Increased vascular responsiveness to α₂-adrenergic stimulation during NOS inhibition-induced hypertension

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Kanagy, Nancy L. Increased vascular responsiveness to α₂-adrenergic stimulation during NOS inhibition-induced hypertension. Am. J. Physiol. 273 (Heart Circ. Physiol. 42): H2756–H2764, 1997.—Increased vascular resistance during systemic nitric oxide synthase (NOS) inhibition is dependent on adrenergic vasoconstriction. This study tested the hypothesis that increased vascular sensitivity to adrenergic agonists contributes to this vasoconstriction. Superior mesenteric arteries and thoracic aortae from male Sprague-Dawley rats drinking water containing N-ω-nitro-L-arginine (L-NNA; 14 days, 60 mg·kg⁻¹·day⁻¹) and control rats were cut into helical strips, and endothelium was removed for contractile experiments. L-NNA arteries were more sensitive to UK-14304 (α₂-adrenergic agonist) and norepinephrine (NE), whereas responses to phenylephrine (PE) were not different [concentration causing 50% maximal response (EC₅₀) L-NNA vs. control: UK-14304, 0.071 vs. 0.71 μmol/l; NE, 1.15 vs. 9.95 nmol/l]. Yohimbine, an α₂-selective antagonist, caused a concentration-dependent inhibition of contraction to NE only in L-NNA arteries (EC₅₀ = 6.3 vs. 1.6 nmol/l at 1 nmol/l yohimbine), whereas prazosin shifted NE curves similarly in arteries from both groups. Yohimbine (10 nmol/l) inhibited contractions to UK-14304 (EC₅₀ = 59 μmol/l vs. 17 μmol/l) but not contractions to PE, whereas prazosin inhibited both. These data indicate that L-NNA-induced hypertension leads to increased sensitivity of prazosin-sensitive α₂-adrenoceptors, an upregulation that could cause the increased vasoconstrictor response to NE in this model of hypertension.

nitric oxide; nitric oxide synthase; α₂-adrenoceptors; UK-14304; vascular smooth muscle

THE ROLE OF NITRIC OXIDE (NO) IN CHRONIC BLOOD PRESSURE REGULATION HAS BEEN CONCLUSIVELY ESTABLISHED BOTH IN PHARMACOLOGICAL ANTAGONISM STUDIES (4, 23, 30) AND IN GENETIC DELETION STUDIES (9, 35). IN ADDITION, IT IS APPARENT THAT ACTIVATION OF THE SYMPATHETIC NERVOUS SYSTEM PARTICIPATES IN THE HYPERTENSION THAT Develops DURING CHRONIC Blockade OF NO PRODUCTION (23, 30). However, it is not clear whether the increased sympathetic vasoconstrictor tone depends entirely on elevated sympathetic outflow or elevated vascular sensitivity to sympathetic discharge may also play a role. Previous studies examining vascular reactivity to adrenergic vasoconstrictors in this model of hypertension showed that endothelium-intact aortic rings have profound inhibition of relaxation to acetylcholine but no change in contractile response to norepinephrine (see Ref. 11). Another study also in endothelium-intact aortic rings found decreased force generation at maximal concentrations of phenylephrine and angiotensin II, suggesting that elevated vasoconstriction through these systems might downregulate the maximal contractile responses (7). However, neither of these previous studies examined contractile responses in the absence of endothelium, and it is not clear whether the changes were at the level of the endothelium or the smooth muscle. The current study was designed to determine whether the hypertension induced by inhibition of NO synthase (NOS) alters vascular smooth muscle sensitivity to adrenergic vasoconstrictors, independently of the loss of endothelial vasodilation.

To test this hypothesis, the contractile responses to α₂-adrenergic agonists were compared in superior mesenteric artery and thoracic aorta from NOS-inhibitor-treated and control rats. These studies examined the hypothesis that increased sensitivity to α₂-adrenoceptor (AR) stimulation contributes to the elevated vascular tone in NOS-inhibited rats.

METHODS

Animals

One hundred male Sprague-Dawley rats (250–300 g) were divided into two groups, treated and control. The treated group drank water containing the NOS inhibitor N-ω-nitro-L-arginine (L-NNA, 0.5 g/l), whereas control rats drank tap water. Systolic blood pressure was measured every 3–4 days using an indirect tail cuff method (plethysmographic detection, IITC, Woodland Hills, CA), and daily water intake was monitored. All procedures were approved by the animal use committee at the University of New Mexico and conform to National Institutes of Health guidelines for animal use.

Tissue Preparation

On day 14 of treatment, animals were anesthetized with pentobarbital sodium (50 mg/kg) and exsanguinated. The thoracic aorta and superior mesenteric artery were removed and placed in physiological saline solution containing (in mmol/l) 130 NaCl, 4.7 KCl, 1.18 KH₂PO₄, 1.17 MgSO₄ · 7H₂O, 14.9 NaHCO₃, 5.5 dextrose, 0.026 CaNa₂-EDTA, and 1.6 CaCl₂, pH 7.3. Vessels were cleaned, cut into helical strips, and denuded of endothelium by gently rubbing the lumen side of the strip with a moistened cotton-tipped applicator. The denuded strips were mounted in tissue baths filled with physiological saline solution (PSS) maintained at 37°C and aerated with 95% O₂:5% CO₂. The removal of the endothelium was confirmed by failure of contracted strips (10⁻⁵ mol/l phenylephrine) to relax to acetylcholine (10⁻⁶ mol/l, <5% relaxation). Two strips were cut from each artery for use in the antagonist studies, and n indicates the number of animals used. One segment from an L-NNA-treated rat and one from a control rat were paired in each bath, and isometric force generation was recorded with FT03 Grass transducers (Quincy, MA) connected to Gould recorders (Harvard, MA). Mesenteric artery strips were stretched with 700-mg passive force and aortic strips with 1,200-mg force to allow a maximum detection of contraction. After the stretch, strips were equilibrated 1 h with washing every 15 min before experiments were performed.
Experimental Protocols

Sensitivity to adrenergic agonists. After equilibration, cumulative concentration-response curves were generated for each of three different adrenergic agonists. These studies were conducted to evaluate the ability of selective α1- and α2-adrenergic agonists to contract mesenteric arteries via postsynaptic receptors. Contractile responses to norepinephrine (nonselective), phenylephrine (α1-selective), and UK-14304 (α2-selective) were evaluated by adding increasing concentrations of agonist to the tissue baths and recording the tension generated. Contractions are expressed as milligrams of developed force.

Sensitivity to adrenergic antagonists. A second set of experiments using mesenteric artery strips evaluated the contribution of α1- and α2-adrenergic activation to norepinephrine contraction in arteries from L-NNA-treated and control rats. Cumulative concentration-response curves to norepinephrine were obtained in the absence and presence of increasing concentrations of either the α1-selective antagonist prazosin (0.1, 1.0, 10 nmol/l) or the α2-selective antagonist yohimbine (1.0, 10, 100, 1,000 nmol/l). Vehicle-treated tissues were used to evaluate the ability of mesenteric arteries to develop consistent force during repeated concentration-response curves. Contractions are expressed as milligrams of developed force.

Specificity of UK-14304. A third series of experiments in mesenteric arteries evaluated the specificity of UK-14304 to cause contraction through activation of α2-ARs. Tissues were treated with prazosin (1 nmol/l), yohimbine (100 nmol/l), or vehicle (distilled H2O), and then cumulative concentration-response curves to either UK-14304 or phenylephrine were generated.

Role of voltage-sensitive Ca2+ influx. The following two protocols were designed to evaluate sensitivity to membrane depolarization and to changes in intracellular Ca2+. First, aortic strips were placed in normal PSS containing 1.6 mmol/l CaCl2. Increasing concentrations of KCl were added to the bath to depolarize the membrane by disrupting the K+ gradient across the cell membrane to stop K+ efflux from the cell and raise the membrane potential toward the activation potential for voltage-sensitive Ca2+ channels (8). An increased sensitivity to K+ depolarization would suggest that the membrane already exists in a depolarized condition. The second protocol placed arterial segments in a depolarizing solution (high K+ concn) that was Ca2+ free. Ca2+ was then added, and contractile responses were recorded. An increased sensitivity to Ca2+ under depolarizing conditions would suggest that there is either a difference in permeability to Ca2+, such as more available channels or larger conductance channels, or increased sensitivity to Ca2+ at the level of the contractile proteins.

Data Analysis and Statistics

Data are reported as means ± SE. Responses were expressed as a percentage of the maximum, and a log-log transformation was performed for calculation of EC50 (concentration that caused 50% maximal response) values. Transformed data were curve fitted using an unweighted least-squares linear regression. Individual points on concentration-response curves for the two groups were compared using a one-way analysis of variance with the Kruskal-Wallace post hoc test of significance. Unpaired Student’s t-tests was used to compare systolic blood pressures, absolute force measurements, threshold values, and EC50 values of the transformed data between animal groups. When multiple t-tests were used for comparisons between groups, the Bonferroni adjustment for multiple testing was employed. A P value ≤ 0.05 was considered statistically significant.

RESULTS

Animals

Systolic blood pressure was significantly increased by day 4 (L-NNA = 178 ± 7 mmHg, control = 138 ± 3 mmHg) and remained elevated through day 14 (L-NNA = 217 ± 7 mmHg, control = 140 ± 2 mmHg) (Table 1). The addition of L-NNA to the drinking water caused a slight decrease in daily water intake (L-NNA = 40 ± 0.4 ml, control = 44 ± 0.4 ml), but intake was still in the normal range and drug intake averaged 63 ± 0.7 mg·kg−1·day−1 throughout the treatment period (Table 1). These data show that L-NNA causes sustained hypertension and that rats were hypertensive for at least 10 days before contractile experiments.

Sensitivity to Adrenergic Agonists

There was no difference in the sensitivity to phenylephrine in arterial segments from the two groups (Fig. 1). In contrast, the sensitivity to norepinephrine was significantly increased in the L-NNA arteries, evidenced as a rightward shift at low concentrations of the nonselective agonist (Fig. 2). The α2-agonist UK-14304 caused a concentration-dependent contraction in all of the tissues (Fig. 3), but only the artery segments from L-NNA rats responded at concentrations <0.1 μmol/l. This indicates that the response to UK-14304 in the control tissues might have been caused by nonspecific activation of α1-ARs (31). The response to this α2-agonist was shifted leftward and upward in the L-NNA arteries, indicating increased sensitivity to this contractile agent (Fig. 3 and Table 2).

Table 1. Body weight, blood pressure, water intake, and L-NNA intake

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Weight, g</th>
<th>Systolic Blood Pressure, mmHg</th>
<th>Water Intake, ml/day</th>
<th>L-NNA Intake, mg·kg−1·day−1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>day 1</td>
<td>273 ± 4</td>
<td>143 ± 2</td>
<td>33 ± 3</td>
<td>NA</td>
</tr>
<tr>
<td>day 4</td>
<td>299 ± 9</td>
<td>139 ± 2</td>
<td>41 ± 1</td>
<td>NA</td>
</tr>
<tr>
<td>day 7</td>
<td>312 ± 4</td>
<td>138 ± 2</td>
<td>45 ± 1</td>
<td>NA</td>
</tr>
<tr>
<td>day 14</td>
<td>331 ± 4</td>
<td>140 ± 1</td>
<td>43 ± 1</td>
<td>NA</td>
</tr>
<tr>
<td>L-NNA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>day 1</td>
<td>271 ± 5</td>
<td>140 ± 2</td>
<td>35 ± 1</td>
<td>63 ± 2</td>
</tr>
<tr>
<td>day 4</td>
<td>287 ± 5</td>
<td>172 ± 5*</td>
<td>38 ± 1</td>
<td>65 ± 2</td>
</tr>
<tr>
<td>day 7</td>
<td>300 ± 3*</td>
<td>192 ± 3*</td>
<td>43 ± 2</td>
<td>72 ± 3</td>
</tr>
<tr>
<td>day 14</td>
<td>315 ± 4*</td>
<td>210 ± 3*</td>
<td>43 ± 2</td>
<td>69 ± 3</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 50 rats/group. N-nitro-L-arginine (L-NNA)-treated group had a significant increase in blood pressure by day 4, and systolic pressure continued to rise until day 7, when it reached a plateau and remained elevated for duration of treatment period. Although control rats tended to be heavier than treated rats, difference was not statistically significant. Water intake was initially decreased in L-NNA rats but returned toward control, and there were no differences between the 2 groups by final day. Drug intake averaged 63 ± 0.7 mg·kg−1·day−1 throughout treatment period. *Significant differences between L-NNA and control groups. NA, not administered.
These data indicate that there is a selective increase in sensitivity to $\alpha_2$- compared with $\alpha_1$-agonists in arterial segments from L-NNA-treated rats.

**Sensitivity to Adrenergic Antagonists**

Yohimbine (0.001–1 µmol/l) inhibited norepinephrine contraction in all tissues, but a 100-fold higher concentration was required to shift the concentration-response curves in arterial segments from control rats. Only at 1 µmol/l was there a significant increase in the EC50 value for the control arteries (Fig. 4A). In contrast, prazosin (0.1–10 nmol/l) caused similar concentration-dependent rightward shifts in norepinephrine curves in both control and L-NNA arteries (Fig. 5, A and B, respectively). These data provide further support of a selective increase in sensitivity of $\alpha_2$-AR after L-NNA treatment in vivo.

**Specificity of UK-14304**

To determine whether contraction to the adrenergic agonist UK-14304 was mediated by $\alpha_1$- or $\alpha_2$-ARs, cumulative concentration-response curves to both phenylephrine and UK-14304 were constructed in the presence of 10 nmol/l yohimbine or 1 nmol/l prazosin. Yohimbine (10 nmol/l) did not inhibit contractions to phenylephrine (Fig. 6, A (control) and B (L-NNA)) but did cause a rightward shift in the UK-14304 curves in L-NNA tissues (Fig. 6, C (control) and D (L-NNA)). This provides additional evidence that $\alpha_2$-AR mediate a portion of the contraction to norepinephrine only in L-NNA tissues and that the augmented response to UK-14304 in arteries from L-NNA-treated rats is mediated by $\alpha_2$-AR.

The response to phenylephrine in the presence of prazosin was shifted to the right in a parallel manner (Fig. 7, A (control) and B (L-NNA)), illustrating the ability of this concentration of prazosin to inhibit $\alpha_1$-mediated contraction. In addition, the contractile response to UK-14304 was also significantly inhibited by prazosin (Fig. 7, C (control) and D (L-NNA)). This indicates that UK-14304 causes contraction through an $\alpha$-AR that is sensitive to prazosin. This includes $\alpha_1$-AR and $\alpha_{2B}$- and $\alpha_{2C}$-AR (24).

**Role of Voltage-Sensitive Ca^{2+} Influx**

Arteries from L-NNA rats were extremely sensitive to KCl, and the addition of 0.5 mmol/l KCl caused a large contraction in arterial strips from L-NNA rats (Fig. 8, A and B), whereas much higher concentrations were required to contract control arteries. In contrast, arteries from both groups displayed similar contractile responses to increasing concentrations of Ca^{2+} under depolarizing conditions (Fig. 8, C and D). This suggests that membrane potential may be altered in arterial segments from L-NNA tissues (Fig. 6, C (control) and D (L-NNA)). This provides additional evidence that $\alpha_2$-AR mediate a portion of the contraction to norepinephrine only in L-NNA tissues and that the augmented response to UK-14304 in arteries from L-NNA-treated rats is mediated by $\alpha_2$-AR.
smooth muscle during L-NNA-induced hypertension but that Ca²⁺ sensitivity is not altered (8).

**DISCUSSION**

Chronic inhibition of NOS causes an increase in blood pressure that is dependent on elevated total peripheral resistance (4). Previous studies suggest that this increased vasoconstriction requires an intact sympathetic nervous system (30) and is mediated by increased adrenergic vascular tone (23), which can be caused by either increased release of adrenergic catecholamines or by increased sensitivity of vascular smooth muscle to catecholamines. The current study tested the hypothesis that NOS-inhibition hypertension increases the sensitivity of vascular smooth muscle to α-adrenergic agonists. The results revealed that after 2 wk of systemic NOS inhibition, there is an increased sensitivity to the nonselective adrenergic agonist norepinephrine in endothelium-denuded thoracic aorta and superior mesenteric artery. Furthermore, this increased sensitivity appears to be mediated by upregulation of α₂-AR without a change in α₁-AR.

The thoracic aorta is not innervated, whereas the superior mesenteric artery is well innervated by sympathetic fibers, yet the two arteries showed similar changes in sensitivity to adrenergic stimulation. The similarity in the changes in the two arteries suggests that sympathetic innervation in not responsible for, nor does it modulate, the vascular changes observed (i.e., increased norepinephrine release acting locally on the smooth muscle does not cause the change). Rather, local inhibition of endothelial NOS, circulating factors (such as epinephrine or endothelin), or the elevated pressure per se caused the changes. The current study does not differentiate between these possibilities, but future studies to address these points would be of great interest.

In contrast to the current findings, two previous studies in this model of hypertension observed either no change (11) or a decreased (7) contractile response to adrenergic stimulation in the thoracic aorta. These two previous studies used endothelium-intact aortic rings, so it appears that there may be an increased endothelium-dependent vasodilation counteracting contraction after chronic NOS inhibition. In addition, the other two studies both used a different strain of rat (Wistar or Wistar-Kyoto rats compared with Sprague-Dawley rats in the current study), so there may be some strain-specific differences. Finally, the other two studies treated rats for 21 rather than 14 days, so the decreased contractility may be a later consequence of the hypertension. The influence of endothelium is the most obvious difference between the studies. In the study by Küng et al. (11), acute incubation of the endothelium-intact aortic rings with N-nitro-L-arginine methyl ester (L-NAME) normalized contractile responses so that the inhibition appears to be dependent on endothelial NO production. Therefore, during the in vivo condition of L-NAME inhibition, it is not likely that there was a suppressed contractile response. In addition, our study suggests that during the in vivo condition, there would actually be an enhanced response to contractile agents.

Table 2. **EC₅₀ values in thoracic aorta and superior mesenteric artery**

<table>
<thead>
<tr>
<th>Group</th>
<th>Aorta (Norepinephrine)</th>
<th>SMA (Norepinephrine)</th>
<th>Aorta (Phenylephrine)</th>
<th>SMA (Phenylephrine)</th>
<th>UK-14030 Aorta</th>
<th>UK-14030 SMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>EC₅₀ -8.94 ± 0.13</td>
<td>-8.02 ± 0.08</td>
<td>-7.91 ± 0.10</td>
<td>-7.62 ± 0.05</td>
<td>-6.64 ± 0.12</td>
<td>-6.15 ± 0.23</td>
</tr>
<tr>
<td></td>
<td>Max F 968 ± 55</td>
<td>478 ± 40</td>
<td>1,013 ± 160</td>
<td>617 ± 89</td>
<td>742 ± 112</td>
<td>473 ± 32</td>
</tr>
<tr>
<td>L-NNA</td>
<td>EC₅₀ -9.45 ± 0.12*</td>
<td>-8.94 ± 0.19*</td>
<td>-7.99 ± 0.10</td>
<td>-7.83 ± 0.09</td>
<td>-7.46 ± 0.24*</td>
<td>-7.15 ± 0.12*</td>
</tr>
<tr>
<td></td>
<td>Max F 916 ± 46</td>
<td>490 ± 30</td>
<td>943 ± 160</td>
<td>561 ± 60</td>
<td>719 ± 73</td>
<td>401 ± 35</td>
</tr>
</tbody>
</table>

Values (means ± SE; n = 5–9 rats/group) were calculated from straight portion of curve using unweighted least-squares linear regression analysis. Log values were compared using a Student’s t-test. SMA, superior mesenteric artery; EC₅₀, concentration causing 50% of maximal response; F, force (in mg). *Significant differences between control and L-NNA groups.
From these studies, the increased sensitivity to \( \alpha_2 \)-AR stimulation appears to depend completely on a change in the arterial smooth muscle. Indeed, UK-14304 does not cause a contraction in the presence of endothelium in tissues from normotensive rats (unpublished observations).

Although the role of postsynaptic \( \alpha_2 \)-AR in regulating arterial pressure is presently undetermined, there is evidence that \( \alpha_2 \)-AR contribute to vasoconstriction in vivo. First, \( \alpha_2 \)-AR have been found in many vascular beds. PCR amplification detected \( \alpha_1 \) and \( \alpha_2 \)-AR mRNA in rat mesenteric artery and aorta (22), and \( \alpha_2 \)-binding sites were observed in rat tail artery (5, 15) and in dog mesenteric artery (26). In contrast, several autoradiographic studies detected only \( \alpha_1 \)-binding sites in arterial segments from normotensive rats (37). Therefore, \( \alpha_2 \)-AR appear to be present in most arteries, but expression may be heterogeneous between vascular beds and at lower levels than \( \alpha_1 \)-AR.

The contribution of \( \alpha_2 \)-AR to vasoconstriction is also unresolved. There are several reports that \( \alpha_2 \)-agonists elicit contraction in veins but not arteries (3, 6), whereas others observe significant contraction of arteries with \( \alpha_2 \)-agonists (15, 16). Most evidence from in vitro studies indicates that \( \alpha_2 \)-AR are the primary mediators of vasoconstriction in large arteries, whereas \( \alpha_2 \)-AR contribute to contraction in small arteries and in veins (14, 17, 20). In vivo recordings, however, have shown that \( \alpha_2 \)-AR mediate most of the arterial contraction in certain vascular beds such as the rabbit knee joint (19).

In addition, \( \alpha_2 \)-agonists, which are not very potent as contractile agents alone, can act synergistically with other vasoconstrictors to facilitate contraction. In the dog mesenteric artery, the contraction to \( \alpha_2 \)-agonist UK-14304 was leftward shifted after treatment with either KCl or endothelin (27), whereas the \( \alpha_2 \)-agonists UK-14304 and BHT-920 enhanced contraction to \( \alpha_1 \)-AR agonists (31). Finally, exercise-induced coronary vasoconstriction has an \( \alpha_2 \)-AR component in the presence of OS inhibition (10). These observations suggest that postsynaptic \( \alpha_2 \)-AR are important mediators of peripheral sympathetic vasoconstriction under certain conditions.

In hypertension, there is evidence both supporting and refuting the hypothesis that \( \alpha_2 \)-AR contribute to elevated adrenergic vasoconstriction. A significant portion of the constriction in tail arteries from spontaneously hypertensive rats depends on \( \alpha_2 \)-AR stimulation after neural stimulation and norepinephrine infusion.
Fig. 6. A and B: Yoh blockade of PE contractile responses. Yoh (0.1 µmol/l) did not inhibit contractile response to the $\alpha_1$-agonist in either control (A) or L-NNA-treated (B) tissues. This indicates that PE contracts arteries from both L-NNA and control rats by activating $\alpha_1$-AR.

C and D: Yoh blockade of UK-14304 contractile responses. Yoh (10 nmol/l) caused significant rightward shift in cumulative concentration-response curve for the $\alpha_2$-agonist UK-14304 in L-NNA-treated tissues (D) but did not affect the contraction in control tissues (C). This indicates that UK-14304 contracts arteries from L-NNA rats by stimulating $\alpha_2$-AR but contracts arteries from control rats by activating $\alpha_1$-AR.

n, No. of animals.

Fig. 7. A and B: Praz blockade of PE contractile responses. The $\alpha_1$-antagonist (1 nmol/l) inhibited contractile response to PE in both control (A) and L-NNA-treated (B) tissues. This indicates that PE contracts arteries from both L-NNA and control rats by activating $\alpha_1$-AR.

C and D: Praz blockade of UK-14304 contractile responses. This concentration of the $\alpha_1$-antagonist significantly inhibited contractile response to the $\alpha_2$-agonist UK-14304 in both control (C) and L-NNA-treated (D) tissues. This indicates that UK-14304 contracts arteries from both control rats and L-NNA rats by stimulating Praz-sensitive $\alpha$-AR.
whereas that in normotensive controls does not (15, 36). Antagonists of $\alpha_2$-AR lower blood pressure only in hypertensives (24), and both hypertensives and their prehypertensive offspring are characterized by increased platelet $\alpha_2$-AR expression (16). However, Van Zweiten and co-workers (33) observed no differences in vascular $\alpha_2$-AR-mediated forearm blood flow in normotensive and hypertensive patients, so that the contribution of altered $\alpha_2$-AR in hypertension is still undecided.

In the current study, UK-14304 elicited a contraction in arteries from control rats only at concentrations previously shown to activate $\alpha_1$-AR $[-\log EC_{50}$ (pD$_2$) value $= 5.9$, Refs. 27 and 31]. In mesenteric arteries from L-NNA treated rats, however, the pD$_2$ value was 6.9, a value close to that reported for $\alpha_2$-AR in veins (2, 26). In addition, the contraction to UK-14304 in arteries from the L-NNA rats was sensitive to yohimbine, further supporting the assertion of increased sensitivity at $\alpha_2$-AR. In contrast, the pD$_2$ values for the $\alpha_1$-agonist phenylephrine were not different between the two groups (7.6 vs. 7.8), and norepinephrine sensitivity to prazosin was the same in both groups. These data indicate that the contractile response to $\alpha_2$-AR activation in arteries from L-NNA rats was potentiated, even in the absence of other contractile agents. Therefore, even normal levels of NE acting on $\alpha_2$-AR could contribute to the elevated sympathetic tone evident in this model of hypertension (38).

A recent study using genetically altered mice indicates that $\alpha_2$-agonists cause vasoconstriction through $\alpha_{2B}$-AR, because UK-14304 does not raise arterial pressure in the absence of functional $\alpha_{2B}$-AR but does in the absence of either $\alpha_{2C}$- or $\alpha_{2A}$-AR (13). Therefore, changes in $\alpha_{2B}$-AR should lead to enhanced vasoconstriction such as that observed in the present study. Because $\alpha_{2B}$-AR are sensitive to yohimbine and prazosin, whereas $\alpha_{2A}$-AR are insensitive to prazosin (2), the current observation that UK-14304 contraction of L-NNA arteries was shifted leftward by both antagonists suggests that $\alpha_{2B}$-AR mediate this response. In contrast, as previously observed (12), UK-14304 in control tissues was insensitive to both antagonists, confirming that only L-NNA arteries exhibit $\alpha_{2}$-AR contraction.

The signal cascade for $\alpha_2$-AR in vascular smooth muscle has not been absolutely determined but appears to depend almost entirely on Ca$^{2+}$ entry through sarcolemmal channels (3, 21, 32). In contrast, $\alpha_1$-AR primarily couple to phospholipase C (18) and depend on release of Ca$^{2+}$ from sarcoplasmic reticulum stores followed by sustained Ca$^{2+}$ entry (3, 18). Because $\alpha_2$-AR are totally dependent on activation of Ca$^{2+}$ influx, the $\alpha_2$-AR response is maximally effected by changes in membrane potential (31). Therefore, the increased sensitivity to UK-14304 in the present study could be caused by partial membrane depolarization as observed in other models of hypertension (25). Indeed, the increased responsiveness to extracellular K$^+$ suggests that L-NNA treatment may lead to partial depolarization of vascular smooth muscle cells. Further support comes from a previous study in L-NNA-treated rats, in which mesenteric artery had increased sensitivity to the Ca$^{2+}$ channel activator BAY K 8644 (34). Indeed, there are numerous indications in the literature that NO alters membrane potential through its unique effects on K$^+$ channels. Specifically, blockade of NOS decreases guanosine 3',5'-cyclic monophosphate (cGMP)
production and the activity of cGMP-dependent protein kinase (PKG). Both L-type Ca channels (28) and Ca²⁺-sensitive K⁺ channels (1) are phosphorylated by PKG, inhibiting Ca²⁺ entry (29). Although not tested directly, NOS inhibition may have decreased K⁺ current, to produce the changes observed.

In summary, the current study found that there is an increase in α₂-AR-mediated contraction of artery segments after 2 wk of systemic NOS inhibition. Although this study was performed in large arteries, previous studies confirm that contractile changes in large arteries parallel changes in smaller resistance vessels and in whole animal responses (35). Therefore, the observed upregulation suggests that changes in vascular α₂-AR could contribute to elevated peripheral resistance in this model of hypertension, and the mechanism responsible for this upregulation is an intriguing area for future studies.

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