Polyamines, Vascular Smooth Muscle, and Deoxycorticosterone Acetate–Salt Hypertension

Edward E. Soltis, Pamela S. Newman, and Jack W. Olson

This study was performed to determine if an alteration in vascular polyamine contents is associated with the development of deoxycorticosterone acetate–salt hypertension. The effects of chronic administration of α-difluoromethylornithine, a specific irreversible inhibitor of ornithine decarboxylase and thus polyamine biosynthesis, on vascular polyamine contents, structure, and function as well as the development of hypertension was studied. Control and deoxycorticosterone acetate–salt rats received either tap water or a drinking solution containing α-difluoromethylornithine for 6 weeks, during which period systolic blood pressures were recorded. Vascular reactivity studies were performed on rings of aorta and tail artery. Medial thickness, vessel weight, and vascular polyamine contents were also assessed in these arteries. α-Difluoromethylornithine treatment had no significant effect on either systolic blood pressure or vascular structure, function, and polyamine contents of control animals. The elevation in blood pressure and the increase in medial thickness, ring weight, and vascular polyamine contents as well as altered vascular reactivity observed in deoxycorticosterone acetate–salt rats was significantly attenuated by α-difluoromethylornithine treatment. These results are the first to demonstrate that vascular polyamine contents are elevated in the deoxycorticosterone acetate–salt rat and that chronic α-difluoromethylornithine treatment prevents the rise in vascular polyamines as well as the elevation in blood pressure and attendant changes in the vasculature. Thus, the increase in vascular polyamines may comprise a critical link between the initiating stimuli and the alterations in vascular structure and function implicated in the pathogenesis of deoxycorticosterone acetate–salt hypertension. (Hypertension 1991;18:85–92)

Polyamines are found in all mammalian cells.1–3 A considerable body of evidence supports the view that these organic cations are essential for a number of cellular activities including growth, differentiation, and stimulus–response coupling involving transmembrane calcium fluxes.2–5 Ornithine decarboxylase (ODC), the initial and generally rate limiting enzyme involved in the biosynthesis of polyamines,1–3 is regulated by a variety of stimuli including neural and hormonal inputs.6,7 Recent studies have suggested an important role for ODC or polyamines, or both, in various pathological conditions such as pulmonary and systemic hypertension.8–15

Functional (hyperresponsiveness) as well as structural (hypertrophy or hyperplasia) changes of the vasculature have been implicated as primary pathogenic factors in deoxycorticosterone acetate–salt (DOCA-salt) hypertension.16–20 A number of mechanisms, including defective regulation of transmembrane calcium movements,16,17 have been proposed to explain the alterations in vascular responsiveness in this model. The vascular structural changes are thought to be due, at least in part, to the trophic effects of enhanced sympathetic nervous system innervation of the smooth muscle.21 These trophic effects may be mediated by norepinephrine's (NE) ability to increase ODC activity.14 Interestingly, an increase in ODC activity has been observed in vascular smooth muscle of the DOCA-salt rat15 as well as in rats made hypertensive with a chronic infusion of NE.22 It is possible that the increase in ODC activity and the subsequent elevation in polyamines results in vascular smooth muscle hypertrophy or hyperplasia as well as an increase in vascular reactivity in DOCA-salt hypertension. Preventing the increase in vascular ODC activity may attenuate the rise in blood pressure by preventing the increase in vascular poly-
amine contents and thus, changes in the vasculature that contribute to an increase in peripheral vascular resistance. The present study was performed to determine if alterations in vascular polyamine content are associated with the development of DOCA-salt hypertension and the attendant changes in vascular structure and function. The effects of chronic administration of \textit{a}-difluoromethylornithine (DFMO), a highly specific enzyme-activated irreversible inhibitor of ODC\textsuperscript{1–3} on vascular polyamine content, structure, and function as well as the development of DOCA-salt hypertension was also studied.

**Methods**

Forty-two male Sprague-Dawley rats (8 weeks old, 243–301 g weight range) (Harlan Sprague Dawley, Indianapolis, Ind.) were housed in a temperature- and humidity-controlled room with a 12-hour light/ dark cycle from 6:00 AM to 6:00 PM. Standard rat chow diet (0.71% sodium chloride, Ralston Purina Company, Richmond, Ind.) and tap water were provided ad libitum before initiation of the study. Experimental protocols involving the animals were approved by the Institutional Animal Care and Use Committee at the University of Kentucky.

The 42 rats were divided into two equal groups designated control and DOCA-salt. Sham right uninephrectomy was performed on control animals under ether anesthesia. Rats from the control group received either tap water, a 1% DFMO solution, or a 2% DFMO solution for drinking and continued on the standard rat chow diet. Rats in the DOCA-salt group underwent right uninephrectomy, received a subcutaneous DOCA implant (100 mg/kg) and were fed a 4% sodium chloride rat chow diet (Ralston Purina). These animals received either tap water, a 0.5% DFMO solution, or a 1% DFMO solution for drinking. These concentrations of DFMO were chosen as a result of initial studies showing that DOCA-salt rats drank approximately twice as much of the solutions as control rats. Thus, as a result, both control and DOCA-salt animals in this study will have consumed similar amounts of DFMO on a per kilogram basis. A postoperative injection of penicillin was administered to all animals.

Systolic blood pressures (SBP) were determined before and once each week after surgery and initiation of the dietary regimens using a standard tail-cuff technique on ether-anesthetized animals. Measurements were recorded using a pneumatic pulse transducer (Narco Bio-Systems, Houston, Tex.) and Model 79D polygraph (Grass Instruments Co., Quincy, Mass.). Body weights were recorded at the time of SBP measurements. Food and water intakes were measured daily in each animal. Since DOCA-salt rats have a considerably higher fluid intake than control animals and since varying the concentration of DFMO in the drinking solution may affect the water intake, a paired-watering procedure was followed such that all DOCA-salt rats were offered the same amount of drinking solution to match the DOCA-salt group that drank the least amount the preceding day, and all control rats were offered the same amount of drinking solution to match the control group that drank the least amount the preceding day. A similar paired-feeding procedure was also followed since DOCA-salt rats in general tend to ingest slightly less of their diet.

After the 6-week treatment period, rats were anesthetized with an intraperitoneal injection of ketamine (120 mg/kg) and acepromazine (1.2 mg/kg) and were implanted with an abdominal aortic cannula via the left femoral artery. Direct mean arterial pressure (MAP) and heart rate (HR) (determined from the pulsatile pressure recordings) were measured the following day in conscious, unrestrained animals. The animals were then killed by an intraperfusion injection of 100 mg/kg ketamine and the heart was removed, cleaned, and weighed.

At the time of termination the entire thoracic aorta and a 40 mm segment of tail artery were removed from the animal and placed in a physiological salt solution (PSS). The vessels were cleaned of excess fat and connective tissue, and a ring was cut from each for vascular reactivity studies (aorta, 4 mm; tail artery, 3 mm). Another segment of each artery (2 mm in length) was placed in a buffered formalin solution for histological preparation and morphometric evaluation at a later date. The remaining segment from each artery was frozen on dry ice and stored at −70°C until the time of polyamine assay. For vascular reactivity studies, two stainless steel or tungsten wires of appropriate diameter to cause minimal damage to the endothelium, were threaded carefully through the lumen of each ring. One wire was attached to a Grass FT.03 force transducer (Grass Instruments) and the other to a fixed base. Tissues were suspended in a water-jacketed muscle bath containing oxygenated (95% O\textsubscript{2} and 5% CO\textsubscript{2}) PSS and maintained at 37°C. A passive force of 2 g was placed on the aorta and 1 g on the tail artery. Cumulative dose–response curves to NE, serotonin (5-HT), acetylcholine (ACH), and sodium nitroprusside (SNP) were generated in the aorta. Relaxation responses to ACH and SNP were obtained after contraction of the vessels with KCl. A cumulative dose–response curve to NE as well as a contractile response curve to a 30-minute exposure to potassium-free (K-free) PSS were obtained in the tail artery. Dose–response curves were generated in random fashion. After each experiment the vessels were blotted dry, and a wet weight was recorded to normalize responses to the weight of the tissue. Effective doses that resulted in 50% of the maximal response (ED\textsubscript{50}) were determined after logit transformation of normalized dose–response curves. Composition of the PSS was as follows (mM): NaCl 130, KCl 4.7, KH\textsubscript{2}PO\textsubscript{4} 1.18, MgSO\textsubscript{4}-7H\textsubscript{2}O 1.17, CaCl\textsubscript{2}·6H\textsubscript{2}O 1.6, NaHCO\textsubscript{3} 14.9, dextrose 5.5, CaNa\textsubscript{2}-EDTA 0.03.

Segments of aorta and tail artery to be used for morphometric evaluation were embedded in paraffin after dehydration of the vessel. Cross-sections 5 μm thick were then cut and placed on glass slides. The
sections were deparaffinized and stained with elastic trichrome. Medial thickness was determined using the Micro-plan II digitizing system (Laboratory Computer Systems, Inc., Cambridge, Mass.) with the aid of a Nikon Optiphot microscope (Fryer Company, Inc., Cincinnati, Ohio) and PC 2100 computer (PC Craft, Inc., Des Plaines, Ill.). Four random measurements were made on four to five sections of each vessel and averaged to obtain the final medial thickness for each artery.

Polyamine contents (putrescine, spermidine, and spermine) were determined as previously reported with slight modifications of the procedures originally described by Steffanelli and colleagues and Kabra et al. In brief, frozen aortic and tail arteries were homogenized in 200 μl of 0.2V HClO₄ with an Ultra-Turrax homogenizer (Tekmar Co., Cincinnati, Ohio) at full speed for three bursts of 20 seconds each, and then the homogenates were centrifuged at 40,000g for 20 minutes. The resulting supernatants were derivatized with dansyl chloride and were transferred to a Bond-Elut C₁₈ column (Analytichem International, Harbor City, Calif.), and after elution with 1,500 μl methanol, the dansylated polyamines were concentrated by evaporation under N₂ and then were dissolved in 200 μl methanol. Samples (25 μl) were then injected onto a 5 μm Beckman ODS Ultrasphere HPLC column (4.6 mm×25 cm) (Beckman Instruments Inc., Palo Alto, Calif.). The dansylated polyamines were separated by gradient elution at a flow rate of 1 ml/min using acetonitrile:water (5:3:2) as solvent A and acetonitrile:methanol (3:2) as solvent B. The gradient consisted of linear increases in solvent B from 10% to 95% within 29 minutes followed by a decrease from 95% to 10% in solvent B from 37–39 minutes and then a column reequilibration period of 7 minutes. Polyamines were quantitated using a Shimadzu model RF-535 fluorescence detector (Shimadzu Scientific Instruments Inc., Columbia, Md.) with wavelengths set at 340 nm and 515 nm for excitation and emission, respectively. The limit of detection of the polyamines was 1 pmol. Polyamine contents were normalized to the weight of the arterial segment.

Drugs were purchased from Sigma Chemical Co., St. Louis, Mo. DFMO was supplied by Merrell Dow Research Institute, Cincinnati, Ohio. Data were analyzed by analysis of variance. When significance was indicated (p<0.05), the Newman-Keuls test was used to determine differences between individual groups.

**Results**

Body weight and SBP (Figures 1A and 1B, respectively) as well as MAP, HR, and heart weight measurements (Table 1) in control animals were unaffected by DFMO treatment. No difference in food or drinking solution intake was observed in the three control groups: (average over 6 weeks) control-water group, 21.08±0.66 g, 47.88±2.38 ml; control-1% DFMO group, 21.81±0.46 g, 48.03±0.81 ml; control-2% DFMO group, 20.69±0.78 g, 46.64±1.02 ml. Calculating the approximate amount of DFMO the animals received on a daily basis over the 6-week period resulted in a dose of 1.47 g/kg/day for the control-1% DFMO rats and 2.87 g/kg/day for the control-2% DFMO rats.

DOCA-salt animals receiving tap water to drink were considered hypertensive (SBP 140 mm Hg or greater) by week 4 of the study and had a final SBP of 176±7 mm Hg (Figure 1B). Hypertension development was unaffected by the 0.5% DFMO treatment; however, administration of 1% DFMO significantly attenuated the rise in blood pressure in
DOCA-salt-treated animals. The direct MAP measurements support these observations (Table 1). Although body weight was slightly less in all DOCA-salt animals when compared with controls, no difference was observed between the three DOCA-salt–treated groups (Figure 1A). Food and drinking solution intake was similar among the DOCA-salt groups: (average over 6 weeks) DOCA-salt-water group, 17.55±0.62 g, 76.31±2.18 ml; DOCA-salt-0.5% DFMO group, 17.24±0.72 g, 74.45±2.11 ml; DOCA-salt-1% DFMO group, 17.83±0.63 g, 72.55±1.70 ml. On a daily basis the DOCA-salt-0.5% DFMO rats received approximately 1.26 g/kg/day DFMO and the DOCA-salt-1% DFMO rats received approximately 2.46 g/kg/day DFMO. HR in DOCA-salt rats was not affected by DFMO treatment (Table 1). Heart weight was significantly increased in DOCA-salt animals receiving tap water, and DFMO treatment significantly decreased this value toward control levels (Table 1).

Vascular reactivity dose–response curves to the various pharmacological agents in aorta and tail artery ring preparations are presented only for the control-0% DFMO, control-2% DFMO, DOCA-salt-0% DFMO, and DOCA-salt-1% DFMO groups since the 2% DFMO intake in controls matches closely with the 1% DFMO intake in DOCA-salt rats. In addition, only the 1% DFMO solution affected the hypertension development in the DOCA-salt animals. DFMO treatment had no effect on vascular responsiveness (contractile or relaxation) in control animals (Figures 2–4 and Tables 2–4). An increase in the sensitivity to 5-HT (aorta, Figure 2A) and NE (aorta, Figure 2B; tail artery, Figure 3B) was observed in vessels from the hypertensive DOCA-salt rats receiving tap water when compared with controls (see Table 2 also for ED50 values). DFMO treatment attenuated the enhanced responsiveness to these agents. Tail arteries from the hypertensive DOCA-salt rats exhibited a faster rate of contraction in response to K-free PSS (Figure 3A). The time (in minutes) to reach a half maximal contraction in response to the K-free solution was significantly less (p<0.05) in the DOCA-salt rats receiving tap water: DOCA-0% DFMO group, 9.6±0.2; DOCA-1% DFMO group, 12.2±0.4; control-0% DFMO group, 11.9±0.3; control-2% DFMO group, 12.8±0.5. No significant difference was observed in the maximal contractile response among the four groups to any of the agents (Table 3). A decrease in the relaxation response to SNP and ACH was seen in the hypertensive DOCA-salt rats (Figure 4 and Table 4).
As seen with the contractile responses, DFMO treatment shifted these relaxation responses back toward control values.

Table 5 lists the data for aortic and tail artery ring weights as well as medial thickness measurements. DFMO treatment had no effect on ring weight or medial thickness of either aorta or tail artery from control animals. A significant increase in ring weight and medial thickness was observed in aorta and tail artery from hypertensive DOCA-salt rats when compared with controls. Although DFMO treatment significantly attenuated the increase in ring weight and medial thickness, these values were still elevated in the DOCA-salt-1% DFMO rats when compared with controls.

A significant increase in the contents of putrescine and spermine, but not spermidine, was observed in aorta from hypertensive DOCA-salt rats when compared with controls (Table 6). Although putrescine was undetectable in tail arteries, spermidine and spermine were found to be elevated in tail arteries from DOCA-salt rats. DFMO treatment (1%) in DOCA rats returned the relaxation responses toward control values. n=7 for all groups.

Figure 3. Line graphs show time-dependent contractile responses, expressed as active tension (mg force/mg tissue weight; mg F/mg TW), to a potassium-free (K-free) physiological solution (panel A), and concentration–response curves to norepinephrine (NE), expressed as a percent of the maximal response to NE (panel B) of rings of tail artery from control rats (0% DFMO or 2% DFMO drinking solution) and DOCA rats (0% DFMO or 1% DFMO drinking solution). A significant increase in the contractile response of tail arteries from DOCA-0% DFMO rats to K-free was observed in the first 10–15 minutes of exposure ($p<0.05$). Tail arteries from DOCA-0% DFMO rats also exhibited an enhanced sensitivity to NE (ED$_{50}$ value, see Table 2). DFMO treatment (1%) in DOCA rats significantly attenuated these enhanced responses. n=7 for all groups.

Figure 4. Line graphs show concentration–response curves to sodium nitroprusside (SNP) (panel A) and acetylcholine (ACH) (panel B), expressed as a percent of the KCl-induced contraction, of rings of aorta from control rats (0% DFMO or 2% DFMO drinking solution) and DOCA rats (0% DFMO or 1% DFMO drinking solution). A significant decrease in the relaxation response (ED$_{50}$ value, see Table 4) to both SNP and ACH was seen in aortas from DOCA-0% DFMO rats. DFMO treatment (1%) in DOCA rats returned the relaxation responses toward control values. n=7 for all groups.
Contents were significantly elevated in this vessel from the hypertensive animals. DFMO treatment had no effect on polyamine contents in vascular tissues from controls; however, the rise in polyamines observed in DOCA-salt rats was either attenuated or prevented.

**Discussion**

Results from this study provide the first evidence that polyamines may be involved in the pathogenesis of DOCA-salt hypertension. The increase in blood pressure and alterations in vascular structure and function observed in this model were associated with elevated quantities of vascular polyamines. Furthermore, in addition to preventing the increase in vascular polyamines, DFMO treatment also attenuated the rise in blood pressure and the altered vascular structure and function in the DOCA-salt rat.

The inhibitory effect of DFMO on tissue ODC activity has been substantiated in vivo in the rat. It has been demonstrated previously that administration of DFMO in drinking water significantly attenuates the rise in lung content of polyamines observed in pulmonary hypertension. Actions of DFMO other than its direct effects on ODC have not been reported. Thus, blockade of the elevation in vascular polyamines observed in DOCA-salt rats by DFMO treatment appears to be due to the specific actions of this enzyme inhibitor. The concentrations of DFMO used in this study were based on the previous experiments performed by Olson et al. Variations in the concentration of DFMO administered to control and DOCA-salt rats were due to the differences in water intake between the two groups. The end result, however, was that rats from both groups received similar daily doses of DFMO. Importantly, a significant antihypertensive effect along with the reduction in vascular polyamine contents in response to DFMO treatment was observed in the DOCA-salt animal. Interestingly, it has been shown previously that vascular smooth muscle ODC activity is increased in the early stages of DOCA-salt hypertension. Thus, although it is possible that the elevation in vascular polyamines is only associated with the hypertension, it is believed that an important role may exist for the increase in vascular polyamines via alterations in ODC activity in the pathogenesis of this model of hypertension.

It is evident from the present study that a significant increase in arterial medial thickness occurred in the aorta and tail artery of DOCA-salt rats and that DFMO treatment attenuated this structural change. Several possible explanations exist for the reversal by DFMO of the medial thickening seen in DOCA-salt rats. First, an increase in sympathetic nervous system innervation or activity to the vasculature has been demonstrated in DOCA-salt hypertension. This increased innervation could have a trophic influence on the vasculature through the ability of NE to increase ODC activity and subsequent polyamine levels. A second possible explanation involves the increased expression of growth factors in vascular tissue such as the elevation of transforming growth factor-β observed in aortic smooth muscle from DOCA-salt animals. The hypertrophic effects of growth factors may be mediated through polyamines. Thyberg and Fredholm have demonstrated that the mitogenic response of arterial smooth muscle cells to platelet-derived growth factor is dependent on the induction of ODC and synthesis of putrescine and that DFMO blocks these effects. Although entirely speculative, a third possible explanation is that the mineralocorticoid or salt excess may also have direct

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**Table 2.** ED₅₀ Values for Contractile Responses to Serotonin and Norepinephrine in Aorta and Norepinephrine in Tail Artery From Control and Deoxycorticosterone Acetate–Salt Rats Receiving Varying Concentrations of α-Difluoromethylornithine in the Drinking Water

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>5-HT (M)</th>
<th>NE (M)</th>
<th>Tail artery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control-0% DFMO</td>
<td>1.6±0.2×10⁻⁶</td>
<td>8.2±1.2×10⁻⁸</td>
<td>8.5±0.9×10⁻⁸</td>
</tr>
<tr>
<td>Control-2% DFMO</td>
<td>2.0±0.4×10⁻⁶</td>
<td>9.1±1.5×10⁻⁸</td>
<td>10.1±1.7×10⁻⁸</td>
</tr>
<tr>
<td>DOCA-0% DFMO</td>
<td>9.1±2.2×10⁻⁷</td>
<td>3.0±0.7×10⁻⁸</td>
<td>3.7±0.8×10⁻⁸</td>
</tr>
<tr>
<td>DOCA-1% DFMO</td>
<td>1.7±0.3×10⁻⁶</td>
<td>6.3±1.3×10⁻⁸</td>
<td>8.4±1.5×10⁻⁸</td>
</tr>
</tbody>
</table>

n=7 for all groups. 5-HT, serotonin; NE, norepinephrine; DFMO, α-difluoromethylornithine; DOCA, deoxycorticosterone acetate-salt.

*Significantly different from all other groups (p<0.05).

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**Table 3.** Maximal Contractile Responses to Serotonin and Norepinephrine in Aorta and to Norepinephrine and a Potassium-Free Physiological Solution in Tail Artery From Control and Deoxycorticosterone Acetate–Salt Rats Receiving Varying Concentrations of α-Difluoromethylornithine in the Drinking Water

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>5-HT</th>
<th>NE</th>
<th>NE</th>
<th>K-free</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control-0% DFMO</td>
<td>240±25</td>
<td>339±42</td>
<td>1,936±171</td>
<td>1,710±188</td>
</tr>
<tr>
<td>Control-2% DFMO</td>
<td>271±21</td>
<td>298±27</td>
<td>2,036±109</td>
<td>1,720±160</td>
</tr>
<tr>
<td>DOCA-0% DFMO</td>
<td>287±40</td>
<td>305±25</td>
<td>2,133±31</td>
<td>2,047±145</td>
</tr>
<tr>
<td>DOCA-1% DFMO</td>
<td>233±32</td>
<td>277±40</td>
<td>2,108±111</td>
<td>1,988±170</td>
</tr>
</tbody>
</table>

Values are in milligrams of force per milligram of tissue weight. n=7 for all groups. 5-HT, serotonin; NE, norepinephrine; DFMO, α-difluoromethylornithine; DOCA, deoxycorticosterone acetate-salt.
TABLE 4. ED₅₀ Values for Relaxation Responses to Acetylcholine and Sodium Nitroprusside in Aorta From Control and Deoxycorticosterone Acetate–Salt Rats Receiving Varying Concentrations of α-Difluoromethylornithine in the Drinking Water

Treatment group | ACH (M) | SNP (M)
--- | --- | ---
Control-0% DFMO | 8.3±1.0×10⁻⁶ | 3.2±0.6×10⁻⁵
Control-2% DFMO | 9.2±1.3×10⁻⁶ | 2.7±0.5×10⁻⁵
DOCA-0% DFMO | 18.0±4.1×10⁻⁶ | 13.5±3.3×10⁻⁵
DOCA-1% DFMO | 10.3±1.9×10⁻⁵ | 5.1±2.2×10⁻⁵

n=7 for all groups. ACH, acetylcholine; SNP, sodium nitroprusside; DFMO, α-difluoromethylornithine; DOCA, deoxycorticosterone acetate–salt.

*Significantly different from all other groups (p<0.05).

Effects on ODC activity in the vascular smooth muscle cell to increase polyamine levels. Regardless of the initiating factor, the vasculature exhibits an increase in medial thickness that presumably results in an increase in peripheral vascular resistance and an elevation in blood pressure. Attenuating the elevation in vascular polyamines by DFMO may prevent any or all of the above possibilities from occurring and thus prevent the arterial medial thickening. A final consideration, however, must be given for the role of pressure overload in causing the medial thickening. The vascular structural alterations observed in hypertension have long been thought by some to be an adaptive response to the chronic elevation in blood pressure. In this particular case, the elevation in vascular polyamines may occur as a result of the increase in blood pressure and not be a primary causative factor. It is possible that DFMO may attenuate the development of hypertension by an alternate mechanism yet to be defined and, thus, the increase in vascular polyamines and altered vascular structure are prevented by the subsequent lack of a significant increase in blood pressure.

Alterations in vascular responsiveness in DOCA-salt hypertension may also be mediated, at least in part, by enhanced polyamine contents. As with the structural component, it is clear that the altered vascular reactivity observed in DOCA-salt rats in this study was associated with elevated quantities of vascular polyamines. DFMO treatment, which lowered polyamine contents to control values, prevented these changes. It has been demonstrated previously that DFMO treatment attenuates monocrotaline-induced pulmonary vascular hyperresponsiveness, reduces the positive inotropic effects of NE, ouabain, and calcium, and inhibits isoproterenol-induced calcium influx into rat ventricular myocytes. As mentioned above, vascular ODC activity is elevated in the early stages of DOCA-salt hypertension. Previous studies in the DOCA-salt model of hypertension have demonstrated alterations in vascular responsiveness before a significant elevation in blood pressure. Thus, polyamines may be involved in the altered vascular responsiveness implicated in the initiation of DOCA-salt hypertension. Based on previous studies, the primary mechanism by which polyamines may be involved in the enhanced vascular responsiveness is through altered transmembrane calcium fluxes. Indeed, defective regulation of transmembrane calcium movements has been suggested as a major contributor to the altered vascular responsiveness observed in DOCA-salt hypertensive rats.

In summary, hypertension and the attendant changes in vascular structure and function observed in DOCA-salt rats was associated with elevated...

TABLE 5. Ring Weights and Medial Thickness Measurements of Aorta and Tail Artery From Control and Deoxycorticosterone Acetate–Salt Rats Receiving Varying Concentrations of α-Difluoromethylornithine in the Drinking Water

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Aorta</th>
<th>Tail artery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RW (mg)</td>
<td>MT (μm)</td>
</tr>
<tr>
<td>Control-0% DFMO</td>
<td>3.6±0.2</td>
<td>72±4</td>
</tr>
<tr>
<td>Control-2% DFMO</td>
<td>3.6±0.2</td>
<td>77±2</td>
</tr>
<tr>
<td>DOCA-0% DFMO</td>
<td>5.2±0.4*</td>
<td>113±7*</td>
</tr>
<tr>
<td>DOCA-1% DFMO</td>
<td>4.1±0.2†</td>
<td>90±4†</td>
</tr>
</tbody>
</table>

n=7 for all groups. RW, ring weight; MT, medial thickness; DFMO, α-difluoromethylornithine; DOCA, deoxycorticosterone acetate–salt.

*Significantly different from all other groups (p<0.05).
†Significantly different from control groups (p<0.05).

TABLE 6. Aortic and Tail Artery Polyamine Contents From Control and Deoxycorticosterone Acetate–Salt Rats Receiving Varying Concentrations of α-Difluoromethylornithine in the Drinking Water

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Aorta</th>
<th>Tail artery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PUT</td>
<td>SPD</td>
</tr>
<tr>
<td>Control-0% DFMO</td>
<td>1.6±0.1</td>
<td>44±4</td>
</tr>
<tr>
<td>Control-2% DFMO</td>
<td>2.1±0.2</td>
<td>39±4</td>
</tr>
<tr>
<td>DOCA-0% DFMO</td>
<td>2.9±0.1*</td>
<td>37±3</td>
</tr>
<tr>
<td>DOCA-1% DFMO</td>
<td>1.7±0.1</td>
<td>26±2*</td>
</tr>
</tbody>
</table>

Values are in nanomoles per gram of tissue. n=7 for all groups. PUT, putrescine; SPD, spermidine; SPM, spermine; DFMO, α-difluoromethylornithine; DOCA, deoxycorticosterone acetate–salt.

*Significantly different from all other groups (p<0.05).
contents of vascular polyamines. Chronic administration of the specific ODC enzyme inhibitor DFMO resulted in a decrease in polyamine contents and an attenuation of the development of hypertension as well as the changes in vascular structure and function. Thus, the present study provides the first evidence for a significant role of polyamines in the pathogenesis of this model of hypertension. Although altered vascular structure and function is considered one of the primary pathogenic factors involved in DOCA-salt hypertension, the specific mechanisms through which vascular polyamines are altered or cause these changes remain to be determined.

Acknowledgments

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Key Words: vascular smooth muscle • deoxycorticosterone • polyamines • blood pressure • vascular reactivity
Polyamines, vascular smooth muscle, and deoxycorticosterone acetate-salt hypertension.
E E Soltis, P S Newman and J W Olson

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