SUMMARY We studied the effects of anteroventral third ventricle (AV3V) lesions on the vascular Na⁺-pump activity of deoxycorticosterone acetate-salt (DOCA-salt) treated rats. Blood pressures and Na⁺-pump activity of the isolated tail arteries, measured as ouabain-sensitive ⁸⁶Rb⁺-uptake, were determined in untreated control rats, DOCA-salt treated rats, rats with AV3V lesions, and rats with AV3V lesions which were treated with DOCA-salt. Control rats receiving DOCA treatment developed higher blood pressures than rats receiving no DOCA treatment. Placement of AV3V lesions prior to administration of DOCA prevented the increase in blood pressure. Vascular Na⁺-pump activity in the DOCA-treated group was reduced by 20% compared to all other groups. The AV3V lesions prevented the suppression of Na⁺-pump activity caused by DOCA treatment.

Suppression of vascular Na⁺-pump activity was due to a humoral substance since Na⁺-pump activity of tail arteries from control rats incubated in plasma from DOCA-salt treated rats was suppressed by 25% when compared to those incubated in control plasma. Our findings support the hypothesis that a circulating pressor substance is at least partially responsible for the development of DOCA-salt hypertension and that the mechanism by which AV3V lesions prevent DOCA hypertension may be through the interruption of secretion, transport, or synthesis of this factor.

(Hypertension 4: 575-580, 1982)

KEY WORDS • hypertension • blood pressure • Na⁺-pump activity • Rb-uptake • tail artery • AV3V lesion

It is well known that digitalis or exposure to media low in potassium inhibits vascular Na⁺, K⁺-ATPase, the biochemical correlate of the cellular Na⁺-pump. Inhibition of the vascular Na⁺ pump produces vasoconstriction and increases vascular sensitivity to vasoconstrictor agents. These vascular changes are present in several forms of hypertension and suggest that a depressed Na⁺-pump activity is involved in high blood pressure. In fact, vascular Na⁺-pump activity is suppressed in several forms of experimental hypertension and sodium transport is reduced in blood cells from patients with essential hypertension.

As early as 1940, a suggestion was made that a slowly acting pressor agent is present in the blood of hypertensive animals. Similar findings were subsequently reported for humans with certain types of hypertension. More recently Gruber et al. have found a material in plasma of volume expanded dogs and hypertensive monkeys which binds specifically with antibodies to digoxin and inhibits Na⁺, K⁺-ATPase activity. Additionally, Pampanini et al. have shown that the supernatant of boiled plasma from dogs with one-kidney, one-wrapped hypertension decreased Na⁺-pump activity of tail arteries from normotensive rats. Thus a blood-borne substance, functionally similar to digitalis glycosides, may be responsible for impaired sodium and potassium transport in the vascular smooth muscle and blood cells of hypertensive subjects. This substance may be central in origin as several investigators have reported the isolation of a substance from whole guinea pig brains or bovine hypothalamus which inhibits active sodium transport and ouabain binding to Na⁺, K⁺-ATPase.

A variety of types of experimental hypertension in rats are prevented by a selective lesion in the periventricular tissue of the anterior and ventral portion of the third ventricle (AV3V).

Interestingly, depressed vascular Na⁺-pump function has also been demonstrated in many of these same...
hypertensive states. Deoxycorticosterone acetate-salt (DOCA-salt) hypertension is an experimental form prevented by AV3V lesions and also shows suppressed vascular Na⁺-pump activity, but the effects of AV3V lesions on Na⁺-pump activity in rats given DOCA-salt treatment are not known. The purpose of the present study was to determine if the rats with AV3V lesions that fail to develop DOCA-salt hypertension also fail to show altered Na⁺-pump activity. Indeed, our results showed that rats with this lesion fail to show depressed Na⁺-pump activity as well as a failure to develop hypertension following DOCA treatment and that Na⁺-pump inhibition is due to a blood-borne factor. These findings suggest that failure of development of DOCA-salt hypertension in rats with AV3V lesions is due in part to interruption of secretion, transport, or synthesis of a humoral substance which suppresses Na⁺-pump activity.

Methods

Anteroventral Third Ventricle (AV3V) Lesions

Male Sprague-Dawley rats weighing 170 to 200 g, purchased from Harlan Company (Madison, Wisconsin) were used throughout these experiments. Rats were given electrolytic lesions in the AV3V region of the brain or underwent control surgery. This procedure has been previously described in detail. Briefly, under ether anesthesia, rats were given an anodal lesion (rectal cathode) of the AV3V periventricular tissue by passing a 3 mA DC current for 20 seconds through a Nichrome wire (26 ga) electrode placed stereotaxically into the AV3V region. This region is 0.3 mm posterior to the bregma referent, on the midline, and 7.3 mm below the dura mater. Control operated rats underwent identical procedures except the electrodes were lowered only 5.5 mm and no current was passed.

All rats received a single injection of penicillin G, 60,000 U/rat, i.m. following surgery. Water consumption was measured the night following the surgery, and those rats which were adipsic (<10 ml water consumed) were given 5% sucrose solution the next day to induce drinking. After one day, all animals were returned to water. The extent and the location of the lesions were verified histologically upon completion of the experiments, and data from rats which did not have bilateral periependymal damage in the AV3V region were discarded.

Deoxycorticosterone Acetate-Salt (DOCA-Salt) Treatment

After 1 week of recovery from surgery, left kidneys of all the rats were removed under light ether anesthesia. Rats were then allowed to recover from uninephrectomy for a week and Silastic capsules containing deoxycorticosterone acetate (Sigma Chemical Company, St Louis, Missouri, D-7000, 100 mg/kg), prepared according to Smith et al. were implanted subcutaneously (s.c.) between the scapulae. Control rats received similar capsules containing no drug. Immediately following capsule implantation, all rats were given only 1% NaCl + 0.2% KCl solution to drink. This treatment resulted in a steady rise in systolic blood pressure reaching values of 150 mm Hg within three weeks, and 180 to 190 mm Hg within five to six weeks. The ⁶¹Rb⁺-uptake measurements were made between weeks five and six. A separate group of rats received no treatment for this same period.

Blood Pressure Measurement: Tail-Cuff Plethysmography

Blood pressure was measured prior to treatment, and then once weekly throughout the experiment. Conscious rats were heated to 37°C for 15 minutes and then placed in restraining cages for measurement of blood pressure using tail-cuff plethysmography (Narco Biosystems, Inc.). Systolic blood pressures exceeding 150 mm Hg were considered hypertensive.

Measurement of Na⁺-pump Activity: ⁶¹Rb⁺-uptake of Isolated Rat Tail Artery

The Na⁺-pump activity of the isolated tail arteries was assessed by measuring ⁶¹Rb⁺-uptake with methods similar to those described by Overbeck et al. Rats were anesthetized with pentobarbital (50 mg/kg) i.p. and the tail arteries were dissected and cleaned within 10 minutes in Krebs-Henseleit buffer (Composition, in mM: NaHCO₃, 27.2; NaCl, 117.0; NaH₂PO₄ • H₂O, 1.0; MgSO₄ • H₂O, 1.2; CaCl₂ • 2 H₂O, 2.5; dextrose, 11.0; KCl, 5.0). Arteries were then cut into 5 to 6 pieces of approximately the same size and transferred into beakers containing ice-cold (4°C), K⁺-free Krebs-Henseleit buffer for a 10 minute preincubation. This procedure reduces the Na⁺-pump activity, allowing the tissue to accumulate Na⁺, and enhances ⁶¹Rb⁺-uptake during subsequent incubation. At the end of the preincubation period, pieces of tail artery were transferred into incubation tubes containing 1 ml of the rat's own plasma obtained from the abdominal aorta immediately following the tail artery dissection. Incubation tubes (with or without ouabain, final concentration 1.0 mM) containing the pieces of tail artery were aerated with 95% O₂ + 5% CO₂ for 30 seconds at room temperature and warmed up for 2 minutes at 37°C in a shaking bath. The isotope (⁶¹RbCl, 0.1 mM final, 10⁴CPM/ml) was added and the artery incubated for an additional 15 minutes.

At the end of the incubation period, pieces of artery were washed in four consecutive beakers containing 100 ml of Krebs-Henseleit buffer, blotted dry and weighed. Tissue pieces were placed into polyethylene test tubes and radioactivity counted by a γ-counter (Searle Radiographics, DesPlaines, Illinois). Three determinations were obtained for each artery. Specific uptake of ⁶¹Rb⁺ was determined (in nmol/mg wet weight/15 min) as the difference between uptake in the presence of 1.0 mM ouabain and uptake in the absence of ouabain. ⁶¹Rb⁺-uptake in the presence of ouabain reflects the distribution of ⁶¹Rb⁺ in extracellular spaces and passive penetration into the cells.

Results were expressed on wet weight basis because wet weight to dry weight ratio of the vascular tissue was found to be the same in each group. Flame photometry (Instrumentation Laboratories, Watertown
Massachusetts, Model 443) was used to assess the plasma potassium ion concentrations. Data were reported as means ± se. The Student's t test was used for comparing the means; differences were considered significant if p < 0.05.

Procedures

Experiment 1

Blood pressure and vascular Na\(^+\)-pump activity were measured in five groups of rats 5 and 6 weeks after initiation of DOCA-salt treatment. One group of rats received AV3V lesions and were treated with DOCA (Group LD). A second group also received AV3V lesions, but no DOCA treatment (Group LC). A third group underwent a control surgery but did not receive AV3V lesions, and these rats were treated with DOCA (Group SD). Members of the fourth treatment group also underwent control surgery and received no DOCA treatment (Group SC). A fifth group of rats received no treatment (Group UC).

Experiment 2

To determine any effects on Na\(^+\)-pump activity due to a humoral agent, tail arteries from untreated control rats were incubated in plasma from DOCA-treated rats. In addition, tail arteries from DOCA-treated rats were incubated in plasma from untreated control rats. One part of each vessel was incubated in the plasma obtained from the same animal and the other half in the plasma of a rat from the other group. The \(^{86}\text{Rb}^+\)-uptake was determined as previously described.

Suppression of Na\(^+\)-pump activity may be merely an immune response to the incubation of an artery in another rat's plasma rather than a specific humoral Na\(^+\)-pump inhibitor. To assess this, one-half of a tail artery from an untreated rat was incubated in the plasma of another untreated rat. The other half of the artery was incubated in the animal's own plasma. Uptake of \(^{86}\text{Rb}^+\) was determined in both pieces of tissue.

Results

Experiment 1

Rats receiving DOCA treatment following sham lesions (Group SD) developed significantly higher systolic blood pressures than control operated rats receiving no DOCA (Group SC) (fig. 1). Placement of AV3V lesions prior to administration of DOCA (Group LD) prevented the increase in blood pressure normally caused by DOCA treatment. In addition, rats with lesions in the AV3V region which received no DOCA (Group LC) had blood pressure similar to Group SC indicating the AV3V lesions alone do not alter blood pressure. Initial, pretreatment systolic blood pressure values were similar in all groups (combined mean: 109 ± 2 mm Hg, N = 41).

Figure 2 illustrates mean specific vascular \(^{86}\text{Rb}^+\)-uptake in the five experimental groups of rats. Vascular Na\(^+\)-pump activity in Groups SD was reduced by 20% (p < 0.05) compared to Group SC. The Na\(^+\)-pump activity in Group LD was the same as Group SC indicating that the AV3V lesion prevented the suppression of Na\(^+\)-pump activity caused by DOCA treatment. Neither AV3V ablation, nor uninephrectomy and drinking of salt water altered basal vascular Na\(^+\)-pump activity, as values for Groups SC and LC were the same as Group UC. Ouabain insensitive or nonspecific \(^{86}\text{Rb}^+\)-uptake was the same in all groups (table 1).

Since DOCA treatment can result in hypokalemia and since low potassium can suppress Na\(^+\)-pump activity, we measured plasma potassium ion concentrations in all treatment groups to determine if a correlation existed between \(^{86}\text{Rb}^+\)-uptake and plasma potassium concentrations. No differences in plasma potassium concentrations were observed (Table 1). However, suppression of the Na\(^+\)-pump activity was noted only in group SD. These data indicate that the depressed Na\(^+\)-pump activity cannot be due to a reduction in plasma potassium levels.
TABLE 1: Plasma Potassium Ion Concentrations and Specific and Nonspecific $^{86}$Rb$^+$ Uptake in Five Experimental Groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>$K^+$ (mEq/liter)</th>
<th>Specific $^{86}$Rb$^+$-uptake (nmole/mg/15 min)</th>
<th>Nonspecific $^{86}$Rb$^+$-uptake (nmole/mg/15 min)</th>
<th>No. of rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC</td>
<td>3.3 ± 0.1</td>
<td>0.301 ± 0.018</td>
<td>0.047 ± 0.004</td>
<td>7</td>
</tr>
<tr>
<td>SD</td>
<td>3.1 ± 0.2</td>
<td>0.243 ± 0.023*</td>
<td>0.051 ± 0.002</td>
<td>8</td>
</tr>
<tr>
<td>LC</td>
<td>3.4 ± 0.2</td>
<td>0.301 ± 0.032</td>
<td>0.050 ± 0.003</td>
<td>8</td>
</tr>
<tr>
<td>LD</td>
<td>2.9 ± 0.1</td>
<td>0.302 ± 0.028</td>
<td>0.050 ± 0.002</td>
<td>8</td>
</tr>
<tr>
<td>UC</td>
<td>3.5 ± 0.1</td>
<td>0.299 ± 0.012</td>
<td>0.057 ± 0.004</td>
<td>10</td>
</tr>
</tbody>
</table>

$^*$p < 0.05 compared to "SC'.

Abbreviations: SC = sham-operated control rats, no DOCA treatment; SD = sham-operated, DOCA-treated rats; LC = AV3V-lesioned control rats, no DOCA treatment; LD = AV3V lesioned and DOCA-treated rats; UC = untreated controls. Values are expressed as means ± SE.

TABLE 2: $^{86}$Rb$^+$ Uptake of Tail Arteries from Untreated Control and DOCA-Treated Rats

<table>
<thead>
<tr>
<th>Incubations</th>
<th>$^{86}$Rb$^+$-uptake (nmole/mg/15 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control artery in control plasma</td>
<td>0.408 ± 0.021</td>
</tr>
<tr>
<td>Control artery in DOCA plasma</td>
<td>0.305 ± 0.017*</td>
</tr>
<tr>
<td>DOCA artery in DOCA plasma</td>
<td>0.346 ± 0.027†</td>
</tr>
<tr>
<td>DOCA artery in control plasma</td>
<td>0.328 ± 0.026‡</td>
</tr>
</tbody>
</table>

$^*$p < 0.0005 compared to "Control artery in control plasma." †p < 0.05 compared to "Control artery in control plasma." ‡Not significantly different compared to "DOCA artery in DOCA plasma." Values are expressed as means ± SE; n = 10 for all groups.

Experiment 2

Results of experiments in which tail arteries from untreated control rats were incubated in plasma from DOCA-treated rats, and tail arteries from DOCA-treated rats incubated in plasma from untreated control rats are presented in table 2. The uptake of $^{86}$Rb$^+$ by tail arteries from control rats incubated in plasma from DOCA-hypertensive rats was suppressed by 25% when compared to incubations in control plasma. The uptake of $^{86}$Rb$^+$ by tail arteries from DOCA-treated rats remained low when incubated in the plasma from a control rat, possibly due to a tight binding of a "digitalis-like factor" to the vascular smooth muscle. As found in experiment 1, $^{86}$Rb$^+$-uptake of arteries from DOCA-treated rats incubated in their own plasma was lower than values obtained from arteries of control rats incubated in their own plasma. The uptake of $^{86}$Rb$^+$ in tail artery segments incubated in the plasma taken from the same untreated rat was compared to that of segments incubated in plasma from another control rat and found to be the same (0.380 ± 0.026 and 0.376 ± 0.015 nmole/mg/15 min respectively; N = 6). This indicates that suppression of Na$^+$-pump activity was not due to an immune response.

Discussion

We have demonstrated that both suppression of vascular Na$^+$-pump activity and the development of hypertension in DOCA-treated rats are prevented by placement of electrolytic lesions in the AV3V area of the brain. It has been previously shown that vascular Na$^+$-pump activity is suppressed in DOCA-salt hypertension and that this hypertension is prevented by AV3V lesions. Reductions in the vascular Na$^+$-pump activity in hypertension may be due to a blood-borne factor since arteries isolated from dogs with one-kidney perinephritic hypertension showed suppressed $^{86}$Rb$^+$-uptake when incubated in the dogs' own plasma but was not when incubated in Krebs-Henseleit medium. In addition, the supernatant of boiled plasma from dogs with one-kidney, one-wrapped hypertension inhibited Na$^+$-pump activity in the tail arteries of normotensive rats. Our findings support the hypothesis that a circulating pressor substance is at least partially responsible for the development of DOCA-salt hypertension and the mechanism by which AV3V lesions prevent DOCA hypertension may be through the interruption of secretion, transport, or synthesis of this.
factor. Suppression of Na⁺-pump activity was not due to the low plasma potassium concentration, since plasma potassium concentrations of the control and DOCA-hypertensive rats were not significantly different (table I). In addition, depressed Na⁺-pump activity was probably not due to differences in intracellular sodium concentrations between the controls and the hypertensive rats, since it has been reported by Brock and Overbeck²⁵ that the intracellular sodium concentrations of DOCA-salt treated rats were the same as their controls.

Lesions of the AV3V region have been shown to alter Na⁺-pump activity. Pamnani et al.²⁶ found that during acute volume expansion, ⁸⁸Rb⁺-uptake was 48% higher in AV3V-lesioned rats than in similarly treated sham-lesioned rats. Additionally, rats with AV3V lesions have a deficit in natriuresis when volume expanded with i.v. saline compared to controls. This may be due to inhibition of a circulating "natriuretic factor."²⁷ Since plasma samples of these lesioned rats did not inhibit sodium transport in the toad urinary bladder as did plasma from control rats.²⁷ These two studies suggest that a humoral substance related to sodium excretion alters vascular Na⁺-pump activity. It has been proposed that such a natriuretic factor is the "circulating pressor substance" and may play an important role in the etiology of volume expanded hypertension.²⁵ Since AV3V lesions prevent both the natriuresis following volume expansion²⁷ and the forms of hypertension which display suppressed vascular Na⁺-pump activity,²⁸ it appears that the natriuretic factor is the same as the circulating pressor substance.

Several mechanisms have been proposed to explain how AV3V lesions prevent the development of hypertension in this and other experimental models. Brody and Johnson²⁹ suggested that interruption of angiotensin pressor mechanisms contributes to the protective effect of the lesion since pressor responses to centrally and peripherally administered angiotensin II are attenuated by AV3V ablation. Berecek et al.³⁰ proposed that altered release of vasopressin, or interference with pathways required for the mechanism of action of vasopressin, account for the protective effect of the AV3V lesions on DOCA-salt hypertension. The present experiments indicate that interruption of release or synthesis of a humoral pressor factor may be also involved.

Considerable controversy exists in the literature concerning the state of vascular Na⁺-pump activity in several natural and experimental forms of hypertension. Although it was originally suggested by Haddy and Overbeck²⁵ that only blood volume expanded types of hypertension display suppressed vascular Na⁺-pump activity, this characteristic is not unique to such forms. For example, uncomplicated essential hypertensive patients show attenuated vasodilatory responses to intraarterial infusion of potassium, suggesting suppressed vascular Na⁺-pump activity.³¹ This form of hypertension is not accompanied by volume expansion, on the contrary, most investigators report decreased plasma volume in such patients.³²,³³ More-over, Overbeck and coworkers³⁴ recently showed that Dahl salt-sensitive rats, which have increased vascular Na⁺-pump activity, actually have expanded plasma volume. More recent reports from the same laboratory showed that when incubated in Krebs-Henseleit buffer, the tail arteries from DOCA-salt, one-kidney Goldblatt and one- and two-kidney Goldblatt hypertensive rats³⁵ displayed an increased ouabain-sensitive ⁸⁸Rb⁺-uptake compared to their controls. The apparent discrepancy between our results and the findings of Brock and Overbeck²⁵ may be due to the different incubation conditions. They incubated the tail arteries in Krebs-Henseleit buffer whereas we incubated the arteries in the rats' own plasma. Neither findings, ours or theirs,³⁵,³⁶ exclude the possibility of a circulating digitalis-like plasma factor in hypertension. The increased Na⁺-pump activity observed by Brock and Overbeck²⁵ following Krebs-Henseleit incubation may be due to either formation of more Na⁺, K⁺-ATPase molecules or an allosteric activation of this enzyme in the smooth muscle sarcolemma due to long-term exposure to a circulating inhibitor. However, when these arteries are incubated in plasma, the otherwise heightened activity may be suppressed even below control levels due to a continuous exposure to the plasma-borne factor during incubation. Such an increase in Na⁺, K⁺-ATPase activity has been observed in the isolated erythrocytes of patients chronically treated with digoxin.³⁶ In addition, an increased active transport of sodium and potassium ions has been observed in arterial smooth muscle from DOCA-salt hypertensive rats.³⁷,³⁸ It has been suggested that these increases in vascular Na⁺-pump activity result from elevations in the concentration of intracellular sodium associated with increased passive permeability of the cell membrane to this ion.³⁹,⁴⁰ Interestingly, we have preliminary evidence suggesting that during the early stages of DOCA-salt hypertension vascular Na⁺-pump activity is enhanced. This issue requires further investigation.

Recent studies have supported the existence of a digitalis-like, blood-borne substance in hypertensive states. Gruber et al.,¹⁹ using radioimmunoassay, have demonstrated higher levels of a blood-borne component which cross reacts specifically with antibodies to digoxin in plasma from monkeys with spontaneous hypertension and two-kidney, one clip Goldblatt hypertension than was found in normotensive controls. This digoxin-like substance has been termed "endoxin" and exists in normotensive controls in much smaller concentrations. Also recently, Poston et al.¹¹ have demonstrated that serum from patients with essential hypertension impairs sodium transport in the white cells of normotensive subjects. The impairment in sodium transport was due to a fall in the ouabain-sensitive component of the total sodium efflux rate-constant indicating that serum of patients with essential hypertension possesses a digitalis-like substance. Our own data indicate the existence of a substance in the plasma of DOCA-hypertensive rats which, like digitalis, inhibits ⁸⁸Rb⁺-uptake in the tail arteries isolated from normotensive rats.
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