Enhanced Release of Endothelium-Derived Relaxing Factor in Mineralocorticoid Hypertension


Ring segments of superior mesenteric arteries studied in vitro were used to determine the role of the vascular endothelium in regulating vascular contractile and relaxant sensitivity in deoxycorticosterone acetate (DOCA)-salt hypertension. Wistar rats were given DOCA (20 mg/kg s.c. twice per week) and 1% NaCl drinking water for 5 weeks. In ring segments containing endothelium, there was a decrease in contractile sensitivity to arginine vasopressin, no change in contractile sensitivity to norepinephrine and KCl, and no change in relaxant sensitivity to acetylcholine or isoproterenol in arteries from hypertensive rats compared with normotensive controls. Removal of the vascular endothelium by rubbing had no effect on the contractile response to arginine vasopressin and KCl or the relaxant response to isoproterenol in control arteries. In arteries without endothelium, DOCA-salt hypertension caused a threefold increase in contractile sensitivity for arginine vasopressin, norepinephrine, and KCl; a 50% reduction in maximal relaxation to isoproterenol; and a threefold decrease in relaxant sensitivity to sodium nitroprusside. Indomethacin (10 μM) had no effect on contraction or relaxation. However, N-monomethyl L-arginine unmasked altered contractile sensitivity to norepinephrine in arteries from DOCA-salt hypertensive rats. These data show that the endothelium compensates for increased contractile and reduced relaxant responses of vascular muscle in DOCA-salt hypertension by increasing the release of endothelium-derived relaxing factor. These data suggest that altered vascular responsiveness is masked by the endothelium, thus preventing these alterations from contributing to increased peripheral resistance during the development of DOCA-salt hypertension. (Hypertension 1992;20:304–313)

KEY WORDS • endothelium • hypertension, mineralocorticoid • endothelium-derived relaxing factor • muscle, smooth, vascular

It is now known that the endothelium is required for the relaxant action of many vasodilators. Acetylcholine is an endothelium-dependent vasodilator because its relaxant effect is mediated by its interaction with the endothelium to cause the release of an endothelium-derived relaxing factor (EDRF). EDRF, a diffusible nonprostanoid substance, stimulates soluble guanylate cyclase in vascular smooth muscle to cause cyclic 3',5'-guanosine monophosphate (cGMP)-dependent relaxation. Endothelium-independent nitrovasodilators such as sodium nitroprusside are converted into nitric oxide (NO), which also acts by stimulating the cGMP-dependent vasodilator pathway. There is good evidence that EDRF is NO. Impaired relaxation to both endothelium-dependent and endothelium-independent vasodilators has been reported in various models of experimental hypertension.

An increase in sensitivity to vasoconstrictors of blood vessels from deoxycorticosterone acetate (DOCA)-salt hypertensive animals is also a common finding. These results have been interpreted to mean that the initiation or maintenance, or both, of the hypertension is caused by an enhanced contractile sensitivity or reduced relaxation of the blood vessel. However, we have recently reported that contractile sensitivity to arginine vasopressin (AVP) and lysine vasopressin is reduced in arteries from the DOCA-salt hypertensive rat. In addition, we found that the reduced contractile sensitivity to AVP is not a result of a decreased vascular vasopressin receptor affinity.

Both AVP- and norepinephrine-induced contractions are attenuated by the presence of the endothelium, presumably because of the vasoconstrictor-induced release of EDRF. In DOCA-salt hypertension, it is likely that endothelial cell modulation of contractile responses may be important in the altered vascular sensitivity. The aim of the present study was to determine the role of the endothelium in modulating vascular sensitivity in DOCA-salt hypertension and to test the hypothesis that the endothelium is masking an increased contractile sensitivity and a reduced vasodilator sensitivity in blood vessels from DOCA-salt hypertensive rats.

Methods

DOCA-Salt Treatment and Blood Pressure Measurement

Male Wistar rats (Harlan, Indianapolis, Ind.) (weight, 175–200 g) were used in these studies. The rats were not
uninephrectomized. Systolic blood pressure was measured with a tail cuff and a pneumatic pulse transducer (Narco BioSystems, Austin, Tex.). Twice-weekly blood pressure measurements were begun 2 weeks before DOCA-salt treatment was started. Pretreatment blood pressure measurements were obtained, and the animals were divided into control and experimental groups. The experimental group received DOCA (20 mg/kg s.c. in olive oil) twice weekly for 4.5 weeks and 1% NaCl in their drinking water. For each rat, at least five blood pressure readings were averaged to obtain the blood pressure on that day. After 5 weeks of treatment, control and hypertensive animals were decapitated.

**Contraction and Relaxation Measurements**

The superior mesenteric artery was carefully removed, placed in Krebs solution (composition in mM: NaCl 120, KCl 5.5, CaCl2 2.5, NaH2PO4 1.4, MgCl2 1.2, NaHCO3 20, dextrose 11.1, and CaNa2EDTA 0.027), and cut into 3-mm-long ring segments. The length of the ring was carefully controlled with a calibrated ocular micrometer. Either adjacent segments were left intact or the intima was rubbed with a wooden probe to disrupt the endothelium. The ring segments were then mounted between two stainless steel pins passed through the vessel lumen for the measurement of isometric contraction and relaxation. The arteries were placed in glass muscle chambers containing Krebs solution gassed with 95% O2-5% CO2 mixture and maintained at 37°C. Isometric contractions and relaxations were measured with FT.03 force transducers connected to a Grass polygraph (Grass Instrument Co., Quincy, Mass.). All tissues were equilibrated for 1 hour at a resting tension of 400 mg, which was determined to be optimal in preliminary length-tension experiments, and then contracted with 10 μM norepinephrine. The arteries were thoroughly washed for 45 minutes, and the tissues were contracted again with norepinephrine. After washing, each segment was contracted to one half of the maximal response to norepinephrine followed by addition of 1 μM acetylcholine to cause relaxation. Arteries with endothelium relaxed to the precontraction resting tone in response to acetylcholine. Arteries in which the endothelium was rubbed did not relax in response to acetylcholine. After a final 45 minutes of thorough washing, cumulative concentration–response curves for agonist-induced contraction or relaxation were generated. After the experiment, tissue wet weight was determined on a Mettler H51 microbalance. The mass of the ring segments was calculated by dividing the wet weight of the ring by the length.

To measure isoproterenol-induced relaxation, concentration–response curves were obtained after ring segments were precontracted to one-half maximal response with norepinephrine and allowed to reach a stable level of tone. Concentration–response curves for acetylcholine and sodium nitroprusside were generated after ring segments were precontracted with a maximal concentration of norepinephrine (10 μM) and allowed to reach a stable level of tone. Relaxation–response curves were plotted as percent maximal relaxation to baseline, where baseline is resting tension before precontraction with norepinephrine.

Experiments using the cyclooxygenase inhibitor indomethacin were performed by adding 10 μM indomethacin to the Krebs solution and incubating the tissues for 1 hour before obtaining concentration–response curves. The same protocol was used in experiments with the competitive inhibitor of EDRF production, N-monomethyl L-arginine (L-NMMA), except that 3 mM L-NMMA was used.

**Data Analysis**

EC50 values were calculated from the concentration–response curves by linear regression of all points between 20% and 80% of the maximal response. Data were analyzed by Student’s t test or an analysis of variance (ANOVA) for multiple comparisons as appropriate. When the ANOVA indicated significant differences among groups, the Newman-Keuls test was used to make specific comparisons. In all cases, the 95% confidence level was accepted as showing a significant difference among groups.

**Results**

**Blood Pressure, Body Weight, and Artery Cross-sectional Area**

Figure 1 presents the change in systolic blood pressure over time in control and DOCA-salt–treated rats. Mean pretreatment systolic blood pressure for all groups was 124±2 mm Hg. Blood pressure in the treated group remained below 140 mm Hg until day 25. After 32 days of DOCA-salt treatment, the average blood pressure was 171±4 mm Hg in the DOCA group and 123±2 mm Hg in the control group. Animals were killed between day 32 and day 35. This mild course of hypertension did not affect the body weight or cause the mesenteric arterial segments to hypertrophy (as measured by cross-sectional area of ring segments). On the day they were killed, the average body weight of animals from the hypertensive group was 441 ±12 g (n = 16) compared with 468±11 g (n = 15) in the normotensive group. Mean cross-sectional area of ring segments was 0.40±0.05 mm2 for both hypertensive and normotensive controls.

**Contractile Sensitivity of Arteries With Endothelium**

The effect of DOCA-salt hypertension on contractile sensitivity (EC50) of isolated mesenteric arteries with an intact endothelium was examined by generating concentration–response curves for the contractile agonists AVP, norepinephrine, and KCl. Table 1 lists the mean EC50 values and maximal responses for these agonists. An increase in contractile sensitivity is indicated by a smaller EC50, whereas a larger EC50 indicates a reduced contractile sensitivity in the hypertensive compared with the control groups. In arteries with endothelium, DOCA-salt hypertension did not affect the contractile sensitivity (EC50) or maximal response of arteries to norepinephrine or KCl. The contractile sensitivity to AVP of arteries with endothelium from hypertensive rats was reduced by 1.4-fold compared with arteries from normotensive rats. DOCA-salt hypertension did not affect the maximal response to AVP in arteries with endothelium.

**Endothelial Regulation of Contraction**

The role of the endothelium in modulating AVP-induced contraction of superior mesenteric arteries...
from normotensive and DOCA-salt hypertensive rats is illustrated in Figure 2. In arteries from normotensive rats, the concentration–response curve for AVP was unaffected by removal of the endothelium. In contrast, arteries from hypertensive rats without endothelium exhibited a fourfold leftward shift in the concentration–response curve (increased contractile sensitivity) for AVP compared with arteries with an intact endothelium. Removal of the endothelium also caused a significant increase in maximal contraction to AVP only in arteries from hypertensive animals (Table 1). Figure 3 illustrates the effect of endothelium removal on concentration–response curves for norepinephrine. In arteries from normotensive rats, removal of endothelium caused a 12-fold leftward shift of the concentration–response curve for norepinephrine compared with arteries with endothelium. A similar pattern was found in arteries from DOCA-salt hypertensive rats. However, there was a significantly larger (34-fold) leftward shift of the concentration–response curve for norepinephrine in arteries without endothelium than in arteries with endothelium from hypertensive rats. Removal of endothelium had no significant effect on maximal contraction caused by norepinephrine in arteries from either normotensive or DOCA-salt hypertensive animals (Table 1).

Endothelium modulation of KCl-induced contraction is illustrated in Figure 4. Removal of endothelium had no effect on the concentration–response curve or maximal contraction for KCl in arteries from normotensive rats (Table 1). In contrast, removal of the endothelium from arteries of hypertensive rats caused a significant fivefold leftward shift of the concentration–response curve for KCl compared with arteries with an intact endothelium. Maximal contraction to KCl of arteries from hypertensive rats was not affected by removal of endothelium (Table 1). These experiments, as well as those using AVP and norepinephrine, show that the presence of the endothelium compensates for the underlying contractile supersensitivity of mesenteric arteries from DOCA-salt hypertensive rats.

Effect of Hypertension and Endothelium on Relaxation

Relaxation responses for acetylcholine, isoproterenol, and sodium nitroprusside of isolated mesenteric arteries from DOCA-salt hypertensive rats were also examined. Table 2 lists the mean EC50 values and maximal responses for these agonists. DOCA-salt hypertension did not affect relaxation responses to acetyl-
TABLE 1. Effect of Deoxycorticosterone Acetate–Salt Hypertension and Endothelin on \( EC_{50} \) and Maximal Contraction for Arginine Vasopressin, Norepinephrine, and KCl in Superior Mesenteric Arteries

<table>
<thead>
<tr>
<th>Drug</th>
<th>Treatment</th>
<th>( EC_{50} ) (nM)</th>
<th>Max (mg)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>AVP</td>
<td>Control+ENDO</td>
<td>2.0±0.1</td>
<td>402±83</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>DOCA+ENDO</td>
<td>2.8±0.2*</td>
<td>430±91</td>
<td>14</td>
</tr>
<tr>
<td>AVP</td>
<td>Control–ENDO</td>
<td>1.9±0.2</td>
<td>647±290</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>DOCA–ENDO</td>
<td>0.7±0.2*</td>
<td>1,238±203*</td>
<td>6</td>
</tr>
<tr>
<td>KCl</td>
<td>Control+ENDO</td>
<td>27±2</td>
<td>1,122±182</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>DOCA+ENDO</td>
<td>27±2</td>
<td>800±130</td>
<td>6</td>
</tr>
<tr>
<td>KCl</td>
<td>Control–ENDO</td>
<td>15±2</td>
<td>938±43</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>DOCA–ENDO</td>
<td>6.0±2*</td>
<td>668±51</td>
<td>6</td>
</tr>
<tr>
<td>NE</td>
<td>Control+ENDO</td>
<td>320±50</td>
<td>1,051±164</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>DOCA+ENDO</td>
<td>300±70</td>
<td>1,358±311</td>
<td>9</td>
</tr>
<tr>
<td>NE</td>
<td>Control–ENDO</td>
<td>27±3*</td>
<td>1,950±117</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>DOCA–ENDO</td>
<td>8.9±2*</td>
<td>1,605±151</td>
<td>7</td>
</tr>
<tr>
<td>NE (- L-NMMA)</td>
<td>Control+ENDO</td>
<td>290±90</td>
<td>381±120</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>DOCA+ENDO</td>
<td>260±100</td>
<td>632±210</td>
<td>4</td>
</tr>
<tr>
<td>NE (+ L-NMMA)</td>
<td>Control+ENDO</td>
<td>150±60</td>
<td>1,250±170‡</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>DOCA+ENDO</td>
<td>32±10‡</td>
<td>1,675±250‡</td>
<td>4</td>
</tr>
</tbody>
</table>

AVP, arginine vasopressin; DOCA, deoxycorticosterone acetate; +ENDO, –ENDO, with and without endothelium, respectively; NE, norepinephrine; –L-NMMA, +L-NMMA, without and with \( N \)-monomethyl \( L \)-arginine, respectively. Values for \( EC_{50} \) and maximal contraction (Max) are mean±SEM. Statistical comparisons were made with an analysis of variance followed by the Newman-Keuls test to determine significant differences among groups.

*Significantly different from all other values for the same agonist, \( p<0.05 \).
†Significantly different from values in the presence and absence of L-NMMA, \( p<0.05 \).
‡Significantly different from values in the absence of L-NMMA, \( p<0.05 \).

choline (Figure 5) or isoproterenol (Figure 6) in arteries with endothelium. Removal of the endothelium did not change relaxation responses to isoproterenol in arteries from normotensive rats. In contrast, arteries without endothelium from DOCA-salt hypertensive rats showed a 50% reduction in maximal relaxation to isoproterenol. These results show that the endothelium compensates for the altered relaxation to isoproterenol in arteries from DOCA-salt hypertensive rats. DOCA-salt hypertension also caused a threefold rightward shift in the concentration–response curve for sodium nitroprusside in arteries with and without endothelium (Figure 7). Removal of the endothelium caused a similar 10-fold leftward shift in the concentration–response curve for sodium nitroprusside in arteries from both DOCA-salt hypertensive rats and normotensive rats.

**Effect of Indomethacin on Contraction and Relaxation**

We used indomethacin to test the involvement of cyclooxygenase-derived prostanoids in the endothelium-dependent compensation for altered vascular responses in DOCA-salt hypertension. Figure 8 presents concentration–response curves for norepinephrine of isolated mesenteric arteries from DOCA-salt hypertensive and normotensive rats in the presence of 10 \( \mu M \) indomethacin. In the presence of indomethacin, there is no difference in contractile sensitivity of arteries with endothelium from DOCA-salt hypertensive rats compared with arteries with endothelium from normotensive rats (\( EC_{50} \) values are 170±30 nM and 180±60 nM, respectively). In the presence of indomethacin, removal of the endothelium caused a fourfold increase in contractile sensitivity of arteries from DOCA-salt hypertensive rats compared with arteries without endothelium from normotensive rats (\( EC_{50} \) values are 4.1±1 nM and 19±4 nM, respectively). Indomethacin had no effect on maximal responses in any of the groups studied (data not shown). These results are the same as those found for norepinephrine-induced contraction in the absence of indomethacin (Figure 3), suggesting that cyclooxygenase-derived prostanoids are not involved in endothelium-dependent compensation.
The effect of indomethacin on isoproterenol-induced relaxation of arteries with endothelium from DOCA-salt hypertensive and normotensive rats is illustrated in Figure 9. Indomethacin had no effect on isoproterenol-induced relaxation in arteries from either DOCA-salt hypertensive or normotensive rats, suggesting no role for cyclooxygenase-derived prostanoids in endothelium-dependent compensation. Additionally, indomethacin had no effect on nitroprusside or acetylcholine-induced relaxation of mesenteric arteries from control and DOCA-salt hypertensive rats (data not shown).

We also used the competitive inhibitor of EDRF/NO production L-NMMA to test whether EDRF/NO played a role in the endothelium-dependent compensation in DOCA-salt hypertension. The effect of 3 mM L-NMMA on norepinephrine-induced contraction of arteries with endothelium from DOCA-salt hypertensive rats and normotensive rats is presented in Figure 10. L-NMMA alone did not cause contraction of the arteries at resting tension. There was no difference in contractile sensitivity to norepinephrine of arteries from DOCA-salt hypertensive rats compared with arteries from normotensive rats in the absence of L-NMMA. In contrast, L-NMMA caused a fivefold increase in contractile sensitivity to norepinephrine of arteries from DOCA-salt hypertensive rats compared with arteries in the presence of L-NMMA from normotensive rats (Table 1). The pattern of responses to norepinephrine in the presence of L-NMMA was similar to those caused by removal of the endothelium (Figure 3). Thus, L-NMMA unmasked the altered contractile sensitivity to norepinephrine in arteries from DOCA-salt hypertensive rats, indicating that EDRF/NO is responsible for endothelium-dependent compensation in the DOCA-salt hypertensive rat.

**Discussion**

The results from the present study show that the endothelium masks a nonspecific increase in contractile sensitivity of arteries from DOCA-salt hypertensive rats. Mesenteric arteries with endothelium from DOCA-salt hypertensive rats exhibited a reduced or unchanged contractile sensitivity, depending on the agonist studied. After removal of the endothelium, however, arteries from hypertensive rats were significantly more sensitive to contractile agonists than were arteries without endothelium from normotensive rats. This effect of unmasking smooth muscle supersensitivity by removing endothelium was also produced by treating endothelium-intact arteries with the competitive inhibitor of EDRF/NO production L-NMMA. Taken together, these results suggest that the endothelium com-

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**Figure 3.** Line graph shows mean (log M) norepinephrine (NE) concentration-response curves in superior mesenteric arteries from normotensive (CONT) and deoxycorticosterone acetate–salt hypertensive (DOCA) rats with (+ENDO) and without (-ENDO) endothelium. There was a threefold increase in contractile sensitivity to norepinephrine in arteries from hypertensive compared with normotensive rats only in the absence of endothelium.

**Figure 4.** Line graph shows mean KCl concentration-response curves in superior mesenteric arteries from normotensive (CONT) and deoxycorticosterone acetate–salt hypertensive (DOCA) rats with (+ENDO) and without (-ENDO) endothelium. There was a threefold increase in contractile sensitivity to KCl in arteries from hypertensive compared with normotensive rats only in the absence of the endothelium.
TABLE 2. Effect of Deoxycorticosterone Acetate–Salt Hypertension and Endothelium on EC50 and Maximal Relaxation for Isoproterenol, Sodium Nitroprusside, and Acetylcholine in Superior Mesenteric Arteries

<table>
<thead>
<tr>
<th>Drug</th>
<th>Treatment</th>
<th>EC50 (nM)</th>
<th>Max ( % relaxation )</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACh</td>
<td>Control+ENDO</td>
<td>7.1±1</td>
<td>94±1</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>DOCA+ENDO</td>
<td>9.3±1</td>
<td>89±3</td>
<td>9</td>
</tr>
<tr>
<td>ISO</td>
<td>Control+ENDO</td>
<td>51±6</td>
<td>74±3</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>DOCA+ENDO</td>
<td>27±4</td>
<td>90±3</td>
<td>6</td>
</tr>
<tr>
<td>ISO</td>
<td>Control-ENDO</td>
<td>48±10</td>
<td>83±2</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>DOCA-ENDO</td>
<td>47±10</td>
<td>42±10*</td>
<td>7</td>
</tr>
<tr>
<td>SNP</td>
<td>Control+ENDO</td>
<td>37±9*</td>
<td>93±3*</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>DOCA+ENDO</td>
<td>110±20*</td>
<td>82±2*</td>
<td>5</td>
</tr>
<tr>
<td>SNP</td>
<td>Control-ENDO</td>
<td>2.9±0.5*</td>
<td>99±1</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>DOCA-ENDO</td>
<td>9.7±1*</td>
<td>97±1</td>
<td>13</td>
</tr>
</tbody>
</table>

ACh, acetylcholine; DOCA, deoxycorticosterone acetate; +ENDO, with endothelium; -ENDO, without endothelium. Values for EC50 and maximal relaxation (Max) are mean±SEM. Maximal relaxation values are percentage of relaxation to the baseline level of tone present before contracting with norepinephrine. Statistical comparisons were made with Student's t test (ACh) or analysis of variance followed by a Newman–Keuls test (ISO and SNP) to determine significant differences among groups.

Figure 5. Line graph shows mean (log M) acetylcholine (ACh) concentration–response curves in mesenteric arteries from normotensive (CONT) and deoxycorticosterone acetate–salt hypertensive (DOCA) rats with endothelium (+ENDO). There was no significant difference in relaxant sensitivity or maximal response to acetylcholine between the two groups.

pensates for an increased contractile sensitivity of vascular smooth muscle in DOCA-salt hypertension by enhancing the production of EDRF/NO.

Our results are consistent with the findings of King and Webb, who showed that if the endothelium is intact, enhanced reactivity to norepinephrine could be partially masked in the isolated perfused mesentery of DOCA-salt hypertensive rats. Although we found that the endothelium completely masks the increased sensitivity of mesenteric arteries from the DOCA-salt hypertensive rat, both studies demonstrate that in the presence of the endothelium, normal function of the blood vessel is preserved despite the underlying supersensitivity of the vascular smooth muscle. Arterial strips and perfused vascular beds have been widely used to examine the contractile sensitivity of blood vessels from DOCA-salt hypertensive animals. Many of these studies were undertaken before it was known that the endothelium could modify the action of contractile agonists. It is possible that the endothelium may have been damaged in earlier studies. Thus, the condition of the endothelium in the arterial preparations used to characterize contractile sensitivity in DOCA-salt hypertension is one factor likely to contribute to the diverse findings in the literature.

The role of endothelial modulation of altered contractile responsiveness may change over the time course of development of hypertension. For example, it has been shown that morphological changes in the blood vessel wall occur in response to a sustained elevated blood pressure. It is likely that damage to the endothelium would occur during this stage of hypertension,
which would unmask the underlying supersensitivity of the vascular smooth muscle. An enhanced vasoconstrictor sensitivity has also been shown before a change in blood pressure, suggesting a role for increased vascular sensitivity in the initiation of DOCA-salt hypertension. However, the role of the endothelium in the prehypertensive stage of DOCA-salt hypertension has not been studied. Whether one finds changes in vascular sensitivity in DOCA-salt hypertension depends on the point during the time course of hypertension at which the studies were done. These factors may also cause different results when vascular sensitivity in hypertension is studied. Further studies are needed to more clearly define the role of the endothelium in modulating vascular sensitivity over the entire time course of DOCA-salt hypertension.

In the present study, we measured vascular responses in the superior mesenteric artery only. Many other large arteries, as well as small resistance vessels as measured in perfused vascular beds, have been used to assess vascular sensitivity in DOCA-salt hypertension. Heterogeneity of vascular responses in large compared with small resistance arteries and heterogeneity among different regions of the vasculature may also account for the differences in our results compared with other reports. Factors such as duration and severity of hypertension, integrity of the endothelium, and vascular bed studied are likely to account for the conflicting data on the effects of DOCA-salt hypertension on vascular sensitivity.

Reduced isoproterenol-induced relaxation has also been reported in arteries from DOCA-salt hypertensive rats, suggesting that alterations in β-adrenergic receptor-mediated relaxation may contribute to the development or maintenance, or both, of hypertension. However, the presence or absence of the endothelium was not determined in those studies. We demonstrated that DOCA-salt hypertension did not affect the relaxant sensitivity or the maximal relaxation to isoproterenol in isolated mesenteric arteries with an intact endothelium. Removal of the endothelium from arteries of normotensive rats also had no effect on isoproterenol-induced relaxation, consistent with previous reports showing that isoproterenol-induced relaxation is independent of the endothelium. In contrast, removal of the endothelium caused a reduction in maximal response to isoproterenol in arteries from DOCA-salt hypertensive rats. These results show that DOCA-salt hypertension may change the vasodilatory action of isoproterenol from endothelium-independent to endothelium-dependent, allowing the endothelium to compensate for a diminished relaxant response. Similar findings have been observed in vivo. The implications of these observations are discussed in the following section.
reported by Konishi and Su\textsuperscript{16} in arteries from spontaneously hypertensive rats. These investigators found that only after removal of the endothelium was the maximal response to isoproterenol reduced in arteries from spontaneously hypertensive rats compared with controls. The authors proposed that the endothelium compensates for the diminished relaxant responsiveness of arteries from the hypertensive rat. Our finding that the reduction in maximal relaxation to isoproterenol in arteries from DOCA-salt hypertensive rats is masked by the endothelium is consistent with this interpretation. A similar role for the endothelium in two different experimental models of hypertension suggests a possible common mechanism for the endothelium to compensate for altered vascular responses in hypertension.

Relaxation responses were also measured for the endothelium-dependent vasodilator acetylcholine and the endothelium-independent vasodilator sodium nitroprusside. Using sodium nitroprusside to study the action of EDRF/NO, we tested the hypothesis that an enhanced action of EDRF/NO explains endothelium-dependent compensation in arteries from DOCA-salt hypertensive rats. Relaxant sensitivity to sodium nitroprusside was reduced in the hypertensive rat, indicating that the response of the vascular smooth muscle to EDRF/NO is less in DOCA-salt hypertension. Others have also shown reduced relaxant sensitivity to nitroprusside in arteries from hypertensive rats.\textsuperscript{5} This reduced response to EDRF/NO is similar to the well-known desensitization caused by chronic exposure of the vasculature to nitrovasodilators. Nitrovasodilator-induced desensitization could explain our results if there is an enhanced release of EDRF/NO from the endothelium in DOCA-salt hypertension. Therefore, we used acetylcholine to examine the release of EDRF/NO and to test the hypothesis that an enhanced release of EDRF/NO explains endothelium-dependent compensation in arteries from DOCA-salt hypertensive rats. Acetylcholine-induced relaxation is caused by both the release of and the action of EDRF/NO. DOCA-salt hypertension did not affect the overall relaxation caused by acetylcholine-induced stimulation of the EDRF/NO pathway. However, our results using sodium nitroprusside show that the action of nitrovasodilators such as EDRF/NO is reduced in DOCA-salt hypertension. Because acetylcholine-induced relaxation was not affected by DOCA-salt hypertension, the endothelium must compensate for the reduced action of EDRF/NO by

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure8}
\caption{Line graph shows mean (log M) norepinephrine (NE) concentration–response curves in the presence of 10 \textmu M indomethacin of mesenteric arteries from normotensive (CONT) and deoxycorticosterone acetate–salt hypertensive (DOCA) rats with (+ENDO) and without (−ENDO) endothelium. There was no effect of indomethacin on the endothelium-dependent compensation for increased contractile sensitivity to norepinephrine. Each curve represents the mean of four or five experiments.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure9}
\caption{Line graph shows mean (log M) isoproterenol (ISO) concentration–response curves in the presence (+INDO) and the absence of 10 \textmu M indomethacin of mesenteric arteries with endothelium from normotensive (CONT) and deoxycorticosterone acetate–salt hypertensive (DOCA) rats. There was no effect of indomethacin on responses to isoproterenol. Each curve represents the mean of four experiments.}
\end{figure}
increasing the release of EDRF/NO in response to stimulation by acetylcholine. Our results are consistent with an enhanced release of EDRF/NO in DOCA-salt hypertension. However, bioassay studies have not shown a difference in acetylcholine-induced release of EDRF in DOCA-salt hypertension. The reasons for these differences may be related to the time when hypertension was studied, the degree of hypertension, unintentional damage to the endothelium, different vascular preparations studied, or other factors.

King et al. have demonstrated that the vasculature of the DOCA-salt hypertensive rat responds to the increase in arterial blood pressure by augmenting L-NMMA-inhibitable EDRF production in vivo. We used the competitive inhibitor of EDRF production L-NMMA to test whether enhanced agonist-induced release of EDRF was the mechanism to explain endothelium-dependent compensation in arteries from DOCA-salt hypertensive rats. L-NMMA did not cause contraction in arteries from hypertensive or normotensive rats at resting tension. This suggests that basal release of EDRF does not play a role in modulating vascular responsiveness in rat superior mesenteric arterial ring segments. However, L-NMMA did unmask the enhanced contractile sensitivity to norepinephrine in arteries from DOCA-salt hypertensive rats. These findings indicate that agonist-induced release of EDRF is increased in arteries from DOCA-salt hypertensive rats. It is likely that enhanced release of EDRF in arteries from DOCA-salt hypertensive rats is the mechanism for the endothelium-dependent compensation described in the present study. Potential mechanisms that may trigger enhanced release of EDRF include an endothelial cell response either to the underlying supersensitivity of the blood vessel or to elevations in blood pressure or both. A direct effect of mineralocorticoids on vascular endothelial cells may also be involved in the mechanism for enhanced release of EDRF in DOCA-salt hypertension.

EDRF is not the only vasoactive substance released from endothelial cells. Cyclooxygenase products are also released from endothelial cells and have been shown to play a role in hypertension. We tested whether the cyclooxygenase inhibitor indomethacin would affect contractile and relaxant responses of blood vessels from DOCA-salt hypertensive and normotensive rats. Indomethacin had no effect on the responses to norepinephrine, isoproterenol, nitroprusside, or acetylcholine. These results suggest that cyclooxygenase products do not play a role in the endothelium-dependent compensation for altered vascular responsiveness under the conditions of our experiments.

We hypothesize that after DOCA-salt hypertension has developed, altered vascular responsiveness does not contribute to the increased peripheral vascular resistance because the endothelium masks increased contractile sensitivity and reduced relaxation. This raises the question of whether altered sensitivity of the vasculature plays a role during the initiation or malignant, or both, stage of hypertension. It is well established that plasma concentrations of AVP are increased and that the morphology of the endothelium is altered during the malignant stage of DOCA-salt hypertension. Thus, it is likely that the compensation by the endothelium for the increased contractile sensitivity to AVP and norepinephrine may be absent or impaired after chronic elevation in blood pressure, allowing the increased vascular responsiveness to play a role in the maintenance of high blood pressure. DOCA-salt treatment has also been reported to increase contractile sensitivity to AVP and norepinephrine in isolated perfused kidneys during the early stages of hypertension, suggesting that enhanced vascular sensitivity may contribute to the initiation of the hypertension. This increased vascular responsiveness may also stimulate the endothelium to elicit the compensatory response.

In conclusion, the superior mesenteric artery from the DOCA-salt hypertensive rat exhibits an enhanced contractile sensitivity as well as reduced relaxation only when the endothelium is experimentally removed. When the endothelium is left intact, there is either no change or reduced contractile sensitivity and no change in relaxation. These data show that the endothelium masks the altered vascular responsiveness and may prevent the altered responsiveness from contributing to the increased peripheral resistance during the development of DOCA-salt hypertension.

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