Sodium Pump Activity and Calcium Relaxation in Vascular Smooth Muscle of Deoxycorticosterone Acetate–Salt Rats

EDWARD E. SOLTIS AND F. PETER FIELD

SUMMARY The Na⁺-K⁺ pump activity was determined in femoral arterial smooth muscle from deoxycorticosterone acetate (DOCA)-salt hypertensive rats using potassium relaxation and ouabain-sensitive Rb⁺ uptake as indices. The membrane-stabilizing effect of calcium and its relation to Na⁺-K⁺ pump activity also were examined. Femoral arteries from DOCA-salt rats exhibited a greater relaxation in response to potassium addition after contraction with norepinephrine in a low potassium (0.6 mM) Krebs solution. The concentration of potassium required to produce a 50% relaxation was significantly less in DOCA-salt rats. Ouabain-sensitive Rb⁺ uptake was significantly greater at 3, 10, and 20 minutes of Rb⁺ incubation in femoral arteries from DOCA-salt rats. Linear regression analysis revealed a significant correlation between the uptake of Rb⁺ and time of incubation in both control and DOCA-salt rats. A significant difference in the slopes of the regression lines showed that the rate of uptake was greater in DOCA-salt rats. No difference was observed in ouabain-insensitive Rb⁺ uptake. A dose-dependent relaxation in response to increasing concentrations of calcium following contraction to norepinephrine was observed in femoral arteries from control and DOCA-salt rats. The relaxation was directly dependent on the level of extracellular potassium and was blocked by ouabain. Femoral arteries from DOCA-salt rats relaxed to a significantly greater extent in response to calcium at each level of potassium when compared with controls. These results provide further evidence for an increase in Na⁺-K⁺ pump activity in vascular smooth muscle from DOCA-salt hypertensive rats. In contrast to previous reports, however, calcium-induced relaxation was shown to be increased in the DOCA-salt rat. This effect is directly dependent on the activity of the Na⁺-K⁺ pump and supports observations of increased potassium relaxation and Rb⁺ uptake. (Hypertension 8: 1032-1039, 1986)

KEY WORDS • potassium relaxation • rubidium-86 uptake • femoral arterial smooth muscle • membrane stabilization

ALTERATIONS in both the active and passive transport of monovalent ions (sodium, potassium) across the vascular smooth muscle membrane have been implicated in the pathogenesis of deoxycorticosterone acetate (DOCA)-salt hypertension. However, there is disagreement with regard to the direction of these changes. Some investigators have reported a suppressed activity of the Na⁺-K⁺ pump, whereas others have observed an increase. A similar discrepancy in the passive movement of monovalent ions has been noted. That is, an increase or no change in the permeability of the vascular smooth muscle membrane to these ions has been reported. It has been suggested that the alterations in monovalent ion transport in vascular smooth muscle from DOCA-salt hypertensive animals is due to a decreased ability of calcium to stabilize the membrane. This stabilizing effect is apparently mediated through the Na⁺-K⁺ pump. The present study was performed to determine whether Na⁺-K⁺ pump activity is increased or decreased in femoral arterial smooth muscle of DOCA-salt rats and whether the calcium-stabilizing effect coincides with these changes in Na⁺-K⁺ pump activity. Potassium-induced relaxation and Rb⁺ uptake were used to determine sodium pump activity. The membrane-stabilizing effect of calcium was investigated.
using a method similar to that reported by Webb and Bohr,\textsuperscript{12} in which high levels of calcium induce a relaxation of already contracted vascular smooth muscle.

**Materials and Methods**

**Animals and General Procedures**

Male Sprague-Dawley rats (6 weeks old; 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, St. Louis, MO, USA) and tap water ad libitum unless otherwise specified. One half of the rats were anesthetized with ether, the right kidney was removed, and a pellet (approximately 75 mg) of DOCA was implanted in the nape of the neck. These rats were fed a 1% NaCl drinking solution. Control rats were not uninephrectomized, did not undergo sham operation, and received tap water to drink. 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). The amount of DOCA delivered to each animal per day was calculated by subtracting the weight of the pellet at the time of the animal's death from the initial weight at implantation and dividing by the mean body weight of the animal over the 4-week treatment period. This value was 2.55 ± 0.08 mg/kg/day.

After 4 weeks of treatment one 3-mm ring was cut from each femoral artery for studies on vascular responsiveness using standard smooth muscle bath techniques.\textsuperscript{13} Isometric contractions were recorded using an F-50 microdisplacement myograph transducer and 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% O\textsubscript{2}, 5% CO\textsubscript{2} 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; CaCl\textsubscript{2}, 2.5; KH\textsubscript{2}PO\textsubscript{4}, 1.2; MgCl\textsubscript{2}, 1.2; NaHCO\textsubscript{3}, 12.5; dextrose, 11.5; disodium ethylenediaminetetraacetic acid, 0.01.

**Potassium Relaxation**

Rings of femoral artery from control and DOCA-salt rats were equilibrated for 90 minutes at 1 g of preload force\textsuperscript{13} in a low potassium (0.6 mM) Krebs physiological solution to depress the Na\textsuperscript{+}-K\textsuperscript{+} pump and load the vascular smooth muscle cells with sodium. An equimolar concentration of Na\textsubscript{2}H\textsubscript{2}PO\textsubscript{4} was substituted for K\textsubscript{2}HPO\textsubscript{4} in the Krebs solution. The concentration of KCl was decreased from 4.7 to 0.6 mM. Following the equilibration period the rings of femoral artery were maximally stimulated with 10\textsuperscript{-4} M norepinephrine (NE). After the contractions had plateaued (approximately 5 minutes), KCl was added back in increments of 0.8 mM to obtain a dose-response curve (1.4–5.4 mM total potassium). This procedure has been shown to cause a stimulation of the Na\textsuperscript{+}-K\textsuperscript{+} pump that hyperpolarizes the smooth muscle resulting in a relaxation.\textsuperscript{14}

\textsuperscript{*}Rb Uptake

Both femoral arteries from control and DOCA-salt rats were placed in aerated Krebs physiological solution at room temperature following removal from the animal. After the arteries were cleaned of fat and connective tissue, they were incubated at 4°C in a potassium-free Krebs solution for 10 minutes to depress Na\textsuperscript{+}-K\textsuperscript{+} pump activity and load the cells with sodium. One artery from each animal was then incubated in a potassium-free Krebs solution with "cold" RbCl (2 mM), \textsuperscript{*}RbCl (0.1 mM; specific activity, 2.31 mCi/mg), and ouabain (10\textsuperscript{-4} M) at 37°C. The other artery was incubated in a similar medium but without ouabain. The tissues were incubated for either 3, 10, or 20 minutes to obtain a time course of \textsuperscript{*}Rb uptake. Following the incubation period the tissues were washed for 30 seconds with a potassium-free Krebs solution containing 2 mM RbCl. The tissues were allowed to dry to a constant weight, weighed, placed in disposable test tubes, and counted using a Model 1195 Searle gamma counter (Des Plaines, IL, USA). The Na\textsuperscript{+}-K\textsuperscript{+} pump activity (ouabain-sensitive \textsuperscript{*}Rb uptake) was determined by subtracting the amount of \textsuperscript{*}Rb taken up in the presence of ouabain from the amount taken up in the absence of ouabain. The Na\textsuperscript{+}-K\textsuperscript{+} pump activity was expressed as picomoles of \textsuperscript{*}Rb taken up per milligram of dry tissue weight.

**Calcium Relaxation**

Rings of femoral artery from control and DOCA-salt rats were equilibrated for 90 minutes at 1 g of preload force\textsuperscript{13} in a low calcium (0.25 mM) Krebs solution with either low (3.0 mM), normal (5.9 mM), or high (10.0 mM) potassium. Adjustments in the concentration of potassium were made by adding or deleting the required amount of KCl. Following the equilibration period the rings of femoral artery were maximally stimulated with 10\textsuperscript{-4} M NE. After the contractions had plateaued, a dose-response curve to calcium (2.25–22.25 mM) was generated. In one series of experiments, the rings of femoral artery were equilibrated in the low calcium, normal potassium Krebs solution and, 1 minute before the NE addition, ouabain was added to the muscle bath. The dose-response curve to calcium was then performed following the addition of NE.

**Statistical Analysis**

All data are expressed as the mean ± SEM. Data were analyzed by unpaired Student's t test where appropriate. Analysis of the rate of \textsuperscript{*}Rb uptake was performed using linear regression and analysis of covariance to determine differences in the slope of the regression lines. A p value of less than 0.05 was considered to be significant.
Drugs

The NE HCl, ouabain octahydrate, and DOCA were purchased from Sigma Chemical (St. Louis, MO, USA). The *RbCl was purchased from New England Nuclear (Boston, MA, USA). Solutions were made fresh each day in double-distilled water.

Results

Blood Pressure

Systolic blood pressure increased significantly during the 4-week period in DOCA-salt rats and was 186 ± 5 mm Hg at the time of experimentation (Table 1). Control rats remained normotensive throughout the treatment period and were 123 ± 3 mm Hg at termination. Body weights, ventricular heart weights, and femoral ring weights are presented in Table 1.

Potassium Relaxation

Figure 1 shows a representative tracing of potassium-induced relaxation of rings of femoral artery from control and DOCA-salt rats. Although the maximum contractile response to NE (force in grams) was depressed following incubation in the low potassium Krebs solution when compared with responses in a normal potassium medium, there was no difference in the response of femoral arterial smooth muscle between control and DOCA-salt rats (1.18 ± 0.08 g and 1.14 ± 0.07 g, respectively). Femoral arterial smooth muscle from DOCA-salt rats exhibited a significantly greater relaxation in response to the addition of potassium following NE-induced contraction in 0.6 mM potassium Krebs solution. The results are summarized in Figure 2 as a dose-response curve to KCl and expressed as the percentage of change in response from the NE contraction in 0.6 mM potassium Krebs solution. Rings of femoral artery from DOCA-salt rats relaxed 80% in response to the addition of 0.8 mM KCl (1.4 mM total potassium), whereas those from controls relaxed only 17%. At 2.2 mM KCl, femoral arterial smooth muscle from DOCA-salt rats had relaxed 105%, and that from controls 55%. The rings of femoral artery from DOCA-salt rats continued to relax beyond 100% at KCl concentrations greater than 2.2 mM and exhibited maximal relaxation (119%) at 3.8 mM KCl. The response of femoral arterial smooth muscle from controls achieved maximal relaxation (100%) at 5.4 mM KCl. The concentration of KCl

![Figure 1](image1)

**Figure 1.** Representative tracing of potassium-induced relaxation of femoral arterial smooth muscle from control and DOCA-salt hypertensive rats. Rings of femoral artery were maximally contracted with 10^-4 M norepinephrine in a 0.6 mM potassium Krebs solution. KCl was then added back in increments of 0.8 mM up to 5.4 mM at the designated points. R = rinse.

![Figure 2](image2)

**Figure 2.** Dose-response curves of potassium-induced relaxation in rings of femoral artery from control (n = 6) and DOCA-salt hypertensive (n = 6) rats. Protocol is described in Figure 1. Asterisks indicate values in DOCA-salt rats significantly different from those in controls (p < 0.001).

<table>
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<th>Treatment group</th>
<th>Systolic blood pressure (mm Hg)</th>
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<th>Heart weight (mg/100 g body wt)</th>
<th>Femoral ring weight (mg dry wt)</th>
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<td>Weeks of treatment</td>
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<td>119 ± 2</td>
<td>125 ± 2</td>
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<td>125 ± 2</td>
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Values are means ± SEM.

*p < 0.01, compared with control values.
required to produce a 50% relaxation was significantly less in femoral arterial smooth muscle from DOCA-salt hypertensive rats when compared with controls (1.13 ± 0.1 vs 2.10 ± 0.11 mM; p < 0.001). The 50% effective dose values were determined graphically from individual curves. The concentration of KCl required to produce a maximal relaxation was also significantly less in femoral arterial smooth muscle from DOCA-salt hypertensive rats (3.62 ± 0.09 mM for DOCA-salt rats and 4.72 ± 0.23 mM for control rats; p < 0.05).

\[ ^{86}\text{Rb} \text{ Uptake} \]

Sodium pump activity as determined by the \(^{86}\text{Rb}\) uptake technique is shown in Figure 3. A significant increase in ouabain-sensitive uptake was observed in femoral arterial smooth muscle from DOCA-salt rats at all three incubation periods when compared with controls. Linear regression analysis showed a significant correlation between the rate of ouabain-sensitive and ouabain-insensitive \(^{86}\text{Rb}\) uptake by femoral arterial smooth muscle from both control and DOCA-salt rats and the length of incubation time. Analysis of covariance revealed a significant difference in the slopes of the ouabain-sensitive regression lines, with the rate of ouabain-sensitive \(^{86}\text{Rb}\) uptake being greater in femoral arterial smooth muscle from DOCA-salt rats. No difference in ouabain-insensitive \(^{86}\text{Rb}\) uptake was observed between the two groups.

\[ \text{Calcium Relaxation} \]

A representative tracing of calcium-induced relaxation of rings of femoral artery from control and DOCA-salt rats is shown in Figure 4. A contraction occurred in response to the addition of 2.0 mM CaCl\(_2\) (2.25 mM total CaCl\(_2\)) in femoral arterial smooth muscle from both control and DOCA-salt rats. Relaxation occurred in response to higher concentrations of CaCl\(_2\). No difference was observed in the response to 2.25 mM CaCl\(_2\) between control and DOCA-salt rats (1.58 ± 0.12 g and 1.52 ± 0.13 g, respectively); therefore, dose-response curves are expressed as the percentage of change in the response to the NE contraction at 2.25 mM CaCl\(_2\) (Figure 5). In a normal Krebs physiological solution (5.9 mM potassium) femoral arterial smooth muscle from DOCA-salt rats exhibited a significantly greater relaxation in response to CaCl\(_2\) when compared with controls (see Figure 5). Ouabain blocked the response similarly in both groups. The significantly greater relaxation of rings of femoral artery from DOCA-salt rats in response to CaCl\(_2\) was also evident in the low (3.0 mM) as well as high (10 mM) potassium Krebs solution (see Figure 5). These data show that the response to CaCl\(_2\) is mediated by the
Figure 5. Dose-response curves of calcium-induced relaxation in rings of femoral artery from control and DOCA-salt hypertensive rats in a low potassium (3.0 mM), normal potassium (5.9 mM), or high potassium (10 mM) Krebs solution. The dose-response curve to calcium in the presence of ouabain (10^-4 M) was performed in 5.9 mM potassium Krebs solution. Ouabain was added 1 minute before the addition of norepinephrine. Protocol is described in Figure 4. The response of femoral arteries from DOCA-salt rats to calcium was significantly greater than that of control rats at each level of potassium (p < 0.05). No difference was seen between the two groups when arteries were treated with ouabain (n = 6 for each curve).

Discussion

The present study provides further evidence in support of the hypothesis that Na^+-K^+ pump activity is increased in vascular smooth muscle from DOCA-salt hypertensive rats treated for 4 weeks. Both potassium relaxation and ouabain-sensitive ^86Rb uptake were significantly increased in femoral arterial smooth muscle from DOCA-salt rats.

The potassium relaxation experiments were performed such that the dose-response curves were generated beginning at low concentrations of potassium and progressing up to physiological levels. This protocol would give an indication of pump activity under physiological concentrations of potassium. The most apparent observation is the high level of Na^+-K^+ pump activity seen in femoral arterial smooth muscle from DOCA-salt rats at the low concentrations of potassium. Although the threshold sensitivity to potassium (dose at which the initial measurable response can be recorded) was not obtained, a large shift in the mid-range sensitivity was seen, demonstrating the increased Na^+-K^+ pump activity that occurs in vascular smooth muscle in this model of hypertension. Maximal pump activity, as measured by maximal potassium relaxation, occurred within a normal physiological range of extracellular potassium in control rats. On the other hand, maximal relaxation in femoral arterial smooth muscle from DOCA-salt rats occurred at a lower range of extracellular potassium. Interestingly, whereas vascular smooth muscle from controls maximally relaxed to 100% on the average, vascular smooth muscle from DOCA-salt rats invariably relaxed beyond this by approximately another 20%. This observation further emphasizes the increased pump activity of vascular smooth muscle from DOCA-salt hypertensive rats.

In support of the potassium relaxation experiments, ouabain-sensitive ^86Rb uptake was significantly increased in femoral arterial smooth muscle from DOCA-salt rats. A time course of ^86Rb uptake was obtained to determine the characteristics of the uptake. It was seen that both ouabain-sensitive and ouabain-insensitive uptake over the 20-minute period were linear in both control and DOCA-salt rats. However, the rate of ouabain-sensitive uptake, as determined by the slopes of the regression lines, was greater in vascular smooth muscle from DOCA-salt rats, suggesting an increase in Na^+-K^+ pump activity. Although the rate of relaxation induced by potassium was not quantified in this study, it would appear from the ^86Rb uptake experiments that vascular smooth muscle from DOCA-salt rats relaxes at a faster rate than that from controls.
The increase in Na\(^{+}\)-K\(^{+}\) pump activity has been attributed to several factors, one of which is an increase in the permeability of the vascular smooth muscle membrane to sodium.\(^2\) Equilibration of femoral arteries from DOCA-salt rats in low potassium (0.6 mM potassium in the potassium relaxation experiments) or zero potassium (\(^{86}\)Rb uptake experiments) would result in a greater accumulation of sodium intracellularly because of the increase in membrane permeability. Upon activation of the Na\(^{+}\)-K\(^{+}\) pump with the increase in extracellular potassium (or \(^{86}\)Rb), the relaxation (or uptake of \(^{86}\)Rb) would be greater as a result of the increase in intracellular sodium. However, ouabain-insensitive \(^{86}\)Rb uptake (a measure of passive ion movement into the cell) was not increased in femoral arterial smooth muscle from DOCA-salt rats. Similar results have been reported in tail arteries from DOCA-salt rats using the \(^{86}\)Rb uptake technique.\(^3\) Brock et al.\(^3\) observed an increase in ouabain-sensitive \(^{86}\)Rb uptake in aortas from DOCA-salt rats, even though no difference in total cell sodium in either freshly excised aortas or aortas that were stored overnight at 4°C, was seen when compared with controls. Furthermore, the use of monensin to increase membrane permeability and elevate cell sodium in vascular smooth muscle from normotensive rats did not result in an increase in pump activity. Based on these results, they suggested that the increase in pump activity in vascular smooth muscle from DOCA-salt rats is not due to an increase in cell sodium. In addition, Overbeck and Grissett\(^5\) have shown that the amount of total cell sodium in freshly excised aorta from DOCA-salt rats is sufficient to maximally stimulate the Na\(^{+}\)-K\(^{+}\) pump and that maximal pumping in sodium-loaded arteries from normotensive controls remains lower than in hypertensive arteries. However, since membrane permeability to Rb may not accurately reflect the permeability to sodium, we cannot state conclusively that membrane permeability to sodium is not altered in femoral arterial smooth muscle from DOCA-salt rats.

A second hypothesis for the increase in Na\(^{+}\)-K\(^{+}\) pump activity in vascular smooth muscle from DOCA-salt rats is that an increase in the number of pump sites occurs because of the involvement of endogenous substances that induce the formation of new pump molecules. These include mineralocorticoids (DOCA).\(^7\)\(^16\) or a circulating digitalislike inhibitor.\(^7\) Although purely speculative, it has been suggested that DOCA may have a direct effect on the vascular smooth muscle by inducing the de novo synthesis of new pump molecules.\(^16\) The circulating inhibitor, on the other hand, has been proposed to increase the number of pump sites through a compensatory mechanism in which the vascular smooth muscle attempts to maintain normal cell sodium concentration. In the in vitro situation of our studies, the inhibitor could be washed from the tissue and these extra pump sites would be exposed. This could explain the greater ouabain-sensitive \(^{86}\)Rb uptake and potassium relaxation in the face of, theoretically, no increase in membrane permeability to sodium, based on the observation of no increase in ouabain-insensitive \(^{86}\)Rb uptake in the present study.

Discrepancies in the literature over whether the activity of the Na\(^{+}\)-K\(^{+}\) pump is increased or decreased in vascular smooth muscle from DOCA-salt hypertensive rats have centered around the proposed circulating digitalislike factor. Whereas some investigators have shown a decrease in pump activity,\(^3\)\(^4\) others have observed an increase.\(^3\)\(^5\) These differences have been attributed to differences in experimental design. However, more recent data suggest that the activity of the vascular Na\(^{+}\)-K\(^{+}\) pump actually changes with the duration of the hypertension.\(^8\) A decrease in pump activity was observed in the first week of treatment and was followed by an increase up to control levels in the second week. Measurements at 2 and 4 weeks of treatment showed significant increases in pump activity. At approximately 7 weeks of treatment pump activity was significantly decreased such that it was similar to that observed at 1 week of treatment. These changes were not dependent on the level of blood pressure. In the present study Na\(^{+}\)-K\(^{+}\) pump measurements were made on rats treated for 4 weeks. The observations of increased pump activity in our study coincide with the results of the above-mentioned study.\(^8\)

A role may exist for a digitalislike factor in these phasic changes in vascular Na\(^{+}\)-K\(^{+}\) pump activity, as it has been demonstrated that elevated levels of a digitalislike substance occur in the plasma of DOCA-salt rats after 5 days of treatment (C.H. Metzler, J.F. Hennessey, V.M. Buckalew, unpublished observation, 1982). The level of this substance decreased to control levels after 11 days. These data correlate well with the altered pump activity observed by Songu-Mize et al.\(^8\)

Alterations in vascular smooth muscle calcium metabolism have been implicated in the pathogenesis of DOCA-salt hypertension. One of these mechanisms involves the membrane-stabilizing effect of calcium. A decrease in the ability of calcium to control ion permeability has been shown to occur in vascular smooth muscle from DOCA-salt rats.\(^8\) In addition, calcium-induced relaxation (stabilization) of already contracted vascular smooth muscle has been shown to be reduced in DOCA-salt hypertension.\(^9\)\(^11\)

The mechanism by which calcium causes relaxation of vascular smooth muscle appears to involve the Na\(^{+}\)-K\(^{+}\) pump.\(^12\) In the present study we wanted to substantiate that calcium-induced relaxation is mediated by the Na\(^{+}\)-K\(^{+}\) pump and to determine if changes in the activity of the Na\(^{+}\)-K\(^{+}\) pump, as previously determined by potassium relaxation and \(^{86}\)Rb uptake in vascular smooth muscle from DOCA-salt hypertensive rats, coincided with changes in calcium relaxation.

Calcium-induced relaxation of femoral arterial smooth muscle from both control and DOCA-salt rats was blocked by ouabain and was directly dependent on the extracellular potassium concentration, suggesting that this response is indeed mediated by the Na\(^{+}\)-K\(^{+}\) pump. Interestingly, the response to calcium was greater in vascular smooth muscle from DOCA-salt rats at all levels of extracellular potassium, corroborating the potassium relaxation and \(^{86}\)Rb experiments.
Two other important observations from the calcium relaxation experiments coincide with the potassium relaxation experiments. First, the concentration of potassium required for calcium to produce an equivalent response in the two groups was approximately twice as much for controls as for DOCA-salt rats; that is, the response in 3.0 mM potassium for DOCA-salt rats was similar to that in 5.9 mM potassium for controls (see Figure 5). Similarly, approximately twice as much KCl was required for vascular smooth muscle from controls to achieve a given level of relaxation when compared with vascular smooth muscle from DOCA-salt rats in the potassium relaxation experiments (see Figure 2). Second, calcium relaxation was no greater at 10 mM potassium than at 5.9 mM potassium in controls. However, this response was greater in vascular smooth muscle from DOCA-salt rats. These observations suggest that maximal pump stimulation occurs at or around physiological levels of potassium in control vascular smooth muscle but there exists a greater number or activity, or both, of Na⁺-K⁺ pumps in vascular smooth muscle from DOCA-salt rats that can be activated over a much larger range of extracellular potassium.

The discrepancies in our findings, with regard to calcium relaxation, and those of others are difficult to identify. Differences in the age, sex, and strain of animal used are possible explanations for these divergent observations. The duration of the DOCA-salt treatment may also be an important factor, and the differences appear to depend on this possibility. As discussed earlier, the activity of the Na⁺-K⁺ pump plays a role in the stabilizing action of calcium. Therefore, it may be quite possible that the differences in calcium relaxation seen in this study compared with those in previous studies are the result of the duration of DOCA-salt treatment. Moreland et al. using NE as the contractile agent (as in the present study), showed a decrease in calcium-induced relaxation after 8 weeks of DOCA-salt treatment. This observation coincides with the decrease in pump activity observed after 7 to 8 weeks, as demonstrated by Songu-Mize et al. Differences in the experimental protocol may also be likely reasons for the differences observed. Holloway and Bohr and Hansen and Bohr observed a decrease in calcium relaxation after 4 weeks of DOCA-salt treatment (same time period as in the present study). However, two differences in the experimental design between their studies and the present study may contribute to the differences in observations. First, KCl was used as a contractile agent in their studies to assess calcium stabilization. Preliminary experiments (E. E. Solits and D. F. Bohr, unpublished observations, 1986) showed that the relaxation induced by calcium was significantly greater in tissues contracted with NE than in tissues contracted with KCl. This seemingly small difference in experimental protocol may be an important factor in this response, as several parameters such as calcium flux and membrane potential are likely to be affected differently by the contractile agents used.

A second difference in experimental protocol is the initial concentration of calcium in which the tissues were equilibrated prior to the calcium relaxation responses. The tissues were equilibrated in 1.6 mM calcium in the studies by Bohr and colleagues, whereas 0.25 mM calcium was used in the present study. It is possible the low level of calcium used during the equilibration period had an effect on the membrane of the vascular smooth muscle from the DOCA-salt rat such that the subsequent response to high levels of calcium was increased and not decreased. This does not appear to be the case, as preliminary studies (E. E. Solits and D. F. Bohr, unpublished observations, 1986) have shown no difference in the response whether 1.6 or 0.25 mM calcium was used during the equilibration period.

The apparent increase in membrane stability, as defined by the increase in calcium-induced relaxation in femoral smooth muscle from DOCA-salt rats, correlates well with the observations that calcium relaxation is dependent on the Na⁺-K⁺ pump and that vascular Na⁺-K⁺ pump activity is increased in DOCA-salt hypertensive rats after 4 weeks of treatment. Recent studies on the phasic changes in vascular Na⁺-K⁺ pump activity and the duration of DOCA-salt treatment support our observations as well as those of others. However, these coincidental findings are not conclusive, and further studies on the time course of the changes in Na⁺-K⁺ pump activity and calcium stabilization are required. The present study provides support for an important interaction of the Na⁺-K⁺ pump and the membrane actions of calcium in the alterations that occur in the vascular smooth muscle of DOCA-salt hypertensive rats. It may well be that the alterations in Na⁺-K⁺ pump activity and the stabilizing actions of calcium observed in the present study are compensatory in nature at this stage of the hypertensive process. A decrease in Na⁺-K⁺ pump activity and, therefore, a decrease in calcium membrane stabilization in the early stages of DOCA-salt treatment may be important factors in altered vascular responsiveness and development of hypertension in this model.

In summary, the present study has provided further evidence that the activity of the Na⁺-K⁺ pump in vascular smooth muscle of DOCA-salt hypertensive rats (treated for 4 weeks) is increased. Furthermore, we have shown that calcium-induced relaxation is mediated by the Na⁺-K⁺ pump and that, in opposition to previous reports, this response is increased, not decreased, in vascular smooth muscle from DOCA-salt rats and coincides well with the alteration in Na⁺-K⁺ pump activity as measured by potassium relaxation and ⁸⁶Rb uptake.

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