Effect of Vasopressin Antagonism on Structure and Mechanics of Small Arteries and Vascular Expression of Endothelin-1 in Deoxycorticosterone Acetate–Salt Hypertensive Rats

Hope D. Intengan, Gang He, Ernesto L. Schiffrin

Abstract—The structural and mechanical properties of small arteries are altered in rat models of hypertension. The precise role of humoral factors in these changes has not been determined. In deoxycorticosterone acetate (DOCA)–salt hypertension, endothelin-1 (ET-1) peptide content and gene expression are enhanced in mesenteric resistance arteries. These vessels also present augmented vasoconstrictor responsiveness to vasopressin versus control uninephrectomized rats. To determine whether an interaction exists between vasopressin and ET-1 in the pathogenesis of small-artery structural alterations in DOCA-salt rats, we examined the effect of chronic V₁ vasopressin receptor antagonism (OPC-21268, 30 mg/kg BID) on the structure and mechanical properties of mesenteric resistance arteries using a pressure myograph and the effect on preproendothelin-1 (preproET-1) gene expression, determined by Northern blot analysis of preproET-1 mRNA. Tail-cuff systolic pressures were elevated in DOCA-salt (200±11 mm Hg) versus uninephrectomized rats (109±4 mm Hg) and decreased slightly but significantly by OPC-21268 to 187±7 mm Hg (P<0.01). Treatment with DOCA-salt increased vascular media-lumen ratios and media cross-sectional areas and reduced both stress and incremental elastic modulus for a given pressure. However, there was no change in distensibility or incremental elastic modulus versus media stress. OPC-21268 partially attenuated the vascular growth in DOCA-salt rats. PreproET-1 mRNA was increased 2-fold in mesenteric arteries of DOCA-salt rats versus uninephrectomized rats, an effect abrogated by OPC-21268. Thus, DOCA-salt hypertension is associated with altered morphology of the small-arterial wall, without altering stiffness of the arterial wall components. OPC-21268 regressed in part these changes, suggesting the involvement of vasopressin. The concomitant attenuation of enhanced ET-1 expression by OPC-21268 suggests that ET-1 may be involved in mediating in part the vascular effects of vasopressin in DOCA-salt hypertensive rats. (Hypertension. 1998;32:770-777.)

Key Words: resistance ■ mechanics ■ growth ■ remodeling ■ elastic modulus ■ endothelin ■ vasopressin

Hypertension is associated with altered structural and mechanical properties of small arteries. In deoxycorticosterone acetate (DOCA)–salt hypertension, the structural changes present in small resistance arteries have been extensively documented. Within 2 weeks of developing hypertension, these vessels exhibit a reduction in lumen and external diameters, consistent with remodeling, as well as increases in media width and cross-sectional area (CSA), consistent with growth.¹

Recent studies have suggested that distensibility may be increased in small arteries in hypertension, including small cerebral arterioles from stroke-prone spontaneously hypertensive rats³ and small mesenteric arteries from adult spontaneously hypertensive rats.⁴ In addition to morphological alterations, changes in wall mechanics (stiffness) of small arteries may also influence pressure-diameter relationships of blood vessels,³ thereby modulating peripheral resistance and blood pressure. Indeed, it has been suggested that arterial distensibility must be considered in studies of vascular remodeling.³⁵ Thus, in addition to studying small-artery morphology, we also determined the mechanical properties of resistance arteries from DOCA-salt rats, which to date remain unreported.

Humoral and/or functional abnormalities have been observed in DOCA-salt rats. For example, active pressure responses to vasopressin are enhanced in mesenteric resistance arteries from DOCA-salt rats versus those from uninephrectomized (U-Nx) rats, as detected on a wire myograph.¹ The production of the potent vasoconstrictor endothelin-1 (ET-1) is also reportedly elevated in DOCA-salt hypertension. Despite unchanged plasma levels of immunoreactive ET (ir-ET), ET-1 peptide levels were increased in endothelial...
cells of mesenteric arteries. Likewise, preproendothelin-1 (preproET-1) mRNA levels were elevated in vessels from DOCA-salt rats as detected by Northern blot analysis and in situ hybridization. Accordingly, pharmacological antagonism of the endothelin system blunted elevation of blood pressure in DOCA-salt rats by 20 mm Hg and resulted in attenuation of the vascular growth and remodeling previously described in mesenteric arteries from this model.

The present study was designed to first determine the interaction of vasopressin and ET-1 in the pathogenesis of the structural alterations of small mesenteric arteries from DOCA-salt rats compared with control U-Nx rats. Using the V1 vasopressin antagonist OPC-21268, we examined the role that vasopressin may be playing in DOCA-salt–related vascular abnormalities. To study the involvement of vasopressin on ET-1 overexpression, we measured the effects of OPC-21268 on vascular preproET-1 gene expression. Because changes detected in resistance arteries may be the result of alterations in the mechanical properties of the vessel wall, it has been proposed that these must be known to understand the type of remodeling that has occurred. There are no studies of the mechanics of small arteries in DOCA-salt rats, and therefore these were studied in detail.

Methods

Materials

OPC-21268 was kindly provided by Dr J.F. Liard (Otsuka America Pharmaceutical, Inc). OPC-21268 has been shown to be an orally effective, selective, competitive antagonist at the V1 vasopressin receptor with little effect at V2 vasopressin receptors. This has been substantiated by radioligand displacement studies in which OPC-21268 potently displaced a selective V1 ligand from liver and kidney membranes with an IC50 <50 nmol/L, whereas it displayed an IC50 >0.1 nmol/L at V2 vasopressin receptors.

Animal Experiments

The study protocol was approved by the Animal Care Committee of the Clinical Research Institute of Montreal and was conducted in accordance with the recommendations of the Canadian Council of Animal Care. Rats were housed under conditions of constant humidity (60%) and temperature (22°C) and subjected to 12-hour light/dark cycles.

DOCA-salt hypertension was induced in Sprague-Dawley rats by the method of Ormsbee and Ryan. Briefly, male Sprague-Dawley rats (Charles River, St Constant, Quebec, Canada) weighing 200 g were unilaterally nephrectomized under sodium pentobarbital anesthesia (40 mg/kg). Silicone rubber impregnated with DOCA (200 mg/kg) was given in the drinking water at night. The precise concentration was adjusted daily according to the volume of water (100 mL) that each individual rat drank the previous day. This dose and route of administration of OPC-21268 were shown to effectively block the pressor effects of intravenous vasopressin (20 nmol/kg) in anesthetized Sprague-Dawley rats after 7 days of treatment. In untreated Sprague-Dawley rats, vasopressin increased mean arterial pressure by 52 ± 10 mm Hg, whereas in OPC-21268–treated Sprague-Dawley rats, vasopressin only increased mean arterial pressure by 8 ± 5 mm Hg (n = 3; P < 0.05, Student’s t test for unpaired data).

Rats were studied 4 weeks after surgery. On the day of the experiment, rats were killed by decapitation. The complete mesenteric bed was removed and dissected free of fat. Small arteries were dissected as described below, and tissues were then frozen with dry ice and stored at −70°C until extraction of total RNA was performed.

Preparation of Small Arteries

Superior mesenteric arteries were taken from the part of the mesenteric vascular bed that feeds the jejunum 8 to 10 cm distal to the pylorus and placed in cold physiological salt solution (PSS) of the following composition (mmol/L): NaCl 120, NaHCO3 25, KCl 4.7, KH2PO4 1.2, MgSO4 1.2, CaCl2 2.5, EDTA 0.026, and glucose 5.5. A third-order branch of the mesenteric arterial tree (~2 mm in length) was carefully dissected 1 mm from the intestine and cleaned of all adherent connective tissue under a dissecting microscope. The arterial segments were mounted in a pressure myograph chamber as previously described and slipped onto 2 glass microcannulas. One cannula was adjustable, whereas the other was fixed. Both ends of the arterial segment were secured to the microcannulas with nylon ties. The axial length of the arterial segment was adjusted by carefully positioning the cannula until vascular walls were parallel without any stretch. Intraluminal pressure was set to 45 mm Hg with a servocontrolled pump. Vessels were then equilibrated for 1 hour with PSS that was bubbled with 95% air and 5% CO2 to give a pH of 7.4 to 7.45 and heated to 37°C.

Arterial segments were considered viable and used in the study if they constricted more than 50% of their resting lumen diameter in response to an extramural application of high-potassium (125 mmol/L KCl) PSS containing 10−5 mol/L norepinephrine and to similar application of 10−3 mol/L norepinephrine in PSS. Endothelial integrity was confirmed if precontracted segments dilated in response to acetylcholine (10−5 mol/L) in PSS.

Experimental Protocol for Small Arteries

After each activation, the arterial segments were perfused with PSS and allowed to regain their resting diameter. Mesenteric resistance arteries were then deactivated by extramural perfusion with Ca2+-free PSS containing 10 mmol/L EGTA for 30 minutes, after which the intraluminal pressure was raised to 140 mm Hg 3 times. This resulted in axial lengthening of the arterial segments. Thus, at an intraluminal pressure of 140 mm Hg, the cannula was adjusted until the artery was unbulcked. To obtain pressure–lumen diameter relationships, the servocontrolled pump was used to increase intraluminal pressure in 10-mm Hg steps (to an intraluminal pressure of 40 mm Hg) and then in 20-mm Hg steps (to an intraluminal pressure of 140 mm Hg). We used 3 mm Hg as the initial diameter because at pressures below this, the vessel invariably collapsed. In vessels in which dimensions could not be measured even at 3 mm Hg, lumen diameter was estimated by fitting the intraluminal pressure–lumen diameter data to a third-order polynomial equation. Using a microcomputer-based video imaging system, we measured media thickness and lumen diameter at 5 points along the vessel for each pressure point, the mean of which was used in further calculations. The precision of the system, as determined with a micrometer, was 0.7 μm.

Measurements

Media CSA was obtained by subtraction of the internal CSA from external CSA: CSA = π(Do−2−Dw)/4, where Dw was the external diameter and Dm was the internal (lumen) diameter of blood vessels.

Incremental distensibility was calculated from the change in lumen diameter for a given change in intraluminal pressure: Incremental Distensibility = [Δ(P) / Δ(D/D)] 100, where ΔD/D was the fractional change in lumen diameter for each change in intraluminal pressure (ΔP).
Circumferential strain was calculated as \( \varepsilon = (D - D_o)/D_o \), where \( D \) was the observed lumen diameter for a given intraluminal pressure and \( D_o \) was the original diameter measured at 3 mm Hg intraluminal pressure.

Circumferential stress was calculated as \( \sigma = (PD)/(2WT) \), where \( P \) was the intraluminal pressure and \( D \) and \( W \) were the lumen diameter and wall thickness, respectively. Pressure was converted from millimeters of mercury to dynes per square centimeter (1 mm Hg = 1.334 × 10^5 dyne/cm^2).

Elastic modulus describes the intrinsic elastic properties of the wall material, independent of vessel geometry. It was obtained by fitting the stress-strain data from each vessel to an exponential curve \( y = ae^{\beta x} \); \( \sigma = \sigma_r e^{\beta s} \), where \( \sigma_r \) is the stress at the original diameter and \( \beta \) is a constant related to the rate of increase of the stress-curve shape. The tangential elastic modulus (ET) was calculated at several values of stress from the derivative of the exponential curve: \( \text{ET} = d\sigma/dx = \beta \sigma e^{\beta x} \).

The remodeling index was defined as the percentage of the observed difference between the lumen diameter of hypertensive and normotensive vessels that can be accounted for by remodeling of the wall material, independent of vessel geometry. It was obtained by fitting the stress-strain data from each vessel to an exponential curve \( y = (D o - D n)/D n \): \( \varepsilon \sigma_r = (D o - D n)/D n \), where \( (D o) \) and \( (D n) \) were the lumen diameters of normotensive and hypertensive vessels, respectively, and \( (D_m) \) was the remodeling lumen diameter. \( (D_m) = (D o)^2 - (4 \cdot \text{CSA}_n/\pi)^{1/2} \), where \( (D_m) \) was the external diameter of hypertensive vessels and \( \text{CSA}_n \) was the cross-sectional area of normotensive vessels. The growth index was calculated as \( \text{Growth Index} = \text{CSA}_h/\text{CSA}_n \), where \( \text{CSA}_h \) and \( \text{CSA}_n \) were the cross-sectional areas of normotensive and hypertensive vessels, respectively.

### Northern Analysis of PreproET-1 mRNA

Total RNA was extracted from the frozen mesenteric vasculature using the method of Trizol (Life Technologies; Gibco-BRL). Total RNA samples (20 \( \mu \)g) were electrophoresed on a 1% agarose gel containing 1× MOPS running buffer (20 mmol/L MOPS, 5 mmol/L sodium acetate, 1 mmol/L EDTA) for 2.5 hours at 9 V/cm gel. Samples were transferred from the gel to a nylon membrane (Hybond-N; Amersham) by capillary action with 3 mol/L NaCl and 0.3 mol/L sodium citrate (20× SCC). Membranes were air dried and ultraviolet cross-linked by an autocross-linker (UV Stratmlinker 2400, Stratagen) and then prehybridized at 65°C for 4 hours in buffer containing 4× SET (120 mmol/L Tris, 8 mmol/L EDTA, and 0.6 mol/L NaCl), 0.1% pyrophosphate, 0.2% SDS, and 100 \( \mu \)g/mL heparin. Hybridization with \[^{32}P\]labeled probes was performed for 18 to 20 hours at 65°C in buffer containing 4× SET, 0.1% sodium phosphate, 0.2% SDS, 500 \( \mu \)g/mL heparin, and 10% dextran sulfate. Membranes were washed twice in 2× SET/0.1% SDS at 65°C for 5 minutes and once in 0.5× SET/0.2% SDS at 65°C for 15 minutes. Membranes were then exposed to a phosphor screen (Storage phosphor screen, Molecular Dynamics) for 2 days and developed by a PhosphorImager System (Molecular Dynamics).

A rat preproET-1 cDNA probe prepared from rat lung as previously described and cloned into a pGem-7zf+ (+) plasmid (Promega) was used. A rat GAPDH probe was used that was the 1.2-kb PstI-XbaI fragment of the rat GAPDH cDNA. Probes were labeled with \[^{32}P\]dCTP (Amersham) with the use of a Prime-a-Gene labeling system (Promega) and then purified by chromatography with the use of a Sephadex G-50 column (Pharmacia).

### Measurement of Plasma ir-ET and Plasma Renin Activity

Plasma ir-ET was measured by radioimmunoassay after plasma extraction by passage through c18 Sep-Pak cartridges, as described previously. Plasma renin activity was measured by radioimmunoassay of angiotensin I produced after a 2-hour incubation of plasma at 37°C and pH 6.5, as previously described.

### Data Analysis

Data are presented as mean±SE. Comparisons of stress at original pressure (3 mm Hg) and slope of the elastic modulus were performed with ANOVA (or Kruskal-Wallis test when SDs were significantly different) followed by a Tukey’s multiple comparison test. Relationships between intraluminal pressure and media-lumen ratio, media CSA, lumen diameter, external diameter, incremental distensibility, and incremental elastic modulus, as well as relationships between stress and strain, strain and incremental elastic modulus, and stress and incremental elastic modulus were compared with ANOVA for repeated measures. Interaction means were analyzed for “simple main effects” by a Tukey’s multiple comparison test. A value of \( P<0.05 \) was considered statistically significant.

### Results

#### Blood Pressure, Tibia Lengths, and Body and Heart Weights

After 4 weeks of treatment with DOCA-salt, systolic blood pressure was greater in DOCA-salt rats than in control U-Nx rats. OPC-21268 slightly but significantly decreased blood pressure.

### Table 1. Systolic Blood Pressure, Tibia Lengths, Body and Heart Weights, Plasma Renin Activity, and Plasma ir-ET in U-Nx, DOCA-Salt, and DOCA-Salt+OPC-21268 Rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>U-Nx</th>
<th>DOCA-Salt</th>
<th>DOCA-Salt+OPC-21268</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>7</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>109±4</td>
<td>200±11†</td>
<td>187±7§</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>345±6</td>
<td>274±8†</td>
<td>278±4†</td>
</tr>
<tr>
<td>Tibia length, mm</td>
<td>39.7±0.4</td>
<td>39.9±0.2</td>
<td>39.5±0.3</td>
</tr>
<tr>
<td>Wet heart weight, g</td>
<td>0.995±0.02</td>
<td>1.329±0.02†</td>
<td>1.094±0.03§</td>
</tr>
<tr>
<td>Wet heart weight/tibia length, g/mm</td>
<td>0.026±0.001</td>
<td>0.033±0.001†</td>
<td>0.028±0.001§</td>
</tr>
<tr>
<td>Wet heart weight/100 g body wt</td>
<td>0.289±0.009</td>
<td>0.490±0.020†</td>
<td>0.393±0.007§</td>
</tr>
<tr>
<td>Dry heart weight, g</td>
<td>0.240±0.007</td>
<td>0.290±0.006†</td>
<td>0.267±0.005*</td>
</tr>
<tr>
<td>Dry heart weight/tibia length, g/mm</td>
<td>0.006±0.0004</td>
<td>0.007±0.0001*</td>
<td>0.007±0.0001*</td>
</tr>
<tr>
<td>Dry heart weight/100 g body wt</td>
<td>0.070±0.002</td>
<td>0.107±0.004†</td>
<td>0.096±0.001‡</td>
</tr>
<tr>
<td>Plasma renin activity, ng angiotension I/mL h</td>
<td>1.61±0.19</td>
<td>0.20±0.06</td>
<td>Nondetectable</td>
</tr>
<tr>
<td>Plasma ir-ET, fmol/mL</td>
<td>4.43±0.32</td>
<td>4.45±0.64</td>
<td>3.74±0.40</td>
</tr>
</tbody>
</table>

\*\( P<0.05 \), †\( P<0.01 \) vs U-Nx

\‡\( P<0.05 \), §\( P<0.01 \) vs DOCA-salt.
pressure in DOCA-salt rats to 187±7 mm Hg (Table 1). The body weights of DOCA-salt rats and OPC-21268–treated DOCA-salt rats were similar, but both weighed less than U-Nx control rats (P<0.01). However, tibia lengths were similar, suggesting that growth was unaffected and that difference in body weight may be related to changes in body fluids. Wet and dry weights of the heart normalized for body weight were greater in DOCA-salt rats than in U-Nx rats. OPC-21268 produced modest decreases toward normal of both wet and dry heart weight per 100 g body weight (Table 1).

Plasma Renin Activity and Plasma ir-ET
As expected, plasma renin activity was significantly depressed in DOCA-salt rats, without normalization by OPC-21268. Plasma ir-ET was similar in U-Nx, DOCA-salt, and OPC-21268–treated DOCA-salt rats (Table 1).

Morphological Characteristics of Relaxed Resistance Arteries: Effects of OPC-21268
Figure 1 shows the profile of media-lumen ratio and media CSA–intraluminal pressure curves in relaxed mesenteric arteries from U-Nx, DOCA-salt, and OPC-21268–treated DOCA-salt rats (n ≥7). Error bars indicate SE. *P<0.05 vs U-Nx; †P<0.05 vs DOCA-salt.

Figure 2. Lumen diameter–intraluminal pressure and media external diameter–intraluminal pressure curves in relaxed mesenteric arteries from U-Nx, DOCA-salt, and OPC-21268–treated DOCA-salt rats (n ≥7). Error bars indicate SE.
TABLE 2. Morphological Characteristics of Relaxed Mesenteric Resistance Arteries From U-Nx, DOCA-Salt, and DOCA-Salt+OPC-21268 Rats at Intraluminal Pressure of 60 mm Hg

<table>
<thead>
<tr>
<th>Parameter</th>
<th>U-Nx</th>
<th>DOCA-Salt</th>
<th>DOCA-Salt+OPC-21268</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>7</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Lumen diameter, μm</td>
<td>230.6±6.7</td>
<td>217.9±12.5</td>
<td>240.0±11.6</td>
</tr>
<tr>
<td>Media thickness, μm</td>
<td>10.8±0.7</td>
<td>16.0±1.0</td>
<td>13.7±0.6*</td>
</tr>
<tr>
<td>Media-lumen ratio, %</td>
<td>4.7±0.3</td>
<td>7.4±0.4†</td>
<td>5.8±0.2‡</td>
</tr>
<tr>
<td>Media CSA, μm²</td>
<td>8230±701</td>
<td>11 879±1327*</td>
<td>10 996±875*</td>
</tr>
</tbody>
</table>

*P<0.05, †P<0.01 vs U-Nx.
‡P<0.01 vs DOCA-salt.

with OPC-21268 produced partial regression of media thickness and media-lumen ratio in DOCA-salt vessels toward normal. The remodeling and growth indices of small arteries in DOCA-salt hypertensive rats versus vessels from U-Nx rats were 21% and 44%, respectively. OPC-21268 resulted in reversal of these processes, in which the remodeling index equals −88% and the growth index equals −7%.

Vascular Mechanics

The morphometric data measured from increasing pressures demonstrated that the capacity for maximal passive dilatation was unchanged in vessels by chronic DOCA-salt treatment. Likewise, incremental distensibility was superimposable between arteries from U-Nx, DOCA-salt, and DOCA-salt+OPC-21268 rats (Figure 3).

At 3 mm Hg intraluminal pressure, media stress was significantly lower in relaxed arteries from DOCA-salt rats than in those from U-Nx rats (P<0.01), although this was normalized by OPC-21268 (P<0.01) (Table 3). Increasing intraluminal pressure increased media stress in arteries from U-Nx significantly more than in DOCA-salt rats (P<0.05). This difference was regressed toward normal by OPC-21268 (Figure 4). In contrast, the relationship between media stress and circumferential strain (that is, isometric stress) was unaltered in DOCA-salt hypertension.

![Image](http://hyper.ahajournals.org/)

**Figure 3.** Incremental distensibility—intraluminal pressure curves in relaxed mesenteric arteries from U-Nx, DOCA-salt, and OPC-21268–treated DOCA-salt rats (n>7). Error bars indicate SE.

![Image](http://hyper.ahajournals.org/)

**Table 3. Baseline Mechanical Characteristics of Relaxed Mesenteric Resistance Arteries From U-Nx, DOCA-Salt, and DOCA-Salt+OPC-21268 Rats**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>U-Nx</th>
<th>DOCA-Salt</th>
<th>DOCA-Salt+OPC-21268</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>7</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Lumen diameter at 3 mm Hg, μm</td>
<td>141.8±11.4</td>
<td>136.0±9.3</td>
<td>148.3±7.0</td>
</tr>
<tr>
<td>Stress at 3 mm Hg, 10⁶ dyne/cm²</td>
<td>0.02±0.002</td>
<td>0.01±0.001*</td>
<td>0.02±0.001†</td>
</tr>
<tr>
<td>Slope of elastic modulus vs stress</td>
<td>5.9±0.9</td>
<td>5.8±0.4</td>
<td>5.7±0.3</td>
</tr>
</tbody>
</table>

*P<0.01 vs U-Nx.
†P<0.01 vs DOCA-salt.

Incremental elastic modulus, when plotted versus strain or stress, was also unaltered in small arteries from DOCA-salt rats compared with those from U-Nx rats. However, when plotted versus intraluminal pressure (that is, isobarically), incremental elastic modulus was significantly lower in resistance arteries from DOCA-salt rats. OPC-21268 attenuated this decrease in isobaric elastic modulus observed in DOCA-salt rats (Figure 5).

Vascular ET-1 mRNA

Figure 6 shows a Northern blot analysis of RNA extracted from the mesenteric arterial bed of all groups of rats. The 2.3-kb band that corresponds to the preproET-1 mRNA showed an approximately 2-fold greater intensity in lanes from arteries of DOCA-salt compared with U-Nx. The abundance of preproET-1 mRNA in mesenteric arteries of DOCA-salt rats treated with OPC-21268 was similar to that of arteries from U-Nx rats.

Discussion

The present results demonstrate that the DOCA-salt model of mineralocorticoid hypertension is associated with vascular growth (increased media width, media-lumen ratio, and a growth index of 44%) in the absence of changes in vascular distensibility. Isobaric incremental elastic modulus is decreased in small mesenteric arteries from DOCA-salt hypertensive rats. These changes appear to entail a mechanism with a humoral component. Vasopressin may be involved, perhaps by stimulating expression of ET-1 in blood vessels of the DOCA-salt hypertensive rat. This is evidenced by the ability of the V₁ vasopressin receptor antagonist OPC-21268 to attenuate both changes in media-lumen ratio and the enhanced expression of preproET-1 mRNA. Interestingly, these actions of OPC-21268 were apparent even though its effect on blood pressure was modest.

The morphological changes in the structure of DOCA-salt small mesenteric arteries include an increase in media width and CSA, both indicators of vascular growth. To date, there have been no data regarding the mechanical properties of the vascular wall in these hypertensive rats, changes of which could be a possible explanation for the previously reported decreases in lumen diameter in this model.¹ In fact, the necessity of considering changes in distensibility in the definition of vascular remodeling has been argued.²,³ We
clearly demonstrate in the present study that despite thickening of the vessel wall, distensibility is unchanged in small mesenteric arteries from DOCA-salt hypertensive rats compared with control U-Nx rats. Thus, the changes found in vessels from DOCA-salt rats occurred with very little contribution of changes in distensibility. Indeed, the growth index was 44% and the remodeling index was 21%. Reverse, outward hypotrophic remodeling occurred in vessels from OPC-21268–treated DOCA-salt rats, in which the remodeling index was −88% and growth index was −7%.

The effect of V1 vasopressin receptor antagonist on small arterial structure suggests that in DOCA-salt hypertension, vasopressin may play a role in mediating these alterations. Indeed, abnormalities of the vasopressin system have been described previously in this model of mineralocorticoid hypertension. Plasma levels of vasopressin are elevated in DOCA-salt rats.20,21 Pressor responses to vasopressin are also enhanced in the mesenteric bed of DOCA-salt rats and may be due to differential regulation of receptors and postreceptor mechanisms and/or the altered vessel structure.1,20,22 Vaso-pressin V1 receptors were decreased in the mesenteric vasculature together with a rise in plasma vasopressin.20 This suggested the potentiation of postreceptor mechanisms and may be related to enhanced inositol triphosphate production, as found in spontaneously hypertensive rats.21 Postreceptor amplification of vasopressin responses could also be the consequence of altered prostanoid production.22

With respect to DOCA-salt–induced hypertension, vasopressin has been implicated in the blood pressure elevation, although whether exerting these actions predominantly through V1 vasopressin receptors or V2 vasopressin receptors is still unclear.24,25 In this study chronic blockade of V1 vasopressin receptors reduced systolic blood pressure slightly by ≈13 mm Hg, suggesting that V1 vasopressin receptors may play a role in a fraction of the blood pressure elevation in these rats. This modest reduction is consistent with the antihypertensive effects of endothelin receptor antagonism previously demonstrated in this model in the past, in the order of 14 to 20 mm Hg.9 Thus, the elevated blood pressure in DOCA-salt hypertension is in part mediated by ET-1, and, as would be expected, other mechanisms are clearly involved.

Vasopressin appears to modulate vascular structure by a mechanism independent of blood pressure elevation since OPC-21268 could attenuate these changes without dramatically reducing blood pressure. A possible explanation lies in our observation that OPC-21268 depressed the activated endothelin system in DOCA-salt hypertensive rats, as shown by the reduction in enhanced vascular expression of preproET-1 mRNA. This finding is consistent with the previous report that in Wistar rats, vasopressin markedly and dose-dependently induced the expression of preproET-1

![Figure 4. Media stress–intraluminal pressure and media stress–media strain curves in relaxed mesenteric arteries from U-Nx, DOCA-salt, and OPC-21268–treated DOCA-salt rats (n = 7). Error bars indicate SE. *P < 0.05 vs U-Nx.](image)

![Figure 5. Incremental elastic modulus–media strain, elastic modulus–intraluminal pressure, and elastic modulus–media stress curves in relaxed mesenteric arteries from U-Nx, DOCA-salt, and OPC-21268–treated DOCA-salt rats (n = 7). Error bars indicate SE. *P < 0.05 vs U-Nx.](image)
mRNA in the isolated intact mesenteric arterial bed and ET-1 peptide secretion into the perfusate. Vasopressin could also induce quiescent cultured aortic smooth muscle cells from spontaneously hypertensive rats to synthesize ET-1 mRNA, accompanied by release of ET-1 peptide into the medium.

However, in the mesenteric bed of DOCA-salt rats, increased ET-1 peptide levels were localized to the endothelial cells. Indeed, bovine carotid arterial endothelial cells can be stimulated by vasopressin to increase ET-1 gene expression. The influence of vasopressin on small arterial structure in DOCA-salt hypertension may consequently be in part indirect and involve activation of the endothelin system. A component of the changes in vascular media-lumen ratios in DOCA-salt hypertension does not correlate with the extent of blood pressure elevation in these rats. We had previously suggested that ET-1 could mediate, at least in part, this pressure-independent change in vessel structure. ET-1 gene expression and peptide levels are elevated in vessels from DOCA-salt hypertensive rats. Moreover, when these rats are treated with the endothelin receptor antagonist bosentan, regression of the vascular growth resulted so that the remaining hypertrophy then correlated with systolic pressure.

The link between vasopressin and ET-1 may be a further clue as to the mechanism involved. Here, the results indirectly support the suggestion that ET-1 has a direct action on vascular growth, since OPC-21268 exerted its effects on vascular structure and ET-1 expression in the absence of a major change in blood pressure. The putative direct effect of ET-1 on vascular biology is not surprising, since ET-1 has hypertrophic and mitogenic effects on vascular smooth muscle cells. The participation of ET-1 in mediating vascular growth in response to vasopressin in this model remains to be elucidated. In this regard, a seemingly discrepant observation may also help to illuminate the precise role of vasopressin in the vascular changes in DOCA-salt hypertensive rats. Despite complete abrogation of the overexpression of preproET-1 mRNA in the mesenteric vasculature, we found that OPC-21268 did not appear to regress the structural changes to the same extent as did endothelin receptor antagonism with bosentan. This disparity may be due to different kinetic characteristics of a direct endothelin receptor antagonist versus a vasopressin antagonist that indirectly inhibits vasopressin-stimulated endothelin production. This may explain why at the end of 4 weeks of treatment, vasopressin antagonism abrogated preproET-1 gene overexpression, whereas the effect on vascular growth is only starting to become evident. This would manifest as a lesser effect of OPC-21268 on endothelin-dependent vascular growth than that of bosentan, which blocks the effects of ET-1 directly. An alternate explanation may be that OPC-21268 only blocked the overexpression of the ET-1 gene without affecting basal levels. In DOCA-salt hypertensive rats, normal levels of vascular ET-1 in conjunction with elevated blood pressure could also have effects on the vasculature, independent of vasopressin.

A critical finding from these experiments was the lack of difference between relaxed resistance arteries from DOCA-salt hypertensive rats and from U-Nx rats when incremental elastic modulus was examined in relation to media stress. Elastic modulus in relation to wall stress, which is independent of vessel size or geometry, describes the stiffness of the blood vessel wall components. These include more distensible components such as elastin, smooth muscle cells, and endothelial cells and relatively stiffer and less distensible components such as collagen. The slopes of the lines describing tangential elastic modulus versus media stress were similar in the small arteries of the hypertensive and normotensive rats (5.9 ± 0.9 and 5.8 ± 0.4 for U-Nx and DOCA-salt, respectively) (Table 3). This indicates that the intrinsic stiffness of the vessel wall components of U-Nx rats is similar to that in DOCA-salt hypertension. Instead, since these vessels present a thicker media, this has the consequence that, in relation to intraluminal pressure, incremental elastic modulus was lower in relaxed vessels from DOCA-salt hypertensive rats. The ability of a vessel to buffer changes in pressure is dependent on both the geometry of the vessel wall (which affects wall stress) and the stiffness of its wall components. From a physiological viewpoint, these collective findings, that the arterial wall of resistance vessels from DOCA-salt hypertensive rats exhibits unchanged distensibility and lower isobaric elastic modulus and wall stress, indicate that in DOCA-salt hypertension the vessel wall has adapted structurally to preserve its blood pressure–buffering capacity. This potentially limits damage to the wall induced by the elevated intraluminal pressures to which these vessels are exposed. These modifications, while protecting the vessel wall from excessive increases in stress as a result of elevated blood pressure, likely have a net detrimental effect, however, wherein tissue perfusion may be compromised. As the thickened media encroaches into the
lumen, the reduction in luminal diameter compromises blood flow and tissue oxygen availability and nutrition. As with the vasculature, cardiac hypertrophy is also evident in DOCA-salt hypertensive rats. The ratios of both wet weight and dry weight of the heart to body weight were elevated in DOCA-salt hypertension. Both ratios regressed toward normal in OPC-21268–treated DOCA-salt rats (Table 1), despite minimal lowering of blood pressure. This suggests that vasopressin and perhaps ET-1 are involved in cardiac hypertrophy in this model. Previous studies have suggested, however, that ET-1 is not involved as a hypertrophic factor in the myocardium since bosentan did not produce regression of cardiac hypertrophy. Whether the role of vasopressin on cardiac hypertrophy is mediated directly or indirectly through endothelins will require further study. These studies provide pharmacological evidence that vasopressin plays a critical role in the vascular changes associated with this model of hypertension. This is the first report that suggests that V1 vasopressin receptor–mediated activation of the endothelin system occurs in DOCA-salt hypertension. The present study demonstrates that the structural remodeling and growth of the vasculature associated with DOCA-salt hypertension are characterized by lower isobaric elastic modulus and wall stress. These latter modifications may be an adaptive mechanism to protect the vessel wall in the face of remarkably elevated blood pressures characteristic of DOCA-salt hypertension. Importantly, distensibility and elastic modulus versus stress (that is, wall stiffness) were not altered in DOCA-salt rat vessels, despite dramatic vascular growth. The previous findings in DOCA-salt rats that endothelin plays a role in resistance artery remodeling, together with the present data showing that vasopressin also mediates endothelin overexpression and vascular remodeling without altering wall stiffness, collectively suggest that there is a link between vasopressin, endothelin, and small-artery remodeling and growth of the vasculature associated with DOCA-salt hypertension. The present study demonstrates that the structural remodeling of rat and human glyceraldehyde-3-phosphate-dehydrogenase gene expression in rat tissues. Nucleic Acids Res. 1984;12:6951–6960. Tso FY, Sun XH, K Bart SB, Reece KS, Wu R. Isolation and characterization of rat and human glyceraldehyde-3-phosphate-dehydrogenase cDNAs: genomic complexity and molecular evolution of the gene. Nucleic Acids Res. 1985;13:2485–2502.


Effect of Vasopressin Antagonism on Structure and Mechanics of Small Arteries and Vascular Expression of Endothelin-1 in Deoxycorticosterone Acetate–Salt Hypertensive Rats
Hope D. Intengan, Gang He and Ernesto L. Schiffrin

Hypertension. 1998;32:770-777
doi: 10.1161/01.HYP.32.4.770

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1998 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

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/32/4/770

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/