Noradrenergic Vascular Hyper-Responsiveness in Human Hypertension Is Dependent on Oxygen Free Radical Impairment of Nitric Oxide Activity

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Background—Noradrenergic vascular hyper-responsiveness is a hallmark of essential hypertension. To evaluate whether nitric oxide plays a role in the enhanced vascular response to norepinephrine in hypertension, we examined 32 hypertensives and 28 normotensives who were distributed in 3 experimental series.

Methods and Results—In the first series, we measured the forearm blood flow (FBF) response to a norepinephrine infusion under control conditions and during the infusion of L-N-monomethylarginine (L-NMMA). Norepinephrine evoked dose-dependent vasoconstriction that was greater in hypertensives than in normotensives (maximum FBF, $-61 \pm 1$ versus $-51 \pm 1$%; $P<0.01$). During L-NMMA infusion, norepinephrine vasoconstriction was not modified in hypertensives; however, it was potentiated in normotensives (maximum FBF, $-64 \pm 2$%; $P<0.01$). In the second series, we tested whether norepinephrine vasoconstriction could be affected by an antioxidant such as ascorbic acid. Norepinephrine vasoconstriction was blunted by ascorbic acid administration only in hypertensives (maximum FBF, $-49 \pm 3$ versus $-63 \pm 2$%; $P<0.01$); the vasoconstriction became similar to that observed in normotensives. During ascorbic acid plus L-NMMA administration, the vascular response to norepinephrine increased to a similar extent in both study groups. To rule out the possibility that the effect of ascorbic acid on norepinephrine vasoconstriction could depend on adrenergic receptor–induced nitric oxide release, in the last series we inhibited endogenous nitric oxide and replaced it with an exogenous nitric oxide donor (sodium nitroprusside). Even in these conditions, ascorbic acid attenuated norepinephrine vasoconstriction only in hypertensives (maximum FBF, $-50 \pm 2$ versus $-62 \pm 1$%; $P<0.01$).

Conclusions—Our data demonstrate that noradrenergic vascular hyper-responsiveness in hypertension is dependent on an impairment of nitric oxide activity that is realized through norepinephrine-induced oxygen free radical production. (Circulation. 2000;102:552-557.)

Key Words: norepinephrine ■ hypertension ■ blood flow ■ endothelium ■ antioxidants

Increased cardiovascular reactivity to the sympathetic nervous system plays a major role in the development and worsening of several human diseases, including hypertension and diabetes.1–6 In hypertension, both exaggerated sympathetic nervous activity and an enhanced vascular responsiveness to norepinephrine, the main sympathetic neurotransmitter, have been reported. On this latter issue, several studies have documented greater vasoconstriction to infused norepinephrine in hypertensive patients when compared with normotensive subjects,7–11 and similar findings are also observed in genetic animal models of hypertension.12

Morphological vascular changes and defects in adrenergic signaling pathways have been proposed as major candidates for the abnormal vascular adrenergic hyper-responsiveness found in hypertension.7,9,11,13–15 However, the observation that norepinephrine vasoconstriction becomes similar in hypertensive and normotensive rats after endothelial denudation or the inhibition of nitric oxide synthesis16 led us to hypothesize that an endothelial nitric oxide mechanism may also be involved in the increased vascular adrenergic responsiveness seen in human hypertension. We previously demonstrated that adrenergic vasoconstriction is the balance of a direct vasoconstrictive effect on smooth muscle and an indirect vasorelaxant action through $\alpha_2$- and $\beta$-adrenergic endothelial receptor–triggered nitric oxide release.17 Thus, a derangement of this balance may also be involved in the enhanced vascular adrenergic responsiveness seen in hypertension.

However, some evidence exists that hypertensive patients have endothelial nitric oxide dysfunction.18–20 Actually, endothelial nitric oxide–dependent vasodilatation is reduced in...
hypertensives compared with normotensives, and it has been proposed that such a defect may depend on increased oxygen-derived free radical production, which impairs the biological action of nitric oxide.21–26

Therefore, it would be noteworthy to explore whether the increased adrenergic vascular responsiveness seen in hypertension is a consequence of a defect in the endothelial nitric oxide modulation of norepinephrine vasoconstriction and, eventually, to clarify whether the use of antioxidant agents that are capable of scavenging oxygen free radicals can alleviate the abnormal vascular adrenergic responsiveness of patients with essential hypertension.

Thus, this study was planned to evaluate the influence of nitric oxide on the forearm blood flow (FBF) response to the intra-arterial infusion of increasing doses of norepinephrine in hypertensives and normotensives and, subsequently, to test the impact of an antioxidant, such as ascorbic acid, on the norepinephrine vascular response.

**Methods**

**Subjects**

The study groups consisted of 32 patients with essential hypertension and 28 matched normotensive subjects. The hypertensives were recruited from the newly diagnosed patients in our outpatient clinic on the basis of the following criteria: age of 20 to 40 years; diastolic blood pressure >95 mm Hg on ≥3 separate occasions in a month; no signs of metabolic, endocrine, renal, or other cardiovascular disease; no presence of secondary forms of hypertension; and never having been treated with any anti-hypertensive medication. The secondary endpoints were: normal FBF, heart rate, and blood pressure of hypertensives compared with normotensives. The hypertensives were recruited from the newly diagnosed patients in our outpatient clinic and 28 matched normotensive subjects. The hypertensives were treated with any anti-hypertensive medication. The secondary endpoints were: normal FBF, heart rate, and blood pressure of hypertensives compared with normotensives.

**Experimental Procedure**

The study began at 8 AM in a quiet room with a constant temperature of 22°C to 24°C. All subjects were studied in a postabsorptive state and, throughout the study sessions, data were analyzed in terms of changes in FBF. Because mean arterial pressure and heart rate did not change significantly throughout the study sessions, data were analyzed in terms of changes in FBF. Because L-NMMA altered resting FBF, data were analyzed as a percent change from baseline. Clinical characteristics of study subjects shown in the Table were compared.

The study was performed in accordance with institutional guidelines for human research. Written informed consent was obtained from all participants.

**Series 1: Effects of L-NMMA on Norepinephrine Vascular Response**

To explore the role of nitric oxide in the norepinephrine-evoked vascular response, we assessed a dose-response curve to norepinephrine in control conditions (during the intrabrachial infusion of saline) and after L-NMMA administration in 12 hypertensives and 11 normotensives (Figure 1).

**Series 2: Effects of Ascorbic Acid on Norepinephrine Vascular Response**

To evaluate whether ascorbic acid influences norepinephrine vasoconstriction, we assessed the dose-response curve to norepinephrine in control conditions, during ascorbic acid administration, and during the concomitant infusion of ascorbic acid plus L-NMMA in 10 hypertensives and 9 normotensives (Figure 1).

**Series 3: Effects of Ascorbic Acid on Norepinephrine Vascular Response During the Clamp of Nitric Oxide Activity**

To clarify whether the effect of ascorbic acid on norepinephrine vascular response is related to a scavenger action on oxygen free radicals and to rule out possible influences on nitric oxide release, we assessed a dose-response curve to norepinephrine in control conditions and during ascorbic acid exposure in 10 hypertensives and 8 normotensives after clamping nitric oxide availability by the coadministration of L-NMMA and sodium nitroprusside (Figure 1).

**Data Analysis**

Because mean arterial pressure and heart rate did not change significantly throughout the study sessions, data were analyzed in terms of changes in FBF. Because L-NMMA altered resting FBF, data were analyzed as a percent change from baseline. Clinical characteristics of study subjects shown in the Table were compared.
by unpaired Student’s t test. Responses to norepinephrine were analyzed by ANOVA for repeated measures; post hoc simultaneous multiple comparisons were done by Bonferroni’s analysis. Results are presented as mean ± SEM.

Results
The Table shows the main characteristics of the study groups. The prevalence of alcohol drinkers was comparable between normotensive and hypertensive groups (6 of 28 versus 8 of 32 subjects). As expected, systolic and diastolic blood pressures were significantly higher in hypertensives compared with normotensives, whereas no change was detected for the other considered variables. Blood pressure, heart rate, and contralateral FBF were not significantly modified during the study sessions (data not shown).

Series 1: Effects of L-NMMA on Norepinephrine Vascular Response
The norepinephrine infusion evoked a dose-dependent FBF decrease that was greater in hypertensives compared with normotensives (−30±1, −50±2, and −61±1 versus −22±1, −39±2, and −51±1%, respectively, for the 3 doses of norepinephrine; P<0.01). L-NMMA administration induced a comparable reduction of FBF in both study groups (−30±2 versus −31±1%; P=NS). During L-NMMA infusion, the response to norepinephrine remained unmodified in hypertensives, but it was significantly enhanced in normotensives (Figure 2).

Series 2: Effects of Ascorbic Acid on Norepinephrine Vascular Response
Ascorbic acid administration did not affect basal FBF in either study group. However, it attenuated the norepinephrine vascular response in hypertensives but not normotensives, so that norepinephrine vasoconstriction became similar between hypertensives and normotensives. Finally, during the coadministration of ascorbic acid and L-NMMA, the norepinephrine vascular response increased to a similar extent in hypertensives and normotensives (Figure 3).

Series 3: Effects of Ascorbic Acid on Norepinephrine Vascular Response During the Clamp of Nitric Oxide Activity
The exposure to L-NMMA reduced basal FBF similarly in hypertensives (23.07±1.4 versus 15.63±1.1; P<0.01) and normotensives (24.16±1.3 versus 16.17±0.8; P<0.01); however, the dose of sodium nitroprusside necessary to restore basal FBF was markedly greater in hypertensives compared with normotensives (0.94±0.05 versus 0.41±0.03 ng min⁻¹ mL⁻¹; P<0.01). During nitric oxide clamp, norepinephrine vasoconstriction was higher in hypertensives compared with normotensives, and the subsequent exposure to ascorbic acid could blunt FBF response to norepinephrine in hypertensives but not in normotensives (Figure 4).

Discussion
In this study, we evaluated the role of nitric oxide in the forearm vascular response to norepinephrine, the main sympathetic neurotransmitter, in normotensives and hypertensives. The major observations were that nitric oxide modulation of norepinephrine-evoked vasoconstriction is impaired in hypertensive patients, and this alteration is corrected by an antioxidant agent, such as ascorbic acid. Moreover, in patients with essential hypertension, ascorbic acid selectively

Clinical Characteristics of Study Groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normotensives</th>
<th>Hypertensives</th>
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<tbody>
<tr>
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<td>32</td>
</tr>
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<td>Age, y</td>
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<td>CHOL, mg/dL</td>
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<td>FBF, mL min⁻¹ mL⁻¹</td>
<td>23.5±0.8</td>
<td>22.1±0.7</td>
</tr>
</tbody>
</table>

SBP indicates systolic blood pressure; DBP, diastolic blood pressure; GLU, plasma glucose; CHOL, plasma total cholesterol; and BMI, body mass index.
affects norepinephrine vascular response without influencing basal FBF.

Nitric oxide takes part in the regulation of basal vascular tone, but it is also further produced during complex vascular responses to selective agonists, such as norepinephrine. Therefore, a tonic release of nitric oxide is involved in basal vascular tone and a phasic release of nitric oxide modulates the vascular response evoked by diverse stimuli. Our study demonstrates, for the first time in humans, that a nitric oxide–dependent mechanism is involved in the enhanced vascular responsiveness to norepinephrine in essential hypertension. In particular, the inhibition of nitric oxide production potentiates norepinephrine-evoked vasoconstriction in normotensive subjects; however, it does not influence the vascular response to the sympathetic neurotransmitter in hypertensive patients. These findings suggest that the phasic nitric oxide component involved in the norepinephrine vascular response is defective in essential hypertension. More importantly, in the nitric oxide–free forearm, the vasoconstriction evoked by norepinephrine is similar in normotensives and hypertensives, indicating that nitric oxide dysfunction may be crucial for the development of the vascular hyper-responsiveness to norepinephrine.

The current data agree with previous observations showing that hypertensive patients have an impairment of phasic endothelial nitric oxide activity, as demonstrated by the reduced vasodilatation to acetylcholine. These data also extend previous observations; they delineate the significance of endothelial nitric oxide dysfunction in the context of complex vascular responses, such as that to adrenergic stimuli. Actually, because the hypertensives enrolled in the current study were mainly young and had minimal cardiovascular organ damage and, thus, had increased adrenergic tone, the results of the current study may also indicate that the dysfunction in phasic endothelial nitric oxide activity contributes to the adrenergic vascular hyper-responsiveness typical of this hypertensive condition.

The dysfunction of endothelial nitric oxide in hypertension could involve a decreased endothelial nitric oxide synthesis or an increased inactivation of nitric oxide by superoxide anions. Because endothelial nitric oxide dysfunction in hypertension is not corrected by the increased availability of substrates for nitric oxide synthesis but is improved by the acute administration of ascorbic acid, we extended our study to explore whether such an antioxidant agent may also influence the abnormal vascular response to norepinephrine. In hypertensives, ascorbate treatment decreased norepinephrine vasoconstriction, which became similar in magnitude to that observed in normotensives. In addition, during ascorbic acid administration, the inhibition of nitric oxide potentiated the vascular response to norepinephrine to a similar extent in both normotensives and hypertensives. Thus, acute treatment with ascorbic acid can save the vascular hyper-responsiveness to norepinephrine in essential hypertension, restoring the nitric oxide modulation of norepinephrine vasoconstriction. These findings further support the concept of a functional component that is critical for the elevated vascular resistance in patients with essential hypertension.

The mechanism underlying the beneficial effect of ascorbic acid on endothelial nitric oxide may involve a scavenger...
action on oxygen free radicals, which protect nitric oxide from inactivation. Thus, the evidence that ascorbic acid does not affect the norepinephrine vascular response in normotensive subjects but attenuates the vascular response to norepinephrine only in patients with essential hypertension indicates that only in this pathological condition can ascorbate exert its scavenger action. This suggests that in hypertensives, norepinephrine itself may have a major impact on oxygen free radical metabolism, which impairs phasic nitric oxide efficacy.

However, it was recently demonstrated that ascorbic acid can also potentiate agonist-induced nitric oxide production in human endothelial cells. Therefore, the ascorbate-induced normalization of the norepinephrine vascular response in hypertension could also be ascribed to a facilitating action on adrenergic receptor–evoked nitric oxide production. To clarify this issue, we inhibited endogenous nitric oxide production and replaced it with an exogenous nitric oxide donor (sodium nitroprusside), such that the putative effect of ascorbic acid on norepinephrine-induced nitric oxide production was abolished. In these experimental conditions, ascorbate acts mainly as a scavenger and, therefore, the persistence of its beneficial effect on norepinephrine vasoconstriction only in hypertensive patients indicates that norepinephrine per se can produce oxidative stress that influences its vascular response. Furthermore, the attenuation of the norepinephrine vasoconstriction induced by ascorbate only in hypertensives also suggests that the vascular hyper-responsiveness to norepinephrine in hypertensive patients is due to an enhanced inactivation of nitric oxide realized by oxygen free radicals specifically produced by norepinephrine.

Our findings are consistent with the results of Wu et al, who recently demonstrated that an endogenous antioxidant, such as superoxide dismutase, significantly blunts norepinephrine-induced contraction in the aorta of hypertensive but not normotensive rats. They also demonstrated that the hypertensive rat strain also has a lower cellular content of antioxidants. Furthermore, Laursen et al reported that oxygen free radicals are not involved in the blood pressure increase induced by norepinephrine in normotensive rats; this is in keeping with our observations that in a normotensive background, oxygen free radical metabolism does not participate in the hemodynamic action of norepinephrine. Therefore, it seems reasonable to speculate that in essential hypertension, norepinephrine may create an imbalance between the antioxidant/pro-oxidant cellular mechanisms, thus increasing nitric oxide inactivation and resulting in vascular hyper-responsiveness to the main sympathetic neurotransmitter.

Regarding tonic nitric oxide activity, the results of our study show that L-NMMA evokes a similar degree of vasoconstriction between hypertensives and normotensives, which suggests that nitric oxide release is not impaired in our hypertensive population. On this particular issue, some authors have reported results similar to ours, whereas several other groups of investigators have demonstrated that L-NMMA evokes reduced vasocostriction in hypertensives only. A careful analysis suggests that these conflicting reports may be explained by the fact that the latter studies were performed in older populations of hypertensive patients; a further worsening of endothelial nitric oxide activity has been demonstrated in such patients.

Although hypertensive patients have both basal blood flow and hemodynamic responses to L-NMMA similar to those observed in normotensives, they need a higher amount of exogenous nitric oxide to clamp blood flow to its basal level, suggesting that hypertensives have reduced sensitivity to nitric oxide. This conclusion is in keeping with the findings of Preik et al, who reported impaired effectiveness of nitric oxide donors in the forearm of hypertensive patients. The observation reported by other authors that sodium nitroprusside produces a similar vasorelaxation in normotensives and in hypertensives could seem to conflict with the reduced sensitivity to nitric oxide in hypertensives that was observed in our study. However, we administered sodium nitroprusside under conditions and at a dose completely different from those of previous studies. In particular, we infused sodium nitroprusside into a forearm free of endogenous nitric oxide and at a final dose 8 to 10 times less than the minimal amount used in other studies. Furthermore, the dose of sodium nitroprusside used in our study is effective in the range of physiological vasoconstrictor changes. Therefore, our results indicate that hypertensive patients have a resistance to sodium nitroprusside in the physiological operative range of endogenous nitric oxide, which can be offset by higher amounts of nitric oxide.

In summary, this study demonstrates that in human hypertension, a nitric oxide defect enhances norepinephrine vasoconstriction; this alteration is corrected by acute ascorbic acid administration. The clinical relevance of our results is that antioxidant therapy may be able to restrain the effect of the
systolic arterial pressure on vascular tissues through increased nitric oxide availability in hypertensives. In addition, because several epidemiological studies have revealed that a high intake of antioxidants reduces cardiovascular morbidity and mortality,41–44 we can hypothesize that part of this beneficial effect may be due to the reduced vascular impact of the sympathetic nervous system, which represents a major cardiovascular risk factor.

References


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