Studies on the Physiological Function of Glandular Kallikrein by Radioimmunoassay

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ABSTRACT

It was shown by a radioimmunoassay that pig pancreatic kallikrein can be absorbed in the rat intestinal tract. The absorption seems to occur both by way of lymphatics and mesenteric vein route. Glandular kallikrein was shown to be present in the serum of pig and man in concentrations of 10 - 20 ng/ml by employing radioimmunoassays.

KEYWORDS

Radioimmunoassay, pig pancreatic kallikrein, human urinary kallikrein; intestinal absorption, glandular kallikrein in serum.

INTRODUCTION

The physiological role of tissue kallikreins is still largely unknown, the same holds true for the mechanism of action by which administered kallikrein causes the many effects which have been observed. Such effects are, for example, the stimulation of intestinal absorption of various substances, like glucose, amino acids and vitamin B 12 (Caspary and Creutzfeldt, 1973; Rumpeltes, Koeppe and Pribilla, 1975), the stimulation of cell proliferation (Schütte and Lindner, 1977), the stimulation of both number and motility of spermatozoa in patients with asthenozoospermia and oligozoospermia after oral or parenteral administration (Schill, 1977; Schirren, 1977), and an enhancing effect on muscular glucose uptake in patients with maturity onset diabetes (Wicklmayr and Dietze, 1977). Thus, a large variety of effects of exogenous kallikrein is known, however, the knowledge on what happens to kallikrein between the administration and the observed effect is rather limited.

In our studies on the fate of administered kallikrein and the physiological role of glandular kallikreins we apply the radioimmuno-

assay technique which, because of its sensitivity and high specificity, should allow to get new insight into the action and fate of glandular kallikrein in the organism.

MATERIALS AND METHODS

Materials. Pig pancreatic kallikrein used in the radioimmunoassay for radioiodination and as a standard was a highly purified, neuraminidase-treated preparation (Fiedler, 1976) kindly provided by Dr.F.Fiedler, Munich; guinea pig coagulation gland and guinea pig submandibular gland kallikreins were gifts from Dr.C.Moriwaki, Tokyo and Dr.M.Lemon, Bristol. Pig urinary kallikrein and pig submandibular gland kallikrein preparations were isolated as described (Tschesche and others, 1976; Lemon, Förg-Brey and Fritz, 1976). Pig pancreatic kallikrein used in experiments on intestinal absorption (1 180 KU/mg) was a gift of Bayer AG, Wuppertal. Sephacryl S-200, Pharmacia.

Radioimmunoassays. The radioimmunoassays for pig pancreatic kallikrein and human urinary kallikrein are described elsewhere (Fink and Güttel, 1978; Mann and Geiger, 1977).

Gel filtration. Samples of sera and lymphatic fluid were subjected to gel filtration on Sephacryl S-200, column size: 0.9 x 95 cm; elution buffer: 0.015 mol/l NaH_2PO_4, 0.15 mol/l NaCl, 0.01 mol/l EDTA, pH 7.4; flow rate: 3 - 4 ml/h. The effluent was monitored at 254 nm.

Experiments on intestinal absorption. The ductus thoracicus of albino rats was canulated with a U-like polythylene tube (Seifert, 1976;Bollmann, Cain and Grindlay, 1948) and the lymphatic fluid collected into tubes which were changed at 30 or 60 min intervals, at the same times blood samples were obtained from the tail vein. After an initial control period of 1 h 10 mg pig pancreatic kallikrein, disolved in 1 ml 0.9 % saline, were injected into the lumen of the duodenum. The samples of lymphatic fluid and the serum samples, collected for up to 6 h after injection, were examined for kallikrein by radioimmunoassay.

RESULTS AND DISCUSSION

An important prerequisit for investigations on the physiological role of glandular kallikreins is a sensitive and specific assay method. The determination of kallikrein is generally achieved by measuring the rate of hydrolysis either of synthetic substrates or of the natural substrate kininogen. These assays are not specific for kallikrein because they also detect other proteases. For physiological studies, which include the measurement of kallikrein in tissues and body fluids, where other proteases may be present, a more specific assay is required. This requirement is met by the radioimmunoassay technique.

Radioimmunoassay. With the radioimmunoassay developed in our laboratory (Fink and Güttel, 1978) the lower detection limit for pig pancreatic kallikrein is 40 - 300 pg corresponding to a concentration of 0.2 - 1.5 ng/ml. This sensitivity is at least equal to that of the most sensitive enzymatic assays.

The assay is highly specific for pig glandular kallikreins, no crossreactivity could be found for porcine trypsin, bovine trypsin and chymotrypsin and kallikrein of guinea pig submandibular glands and guinea pig coagulation glands, whereas pig urinary and pig submandibular kallikreins show an immunochemical reactivity indistinguishable from that of pig pancreatic kallikrein under the radioimmunoassay conditions as indicated by the prallel dose-response curves (Fig. 1).

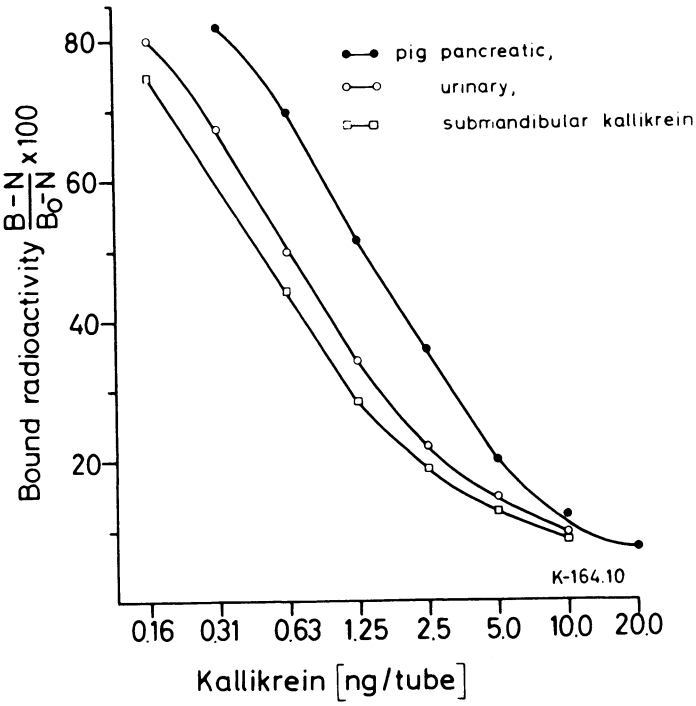

Fig 1. Dose-response curves of pig glandular kallikreins

Intestinal absorption. The radioimmunoassay was employed to investigate wheter kallikrein can be absorbed in the intestinal tract using the rat as a model. Kallikrein (10 mg dissolved in 1 ml 0.9 % NaCl) was injected into the lumen of the duodenum of anesthetized albino rats. When samples of lymphatic fluid obtained from the thoracic duct and blood samples were assayed by radioimmunoassay, kallikrein was detected in concentrations up to 200 ng/ml within 4 h after injection. However, the time dependence of the concentration increase of kallikrein in lymph and blood was highly variable within series of experiments both with fasted and with unfasted rats. It was not yet possible to standardize the experiments in a way that would make the kinetics of absorption in different animals comparable. Fig.2 demonstrates the highly different absorption kinetics, the two experiments were done under identical conditions with fasted rats.

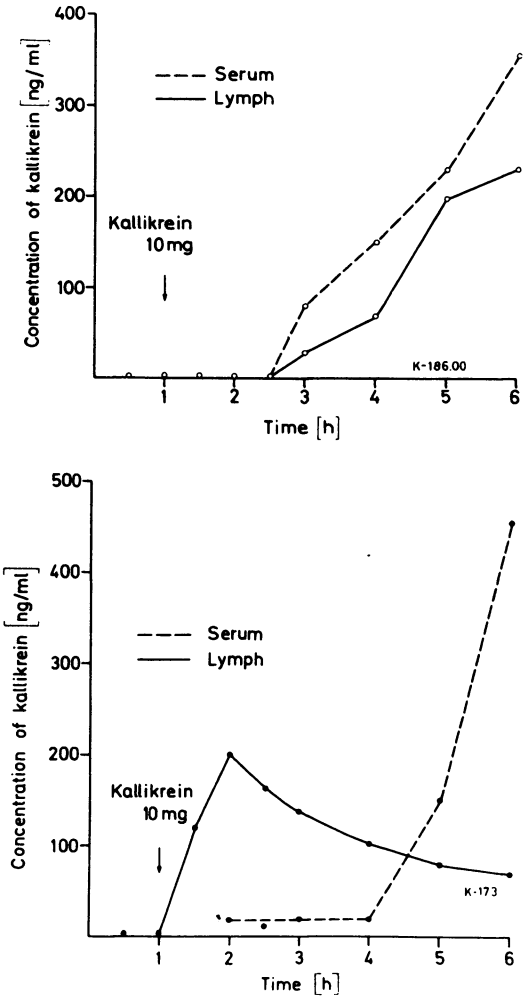

Fig 2. Kinetics of intestinal absorption of pig pancreatic kallikrein into serum and lymph of rats after intraduodenal injection

In Table 1 those two rats of the fasted and unfasted groups are compared which showed the highest absorption.
The data suggest that fasting has a stimulating effect on the intestinal absorption of kallikrein.

In order to clarify whether or not the radioimmunoassayable material consisted of low molecular degradation products, samples of lymphatic fluid were subjected to gel filtration on Sephacryl S-200 and the eluate was tested by radioimmunoassay. Two main peaks of immunochemically reactive material were found, the one in an elution position corresponding to the molecular weight of pig pancreatic kallikrein, the other in a position of a molecular weight of about 80 000. These findings indicate that undegraded molecules of kallikrein were absorbed in the rat intestine and that the ab-

sorbed kallikrein is partly bound to a plasma protein, presumably to α_1-antitrypsin.

Table 1 Intestinal Absorption of Pig Pancreatic Kallikrein in the Rat.

	Rat A (without fasting)	Rat B 24 h fasting
Dose administered	10 mg	10 mg
Total amount of kallikrein in lymphatic fluid	300 ng (30 ppm)	15 000 ng (1 500 ppm)
Maximal concentration in lymphatic fluid	800 ng/ml	33 000 ng/ml
Maximal concentration in serum	700 ng/ml	4 700 ng/ml

In two samples of lymphatic fluid, which had been stored frozen for several months, one peak of immunoreactive material was found in the position of a molecular weight near 80 000 as described above whereas the second peak was in a position near the void volume of the column. We assume that this high molecular material represents kallikrein unspecifically bound to high molecular proteins, possibly an artefact as a result of prolonged storage. A complex with α_2-macroglobulin seems improbable because of the finding of Hamberg (Vathera and Hamberg,1976) that kallikrein is not inhibited by α_2-macroglobulin.

In spite of the high variability discussed above our results demonstrate that glandular kallikrein can be absorbed by the intestine without detectable degradation. The partial binding to a serum protein, probably α_1-antitrypsin, suggests that at least some of the absorbed kallikrein is enzymatically active. Furthermore, the results suggest a significant role of lymphatics in the intestinal absorption of pancreatic kallikrein in addition to the absorption via portal vein routes. Absorption via the mesenteric vein route was demonstrated earlier by Moriwaki and co-workers (1973) using a mesenteric perfusion system.

Glandular kallikrein in blood. The radioimmunoassay was further applied to investigate, whether endogenous glandular kallikrein is present in the blood. We found glandular kallikrein in pig serum in concentrations of 10 - 20 ng/ml. In order to ascertain that the radioimmunoassayable substance was not a low molecular degradation product, serum samples were subjected to gel filtration and the fractions tested by radioimmunoassay. One peak was found in[a] position which would correspond to the complex with α_1-antitrypsin, in several samples a second peak was detected in the elution position of free kallikrein (Fig. 3).
For boar seminal plasma mainly one peak was found corresponding to free kallikrein.

Similar experiments, employing a radioimmunoassay for human urinary kallikrein (Mann and Geiger, 1977), demonstrated the presence of glandular kallikrein also in human serum in a concentration

of 10 - 15 ng/ml using our human urinary kallikrein preparation as a standrad.

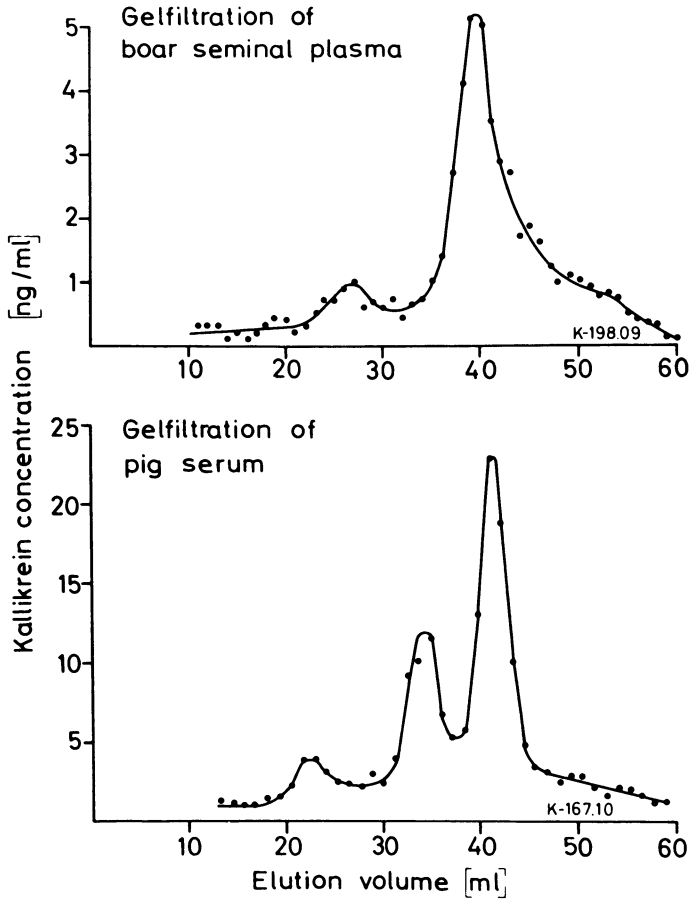

Fig 3. Gel filtration of boar seminal plasma and pig serum through Sephacryl S-200. The fractions were assayed by a radioimmunoassay for pig pancreatic kallikrein.

After gel filtration one peak was detected in the position of a molecular weightof 80 000 whereas our kallikrein preparation was eluted in the position of 50 000. Nustad and co-workers (1978) published recently that also in rat serum an antigen can be detected by a radioimmunoassay for rat submandibular kallikrein which is immunologically identical to glandular kallikrein but appears to be complexed with plasma proteins. Thus, the occurrence of glandular kallikreins in the blood is now established for three species. The exact origin of the glandular kallikrein in blood is unknown, a

discrimination by radioimmunoassay is impossible because of the immunological crossreactiivity of the various tissue kallikreins.

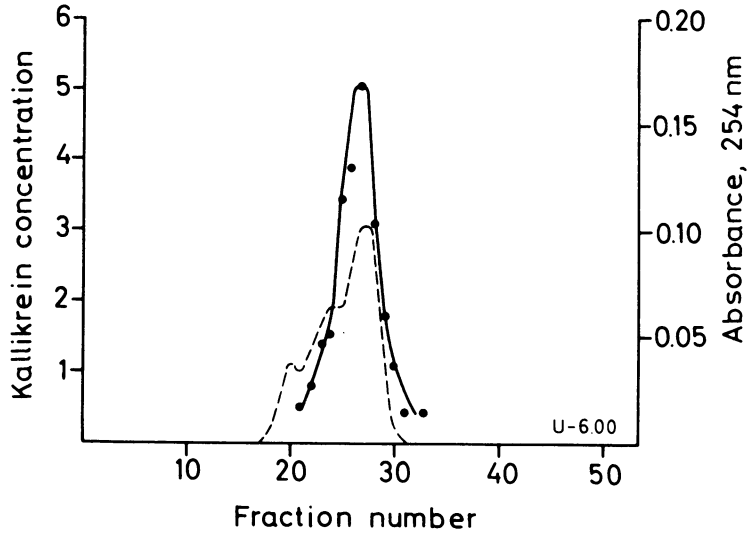

Fig 4. Gel filtration of human serum through Sephacryl S-200. The fractions were assayed by a radioimmunoassay for human urinary kallikrein.

Our findings and the findings of other authors suggest that glandular kallikreins have access to the circulation via intestinal absorption and via release into the glandular circulation. The presence of glandular kallikrein in blood leads to the assumption that renal filtration might contribute to some extent to the amount of kallikrein found in urine.

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