STUDIES ON THE BIOLOGICAL FUNCTION OF GLANDULAR KALLIKREIN

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INTRODUCTION

The physiological role of glandular kallikrein is still a matter of speculation. The same holds true for the mechanism of action by which administered kallikrein produces the many biological effects which have been observed (Haberland et al., 1977). Investigations which might contribute to the clarification of these problems include the cellular localization of glandular kallikrein, the measuring of physiological concentrations in tissues and body fluids and the tracing of administered kallikrein within the organism by sensitive and specific assay methods. In our studies on the physiological role of endogenous glandular kallikrein and the fate of administered kallikrein we applied immunofluorescent methods for the cellular localization and the radioimmunoassay technique for the sensitive and highly specific measurement of kallikrein in biological samples.

MATERIALS AND METHODS

Materials

Pig pancreatic kallikrein used in cellular localization studies and in the radioimmunoassay was a highly purified, neuraminidase treated preparation (Fiedler, 1976) kindly provided by

Dr. F. Fiedler, Munich, guinea pig coagulation gland kallikrein was a gift of Dr. C. Moriwaki, Tokyo, guinea pig submandibular gland and pig submandibular gland kallikreins (Lemon et al., 1976) and pig urinary kallikrein (Tschesche et al. 1976) were kindly provided by Dr. M. Lemon, Bristol and Dr. G. Mair, Munich. Pig pancreatic kallikrein used in experiments on intestinal absorption (1 180 KU/mg) and antisera and immunoglobulin G directed against pig pancreatic kallikrein were gifts of Bayer AG, Wuppertal.

Radioimmunoassay

The radioimmunoassay for pig pancreatic kallikrein is described elsewhere (Fink and Guttel, 1978).

Gel Filtration

For gel filtration experiments Sephacryl S 200, Pharmacia, was used. 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, 200 mg/l NaN_3, pH 7.4, flow rate: 3-4 ml/h.

Cellular Localization

Production of kallikrein antibodies, tissue preparation and indirect immunofluorescent technique are described elsewhere (Dietl et al., 1978).

RESULTS AND DISCUSSION

Radioimmunoassay

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, since the substrates are hydrolyzed also by 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.

With the radioimmunoassay developed in our laboratory (Fink and Guttel, 1978) the lower detection limit for pig pancreatic kallikrein is 40 - 300 pg, corresponding to concentrations of 0.2 - 1.5 ng/ml. The assay is highly specific for pig glandular kallikreins. Pig urinary and pig submandibular kallikreins show an immunological reactivity indistinguishable from that of pig pancreatic kallikrein under radioimmunoassay conditions as indicated by the parallel dose-response curves (Fig. 1). No cross-reactivity was detected with porcine trypsin, bovine trypsin and chymotrypsin

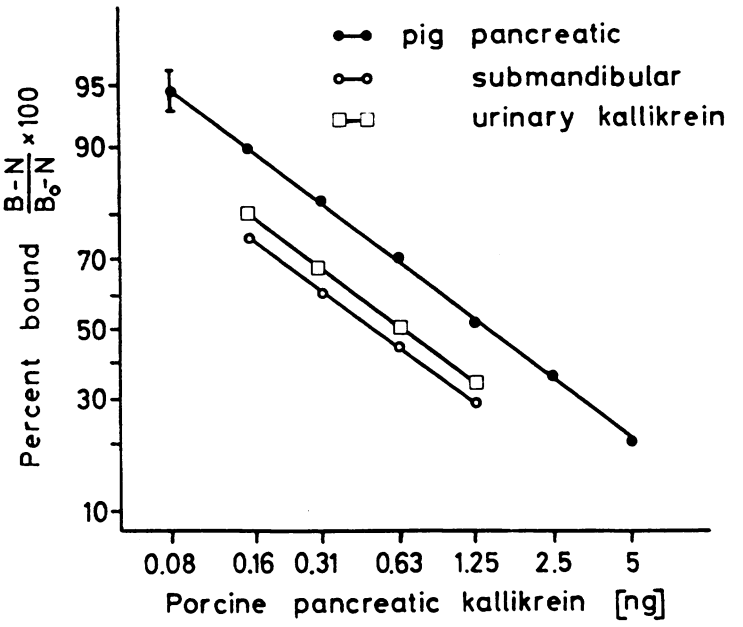

Fig. 1. Dose-response curves of pig glandular kallikrein.

and kallikreins of guinea pig submandibular glands and guinea pig coagulation glands.

Intestinal Absorption

The radioimmunoassay was employed to investigate whether kallikrein can be absorbed in the intestinal tract using the rat as a model (Fink et al., 1978; Fink et al., in press). The experiments were done both with rats which had fasted for 24 hr and with unfasted rats. 10 mg hog pancreatic kallikrein dissolved in 1 ml 0.9% saline were injected into the lumen of the duodenum of anesthetized rats. Lymphatic fluid, collected from the thoracic duct, and blood samples, drawn from the tail vein, were measured by radioimmunoassay. Kallikrein was detected in the samples in concentrations up to 200 ng/ml within 4 hr after injection, demonstrating that intestinal absorption had taken place. However, the absorption kinetics were highly variable within series of identical experiments, both with fasted and unfasted rats, in some experiments even no absorption at all was observed. Figure 2 gives an example of two completely different absorption kinetics, the two experiments were done under identical conditions with fasted rats.

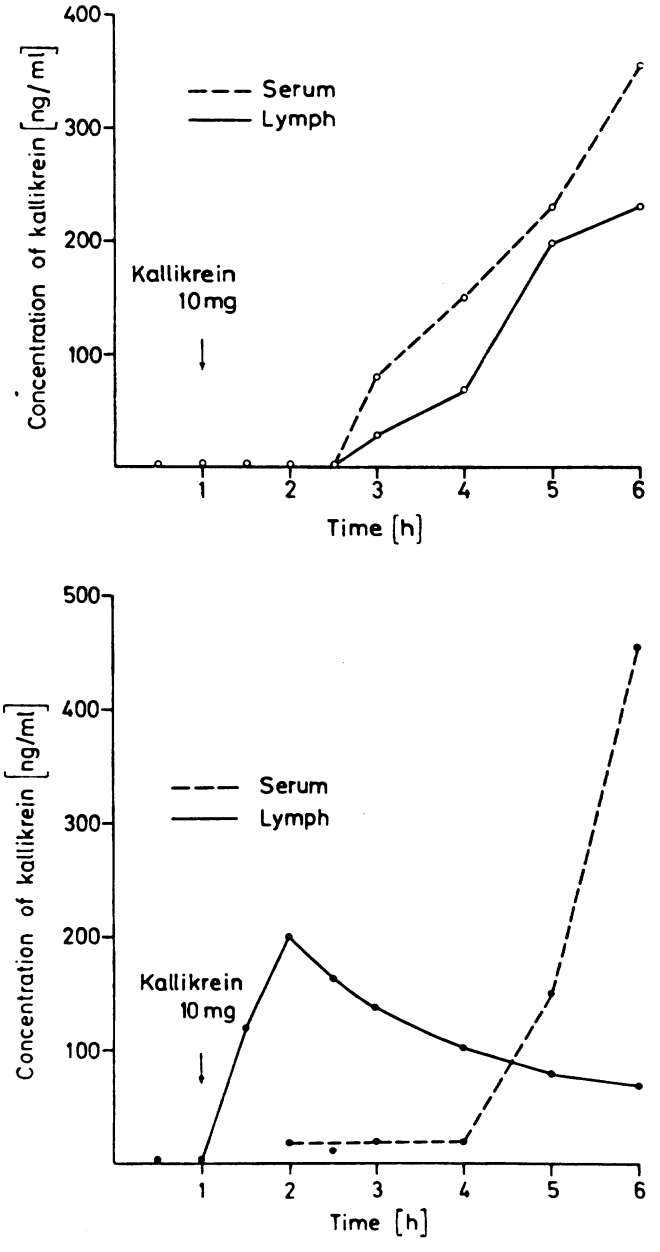

Fig. 2. Kinetics of intestinal absorption of pig pancreatic kallikrein into serum and lymph of rats after intraduodenal administration.

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

Table 1.

INTESTINAL ABSORPTION OF PIG PANCREATIC KALLIKREIN IN THE RAT

	Rat A (without fasting)	Rat B (24 hr 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 order to clarify, whether or not the radioimmunoassayable material consisted of low molecular weight degradation products, samples of lymphatic fluid were subjected to gel filtration and the eluate was tested by radioimmunoassay (Fig. 3). Two main peaks of immunochemically reactive material were found, the smaller one in an elution position corresponding to the molecular weight of about 80,000, indicating that most of the absorbed kallikrein was bound to a plasma protein, probably α_1 -antitrypsin. Immunochemically reactive material could not be found in the low molecular weight region where degradation products are to be expected.

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 - presumably - α_1-antitrypsin suggests that a large part of the absorbed kallikrein is enzymatically active. Furthermore, the results indicate a significant role of the lymphatics in the intestinal absorption of pancreatic kallikrein, in addition to the absorption via mesenteric vein routes. Absorption via mesenteric vein route was demonstrated also by Moriwaki et al. (1973) using a mesenteric perfusion system.

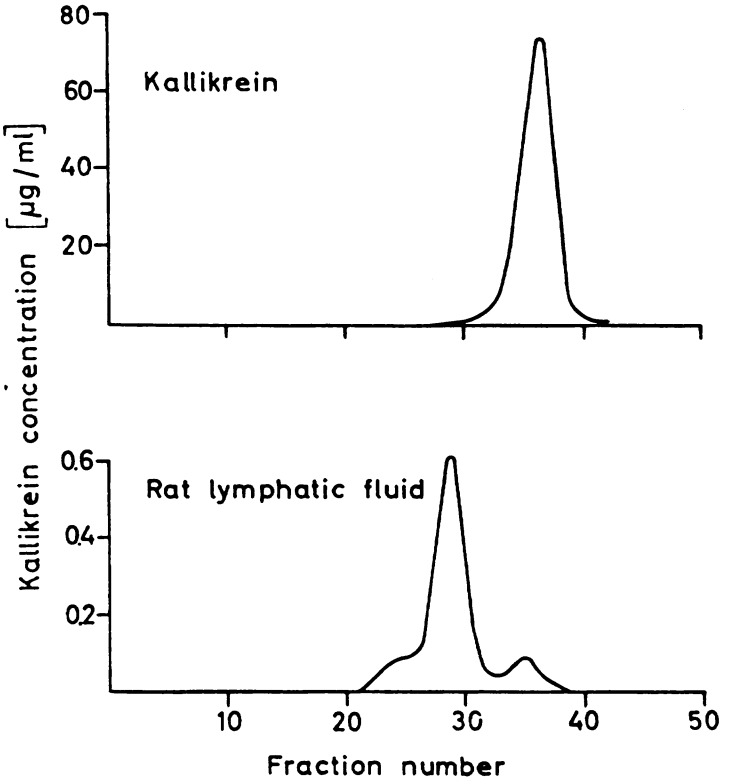

Fig. 3. Gel filtration of pig pancreatic kallikrein and rat lymph (from experiments on intestinal absorption) on Sephacryl S 200. The fractions were assayed for pig pancreatic kallikrein by radioimmunoassay.

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 (Fink et al. 1978; Fink et al., in press). In order to ascertain that the radioimmunoassayable substance was not a low molecular weight degradation product, serum samples were subjected to gel filtration and the fractions tested by radioimmunoassay. Generally two peaks were found, one in the elution position of kallikrein, the other one in a position which would correspond to the complex with α_1-antitrypsin (Fig. 4). In similar experiments with boar seminal plasma mainly one peak was detected corresponding

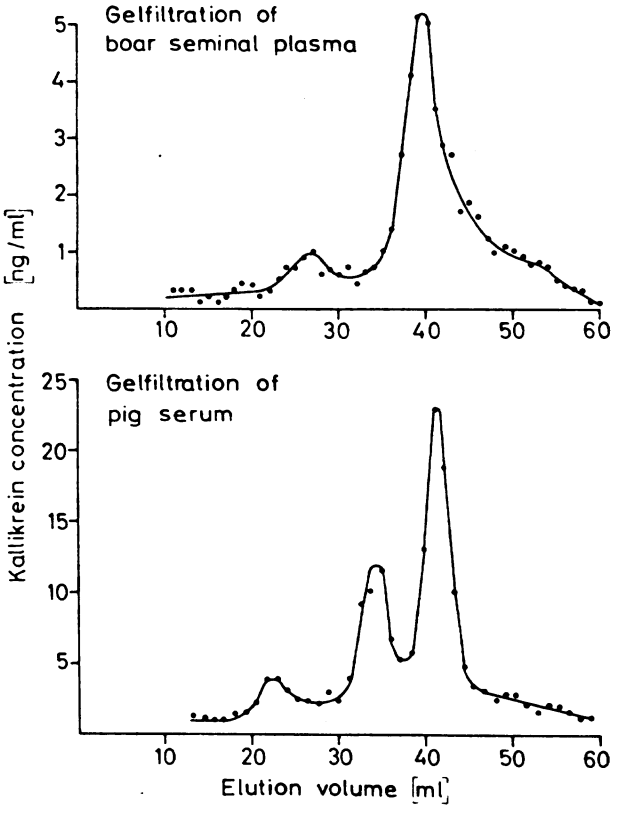

Fig. 4. Gel filtration of boar seminal plasma and pig serum on Sephacryl S 200. The fractions were assayed by a radioimmunoassay for pig pancreatic kallikrein.

to free kallikrein. Comparable results were found both for human blood (Fink et al. 1978; Geiger et al., this volume) and for rat blood (Nustad et al. 1978). Hence, the occurrence of glandular kallikreins in the blood is established now for three species. The exact origin of the glandular kallikrein in blood is unknown. The discrimination by radioimmunoassay is impossible because of the immunological cross-reactivity of the various glandular kallikreins (Fig. 1). The findings of other authors and our observations suggest that glandular kallikreins have access to the circulation via intestinal absorption and/or via release into the local glandular circulation.

Renal Excretion of Glandular Kallikrein

The presence of glandular kallikrein in blood leads us to the assumption that renal excretion might contribute to some extent to the amount of kallikrein present in urine. Some recent experiments on the renal excretion of pig pancreatic kallikrein after intravenous infusion into dogs corroborate this assumption.

0.5 mg pig pancreatic kallikrein, dissolved in 250 ml saline, were infused over a period of 2 hr into the femoral vein of anesthetized dogs. Blood samples were drawn every 15 or 30 min from the femoral artery. Urine was collected from the two catheterized ureters. The results of one experiment are summarized in Figure 5.

The plasma level of pig pancreatic kallikrein during the infusion (about 45 ng/ml) was comparable to the physiological concentration in porcine blood (10-20 ng). The fact that the kallikrein concentration in the urine samples was twice to three times higher than in plasma, demonstrates that the kallikrein found in urine was excreted by the kidney, and did not originate from the small amount of blood by which the urine samples were contaminated. The lower panel in Figure 5 shows the amounts of kallikrein excreted during each urine collection period. The total excreted kallikrein amounted to 2% of the administered dose.

These results demonstrate that glandular kallikrein can be excreted by the kidney. This is not surprising considering that glomerular filtration of proteins with molecular weights up to 70,000 is possible, and that the molecular weight of pig pancreatic kallikrein is only about 30,000 (for other glandular kallikreins, values of 25,000 - 45,000 are reported). Taking into account that glandular kallikrein is physiologically present in the blood, as demonstrated by radioimmunoassays for man, pig and rat, it seems very likely to us that urinary kallikrein is not pure kidney kallikrein but contains also other glandular kallikrein(s).

Cellular Localization

For cellular localization studies we employed the indirect immunofluorescence technique on cryosections of pig submandibular gland, kidney and pancreas (Dietl et al. 1978).

Submandibular gland. Strong and specific fluorescence indicates location of kallikrein in striated and also collecting ducts of the submandibular gland (Fig. 6). Kallikrein is present there in the apical portion of the duct cells, where the zymogen granules are located. Our results are in agreement with the

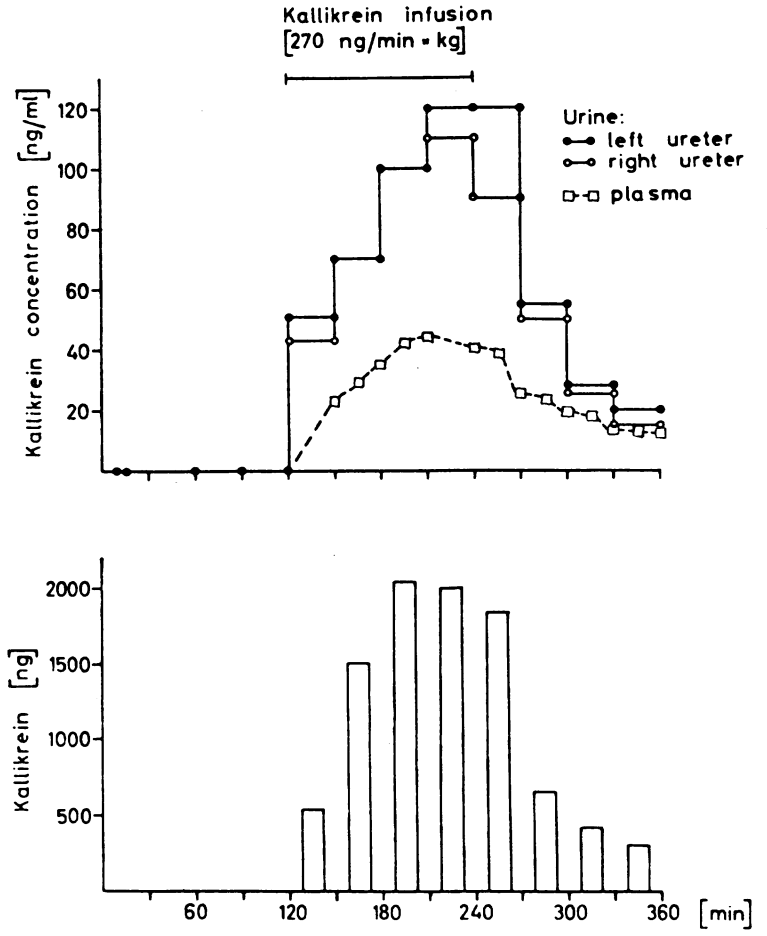

Fig. 5. Renal excretion of pig pancreatic kallikrein infused into the femoral artery of a dog. The samples were assayed for pig pancreatic kallikrein by radioimmunoassay.

location of kallikrein in submandibular glands of rat and cat as described by other groups (Berg Ørstavik et al. 1975; Brandtzaeg et al. 1976; Hojima et al. 1977). Only in guinea pigs additional fluorescence was found in acinar cells (Bhoola et al. 1977).

Pancreas. In the pancreas strong and specific fluorescence could be observed in the acinar cells, mainly in the apical regions (Figure 7a and b). In contrast to the submandibular gland, we observed no specific fluorescence in the interlobular ducts of the pancreas. The islets of Langerhans were also unstained. This

Fig. 6. Localization of kallikrein in porcine submandibular gland as revealed by immunofluorescence (x 400)(Dietl et al. 1978).

indicates that pancreatic kallikrein is, like other proteases, located in the exocrine cells. We suppose, therefore, that the zymogen granules in the apical region of the acinar cells contain the kallikrein.

Kidney. Differing from the results in the rat kidney, where kallikrein was found in the distal tubular cells (Ørstavik et al. 1976) by direct fluorescence-antibody technique, no proper localization of kallikrein was achieved by us in the porcine kidney: Specific fluorescence could not be observed by employing the same technique as for porcine pancreas and submandibular glands. This might be due to the much lower amount of kallikrein present in the kidney compared to pancreas and submandibular gland or to a special mode of binding of kallikrein to kidney organelles so that its antigenic sites are inaccessible to the antibody.

Immunological crossreactivity. In immunodiffusion experiments precipitation patterns of identity were obtained for porcine kallikreins from pancreas, submandibular gland and urine with antibodies directed against each of the kallikreins. When we applied the three antibodies in the localization studies, each of them yielded the same immunofluorescence pattern in the submandi-

bular gland and in the pancreas, respectively. This finding demonstrates that the tissue-bound kallikreins, probably prekallikreins, also crossreact with antibodies produced against the three active enzymes.

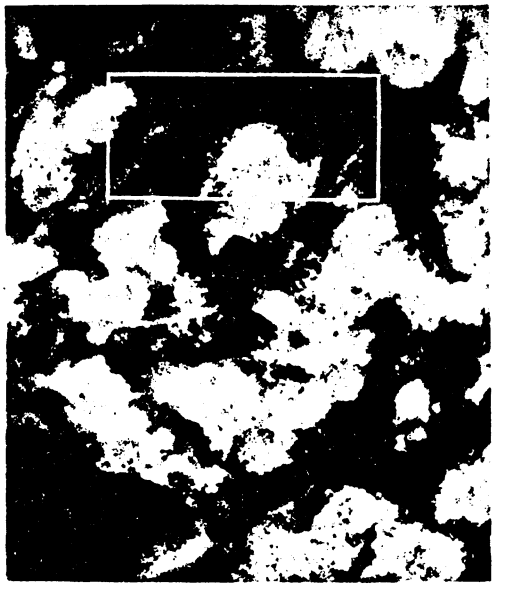

Fig. 7a. Localization of kallikrein in porcine pancreas as revealed by immunofluorescence (x 400) (Dietl et al. 1978).

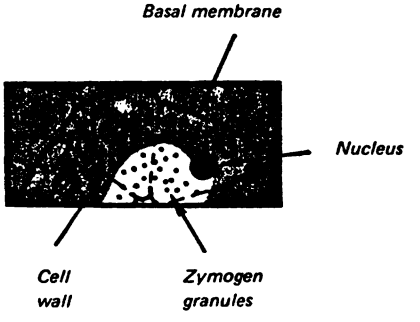

Fig. 7b. Schematic drawing of acinar cells. Nucleus and basal membranes, but not cell walls, are visible in Fig. 7a. Zymogen granules are located in the region of fluorescence (Dietl et al. 1978).

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