Biochemical, Pharmacological, and Functional Aspects of Glandular Kallikreins

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Kallikreins

Kallikrein (EC 3.4.21.8) specifies those serine proteinases which liberate kinins from kininogens by limited proteolysis and have no or only little proteolytic activity on other proteins (for review, see refs. 2,3,21). Unfortunately, this definition fits two totally different groups of proteinases: the glandular, tissue or organ kallikreins on the one hand, and the plasma or serum kallikrein on the other. The two types differ (a) in physicochemical properties; (b) in the type of kinin they release (plasma kallikreins: bradykinin; glandular kallikreins: kallidin = lysyl-bradykinin); (c) in immunochemical properties (no cross-reactivity of the one type with antiserum directed against the other type has ever been observed); and (d) in susceptibility to inhibition by natural proteinase inhibitors.

The plasma kallikrein plays an important role in the coagulation/fibrinolysis system (21). The physiological function of the glandular kallikreins is still to a large degree a matter of speculation. Glandular kallikreins are found in pancreas, salivary and sweat glands, intestines, kidney, and urine.

PROPERTIES OF PIG GLANDULAR KALLIKREINS

The biochemically best characterized glandular kallikreins are the urinary, submandibular, and especially pancreatic kallikrein of the pig (12). These three glandular kallikreins have been isolated and highly purified. On isoelectric focusing the pure enzymes show numerous multiple forms with slightly different isoelectric points close to pH 4. Another type of heterogeneity is revealed by SDS-polyacrylamide gel electrophoresis of reduced submandibular and urinary kallikrein. With both enzymes two protein bands are obtained. In analogy to the two forms of pancreatic kallikrein originally described by Habermann (13) the faster migrating component is called "A-form" and the slower one "B-form." For pancreatic kallikrein it was shown that the two forms differ in their carbohydrate content: the A-form contains about 5.6%, the B-form about

11.5% carbohydrate. It is assumed that also the differences of the A- and B-forms of submandibular and urinary kallikrein are a reflection of different carbohydrate contents (Table 1).

Pig pancreatic kallikrein B, isolated from autolyzed pancreas, consists of two polypeptide chains connected by disulfide bridges. This form was denoted β-kallikrein (Table 1). By comparing the amino acid compositions of the two-

TABLE 1. *Molecular forms of pig glandular kallikreins*

Designation	Definition
Kallikrein A Kallikrein B	A and B differ in their carbohydrate content
α-Kallikrein β-Kallikrein	Single polypeptide chain Two polypeptide chains linked by disulfide bridges

chain pancreatic β-kallikrein and of the single-chain submandibular and urinary α-kallikreins, a difference of three amino acids is revealed: Obviously, the pancreatic β-kallikrein has lost, by limited proteolytic cleavage during tissue autolysis, a tripeptide which originally formed the linkage between the two chains (4,28).

The apparent molecular weights of the pure kallikrein preparations were determined by SDS-polyacrylamide gel electrophoresis and ultracentrifugation (12) (Table 2). For the neuraminidase-treated pancreatic β-kallikrein B, a somewhat lower molecular weight was found than for urinary and submandibular kallikrein, probably reflecting differences in the carbohydrate moieties of the three enzymes (12).

The amino acid sequences of the two chains of pancreatic β-kallikrein have been elucidated by Fiedler et al. (4) and Tschesche et al. (27). The comparison

TABLE 2. *Molecular weights of pig glandular kallikreins*

Kallikrein	β-Pancreatic[a]	Submandibular	Urinary
	SDS-polyacrylamide gel electrophoresis		
M_r app M_r app	34,600[b] form B	35,900 (form A) 39,600 (form B)	36,100 (form A) 39,600 (form B)
	Ultracentrifugation[c]		
M_r	28,000 ± 1000	38,000 ± 1000	40,000 ± 1500

[a] Neuraminidase-treated.
[b] Total of the molecular weights of the two chains of the β-kallikrein.
[c] The results were obtained by high speed sedimentation equilibrium runs and by active enzyme centrifugation (12).

TABLE 3. *Relative rates of ester hydrolysis catalyzed by pig glandular kallikreins*

Substrate	Kallikrein		
	β-pancreatic, B	Submandibular	Urinary
Ac-Phe-Arg-OMe	578	257	266
Bz-Arg-OMe	112	87.3	95.5
Bz-Arg-OEt	95.5	69.8	73.6
Tos-Arg-OEt	2.73	2.57	2.99
Bz-Lys-OMe	4.23	0.86	1.19
Ac-Tyr-OEt	0.25	0.21	0.19
Bz-Met-OMe	0.18	0.12	0.12

The hydrolysis rates were measured on the autotitrator and related to rate = 100 for each kallikrein at 10 mM Bz-Arg-OEt. Conditions: 1 mM substrate, 0.1 M NaCl, 0.1 mM ethylenediaminetetraacetic acid, pH 8.0, at 25°C.
Data from Fritz et al., ref. 12.

with the amino acid sequence of porcine trypsin revealed a degree of homology of 51%.

Evidence for identical amino acid sequences of the three pig glandular kallikreins was obtained from studies of the amino terminal sequences of urinary and submandibular kallikreins. The sequences of the first 10 amino acids of submandibular and of the first 28 amino acids of urinary kallikrein are identical with the corresponding sequence of pancreatic kallikrein (27).

The comparison of hydrolysis rates of synthetic substrates (Table 3) as well as the accessibility to inhibition by natural and synthetic inhibitors (1,12) (Table 4) measured for the three kallikreins indicates a high degree of similarity of the single-chain submandibular and urinary α-kallikreins, whereas the two-chain pancreatic β-kallikrein differs distinctly. These differences observed for the pancreatic kallikrein are in all probability due to the intrachain split. For single-chain pancreatic kallikrein, presently under investigation by Dr. Fiedler in our department, such differences in enzymatic properties should not exist.

TABLE 4. *Apparent dissociation constants* K_I *(nmoles/l) of pig glandular kallikrein–inhibitor complexes*

Inhibitor from:	Kallikrein		
	Pancreatic	Submandibular	Urinary
Bovine lung (TKI)	0.8	0.9	1.0
Sea anemone (SAI-5-II)	0.8	0.6	0.8
Snake venom (NNV-II)	1.1	1.3	1.3
Snake venom (HHV-II)	8.3	210	109
Snail (HPI$_K$)	27.7	210	204

Abbreviations: TKI, aprotinin (Trasylol®); SAI-5-II, fraction 5-II from *Anemonia sulcata;* NNV-II, fraction II from snake *(Naja naja)* venom; HHV-II, fraction II from snake *(Hemachatus hemachatus)* venom; HPI$_K$, isoinhibitor K from *Helix pomatia*. The five inhibitors are structurally homologous (1).

The antigenic relationship between the three pig glandular kallikreins was first studied by double-diffusion experiments (12). The precipitin lines formed during diffusion of the three kallikreins against rabbit antisera to any of the three enzymes were completely confluent, indicating immunological identity of the three kallikreins. Immunoelectrophoretic studies demonstrated clearly different electrophoretic mobilities, but also immunological identity of the three kallikreins (12).

Hence, all evidence accumulated from physicochemical, immunochemical, enzyme-kinetic, inhibition, and structural studies indicates a high degree of similarity or even identity of the polypeptide chain of the three pig glandular kallikreins. Existing differences obviously reflect differences in the carbohydrate moieties of these glycoproteins and different stages of limited proteolytic degradation.

STUDIES ON THE BIOLOGICAL ROLE OF GLANDULAR KALLIKREINS

Binding of Glandular Kallikreins to Serum Proteins

The only serum protein so far known to react with glandular kallikrein is α_1-antitrypsin (11). For α_2-macroglobulin, Vathera and Hamberg (28) demonstrated convincingly that it does not bind urinary and pancreatic kallikrein. Recently we began to examine if, in addition to α_1-antitrypsin, other kallikrein binding proteins are present in human serum.

In these studies pig pancreatic ^{125}I-kallikrein was incubated with human serum at 37°C. After 10 min, 30 min, and 14 hr incubation, approximately 25, 39, and 66%, respectively, of the labeled kallikrein was bound to serum proteins, as determined by gel filtration of the incubation mixture and subsequent measuring of the radioactivity in the eluted fractions. As these data demonstrate, the binding of active kallikrein to plasma protein(s) occurs relatively slowly—at least in the pig pancreatic kallikrein and human serum systems—even after 14 hr the reaction is still incomplete.

To discriminate between true and unspecific binding, ^{125}I-kallikrein inactivated by diisopropylphosphorofluoridate (DFP) was employed. In this case, after 14 hr incubation 30% of the radioactivity was bound to serum proteins. Obviously, in addition to the interaction of kallikrein with inhibitor(s) an unspecific binding of glandular kallikrein to serum proteins also takes place, which is not dependent on the enzymatic activity.

Partial identification of the kallikrein binding proteins was achieved by crossed immunoelectrophoresis and subsequent staining and autoradiography of the electrophoretograms. α_1-Antitrypsin was clearly the prevailing inhibitor of pig pancreatic kallikrein. In addition, some other serum proteins are able to bind the enzyme: for example, α_2-macroglobulin, α_1-antichymotrypsin, the C1-inactivator, and antithrombin III. From our experiments it is not yet clear to what

extent these interactions are specific, because some binding also occurred in the control with DFP-inactivated kallikrein; for example, to α_2-macroglobulin and especially to antithrombin III, yet almost no binding of DFP-inactivated kallikrein to α_1-antitrypsin was observed. In order to distinguish between specific and nonspecific binding to the various serum proteins, more studies will be necessary using quantitative identification methods and purified preparations of serum proteins.

RADIOIMMUNOASSAY

The radioimmunoassay technique is highly suitable for studies on the fate and the functions of exogenous and endogenous glandular kallikreins in the organism because of the high sensitivity and high specificity of this method.

At first a radioimmunoassay for pig pancreatic kallikrein was developed (5,8) which is highly specific for the pig glandular kallikreins. With the available antiserum to pig pancreatic kallikrein, no cross-reactivity was detected for porcine trypsin, bovine trypsin, and chymotrypsin, and kallikreins of guinea pig submandibular and coagulation glands. In contrast, dose-response curves of pig submandibular and urinary kallikrein paralleled the pancreatic kallikrein standard curve, again indicating immunochemical identity of the three pig glandular kallikreins (Fig. 1). Remarkably, dose-response curves were also obtained for human urinary kallikrein which nearly paralleled the pig pancreatic kallikrein standard curve (Fig. 2). The cross-reactivity calculated from the 50% intercept was approximately 4%, i.e., the amount of human urinary kallikrein yielding
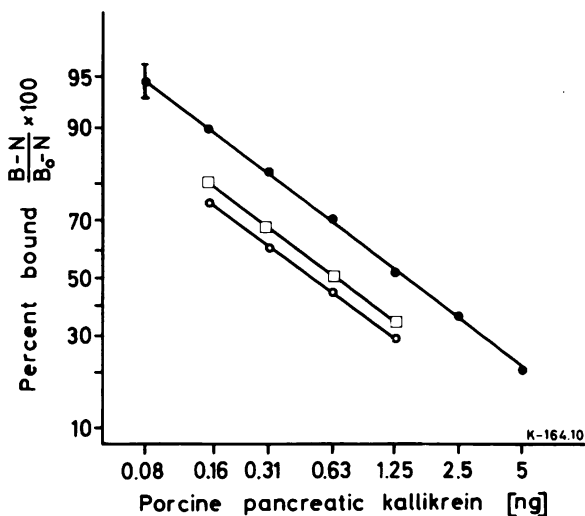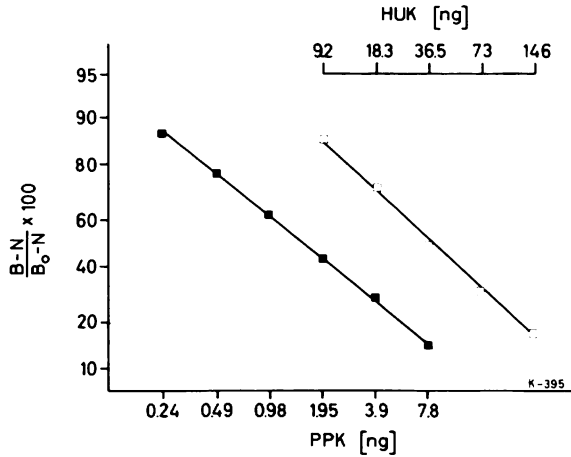

FIG. 1. Dose-response curves of pig glandular kallikreins. *Open circles,* submandibular; *closed circles,* pig pancreatic; *squares,* urinary kallikrein.

FIG. 2. Demonstration of cross-reactivity of human urinary kallikrein (HUK) with antiserum to pig pancreatic kallikrein (PPK) under radioimmunoassay conditions. Dose-response curve of PPK *(closed squares)* and HUK *(open squares).*

a 50% inhibition of binding of pig pancreatic ^{125}I-kallikrein to the antibody is 25-fold higher for human urinary kallikrein than for pig pancreatic kallikrein. Obviously, there is a much closer immunochemical similarity between glandular kallikreins of different species than between glandular kallikreins and structurally related serine protease like trypsin of the same species. The lower detection limit of pig pancreatic kallikrein in the radioimmunoassay is approximately 20 pg corresponding to a concentration of 200 pg/ml.

Intestinal Absorption

During the past few years evidence has accumulated that enteral and parenteral administration of pig pancreatic kallikrein can normalize several pathologic disorders: Schill (24) demonstrated in double-blind studies an increase both in sperm count and motility in patients with asthenozoospermia and oligozoospermia after intramuscular and oral administration of pig pancreatic kallikrein. Wicklmayr and Dietze (29) found an enhancing effect on muscular glucose uptake in maturity onset diabetics. Overlack and co-workers (20) showed that in a group of patients with essential hypertension and subnormal excretion of urinary kallikrein both parameters normalized after oral administration of pig pancreatic kallikrein. Since the observed effects were similar both after intramuscular injection and after oral administration of stomach-resistant tablets (23), not a mediator function of the intestinal tract but access to the blood stream seems to be obligatory for the action of the administered kallikrein.

As a first step towards clarification of what happens between oral intake of

kallikrein and the observed effect, we investigated if pig pancreatic kallikrein can be absorbed by the intestine using the rat as a model (6,7,9,10).

Ten milligrams pig pancreatic kallikrein, dissolved in 1 ml 0.9% saline, was injected into the lumen of the duodenum of anesthetized rats. Lymph was collected from the thoracic duct and blood samples were drawn from the tail vein. All samples were measured by radioimmunoassay. Pig pancreatic kallikrein was detected in the samples in concentrations of 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 a series of identical experiments, with both fasted and unfasted rats. In some experiments no absorption at all could be observed.

The two experiments shown in Fig. 3 were selected to demonstrate the high variability. The experiments were carried out under identical conditions. The highest absorption into lymph that we ever found was 1.5 ppm of the administered dose.

In order to clarify if the radioimmunoassayable material consisted of low molecular weight degradation products, samples of lymphatic fluid were subjected to gel filtration and the eluted fractions tested by radioimmunoassay. Two peaks of immunochemically active material were found, the smaller one
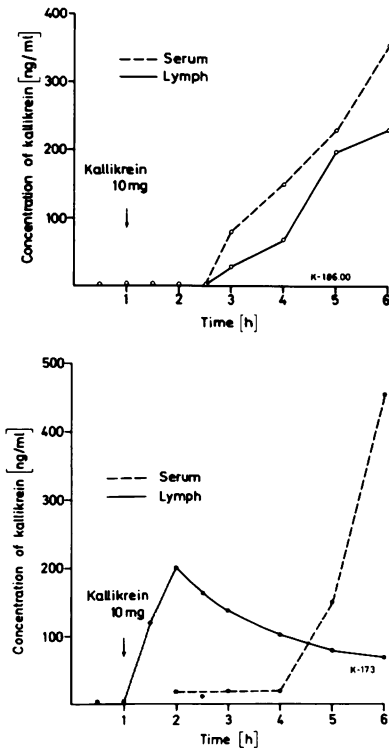
FIG. 3. Kinetics of intestinal absorption of pig pancreatic kallikrein into serum and lymph of rats after intraduodenal administration.

in the elution position of pig pancreatic kallikrein, the larger one in the position of a molecular weight of about 80,000, indicating that most of the intestinally absorbed kallikrein was bound to a plasma protein, probably α_1-antitrypsin. No immunoreactive material at all was found in the region below the molecular weight of pig pancreatic kallikrein where degradation products are to be expected.

In spite of the high variability the experimental results demonstrate that undegraded glandular kallikrein can be absorbed by the intestine. The partial binding to, presumably, α_1-antitrypsin suggests that most of the absorbed kallikrein was enzymatically active. Furthermore, the results indicate a significant role of the lymphatics in the intestinal absorption of kallikrein in addition to the absorption by the mesenteric vein route which was demonstrated earlier by Moriwaki and co-workers (18) using a mesenteric perfusion system. The intestinal absorption of undegraded kallikrein is not a singularity, a whole variety of other proteins is known to be absorbed as intact molecules, for example, insulin, immunoglobulin, chymotrypsin, chymotrypsinogen, trypsin, lipase, and albumin (see ref. 15, 26).

GLANDULAR KALLIKREIN IN BLOOD

The radioimmunoassay was further applied to investigate if endogenous glandular kallikrein is present in the blood (6,7,9,10). When samples of pig serum or plasma were assayed, concentrations of up to 20 ng/ml were found. Unfortunately, the dose-response curves did not parallel the standard curve; therefore, an exact quantitative measurement of glandular kallikrein in blood is not yet feasible. Yet the results indicate strongly the presence of a glandular kallikrein-like antigen in blood.

In order to further characterize the kallikrein-like antigen gel filtration experiments were performed. Serum and urine samples of the same pig obtained at the same time were gel filtrated on Ultragel ACA 44 or Sephacryl S 200 and the eluted fractions assayed by radioimmunoassay (Fig. 4). The similar elution positions of the radioimmunoassayable material indicate that the molecular weight of the kallikrein-like antigen in blood is very close to that of urinary kallikrein.

In this connection it has to be regarded that, in the radioimmunoassay, any substance reacting with the labeled antigen in a way that reduces the binding to the antibody would simulate the presence of antigen. Especially for the radioimmunoassay of proteinases in serum where proteinase inhibitors are present this type of interference has to be considered. However, α_1-antitrypsin, as determined by a trypsin inhibition assay, was eluted at a position corresponding to a higher molecular weight than that of the kallikrein-like antigen. No other plasma inhibitor of glandular kallikrein with a molecular weight similar to that of glandular kallikrein is known. Therefore, it seems highly unlikely that the peak of radioimmunoassayable material detected in the gel filtration eluate of the serum sample is caused by such a substance.
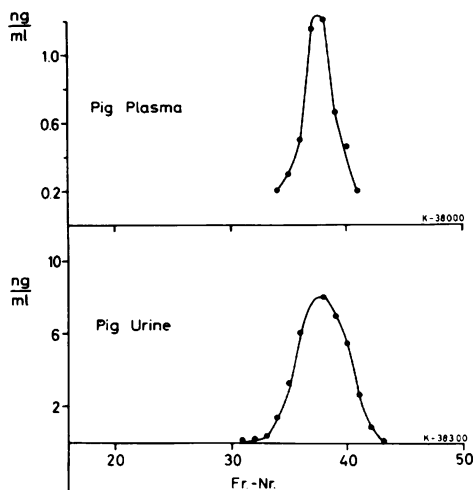
FIG. 4. Gel filtration of pig plasma and pig urine. The eluted fractions were measured by radioimmunoassay for pig pancreatic kallikrein.

Gel filtration experiments were also done with human serum. In this case the peak of antigenic activity (employing antiserum to human urinary kallikrein in the radioimmunoassay) was eluted in the range of 60,000 to 80,000 dalton (α_1-antitrypsin, for comparison, 54,000 dalton), whereas the elution position of the purified human urinary kallikrein corresponded to about 50,000 dalton. Therefore, the evidence for the presence of glandular kallikrein in human blood is not yet as conclusive as for porcine blood. However, in analogy to our results with porcine blood, the results of other groups with the rat (19,22; Rabito et al., *this volume*) and the tendency of human urinary kallikrein to associate with hydrophobic substances (R. Geiger et al., *unpublished*) lead us to conclude that glandular kallikrein is also present in human blood. Final proof for the identity of the kallikrein-like antigen in blood with glandular kallikrein will only be possible after its isolation and characterization.

In addition to all the evidence accumulated so far regarding the occurrence of glandular kallikrein in blood of various species, the fact that other pancreatic enzymes such as lipase, amylase, chymotrypsin, chymotrypsinogen, trypsin, pancreatic elastase, and carboxypeptidase B were found in blood suggests that glandular kallikrein, at least pancreatic kallikrein, should also be present. Two ways of access are possible: by release into the local glandular circulation, directly or via the lymphatic route, and/or via intestinal absorption. The exact origin of the kallikrein-like antigen in blood is not yet known, and also, the question of whether the antigen is enzymatically active kallikrein or inactive prekallikrein cannot yet be answered.
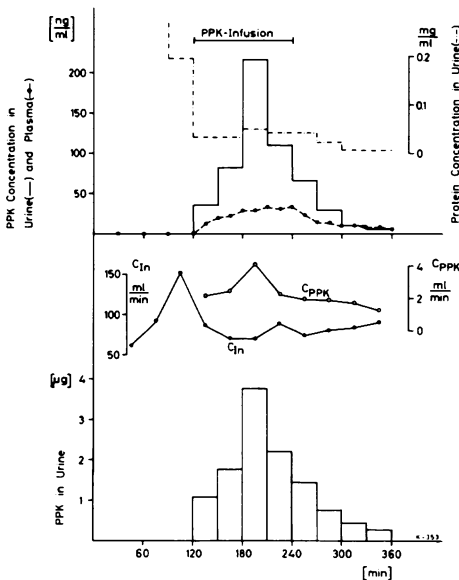
RENAL EXCRETION OF GLANDULAR KALLIKREIN

The presence of glandular kallikrein in blood led us to the assumption that glomerular filtration might partly be responsible for the occurrence of glan-

dular kallikrein in urine. Experiments on the renal excretion of pig pancreatic kallikrein during intravenous infusion into dogs corroborate this assumption. Pig pancreatic kallikrein, 0.5 mg dissolved in 250 ml saline, was infused over a 2-hr period into the femoral vein of anesthetized dogs. Blood samples were drawn from the femoral artery and urine was collected in 30-min periods from the bladder.

During the infusion a broad maximum of the kallikrein concentration in the plasma was reached, being of the same order of magnitude as the concentration measured by radioimmunoassay in porcine blood (Fig. 5, upper panel). The concentration in urine, estimated also by radioimmunoassay, was up to fivefold higher than the plasma concentration, thus proving that the pig pancreatic kallikrein was indeed excreted by the kidney and did not originate from the small amounts of blood by which some urine samples were contaminated. The middle part of Fig. 5 shows the clearances determined for inulin and pig pancreatic kallikrein for intervals of 30 min. The relative clearance of kallikrein, related to the inulin clearance, was approximately 4%, which is in the same range as the amylase clearance determined in man (16). The lower panel of Fig. 5 shows the amounts of kallikrein excreted during the single urine collection periods. The total excreted kallikrein in five experiments was 1 to 3% of the administered dose.

In order to ascertain that not only kallikrein of a different species can be excreted, so far one experiment with a pig was carried out. Since the endogenous pig urinary kallikrein cannot be distinguished from the exogenous pig pancreatic kallikrein by radioimmunoassay, high doses of kallikrein (300–1,000 μg) were

FIG. 5. Renal excretion of pig pancreatic kallikrein (PPK) in the dog. C_{In}, clearance of inulin; C_{PPK}, clearance of PPK. For details see text.

administered and the excretion rate of kallikrein determined. After the administration of the high doses the renal excretion rate increased up to 20-fold. The most likely explanation is that the increase was due to excretion of the administered kallikrein, since an increase of synthesis and secretion of kidney kallikrein after administering exogenous glandular kallikrein has never been observed.

The experiments with both dog and pig demonstrate that glandular kallikrein can be transferred from the blood stream to the urine by the kidney, most likely by glomular filtration since the kidney was functioning adequately as judged by the inulin clearance and the protein excretion (Fig. 5). The results reported by Mills et al. (17) do not contradict ours: Mills and co-workers found that after intravenous injection of pig pancreatic ^{125}I-kallikrein into rabbits, about 8% of the administered radioactivity was excreted in the urine as kallikrein and 32% as low molecular weight degradation products. The authors concluded that glomerular filtration would not contribute significantly to the renal excretion of kallikrein into the urine. However, calculated from the clearance of pig pancreatic kallikrein found in the dog (approximately 2.5 ml/min) and the (preliminary) concentration of glandular kallikrein in blood (10–20 ng), a daily excretion of about 50 μg of glandular kallikrein would result, which is in the range of several ten percent of the daily excretion of urinary kallikrein in man. Therefore, since kallikrein is present in blood, as indicated by the results described above and by the findings of other authors (19,22; Rabito et al., *this volume*), it has to be assumed that it can be transferred into the urine by glomerular filtration. Only if glandular kallikrein were handled by the kidney in a uniquely different way from other proteins of similar molecular weight (i.e., no glomerular filtration or complete reabsorption in the tubule) would urinary kallikrein represent exclusively kallikrein synthesized and secreted by the kidney. However, to our knowledge no evidence is available suggesting a uniquely different handling of kallikrein by the kidney.[1] Therefore, it seems very likely to us that urinary kallikrein does not originate exclusively from the kidney but also contains kallikrein of different origin.

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[1] *Note added after the Workshop Conference:* The stop-flow experiments (25) to which Dr. Carretero referred in his discussion remark [see *Discussion,* this chapter] do not contradict our results. In stop-flow experiments similar results are to be expected for proteins, which are glomerularly filtrated and then partly reabsorbed in the proximal tubule, and for proteins, which are secreted into the urine in the distal tubule. In both cases the highest concentration of the protein would be found in the urine fractions corresponding to the distal tubule.

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DISCUSSION

Dr. Carretero: I think you have to differentiate between what happens in a normal situation and what happens with the infusion of kallikrein. In our stop-flow studies, we never found kallikrein in the proximal tubule, indicating that the kallikrein was not filtered. A kallikrein peak was found in the distal nephron, indicating that the kallikrein in the urine was coming from this part of the kidney. Of course, I would not say that this occurs also in the case of pancreatitis, where a considerable amount of kallikrein enters the circulation. When kallikrein is injected, it binds to inhibitors that have a high molecular weight and will not be filtered in the kidney. However, you assume that kallikrein is circulating freely even though you demonstrated that kallikrein is bound to the inhibitors and that it is inactive. Are you proposing a renal mechanism for cleaving kallikrein from the inhibitor and then filtering it?

Dr. Fink: The slide with the gel filtration of rat serum and rat lymph showed two peaks of pig pancreatic kallikrein, the smaller peak was unbound kallikrein. This demonstrates that injected kallikrein is partly bound to α_1-antitrypsin or whatever the inhibitor is, but some of the kallikrein is still present in the free form. The complex formation, at least *in vitro,* occurs relatively slowly. Therefore, if kallikrein is infused intravenously, part of it should be present in the free form during the infusion.

Dr. Carretero: How do you explain then that, after intravenous injection of kallikrein, the depressor effect disappears almost immediately? What proportion of the urinary kallikrein do you feel comes from organs other than the kidney?

Dr. Fink: Kallikrein, which circulates in the blood, may still be of renal origin. It may be secreted into the blood first and then filtered in the glomeruli, so it still may be kidney kallikrein.

Dr. Carretero: Let me put it another way. What proportion of the kallikrein that you find in the urine was secreted directly into the lumen of the distal tubule, and what proportion was filtered through the glomeruli?

Dr. Fink: We calculated that in these experiments, at least up to 50 μg of the human urinary kallikrein is filtered in the kidney, maybe even more, but at least 50 μg.

Dr. Carretero: When the inhibitor takes 24 hr to bind kallikrein, how do you explain then that, after injection of kallikrein, the depressor effect does not last, but disappears immediately?

Dr. Fink: If you inject relatively high doses of kallikrein, the lowering of blood pressure lasts for several hours; if you inject only small doses, as for measuring kallikrein concentrations by the response of blood pressure, this response lasts only for a very short period.

Dr. Heimburger: Concerning the interaction, respectively the unspecific binding of kallikrein by antithrombin III: it is well known that antithrombin III is rich in carbohydrates, and we found that antithrombin III combines with lectines of a defined carbohydrate composition. I presume that the nonspecific binding is due to an interaction of the carbohydrates of kallikrein and antithrombin III, and I think the carbohydrate composition might also be of importance for the distribution of kallikrein in various organs and in the body fluids. You should be able to check it by treatment of the inhibitor, kallikrein, or both with neuraminidase.

Dr. Vogt: In your immunodiffusion experiment, the three kallikrein preparations gave completely fusing precipitation lines with antiserum directed against one of them. Did you repeat the experiment with antisera raised against one of the other two kallikrein preparations? If so, was there again complete fusion or did spurring occur, indicating only partial immunochemical identity?

Dr. Fink: Such experiments were done, there was again complete fusion.

Dr. Fritz: Dr. Fink showed that, with the amount of kallikrein he injected, the kallikrein concentration in dog serum never rose above the normal level measured in pig serum. Nevertheless, he found kallikrein excreted in the urine. In the pig, this experimental approach was impossible because of the immunological identity of the exogenous and endogenous glandular kallikreins. He had to apply, therefore, higher kallikrein doses in the pig. I think, however, when a blood-kallikrein level within the physiological range— as it was the case in the dog experiments—leads to an excretion of kallikrein in the urine, this could also occur under normal conditions.

Dr. Mills: If I could come back to the point that Dr. Carretero was mentioning: If you inject enough kallikrein to have any measurable effect on blood pressure, you increase vascular permeability and this effect on vascular permeability far outlasts the length of time for which the blood pressure changes. With this increased vascular permeability, large proteins escape, even globulins, and I think it is quite likely that the glandular kallikrein might then be filtered in the glomerulus.

Dr. Fink: The only control we did with the dog was the determination of protein in the urine. Since the urine was quite diluted, we found a lowering of protein concentration. We tried to determine amylase activity but, obviously, the dog does not have amylase in the urine. We plan to do control experiments with added proteins of lower molecular weight. However, I do not think that the renal excretion of kallikrein is an artifact. Just from the physiological point of view, the barrier for glomerular filtration is about 70,000 dalton and one has to expect that kallikreins with a molecular weight of 30,000, 40,000 can be filtered.

Dr. Mills: When you put in labeled kallikrein in such a small amount that the blood pressure does not change, then we find less than 8% comes out in the fraction which contains active kallikrein.

Dr. Fink: I found up to 3%, so it was still a small amount, but enough to explain a large part of the urinary kallikrein.

Dr. Erdös: You have shown that only the pancreatic kallikrein has two chains, while the others have a single chain. Is that due to cleavage of the single peptide bound during extraction, or is it the native form of the pancreatic kallikrein?

Dr. Fink: It is obviously an artifact caused during the isolation procedure. The pancreatic kallikrein which we use is isolated from autolyzed pancreas. Recently, Drs. Fiedler and Gebhardt isolated prekallikrein, which is not yet pure. The prekallikrein is obviously a

single chain and all of the activated prekallikrein is a single chain. The two-chain kallikrein obviously has lost a peptide of three-amino acids. This is the difference between the amino acid compositions of the pancreatic kallikrein and the submandibular and urinary kallikrein.

Dr. Phillips: I heard this morning that there are kinins in the brain. Does kallikrein pass through the blood-brain barrier, or is there an endogenous source in the brain?

Dr. Fritz: The presence of kininogenases and kininases in brain tissue was described in earlier experiments by Werle, Frey, and Kraut. We know that various tissue cells contain kininogenases as well as kininases, e.g., mast cells, fibroblasts, etc. As far as I know these are not kallikreins in the strict sense as they are present in pancrease and submandibular gland. For example, cathepsin D, which is present in the brain, is also a potent kininogenase. It liberates leucokinins which are very similarly to normal kinins, from leucokininogen, probably also present in the brain.

Dr. Carretero: Kininogenase activity in the arterial wall has recently been described by Dr. Nolly. Unfortunately, this kininogenase is only active at acid pH, indicating that it may be a cathepsin.

Discussion Reference

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