Nitric Oxide Opposes Myogenic Pressure Responses Predominantly in Large Arterioles In Vivo

Cor de Wit, Bernhard Jahrbeck, Christian Schäfer, Steffen-Sebastian Bolz, Ulrich Pohl

Abstract—A myogenic vasoconstriction may amplify the effects of circulating vasoconstrictors. In cremaster arterioles, the contribution of a myogenic component to the constriction on intravenous infusion of norepinephrine (NE) or angiotensin II (Ang II) was studied. Second, the role of endothelium-derived nitric oxide (NO) in the control of these myogenic constrictions and its site of action in the resistance vascular bed was investigated. In 30 anesthetized (pentobarbital) hamsters, the cremaster was prepared for intravital microscopy, and a pneumatic vessel occluder was placed around the aorta to vary blood pressure in the hindquarter of the animal. Intravenous infusion of NE (0.5 nmol/min) increased the systemic blood pressure by 52±2 mm Hg. Simultaneously, constrictions of up to 33±6% were observed in the small arterioles (SAs; maximal inner diameter, 36 to 65 μm). The constrictions were not significantly altered by a local adrenergic blockade but were abolished when the pressure elevation in the cremaster arterioles was blocked by partial occlusion of the abdominal aorta. Diameters in large arterioles (LAs; maximal inner diameter, 65 to 127 μm), however, did not change significantly on NE infusion. Similar responses in the arterioles were observed when the local pressure was increased stepwise from 60 to 120 mm Hg by partial opening of the aortic occluder. However, after treatment of the cremaster tissue with the inhibitor of the NO synthase, Nω-nitro-L-arginine (L-NNA, 30 μmol/L), a significant pressure-induced constriction of up to 16±3% occurred in LAs, whereas the magnitude of the constriction in SAs remained unchanged. L-NNA also abolished the increases in blood flow that were observed with increments in pressure in control animals. Similar results were obtained when Ang II was used to increase blood pressure. We conclude that a myogenic constriction of SAs contributes markedly to the overall response of cremaster arterioles to circulating vasoconstrictors. NO effectively opposes the myogenic response in LAs, thus preventing myogenic constrictions in a vascular region where constriction cannot be fully controlled by metabolic dilation. If this attenuating effect of NO on myogenic constriction also takes place in other organs, it might be a decisive mechanism in controlling changes of total peripheral vascular resistance elicited by vasoconstrictors. (Hypertension. 1998;31:787-794.)

Key Words: endothelium • angiotensin II • norepinephrine • arterioles • blood pressure • microscopy

The Bayliss effect, a myogenic vasoconstriction in response to an increase in transmural pressure and vice versa, is thought to be an important mechanism in keeping capillary filtration pressure constant and in contributing to the autoregulation of blood flow.1 It has also been demonstrated that part of the peripheral vasoconstriction in response to infusions of vasoconstrictors can be attributed to the resulting increase in pressure rather than to a direct pharmacological effect of the compound.2–4 This finding suggests that the myogenic vasoconstriction could represent a mechanism that enhances increases in blood pressure by further increasing peripheral resistance. This potential positive feedback mechanism might lead to instability in the intact circulation and therefore requires control through opposing mechanisms.

A well-adjusted endothelial release of NO can principally exert such a control function. Several studies on isolated vessels,5–7 as well as on isolated organs,8 have demonstrated that a shear stress–dependent augmentation of endothelial NO release can effectively oppose pressure-induced constrictions. At least in certain sections in the microvascular tree, an increase in pressure with simultaneous myogenic response should elevate WSR and eventually wall shear stress. In fact, recent analyses of WSR in skeletal muscle microcirculation have demonstrated that it tended to increase with augmentation in blood pressure.9

A myogenic constriction occurs irrespective of the size or generation in isolated arterial vessels,10,11 whereas in vivo usually only the smallest arterioles react to changes in transmural pressure by vasoconstriction.12 This different behavior suggests the existence of opposing mechanisms acting in vivo with different efficacies along the vascular tree. The exact localization of these potentially counterbalancing mechanisms would be an important prerequisite to understand the processes leading to hypertension after impairment of endothelial function. It is of particular interest to study this question in skeletal muscle arterioles, because skeletal muscle vasculature plays an important role in the control of total peripheral resistance.

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The aim of this study was to investigate the extent of the contribution that myogenic responses make to the vasoconstriction induced by infusion of NE or Ang II in the hamster cremaster in vivo. The second aim of our study was to identify the major site of myogenic constrictions within certain vascular segments. Third, we examined whether the endothelium, through the release of NO, could counterbalance these constrictions and how its action was distributed throughout the vascular tree.

### Methods

#### Preparation

Male golden Syrian hamsters (80 to 150 g body weight) were anesthetized by intraperitoneal injection of pentobarbital (75 mg/kg), followed by continuous administration of the anesthetic (5 to 10 mg·kg\(^{-1}·h\) ) through a jugular vein catheter throughout the experiment. The animals were artificially ventilated (7025 Rodent Ventilator, Hugo Sachs Elektronik) to achieve physiological arterial O\(_2\) and CO\(_2\) partial pressures. Arterial blood samples taken at the end of the experiment revealed a pH of 7.39±0.02, and arterial PO\(_2\) and PCO\(_2\) were 123±15 and 37±2 mm Hg, respectively. The abdominal aorta was exposed distal to the renal arteries through an abdominal midline incision. A pneumatic occluder (Jones) connected to a syringe was placed around the aorta. The left carotid and femoral arteries were cannulated to measure pressure proximal and distal to the vascular occluder by means of pressure transducers (Statham). The pressures were monitored by means of a pen recorder and stored on computer disk for later analysis. The right cremaster muscle was prepared for intravital microscopy as described previously. In some animals, the care of the animals and the conduct of the experiments were in strict accordance with the rules of the German animal protection laws.

#### Experimental Setup

The muscle was superfused with warmed (34°C, physiological for cremaster tissue) bicarbonate-buffered salt solution at a rate of 8 mL/min. The superfusion fluid had a pH of 7.35±0.01, a PO\(_2\) of 33±2.16 mm Hg, and a PCO\(_2\) of 38.8±0.7 mm Hg as measured in samples taken at the edge of the cremaster. Three to five arterioles of different sizes and vascular generation were studied in one single animal. The arterioles were located in adjacent microscopic fields and were examined in each animal consecutively. The same arterioles were studied subsequently under the same protocol. In a subset of experiments (four animals), the increase in pressure on intravenous infusion of NE in the vessels supplying the cremaster was blocked (as monitored in the femoral artery) by partial occlusion of the abdominal aorta. A rapid deflation of the vascular occluder after 3 minutes allowed us to investigate the arteriolar responsiveness on a sudden rise in the inflow pressure.

In a second set of experiments in different animals, arteriolar diameters and RBC velocities on stepwise (20 mm Hg) increases in inflow pressure were measured. The inflow pressure to the cremaster (as measured in the femoral artery) was initially adjusted to 60 mm Hg by partial aortic occlusion and then raised to 80, 100, and 120 mm Hg by stepwise deflation of the occluder under continuous infusion of NE. Each pressure level was maintained for 1 minute. Before the next arteriole was investigated, the infusion of NE was stopped, and a recovery period of 15 minutes was allowed. Three to four arterioles were examined in each animal consecutively. The same arterioles were then investigated according to the same protocol 30 minutes after the NOS inhibitor L-NNA (30 μmol/L) was added continuously to the superfusion buffer. This concentration has been shown to be sufficient to block basal and ACh-induced NO release. In some animals, Ang II (0.47 mmol/min IV) was used instead of NE to increase blood pressure. The AT\(_1\) receptor blocker S4509 (0.5 mmol/L) was applied locally in these animals instead of the ARB. The maximum diameters of the vessels were determined at the end of each experiment by superfusion of the preparation with a combination of the vasodilators adenosine (100 μmol/L), SNP (1 μmol/L), and papaverine (300 μmol/L). Finally, blood was withdrawn from the carotid artery for blood gas analysis.

Because we infused vasoconstrictors to increase pressure, receptor blockers had to be used to differentiate between the direct constrictor effect and the effect of the elevated arterial pressure. To test the efficacy of ARBs, arteriolar responses on bolus injections of NE (0.1 mmol, 10 μL in volume) into the femoral artery (from which the cremaster arterioles emerge) were investigated in three animals. The dilution of the blood due to the injected bolus was clearly visible in all vessels investigated. The bolus injections were performed both before and during the local superfusion of ARB. Prior experiments have revealed that the injection of 10 μL of saline alone did not change the diameters of the cremaster arterioles significantly (not shown). Additionally, in each experiment investigating pressure effects, the efficacy of the receptor blockade was tested by local superfusion of NE (0.1 mmol/L) or Ang II (10 mmol/L) before and during the addition of ARB or S4509.

#### Solutions and Drugs

The salt buffer used for superfusion was of the following composition (in mmol/L): Na\(^+\) 143, K\(^+\) 6, Ca\(^{2+}\) 2.5, Mg\(^{2+}\) 1.2, Cl\(^-\) 128, HCO\(_3\)\(^-\) 25, SO\(_4\)\(^2-\) 1.2, and H\(_2\)PO\(_4\)\(^-\) 1.2. ACh, SNP, adenosine, prazosin, and angiotensin were obtained from Sigma Chemical Co, yohimbine and propranolol from ICN Biochemicals, and L-NNA from Serva. S4509 and NE were generous gifts from Hoechst (Frankfurt, Germany). Propranolol (10 mmol/L) and yohimbine (1 mmol/L) were dissolved in 1 mol/L acetic acid, and prazosin (1 mmol/L) in 3:1 ethanol/ascorbic acid (1 mmol/L). These stock solutions were stored using a modified dual-slit cross-correlation method, which was described in detail elsewhere.  

### Experimental Protocol

After the surgical preparation, the hamsters were allowed to recover for 30 minutes before control diameters were taken. Vascular vasodilator reactivity was tested by consecutive local superfusions (1 to 3 minutes) of the endothelium-independent NO-donor SNP (1 μmol/L) or the endothelial stimulator ACh (10 μmol/L). Arteriolar diameters were measured before and during the application of the respective vasoreactive agent. Thereafter, the diameter and RBC velocities were monitored continuously in one arteriole before and during the intravenous infusion of NE (0.5 mmol/min) for 8 to 10 minutes. The recovery period before the next arteriole was studied under the same conditions was at least 15 minutes. The same protocol was thereafter repeated in the presence of the ARB prazosin, yohimbine (0.1 μmol/L each), and propranolol (1 μmol/L), which were added to the superfusion continuously. In a subset of experiments (four animals), the increase in pressure on intravenous infusion of NE in the vessels supplying the cremaster was blocked (as monitored in the femoral artery) by partial occlusion of the abdominal aorta. A rapid deflation of the vascular occluder after 3 minutes allowed us to investigate the arteriolar responsiveness on a sudden rise in the inflow pressure.
at −20°C until use and further diluted in the superfusion buffer. SNP (10 mmol/L) was dissolved in 1 mmol/L sodium acetate on the day of the experiment. For all other solutions and further dilutions, freshly prepared superfusion buffer was used. All locally applied drugs (concentrated 100-fold over the final concentration) were added to the superfusion fluid by means of a roller pump at 1/100th of the total superfusion rate (0.08 mL/min) to obtain the final concentrations indicated above.

Statistics and Calculations
Vascular tone is expressed as quotient of the vessel’s resting diameter divided by its maximal diameter. Changes of the arteriolar inner diameter on treatment were calculated as a percentage of the respective control diameter; % change=100×[(Dtr−Dco)/Dco], where Dtr represents the diameter after treatment and Dco the control diameter before treatment. The mean RBC velocity (Vw) was calculated from the measured centerline velocity divided by an empirical correction factor (1.6).15 Arteriolar blood flow, Q, was calculated from the formula Q=Vw×D²×π/4 and is expressed as nanoliters per second. WSR was calculated according to the formula WSR=8×Vw/D. Comparisons within groups were performed with paired t tests, and those between groups with ANOVA. For multiple comparisons, the probability values were corrected according to Bonferroni. Differences were considered significant at a corrected error probability of P<.05.

Results
Responses on Local Application of Vasoactive Substances
A total of 114 arterioles with maximal luminal diameters between 32 and 127 µm (66.4±1.8 µm) were studied in 30 animals. The vessels exhibited varying degrees of spontaneous tone, ie, the quotient of resting to maximal diameter ranged from 0.38 to 0.96 (0.73±0.01). This quotient was significantly lower in SAs (0.68±0.02, n=57) than in LAs (0.77±0.02, n=57), indicating a higher basal tone in small vessels. SAs belonged to A3 and A4 and LAs belonged to A1 and A2 generation of the arteriolar tree.10 Local superfusion of SNP (1 µmol/L) or ACh (10 µmol/L) dilated the arterioles by 30.6±3.4% and 42.8±2.9% (Table 1).

Superfusion of the arterioles with NE (0.1 µmol/L) induced a arteriolar constriction by 33.9±2.0%, which was virtually abolished after addition of prazosin, yohimbine (0.1 µmol/L each), and propranolol (1 µmol/L, ARB) to the superfusate. In addition, these blockers led to a slight increase of arteriolar diameters (by 4.2±1.6%, P<.05, Table 1). To test the efficacy of the ARB to inhibit effects of circulating NE, a bolus of NE was locally applied from the luminal side of the arteriole. This bolus injection of NE (0.1 nmol) raised arterial pressure only from 85±2 to 89±2 mm Hg (P<.05). Nevertheless, it induced a vasoconstriction by 10.7±2.3% in LAs (n=8) and by 30.1±6.2% in SAs (n=7) within 1 minute. After addition of ARB to the superfusion, the arteriolar constrictions on injected NE were completely abolished (LAs, −1.3±0.8%; SAs, −2.0±3.2%, Table 1). Similarly, the constriction induced by addition of Ang II (10 nmol/L) to the superfusion fluid (−18.6±2.9%) was completely abolished in the presence of the AT1 receptor blocker S4509 (0.5 µmol/L), which induced a small increase of arteriolar diameter (by 7.0±1.9%, P<.05, Table 1).

Arteriolar Responses on Increases in Pressure
Intravenous infusion of NE (0.5 nmol/min) increased the arterial blood pressure within 50±3 seconds from 75±2 to 125±2 mm Hg, which was maintained until the infusion of NE was stopped. The HR decreased slightly from 333±5 to 321±7 bpm (P<.05) during this infusion. The cremaster arterioles exhibited different behavior on the intravenous infusion of NE: The SAs constricted significantly (by −23.8±9.3%), and LAs had no significant change in diameter. Local blood flow remained unchanged in SAs and tended to increase in LAs (data not shown), whereas the WSR increased in both groups (Fig 1). Local ARB affected neither the NE-induced increase in blood pressure nor the absolute (Fig 1) or relative diameter changes in SAs (−29.1±8.1 versus −32.7±5.7%, respectively) or LAs (−12.2±8.9 versus −8.8±7.1%, respectively) on intravenous infusion of NE.

In a separate series (four animals, local ARB), arterial pressure was raised to a similar level (124±6 mm Hg) by intravenous infusion of NE. In these animals, when the increase in pressure in the hindquarter was blocked by partial aortic occlusion, no diameter changes occurred (Fig 1). However, the sudden release of the aortic occlusion, which increased the inflow pressure rapidly by 49±4 mm Hg, led to a significant constriction in both vessel types (LAs, −10.5±3.5%; SAs, −34.6±5.2%). The reinflation of the aortic occluder brought the inflow pressure back to the initial level and dilated the arterioles again back to control diameter despite the ongoing intravenous application of NE (Fig 2). Opening or closing the aortic occluder changed systemic blood pressure by no more than 6 mm Hg.

To study whether pressure-induced constrictions could be elicited over the whole pressure range investigated here, we increased the inflow pressure stepwise in another series. After systemic blood pressure had reached a plateau of

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**TABLE 1. Reactivity of Arterioles to Local Application of Vasomotor Stimuli**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Resting Diameter, µm</th>
<th>Treated Diameter, µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extravasal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SNP (1)</td>
<td>99</td>
<td>47.8±2.0</td>
<td>59.8±1.8*</td>
</tr>
<tr>
<td>ACh (10)</td>
<td>99</td>
<td>45.2±1.6</td>
<td>60.3±1.6*</td>
</tr>
<tr>
<td>NE (0.1)</td>
<td>67</td>
<td>48.0±2.2</td>
<td>32.2±2.0*</td>
</tr>
<tr>
<td>ARB</td>
<td>67</td>
<td>44.1±1.9</td>
<td>45.9±1.9*</td>
</tr>
<tr>
<td>NE (0.1)+ARB</td>
<td>67</td>
<td>44.3±1.9</td>
<td>43.5±2.0*</td>
</tr>
<tr>
<td>Ang II (0.01)</td>
<td>32</td>
<td>44.6±2.4</td>
<td>35.8±2.2*</td>
</tr>
<tr>
<td>S4509 (0.5)</td>
<td>32</td>
<td>43.7±2.5</td>
<td>46.1±2.5*</td>
</tr>
<tr>
<td>Ang II (0.01)+S4509</td>
<td>32</td>
<td>45.1±2.6</td>
<td>44.8±2.6</td>
</tr>
<tr>
<td>Intravasal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NE (bolus injection)</td>
<td>15</td>
<td>50.5±5.2</td>
<td>41.4±4.5†</td>
</tr>
<tr>
<td>NE (bolus injection)+ARB</td>
<td>15</td>
<td>47.7±5.5</td>
<td>46.7±5.3†</td>
</tr>
</tbody>
</table>

Substances were applied from the adventitial (extravasal) or the luminal (intravasal) side of the arteriole. Diameters were measured before (resting) and 2 minutes after local addition of the respective substances to the superfusion (treated). Numbers in parentheses give the concentration in µmol/L. For intravasal application, NE was injected as a single bolus (0.1 mmol) into the femoral artery from which the cremaster vessels emerge.

*P<.05 vs resting diameter.
†Peak response on bolus injection.
inflow pressure back to baseline led to a dilation by which the arterioles reattained their control diameter despite the continuous infusion of NE (Table 2, Fig 3). Systemic blood pressure increased by 3.4±0.8 mm Hg and HR from 409±5 to 415±5 bpm after inflation of the occluder.

**Effects of NOS Inhibition**

Addition of L-NNA to the superfusate (30 μmol/L) decreased arteriolar diameters by 9.1±1.3% \((P<.05)\) and also significantly reduced ACh (10 μmol/L)–induced dilations (from 44.0±3.6% to 29.6±2.2%) but not SNP-induced dilations (27.4±4.0% versus 25.4±5.4%). In contrast to control conditions, LAs constricted significantly now on each increase in inflow pressure, whereas the constrictions of SAs were not different from control animals. The local blood flow no longer increased on pressure elevations, whereas augmentations of RBC velocity and WSR were still found. Again, the reduction in inflow pressure back to baseline brought the arteriolar diameters back to their respective control values despite continuous NE infusion (Table 2, Fig 3), whereas systemic blood pressure increased (by 5.3±0.9 mm Hg), and HR remained unchanged (380±4 versus 387±4 bpm).

**Stepwise Increases in Pressure During Ang II Infusion**

In different experiments (seven animals), Ang II instead of NE was infused intravenously to increase arterial pressure in the local presence of the AT1 receptor blocker S4509 (0.5 μmol/L). The blood pressure increased from 70.2±1.4 to 162±2 mm Hg. Thus, four increments in inflow pressure of 20 mm Hg each were investigated. The stepwise increases in pressure elicited virtually the same diameter constrictions in SAs \((n=12)\) as were found with similar increments in pressure under NE. Only at the last increment, which was not obtained in the NE group, did the vessels not constrict further. Again, there were no significant
TABLE 2. Vascular Diameters and RBC Velocities at Increasing Inflow Pressures During NE-Induced Blood Pressure Rise in LAs and SAs

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pressure, mm Hg</th>
<th>Diameter, μm</th>
<th>Velocity, mm/s</th>
<th>Pressure, mm Hg</th>
<th>Diameter, μm</th>
<th>Velocity, mm/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>60±0.3</td>
<td>62±4.3</td>
<td>7.0±0.8</td>
<td>59±0.3</td>
<td>37±2.3</td>
<td>4.3±0.5</td>
</tr>
<tr>
<td></td>
<td>79±0.3</td>
<td>62±4.9</td>
<td>9.7±1.0*</td>
<td>79±0.3</td>
<td>35±2.5*</td>
<td>5.8±0.7*</td>
</tr>
<tr>
<td></td>
<td>98±0.5</td>
<td>62±5.2</td>
<td>12.1±1.6*</td>
<td>98±0.4</td>
<td>32±2.7*</td>
<td>7.7±1.1*</td>
</tr>
<tr>
<td></td>
<td>114±0.2</td>
<td>61±5.9</td>
<td>13.9±1.9</td>
<td>116±0.1</td>
<td>29±2.7*</td>
<td>10.3±1.4*</td>
</tr>
<tr>
<td></td>
<td>58±0.7</td>
<td>63±3.6</td>
<td>7.0±0.8*</td>
<td>59±0.3</td>
<td>37±1.9*</td>
<td>4.6±0.6*</td>
</tr>
<tr>
<td>L-NNA 30 μmol/L</td>
<td>59±0.4</td>
<td>54±4.3</td>
<td>8.3±0.7</td>
<td>59±0.3</td>
<td>32±1.7</td>
<td>3.7±0.5</td>
</tr>
<tr>
<td></td>
<td>80±0.5</td>
<td>51±4.7*</td>
<td>10.1±1.0*</td>
<td>79±0.2</td>
<td>28±1.8*</td>
<td>4.3±0.6*</td>
</tr>
<tr>
<td></td>
<td>98±0.6</td>
<td>48±5.0*</td>
<td>12.6±1.0*</td>
<td>98±0.5</td>
<td>26±2.0*</td>
<td>5.2±0.7*</td>
</tr>
<tr>
<td></td>
<td>117±1.4</td>
<td>46±4.9*</td>
<td>14.2±1.0*</td>
<td>115±0.6</td>
<td>25±2.1*</td>
<td>6.6±0.9*</td>
</tr>
<tr>
<td></td>
<td>58±0.4</td>
<td>52±4.3*</td>
<td>7.4±0.6*</td>
<td>57±0.6</td>
<td>32±1.8*</td>
<td>2.7±0.4*</td>
</tr>
</tbody>
</table>

Pressure was measured in the femoral artery (distal to a vascular occluder) and represents the inflow pressure into the cremaster. Pressure was increased in three steps and thereafter brought back to the initial value. Diameter and RBC velocity were measured in the steady-state period (1 minute after each step). n indicates number of arterioles. Values were obtained in 7 animals and are presented as mean±SEM. LA maximal diameter >65 μm; SA maximal diameter <65 μm.

*P<.05 vs previous pressure level.

The experiments demonstrate that a pressure-induced vasoconstriction contributes markedly to the arteriolar response on intravenous infusion of a vasoconstrictor in the cremaster vascular bed. They also show that this response occurs predominantly in SAs, whereas LAs respond to such pressure elevations only weakly. The major finding of this study is that myogenic constriction is extended to LAs after inhibition of NOS. This finding suggests that one of the basic mechanisms by which NO reduces blood pressure and enhances organ blood flow is the blockade of a pressure-induced constriction in LAs.

Because we were interested in myogenic reactivity in the high normal and hypertensive blood pressure range, we infused vasoconstrictors. The intravenous infusion of NE induced an

**Influence of Vascular Tone on Myogenic Responses**

Since the graded pressure increments during intravenous infusion of NE or Ang II elicited virtually the same arteriolar constrictions, these groups were analyzed together to deduce the role of initial vascular tone on the myogenic responsiveness. The arterioles were grouped according to their tone (quotient of actual and maximal diameter) at the baseline pressure of 60 mm Hg, i.e., just before the increase in pressure. Under control conditions, significant vasoconstrictions on increases in pressure (40 or 60 mm Hg applied in 20 mm Hg increments) were found only in arterioles with high tone (quotient from 0.55 to 0.70) but not in those with moderate (quotient from 0.70 to 0.85) or low (quotient from 0.85 to 1.00) tone. In contrast, after L-NNA, significant vasoconstrictions were observed in all groups, i.e., also in those large vessels that exhibited moderate or low tone after NOS inhibition (Fig 5). SAs behaved similarly before and after L-NNA (data not shown).

**Discussion**

Constrictions in LAs (n=20) except for the pressure change from 80 to 100 mm Hg. The constriction was much weaker here than in small vessels (P<.05). At most pressure increments, RBC velocity and WSR increased in LAs and SAs, whereas local blood flow increased only in LAs. These changes were completely reversible if the inflow pressure was returned to 60 mm Hg, despite the continued Ang II infusion (Table 3, Fig 4). On the inflation of the occluder, small increases in systemic blood pressure (by 3.3±1.0 mm Hg) but no changes in HR (389±5 bpm) were observed. In the presence of L-NNA, each pressure elevation led to significant constrictions in LAs, and local blood flow no longer increased, as observed in control animals. The vasoconstrictions in SAs were unaltered compared with control animals, as found in the NE group (Table 3, Fig 4).

**Figure 3.** Effect of NO inhibition on pressure-induced changes of microvascular parameters during infusion of NE. Small stepwise increases in inflow pressure (as measured in the A. femoralis [A.fem.]) during NE infusion induced myogenic constrictions in SAs (right, n=23), but not in LAs (left, n=14) under control conditions (open symbols). L-NNA (solid symbols) unmasked a pressure-dependent vasoconstriction in LAs, whereas it did not modify it in SAs. Arteriolar blood flow increased only under control conditions with pressure; nevertheless, the WSR increased under both conditions. Data were obtained in six animals under local superfusion with ARB. *P<.05 vs previous pressure level. Control values, see Table 2.
TABLE 3. Vascular Diameters and RBC Velocities at Increasing Inflow Pressures During Ang II–Induced Blood Pressure Rise in LAs and SAs

<table>
<thead>
<tr>
<th>Treatment</th>
<th>LAs (n=20)</th>
<th>SAs (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pressure, mm Hg</td>
<td>Diameter, μm</td>
</tr>
<tr>
<td>Control</td>
<td>63.6±0.5</td>
<td>67.4±4.8</td>
</tr>
<tr>
<td></td>
<td>83.5±0.5</td>
<td>66.5±5.4</td>
</tr>
<tr>
<td></td>
<td>102.1±0.7</td>
<td>64.1±5.8*</td>
</tr>
<tr>
<td></td>
<td>121.9±0.5</td>
<td>63.7±5.9</td>
</tr>
<tr>
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<td>145.8±1.9</td>
<td>63.1±5.9</td>
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<td></td>
<td>61.5±0.4</td>
<td>66.6±3.8</td>
</tr>
<tr>
<td>L-NNA 30 μmol/L</td>
<td>62.3±0.4</td>
<td>55.1±3.6</td>
</tr>
<tr>
<td></td>
<td>82.9±0.5</td>
<td>52.3±3.6*</td>
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<td>102.7±0.4</td>
<td>49.5±3.5*</td>
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<td>123.2±0.5</td>
<td>45.8±3.2*</td>
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<td>147.3±1.7</td>
<td>43.2±2.9*</td>
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<td>62.4±0.4</td>
<td>55.7±2.1*</td>
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Pressure was measured in the femoral artery and represents the inflow pressure into the cremaster. Diameter and RBC velocity were measured 1 minute after each pressure change (steady state). n indicates number of arterioles. Data were collected in six animals and are presented as mean±SEM. LA maximal diameter >65 μm; SA maximal diameter <65 μm.

*P<.05 vs previous pressure level.

Figure 4. Effect of NO inhibition on pressure-dependent changes of microvascular parameters during infusion of Ang II. During stepwise increases in pressure under infusion of Ang II, there was, in contrast to the NE infusion, a weak vasoconstriction of LAs (left) already under control conditions (open symbols). L-NNA (solid symbols) enhanced the vasoconstriction in these LAs but not in SAs. Data were obtained in seven animals: 19 LAs and 13 SAs. All experiments were done in the presence of AT1 receptor blockade (S4508, 0.5 μmol/L, local superfusion). *P<.05 vs previous pressure level. Control values, see Table 3.

A.fem. indicates A. femoralis.
myogenic constriction occurred predominantly in SAs, whereas LAs responded only weakly and only when the increase in pressure was rapid and large. It has to be kept in mind that additional, ie, non–pressure-related, mechanisms might have contributed to the observed responses in these vessels. In particular, metabolic signals have been implicated in this regulation. SAs have been shown to be more sensitive to metabolic stimuli.\textsuperscript{17,18} Thus, it cannot be ruled out that the exhibited constriction in the SAs has emerged partially because of these metabolic regulatory mechanisms, because flow increased with pressure. However, the capability of LAs to constrict in response to metabolic stimuli seems to be weak.\textsuperscript{17,18} Therefore, at least in large vessels, the observed responses are likely to be myogenic.

In contrast to our in vivo experiments, LAs and even small arteries reveal significant myogenic responses to small changes in pressure when studied in vitro.\textsuperscript{6,10,11,19,20} However, after inhibition of NOS, we found significant vasoconstrictions in LAs on graded increases in pressure in vivo as well, and thus, the release of NO represents part of the mechanism that controls myogenic responsiveness of these vessels. This NO release was not due to a receptor-dependent endothelial stimulation by NE or Ang II as previously reported,\textsuperscript{21} because these receptors were blocked. Inhibition of NOS led to an augmentation of vascular tone, and merely an enhanced vascular tone might increase myogenic responsiveness. Therefore, responses in LAs with similar tone under control conditions and after inhibition of NOS were compared. These data demonstrated that arterioles with a comparable degree of activation constricted significantly in response to an increase in pressure only in the presence of L-NNA (Fig 5). Thus, the effect of inhibition of NOS cannot be attributed solely to the augmentation of vascular tone, but rather, NO can be implicated in playing a specific role in preventing myogenic responses. Likewise, studies in isolated organs have demonstrated that the same increase in basal vascular tone led to different myogenic responses when endothelin or NOS inhibitors were used.\textsuperscript{9}

Several in vitro studies have demonstrated an influence of NO on myogenic constriction only when the vessels were perfused and are therefore under conditions of shear stress.\textsuperscript{5–7,22} As shown here, and in a recently published study,\textsuperscript{9} the WSR, and thus the wall shear stress, increases roughly linearly with elevations in blood pressure, and elevations in wall shear stress have been shown to release NO.\textsuperscript{15} Consistent with shear stress–dependent augmentation of NO release, our data show the strongest effect of L-NNA at the highest WSR (Figs 3 and 4). Therefore, the shear stress–induced NO release may counteract the pressure-induced vasoconstriction in these LAs. In accordance with this view, myogenic constrictions in the isolated rabbit ear were unmasked only after inhibition of NOS.\textsuperscript{23} Furthermore, a recent study in the perfused rat kidney demonstrated that vasoconstrictions in afferent arterioles induced by increases in pressure coincided with a prolonged increase in endothelial intracellular free calcium,\textsuperscript{24} a signal that activates the endothelial NOS.\textsuperscript{25}

Despite its very marked effect on LAs, L-NNA failed to enhance the apparent myogenic responses in SAs. The reasons for this are not clear. One plausible explanation is that L-NNA treatment and the subsequent myogenic constriction of LAs caused an increased drop in pressure along these LAs. Thus, further downstream, the increase in transmural pressure would be attenuated. The apparent constancy of the myogenic response in the SAs would reflect the net result of two compensatory effects. These effects are an enhanced constriction in response to increases in pressure (as observed in LAs) and, concomitantly, a reduced increase in transmural pressure. Furthermore, metabolic counterregulation may contribute to an attenuation of the constrictions, because these SAs are controlled by metabolic signals.\textsuperscript{17,18}

The most important functional implication of this study is that NO inhibits the myogenic response of LAs. These vessels are influenced differently from small ones, because they are less tightly controlled by metabolic signals. This finding is partially due to the different distribution of \(\alpha\)-receptor subtypes along vessels of different generations.\textsuperscript{17,18} Therefore, any impairment of endothelial NO release will extend the myogenic reactivity to the LAs, thereby enhancing the overall myogenic responsiveness of a vascular bed. Consistent with these results, it has previously been demonstrated that (myogenic) autoregulation

\textbf{Figure 5.} Enhanced myogenic constrictions after inhibition of NOS in arterioles with similar initial tone. Diameter changes (percentage change of the diameter at 60 mm Hg) after two (top) or three (bottom) increments in inflow pressure (20 mm Hg each) of LAs at different initial vascular tone (ie, tone at the baseline pressure). Arterioles with moderate or low tone did not constrict significantly on these increases in pressure under control conditions (open bars). In contrast, in the presence of L-NNA (30 \(\mu\)mol/L, hatched bars) a significant constriction in arterioles with similar tone was observed. Digits within bars indicate number of arterioles. Data include pressure elevations by NE and Ang II infusion in the local presence of the respective blockers. \(\textsuperscript{P}<.05\) vs respective control diameter.
is enhanced after inhibition of NOS in isolated perfused hearts. Furthermore, the adaptive increase in blood flow is impaired during reactive and active hyperemia, conditions that are associated with (re-)increases in blood pressure. It is also tempting to speculate that the amplification of the vasoconstrictor effects on peripheral resistance described above will be further enhanced after inhibition of NOS. It remains to be established whether, under conditions of impaired NO release, a shift of myogenic responsiveness to LAs contributes to the elevated peripheral resistance observed in many hypertensive patients. It would be particularly interesting to examine these myogenic effects on peripheral resistance in hypertensive patients, who respond with a decrease in blood pressure on infusion of the NOS substrate L-arginine.

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References


Nitric Oxide Opposes Myogenic Pressure Responses Predominantly in Large Arterioles In Vivo
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