Tissue kallikrein synthesis and its modification by testosterone or low dietary sodium

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A method has been developed to measure the relative rate of rat tissue kallikrein synthesis which employs a specific antiserum raised against a purified rat urinary kallikrein. Incorporation of [^{35}S]methionine into kallikrein and protein 20 min after intraperitoneal injection was measured in submaxillary gland, pancreas, kidney and descending colon. Kallikrein content was measured with a direct radioimmunoassay, and kallikrein-specific incorporation of [^{35}S]methionine measured after immunoprecipitation. Kallikrein specific radioactivity (c.p.m./mg of enzyme) was about 100-fold greater than that in total protein in both kidney and colon. In contrast, in pancreas the incorporation into the enzyme was only 5-fold higher than into protein, and in submaxillary gland the incorporation was equivalent. Measured as kallikrein-specific radioactivity relative to total protein radioactivity incorporated in 20 min, kallikrein represents 0.18% of total protein synthesis in the kidney, 0.34% in the pancreas, 0.41% in the colon, but 7.29% in the submaxillary gland. Dietary Na^+ restriction increased the relative rate of kallikrein synthesis 1.8-fold in the kidney without a comparable effect in submaxillary gland. In contrast, testosterone increased the relative rate of synthesis 2.3-fold in submaxillary gland, but decreased it in kidney. The data show that endogenous kallikrein synthesis differs markedly in various tissues, and that interventions which are known to change kallikrein content or excretion also change the relative rate of enzyme synthesis.

Tissue kallikrein (EC 3.4.21.8) is a serine proteinase found in renal cortex, salivary glands, exocrine pancreas and gastrointestinal mucosa, and in excretory products and secretions such as sweat, saliva and urine (Schachter, 1980; Nustad *et al.*, 1978; Zeitlin, 1972; Seki *et al.*, 1972). Its established activity is to liberate kinin peptide from kininogen substrates, although additional enzymic properties have been suggested (Chao *et al.*, 1981; Arakawa & Marita, 1980; ole-Moi Yoi *et al.*, 1979). Although enzyme activity or quantity is known to be altered at some sites with various dietary manœuvres, drugs or pathological circumstances such as hypertensive or renal parenchymal diseases (Margolius *et al.*, 1974; Margolius & Chao, 1980; Orce *et al.*, 1980; Mitas *et al.*, 1978;

Cuthbert & Margolius, 1982; Carretero & Scicli, 1980; Holland *et al.*, 1980), nothing is known of how these changes are effected. Changes in the rate of enzyme synthesis, activation, secretion or degradation could all possibly be involved.

To investigate the mechanism of these changes, we have begun studies of tissue kallikrein metabolism by measuring its synthesis, simultaneously at several sites, in live animals. Since tissue kallikrein concentrations are known to change in rats either kept on a low-sodium diet (Mimran *et al.*, 1977; Johnston *et al.*, 1975), or to which testosterone has been chronically administered (Chao *et al.*, 1982), these interventions were employed to examine whether changes in kallikrein levels are associated with changes in kallikrein synthesis rate. Previous studies with tissue kallikrein, furthermore, have emphasized either one tissue or another, but integrative studies, where simultaneous effects on the system in different tissues were examined, have not been reported. The present experiments,

Abbreviations used: PBS, phosphate-buffered saline (0.14 M-NaCl/0.01 M-phosphate, pH7); Chaps, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulphonic acid.

therefore, were also designed to see whether different tissues respond to these interventions in the same way. Our results establish that changes in the rate of kallikrein synthesis are an important mechanism in the regulation of the system, but that the nature of the changes is different in different tissues.

Experimental

Preparation of experimental animals

Male Sprague-Dawley rats, 200–300 g, were used. Most were obtained from a local supplier, but castrated animals came from Sprague-Dawley, Madison, WI, U.S.A. Animals were housed in groups of four in a light-controlled (12 h light/12 h dark), constant-temperature (25°C) room, allowed free access to tap water and fed a normal lab chow (0.18 m-equiv. of Na⁺/g, 0.20 m-equiv. of K⁺/g; Wayne Pet Food Division, Chicago, IL, U.S.A.). For the low-dietary-Na⁺ study, groups of rats ate a control, normal Na⁺, diet (0.10 m-equiv. of Na⁺/g, 0.33 m-equiv. of K⁺/g; Teklad Industries, Madison, WI, U.S.A.) for at least 7 days while drinking deionized water. Then half the animals were given the low-Na⁺ diet (no Na⁺, 0.33 m-equiv. of K⁺/g) for 21–28 days before labelling. For the testosterone experiments, the castrated rats were maintained on the lab-chow diet and tap water. At 2 weeks after castration, groups of rats received subcutaneous injections of 17 α -methyltestosterone (Sigma Chemical Co., St. Louis, MO, U.S.A.) in sesame oil, 4.0 mg/100 g body wt., or vehicle, every other day for 10 days before labelling.

Labelling procedure

[³⁵S]Methionine (sp. radioactivity ~400 Ci/mmol; New England Nuclear Corp., Bedford, MA, U.S.A.) was diluted with 0.15 M-NaCl to a volume of 0.3–0.5 ml and injected intraperitoneally at a dose between 0.5 and 1.0 μ Ci/g body wt., depending upon the experiment. The rats were killed after 20 min. Preliminary experiments showed that [³⁵S]methionine incorporation into kallikrein and total protein was linear for at least 45 min. Incorporation was detectable by 10 min, and labelling *in vivo* was routinely terminated at 20 min in order to preclude significant secretion of the labelled material (Palade, 1975).

Sample preparation

After the 20-min labelling period, rats were anaesthetized with ether and tissues removed in the following order: submaxillary glands, pancreas, kidneys and descending colon. Sublingual glands were dissected away, discarded, and the submaxillary glands frozen. Kidney pairs were perfused via the renal arteries with 10–20 ml of PBS.

Pancreas and descending colon were rinsed free of debris, and all samples were frozen at –20°C. Subsequent steps were begun within 1 week of the labelling procedure. After thawing, submaxillary and pancreatic tissue were minced and homogenized in 3.0 ml of ice-cold PBS with a Potter-Elvehjem glass homogenizer and Teflon pestle, whereas kidney pairs or colonic tissue were minced and homogenized in 5.0 ml of ice-cold PBS. Sodium deoxycholate was added to each homogenate to a final concentration of 0.5%. After being left at room temperature for 30 min with occasional mixing, homogenates were spun at 24000 g at 4°C for 30 min in a Beckman JA-20 rotor. Supernatants were then spun through a column (2.5 cm $\times$ 4.5 cm) of Sephadex G-25 (fine grade) previously equilibrated with 0.01 M-Tris/HCl, pH 8.0, in a centrifugal filter holder (Gelman Instrument Co., Ann Arbor, MI, U.S.A.) to remove salts and excess deoxycholate. Filtrates were stored at –20°C.

Kallikrein and total-protein assays

Immunoreactive kallikrein in the prepared homogenates was measured by using the direct radioimmunoassay described by Shimamoto *et al.* (1979). The antibody was raised against purified rat urinary kallikrein (Chao, 1978; Chao & Margolius, 1979), which was also used as the radio-labelled competing ligand in the assay. The specificity of the antibody has been reported (Shimamoto *et al.*, 1979), and it does not cross-react with human or dog urinary kallikrein, collagenase or the serine proteinases plasmin, trypsin, urokinase and rat urinary esterase A. Plasma kallikrein is also unlikely to interfere with the assay, since it has been shown in other laboratories not to cross-react with rat urinary kallikrein antisera (Lawton *et al.*, 1981; Rabito *et al.*, 1982). Protein concentration of prepared homogenates was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

Determination of [³⁵S]methionine incorporation into total protein

Duplicate 20 μ l portions of the prepared homogenates were treated with 50 μ l of 0.1 M-NaOH at 37°C for 30 min. Bovine serum albumin and casein hydrolysate (type I, Sigma) were then added to final concentrations of 0.1% and 0.2% respectively. Protein was precipitated with 0.5 ml of 10% (w/v) trichloroacetic acid and the tubes were centrifuged at 4000 g for 10 min. The pellet was washed twice with 1.0 ml of 5% trichloroacetic acid containing 0.2% casein hydrolysate, dissolved in 0.2 ml of 0.1 M-NaOH, and transferred to a liquid-scintillation radioactivity-counting cocktail for aqueous samples. The tubes were rinsed with 0.3 ml of

water, which was added to the cocktail before counting for radioactivity.

Determination of [^{35}S]methionine incorporation into kallikrein

For the submaxillary-gland samples, duplicate portions (10–50 μl) of the prepared homogenates known to contain 15 μg of immunoreactive kallikrein, on the basis of direct radioimmunoassay of the samples, were added to an incubation buffer of PBS containing 0.1% bovine serum and 10 mM-Chaps to total volumes of 425 μl . Pilot experiments were carried out to determine optimal conditions for maximal immunoprecipitation of kallikrein. Incubation time was 18 h at 4°C. It was found that a ratio of 2.5–10 μl of undiluted antiserum per μg of immunoreactive kallikrein in the samples resulted in maximal immunoprecipitation of native kallikrein or added trace amounts of ^{125}I -kallikrein. A 5 μl portion of antiserum/ μg of kallikrein was therefore used for immunoprecipitation. Equal amounts of non-immune sheep serum were added to duplicate tubes containing the same amounts of prepared homogenates to measure non-specific precipitation. After the 18 h incubation, tubes were centrifuged at 5000 g in a Sorvall HS-4 rotor for 30 min at 4°C. The pellets were washed twice with 1.0 ml of the incubation buffer. The Chaps concentration was reduced to 1.0 mM in this washing buffer. Pellets were then dissolved in 0.2 ml of 0.1 M-NaOH and transferred to the radioactivity-counting cocktail. The tubes were rinsed with 0.3 ml of water, which was added to the cocktail before counting for radioactivity.

The kidney, colon and pancreatic samples required more extensive preparation, because of higher levels of non-specific precipitation, possibly caused by the greater tendency of the proteins in these homogenates to aggregate after freezing and thawing. The thawed, prepared homogenates were initially centrifuged at 24000 g for 90 min at 4°C. The supernatants were centrifuged again at 181000 g in a Beckman 75 Ti rotor for 45 min at 4°C. Triton X-100 and Chaps were added to the supernatants to final concentrations of 1% and 10 mM respectively. The lower concentrations of kallikrein in the kidney and colon samples required the addition of up to 15 μg of carrier kallikrein in the form of an unlabelled submaxillary-gland prepared homogenate to aid precipitation. Pancreatic samples required carrier kallikrein when immunoassayed kallikrein content was less than 5 μg /sample. These prepared tissue homogenates were divided into four equal portions, two of which were treated with antiserum and two with the non-immune sheep serum. The remaining steps of incubation, centrifugation and counting were

identical with those used for the submaxillary-gland samples.

To insure that kallikrein was precipitated from the various prepared homogenates in the same way, ^{125}I -labelled rat urinary kallikrein in tracer amounts was added to the homogenates before the precipitation procedure described above. The tracer-labelled kallikrein was carried down from each homogenate with equal efficiency, demonstrating that, under these conditions, no tissue-specific interference to the immunoprecipitation occurs.

Polyacrylamide-gel electrophoresis

Antiserum precipitates from labelled submaxillary-gland prepared homogenates were examined in slab gels containing 12.5% acrylamide cross-linked with 0.33% *NN'*-methylenebisacrylamide, with buffers as described by Laemmli (1970). Immunoprecipitates containing 18 μg of kallikrein were dissolved in 0.06 ml of a solution containing 0.0625 M-Tris/HCl, pH 6.8, 2.0% sodium dodecyl sulphate and 4% 2-mercaptoethanol, with a drop of added glycerol before layering the sample on gels. Gels were run at 30 mA for 4 h and fixed and stained in a solution containing 10% (v/v) acetic acid, 45% (v/v) methanol and 0.1% Coomassie Blue (R-250). After destaining in 10% acetic acid, gels were cut into 3-mm slices. Slices were dissolved in 30% (v/v) H_2O_2 (Goodman & Matzura, 1971) and counted for radioactivity.

Interpretation of data and statistical analysis

The rate of kallikrein synthesis was assessed by calculating the ratio of kallikrein-specific radioactivity of the immunoprecipitate to total incorporated radioactivity of the trichloroacetic acid precipitate per mg of protein. The measure is a relative one, the rate of kallikrein synthesis being compared with that of total protein synthesis within the same animal, and is similar to that used in studies of glucuronidase induction in mice (Swank *et al.*, 1973). Normalizing the data in this manner eliminated variability of total labelling from animal to animal. However, this variability was not so large that effects on the ratio, due to effects on total protein labelling, could not be determined.

All data are presented as the means $\pm$ S.E.M. Student's *t* test for unpaired data was used to assess significance.

Results

Evaluation of antiserum specificity

Antiserum precipitates were generated from [^{35}S]methionine-labelled submaxillary-gland hom-

ogenates and run on sodium dodecyl sulphate/polyacrylamide gels as described in the Experimental section. Fig. 1 shows that essentially all precipitate radioactivity migrated as a single peak slightly behind the purified active urinary kallikrein B (Chao & Margolius, 1979) run in an adjacent lane. This result, in the tissue most likely to contain proteinases immunologically related to tissue kallikrein, but of various molecular weights (Bothwell *et al.*, 1979; Lazure *et al.*, 1981; Shenkein *et al.*, 1979), suggests that kallikrein, perhaps as kallikrein A (Chao & Margolius, 1979), or in precursor or variably glycosylated forms, is precipitated with high specificity. The possibility, however, that proteins of nearly identical electrophoretic mobility in these gels, having similar immunological specificities, might also be precipitated cannot be eliminated.

Tissue-dependent differences in kallikrein content and labelling

Table 1 shows that immunoreactive kallikrein content varies widely. Higher concentrations in submaxillary gland and pancreas presumably reflect the existence of kallikrein in storage granules in these tissues (Simson *et al.*, 1979; Bhoola & Dorey, 1971; Ørstavik *et al.*, 1975). Kallikrein specific radioactivity (kallikrein c.p.m./mg of kallikrein) also varies widely, being significantly higher ($P < 0.05$) in colon or kidney than in submaxillary gland or pancreas (Table 1). This finding is of interest, since total protein specific radioactivity (total-protein c.p.m./mg of protein) is almost constant in three of the tissues. Higher total-protein specific radioactivity in pancreas is expected because of the high rate of protein synthesis in that tissue (Hansson, 1959; Kukral *et al.*, 1965). The ratio of kallikrein specific radioactivity to total protein specific radioactivity, in

fact, varies from 1.36 to 89.5 (Table 1). These higher ratios derived from the higher kallikrein specific radioactivity in kidney and colon, along with low kallikrein content, imply a higher rate of degradation or secretion in these tissues. From the same data, relative rates of kallikrein synthesis in the four tissues can be determined. Measured in this way, kallikrein accounts for from 0.178 to 7.29% of total protein synthesis for the experiment depicted in Table 1. This measurement of the relative rate of kallikrein synthesis makes it possible to study whether changes in kallikrein levels brought on by the effects of dietary intake, drugs or other interventions, are associated with changes in kallikrein synthesis.

Fig. 1. Polyacrylamide-gel electrophoresis of a tissue kallikrein antiserum precipitate from a submaxillary-gland tissue homogenate

The samples were electrophoresed through sodium dodecyl sulphate-containing gels and analysed as described in the Experimental section. The top of the gel is at the left. The arrow indicates the position of purified rat urinary kallikrein (RUK) run in an adjacent lane.

Table 1. *Tissue content and labelling of tissue kallikrein*

Male rats on a normal lab chow diet were labelled with [^{35}S]methionine for a period of 20 min before being killed. Kallikrein content and incorporation of [^{35}S]methionine were measured as described in the Experimental section. All values are means $\pm$ S.E.M. ($n = 8$).

	Submaxillary gland	Pancreas	Descending colon	Kidney
(a) Content				
Kallikrein/mg of protein	$55.7 \pm 6.6 \mu\text{g}$	$781 \pm 49 \text{ ng}$	$47.1 \mu\text{g} \pm 6.2 \text{ ng}$	$20.3 \pm 1.4 \text{ ng}$
(b) [^{35}S]Methionine labelling				
(1) Kallikrein c.p.m./mg of kallikrein	9452 ± 1412	232848 ± 49515	839795 ± 240250	532523 ± 55066
(2) Total-protein c.p.m./mg of protein	7100 ± 1063	49913 ± 6268	9210 ± 699	5964 ± 565
(3) Ratio (1)/(2)*	1.36 ± 0.13	4.36 ± 0.54	87.4 ± 13.0	89.5 ± 4.9
(4) Kallikrein c.p.m./mg of protein	478 ± 46	179 ± 37	40.2 ± 11.7	10.5 ± 0.9
(5) $10^3 \times$ Ratio (4)/(2)†	72.9 ± 7.1	3.39 ± 0.43	4.14 ± 1.12	1.78 ± 0.09

* Ratio of kallikrein-specific radioactivity to total-protein radioactivity.

† Rate of kallikrein synthesis relative to total-protein synthesis.

Fig. 2. Effect of dietary sodium restriction on renal kallikrein content and relative rate of synthesis

Male Sprague-Dawley rats were kept on either a control or a low-sodium diet, then injected with [³⁵S]methionine 3–4 weeks later and analysed as described in the Experimental section. Means $\pm$ S.E.M. are shown ($n = 8$). (a) Kallikrein content of the renal homogenates ($P < 0.001$); (b) relative rate of kallikrein synthesis ($P < 0.01$).

Fig. 3. Lack of effect of dietary sodium restriction on submaxillary-gland kallikrein content and rate of synthesis

Homogenates of submaxillary glands taken from the same animals studied in Fig. 2 were prepared and analysed. Means $\pm$ S.E.M. are shown ($n = 8$). (a) Kallikrein content of the submaxillary-gland homogenates; (b) relative rate of kallikrein synthesis. There are no significant differences.

Relative rates of kallikrein synthesis with dietary sodium restriction

It is known that both urinary and renal kallikrein levels are increased in rats maintained on a low-sodium diet (Mimran *et al.*, 1977; Johnston *et al.*, 1975). Fig. 2 confirms this finding and extends it to show that the relative rate of kallikrein synthesis also significantly increased. In contrast, neither kallikrein content nor relative synthesis rate was increased by this diet in the submaxillary glands of the same rats (Fig. 3).

Relative rates of kallikrein synthesis with testosterone treatment

Testosterone was shown previously to increase submaxillary-gland immunoreactive kallikrein content in castrated male rats (Chao *et al.*, 1982). Fig. 4 shows that there is a corresponding increase in the relative rate of kallikrein synthesis in this

Fig. 4. Effect of testosterone on submaxillary-gland kallikrein content and relative rate of synthesis

Castrated male Sprague-Dawley rats were treated with either 17 α -methyltestosterone or the sesame-oil vehicle over a period of 10 days, injected with [³⁵S]methionine and analysed as described in the Experimental section. Means $\pm$ S.E.M. are shown ($n = 6$). (a) Kallikrein content of the submaxillary-gland homogenate ($P < 0.005$); (b) relative rate of kallikrein synthesis ($P < 0.001$).

Fig. 5. Effect of testosterone on renal kallikrein content and relative rate of synthesis

Homogenates of kidneys taken from the same animals studied in Fig. 4 were prepared and analysed. Means $\pm$ S.E.M. are shown ($n = 6$). (a) Kallikrein content of the renal homogenates ($P < 0.005$); (b) relative rate of kallikrein synthesis ($P < 0.005$).

tissue. However, in the kidney of these same animals, both kallikrein content and relative synthesis rate are significantly decreased by testosterone (Fig. 5). There was always significant correlation between kallikrein content and relative rate of synthesis in kidney or submaxillary gland with either sodium restriction or testosterone treatment. Linear-regression analysis of the values of Fig. 2, for example, yielded a line described as:

$$y = 2.05x + 7.19 \quad (r = 0.91; P < 0.01)$$

Discussion

We have developed a method to measure the relative rate of rat tissue kallikrein synthesis *in vivo* and have shown that this rate changes in response to manoeuvres previously shown to change tissue

kallikrein content. [^{35}S]Methionine incorporation into kallikrein is expressed in relation to incorporation into total protein to correct for variations in the efficiency of protein labelling among animals. The response of both kallikrein and total protein to interventions can be examined separately, so that non-specific effects can be appreciated. The effects of dietary sodium restriction or testosterone on the concentrations of free methionine in the intracellular pools have not been measured, but if pool effects were of major importance in these experiments, total protein synthesis would have been affected, and this was not observed.

Some interesting inferences can be drawn from the wide range of kallikrein contents and specific radioactivities in the four sites (Table 1). Although previous studies have shown that kallikrein-like activity is relatively higher in submaxillary gland or pancreas than in renal cortex or colon, this conclusion is the result of several different studies in various mammalian species using different techniques of measurement (Webster, 1970). Here, this general conclusion is reaffirmed with quantitative measurements of immunoreactive kallikrein within the same experiment. Submaxillary-gland kallikrein levels are 70-fold higher than pancreatic, and more than 1000-fold higher than kidney or colon levels. It is interesting to note that the kidney and colon have the highest kallikrein specific radioactivities (kallikrein-specific c.p.m./mg of kallikrein) and the submaxillary gland the lowest. When this kallikrein labelling is expressed relative to total protein labelling, kidney and colon have specific radioactivities that are nearly 90-fold higher than for total protein, whereas submaxillary gland is roughly the same. In the submaxillary gland, therefore, it seems that kallikrein turnover is nearly equivalent to that of total protein, whereas in kidney and colon it is much faster. To determine whether this is the case will now require direct measurements of kallikrein turnover. It should then be possible to consider the relations between turnover rate and functional responsibilities at the different tissue sites, as well as the significance of modulation of these rates by various hormones, drugs or diseases. Although it seems probable that these high specific radioactivities are reflections of a high turnover rate, the relative contributions of kallikrein synthesis, secretion or degradation to this rate also remain to be determined.

Low-sodium diets result in increased urinary kallikrein excretion and renal kallikrein content. The present data show that these changes are accompanied by a significant increase in the relative rate of kallikrein synthesis in kidney, but not in submaxillary gland. Submaxillary-gland kallikrein content was also unchanged by the

chronic low-sodium diet. This result is interesting in the light of recent work which clearly established that human salivary kallikrein excretion rates are increased by low sodium diet or sodium-retaining steroids (Horwitz *et al.*, 1982). In their study, only parotid saliva was collected. Immunohistochemical studies have suggested that parotid kallikrein is likely to be detected as an intense apical (luminal) rim of the striated duct cells, quite similar to that seen in the renal tubule (Simson *et al.*, 1979). On the other hand, submaxillary-gland kallikrein localization by immunohistochemistry has always noted heavy staining associated with cytoplasmic granules (Simson *et al.*, 1979). We question whether these differences in immunohistochemical enzyme localization, as well as differences in tissue content or relative synthesis rate in response to chronic low-sodium diet, have some implication in differences in functional responsibility of the enzyme in kidney and parotid versus submaxillary gland.

This question is perhaps reinforced by the results of the testosterone studies. There was not only clearly increased relative kallikrein synthesis rate and content in submaxillary gland, but concomitantly reduced content and synthesis in the kidney of castrated male rats. The former findings in submaxillary gland are consistent with previous work suggesting that this enzyme is subject to androgenic stimulation (Chao *et al.*, 1982). The meaning of the latter finding is unclear, and it is possible that reduced renal kallikrein synthesis only reflects an indirect response of this process to the primary effects of androgen elsewhere.

In any case, it is clear that kallikrein content and synthesis rate are regulated differently in different tissues. Independent control of an enzyme among tissues has been previously reported, as, for example, renin content in the kidney versus the submandibular gland in genetically different strains of the mouse (Tanaka *et al.*, 1980), or the response to dihydrotestosterone of glucuronidase in the kidney versus various other tissues, also in the mouse (Swank *et al.*, 1973). It is possible that the tissue-specific responses of kallikrein to the interventions reported here are similar in nature in that, for unknown reasons, the same enzyme is independently regulated among tissues. It is also conceivable that we are measuring different gene products, closely related members of the kallikrein gene family that cannot be distinguished by our antibody (Mason *et al.*, 1983). In either case, the importance of the question as to whether there are tissue-specific functional responsibilities of this enzyme, or enzyme family, is re-emphasized.

In summary, we have devised a reliable and sensitive method to measure relative rates of kallikrein synthesis in several rat organs simultaneous-

ly. These rates can be changed with dietary or hormonal treatment, directly demonstrating for the first time that changes in kallikrein synthesis rate are an aspect of the metabolic regulation of this enzyme. Kallikrein-specific [^{35}S]methionine incorporation per mg of kallikrein also varies widely in the different organs in a way that raises many questions about the metabolism of endogenous tissue kallikreins. Some of these questions concern relationships between these measures of metabolism and how they are regulated in connection with functional responsibilities of kallikreins at various sites and in various disease states.

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