Phasic Vascular Sodium Pump Changes in Deoxycorticosterone-Hypertensive Rats

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SUMMARY. We determined the sodium pump activity, measured as ouabain-sensitive $^{86}$Rb$^+$ uptake, in the tail arteries of rats treated with deoxycorticosterone and sodium chloride for 6, 9, 14, 28, and 50 days. Systolic blood pressures, plasma sodium, potassium, and creatinine concentrations were measured, and the body weights were recorded. Vascular sodium pump activity was suppressed (by 27%) at the 6th day of deoxycorticosterone and sodium chloride treatment, a prehypertensive state. By the 9th day, blood pressure of deoxycorticosterone-treated and sodium chloride rats had increased, but sodium pump activity was not different from that of control animals. However, increases in sodium pump activity were noted after 14 and 28 days of deoxycorticosterone and sodium chloride treatment (18 and 21%, respectively). By 28 days, a fully developed hypertension was noted. At 50 days, rats displayed lower vascular sodium pump activity (by 23%) than the controls. These rats, although hypertensive, had significantly lower systolic blood pressures than the rats treated for 28 days. They had high plasma creatinine levels, low potassium and sodium concentrations, and low body weights compared to the controls, suggesting the presence of a malignant state. Our data indicate that there are time-related changes in the vascular sodium pump activity with this type of hypertension. (Circ Res 55: 304-308, 1984)

SODIUM, potassium-ATPase, the biochemical correlate of the electrogenic Na$^+$ pump, contributes to vascular smooth muscle tone by affecting the cellular membrane potential (Thomas, 1972; Fleming, 1980). Alterations in Na$^+$ pump activity and abnormalities of transmembrane monovalent cation transport have been reported in hypertensive animal models (Pamnani et al., 1981) and human essential hypertension (Tosteson et al., 1981), but there is disagreement regarding the direction of these changes.

Some investigators have reported suppressed vascular Na$^+$ pump activity in a number of experimental models of hypertension (Pamnani et al., 1978, 1981; Huot et al., 1983), including deoxycorticosterone acetate (DOCA)-salt hypertension (Pamnani et al., 1978). We also have shown that, at an advanced stage (5–6 weeks) of DOCA-salt hypertension, vascular Na$^+$ pump activity was depressed in rats (Songu-Mize et al., 1982). However, other laboratories have reported that vascular Na$^+$ pump activity was increased in rats treated with DOCA-salt for a 3- to 4-week period (Friedman, 1974; Jones, 1980; Brock et al., 1982). Although DOCA-salt hypertension is generally accepted to be a volume-expanded model (Laragh, 1973; Haddy, 1980), rats treated with DOCA-salt show expansion of fluid volume only during the benign phase of hypertension as a result of sodium retention (Gavras et al., 1975; Möhring et al., 1977; Yamamoto et al., 1983). The malignant phase of DOCA-salt hypertension, starting about the 5th week of treatment, is characterized by negative Na$^+$ balance and loss of body weight, suggesting loss of body fluid (Gavras et al., 1975; Möhring et al., 1977; Yamamoto et al., 1983).

To determine whether the differences among reported findings could be due to time-related alterations in the vascular Na$^+$ pump activity during the development and maintenance of DOCA-salt hypertension, we studied the specific uptake of $^{86}$Rb$^+$ in the tail arteries of rats during a prehypertensive and an early stage (6 and 9 days), two intermediate stages (14 and 28 days), and a late stage (50 days) of DOCA-salt treatment. During the prehypertensive stage, the vascular Na$^+$ pump activity was suppressed. However, it was increased at days 14 and 28 of treatment when hypertension had developed. At a late and possibly malignant stage of hypertension, rats displayed a suppressed vascular Na$^+$ pump activity. Our data indicate that changes in vascular Na$^+$ pump activity are related to the length of time animals undergo DOCA-salt treatment, but do not correspond to changes in the arterial blood pressure.

Methods

Sprague-Dawley rats (Harlan Co.) weighing 170–200 g were used for these experiments. Left kidneys of all the rats were removed under chloral hydrate anesthesia (350 mg/kg). After a week of recovery from uninephrectomy, the rats received weekly injections of deoxycorticosterone acetate.
acetate (DOCA, 25 mg/kg, Sigma Chemical Co.), suspended in peanut oil. The control rats received peanut oil injections. All rats were restricted to a 1% NaCl-0.2% KCl drinking solution throughout the treatment period. Blood pressures were measured by tail cuff plethysmography prior to treatment, and once weekly until the day of uptake measurements were made.

The Na+ pump activity of the isolated tail arteries was assessed by incubating the artery in plasma obtained from the same animal by aortic puncture, and measuring the uptake of 86Rb+ taken up by the tissue (Songu-Mize et al., 1983). Rats were anesthetized with ip chloral hydrate (350 mg/kg), and tail arteries were isolated and placed in Krebs-Henseleit buffer (composition in mm: NaHCO3, 27.2; NaCl, 117.0; NaH2PO4, 1.0; MgSO4, 1.2; CaCl2, 2H2O, 2.5; dextrose, 11.0; KCl, 5.0). Arteries were cleaned in this solution, and cut into five to six pieces of approximately the same length. This procedure was completed in less than 5 minutes, and took place at room temperature. The pieces of artery were then transferred into beakers containing ice-cold (4°C) K+-free Krebs-Henseleit buffer, for a 10-minute preincubation. This procedure loads the cells with Na+ and optimizes the Na+ pump activity. At the end of the preincubation period, tissue pieces were transferred into incubation tubes containing 1 ml of the rats' own plasma obtained from the abdominal aorta immediately following the tail artery isolation. 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, stoppered, and warmed up for 2 minutes at 37°C in a shaking bath. The isotope (86RbCl, 0.1 mM and 10 counts/min per 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 was counted by a y-counter (Searle Radiographics). Three determinations were obtained for each artery. Since the percentage of tissue water, calculated as the ratio of dry to wet weight of the tissue, was found to be the same in each group (% tissue water in treatment vs. control groups; 6-day: 71.55 ± 0.27 vs. 70.91 ± 0.40, 28-day: 72.10 ± 0.60 vs. 71.55 ± 0.42, and 50-day: 68.5 ± 0.78 vs. 70.07 ± 0.52, n = 5–8), results were expressed on a wet weight basis. Specific uptake of 86Rb+ (nmol/mg wet weight per 15 minutes) was determined as the difference between 86Rb+ uptake in the absence and presence of 1.0 mM ouabain. 86Rb+ uptake in the presence of ouabain, termed nonspecific uptake, reflects the distribution of 86Rb+ in extra-cellular spaces and passive penetration into the cells, whereas ouabain-specific 86Rb+ uptake reflects activity of the Na+ pump. Plasma sodium and potassium concentrations were measured by flame photometry (model 343, Instrumentation Laboratories). Plasma creatinine measurements were made to determine whether kidney impairment occurred.

Our previous study has shown that hypokalemia can result from chronic DOCA-salt treatment (Songu-Mize et al., 1982). Therefore, the potassium concentration of plasma from rats treated with DOCA-salt for 50 days was adjusted with KCl to match that of plasma from the matched control rats. Adjustment of plasma potassium concentration did not take more than 5 minutes and did not disrupt the design of the experiment. This adjustment of potassium assured that any suppression of Na+ pump activity noted in this group was not secondary to the low plasma potassium concentration.

Data are reported as means ± SE. Student’s t-test was applied for comparison of the groups. A P value of <0.05 was considered significant.

Results

Changes in Systolic Blood Pressure and Vascular Na+ Pump Activity

A summary of the changes noted in the systolic blood pressure and vascular Na+ pump activity is presented in Figure 1. Different groups of rats were studied for every time point during DOCA-salt treatment. Uninephrectomized rats drinking saline solution served as controls for every treatment group; our previous studies indicate no difference in vascular Na+ pump activity between the untreated rats drinking tap water and the uninephrectomized rats drinking saline solution (Songu-Mize et al., 1982). At a prehypertensive stage, 6 days after the initiation of DOCA-salt treatment there was no difference in the systolic blood pressure (128 ± 3 mm Hg) compared to the controls (131 ± 3 mm Hg). By the 9th day of treatment, blood pressure had begun to rise (8% increase compared to the 9-day controls), and at day 14, the systolic blood pressure was 148 ± 1 mm Hg (control group: 121 ± 2 mm Hg). Systolic blood pressure peaked at day 28 of DOCA-salt treatment (210 ± 8 mm Hg). At an advanced stage of hypertension, 50 days of treatment with DOCA-salt, the mean systolic blood pressure of the rats was significantly lower (156 ± 6 mm Hg) compared to the blood pressure of rats treated for 28 days, but was still higher than the controls (Table 1).

Vascular Na+ pump activity was suppressed by 27% on the 6th day of DOCA-salt treatment com-
Body Weights

Effect of Duration of DOCA-Salt Treatment on Body Weights

The mean starting body weights for control and treatment groups were 189 ± 3 g (n = 8) and 184 ± 3 g (n = 8), respectively. At the end of 28 days of treatment with DOCA-salt, rats weighed 362 ± 31 g (n = 4), the same as their salt-drinking controls, 363 ± 10 g (n = 4). However, at the end of 50 days of treatment, rats weighed 376 ± 11 g (n = 4), significantly less than the 50-day controls, 428 ± 29 g (n = 4).

Discussion

It is now believed that Na⁺,K⁺-ATPase is the biochemical counterpart of Na⁺ pump across cell membranes (Wallick et al., 1979), and that inhibition of Na⁺ pump activity in heart and blood vessels increases the contractility of cardiac muscle (Brace et al., 1974) and the tone of the vascular smooth muscle (Anderson, 1976). Interestingly, during hypertension, alterations in vascular Na⁺ pump activity have been reported in animal models of hypertension (Pamnani et al., 1981).

It has been proposed that alterations of the Na⁺ pump in cardiovascular tissues associated with certain experimental and human types of hypertension are due to a circulating Na⁺ pump inhibitor (Haddy and Overbeck, 1976; de Wardener and MacGregor, 1980; Gruber et al., 1982), and DOCA-salt, a low renin model of experimental hypertension, is one of these models (Pamnani et al., 1978). We have previously shown that rats treated with DOCA-salt for 5–6 weeks exhibit a suppressed vascular Na⁺ pump activity. In addition, plasma from these rats de-
pressed the Na\textsuperscript{+} pump activity of vascular tissue obtained from untreated control rats, indicating that a plasma factor is responsible (Songu-Mize et al., 1982). However, Brock et al. (1982), using a similar technique, found increased vascular Na\textsuperscript{+} pump activity in rats at the 4th week of DOCA-salt treatment. Additionally, Metzler et al. (1982) have measured, by radioimmunoassay, elevated levels of a digoxin-like substance in the plasma after 5 days of treatment with DOCA-salt, which would be expected to decrease Na\textsuperscript{+} pump activity. Levels of this digoxin-like substance returned to control levels on the 11th day of DOCA-salt treatment. Similarly, in our present experiments, the vascular Na\textsuperscript{+} pump activity, noted to be suppressed on day 6 of DOCA-salt treatment, was at control levels on the 9th day.

Realizing that these reported differences in Na\textsuperscript{+} pump activity may be related to the length of treatment with DOCA-salt, we were prompted to examine Na\textsuperscript{+} pump activity over a broad time span of treatment, between 6 and 50 days. Our findings indicate that there are indeed phasic changes in the vascular Na\textsuperscript{+} pump activity during the development, established, and advanced stages of DOCA-salt hypertension. At an early normotensive stage, 6 days after the beginning of treatment, vascular Na\textsuperscript{+} pump activity was decreased, compared to the values obtained with control rats, whereas Na\textsuperscript{+} pump activity was high compared to the corresponding control groups at days 14 and 28, when hypertension had fully developed. At day 50 of treatment, there was, again, a suppression in the vascular Na\textsuperscript{+} pump activity compared with the saline-drinking controls. At this late time, elevated plasma creatinine levels, weight loss, and lowered plasma sodium and potassium concentrations indicated a malignant stage of hypertension (Gavras et al., 1975; Möhring et al., 1977). Our present findings show that differences in vascular Na\textsuperscript{+} pump activity do exist at different time points during DOCA-salt treatment; the direction of these changes appears to be consistent temporally with several independent reports coming from different laboratories that studied vascular Na\textsuperscript{+} pump activity following DOCA-salt treatment of varying duration (Friedman, 1974; Brock et al., 1982; Pamnani et al., 1978). These seemingly inconsistent reports may be the product of the time period of treatment chosen for study.

Although suppression of the vascular Na\textsuperscript{+} pump seen at certain stages of DOCA-salt hypertension may be due to a specific inhibitor of the Na\textsuperscript{+},K\textsuperscript{+}-ATPase, as we have also demonstrated at an advanced stage (Songu-Mize et al., 1982), it is possible that other factors may affect the activity of the vascular smooth muscle Na\textsuperscript{+} pump during the development of this complex model of hypertension. The early suppression of pump activity may be due to increased plasma levels of a digitalis-like Na\textsuperscript{+} pump inhibitor released as a response to acute volume expansion (Gruber et al., 1980; Pamnani et al., 1981); an acute volume expansion is expected after the initiation of DOCA-salt treatment (Haddy, 1980). This hypothesis is supported by Gruber et al. (1980), who have shown that acute volume expansion in dogs results in the appearance of a plasma factor which competes with digoxin for specific antibodies and is an inhibitor of Na\textsuperscript{+},K\textsuperscript{+}-ATPase. Furthermore, Metzler et al. (1982) have reported an elevation of a digoxin-like immunoreactive substance in rat plasma after 5 days of treatment with DOCA-salt.

The elevation of Na\textsuperscript{+} pump activity noted during the intermediate stages of DOCA-salt hypertension may result from increased membrane permeability to sodium, as suggested by Friedman and Friedman (1976) and Jones and Hart (1975). By increasing intracellular sodium concentration, this "leakiness" would be expected to stimulate active transport. In our experiments, however, nonspecific \textsuperscript{86}Rb\textsuperscript{+} uptake, which would be expected to be elevated with any increase in permeability, was not altered. Another possibility, suggested by Overbeck et al. (1982), is that the increased pumping ability of the vascular tissue seen during the established stage of hypertension results from increased number of pump molecules in the vascular smooth muscle induced by a prior exposure to a circulating, digitalis-like pump inhibitor. If Na\textsuperscript{+},K\textsuperscript{+}-ATPase is indeed the receptor for a circulating "digitalis-like" substance, it is possible that the number or density of Na\textsuperscript{+},K\textsuperscript{+}-ATPase molecules is regulated by the plasma level of inhibitor. In fact, an increase in Na\textsuperscript{+},K\textsuperscript{+}-ATPase activity has been reported in guinea pig heart after chronic treatment with digitalis (Bluschke et al., 1976). Additionally, chronic digitalis treatment (2–117 months) in human patients caused an increase in number of enzyme molecules (Cumberbatch et al., 1981). Finally, mineralocorticoids, e.g., DOCA, may induce synthesis of new Na\textsuperscript{+},K\textsuperscript{+}-ATPase molecules by stimulating protein synthesis (Knox and Sen, 1974; Friedman and Friedman, 1976). The strain of rats used or other differences in experimental protocol may also account for some discrepancies noted between the results from different laboratories.

Interpretation of the findings at the late stage of DOCA-hypertension is complicated by the presence of biochemicals in plasma that could affect activity of the vascular Na\textsuperscript{+} pump. Kidney impairment, for example, might introduce other chemicals into the plasma which could inhibit the Na\textsuperscript{+} pump. We have observed an increase in plasma creatinine after 50 days of DOCA treatment, indicating renal insufficiency. The inhibition of the vascular Na\textsuperscript{+} pump activity may be due to agents such as urea or methylguanidine, which are reported to inhibit cell membrane Na\textsuperscript{+},K\textsuperscript{+}-ATPase (Skou and Hilberg, 1965). It appears, however, that the possibility that hypokalemia is responsible for suppressed vascular Na\textsuperscript{+} pump activity can be ruled out. In the present study, when potassium concentration in incubation
plasma from rats treated with DOCA for 50 days was adjusted to match that of control rats, vascular Na\(^+\) pump activity of the DOCA-treated rats was decreased by 23%. This is similar to the 19% decrease noted in an earlier study (Songu-Mize et al., 1982) in which potassium concentration of the incubation plasma from the DOCA-treated rats was not adjusted to match that of control rat plasma.

In conclusion, our results do indicate phasic changes in the vascular Na\(^+\) pump activity during developmental and advanced stages of DOCA-salt hypertension: it was suppressed at an early prehypertensive stage, elevated during the established stage, and suppressed again at a late stage. These changes in vascular Na\(^+\) pump function did not correlate with the arterial blood pressure. These alterations in vascular Na\(^+\) pump activity may contribute to or oppose other hypertensive mechanisms.

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