Area Postrema Ablation and Vascular Reactivity in Deoxycorticosterone-Salt-Treated Rats

CATHY A. BRUNER, MICHAEL L. MANGIAPANE, GREGORY D. FINK, AND R. CLINTON WEBB

SUMMARY In rats, central administration of the neurotoxin 6-hydroxydopamine prevents hypertension and certain functional vascular changes after deoxycorticosterone (DOC)-salt treatment. In this study, the effect of electrolytic ablation of the area postrema on blood pressure and vascular reactivity in DOC-salt-treated rats was examined. Four treatment groups of rats were studied (n = 5 in each): area postrema lesion, DOC-salt (DOC pivalate, 5 mg/wk s.c. for 5 weeks); sham lesion, DOC-salt; area postrema lesion, control; and sham lesion, control. Helically cut strips of carotid artery, aorta, and mesenteric artery were prepared for isometric force recording. Area postrema lesion attenuated hypertension in DOC-salt rats (mean arterial pressure, 107 vs 123 mm Hg in area postrema lesion and sham lesion rats, respectively; chronic aortic catheter). Vascular strips from sham lesion-DOC-salt rats were more sensitive to KCl, ouabain, and serotonin than were those from sham lesion-control rats. These changes in vascular reactivity also were observed in area postrema lesion–DOC-salt rats. DOC treatment in rats on a normal sodium intake did not result in hypertension or increased vascular reactivity. In summary, integrity of the area postrema is necessary for hypertension, but not for changes in vascular reactivity, in DOC-salt rats. It appears that 1) changes in vascular reactivity may be necessary, but they are not sufficient to produce DOC-salt hypertension, and 2) if these vascular changes are secondary to a central nervous system effect, they are mediated by a pathway distinct from the area postrema. (Hypertension 11: 668–673, 1988)

KEY WORDS hypertension • aorta • mesenteric artery • carotid artery • ouabain • serotonin • arterial pressure

ADMINISTRATION of the mineralocorticoid deoxycorticosterone (DOC) in combination with high NaCl intake produces hypertension in rats. Although the precise mechanisms involved in the pathogenesis of DOC-salt hypertension are uncertain, it is becoming increasingly clear that the central nervous system plays an important role in this form of hypertension. Electrolytic destruction of periventricular hypothalamic structures (anteroventral third ventricle) prevents the development of DOC-salt hypertension in rats. More recently, it has been demonstrated that ablation of the area postrema (AP), a high vascular circumventricular organ in the medulla, also prevents DOC-salt hypertension in rats. Furthermore, central catecholamine depletion by administration of the neurotoxin 6-hydroxydopamine (6-OHDA) has been shown to prevent hypertension in this model.

Changes in reactivity of vascular smooth muscle to vasoconstrictors have been postulated to contribute to DOC-salt, as well as many other forms, of hypertension. Interestingly, central 6-OHDA treatment prevents not only elevated arterial pressure, but also the increased vascular sensitivity to vasoconstrictors characteristic of DOC-salt hypertension. Based on studies of this type, it may be hypothesized that the increase in vascular reactivity in DOC-salt hypertensive rats is not due to a direct effect of DOC-salt on the vasculature, but rather to an effect of DOC-salt on the brain. As central 6-OHDA treatment produces catecholamine depletion in many brain regions, it is not possible from these studies to identify specific areas that are involved in the genesis of hypertension or in
changes in vascular reactivity in response to DOC-salt treatment. The purpose of the current study was to
determine whether discrete ablation of the AP in rats (which has been shown to prevent DOC-salt hyperten-
sion) would prevent the development of changes in vascular reactivity in response to DOC-salt treatment.

Materials and Methods

Male Sprague-Dawley rats (weight, 300–400 g; Sasco, Omaha, NE, USA) were used in all experi-
ments. In the first study, the effect of AP ablation (APX) on blood pressure and vascular reactivity was
assessed. All rats underwent operation for electrolytic ablation of the AP. Details of this procedure are
described elsewhere. Briefly, rats were anesthetized with a mixture of pentobarbital and chloral hydrate and
placed in a stereotaxic apparatus (David Kopf, Tujunga, CA, USA). The AP was exposed on the surface
of the medulla, and 700 μA of anodal current was delivered to the surface of the AP by a tungsten elec-
trode for 9 to 12 seconds (6.3–8.4 mC). In sham-operated rats, the electrode was placed on the AP, but
no current was passed.

Rats were uninephrectomized under pentobarbital anesthesia (50 mg/kg i.p.). One week later indwelling
catheters were placed in the abdominal aorta and vena cava through the femoral vessels (with the rats under
pentobarbital anesthesia). Rats were treated with DOC pivalate (DOC; 5 mg/rat/ wk s.c.) and given 0.9%
NaCl to drink for 5 weeks. Details and results of this protocol have been described previously. Four treat-
ment groups of rats were studied: APX, DOC-salt; sham lesion, DOC-salt; APX, control; and sham le-
son, control (n = 5/group).

For vascular reactivity studies, rats were killed with an i.p. injection of pentobarbital and the thoracic aorta,
superior mesenteric artery, and one common carotid artery were removed and placed in cold physiological
salt solution (PSS). Brains were removed and placed in 10% buffered formalin for subsequent histological
evaluation of APX. Arteries were cleaned of excess fat and connective tissue and cut into helical strips (aorta: 1.5 × 15 mm; carotid artery: 1 × 10 mm; mesenteric artery: 0.7 × 8 mm). Vascular strips were mounted on
metal tissue holders and placed in 50-ml tissue baths filled with warmed (37°C), aerated (95% O2, 5% CO2)
PSS. The upper end of each strip was connected to a Grass FT.03 force transducer (Quincy, MA, USA) for
the measurement of isometric force. Recordings were made on a Grass polygraph. The composition of the
PSS was as follows (in mM): NaCl, 130; KCl, 4.7; MgSO4·7H2O, 1.17; KH2PO4, 1.18; NaHCO3, 14.9;
dextrose, 5.5; NaCa2EDTA, 0.03; CaCl2·H2O, 2.5 for aorta and carotid artery, 1.6 for mesenteric artery.

Vascular strips were allowed to equilibrate for 90 minutes under a constant passive force of 1.5 g for
aortas and 500 mg for mesenteric and carotid arteries. Aortic strips were then exposed to 130 mM KCl PSS
(made by equimolar substitution of KCl for NaCl in the PSS) until a steady contractile response was attained.
After a 1-hour recovery period, a cumulative concen-
tration-response curve to ouabain (10^-6 to 10^-3 M) was obtained. Aortic strips were exposed to each concen-
tration of ouabain for 15 minutes.

Cumulative concentration-response curves to KCl (in the presence of 10^-5 M phenolamine) were deter-
dined in carotid artery strips. KCl concentration in the tissue bath was increased by addition of appropriate
volumes of a 3 M KCl stock solution. In mesenteric artery strips, cumulative concentration-response
curves to serotonin (2.6 × 10^-9 to 2.6 × 10^-3 M) were obtained.

In a separate set of experiments, the effect of DOC treatment on blood pressure and vascular reactivity in
rats on normal sodium intake (drinking tap water instead of 0.9% NaCl) was determined. Rats were uni-
nephrectomized and treated with DOC pivalate as already described, but they were maintained on tap
water. One week before the end of the 5-week treatment period, rats were instrumented with an abdomi-
nal aortic catheter for the measurement of blood pressure. Mean arterial pressure was determined in
conscious rats 3 days after catheter placement. Rats were then killed, and vascular responses to ouabain,
KCl, and serotonin were determined as just described. Two treatment groups of rats were studied in this
protocol: normal sodium–DOC (n = 7) and normal sodium–control (n = 6).

Values are expressed as means ± SEM. For calculation of ED16 values to KCl and ED90 values to serotonin
(concentrations that produced 16% and 50% of the maximal response, respectively), contractile responses
were expressed as a percentage of the maximal re-
sponse and a probit analysis was performed. These
measures of vascular responsiveness have been shown
to be indices of increased vascular reactivity in DOC-
salt hypertension in rats. Statistical analysis was
performed on the negative log of the ED16 and ED90
values. Contractile responses to 10^-3 M ouabain were
expressed as a percentage of the response to 130 mM
KCl solution. The contractile response to 10^-3 M oua-
ibain was used as an index of sensitivity, since maximal
contractile responses to the glycoside cannot be ob-
tained. The arc sin square root transformation of
these percentages was then used in subsequent statisti-
cal analysis. Data from the APX–DOC-salt experi-
ment were analyzed using a two-way analysis of vari-
cation (ANOVA). Factor 1 was steroid treatment (DOC
vs control), whereas Factor 2 was the lesion factor
(APX vs sham lesion). Data from the normal sodium–
DOC experiment were analyzed using a Student’s t test
(normal sodium–DOC vs normal sodium–control). A
p value of 0.05 was the criterion for statistical signifi-
cance.

Results

As shown in Figure 1, mean arterial pressure was
significantly higher in sham lesion–DOC-salt rats than
in sham lesion–controls. Rats with AP lesion did not
become hypertensive in response to DOC-salt treat-
manship for carotid artery strips to KCl was sig-
FIGURE 1. Mean arterial pressure of sham lesion and area postrema lesion (APX) rats treated with DOC-salt or high salt alone (control). Sham lesion–DOC-salt rats had significantly elevated blood pressures when compared with sham lesion–control rats (p < 0.05, by ANOVA and least significant difference test). Mean arterial pressures of APX-DOC-salt rats were not different from APX-control rats (p > 0.05, by ANOVA and least significant difference). There also were no significant differences in blood pressure between normal sodium-control and normal sodium–DOC rats (p > 0.05, by t test).

As shown in Figure 3, mesenteric artery strips from sham lesion–DOC-salt rats display an increased sensitivity to serotonin when compared with those from sham lesion–control rats. APX-DOC-salt rats also exhibited increased sensitivity to serotonin when compared with APX-control rats (ED50 values reported in Table 1). F values from the two-way ANOVA for log ED50 values to serotonin were 4.67 for FDOC (p < 0.05), 0.29 for FAPX (p = NS), and 0.17 for FDOC × APX (p = NS). Maximal contractile responses to serotonin did not differ between groups (sham lesion–control: 476 ± 23 mg; APX-control: 488 ± 49 mg; sham lesion–DOC-salt: 548 ± 50 mg; APX-DOC-salt: 490 ± 32 mg).

Contractile responses to 10^{-3} M ouabain were significantly greater in aortas from sham lesion–DOC-salt rats when compared with aortas from sham lesion–control rats (Figure 4; see Table 1). Again, APX–DOC-salt rats exhibited an increased sensitivity to ouabain when compared with APX-control rats. F values from the two-way ANOVA for the contractile responses to 10^{-3} M ouabain (arc sin square root transformation of the percentage of contraction to 130 mM KCl PSS) were 10.7 for FDOC (p < 0.05), 0.81 for FAPX (p = NS), and 0.20 for FDOC × APX (p = NS). The contractile response to 130 mM KCl PSS did not differ between groups (sham lesion–control: 1.66 ± 0.09 g; APX-control: 1.57 ± 0.13 g; sham lesion–DOC-salt: 1.68 ± 0.10 g; APX–DOC-salt: 1.65 ± 0.04 g).

As shown in Figure 1, DOC treatment did not produce a significant increase in mean arterial pressure in rats on a normal sodium intake (normal sodium–control = 108 ± 3 mm Hg; normal sodium–DOC = 113 ± 2 mm Hg). Vascular contractile re-

![Graph](https://i.imgur.com/3Q5J5Q.png)

**Figure 2.** Concentration-response curves to KCl in carotid arteries from sham lesion–DOC-salt (●), area postrema lesion (APX)–DOC-salt (■), APX-control (●), and sham lesion–control rats (●). Carotid arteries from sham lesion–DOC-salt rats exhibited an increased sensitivity to KCl when compared with those from sham lesion–control rats. This relationship was not altered in APX rats. $F_{DOC}$ × APX (p = NS). Maximal contractile responses to serotonin did not differ between groups (sham lesion–control: 476 ± 23 mg; APX-control: 488 ± 49 mg; sham lesion–DOC-salt: 548 ± 50 mg; APX–DOC-salt: 490 ± 32 mg).

<table>
<thead>
<tr>
<th>Rat group</th>
<th>Carotid artery: KCl sensitivity (ED50, mM)</th>
<th>Mesenteric artery: serotonin sensitivity (ED50, M)</th>
<th>Aorta: 10^{-3} M ouabain sensitivity (KCl max, % KCl max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham lesion–DOC-salt (n = 5)</td>
<td>13 ± 2</td>
<td>3.5 (± 0.06) × 10^{-7}</td>
<td>55 ± 14</td>
</tr>
<tr>
<td>AP lesion–DOC-salt (n = 5)</td>
<td>16 ± 4</td>
<td>3.2 (± 0.05) × 10^{-7}</td>
<td>40 ± 10</td>
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<tr>
<td>Sham lesion–control (n = 5)</td>
<td>25 ± 1</td>
<td>9.4 (± 3.4) × 10^{-7}</td>
<td>20 ± 10</td>
</tr>
<tr>
<td>AP lesion–control (n = 5)</td>
<td>23 ± 1</td>
<td>5.9 (± 1.3) × 10^{-7}</td>
<td>13 ± 4</td>
</tr>
<tr>
<td>Normal Na–control (n = 6)</td>
<td>25 ± 3</td>
<td>5.9 (± 1.2) × 10^{-7}</td>
<td>22 ± 8</td>
</tr>
<tr>
<td>Normal Na–DOC (n = 7)</td>
<td>21 ± 3</td>
<td>7.1 (± 1.3) × 10^{-7}</td>
<td>25 ± 6</td>
</tr>
</tbody>
</table>

Values are means ± SEM. See text for description of statistical analysis.

ED50 = concentration that produced 16% of the maximal response; % KCl max = percentage of contractile response to 130 mM KCl physiological salt solution; AP = area postrema.
FIGURE 3. Concentration-response curves to serotonin in mesenteric arteries from area postrema lesion (APX)-DOC-salt (■), sham lesion–DOC-salt (○), APX-control (●), and sham lesion–control rats (○). The increased sensitivity to serotonin in mesenteric arteries from DOC-salt rats was not altered by APX.

FIGURE 4. Concentration-response curves to ouabain in aortas from area postrema lesion (APX)-DOC-salt (■), sham lesion–DOC-salt (○), APX-control (●), and sham lesion–control rats (○). % KCl maximum = percentage of contractile response to 130 mM KCl PSS. The increased contractile response to 10^{-5} M ouabain in mesenteric arteries from DOC-salt rats was not altered by APX.

FIGURE 5. Concentration-response curves to KCl (top panel), serotonin (middle panel), and ouabain (bottom panel) in control and normal sodium–DOC rats. There were no differences in sensitivity to these agonists between rat groups.

Discussion

In this study, the importance of the AP in the development of hypertension and changes in vascular reactivity in response to DOC-salt treatment was assessed in rats. In rats with lesions of the AP, hypertension did not result after 5 weeks of DOC-salt treatment. Despite the ability of APX to prevent the rise in arterial pressure in response to DOC-salt treatment, APX was without effect on the increased sensitivity to serotonin, KCl, and ouabain observed in isolated vascular strips from DOC-salt rats. Thus, a dissociation between the effects of APX on blood pressure and vascular reactivity in DOC-salt rats was observed in these studies.

The results of several published reports are consistent with the hypothesis that both hypertension and changes in vascular reactivity in DOC-salt rats are dependent on an intact central nervous system. Increases in norepinephrine and vasopressin sensitivity observed in the renal and mesenteric vasculature of DOC-salt rats are prevented by intracerebroventricular administration of 6-OHDA. Similarly, increases in sensitivity of isolated vascular strips to norepinephrine, serotonin, arachidonic acid, and ouabain observed in DOC-salt rats are prevented by prior central 6-OHDA administration. It is unlikely that these changes in vascular reactivity are secondary to increases in wall tension resulting from elevated blood pressure, since antihypertensive therapy and protection of a vascular bed from elevated pressure by arterial ligation in DOC-salt rats did not prevent the development of increased vascular sensitivity. Furthermore, changes in renal vascular sensitivity to norepinephrine, vasopressin, and angiotensin II occur prior to detectable increases in blood pressure after the initiation of DOC-salt treatment. Clearly, the results of the present experiments in which changes in vascular reactivity were present in normotensive APX–DOC-salt rats argue against the hypothesis that these changes in vascular reactivity are pressure-dependent.

One possible interpretation of the results of the present experiments is that DOC-salt acts directly on the vasculature to produce changes in reactivity. Mineralocorticoids have been shown to have a variety of
direct effects on vascular smooth muscle in vitro. These actions include a decrease in free intracellular sodium secondary to stimulation of Na\(^+\),K\(^+\)-adenosine triphosphate (ATPase) and potentiation of contractile responses to catecholamines, probably by inhibiting extraneuronal uptake. In the current studies, DOC treatment alone, in the absence of high salt intake, did not produce hypertension or functional vascular changes in rats. These results support the contention that increases in vascular sensitivity in APX-DOC-salt rats are not due to a direct effect of DOC on the vasculature. Furthermore, since vascular reactivity is normal in DOC-salt rats pretreated with central 6-OHDA, the combination of DOC plus high salt intake does not appear to have a direct effect on smooth muscle to produce increases in vascular sensitivity.

The particular contractile agonists used in these studies (serotonin, KCl, and ouabain) are of interest since mechanisms responsible for increases in sensitivity to these agents have been implicated as possible contributors to elevated peripheral vascular resistance in hypertension. Vascular sensitivity to serotonin in hypertension is increased to a greater degree than is the sensitivity to other vasoconstrictors such as angiotensin II or norepinephrine. The increased in vascular sensitivity to serotonin in DOC-salt rats does not appear to relate to a change in receptor affinity or to an alteration in transmembrane movement of calcium following receptor activation, but rather to an altered mobilization of calcium from an intracellular store. Contraction in response to KCl is due to depolarization secondary to a reduction in the potassium gradient. The specific cellular mechanism responsible for the difference in KCl sensitivity between DOC-salt hypertensive rats and normotensive rats is not known. Possibilities include alterations in membrane potassium conductance or in the threshold for opening of potential-sensitive calcium channels. The contractile response to ouabain is due to inhibition of the electrogenic Na\(^+\),K\(^+\)-ATPase. The increased contractile sensitivity to ouabain observed in isolated vascular smooth muscle in DOC-salt hypertension is generally thought to be secondary to either 1) elaboration of a humoral inhibitor of Na\(^+\),K\(^+\)-ATPase, which causes up-regulation of Na\(^+\),K\(^+\) pump sites, or 2) a primary increase in membrane permeability to sodium, which results in activation of the Na\(^+\),K\(^+\) pump. It has been hypothesized that one mechanism by which anteroventral third ventricle ablation prevents DOC-salt hypertension in rats is by inhibition of the production of a humoral Na\(^+\),K\(^+\)-ATPase inhibitor. In the present experiments, no direct attempt was made to quantify a Na\(^+\),K\(^+\)-ATPase inhibitor in the plasma. However, if production of a humoral Na\(^+\),K\(^+\) pump inhibitor in vivo does translate into a greater vascular contractile response to ouabain in vitro, then AP lesion probably does not prevent DOC-salt hypertension by interfering with production of the Na\(^+\),K\(^+\) pump inhibitor (increased contractile sensitivity to ouabain was present in aortas from both sham lesion-DOC-salt and APX-DOC-salt rats).

The precise mechanisms by which APX prevents DOC-salt hypertension are not clear. There is much evidence in the literature that sympathetic nervous system activity is elevated in DOC-salt hypertensive rats. The depressor response to ganglion blockade, which has been used as an index of cardiovascular neurogenic tone, is increased in DOC-salt rats. In rats with AP lesions, DOC-salt treatment does not result in an augmented depressor response to ganglion blockade. These data suggest that APX may interfere with an action of DOC-salt to modulate sympathetic outflow.

In summary, electrolytic ablation of the AP prevented hypertension, but not changes in vascular reactivity to serotonin, KCl, or ouabain in isolated blood vessels from DOC-salt rats. Regardless of the mechanism by which APX prevents the development of hypertension, integrity of fibers originating in or passing through the AP is not necessary for DOC-salt treatment to produce changes in vascular reactivity. Since central 6-OHDA treatment prevents both hypertension and changes in vascular reactivity, APX treatment may produce these vascular changes by acting at a central site other than the AP. Finally, in the neurally intact rat, changes in vascular reactivity may be necessary for DOC-salt hypertension to develop, but these vascular changes alone are not sufficient to produce a rise in blood pressure.

References

Area postrema ablation and vascular reactivity in deoxycorticosterone-salt-treated rats.
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