Vascular Reactivity in the Spontaneously Hypertensive Rat
Effect of High Pressure Stress and Extracellular Calcium

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SUMMARY The role of extracellular calcium and high blood pressure stress in altered vascular adrenergic responsiveness in rings of femoral artery from spontaneously hypertensive rats (SHR) was investigated. A model in which partial ligation of the external iliac artery prevents the increase in blood pressure to the ipsilateral femoral artery was used to assess the effect of the increase in pressure stress on these alterations. Age-matched (5-week-old) male Wistar-Kyoto rats (WKY) and SHR were used in the study. Partial ligation was performed before a substantial increase in blood pressure occurred (6 weeks of age), and studies on vascular reactivity were carried out at 10 to 12 weeks of age when the SHR were considered hypertensive (indirect systolic blood pressure > 150 mm Hg). Maximal contractility of rings of unprotected femoral artery from the SHR in response to KCl in either a normal (2.5 mM) or low (0.25 mM) calcium Krebs solution was significantly greater (p < 0.05) than was that of protected vessels from the SHR or protected and unprotected vessels from the WKY; however, no difference in the sensitivity to KCl was observed. Isoproterenol-induced relaxation was significantly attenuated in rings of vascular smooth muscle from unprotected femoral arteries of the SHR (p < 0.05), while the responses of protected vessels from the SHR were similar to controls. Equilibration of vascular smooth muscle in a low calcium Krebs solution resulted in an increase in β-adrenergic mediated relaxation in all groups. The response of unprotected vessels from the SHR was still attenuated when compared with those of the WKY. Unprotected vessels from the SHR exhibited an enhanced response to norepinephrine stimulation in a normal calcium Krebs solution when compared with protected vessels from the SHR as well as with protected and unprotected vessels from the WKY. The response of rings of vascular smooth muscle from unprotected femoral arteries of the SHR to norepinephrine in a low calcium Krebs solution, however, was similar to both vessels from the WKY and protected vessels from the SHR. The results of this study suggest that: (1) the alterations of vascular α- and β-adrenergic responsiveness as well as maximal KCl-induced contraction in the SHR are a result of the increase in blood pressure, as protection of the vasculature from the increase in pressure stress prevented these changes; (2) there exists a role for extracellular calcium in the increase in norepinephrine sensitivity of vascular smooth muscle from the SHR that is dependent on the increase in pressure stress; (3) there does not appear to be a role for extracellular calcium in the decrease in β-adrenergic mediated relaxation, as the response to isoproterenol in low calcium Krebs solution was still attenuated when compared with controls. (Hypertension 7: 228-235, 1985)

KEY WORDS • spontaneously hypertensive rats • extracellular calcium • high blood pressure stress • vascular reactivity • α- and β-adrenergic responsiveness

THE increase in peripheral vascular resistance observed in spontaneously hypertensive rats (SHR) has been attributed to several factors, including an increase in sympathetic nervous activity, structural alterations in the blood vessel, and an increase in vascular smooth muscle reactivity. Although most investigators agree that an increase in reactivity occurs in the vasculature of the SHR, it is yet to be determined whether this alteration is a primary cause of the increase in peripheral resistance or is a result of vascular structural changes.

More recently it has been suggested that the increase in responsiveness of vascular smooth muscle from the SHR to norepinephrine is due to an increase in the permeability of the cell membrane to calcium. In addition, Mulvany and colleagues have shown that antihypertensive treatment prevents the increase in arterial media thickness but does not prevent the increase...
in norepinephrine sensitivity and calcium sensitivity of mesenteric resistance vessels from SHR. They suggested that the altered vascular reactivity in SHR is an intrinsic defect of the vascular smooth muscle cell independent of blood pressure.

As opposed to the large number of studies done on vascular α-adrenergic responsiveness in the SHR, relatively few reports have appeared in the literature dealing with the β-adrenergic system. Furthermore, the results of these studies have been inconsistent. While Spector and co-workers reported an enhanced relaxation, Cohen and Berkowitz observed an attenuation of β-adrenergic mediated relaxation of vascular smooth muscle. Cheng and Shibata also have shown a decrease in the response of vascular smooth muscle from SHR to isoproterenol. They suggest that this alteration is not a consequence of hypertension because prehypertensive SHR treated with reserpine to prevent the increase in blood pressure still exhibited an attenuated response. They believe this decreased relaxation is a result of an alteration in calcium handling by the smooth muscle cell.

Because an increase in peripheral resistance may be due to an increase in α-adrenergic vasoconstrictor activity or a decrease in β-adrenergic vasodilator activity, or both, it is important to determine alterations in both systems. It has been suggested that alterations in both systems may be due to a defect in the handling of calcium by the vascular smooth muscle cell. In addition, it has been suggested that this may be an intrinsic defect independent of the increase in blood pressure. Therefore, the present study was designed to investigate the role of extracellular calcium in the alterations of both α- and β-adrenergic responsiveness seen in the vasculature of the SHR and to determine if the increase in blood pressure, per se, contributes to this defect. To conduct these studies an animal model similar to that employed by Hansen and Bohr was used — one femoral artery was protected from the increase in blood pressure by partial ligation of the ipsilateral external iliac artery. The responsiveness of rings of femoral artery to α- and β-adrenergic stimulation in normal and low calcium Krebs solution was then investigated.

**Methods**

Thirty 5-week-old male Wistar-Kyoto rats (WKY) and thirty 5-week-old male SHR (Charles River Breeding Laboratories, Inc., Wilmington, MA) were housed in groups of two in hanging stainless steel cages in a room illuminated from 0700 to 1900 hours and maintained at 24 ± 1°C. All animals were provided food (Purina Laboratory Chow,Ralston-Purina Co., St. Louis, MO) and tap water ad libitum.

At 6 weeks of age each animal was anesthetized with ether, an incision was made into the ventral surface of the left hindlimb, and the external iliac artery was exposed. Partial ligation of the artery was performed by firmly tying a stainless steel wire (0.015 inches in diameter) against the side of the vessel with 3-0 silk suture, which resulted in complete occlusion of the vessel lumen. The stainless steel wire was then removed, leaving the silk suture ligature intact and allowing the vessel to expand to the approximate diameter of the stainless steel wire. Femoral artery cannulation (PE-10 tubing) was performed on eight randomly chosen rats, and direct arterial pressure was measured (Mark IV physiograph and P-1000B pressure transducer, Narco Bio-Systems, Houston, TX) at the time of ligation and at 10 weeks of age to determine the effectiveness of the ligature in reducing arterial blood pressure in the ipsilateral versus contralateral vasculature. These animals were not used in the vascular reactivity studies. Indirect systolic blood pressures also were recorded weekly on lightly anesthetized (ether) animals by a standardized tail cuff technique with a Narco Bio-Systems pneumatic pulse transducer and Mark IV physiograph. Studies on vascular reactivity were done when the animals were 10 to 12 weeks of age.

Whenever isolated vascular smooth muscle was studied, the animal was anesthetized with ether and each femoral artery quickly removed and placed in an aerated modified Krebs solution at 26°C. The vessels were cleaned of excess fat, connective tissue, and blood, and one 3-mm ring was cut from each vessel with a cutter consisting of two stainless steel blades mounted on an aluminum block 3 mm wide. The rings were suspended between two stainless steel hooks (250 μm in diameter) inserted through the lumen (approximately 750 μm inside diameter) to record circular smooth muscle contraction. Each isolated tissue was mounted individually in a 20-ml temperature controlled (water jacketed) muscle bath containing modified Krebs solution and bubbled with an O2/CO2 (95:5) gas mixture to maintain the pH at 7.3 ± 0.1. The temperature was controlled at 37 ± 1°C with a heater/pump (Haake) and reservoir system. Isometric contractions were recorded with an F-50 microdisplacement myograph transducer with a Model DMP-4B physiograph recorder (Narco Bio-Systems). After each experiment, all tissues were allowed to dry and were then weighed to a constant weight on a Cahn (Cerritos, CA) electrobalance. Unless otherwise stated, composition of the modified Krebs solution in double distilled water was (in mM): NaCl, 118; KCl, 4.7; CaCl2·2H2O, 2.5; MgCl2·6H2O, 1.2; KH2PO4, 1.2; NaHCO3, 12.5; dextrose, 11.5; disodium EDTA, 0.01.

**Vascular Reactivity Studies**

Twelve WKY and twelve SHR were used for each of the two levels of calcium tested. Femoral rings were prepared as described and equilibrated for 90 minutes in either a normal (2.5 mM) or low (0.25 mM) calcium Krebs solution at 1 g of preload force. This preload force was chosen based on previous studies of force-active tension analysis of rings of protected and unprotected femoral arteries from SHR and WKY. Following the equilibration period a KCl dose-response curve (8–80 mM) was generated for each tissue. After the last addition of KCl, the baths were...
rinsed with fresh Krebs solution and the tissues were allowed to relax to baseline tension. The α-adrenergic activity of the vascular smooth muscle was blocked by incubation with phentolamine (10^{-5} M) for 45 minutes. The bath solutions were changed every 15 minutes, and phentolamine was added each time. After the 45-minute incubation period sufficient KCl was added to each bath to produce a half-maximal contraction. After the tension generated reached a plateau, a cumulative isoproterenol dose-response curve (10^{-9}—10^{-4} M) was obtained, which was followed by a cumulative dose-response curve to sodium nitrite (NaN02, 1.5 × 10^{-5} to 1.5 × 10^{-3} M; isoproterenol still present in the bath). After the last dose of NaNO2 was added, the bath solutions were changed and the tissues were allowed to return to baseline conditions. The bath solutions were changed every 15 minutes for 1 hour, after which a cumulative norepinephrine dose-response curve (10^{-10}—10^{-6} M) was generated. The tissues remained in contact with each concentration of drug for a sufficient period of time to produce a maximal response (3 minutes for KCl, isoproterenol and NaNO2; 5 minutes for norepinephrine). We have previously found (unpublished data) that no long-lasting interaction occurs between these drugs when this protocol is used to test α- and β-adrenergic responsiveness. Tissue viability (contractility) was determined at the end of each experiment by stimulation with 60 mM KCl.

Isoproterenol HCl, NaNO2, and norepinephrine HCl were purchased from Sigma Chemical Co. (St. Louis, MO), and the phentolamine (Regitine) was graciously donated by Ciba-Geigy Laboratories (Summit, NJ). All drugs were prepared fresh every day in double-distilled water.

Comparisons between unprotected and protected vessels of WKY and SHR were analyzed by two-way analysis of variance (ANOVA). Significance was set at the p < 0.050 level.

Results

Partial ligation of the left external iliac artery of 6-week-old WKY and SHR resulted in a slight decrease in femoral arterial pressure at the time of ligation. Although blood flow remained, the ligation prevented an increase in blood pressure to the protected femoral artery, as shown by direct mean arterial pressure measurements at 10 weeks of age (Table 1). No difference in indirect systolic blood pressure was observed between WKY and SHR at the time of ligation; however, blood pressure increased thereafter in the SHR, and they were considered hypertensive (systolic > 150 mm Hg) at 9 weeks of age.

The development of active tension by protected and unprotected rings of femoral arterial smooth muscle from WKY and SHR in response to cumulative addition of KCl is shown in Figure 1. A significant increase

<table>
<thead>
<tr>
<th>Parameters</th>
<th>At ligation</th>
<th>At sacrifice</th>
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<tbody>
<tr>
<td></td>
<td>WKY</td>
<td>SHR</td>
</tr>
<tr>
<td>Age (weeks)</td>
<td>6(24)</td>
<td>6(24)</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>88 ± 3</td>
<td>95 ± 2</td>
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<tr>
<td></td>
<td>(24)</td>
<td>(24)</td>
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<tr>
<td>Heart weight</td>
<td>—</td>
<td>—</td>
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<tr>
<td>(ventricles, mg/100 g body weight)</td>
<td>—</td>
<td>—</td>
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<tr>
<td>Indirect systolic blood pressure (mm Hg)</td>
<td>106 ± 4 (24)</td>
<td>111 ± 3 (24)</td>
</tr>
<tr>
<td>Direct mean blood pressure (mm Hg)</td>
<td>73 ± 6 (6)</td>
<td>78 ± 8 (6)</td>
</tr>
<tr>
<td>Unprotected</td>
<td>61 ± 4 (6)</td>
<td>68 ± 7 (6)</td>
</tr>
<tr>
<td>Protected</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Femoral ring weight (mg)</td>
<td>—</td>
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Values in parentheses represent number of animals.

SHR = spontaneously hypertensive rats; WKY = Wistar-Kyoto rats.

*Significantly different from WKY (p < 0.05).
†Significantly different from respective value at time of ligation (p < 0.05).
‡Significantly different from protected value of either group (p < 0.05).

Results expressed as means ± SEM.
FIGURE 1. Dose-response relationship between concentration of KCl and development of active tension of protected (•, △) and unprotected (○, ▽) rings of femoral artery from 10-week-old WKY and SHR in either 2.5 mM (A) or 0.25 mM (B) calcium Krebs solution. Each point represents the mean and standard error of 12 rings. * = SHR significantly different from all other groups (p < 0.05).

(p < 0.05) in the response to high concentrations of KCl was observed in the unprotected vessels of the SHR in both normal and low calcium Krebs solution when compared with the vessels of the WKY. This increase in contractility was prevented when the vasculature of the SHR was protected from the increase in blood pressure. Ligation of the left external iliac artery of the WKY had no effect on femoral artery response to KCl stimulation. Equilibration of femoral arterial rings in the low calcium Krebs solution did not alter the maximal response but did result in a decrease in the sensitivity of all vessels to low doses of KCl when compared with responses in the normal calcium Krebs buffer. Expressing the data for both levels of calcium as a percentage of the maximal KCl response (Figure 2) reveals no difference in the dose-response curves of protected and unprotected vessels to either low or high concentrations of KCl.

Unprotected femoral arterial rings from the SHR exhibited a significant increase (p < 0.05) in the adrenergic responsiveness to norepinephrine stimulation in both normal and low calcium Krebs solution when compared with rings of femoral smooth muscle from the WKY (Figure 3). In addition, protection of the vasculature of the SHR from the increase in blood pressure prevented this increase in responsiveness. Expressing the data as a percentage of the maximal norepinephrine response reveals that the unprotected vessels of the SHR still exhibit an enhanced sensitivity (p < 0.05) to norepinephrine (5 × 10^{-10} to 10^{-8} M) in normal calcium Krebs solution (Figure 4), while no difference is observed between protected and unprotected vessels of either WKY or SHR in the presence of low extracellular calcium (Figure 4).

Isoproterenol-induced relaxation of the unprotected vessels of the SHR was significantly attenuated (p < 0.05) in both normal and low calcium Krebs solution when compared with that of controls (Figure 5). The protected vessels of the SHR responded in a similar fashion to the vessels of the WKY. Maximal relaxation occurred at 10^{-3} M isoproterenol for all vessels in the normal calcium Krebs solution and for the unprotected vessels of the SHR in the low calcium Krebs, while the protected vessels of the SHR and both vessels of the WKY continued to relax at 10^{-4} M isoproterenol in the presence of low levels of calcium. Relaxation in response to NaNO_2 was similar for all vessels in both normal and low calcium Krebs solution. All vessels showed an enhanced response to isoproterenol in the low calcium Krebs solution when compared with responses in the normal calcium Krebs solution.

Discussion

Systolic blood pressures of SHR were not significantly different from those of control WKY at the time of partial ligation of the external iliac artery. Although arterial pressure in the SHR was slightly decreased, the partial ligation still allowed some pulsatile blood flow, and direct blood pressure measurements when the SHR were 10 weeks of age demonstrated that the arterial...
Figure 2. Dose-response relationship between concentration of KCl and percentage of maximal KCl response of protected (●, △) and unprotected (○, △) rings of femoral artery from 10-week-old WKY and SHR in either 2.5 mM (A) or 0.25 mM (B) calcium Krebs solution. Responses are calculated from the data in Figure 1 and expressed as a percent of the maximum active tension generated. Each point represents the mean and standard error of 12 rings.

Figure 3. Dose-response relationship between concentration of norepinephrine and development of active tension of protected (●, △) and unprotected (○, △) rings of femoral artery from 10-week-old WKY and SHR in either 2.5 mM (A) or 0.25 mM (B) calcium Krebs solution. Each point represents the mean and standard error of 12 rings. * = SHR significantly different from all other groups (p < 0.05).
FIGURE 4. Dose-response relationship between concentration of norepinephrine and percentage of maximal norepinephrine response of protected (•, △) and unprotected (○, △) rings of femoral artery from 10-week-old WKY and SHR in either 2.5 mM (A) or 0.25 mM (B) calcium Krebs solution. Responses are calculated from the data in Figure 4 and expressed as a percentage of maximum active tension generated. Each point represents the mean and standard error of 12 rings. * = SHR significantly different from all other groups (p < 0.05).

FIGURE 5. Dose-response relationship between concentration of isoproterenol and sodium nitrite and relaxation, expressed as a percentage of KCl contraction, of protected (•, △) and unprotected (○, △) rings of femoral artery from 10-week-old WKY and SHR in either 2.5 mM (A) or 0.25 mM (B) calcium Krebs solution. Each point represents the mean and standard error of 12 rings. * = significantly different from all other groups (p < 0.05).
blood pressure did not increase in the protected femoral artery. Therefore, the physical stress on femoral smooth muscle, which results from the increase in blood pressure, was prevented. This allowed for an examination of the effect of increased pressure per se on vascular reactivity and for a determination of the role of extracellular calcium in the altered \(\alpha\) and \(\beta\)-adrenergic responsiveness.

One test to determine a possible role for extracellular calcium in the increased responsiveness of vascular smooth muscle from the SHR is carried out by altering the concentration of calcium in the media. In the present study an increase in the maximal contractility of unprotected vessels of the SHR to KCl was observed in a normal calcium Krebs solution, while no difference in the sensitivity (response to low doses) was seen. By decreasing the concentration of extracellular calcium one might expect a decrease in the response. This was not the case, however, as the maximal response to KCl was not altered. Although it was apparent that the sensitivity to low doses of KCl was decreased, the shift in the dose-response curves was similar for all groups. That is, no difference was observed between the unprotected and protected vessels of either the WKY or SHR. This point is further emphasized when the responses are normalized for the differences in maximal contractility and expressed as a percentage of the maximal KCl response. The increase in maximum tension generated by the unprotected vessels of the SHR in response to KCl is, therefore, most likely due to structural alterations such as hypertrophy or hyperplasia (or both) of the smooth muscle cells. These structural alterations could explain the higher ring weights of the unprotected vessels. In addition, protection of the vasculature prevented the increase in ring weight and increase in maximum active tension while it did not affect the sensitivity of the vessels. It appears then that the differences observed in the response to KCl are a result of structural changes in response to the increase in blood pressure stress and are not due to alterations in the calcium sensitivity. In support of these findings, Mulvany and Nyborg observed substantial structural changes with no difference in the calcium sensitivity of mesenteric resistance vessels from SHR in response to KCl when compared with those of controls. They suggested that there is no alteration in the potassium (potential)-dependent calcium permeability of vascular smooth muscle from the SHR.

Several hypotheses have been put forth to explain the increase in \(\alpha\)-adrenergic responsiveness in vascular smooth muscle from SHR. One of these involves a role for an increase in the permeability of the smooth muscle cell to calcium. In the present study an increase in the sensitivity and contractility of unprotected femoral artery from SHR in response to norepinephrine was observed in normal and low calcium Krebs solution when compared with those of protected vessels of the SHR and both vessels of the WKY. As with the KCl dose-response curves, the increase in responsiveness may reflect structural changes; however, when the data are normalized for the difference in maximal contractility and expressed as a percentage of the maximal norepinephrine response in normal calcium Krebs solution, the unprotected vessels from the SHR still exhibit an enhanced sensitivity to norepinephrine. Furthermore, it appears that calcium is important for the increase in norepinephrine sensitivity, as the increased sensitivity was not seen in the low calcium Krebs solution. This finding is in agreement with Mulvany and Nyborg, who observed an increase in the calcium sensitivity of mesenteric resistance vessels from SHR to norepinephrine stimulation. Mulvany and colleagues further suggested that this alteration is an intrinsic defect of vascular smooth muscle from the SHR that is independent of the increase in blood pressure, as antihypertensive treatment of prehypertensive SHR did not prevent the increase in norepinephrine sensitivity or calcium sensitivity. The present study, however, is in opposition to this hypothesis as the response of protected vessels from the SHR was similar to the response of vessels from the WKY in either a normal or low calcium Krebs solution. This finding suggests that the increase in norepinephrine responsiveness of vascular smooth muscle from SHR is due to an increase in the calcium sensitivity, which is a result of the increase in blood pressure.

Because a decrease in vascular \(\beta\)-adrenergic responsiveness, as well as an increase in \(\alpha\)-adrenergic responsiveness, may contribute to the increase in peripheral vascular resistance, alterations in \(\beta\)-adrenergic mediated relaxation of rings of femoral artery from SHR were investigated. In addition, a role for calcium and the effect of high blood pressure per se were examined. Unprotected vessels from SHR showed a decrease in relaxation in response to isoproterenol, which suggests that the increase in blood pressure causes the decrease in \(\beta\)-adrenergic responsiveness. This finding is in agreement with Cheng and Shibata, who also showed a decrease in the response to isoproterenol in thoracic aorta of the SHR. They suggested that the decrease in responsiveness is not a result of the increase in blood pressure as prehypertensive SHR treated with reserpine still exhibited a decrease in \(\beta\)-adrenergic relaxation. They concluded that this decrease is a result of a defect in calcium handling by the vascular smooth muscle cell. In the present study the response to isoproterenol in low calcium Krebs solution was increased in all vessels when compared with responses in normal calcium Krebs solution; however, unprotected rings of femoral artery from SHR still exhibited an attenuated response. It appears then that the decrease in responsiveness is due to an inability of the vascular smooth muscle cell to sequester calcium in response to \(\beta\)-adrenergic stimulation and is a result of the increase in blood pressure. This decrease in relaxation appears to be the result of a defect in the \(\beta\)-adrenergic system specifically, as the response to sodium nitrite, a nonspecific smooth muscle relaxant, was similar in all groups. Therefore, an alteration in the \(\beta\)-adrenergic receptor itself, or in the sequence of events following \(\beta\)-adrenergic receptor activation, which results in the sequestration of calcium, is implicated.
Conclusion

The results of this study suggest that alterations in vascular α- and β-adrenergic responsiveness of SHR are a result of the increase in blood pressure, as protection of the vasculature from the increased physical stress prevented these changes. In addition, the increase in sensitivity of rings of femoral artery from the SHR to norepinephrine appears to be the result of an increase in the calcium sensitivity, as vascular smooth muscle reactivity to norepinephrine was decreased in a low calcium Krebs solution so that the response was similar to controls. The decrease in β-adrenergic responsiveness appears to be the result of a defect in the β-adrenergic system specifically and not an alteration in the ability of the vascular smooth muscle cell to sequester calcium.

References

Vascular reactivity in the spontaneously hypertensive rat. Effect of high pressure stress and extracellular calcium.
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