Kallidin Effect on Renal Tubular Function in Meclofenamate- and Vehicle-Pretreated Rats (42991)

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Abstract. **The effect of kallidin (lysyl-bradykinin) on the urinary recovery of sodium-22 was examined in anesthetized, volume-expanded rats. Sodium-22 was microinfused into the lumen of late proximal convoluted tubules with and without kallidin (100 pg/ml). Kallidin enhanced mean sodium-22 recovery from a control of 2.24 ± 0.29% to 6.22 ± 1.30% (Δ = 3.98 ± 1.31%, $P < 0.005$). The urinary recovery of simultaneously microinfused inulin, mean blood pressure, urine flow, and the rate of tubular infusion were similar during control and kallidin microinfusions.**

Pretreatment of rats with meclofenamate (3.0 mg/kg) to inhibit renal prostaglandin synthesis blunted, but did not abolish, the effect of kallidin to promote sodium-22 recovery. The changes in sodium recovery induced by kallidin represent a 175 ± 47% and a 58 ± 11% increase from control values in vehicle- and meclofenamate-pretreated rats, respectively. The results indicate that kallidin, microinfused in high doses into the lumen of late proximal tubules, may lower sodium efflux in that nephron. Inhibition of prostaglandin synthesis reduced the tubular effect of kallidin, suggesting that enhanced prostaglandin synthesis may contribute to the natriuretic effects of kallidin. Alternatively, meclofenamate may directly oppose the tubular effect of kallidin.

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The kallikrein-kinin system is highly active in the kidneys and it may play a role in the regulation of renal hemodynamic and excretory functions (1, 2). Previous work from our laboratory suggests that kinins may enhance the urinary excretion of salt and water. In one of these studies, administration of bradykinin into the lumen of the rat nephron at late proximal sites increased the urinary recovery of simultaneously microinfused sodium-22 (3). In another investigation, vasopressin enhanced the low baseline kinin excretion in Brattleboro, DI rats (4). In these studies (4), kinin excretion varied directly with urinary vasopressin but independently of urine flow, osmolality, sodium, and kallikrein excretion. In a further set of experiments, reduced kinin activity, resulting from inhibition of kallikrein by aprotinin administration, intensified the antidiuretic effect of vasopressin in DI rats and also blocked the vasopressin induced rise in kinin excretion (5). Aprotinin has also been shown by us to blunt the diuretic response to volume expansion perhaps consequent to elevated tubular reabsorption of filtrate in distal nephron segments (6). Other investigators have suggested that kinins may exert their natriuretic and diuretic actions by promoting renal prostaglandin synthesis which in turn lowers the reabsorption of filtrate (7, 8).

The purpose of these investigations was to examine the influence of an inhibition of prostaglandin synthesis on kallidin (lysylbradykinin)-induced changes in tubular sodium reabsorption. Sodium-22 was infused into late proximal tubules with or without kallidin in vehicle- and meclofenamate-pretreated rats expanded with the infusion of a saline-glucose solution. Kallidin significantly increased the urinary recovery of sodium-22. This effect of kallidin was lowered but not abolished by meclofenamate pretreatment, suggesting a contribution of enhanced prostaglandin synthesis to the renal action of kinins to inhibit tubular sodium efflux.

Materials and Methods

Experiments were performed in 14 male Sprague-Dawley rats with an average body weight of 326 ± 12

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g and a mean age of 76 days (Sasco, Omaha, NE). Rats were anesthetized by intraperitoneal injection of Inactin in a dose of 100 mg/kg body wt. Cannulas were placed in the trachea to maintain an open airway, in the right external jugular vein for infusions, in the right common carotid artery for blood pressure recordings, and into the left ureter for quantitative urine collections. The animals were placed on a thermostatically controlled, micropuncture table to maintain body temperature at 36–37°C. The rats were expanded with an intravenous infusion of 2.5% glucose in 0.45% NaCl in an amount of 7.5% of body weight. A maintenance infusion of the same solution was given continuously at a rate of 5.0 ml/hr. Six rats were pretreated with meclofenamate (3.0 mg/kg iv) after volume loading to inhibit renal prostaglandin synthesis. An equilibration period of 90 min was allowed to assure sufficient time for the inhibition of prostaglandin synthesis prior to the start of microinfusions. Eight rats received only a vehicle infusion of the saline-glucose solution. The temporal course of surgical preparation, volume expansion, equilibration period, and tubular miocroinfusions were similar in all rats.

The left kidney was exposed for micropuncture, bathed in mineral oil, and illuminated with a fiber optic light source as described previously (3, 6). Late proximal tubular segments were identified on the surface of the kidney after an intravenous bolus injection of a green dye (100 μl of a 5% solution, FD and C Green #3; Keystone, Chicago, IL). Tubular transit times from the glomerulus to the site of microinfusion were measured in the same manner. Tubular microinfusions (Fig. 1) were performed with sharpened, silicone-treated, calibrated glass volumetric micropipettes with an average outer tip diameter of 3–4 μm and a capacity of 25–35 nl. The infusion rate (7–17 nl/min) was adjusted to approximately 20% of tubular flow, to prevent retrograde flow and to maintain constant tubular diameter. Paired control and kallidin-containing infusions were made into the same nephron at the same puncture site. The sequence of the two infusions were randomized and one to three infusion pairs were made in each rat. The infusion solution contained [^{3}H]inulin and was colored with FD and C green dye to allow detection of leakage or technically unsuccessful infusions (see Fig. 1). An infusion was considered technically successful if inulin recovery was 97.5–102.5%. After each microinfusion urine was collected continuously for 18 min into vials containing liquid scintillation counting fluid. Collection time was 1 min each for the initial and final five samples, and 30 sec each for midcollections. Reference standards were prepared by depositing a volume of radioactive infusate, equal to the subsequently microinfused volume, directly into counting vials. All samples were analyzed for ^{3}H and ^{22}Na activities in a liquid scintillation spectrometer with quench correction and external standardization. Appropriate corrections were made for ^{22}Na overlap into the ^{3}H channel which was less than 5%. The urinary recoveries of [^{3}H]inulin and ^{22}Na were calculated with formulas shown in Figure 1. Control and kallidin infusion data were averaged for each rat before calculating mean recovery rates and statistical analysis of the results. Results are presented as mean ± SE. Statistical significance was evaluated using paired Student's t test and one-way analysis of variance as appropriate. A $P < 0.05$ was considered significant.

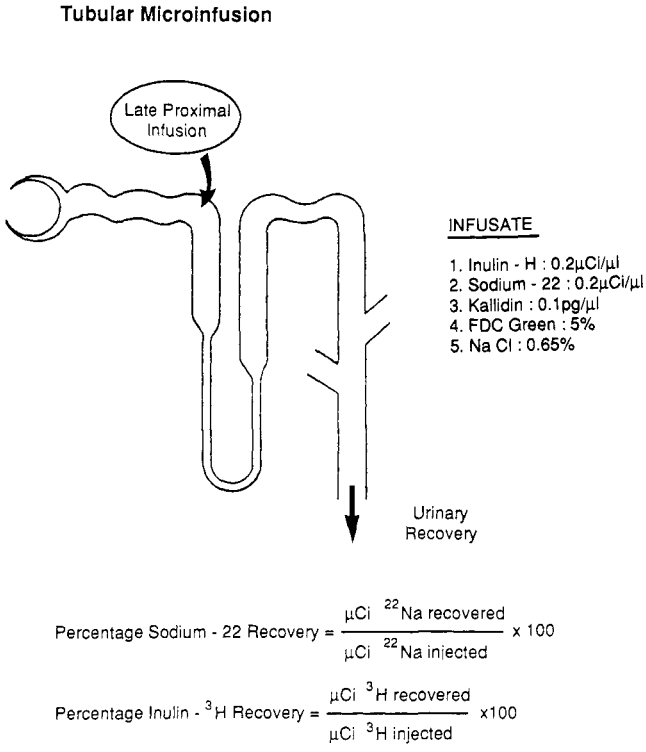

Figure 1. Renal tubular microinfusions, composition of infusate, and calculation of urinary recovery of infused radioactive sodium and inulin.

Results

A total of 52 technically successful tubular microinfusions was made into the lumen of late proximal surface convolutions of rat nephrons. Twenty-six of these microinfusions were performed in eight rats pretreated with a vehicle infusion. Thirteen of the 26 infusions served as controls and 13 contained kallidin. Another 26 microinfusions were made in six rats pretreated with meclofenamate. Half of these served as controls and the other half were paired kallidin-containing infusion. Therefore, an equal number of paired microinfusions were performed in vehicle- and meclofenamate-pretreated rats (13 pairs each). The general condition of the animals and the circumstances of microperfusions were similar in both groups of rats during control and kallidin-containing infusions (Table I). Urine flow and the rate of microinfusion, the two primary factors that may introduce errors into the

Table I. Mean Blood Pressure, Urine Flow, Infusion Rate, and Tubular Transit Times during Control and Kallidin Microinfusions into Late Proximal Segments of Rat Nephron[a]

	Control	Kallidin	P
Mean blood pressure (mm Hg)			
Vehicle (n = 8)	123 ± 5	124 ± 6	NS
Meclofenamate (n = 6)	141 ± 2[b]	141 ± 2[b]	NS
Urine flow ($\mu l \cdot min^{-1} \cdot 100\ g^{-1}$)			
Vehicle (n = 8)	32.1 ± 3.4	33.0 ± 4.1	NS
Meclofenamate (n = 6)	33.2 ± 0.9	33.7 ± 1.0	NS
Infusion rate (nl/min)			
Vehicle (n = 8)	12.1 ± 1.3	13.9 ± 1.8	NS
Meclofenamate (n = 6)	12.5 ± 1.2	11.0 ± 1.2	NS
Late proximal tubular transit times (sec)			
Vehicle (n = 8)	14.5 ± 0.9	14.3 ± 0.8	NS
Meclofenamate (n = 6)	12.4 ± 0.2[b]	12.4 ± 0.2[b]	NS

[a] Values are mean ± SE. Values were recorded during each control and kallidin microinfusion period. They were averaged for each rat before statistical analysis of results. P values are for control versus kallidin microinfusion periods using one-way analysis of variance. NS, not significant, $P > 0.05$.
[b] $P < 0.05$ (one-way analysis of variance) vehicle versus meclofenamate.

urinary recovery of substances administered directly into the lumen of the nephron, were similar during control and kallidin infusions in both vehicle- and meclofenamate-pretreated rats (Table I). Tubular transit time to the site of micropuncture were also similar during control and kallidin infusions (Table I). In the meclofenamate-pretreated rats, mean late proximal transit time was significantly lower than in vehicle-treated rats during both control and kallidin infusion periods (Table I). The reason for this is not apparent. In rats receiving meclofenamate, the recovery of [^{3}H] inulin was 98.6 ± 0.8% and 99.7 ± 0.7% during control and kallidin infusions, respectively. In the rats receiving the vehicle, these values were 99.5 ± 0.4% and 100.4 ± 1.5%. None of these mean values are statistically significantly different ($P > 0.05$, paired t test).

Mean urinary recovery of sodium-22 was significantly higher during kallidin infusions (6.22 ± 1.30%) than control infusions (2.24 ± 0.29%) in the vehicle-pretreated rats (Fig. 2). The difference (3.98 ± 1.31%) is highly significant ($P < 0.005$, paired t test). In the meclofenamate-pretreated rats, sodium-22 recovery averaged 1.86 ± 0.23% during control and 2.98 ± 0.29% during kallidin microinfusions. The difference (1.08 ± 0.13%), although smaller than in the vehicle group, is also highly significant ($P < 0.001$, paired t test). The effect of kallidin to increase sodium-22 recovery was significantly higher in the vehicle-treated than in the meclofenamate-treated rats whether the differences were expressed as fractional sodium-22 recovery or as percentage of control recovery (Fig. 3).

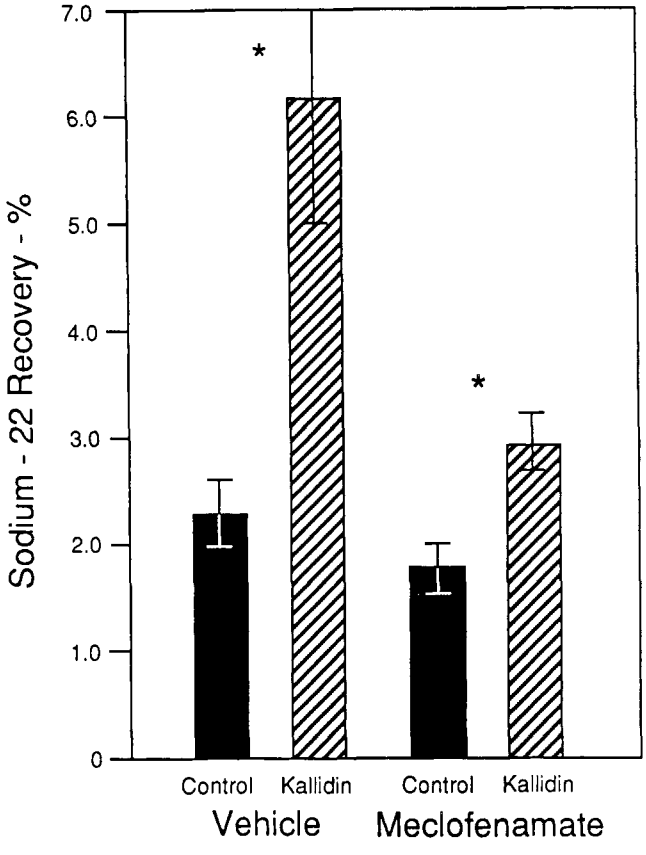

Figure 2. Percentage of urinary recovery of radioactive sodium after its microinfusion into late proximal surface convolutions of rat nephron. * Indicates significant difference between mean control and kallidin infusion values ($P < 0.05$, paired t test).

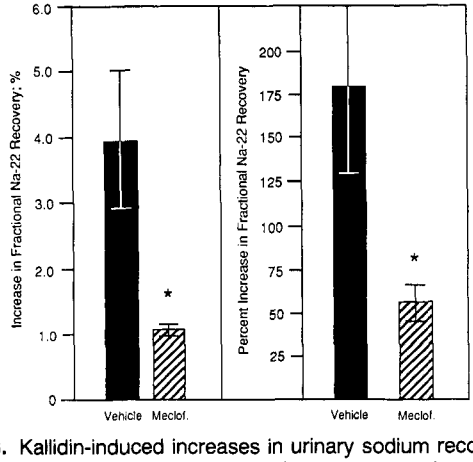

Figure 3. Kallidin-induced increases in urinary sodium recoveries in vehicle- and meclofenamate-pretreated rats expressed as absolute increase in fractional sodium recovery (left panel) and as percentage of increase from control (right panel). An asterisk indicates a significant difference between vehicle- and meclofenamate-treated rats. The kallidin-induced increase in sodium recovery was significantly lower in the meclofenamate-pretreated rats than in the vehicle-treated rats ($P < 0.005$, one-way analysis of variance).

Discussion

Kallidin, applied to the luminal side of the rat nephron, increased significantly the urinary recovery of simultaneously administered radiolabeled sodium. Tubular transit time, urine flow, and the rate of microinfusions were similar during control and kallidin infusions and the infusions were made randomly at the same puncture sites. Therefore, the enhanced sodium recovery may best be attributed to its reduced net transtubular efflux during kallidin administration. In vehicle-pretreated rats, the nephron segments beyond the site of infusion removed more than 97% of radioactive sodium. Kallidin more than tripled the urinary recovery of sodium in these rats, indicating that the peptide markedly reduced the reabsorption of sodium in distal nephron segments. The results imply that kallidin may enhance sodium excretion from about 2.3% of distal delivery to more than 6.2% and suggest that the renal kallikrein-kinin system may play an important role in the regulation of the tubular transport of this cation.

Inhibition of renal prostaglandin synthesis by meclofenamate pretreatment markedly reduced the ability of kallidin to inhibit the tubular reabsorption of sodium. However, sodium recovery remained higher in the presence of the peptide than in its absence. Therefore, it appears that prostaglandins may play a role in the mediation of the natriuretic response to kinins in the tubular fluid. Meclofenamate has previously been shown to inhibit renal prostaglandin synthesis by 90% in a dose of 2.0 mg/kg under similar experimental conditions (7). Results from previous experiments have also suggested that the renal actions of kinins may, at least in part, be mediated by prostaglandins. The two compounds appear to act in concert (8). Meclofenamate has been shown to suppress the bradykinin-induced natriuresis in dogs (9) and enhance chloride transport in the cortical collecting ducts (10). Prostaglandin E_2 has been shown to inhibit chloride and sodium reabsorption in this nephron segment (11). Under experimental conditions similar to those used in the present study, addition of prostaglandin E_2 to the tubular fluid has also been shown to reduce the tubular efflux of sodium (12). It is therefore possible that luminal kinins may reduce sodium and water reabsorption in distal nephron segments partly through stimulation of prostaglandin synthesis. Alternatively, the effects of prostaglandins and kinins on sodium transport may be independent. Meclofenamate in these experiments may have blunted the effect of kallidin by increasing sodium transport downstream from the site of kinin action as a consequence of removal of the inhibitory effect of prostaglandins in the collecting tubules. The significant increase in mean blood pressure after meclofenamate administration (Table I) is consistent with a frequently observed antihypertensive effect of vasodilator prostaglandins, the removal of which should elevate peripheral resistance.

In the present studies kallidin most likely acted on nephron segments downstream from the site of microinfusion, i.e., in the loop of Henle, distal convoluted tubule, and collecting duct. Previous studies identified the distal nephron as the site of luminal entry of both kinin and kallikrein (1, 2). Studies in isolated, *in vitro* perfused tubules identified the cortical collecting duct as the primary site of action of bradykinin on sodium and chloride transport (13). Aprotinin, an inhibitor of kallikrein, has been shown in *in vivo* micropuncture experiments to enhance reabsorption primarily in distal nephron segments (6). Therefore, the present data are consistent with previous findings that kinins have access to and probably act on distal nephron segments.

Whether the inhibitory action of kinins on sodium reabsorption is expressed from the luminal or the peritubular side of the nephron is not certain. Intratubular infusion of kallidin in these experiments would be expected to act from the luminal side. However, previous studies in isolated, perfused rabbit cortical collecting tubules suggest that kinins are effective only when they reach the basolateral side of the tubular cells (14). The estimated concentration of kallidin reaching the distal nephron sites in the present studies (15–30 pg/ml) was about 10 times lower than in previous studies (3, 14) but still about 10–25 times higher than what the expected endogenous luminal kinin levels may be. Although it is difficult to estimate renal tubular kinin concentration under varied physiologic conditions, measured urinary kinin levels (4) and an assumed partial metabolism would suggest that it may be in the range of 1.0–1.5 pg/ml. Because of the high concentration gradient, in this study, kallidin may have exited the tubule in sufficient quantities to exert an effect from the peritubular side. Therefore, the present studies do not allow a clear distinction between a luminal or peritubular side of action of kallidin.

The mechanism of action of kallidin on tubular sodium efflux is also uncertain. The results are compatible with either blockade of active sodium transport or a reduction of tubular permeability to sodium. It is likely that kallidin acted directly on the tubular epithelium to reduce sodium efflux. However, an indirect, hemodynamically mediated action cannot be excluded. Tubular kinins may influence renal hemodynamic events after reaching the afferent and efferent arterioles *via* the tubular route (15 and personal communication). A possible renal hemodynamic action of kinins is also indicated by the studies of Roman *et al.* (16) who observed marked increases in papillary blood flow after the administration of phosphoramidon, an inhibitor of neutral endopeptidase, the enzyme that is primarily responsible for the metabolism of renal kinins. A kinin antagonist in these studies returned papillary blood flow to normal but failed to reduce sodium excretion, sug-

gesting that endogenous kinins may act predominantly on renal hemodynamics and that their tubular effects may be secondary to altered medullary and papillary physical forces for capillary fluid uptake (17). A more likely reason of tubular effect of the kinin antagonist is its failure to reach the distal nephron intact (16). Therefore, a hemodynamically mediated tubular action of kinins remains an unproven possibility which may contribute to the differences between the results of *in vivo* and *in vitro* experiments.

The significance of the present observation in relation to the role of the renal kallikrein-kinin system in the regulation of salt and water excretion is not apparent. Several lines of investigations suggest that high endogenous renal kinin activity is associated with natriuresis and diuresis while a reduction in kinin activity leads to reduced renal excretory functions (1, 2, 10, 11). The results of the present studies are consistent with the proposal that renal kinins may act as paracrine hormones that exert an antihypertensive effect by increasing salt and water excretion.

Kallidin, microinfused in high doses into late proximal tubules, enhanced the urinary recovery of simultaneously infused sodium perhaps by inhibiting its reabsorption. In vehicle-pretreated rats, kallidin enhanced fractional sodium recovery from 2.24 to 6.22% (a change of 175 ± 47% from the control value). In meclofenamate-pretreated rats the effect of kallidin to enhance sodium recovery was blunted. In these rats, kallidin only increased fractional sodium recovery from 1.86 to 2.94% (a change of only 54 ± 11% from the control value). Inhibition of renal prostaglandin synthesis reduced but did not abolish the ability of kallidin to inhibit sodium reabsorption, suggesting that enhanced prostaglandin synthesis may contribute to the natriuretic effect of kallidin.

The results are consistent with the hypothesis that the renal kallikrein-kinin system is a component of a diuretic-antihypertensive mechanism that enhances sodium and water excretion acting either directly on the tubules or indirectly through changes in regional renal blood flow distribution.

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