Site of Entry of Kininase II into Renal Tubular Fluid

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SUMMARY The stop-flow technique was used to determine the site of entry of kininase II into tubular fluid in dogs. Stop-flow patterns were constructed for kininase II, p-aminohippurate, sodium, and potassium. The proximal tubule was localized by the peak of p-aminohippurate concentration and the distal tubule by the minimum sodium concentration. In the stop-flow pattern for kininase II, three peaks (a, b, and c) were observed. A main peak (a), located 2.25 ± 0.45 ml distal to the p-aminohippurate peak (p < 0.01) and 3.75 ± 0.31 ml proximal to the minimum sodium concentration (p < 0.001), was observed in all experiments. Peak c, located 2.6 ± 0.4 ml (p < 0.01) proximal to the p-aminohippurate peak, was observed in five dogs. Peak b appeared in five dogs and was always located 2.0 ml distal to the minimum sodium concentration. This peak was coincident with the potassium peak. Only two of eight experiments showed all three peaks. These results showed that the major kininase II entry into the tubular fluid is near the p-aminohippurate peak and that distal entry occurred in 63% of the dogs. (Hypertension 11 [Suppl I]: I-66-I-68, 1988)

KEY WORDS • stop-flow experiments • urinary converting enzyme • renal kallikrein-kinin system • renal renin-angiotensin system • renal function

It is known that kininase II, which inactivates kinins and converts angiotensin I to angiotensin II, is present in urine. When infused into the renal artery, kinins do not appear in the urine, and it has been shown that inactivation of these peptides occurs at the level of the proximal tubule.

Through immunocytochemical studies, Caldwell et al. localized kininase II in the proximal tubule, glomerular tuft, and intertubular capillary network of the rabbit kidney. Using the same technique in swine, other authors showed that this enzyme was localized along all the nephron, especially in the proximal tubule. In rat and mouse kidneys, kininase II was localized in the brush border of the proximal tubule, the endothelial cells of the renal arterial tree, some capillaries, and a few peritubular capillaries. High specific activity of kininase II in proximal tubule brush border preparations has been also demonstrated.

The aim of the present work was to detect the sites of entry of kininase II into the tubular lumen in dogs by using the stop-flow technique.

Materials and Methods

Eight mongrel dogs weighing 9 to 15 kg were anesthetized with sodium pentobarbital (30 mg/kg, i.v.). A tracheal tube was inserted to ensure adequate ventilation. The right femoral artery and the right femoral vein were cannulated for infusions and blood collections, respectively. The left ureter was exposed through a flank incision, a PE-200 catheter was inserted into the ureter, and its tip was advanced into the renal pelvis. The dead space of the catheter was 0.5 to 0.6 ml. The kidney was not manipulated during the experiment.

Osmotic diuresis was established by a continuous infusion of Ringer's solution to which was added mannitol, 20 g/dl; creatinine, 120 mg/dl; and p-aminohippurate (PAH), 30 mg/dl. The rate of infusion was 0.5 ml·min⁻¹·kg⁻¹. Prior to this continuous infusion the dogs received a priming dose of creatinine (50 mg/kg) and PAH (40 mg/kg). As soon as urinary flow reached a stable value (approximately 45 minutes), one free-flow urine sample was collected immediately before the ureter was clamped. The ureter was kept clamped for 5 minutes. Immediately after removing the clamp, 15 samples of urine of 1 ml each were serially collected at 4°C in individual plastic tubes.

Plasma samples were collected 3 minutes before and
3 minutes after ureter clamping. Free-flow urine, urine fractions, and plasma samples were analyzed for creatinine, PAH, sodium, and potassium.

Kininase II activity was measured in the urine samples by the method of Santos et al. Urine (25 μl) was incubated with 500 μl of assay solution containing 5 mM Hip-His-Leu in 0.4 M sodium borate buffer, pH 8.3, and 0.9 M NaCl for 2 hours at 37°C. The reaction was stopped by the addition of 1.2 ml of 0.34 N NaOH. The product, His-Leu, was measured fluorometrically after the addition of 100 μl of o-phthalaldehyde (20 mg/ml) in methanol, which was followed 10 minutes later by the addition of 200 μl of 3 N HCl and by centrifugation at 800 g for 5 minutes at room temperature. Blanks were prepared by reversing the order of the addition of enzyme and NaOH. All measurements were made in duplicate.

Kininase II activity was tested by the in vitro addition of a specific inhibitor, MK 422, to the assay. Creatinine was determined by the method of Bonsnes and Taussky and PAH determinations were made according to Smith et al. Sodium and potassium were assayed by flame photometry with lithium as the internal standard.

**Results**

At free-flow the urine flow was 7.6 ± 0.5 ml/min and the urinary kininase II activity was 0.085 ± 0.015 nmol·min⁻¹·ml⁻¹.

Figure 1 shows the patterns of kininase II, potassium, sodium, and PAH. The values are the means ± SEM of experiments in eight dogs. The samples with the highest PAH concentration indicate the proximal tubule and the samples with the lowest sodium concentration indicate the distal tubule.

In the kininase II stop-flow pattern the ratio of (kininase II sample/kininase II free-flow) to (creatinine sample/creatinine free-flow) was used. When this ratio is above one it means that the substance was secreted, because this ratio corrects for the effect of water reabsorption on tubular solute concentration.

The kininase II stop-flow pattern shows three peaks, which means that kininase II had access to tubular fluid at three different tubular points. A main peak (a) of kininase II entry into tubular fluid was observed in all dogs. This peak was localized 2.25 ± 0.45 ml distal to the PAH peak (p < 0.01) and 3.75 ± 0.31 ml proximal to the minimum sodium concentration (p < 0.001). Peak b appeared only in five experiments, and it was localized 2.0 ml distal to the minimum sodium and was always coincident with the potassium peak. Peak c was observed in five experiments and was more proximal than the PAH peak, 2.6 ± 0.4 ml (p < 0.01). Only two of the eight experiments showed all three peaks.

When 25, 50, and 100 μg of MK 422 were added to proximal and distal pools of urine samples, kininase II activity was inhibited by 76.1 ± 2.64% (n = 3), 94.1 ± 1.9% (n = 3), and 95.7 ± 2.36% (n = 3), respectively.

**Discussion**

The stop-flow technique was used in the experiments because it allows determination, in relation to markers, of where secretion or reabsorption occurs along the nephron. The present experiments show that kininase II may enter the tubular fluid at three sites. The largest site of entry (Peak a), observed in all experiments, was proximal, which is in agreement with immunocytochemical and microperfusion studies that localized the enzyme mainly at the proximal tubule level. A fact to be considered is that this peak was significantly distal (2.25 ± 0.45 ml) to the PAH peak. Samples of the PAH peak are considered the most representative of the fluid that stayed in contact with the proximal tubular cells during the interruption of urine flow.

A second peak (b) appeared in 63% of the dogs. It was located distal (2.0 ml) to the samples with the lowest sodium concentration. These samples are con-
sidered the most representative of the fluid that stayed
in contact with the distal tubule during occlusion of the
ureter. Peak b was coincident with the potassium peak.
Scicli et al.\textsuperscript{13} suggested the presence of kininase II
in the distal tubule because they observed in stop-flow
experiments an increase in the kinins peak when the
kininase II inhibitor BPP 9a was present in tubular
fluid. The presence of Peak b observed in the present
experiments confirms this suggestion.

As kininase II is present in the glomerular tuft\textsuperscript{3,4} and
in the endothelial cells of the renal arterial tree,\textsuperscript{5} it is
possible that Peak c is of glomerular origin.

Although the site of entry of kininase II into the
tubular fluid was clearly determined, its physiological
role in renal function needs further investigations.

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