Interrelations of the Renal Kallikrein-Kinin System and Renal Prostaglandins in the Conscious Rat

Influence of Mineralocorticoids

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SUMMARY To investigate possible relationships between mineralocorticoids, the renal kallikrein-kinin system, and renal prostaglandins, we studied the effects of aldosterone and deoxycorticosterone acetate (DOCA) and of an inhibitor of kallikrein, aprotinin, on the urinary excretion of kallikrein and prostaglandin E-like substance (PGE) by the conscious rat. Aldosterone (0.25 mg/day, sc), injected into six rats for 14 consecutive days, increased PGE and kallikrein excretion from 52.3 ± 8.7 (mean ± SE) ng/day and 29.8 ± 3.0 U/day to 141.5 ± 30.7 ng/day (P < 0.02) and 105.6 ± 28.1 U/day (P < 0.05), respectively. Similarly, injections of DOCA (5 mg/day) into 14 rats increased the excretion of PGE and kallikrein, measured before and after 10 days of treatment, from 41.6 ± 3.9 ng/day and 39.4 ± 4.9 U/day to 194.3 ± 20.7 ng/day (P < 0.001) and 90.6 ± 14.7 U/day (P < 0.001), respectively. Injections of aprotinin for 4 days (50,000 KIU twice daily, sc) in conjunction with DOCA into eight rats pretreated with the steroid for 10 days decreased the urinary excretion of kallikrein and PGE, measured on the 4th day of aprotinin administration, by 61% (P < 0.01) and 80% (P < 0.001), respectively. Urinary potassium excretion decreased throughout the course of aprotinin treatment, whereas sodium excretion and urine volume decreased during the first 2 days but subsequently returned toward control values. This study demonstrates that mineralocorticoids enhance the urinary excretion of PGE, and this effect appears to be a consequence of activation of the renal kallikrein-kinin system by the steroids. Thus, changes in the intrarenal activity of the kallikrein-kinin system may modulate renal prostaglandin release.

THE kidney contains kallikrein(s) which releases the decapeptide lysyl-bradykinin (kallidin) from protein substrate(s). Recent studies indicate that interactions occur between the renal kallikrein-kinin system and two classes of hormones, mineralocorticoids and renal prostaglandins, which could affect renal function. That mineralocorticoids enhance the activity of the renal kallikrein-kinin system is inferred from the finding of increased urinary kallikrein excretion in patients with primary aldosteronism and in patients and animals receiving sodium-retaining steroids. The kallikrein-kinin system in turn influences renal prostaglandin release. Thus, bradykinin stimulates release of a prostaglandin E-like substance into renal blood, and kinins generated intrarenally increase the venous and urinary effluxes of E prostaglandins from the isolated rabbit kidney perfused with Krebs solution. The renal kallikrein-kinin and prostaglandin systems may be instrumental in buffering the sodium-retaining action of mineralocorticoids for the following reasons. First, kinins and prostaglandin E2 (PGE2), the major product of prostaglandin synthesis in the renal medulla, share the ability to effect natriuresis and renal vasodilation. Second, PGE2 inhibits sodium transport in isolated cortices and outer medullary collecting tubules taken from deoxycorticosterone-treated rabbits. A corollary of these observations, if one assumes tonic activity of the renal kallikrein-kinin system on prostaglandin production by the kidney in vivo, is that activation of the kallikrein-kinin system by sodium-retaining steroids may enhance renal PGE2 production, and this in turn could influence the tubular actions of mineralocorticoids. To test the first component of the hypothesis, i.e., that activation of the renal kallikrein-kinin system by mineralocorticoids augments prostaglandin production by the kidney, we studied the effects of two steroids, aldosterone and deoxycorticosterone, and of an inhibitor of kallikrein, aprotinin, on the urinary excretion of kallikrein and prostaglandin E-like substance (PGE) by the rat.

Methods

Thirty-eight male Sprague-Dawley albino rats (Southern Animal Farms) weighing 220–270 g were housed in individual metabolism cages in a temper-
ature-controlled room (24°C) that was illuminated from 6:00 a.m. to 6:00 p.m. The rats were fed pellets of a modified sodium-deficient rat diet (Nutritional Biochemicals) and had access to two bottles to drink, one containing a fixed allotment of 0.15 m NaCl (10 ml) and the other deionized water (100 ml). After adaptation to the metabolism cage, each rat drank all the allocated saline solution (10 ml), which fixed the intake of sodium at 1.50 meq/day, plus variable amounts of deionized water.

Experimental Protocol

The studies were performed 10–12 days after the rats had become acclimated to the metabolism cages and to the feeding and drinking conditions. Following a 3- to 5-day control period, the rats were divided into five groups. Group I: six rats were injected with d-aldosterone-21-acetate (Sigma Chemical Co), 0.25 mg/day, for 14 consecutive days. Group II: 14 rats received deoxycorticosterone acetate (DOCA, Sigma), 5 mg/day, for 14 days. On the 10th day of DOCA treatment, the rats were divided into two subgroups; eight were injected with the kallikrein inhibitor, aprotinin (50,000 kallikrein inhibitor units, KIU, twice daily) (Trasylol; Farbenfabriken Bayer) in conjunction with DOCA for 4 consecutive days; the six remaining rats served as controls and received DOCA and the carrier of aprotinin only. Group III: six rats were injected with sesame oil (0.20 ml/day), the vehicle for aldosterone and DOCA, for 16 days. Groups IV and V included rats receiving only aprotinin (50,000 KIU twice daily, n = 6) and vehicle (5% dextrose, 0.20 ml, twice daily, n = 6), respectively, for 4 consecutive days. Each dose of steroids, suspended in 0.20 ml of sesame oil, and aprotinin, dissolved in 0.20 ml of 5% dextrose in water, was injected by the subcutaneous route.

Every day, beginning at 8:00 a.m., each rat was induced to empty its bladder by sniffing ether and by applying pressure on the suprapubic region. Urine was collected for 24 hours under mineral oil except when measuring prostaglandins and kallikrein, in which case the urine was received in plastic bottles surrounded by dry ice to keep the urine frozen throughout the collection period. Prostaglandins and kallikrein in urine were measured at intervals before and after an experimental intervention. Twenty-four hour fluid intake, urine volume, and urinary excretion of sodium and potassium were determined daily. Body weight and systolic blood pressure, the latter measured by tail sphygmography under light ether anesthesia, were determined twice weekly.

At the conclusion of each experiment, the rats were anesthetized with ether and blood was drawn through a cannula in the abdominal aorta to measure the concentration of serum sodium and potassium. Before killing the rats, we consecutively occluded the aorta above the renal arteries, cut both renal veins and ureters, flushed the kidneys in situ with cold 0.15 m NaCl (100 ml) to remove the blood, and stored the kidneys (~20°C) for tissue kallikrein assay.

Analytical Methods

Determination of Kallikrein

Kallikrein in urine and kidney tissue was measured by the methods of Marin-Grez and Carretero12 and Carretero et al.,12 respectively, with modifications. These procedures are based on the measurement of kinins formed when a sample containing kallikrein is incubated with an excess of kinogen, the protein precursor of kinins, in the presence of inhibitors of kininases. Kininogen was obtained from pooled canine plasma, collected in the presence of sodium citrate (3.1 mg/ml) and hexadimethrine (0.4 mg/ml), by a procedure involving heat treatment (62°C for 2 hours) to inactivate both kinin-forming and kinin-inactivating enzymes, ammonium sulphate fractionation (1.9 M), dialysis, and lyophilization.6

The lyophilized substrate was dissolved to a concentration of 50 mg/ml in a solution containing 0.15 m NaCl, 0.003 m 1,10 phenylenediaminetetraacetate (EDTA); subsequently, the kininogen solution was adjusted to pH 8.5 with 5 n NaOH. To measure urine kallikrein activity, we incubated 2 μl of urine with 1 ml of prewarmed (37°C) kininogen solution (50 mg/ml) for 20 minutes at 37°C and pH 8.5. The enzymic reaction was terminated after addition of 0.02 malonate buffer, pH 5.5 (1 ml), by heating in a boiling water bath for 5 minutes. Following centrifugation, the supernatant fluid was neutralized (pH 7.0) and assayed for kinins in the canine hindlimb preparation with bradykinin as reference standard (Sandoz).13 Urinary kallikrein activity is expressed in units of enzyme per milliliter of urine (units/ml). Kallikrein excretion (kallikrein activity × 24 hour urine volume) is expressed as units of enzyme excreted per day (units/day). Arbitrarily, we defined 1 unit of kallikrein as the amount of enzyme that, under the assay conditions described above, releases from canine kinogen 1 μg of bradykinin equivalent per minute of incubation. To measure kidney kallikrein activity,12 the whole kidney was homogenized in 0.012 m deoxycholic acid, kept for 60 minutes at 3–5°C, and centrifuged. Subsequently, 0.05 ml of supernatant fluid corresponding to 10 mg of wet kidney tissue was incubated for 10 minutes at pH 8.5 and 37°C with 1 ml of kininogen solution (50 mg/ml) containing the kininase II inhibitor Pyr-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro (10 μg/ml) SQ-20881, Squibb). The enzymatic reaction was terminated after addition of 0.02 m malonate buffer, pH 8.5 (1 ml), by heating in a boiling water bath. Following centrifugation, the supernatant fluid was neutralized (pH 7.0) and assayed for kinins in the canine hindlimb preparation with bradykinin as reference standard.13 Kidney kallikrein activity...
is expressed in units per gram of wet kidney (U/g).

The initial concentration of kininogen in the assay mixtures was equivalent to 6000 ng of bradykinin/ml; incubation with either urine (2 μl) or kidney homogenate (10 mg) resulted in a consumption of substrate that did not exceed 5% of the initial amount. Kinin formation was directly proportional to both the duration of incubation (0–60 min) and the amount of enzyme. Complete inhibition of kininogen during incubations was demonstrated by almost complete recovery (96%) of the bradykinin (50 ng) incubated with either urine, kidney homogenate, or kininogen, in the presence of kininase inhibitors (1,10-phenanthroline, EDTA, SQ-20881). Addition of aprotinin (100 KIU) to incubation mixtures containing kininogen and either urine (5 μl) or kidney homogenate (10 mg) inhibited kinin formation.

Kinin formed by kidney and urinary kallikrein was bioassayed in the hindlimb of the dog by bracketing the increase in femoral blood flow produced by the unknown sample between the increases elicited by synthetic bradykinin, the reference standard. The mean of nine replicate assays of kallikrein in a sample of rat urine was 1.10 ± 0.05 (se) U/ml.

**Determination of Prostaglandins**

The content of prostaglandin-like substance in urine was determined as follows. Samples (10–15 ml) were combined with 5 volumes of acetone, passed through a fritted glass funnel (pore size 4–5.5 μm), and evaporated to 4–8 ml under reduced pressure at 35°C. The aqueous extract was adjusted to 10–12 ml with distilled water, acidified to pH 3.0 with formic acid, and extracted three times with an equal volume of ethyl acetate. Lipids in the ethyl acetate phase were extracted subsequently with 0.1 M potassium phosphate buffer, pH 8.0; the aqueous phase was separated, acidified to pH 3.0 with formic acid, and extracted with chloroform. The chloroform phase was evaporated to dryness and the lipid residue was dissolved in a minimum amount of chloroform-methanol (4:1, vol/vol), applied as a band to a silica gel thin layer chromatography plate (Silica Gel S-254, 0.5 mm, Brinkman Instruments), and chromatographed using the solvent system chloroform-methanol-acetic acid (18:2:1, by volume). Zones of the preparative plate corresponding to the position of PGE2 (Rf 0.44), PGF2α (Rf 0.26) in some experiments, and the area in between them were scraped off and eluted with chloroform-methanol (4:1, vol/vol). Eluates were dried in nitrogen, reconstituted in 0.15 M NaCl, and bioassayed for their content of prostaglandin E- (PGE) and F- (PGF) like material using PGE2 and PGF2α, respectively, as reference standards.

The concentration of prostaglandin-like substance in samples was determined by bracket assay on strips of rat stomach superfused with Krebs solution. A rat colon and a chick rectum were included in the assay system to obtain additional evidence in support of the tentative identification of prostaglandins in the samples. The reconstituted eluate of the plate at the zones corresponding to the migration of prostaglandins E2 and F2α standards contained material which contracted all three assay organs in a manner indistinguishable from the effects of PGE2 and PGF2α, respectively.

Other products of renal prostaglandin synthesis having chromatographic mobility similar to that of PGE2 in the solvent system chloroform-methanol-acetic acid (18:2:1), e.g., PGE1 and 6-keto-PGF1α, could contribute to the myotropic activity eluted from the “PGE” zone of the chromatography plate. However, several arguments minimize the significance of such a contribution. First, PGE1 is a minor product of renal prostaglandin synthesis in the rat and is excreted into urine at a rate 1/100 that of PGE2. Second, the stable derivative of PGI2, 6-keto-PGF1α, has only 1/500 the musculotropic activity of PGE2 on strips of rat stomach. Third, like PGE2, the myotropic activity eluted from the plate at a zone corresponding to the migration of authentic PGE2 vanished after alkali treatment (pH 12 at 100°C for 5 minutes). This behavior is compatible with a prostaglandin of the E series rather than with 6-keto-PGF1α, which is resistant to alkali treatment.

Excretion rate of PGE and PGF-like substance (24-hour urine volume × prostaglandin concentration) is expressed as nanograms of PGE2 and PGF2α equivalents excreted per day (ng/day), respectively. Excretion values are uncorrected for losses incurred in extraction and chromatographic purification. Recovery values of authentic PGE2 and PGF2α added (50 ng each) to rat urine averaged 64.7 ± 5.0% and 58.4 ± 5.5% (se), respectively (n = 8). The means ± se of six replicate assays of PGE2- and PGF2α-like substance in pooled rat urine were 4.6 ± 0.2 and 5.9 ± 0.4 ng/ml of PGE2 and PGF2α equivalents. There is evidence that urinary prostaglandins originate in the kidney. Contamination of urine with prostaglandins of seminal fluid origin is unlikely in the rat since vasectomy did not affect the excretion of PGE into urine (44.3 ± 4.5 ng/day 7 days after vasectomy, n = 5, vs. 42.5 ± 5.1 ng/day in sham-operated rats, n = 5).

**Determination of Electrolytes**

Serum and urine determinations of sodium and potassium were made by flame photometry using lithium as internal standard.

All results are expressed as mean ± se. Statistical significance was determined by Student’s t-test.

**Results**

**Effects of Vehicle for Steroids (Fig. 1)**

In six rats, we injected sesame oil (0.2 ml/day) for 16 consecutive days. The excretion rate of kallikrein and PGE during the control period averaged...
42.1 ± 10.0 U/day and 44.7 ± 12.2 ng/day, respectively. Repeated injections of sesame oil affected neither kallikrein nor PGE excretion rates. Values of urinary sodium, urinary potassium, urine volume, and systolic blood pressure during vehicle administration did not differ from pretreatment values.

Effects of Aldosterone (Fig. 2)

Aldosterone (0.25 mg/day, sc), injected into six rats for 14 consecutive days, increased urinary PGE excretion from 52.3 ± 8.7 ng/day to 89.7 ± 19.7 (P < 0.05) and 141.5 ± 30.7 (P < 0.02) ng/day on the 7th and 14th day of treatment, respectively. Concomitantly, urinary kallikrein excretion rose to 73.8 ± 8.4 U/day (P < 0.01) on day 7 and 105.6 ± 28.1 U/day (P < 0.05) on day 14 from a mean control value of 29.8 ± 3.0 U/day. Kidney kallikrein activity in aldosterone treated rats (2.34 ± 0.10 U/g) was higher (P < 0.01) than in control rats treated with vehicle (1.63 ± 0.13 U/g) (Table 1).

On the first day of treatment with aldosterone, urinary sodium excretion decreased by 17% (P < 0.001), from 1.30 ± 0.12 to 1.07 ± 0.11 mEq/day, and potassium excretion increased by 10% (P < 0.05), from 3.29 ± 0.05 to 3.63 ± 0.12 mEq/day. On the next day, the second day of steroid treatment, sodium excretion (1.42 ± 0.07 mEq/day) returned to near control level whereas potassium excretion (3.57 ± 0.07 mEq/day, P < 0.01) remained elevated; thereafter, neither sodium nor potassium excretion differed significantly from control. Serum potassium concentration in aldosterone-treated rats (3.53 ± 0.23 mEq/liter) was lower than in control rats injected with vehicle (4.34 ± 0.08, P < 0.01), but there was no difference in serum sodium concentration (Table 1). Urine volume increased gradually from 12.6 ± 0.8 ml/day to 32.4 ± 3.6 ml/day (P < 0.01) after 14 days of steroid treatment. Aldosterone tended to increase systolic blood pressure slightly, but the change was not significant. Body weight gain in rats receiving the steroid (1.2 ± 0.2 g/day) was lower than in vehicle-treated controls (2.4 ± 0.1 g/day, P < 0.001).

Effects of Deoxycorticosterone (Fig. 3)

Control urinary PGE and PGF excretion in 14 rats injected with DOCA (5 mg/day, sc) was 41.6 ± 3.9 ng of PGE2 equivalents per day and 33.3 ± 3.5 ng of PGF2α equivalents per day, respectively. PGE excretion rate did not change on the first day of steroid treatment, (48.0 ± 5.2 ng/day, P > 0.05), but increased to 97.4 ± 10.3, (P < 0.001), 142.1 ± 32.3, (P < 0.01) and 194.3 ± 20.7 ng/day (P < 0.001) after 4, 7, and 10 days of treatment, respectively. PGF excretion rate also increased on days 4 and 10 of DOCA administration (51.4 ± 8.5 ng/day, P < 0.05 and 60.1 ± 7.4 ng/day, P < 0.01, respectively), but to a lesser extent than PGE. Urinary kallikrein excretion averaged 34.6 ± 4.9 and 39.4 ± 4.9 U/day on two different control days and 48.1 ± 11.2 U/day (P < 0.2) on the first day of steroid treatment. Thereafter, it increased to 75.0 ± 9.8 (P < 0.001), 98.6 ± 12.0 (P < 0.01), and 90.6 ± 14.7 U/day (P < 0.001) on days 4, 7, and 10 of DOCA treatment, respectively. Kidney kallikrein activity in DOCA-treated rats (2.27 ± 0.22 U/g) was higher than in
vehicle-treated controls (1.63 ± 0.13 U/g, P < 0.05). Urinary sodium excretion fell by 53%, from 1.29 ± 0.05 to 0.69 ± 0.07 mEq/day (P < 0.001), the first day of steroid treatment but on the following day it returned to pretreatment levels. Urinary potassium excretion increased during the first 3 days of DOCA administration, but thereafter it returned to pretreatment values. Serum potassium concentration in DOCA-treated rats was lower (P < 0.001) than in controls treated with vehicle, but serum sodium concentration remained unchanged (Table 1). Urine volume and fluid intake increased gradually from 12.5 ± 1.2 ml/day and 28.2 ± 2.2 ml/day to 27.3 ± 2.3 ml/day (P < 0.001) and 35.4 ± 2.7 ml/day (P < 0.05), respectively, after 10 days of steroid treatment. DOCA administration did not affect systolic blood pressure. Body weight gain in DOCA-treated rats (3.3 ± 0.3 g/day) was not significantly higher than weight gain in vehicle-treated controls (2.4 ± 0.1 g/day, P < 0.1).

**Effects of Aprotinin in DOCA-Treated Rats**

In rats pretreated with DOCA (5 mg/day) for 10 days, we injected either aprotinin (50,000 KIU, sc, twice daily) (n = 8) or its vehicle (5% dextrose, 0.2 ml) (n = 6) in conjunction with the steroid for 4 consecutive days. Repeated injections of vehicle had no discernible effect on the urinary excretion of kallikrein, prostaglandins, and electrolytes (Table 2). In contrast, treatment with aprotinin, an inhibitor of kallikrein,11 clearly affected the kallikrein activity of kidney and urine and the excretion rate of prostaglandins (Table 2). Kidney kallikrein activity in rats receiving DOCA and aprotinin (1.10 ± 0.10 U/g) was lower than in rats treated with sesame oil (1.63 ± 0.13 U/g, P < 0.01) or DOCA alone (2.27 ± 0.22 U/g, P < 0.001) (Table 1). Urinary kallikrein in aprotinin-treated rats decreased by 43% (P < 0.05) and 61% (P < 0.01) after 2 and 4 days of treatment (Table 2). Concomitantly, PGE...
excretion decreased by 67% ($P < 0.001$) on the second, and by 80% ($P < 0.001$) on the fourth day of aprotinin treatment, whereas PGF excretion rate diminished slightly (21%, $P < 0.05$) on the last day only (Table 2).

Urinary potassium excretion decreased throughout the course of aprotinin treatment, whereas sodium excretion fell during the first 2 days but subsequently increased to a level exceeding pretreatment values (Table 2). Serum potassium in rats receiving DOCA and aprotinin (3.57 ± 0.14 mEq/liter) was lower than in rats injected with sesame oil (4.34 ± 0.08 mEq/liter, $P < 0.001$) and slightly higher than in rats given only DOCA (3.10 ± 0.17 mEq/liter, $P < 0.1 > 0.05$) (Table 1). Serum sodium was not affected by aprotinin (Table 1). Urine volume decreased by 55% ($P < 0.001$) on the first day after giving the inhibitor of kallikrein but gradually returned to near pretreatment values on the fourth day of treatment. Fluid intake decreased in six of eight rats treated with aprotinin, but the change was not significant ($P > 0.1$) (Table 2). The kallikrein inhibitor did not affect systolic blood pressure. Body weight gain in aprotinin-treated rats (3.7 ± 0.7 g/day) was not different from weight gain in vehicle-treated controls (2.5 ± 0.5 g/day) (Table 2).

### Effects of Aprotinin in Normal Untreated Rats

In normal untreated rats, we injected either aprotinin (50,000 KIU, sc, twice daily, $n = 6$) or its vehicle (5% dextrose, sc, 0.2 ml, $n = 6$) for four consecutive days. The effects of kallikrein inhibitor in normal rats resembled those in DOCA-treated animals. Kidney kallikrein activity in rats given aprotinin (0.83 ± 0.08 U/g) was 48% lower than in vehicle-treated rats (1.59 ± 0.16 U/g, $P < 0.01$) (Table 1). Urinary kallikrein in rats receiving the enzyme inhibitor decreased by 24% ($P < 0.01$) on the first, 42% ($P < 0.01$) on the third, and 60% ($P < 0.01$) on the fourth day of treatment (Table 3). Simultaneously, the excretion rate of PGE decreased by 63% ($P < 0.01$) and 67% ($P < 0.01$) after 1 and 3 days of aprotinin treatment, respectively (Table 3).

Urine sodium excretion fell from 1.37 ± 0.04 mEq/day to 0.91 ± 0.15 mEq/day ($P < 0.05$) the first day after aprotinin but returned to near-pretreatment values thereafter. Potassium excretion was not affected by the kallikrein inhibitor during the first days of treatment but decreased by 24% ($P < 0.01$) on the fourth day (Table 3). Aprotinin did

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**Table 2 Effects of Aprotinin on the Urinary Output of Kallikrein and of Prostaglandin-like Substances in Rats Pretreated with Deoxycorticosterone (5 mg/day)**

<table>
<thead>
<tr>
<th>Days of DOCA treatment</th>
<th>Kallikrein (U/day)</th>
<th>PGE (ng/day)</th>
<th>PGF (ng/day)</th>
<th>UN.V (mEq/day)</th>
<th>UKV (mEq/day)</th>
<th>Urine volume (ml/day)</th>
<th>Fluid intake (ml/day)</th>
<th>Arterial pressure (mmHg)</th>
<th>Body weight (g)</th>
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<tr>
<td>10</td>
<td>99.8 ± 5.1</td>
<td>224.7 ± 15.3</td>
<td>56.8 ± 3.7</td>
<td>6.4 ± 0.1</td>
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<td>123 ± 273</td>
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<td>11</td>
<td>0.75 ± 0.10</td>
<td>2.78 ± 0.11</td>
<td>10.5 ± 1.6</td>
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<td>12</td>
<td>0.76 ± 0.13</td>
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<td>13</td>
<td>1.89 ± 0.13</td>
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<td>16.8 ± 1.6</td>
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<td>14</td>
<td>3.9 ± 0.10</td>
<td>4.53 ± 1.6</td>
<td>21.7 ± 1.9</td>
<td>27.2</td>
<td>126 ± 2.5</td>
<td>2.9 ± 1.6</td>
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A. Aprotinin-treated group ($n = 8$)

B. Vehicle-treated group ($n = 6$)

Abbreviations: PGE and PGF = Excretion rate of prostaglandin E-like and F-like substances; UN.V = sodium excretion; UKV = potassium excretion; $n$ = number of animals. Sodium intake was 1.5 mEq/day throughout the experiment. Aprotinin and vehicle were injected on each of four consecutive days. Values are means ± SE. Significance of difference from control (paired Student’s Mest): * $P < 0.01$; † $P < 0.001$; ‡ $P < 0.05$; § $P < 0.02$. 

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Effects of Aprotinin in Normal Untreated Rats

In normal untreated rats, we injected either aprotinin (50,000 KIU, sc, twice daily, $n = 6$) or its vehicle (5% dextrose, sc, 0.2 ml, $n = 6$) for four consecutive days. The effects of kallikrein inhibitor in normal rats resembled those in DOCA-treated animals. Kidney kallikrein activity in rats given aprotinin (0.83 ± 0.08 U/g) was 48% lower than in vehicle-treated rats (1.59 ± 0.16 U/g, $P < 0.01$) (Table 1). Urinary kallikrein in rats receiving the enzyme inhibitor decreased by 24% ($P < 0.01$) on the first, 42% ($P < 0.01$) on the third, and 60% ($P < 0.01$) on the fourth day of treatment (Table 3). Simultaneously, the excretion rate of PGE decreased by 63% ($P < 0.01$) and 67% ($P < 0.01$) after 1 and 3 days of aprotinin treatment, respectively (Table 3). Urinary sodium excretion fell from 1.37 ± 0.04 mEq/day to 0.91 ± 0.15 mEq/day ($P < 0.05$) the first day after aprotinin but returned to near-pretreatment values thereafter. Potassium excretion was not affected by the kallikrein inhibitor during the first days of treatment but decreased by 24% ($P < 0.01$) on the fourth day (Table 3). Aprotinin did
not affect the concentration of serum sodium and potassium (Table 1). Urine flow and fluid intake were not affected by the kallikrein inhibitor during the first 3 days of treatment but both increased significantly on the fourth day (Table 3). Body weight gain in aprotinin-treated rats (3.3 ± 0.5 g/day) did not differ from weight gain in vehicle-treated controls (4.0 ± 0.5 g/day) (Table 3).

### Table 3 Effects of Aprotinin on the Urinary Output of Kallikrein and of Prostaglandin E-like Substance in Untreated Rats

<table>
<thead>
<tr>
<th>Day</th>
<th>Kallikrein (U/day)</th>
<th>PGE (ng/day)</th>
<th>U_{ser}V (mEq/day)</th>
<th>U_{ur}V (mEq/day)</th>
<th>Urine volume (ml/day)</th>
<th>Fluid intake (ml/day)</th>
<th>Body weight (g)</th>
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<td>3.45 ± 0.17</td>
<td>10.2 ± 1.0</td>
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<td>3.80 ± 0.13</td>
<td>9.7 ± 0.8</td>
<td>23.5 ± 2.8</td>
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<td>3.52 ± 0.15</td>
<td>11.7 ± 1.3</td>
<td>27.8 ± 2.8</td>
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Aprotinin, 50,000 KIU, sc, twice daily

<table>
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<tr>
<th>Day</th>
<th>Kallikrein (U/day)</th>
<th>PGE (ng/day)</th>
<th>U_{ser}V (mEq/day)</th>
<th>U_{ur}V (mEq/day)</th>
<th>Urine volume (ml/day)</th>
<th>Fluid intake (ml/day)</th>
<th>Body weight (g)</th>
</tr>
</thead>
<tbody>
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<td>1</td>
<td>37.3 ± 4.2</td>
<td>18.5 ± 4.9</td>
<td>0.91 ± 0.15</td>
<td>11.1 ± 1.5</td>
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<tr>
<td>2</td>
<td>1.20 ± 0.08</td>
<td>3.17 ± 0.45</td>
<td>12.7 ± 2.2</td>
<td>28.2 ± 1.9</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>3</td>
<td>28.5 ± 6.1*</td>
<td>16.2 ± 2.9*</td>
<td>1.33 ± 0.07</td>
<td>15.5 ± 2.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>19.4 ± 4.4*</td>
<td>2.68 ± 0.14*</td>
<td>1.63 ± 0.10</td>
<td>28.3 ± 2.5</td>
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</table>

In control rats, repeated injections of vehicle for 4 days did not affect the urinary excretion of kallikrein, prostaglandins, and electrolytes (Table 3).

### Discussion

Renal prostaglandins are synthesized in the medulla and at a lesser degree in the cortex and are released into venous blood and tubular fluid. Increased urinary prostaglandin excretion induced by mineralocorticoids may be related to one or more electrolyte and hormonal disturbances associated with administration of sodium-retaining steroids, viz, sodium retention, potassium depletion, increased plasma ADH levels, and increased kidney kallikrein activity. Support for this interpretation includes: (1) reports that an acute saline load increases the concentration of PGE in renal venous blood (2) the demonstration that depletion of potassium in the dog augments urinary PGE, (3) evidence that ADH stimulates the synthesis and the release of kidney prostaglandins, and (4) observations suggesting that a product of kidney kallikrein activity stimulates output of renal PGE.

The present studies, however, suggest that disturbances in the activity of the kallikrein-kinin system have primacy in bringing about the increase of urinary prostaglandins, PGE in particular, evoked by mineralocorticoids. Support for this conclusion derives from two findings: first, aldosterone and DOCA increase urinary kallikrein excretion and renal kallikrein activity, effects which are nullified by treatment with aprotinin, an inhibitor of kallikrein; second, aprotinin cancels the increment of urinary prostaglandin excretion produced by DOCA. The renal activity of the kallikrein-kinin system probably is a function of both kinin-generating and kinin-inactivating enzymes. Consequently, mineralocorticoids which increase kidney and urinary kallikrein may augment the activity of the system by promoting renal production of kinins, which in turn stimulate release of prostaglandins. Conversely, aprotinin which inhibits kidney and urinary kallikrein may decrease the activity of the kallikrein-kinin system by reducing renal production of kinins, which in turn results in diminished release of prostaglandins. Aprotinin occurs in the kidney and to a lesser extent in the urine of rats injected with the enzyme inhibitor, and this may account for our finding of reduced kidney and urinary kallikrein activity in such rats. However, measurement of urinary kallikrein activity in vitro may not accurately reflect the effectiveness of aprotinin as an inhibitor of kallikrein in vivo. Thus, the binding of aprotinin to kallikrein is reversible and could be affected by the pH and composition of the urine intrarenally.
Other interpretations of the actions of mineralocorticoids and aprotinin on prostaglandin excretion have little support. Thus, neither aldosterone nor fludrocortisone affected prostaglandin synthesis by kidney microsomes. Further, a direct effect of mineralocorticoids or of aprotinin, unrelated to activation of the kallikrein-kinin system, on either the synthesis, release, or inactivation of renal prostaglandins is improbable since such effects are not likely to be impaired by aprotinin. We do not exclude the possibility that inhibition by aprotinin of plasma kallikrein and, less likely, of other extrarenal kininogenases, e.g., trypsin and plasmin, contributes to reduce urinary prostaglandins by decreasing formation of kinins in blood. However, such an effect resulting from inhibition of extrarenal kininogenases is probably short-lived because of the short plasma half-life of aprotinin which accumulates selectively in kidney tissue.

Thus, the conclusion emerging from these studies is that a product of kidney kallikrein activity is instrumental in stimulating urinary output of prostaglandins, PGE especially, in rats receiving mineralocorticoids. Also, the data suggest that the renal kallikrein-kinin system influences PGE excretion in the untreated rat. These conclusions are in accord with (1) the demonstration that bradykinin stimulates kidney PGE release in vivo and in vitro, and (2) evidence that kinins liberated by kidney kallikrein from kininogen releases PGE in the isolated kidney of rabbit. Stimulation by kinins of prostaglandin release in kidney and in other organs denotes stimulation of prostaglandin production. Kinins promote prostaglandin biosynthesis by increasing the tissue level of free arachidonic acid, the substrate of a membrane-bound cyclo-oxygenase, consequent to activation of an acyl hydrolase. The products of cyclo-oxygenase activity, the prostaglandin endoperoxides, PGG₂ and PGH₂, subsequently are transformed in the kidney to prostaglandins E₂, F₂α, and D₂. Kinins may influence the production of PGF₂₀ by stimulating PGE₂ 9-keto-reductase. Additional effects of kinin peptides influencing the production of PGI₂ and thromboxane A₂ cannot be excluded.

The finding that aldosterone and DOCA increase kidney and urinary kallikrein confirms reports that mineralocorticoids stimulate the production and release of kidney kallikrein which is synthesized in the cortex by distal tubula cells. There is evidence of formation of kinins intrarenally. However, the exact site(s) of formation is not established conclusively, nor is the route followed by the peptide product of renal kallikrein, predominantly a cortical enzyme, to the sites of prostaglandin synthesis in the kidney medulla. However, stop-flow studies in the dog suggest that kallikrein and kinins enter the tubular fluid in the distal nephron. From these observations we could infer that kallikrein(s) synthesized in distal tubule cells acts on kininogen to produce lysyl-bradykinin which travels, via the tubule, to the renal medulla where the peptide stimulates production of prostaglandins in cellular elements associated with the collecting tubules, the medullary interstitium, and blood vessels.

In the present study, rats receiving aldosterone and DOCA exhibited the characteristic abnormalities of electrolyte and fluid balance, i.e., urinary sodium retention, potassium depletion, and polyuria, produced by mineralocorticoids. Mineralocorticoids produce sodium retention only transiently, because the kidney escapes from the sodium-retaining actions of this class of steroids due to reduced sodium reabsorption in the distal nephron. Our finding of reduced urinary sodium excretion, coincident with diminished excretion of kallikrein and PGE, during the first 2 days of aprotinin administration, suggests that the renal activities of the renal kallikrein-kinin and prostaglandin systems favor sodium excretion and, thereby, could contribute to the mineralocorticoid-escape mechanism(s).

This interpretation is compatible with the following observations. (1) Enhanced intrarenal activity of the kallikrein-kinin system consequent to kininase II inhibition is associated with augmented sodium excretion. (2) Specific bradykinin antibodies, which block the vascular actions of kinins, were reported to attenuate the natriuretic response to an acute saline load. (3) Renal artery infusion of arachidonic acid stimulates renal prostaglandin production and increases urinary sodium excretion. (4) Blockade of renal prostaglandin synthesis in the rat is associated with increased concentration of NaCl in the renal medulla, which is compatible with exaggerated tubular reabsorption of sodium. (5) PGE₂ inhibits sodium transport in the distal nephron of the rat and in the cortical and outer medullary collecting tubules of the rabbit treated with DOCA.

Polyuria in animals receiving mineralocorticoids reflects a defect in urine-concentrating mechanisms which could be attributed, rather speculatively, to associated alterations in the renal activity of the kallikrein-kinin and prostaglandin systems. Our finding that aprotinin reduces urine volume transiently in DOCA-escaped animals but not in untreated rats is compatible with that view. Thus, kinins and PGE₂ promote excretion of free water. This effect, in the case of PGE₂, appears to be due to inhibition of the actions of ADH on the collecting tubule. Further, inhibition of renal prostaglandin synthesis with indomethacin enhances the effects of ADH on water excretion and restores ability to concentrate urine in potassium-depleted dogs.

The effect of aprotinin to reduce urinary potassium excretion, particularly in rats given DOCA, may denote an influence of the renal kallikrein-kinin and prostaglandin systems on potassium se-
cretion by distal nephron cells. This possibility is consistent with reports that renal arterial infusion of either bradykinin, PGF₂α, or arachidonic acid increases, whereas inhibition of prostaglandin synthesis with indomethacin and meclofenamate decreases urinary excretion of potassium. In summary, the present studies suggest relationships between mineralocorticoids, renal prostaglandins, and the renal kallikrein-kinin system. Although our results do not permit a definite conclusion about the physiological significance of these relationships, they do suggest that a product of kidney kallikrein activity links the components of a system, the aldosterone-kallikrein-kinin-prostaglandin system, that is important in the regulation of renal excretory events.

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