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Development of a Radioimmunoassay for Pig Pancreatic Kallikrein and its Application in Physiological Studies

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Entwicklung eines Radioimmunoassay für Schweinepankreaskallikrein und seine Anwendung in physiologischen Studien

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The generally employed assays for pig pancreatic kallikrein are based on the hydrolytic activity of the enzyme towards synthetic substrates or the natural substrate kininogen. None of these assays is specific for kallikrein since these substrates are hydrolyzed by other proteases, too. For physiological studies, which require the measurement of pig pancreatic kallikrein in tissues and body fluids, a more specific assay would be highly desirable. Since methods, based on immunochemical properties can be expected to be highly specific we developed a radioimmunoassay.

Purified pig pancreatic kallikrein [2] was labelled with iodine-125 employing the chloramine-T method [3] to a specific radioactivity of 80 μCi/μg. The labelled kallikrein was diluted with buffer A (0.015 M NaH_2PO_4, 0.15 M NaCl, 0.02 % Merthiolat, 0.2 % bovine serum albumin, 0.01 M EDTA, pH 7.4) to a concentration of 250000 cpm/ml, 70 μg/ml of rabbit IgG were added. Anti-kallikrein serum produced in rabbits was diluted 1:300000 to 1:500000 with buffer B (composition like buffer A but 4% bovine serum albumin). Incubation conditions: 100 μl of buffer A, 100 μl of standard sample or unknown, 100 μl of antiserum dilution and 100 μl of tracer were incubated for 24 – 48 h at room temperature, then 100 μl of anti-rabbit IgG serum dilution and, after additional 2 h, 0.5 ml of buffer A were added. After centrifugation (10 min, 6000 g) the supernatant was aspirated and the radioactivity of the precipitates measured in a gamma counter. The assays were evaluated by the spline approximation method [4]. The lower detection limit is generally between 0.4 and 3 ng/ml. The intraassay precision of quadruplicates is 6 – 10%, the intraassay precision of 5 consecutive assays was 6 – 15 %. The specificity of the assay was examined by measuring the capacity of a series of serine protease to inhibit the binding of ^{125}I-labelled kallikrein to the antibody. Porcine trypsin, bovine trypsin and chymotrypsin, guinea pig coagulation gland and submandibular gland kallikrein had no effect on the binding of ^{125}I-kallikrein to the antibody whereas porcine urinary and porcine submandibular gland kallikreins showed an immunochemical reactivity identical with pig pancreatic kallikrein in the radioimmunoassay.

The radioimmunoassay was employed to investigate whether pig pancreatic kallikrein is absorbed by the intestine. The ductus thoracicus of albino rats was canulated with a U-like polyethylene tube [1] and the lymphatic fluid collected into tubes which were changed at regular intervals. After an initial control period 10 mg of pig pancreatic kallikrein dissolved in 1 ml of 0.9 % saline were injected into the lumen of the duodenum. The fractions of lymphatic fluid collected for up to 6 h after injection were examined for kallikrein by means of the radioimmunoassay. Kallikrein was detected in the samples within 4 h in concentrations up to 200 ng/ml. Several samples of lymphatic fluid were subjected to gel filtration. When the eluate was checked by the radioimmunoassay two peaks of kallikrein were detected, one in the normal elution position of pig pancreatic kallikrein, the other in a position corresponding to a molecular weight of ~ 80000. These findings indicate that intact molecules of kallikrein are absorbed and that the absorbed kallikrein is partly bound to a plasma protein, presumably α_1-antitrypsin.

Since, under physiological conditions, pancreatic kallikrein is secreted into the duodenum from where it can be adsorbed, it seemed possible that pancreatic kallikrein might be present in blood. When be examined samples of pig serum by the radioimmunoassay we found kallikrein in concentrations up to 200 ng/ml. If pig serum was subjected to gel filtration two peaks of kallikrein were detected in elution positions as described above for rat lymphatic fluid.

These results demonstrate – to our knowledge for the first time – the presence of glandular kallikrein in blood. Its origin remains unclear since by our radioimmunoassay it is not possible to discriminate between the different glandular kallikreins. The question arises whether urinary kallikrein is synthesized by the kidney as generally assumed or whether it is only filtrated from the blood.

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