STUDIES ON THE INTESTINAL ABSORPTION OF TISSUE KALLIKREIN

Edwin Fink*, Xu-Chang Feng** and Elmar Richter**

Abteilung für Klinische Chemie und Klinische Biochemie in der Chirurgischen Klinik Innenstadt* and Walther-Straub-Institut für Pharmakologie and Toxikologie** der Universität München

INTRODUCTION

It has recently been reported that enteral administration of tissue kallikrein (pig pancreatic kallikrein) can have an improving effect in several pathologic disorders such as an increase in sperm output and sperm motility in asthenozoospermia and oligozoospermia (1) and a decrease of blood pressure in essential hypertension (2). These findings suggest that tissue kallikrein can be absorbed intestinally in a biologically active form. That this is indeed the case was recently shown by other groups and by us (3,4,5). Here we present results on the intestinal absorption of enzymatically active tissue kallikrein obtained with a new experimental model.

METHODS

Perfusion of Intestinal Segments

Female Sprague-Dawley rats (270-310 g body weight) fasted for 18 h were operated as described by Windmueller and Spaeth (6). Briefly, the rats were anesthetized with urethane (0.8 g/kg, i.p.) and the mesenterial vein draining a 8-10 cm segment of ileum as well as the jugular vein and the carotid artery were cannulated. Ligatures were secured around each end of the selected segment. 0.2, 1.0 or 5.0 mg pig pancreatic kallikrein in 0.5 ml saline was injected into the closed loop. In control experiments C-14 labeled polyethylene glycol 4000 (50 nCi) was administered together with 0.5 ml saline or 5 mg kallikrein. Blood was collected from the intestinal vein for 60 min in 5 min fractions. The blood lost was replaced by infusion of heparinized blood of donor rats into the jugular vein.

Analytical Procedures

Immunoreactive porcine pancreatic kallikrein was determined by a radioimmunoassay (7). Kininogenase activities were measured by assaying the kinin released upon incubation of an aliquot of the respective sample with partially purified kininogen (8).

Gel filtration experiments were performed using an Ultrogel AcA 44 column, 0.9 x 115 cm, eluted at a flow rate of 4.0 ml/h with 15 mM NaH_2PO_4,

0.15 M NaCl, 0.01 M EDTA, 2 g NaN_3/l, pH 7.4. Fractions of 0.9 ml were collected and analyzed for kininogenase activity and immunoreactive pig pancreatic kallikrein.

RESULTS

Complex Formation of Pig Pancreatic Kallikrein (PPK) with Rat Plasma Components

Single plasma samples obtained during the absorption experiments were subjected to gel filtration. Immunoreactive PPK was eluted in three peaks (Fig. 1). The peak maxima at fractions 32, 40 and 54 correspond to molecular masses of >130, 82 and 32 kDa. Since PPK has a molecular mass around 32 kDa the two peaks corresponding to higher molecular masses obviously represent complexes of PPK with components of rat plasma.

The immunoreactivities of the three species of PPK were not identical: when serial dilutions of single fractions of the three peaks were measured by radioimmunoassay the slopes of dose-response curves of the two complexes were not identical and they were also different from the slope of the standard curve.

The relative amounts of immunoreactive PPK eluted in the three peaks varied when different plasma samples were subjected to gel filtration. It seems that the complex formation depends on different concentrations of the complex forming components of the individual rat plasmas or on not controllable conditions during sample preparation.

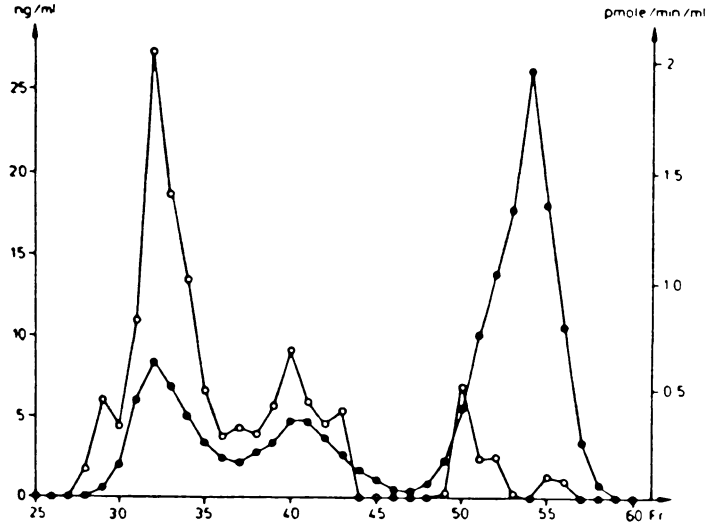

Fig. 1 Gel filtration of a sample of mesenteric venous plasma containing absorbed pig pancreatic kallikrein. The fractions were assayed for immunoreactive kallikrein (filled circles) and for kininogenase activity (open circles).

Kininogenase activity was also eluted in three peaks (Fig. 1), the highest activity was contained in the immunoreactivity peaks corresponding to the complexes, almost no activity was found in the peak of presumably free PPK (around fraction 54). The "free" PPK present in the sample obviously represents to a high degree an enzymatically inactive form, the active PPK is almost completely bound to plasma components. No kininogenase peaks were detectable when an identical gel filtration experiment was performed with a plasma sample which did not contain pig pancreatic kallikrein.

Radioimmunoassay of PPK in Rat Plasma

As a consequence of the complex formation the concentrations of PPK measured by radioimmunoassay are too low. This was shown by recovery experiments.

PPK was incubated with rat plasma in concentrations between 2 and 50 ng/ml and the PPK concentration determined by radioimmunoassay. The recoveries ranged between 25 and 8 percent. Thus, since the recoveries are dose dependent, a correction of the values is not possible.

Taken together, PPK and rat plasma components react under formation of complexes which have immunoreactivities different from and lower than free PPK. As a result, the value obtained as PPK concentration by radioimmunoassay varies with the dilution at which the sample is assayed and is significantly lower than the true PPK concentration in the sample. In addition, the ratio of the amounts of the three immunoreactive PPK species may vary from sample to sample, thus, even if the true concentrations of PPK in different plasma samples are identical the concentrations determined by radioimmunoassay may still be different.

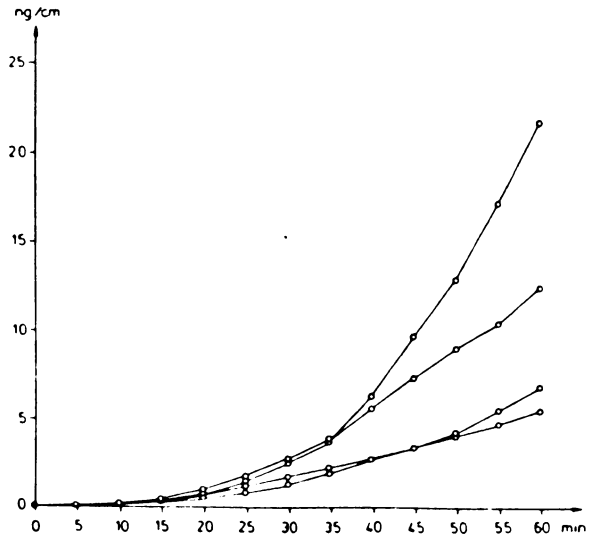

Fig. 2 Cumulative amount of pig pancreatic kallikrein in mesenterial venous blood of an intestinal segment after administration of 1.0 mg (four identically performed experiments, the amount of absorbed kallikrein is calculated per cm of the intestinal segment).

At present, these difficulties cannot be overcome and no [illegible], more reliable method to determine low concentrations of PPK in rat plasma is at hand. Therefore, in spite of the problems just presented we used the radioimmunoassay procedure in our studies. However, one has to keep in mind that the concentrations determined represent "immunoreactivity equivalents" and not the true PPK concentrations. The true PPK concentrations will be significantly higher (certainly by a factor of ten) than the values presented here.

Intestinal Absorption of Porcine Pancreatic Kallikrein

Porcine pancreatic kallikrein was administered at dose levels of 0.2, 1.0 and 5.0 mg. When 0.2 mg were administered, only in one out of six experiments absorption of PPK was detectable whereas at doses 1.0 mg and 5.0 mg only one experiment out of eight and nine, respectively, was negative. The amount absorbed during one hour per 1 cm of ileum was 2.6 ng for dose 0.26 mg, up to 22 ng for dose 1.0 mg and up to 248 ng for dose 5.0 mg. Thus, in spite of the high variability of absorption a dose dependency is clearly recognizable. The time course of intestinal absorption for dose levels 1.0 and 5.0 mg is shown in Figs. 2 and 3.

The highest amount absorbed in one of the experiments was 0.05 % of the administered dose. However, one has to consider that this amount was absorbed by only 10 cm of ileum whereas the total length of rat small intestine is about 100 cm and that the shape of the curves clearly demonstrates that the absorption was still in progress at the end of the experiment. In addition, as discussed above, the PPK concentrations in the plasma samples are highly underestimated by the radioimmunoassay.

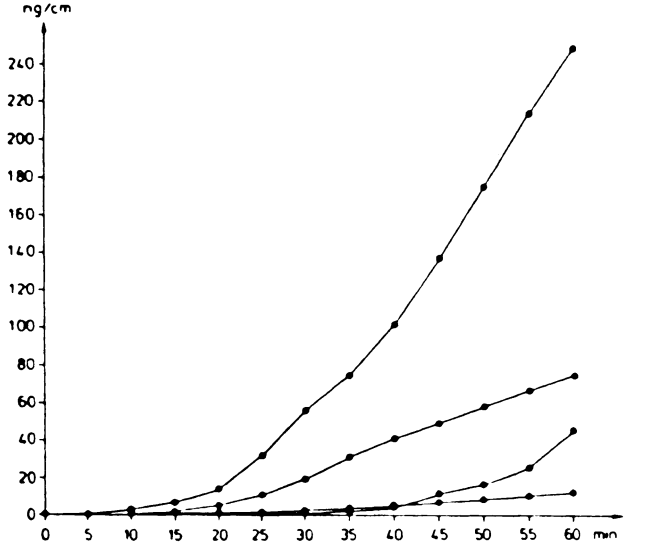

Fig. 3 Cumulative amount of pig pancreatic kallikrein in mesenterial venous blood of an intestinal segment after administration of 5.0 mg (four identically performed experiments, the amount of absorbed kallikrein is calculated per cm of the intestinal segment).

In a control experiment C-14 labelled PEG 4000 was administered together with 5 mg kallikrein. Only about 0.8 % of the radioactivity were found in the blood which is in the same range as in the absence of kallikrein. Thus, neither the experimental procedure nor the presence of the tissue kallikrein caused any significant unspecific permeability increase of the intestinal wall for high molecular substances.

DISCUSSION

Earlier investigations (3-5) have shown that intestinal absorption of tissue kallikrein is possible and some evidence has been presented suggesting that the absorbed kallikrein is to some degree still enzymatically active.

The experimental model employed here will allow to study the intestinal absorption of tissue kallikrein on a fully quantitative basis as soon as the problems of the radioimmunoassay can be overcome. The model is well established for studies on the absorption of small molecules and seems to be equally well suited for investigations on the tissue kallikrein absorption though further control experiments will be necessary to confirm that the observed absorption is not an artefact of the experimental setup.

The present studies corroborate the earlier findings (3-4) and demonstrate clearly that tissue kallikrein can indeed be absorbed in an enzymatically active form. Virtually all of the active tissue kallikrein is bound to plasma components, but these kallikrein complexes still display kininogenase activity. It is not clear whether this kininogenase activity is intrinsic or due to a partial dissociation of the complexes under the conditions of our assay.

At present, nothing is known on the nature and the physiological function of the complex forming proteins. One might speculate that they act both as inhibitors and as carriers for tissue kallikreins preventing undesirable kinin release during the transport to the target site.

Compared to the administered dose the fraction of absorbed tissue kallikrein was quite low and it may be regarded as questionable whether such small amounts can exert the pharmacological effects observed in human. However, for the treatment of asthenozoospermia and oligozoospermia as well as of essential hypertension the tissue kallikrein is administered orally for at least several weeks and under these conditions a pharmacologically active level may build up.

ACKNOWLEDGMENTS

This work was supported by the Deutsche Forschungsgemeinschaft, Sonderforschungsbereich 207, B2. Pig pancreatic kallikrein was kindly provided by Dr. P. Huber, Bayer AG, Wuppertal, FRG. We are very grateful to Mrs. G. Godec and Mrs. C. Hirschauer for their excellent technical assistance.

REFERENCES

1. Schill, W.-B., Kinin-releasing pancreatic proteinase kallikrein. In: "Treatment of male infertility", J. Bain, W.-B. Schill and L. Schwarzstein, eds., Springer-Verlag, Heidelberg, pp.125-142, (1982).

2. Overlack, A., K. O. Stumpe, R. Kolloch, C. Ressel, and F. Krueck, Antihypertensive effect of orally administered glandular kallikrein in essential hypertension. Results of a double blind study. Hypertension 3, Supp. 1:18-21, (1981).

3. Moriwaki, C., K. Yamaguchi, and H. Moriya, Studies on kallikreins. III. Intra-intestinal administration of hog pancreatic kallikrein and its appearance in the perfusate from the mesenteric vein. Chem. Pharm. Bull. 22:1975-1980, (1980).

4. Fink, E., R. Geiger, J. Witte, S. Biedermann, J. Seifert, and H. Fritz, Biochemical, pharmacological, and functional aspects of glandular kallikreins. In: "Enzymatic release of vasoactive peptides", F. Gross and G. Vogel, eds., Raven Press, New York, pp. 101-115, (1980).

5. Overlack, A., A. G. Scicli, and O. Carretero, Intestinal absorption of glandular kallikrein in the rat. Am. J. Physiol. 244:G689-G694, (1983).

6. Windmueller, H. G., and A. E. Spaeth, Vascular autoperfusion of rat small intestine in situ. Meth. Enzymol. 77:120-129, (1981).

7. Fink, E., and C. Güttel, Development of a radioimmunoassay for pig pancreatic kallikrein. J. Clin. Chem. Clin. Biochem. 16:381-385, (1979).

8. Fink, E., W.-B. Schill, F. Fiedler, F. Krassnigg, R. Geiger, and K. Shimamoto, Tissue kallikrein of human seminal plasma is secreted by the prostate gland. Biol. Chem. Hoppe-Seyler 366:917-924, (1985).