Reactivity of Vascular Smooth Muscle in Hypertensive Rats

By Edwin T. Holloway and David F. Bohr

ABSTRACT
The reactivity of vascular smooth muscle in helical strips from femoral arteries of normotensive, spontaneously hypertensive, renal hypertensive, and deoxycorticosterone acetate- (DCA-) hypertensive rats was studied. Spontaneous rhythmic contractions occurred in 25 of the 30 strips from the three groups of hypertensive rats and in only 2 of the 10 strips from normotensive rats. Strips from renal and DCA-hypertensive rats had lower thresholds to epinephrine and potassium chloride (KCl) than did strips from spontaneously hypertensive and normotensive rats. Lanthanum (2.5 mM) caused contraction of all 10 strips from spontaneously hypertensive rats but failed to cause contraction of any strip from the other three groups of rats. Strontium (5 mM) caused contraction in 8 of 10 strips from spontaneously hypertensive rats but caused contraction in only 7 of the 30 strips from the other three groups. The optimal calcium concentration for tension development in response to a KCl stimulus was approximately twice as high for strips from hypertensive rats as it was for strips from normotensive rats. Strips from DCA-hypertensive rats showed less tachyphylaxis to angiotensin II than did strips from the other three groups of rats. These results quantify our earlier observation that the reactivity of vascular smooth muscle from hypertensive rats is importantly different from that of normotensive rats. In addition, the study delineates individuality in vascular smooth muscle reactivity in different types of experimental hypertension. The results suggest that the cell membrane of the vascular smooth muscle in the hypertensive rat is more labile than that in the normal rat.

KEY WORDS
renal hypertension DCA-hypertension angiotensin strontium

The increased total peripheral resistance responsible for the elevated blood pressure of established clinical or experimental hypertension could be the result of (1) increases in neural or humoral vasoconstrictor influences, (2) passive structural alterations of the blood vessel, or (3) intrinsic changes in reactivity of the vascular smooth muscle cells. Existing evidence does not permit a comfortable conclusion about which of these mechanisms is responsible.

From perfusion studies with several vascular beds, Folkow (1) and Folkow et al. (2) have concluded that there is no intrinsic change in vascular smooth muscle reactivity in spontaneously hypertensive rats and that a passive structural change (increased wall-lumen ratio) is responsible for the increase in total peripheral resistance. Other investigators (3-6), using perfused preparations from spontaneously hypertensive and deoxycorticosterone acetate- (DCA-) hypertensive rats, have found increased vascular reactivity to various stimulating agents. Folkow (1) also observed an increase in vascular reactivity indicated by the greater pressor response to norepinephrine. He calculated that this increase in vascular reactivity could have been caused entirely by an increase in wall thickness, and from this calculation he developed his argument that there is no increase in intrinsic reactivity of vascular smooth muscle in the spontaneously hypertensive rat.

It is important to emphasize that an increase in vascular reactivity indicated by a greater pressor response of a perfused vascular bed does not, in itself, permit a differentiation between increased wall thickness and increased intrinsic vascular smooth muscle reactivity. However, the contractile responses of helical strips of the blood vessel wall can be interpreted more directly in terms of intrinsic vascular smooth muscle reactivity. Studies (7-9) of the reactivity of strips of vascular smooth muscle from hypertensive animals have given...
conflicting results; however, recent studies have been consistent in the finding that the reactivity of femoral artery strips from renal hypertensive rats (10) and DCA-hypertensive rats (11, 12) is greater than that of strips from normotensive rats.

The study presented in this paper provides a descriptive survey of the responses of strips of femoral arteries from normotensive, renal hypertensive, DCA-hypertensive, and spontaneously hypertensive rats to various agents. Similarities and differences between the vascular reactivities of the several experimental models of hypertension are defined, permitting inferences as to whether a given change in reactivity is related to a specific model of hypertension or is a characteristic common to vascular smooth muscle of all types of hypertension.

Methods

Male Sprague-Dawley rats were used as controls and for the production of renal and DCA-hypertensive. Sodium pentobarbital (50 mg/kg, ip) was the anesthetic used for surgical procedures.

Renal Hypertension.—Silver blocks with a slit 0.25 mm wide were applied as described by Leenen and de Jong (13) to left renal arteries of rats (150-200 g). Postoperatively, the rats received a standard diet and tap water ad libitum. These rats were killed 1-3 weeks after application of the clip, and sections of their femoral arteries were removed for study.

Doxycorticosterone Acetate — Hypertension. — Rats weighing 200-250 g were subjected to left nephrectomy, and two 20-mg pellets of DCA were implanted subcutaneously. Postoperatively, these rats received a standard laboratory diet but were given tap water. Two weeks after the operation an additional 20-mg pellet of DCA was implanted. Approximately 2 weeks later the vascular smooth muscle was studied.

Spontaneous Hypertension.—Male albino rats from the Okamoto and Aoki (14) strain were used. These rats also received a standard diet and tap water.

On the day of an experiment, one rat from each of the four groups was anesthetized, and a carotid artery of each was cannulated for direct blood pressure recording. The femoral arteries were then removed and dissected free of loose connective tissue; these vessels had an outside diameter of approximately 800 µ and a wall thickness of 75-100 µ. Helical strips, approximately 700 µ wide and 4 mm long, were cut, and four strips, one from each of the four groups of rats, were mounted in a common bath between a fixed base and four force-displacement transducers (Grass FT-03). The outputs of which were recorded on a Grass polygraph. Each strip was stretched to give a resting force of about 300 mg. This degree of passive stretch of strips from both normotensive and hypertensive rats is nearly optimal for active tension development. Length—passive tension studies failed to demonstrate differences in passive stiffness between strips from the normotensive and hypertensive rats. Strips were allowed to equilibrate for 2 hours before experimental procedures were begun. The millimolar composition of the physiological salt solution used in this study was: NaCl 119, KCl 4.7, KH₂PO₄ 1.18, MgSO₄ · 7H₂O 1.17, CaCl₂ 1.6, NaHCO₃ 14.9, dextrose 5.5, sucrose 50, and CaNa₂ versenate 0.026. The muscle bath was maintained at 37°C with pH between 7.3 and 7.4 and was aerated with 95% O₂-5% CO₂.

Results

Group average weights and mean blood pressures at death are given in Table 1. The spontaneously hypertensive rats were approximately 6 months old and, according to studies by Okamoto et al. (14), probably had had elevated blood pressures since the age of 10 weeks. Duration of hypertension was 2-3 weeks for DCA-hypertensive rats and 3-17 days for renal hypertensive rats. Mean blood pressure was under 130 mm Hg in all control rats and above 150 mm Hg in all hypertensive rats.

Rhythmic Contractions.—Spontaneous rhythmic contractions (Fig. 1) were seen far more frequently in femoral artery strips from spontaneously hypertensive rats (ten of ten), DCA-hypertensive rats (eight of ten), and renal hypertensive rats (seven of ten) than in strips from normotensive rats (two of ten). These phasic contractions were variable in magnitude, frequency, and stability, but rhythmicity was clearly more evident in strips from hypertensive rats than it was in strips from normotensive rats. This rhythmicity disappeared immediately when a calcium-free solution replaced the control physiological salt solution in the bath.

Response to Constrictor Agents.—Cumulative concentration—response curves for KCl and for epinephrine were determined (Fig. 2). Because the

### Table 1: Group Average Weight and Mean Blood Pressure (Direct Recording) at Death

<table>
<thead>
<tr>
<th></th>
<th>Number of rats</th>
<th>Weight (g)</th>
<th>Mean blood pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normotensive rats</td>
<td>10</td>
<td>320 ± 40</td>
<td>110 ± 15</td>
</tr>
<tr>
<td>Spontaneously hypertensive rats</td>
<td>10</td>
<td>400 ± 42</td>
<td>185 ± 13</td>
</tr>
<tr>
<td>Renal hypertensive rats</td>
<td>10</td>
<td>260 ± 32</td>
<td>160 ± 5</td>
</tr>
<tr>
<td>DCA- hypertensive rats</td>
<td>10</td>
<td>300 ± 12</td>
<td>173 ± 16</td>
</tr>
</tbody>
</table>

All values are means ± SD.

1Dr. L. T. Blouin of Parke Davis and Company kindly supplied the spontaneously hypertensive rats.

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Normotensive

Spontaneous Hyp.

DCA Hyp.

Renal Hyp.

CaCl$_2$

1.6mM 3.2mM

KCl

4.7mM 40mM

FIGURE 1

Responses of four femoral artery strips to 3.2 mM CaCl$_2$ and to 40 mM KCl. Spontaneous rhythmic contractions are seen in strips from the three types of hypertensive rats but are not seen in the normotensive rat strips. Strips from DCA- and renal hypertensive rats contracted in response to 3.2 mM CaCl$_2$, but strips from normotensive and spontaneously hypertensive rats did not. All strips responded to the KCl stimulus.

maximum responses varied with the size and the origin of the strip, the curves were normalized by expressing each response as a percent of the greatest response of that strip to the specific agonist.

When KCl was the stimulant, strips from the DCA- and renal hypertensive rats gave significantly (P < 0.001) greater responses to concentrations of 15, 25, 35, and 45 mM than did strips from the normal group. The strips from the spontaneously hypertensive rats were significantly more reactive only at 25 mM KCl. In five of the ten studies the threshold concentration for contraction was lower for the strip from the spontaneously hypertensive rat than it was for the accompanying strip from the control rat; in the remaining five studies the threshold concentrations were the same.

When epinephrine was the stimulating agent, strips from the DCA- and renal hypertensive rats again appeared to be more responsive than did those from the spontaneously hypertensive and control rats, but few differences were statistically significant. This relative reactivity among the four groups is supported by their ability to contract in response to an extremely low concentration of epinephrine (10$^{-11}$ g/ml): control two of ten, spontaneously hypertensive three of ten, renal hypertensive seven of ten, and DCA-hypertensive four of nine.

The same data were used to obtain a more rigorous measure of vascular smooth muscle reactivity (Fig. 2). With responses normalized in this way, we interpret a lower concentration of agonist for half-maximum response (ED$_{50}$) as indicating an increase in reactivity, regardless of any difference in maximum force developed. Values for ED$_{50}$ were calculated using the method of probit analysis described by Goldstein (15). In Table 2, the mean ED$_{50}$ for each group is presented and shown as a percent of the ED$_{50}$ for the normotensive group.
Normalized (see text) cumulative concentration-response curves. **Left**: KCl as agonist. Curves for all hypertensive groups (ten in each group) are shifted to the left. The difference of these curves from that of the normotensive rats is greatest for DCA-hypertensive rats and successively less for renal and spontaneously hypertensive rats. The values of ED50 for the first two groups are significantly different from that of the normal group (P < 0.001, double asterisks) and that for the spontaneously hypertensive group (P = 0.05, asterisk). Brackets indicate SE.

**Right**: Epinephrine as agonist. Curves for DCA- and renal hypertensive groups (ten in each group) are shifted somewhat to the left. Only the ED50 of the renal hypertensive rats is significantly different (P < 0.05).

The observed differences indicate that vascular smooth muscle reactivity to KCl was increased in renal and DCA-hypertensive rats more than it was in spontaneously hypertensive rats. Although the percent shift in ED50 to KCl was less than that to epinephrine, the increase in reactivity to KCl is statistically more convincing than is the increase in reactivity to epinephrine.

Studies with Altered Calcium Concentration.—

The effect of calcium concentration on the response of the strips to KCl and to epinephrine was studied by adding small amounts of 1 mM CaCl2 to the muscle bath during the plateau period of the response to each of these stimulating agents. In the experiment with KCl (Fig. 3), an increase in the calcium concentration from the control level of 1.6 mM to 4.1 mM caused a slight decrease in the force of contraction of the strip from the normotensive rat, a negligible change in the response of the strip from the spontaneously hypertensive rat, and an increase in the response of strips from the DCA- and renal hypertensive rats. This increase in calcium concentration produced a decrease in contractile force in six of nine strips from control rats and in zero of nine strips from each of the three groups of hypertensive rats. Increasing the calcium concentration to 9.1 mM caused a decrease in contractile force in eight of nine strips from control rats, in four of eight strips from spontaneously hypertensive rats, and in only one of eight strips from the DCA-hypertensive rats and the renal hypertensive rats. In Figure 4 these data are normalized by assigning the value of 100% to the greatest contractile force developed by each strip at any calcium concentration. It is clear that the concentration of calcium required to depress the response to KCl is higher for vascular smooth muscle from the three groups of hypertensive rats than it is for muscle from the normotensive group.

In similar studies in which epinephrine was used as the stimulating agent instead of KCl, there appeared to be no difference in the optimal concentration of calcium for strips from the normotensive and the spontaneously hypertensive groups. The optimal concentration of calcium was clearly higher for the DCA- and renal hypertensive groups, but the difference was not as great as that when KCl was used as the stimulating agent.

Responses to Calcium, Strontium, and Lanthanum.—An increase in calcium concentration in the bath from 1.6 mM to 3.2 mM caused contraction of some strips (Fig. 1): normotensive one of ten, spontaneously hypertensive one of ten, renal hypertensive three of ten, and DCA-hypertensive six of ten.

### TABLE 2

<table>
<thead>
<tr>
<th></th>
<th><strong>KCl</strong></th>
<th><strong>Epinephrine</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ED50 (mM)</td>
<td>% of norm</td>
</tr>
<tr>
<td>Normotensive rats</td>
<td>34.6 ± 1.0</td>
<td>100</td>
</tr>
<tr>
<td>Spontaneously hypertensive rats</td>
<td>30.8 ± 2.0</td>
<td>87</td>
</tr>
<tr>
<td>DCA-hypertensive rats</td>
<td>23.0 ± 2.6</td>
<td>68</td>
</tr>
<tr>
<td>Renal hypertensive rats</td>
<td>26.6 ± 1.3</td>
<td>77</td>
</tr>
</tbody>
</table>

All values for ED50 are means ± se. P denotes significance of difference from control.

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The most consistent difference between the strips from the spontaneously hypertensive rats and those from the other three groups was in their response to 2.5 mM lanthanum chloride (LaCl₃). This cation caused contraction of ten of ten strips from these hypertensive rats but caused no contraction in ten strips from each of the other three groups.

When 5 mM strontium chloride (SrCl₂) was added to the bath, strips from the spontaneously hypertensive rats again contracted more regularly (eight of ten) than did those from the DCA-hypertensive (four of ten), renal hypertensive (three of ten), or normotensive (one of ten) rats.

Tachyphylaxis to Angiotensin II.—Strips from DCA-hypertensive rats showed much less tachyphylaxis to angiotensin II than did strips from any of the other three groups (Fig. 5). In each of the four tests, the strip from the DCA-hypertensive rat responded consistently to repeated angiotensin stimulation whereas the strips from the other three groups showed great tachyphylaxis.

Discussion

Studies of isolated strips of arterial smooth muscle permit a comparison of the intrinsic contractile properties of this muscle in different types of hypertension. Admittedly, the muscle has been subjected to a degree of trauma and the environment in which it is studied is quite different from that in which it functions in situ. However, the fact that there are consistent differences between smooth muscle from normotensive and hypertensive rats and, for that matter, between smooth muscle from rats with different types of hypertension argues that the differences in vascular smooth muscle from these different types of rats are intrinsic and of a magnitude that is not obliterated by tissue trauma or by placing the smooth muscle from different sources in a common environment.

The current study confirms and extends previous observations (10-12) made on strips of vascular smooth muscle from the femoral artery of hypertensive rats; the observations differ from those obtained with strips of aorta (7-9) or portal vein (7). This difference appears to reflect an individuality of vascular smooth muscle from different sites in the body.

One unsatisfactory feature of the current study is that the duration and the magnitude of the hypertension were not the same in the three groups. It may be that the observed differences between these groups are attributable to differences in these two variables. This possibility seems unlikely.
however, since the spontaneously hypertensive rats, whose pressure elevation was greatest and whose blood pressure had been elevated for the longest time, showed the smallest increase in vascular reactivity. In any case, the fact that vascular smooth muscle from all hypertensive groups differed from that from the normal group argues that there are important and persistent changes in intrinsic vascular smooth muscle reactivity during hypertension in the rat.

**VASCULAR REACTIVITY**

Although increased smooth muscle reactivity of the femoral artery in hypertensive rats has been reported previously, such differences have not been quantified in terms of shifts of a concentration-response curve nor has reactivity of this smooth muscle from the three models of hypertension been evaluated in a single study. Using the ED$_{50}$ as an index, it is clear that the vascular reactivity to KCl in renal and DCA-hypertensive rats is greater than that in normotensive rats. There is no apparent difference in the degree of increase in reactivity in these two types of hypertension.

Judged by the relative degrees of significance, the increase in reactivity to KCl is more consistent than is the increase in reactivity to epinephrine. Possibly this difference between the reactivity shift to KCl and that to epinephrine supports the hypothesis that a basic change which occurs in vascular smooth muscle with the development of hypertension is an increase in cell membrane permeability.

**FIGURE 4**

Normalized (see text) average of responses to 40 mM KCl at various calcium concentrations. The greatest response of strips (eight or nine each group) from the normotensive rats was at 1.6 mM Ca, from the spontaneously hypertensive rats it was 4.1 mM, and from the DCA- and renal hypertensive rats it was at 6.6 mM. Brackets indicate SE, and an asterisk indicates a significant difference from normotensive value at $P < 0.05$.

**FIGURE 5**

Responses to repeated stimulation with angiotensin II ($10^{-8}$ g/ml). The strip from the DCA-hypertensive rat showed much less tachyphylaxis than did those from the other three groups. Spontaneous rhythmic activity is seen only in strips from the three hypertensive groups.

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to calcium. Numerous observers (16-19) have established that the contractile response of vascular smooth muscle to an increase in KCl concentration depends more on an influx of extracellular calcium through the plasma membrane than does the response to epinephrine, which may be effected by a release of intracellularly sequestered calcium. This finding agrees with earlier reports of Hinke (4) and Holloway et al. (12) that vascular smooth muscle membrane permeability to calcium is greater in the hypertensive rat than it is in the normotensive rat. The greater incidence in contractile response to doubling the concentration of calcium (Fig. 1) may reflect a difference in membrane permeability to calcium between the vascular smooth muscle of normotensive and spontaneously hypertensive rats, on the one hand, and that of DCA- and renal hypertensive rats on the other hand.

On the basis of these same criteria of shifts in ED_{50} of the responses of vascular smooth muscle to KCl and to epinephrine, it appears that there is negligible difference between the reactivity of vascular smooth muscle from normotensive rats and that from spontaneously hypertensive rats. Since these spontaneously hypertensive rats had had the increased pressure for many months, it is possible that the vascular smooth muscle cells had undergone an adaptive change which corrected the abnormally high vascular reactivity seen in the acutely developed hypertension of the first month of renal and DCA-hypertension.

The incidence of spontaneous rhythmic activity is greater than normal in smooth muscle from the three groups of hypertensive rats. Since this rhythmic smooth muscle contraction depends on the existence of myogenic pacemakers and on cell-to-cell propagation of excitation and since these cellular phenomena depend on properties of the cell membrane, this observation is also evidence that there is a difference in the functional properties of the cell membrane of vascular smooth muscle in the hypertensive rat.

Further evidence bearing on the lability of the vascular smooth muscle membrane in the hypertensive rat can be deduced from the studies of the effect of increased calcium concentration on the magnitude of the contractile response of this tissue. Calcium has a biphasic effect on vascular smooth muscle contraction (20). Low concentrations are necessary to effect excitation-contraction coupling and thereby bring about contraction, and higher concentrations stabilize the membrane, inhibiting its excitation and thereby depressing contraction. Rothstein (21) has reviewed extensive experimental evidence demonstrating this membrane-stabilizing effect of calcium. We have previously reported (12) that the concentration of calcium required to inhibit the contraction of vascular smooth muscle from rats with DCA-hypertension is significantly higher than that required to inhibit muscle from normotensive rats. The current study was carried out to determine if there were differences in this calcium requirement for membrane stabilization between muscles obtained from rats with different types of hypertension. Interestingly, using this index for increased lability of the cell membrane, the observations in this study indicate that cell membrane lability is increased in all three types of hypertension studied.

**RESPONSE TO STRONTIUM AND LANTHANUM**

Shibata and Kurahashi (22) have reported that vascular smooth muscle from spontaneously hypertensive rats contracts in response to strontium and lanthanum whereas that from normotensive rats does not. We repeated this study in the present experiment to determine whether this individuality was a characteristic common to vascular smooth muscle from rats with increased blood pressure produced by any intervention or whether it was a specific individuality of vascular smooth muscle from the spontaneously hypertensive rat. Our results indicate clearly that this response occurs infrequently in smooth muscle from the normotensive rat and the renal or DCA-hypertensive rat. Therefore, it represents an interesting characteristic of the smooth muscle from the spontaneously hypertensive rat alone. The mechanistic significance of this observation and its possible etiological role remain obscure.

**ANGIOTENSIN TACHYPHYLAXIS**

Tachyphylaxis of isolated vascular smooth muscle to angiotensin has been extensively studied (23-25), and its mechanism has been postulated to be one of receptor occupancy. Our observations suggest that tachyphylaxis is pronounced in the normotensive, spontaneously hypertensive, and renal hypertensive rats but that it is greatly attenuated in the DCA-hypertensive rat. Of the four groups of rats, this group is the one which would be expected to have a low circulating angiotensin level. If this low angiotensin level is the cause of the attenuated tachyphylaxis, then it may be postulated that a protracted angiotensin deficiency leads to a change in the receptors that persist after the smooth muscle is...
removed from the angiotensin-containing in situ environment.

References
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