Increased sympathetic venoconstriction and reactivity to norepinephrine in mesenteric veins in anesthetized DOCA-salt hypertensive rats

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Xu H, Fink GD, Galligan JJ. Increased sympathetic venoconstriction and reactivity to norepinephrine in mesenteric veins in anesthetized DOCA-salt hypertensive rats. Am J Physiol Heart Circ Physiol 293: H160–H168, 2007. First published February 23, 2007; doi:10.1152/ajpheart.01414.2006.—Increased sympathetic nervous activity (SNA) elevates venomotor tone in deoxycorticosterone acetate (DOCA)-salt hypertension. We studied the mechanisms by which the SNA increases venomotor tone in DOCA-salt hypertension by making in situ intracellular recordings of venous smooth muscle cell (VSMC) membrane potential ($E_m$) and measurement of outside diameter (OD) in mesenteric veins (MV) and mesenteric arteries (MA) of anesthetized rats. We also studied norepinephrine (NE)- and endothelin-1 (ET-1)-induced increases in MA or MV perfusion pressure (PP) in vitro. $E_m$ in DOCA-salt MV was depolarized compared with sham MV. Prazosin hyperpolarized VSMC $E_m$ in DOCA-salt but not in sham MV. NE concentration-response curves (CRCs) for OD decreases in MV from DOCA-salt rats were left-shifted with an increased maximum response ($E_{max}$) compared with sham MV. NE CRCs for OD decreases in MA were right-shifted with reduced $E_{max}$ in DOCA-salt compared with sham rats. ET-1 CRCs were similar in DOCA-salt and sham MV but were right-shifted with reduced $E_{max}$ in DOCA-salt MA. NE CRCs for MAPP increases were left-shifted without a change in $E_{max}$ in DOCA-salt rats. NE did not change MVPP. MAPP and MVPP for ET-1 CRCs were similar in sham and DOCA-salt rats, but $E_{max}$ for MAPP was reduced in DOCA-salt rats. Hematoxylin staining revealed hypertrophy in DOCA-salt MA but not in MV. We conclude that there is increased reactivity to NE released from the sympathetic nervous system in DOCA-salt MV that causes VSMC depolarization and increased venomotor tone. In DOCA-salt rats, in vivo ET-1 reactivity is maintained in MA, but reduced in MA.

Vascular capacitance is the change in intravascular volume resulting from a change in intravascular pressure (8, 28, 32). Any change in contained volume and distending pressure of a segment of the vasculature could affect vascular capacitance. The venous system contains ~70% of the blood volume, most of it in small veins and venules. The majority of blood storage in mammals is in the veins of splanchnic organs. The splanchnic region is an important venous bed because it is richly innervated by sympathetic nerves; highly compliant; and contains ~25% of total blood volume (28, 32, 34). Increased venomotor tone and reduced compliance in peripheral veins shifts blood to the small arteries, increasing arterial blood pressure (BP). Hypertensive humans and experimental animals have decreased venous capacitance because of reduced compliance of extrathoracic veins, particularly those in the splanchnic bed (28, 35). Venomotor tone is increased in established deoxycorticosterone acetate (DOCA)-salt hypertension (6, 47), and it also occurs during hypertension development in spontaneously hypertensive rats (SHRs), aldosterone, Goldblatt, and ANG II-induced experimental hypertension models (12, 18, 27, 46). Increases in venomotor tone can be because of increases in sympathetic nerve activity (SNA) (6, 12, 18, 38, 43), the effects of circulating and locally produced hormones (9, 11, 12, 15, 22, 39), or vascular remodeling (19).

There is increased venomotor tone in DOCA-salt hypertensive rats (6), and DOCA-salt hypertension depends on increased SNA and on locally acting hormones, including endothelin-1 (ET-1). One consequence of increased SNA in DOCA-salt rats is enhanced contraction of venous smooth muscle cells (VSMC). Depolarization contributes to increased vascular tone by increasing the open probability of L-type Ca$^{2+}$ channels causing an elevation of intracellular Ca$^{2+}$ (23).

Based on the observation discussed above, we made in situ intracellular recordings of membrane potential ($E_m$) of VSMC in small mesenteric veins (MV) from DOCA-salt and sham (normotensive) rats to determine if sympathetic venoconstriction and reactivity to norepinephrine (NE) contribute to increased venomotor tone in DOCA-salt hypertension. Vascular reactivity for exogenous NE, ET-1, and KCl were also measured in anesthetized sham and DOCA-salt rats and in isolated mesenteric preparations from sham and DOCA-salt rats. Vascular morphology was used to determine if there are changes in venous wall structure in DOCA-salt rats.

MATERIALS AND METHODS

Animals. Animal use protocols were approved by the Institutional Animal Care and Use Committee at Michigan State University. Sham-operated and DOCA-salt hypertensive rats (male Sprague-Dawley, 170–225 g; Charles River Laboratories, Portage, MI) were prepared as previously described (44, 45). BP was measured by tail-cuff plethysmography 3–4 wk after DOCA implantation in animals that would provide tissues for in vitro studies.

In situ measurement of $E_m$ and vascular reactivity in anesthetized rats. Heart rate (HR), BP, mean arterial blood pressure (MAP), and mesenteric artery outer diameter (MAOD) or mesenteric vein outer diameter (MVOD) were recorded as previously reported (45). An ileal loop was drawn out and fixed to a Silastic chamber perfused with Krebs’ solution (37°C). The Krebs solution contained (mmol/l) 118 NaCl, 4.7 KCl, 1.1 MgCl$_2$, 1.2 Na$_2$HPO$_4$, 2.5 CaCl$_2$, 25 NaHCO$_3$, and 11 glucose equilibrated with compressed air (5% CO$_2$-21% O$_2$-74% N$_2$). Mesenteric arteries (MA) and MV [outside diameter (OD) ~300

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µm] were isolated and cleared. The output of a black-white video camera (DAGE-MTI 100) attached to a microscope (Nikon SMZ 1000) was fed to a frame grabber card (Picolo; Euresys) mounted in a personal computer. Video images were analyzed using Diamtrak software (Diamtrak, Adelaide, Australia). The digitized signal was converted to an analog output (NuDAQ+ 6208; ADLINK Tech).

For measurements of $E_{m}$, glass microelectrodes filled with 3 M KCl (tip resistance: 50–100 MΩ) were used. Microelectrodes were suspended on a headstage (7001; Dagan, Minneapolis, MN) and connected to a bridge amplifier (I2X-700; Dagan). Electrical signals were monitored simultaneously with an oscilloscope. Successful impalements were defined as an abrupt and stable negative potential greater than −35 mV maintained for 6 s or more without progressive decline. Measurements of $E_{m}$ were made only in MV, since pulse pressure in MA routinely dislodged impalements.

Analog signals for HR, BP, MAP, MAOD or MVOD, and $E_{m}$ were digitized (Digidata 1200; Axon Instruments) and displayed using the Clampex acquisition routine in the pClamp 7.0 acquisition and analysis software suite (Axon Instruments).

Measurement of perfusion pressure in isolated MA and MV in vitro. Changes in perfusion pressure (PP) in isolated MA and MV in vitro were also studied using methods described previously (45). Briefly, the mesenteric bed was removed from rats, and the superior MA and MV were cannulated and placed in a chamber superfused with Krebs solution (37°C; see above). The arterial and venous trees were perfused with Krebs solution. The flow rate was adjusted to keep MAP at 30–40 mmHg and MVPP at 6–8 mmHg. MAP and MVPP were monitored with a Statham pressure transducer and a strain gauge amplifier (CP122; Grass Instruments). Data were displayed and analyzed using Polyview software (Grass Instruments).

Vascular morphology. The mesenteric bed and associated intestine were removed from anesthetized rats, and MA and MV (OD ≈300 µm) were isolated and fixed immediately using 10% formaldehyde. The segments were embedded using paraffin, cut into 8-µm cross sections, and stained using hematoxylin. Images were obtained using a microscope (model TE 2000-U; Nikon), brightfield illumination, and a ×40 objective. Images were processed using Meta imaging Series 6.1. Media and lumen area were measured in MA and MV sections using Image J (National Institutes of Health) software. VSMC numbers were also counted from the sections.

Data acquisition. $E_{m}$ measurements were discarded if changes in microelectrode impedance occurred during the penetration or if the potential did not return abruptly to baseline on electrode withdrawal. Each $E_{m}$ (±SE) value reported at each experimental point in the various protocols is the mean $E_{m}$ value, where each average $E_{m}$ value, in turn, was determined from multiple impalements (at least 3 impalements) per animal or vessel. Thus the replication factor “n” for each mean value of $E_{m}$ or $E_{m}$ difference (±SE) is defined as the number of animal preparations for in situ measurements. Constrictions of blood vessels to the different treatments are expressed as percentage constriction (percentage reduction from the resting diameter). Half-maximal effective agonist concentration (EC50) and maximum potential ($E_{max}$) were calculated using the logistic curve-fitting routine in Origin 7.0 (Origin-Lab, Northampton, MA).

Statistics. Data are reported as means ± SE from n rats. Paired and unpaired t-tests were used to make single-point comparisons. Comparison of multiple points generated in dose-response curves was tested by two-way ANOVA with repeated measures, followed by the Student-Newman-Keuls test. Significance was accepted at $P < 0.05$.

RESULTS

Thirty-two rats (16 sham and 16 DOCA-salt) were used for in vivo studies. Forty-eight sham and 44 DOCA-salt rats were used for in vitro studies. The average systolic BP was higher in DOCA-salt than sham rats (195 ± 4 vs. 120 ± 5 mmHg, $P < 0.05$). At the time of the experiments, the body weight of sham rats was 420 ± 8 g and 320 ± 7 g in DOCA-salt rats ($P < 0.05$).

Depolarized $E_{m}$ values in anesthetized DOCA-salt MV in vivo. $E_{m}$ values from sham and DOCA-salt MV were observed before and after prazosin (0.1 µmol/l) treatment. Resting $E_{m}$ values were depolarized in DOCA-salt MV compared with sham MV (Fig. 1, A and B). The $E_{m}$ values were $−40 ± 2$ mV in DOCA-salt and $−46 ± 1$ mV in sham MV ($P < 0.05$; Fig. 1C). The difference in $E_{m}$ values between sham and DOCA-salt MV was reduced by prazosin treatment ($−44 ± 2$ in DOCA-salt and $−48 ± 2$ mV in sham rats, $P > 0.05$; Fig. 1C). MAOD and MVOD were also studied before and after prazosin treatment. Resting MAOD was smaller than MVOD, but there were no differences between sham and DOCA-salt rats (Table 1). Prazosin completely blocked NE (1–10 µmol/l)-induced MA and MV constriction, and prazosin superfusion over the exposed mesentery for 1 h caused a reduction in HR and BP in DOCA-salt but not sham rats (Table 1). HR and BP did not change significantly in time control studies done in DOCA-salt rats without prazosin treatment ($n = 3$). Prazosin also caused a slight vasodilation in MA and MV from sham and DOCA-salt rats that did not reach statistical difference (Table 1). In the same groups of rats, vasodilation caused by sodium nitroprusside (SNP; 0.1 µmol/l) was 19 ± 6% in sham and 29 ± 7% in DOCA-salt MA ($n = 4$, $P > 0.05$). In sham and DOCA-salt MV, vasodilation caused by SNP was 8 ± 5 and 10 ± 6%, respectively ($n = 4$, $P > 0.05$).

Increased reactivity to NE in MV and impaired reactivity to ET-1 in MA in anesthetized DOCA-salt rats in situ. To determine if reactivity to vasoconstrictor treatments was changed in DOCA-salt rats, concentration-response curves (CRCs) for NE (0.001–10 µmol/l) and ET-1 (0.001–100 nmol/l) on MAOD and MVOD were studied. To avoid intestinal ischemia caused by vasoconstriction, NE was applied by superfusion, and increasing concentrations were applied in a single-dose manner with an interdose interval of 30 min. ET-1 was applied by superfusion, and increasing concentrations were applied in a cumulative manner because the effects of ET-1 on blood vessels are only slowly reversible.

NE decreased MAOD and MVOD without changing HR or MAP (Fig. 2, A and B). NE caused a stable vasoconstriction in MA, but this response desensitized rapidly in MV (Fig. 2, A and B). NE CRCs in DOCA-salt MV were left-shifted with an elevated $E_{max}$ compared with sham MV (Fig. 2C and Table 2), whereas NE CRCs in DOCA-salt MA were right-shifted compared with sham MA, with a smaller $E_{max}$ in DOCA-salt MA (Fig. 2C and Table 2). MV were more sensitive to NE than MA in sham and DOCA-salt rats, but the $E_{max}$ in MV was smaller than MA (Fig. 2C and Table 2). $E_{max}$ was produced by 10 µmol/l of NE in MA and 1 µmol/l in MV.

ET-1 CRCs were similar in sham and DOCA-salt MV (Fig. 2D and Table 2). However, the ET-1 CRCs in DOCA-salt MA were right-shifted compared with sham rats (Fig. 2D). MV were more sensitive to ET-1 than MA, and the $E_{max}$ in MV was larger than in MA (Fig. 2D and Table 2). The $E_{max}$ for ET-1 in DOCA-salt MA was less than that measured in sham MA (Fig. 2D and Table 2).
Increased reactivity to NE and impaired reactivity to ET-1 in MA in DOCA-salt rats in isolated and perfused mesentery. Vasoreactivity to NE and ET-1 was determined simultaneously in isolated and perfused MA and MV from sham and DOCA-salt rats. NE was applied by superfusion, and increasing concentrations were applied in a single-dose manner; increasing concentrations of ET-1 were applied in a cumulative manner. NE increased MAPP (Fig. 3A), and NE CRCs in DOCA-salt MA were left-shifted compared with sham MA (Fig. 3B), whereas the $E_{\text{max}}$ values were similar in DOCA-salt and in sham MA (Table 3). NE did not change MVPP in tissues from sham or DOCA-salt rats (Fig. 3, A and B, and Table 3). ET-1 caused a concentration-dependent increase in MAPP and MVPP (Fig. 3C), and ET-1 was a more potent and efficacious constrictor of MV compared with MA (Fig. 3D and Table 3). The $pD_2$ ($-\log \text{EC}_{50}$) values for ET-1 were similar in sham DOCA-salt MA (Fig. 3D and Table 3), but the $E_{\text{max}}$ for ET-1 was reduced in DOCA-salt MA (Fig. 3D and Table 3). ET-1 CRCs were similar in sham and DOCA-salt MV (Fig. 3D and Table 3).

Unchanged reactivity for KCl in MA and MV in DOCA-salt rats in situ and in isolated and perfused mesentery. KCl (5–80 mmol/l) was applied to the exposed mesentery of anesthetized rats by superfusion, and increasing concentrations were applied in a single-dose manner with an interdose interval of 30 min because responses desensitized in MV (Fig. 4A). KCl CRCs in DOCA-salt MA and MV were similar to sham MA and MV (Fig. 4B and Table 2). However, KCl CRCs in MV were

Table 1. HR, MAP, and OD changes before and after superfusion of prazosin (0.1 μmol/l for ~60 min) in situ in anesthetized DOCA-salt and sham rats

<table>
<thead>
<tr>
<th>Group</th>
<th>HR, beats/min</th>
<th>MAP, mmHg</th>
<th>MAOD, μm</th>
<th>MVOD, μm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>PR</td>
<td>Control</td>
<td>PR</td>
</tr>
<tr>
<td>Sham (n = 5)</td>
<td>358±20</td>
<td>327±29</td>
<td>96±4</td>
<td>88±3</td>
</tr>
<tr>
<td>DOCA-salt (n = 5)</td>
<td>392±17</td>
<td>347±10*</td>
<td>147±6†</td>
<td>114±9†</td>
</tr>
</tbody>
</table>

Data were obtained from the indicated number of animals (n) and are means ± SE. HR: heart rate; MAP: mean arterial pressure; PR: treatment with prazosin; MA: mesenteric artery; MV: mesenteric vein; OD: outside diameter; DOCA: deoxycorticosterone acetate. $P < 0.05$, prazosin vs. control (*) and DOCA-salt vs. sham (†) rats.
shifted to the left compared with MA (Fig. 4, A and B). The KCl $E_{\text{max}}$ values in MA and MV were similar in DOCA-salt and sham rats (Table 3).

In isolated, perfused mesentery preparations, increasing concentrations of KCl were applied in a cumulative manner. In these experiments, KCl caused a concentration-dependent increase in MAPP, and there was no difference in responses in preparations from sham or DOCA-salt rats (Fig. 4C and Table 3). KCl did not change MVPP in preparations from either sham or DOCA-salt rats (Fig. 4C and Table 3).

Hypertrophy occurs in DOCA-salt MA but not MV. The media was clearly thicker in MA from DOCA-salt rats compared with that in sham rats (Fig. 5A); however, there were no obvious differences in medial thickness in MV from sham and DOCA-salt rats. Vascular media-to-lumen ratios were significantly increased in DOCA-salt MA, but not in MV, compared with sham MV (Fig. 5B). Cell counts per unit area in MA and MV from DOCA-salt rats did not differ from counts in sham MA and MV (Fig. 5C).

**DISCUSSION**

Increased sympathetic tone in DOCA-salt MV. Changes in sympathetic neuroeffector mechanisms associated with hypertension occur in arteries (1, 24), but there have been few comparable studies in veins. Early studies using in situ recording of $E_m$ in SHRs, or reduced renal mass hypertensive rats, showed that $E_m$ was depolarized in MV from hypertensive but not control rats (Fig. 2). Reactivity for norepinephrine (NE) and endothelin-1 (ET-1) in situ. Recordings of HR, BP, MAP, mesenteric artery outside diameter (MAOD; A), and MVOD (B) in an anesthetized sham rat. In situ application of NE caused a stable mesenteric artery (MA) constriction, but the constriction in MV was transient. Constriction caused by NE (10 $\mu$mol/l) in MV was smaller than MA. C. NE concentration-response curves (CRCs) in MA and MV from anesthetized sham and DOCA-salt rats in situ. CRCs in MV were left-shift compared with MA in sham and DOCA-salt rats. D: ET-1 CRCs in MA and MV in situ. CRCs in DOCA-salt MA were right-shifted compared with sham MA, whereas the CRCs in sham and DOCA-salt MV were similar. CRCs in MV were left-shift compared with MA. $P < 0.05$, significantly different from sham rats (*) and significantly different from MA (#).

Table 2. **NE, ET-1, and KCl pD2 and $E_{\text{max}}$ values measured in situ in MA and MV from anesthetized sham and DOCA-salt rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>MA</th>
<th>MV</th>
<th>DOCA-Salt</th>
<th>MA</th>
<th>MV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$pD_2$</td>
<td>$E_{\text{max}}$</td>
<td>$pD_2$</td>
<td>$E_{\text{max}}$</td>
<td>$pD_2$</td>
</tr>
<tr>
<td>NE</td>
<td>7.9±0.1</td>
<td>−53±3</td>
<td>8.1±0.2</td>
<td>−26±3†</td>
<td>7.7±0.1</td>
</tr>
<tr>
<td>ET-1</td>
<td>9.5±0.1</td>
<td>−52±2</td>
<td>9.6±0.2</td>
<td>−79±4*</td>
<td>8.9±0.1</td>
</tr>
<tr>
<td>KCl</td>
<td>1.3±0.1</td>
<td>−54±2</td>
<td>1.7±0.1†</td>
<td>−47±4</td>
<td>1.3±0.1</td>
</tr>
</tbody>
</table>

Data are means ± SE; $n = 5$–6 animals/group. NE, norepinephrine; $pD_2$, −log $EC_{50}$; $E_{\text{max}}$, %change in OD; ET-1, endothelin-1. $E_{\text{max}}$ for NE was measured at 10 $\mu$mol/l for MA and at 1 $\mu$mol/l for MV. *Significantly different from sham levels. †Significantly different from MA.
(39, 40, 43), suggesting that there is a tonic elevation of sympathetic input to MV to cause depolarization in hypertension. \(E_m\) depolarization would activate voltage-dependent \(Ca^{2+}/H^{+}\) channels, and this could contribute to tonic venous contraction (14). In the present study, \(E_m\) depolarization was also detected in DOCA-salt MV. Prazosin treatment hyperpolarized \(E_m\) in DOCA-salt but not sham MV. These results suggest that there is a tonic elevation of SNA input to veins that depolarizes VSMC in DOCA-salt hypertension. This suggestion is supported by data from studies of the effects of ganglion blockade on mean circular filling pressure (MCFP), an index of integrated venomotor tone, in DOCA-salt (6), and in ANG II-induced hypertensive rats (12). These studies showed that ganglion blockade caused a larger fall in MCFP and BP in hypertensive vs. control rats (6, 12, 18). It should be pointed out that in situ application of prazosin caused a small dilation of MA and MV (although this did not reach statistical significance), suggesting that there was a tonic prazosin-sensitive constrictor input to these blood vessels. Our data suggest that measurements of \(E_m\) values in situ to study vascular tone, particularly in small veins, may be an indicator of the strength of SNA input. \(E_m\) measurements might reflect differing SNA input in vivo resulting from neural factors relevant to maintenance of hypertension, since depolarized \(E_m\) disappeared in vitro or after sympathetic nerves were cut in vivo (43). In view of the steep and positive relationship between resting \(E_m\) and vascular diameter at these voltages, even a loss of a few millivolts in \(E_m\) would activate L-type \(Ca^{2+}/H^{+}\) channels and accentuate voltage-gated \(Ca^{2+}\) influx in VSMC to cause graded vascular tone (25). Therefore, changes in resting \(E_m\) contribute to venous tone in DOCA-salt hypertension, and decreased \(E_m\) could reflect increased SNA input to DOCA-salt MV. We also found that prolonged local application (1 h) of prazosin to the mesentery reduced MAP and HR in DOCA-salt but not in sham rats. These systemic effects of prazosin likely occurred following intestinal absorption of the drug in the circulation.

Previous in vitro studies showed that neurogenic constrictions of MV from DOCA-salt and sham rats were similar,

Table 3. \(NE, ET-1, and KCl\) \(pD_2\) and \(E_{max}\) values measured in isolated perfused MA and MV from sham and DOCA-salt rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Sham MA</th>
<th>Sham MV</th>
<th>DOCA-Salt MA</th>
<th>DOCA-Salt MV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(pD_2)</td>
<td>(E_{max})</td>
<td>(pD_2)</td>
<td>(E_{max})</td>
</tr>
<tr>
<td>NE</td>
<td>5.8±0.1</td>
<td>131±12</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>ET-1</td>
<td>7.7±0.1</td>
<td>121±12</td>
<td>9.2±0.1†</td>
<td>27±3†</td>
</tr>
<tr>
<td>KCl</td>
<td>1.3±0.1</td>
<td>124±6</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Data are means ± SE; \(n = 5–6\) animals/group. *Significantly different from control (sham) levels. †Significantly different from MA. NA, not available.
but there were decreased basal NE content and increased NE transporter levels in DOCA-salt MV. Those data suggest that there is an increased release of NE from sympathetic nerves associated with DOCA-salt MV (16). Impaired \(\alpha\)-adrenergic autoreceptor function in sympathetic nerves associated with arteries and veins from DOCA-salt rats could be responsible for increased NE release (17). We found that \(E_m\) values were depolarized in DOCA-salt MV compared with sham MV and that, after prasozin treatment, VSMCs were hyperpolarized in DOCA-salt but not sham MV. Collectively, these data are consistent with increased SNA in veins that leads to increased venomotor tone in DOCA-salt hypertension. The increased SNA input could be because of increased NE release or increased reactivity of MV to neurogenically released NE. This second possibility was investigated in the next set of experiments.

Increased reactivity to NE in DOCA-salt MV. We found that reactivity to in situ application of NE to the exposed mesentery was increased in DOCA-salt MV. We also found that in situ constrictions in MA caused by NE were reduced in DOCA-salt rats. These results show that reactivity for NE is increased in MV but reduced in MA in situ in DOCA-salt rats. These in situ data differ markedly from data obtained in vitro using the isolated perfused mesenteric preparation. In the in vitro studies, NE did not constrict MV. This negative result is probably because of desensitization of \(\alpha\)-adrenergic receptors in MV. To keep MVPP at near-physiological levels, flow of buffer through the venous circulation was maintained at a very slow rate. Because NE was applied by addition to the perfusion buffer, a slow rise in NE concentration may have desensitized the \(\alpha\)-adrenergic receptors before measurable receptor activation occurred. The data obtained in MA in previous in vitro studies (16) differ from those obtained in the present study when measuring MAPP. In the MAPP studies, it was found that the NE CRCs in DOCA-salt MA were shifted to the left of that obtained in sham MA. These results are similar to those obtained in tension recording experiments in vitro (42). In these studies, it was found that the aorta from DOCA-salt rats was more sensitive (lower \(pD_2\)) to the constrictor effects of NE than the aorta from sham rats, whereas the \(E_{max}\) was not altered in DOCA-salt rats (42). It was also found that efficacy and potency of NE were not altered in DOCA-salt vena cava (42). Other in vitro studies measuring diameter changes showed that NE CRCs in MA or MV from sham and DOCA-salt rats (16) and mice (29) were similar. The different results are likely based on differences in the specific blood vessels studied (aorta, vena cava, MA, and MV) and also on different techniques for measuring vasomotor responses (PP vs. wall tension vs. OD measurements).

Increased reactivity to NE in DOCA-salt MA (this was observed in vitro but not in situ) and MV (this was observed in situ but not in vitro) in the present study is not caused by structural changes or vascular hypertrophy. This is the first study to look at hypertrophy of small MV in DOCA-salt hypertension. Our results showed that hypertrophy only occurred in DOCA-salt MA but not MV. Therefore, venous hypertrophy does not contribute to increased venous reactivity to NE. In addition, KCl-induced constrictions of DOCA-salt MA and MV did not differ from those in sham MA or MV, suggesting that there is not a general change in vascular...
reactivity in MV and MA from DOCA-salt rats. Taken together, these data indicate that sympathetic neurotransmission may be more effective in MV compared with MA, and this difference is enhanced in DOCA-salt hypertension.

**ET-1 reactivity is maintained in MV but reduced in MA in anesthetized DOCA-salt rats.** ET-1 contributes to the pathogenesis of hypertension in DOCA-salt rats (5, 26, 20) and in humans (3, 9) and reduces renal mass hypertension (30). Studies of the contributions of ET-1 to the pathogenesis of hypertension have focused on arteries (31, 36). However, data from human (2, 9) and animal (6, 10, 11, 40) studies indicate that ET-1 is an important determinant of venous tone in hypertension. Veins are more sensitive to ET-1-induced constriction than arteries (4, 42). ET-1-induced venoconstriction is selectively enhanced in patients with essential hypertension (9), and ET<sub>A</sub> receptor antagonists reduce BP in hypertensive patients (7, 13) and in DOCA-salt hypertensive rats (2, 27, 33). ET<sub>A</sub> receptor antagonists also decrease BP and MCFP more in hypertensive than in normotensive rats, indicating a role for ET-1 in increased venomotor tone in DOCA-salt hypertension.

We found that ET-1 reactivity is maintained in DOCA-salt MV in situ but reduced in MA, as also occurs in vitro (21, 42). Our data are important because, in our in situ studies, intravascular pressures in MA and MV were at physiological levels set by endogenous hemodynamic regulatory mechanisms. The in situ CRCs for ET-1 in MV and MA in sham and DOCA-salt rats were identical to those obtained in vitro using the same blood vessels but in an unpressurized state (11). In addition, ET-1 responses studied in situ were maintained in MV, but not MA, of DOCA-salt rats as also occurs in unpressurized blood vessels in vitro (11, 42). These data indicate that differences in MA and MV ET-1 reactivity are not an artifact arising from in vitro assessment of ET-1 vascular reactivity. Vascular endothelial cell ET-1 mRNA expression is elevated in arteries, but not veins, from DOCA-salt rats, and veins have a higher ET-1 content than arteries, but ET-1-increased venomotor tone is not the result of a higher ET-1 content (11, 41, 42).

We found that ET-1 reactivity in MA is reduced in DOCA-salt rats. Similar reductions in ET-1 reactivity in MA and aorta of hypertensive rats have been reported previously (21, 42). This previous work demonstrated that reduced ET-1 arterial constrictions in hypertensive rats are not the result of reduced expression of ET<sub>A</sub> receptors or L-type Ca<sup>2+</sup> channels, since ET<sub>A</sub> receptor and L-type Ca<sup>2+</sup> channel protein are increased in DOCA-salt arteries (21, 42). Therefore, decreased ET-1-induced arterial constriction may be the result of changes in signaling pathways downstream from ET<sub>A</sub> receptor activation. Alternatively, ET<sub>A</sub> and ET<sub>B</sub> receptor activation contributes to ET-1-induced venoconstriction, whereas arterial constriction is mediated by ET<sub>A</sub> receptors only (10, 11). Selective venous expression of ET<sub>A</sub> and ET<sub>B</sub> receptors may contribute to the increased sensitivity of veins to the constrictor effects of ET-1 and to the maintained venous ET-1 reactivity in DOCA-salt hypertension.

In conclusion, we showed that MV are more sensitive to the constrictor effects of NE, KCl, and ET-1 than MA. Therefore, veins would be more sensitive than arteries to pathophysiological changes that contribute to hypertension. These changes

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**Fig. 5. Morphological studies in MA and MV.**

A: images of hematoxylin-stained cross sections of sham and DOCA-salt MA and MV. B: summary data for MA and MV media-to-lumen ratio. C: summary data for MA and MV showing cell density. Each value (±SE) is the mean of an average of measurements made on (3–4) sections obtained on a blood vessel from an individual animal. Thus the n for each mean ± SE is the no. of animals from which the mean was calculated. Media-to-lumen ratio was increased in DOCA-salt MA but not in MV. Cell density did not change. *P < 0.05, significantly differ from sham.
could include increased SNA, which would cause a chronic increase in venomotor tone. Increased venomotor tone would be due in part to the chronic depolarization of MV caused by NE released from sympathetic nerves in DOCA-salt hypertension. We did not detect hypertrophy or remodeling in MV of DOCA-salt rats, so changes in venous wall structure cannot account for increased reactivity to NE in MV of DOCA-salt rats. In DOCA-salt rats, in vivo, ET-1 reactivity is unchanged in MV but reduced in MA as also occurs in vitro. Hence, increased arterial tone would be caused by increased reactivity to NE, but not ET-1, in DOCA-salt hypertension. It is important to note that the changes reported here may be specific for the mesenteric circulation, and their alterations in neural and humoral control may only partly contribute to systemic changes in BP. VSMC depolarization and increased sympathetic nerve activity, NE, and ET-1 reactivity in other veins and arterial beds are also likely to make important contributions to the hemodynamic changes that occur in DOCA-salt hypertension.

REFERENCES

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