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Renal Effects of Aprotinin, a Kallikrein Inhibitor in Rats in Saline Diuresis (42002)

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Abstract. Administration of aprotinin, a kallikrein inhibitor, to anesthetized rats infused with 0.9% saline solution to expand the extracellular fluid volume resulted in blunted natriuresis and diuresis. Urine flow declined from 27.1 ± 2.6 to 8.0 ± 0.9 μl/min/100 g body wt while sodium and potassium excretion were reduced 63 and 45%, respectively ($P < 0.01$). Mean blood pressure and glomerular filtration rate were not significantly altered by aprotinin. Acute or chronic pretreatment with DOCA, to enhance kinin synthesis, failed to modify the renal excretory response to aprotinin suggesting that saline loading alone was able to induce kinin generation fully in these rats. The results indicate that aprotinin enhanced the reabsorption of filtrate in rats expanded with isotonic saline and imply an influence of renal kinins on the tubular transport of salt and water.

The kidneys contain acidic glycoproteins, kininogenases or kallikreins. These proteolytic enzymes generate kinins, the active peptides from precursor kininogens. The role of this renal kallikrein–kinin system is not adequately defined. Several lines of investigations indicate that it may influence the tubular transport of salt and water (1, 2). Infusion of kinins leads to increased urinary excretion of sodium and water without changes in glomerular filtration rate (3, 4). Microinjection of bradykinin into the lumen of late proximal tubules reduces the tubular efflux of simultaneously administered sodium-22 in more distal nephron segments (5) while bradykinin antibody attenuates the diuretic response to isotonic expansion of the extracellular fluid volume (6). Additionally, enhanced kinin activity, resulting from inhibition of its degrading enzyme, kininase II, promotes diuresis and natriuresis (7).

Aprotinin is a polyvalent inhibitor of kallikrein (8) capable of significantly lowering renal excretion of kinins without altering hemodynamic functions (9). The present investigations were designed to evaluate the renal excretory response to aprotinin in anesthetized rats to gain information about the possible influence of the kallikrein–kinin system on tubular transport processes. The animals were made diuretic by infusion of 0.9% saline or were pretreated with the mineralocorticoid, DOCA, in order to enhance renal kinin synthesis and, therefore, amplify the observable effects of aprotinin. Although aprotinin is a complex drug and is not a specific inhibitor of renal glandular kallikrein, it provides a limited but useful model for the study of kinin effects on the kidney. It changes the activity of renal kinins at their normal site in the distal nephron, unlike infusion of bradykinin, which may influence hemodynamic functions as well as tubular transport along the entire nephron.

In the present study, the tubular reabsorption of salt and water was enhanced by aprotinin. The results are consistent with the suggestion that renal kinins may serve to increase the excretion of salt and water during saline diuresis.

Methods. Experiments were performed on male Sprague–Dawley rats with an average body weight of 346 ± 9 g. The animals had free access to food and water except for the night before the experiment when their food intake was restricted to about half of normal intake to reduce abdominal mass. Rats were anesthetized with ip Inactin (100 mg/kg body wt). The animals were placed on a thermostatically controlled, heated board to maintain body temperature at 36.5°C and prepared surgically as previously described (5). Polyethylene cannulae were inserted into the right external jugular vein (PE-10) for infusion of

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saline and aprotinin, and into the right common carotid artery (PE-50) for recording of systemic blood pressure. The left ureter was exposed through a midline abdominal incision and cannulated with a tapered tip PE-50 tubing for quantitative urine collections. After completion of surgery, a 45- to 60-min equilibration period was allowed. An isotonic saline infusion (0.9%) was begun soon after the insertion of the jugular cannula. The initial saline load of 7% of body wt was given at a rate of 15 ml/hr followed by a maintenance infusion at 4–6 ml/hr. After two to three control urine collections of 20–30 min each, the animals received an iv infusion of aprotinin, (Trasylol; Farbenfabriken Bayer) 20,000 KIU/kg, in 20 min. After completion of aprotinin infusion, an additional period of 20–30 min was observed and was followed by two to three experimental urine collection periods.

The rats were divided into five treatment groups. The first group of rats received only 0.9% saline infusion (saline diuretic group). In the second group, water soluble DOCA was given continuously with the saline infusion at a rate of 0.2 mg/hr per rat or about 0.7 mg/hr/kg body wt (DOCA infused group). The third group of rats was pretreated for 10–14 days with DOCA in sesame oil, 5.0 mg/day per rat, and received saline infusion to induce diuresis (DOCA pretreated group). The fourth group was similarly pretreated with DOCA but was not given a saline load on the day of the experiment (nondiuretic, DOCA pretreated group). The fifth group received saline and vehicle infusion without aprotinin (time-control group).

The concentration of kinins in urine was measured by radioimmunoassay after extraction, as previously described (9). Urine samples were analyzed for sodium and potassium concentration using a flame photometer. Data were evaluated using analysis of variance and a P value of less than 0.05 was considered statistically significant. Individual control and experimental clearance measurements varied less than ± 12% of mean. Values obtained during several control and experimental periods from the same animal were averaged before data analysis.

Results. Data from the first three treatment groups are presented in Figs. 1 and 2. In 10 rats with an average body weight of 338 ± 19 g, aprotinin was superimposed on isotonic saline diuresis (group 1). Urine flow declined from 27.83 ± 3.27 to 6.74 ± 0.83 μl/min/100 g body wt. Sodium and potassium excretion was reduced 67 and 60%, respectively. Aprotinin did not significantly alter systemic blood pressure in this or any of the other treatment groups (Fig. 1). Continuous iv infusion of DOCA along with the saline load (group 2) failed to alter the renal excretory response to aprotinin in five rats weighing 343 ± 6 g. Urine flow decreased 73% while sodium and potassium excretion were lowered 65 and 38%, respectively (Fig. 2). The results from seven DOCA pretreated rats (group 3) with an average body wt of 326 ± 14 g were also similar. After aprotinin, urine flow, and sodium excretion were reduced significantly; 57 and 58%, respectively. The only statistically significant difference in the aprotinin response of these three groups was in potassium excretion. It was reduced significantly after aprotinin infusion in the saline diuretic group (59%), but not in the two DOCA treated groups (38% each $P > 0.05$).

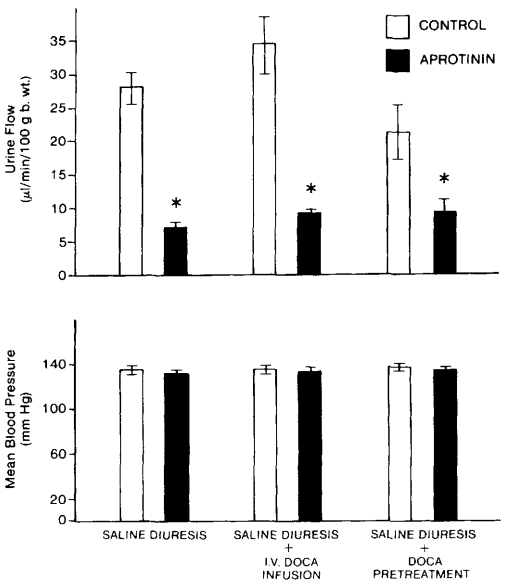

FIG. 1. Effect of aprotinin on urine flow and systemic blood pressure in saline diuretic (N = 10), DOCA-infused (N = 5), and DOCA pretreated (N = 7) rats. Mean values ± SE are presented. Asterisks indicate significant change from control; $P < 0.05$.

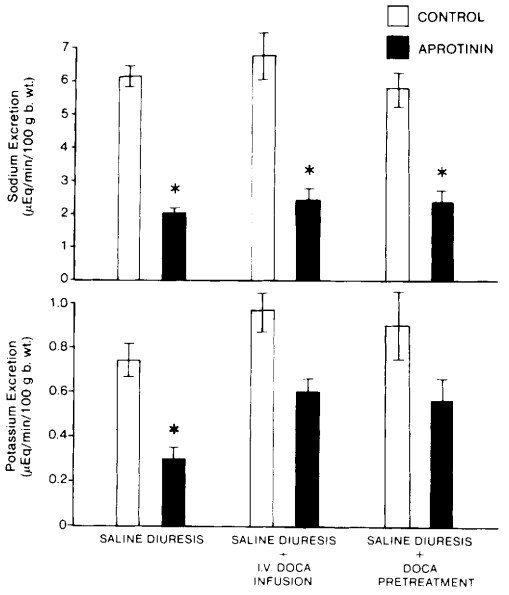

FIG. 2. Effect of aprotinin on sodium and potassium excretion in saline diuretic (N = 10), DOCA-infused (N = 5), and DOCA pretreated (N = 7) rats. Mean values ± SE are presented. Asterisks indicate significant change from control; $P < 0.05$.

Only limited data were obtained from the fourth treatment group (DOCA pretreated, nondiuretic). In three of the eight rats in this group, renal excretory response was similar to those infused with saline. Fractional water excretion was reduced 34% by aprotinin while urine flow declined 56%. In the remaining five rats, postaprotinin data could not be obtained because of oliguria and anuria. Glomerular filtration rate, which was calculated as the clearance of [^{3}H]inulin, and mean blood pressure were not significantly affected by the kallikrein inhibitor. In the five time-control rats, infusion of 0.9% saline caused diuresis which was not significantly altered by vehicle administration. Urine flow declined slightly (4%) but not significantly with time (28.35 ± 2.18 and 27.22 ± 2.20 μl/min/100 g body wt, respectively). Sodium excretion was 5.93 ± 0.77 and 5.77 ± 0.82 μeq/min/100 g body wt during control and vehicle infusion periods. Potassium excretion also remained unchanged with time in these rats (0.66 ± 0.07 and 0.68 ± 0.08 μeq/min/100 g body wt, respectively). None of these values were significantly different from each other or from the mean control values of the first group.

Since the response to aprotinin was similar in all three of the saline infused groups, the data were combined and analyzed as overall change. The results are presented in Table I. Inhibition of kinin generation produced a 70% reduction in urine flow while sodium and potassium excretions declined 63 and 45%, respectively. Mean blood pressure and glomerular filtration rate remained unaltered (Table I). Urinary kinin excretion declined from 246 ± 83 to 98 ± 60 pg/min/kg body wt after aprotinin administration (N = 5, $P < 0.05$) in the saline diuretic rats (first group) while it was not significantly altered by vehicle infusion (time-control group; 261 ± 91 and 250 ± 90 pg/min/kg body wt, respectively).

Discussion. In the present studies, acute iv administration of aprotinin in rats expanded with 0.9% saline infusion resulted in a reduction in urine flow and sodium excretion to 30 and 37% of their respective control values while potassium excretion decline to half. Glomerular filtration rate and mean blood

TABLE I. EFFECT OF APROTININ (20,000 KIU/kg, iv) ON RENAL FUNCTION IN RATS

	Control	Aprotinin	Δ	P
Urine flow (μl/min/100 g body wt)	27.09 ± 2.60	8.03 ± 0.89	19.06 ± 1.09	<0.01
Sodium excretion (μeq/min/100 g body wt)	6.08 ± 0.41	2.23 ± 0.23	3.85 ± 0.31	<0.01
Potassium excretion (μeq/min/100 g body wt)	0.86 ± 0.08	0.47 ± 0.05	0.39 ± 0.06	<0.01
Mean blood pressure (mm Hg)	130 ± 2	124 ± 2	6 ± 2	NS
Glomerular filtration rate (μl/min/100 g body wt)	787 ± 56	779 ± 56	8 ± 43	NS

pressure remained unaltered suggesting that the influence of the drug on tubular reabsorption of electrolytes and water was not secondary to hemodynamic alterations.

The present findings are consistent with previously observed antidiuretic and antinatriuretic effects of aprotinin in conscious rats pretreated with DOCA or maintained on high salt balance by drinking 0.9% saline solution (2, 10, 11) although in those studies, aprotinin also tended to alter renal hemodynamics.

The mechanism of aprotinin-induced reduction in salt and water excretion is not apparent from the study. The hypothesis that the observed changes in renal excretory function in these rats were secondary to inhibition of the activity of the renal kallikrein–kinin system is supported by the following observations. First, the principal pharmacologic effect of aprotinin is to inhibit kinin generating enzymes (12). Second, the drug is known to accumulate in the kidneys (8) and therefore is expected to act primarily on the glandular renal kallikrein. Third, the compound reduced the urinary excretion of both kallikrein (2) and kinin (9). Furthermore, the action of aprotinin on renal excretory function is most apparent when it is superimposed on high kallikrein–kinin activity states (10, 13).

Acute and chronic pretreatment with DOCA failed to modify the antidiuretic response to aprotinin in these rats. Therefore, the present study does not distinguish between the two mechanisms proposed as activators of the renal kallikrein–kinin system, positive sodium balance (1) and high mineralocorticoid levels (14). Apparently, kinin generation was fully induced by 0.9% saline infusion alone and DOCA did not enhance it further. The present data suggest maximal activation of the system by either high salt or high mineralocorticoids. The oliguria induced by aprotinin in DOCA pretreated hydropenic rats implies a supportive role for renal kinins in the antidiuretic state.

Renal excretion of kinin was reduced markedly by aprotinin, indicating that the drug inhibited the activity of the intrarenal kallikrein–kinen system. Although aprotinin has varied pharmacological actions and is not a selective kallikrein inhibitor (8), its use provides us with a model that is more appropriate for the study of the tubular action of kinins than intrarenal infusion of bradykinin which may produce hemodynamically mediated as well as direct effects on transport processes along the entire nephron. Aprotinin alters only the endogenous renal kinin activity which appears to be limited to the distal nephron.

The present results are consistent with the hypothesis that the renal kallikrein–kinin system influences the tubular transport of salt and water when the extracellular fluid volume is expanded, and that its blockade enhanced the reabsorption of filtrate while its activation promotes diuresis and natriuresis. Alternatively, aprotinin may act as a nonspecific serine protease inhibitor and may increase salt and water reabsorption by an unidentified pharmacologic mechanism.

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