ISOLATION OF PORCINE URINARY KALLIKREIN

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Kallikrein from porcine urine was isolated by aceton precipitation, Sephadex G-75 gel filtration and affinity chromatography on a column of bovine trypsin-kallikrein inhibitor bound to BrCN-activated Sepharose 4 B. The preparation was further purified by Sephadex G-50 gel filtration and characterized by ion exchange chromatography, gel electrophoresis, molecular weight determination (gel filtration method), amino acid and carbohydrate composition and biological activity in the ileum test.

A SENSITIVE KININ LIBERATING ASSAY FOR KININOGENASE IN RAT URINE, ISOLATED GLOMERULI AND TUBULI OF RAT KIDNEY

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There is no Kinin liberating assay for rat urine kininogenase available, which is sensitive enough to measure the enzyme activity in isolated structures of the kidney.
First of all we developped a well reproduceable rat uterus test, which allows to measure Bradykinin equivalents from 0,4 ng to 4 ng and doses differences of 0,13 ng. The variation coefficient of the method is about 5%.
Substrate was prepared in a modification of the method, suggested by Habal, Movat (Biochem. Pharmacol. 23, 2291, 1974) and Spragg, Austen (Biochem. Pharmacol. 23,721, 1974). A fairly simple one-step procedure yields a human HMW-Kininogen, sufficient purified for a sensitive assay. "Spontaneous Kinin forming activity" is about 1% of total Kinin liberating activity (150 ng/mg protein). α_2-macroglobulin Cl inactivator and α_1-Antitrypsin could not be detected in Ouchterlony-double diffusion test. Kininases were completely inhibited by Na_2 EDTA ($4x10^{-3}$ M) and 8-Hydroxychinolin (1×10^{-2} M).
The kininogenase activity of rat urine was determined by incubating 0,1 – 1,2 µl with 600 ng substrate in a total volume of 250 µl for 4 hours. The Kinin formation was directly proportional to substrate – enzyme concentration and time. Optimal conditions: pH 8,6; ion strength 0,15 M, temperature 37°C.

Isolation of glomeruli was done by microdissection. Tubuli were isolated after "dissociative" treatment with collagenase, according to Guder. (Hoppe-Seyler's 2. Physiol.Chemie 352,1319 1971). We observed a linear dose dependency of enzyme in microdissected glomeruli and in isolated tubuli. Enzyme activity of 80 glomeruli was the lowest measurable. 90% in inhibition of Kinin formation was observed by the use of Trasylol. Carboxypeptidase B completey destroyed the uterus contracting material.
There was no effect on rat blood pressure even after application of an incubation mixture with high doses of urine glomeruli and tubuli. Renin and Angiotensin II was not detectable.
There were several hints of a tubular localisation of renal Kininogenase. Now it is evident, that renal Kininogenase is located in glomeruli as well as in tubuli.

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CONFORMATIONS OF BRADYKININ IN RELATION TO SOLVENT ENVIRONMNET

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Bradykinin has different spectroscopic properties in polar compared to non-polar environments. These spectroscopic differences are assumed to reflect a reorientation of the amide dipoles with respect to each other and with respect to the solvent environment. This assumption is based on analogy with spectroscopic changes known to occur in model polypeptides such as poly-γ-benzyl-L-glutamate in solvents of differing polarity. Strong evidence has been accumulated from biophysical studies which indicates that the conformations of cyclic depsipeptides differ in polar and non-polar environments and also when complexed with a cation. These transitions have been related to the ion-transport properties of the depsipeptides indicating conformational changes of the depsipeptides upon association with the hydrophobic environment in a membrane. Similarly, the apparent conformation changes of bradykinin associated with the change from polar to non-polar solvents may reflect possible changes involved in its pharmacological effects. In addition we have found that sodium dodecyl sulfate (SDS) induces changes in the circular dichroism (CD) spectrum of bradykinin equivalent to those found in non-polar solvents. SDS in association with proteins has been suggested by Reynolds and Tanford (J. Biol. Chem., 1970, 245, 5161) to provide a model system for studies of the conformational properties of membrane-associated proteins. Analogs of bradykinin where small modification have been carried out on the proline residue in the third position from the α-amino terminal demonstrate different aqueous CD spectra from the parent