetylcholine, and 10^{-7} mol/L acetylcholine were infused in this order. Finally, 10^{-4} mol/L N^{o}-monomethyl-L-arginine (L-NMMA) was added to 10^{-4} mol/L acetylcholine. During the infusions RPP and chemiluminescence were continuously monitored. After the study the perfusate was switched to 20% glutaraldehyde, and the tissue was examined histologically. Glomeruli and arteries were examined with a light microscope after periodic acid-Schiff staining, and the vascular endothelium was observed with an electron microscope. Systolic BP in conscious animals was measured weekly with the tail-cuff method throughout the period after uninephrectomy.

Plasma L-Arg concentrations were measured in the other rat groups. Rats were anesthetized with ether, and heparinized blood was collected. L-Arg concentrations in plasma were determined with an amino acid autoanalyzer.

The acute effects of intravenous L-Arg infusion on acetylcholine-induced vasorelaxation and NO release were also examined in kidneys isolated from DOCA-salt and control rats. After an equilibration period, 0.1 mmol/L L-Arg was infused, after which vehicle, 10^{-8} and 10^{-7} mol/L acetylcholine, and 10^{-4} mol/L L-NMMA were added sequentially to the renal perfusate in the two rat groups. These rats were also uninephrectomized at 6 weeks of age and given DOCA and 0.9% saline or tap water for 8 weeks.

To make the NO signal clearer, we reduced the perfusion flow to 5 mL/min. However, DOCA-salt rat kidney weight is approximately twice as heavy as that of control rats, so the same perfusion flow may lead to hypoperfusion and less shear stress in the kidneys of DOCA-salt rats. To examine the effect of differences in perfusion flow on the response of RPP and NO release, we compared the responses to acetylcholine and L-NMMA with perfusion flow at 5 and 10 mL/min (n=6).

Statistical Analysis
Values are expressed as mean±SEM. The effects of the agents tested were assessed by ANOVA for repeated measures followed by Dunnett’s test. Comparisons among the four rat groups were tested by the Bonferroni method on the basis of one-way ANOVA. A value of P<.05 was considered statistically significant.

Results
The Table shows baseline variables measured in the four rat groups. L-Arg administration did not influence the time course changes in systolic BP during the 8-week period in DOCA-salt or control rats. Heart and kidney weights were greater in DOCA-salt than in control rats, whereas body weight was comparable be-

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BP indicates blood pressure; BW, body weight; RPP, renal perfusion pressure; L-Arg, L-arginine; and DOCA, deoxycorticosterone acetate. Data are mean±SEM.

*P<.01 vs their normotensive controls.
Fig 2. Representative tracings show nitric oxide (NO) release rate into renal perfusate and renal perfusion pressure (RPP) in control rats (A), deoxycorticosterone acetate (DOCA)-salt rats (B), and DOCA-salt rats with L-arginine (L-Arg) (C) during administration of acetylcholine (ACh) and \( N^\circ\)-monomethyl-L-arginine (NMMA). KW indicates kidney weight.

Fig 3. Bar graphs show effects of acetylcholine (ACh) and \( N^\circ\)-monomethyl-L-arginine (LNMMA) on renal perfusion pressure (RPP) and nitric oxide (NO) release rates into perfusate from kidney of deoxycorticosterone acetate (DOCA)-salt and control (Cont) rats with or without L-arginine (Arg). KW indicates kidney weight.

Plasma L-Arg concentrations in rats with L-Arg were significantly higher than in those without L-Arg (control, 0.115±0.005 mmol/L; control with L-Arg, 0.167±0.011 mmol/L; DOCA-salt with L-Arg, 0.188±0.015 mmol/L, both \( P<.01 \) versus control).

Because oral administration of L-Arg increased the plasma L-Arg level by approximately 0.06 mmol/L, we examined the effect of acetylcholine in the presence of 0.1 mmol/L L-Arg. Acute increases in the perfusate L-Arg concentration did not cause any changes in baseline RPP or NO signals in control or DOCA-salt rats. Similarly, acetylcholine-induced changes in RPP or NO were not influenced in either rat group, although the effect of L-NMMA was attenuated (Fig 4).

Histological analysis revealed that focal glomerular sclerosis, interstitial changes, and vascular hypertrophy were often observed in DOCA-salt rats. DOCA-salt rats drinking L-Arg also showed these changes in the kidney. Although endothelial cells were not detached, subendothelial spaces were thickened, and endothelial vacuolar changes were increased in DOCA-salt rats. Such electron microscopic findings of endothelial damage did not improve in DOCA-salt rats given L-Arg (data not shown).

Fig 5 demonstrates the relation among BP, kidney weight, renal perfusion flow, and the responses to
Acetylcholine. When the perfusion flow to DOCA-salt rats was increased to 10 mL/min, which was in proportion to the larger kidney weight, the increase in NO release and decrease in RPP by 10^{-8} mol/L acetylcholine were similar to those for 5 mL/min perfusion and still smaller than in control rats. Similarly, responses to 10^{-7} mol/L acetylcholine in DOCA-salt rats did not differ between perfusion flows of 5 and 10 mL/min (%ARPP: 5 mL/min, -24.0±2.0% versus 10 mL/min, -31.6±2.2%, P=NS; ANO: 5 mL/min, +3.0±0.5 versus 10 mL/min, +3.1±0.6 fmol/min per gram kidney weight, P=NS).

**Discussion**

In the present study NO release from the isolated kidney was detected with the use of a highly sensitive chemiluminescence method. We have reported that the ultimate reactant in this system is peroxynitrite. Although this reaction may not be specific for NO, the chemiluminescent signal was intensified by endothelium-dependent vasodilators and diminished by the NO synthesis antagonist L-NMMA, whereas L-Arg administration restored the signal. Furthermore, chemical endothelectomy also diminished an acetylcholine-induced increase in the NO signal. These findings suggest that most of the chemiluminescence may reflect the presence of EDNO in the isolated perfused organ system. In DOCA-salt hypertensive rats the factors responsible for the reduced endothelium-dependent vasodilation have not been fully investigated. The present study provides strong evidence that attenuated endothelium-dependent renal vasodilatation in DOCA-salt rats is mainly due to the decrease in EDNO release. This is compatible with our previous report that renal release rates of the NO metabolites NO_{2−} and NO_{3−} were less in DOCA-salt than in control rats.

To confirm the adequacy of the perfusion method, we examined the effects of differences in BP, kidney weight, and renal perfusion flow on RPP and NO release. Even when the relative hypoperfusion to kidneys with DOCA-salt hypertension was corrected by the increase in perfusion flow in proportion to kidney weight, the hyporesponsiveness of the kidneys in DOCA-salt rats to acetylcholine was not influenced. Accordingly, we compared DOCA-salt and control rats under the same perfusion flow, which provided a more precise means with which to determine the NO concentration in the perfusate.

The acute increase in the perfusate L-Arg concentration, which corresponded to the increase in the plasma L-Arg level in rats given L-Arg, did not improve acetylcholine-induced vasodilation or NO release in DOCA-salt rats. This may be due to an insufficient increase in the endothelial L-Arg level. However, 8-week oral administration of L-Arg improved acetylcholine-induced vasorelaxation and NO release in DOCA-salt hypertensive rats despite the lack of obvious effects on BP or vascular damage. In this study we administered 0.5 g/L L-Arg in drinking water. Chen and
Sanders\(^7\) used 1.25 g/L L-Arg and observed BP-lowering effects in Dahl rats. The lower L-Arg concentration used in the present study may be the reason for the ineffectiveness of L-Arg on BP because it is possible that hypertension caused by DOCA-salt treatment is so severe that mild antihypertensive agents are not effective. In our preliminary study the higher dose of L-Arg decreased the water intake of rats, most likely because of its bitter taste. Normal water intake by DOCA-implanted rats is critical for BP elevation, so we chose this L-Arg concentration. As a result, we were able to observe the effects of L-Arg administration on endothelial function independent of BP changes.

Under normal conditions the NO synthase substrate L-Arg is present in excess, and therefore its availability is not rate limiting for NO production. In fact, L-Arg did not influence endothelium-dependent vasorelaxation in normotensive rats.17,18 These findings suggest that damaged endothelial cells may have defects with respect to the availability of the substrate. L-Arg supplements appear to compensate for this defect, although it is possible that L-Arg actually protected vessels from the endothelial damage, the changes in which could not be detected by the morphological study used here.

Although L-Arg infusion acutely lowered BP in healthy humans,\(^3\),4 most reports using normal animals showed that L-Arg did not relax precontracted normal vessels. However, it has been shown that prolonged incubation of normal vessels in physiological buffers sensitizes them to L-Arg. After 6 to 24 hours of incubation, L-Arg has been shown to cause endothelium-dependent vasorelaxation and enhanced response to acetylcholine.20,21 This was accompanied by a reduction in tissue levels of L-Arg.22 These findings suggest that prolonged incubation of the vessels under tension depleted L-Arg, the substrate of EDNO. Moreover, incubation with L-Arg for approximately 40 minutes restored endothelium-dependent vasodilation in vessels from animals with hypercholesterolemia and those with cardiomyopathy.5-8 Therefore, it seems likely that the renal vessels in DOCA-salt rats under excessive tone for a long time are also deprived of L-Arg.

In conclusion, we developed a sensitive assay method for EDNO and found that long-term L-Arg administration improved agonist-induced EDNO release in DOCA-salt rats. This is a direct effect of L-Arg and not due to BP changes, suggesting that hypertensive vessels seem to be depleted and/or have defects related to the availability of L-Arg for EDNO synthesis. This may be one of the mechanisms for decreased NO release in DOCA-salt rats.

Acknowledgments

This study was supported in part by a Grant-in-Aid for Scientific Research on Priority Areas ("Endothelium-Smooth Muscle Coupling") and by Grant-in-Aid No. 05670948 from the Ministry of Education, Culture and Science of Japan.

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*Hypertension*. 1994;23:752-756
doi: 10.1161/01.HYP.23.6.752

*Hypertension* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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