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The Involvement of Urinary Kallikrein in the Renal Escape from the Sodium Retaining Effect of Mineralocorticoids

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It is well known that the normal kidney "escapes" the sodium retaining effect of mineralocorticoids. However, the mechanism that mediates this "escape" is not understood. The possible role of kallikrein in this "escape" phenomenon was investigated by placing seven dogs in metabolic cages and giving them a constant sodium diet. After they had been on this diet three days, urine was collected for two 24-hour periods. DOCA (25 mg/day) was then given intramuscularly for five days. Urine was collected daily during this DOCA period and for two additional 24-hour periods. Urine volume, sodium, potassium, protein, and kallikrein excretion were then measured. Urinary kallikrein increased from 251.9 ± 34.8 (mean ± SE) in the second day of the control period to 639.8 ± 110.1 μg/day ($P < .01$) by the third day of treatment. It remained elevated two days after DOCA was discontinued. Sodium excretion decreased significantly on the first day of DOCA treatment, returning to the previous values thereafter. Urine protein excretion remained constant. The enhanced urinary kallikrein during the "escape" suggests that the kallikrein system could be involved in the regulation of sodium metabolism by acting as a natriuretic factor, or perhaps by regulating the renal blood flow.

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ALTHOUGH it is well-known that the kidney "escapes" the sodium retaining effects of mineralocorticoids,[1] the nature of the changes responsible for the "escape" is not known. Changes in glomerular filtration rate (GFR), renal nerve tone, renal venous pressure and plasma renin do not determine the appearance of the "escape".[2-4]. Free flow micro-puncture studies [5] have shown that sodium reabsorption is reduced in the proximal tubules of dogs during the "escape" from the mineralocorticoid effect of the deoxycorticosterone-acetate (DOCA). Consequently, a larger volume of sodium and water is delivered from the proximal tubule, probably contributing to the "escape". The postulated hormone (third factor) [2,5] which is released by the expansion of the extracellular fluid volume (ECF), is a possible mediator of the diminished proximal reabsorption and of the "escape" from the sodium retaining effects of DOCA. The observation that urinary kallikrein as well as plasma kinins are increased during elevated natriuresis[6-8] suggests that renal kallikrein might also be involved in the "escape" from mineralocorticoids. To test this hypothesis, we measured urinary kallikrein before, during and after the "escape" in dogs treated with DOCA.

Materials and Methods

Seven female mongrel dogs, weighing between 7-14 Kg, were placed in metabolic cages for 15 days prior to the experiment. During this period the animals were trained for daily bladder catheterization and received commercial dog chow (Purina) and tap water *ad libitum*. The dogs were then given 1.5 lb/day of a sodium deficient diet (Prescription Diet, H/d, Riviana Foods Inc., Hills Division, Topeka, Kansas) which was supplemented with 5.5 mEq/Kg/dog/day of sodium chloride. The animals ate the total amount of food. Following a habituation period of 72 hours, urine samples were collected for a control period of 48 hours. Subsequently, 25 mg/day of DOCA in oil was given intramuscularly for five consecutive days and urine samples collected. This DOCA period was followed by another control period of 48 hours. During each of these periods urine was individually collected under mineral oil in plastic bottles at room temperature. The 24-hour collection periods were terminated by catheterization. Urine volume was measured and an aliquot was frozen and stored at -20° C for subsequent determination of electrolytes, proteins, and kallikrein activity. The daily excretion of each was calculated on the basis of the urine volume. Sodium and potassium ion concentration was determined with a flame photometer* using internal standard. The protein concentration was determined by the Lowry procedure[9] using human serum albumin (4 times crystallized, Nutritional Biochemical Corp., Cleveland, Ohio) as a standard. The urine samples were dialyzed against running water for 72 hours prior to the determination of protein concentration. Appropriate corrections were made for the dilution during dialysis.

Urinary kallikrein was measured by a modification of the method of Marin-Grez and Carretero[10] in which 1 μl of urine was incubated for 20 minutes at 37° C with 5 mg of a substrate preparation. The substrate was prepared by heating dog plasma for 3 hours at 58°-60° C. This plasma was then precipitated with ammonium sulfate, and the fraction obtained between 1.51-2.27 M concentration was dissolved in distilled water, dialyzed overnight against run-

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ning water, lyophilized, and stored in powder form.

The peptidases were inhibited by adding 0.3 ml of a 0.01 M 1,10-phenanthroline solution to the incubation mixture. Volume was brought to 2 ml with a 0.068 M phosphate buffer of pH 8.5. The reaction was terminated by the addition of 4 ml of cold absolute ethanol. The precipitate was separated by centrifugation (1820 g for 15 minutes) and the supernatant evaporated to 0.5 ml in a water bath at 45° C under a nitrogen current. The volume was then brought to 2 ml with a 0.2 M phosphate buffer, pH 7.4.

The samples were bioassayed in the perfused hindleg of the dog,[10] using a peristaltic pump (Sigmamotor, Middleport, New York) which circulated blood at a constant rate from the proximal end of one femoral artery to the distal end of the opposite femoral artery. Tygon tubing was used for the extracorporeal circuit. The changes in perfusion pressure caused by samples and standards (Bradykinin, Sandoz, Hanover, New Jersey) were recorded by means of a Sanborn pressure transducer and a Sanborn 150 preamplifier and recorder. The activity of the unknown sample was determined by bracketing its depressor effect between two close doses of the standard, and calculated by interpolation. Kallikrein activity was expressed as μg of bradykinin released per milliliter of urine and per minute of incubation.

Results

A marked decrease in sodium excretion was observed only on the first day of DOCA treatment. The excretion then returned to the previous level, although DOCA treatment was continued (Figure 1). The potassium excretion increased from a control level of 2.92 ± .26 (SEM) to 3.35 ±.24 mEq/Kg/day on the first day of treatment. Thereafter, the potassium excretion returned to the control level.

The urine volume tended to increase slightly. However, the kallikrein excretion was significantly higher than the level which would be due to the increased urine volume. Thus, on a quantitative basis, the enhancement in the excretion of enzymic protein was quite apparent. The elevation of urinary kallikrein excretion was significant at the 1% level when the third day of DOCA treatment was compared to the first control day (T-test for pair of dependent samples). Protein excretion remained unchanged throughout the experiment (Figure 1).

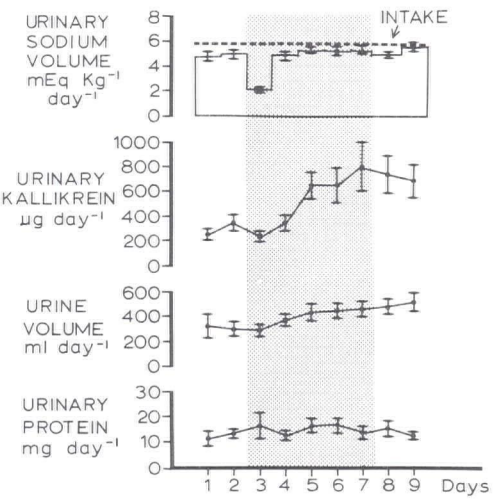

Figure 1

Effect of DOCA on sodium excretion, urinary kallikrein, urinary volume, and urinary protein. The broken line on top indicates sodium intake. Sodium excretion and intake is expressed in mEq/Kg dog/day. The shaded area indicates the period during which DOCA was injected (25 mg/day). Vertical bar indicates SEM.

Discussion

The observation that kallikrein excretion increases during DOCA treatment, while total protein excretion remains the same, could suggest the involvement of the kallikrein system in the "escape" to mineralocorticoids. The increased kallikrein excretion, however, was gradual and reached its maximum after the "escape" had occurred. This gradual increase of urinary kallikrein could be the reflection of the gradual expansion of the ECF, which somehow stimulates the activation and release of the enzyme. Kallikrein could, therefore, through the release of kinins, produce a vasodilatation and, either by redistribution of the intrarenal blood flow, or by increasing the peritubular pressure, decrease the sodium reabsorption [11-13] and contribute to the "escape". It could also be that the kinins act directly on the proximal or distal tubules by inhibiting sodium reabsorption.

Another explanation for this gradual increase of kallikrein excretion could be that the kallikrein excretion is the consequence rather than the cause of the "escape". If this is the case, it could be postulated that the kallikrein excretion in the urine represents a spillover of the enzyme released in the renal tissue and that it only appears in the urine after a certain delay. If kallikrein increases as a consequence of the natriuresis induced by the expansion of the ECF produced by the DOCA injection, it could still contribute to the natriuresis by increasing the renal blood flow and producing additional sodium excretion.

Adetuyibi and Mills[14] found results similar to ours in humans treated with Fludrocortisone. Gellar et al[15] also found an increase in kallikrein excretion after treating animals with DOCA. However, they correlated this increase, not with the "escape" phenomenon, but with the direct effect of the mineralocorticoids in the kidney, independent of the sodium excretion. They made this assumption on the basis that the sodium excretion was the same in the control animals as in the DOCA treated animals. However, the mechanism by which these animals reached a sodium balance should be different since the DOCA treated rats have to "escape" the sodium retaining effect of the mineralocorticoids.

Margolius et al[16] also found an increase in urinary kallikrein excretion in patients with primary aldosteronism. It is reasonable to assume that these patients were in the "escape" phase, since patients with primary aldosteronism do not develop edema. We have been able to confirm these results with a larger series of patients (unpublished data).

The exact relationship between renal kallikrein and sodium metabolism remains to be clarified. The present data, however, suggests an activation of the system by ECF expansion.

Further, the hypothesis that kallikrein could participate in the regulation of sodium excretion is supported by the following facts: that kinins produced natriuresis,[17-19] that kallikrein is formed and excreted by the kidney itself,[20-22] and that the postulated natriuretic hormone may also be localized in the kidney.[23-24]

Acknowledgments

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