GLANDULAR KALLIKREIN IN PLASMA AND URINE: EVALUATION OF A DIRECT RIA FOR ITS DETERMINATION*

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SUMMARY

To determine whether there is glandular kallikrein in plasma, untreated as well as acetone-treated and heated-acidified rat plasmas together with rabbit anti-rat urinary kallikrein were used in counterimmunoelectrophoresis. Precipitation bands were observed with untreated and acetone-treated plasma, suggesting that glandular kallikrein is present in plasma. This enzyme, however, cannot be quantified in the untreated plasma by a new direct RIA since kallikrein inhibitors present in plasma appear to interfere with this assay. Destroying the inhibitors by acetone treatment or by heat and acidification of the plasma partially solves this problem. In the second part of the study, this RIA as well as a kininogenase and an esterase assay were used to measure urinary kallikrein in DOCA-salt treated rats and in control rats. There is a significant correlation between urinary kallikrein measured by the direct RIA and by a kininogenase method ($r = 0.75$, $p < 0.001$) in both DOCA-salt treated and in the control rats. Although the results obtained by the direct RIA and an esterase method significantly correlate in the control rats ($r = 0.67$, $p < 0.001$), they did not in the DOCA-salt rats ($r = -0.048$, $p > 0.1$). This suggests that part of the urinary esterase activity in the DOCA-salt rats is due to urinary enzymes other than kallikrein and that the esterase assay is not reliable for the determination of urinary kallikrein in pathological situations. However, the direct RIA and the kininogenase assay are suitable for this purpose.

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INTRODUCTION

Urinary kallikrein has been implicated in the regulation of local blood flow (13,15), blood pressure and sodium balance (1,18,11), as well as in the pathogenesis of human and experimental hypertension (7,19,20,17,6) and renal diseases (6,12). However, it is not clear whether renal and other glandular kallikreins are excreted only into the exocrine secretion of the gland or whether they are also secreted, or reabsorbed, into the vascular compartment where blood pressure and local blood flow could be more directly affected.

In the first part of this study, the counterimmunoelectrophoresis technique was used with rabbit anti-rat urinary kallikrein and rat plasma to see if there is glandular kallikrein in plasma. In addition, the possibility of measuring glandular kallikrein in rat plasma by using a direct RIA for kallikrein, recently developed in our laboratory (3), was investigated. This assay measures absolute concentration of the enzymic protein (antigen). Since it is well known that plasma has kallikrein inhibitors, the effect of destroying these inhibitors and the effect of the kallikrein inhibitors aprotinin and benzamidine on the direct RIA for kallikrein were also studied.

In the second part of the study, this direct RIA as well as a kininogenase and an esterase assay were used to measure urinary kallikrein in DOCA-salt treated rats and in control rats. The results obtained with these three methods were compared for the purpose of determining whether the conflicting results reported on urinary kallikrein (5) could be explained, in part, by the fact that different methods based on different principles were used to determine urinary kallikrein.

MATERIALS AND METHODS

Glandular kallikrein in plasma. Blood was collected from normal rats in plastic tubes containing disodium ethylenediaminetetraacetate (EDTA). After centrifugation, the plasma was separated and kept frozen until its use. One aliquot was then acidified to pH 2.0 with 5N HCl, incubated at room temperature for one hour, brought back to pH 7.4 with 5N NaOH, and then heated at 56°C for one hour (heated-acidified plasma). A second aliquot was treated with acetone (20% v/v) overnight at 4°C, and the solvent was then evaporated under N2 (acetone-treated plasma). A third aliquot was used without pre-treatment (untreated plasma). An aliquot of each plasma was dialyzed against phosphate-buffered saline pH 7.4. These three plasmas, both dialyzed and non-dialyzed, were used with rabbit anti-rat