Nonendothelial NO Blunts Sympathetic Response of Normotensive Rats but not of SHR

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Abstract—The inhibitory role of NO on sympathetic-induced contraction of resistance vessels of spontaneously hypertensive rats (SHR) has not been defined. Accordingly, we investigated the effect of endothelial removal or NO synthase inhibition on vasoconstrictor responses to sympathetic stimulation or phenylephrine in perfused mesenteric beds isolated from normotensive rats (NR) and SHR. Electrical stimulation (10 to 64 Hz) of perivascular nerves elicited a frequency-dependent increase in perfusion pressure that was greater in preparations from SHR (maximal effect: 223.4 ± 8.4 versus 117.6 ± 10.3 mm Hg in NR, n = 6, P < 0.001), and endothelium removal did not affect these responses in arteries from NR but caused a significant shift to the left of the frequency-response curve in arteries from SHR. In arteries with endothelium, inhibition of NO synthase with Nω-nitro-L-arginine (L-NNA, 50 μmol/L) augmented the vasoconstrictor responses to sympathetic stimulation in both NR and SHR preparations. In preparations that had the endothelium removed, however, L-NNA potentiated only the responses to sympathetic stimulation of NR arteries. Vasoconstrictor responses to phenylephrine was potentiated by endothelium removal and in the presence of L-NNA only when the endothelium was intact in both NR and SHR arteries. The number of NADPH-diaphorase–positive cells in the superior mesenteric sympathetic ganglion of SHR was significantly less compared with that of NR. In conclusion, these data suggest a prejunctional inhibitory action of non–endothelial-derived NO, most probably neuronal-derived NO, on sympathetic-mediated vasoconstriction in SHR arteries elicited by L-NNA can be attributed to inhibition of endothelial-derived NO. (Hypertension. 2001;38[part 2]:565-568.)

Key Words: rats, inbred SHR ■ mesenteric arteries ■ nitric oxide synthase ■ endothelium ■ prostaglandins

Endogenous NO, released basally and after receptor-mediated stimulation of endothelial cell, is an important mediator of vasodilatation and may additionally modulate vasoconstrictor effects. Inhibition of NO synthesis enhances contractile responses induced by agonists or sympathetic stimulation in a number of preparations, including dog mesenteric artery,1 rat caudal and mesenteric arteries,2–4 and human arteries.5 Endothelium-derived NO has been proposed to participate in the enhancement of the vasoconstrictor responses to perivascular nerve stimulation by NO synthase (NOS) inhibition.3,6 However, an inhibitory action for non–endothelial-derived NO on sympathetic vasoconstrictor response has also been reported.7 NOS, and therefore presumably NO, also occurs in the autonomic nerves in the outer adventitial layers of various blood vessels7,8 and in preganglionic sympathetic neurons.9,10 Augmented vasoconstrictor responses to perivascular nerve stimulation have been consistently reported in isolated arteries of spontaneously hypertensive rats (SHR).11–13 However, the inhibitory role of endothelium and NO in the sympathetic-induced contraction of resistance vessels of hypertensive rats has not been defined. Accordingly, the present study was performed to examine the influence of endothelium and NO on neurally induced or phenylephrine-induced vasoconstrictor responses of isolated mesenteric arterial bed from Wistar normotensive rats and SHR. Because endothelium may also release cyclooxygenase (COX) products that can modulate the sympathetic-mediated vasoconstriction, the effect of COX inhibition was also investigated. In addition, histochemical localization of NADPH diaphorase staining9 was also performed in superior mesenteric ganglion of normotensive and SHR.

Methods

Experiments were performed using female (body weight, 180 to 240 g) normotensive Wistar rats (NR) and SHR (inbred SHR originally obtained from Taconic Farms Inc, Germantown, NY) with free access to standard rat chow and tap water. All experiments were conducted in accordance with institutional guidelines on the use of animals in research.

Arterial pressure was measured in unanesthetized NR and SHR by the tail-cuff method. Following that, the rats were anesthetized with tribromoethanol 2% (1 mL/100 g IP), and the mesenteric bed was removed and prepared for perfusion in a water-jacket organ bath.
maintained at 37°C, as previously described. In brief, the mesenteric arteries were perfused with a modified Kreb’s solution (in mmol/L: NaCl 120.0, KCl 4.7, NaHCO 3 25.0, CaCl 2 0.2H 2 O 3.0, MgCl 2 0.6H 2 O 1.4, KH 2 PO 4 1.2, glucose 11.0, and EDTA 0.03) equilibrated with 95% O 2/5% CO 2 mixture (pH 7.4) at 37°C at constant flow of 4 mL/min (LKB 2215 multiperfus pump). The mesenteric perfusion pressure was monitored (Beckman recorder) with a pressure transducer (Grass P23XL) connected to a sidearm of the mesenteric artery cannula. Electrical stimulation of periarterial nerves was achieved through 2 bipolar platinum ring electrodes placed around the superior mesenteric artery, and consisted of rectangular pulses (34 V, 3 milliseconds) and variable frequency (10 to 64 Hz) applied for 20 seconds at 3-minute intervals. After the perfused mesenteric arterial bed was allowed to equilibrate for 15 minutes, electrical stimulation (10 to 64 Hz) was applied, and the increases in perfusion pressure were recorded. Guanethidine (5 μmol/L) or tetrodotoxin (1 μmol/L) added to the solution at the beginning of the perfusion period completely abolished the vasoconstrictor responses to electrical stimulation (data not shown), indicating their sympathetic origin, as previously described. In some preparations, the endothelium was disrupted by infusing 2 mL of sodium deoxycholate solution (1 mg/mL) before the equilibration period; the successful disruption of the endothelium was confirmed in each preparation by the failure of acetylcholine (20 pmol, bolus injection) to elicit relaxation in the mesenteric arteries precontracted with phenylephrine. Administration of acetylcholine (20 pmol) in preparation of NR and SHR with intact endothelium induced a decrease in perfusion pressure of ~75% and 60% of maximal dilatory response, respectively. To investigate the influence of NO or prostanoids on the vasoconstrictor responses elicited by sympathetic stimulation, the NOS inhibitor N^3-nitro-L-arginine (L-NNA, 50 μmol/L) or the COX inhibitor indomethacin (10 μmol/L) was added to the solution perfusion at the beginning of the experiment in preparations with or without intact endothelium.

Because vasoconstriction induced by electrical stimulation of perivascular nerves was abolished by prazosin (1 μmol/L, data not shown), the effect of endothelium removal or L-NNA on the vasoconstrictor responses induced by phenylephrine (2.5 to 400 nmol, bolus injection) was also investigated in preparations of NR and SHR.

In a separate series of experiments, anesthetized SHR and NR were intracardially perfused with 200 mL saline initially, then with 100 to 150 mL of 4% paraformaldehyde in 0.1 mol/L phosphate buffer. At the end of the perfusion, the superior mesenteric ganglia was removed and stored in 15% sucrose in 0.1 mol/L phosphate buffer overnight, frozen in liquid nitrogen, and stored at −70°C. Twenty-μm sections were then cut with a cryostat. The sections were mounted on gelatin-coated slides and processed for NADPH diaphorase histochemistry as follows: sections were incubated for 60 minutes at 37°C with a solution containing 0.1 mol/L phosphate buffer (pH 7.4), 0.3%Triton X-100, 0.1 mg/mL nitroblue tetrazolium and 1.0 mg/mL β-NADPH; washed with distilled water; and mounted for microscopic observation. The sections were examined by a computerized image analysis system. Images were captured from slides using an Olympus BX50 microscope and Sony DXC 107A camera together with Image Pro Plus (version 4.0, Media Cybernetics). For quantification of NADPH diaphorase cell number, the average number (n=3) from a fixed size/area (35.8 μm 2) was measured.

Results are expressed as mean±SE; however, frequencies that elicited 50% of the maximal response (F 50) are reported as geometric means with their respective 95% confidence limits (95% CIs). The F 50, P D 2, and maximal effect (Emax) values were calculated from regression analysis from the complete frequency- or dose-response curves using the GraphPad Inplot (version 4.0) and were analyzed by ANOVA (Instat GraphPad). If differences were observed, the data were compared using Student’s t test with Bonferroni correction. Arterial pressure and number of NADPH-positive cells were analyzed by unpaired t test. Differences were considered significant when P<0.05.

Figure 1. Vasoconstrictor effect induced by perivascular electric stimulation (top panels) and phenylephrine (bottom panels) in intact (E+) and disrupted (E−) endothelium preparations of mesenteric arterial bed isolated from NR (A and C) and SHR (B and D) and perfused with Kreb’s solution in the absence or presence of L-NNA 50 μmol/L. Data are presented as mean±SE (n=5 to 6). MPP indicates mesenteric perfusion pressure.

Arterial pressure of SHR was significantly higher than that of NR (177±3 mm Hg versus 116±2 mm Hg, n=37; P<0.001). The basal perfusion pressure of isolated mesenteric arteries with endothelium was higher in SHR (20.7±1.4 versus 14.5±0.9 mm Hg in NR, n=6 each; P<0.01). Electrical stimulation (10 to 64 Hz) of perivascular nerves elicited frequency-dependent vasoconstrictor responses of the mesenteric beds isolated from NR (Figure 1A) and SHR (Figure 1B). However, the frequency of stimulus required for eliciting F 50 was lower (P<0.001) in preparations with endothelium from SHR (mean F 50=18.7 Hz [95% CI, 18.4 to 19.1 Hz], n=6) than from NR (20.7 Hz [95% CI, 20.1 to 21.0 Hz], n=6), whereas the maximal vasoconstrictor response was significantly higher in the former group (Emax=223.4±8.4 versus 117.6±10.3 mm Hg in NR; P<0.01).

Endothelium removal and/or L-NNA did not affect basal perfusion pressure of preparations from both SHR and NR. The effects of endothelium removal and/or L-NNA (50 μmol/L) on the vasoconstrictor responses of mesenteric arteries from NR and SHR to perivascular stimulation are also shown in Figure 1. Endothelium removal did not affect the responses in the NR group (F 50=20.3 Hz [95% CI, 19.5 to 21.1 Hz], n=6) (Figure 1A) but caused a significant shift to the left of the frequency-response curve in the SHR group (F 50=14.1 Hz [95% CI, 11.4 to 17.5 Hz], n=6; P<0.01) (Figure 1B). In addition, the maximal vasoconstrictor effect was not influenced by the endothelium in both groups (219.8±3.9 mm Hg in SHR and 119.9±5.8 mm Hg in NR). In arteries with endothelium, inhibition of NOS with L-NNA, however, induced a significant (P<0.001) shift to the left of the frequency-response curve in both NR (F 50=16.1 Hz [95% CI, 15.7 to 16.5 Hz]) and SHR (F 50=10.5 Hz [95% CI, 10.1
to 10.8 Hz) groups (n=6 each). L-NNA caused an increase in the maximal vasoconstrictor response induced by electrical stimulation only in arteries from NR (175.9±7.7 mm Hg; P<0.01). L-NNA also potentiated the responses of mesenteric arteries that had the endothelium removed in the NR group (F0=14.1 Hz [95% CI, 13.3 to 14.9 Hz]; Emax=160.9±11.5 mm Hg; P<0.01; n=6) but induced no further effect in arteries from SHR without endothelium (13.5 Hz [95% CI, 13.4 to 13.6 Hz]; 233.6±7.7 mm Hg; n=6).

Phenylephrine elicited a dose-dependent increase in perfusion pressure of mesenteric arteries of NR and SHR (Figure 1C and 1D). Hypertension was associated with a significant increase in phenylephrine-induced maximal vasoconstrictor effect (210.9±11.9 versus 80.0±8.7 mm Hg in NR, P<0.0001) and similar pd1 values compared with those of the NR group (7.07±0.02, n=5, versus 7.17±0.03, n=7). In the presence of L-NNA or after endothelium removal, dose-response curve to phenylephrine was significantly (P<0.001) shifted to the left in both NR (n=5) and SHR (n=6) preparations, but maximal response was increased (P<0.01) only in NR group. In preparations without endothelium, L-NNA did not enhance vasoconstrictor responses to phenylephrine in both groups.

Inhibition of COX with indomethacin (10 μmol/L) significantly (P<0.05) reduced basal perfusion pressure in arteries from NR and SHR with (10.5±0.3 and 13.5±1.1 mm Hg, n=5) or without (8.6±1.0 and 13.9±2.1 mm Hg, n=5) endothelium. Indomethacin did not affect the vasoconstrictor responses induced by electrical stimulation in arteries of NR (Figure 2A), but induced a significant shift to the right of the frequency-response curve of SHR arteries with (22.9 Hz [95% CI, 22.3 to 23.5 Hz], n=5; P<0.01) or without endothelium (20.1 Hz [95% CI, 19.7 to 20.6 Hz], n=5; P<0.001) (Figure 2B).

In superior mesenteric ganglia, ≈50% of the postganglionic neurons were surrounded by a very dense pericellular network of NADPH diaphorase-containing terminals, as described by Anderson et al.9 The number of neurons surrounded by NADPH-positive fibers was significantly (P<0.01) diminished in superior mesenteric ganglia of SHR than of NR (1.04±0.08 versus 1.68±0.09 cells/103 μm2, n=8).

**Discussion**

The present study shows that inhibition of NOS enhances constrictor responses of mesenteric resistance vessels induced by perivascular nerve stimulation in preparations from both NR and SHR. Removal of endothelium enhances vasoconstrictor responses of SHR but not of NR preparations. Moreover, in mesenteric arteries in which the endothelium had been removed, inhibition of NOS induces an increase in constrictor responses to electrical stimulation in preparation of NR but not of SHR. In our study, we have confirmed that the vasoconstrictor response induced by perivascular nerve activation is mediated by sympathetic nerves, because guanethidine abolished these responses. Therefore, the effect of L-NNA on sympathetic stimulation-induced vasoconstriction indicates that NO has an inhibitory influence on the responses to sympathetic nerve activation in mesenteric arteries of NR and SHR.

Augmentation of vasoconstrictor effect induced by perivascular stimulation by inhibitors of NOS has been reported previously in different isolated vessels.1–6 Although some reports have described that endothelium-derived NO is involved in this enhanced response to inhibitors of NOS,3,6 in the present study endothelium removal had no effect on the vasoconstrictor responses to sympathetic stimulation in preparations from NR. The fact that L-NNA also enhanced the vasoconstrictor responses in preparations in which the endothelium had previously been removed suggests a nonendothelial source of NO in mesenteric arteries of NR. In contrast, the enhancement of the vasoconstrictor responses to phenylephrine elicited by L-NNA in mesenteric arteries of NR was endothelium dependent. The fact that L-NNA enhanced the response to sympathetic stimulation without enhancing vasoconstriction to phenylephrine in mesenteric arteries denuded of endothelium suggests that NO exerts its inhibitory effects via a prejunctional action. These findings are entirely consistent with those reported for the rabbit renal artery.7 Because dexamethasone, an inhibitor of the inducible NOS, had no effect on the responses to perivascular stimulation (data not shown), NO could be released by electrical stimulation from perivascular nerves containing NOS. In fact, NOS has been found in autonomic nerves of various blood vessels.7,9 In SHR arteries, however, endothelium removal enhanced the vasoconstrictor responses to both perivascular nerve stimulation and phenylephrine, and the effect of NOS inhibitor was endothelium dependent. These findings indicate that endothelium-derived NO, under basal or stimulated conditions, is the main source of NO in modulating sympathetic vasoconstrictor responses in preparations from SHR. Paradoxically, impaired endothelial-dependent relaxations have been described in vessels from SHR, suggesting that hypertension is associated with an apparent decrease in the production of bioactive NO.16 The effect of hypertension on NOS expression and activity is still not fully understood, but an increase in constitutive NOS activity was reported in cardiac endothelium of SHR.17 Consequently, the mechanism involved in the release of NO by sympathetic stimulation is altered in arteries of SHR and differs from that involved in the response to endothelium-dependent vasodilators. In fact, the pharmacological profile of eNOS activation elicited by smooth muscle contraction is remarkably distinct from that of acetylcholine or other receptor-dependent agents but similar to that of fluid shear stress and tyrosine phosphatase inhibitors.18
Although in arteries from NR nonendothelium NOS plays an important role in modulating the constrictor responses elicited by electrical stimulation, L-NNA fails to affect the vasoconstrictor responses to sympathetic stimulation in arteries from SHR in which endothelium has been removed. This indicates that nonendothelium NOS, most probably neuronal NOS, activity is suppressed in SHR preparations. In the present study, NADPH diaphorase staining, used as a marker for neurons containing NOS, is significantly diminished in superior mesenteric ganglion of SHR. Similarly, a decrease in NADPH diaphorase positive fibers in the sympathetic preganglionic neurons of the upper thoracic cord was also reported in SHR, indicating that probably NO is a modulator of ganglionic transmission, and it may have a role in sympathetic tone in vivo.

Indomethacin did not influence the sympathetic constrictor responses in arteries of normotensive rats but caused a rightward shift in the frequency-response curve of arteries from SHR. Because indomethacin decreased the sensitivity of SHR arteries with or without intact endothelium, the origin of such a constrictive product of COX may be the smooth muscle.

In conclusion, the present study has suggested an inhibitory action for NO on sympathetic vasoconstriction of the rat mesenteric arteries. A prejunctional inhibitory action of non-endothelial-derived NO, most probably neuronal-derived NO, modulates sympathetic constrictor responses in arteries from NR, whereas the enhancement of the response to sympathetic stimulation by L-NNA can be attributed only to inhibition of endothelium-derived NO in mesenteric arteries of SHR.

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References

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