Extracellular Calcium and Altered Vascular Responsiveness in the Deoxycorticosterone Acetate–Salt Rat

EDWARD E. SOLTIS AND F. PETER FIELD

SUMMARY This study investigated the effects of altered extracellular Ca\(^{2+}\) on in vitro femoral arterial smooth muscle responsiveness in deoxycorticosterone acetate (DOCA)–salt hypertensive rats. Compared with controls, femoral arteries from DOCA-salt rats showed a significant increase in sensitivity to KCl and norepinephrine in normal Ca\(^{2+}\) (2.5 mM). Although no difference in maximal contractile response to KCl was observed between groups, there was a significant difference in maximal response to norepinephrine. Dose-response curves in low Ca\(^{2+}\) (0.25 mM) resulted in a significant decrease in the sensitivity of femoral arteries from DOCA-salt rats to KCl and NE so that the responses were similar to those of controls. Relaxation of femoral arteries from DOCA-salt rats after washout of the KCl contraction was significantly slower than that of controls in both low and normal Ca\(^{2+}\). Isoproterenol-induced relaxation of femoral arteries from DOCA-salt rats was significantly attenuated in normal Ca\(^{2+}\). Sensitivity of femoral arteries from DOCA-salt rats to isoproterenol increased in low Ca\(^{2+}\), but maximal relaxation was unaltered. Whereas no difference in maximal relaxation to NaNO\(_2\) was seen in femoral arteries from either group in normal Ca\(^{2+}\), a significant decrease in sensitivity to NaNO\(_2\) was observed in femoral arteries from DOCA-salt rats. In low Ca\(^{2+}\), the response of femoral arteries from DOCA-salt rats to NaNO\(_2\) was similar to that of controls. These results suggest that the increased vascular smooth muscle responsiveness to KCl and norepinephrine seen in DOCA-salt hypertension is due to increased sensitivity of the vascular smooth muscle to Ca\(^{2+}\). Extracellular Ca\(^{2+}\), however, plays only a minor role in the decreased vasodilator responsiveness seen in this form of hypertension. (Hypertension 8: 526–532, 1986)

KEY WORDS • hypertension • femoral smooth muscle • potassium chloride • norepinephrine • isoproterenol • sodium nitrite • vascular reactivity

STUDIES on perfused vessels as well as isolated ring and strip preparations of vascular smooth muscle (VSM) from hypertensive animals have demonstrated alterations in the response to vasoconstrictor and vasodilator stimuli.\(^1\),\(^2\) Calcium ions play a pivotal role in the contraction and relaxation processes of VSM, and an alteration in Ca\(^{2+}\) handling by the VSM cell has been suggested to explain some of the functional changes that occur in the vasculature in hypertension.\(^3\) Few reports have appeared in the literature on the role of extracellular Ca\(^{2+}\) in the altered vascular responsiveness observed in the deoxycorticosterone acetate (DOCA)–salt hypertensive rat, and these reports are conflicting. Whereas Hinke\(^4\) has shown an increased vasoconstrictor-sensitivity of VSM to Ca\(^{2+}\), others have shown either no change\(^5\) or a decreased response.\(^6\)

The present study investigated the effect of altered extracellular Ca\(^{2+}\) concentration on the responsiveness of rings of femoral artery from DOCA-salt hypertensive rats to KCl depolarization and norepinephrine (NE) stimulation. In addition to the increased vasoconstrictor response, a decrease in the vasodilator responsiveness has been reported in this model of hypertension.\(^7\) Therefore, the effect of altered extracellular Ca\(^{2+}\) on the relaxation of VSM in response to the washout of KCl as well as isoproterenol (ISO)-induced and NaNO\(_2\)-induced relaxation was investigated.

Subjects and Methods

Thirty-six 6-week-old male Sprague-Dawley rats (Blue Spruce Farms, Altamont, NY, USA) were used. Animals were housed in groups of two in hanging stainless steel cages in a room maintained at 26 ± 1 °C.
and illuminated from 0700 to 1900. All rats were fed Purina Laboratory Chow (Ralston Purina Co., St. Louis, MO, USA) and tap water ad libitum unless otherwise specified. Under ether anesthesia, 18 of the rats had the right kidney removed and a pellet (approximately 75 mg) of DOCA implanted subcutaneously in the nape of the neck. These rats were fed a 1% NaCl drinking solution. Control rats were not uninephrectomized and did not undergo sham nephrectomy. Systolic blood pressures were recorded weekly with a tail cuff technique using a pneumatic pulse transducer and Physiograph Four-A (Narco Bio-Systems, Houston, TX, USA).

After 4 weeks of treatment, one 3-mm ring was cut from each femoral artery for studies on vascular reponsiveness using standard smooth muscle bath techniques. Isometric contractions were recorded using an F-50 microdisplacement myograph transducer and Model DMP-4B physiograph recorder (Narco Bio-Systems). Tissues were bathed in a modified Krebs physiological solution at 37 ± 1 °C and aerated with a 95% O₂, 5% CO₂ gas mixture to maintain the pH at 7.4 ± 0.1. Unless otherwise specified, the composition of the Krebs solution was (in mM) NaCl, 118; KCl, 4.7; CaCl₂, 2.5; KH₂PO₄, 1.2; MgCl₂, 1.2; NaHCO₃, 12.5; dextrose, 11.5; and disodium ethylenediaminetetraacetic acid, 0.01. At the conclusion of each experiment the rings of femoral artery were allowed to dry and weighed to a constant weight on a Cahn electrobalance (Cahn Instruments, Cerritos, CA, USA).

Rings of femoral artery from six control and six DOCA-salt hypertensive rats were prepared and equilibrated for 90 minutes at the preload force that results in maximum force generation as determined in the force-tension studies. Cumulative dose-response curves to ISO (10⁻⁹–10⁻⁴ M), NaNO₂ (1.5 × 10⁻⁵ to 1.5 × 10⁻³ M), KCl (8–80 mM), and NE (10⁻₁⁰–10⁻⁴ M) were generated in the presence of a normal (2.5 mM) or a low (0.25 mM) Ca²⁺ Krebs solution. Washout time (relaxation) following the KCl response also was determined. Sufficient time (approximately 30 minutes) was allowed between each dose-response curve to allow washout of the drugs. Dose-response curves to the various agents were performed in a randomized order except that the KCl response was always obtained first. A similar KCl dose-response curve was generated at the end of each experiment to determine tissue viability. No difference in the initial and final dose-response curves was seen (data not shown). The tissues were exposed to each concentration of drug for a period that resulted in a maximal response (approximately 3 minutes). Previous experiments have shown that the endothelium remains intact in our ring preparation as determined by acetylcholine-induced relaxation (E.E. Soltis and F.P. Field, unpublished observation, 1985).

Norepinephrine HCl, ISO HCl, NaNO₂, and DOCA were purchased from the Sigma Chemical Company (St. Louis, MO, USA). Solutions were made fresh each day in double-distilled water.

Student’s t test was used to determine statistical significance between responses of VSM from control and DOCA-salt hypertensive animals and the effect of the extracellular Ca²⁺ concentration within groups. Significance was set at the 95% confidence limit.

**Results**

Systolic blood pressure was significantly elevated in DOCA-salt hypertensive rats after 1 week of treatment and continued to rise to a level of 191 ± 8 mm Hg at the time of experimentation (Table 1). Body weights, heart weights, and femoral ring weights are summarized in Table 1.

Rings of femoral artery from both control and DOCA-salt hypertensive rats showed a progressive increase in active tension development with increasing preload force (Figure 1). Maximum active tension occurred at approximately 1000 mg of preload force in both groups. This level of preload force was used in subsequent studies on vascular reactivity.

### Table 1. Systolic Blood Pressure, Body Weight, Heart Weight, and Femoral Ring Weight of Control and DOCA-Salt Hypertensive Rats

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Weeks of treatment</th>
<th>Systolic blood pressure (mm Hg)</th>
<th>Body weight (g)</th>
<th>Heart weight (mg/100 g body wt)</th>
<th>Femoral ring weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1</td>
<td>121 ± 3</td>
<td>305 ± 8</td>
<td>275.79 ± 6.68</td>
<td>0.2154 ± 0.0056</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>121 ± 2</td>
<td>275.79 ± 6.68</td>
<td>0.2154 ± 0.0056</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>124 ± 4</td>
<td>138 ± 3*</td>
<td>154 ± 5*</td>
<td>168 ± 8*</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>123 ± 2</td>
<td>191 ± 8*</td>
<td>278 ± 10*</td>
<td>392.73 ± 14.51*</td>
</tr>
<tr>
<td>DOCA-salt</td>
<td>1</td>
<td>112 ± 3</td>
<td>305 ± 8</td>
<td>275.79 ± 6.68</td>
<td>0.2154 ± 0.0056</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>112 ± 2</td>
<td>275.79 ± 6.68</td>
<td>0.2154 ± 0.0056</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>124 ± 4</td>
<td>138 ± 3*</td>
<td>154 ± 5*</td>
<td>168 ± 8*</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>123 ± 2</td>
<td>191 ± 8*</td>
<td>278 ± 10*</td>
<td>392.73 ± 14.51*</td>
</tr>
</tbody>
</table>

Values are means ± SEM.

*p < 0.05, compared with control values.
Figure 1. Effect of increasing preload force on development of active tension by rings of femoral artery from control and DOCA-salt hypertensive rats in response to 60 mM KCl stimulation. Each point represents the mean ± SE of 12 rings.

Figure 2 shows the results of dose-response curves to KCl in either normal (2.5 mM) or low (0.25 mM) Ca²⁺ Krebs solution. A significant increase in the sensitivity (response to low doses of KCl) was observed in femoral arterial smooth muscle from the DOCA-salt hypertensive rat in the normal Ca²⁺ media. This increased sensitivity also was reflected in the lower 50% effective dose (ED₅₀) value: 22.5 ± 1.5 mM for DOCA-salt versus 29.5 ± 0.8 mM for control. Maximal contractility was similar in the two groups. In the low Ca²⁺ media the KCl dose-response curves for both control and DOCA-salt rats were shifted significantly down and to the right. Maximal contractility was decreased 66% in DOCA-salt hypertensive rats and 43% in controls. A greater decrease in the response to lower doses of KCl (sensitivity) also occurred in DOCA-salt hypertensive rats than in control rats when compared with responses in normal Ca²⁺. The ED₅₀ values were similar for control and DOCA-salt hypertensive rats in the low Ca²⁺ media (35.7 ± 2.2 and 35.8 ± 2.5 mM, respectively), although the shift was greater in the DOCA-salt hypertensive rat.

Relaxation of the tissues following washout of KCl was quantified as the time (in seconds) to reach 50% of the maximum active tension generated (tₓ). The tₓ values in the normal Ca²⁺ media were 30 ± 2 and 327 ± 73 seconds for control and DOCA-salt rats, respectively. Washout time in the low Ca²⁺ media for controls (tₓ = 28 ± 1 seconds) was similar to that in normal Ca²⁺; however, washout time for DOCA-salt hypertensive rats was significantly less in the low Ca²⁺ media (tₓ = 139 ± 36 seconds) when compared with the response in normal Ca²⁺ media.

A significant increase in the sensitivity as well as maximal contractility to NE was observed in femoral arterial smooth muscle from the DOCA-salt hypertensive rats in the normal Ca²⁺ media when compared with that in controls (Figure 3). The ED₅₀ value for DOCA-salt hypertensive rats was significantly less than that of controls (6.8 ± 0.9 x 10⁻⁸ M vs 18.8 ± 2.3 x 10⁻⁸ M). The maximal contractile response to NE in the low Ca²⁺ media was decreased by 42% in controls and 64% in DOCA-salt hypertensive rats. No change in sensitivity to NE occurred in femoral arterial smooth muscle from controls in the low Ca²⁺ media; however, a significant decrease was observed in DOCA-salt hypertensive rats. The ED₅₀ values for

Figure 2. Dose-response relationship between concentration of KCl and development of active tension by rings of femoral artery from control and DOCA-salt hypertensive rats in either normal (2.5 mM) or low (0.25 mM) calcium Krebs physiological solution. Each point represents the mean ± SE of 12 rings. Single asterisks indicate DOCA-salt significantly different from control (p < 0.05). Double asterisks indicate control response in low calcium significantly different from control response in normal calcium (p < 0.05). Triple asterisks indicate DOCA-salt response in low calcium significantly different from DOCA-salt response in normal calcium (p < 0.05).

Figure 3. Dose-response relationship between concentration of norepinephrine (NE) and development of active tension by rings of femoral artery from control and DOCA-salt hypertensive rats in either normal (2.5 mM) or low (0.25 mM) calcium Krebs physiological solution. Each point represents the mean ± SE of 12 rings. Symbols for statistical significance are the same as those in Figure 2.
control and DOCA-salt hypertensive rats in low Ca\(^{2+}\) media were 22.6 (± 3.4) \times 10^{-8}\ M and 22.1 (± 3.9) \times 10^{-8}\ M, respectively. Although the data are not shown, the characteristics of the KCl and NE dose-response curves were altered in different fashions in the low Ca\(^{2+}\) media. In low Ca\(^{2+}\) media the response to each dose of KCl was simply depressed, since the contractile response to KCl is entirely dependent on extracellular Ca\(^{2+}\). In the NE dose-response curve, however, the fast or phasic component was not altered but the tonic or maintained component was diminished for each dose of NE, because the phasic component is dependent on intracellular release of Ca\(^{2+}\) and the tonic component is dependent on the influx of extracellular Ca\(^{2+}\). This response occurred in both control and DOCA-salt hypertensive rats and explains the observed decrease in maximum force generation since the response to each dose of NE was recorded after 3 minutes during the plateau phase of the contraction.

Whereas the maximal contractile response of femoral arterial smooth muscle from DOCA-salt hypertensive rats to NE was similar to the maximal response to KCl, the maximum response of femoral smooth muscle from controls to NE was less than that to KCl. This decreased maximum response to NE appears to be due to activation of \(\beta\)-adrenergic receptors, since relaxation occurred at high doses of NE. This relaxation could be blocked by propranolol and resulted in a maximal contraction similar to that in DOCA-salt hypertensive rats (data not shown). The lack of this response in the DOCA-salt hypertensive animal is reflected in the ISO dose-response curves.

The ISO-induced relaxation was significantly attenuated in femoral arterial smooth muscle from the DOCA-salt hypertensive rat in normal Ca\(^{2+}\) Krebs solution when compared with that in controls (Figure 4). A decrease in both sensitivity and maximal relaxation to ISO was observed. Contraction to high doses of ISO was evident in femoral arterial smooth muscle from both groups and was significantly greater in the DOCA-salt hypertensive rat (27 ± 4 vs 13 ± 3% increase in active tension). This response has been shown to be mediated by \(\alpha\)-adrenergic activation since it can be blocked by phentolamine. The increased response in the DOCA-salt hypertensive rat to high doses of ISO is most likely due to the increased \(\alpha\)-adrenergic sensitivity of the femoral arterial smooth muscle, as evidenced by the increased NE responsiveness. A significant increase in the sensitivity to ISO was observed in femoral arterial smooth muscle from DOCA-salt hypertensive rats in the low Ca\(^{2+}\) media when compared with responses in normal Ca\(^{2+}\) media. A slight but nonsignificant increase occurred in controls. No changes in the maximal relaxation response or contractile response to ISO were observed in either group in low Ca\(^{2+}\) media.

Maximal relaxation in response to NaNO\(_2\) was similar for both groups in either normal or low Ca\(^{2+}\) media (Figure 5). However, a decrease in the sensitivity to NaNO\(_2\) was observed in femoral arterial smooth muscle from the DOCA-salt hypertensive rat in normal Ca\(^{2+}\) media. Relaxation responses in the low Ca\(^{2+}\) media were unaltered in the control animals. A significant increase in the sensitivity to NaNO\(_2\) of femoral arterial smooth muscle from DOCA-salt hypertensive rats was seen such that the response was similar to that of controls.

**Discussion**

An increase in VSM responsiveness to various vasoconstrictor agents has been shown to occur in the DOCA-salt hypertensive rat. The response to vasodilator agents also appears to be reduced in this model.
of hypertension. The present study was performed to determine the importance of extracellular Ca\textsuperscript{2+} in these altered responses. Our results suggest that the increased vascular sensitivity to KCl depolarization and NE stimulation (as determined by a decrease in the ED\textsubscript{50} values) is due to increased Ca\textsuperscript{2+} sensitivity of the VSM. The decrease in vasodilator responsiveness of VSM from the DOCA-salt hypertensive rat also appears to be related to altered extracellular Ca\textsuperscript{2+} handling but to a lesser degree than the increased vasconstrictor response.

Prior to the studies on vascular responsiveness, a force-tension analysis was performed on rings of femoral arterial smooth muscle. Numerous investigators have used a strip preparation of this VSM and determined that the length-tension characteristics between control normotensive rats and DOCA-salt hypertensive rats are similar. The findings in this study confirm these previous observations in that no difference in the force-tension curves between control and DOCA-salt hypertensive rats was seen.

An increase in the response of rings of femoral arterial smooth muscle from DOCA-salt hypertensive rats to KCl depolarization and NE stimulation was observed in the present study. Holloway and Bohr\textsuperscript{13} and Hagen and Webb\textsuperscript{14} also have observed an enhanced responsiveness to both KCl and NE in strips of femoral and coronary artery from DOCA-salt hypertensive rats. In the present study, decreasing the concentration of extracellular Ca\textsuperscript{2+} resulted in a decrease in the response of VSM from control as well as DOCA-salt hypertensive rats to KCl depolarization. This decrease in responsiveness was expected since KCl-induced contractions are dependent on extracellular Ca\textsuperscript{2+}. The effect of reducing extracellular Ca\textsuperscript{2+} was greater in the DOCA-salt hypertensive rat and resulted in a response similar to that of control rats; this response is reflected in the similar ED\textsubscript{50} values. Thus, the increased sensitivity of femoral arterial smooth muscle from the DOCA-salt hypertensive rats to KCl was due to an increase in Ca\textsuperscript{2+} sensitivity. This conclusion is in agreement with an earlier study of Hinke.\textsuperscript{4} who observed increased responsiveness of isolated perfused tail artery from DOCA-salt hypertensive rats to Ca\textsuperscript{2+} in the presence of a high potassium physiological solution.

Contraction of VSM by NE involves both an influx of extracellular Ca\textsuperscript{2+} and release of Ca\textsuperscript{2+} from intracellular stores. The present study examined the role of extracellular Ca\textsuperscript{2+} in the enhanced responsiveness of femoral arterial smooth muscle from DOCA-salt hypertensive rats to NE. Although the maximal response of femoral arterial smooth muscle from control rats to NE in low Ca\textsuperscript{2+} media was reduced, no change in the ED\textsubscript{50} value was observed. As with the KCI dose-response curves, the response of femoral arterial smooth muscle from DOCA-salt hypertensive rats to NE in low Ca\textsuperscript{2+} media was affected more than controls. The ED\textsubscript{50} value for DOCA-salt hypertensive rats increased significantly so that it was similar to the control value. Thus, the increase in NE sensitivity of femoral arterial smooth muscle from DOCA-salt rats was largely due to an increase in the sensitivity of the VSM to Ca\textsuperscript{2+}. This role for extracellular Ca\textsuperscript{2+} in the increased NE responsiveness is in agreement with the findings of Hinke.\textsuperscript{4} Conversely, Mecca and Webb\textsuperscript{5} have shown no change in the Ca\textsuperscript{2+} sensitivity of VSM from DOCA-salt hypertensive rats to NE stimulation. This lack of change may be due to the fact that no change in the sensitivity to NE itself was observed. Moreland et al.\textsuperscript{6} however, have observed an increase in NE responsiveness of VSM from DOCA-salt hypertensive rats, which they suggest is mediated by an increase in the membrane stores of Ca\textsuperscript{2+}.

Although the exact mechanism (or mechanisms) by which the Ca\textsuperscript{2+} sensitivity is increased in VSM from DOCA-salt hypertensive rats cannot be determined from this study, two possible general mechanisms are suggested. The first can be classified as a membrane defect, which may include alterations in receptor number or affinity, receptor- or voltage-operated Ca\textsuperscript{2+} channels, Ca\textsuperscript{2+} sequestration and extrusion mechanisms, Ca\textsuperscript{2+} permeability, and Ca\textsuperscript{2+} stabilization. The second can be classified as a defect in the components directly involved in the contractile response, such as calmodulin, myosin light chain kinase, and the contractile proteins.

Few studies have been performed with respect to the second classification, but these mechanisms do not appear to be involved in the altered Ca\textsuperscript{2+} sensitivity. Calmodulin levels are not altered in VSM from DOCA-salt hypertensive rats,\textsuperscript{15} and the actomyosin content and actin-to-myosin ratio remain unchanged.\textsuperscript{8} To our knowledge, studies on other components involved in the contractile process of VSM from hypertensive animals, such as myosin light chain kinase, have not been performed.

Defects in membrane properties have been studied extensively in various forms of experimental hypertension, and alterations in various membrane functions may contribute to the enhanced VSM responsiveness seen in DOCA-salt hypertension. Although alterations in both receptor- and voltage-operated Ca\textsuperscript{2+} channels may be involved in the enhanced responsiveness to NE and KCl, respectively, a generalized increase in the permeability of the membrane to Ca\textsuperscript{2+} also may be implicated.\textsuperscript{12} Alterations in both Ca\textsuperscript{2+} extrusion and sequestration mechanisms have been shown to occur in VSM from DOCA-salt hypertensive rats.\textsuperscript{16} In the face of an increase in membrane Ca\textsuperscript{2+} permeability or receptor- or voltage-operated Ca\textsuperscript{2+} influx, a decrease in Ca\textsuperscript{2+} extrusion or sequestration could lead to an increased myogenic tone and enhanced VSM responsiveness. Another aspect of altered Ca\textsuperscript{2+} handling by the VSM in DOCA-salt hypertension is its decreased ability to stabilize the membrane.\textsuperscript{9} Again, this could result in an enhanced responsiveness of the VSM to various stimuli.

As mentioned previously, the response of VSM to NE is dependent on the release of intracellular Ca\textsuperscript{2+} and the influx of extracellular Ca\textsuperscript{2+}, which contribute to the phasic and tonic components of the contraction,
respective. No difference in the phasic component was observed in either the normal or low Ca\(^2+\) media in DOCA-salt hypertensive or control rats. However, the tonic component was diminished so that it was similar in both groups in the low Ca\(^2+\) media. Since no difference was observed in the phasic response, it is unlikely that the low Ca\(^2+\) media had an effect on intracellular Ca\(^2+\) stores or on the mechanisms responsible for the contractile response. Therefore, the enhanced response to NE as well as KCl in femoral arterial smooth muscle from DOCA-salt rats appears to be due to an alteration in one or more of the membrane mechanisms, which results in an increase in the Ca\(^2+\) sensitivity of the VSM.

Relaxation following washout of a vasoconstrictor agent or induced by a vasodilator agent may involve extrusion of Ca\(^2+\) from the cell or sequestration of Ca\(^2+\) by intracellular stores. As stated earlier, alterations in these mechanisms may be important in the altered VSM responsiveness in hypertension. A decrease in the activity of the mechanisms responsible for relaxation or maintaining basal levels of intracellular Ca\(^2+\) in a nonstimulated VSM could be further compromised by an increased permeability or sensitivity of the VSM cell membrane to extracellular Ca\(^2+\). Therefore, the effect of an altered extracellular Ca\(^2+\) on the relaxation of femoral arterial smooth muscle from DOCA-salt hypertensive rats was investigated.

Relaxation following the washout of KCl was significantly slower in femoral arterial smooth muscle from the DOCA-salt hypertensive rat. Decreasing extracellular Ca\(^2+\) did not affect the response of control VSM but did decrease the washout time of VSM from DOCA-salt hypertensive rats. The finding that the washout time remained much greater in the DOCA-salt rat in low Ca\(^2+\) media suggests a defect in the DOCA-salt hypertensive rat’s ability to extrude or sequester Ca\(^2+\) in VSM. Kwan and Grover have shown a decrease in the adenosine triphosphate-dependent Ca\(^2+\) accumulation of microsomal fractions of VSM from DOCA-salt hypertensive rats, which could explain the slower relaxation observed in femoral arterial smooth muscle from the DOCA-salt hypertensive rat following washout of KCl. Nonetheless, extracellular Ca\(^2+\) plays some role in this decreased response.

The role of extracellular Ca\(^2+\) in the decreased response of femoral arterial smooth muscle from DOCA-salt rats to ISO is less evident. Whereas the sensitivity to ISO was increased in VSM from DOCA-salt hypertensive rats in low Ca\(^2+\), the maximum relaxation remained significantly attenuated when compared with that in controls. The alteration in extracellular Ca\(^2+\) handling may be important for the decreased sensitivity to ISO, but another mechanism appears to be involved in the decreased maximum relaxation. Since \(\beta\)-adrenergic relaxation is mediated by cyclic adenosine 3',5'-monophosphate, which stimulates Ca\(^2+\) uptake in microsomal fractions of VSM, the decreased response may be the result of a reduced ability to sequester or extrude Ca\(^2+\). Alterations at the site of the receptor also may be involved. Woodcock et al. have shown a decrease in the number of \(\beta\)-adrenergic receptors in VSM from DOCA-salt hypertensive rats. This decrease is most likely the major cause of the decrease in maximal relaxation, since the intrinsic capability of the VSM to respond maximally to a vasodilator agent remained intact. This conclusion is evidenced by the finding that the maximal relaxation response to NaNO\(_2\) (a nonspecific smooth muscle relaxant) was not altered in VSM from the DOCA-salt hypertensive rat. Hagen and Webb have observed a similar decrease in maximal relaxation in response to ISO with no change in the response to nitroprusside, which is similar to NaNO\(_2\), in its actions. The sensitivity to NaNO\(_2\) was decreased, however, and as with the response to ISO, this decreased response appears to be influenced by extracellular Ca\(^2+\), since the response was similar to that of controls in low Ca\(^2+\) media.

In summary, an increased sensitivity to vasoconstrictor stimuli as well as a decreased response to vasodilator agents was observed in femoral arterial smooth muscle from DOCA-salt hypertensive rats. The increased vasoconstrictor sensitivity appears to be largely dependent on an increase in the sensitivity of the VSM to extracellular Ca\(^2+\). Only a minor contributory role for extracellular Ca\(^2+\) is suggested in the decreased vasodilator responsiveness, since alterations in other mechanisms required to extrude or sequester Ca\(^2+\) appear to be involved.

Acknowledgment
The authors acknowledge Rosemarie Renzi for her assistance in preparing this manuscript.

References
Extracellular calcium and altered vascular responsiveness in the deoxycorticosterone acetate-salt rat.
E E Soltis and F P Field

Hypertension. 1986;8:526-532
doi: 10.1161/01.HYP.8.6.526

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/8/6/526