THE ASSOCIATION OF A HUMORAL SODIUM TRANSPORT INHIBITORY ACTIVITY WITH RENAL ESCAPE FROM CHRONIC MINERALOCORTICOID ADMINISTRATION IN THE DOG

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SUMMARY

1. Previous studies in dogs given large intravenous saline infusions had shown the presence of an inhibitor of toad bladder sodium transport in jugular venous plasma. In the present study plasma of six dogs on a constant sodium intake was tested for this humoral sodium transport inhibitory (HSTI) activity before and during chronic administration of desoxycorticosterone acetate (DOCA).

2. Renal escape from mineralocorticoid antinatriuresis occurred 2–7 days after beginning treatment with DOCA. No HSTI activity was present in plasma drawn at 9 a.m. during a control period before DOCA administration but it was found in every case on the morning of the day on which escape occurred. A positive correlation was shown between HSTI activity of 9 a.m. plasma and the rate of sodium excretion for the subsequent 24 h period ($r = 0.66, P<0.002$).

3. When DOCA administration was continued for several days after escape had occurred in three dogs, oscillations in HSTI activity correlated with oscillations in renal sodium excretion.

4. The degree of antinatriuresis on any given day correlated with the amount of HSTI activity the following morning ($r = 0.61, P<0.0002$). This finding suggests that extracellular fluid volume expansion resulting from DOCA-induced antinatriuresis leads to HSTI activity. These results fit the hypothesis that HSTI activity is a natriuretic hormone which plays a role in the mechanism of DOCA escape.

The renal response to chronic mineralocorticoid administration is characterized by transient antinatriuresis lasting several days; subsequently, sodium excretion returns to intake values and external sodium balance is maintained in the presence of an expanded extracellular fluid volume (Davis, Johnston, Howards & Wright, 1967; August, Nelson & Thorn, 1958). The mechanism of this so-called steroid ‘escape’ has been studied by numerous investigators in both man and dog. Rovner, Conn, Knopf, Cohen & Hseuh (1965) demonstrated in man that escape could occur independent of changes in glomerular filtration rate or renal blood flow. Correspondence: Dr V. M. Buckalew, Jr, 69 Butler Street, S.E., Atlanta, Georgia 30303, U.S.A.
flow. Davis, Urquhart, Higgins, Johnston & Brown (1966) showed that escape took place in dogs in which renal blood flow and glomerular filtration rate had been experimentally decreased by constriction of the renal artery. The implication of these studies is that steroid escape is at least partly due to decreased tubular sodium reabsorption. This has recently been confirmed by Knox, Schneider, Dresser & Lynch (1970) using micropuncture techniques in the dog. Other investigators have shown that the escape phenomenon is independent of the level of systemic arterial blood pressure (Higgins, 1970) of renal venous pressure or the presence of renal nerves (Davis, Holman, Carpenter, Urquhart & Higgins, 1964) the renin–angiotensin system (Johnston, Davis, Robb & Mackenzie, 1968) and changes in the rate of secretion of adrenal cortical steroids other than aldosterone (Davis et al., 1964). Inability to demonstrate an obvious mechanism has led to the suggestion that a humoral natriuretic agent may in part be responsible for the decreased tubular sodium reabsorption in steroid escape (Davis et al., 1964). This suggestion was supported by cross circulation studies which showed a transferable natriuretic agent in DOCA escape dogs whose extracellular fluid volume had been expanded with saline (Davis & Johnston, 1966).

No clearly identifiable natriuretic hormone has yet been isolated, although several laboratories have recently reported the demonstration of humoral natriuretic factors which could be hormonal in nature (Cort, Dousa, Pliska, Lichardus, Safarova, Vranesic & Rudinger, 1968; Bricker, Bourgoignie & Klahr, 1970; Sealey, Kirshman & Laragh, 1969). In attempting to demonstrate the presence of the postulated natriuretic hormone, we have recently reported that ultrafiltrates and dialysates of jugular vein plasma of dogs given intravenous infusions of saline inhibit sodium transport by the toad bladder in vitro (Buckalew, Martinez & Green, 1970). If this inhibitory activity is a natriuretic hormone involved in regulating the day-to-day excretion of sodium by the kidney, it might be expected to play a role in the steroid escape mechanism. The present study was performed to examine the relationship between the previously demonstrated humoral sodium transport inhibitory (HSTI) activity and renal sodium excretion during the chronic administration of desoxycorticosterone acetate (DOCA) in the dog.

MATERIALS AND METHODS

One male and four female mongrel dogs weighing 13–20.9 kg were kept in individual metabolism cages, allowed water ad libitum and trained to lie quietly during the drawing of jugular venous blood. They were fed a constant diet consisting of one can of a commercially prepared dog food per day (Ken-L-Ration, Quaker Oats, Chicago, Ill.) Samples of food were ashed in conc. HCl and analysed for sodium and potassium content. The mean sodium content was 33 mmol and the mean potassium content 29 mmol with a variation of less than 10% from can to can.

Six experiments were performed on these five dogs on an intake of 33 mmol of sodium per day, and in three of these potassium chloride was added to provide an intake of 67 mmol daily. The same dog was used in experiments 1 and 5 (Table I), with 17 days between the two experiments.

The protocol of the study was as follows. The animals were allowed to void spontaneously, and urine was collected from 9 a.m. to 9 a.m. daily in vessels containing 1 ml of conc. HCl to prevent bacterial growth. At 9 a.m. each day 15–20 ml of jugular venous blood was collected
and the dog given its daily ration of food. After an equilibration period of 2–3 days control blood and urine samples were collected for 1–3 days, after which 15 mg of DOCA in oil was administered intramuscularly each day at the time of feeding. Urine and plasma were analysed for sodium by flame photometry. Plasma total protein concentration was determined with a Technicon AutoAnalyzer (Technicon Instrument Corp., Chauncey, N.Y.) by the Stevens modification of the Weichselbaum procedure (Weichselbaum, 1946).

Ultrafiltrates of jugular vein plasma, prepared with Amicon (Amicon Corporation, Lexington, Mass.) models 10 and 12 stirred pressure cells, were assayed for effect on toad bladder short circuit current by methods previously described, assuming that the short circuit current approximately equalized the rate of active sodium transport (Buckalew et al., 1970). Toad hemibladders were placed in a two-compartment lucite chamber which divides the tissue into two halves. One side was used for testing unknown ultrafiltrate and the other side as a control. Bladders were kept open-circuited and trans-membrane potential difference and short circuit current were monitored intermittently. After a stable baseline potential difference and short circuit current were attained, assays were performed by aspirating the contents of the serosal bath of both quarterbladders (4 ml), and replacing one bath with 4 ml of ultrafiltrate diluted to isotonicity with amphibian Ringer solution, the other with 4 ml of fresh Ringer solution. Short circuit current values 30 min after exposing the bladder to the ultrafiltrate were compared with values obtained during the steady-state baseline before the assay. Humoral sodium transport inhibitory (HSTI) activity was expressed as percentage change in short circuit current corrected for any spontaneous change on the control side. After individual assays the contents of both serosal chambers were aspirated and replaced with fresh Ringer solutions, and when a stable short circuit current was re-established, additional assays were performed on the same bladder by using alternate quarterbladders as test and control sides. The technician performing the bioassay had no knowledge of which blood specimen was being tested.

Standard statistical analyses were preformed by using programs prepared by the Emory University Department of Biometry for the University Computer Centre RCA Spectra-70 computer.

RESULTS

The results of the six experiments are summarized in Table 1. It can be seen that during control periods urinary sodium excretion averaged 21 mmol per day. The difference of 12 mmol between the daily sodium intake and the mean output is an approximation of the average daily stool sodium excretion, a value that agrees with results found for normal dogs (Davis, Lindsay & Southworth, 1952). Stool sodium was not measured but was assumed to remain constant from day to day and to be lower during DOCA administration than during control periods (Davis et al., 1966). HSTI activity is shown for the morning on which DOCA treatment was begun, and for the morning of the day on which escape occurred. "Escape" was judged to have taken place on the day that urine sodium excretion first equalled or exceeded mean sodium excretion values obtained during control periods. This was observed on the second day of DOCA administration in one experiment, day 4 in three, day 5 in one and day 7 in one (Table 1). In each experiment (in number 3 the control blood was lost) HSTI activity was greater on the day of escape than on the control
day, the mean value for HSTI activity of $-18 \pm 0.7\%$ (mean $\pm$ SE) being significantly different from the mean control value before DOCA of $-2 \pm 2.0\%$ ($P < 0.001$). Values for plasma total protein were lower on the day of escape than during the control period, as expected; however, the variability among the dogs was such that no correlation was observed between absolute total protein concentration, HSTI activity, or daily sodium excretion.

**Table 1. DOCA escape in normal dogs ingesting 33 mmol of sodium /day**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Control</th>
<th>Escape$\dagger$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$U_{Na}V$ * (mmol/day)</td>
<td>TP $\dagger$ (g %)</td>
</tr>
<tr>
<td>1</td>
<td>20 (1)</td>
<td>6.7</td>
</tr>
<tr>
<td>2$\parallel$</td>
<td>16 (1)</td>
<td>5.3</td>
</tr>
<tr>
<td>3$\parallel$</td>
<td>19 (2)</td>
<td>5.7</td>
</tr>
<tr>
<td>4$\parallel$</td>
<td>20 (2)</td>
<td>6.4</td>
</tr>
<tr>
<td>5</td>
<td>25 (2)</td>
<td>6.7</td>
</tr>
<tr>
<td>6</td>
<td>25 (3)</td>
<td>5.3</td>
</tr>
<tr>
<td>Mean</td>
<td>21</td>
<td>1.0</td>
</tr>
<tr>
<td>SE</td>
<td>1.4</td>
<td>1.2</td>
</tr>
</tbody>
</table>

$TP = total$ $plasma$ $protein; PD = potential$ $difference$ $and$ $SCC = short$ $circu$ $t current$ $in$ $toad$ $bladder$ $preparation.$

* Average for number of days indicated in parentheses.
† Value for morning of day on which DOCA was begun.
‡ Values for day on which escape occurred.
§ Number of days of DOCA administration when escape occurred.
$\parallel$ Potassium supplement of 34 mmol/day added to diet.
$\dagger$ Probability that difference in PD and SCC between control and escape values are due to chance alone.

The number of days of antinatriuresis and estimated amount of sodium retained during DOCA administration was similar to previously reported studies (Davis $et$ $al.,$ 1967) with one exception. In experiment 1 (Table 1) antinatriuresis lasted one day only and escape occurred on the second day of DOCA administration in association with HSTI activity of $-20\%$.

A typical experiment (number 5, Table 1) is shown in Fig. 1. HSTI activity is plotted between values for urinary sodium excretion on the days before and after its collection. During a control period of 2 days, sodium excretion was 25 mmol/day and HSTI activity was $-1$ and $-3\%$. On administration of DOCA profound antinatriuresis lasted for 2 days, after which sodium excretion gradually increased with complete escape on day 5. The increase in sodium excretion was accompanied by increasing HSTI activity which was $-16\%$ on the morning of the day of complete escape. DOCA administration was continued and on the morning of day 6 HSTI activity was absent ($+2\%$), renal sodium retention recurring on that day with $U_{Na}V$ falling to 12 mmol. On the morning of day 7 HSTI activity reappeared ($-24\%$) and on that day sodium excretion returned to the control value of 26 mmol. These results show an oscillation in urine sodium excretion on
the same day as an oscillation of HSTI activity. In two other experiments (numbers 2 and 4, Table 1) observations were made beyond the period of initial DOCA escape and in each of these simultaneous oscillations in \( U_{Na^+} \) and HSTI activity were observed (Fig. 1).

![Graph showing DOCA escape and \( U_{Na^+} \) over days.]

**Fig. 1.** Results of a typical experiment (Expt. 5, Table 1) showing relationship between daily sodium excretion (\( U_{Na^+} \)) and humoral sodium transport inhibitory activity expressed as % change in short circuit current (SCC) during chronic DOCA administration. C = control. DOCA was begun at the arrow, on day 1, and continued throughout the remainder of the experiment. Values for SCC on 9 a.m. blood samples are plotted between values for \( U_{Na^+} \) for 24 h periods before and after blood collection.

The correlation between DOCA escape and HSTI activity is further demonstrated in Fig. 2, where values for 9 a.m. HSTI activity for each day of DOCA administration are plotted as a function of \( U_{Na^+} \) for that same day. Only those values up to the initial escape are included. A significant linear correlation is apparent with a calculated regression equation of \( y = -0.69x - 1.0 \) (\( r = 0.66, P < 0.002 \)).

The oscillation in HSTI activity and \( U_{Na^+} \) observed in these experiments and illustrated in Fig. 1 suggests that HSTI activity was present only after antinatriuresis had taken place and was independent of the absolute amount of the extracellular fluid volume. To test this hypothesis further the degree of antinatriuresis for each day was calculated as the fraction of the daily intake of sodium not excreted in the urine and plotted against HSTI activity for the subsequent morning. Thus, if the morning value of HSTI activity is the result of volume expansion during the preceding day, there should be a direct relationship between HSTI activity and antinatriuresis. Fig. 3 shows the results of plotting these results for all forty-one days of DOCA administration in the six experiments. A significant correlation was present
with a linear regression calculated by the method of least squares of \( y = -0.15x + 0.2 \) \( (r = 0.61, P < 0.0002) \). On the morning after 11 days with an antinatriuretic index greater than 40 there was HSTI activity of less than \(-10\%\). As shown in Fig. 3, eight of these eleven days represented either the first or second day of DOCA-induced antinatriuresis, or the first day of antinatriuresis during an oscillation subsequent to the escape. These findings indicate that antinatriuresis on any given day was not invariably accompanied by HSTI activity on the subsequent day, and that lack of HSTI response was more likely to occur on the first two days after the onset of DOCA-induced sodium retention. HSTI activity, when present, was statistically related to the degree of antinatriuresis on the preceding day. This conclusion is further emphasized by calculating a correlation coefficient for the results in Fig. 3 excluding the eleven points in which antinatriuresis was followed by little or no HSTI activity. A regression equation of \( y = -0.21x -1.3 \) was obtained and the \( r \) value increased to 0.84 \( (P < 0.0001) \).

**DISCUSSION**

The present experiments demonstrate the well-characterized alterations in renal sodium excretion during chronic mineralocorticoid administration in the dog. In six animals on sodium intake of 33 mmol antinatriuresis was transient and after a period of 2–7 days,
sodium excretion returned to control values. Our studies were designed to determine
whether there was any correlation between the renal escape from DOCA and the sodium
transport inhibitory activity in plasma which we have previously reported in dogs given
large infusions of saline intravenously (Buckalew et al., 1970). HSTI activity was deter-
mined daily on plasma obtained at 9 a.m., at the time of feeding, and correlated with
sodium excretion during the subsequent 24 h period. Previous work has shown that most
urine sodium is excreted during the first 12 h after a meal in the dog and that this pattern is
unchanged during DOCA administration (Davis et al., 1966). Thus, if the previously

demonstrated HSTI activity is a natriuretic hormone regulating renal sodium excretion,
the level present at 9 a.m. might be expected to have an influence on the amount of sodium
excreted during that day.

HSTI activity was significantly greater on the morning of the day of escape than during a
control period before DOCA administration. In addition, a correlation existed between
HSTI activity and $U_{Na}V$ at 9 a.m. for the subsequent day over the entire range of sodium
excretion observed during the escape process. This relationship is illustrated in an in-
dividual experiment in which HSTI activity was shown to be absent until the morning of

![Graph](attachment://graph.png)

Fig. 3. Relationship during DOCA administration between humoral sodium transport in-
hibitory activity expressed as % change in SCC on 9 a.m. blood sample and the fraction of daily
sodium intake not excreted in urine ($I_{Na} =$ sodium intake, $U_{Na}V =$ renal sodium excretion)
for 24 h period immediately before blood collection. □, Values for SCC obtained after the first
day of DOCA-induced antinatriuresis, or after the first day of antinatriuresis during an
oscillation subsequent to escape. △, Values for SCC obtained after the second day of DOCA-
induced antinatriuresis. ●, SCC on all other days. The estimated regression line calculated by
the method of least squares $\pm 1$ SE of the estimate is shown [SCC = $-0.15(I_{Na} - U_{Na}V/I_{Na})$
$+ 0.2; r = 0.61, P < 0.0002]$. 

Sodium transport inhibitor in DOCA escape 75
the third day of DOCA administration (Fig. 1). On that day urine sodium excretion began to recover and on subsequent days increased in a step-wise fashion concomitant with increasing levels of HSTI activity, until complete escape occurred on the fifth day.

Two additional observations further emphasize the close relationship between escape from antinatriuresis and HSTI activity. In three of the six experiments in which DOCA was continued after escape had taken place, antinatriuresis recurred followed by secondary escape. Similar findings are evident in the results of most investigators in both dog and man (Davis et al., 1966; August et al., 1958), and in our experiments these oscillations in urine sodium excretion occurred on the same day as oscillations in the amount of HSTI activity. In addition, in one experiment (Table 1) escape occurred on the second day of DOCA administration after no more than 20–25 mmol of sodium had been retained. This unusually early escape was associated with HSTI activity of −20% on the morning of the second day of DOCA administration.

These results suggest that HSTI activity at 9 a.m. had a modulating influence on the rate of sodium excretion during the subsequent 24 h. In addition, the results imply that lack of sodium excretion on any given day caused an increase in the amount of HSTI activity on the subsequent day (Fig. 3).

We have recently reported results on the relationship between HSTI activity and renal sodium excretion in normal dogs on a constant sodium intake (Buckalew & Lancaster, 1971). Oscillations in urine sodium excretion were common and a direct correlation was also demonstrated between the degree of antinatriuresis on any given day and the amount of HSTI activity the following morning. The relationship between antinatriuresis and HSTI activity was independent of the absolute sodium intake. The results of the present experiments agree with these observations, and in addition, the slope of the regression in Fig. 3 of −0.15 during DOCA administration is not significantly different from the slope of −0.13 found in normal dogs (P>0.5). These results demonstrate that the correlation between HSTI activity and preceding antinatriuresis is present whether the latter occurs spontaneously or is experimentally induced. Normal and DOCA-treated dogs are also similar in that antinatriuresis on any given day is not always followed by HSTI activity on the subsequent day. The results of the present experiments suggest that sustained antinatriuresis lasting 2–3 days is more likely to be followed by HSTI activity, which frequently does not appear on the first or second day after the onset of antinatriuresis (Fig. 3).

The oscillations in urine sodium excretion in normal dogs and in those receiving a mineralocorticoid chronically can be viewed as part of a negative feedback control system in which an increase in plasma or extracellular fluid volume leads to an increased renal sodium excretion due at least in part to a decrease in tubular sodium reabsorption (Wesson, 1969). The present studies are compatible with the hypothesis that HSTI activity is one of the effector mechanisms by which volume expansion results in decreased tubular sodium reabsorption. The fact that HSTI activity reaches undetectable values when antinatriuresis is absent, regardless of the absolute level of extracellular fluid volume, suggests that HSTI release is not controlled by the absolute volume of the plasma or extracellular fluid, but by a change from any level to a larger volume. If HSTI activity is natriuretic, the results imply that it acts primarily to facilitate renal sodium excretion in the presence of either spontaneous or experimentally induced antinatriuresis and that other factors are more important in regulating renal sodium excretion in the absence of antinatriuresis. For example, sodium
excretion could be facilitated in some circumstances in normal subjects by the absence of poorly defined antinatriuretic factors, as previously suggested by Smith (1957).

Potential natriuretic factors which have been identified during DOCA escape include decreased plasma renin concentrations (Johnston et al., 1968), increased glomerular filtration rate and renal plasma flow (Davis & Howell, 1953). However, escape has been shown to occur in the absence of changes in any of these variables, indicating that none of them is critical to the escape mechanism (Davis et al., 1966; Johnston et al., 1968). Other potential natriuretic factors that could be present during DOCA escape include decreased oncotic pressure and redistribution of blood flow to shorter cortical nephrons with lower reabsorptive capacity (Earley & Daugharty, 1969). If HSTI activity is indeed a natriuretic factor, its presence with other potential natriuretic forces during DOCA administration would facilitate escape. The fact that under the conditions of the present studies escape did not occur in the absence of HSTI activity provides circumstantial evidence supporting the concept that it is a natriuretic hormone which may be critical in the escape mechanism.

In this regard it is noteworthy that escape from mineralocorticoid administration fails to occur in several circumstances, notably in dogs with inferior vena cava ligation (Davis et al., 1967) and with large arteriovenous fistulae (Johnston et al., 1968). Although we have no observations in dogs with cava ligation during DOCA administration, we have recently reported that HSTI activity is absent in these dogs during the administration of saline intravenously, in contrast with the findings in normal dogs (Buckalew & Lancaster, 1971). This suggests the possibility that dogs with partial ligation of the inferior vena cava fail to escape from DOCA-induced antinatriuresis at least in part because of the absence of HSTI activity.

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