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Distribution of Kidney Kininogenases (39142)[1]

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Kininogenases (kallikrein) are a group of enzymes that act upon substrates (kininogens) present in plasma and in lymph, releasing peptides known as kinins (bradykinin, lysyl-bradykinin and methionyl-lysyl-bradykinin). The two main groups of kininogenases that have been described are plasma and glandular kininogenases (1). Urinary kallikrein, the first glandular kininogenase discovered (2, 3), is probably produced by the kidney cortex (4–7). However, the exact location and distribution of this enzyme in the renal cortex tissue is unknown. In this work we have studied the distribution of kininogenases in the dog kidney. In addition, glomeruli were isolated from rat kidneys and the kininogenase concentration per unit of protein was measured and compared to the kininogenase concentration in the total cortex.

Materials and method. Distribution of kininogenase in the dog kidney. Six female mongrel dogs were used. The dogs were anesthetized with sodium pentobarbital, 30 mg/kg, and the left renal artery was cannulated. The kidneys were perfused *in situ* with cold Hartman solution (8) until the surface became nearly white in color. At this time, the kidney was excised and placed over crushed ice and the perfusion continued until 1500 ml of fluid had passed through the kidney. Then a cylinder of tissue, going from the cortex to the papilla, was cut using a cork borer number six. The cortical region was sliced in three equal zones from the surface to the juxtamedullary area (outer, middle, and inner cortex). A slice of tissue was also obtained from the medulla and from the papilla. A small piece of tissue at the end of these two areas was discarded to avoid contamination from the adjacent areas.

Kininogenase activity was measured as previously described (9). Briefly, a 100-mg longitudinal wedge of each slice was homogenized in 1 ml of 0.5% deoxycholic acid at pH 8.5. The homogenate was incubated for 30 min at 4°, then centrifuged, and the supernatant used for the kininogenase assay. The kininogenase activity was determined by incubating 0.1 ml of the supernatant, equivalent to 10 mg of tissue, with a semipurified substrate in the presence of 1,10-phenanthrolene and sodium ethylenediamine tetraacetic acid (EDTA) to inhibit kininases. The volume was adjusted to 2 ml with 0.1 *M* phosphate buffer, pH 8.5. The samples were incubated at 37° for 10 min, and the reaction was stopped by immersing the tubes in boiling water. The pH was adjusted to 7.5, and the kinins generated were assayed in the hind leg of the dog. Both the samples and standards (kallidin, Schwarz/Mann) were injected into the femoral artery through an indwelling cannula (PE-60). The increased flow that resulted was recorded by electromagnetic flow meter. The activity of the unknown samples was determined by bracketing their flow effect between two close doses of the standard and calculated by interpolation (Nasjletti, personal communication). Kininogenase activity was expressed as micrograms of kallidin released per gram of wet tissue per minute of incubation. Since a wide variation was found between individual dog kidneys, we also expressed the distribution of kininogenases as a percentage of the activity found in the five fractions.

Kininogenase activity in the isolated glomeruli of the rat. Sprague–Dawley rats weighing between 200 and 300 g were used.

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The rats were anesthetized, and the abdominal aorta was cannulated distal to the renal artery. The aorta and vena cava were completely tied above the renal artery, and a small puncture was made in the vena cava below the ligature to allow the free movement of saline. The kidneys were perfused *in situ* with 100 ml of cold saline until the surface became whitish. The kidneys were then removed and the cortex was separated from the medulla by dissection. The kidney cortices of 10 to 12 rats were pooled and minced over a petri dish cooled with crushed ice. The glomeruli were separated by the use of different sieves as described by Spiro (10). The glomeruli obtained in the last sieve were further purified through a discontinuous sucrose gradient of 50, 55, and 65%. The suspension of glomeruli was layered on the top and centrifuged on a Spinco Ultracentrifuge Rotor SW 25 at 5000 rpm for 30 min. The glomeruli were located at the bottom of the tube. If any contamination was observed, the glomeruli were suspended again in saline and layered over a new gradient of sucrose of 50, 65, and 75% and centrifuged at 8000 rpm for 30 min. The glomeruli appeared at the top of the 75% layer. These glomeruli were generally void of Bowman's capsule. The final preparation was washed with cold saline, centrifuged three times, resuspended in saline and sonified. An aliquot of the total cortex homogenate was also sonified in the same way; protein was determined by the method of Lowry (11). An aliquot of glomeruli or total cortex homogenate was treated with deoxycholic acid solution 0.5% and kininogenases were measured as described for the dog kidney, with the exception that only 1 mg of protein of the total cortex homogenate or glomeruli was used for the incubation; and the incubation time was 60 min. The results are expressed in nanograms per milligram of protein per minute of incubation. Statistical tests were done using the one-sided sign test (12) with an experimental-wise statistical protection of at least 0.05 for the analysis of kininogenase distribution in the dog kidney. The paired Student's t test was used in comparing rat glomeruli with the total cortex kininogenases.

Results. Table I shows kininogenase activity per gram of wet tissue in five different areas of six kidneys. Considerable heterogeneity in the kininogenase activity of each dog was found. Figure 1 shows the same results expressed as a percentage of the activity of the five areas studied. Ninety-one percent of the kininogenase activity was in the cortex. Statistical comparison between the different areas of the kidney across the six dogs was partially thwarted because of the considerable heterogeneity in the kininogenase concentration found among individual dogs. Thus, the nonparametric sign test, which compared the relative amounts of kininogenase per kidney region per dog, was used with an experimental-wise α error of 5%. The results of this test indicated that all six dogs had significantly higher kininogenases in the outer than in the middle cortex and in the inner cortex than in the medulla. Five of the six dogs had higher levels of kininogenase in the middle cortex than in the inner cortex; however, this did not have statistical significance. The overall results indicate that there are higher amounts of kininogenases in the outer as opposed to the middle cortex, in the middle as opposed to the

TABLE I. KININOGENASE ACTIVITY IN THE FIVE DIFFERENT AREAS OF THE DOG KIDNEY.[a]

Dog	Outer cortex	Middle cortex	Inner cortex	Medulla	Papilla
1	0.67	0.59	0.37	0.08	0.08
2	4.52	3.43	3.66	0.32	0.26
3	3.56	2.66	1.14	0.34	0.21
4	1.48	1.17	1.16	0.38	0.50
5	1.53	0.81	0.37	0.16	0.11
6	7.03	3.72	2.78	0.31	0.13
$\bar{X}$ ± SE	3.13 ± 0.98	2.06 ± 0.56	1.58 ± 0.55	0.27 ± 0.05	0.22 ± 0.06

[a] Kininogenase activity is expressed as μg/gm wet tissue/min incubation.

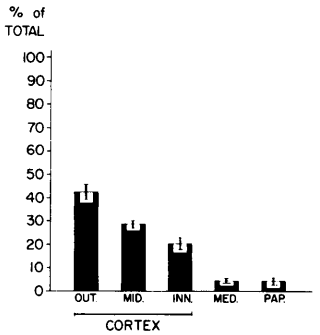

FIG. 1. Kininogenase activity in the outer (OUT.), middle (MID.), and inner (INN.) cortex, and in the medulla (MED.) and papilla (PAP.) of the kidney. Kininogenases are expressed as a percentage of the activity found in the five areas studied. The vertical lines within each bar indicate SE.

inner cortex, and in the inner cortex as opposed to the medulla.

Table II shows the kininogenase activity in the isolated rat glomeruli and in the total rat renal cortex. The activity per milligram of protein in the glomeruli was only 15% of the activity in the total cortex. This difference was statistically significant ($P < 0.006$).

Discussion. These results show that kininogenase activity in the dog kidney is localized primarily in the cortex with minimal activity found in the medulla and papilla, thus confirming earlier findings in rats by Nustad (13). The distribution pattern shows that kininogenase activity decreases from the outer cortex to the medulla. Although kidney kininogenase activity was found to vary considerably among the dogs, the distribution pattern was consistent (Fig. 1). The same distribution pattern also has been found for renin in the dog kidney (14). In the isolated rat glomeruli, the kininogenase activity per milligram was only 15% of that found in the total cortex. This suggests that the main location of kininogenases is somewhere else in the cortical segment of the nephron. However, since some activity was always found in the glomeruli, this could indicate some degree of contamination by the tubules, juxtaglomerular apparatus, or other parts of the nephron, or that the glomeruli actually contain small amounts of kininogenases. Similarly, a very low renin concentration was reported by

TABLE II. KININOGENASE ACTIVITY IN THE RAT GLOMERULI AND IN THE TOTAL CORTEX.[a]

Number	Glomeruli (G)	Total cortex (TC)	(G/TC) × 100
1	3.10	8.40	37
2	0.73	7.80	9
3	1.04	8.10	13
4	1.66	14.40	12
5	0.32	2.09	15
6	0.70	11.30	6
$\bar{X} \pm$ SE	1.25 ± 0.56	8.70 ± 1.80	15.30 ± 4.50

[a] Kininogenases are expressed in ng of kinins generated/mg protein/min incubation.

Bing *et al.* in the isolated glomeruli (15) devoid of juxtaglomerular apparatus. The isolated glomeruli in our preparation were also devoid of juxtaglomerular apparatus.

The function of kininogenases in the kidney remains undetermined. It could be that these enzymes release kinins from the kininogen, and, through the release of this potent vasodilator, they could participate in the local regulation of blood flow. Finally, the possibility exists that there is some relationship between the functional heterogeneity of the nephrons and the distribution of kininogenases in the kidney.

Summary. The distribution of kininogenase activity in the dog kidney was studied. It was found that kininogenase decreased from the outer to the inner cortex and that the medulla and papilla have very little kininogenase activity. Kininogenase activity was also measured in isolated glomeruli devoid of juxtaglomerular apparatus and in the total cortex of rat kidney. In the isolated glomeruli, the kininogenase activity per milligram of protein was only 15% of that found in the total cortex. It was concluded that the main localization of these enzymes is not in the glomeruli, but somewhere else in the cortical segment of the nephron.

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