Kininogenase in Kidney After Ligation of the Ureter and After Experimental Aortic Stenosis

The kininogenases (or kallikreins) are a group of widely distributed proteolytic enzymes with hypotensive activity which is due to their ability to release the peptide, kinin, from a globulin precursor in plasma[1,2]. Urinary and renal kininogenases, which are similar if not identical, have recently become a subject of active investigation, because of their suggested involvement in experimental and human hypertension[3–6].

Whereas renin has been located precisely in the granules of the juxtaglomerular cell of the kidney[7], the type of cell (or cells) in which kininogenase is located is not established. Recent studies have shown that, like renin, the kininogenase in rat kidney is located almost entirely in the renal cortex, particularly the outer part[8,9]. However, whereas renin is found in the heavy mitochondrial and nuclear fractions of homogenates of rat kidney cortex, kininogenase is predominantly a component of the membranes constituting part of the microsomal fraction. From these studies, NUSTAD and RUBIN[9] concluded that 'no subcellular common compartmentalization between a hypertensive and a hypotensive factor exists'.

It seemed desirable to us to study the behaviour of renal kininogenase after various procedures known to affect renin in definite ways. We therefore determined the kininogenase content of the rat's kidney after ligation of the ureter and after ischaemia produced by aortic stenosis.

Methods. Rats of both sexes, weighin 200–300 g, were anaesthetized with sodium pentobarbital (40–50 mg kg^{-1} i.p.). The left ureter was cut between ligatures 1–2 cm from the hilus of the kidney. 1–3 weeks later the rats were again anaesthetized and both kidneys were perfused with cold 0.25 *M* sucrose, minced and freeze-dried. An aqueous extract of this powder was adjusted to pH 2 with HCl for 30 min to inactivate kininase also extracted[10]. The solutions were neutralized with NaOH, centrifuged, and the supernatant solutions were freeze-dried. The kininogenase activity was assayed on the isolated guinea-pig ileum by measuring the kinin released from dog pseudoglobulin adjusted to pH 9[6]. Details of this assay are described by BEILENSON et al.[11]. The renin content of kidney was determined with the extraction method for renin and substrate preparation as described by GROSS et al.[12] with the following differences: Starting material for renin was blood-free, freeze-dried kidney as described above, rather than fresh renal tissue; also, we used the isolated guinea-pig ileum to assay angiotensin. Aortic stenosis was produced by tying a silk thread around the aorta over a platinum wire of 0.4 mm thickness placed between the origins of both renal arteries[13]. The wire was then withdrawn. Synthetic bradykinin (Schwarz Bioresearch Inc.) and val[5]-angiotensin 11-amide (Hypertensin, Ciba) were used.

Results and discussion. We measured both kininogenase and renin in the kidney after ligation of the ureter and found a progressive decrease in the content and concentration of kininogenase which fell to approximately 20% after 2–3 weeks of ligation. The mean values for renin at this time, however, were approximately double those of the contralateral kidney. These results are shown in Table I and those for renin are in agreement with those of previous workers[14,15]. Since ligation of the ureter results in progressive and extensive damage to the tubular system of the kidney whilst the glomeruli are relatively unaffected[14], the marked reduction in kininogenase content in our experiments is consistent with the suggestion of WERLE and VOGEL[16] that renal kininogenase is either present or formed in the tubules. Since in our experiments the renin content was either unaffected or somewhat increased by ligation of the ureter, it would seem that unlike the kininogenase, all the renin in the kidney is associated with the glomeruli.

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Table I. Kininogenase and renin in rat kidney after ligation of the left ureter

Duration of ligation of ureter		Kininogenase[a] (L/R %) Concentration	Kininogenase[a] (L/R %) Total	Renin[a] (L/R %) Concentration	Renin[a] (L/R %) Total
Control	Mean	100	115		
	Range	72–115	87–138		
	n	5	5		
1 week	Mean	37	36		
	Range	31–49	24–50		
	n	4	4		
2–3 weeks	Mean	20	18	171	164
	Range	13–27	11–28	67–500	81–524
	n	8	8	6	6

[a] Kininogenase and renin (see methods) are expressed as bradykinin and angiotensin equivalents. L/R %, is the ratio of enzyme activity of the left and right kidneys.

Table II. Kininogenase in rat kidney after aortic stenosis

Experimental procedure		Blood pressure (mm Hg)	Kininogenase[a] (L/R %) Concentration	Total
Control	Mean	111	96	129
	Range	110–112	45–124	50–183
	n	3	3	3
2-week aortic stenosis	Mean	171	310	135
	Range	155–200	180–739	65–282
	n	8	8	8
2-week aortic stenosis + ligation of left ureter	Mean	162	221	99
	Range	105–200	103–792	25–302
	n	5	5	5

[a] As Table I. Kininogenase ratios expressed in concentrations are greater than totals since the left 'stenotic' kidney becomes reduced in size.

Since JELÍNEK and GROSS[13] found that experimental aortic stenosis in rats increased the renin content of the 'stenotic' kidney approximately 25-fold, we measured renal kininogenase in similar experiments. In distinction to the findings of the above authors for renin, however, the total kininogenase content of the 'stenotic' left kidney did not differ significantly from that of the other kidney. When JELÍNEK and GROSS expressed renin concentrations per g kidney tissue, the concentration in the 'stenotic' kidney was approximately 50 times greater than in the other; when our results are similarly expressed, the kininogenase concentration is only 2–3 times greater. Also, whereas these authors noted further increases in renin concentration of up to 300-fold over the normal kidney if ligation of the ureter was added to the aortic stenosis, similar experiments by us failed to show any additional change in kininogenase content. These results are shown in Table II.

Our experiments support the view that, unlike renin, renal kininogenase is located in the tubular system of the kidney. These enzymes behave very differently in response to various procedures. The role of the renal kininogenase-kinin system in physiology or pathology requires further investigation.

Zusammenfassung. Nachweis, dass Abbinden des Harnleiters von Ratten in 1–3 Wochen eine fortschreitende und ausgesprochene Verminderung der Kininogenase in der Niere verursacht. Experimentelle Verengerung der Aorta kingegen hat entweder keine Wirkung auf die Nieren-Kininogenase oder erhöht sie nur geringfügig.

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Einfluss von elektrischer Stimulation auf Phosphorylase-Aktivität und cAMP-Gehalt in Insektenflugmuskeln von *Locusta migratoria*

In der Leber und möglicherweise im Fettkörper der Insekten wird die Phosphorylase-Aktivität humoral über cAMP[1] reguliert[2]. In Vertebratenmuskeln ist ebenfalls eine hormonelle Steuerung der Phosphorylase-Aktivität durch cAMP möglich[3]. Wichtiger scheint hier dagegen die raschere Aktivierung der Phosphorylase durch die Erhöhung der Calcium-Konzentration im Sarcoplasma zu sein[4]. Bei dieser nervösen Steuerung der Glykogenolyse ist cAMP nicht eingeschaltet, da bei der Kontraktion von Skelettmuskeln nach elektrischer Stimulation kein Anstieg des cAMP-Gehalts festgestellt wurde, wohl aber eine Aktivierung der Phosphorylase[5].

Untersuchungen von HANSFORD und SACKTOR[6] zur Regulation der Phosphorylase-Aktivität in Insektenflugmuskeln haben ebenfalls eine Steuerung der Glykogenolyse über die Ca^{2+}-Konzentration im Sarcoplasma wahrscheinlich gemacht. Bislang wurde nicht untersucht, ob eine Erhöhung der Phosphorylase-Aktivität auch durch cAMP verursacht werden kann.

Um diese Frage zu klären, wurden die Flugmuskeln in Thoraxhalbpräparaten von *Locusta migratoria* durch elektrische Reizung des Bauchmarks[7] zur Kontraktion gebracht und anschliessend die Phosphorylase-Aktivität und der cAMP-Gehalt bestimmt[8]. Es wurden zwei Reizfrequenzen (2 und 20 Doppelimpulse/sec) angewendet, um einen möglichen Zusammenhang zwischen der Muskelarbeit und Veränderungen der Phosphorylase-Aktivität bzw. des cAMP-Gehalts feststellen zu können. Bei der höheren Reizfrequenz trat innerhalb von 5–10 sec Tetanus ein, nach 30–40 sec begannen die Muskeln zu

[1] Abkürzungen: cAMP; zyklisches 3′,5′-Adenosin-Monophosphat; AMP, Adenosin-5′-Monophosphat.

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