Vascular Reactivity in the Spontaneously Hypertensive Stroke-Prone Rat
Effect of Antihypertensive Treatment

EDWARD E. SOLTIS AND DAVID F. BOHR

SUMMARY This study investigated vascular responsiveness in stroke-prone spontaneously hypertensive rats (SHRSP) and the effect of antihypertensive treatment on this responsiveness. Weanling (4-week-old) male and female SHRSP and Wistar-Kyoto rats (WKY) received either the antihypertensive combination treatment of hydralazine plus hydrochlorothiazide in drinking water or tap water alone (controls) for 15 weeks. Whereas the antihypertensive combination prevented the development of hypertension in treated SHRSP (SHRSP-T), blood pressure remained unchanged in treated WKY (WKY-T). Femoral arterial smooth muscle responsiveness to KCl, norepinephrine, and calcium (in the presence of either 40 mM KCl or 1 μM norepinephrine) was not altered in SHRSP when compared with WKY. A significant increase in the sensitivity of femoral arteries to KCl and calcium (in the presence of 40 mM KCl) was seen, however, in SHRSP-T and WKY-T. An increased sensitivity to norepinephrine and calcium (in the presence of 1 μM norepinephrine) was seen only in SHRSP-T. Isoproterenol-induced relaxation was significantly attenuated in both SHRSP and SHRSP-T. Relaxation induced by sodium nitroprusside and calcium (membrane stabilization) was not different between the four groups. These results show that femoral arterial smooth muscle responsiveness to vasoconstrictor stimuli is not altered in SHRSP but that β-adrenergic-mediated relaxation is attenuated. Antihypertensive treatment resulted in an enhanced responsiveness to these vasoconstrictor stimuli but had no effect on the relaxation properties of femoral arterial smooth muscle.

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KEY WORDS • hydralazine • hydrochlorothiazide • femoral artery • calcium sensitivity

Numerous investigators have suggested that the development of hypertension in spontaneously hypertensive rats (SHR) is the result of an increased vascular sensitivity to vasoconstrictor stimuli that leads to an increase in peripheral vascular resistance.¹⁻⁵ Antihypertensive treatment reverses the elevation of blood pressure in SHR but has no effect on the altered vascular responsiveness.⁶ These authors suggest that the increase in vascular sensitivity is associated with genetic factors responsible for and not a consequence of the increase in blood pressure. Unlike the SHR, few studies have been reported on vascular changes in the stroke-prone SHR (SHRSP).⁷ In the present study, we examined contraction and relaxation of isolated strips of femoral arterial smooth muscle to determine whether this muscle of SHRSP differed from that of normotensive Wistar-Kyoto rats (WKY). We also determined the role of the increase in blood pressure on these differences. To this end, half of each group of rats was treated with the antihypertensive combination of hydralazine (Hyd) and hydrochlorothiazide (HCTZ) starting at the time of weaning (approximately 4 weeks of age) and continuing for 4 months.

Materials and Methods

Four-week-old male and female SHRSP and WKY were obtained from colonies maintained at the University of Michigan Department of Anatomy and housed in a pathogen-free environment. Rats from each strain received either the antihypertensive combination treatment of Hyd plus HCTZ (100 mg/L of each drug in drinking water) or tap water ad libitum. At approximately 8 weeks into the study the concentration of each drug in the drinking water was increased to 200 mg/L. Purina rat chow (Ralston Purina, St. Louis, MO, USA)
was provided ad libitum. Systolic blood pressure (SBP) was recorded every 3 weeks with a standard tail cuff technique using a pneumatic pulse transducer (Narco Bio-Systems, Houston, TX, USA) and polygraph (Model 79D; Grass Instruments, Quincy, MA, USA).

After approximately 15 weeks of treatment the rats were anesthetized with pentobarbital (50 mg/kg) and killed by exsanguination. Both femoral arteries were removed and placed in a physiological salt solution (PSS) at room temperature. Helical strips of the femoral arteries (1 × 10 mm) were cut under a dissecting microscope and suspended in a muscle bath containing oxygenated (95% O₂ and 5% CO₂) PSS maintained at 37°C. The upper ends of the strips were connected to a force transducer (Grass FT 0.03), and 500 mg of passive force was placed on each artery. This passive force was found to be optimal for maximum force generation by strips of femoral artery from all four groups in preliminary experiments. The tissues were equilibrated for 90 minutes before studies on vascular responsiveness were performed. Unless otherwise specified, composition of the PSS was (in mM): NaCl, 130; KCl, 4.7; KH₂PO₄, 1.18; MgSO₄-7H₂O, 1.17; NaHCO₃, 14.9; CaCl₂-2H₂O, 1.6; dextrose, 5.5; CaNa₂ EDTA, 0.026.

Dose-response curves were performed to the contractile agents KCl (5–80 mM) and norepinephrine (NE; 10⁻⁶ to 3 × 10⁻⁵ M) were obtained in a cumulative fashion. Relaxation of strips of femoral artery in response to cumulative addition of isoproterenol (10⁻⁹ to 3 × 10⁻⁶ M) and sodium nitroprusside (10⁻¹⁰ to 10⁻⁶ M) were determined in tissues contracted with a dose of KCl that resulted in approximately 50% of the maximal response (ED₅₀).

Calcium sensitivity of both KCl and NE contractions of femoral arteries was determined. Initially, the tissues were exposed to a Ca-free, EGTA (1.0 mM) PSS for 10 minutes. Five minutes into this interval the tissues were stimulated with either 40 mM KCl or 1 μM NE to deplete membrane stores of Ca. After this 10-minute period, the tissues were rinsed with a Ca-free PSS and either 40 mM KCl or 1 μM NE was added to the bath. No contraction occurred in response to either agonist. A Ca dose-response curve (0.01–1.6 mM) was then generated.

The membrane-stabilizing action of Ca also was determined. Following contraction with an ED₅₀ dose of KCl, strips of femoral artery were exposed to increasing concentrations of Ca (2.4–21.4 mM) resulting in a relaxation.

Dose-response curves were performed in random order with the exception of KCl, which was obtained first so as to determine the approximate ED₅₀ concentration to use in relaxation studies. Sufficent time was provided between each dose-response curve to allow washout of the agents. The presence of endothelium was determined by maximal acetylcholine-induced relaxation. No difference in acetylcholine-induced relaxation was seen between the four groups (data not shown).

Hydralazine hydrochloride, HCTZ, NE hydrochloride, isoproterenol hydrochloride, sodium nitroprusside, EGTA, and acetylcholine chloride were purchased from Sigma Chemical (St. Louis, MO, USA). Drinking solutions of the antihypertensive treatment were made fresh each day in tap water. Agents used in the in vitro studies on vascular smooth muscle were dissolved in 0.1% ascorbic acid solution and serially diluted in distilled water each day.

Data are expressed as the mean ± one standard error of the mean. The ED₅₀ values were determined following logit transformation of normalized dose-response curves. Statistical significance between the four groups was determined with a two-way analysis of variance followed by individual comparisons using the Newman-Keuls test. A p value of less than 0.05 was considered significant.

Results

The antihypertensive combination of Hyd plus HCTZ prevented the development of hypertension in treated SHRSP (SHRSP-T; Figure 1). Although SHRSP-T exhibited a progressive rise in SBP of almost 30 mm Hg during the first 6 weeks of treatment, SBP remained at or below the level of 140 mm Hg for the rest of the treatment period and was similar to that in control WKY and in treated WKY (WKY-T) at the time of studies on vascular responsiveness. Antihypertensive treatment had no apparent effect on SBP in normotensive WKY.

No difference was observed in the contractions of femoral arteries from WKY and SHRSP in response to KCl stimulation (Figure 2A). A significant increase in the sensitivity to KCl was seen, however, in WKY-T and SHRSP-T when compared with WKY and SHRSP. The increased sensitivity is reflected in the lower ED₅₀ values (Table 1). Calcium sensitivity in the presence of 40 mM KCl followed a similar pattern (Figure 2B); that is, no difference in the response of
Femoral arteries to Ca in the presence of KCl was seen between WKY and SHRSP. An increased sensitivity was observed in WKY-T and SHRSP-T and is reflected in the lower ED_{50} values (see Table 1). No difference in maximum force generation was seen among the four groups in response to KCl or Ca (see Figure 2).

Femoral arterial smooth muscle responsiveness to NE was not significantly different in SHRSP when compared with WKY (Figure 3A). Antihypertensive treatment had no effect on NE responsiveness in WKY-T, however, SHRSP-T exhibited a significant increase in sensitivity. ED_{50} values were significantly lower in SHRSP-T (see Table 1). The response of femoral arteries to Ca in the presence of NE followed a similar pattern to that seen for NE alone (Figure 3B); that is, whereas no difference was observed among WKY, WKY-T, and SHRSP, a significantly greater sensitivity to Ca was seen in SHRSP-T. Again, this difference is reflected in the lower ED_{50} values (see Table 1). Maximum force generation was similar in the four groups for NE and Ca (see Table 1).

Isoproterenol-induced relaxation of femoral arteries from SHRSP was significantly attenuated when compared with WKY (Figure 4A). Antihypertensive treatment had no effect on this response in either WKY-T or SHRSP-T. Relaxation induced by sodium nitroprusside was not different among the four groups (Figure 4B).

The membrane-stabilizing action of Ca (Ca-induced relaxation) is depicted in Figure 5. No difference in this response was observed between WKY and SHRSP, and antihypertensive treatment had no effect.

**Discussion**

Alterations in vascular responsiveness may be important in the development of hypertension in the SHR. These alterations are not a result of the in-

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**TABLE 1. Half-Maximal (ED_{50}) Values for Contraction and Relaxation Responses**

<table>
<thead>
<tr>
<th>Agonist</th>
<th>WKY (n = 7)</th>
<th>WKY-T (n = 6)</th>
<th>SHRSP (n = 9)</th>
<th>SHRSP-T (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>KCl (mM)</strong></td>
<td>31.5 ± 1.1</td>
<td>26.3 ± 0.6*</td>
<td>29.5 ± 0.7</td>
<td>25.3 ± 0.8*</td>
</tr>
<tr>
<td></td>
<td>(368 ± 36)</td>
<td>(364 ± 47)</td>
<td>(407 ± 23)</td>
<td>(414 ± 37)</td>
</tr>
<tr>
<td><strong>Ca (mM; in 40 mM KCl)</strong></td>
<td>0.32 ± 0.03</td>
<td>0.24 ± 0.02*</td>
<td>0.36 ± 0.01</td>
<td>0.27 ± 0.01*</td>
</tr>
<tr>
<td></td>
<td>(452 ± 41)</td>
<td>(437 ± 35)</td>
<td>(496 ± 40)</td>
<td>(478 ± 25)</td>
</tr>
<tr>
<td><strong>Norepinephrine (M)</strong></td>
<td>5.3 [± 0.6] ± 10^{-7}</td>
<td>6.6 [± 1.3] × 10^{-7}</td>
<td>4.6 [± 0.3] × 10^{-7}</td>
<td>1.8 [± 0.5] × 10^{-7}†</td>
</tr>
<tr>
<td></td>
<td>(371 ± 33)</td>
<td>(579 ± 41)</td>
<td>(632 ± 47)</td>
<td>(633 ± 44)</td>
</tr>
<tr>
<td><strong>Ca (mM; in 1 μM NE)</strong></td>
<td>0.44 ± 0.04</td>
<td>0.42 ± 0.02</td>
<td>0.38 ± 0.01</td>
<td>0.30 ± 0.02†</td>
</tr>
<tr>
<td></td>
<td>(462 ± 39)</td>
<td>(497 ± 50)</td>
<td>(528 ± 33)</td>
<td>(562 ± 41)</td>
</tr>
<tr>
<td><strong>Isoproteranol (M)</strong></td>
<td>7.9 [± 1.5] ± 10^{-8}</td>
<td>9.7 [± 1.3] × 10^{-8}</td>
<td>17.6 [± 1.2] × 10^{-8}‡</td>
<td>18.1 [± 1.3] × 10^{-8}‡</td>
</tr>
<tr>
<td></td>
<td>(571 ± 33)</td>
<td>(579 ± 41)</td>
<td>(632 ± 47)</td>
<td>(633 ± 44)</td>
</tr>
<tr>
<td><strong>SNP (M)</strong></td>
<td>3.0 [± 0.9] ± 10^{-9}</td>
<td>2.4 [± 0.6] × 10^{-9}</td>
<td>3.4 [± 0.8] × 10^{-9}</td>
<td>4.2 [± 0.9] × 10^{-9}</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Numbers in parentheses are maximum force generation (mg). T = treated; NE = norepinephrine; SNP = sodium nitroprusside.

* p < 0.05, significantly different from values for WKY and SHRSP.
† p < 0.05, significantly different from values for the other three groups.
‡ p < 0.05, significantly different from values for WKY and WKY-T.
crease in blood pressure, as antihypertensive treatment or prevention of the increase in high blood pressure stress to an isolated vascular bed do not reverse the changes. Unlike studies in SHR, few studies have examined alterations in vascular responsiveness in the SHRS and the role of blood pressure in these changes. In the present study, SHRS and normoten-sive WKY were treated with the antihypertensive combination of Hyd plus HCTZ. Treatment was begun at weaning (3-4 weeks of age), at which time no significant increase in blood pressure was seen in SHRS. This protocol allowed us to examine the role of blood pressure on vascular changes. Studies of vascular responsiveness were performed after several months when SHRS were in the established stage of hypertension. Our results show that no major alterations in vascular responsiveness to vasoconstrictor stimuli are present at this stage of hypertension. Unexpectedly, antihypertension treatment resulted in a slight but significant increase in vascular responsiveness in both SHRS and WKY.

Although SHRS-T exhibited a rise in blood pressure during the first 6 weeks of treatment, they did not become hypertensive (SBP > 140 mm Hg). The initial rise in SBP in SHRS-T and the leveling off after 6 weeks are most likely due to the treatment regimen. That is, the initial dose of 100 mg/L was enough to attenuate the initial rise; however, increasing the dose to 200 mg/L was required to prevent any further rise in blood pressure. Although accurate measurements of the amount of drinking water consumed by the rats were not made, gross daily observations revealed no differences. As the study was performed over a long period (15-16 weeks), this initial slight rise in blood pressure was probably of no consequence when alterations in vascular responsiveness were eventually examined. The important point is that blood pressure was significantly lowered in SHRS-T and that these values were similar to those in the normotensive WKY.

Unlike the SHR, no increase in vascular sensitivity to KCl, NE, and Ca was seen in SHRS. Furthermore, the membrane-stabilizing action of Ca (Ca re-

FIGURE 3. Dose-response relationships of norepinephrine (NE)-induced contractions (A) and calcium (in the presence of 1 μM NE)-induced contractions (B) in strips of femoral artery from SHRS, SHRS-T, WKY, and WKY-T (T = treated). Data are expressed as the percentage of the maximal contraction to the respective agonist. Statistical comparisons are the same as those in Figure 2.

FIGURE 4. Dose-response relationships of isoproterenol (ISO)-induced relaxation (A) and sodium nitroprusside (SNP)-induced relaxation (B) of strips of femoral artery from SHRS, SHRS-T, WKY, and WKY-T (T = treated). Data are expressed as the percentage of relaxation from the initial contraction to an ED50 dose of KCl. Statistical comparisons are the same as those in Figure 2.
relaxation) also was not altered in the SHRSP, as is usually observed in the SHR. The decrease in the stabilizing action of Ca has been suggested to be an important membrane alteration that is involved in the altered vascular responsiveness seen in hypertension. Perhaps the lack of a change in this membrane function in SHRSP accounts for the lack of a change in the sensitivity of the vascular smooth muscle to vasoconstrictor stimuli investigated in the present study.

The decrease in isoproterenol-induced relaxation and the absence of a change in non-receptor-mediated relaxation (such as sodium nitroprusside or sodium nitrite) have been reported previously in the SHR. It is interesting that a specific alteration in β-adrenergic-mediated relaxation is observed in vascular smooth muscle from SHRSP in the absence of the more commonly seen changes in vasoconstrictor responses in hypertension. What implications this single alteration in vascular responsiveness has with respect to the pathogenesis of hypertension in SHRSP is unknown. However, a reduced β-adrenergic component has been suggested to be important in regulating vascular smooth muscle contraction in SHR.

Although it was of interest to see no change in vascular reactivity in the SHRSP when compared with WKY, it was quite unexpected to observe an increase in vasoconstrictor sensitivity following antihypertensive treatment in SHRSP-T and WKY-T. These alterations probably are due to a direct effect of the antihypertensive treatment on the vasculature and not to a blood pressure-lowering effect, as similar changes occurred in both SHRSP-T and WKY-T.

Little is known about the direct vascular effects of HCTZ, although, Hyd is known to affect several mechanisms involved in vascular smooth muscle contraction. Several investigators have suggested an interference of Hyd with Ca handling by the vascular smooth muscle cell. Others have shown that Hyd has presynaptic inhibitory effects on sympathetic nerves to decrease stimulation-induced NE release. More recently, a role for the endothelium has been demonstrated in the action of Hyd. Finally, a direct action of Hyd on the contractile apparatus of vascular smooth muscle has been proposed, as this antihypertensive agent inhibits the phosphorylation of the myosin P-light chains and subsequent activation of actomyosin adenosine triphosphatase.

Although each of these mechanisms may be involved in the altered response, it would appear from the data that the most likely candidate is an alteration in Ca handling by the vascular smooth muscle cell. Ca sensitivity was similarly altered in both WKY-T and SHRSP-T, and these changes followed the same pattern as the changes in KCl and NE sensitivity. We cannot, of course, rule out the other aforementioned mechanisms.

Another interesting point with regard to the antihypertensive treatment is that only contractions (KCl, NE, and Ca) were affected and not relaxation responses (isoproterenol, sodium nitroprusside, and Ca relaxation). The reason for the dichotomy in this response is not readily apparent.

In summary, the characteristics of vascular reactivity studied were largely unaltered in SHRSP when compared with WKY. Antihypertensive treatment with Hyd and HCTZ resulted in an increased response to vasoconstrictor stimuli (KCl, NE, Ca) that was independent of the level of blood pressure. This finding suggests a direct effect of the antihypertensive agents on the vascular smooth muscle.

References

10. Soltis EE, Field FP. Effect of high blood pressure stress on...
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