Effects of Mineralocorticoids, Altered Sodium Intake, and Adrenalectomy on Urinary Kallikrein in Rats

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ABSTRACT

The urinary excretion of kallikrein was examined in rats whose mineralocorticoid level was increased by the administration of deoxycorticosterone or a low-sodium diet and decreased by adrenalectomy. Administration of deoxycorticosterone by injection or by subcutaneous implantation of pellets resulted in a threefold increase in kallikrein excretion after 10–30 days ($P < 0.01$). Low-sodium intake produced a twofold increase in kallikrein excretion after 2 and 4 weeks ($P < 0.01$). Urine volume and blood pressure were normal and unchanged throughout the study. Sodium excretion was unaltered by deoxycorticosterone but was essentially zero during low-sodium intake. Adrenalectomized rats excreted less than half the kallikrein of sham-operated control rats ($P < 0.01$). These data show a direct relationship between mineralocorticoids and kallikrein excretion and suggest that mineralocorticoids are a primary factor regulating the urinary excretion of kallikrein in rats.

KEY WORDS

- high-sodium diet
- deoxycorticosterone
- urinary sodium
- kallikrein esterase activity
- low-sodium diet

Urinary excretion of kallikrein is altered in hypertensive patients (1, 2) and rats (2, 3); it is significantly increased in patients with primary aldosteronism and in rats with deoxycorticosterone-salt hypertension. These findings suggest a direct correlation between hypertension associated with elevated mineralocorticoid levels and urinary kallikrein excretion. However, it is unclear whether increased kallikrein levels are due to the hypertension induced by the mineralocorticoid and sodium or to the steroid alone. The purpose of this study was to determine if urinary kallikrein excretion was responsive to altered mineralocorticoid levels under conditions that did not result in hypertension. Mineralocorticoid activity was altered directly by administration of deoxycorticosterone or by adrenalectomy and indirectly through alterations in dietary sodium.

Methods

Three different studies were performed using 53 male Sprague-Dawley rats. In the first study, deoxycorticosterone was administered by injection (10-week-old rats, 200–220 g) or by pellet implantation (16-week-old rats, 280–310 g). In the second study, bilateral adrenalectomy was performed on 10-week-old 200–210-g rats. In the third study, 10-week-old rats, 190–210 g, were placed on low-, normal-, or high-sodium diets.

Mineralocorticoid Studies

Two forms of the mineralocorticoid, deoxycorticosterone (DOC), were used: DOC-pivalate, aqueous suspension, 25 mg/ml, for injection, and DOC-acetate pellets, 20 mg, for implantation. After an initial measurement of kallikrein excretion, DOC-pivalate (25 mg) was injected subcutaneously into seven rats, and this dose was repeated 10 days later. Control rats received equal volumes (1 ml) of saline. Two DOC pellets were implanted subcutaneously in four other rats. Urine collections for both groups of DOC-treated rats were obtained at 10-day intervals. All rats
were fed the same laboratory diet and drank distilled water ad libitum.

**ADRENALECTOMIZED RATS**

Seven bilaterally adrenalectomized rats and seven sham-operated control rats were given 1% NaCl to drink ad libitum within 36 hours after surgery and for the first 13 days of the study. Urinary kallikrein excretion was measured on the seventh and fourteenth days. During the final 24-hour urine collection all rats drank distilled water instead of saline.

**ALTERATIONS IN DIETARY SODIUM INTAKE**

Twenty-one rats were fed a normal diet and given distilled water during a 10-day control period. Then they were divided into three groups of seven rats and treated for 4 weeks. The first group received a normal diet with distilled water (normal-sodium diet); the second group received a sodium-deficient diet with distilled water (low-sodium diet); and the third group received a normal diet with 1% NaCl in the drinking water (high-sodium diet). All three groups of rats were then given the normal diet and distilled water for an additional 3 weeks. Diets (in pellet form) were obtained from General Biochemicals, Chagrin Falls, Ohio. The low-sodium diet (GB-no. 170950, Hartroft formula) contained approximately 0.2 mEq Na/100 g diet. The normal diet was made by adding 6 g NaCl/kg to the low-sodium diet and contained approximately 10 mEq Na/100 g diet. Each rat ate 20-30 g of its respective diet per day. Urinary kallikrein excretion was measured before and after the second and the fourth weeks on these diets.

The procedures for the collection of rat urine and for the measurement of urinary kallikrein activity have been previously described in detail (3). Twenty-four-hour urine collections were obtained from individual rats housed in glass metabolism cages. The rats were fasted during the collection period but were allowed free access to distilled water. Blood pressure, measured in unanesthetized rats using the technique of tail plethysmography, and body weight were determined before each urine collection. Urinary kallikrein esterase activity was measured according to the radiochemical method of Beaven et al. (4) with the indicated modifications (3). Purified rat urine kallikrein with known esterolytic activity was used as a standard (5). One kallikrein esterase unit (EU) is defined as the amount of kallikrein which hydrolyzes 1 μmole p-tosyl-L-arginine methyl ester/min at pH 8.0 and 30°C in a titriconic assay. Data are expressed as kallikrein EU excreted per 24 hours. Representative, randomly selected urine samples were also bioassayed on the guinea pig ileum (3) to show that urinary esterase activity was analogous to urinary kinin-generating activity. Urinary sodium was measured with a flame photometer. Data was expressed as means ± se and analyzed for significance with Student's t-test.

**Results**

**EFFECT OF DEOXYCORTICOSTERONE AND ADRENALECTOMY**

Urinary kallikrein excretion increased threefold after administration of DOC by either subcutaneous pellet implantation or injection (Fig. 1). Both pellet implantation and injection increased kallikrein excretion to the same
maximal levels, but pellets produced a more rapid response. The highest amount of kallikrein excretion was seen 10 days after DOC pellet implantation (15.0 ± 2.6 EU/24 hours) and 30 days after DOC injection (15.3 ± 1.3 EU/24 hours). There were no significant changes in urine volume, body weight, or sodium excretion in any of the rats during the study. Blood pressure in all the DOC-treated rats averaged 129 ± 2 mm Hg before DOC administration and 125 ± 4 mm Hg throughout the study.

Adrenalectomized rats excreted only 1.6 ± 0.2 kallikrein EU/24 hours, a reduction of 63% from the excretion of the sham-operated control rats (4.3 ± 0.7 EU/24 hours, P < 0.01). There was no further change in kallikrein excretion in either group (1.6 ± 0.5 vs. 5.3 ± 1.0 EU/24 hours) during the subsequent collection when all rats drank distilled water. There were no differences in body weight or urine volumes. The sham-operated control rats drinking 1% NaCl excreted amounts of kallikrein equal to the levels in rats drinking 1% NaCl in the altered sodium-intake study.

EFFECT OF ALTERED SODIUM INTAKE

Urinary kallikrein excretion increased two-fold (P < 0.01) after 2 and 4 weeks on the low-sodium diet (Fig. 2). This increase was unrelated to urine volume (20 ± 3 ml/24 hours) or blood pressure (125 ± 7 mm Hg), both of which were essentially unchanged throughout the study. At the end of the 4-week period, these rats weighed an average of 35 g less than the rats on the normal diet. Urinary sodium excretion was essentially zero (< 0.01 mEq/24 hours) during the period on the low-sodium diet. In rats on the normal diet, there was a slight decrease in kallikrein excretion after 2 weeks, but at 4 weeks the level was the same as the initial value.

![Figure 2](http://circres.ahajournals.org/)

**Figure 2**

Effect of altered sodium intake on urinary kallikrein excretion in rats. Number of rats is given in parentheses. Values shown are means ± SE. In rats on the low-sodium diet, values at 2 and 4 weeks are significantly different from their control values (P < 0.01).
Kallikrein levels in rats on the normal diet and in rats formerly on the low-sodium diet were identical 7 weeks after the study began. The reason for the slightly lower kallikrein excretion after 2 weeks on the normal diet is unclear. Probably, the moderate increase in kallikrein excretion in the control rats is related to an increase in the body weight of these rats from 192 ± 5 g at the beginning of the study to 289 ± 7 g at 7 weeks. Urine volume and blood pressure were the same for the control rats and the rats on the low-sodium diet and were not altered throughout the study. Rats receiving the high-sodium diet excreted a slightly higher amount of kallikrein than did the other two groups during the control collection. However, these rats showed no change in kallikrein excretion while they were on the high-sodium diet. Urinary sodium excretion averaged 8.4 mEq/24 hours during the high-sodium diet. When kallikrein excretion was calculated as EU/24 hours 100 g⁻¹, rats on the normal and high-sodium diets had approximately the same kallikrein excretion. Rats on the low-sodium diet still showed the same twofold elevation.

Discussion

Urinary kallikrein excretion is elevated in rats with DOC-salt hypertension (2, 3). However, it was unclear if kallikrein excretion was elevated because of DOC, high-sodium intake, or some other effect of hypertension itself. The present data showed that the administration to rats of DOC alone, in the absence of high-salt and hypertension, significantly increased urinary kallikrein excretion. Normotensive rats drinking saline excreted normal levels of kallikrein. Therefore, the elevated kallikrein excretion seen in rats with DOC-salt hypertension was probably caused by a mineralocorticoid excess and not by a high-salt intake or by the development of hypertension. Also, normotensive rats on a low-sodium diet, a stimulus which increases endogenous mineralocorticoid (aldosterone) activity (6, 7), excreted elevated levels of urinary kallikrein. In contrast, adrenalectomy significantly decreased kallikrein excretion. These data, therefore, suggest a direct relationship between mineralocorticoid activity and urinary kallikrein excretion. Furthermore, preliminary studies in our laboratory showed that urinary kallikrein (molecular weight 30,000) excretion is elevated in normal human subjects on a low-sodium diet (9 mEq Na/day) at a time when urinary amylase (molecular weight 50,000) is slightly decreased. Kallikrein excretion is elevated in patients with primary aldosteronism (1, 2).

A recent report suggested that increased kallikrein excretion is directly related to increased urinary sodium excretion in humans (8). Our data in rats contradict these observations. Normal rats with low-sodium intake, excreting no detectable sodium, had significantly elevated levels of kallikrein excretion. Rats with high-sodium intake, excreting elevated amounts of urinary sodium, had normal levels of kallikrein excretion. Also, urinary sodium excretion did not increase in rats treated with DOC when kallikrein excretion was rising. Thus, increased kallikrein excretion is not related to elevated urinary sodium excretion or sodium escape in these experiments on rats.

Possibly a relationship between plasma renin activity and urinary kallikrein excretion exists. Low-sodium intake, in addition to increasing mineralocorticoid activity, elevates plasma renin activity in rats (9); DOC, however, suppresses plasma renin activity (9). In our experiments, urinary kallikrein was elevated under both experimental conditions. Thus, in the present experiments plasma renin activity would seem to be unrelated to urinary kallikrein excretion. Moreover, increased mineralocorticoid activity is associated with increased kallikrein excretion, and removal of mineralocorticoid activity results in decreased kallikrein excretion; therefore, mineralocorticoid activity appears to be a primary factor controlling urinary kallikrein excretion.

The biologic significance of urinary kallikrein alterations in response to mineralocorticoids or to changes in dietary sodium intake is
unclear. Further evidence relating the kallikrein-kinin system to the renin-angiotensin-aldosterone system is needed. The data presented in this paper combined with previous observations suggest a close and important relationship between mineralocorticoids, sodium homeostasis, and urinary kallikrein excretion.

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