Effect of Chronic Nitric Oxide Deficiency on Angiotensin II–Induced Hypertrophy of Rat Basilar Artery

Pierre Moreau, PhD; Hiroyuki Takase, MD; Livius V. d’Uscio, PhD; Thomas F. Lüscher, MD

Background and Purpose—Although in vitro studies suggest that nitric oxide has an inhibitory effect on cellular proliferation and migration, in vivo experiments failed to support this conclusion. The present study was designed to determine the effect of endogenous nitric oxide on angiotensin II–induced hypertrophy of small arteries in vivo.

Methods—Angiotensin II (200 ng/kg per minute), alone or in combination with N\textsuperscript{G}-nitro-L-arginine methyl ester (L-NAME) (60 mg/kg per day), was administered for 2 weeks in normotensive rats. Basilar arteries were harvested, and their geometry was determined in perfused and pressurized conditions.

Results—Angiotensin II increased media thickness, media-lumen ratio, and cross-sectional area of the arteries, confirming the presence of hypertrophic remodeling. The concomitant administration of L-NAME, an inhibitor of nitric oxide synthesis, prevented vascular hypertrophy. The remodeling of the basilar artery geometry in the combined treatment was of eutrophic nature, similar to that observed with the administration of L-NAME alone.

Conclusions—Our results suggest that endogenous nitric oxide does not inhibit angiotensin II–induced vascular hypertrophy in vivo. Nitric oxide may even be a necessary factor for hypertrophy to develop. (Stroke. 1998;29:1031-1036.)

Key Words: angiotensin II ■ basilar artery ■ hypertension ■ nitric oxide ■ vascular remodeling ■ rats

In hypertension, resistance arteries adapt to the increased wall tension by changing their geometry.\textsuperscript{1} Accordingly, a reduced lumen diameter and/or an increased wall thickness can normalize the excessive tension applied on the vessel wall and may then protect the microcirculation against the blood pressure rise. However, this adaptive process may also contribute to the maintenance of hypertension by elevating total peripheral resistance\textsuperscript{2} and, particularly in the cerebral circulation, to the vascular complications of the disease process. Alterations of small-artery structure may be mediated by eutrophic (no increase in CSA) or hypertrophic (CSA increase) remodeling of the vascular wall or by a combination of both processes.\textsuperscript{2,3} The vascular endothelium, by its anatomic position and by releasing several factors, may influence the local vascular environment and modulate the changes of vascular geometry observed in the context of hypertension.

We have previously shown that chronic NO deficiency with the administration of L-NAME produces an elevation of blood pressure that is not associated with hypertrophic remodeling of small arteries of the cerebral\textsuperscript{4} and mesenteric circulations.\textsuperscript{5} Indeed, in this model eutrophic remodeling can be observed, and it is believed to develop in close relation to the elevation of blood pressure.\textsuperscript{4} These findings, which have also been made by other investigators, also apply to the heart, which, despite the elevation of blood pressure, does not become hypertrophied.\textsuperscript{6} These in vivo results are at variance with the general belief, from earlier studies in cell culture systems,\textsuperscript{7,8} that NO donors and cGMP analogues are inhibitors of vascular growth. This hypothesis was reinforced by a recent study showing that local transfection of the NO synthase gene in balloon-injured carotid arteries blunts neointimal formation and prevents the increase in CSA.\textsuperscript{9} However, this effect may be the result of inhibition of VSMC migration rather than proliferation by NO,\textsuperscript{10} since the former process is necessary for neointimal formation.\textsuperscript{2} Furthermore, Garg and Hassid,\textsuperscript{7} who reported the antimitogenic effect of NO donors in passaged cells, more recently showed that the same agents can actually potentiate fibroblast growth factor–induced replication of freshly dissociated VSMC in culture.\textsuperscript{11} This would suggest that during passages, cells acquire the ability for their replication to be inhibited by NO donors, as has been previously suggested.\textsuperscript{12} It can also be postulated that NO may act as a growth promoter or as an enhancer of proliferation in vivo, thus explaining the lack of vascular and cardiac hypertrophy during chronic NO deficiency. A recent study of DOCA-salt hypertension lends support to that postulate, since hypertrophic remodeling of small arteries and heart hypertrophy were prevented by chronic L-NAME treatment.\textsuperscript{13}
Selected Abbreviations and Acronyms

Ang II = angiotensin II
CSA = cross-sectional area
DOCA = deoxycorticosterone acetate
L-NAME = N\'\'-nitro-L-arginine methyl ester
NO = nitric oxide
PRA = plasma renin activity
RAS = renin-angiotensin system
VSMC = vascular smooth muscle cells

To better define the role of endogenous NO in the modulation of hypertrophic remodeling of small arteries, we studied the effects of a chronic administration of L-NAME in a model of vascular hypertrophy induced by the administration of Ang II.\(^\text{14,15}\) We hypothesized that if NO indeed has antiproliferative properties in vivo, we should expect an increased vascular hypertrophy when animals are treated concomitantly with Ang II and L-NAME. In opposition, if NO is a growth promoter in vivo, an inhibition of Ang II–induced hypertrophy should be observed with L-NAME.

Materials and Methods

Wistar-Kyoto rats were purchased from IFFA CREDO (L’Arbresle, France) and treated for 2 weeks from 9 weeks of age. Seven untreated rats served as controls. One group of 7 rats was treated with Ang II that was administered from subcutaneously implanted osmotic pumps (model 2002, Alzet Corp) at a rate of 200 mg/kg per minute. Other groups were treated with L-NAME alone or in combination with Ang II. The dose of L-NAME, calculated from the water intake, was 58±5 mg/kg per day in the L-NAME group and 61±4 mg/kg per day in the Ang II plus L-NAME group (\(P=\text{NS}\)). Before and at the end of the treatment, the rats were weighed and their systolic blood pressure and heart rate were determined by the tail cuff method with the use of a pulse transducer (model LE 5000, Letica). These procedures were approved by the Commission for Animal Research of the Canton of Bern, Switzerland.

Basilar arteries were harvested from the animals that had been previously anesthetized (thiopental 50 mg/kg IP) and prepared under a dissecting microscope in cold Krebs’ solution of the following composition (in mmol/L: control solution): NaCl 118.6, KCl 4.7, CaCl\(_2\) 2.5, KH\(_2\)PO\(_4\) 1.2, MgSO\(_4\) 1.2, NaHCO\(_3\) 25.1, edetate calcium disodium 0.026, glucose 10.1. The arteries were then pulled and sutured on two small glass cannulas positioned in a vessel chamber (Living Systems Instrumentation), allowing for the measurements of lumen diameter (LD) and the external diameter (ED) according to the formulas previously described.\(^\text{2,4}\)

\[
\text{CSA} = (\pi/4) \cdot \{(ED)^2 - (LD)^2\}
\]

Growth Index\(^{\text{4}}\) = [(CSA\(_\text{TRx}\) – CSA\(_\text{Control}\))/CSA\(_\text{Control}\)] \times 100

Remodeling Index\(^{\text{4}}\) = [(LD\(_\text{Control}\) – LD\(_\text{Remodel}\))/(LD\(_\text{Control}\) – LD\(_\text{TRx}\))] \times 100

where

\[
\text{LD}_{\text{Remodel}} = \sqrt{(ED_{\text{TRx}})^2 - 4\cdot\text{CSA}_{\text{Control}}/\pi}
\]

Figure 1. Net increase in systolic blood pressure (SBP) between values obtained before and after 2 weeks of the respective treatments (n=7 per group). SBP was measured with the tail cuff method in conscious rats. *\(P<0.05\) compared with control (Ctl) (ANOVA+Bonferroni). The Ang II + L-NAME group was not significantly different from the Ang II group.

Results

During the treatment period, control rats gained 42.3±3.1 g (initial value, 276.6±8.7 g), while those treated with Ang II and Ang II plus L-NAME gained 21.3±2.6 and 3.7±7.9 g, respectively (\(P<0.05\)). Weight gain was normal in the L-NAME group (40.6±2.8 g). Chronic administration of Ang II, L-NAME, or the combination of both vasoressors induced a similar and significant increase in systolic blood pressure (Figure 1). Final blood pressure values were 136±4, 167±6, 180±6, and 189±7 mm Hg in control, Ang II, L-NAME, and Ang II plus L-NAME groups, respectively. Heart rate was not significantly modified by any chronic treatment (control value, 323±11 beats per minute).

Ang II administration increased media thickness and media-lumen ratio of basilar arteries without modifying the lumen diameter (Table 2). The CSA and growth index were also significantly increased by Ang II (Table, Figure 2). The administration of L-NAME, alone or in combination with Ang II, produced an increase in media thickness and media-lumen ratio comparable to that in the Ang II–treated animals. However, these changes were accompanied by a reduction of lumen diameter, without modification of CSA or growth index (Table, Figure 2). A significant statistical interaction was observed among the groups for the growth index (two-way ANOVA).

There was a strong positive correlation between systolic blood pressure and media-lumen ratio in basilar arteries
In contrast, no correlation was observed between systolic blood pressure and CSA. The distensibility of the basilar artery, as determined by the pressure–lumen diameter curves, was similar in all groups (slope $= 2 \text{ mm/mm Hg}$; data not shown), implying that the modifications of vascular structure could not be accounted for by increased stiffness of the vessel wall.

**Discussion**

In the present study we investigated the in vivo modulation of Ang II–induced vascular hypertrophy by endogenous NO by chronically blocking its synthesis with L-NAME. Our results not only suggest that endogenous NO does not exert a marked antiproliferative effect, but they rather demonstrate that NO seems necessary for hypertrophy to develop in this model of Ang II–induced hypertension.

The chronic administration of an initially subpressor dose of Ang II induced an increased media thickness and media-lumen ratio of basilar arteries. This alteration of the vascular geometry followed what has been described as hypertrophic remodeling, as shown by the significant increase in CSA and growth index. Furthermore, the calculated remodeling index (8%) does not support eutrophic remodeling (rearrangement of the vascular tissue around a smaller lumen) as an important contributor to the remodeling process. Our results therefore confirm those previously reported in this model, which also emphasized that hypertrophy, and not hyperplasia, explains

**Table**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Ang II</th>
<th>L-NAME</th>
<th>Ang+L-NAME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumen diameter, $\mu$m</td>
<td>298±9</td>
<td>287±5</td>
<td>266±6*</td>
<td>265±3</td>
</tr>
<tr>
<td>External diameter, $\mu$m</td>
<td>352±9</td>
<td>351±6</td>
<td>328±7*</td>
<td>326±4†</td>
</tr>
<tr>
<td>Media thickness, $\mu$m</td>
<td>27.1±0.8</td>
<td>31.7±0.6*</td>
<td>30.8±1.3*</td>
<td>30.2±1.0</td>
</tr>
<tr>
<td>Mean CSA, $\mu$m$^2\times10^1$</td>
<td>27.0±0.8</td>
<td>30.9±0.7*</td>
<td>27.9±1.3</td>
<td>27.6±0.7†</td>
</tr>
</tbody>
</table>

*P<0.05 compared with control rats.
†P<0.05 compared with Ang II–treated rats.

See “Materials and Methods” for statistical comparisons selected a priori. Except for mean CSA, the values given were obtained at 35 mm Hg of perfusion pressure, as published in previous studies. Similar statistics were also obtained at 25, 45, and 55 mm Hg.

**Figure 2.** Media thickness–lumen diameter ratio (A) and growth index in basilar arteries (B) of rats treated for 2 weeks with respective treatments ($n=7$ per group). The growth index is calculated as a ratio of the difference between the treatment CSA (CSATRx, Table) and the control CSA (CSA_Control) divided by CSA_Control (CSA_Control). Thus, the control group has a growth index of zero. A, *P<0.05 compared with control (ANOVA+Bonferroni). The Ang II+NAME group was not significantly different from the Ang II group. B, *P<0.05 compared with zero (one-sample analysis); †P<0.05 compared with Ang II alone (ANOVA+Bonferroni). A significant interaction was found for the growth index (two-way ANOVA).

**Figure 3.** Correlation analysis between systolic blood pressure and media-lumen ratio (A) or CSA (B) in the four treatment groups. These results demonstrate a relationship between media-lumen ratio and arterial pressure but not between CSA, an index of vascular hypertrophy, and arterial pressure. Therefore, the pressure-dependent change in media-lumen ratio does not necessarily involve vascular hypertrophy. Indeed, the relationship between media-lumen ratio and CSA was not significant ($r=0.342$; data not shown).
the increased CSA, at least in mesenteric arteries. However, we did not measure VSMC number and size in basilar arteries in this study, and it remains possible that the increased CSA was due to an increased cell number or to an enhanced production of extracellular matrix in these small arteries, although there was no noticeable change in vascular stiffness.

The chronic administration of L-NAME induced an increase in systolic blood pressure and media-lumen ratio comparable to that in Ang II–treated animals. The strong correlation between blood pressure and media-lumen ratio supports the notion that this parameter of vascular morphology is adaptive to the increase in pressure. However, the process involved with L-NAME is eutrophic remodeling (reduced lumen diameter without increase in the CSA; calculated remodeling index for the basilar artery: 97%) instead of hypertrophic remodeling. This confirms our previous reports in L-NAME–induced hypertension. The new finding of this study is that the chronic inhibition of endogenous NO production did not enhance the proliferative efficacy of Ang II, in contrast to what has been suggested from some studies in cultured VSMC. Since we have previously shown that the same chronic dose of L-NAME inhibits as much as 50% of NO synthase activity, we would therefore expect at least part of the antiproliferative properties of NO to be eliminated by L-NAME. In contrast, our results obtained in the basilar artery support the notion that NO may be important to facilitate VSMC proliferation in vivo, as suggested by a recent study with fibroblast growth factor in freshly dissociated VSMC. The significant statistical interaction of the growth index confirms that blockade of NO synthesis inhibits Ang II–induced increase in CSA.

Similar findings have also been reported in the DOCA-salt model of hypertension. However, in this model the RAS is blunted, and Ang II is an unlikely candidate to explain the vascular hypertrophy. Indeed, vascular hypertrophy is not influenced by angiotensin-converting enzyme inhibitors. Nonetheless, chronic L-NAME treatment inhibited hypertrophy of the heart and of the small arteries. In the aorta, however, L-NAME potentiated the hypertrophy. This observation was also recently reported for Ang II, as a chronic L-NAME treatment enhanced Ang II–induced hypertrophy of the aorta. Thus, the role of NO on vascular growth seems to differ between large conduit and small resistance arteries, an observation that was already emphasized for other vascular functions. It is therefore interesting to note that VSMC used in culture systems are normally derived from the aorta. But even with aortic VSMC, culture conditions seem to determine the effect of NO on cellular growth. In vivo, however, NO appears to be necessary for small arteries to proliferate, the stimulus being Ang II or DOCA-salt treatment.

From the present experiments, it is not possible to determine the mechanism by which NO can enhance vascular hypertrophy. However, from the results reported by Hassid et al in freshly dissociated VSMC, it is reasonable to speculate that an increased production of cGMP mediates the effect. Furthermore, cGMP levels have been shown to be reduced in the L-NAME model of hypertension. This reduction could explain the blunted hypertrophy, but this hypothesis needs to be addressed further. There are concerns that L-NAME may actually prevent hypertrophy by an action that is unrelated to NO synthase blockade. One attempt to test this hypothesis was recently presented, and although 1 mmol/L of L-NAME blunted mitogen-induced cellular replication, the same dose was ineffective in inhibiting stimulated protein synthesis in VSMC. Thus, it seems unlikely that L-NAME exerted a direct inhibition of Ang II–induced vascular hypertrophy in the present study, if indeed hypertrophy is the mechanism involved in the increased CSA (see above). High doses of L-NAME have also been shown to antagonize muscarinic receptors in vitro, and although stimulation of these receptors enhances proliferation of glial and prostate cancer cells, the relevance of this antagonism to the modulation of vascular hypertrophy is undetermined.

Although the present study was not designed to determine the involvement of the RAS in L-NAME–induced hypertension, the use of Ang II warrants discussion of this aspect. Measurement of PRA yielded conflicting results regarding the implication of the RAS in this model of hypertension. Indeed, some reports show increased PRA, whereas others do not show any alteration or even show a decrease. And one study shows a different effect depending on the salt diet. To our knowledge, only one study measured plasma Ang II levels directly, and the authors reported a reduced concentration of the peptide. The conflicting data do not seem to be related to the dose of L-NAME or to the duration of treatment. Some have suggested that hypertrophy in this model is proportional to the activity of the RAS. The lack of hypertrophy in our study suggests that RAS activity was not enhanced, although we did not measure PRA to confirm this. In most studies, angiotensin-converting enzyme inhibitors and AT1-receptor antagonists are effective to lower pressure, especially if given chronically. However, it is not known whether this is due to interruption of a hyperactive RAS or to enhancement of endothelium-dependent vasodilation, as suggested by several groups, including ours.

In conclusion, the inhibition of NO synthesis did not enhance Ang II–stimulated vascular growth, arguing against an important antiproliferative action of NO in small arteries in vivo. In contrast, the change in vascular geometry resulting from the combination of L-NAME and Ang II appeared similar to that observed with L-NAME alone and consisted of pressure-dependent eutrophic remodeling. Therefore, in the basilar artery, NO seems to be necessary for Ang II to augment vascular CSA.

Acknowledgments
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References


**Editorial Comment**

The importance of endothelium-derived factors to the regulation of vascular relaxation and constriction has become widely recognized since the original discovery of Furchgott and Zawadzki\(^1\) almost 20 years ago. More recently, endothelium-derived factors also have come to be recognized as potential determinants of vascular structure. Their importance in this regard, however, remains unclear. Thus, any effort to clarify the actions of endothelium-derived factors on vascular structure, as in this study, is particularly welcome and appreciated.

The goal of this study, as stated by the authors, was to determine the effect of endogenous NO on Ang II–induced hypertrophy of small arteries in vivo. Based on the finding that NO inhibits proliferation of VSMC in tissue culture,\(^2\) one might anticipate that a reduction in availability of NO to the vascular wall during treatment with an NO synthase inhibitor would result in increased Ang II–induced hypertrophy of small arteries as a consequence of reduced inhibition of smooth muscle growth. Thus, the finding in this study that L-NAME did not accentuate hypertrophy of basilar artery in Ang II–treated rats, and in fact appeared to prevent hypertrophy, is surprising, if not paradoxical.

One interpretation of this finding is that endogenous NO does not inhibit Ang II–induced hypertrophy in vivo and may even be a necessary factor for hypertrophy to develop. At least one other interpretation is possible, however, NO synthase inhibitors may prevent hypertrophy directly when given in sufficient doses, a possibility suggested by find-
ings in rat VSMCs grown in culture. This possibility is further supported by the recent findings that (1) cerebral arterioles in Sprague-Dawley rats undergo hypertrophy during hypertension induced by L-NAME and (2) carotid clipping does not prevent cerebral arteriolar hypertrophy induced by L-NAME, even though clipping does prevent hypertrophy in cerebral arterioles of stroke-prone spontaneously hypertensive rats.

In conclusion, the present study by Moreau et al provides significant new information with regard to possible effects of NO on vascular structure in vivo. The possibility that NO may be a necessary factor for the development of vascular hypertrophy during chronic hypertension is especially provocative, with important clinical implications regarding reversal or prevention of alterations in vascular structure during treatment of chronic hypertension. In addition, the possibilities and questions raised by this study provide the focus for future investigations in this important area.

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References
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