Endothelium-Dependent and Endothelium-Independent Vasodilation in Resistance Arteries from Hypertensive Rats

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SUMMARY The endothelium-dependent and presumed endothelium-independent vasodilators acetylcholine and sodium nitroprusside, respectively, were used to characterize relaxation responses of mesenteric resistance arteries from stroke-prone spontaneously hypertensive rats (SHRSP) and Wistar-Kyoto rats (WKY). Vessels were preconstricted using concentrations of norepinephrine or 5-hydroxytryptamine, which reduced their diameters by 50 to 60%. Relaxation responses to acetylcholine (10^-8-10^-7 M) were significantly smaller (p<0.05) in vessel segments from SHRSP, but the maximal relaxations at higher concentrations were the same in both strains. However, SHRSP vessels relaxed to a greater extent than did those of the WKY at all concentrations of sodium nitroprusside. Endothelium removal significantly enhanced sodium nitroprusside-induced dilations in both rat strains, and the dilations were significantly greater in segments from SHRSP in the concentration range of 3 x 10^-8 to 10^-6 M. The decreased relaxation to acetylcholine in resistance arteries from adult hypertensive rats compared with those from the normotensive strain suggests that functional alterations in the endothelium may play a role in hypertensive disease.

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KEY WORDS • perfused resistance arteries • hypertension • endothelium • acetylcholine • sodium nitroprusside

Essential hypertension has been characterized by increased peripheral vascular resistance as a consequence of morphological or functional changes, or both, in the arterial wall.1,2 Whether these changes also include the endothelium and the functional coupling between endothelial and smooth muscle cells in resistance arteries remains to be established. A variety of vasodilator agents have been found to require an intact endothelium to exert their effects on vascular smooth muscle.3,4 The relaxant responses to acetylcholine of precontracted thoracic aortas from genetically hypertensive rats have been reported to be smaller than those from normotensive Wistar-Kyoto rats (WKY).5-7 It is also known that endothelial damage leading to intimal proliferation and lesions occurs following chronic hypertensive episodes.8 However, vasodilator responses in resistance arteries from hypertensive animal models have received relatively little study.

The present study was designed to characterize relaxation responses of resistance arteries from normotensive and hypertensive rats using acetylcholine, an endothelium-dependent vasodilator, and sodium nitroprusside, presumed to be an endothelium-independent vasodilator. Increased levels of cyclic guanosine 3',5'-monophosphate in the smooth muscle cells is a common mediator of both acetylcholine-released endothelium-derived relaxing factor and sodium nitroprusside-induced relaxation.9 Relaxation responses after removal of the endothelium were also examined to determine the direct effect of sodium nitroprusside on the smooth muscle cells.

Materials and Methods

Age-matched male WKY and stroke-prone spontaneously hypertensive rats (SHRSP), 16 to 20 weeks of age, were obtained from the colony maintained at the University of Vermont. Systolic blood pressure was measured in the unanesthetized animal using indirect tail-cuff plethysmography. The rats were lightly anesthetized with ether and decapitated. The abdomen was opened immediately, and a branched mesenteric artery
segment proximal to the gut wall (length, 2.0 mm) was gently dissected and cleared of adhering adipose tissue. The vessel was placed in an experimental vessel chamber (arteriograph) and then mounted onto inflow and outflow cannulas. The vessel branch was similarly mounted onto a third cannula attached to a pressure transducer for measurement of transmural pressure at the proximal end of the vessel segment. All cannulas were secured with strands of 10-0 nylon suture. At a transmural pressure of 80 to 90 mm Hg, the inflow cannula was moved axially by means of a micrometer so as to remove any arterial buckle. The arteriograph was placed on the stage of a microscope with a television camera attached to the viewing tube. Physiological salt solution aerated with 95% O₂, 5% CO₂ was superfused continually for a 1-hour equilibration period. The composition of the physiological salt solution was (mM) NaCl, 119; NaHCO₃, 24; KCl, 4.7; KH₂PO₄, 1.18; MgSO₄, 1.17; CaCl₂, 1.6; glucose, 5.5; Na⁺ EDTA, 0.026. Temperature and pH were monitored at all times to maintain the vessel environment at 37.0 ± 0.5 and 7.4 ± 0.05°C, respectively. Lumen diameter was measured automatically by a videoelectronic measurement system (Living Systems Instrumentation, Burlington, VT, USA), and flow through the lumen was determined using a sensitive strain gauge scale to weigh the effluent.10

Vessel responses were measured at a transmural pressure of 40 mm Hg. This pressure was found to be optimum for constriction of mesenteric resistance arteries by testing repeated responses to electrical stimulation (30-second train, 0.2-msec pulse width, 8/second rate) at various transmural pressures. The flow rate was approximately 0.5 ml/min. Following the equilibration period, vessels were preconstricted to 50 to 60% of their initial diameter using appropriate norepinephrine (Sigma) or 5-hydroxytryptamine (Sigma) concentrations. When a steady constrictive tone was established, acetylcholine (Sigma) or sodium nitroprusside (Sigma) was added in cumulative concentrations and vessel diameters were measured. Tissues were then washed with physiological salt solution and rested for 15 to 20 minutes before activations were repeated.

The endothelium was removed by intraluminal perfusion of 0.3% 3-[[(3-cholamidopropyl)-dimethyl-ammonio]-1-propanesulfonate (Sigma), a nonionic, non-denaturing detergent, for 30 seconds followed by perfusion with physiological salt solution for 30 minutes before repeating the activations. This procedure has been shown to remove effectively the endothelium in these arteries.12 In every experiment, endothelium removal was verified by the absence of relaxation to acetylcholine (10⁻⁸-10⁻⁶ M) and in some instances by direct observation of the intimal surface after en face silver staining.

Relaxation was expressed as the percent diameter change referred to the diameter at the level of induced tone. Statistical significance of differences between the individual group means was ascertained by repeated measures of analysis of variance and unpaired Student’s t test. The data were expressed as means ± SE. A p value less than 0.05 was considered statistically significant. In all experiments, n equals the number of rats from which vessel segments were obtained.

**Results**

The body weights and systolic blood pressures of the SHRSP were 308 ± 9 g and 197 ± 9 mm Hg (n = 6) and those of the WKY were 298 ± 7 g and 129 ± 4 mm Hg (n = 6), respectively. The diameter and wall thickness of the segments from SHRSP and WKY were 265 ± 5.7, 28.9 ± 2.3 μm (n = 6), and 260.5 ± 8.5, 23.7 ± 1.9 μm (n = 6), respectively. There was no difference in the initial diameter of the vessel segments before and after removal of the endothelium.

No significant difference existed in the constrictor responses induced by norepinephrine in mesenteric arteries between hypertensive and control rats (Table 1). The tone for relaxation studies was maintained using 10⁻⁶ M norepinephrine, which reduced their diameters by nearly 50%. However, similar concentrations of 5-hydroxytryptamine caused significantly greater (p < 0.05) constrictions in arteries from SHRSP (see Table 1). Thus, the 5-hydroxytryptamine concentrations used to maintain comparable tones were 3 × 10⁻⁷ M in SHRSP and 5 × 10⁻⁷ M in WKY. Constrictor responses to both norepinephrine and 5-hydroxytryptamine were enhanced after removal of the endothelium (see Table 1). Responses to 5-hydroxytryptamine were significantly greater (p < 0.05) in SHRSP compared with WKY before and after removal of the endothelium (see Table 1).

Acetylcholine (10⁻⁸-3 × 10⁻⁶ M) produced dose-

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**TABLE 1. Percent Diameter Constrictor Responses of Mesenteric Arteries from WKY and SHRSP to Norepinephrine and 5-Hydroxytryptamine With and Without Endothelium**

<table>
<thead>
<tr>
<th>Variable</th>
<th>With endothelium</th>
<th>Without endothelium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WKY (n = 6)</td>
<td>SHRSP (n = 6)</td>
</tr>
<tr>
<td>NE</td>
<td>3 × 10⁻⁷ M</td>
<td>12.1 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>8 × 10⁻⁶ M</td>
<td>28.5 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>10⁻⁶ M</td>
<td>44.6 ± 1.0</td>
</tr>
<tr>
<td>5-HT</td>
<td>10⁻⁷ M</td>
<td>11.6 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>3 × 10⁻⁷ M</td>
<td>41.0 ± 2.4</td>
</tr>
</tbody>
</table>

Values are means ± SE. NE = norepinephrine; 5-HT = 5-hydroxytryptamine.

*p < 0.05, †p < 0.01, compared with respective values for WKY.
Dependent relaxations of vessels with intact endothelium preconstricted with either norepinephrine or 5-hydroxytryptamine from both hypertensive and control rats. With 10^-8, 3 X 10^-8, and 10^-7 M acetylcholine, arteries from hypertensive rats relaxed significantly less (p<0.05) than did those from control rats. However, there was no difference at higher concentrations between vessels from hypertensive and control rats (Figures 1 and 2).

Dilations induced by acetylcholine (10^-6—10^-3 M) were abolished after perfusion with 0.3% 3-[3-cholamidopropyl]-dimethyl-ammonio]-1-propanesulfonate in vessel segments from both WKY and SHRSP and provided functional evidence of endothelium removal (Figure 3).

In arterial segments with intact endothelium from SHRSP and WKY and preconstricted with norepinephrine or 5-hydroxytryptamine, sodium nitroprusside induced relaxations in the concentration range of 10^-8 to 10^-3 M. The maximal dilations obtained with sodium nitroprusside were 70 to 80%. Sodium nitroprusside relaxed vessel segments significantly more in arteries from SHRSP than in those from WKY at all concentrations used (Figures 4 and 5).

Endothelium removal significantly enhanced the sodium nitroprusside—induced dilations in segments preconstricted using norepinephrine or 5-hydroxytryptamine in each rat strain. This enhancement of relaxation remained greater in segments from SHRSP in the concentration range of 3 X 10^-8 to 10^-6 M (see Figures 4 and 5).

Discussion

Relaxation responses in these arteries were obtained using a pressurized vessel technique that presents the vessel wall with a true transmural pressure, maintains the wall shape, and does not damage the endothelium. With each dilator used in this study, the mesenteric resistance segments from both hypertensive and control rats were subjected to similar percent diameter constrictions referred to their initial diameters. Previous studies showed that norepinephrine sensitivities were higher in spontaneously hypertensive rats (SHR) compared with WKY after 6-hydroxydopamine treatment in which nerve varicosities were destroyed, but not before treatment, which indicates that there are alterations in uptake mechanisms of the SHR. In this study, we preconstricted vessels using norepinephrine or 5-hydroxytryptamine to exclude possible variability of responses arising from the agonist used. The results show that contractile responses from both SHRSP and WKY to norepinephrine were not different (see Table 1). However, responses to 5-hydroxytryptamine were significantly less in segments from normotensive rats than were the corresponding responses of vessels from hypertensive rats.

Endothelium-dependent, adrenergically mediated responses that counteract the direct stimulating effect of norepinephrine on vascular smooth muscle of mesenteric resistance arteries have been reported. As a consequence, in these blood vessels, removal of the endothelium enhances the constrictor responses of the smooth muscle to norepinephrine activation. In this study, it was found that constrictor responses to 5-hydroxytryptamine were modulated by the endothelium, since responses were enhanced after removal of the endothelium in both hypertensive and control rats.

Our results show that mesenteric resistance arteries from hypertensive rats were significantly less responsive to the endothelium-dependent vasodilator acetylcholine at low concentration ranges compared with segments from normotensive control rats. In addition, the maximal relaxations caused by acetylcholine (10^-6 M) were not different between SHRSP and WKY (see Figures 1 and 2). The decreased relaxation ability of acetylcholine at the lower concentrations may be due to reduced production or release (or both) of endothelium-derived vasodilators, to impaired transduction mechanism coupling between endothelium and smooth muscle cells, or to simultaneous release of contracting substances. Acetylcholine-induced contractions were reported in the thoracic aorta of SHR. In this study, acetylcholine added at baseline or to preconstricted artery did not cause further constriction. Other studies demonstrate decreased acetylcholine-induced relaxations.
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Figure 3. Diameter traces of mesenteric artery from SHRSP preconstricted with $10^{-6}$ M norepinephrine (NE). Left: Intact endothelium, dilation response to acetylcholine (ACH; $10^{-8}-3 \times 10^{-7}$ M). Right: Endothelium removed, absence of dilation to ACH ($10^{-6}-10^{-5}$ M).

Dilation ability of vascular smooth muscle; for example, thoracic aorta from hypertensive animals,$^6$ resistance vessels from two-kidney, one clip Goldblatt hypertensive rats,$^9$ and SHR mesenteric arteries.$^{16}$ However, studies in the blood-perfused renal vasculature$^1$ have shown no difference in the relaxation ability of acetylcholine in deoxycorticosterone acetate hypertensive rats compared with their controls. If endothelium-dependent reactivity operates in vivo, a reduction of endothelium-derived relaxing factors may contribute to depressed vasodilation in established hypertension, consequently augmenting constriction.

Vessel segments of SHRSP showed greater endothelium-independent relaxation responses to sodium nitroprusside. These results are similar to those obtained in tail artery strips from deoxycorticosterone acetate hypertensive rats$^8$ and canine mesenteric arteries.$^{19}$ The greater degree of relaxation to sodium nitroprusside in SHRSP than in WKY may be due to hypertrophy of the media in hypertensive rats,$^2$ which then leads to increased responsiveness to sodium nitroprusside. The mechanism for this response is unknown, but such an enhanced degree of relaxation would be favorable to the use of nitrovasodilators in the treatment of hypertension.

Our results further demonstrate that vasodilator effects of sodium nitroprusside on mesenteric resistance arteries were enhanced after removal of the endothelium, similar to results reported for rat aorta.$^{20}$ This finding suggests that endothelial cells may either par-
participate in the breakdown of sodium nitroprusside or release a constrictor factor that influences the vascular smooth muscle cells to modulate their responsiveness and that these effects are modified with genetic hypertension.

The differences observed between the increased effects of sodium nitroprusside on the one hand and decreased acetylcholine-mediated relaxation on the other hand support the idea that there is a reduced production and release of endothelial relaxing factor to activate guanylate cyclase by the endothelium. If the mode of action of sodium nitroprusside and endothelium-derived relaxing factor(s) is similar, this would suggest that the release of the latter is decreased in hypertensive rats. Since morphological modifications such as intimal thickening and increased volume of the endothelial cell layer develop in vascular endothelium in experimental hypertension, there may be decreased diffusion of endothelial cell vasodilator factors toward the smooth muscle cells.

In conclusion, the decreased endothelium-dependent relaxation response in mesenteric resistance arteries from adult SHRSP compared with those from WKY suggests that endothelial cell alterations may play a role in hypertensive disease or that some aspect of endothelial and vascular smooth muscle interactive function is compromised in hypertension.

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References
Endothelium-dependent and endothelium-independent vasodilation in resistance arteries from hypertensive rats.

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