Regulation of rat urinary and renal kallikrein and prekallikrein by corticosteroids

(kininogenase/dexamethasone/deoxycorticosterone/phospholipase A2/renin)

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ABSTRACT Rats were adrenalectomized and injected for 7 days with dexamethasone (DEX) or deoxycorticosterone. Kallikrein and prekallikrein were assayed in urine and in a basolateral membrane-enriched fraction. The activities of renin and phospholipase A2 were also determined in the fraction. Adrenalectomy significantly decreased active kallikrein in urine. Administration of deoxycorticosterone raised the level of active kallikrein in urine without affecting the concentration of prekallikrein. Rats treated with DEX only had high Na+ and low active kallikrein excretion. The total kallikrein level (active kallikrein together with prekallikrein), however, returned to normal because DEX elevated the prekallikrein level. DEX also increased the prekallikrein concentration in the membrane-enriched fraction. Renin activity in the membrane-enriched fraction was enhanced by adrenalectomy but suppressed by either corticosteroid. The changes in the concentration of plasma renin were qualitatively similar but quantitatively different. The activity of phospholipase A2 in the membrane-enriched fraction was enhanced only by deoxycorticosterone. Thus, both glucocorticoid and mineralocorticoid increased kallikrein excretion in the adrenalectomized animals, but DEX was apparently effective at a lower dose than deoxycorticosterone. DEX increases the prekallikrein concentration in urine and on the basal membrane of distal tubular cells and, in addition, may prevent its conversion by releasing an inhibitor of a prekallikrein activator.

Changes in urinary kallikrein excretion have been studied extensively in experimental animals, mainly because hypertensive patients are reported to excrete less of this hypotensive enzyme (1, 2). In laboratory animals, the excretion of urinary kallikrein increases during low sodium diet or after a mineralocorticoid has been administered for several days (1, 3–6). This effect is presumably due to stimulation of renal kallikrein synthesis (7). Kallikrein has been isolated in membrane-bound form from fractions of homogenized kidney (8–10). It was suggested that the membrane fraction containing kallikrein originates from the distal nephron (11), in accord with the observation that kallikrein in stop-flow experiments enters the nephron from distal tubules (12, 13).

Two kallikreins of different physical properties have been identified in the kidney; one in a plasma membrane-enriched fraction (8–10) and the other in a basolateral membrane (BLM)-enriched fraction (14, 15). Kallikrein derived from apical membrane can be the source of urinary kallikrein excreted at the luminal side of the nephron, while kallikrein and prekallikrein of the BLM fraction can be released into the renal venous blood and lymph (2) from the distal tubular cells (14, 15). This latter (BLM) kallikrein has a higher molecular weight and a lower electrophoretic mobility than rat urinary kallikrein (15).

The aim of the present studies was to determine the consequences of chronic administration of a corticosteroid on kallikrein and prekallikrein both in the urine and in the BLM-enriched fraction of rat kidneys. The animals were adrenalectomized to exclude the effects of an endogenous hormone release. Deoxycorticosterone (DOC) was used because of the above-cited effects of mineralocorticoids on urinary kallikrein. In addition, we also administered dexamethasone (DEX) to establish the actions of a glucocorticoid on active and inactive kallikrein.

We found that although the administration of a mineralocorticoid increased the activity of kallikrein in the urine of adrenalectomized rats, the level of prekallikrein was raised primarily by the administration of a glucocorticoid.

MATERIALS AND METHODS

The steroids were purchased from Sigma. Trasylol was donated by G. Haberland of Bayer AG. Trypsin was from Worthington, and S-2266 was from Kabi (Stockholm). Trypsin was coupled to Sepharose as done previously (14). Phospholipase A2 substrate was synthesized by T. Okazaki (University of Texas Health Science Center). Antiserum to kallikrein was donated by O. A. Carretero (Detroit, MI). Other reagents were laboratory grade obtained from commercial sources.

Experimental Procedures. Male Sprague–Dawley rats (190–250 g) were anesthetized with sodium pentobarbital (50 mg/kg intraperitoneally) and the adrenals were removed. After adrenalectomy, the rats, fed with a commercial standard rat chow, were housed in individual metabolic cages.

Steroid Administration. After the animals had adapted to the metabolic cages, they were divided into four experimental groups, with 10–15 in each group. Steroids were administered starting the 8th to 10th day (1st experimental day) after adrenalectomy. Intramuscular injections were made once a day at 09:00. The groups were as follows: 1 (control; n = 14), received the vehicle (sesame oil) only; 2A and 2B (n = 13 and n = 11), received DEX at 250 and 25 μg; 3A and 3B (n = 15 and n = 10), received DOC at 2,500 and 250 μg; 4 (normal; n = 11), were used as the second set of controls. In addition, five adrenalectomized animals received a combination of 25 μg of DEX and 250 μg of DOC.

All rats were offered 0.9% saline solution as drinking fluid during the study; thus, the sodium intake of both normal and adrenalectomized animals was high. The food was removed from the cages the evening before urine collection. Urine was collected individually for 24 hr from 09:00. Blood samples were collected from the tail vein in 0.01 ml of 3.8% Na3EDTA. Rats

Abbreviations: DEX, dexamethasone; DOC, deoxycorticosterone; BLM, basolateral membrane.

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were decapitated in the morning on the 8th experimental day.  

**Tissue Fractionation.** Rat BLM-enriched fractions were prepared from total kidney cortex homogenates pooled from 2–4 animals by using differential centrifugation in sucrose as previously. The BLM-enriched fraction was obtained after 18 separate washings of the sedimented proteins (14, 15).

**Assays.** Urinary kallikrein was measured with S-2266 (D-Val-Leu-Arg-p-nitroanilide) as substrate (8, 10, 15). The activity of urinary kallikrein is expressed in μmol of S-2266 hydrolyzed per min in urine collected in 24 hr or per mg of creatinine excreted. Similar results were obtained with both methods. Urinary total kallikrein was estimated in 5 μl of rat urine after diluting it to 50 μl and adding 25 μg of trypsin or an equivalent amount of Sepharose-bound trypsin (14, 15) in 10 mM Tris-HCl (pH 7.4) for 30 min at 37°C. The reaction was stopped by addition of 500 μg of lima bean trypsin inhibitor. Active kallikrein was measured in the samples without added trypsin. Prekallikrein activity in the samples was calculated by subtracting the value of active kallikrein from that of total trypsin-activated kallikrein. Urinary active kallikrein and total kallikrein from two animals in each group were also determined by bioassay. Kallikrein contracts rat uterine without added substrate. Samples from two animals from each group before and after activation by trypsin in vitro were assayed on the isolated rat uterus in estrus. The activity is expressed in ng of bradykinin equivalent. Because we obtained excellent correlation with the S-2266 activity (r = 0.95, n = 15, P < 0.001), only the results obtained with S-2266 are reported. The kininogenase activity of tissue kallikrein was measured by radioimmunoassay with [125I]-labeled [Tyr3]bradykinin and synthetic bradykinin as standard (8). Kininogenase activity in tissues is expressed as ng of bradykinin released from heated hog plasma per min per mg of protein. For measuring enzymatic activity, the enzyme in the BLM-enriched fraction was solubilized with 0.1% Triton X-100 for 30 min at 4°C and the mixture was then centrifuged at 60 min at 105,000 × g in Tris·HCl, pH 7.4/0.25 M sucrose. The supernatant was assayed for activity. Prekallikrein was activated in the extracts by incubating it with 10 μg of trypsin in 10 mM Tris·HCl (pH 7.4) for 30 min at 37°C and terminating the reaction with 200 μg of lima bean trypsin inhibitor or, when the insoluble trypsin-Sepharose complex was used, by sedimentation (14, 15).

The activity of renin was determined by radioimmunoassay of angiotensin I released from plasma of nephrectomized rats as reported (8).

Phospholipase A2 was assayed with 1-palmitoyl-2-[1-14C]arachidonoyl-sn-glycero-3-phosphoethanolamine (83.3 μM) in a 0.6 ml reaction mixture containing 0.1 M Tris·HCl (pH 8.0) and 10 mM CaCl2 (16).

Protein content was estimated after alkaline digestion according to Lowry et al. (17) using bovine serum albumin as standard. Urinary sodium and potassium were measured in a flame photometer. Urinary creatinine was measured according to a spectrophotometric method (18).

Urine prekallikrein was separated by gel filtration on a Sephadex G-200 column. Urine was applied directly to the column as reported. Preparative polyacrylamide gel electrophoresis was carried out in 5% gels (15). To establish the specificity of kallikrein, rabbit or sheep antiserum to purified rat urinary kallikrein was used to inhibit it. Aprotinin (Trasylol) was also used as an inhibitor.

**Statistical Methods.** Results are expressed as mean ± SEM. The evaluation of statistical probability was carried out, where appropriate, with Student's t test. To establish the existence of correlation between the different variables studied, linear regression analysis, Kendall's rank correlation test (19), and Spearman's rank correlation test (20) were used. The 5% probability level was used as a criterion for significance.

**RESULTS**

**Effect of Steroids.** Administration of 250 μg of DEX for 7 days (group 2A) increased water and sodium excretion and reduced the weight of the animals (Table 1). There were no significant changes in the DOC-treated rats except in weight (groups 3A and 3B). (The lack of difference in sodium excretion between the intact and adrenalectomized rats was probably due to high sodium intake because both groups of animals received saline instead of drinking water.) Urinary creatinine excretion did not change significantly in any group but was lower than in the normal intact animals (P < 0.05).

**Excretion of Prekallikrein and Kallikrein.** Although rats excreted large amounts of active kallikrein, relatively little prekallikrein (19% of total) was detected in normal rat urine (Fig. 1). Adrenalectomy decreased the urinary active kallikrein level (P < 0.001).

Injection of 25 or 250 μg of DEX for 7 days to adrenalectomized animals (groups 2A and 2B) did not change active urinary kallikrein. However, the urinary excretion of total kallikrein measured after activation in vitro was elevated over the level of control group 1 because DEX increased urinary prekallikrein excretion over all other groups (Fig. 1).

Injection of 250 or 2,500 μg of DOC increased the active kallikrein excretion in groups 3A and 3B but significantly increased the prekallikrein concentration in the urine only at the lower dose level (group 3B), although the effect was less than that of DEX.

The administration of combined low doses of DEX and DOC (data not shown) raised prekallikrein (P < 0.001), but not active kallikrein, over the control significantly; thus, total kallikrein activity increased to 2.0 μmol over 1.0 μmol found in control group.

**Table 1. Effect of corticosteroids on adrenalectomized rats**

<table>
<thead>
<tr>
<th>Rat group</th>
<th>n</th>
<th>Body weight, g</th>
<th>Urinary volume, ml/day</th>
<th>Urinary excretion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td>11</td>
<td>247 ± 9</td>
<td>11.5 ± 2.4</td>
<td>2.0 ± 0.4</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>7.3 ± 0.6*</td>
</tr>
<tr>
<td>Adrenalectomized</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (group 1)</td>
<td>14</td>
<td>208 ± 6*</td>
<td>10.9 ± 1.5</td>
<td>1.5 ± 0.2</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>5.3 ± 0.7</td>
</tr>
<tr>
<td>DEX (250 μg) treated (group 2A)</td>
<td>13</td>
<td>190 ± 5*</td>
<td>30.3 ± 2.5*</td>
<td>4.3 ± 0.4*</td>
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<td></td>
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<td></td>
<td>5.8 ± 0.3</td>
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<tr>
<td>DOC (2,500 μg) treated (group 3A)</td>
<td>15</td>
<td>233 ± 5*</td>
<td>14.8 ± 3.6</td>
<td>1.7 ± 0.3</td>
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<td>6.0 ± 0.4</td>
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<tr>
<td>DOC (250 μg) treated (group 3B)</td>
<td>10</td>
<td>226 ± 4*</td>
<td>10.5 ± 2.1</td>
<td>1.5 ± 0.2</td>
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</tbody>
</table>

*P < 0.05, compared with control.
*P < 0.005, compared with normal.
*P < 0.005, compared with control.
Correlations with Urinary Volume and Sodium. To determine whether active kallikrein and prekallikrein excretion correlated with urinary volume or urinary sodium excretion, the relationship between them was determined. Active kallikrein excretion in normal intact rats correlated positively with urine volume and urinary sodium. Urinary prekallikrein excretion in normal rats did not correlate statistically significantly with these parameters. In adrenalectomized control rats, there was a significant correlation between urinary prekallikrein and urinary volume (r = 0.83, P < 0.001) and between active kallikrein and sodium excretion (r = 0.60, P < 0.05).

In DEX-treated rats (group 2A), a significant correlation was obtained between prekallikrein and urinary sodium excretion (r = 0.73, P < 0.005). In contrast to these results, prekallikrein excretion in DEX-treated rats (group 3A) correlated highly significantly with urinary volume (r = 0.99, P < 0.001) but not with sodium excretion.

Because of the similarity in activation of tissue and urinary prekallikrein, the correlation between urinary and BLM prekallikrein in groups 1, 2A, and 3A was analyzed. In calculating the correlation coefficient, the mean urinary prekallikrein value of each group in each experiment was taken into consideration. A statistically significant correlation was observed between urinary prekallikrein and BLM prekallikrein (Kendall’s rank correlation coefficient r = 0.45, n = 12, P < 0.05; Spearman’s rank correlation coefficient r_s = 0.64, n = 12, P < 0.05).

The BLM Fraction. The effects of adrenalectomy and corticosteroids on kallikrein and prekallikrein concentration in the BLM fraction are shown in Fig. 2. The differences in active kallikrein concentration were not statistically significant. Although the prekallikrein concentration in group 1 animals was apparently below normal, because of the small number of groups used, this difference was not significant either.

In DEX-treated rats (group 2A), the prekallikrein concentration in the fraction increased above the level found in normal intact rats. This value is significantly higher than that found in DEX-treated (group 3A) and control (group 1) rats (P < 0.01 and P < 0.02).

There was no significant difference in kallikrein or prekallikrein concentration in crude renal homogenates of these animals (data not shown).

Phospholipase A_2 in the BLM Fraction. Adrenalectomy did not significantly change the phospholipase A_2 activity in the BLM fraction. Phospholipase A_2 activity in the fraction of group 3A increased 61% over control (15.8 vs. 9.5 (nmol/hr)/mg) after injections of DOC, but DEX (group 2A) had no significant effect on this enzyme.

Renin in Plasma and in the BLM Fraction. The renin concentration was low in the untreated BLM fraction; only 7–12% of total renin activity was detected without Triton. After Triton X-100 was added, the solubilized BLM fraction released 216 ± 12 ng of angiotensin I/hr per mg of protein. This value almost doubled after adrenalectomy (group 1, 425 ± 44), but treatment with DEX or DOC decreased it (group 2A, 193 ± 59, P < 0.02; group 3A, 117 ± 38, P < 0.005). The value in group 3B was also lower than that in control rats (158 ± 11) and administration of the combination of 25 μg of DEX and 250 μg of DOC decreased renin in the BLM fraction to 139 ± 23. Qualitatively similar but quantitatively different changes were found in the plasma of the rats. Adrenalectomy, as expected, enhanced the renin concentration in plasma more than in tissues. Either DEX or DOC decreased it, although DOC was clearly more effective than DEX, because it decreased the value to less than that found in intact normal animals. The plasma renin concentration was 26 ± 4 ng/hr per ml in intact animals, 189 ± 24 in group 1, 53 ± 13 in group 2A, 15 ± 2 in group 3A, and 51 ± 7 in group 3B.

Urinary Kallikrein. Urinary kallikrein of DEX-treated animals of group 2 appeared to be similar to normal urinary kallikrein when analyzed by gel filtration and polyacrylamide gel electrophoresis. It was eluted from a Sephadex G-200 column in a peak corresponding to a molecular weight of about 40,000. In preparative polyacrylamide gel electrophoresis at pH 8.9, it migrated with the front (R_f = 1). Activation with trypsin of the urinary prekallikrein in fractions collected after gel filtration or eluted from polyacrylamide gel slices (15) indicated that prekallikrein had about the same molecular weight and electrophoretic mobility as urinary active kallikrein.

The kininogenerate activity of both kallikrein and trypsin-activated prekallikrein from the BLM fractions was inhibited >95% by antiserum (1:1,000 to 1:3,000 [vol/vol]) to rat urinary kallikrein or by aprotinin (10 international units).

DISCUSSION
It has been known for some time that the higher molecular weight plasma kallikrein occurs as a proenzyme (21), and it has recently
been found that the lower molecular weight tissue or glandular kallikrein is also present as an inactive proenzyme in tissues such as kidney (8) and in urine (22). To conform to the terminology used in the past literature, we refer here to the proenzyme, which is activated by trypsin, as prekallikrein.

We investigated the effects of a glucocorticoid (DEX; groups 2A and 2B) and a mineralocorticoid (DOC; groups 3A and 3B) on urinary and kidney kallikrein in adrenalectomized rats. The activity of renin was also measured.

Efforts have previously been made to find a link between kallikrein excretion, sodium metabolism, and development of high blood pressure (1, 2). A pronounced enhancement of kallikrein excretion in aldosteronism has indeed been observed (1, 2) but aldosterone did not increase kallikrein in the perfusate of kidneys (23). Because part of the renal kallikrein is a plasma membrane-bound enzyme, the finding that chronic administration of high doses of a mineralo- or a glucocorticoid increased the area of basolateral cell membrane of the renal cortical collecting tubules of the rabbit was of particular interest (24). It was also reported that the area of the luminal membrane, the cell number, and the cell volume were not changed significantly by these hormones.

Adrenalectomy leads to decreased urinary kallikrein excretion in rats (3). In our experiments adrenalectomy also decreased the excretion of active urinary kallikrein. When correlation coefficients were calculated, based on determinations done in individual urine samples, it was found that the level of urinary kallikrein correlated well with the excretion of sodium while prekallikrein correlated statistically significantly with the urinary volume in adrenalectomized rats.

The origin of renin in the BLM-enriched fraction is not clear. However, it is present there, although mostly in inactive membrane-bound form, even after an isolation procedure involving 18 different washing steps (15). Adrenalectomy increased renin activity 2-fold in the BLM fraction but more than 7-fold in plasma. A possible cause for the latter effect may be the removal of the source of aldosterone by adrenalectomy.

Effects of DOC. Urinary excretion of sodium in group 3A decreased initially on the 3rd day (data not shown) but, by the 7th experimental day, the excretion of sodium had returned to the control level (group 1) in this group. On the 7th day, this elevation in sodium excretion due to the phenomenon of escape from mineralocorticoids coincided with an increase in kallikrein excretion. At the same time, renin activity decreased in the BLM-enriched fraction. DOC administration did not affect prekallikrein either in urine or in BLM fractions of group 3A. A statistically significant correlation was found between urine volume and prekallikrein excretion in group 3A.

The increased kallikrein activity may lead to increased production of kinins. Kinins can enhance prostaglandin synthesis (25, 26) and, through this mechanism, they may affect renal hemodynamics and urinary electrolyte excretion. It has been reported that mineralocorticoids enhance urinary excretion of E-type prostaglandins in rats as a consequence of activation of the renal kallikrein–kinin system by corticosteroids (6). We found that DEX increased phospholipase A₂ activity 61% in the BLM fraction (group 3A) but DEX had no effect. This implies a hormonal control only by DOC, similar to the effect of aldosterone on the enzyme in toad bladder (27). Since phospholipase A₂ (9, 28) enhances (or activates) membrane-bound kallikrein, possibly via the release of lysocleithin (9, 10, 28), this could be a mechanism whereby mineralocorticoids enhance active kallikrein excretion.

The elevated renin concentration in plasma and in the BLM fraction brought about by removing the adrenals was lowered to less than normal by DOC.

Effects of DEX. The effects of glucocorticoid administration were more complicated. In addition to influences on renal gluconeogenesis, the physiological actions of glucocorticoids on renal handling of salt and water include modulation of the glomerular filtration rate and possibly control of the synthesis of Na⁺/K⁺-ATPase in various target segments (29, 30) removed after chronic administration. Regarding kallikrein in the perfused rat kidney, DEX increased kallikrein release in the venous effluent (23) but decreased active kallikrein excretion in intact rats (31, 32).

We found that adrenalectomized animals indeed do excrete less active kallikrein after DEX injection but, because of the appearance of prekallikrein, which is normally low in rat urine, total kallikrein excretion was higher than in control groups (Fig. 1). Significantly more prekallikrein was found in the BLM fraction of adrenalectomized rats that had been treated with DEX (Fig. 2). Thus, while DOC certainly increases urinary active kallikrein (Fig. 1), prekallikrein appears to be under the influence of DEX.

Prekallikrein was previously found in kidney membrane fractions of rat (10, 28) and in effluents of the perfused kidney (33) but, as mentioned above, its level is very low in urine of normal or adrenalectomized rats. Kallikrein and prekallikrein in urine or DEX-treated animals had a molecular weight of about 40,000 and the same electrophoretic mobility as normal rat urinary kallikrein (15).

It appears that glucocorticoids can enhance the synthesis of prekallikrein, but they inhibit its conversion to active kallikrein. Prekallikrein can be converted to kallikrein by serine proteases such as trypsin or plasmin (14, 15). Administration of a glucocorticoid can lead to inhibition of an intracellular plasminogen activator (34), a serine protease that converts plasminogen to plasmin. DEX may induce the synthesis of such an inhibitor of serine proteases and thereby block the activation of prekallikrein and consequently elevate its level.

In addition, DEX may affect prekallikrein excretion indirectly by its effect on sodium and water excretion (Table 1).

Changes in renin activity in the BLM fraction brought about by steroid treatment of adrenalectomized animals were grossly reflected by changes in plasma renin concentration. The elevated renin activity in plasma (35) or BLM fractions of rats was suppressed by DEX, but in this case administration of DEX was less effective than that of DOC.

In conclusion, it appears that active kallikrein excretion from the luminal side of the distal tubules is controlled primarily by DOC, while DEX controls prekallikrein concentration both in urine and in the basal membrane on the blood side of the nephron. The action of DEX on prekallikrein may include, in addition to stimulation of the synthesis of the proenzyme, induction or release of an inhibitor of a protease-type prekallikrein activator.

Although in these investigations steroids were used mainly in pharmacological doses, a more detailed kinetic analysis of a replacement therapy should be the subject of future studies.

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