Vascular Changes in DOCA Hypertension
Influence of a Low Protein Diet

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SUMMARY The goal of this study was to characterize the influence of a low protein diet on vascular changes induced by deoxycorticosterone acetate (DOCA) hypertension. DOCA hypertensive and control normotensive rats were placed on a low protein (5%) diet for 4 weeks. This intervention blocked the further increase in systolic blood pressure of rats treated with DOCA; systolic blood pressures of control rats were not influenced by the low protein diet. The sensitivity of isolated mesenteric arteries to norepinephrine was increased in DOCA hypertensive rats compared to that in arteries from control rats; arterial strips from rats maintained on the low protein diet were less sensitive to the catecholamine than arteries from their respective control diet group. Vascular sensitivity to calcium was identical in both normotensive and DOCA hypertensive rats, and the low protein diet had no effect on this measure of calcium activation. Calcium-induced relaxation was depressed in arteries from DOCA hypertensive rats, suggesting a decreased stabilizing influence of the cation on the excitable membrane. Arteries from rats maintained on the low protein diet showed enhanced relaxation to calcium compared to those from their respective control diet group. Membrane stores of calcium available for activation by norepinephrine were increased in arteries from DOCA hypertensive rats; the low protein diet decreased the storage capacity of these membrane sites. The total protein content of the aorta was increased in DOCA hypertensive rats and depressed to control level in DOCA rats maintained on low protein diet. No change was observed in actomyosin content nor in the actin-to-myosin ratio during the DOCA hypertension or the addition of a low protein diet. Since one action of DOCA is to increase cellular protein synthesis, the attenuation of these vascular changes in DOCA rats maintained on a protein-deficient diet is probably due to a decrease in available substrate.


KEY WORDS • actomyosin • smooth muscle sensitivity • calcium stabilization • sodium flux

THE importance of the vascular system in the pathogenesis and maintenance of hypertension has been well documented.1–3 Both functional and structural alterations in the vasculature contribute to the development and maintenance of the increased arterial pressure. Since these vascular alterations occur in all forms of hypertension, regardless of the initiating factor, they may be considered to be the final common pathway in the pathogenesis of hypertension.4

The structural alterations are of two types. One type is a thickening of the vessel wall, due to hypertrophy of the smooth muscle layer, with encroachment on the lumen.5, 6 The second structural alteration is a decrease in number of the resistance vessels.7 The importance of this type of structural change is controversial.8

The functional changes are characterized by augmented responses to constrictor agents, a so-called increase in vascular reactivity. The increased reactivity has been demonstrated using many different agonists and many different techniques: isolated vascular strips or rings; perfused vessels or organs, and whole body pressor responses.9,10 The increase in reactivity is due to an increase in vascular smooth muscle sensitivity and not to an increase in force generating ability, since this parameter is often depressed.11 The increase in sensitivity occurs early following the intervention that initiates the hypertension and may actually be responsible for the increase in vascular resistance that causes hypertension.

The increase in vascular smooth muscle sensitivity reflects a change in cell membrane function. Increases in fluxes of Na⁺, K⁺, Ca²⁺, and Cl⁻ have been report-
ed, Jones and Hart have observed an increased rate of $^{42}$K efflux from vascular smooth muscle soon after mineralocorticoid treatment, and preceding the arterial pressure elevation. This observation is compatible with the possibility that the mineralocorticoid has induced the formation of proteins which are inserted into the cell membrane and serve as channels for passive electrolyte fluxes. In the current study, the vascular reactivity of rats with deoxycorticosterone acetate (DOCA) hypertension subjected to a low protein diet was examined. In addition, the protein content of the vessel wall from these same rats was characterized.

Methods

Animal Preparation

All studies were performed on adult, male Sprague-Dawley rats (250 g, n = 20). Half of the rats were uninephrectomized and received subcutaneous implantations of deoxycorticosterone acetate (DOCA, 200 mg/kg, Sigma Chemical Company, St. Louis, Missouri) impregnated in Silastic strips (Dow Corning Corporation, one part DOCA to two parts Silastic, w/w). These animals were given 1% NaCl and 0.2% KCl in their drinking water. Control rats did not undergo sham treatment and received normal tap water for drinking. Twenty-eight days after DOCA implant, the rats were divided into four groups (five rats to a group). Two of these groups (one control and one DOCA) were placed on a low protein diet (Purina test diet No. 5767, 5% protein, 0.29% sodium, 0.75% calcium, 0.05% magnesium, and 0.46% potassium), whereas the other two groups were maintained on standard rat chow (Purina diet No. 5012, 22.5% protein, 0.36% sodium, 1.01% calcium, 0.21% magnesium, and 0.46% potassium). The rats were maintained on these diets for 4 weeks. Body weights and systolic blood pressures (by the indirect tail cuff technique) were determined before and at weekly intervals following diet intervention.

Isolated Vascular Strip Preparation

Helically cut strips (0.8 x 7 mm) of mesenteric arteries (1.0 mm, O.D.) isolated from the four groups of rats were mounted in organ chambers and isometric contractions were recorded, as described previously.

Plasma Analysis and Heart Weight Determination

The abdominal aorta of each rat was cannulated, and 10 ml of blood was drawn into a syringe containing 100 units of sodium heparin. This sample was then centrifuged for 10 minutes at 2500 x g, and the plasma fraction was collected for electrolyte and protein concentration. Plasma electrolytes, Na+, and K+ were determined using an Instrumentation Laboratory Model 443 flame photometer. Plasma and tissue protein concentrations were determined by the method of Lowry et al. using bovine serum albumin as a standard. At the end of the experimental period, the hearts of all animals were excised, the atria and great vessels cut away, and ventricular weights determined. DOCA-treated rats had increased heart weight/body weight ratios (DOCA = 3.16 ± 0.11 g/kg; DOCA low protein = 3.21 ± 0.15 g/kg) compared to the normotensive rats (control = 2.56 ± 0.07 g/kg; control low protein = 2.54 ± 0.14 g/kg).

Gel Electrophoresis

Sections of the thoracic aorta from all rats were cut longitudinally and cleaned of fat and loose connective tissue. The aortic sections were cut to 10 mm lengths and the wet weight was determined. The cross-sectional area of the sections was calculated as wet weight/ (density x length) assuming a density of 1.05 g/cm³. The cleaned strips were homogenized for 2 minutes on ice with a solution composed of 1% SDS (sodium dodecyl sulfate), 10% glycerol, and 20 mM DTT (Dithiothreitol) final concentration. After homogenization, the solution was centrifuged at 2500 g for 10 minutes to pellet the insoluble material. A 0.3 ml aliquot of the supernatant was subjected to SDS-polyacrylamide electrophoresis as previously described.

DNA Content

DNA content was determined in the thoracic aorta from the same rats as those used for electrophoresis. DNA content was determined by the method of Cericott as modified by Hubbard et al. DNA (Sigma Chemical Company) was used as a standard, and the concentration in the stock solution was determined by measuring its optical density at 260 nm.

Statistics

The statistical significance was assessed by Student's t test; p < 0.05 was considered significant. Data are expressed as means ± SEM.

Results

General Characteristics

Body weights and systolic blood pressures during the 4-week dietary intervention are presented in figure 1. Before placing the rats on the test diet, the DOCA rats had systolic blood pressures that were significantly higher than those of the normotensive rats (fig. 1 left). During the 4-week period on the low protein diet, the DOCA-treated rats showed no further increase in systolic blood pressure, whereas the systolic pressures of DOCA rats maintained on the control diet increased to approximately 15% above the values obtained before dietary intervention. The systolic pressures of control rats did not change throughout the 4-week period. The body weights of DOCA-treated rats were significantly less than those of control rats before dietary intervention. All rats gained weight throughout the 4-week period of dietary intervention (fig. 1 right).

The results of the plasma protein concentration and plasma electrolyte determinations are shown in table 1. The low protein diet in normotensive rats had no effect on either plasma Na+, K+, or protein concentration. The administration of DOCA significantly decreased both plasma protein and K+, and increased plasma...
Na⁺. Feeding the low protein diet to the DOCA hypertensive rats further decreased K⁺ concentration. Plasma Na⁺ and protein values did not differ from those of the DOCA-treated rats on a normal diet.

**Vascular Responsiveness**

Cumulative addition of norepinephrine ($10^{-12}$ to $10^{-3}$ g/ml) to the muscle bath produced contractile responses in mesenteric artery strips from all rats (fig. 2). The maximal contractile responses to norepinephrine were: DOCA = 591 ± 67 mg; DOCA low protein = 542 ± 65 mg; control = 688 ± 46 mg; control low protein = 663 ± 30 mg. Arterial strips from DOCA rats were more sensitive to the catecholamine than were those from the control rats; rats maintained on a low protein diet were less sensitive to norepinephrine than their respective control group. The concentrations of norepinephrine necessary to produce a half-maximal response were: DOCA = $3.3 \times 10^{-10}$ g/ml; DOCA low protein = $16.1 \times 10^{-10}$ g/ml; control = $33.4 \times 10^{-10}$ g/ml; control low protein = $48.6 \times 10^{-10}$ g/ml.

To characterize the actions of calcium on vascular responsiveness, we used several different experimental

**Figure 1.** Systolic blood pressure and body weights. The systolic blood pressure (left panel) and body weight (right panel) of each rat was determined before (zero on the horizontal axis) and at weekly intervals after diet intervention. Values are expressed as percent change from measurements made before diet intervention (actual values given above each graph). Each point is the mean ± SEM for five rats.

**Figure 2.** Dose-response to norepinephrine. Helical strips of mesenteric arteries from DOCA, DOCA low protein, control, and control low protein rats were made to contract in response to the cumulative addition of norepinephrine to the muscle bath. Values are the means ± SEM for five rats in each group.
procedures (fig. 3): dose-response to calcium; contraction following calcium-free EGTA; calcium-induced relaxation; and membrane store of calcium.

In the first set of experiments (fig. 3 A, dose-response to calcium), the arterial strips were placed in calcium-free PSS containing 1.0 mM EGTA for 30 minutes. After 15 minutes into this interval, the strips were made to contract in response to $10^{-7}$ g/ml norepinephrine to deplete vascular stores of calcium. Following this 30-minute incubation, the strips were placed in calcium-free PSS without EGTA for 10 minutes. Norepinephrine ($10^{-7}$ g/ml) was then added to the muscle bath; none of the arterial strips responded to this second addition of the catecholamine. Cumulative addition of calcium chloride to the muscle bath produced responses in all strips. The concentration of calcium that elicited a half-maximal response was: DOCA = 0.37 mM; DOCA low protein = 0.30 mM; control = 0.28 mM; control low protein = 0.24 mM.

Vascular responsiveness following treatment with calcium-free EGTA solution was determined (fig. 3 B). Mesenteric arterial strips were contracted with $10^{-7}$ g/ml norepinephrine. After the response had reached a plateau, the norepinephrine was rinsed from the bath with a calcium-free PSS with 1.0 mM EGTA for 10 minutes. At the end of this EGTA treatment, the strips were placed in normal PSS containing either 1.0 or 2.0 mM calcium for 5 minutes. Arterial strips from

![Diagram of vascular responsiveness to calcium](http://hyper.ahajournals.org/)

**Figure 3.** Vascular responsiveness to calcium. A. Dose-response to calcium. Helical strips of mesenteric arteries were made to contract in response to the cumulative addition of calcium chloride in the presence of $10^{-7}$ g/ml norepinephrine following a 30-minute incubation in calcium-free PSS with 1 mM EGTA. B. Contraction following exposure to calcium-free PSS, 1.0 mM EGTA. C. Calcium-induced relaxation. D. Membrane store of calcium for norepinephrine response. Arterial strips from control and DOCA-treated rats were subjected to the procedure of calcium depletion and calcium-loading as described in figure 5. The concentration of calcium added back to the PSS during the loading procedure was varied from 0.05 to 2.0 mM. Values are the means ± SEM for five rats in each group.
DOCA hypertensive rats contracted slowly on exposure to calcium; those from the control rats did not (fig. 3 B). The magnitude of the contractile response to calcium was less in DOCA rats maintained on a low protein diet than it was in those maintained on the control diet.

The presence of an excess of calcium causes relaxation of contracted vascular smooth muscle through a membrane stabilizing influence. This membrane action of calcium was evident in experiments described above (dose response to calcium) when the concentration of added calcium reached 4.6 and 10.0 mM (fig. 3 C and fig. 4). Arterial strips from control rats relaxed to a greater degree than did those from DOCA rats at both concentrations of calcium; arterial strips from DOCA rats maintained on a low protein diet relaxed to a greater degree than DOCA rats maintained on the control diet.

Figure 5 illustrates the technique used to characterize calcium storage in vascular smooth muscle. In this experiment, mesenteric arterial strips from a control and from a DOCA hypertensive rat were mounted in the same muscle bath. Following a 10-minute period of calcium depletion they were exposed for 5 minutes to PSS containing 2.0 mM calcium. This solution was replaced with calcium-free PSS containing 1.0 mM EGTA, and 1 minute later the strips were tested for the amount of available calcium by stimulation with 10^{-7} g/ml norepinephrine. The response of the arterial strip from the DOCA hypertensive rat was greater than that from the normotensive control. Results of these studies are summarized in fig. 3 D for various loading concentrations of calcium in arterial strips from the different groups of rats. The magnitude of the response in this test of calcium loading was greatest in the DOCA rats maintained on the control diet and least in the arterial strips from control rats maintained on the low-protein diet.

Vascular Chemistry

Table 1 lists the influence of DOCA hypertension and a low protein diet on the cross-sectional area and the protein composition of the aorta. The cross-sectional area (table 1 B) was significantly increased during DOCA hypertension. This increase was significantly attenuated by a low protein diet. The total protein measurements showed approximately the same changes as cross-sectional area during DOCA hypertension and a low protein diet. The low protein diet in the normotensive rat significantly decreased total protein content. In the DOCA hypertensive rat, the low protein diet reduced the elevated protein content to control values. Although total protein content was altered by both DOCA hypertension and a low protein diet, actomyosin content was not.

Investigation of the influence of DOCA and a low protein diet on the contractile proteins showed that the ratio of actin to myosin was unchanged in any of the experimental groups (table 1C). This quantitation of
FIGURE 5. Calcium store for norepinephrine response (mesenteric artery). Tracings of vascular responsiveness illustrating the procedure used to characterize calcium in mesenteric arteries (see Methods for details). R = rinse.

Contrast proteins by gel electrophoresis requires linearity of Coomassie Blue staining over a range of protein concentrations, as well as a known concentration of electrophoresed protein. It has been demonstrated that both actin (A) and myosin (M) are stained on electrophoretic gels in a linear manner and purified myosin of a known concentration (Bio-Rad Laboratories) was used to quantitate the results. The final information listed in Table 1 C is DNA content and AM per cell. The values for AM per cell were calculated assuming smooth muscle to be the only cell in the tissue and a value of 6.02 μg DNA per mammalian cell. With these assumptions it was found that neither total DNA content nor actomyosin per cell changed significantly during DOCA hypertension and/or a low-protein diet.

<table>
<thead>
<tr>
<th>Table 1. Changes in DOCA Hypertension and the Influence of a Low Protein Diet</th>
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<tbody>
<tr>
<td>Determinations</td>
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<tr>
<td>A. Plasma concentrations:</td>
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<tr>
<td>Sodium (Na⁺) (mEq/liter, n = 4)</td>
</tr>
<tr>
<td>Potassium (K⁺) (mEq/liter, n = 4)</td>
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<tr>
<td>Protein (g %, n = 5)</td>
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<td>B. Cross-sectional area and protein:</td>
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<tr>
<td>Cross-sectional area (mm², n = 5)</td>
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<tr>
<td>Total protein (TP) (mg/g wet wt, n = 5)</td>
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<tr>
<td>Actomyosin (mg/g wet wt, n = 5)</td>
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<tr>
<td>C. Contractile proteins:</td>
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<tr>
<td>Actin/myosin (A/M) (mg/mg, n = 5)</td>
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<tr>
<td>AM/TP (mg/g, n = 5)</td>
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<tr>
<td>DNA (mg/g wet wt, n = 5)</td>
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<tr>
<td>AM/cell (pg, n = 5)</td>
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All values (except for AM/TP and AM/cell) are means ± SEM.

*Statistically significant difference, relative to controls, at p < 0.05. Values for AM/TP and AM/cell were calculated from values in tables B and C.
DOCA HYPERTENSION: EFFECT OF LOW PROTEIN DIET/Moreland et al.  III-105

Discussion

Numerous variables have been monitored to characterize the vascular changes that occur in mineralocorticoid hypertension. Further insight into the action of mineralocorticoids was obtained by determining the effects of a protein-deficient diet on these variables. This intervention has special relevance in this study because the primary cellular effect of mineralocorticoids is the stimulation of the synthesis of proteins involved in transmembrane electrolyte transport.22 We questioned whether this induction of transport proteins could be diminished by a protein-deficient diet and, if so, whether the vascular changes that occur in mineralocorticoid hypertension would be attenuated.

Aldosterone alters transmembrane sodium flux in kidney, intestine, salivary glands, sweat glands, toad bladder, and other tissues.22 In classical toad bladder studies, Sharp and Leaf22 demonstrated that the increase in sodium transport produced by aldosterone depended on its induction of transport protein. If the toad bladder had been pretreated with puromycin, which interfered with protein synthesis, aldosterone was without effect on sodium transport. Current evidence indicates that the transport protein synthesized in response to aldosterone creates a channel through which sodium and other cations move passively down their electrochemical gradients.24 Jones and coworkers,13 using radioisotope studies, observed that shortly after the administration of DOCA to rats, ion fluxes through the cell membrane of vascular smooth muscle were doubled. Friedman and Friedman,25 using ion sensitive electrodes, observed that DOCA produced similar increases in sodium, potassium, and lithium fluxes through the vascular smooth muscle cell membrane.

In the current study we have observed that DOCA treatment causes an increase in vascular smooth muscle sensitivity to stimulation with norepinephrine. We have recently reviewed evidence dealing with the structural and functional vascular changes in hypertension.3 The variables characterized in the current study are summarized in Table 2. The increase in sensitivity in DOCA hypertension has been shown previously to occur early,11 paralleling and possibly caused by the increase in membrane permeability.13 We observed that the protein-deficient diet significantly reduced the increase in sensitivity produced by DOCA administration. This observation suggests that a protein-deficient diet limits the ability of the mineralocorticoid to induce the synthesis of transport protein. In the DOCA-treated rat a low-protein diet prevented a further increase in arterial pressure. This observation is compatible with the possibility that with this intervention the mineralocorticoid was unable to produce its usual increase in transport proteins, and hence its increase in vascular smooth muscle sensitivity and the resultant increase in vascular resistance.

A difference was also observed between control and hypertensive rats with respect to membrane permeability of the calcium ion. When the membrane of these smooth muscle preparations had been sensitized by incubation in a calcium-free solution, those from the hypertensive rats leaked calcium upon subsequent exposure to this cation, resulting in a contractile response (fig. 3 B). Those from the control animals did not. Mesenteric arteries from DOCA-treated rats maintained on a low-protein diet contracted less than those from the DOCA rats maintained on the control diet, suggesting that the dietary intervention decreased this membrane abnormality. This abnormality may also reflect the presence of excess transport protein. It is unlikely that this membrane defect contributed to the differences in catecholamine sensitivity since it occurred in the absence of receptor activation. However, it may contributed to some aspect of vascular function in the hypertensive animal which resulted in a greater increase in vascular resistance than in the normal animal.

Another possible explanation for the differences in catecholamine sensitivity between the various groups of rats may be associated with the membrane stabilizing action of calcium (fig. 3 C). Presumably, calcium ions can bind to extracellular sites on the cell membrane causing changes in monovalent ion permeability which result in a depressant action on membrane excitability.3 In this study, the membrane-stabilizing influence of calcium was decreased in arteries from hypertensive rats as indicated by the smaller relaxation response when high concentrations of the cation were added to the muscle bath. Interestingly, mesenteric arteries from rats fed a low-protein diet showed in-
creased relaxation in response to calcium compared to those from their respective control diet group. The membrane stabilization appeared to be overcome by an increase in calcium leak in the vascular smooth muscle from the hypertensive rat.

It is clear that the increase in sensitivity to norepinephrine was not due to changes in receptor-activated channels for calcium (fig. 3 A). Dose-response curves to calcium in the presence of norepinephrine were shifted to the right in arterial strips from hypertensive rats compared to those obtained from arterial strips from normotensive rats, indicating decreased sensitivity to the cation in hypertensive blood vessels. Arteries from rats maintained on a low protein diet were more sensitive to calcium than from those rats maintained on the control diet.

A possible explanation for the differences in catecholamine sensitivity is that vascular smooth muscle from hypertensive animals stored calcium more readily in membrane pools that were available for activation by norepinephrine (fig. 3 D). Dietary intervention with low-protein decreased the storage capability of these membrane sites.

The influence of DOCA hypertension alone and in conjunction with a low protein diet on the structural and biochemical properties of the aorta can be discussed in terms of effects on cross-sectional area, total protein, and actomyosin. The increase in vessel wall thickness in a hypertensive state is a well-accepted phenomenon. Arteries from normotensive rats, indicating decreased sensitivity to the cation in hypertensive blood vessels. Arteries from rats maintained on a low protein diet were more sensitive to calcium than from those rats maintained on the control diet.

The changes in total protein content of the aorta seen in this study were similar in direction to the changes in its cross-sectional area. Since the major protein components of the aorta are collagen, elastin, mucopolysaccharides, and the contractile proteins, and since the actomyosin did not change, it may be assumed that the observed differences were in the noncontractile proteins. The failure of a hypertensive state to alter actomyosin did not change, it may be assumed that the expected increase in wall thickness and in addition demonstrated that this hypertrophy was attenuated by a decrease in available protein. Alternatively, the attenuation of vascular hypertrophy is related to the lower blood pressures in this group of animals.

The changes in total protein content of the aorta seen in this study were similar in direction to the changes in its cross-sectional area. Since the major protein components of the aorta are collagen, elastin, mucopolysaccharides, and the contractile proteins, and since the actomyosin did not change, it may be assumed that the observed differences were in the noncontractile proteins. The failure of a hypertensive state to alter actomyosin content has been previously demonstrated in the spontaneously hypertensive rat as it was seen in this study. However, in the spontaneously hypertensive rat, total protein content did not increase as was noted in this study. This difference may be due to the protein anabolic effect of DOCA. This interpretation is supported by our observation that there was a negligible increase in DOCA hypertensive rats fed a low protein diet.

The fact that DNA content per gram wet weight did not change with the interventions, but the total tissue wet weight increased with DOCA hypertension (shown by increased cross-sectional area in aortic strips of equal length), suggests a hyperplasia of the smooth muscle cells. However, if there is an increase in DNA per cell in vascular smooth muscle of DOCA hypertensive rats, as has been reported for spontaneously hypertensive rats, there may not be an increased number of cells. On the other hand, along with DOCA hypertension there was seen an increase in total protein per gram wet weight. This would suggest hypertrophy of the cells within this tissue. Whether hyperplasia and/or hypertrophy occurred cannot be differentiated in the present study. Another possibility is that there was no change in cell size or number but merely an increase in the connective tissue component of the aorta.

Studies measuring vascular reactivity and maximal force development in vessels from hypertensive animals have shown a decreased force generating ability. It has been suggested that this decrease in force development may be due to alterations in either actomyosin content or actin to myosin ratio. This is not supported in this report as neither parameter was altered. It is possible that increase in bulk and noncontractile tissue in the vessel wall may place constraints on its force generating ability.

This study has demonstrated that vascular changes induced by mineralocorticoid excess are attenuated by a protein-deficient diet. It is probable that this attenuation is the result of a decrease in membrane transport protein. Alternatively, the protein-deficient diet decreased membrane protein receptors, which initiate the excitatory process in the vascular smooth muscle cell.

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Vascular changes in DOCA hypertension. Influence of a low protein diet.
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