Norepinephrine Sensitivity and Membrane Potentials of Caudal Arterial Muscle in DOCA-Salt, Dahl, and SHR Hypertension in the Rat

KENT HERMSMEYER, PH.D., PETER W. ABEL, PH.D., AND ANGELO J. TRAPANI, B.S.

SUMMARY Comparison of norepinephrine (NE) sensitivity in caudal arterial muscle of rats with three forms of hypertension showed that there was no increase in either DOCA-salt or Dahl genetic hypertension, in contrast to the increased NE sensitivity found in spontaneously hypertensive rats (SHR). In hypertension induced by deoxycorticosterone acetate (DOCA)-salt treatment, as in Dahl genetic hypertension, there was also no difference in membrane potential (E_m) between hypertensive and normotensive rats. By comparison to the SHR membrane alterations reported previously, any increased NE sensitivity might have been associated with altered E_m electrogenesis which is triggered by a trophic factor of the sympathetic nervous system. SHR have a lower intracellular K^+ free ion concentration and thus a smaller contribution of the ion gradient generated voltage which appears to be compensated for at rest by more active electrogenic ion transport. While SHR show greater depolarization and contraction than Wistar-Kyoto (WKY) rats in response to midrange NE concentrations, DOCA-salt and Dahl hypertensive rat caudal arterial muscle showed neither NE hypersensitivity nor any evidence of altered E_m mechanisms. Ion transport in isolated peripheral arteries in DOCA-salt hypertension may only secondarily be altered in response to primary changes in humoral factors and altered neural control mechanisms. (Hypertension 4 (suppl II): 11-49—11-51, 1982)

KEY WORDS • electrogenic ion transport • intracellular K^+ concentration • caudal arterial muscle • DOCA-salt • Dahl rat • spontaneously hypertensive rat (SHR) • membrane potential electrogenesis • norepinephrine hypersensitivity • sympathetic nervous system • contraction

ALTHOUGH there have been several detailed studies done on ion fluxes and transport enzyme characteristics of deoxycorticosterone acetate (DOCA)-salt induced hypertension (reviewed by Haddy et al.1), there are only a few reports on the norepinephrine (NE) sensitivity of arterial muscle in this particular model of nongenetic hypertension. It has been speculated that there may be a depolarization of arterial muscle from hypertensives resulting from inhibition of the Na^+-K^+ ATPase that is believed to be the transport enzyme.2,4 However, no direct test of the hypothesis that there is depolarization of arteries in DOCA-salt hypertension has appeared.

It is particularly important to study arterial muscle in DOCA-salt hypertension because this form of hypertension is associated with increases in Na^+ movements in peripheral arteries3,5 as well as whole animal Na^+ balance.6 In hypertension initiated by DOCA and salt, there are increases in fluxes of sodium (Na^+), potassium (K^+), and chloride (Cl^-) that precede the development of hypertension and gradually increase simultaneously with increasing blood pressure.3 This study was an investigation of the NE sensitivity and membrane potential (E_m) of arterial muscles in DOCA-salt hypertension to compare characteristics with the Dahl genetic strain of hypertension, which is also induced by salt loading, and with the SHR form of genetic hypertension, which does not require high salt intake.

Methods

Male Sprague-Dawley rats (Bio-Lab) were unilaterally nephrectomized and divided into two groups of 10 each. One group was treated with DOCA at a concentration of 100 mg/kg, as a Silastic implant. Control animals were implanted with Silastic that did not contain DOCA. All animals were then given 0.9% NaCl.
and 0.2% KCl in drinking water for 6 weeks. Blood pressure was measured at weekly intervals by tail cuff plethysmography to determine that all of the DOCA-treated group had developed significant hypertension by 6 weeks. The age of each animal at the start of the DOCA-salt treatment was 6 to 7 weeks.

Animals were anesthetized with pentobarbital (Nembutal), after which the caudal artery was carefully dissected without stretching from the ventral surface of the tail. Spiral strips were cut, mounted in flow-through chambers, and attached to Grass tension transducers for determination of NE sensitivity. All arteries were freed of adrenergic influences by the 6-hydroxydopamine in vitro technique. Vascular muscle strips were suffused with isotonic solution for 8 minutes. In all experiments, arterial segments at rest (37°C) were maintained at normal levels by an upward shift of the Na+ efflux curves. Although NE contraction dose-response curves for aortas from DOCA-hypertensive animals than in controls, a difference that is reduced by treatment with antihypertensives. However, intracellular Na+ is maintained at normal levels by an upward shift of the Na+ efflux curves. Although NE contraction dose-response curves for aortas from DOCA-hypertensive rats do not appear to have been reported, the epinephrine sensitivity of femoral artery in DOCA hypertensive rats was measured in vivo circulation of the DOCA hypertensive pig also showed increased NE sensitivity. It appears possible that the evidence for increased NE sensitivity of small arteries in DOCA hypertension might only be found in the presence of neural and humoral factors that contribute to increased peripheral vascular reactivity. Accumulating data suggest that DOCA-salt hypertension might have different causative mechanisms from the SHR form. Thus, to integrate data from different laboratories, there clearly is a need for more experiments on contraction and Em in DOCA hypertension models and on ion fluxes in SHR.

The lack of a change in NE sensitivity or Em in the DOCA-salt form of hypertension is important in the context of the altered NE sensitivity and Em in SHR. In the SHR genetic model, the cause of the NE sensitivity increase appears to be a different composition of Em, that occurs in response to a trophic influence of the sympathetic nervous system. A comparison of caudal artery NE sensitivity and Em in DOCA, Dahl, and SHR forms of hypertension is shown in table 2. The trophic influence of the sympathetic nervous system is evident in the cross-innervation experiments which demonstrate that the alteration in Em and NE sensitivity follows the sympathetic innervation. In both the Dahl S strain on high salt intake.

Results

Six weeks of treatment with DOCA induced hypertension, giving a mean systolic blood pressure of 164 ± 4 mm Hg as compared with 129 ± 3 mm Hg in the control group. Neither NE sensitivity nor Em of caudal arteries isolated, cut into strips, and perfused without recirculation for at least 2 hours was different in the two groups. The NE dose-response curves for DOCA-salt hypertensive and control groups are shown to completely overlap (fig. 1). The NE EC50 and 95% confidence intervals were 240 nM (170 to 310) for the control and 220 nM (170 to 260) for the DOCA-treated groups. Maximum tension was 800 ± 107 Kdynes/cm² for the control and 730 ± 116 Kdynes/cm² for the DOCA hypertensive groups (not significantly different). Em averaged −56 mV for both the control and DOCA hypertensive groups, as shown in table 1.

Discussion

These experiments demonstrate that neither caudal arterial NE sensitivity nor Em are altered in DOCA-salt hypertension, in contrast to results reported for SHR. It appears that in DOCA-salt hypertension, neural and humoral mechanisms, which have been suggested by the work of others (reviewed by Brody13), are more important contributors to hypertension, at least in the smaller peripheral arteries. On the other hand, increases in K+ flux induced by NE are greater in aortas from DOCA hypertensive animals than in controls, a difference that is reduced by treatment with antihypertensives. However, intracellular Na+ is maintained at normal levels by an upward shift of the Na+ efflux curves. Although NE contraction dose-response curves for aortas from DOCA-salt hypertensive rats do not appear to have been reported, the epinephrine sensitivity of femoral artery in DOCA hypertension is increased, at least under conditions (a nonperfused muscle bath) where an endogenous plasma substance may not have been removed. The in vivo circulation of the DOCA hypertensive pig also showed increased NE sensitivity. It appears possible that the evidence for increased NE sensitivity of small arteries in DOCA hypertension might only be found in the presence of neural and humoral factors that contribute to increased peripheral vascular reactivity. Accumulating data suggest that DOCA-salt hypertension might have different causative mechanisms from the SHR form. Thus, to integrate data from different laboratories, there clearly is a need for more experiments on contraction and Em in DOCA hypertension models and on ion fluxes in SHR.

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**TABLE 1. DOCA-Salt Hypertensive and Control Rat Blood Pressure and Membrane Potentials (Em) of Caudal Arterial Muscle Cells**

<table>
<thead>
<tr>
<th>No.</th>
<th>DOCA</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic pressure (mm Hg)</td>
<td>10</td>
<td>164 ± 4</td>
</tr>
<tr>
<td>Em (mV)</td>
<td>6</td>
<td>−66 ± 1</td>
</tr>
</tbody>
</table>

Values are means ± standard errors with Em from arterial segments at rest (37°C).

Kdynes/cm² for the DOCA hypertensive groups (not significantly different). Em averaged −56 mV for both the control and DOCA hypertensive groups, as shown in table 1.

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**FIGURE 1. Dose-response curves for norepinephrine in control and DOCA hypertensive rat caudal arteries showed complete overlap. Points are means of 10 animals for each point. Standard errors were ± 3 or less for all points except the control point at 7, which was ± 4. There were no significant differences between control and DOCA groups (p < 0.05, Student's t test comparison).**
Comparison of Membrane Properties of Hyper- 
tensive-Normotensive Pairs of Rats

<table>
<thead>
<tr>
<th>Form of hypertension</th>
<th>Hypertensive/ normotensive NE sensitivity</th>
<th>Hypertensive-normotensive ΔE m</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOCA-salt</td>
<td>1.0</td>
<td>0</td>
</tr>
<tr>
<td>Dahl (S vs R)</td>
<td>1.0</td>
<td>−1</td>
</tr>
<tr>
<td>SHR vs WKY</td>
<td>2.25*</td>
<td>−7*</td>
</tr>
</tbody>
</table>

*Significantly different (p < 0.05).

Dahl data are taken from Abel et al. SHR/WKY data are taken from Hermansmeyer. SHR are more sensitive to NE (lower E D0) and have less negative E m at 16° C. DOCA = deoxycorticosterone acetate; SHR = spontaneously hypertensive rat; WKY = Wistar-Kyoto rat (both SHR and WKY are from the genetically matched breeding colony at the University of Iowa); NE = norepinephrine.

and the DOCA-salt models of hypertension, there was no detectable increase in NE sensitivity or change in E m (table 2). In the DOCA-salt hypertensive animals, the increase in Na + influx appears to be compensated by an enhanced ion transport 12 without a contribution to resting E m. The depressed R b + uptake of arteries immediately upon removal may reflect the action of a humoral substance that tonically depresses membrane transport causing a compensatory increase in number of transport sites, to maintain normal intracellular Na +. In contrast, the SHR isolated caudal artery even continuously perfused for up to 12 hours, when all traces of plasma substances are likely to be washed away, shows the altered basis for E m and high NE sensitivity. 17 It is noteworthy that only the SHR form of hypertension is not prevented by lesions of the anteroverentral third ventricle of the brain. 18 Perhaps it is true that in the Dahl, renal, and DOCA-salt forms of hypertension, neural and humoral factors are sufficient to explain the increased vasoconstriction without intrinsically altered vascular muscle membrane mechanisms. 19

These results suggest that the increased K + efflux found in DOCA-salt hypertension is not due to partial depolarization of the vascular muscle cells, at least not in the caudal artery. The present results are in agreement with the suggestion of Jones and Hart 20 and Friedman and Friedman 19 that there is an increase in the ion permeability for both Na + and K + in DOCA-salt hypertension. The increased Na + influx and K + efflux, perhaps due to humoral depression of ouabain-sensitive transport, 1 would result in Na + loading and K + depletion in the vascular muscle cells if there were not increases in ion transport 18 that compensated to maintain the higher K + and lower Na + levels than are found in plasma. If the caudal artery data are representative of small arteries that determine peripheral resistance, the plasma factor that depresses the R b + uptake may well be a principal explanation for increased peripheral resistance in DOCA-salt hypertension. While vascular muscle E m alteration does not appear inherent to DOCA-salt hypertension, possibly important membrane actions of an endogenous digitalis-like substance 1 and the alternate suggestion that reduced Ca ++ accumulation by isolated membrane fractions is an important mechanism 20 make further measurements of vascular muscle membrane properties important.

References
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