Increased Vasodilator Responses to Acetylcholine in Psychosocial Hypertensive Mice

R. Clinton Webb, Arthur J. Vander, and James P. Henry

Summary Responsiveness to endothelium-dependent (acetylcholine and A23187) and endothelium-independent (nitroprusside and 8-bromo cyclic guanosine 3',5'-monophosphate [cGMP]) vasodilators was examined in two vascular preparations from hypertensive and normotensive mice. CBA Agouti mice were made hypertensive by exposure to social stress in a complex population cage. After 2 months, the hindquarter vascular bed was pump-perfused at a constant flow with plasma substitute to evaluate changes in perfusion pressure, and helical strips of aorta were suspended in muscle baths for measurement of isometric force generation. Tissues were treated with methoxamine to induce contractile tone. Threshold dilator responses to acetylcholine were elicited at a significantly lower dose in the hindquarters of hypertensive mice than in those from normotensive mice, indicating increased vasodilator sensitivity. In contrast, vasodilator responsiveness to nitroprusside in hindquarters of hypertensive mice did not differ from that in hindquarters of normotensive mice. Aortas from hypertensive mice were more sensitive (lower ED₅₀) to the relaxant effects of acetylcholine and A23187 than those from normotensive mice. The relaxant effects of nitroprusside and 8-bromo cGMP on aortas from hypertensive mice were not significantly different from those in normotensive aortas. Aortic strips that had been rubbed on the lumen surface with a wooden stick did not relax to acetylcholine or A23187. In aortas that were not initially contracted with methoxamine, acetylcholine and A23187 caused small contractions from baseline. The magnitude of these contractile responses were potentiated after removal of the endothelium, and the potentiation was greater in aortas from hypertensive mice. These results demonstrate an increased responsiveness to endothelium-dependent vasodilators in this psychosocial model of hypertension. (Hypertension 9: 268-276, 1987)

Key Words • vascular smooth muscle • norepinephrine • A23187 • nitroprusside • endothelium-derived relaxing factor

The endothelium plays an important role in the relaxation of isolated arterial segments to several vasodilator drugs (e.g., acetylcholine, A23187, bradykinin, histamine, adenosine triphosphate; see References 1 and 2 for review). Presumably, these drugs interact with the endothelium to cause the release of a factor (endothelium-derived relaxing factor, EDRF) that elicits the inhibitory effect on contracted smooth muscle cells. ¹,² Although the physiological importance of a given endothelium-dependent response in regulating vascular resistance has yet to be determined, the possibility exists that the release of a dilator factor may be altered in pathological states such as hypertension.¹,² Damage to the endothelial lining of blood vessels in hypertensive humans and animals has been observed.³-⁴ However, investigators, using a variety of techniques (isolated arterial segments, perfused vascular beds, intact animals), have found that vascular smooth muscle relaxation or vasodilatation in response to acetylcholine is impaired,⁵-¹⁳ unchanged,⁶,⁹,¹¹,¹⁶,¹⁷ or enhanced¹⁸-²⁰ during hypertension.

In the present study, we examined endothelium-dependent relaxation in response to acetylcholine in vascular preparations from psychosocial hypertensive mice, a model not yet evaluated. To determine if the effects of acetylcholine are specific with regard to the endothelium, the actions of two other vasodilators were characterized. One of these drugs (A23187) acts through the endothelium to cause vascular relaxation,¹,² whereas the other (nitroprusside) relaxes
smooth muscle directly through a mechanism that may be common to the dilator factor released from the endothelium (i.e., increased cyclic guanosine 3′,5′-monophosphate [cGMP]).

**Materials and Methods**

**Animal Preparation**

All studies were performed on adult male mice (weight, 30–35 g) of the CBA Agouti strain (University of Southern California Laboratories, Los Angeles, CA, USA). The protocol for induction of hypertension in these mice by exposure to social stress has been described in detail previously. Briefly, sixteen 4-month-old male mice were placed into a special population cage with an equal number of 4-month-old female mice. The population cage consists of six standard boxes formed into a circle by narrow connecting tubes with a central feeding and watering station connected to each box by tubes placed in a radial spoke pattern. Under these conditions, the male mice become highly aggressive, fail to establish a social hierarchy, and become hypertensive within the first week. Male mice raised in a traditional laboratory manner served as normotensive control animals. Systolic blood pressures were measured in the conscious state by a tail cuff technique described previously. All mice were fed a standard commercial diet (Purina, Richmond, IN, USA) and tap water. The mice were maintained in the special population cage for 54 days before experimentation.

**Hindquarter Vascular Perfusion**

Studies were performed on 18 normotensive and 18 hypertensive mice. On the days of experimentation, the mice were anesthetized with sodium pentobarbital (University of Michigan Pharmacy, Ann Arbor, MI, USA), 35 to 50 mg/kg i.p., and a midline laparotomy was performed. The hindquarters were perfused through a catheter placed in the abdominal aorta proximal to the iliac bifurcation (see Reference 26 for details). The abdominal vena cava was opened to facilitate free exit of perfusate. The mouse was killed by pneumothorax, and the hindquarter vasculature was perfused in situ at a constant flow using a peristaltic pump (Model 1202; Harvard Apparatus, South Natick, MA, USA). Perfusion flows in all preparations were adjusted until perfusion pressure was approximately 50 mm Hg. Preliminary experiments indicated that pressor responsiveness to norepinephrine (50 μg bolus), methoxamine (500 μg bolus), and angiotensin II (5 μg bolus) was maximal at these flow rates (~14 ml/100 g/min; see Reference 26 for details). The perfusate was maintained at 37°C and saturated with 95% O₂, 5% CO₂. The composition of the perfusate was as follows (mM): NaCl, 130; KCl, 4.7; KH₂PO₄, 1.18; MgSO₄·7H₂O, 1.17; CaCl₂·2H₂O, 1.6; NaHCO₃, 14.9; dextrose, 5.5; CaNa EDTA, 0.03; and Ficoll 70 (Pharmacia AB; Uppsala, Sweden), 0.71.

**Isolated Aortic Strips**

Following killing of the mice by pneumothorax, the thoracic aorta was excised and placed in cold physiological salt solution (same composition as that for the perfusate already described, except that Ficoll was not added). The aortas were cleaned of adherent fat and connective tissue and cut helically into strips (0.8 × 10 mm) under a dissecting microscope. The helical strips were mounted vertically on a stainless steel or glass holder in a tissue bath (50 ml volume) containing buffer solution. The upper ends of the strips were connected to force transducers (Model FT.03; Grass, Quincy, MA, USA), and the resting tension of each strip was adjusted so that it developed maximum active force in response to a standard dose of norepinephrine (5.9 × 10⁻⁷ M; see Reference 27 for details). The optimum passive force for maximum response to norepinephrine was similar for aortic strips from hypertensive mice (564 ± 58 mg) and those from normotensive mice (560 ± 36 mg). The bathing medium was maintained at 37°C and aerated with a mixture of 95% O₂ and 5% CO₂. Before the start of experimentation, the strips were allowed to equilibrate in physiological salt solution for 90 to 120 minutes. In each experiment, strips of aorta from a hypertensive mouse and a control mouse were mounted in the same bath, ensuring that the vascular strips had identical stimuli.

In some experiments, the lumen surface of aortic strips was rubbed with a wooden applicator stick to mechanically remove the endothelium. The functional integrity of the endothelium on each vascular segment was evaluated by relaxation responses to a standard dose of acetylcholine (1.6 × 10⁻⁶ M) following contraction induced by methoxamine (10⁻⁷ M).
Aortic responses to methoxamine, acetylcholine, A23187, nitroprusside, and 8-bromo cGMP were examined. Relaxation responses are normalized to a percentage of the contractile force to methoxamine (10⁻⁸ to 10⁻⁴ M) that existed before addition of the vasodilator. In some experiments, the drugs were added to the muscle bath in a cumulative manner. The volume of the added drug was 15 to 50 μl. Responses to each drug concentration were allowed to stabilize before the addition of a subsequent dose of the same drug or another drug. Drug concentrations are expressed as the final bath concentration. Experiments were designed so that the strips were exposed to no more than three vasodilator drugs. The order of drug exposure was randomized, and the period between cumulative treatments was 2 hours.

Statistical Analyses

Data are reported as means ± SEM. For calculation of threshold (ED₅₀) and ED₅₀ values (concentration or dose that caused a 10% or 50% of maximal response, respectively), responses were expressed as a percentage of the maximal response and a logit-log transformation was then performed. Transformed data were curve-fitted using an unweighted least-squares linear regression. An unpaired analysis (Student's t test) was used to compare systolic blood pressures, absolute maximal pressor or contractile responses, threshold values, and ED₅₀ values (expressed as −log values) between animal groups. Dose-response curves (as percentage relaxation in aortic strips or as absolute change in perfusion pressure in hindquarters) were analyzed by two-way analysis of variance, and between-group comparisons were performed using Duncan's multiple range test. A p value less than 0.05 was considered statistically significant. The n values reported in the figures and tables are the number of mice used in each experiment.

Drugs

Norepinephrine (Levophed bitartrate, Breon Laboratories, New York, NY, USA) and methoxamine (Burroughs Wellcome Company, Research Triangle Park, NC, USA) were diluted in physiological salt solution containing 0.1% ascorbic acid. Nitroprusside (Nipride, Abbott Laboratories, North Chicago, IL, USA), 8-bromo cGMP (Sigma Chemical, St. Louis, MO, USA), acetylcholine (Sigma), and papaverine (Sigma) were dissolved in physiological salt solution. A23187 (Calbiochem, San Diego, CA, USA) was dissolved in absolute ethanol; at the concentrations used in these experiments, ethanol alone had no effect on basal tension or contractile responses to methoxamine (10⁻⁷ M) in aortic strips.

Results

Animals and Characteristics of Vascular Preparations

Systolic blood pressures of psychosocial hypertensive mice were significantly higher than those of normotensive mice (154 ± 3 vs 118 ± 2 mm Hg; n = 18 in each group; p < 0.05). Body weights of mice in the two groups were not different (hypertensive mice = 33.0 ± 0.5 g; normotensive mice = 33.6 ± 0.6 g).

Under the conditions of these experiments, both the aortic strip preparation and the hindquarter vasculature were aortic; bolus injections of papaverine into the hindquarter bed or addition of papaverine to the muscle bath had no effect on baseline resting values. Therefore, to examine the effects of vasodilator drugs, methoxamine was used to induce constrictor tone (see the following sections for details of each experimental protocol). At the end of the perfusion experiments, perfused hindquarters weighed 108 ± 3% of nonperfused hindquarters (isolated from mice that were not perfused), indicating minimal edema formation. The weight of perfused hindquarters from hypertensive mice (9.7 ± 0.2 g, n = 18) was similar to that from normotensive mice (9.6 ± 0.2 g; n = 18).

Vasodilator Responses to Acetylcholine

In the first set of experiments, responsiveness to acetylcholine was characterized (Figure 1). Aortic strips were contracted with methoxamine (10⁻⁷ M, a concentration that produced a half-maximal contraction in aortic strips from both groups of mice), and the magnitude of force development in aortic strips from hypertensive mice (unrubbed = 159 ± 27 mg, n = 10; rubbed = 160 ± 32 mg, n = 6) was not significantly different from that in aortic strips from normotensive mice (un rubbed = 168 ± 25 mg, n = 10; rubbed = 163 ± 29 mg, n = 6). Cumulative addition of acetylcholine (5.5 × 10⁻¹⁰ to 5.5 × 10⁻⁴ M) to the muscle bath produced relaxation responses in unrubbed aortic strips from hypertensive and normotensive mice (see Figure 1A). Unrubbed aortic strips from hypertensive mice relaxed to a greater percentage of the response to methoxamine than unrubbed strips from normotensive mice. Rubbed aortic strips from hypertensive and normotensive mice did not relax in response to acetylcholine.

In the hindquarter vasculature, the constant infusion of methoxamine (200 μg/kg/min) produced a pressor response in hindquarters of hypertensive mice (84 ± 9 mm Hg; n = 6) that was not significantly different from that in the hindquarters of normotensive mice (72 ± 7 mm Hg; n = 6). During the methoxamine infusion, bolus injections of acetylcholine (0.0005–50 μg) decreased perfusion pressure in hypertensive and normotensive hindquarter preparations (Figure 1B). Depressor responses to acetylcholine in hypertensive mice were greater than those in normotensive mice at low doses, whereas high doses of the drug (5 and 50 μg) produced similar changes in perfusion pressure in the two groups.

To interpret the results in terms of sensitivity to the dose of the agonist, the dilator response for each dose was normalized to its maximal response and the doses of the drug producing 10% (threshold, ED₅₀) and 50% maximal responses (ED₅₀) were determined. Threshold and ED₅₀ values to acetylcholine were lower in unrubbed aortic strip (Table 1) and hindquarter prep-
FIGURE 1. Vascular relaxation induced by acetylcholine in methoxamine-contracted preparations. A. Mean relaxation responses to acetylcholine in control aortic strips (unrubbed) from hypertensive mice that were significantly greater than those in aortic strips from normotensive mice are denoted by asterisks (p<0.05). Acetylcholine caused small relaxation responses in aortic segments in which the lumen was rubbed with a wooden applicator stick. Daggers indicate a statistically significant difference between rubbed and control aortic segments (p<0.05). B. Mean depressor responses to acetylcholine in the hindquarters of hypertensive mice that were significantly greater than those in the hindquarters of normotensive mice are marked by asterisks (p<0.05). All values are means ± SEM. Values in parentheses are the number of mice.

Dilator responses to acetylcholine were dependent on the magnitude of tone induced by methoxamine in both aortic strip and hindquarter preparations (Figure 2, lower panels). As the contractile force to methoxamine increased, the magnitude of relaxation (expressed as a percentage of the developed force) in response to acetylcholine decreased in aortic strips (see Figure 2A). Similarly, the greater the pressor response to methoxamine, the smaller the dilatation induced by acetylcholine in the hindquarter vasculature. In both vascular preparations, the response to acetylcholine was greater in hypertensive mice than in normotensive mice. Contractile responses to methoxamine in aortic strips from hypertensive mice did not differ from those in aortic strips from normotensive mice (see Figure 2A). In the perfused hindquarters of hypertensive mice, methoxamine-induced vasoconstriction did not differ from that in normotensive mice at the two lower doses, whereas pressor responses to 500 μg/kg/min in hypertensive mice were augmented compared with that in hindquarters of normotensive mice (see Figure 2B).

Relaxation Responses to A23187 in Aortic Strips

As in the experiments with acetylcholine, aortic strips were contracted with methoxamine (10^-7 M) and the magnitude of force development in aortic strips from hypertensive mice (unrubbed = 165 ± 29 mg, n = 6; rubbed = 158 ± 32 mg, n = 6) did not differ significantly from that in aortic strips from normotens-

Table 1. Threshold (ED_{10}) and ED_{50} Values in Methoxamine-Contracted Aortic Strips

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Acetylcholine</th>
<th>A23187</th>
<th>Nitroprusside</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ED_{10} (nM)</td>
<td>ED_{50} (nM)</td>
<td>ED_{10} (nM)</td>
</tr>
<tr>
<td>Unrubbed Aortic Strips</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertensive mice</td>
<td>6.0 (8.223 ± 0.150)*</td>
<td>27.9 (7.556 ± 0.154)*</td>
<td>3.5 (8.453 ± 0.194)*</td>
</tr>
<tr>
<td>Normotensive mice</td>
<td>18.2 (7.740 ± 0.115)</td>
<td>119.7 (6.922 ± 0.069)</td>
<td>9.7 (8.013 ± 0.102)</td>
</tr>
<tr>
<td>Rubbed Aortic Strips</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertensive mice</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Normotensive mice</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

Threshold and ED_{50} values are the antilog of geometric means presented for respective groups. The −log molar values (mean ± SEM) are indicated parenthetically. The number of mice in each experimental group (n) is also indicated parenthetically.

*p < 0.05, compared with respective value in normotensive mice.
sive mice (unrubbed = 172 ± 21 mg, n = 6; rubbed = 157 ± 33 mg, n = 6). Cumulative addition of A23187 (10^-9 to 3 x 10^-6 M) to the muscle bath produced relaxation responses in unrubbed aortic strips from hypertensive and normotensive mice (Figure 3). Unrubbed aortic strips from hypertensive mice relaxed to a greater percentage of the methoxamine-induced tone than did unrubbed strips from normotensive mice. Rubbed aortic strips from hypertensive and normotensive mice did not relax in response to A23187. Threshold and ED50 values to A23187 in unrubbed aortic strips from hypertensive mice were lower than those in unrubbed aortic strips from normotensive mice (see Table 1).

Vascular Responses to Acetylcholine and A23187 in the Absence of Methoxamine-Induced Tone

In a separate set of experiments, the effects of acetylcholine and A23187 on baseline contractile activity were examined in aortic strips. Cumulative addition of either drug to the muscle bath caused contraction in aortic strips from hypertensive and normotensive mice (Figure 4). At the highest concentration of acetylcholine (5.5 x 10^-4 M), the magnitude of force development in unrubbed strips was approximately 5% of the maximal force developed in response to methoxamine (10^-4 M) and contractile force to acetylcholine in unrubbed aortic strips from hypertensive mice was less than that in unrubbed aortic strips from normotensive mice (see Figure 4A). Following removal of the endothelium, the contractile responses to acetylcholine were potentiated and the magnitude of the potentiation was greater in aortic strips from hypertensive mice than in those from normotensive mice. Threshold and ED50 values for acetylcholine in rubbed aortic strips from hypertensive mice (−log ED10 = 6.070 ± 0.098; antilog = 0.9 x 10^-6 M; −log ED50 = 5.342 ± 0.077; antilog = 4.5 x 10^-6 M; n = 6) were significantly lower than those from normotensive mice (−log ED10 = 5.619 ± 0.108; antilog = 2.4 x 10^-6 M; −log ED50 = 4.876 ± 0.056; antilog = 13.3 x 10^-6 M; n = 6), indicating increased smooth muscle sensitivity to the drug.

Contractile responses to A23187 in unrubbed aortic strips were approximately 5% of the maximal force developed in response to methoxamine, and contractile force to A23187 in unrubbed aortic strips from hypertensive mice did not differ from that in unrubbed aortic strips from normotensive mice (see Figure 4B). In aortic strips that had been rubbed, A23187 caused contractions that were approximately 20 to 25% of the maximal response to methoxamine, and the magnitude of contractions to A23187 in rubbed aortic strips from hypertensive mice was greater than that in those from normotensive mice (statistical difference between mouse groups at only 1 concentration: 3 x 10^-6 M).

Vasodilator Responses to Nitroprusside

These experiments characterized responsiveness in hypertensive and normotensive preparations to a vasodilator (nitroprusside) that is not dependent on the endothelium. Aortic strips were contracted with 10^-7 M methoxamine (hypertensive mice: 10 unrubbed = 155 ± 23 mg; 6 rubbed = 171 ± 31 mg; normotensive mice: 10 unrubbed = 156 ± 21 mg; 6 rubbed = 171 ± 34 mg). Cumulative addition of nitroprusside (3.8 x 10^-11 to 3.8 x 10^-6 M) to the muscle bath produced relaxation responses in unrubbed and rubbed aortic strips from hypertensive and normotensive mice (Figure 5A; see Table 1). The magnitude of relaxation to nitroprusside in aortic strips from hypertensive mice did not differ from that in aortic strips from normotensive mice.

In the hindquarter vasculature, the constant infusion of methoxamine (200 μg/kg/min) produced a pressor response in hindquarters of hypertensive mice (88 ± 6 mm Hg; n = 8) that was not significantly different from that in the hindquarters of normotensive mice (83 ± 8 mm Hg; n = 8). During the methoxamine-induced vasoconstriction, bolus injections of nitroprusside (0.0005–500 μg) decreased perfusion pressure in hypertensive and normotensive hindquarter preparations (Figure 5B; see Table 2). Depressor responses to nitroprusside in hypertensive mice were not significantly different from those in normotensive mice.

Relaxation Responses to 8-Bromo cGMP in Aortic Strips

Aortic strips were contracted with methoxamine (10^-7 M), and force development in aortic strips from hypertensive mice (unrubbed = 168 ± 21 mg, n = 6; rubbed = 158 ± 31 mg, n = 6) did not differ significantly from that in aortic strips from normotensive mice (unrubbed = 148 ± 35 mg, n = 6; rubbed = 142 ± 34 mg, n = 6). After the contractile

<table>
<thead>
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<th>Hindquarter vasculature</th>
<th>Acetylcholine (n = 6)</th>
<th>Nitroprusside (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ED10 (ng)</td>
<td>ED50 (ng)</td>
</tr>
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<td>Hypertensive mice</td>
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<td>19.50</td>
</tr>
<tr>
<td></td>
<td>(9.039±0.200)*</td>
<td>(7.710±0.129)*</td>
</tr>
<tr>
<td>Normotensive mice</td>
<td>7.07</td>
<td>184.04</td>
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<tr>
<td></td>
<td>(8.151±0.216)</td>
<td>(6.735±0.122)</td>
</tr>
</tbody>
</table>

Threshold and ED50 values are the antilog of geometric means presented for respective groups. The − log gram values (mean ± SEM) are indicated parenthetically. The number of mice in each experimental group (n) is also indicated parenthetically.

*p < 0.05, compared with values in normotensive mice.
response had reached a plateau, 8-bromo cGMP ($10^{-3}$ M) was added to the muscle bath. This concentration of 8-bromo cGMP caused 100% relaxation, and the interval of time to half-maximal relaxation ($t_{1/2}$) in unrubbed (6.6 ± 0.5 minutes; $n = 6$) and rubbed aortic strips (6.8 ± 0.6 minutes; $n = 6$) from hypertensive mice did not differ significantly from that in unrubbed (6.7 ± 1.1 minutes; $n = 6$) and rubbed aortic strips (6.7 ± 1.2 minutes; $n = 6$) from normotensive mice.

**Discussion**

This study shows that acetylcholine was more effective in producing smooth muscle relaxation in two different preparations (aortic strip and hindquarter vasculature) from psychosocial hypertensive mice than in those from normotensive mice. Aortic strips that had been rubbed on the intimal surface did not relax in response to acetylcholine, demonstrating endothelial dependency. Intact aortic strips from hypertensive mice were also more responsive to the endothelium-dependent dilator properties of the calcium ionophore A23187. Acetylcholine and A23187 caused small contractile responses in intact aortic strips that were not contracted with methoxamine. After removal of the endothelium, contractions to acetylcholine and A23187 were potentiated to a greater extent in hypertensive aortic strips than in those from normotensive mice. Smooth muscle relaxation in hypertensive mice in response to nitroprusside and 8-bromo cGMP did not differ significantly from that in normotensive mice.

Previous studies of psychosocial hypertensive mice have demonstrated increased vasoconstrictor sensitivity to norepinephrine and angiotensin II in the renal and hindquarter vasculature. In the present study,
FIGURE 4. Aortic responses to acetylcholine and A23187 in the absence of methoxamine-induced tone. Contractile responses to acetylcholine (A) and A23187 (B) in control aortic strips (unrubbed) from hypertensive mice were either less than or not significantly different from those in aortic strips from normotensive mice. Contractile responses to the drugs in rubbed aortic segments from hypertensive mice that were significantly greater than those in normotensive aortic strips are denoted by asterisks (p<0.05). Daggers indicate a statistically significant difference between rubbed and control aortic segments (p<0.05). Values are means ± SEM. Values in parentheses are the number of mice in each experimental group.

FIGURE 5. Vascular relaxation induced by nitroprusside in methoxamine-contracted preparations. Relaxation responses to nitroprusside in aortic segments (A) and hindquarters (B) of hypertensive mice were not significantly different from those in respective vascular preparations from normotensive mice. Values are means ± SEM. Values in parentheses are the number of mice in each experimental group.

Vasoconstrictor sensitivity to methoxamine in hypertensive mice did not differ from normotensive values (although the response to the highest dose of methoxamine was greater in the hindquarters of hypertensive mice). An explanation for these findings is not readily apparent, but it may relate to the relative specificity of the adrenergic agonists for specific subtypes of the α-adrenergic receptor. Norepinephrine is a mixed agonist that interacts at both α₁ and α₂ sites, whereas methoxamine is selective for the α₁-adrenergic subtype. Thus, it is possible that a change in the α₂-adrenergic receptor subtype accounts for the increased vasoconstrictor sensitivity to norepinephrine in the psychosocial hypertensive mouse (aortic strips from mice contract to the selective α₂-agonist clonidine; data not shown). In spontaneously hypertensive rats, the increased sensitivity to α-adrenergic agonists in isolated tail arteries is associated with an increased number of α₂-adrenergic receptors on the vascular smooth muscle. Vascular sensitivity to methoxamine in tail arteries of spontaneously hypertensive rats does not differ from that in tail arteries of Wistar-Kyoto rats. An alternative possibility is that norepinephrine sensitivity (but not methoxamine sensitivity) may re-
fect an interaction with \( \beta \)-adrenergic receptors that may be changed in aortic smooth muscle of hypertensive mice.

The increased responsiveness to acetylcholine in hypertensive mice could have been related, theoretically, to the amount of tone (or preload) induced by methoxamine, since the relaxation responses (on a percentage basis) to several vasodilator agents vary with the magnitude of tone induced by a constrictor drug.\(^{28-31}\) This effect was evident in the responses to acetylcholine in both the aortic strip and hindquarter vasculatures used in this study (see Figure 2). The magnitude of the constrictor responses to methoxamine varied with the concentration of the agonists, and the relaxation response to acetylcholine was reduced when the tone of the preparation was large. However, contractile responses to methoxamine in hypertensive mice were not significantly different from those in normotensive mice, and the relaxation response to a single dose of acetylcholine was enhanced in hypertensive mice at all doses of methoxamine.

In the hindquarter vasculature of hypertensive mice, there was a greater depressor activity in response to acetylcholine at low and mid-range doses; at higher doses, vasodilatation was similar in the two groups of mice (see Figure 1B). Analogous results were obtained by Hollenberg and Adams,\(^{19}\) who measured renal blood flow responses to intra-arterial injections of acetylcholine in patients with essential hypertension. It seems possible that the vasodilator action of high doses of acetylcholine was masked by the inability of the vasculature to dilate further because of structural changes (increased wall thickness). Evidence in support of this hypothesis is that in aortic strips, where a structural factor would not play a role, there was a greater percentage of relaxation in segments from hypertensive mice than in aortic strips from normotensive mice, but the responses at high doses in hypertensive mice did not converge toward the responses of the aortic strips from normotensive mice. Evidence against this hypothesis is the observation that vasodilatation in response to nitroprusside in both aortic strips and hindquarter vasculature of hypertensive mice was not significantly different from that in normotensive mice; if a structural change limited the vasodilator properties of acetylcholine in hypertensive mice, then it should also have been apparent with nitroprusside. An alternative possibility is that vasoconstrictor activity may be involved. For example, aortic strips without endothelium from hypertensive mice contracted more in response to acetylcholine than those from normotensive mice (see Figure 4), suggesting an increased contractile activity of acetylcholine on smooth muscle cells in hypertensive mice. A similar hypersensitivity could limit the depressor activity of high doses of acetylcholine in the hindquarter vasculature of hypertensive mice. It is also possible that acetylcholine-induced vasodilatation in the hindquarter involves a nonendothelial component.

Recently published observations suggest that cGMP participates in the relaxation event induced by acetylcholine and other endothelium-dependent vasodilators.\(^1,2,21\) Presumably, EDRF released in response to the vasodilator increases cGMP in the smooth muscle cells, resulting in relaxation. Recent observations by Lockette et al.\(^{18}\) suggest that attenuated responsiveness to acetylcholine in aortas from mineralocorticoid hypertensive rats is correlated with a reduced ability of the smooth muscle cells to synthesize cGMP. However, the enhanced responsiveness to acetylcholine in psychosocial hypertensive mice does not appear to involve the activity of cGMP, because relaxation to the cyclic nucleotide in aortic strips did not differ between hypertensive and normotensive mice. It is possible that a greater amount of cGMP is produced in response to acetylcholine, causing an enhanced response in aortas from hypertensive mice. Alternative explanations for the augmented dilator responsiveness to acetylcholine in hypertensive mice may relate to one or more of the following: 1) an increased sensitivity of the endothelium to acetylcholine; 2) an increased sensitivity of the smooth muscle cells to EDRF; and 3) an increased release of EDRF from endothelial cells in response to acetylcholine.

Regardless of the precise mechanism, the effect seems generalized, since A23187, another endothelium-dependent vasodilator, mimics the effect of acetylcholine. The magnitude of relaxation (as a percentage of the contractile response to methoxamine) to A23187 in aortic strips from hypertensive mice was greater than that in aortic strips from normotensive mice. Furthermore, vascular sensitivity (ED\(_{50}\) value) to the relaxing effect of the calcium ionophore was increased in aortic strips from hypertensive mice, as was vascular sensitivity to acetylcholine.

Several investigators have proposed that impaired vasodilatation may contribute to elevated vascular resistance in hypertension (see Reference 28 for review). Reduced responsiveness to endothelium-dependent vasodilators in hypertension has been reported by a number of laboratories,\(^2-15\) but contrary observations have also been reported.\(^7,8,9,11,16-20\) The results of the current study do not support this concept of impaired vasodilatation, and it seems reasonable that the response of the vasculature to endothelium-dependent vasodilators may represent a compensatory mechanism to dampen augmented vascular reactivity in this psychosocial model of hypertension.

References

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