Endothelium-Derived Relaxing Factor and the Vascular 
Reply to Systemic Hypertension\textsuperscript{1,2}

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ABSTRACT

Endogenous nitric oxide is an important modulator of 
vascular smooth muscle tone. The role of nitric oxide in 
the vascular adaptation to systemic hypertension was examined by using N\textsuperscript{-}monomethyl-L- 
arginine (L-NMMA; 110 \textmu g/kg/min), a competitive inhibitor of the conversion of L-arginine to nitric oxide. 
L-NMMA or saline vehicle (9.6 \textmu L/min) was infused i.v. into several rat models of acute and chronic systemic hypertension. The response to L-NMMA was compared either in uninephrectomized Sprague-Dawley rats treated with deoxycorticosterone on either a high- or low-sodium diet or in untreated uninephrectomized rats on normal chow. Hypertensive deoxycorticosterone rats had a significantly greater pressor response to L-NMMA (139 ± 2 to 169 ± 3 mm Hg; \textit{N} = 9) than did normotensive uninephrectomized rats (112 ± 4 to 129 ± 3 mm Hg; \textit{N} = 7) or deoxycorticosterone treated rats on a low-sodium diet (108 ± 2 to 121 ± 3 mm Hg; \textit{N} = 9). By contrast, hypertension induced by the vasoconstrictor angiotensin II did not have an enhanced response (134 ± 3 to 154 ± 4 mm Hg; \textit{N} = 7) nor did spontaneously hypertensive rats (164 ± 4 to 175 ± 4 mm Hg; \textit{N} = 6). This dose of L-NMMA had minimal effects on renal hemodynamics in the normotensive and hypertensive animals, except for those receiving angiotensin II where it led to substantial reductions of inulin and \textit{para}-aminohippurate clearance. In conclusion, these data point to a role for nitric oxide in the vascular adaptation to volume-mediated hypertension, an effect that was not observed in vasoconstrictor-induced hypertension.

Key Words: Hypertension, EDRF, nitric oxide, L-NMMA, hemodynamics, kidney

The role of the endothelium in the vascular adap 
tation to systemic hypertension remains un 
clear. In response to a variety of mechanical and 
chemical stimuli, endothelial cells elicit both vasoco 
stricting and vasodilating mediators, the balance of which is a determinant of the vascular smooth 
muscle tone (1,2). An overabundance of endothelium 
derived constricting factor(s) or a reduction of vasodila 
tor(s) may lead to an increase in systemic vascular 
resistance and hypertension. Several studies have 
demonstrated impaired endothelial function in a va 
riety of animal models of systemic hypertension as 
well as in human hypertensives (3–8). However, it is 
uncertain whether dysfunctional endothelium is a 
primary factor in the pathogenesis of systemic hy 
pertension or whether it is a secondary effect of 
prolonged hypertension. With the capability of pro 
ducing potent vasodilators such as endothelium-de 
derived relaxing factor (EDRF), these cells have the 
potential to offset the increase in vascular smooth 
muscle tone and to thus moderate the rise in systemic vascular resistance. EDRF is distinguished by its 
short half-life and its ability to freely diffuse across cell membranes, supporting a paracrine function 
illy suited for moment-to-moment modulation of 
vascular smooth muscle tone. EDRF has been iden 
tified to be, at least in part, nitric oxide (NO) derived 
from the guanido nitrogen atom(s) of L-arginine (9). 
Although a wide variety of physical and chemical 
stimuli are capable of altering EDRF production in 
cultured cells and perfused organs, less is known of 
the modulators that are operative \textit{in vitro} (1,2). The 
purpose of the studies presented here is to examine 
the ability of the endothelium to augment NO produc 
tion in response to acute and subacute hypertension 
by using a competitive analog of L-arginine (N\textsuperscript{\text{-}mono 
methyl-L-arginine [L-NMMA]).

METHODS

Adult male Munich-Wistar rats (240 to 290 g) were 
studied except where specified. All animals were
handled in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The deoxycorticosterone (DOCA)-salt model of hypertension was examined with slight modifications of previously published protocols (10). Briefly, Brevital-sedated rats \((N = 31)\) underwent a unilateral nephrectomy \((\text{UNx})\) (flank incision) 3 wk before table studies. One group of rats was placed on standard chow and normal water \((\text{UNx}; N = 7)\). In the remainder of rats \((N = 24)\), a 50-mg pellet of slow-release DOCA (Innovative Research of America, Toledo, OH) was placed s.c. at the time of nephrectomy. Rats were subdivided to receive either normal chow \((0.4 \text{ g of } \text{Na}^+/100 \text{ g of chow})\) and 1% saline drinking water \((\text{UNx/DHS}; N = 15)\) or a Restricted sodium diet \((0.005 \text{ g of } \text{Na}^+/100 \text{ g of chow}; \text{ICN, Cleveland, OH})\) and normal drinking water \((\text{UNx/DLS}; N = 9)\), i.e., HS designates high salt and LS designates low salt.

Rats were anesthetized with Inactin \((100 \text{ mg/kg i.p.})\) and were placed on a temperature-controlled table. The right femoral artery was cannulated, and a baseline sample of blood was collected for determination of hematocrit. This arterial catheter was used for all subsequent blood sampling and for the estimation of mean arterial pressure \((\text{MAP})\) via an electronic transducer connected to a direct-writing recorder. After tracheostomy, bilateral jugular catheters were inserted for infusions of rat plasma, 10% inulin with 0.8% para-aminomipurate \((\text{PAH})\) in 0.9% NaCl \((1.2 \text{ mL/h})\), and experimental agents (such as angiotensin II \([\text{AI}]\)). The left femoral vein was then cannulated for subsequent infusion of L-NMMA \((\text{Calbiochem, San Diego, CA})\) or vehicle. The left ureter was catheterized for urine collections.

Rats prepared in this fashion have been shown to have a 20% reduction in plasma volume \((11)\); thus, the following protocol was used to maintain euolemia: isoncotic rat plasma was infused at 0.1 mL/min in a total amount equal to 1% of the body weight, followed by a reduction in infusion rate to 1.60 mL/kg/h.

After a 1-h equilibration period, two 10-min urine collections with concurrent arterial blood samples \((0.21 \text{ mL})\) were obtained for baseline measurements of \(\text{GFR (inulin clearance } [C_{\text{in}}] \text{ and estimated RPF rate (PAH clearance } [C_{\text{PAH}}]) (Pre). All rats then received a continuous i.v. infusion of either L-NMMA \((3 \text{ mg/mL; 9.6 } \mu \text{L/min})\) or sodium acetate vehicle, which was continued for the remainder of the experiment. Thirty minutes into the infusion, measurements of \(\text{MAP, } C_{\text{in}} \text{ and } C_{\text{PAH}}, \text{ were repeated (Post).}

To assess the role of NO in another model of hypertension, the above protocol was repeated in spontaneously hypertensive rats \((\text{SHR})\) aged 14 wk \((\text{MAP, } 164 ± 4 \text{ mm Hg; } N = 6)\). Finally, a separate group of normal rats received i.v. angiotensin II \([\text{AI}]\); Sigma Chemical Co., St. Louis, MO) 17.9 \mu g/mL; 1.30 \mu L/min) to achieve a \(\text{MAP comparable to that of DOCA-treated rats before receiving L-NMMA (AI plus L-NMMA; } N = 7)\) or vehicle \((\text{AI plus vehicle; } N = 3)\). For comparison, previously published data on the hemodynamic effects of identical doses of L-NMMA and vehicle on normals have been included in Table 1 and have been identified as such. These studies were performed contemporaneously with the studies presented here.

**Analytical**

Inulin concentrations in plasma and urine were measured by a macroanthrone method \((12)\), and PAH concentrations were measured by the method of Smith et al. \((13)\). Plasma protein concentrations were measured by using refractometry.

**Statistical**

Reported values represent means ± SE. Individual baseline and experimental hemodynamic values of rats given vehicle or L-NMMA were compared by paired \(t\)-test, whereas group values were compared by one-way analysis of variance \((\text{Scheffé } F\text{-test). Statistical significance was defined as } P < 0.05.\)

**RESULTS**

**Systemic Hemodynamics**

The effects of L-NMMA on blood pressure in normotensive and hypertensive rats are summarized in Table 1. Rats with DOCA-salt hypertension \((\text{UNx/DHS})\) had a marked pressor response to L-NMMA \((29 ± 3 \text{ mm Hg}), \text{ which was substantially greater in magnitude than that of normotensive controls for uninephrectomy (UNx) and DOCA treatment (UNx/DLS) (16 ± 2 mm Hg; } P < 0.05; \text{ and 13 ± 3 mm Hg; } P < 0.05, \text{ respectively}) (Figure 1). Vehicle had no significant effect on MAP in rats with DOCA-induced hypertension \((2 ± 5 \text{ mm Hg}; \text{ not significant } [\text{NS}]) (Table 1). To assess for similar augmentation of NO output in other forms of hypertension, the effects of L-NMMA were examined in rats with acute hypertension induced by ALL infusion. Baseline MAP of ALL-induced hypertension was not different from that of DOCA-salt hypertensives \((134 ± 3 \text{ versus } 139 ± 2 \text{ mm Hg; } \text{NS). The pressor response to L-NMMA in the UNx/DHS group was significantly greater than that in comparably hypertensive ALL-treated rats } (29 ± 3 \text{ versus } 20 ± 2 \text{ mm Hg; } P < 0.05) (Figure 2). Although values were intermediate between UNx and UNx/DHS, the hypertensive response to L-NMMA in ALL-treated rats was not different from that in normotensive UNx controls \((20 ± 2 \text{ versus } 16 ± 2 \text{ mm Hg; } \text{NS). Finally, the effects of L-NMMA were assessed in the SHR rat, a genetic model of progressive hypertension. Fourteen-week-old SHR rats of comparable size to the UNx/DHS rats had higher baseline MAP than did
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Renal Hemodynamic Effects

The effects of L-NMMA on renal hemodynamics in normotensive and hypertensive rats have been summarized in Table 1. L-NMMA had no significant effect on $C_{in}$ and minimal effects on $C_{PAH}$ in all of the normotensive groups. The modest changes in $C_{PAH}$, in conjunction with the increase in renal perfusion pressure, are reflected in the significant rise in renal vascular resistance for these groups (Table 1). In the DOCA-induced hypertensive, baseline values for $C_{in}$ and $C_{PAH}$ were both significantly higher than those in the other hypertensive groups. L-NMMA had no effect on the hyperfiltration of these UNx/DHS rats (−1 ± 7%), despite a reduction of $C_{PAH}$ (−17 ± 4%). However, the significance of the reduction in $C_{PAH}$ is unclear, because vehicle-treated UNx/DHS rats had a similar fall in $C_{PAH}$ (−15 ± 3%). Thus, in this setting of hyperfiltration and renal hyperemia, the effects of L-NMMA on renal hemodynamics were modest. The response to L-NMMA was markedly different in a preconstricted kidney. Rats receiving All with vehicle had modest declines in $C_{in}$ (−10 ± 1%) and more pronounced reductions in $C_{PAH}$ (−25 ± 12%). Both indices declined further with L-NMMA (All: $C_{in}$: −20 ± 1%; $P < 0.05$ versus baseline; and $C_{PAH}$: −48 ± 3%; $P < 0.05$ versus baseline), suggesting that the renal vasculature augments NO production in response to All. Of interest, L-NMMA had no significant effects on renal hemodynamics in the chronically hypertensive SHR rats.

**DISCUSSION**

In exploring the role of endogenous nitrates in the vascular reply to acute and chronic systemic hypertension, the findings of the study presented here suggest that, at least in some forms of systemic hypertension, the vasculature may respond to an elevation of blood pressure by augmenting NO production. The mild systemic pressor effect induced by L-NMMA in the basal state of the normotensive groups confirms previous findings in the rat, rabbit, and guinea pig and suggests that there is steady-state output of NO under the prevailing experimental conditions of anesthesia and moderate surgery (14–17). Similarly, infusion of L-NMMA into the forearm results in an increase in vascular resistance, indicating that NO production occurs in humans (18). The magnitude of the pressor responses in the normotensive...
Figure 1. Pressor effects of L-NMMA. MAP before (Pre) and after (Post) i.v. L-NMMA infusion in UNx, UNx/DHS, and UNx/DLS rats. Note that the pressor response of the hypertensive group is robust compared with the normotensive rats. Values are means ± SE. *P < 0.05 versus Pre.

Figure 2. Pressor effects of L-NMMA in hypertensive rats. Results are expressed as the absolute change in MAP after infusion of L-NMMA in UNx rats (baseline MAP, 112 ± 4 mm Hg) and in the three hypertensive groups: DHS/DLS rats (baseline MAP, 139 ± 2 mm Hg), All-infused rats (baseline MAP, 134 ± 3 mm Hg), and SHR rats (baseline MAP, 164 ± 4 mm Hg). Values are means ± SE. *P < 0.05 versus UNx.

Rats were unaffected by uninephrectomy UNx or a very low sodium intake UNx/DLS (Table 1). The effect of sodium intake on NO production has never been formally addressed. However, in hypertensive Dahl salt-sensitive rats, endothelium-dependent relaxations are depressed—an effect that was not observed in either salt-resistant rats (high- or low-sodium diet) or in normotensive salt-sensitive rats on a low-sodium diet (19). In addition, pharmacological treatment of the hypertension normalized the response in these rats (20). Of note, dietary potassium supplementation prevents the impairment of acetylcholine-induced relaxation in hypertensive Dahl rats (21).

Similar findings occur in stroke-prone SHR rats, suggesting that serum or tissue electrolytes may be important modifiers of endogenous nitrate production (22).

In the DOCA-salt model of "volume-mediated hypertension," L-NMMA induced a pressor response far exceeding that observed in the normotensive groups, suggesting that the vasculature responds to the chronic rise in transmural pressure by increasing the steady-state production of NO (Figure 1, Table 1). Indeed, disruption of NO production resulted in the transition from moderate to severe hypertension. Evidence that underlying vascular tone may be an important determinant of NO is found in studies of L-NMMA pressor effects in rats devoid of sympathetic tone (23). In pithed rats with hypertension induced by phenylephrine, the L-NMMA pressor response was augmented compared with that in unpithed normotensives (23). Furthermore, the pressor response to L-NMMA was blunted in pithed or ganglionic-blocked rats (reduced baseline MAP) as compared with either that in normals or pithed rats where "normotension" was restored by phenylephrine infusion (23). Several studies of vascular rings corroborate the importance of underlying vascular tone. Phenylephrine-treated vascular rings contract in response to L-NMMA, whereas relaxed rings fail to respond (24,25). In the studies presented here, rats with All-induced hypertension equivalent to that of the DOCA hypertensive group did not have an enhanced pressor response to L-NMMA as compared with normals. Whether this discrepancy with the phenylephrine study reflects differences in underlying sympathetic tone, L-NMMA dosage, or pressor dosage (baseline MAP, 134 ± 2 mm Hg with All and 155 ± 7 mm Hg with phenylephrine) or a difference in the pharmacological effects of...
phenylephrine and All remains unclear. It appears that the mechanism by which vascular resistance is controlled in hypertensives may have an important impact on EDRF release. The study presented here raises the intriguing possibility that there is a difference in the vascular reply to volume-mediated hypertension (DOCA) and vasoconstrictor-induced hypertension (All). Augmented endogenous nitrate production was evident only in the DOCA hypertensives.

A number of studies of small and large vessels from hypertensive animals (including DOCA-salt hypertension) have demonstrated impaired vasorelaxation in response to endothelium-dependent vasodilators, an effect that can be normalized with anti-hypertensive therapy (3–7). However, in the psychosocial model of hypertension in mice, aortic strips demonstrated substantially increased sensitivity to the vasorelaxant effects of endothelium-dependent vasodilators (acetylcholine and A23187) as compared with normotensive controls (26). Similarly, femoral arteries from hypertensive SHR rats were more sensitive to acetylcholine-induced vasodilatation than were femoral arteries from WKY normotensive control rats, whereas the converse was observed in the aortas (27). Most recently, Panza et al. (8) have examined the forearm vasodilatory response of hypertensive patients and normotensive controls to endothelium-dependent (acetylcholine) and endothelium-independent (nitroprusside) vasodilators. Hypertensive patients had an impaired response to acetylcholine and a normal response to nitroprusside, indicating dysfunctional endothelium (8). In the studies presented here, the pressor effects of L-NMMA in the SHR rat were much less than those in the DOCA-salt model. Indeed, the magnitude of the pressor effect tended to be lower than in normotensive animals, suggesting suppressed NO production. The ability of the endothelium to respond to an increase in transmural pressure may be lost in chronic hypertension. Factors such as duration and severity of hypertension, species and model of hypertension, and vascular beds studied are likely to account for the conflicting data on the effects of hypertension on endothelial function.

In the normotensive animals, this dose of L-NMMA had little effect on GFR and induced only mild reductions of C_PAH, findings consistent with those of previous reports (Table 1) (14,28). Others have reported a more pronounced reduction of C_PAH with EDRF blockade, suggesting that renal vasoconstriction is dose related (29). L-NMMA had no major effect on renal hemodynamics in the DOCA-salt hypertensives or in the SHR rats. By contrast, the combination of All and L-NMMA induced substantial reductions of both C_R and C_PAH in normal rats that were significantly greater than the effects of All and vehicle (Table 1). Recent studies of microperfused afferent arterioles suggest that EDRF production is enhanced by All (30). Furthermore, pretreatment with Nω-nitro-L-arginine significantly augmented and prolonged All-induced vasoconstriction in the preglomerular resistance vessels (30). Taken together, these data suggest that there is a compensatory increase in renal NO production in the setting of All-induced renal vasoconstriction that is similar in nature to the well-documented compensatory increase in renal vasodilatory prostanooid production (31).

In summary, these data suggest that in the DOCA-salt model of systemic hypertension the vasculature responds to the increase in arterial pressure by augmenting L-NMMA-inhibitable NO production. Enhanced sensitivity to L-NMMA was not evident in SHR rats or in animals with comparable blood pressures induced by All infusion. This dose of L-NMMA had minimal effects on renal hemodynamics in the normotensive and hypertensive animals, except for those receiving All where it led to substantial reductions of both C_R and C_PAH. In conclusion, these data point to an important role for NO in the vascular adaptation to volume-mediated hypertension, which when disrupted, may result in the transition from moderate to severe systemic hypertension.

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REFERENCES


