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KALLIKREIN AND RENIN IN THE MEMBRANE FRACTIONS OF THE RAT KIDNE--ETC(U)
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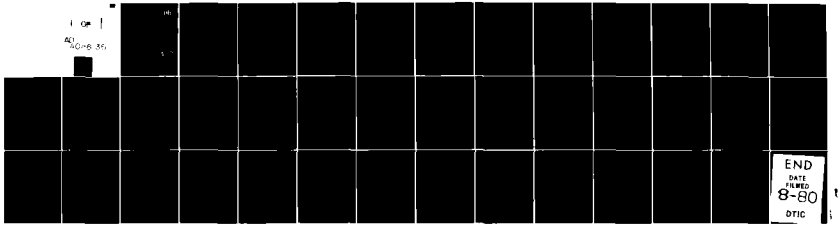

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Technical Report No. 20

Kallikrein and Renin in the Membrane Fractions of the Rat Kidney

Prepared by

Ervin G. Erdös

For Publication In
Hypertension

Departments of Pharmacology and Internal Medicine
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5323 Harry Hines Boulevard
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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Plasma membrane (PM) and endoplasmic reticulum (ER) enriched fractions were isolated from the homogenized rat kidney. Transmission electron micrographs of PM showed empty vesicles, but no granules present in the fraction. Kallikrein activity was detected in the homogenate, in the microsomal and in the PM and ER fractions; it was most enriched in PM fraction. PM-kallikrein released a kinin, cleaved the peptide substrate, S-2266 and a radio-labeled arginine ester. The ester was also hydrolyzed by renal enzymes other than kallikrein. PM-kallikrein was activated by Triton X-100, phospholipase A_2 →

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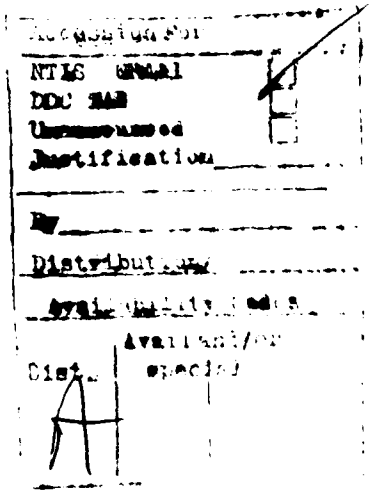
lysolecithin, and by a peptide, melittin. Melittin (2 μM) was most potent, it increased the activity to 750%. Solubilized PM and ER kallikrein were inhibited by antibody to rat urinary kallikrein, but membrane-bound kallikrein was more resistant to inhibition. The Km of S-2266 was higher with renal than with urinary kallikrein. PM and ER fractions also contained renin. Its activity was enhanced thirty-fold or more by activators of kallikrein, e.g. by phospholipase A_2, lysolecithin and melittin. Low sodium diet increased the activity of kallikrein in the homogenate and in the membrane fraction. This diet increased the activity of renin in the homogenate but not in the membrane fraction.

It was suggested that prekallikrein is on PM and it is activated prior to release from the membrane. Membrane bound renin may be a form of renin retained in the kidney.



KALLIKREIN AND RENIN IN THE MEMBRANE FRACTIONS OF THE RAT KIDNEY

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Key Words: plasma membrane · endoplasmic reticulum · membrane-bound enzymes · rabbit uterus · melittin · enzyme activation · lysolecithin · phospholipase A_2 · microsomes · aprotinin · Na^+ diet

Running Head: Membrane-bound Kallikrein and Renin

SUMMARY

Plasma membrane (PM) and endoplasmic reticulum (ER) enriched fractions were isolated from the homogenized rat kidney. Transmission electron micrographs of PM showed empty vesicles, but no granules present in the fraction. Kallikrein activity was detected in the homogenate, in the microsomal and in the PM and ER fractions; it was most enriched in PM fraction. PM-kallikrein released a kinin, cleaved the peptide substrate, S-2266 and a radiolabeled arginine ester. The ester was also hydrolyzed by renal enzymes other than kallikrein. PM-kallikrein was activated by Triton X-100, phospholipase A_2, lysolecithin, and by a peptide, melittin. Melittin (2 μM) was most potent, it increased the activity to 750%. Solubilized PM and ER kallikrein were inhibited by antibody to rat urinary kallikrein, but membrane-bound kallikrein was more resistant to inhibition. The Km of S-2266 was higher with renal than with urinary kallikrein. PM and ER fractions also contained renin. Its activity was enhanced thirty-fold or more by activators of kallikrein, e.g. by phospholipase A_2, lysolecithin and melittin. Low sodium diet increased the activity of kallikrein in the homogenate and in the membrane fraction. This diet increased the activity of renin in the homogenate but not in the membrane fraction.

It was suggested that prekallikrein is on PM and it is activated prior to release from the membrane. Membrane bound renin may be a form of renin retained in the kidney.

Although active kallikrein is excreted in urine, relatively little of this hypotensive enzyme can be detected in the kidney. Much of renal kallikrein is bound to plasma membrane,[1] very likely in the cells of the distal tubules.[2,3] In order to explain the discrepancy inthe activity of renal and urinary kallikrein we studied the mode of activation of bound kallikrein.[4,5] After separation of membrane fractions from homogenized kidney,[6] we found that these fractions contain renin activity in addition to kallikrein. We also found that both membrane-bound kallikrein and renin are activated by detergent, phospholipase A_2, lysolecithin and a basic polypeptide, melittin.[4, 5]

Materials and Methods

Dextran T 70 was purchased from Pharmacia Fine Chemical, Uppsala, Sweden α-N-tosyl-L-argine(^{3}H)methylester(^{3}H-TAMe) was from Biochemical and Nuclear Corp., Burbank, CA, and α-N-tosyl-L-argine methylester (TAMe) from Sigma Chemical Co., St. Louis, Mo. D-Val-Leu-Arg-p-nitroanilide(S-2266) was obtained from Kabi Diagnostica, Stockholm, Sweden. Venom and pancreatic phospholipase A_2 were purchased from Boehringer Mannheim, Germany. Phenylmethylsulfonylfluoride (PMSF), melittin, lecithin, arachidonic acid and soybean trypsin inhibitor were from Sigma Chemical Co., St. Louis, MO. Lysolecithin was purchased from Supelco, Inc., Bellefonte, PA. Aprotinin (trasylol) was obtained from Professor G. Haberland Bayer AG, Wuppertal-Elberfeld, Germany. Anti-sera to rat renal kallikrein and to bradykinin were donated by Dr. O. A. Carretero, Detroit, MI and Dr. H. Margolius of Charleston, SC. Other chemicals

were obtained from Bio-Rad Lab., Richmond, CA, and Eastman Kodak Co., Rochester, NY. Pregnant rabbit uteri were purchased from Pel-Freez Inc., Rogers, AR.

Fractionation

Male Sprague-Dawley rats (200-250 g) were killed by decapitation. 20 g of renal cortex, dissected from eight to ten rats, was pooled and minced. Renal tissue was suspended in 200 ml of 10 mM Tris-HCl buffer, pH 7.4, containing 0.25 M sucrose and homogenized in a Dounce homogenizer. 2 ml of 0.1 M EDTA was added and the homogenate centrifuged at 10,000g for 15 min. The supernatant was then centrifuged at 30,000g for 15 min. The supernatant containing microsomal particles was centrifuged at 100,000g for 60 min in a Beckman L5-65 ultracentrifuge. The microsomal pellet was suspended in 10 mM Tris-HCl buffer, pH 8.6, and recentrifuged at the above speed, then the pellet was resuspended in 1 mM Tris-HCl buffer, pH 8.6, and centrifuged again. The washed microsomal fraction was suspended in 50 ml of 1 mM $MgSO_4$ and 1 mM Tris-HCl buffer, pH 8.6, and dialyzed against the same buffer for 2 h. The microsomal fraction was separated into plasma membrane (PM) and endoplasmic reticulum (ER) enriched subfractions.[1,7] The washed microsomal fraction was carefully layered on 24 ml of 15% w/w dextran T 70 in 1 mM $MgSO_4$ and 1 mM Tris-HCl, pH 8.6, and centrifuged in a Sw-27 swinging bucket rotor at 25,000 rpm for 16 h in the

ultracentrifuge. The PM containing fraction was concentrated at the interface and the pellet was rich in ER. Both fractions were resuspended in 10 mM Tris-HCl buffer,pH 8.6, containing 10 mM $MgSO_4$ and centrifuged at 100,000g for 60 min. The pellets were suspended in 10 mM Tris-HCl buffer, pH 7.4, containing 0.25 sucrose. The samples were kept at -70°C until used. There was no difference in kallikrein activity between the fresh samples and the samples stored frozen. Freshly collected fractions were used for the assay of bound renin. Pregnant rabbit uteri were fractionated the same way as rat kidney as a source of renin. Transmission electron micrographs were taken as previously described.[1]

Fresh rat urine was centrifuged at 3,000g for 5 min and the supernatant was kept at -20°C until used.

Low Na^+ diet

Rats were kept on low (10 mEq Na/kg) or high sodium diet (150 mEq Na/kg plus saline) in groups of six in metabolic cages according to Campbell, et al.[8] The animals on the low sodium diet received a 10 mg/kg dose of furosemide on the first and second day.

Enzyme Assays

Kallikrein activity was assayed initially with ^3H TAMe,[9] but in the majority of studies using isolated membrane fractions S-2266 was the substrate. The hydrolysis of S-2266 was determined in a Cary 118 or Cary 15 recording spectrophotometer either by continuously

recording the increase in absorption at 405 nm or by taking aliquots from incubation mixture at regular time intervals.[10] The reaction mixture contained enzyme (50 µl), 0.1 M Hepes buffer, pH 9.1 (700 µl) and 1 mM S-2266 (50 µl) incubated for 15 min at 37°C. With turbid solutions the following technique was used: the reaction was stopped with 20% (w/w) perchloric acid (300 µl) and centrifuged at 3,000g for 10 min. Then 0.2% (w/v) sodium nitrite (500 µl) was added to the supernatant fluid (500 µl). The solution was kept at 4°C for 10 min to diazotize and 0.5% (w/v) ammonium sulphamate (500 µl) was added to destroy the excess nitrite; subsequently 0.05% naphthylethylenediamine dissolved in methanol (1 ml) was added. After the solution was kept at 37°C in the dark for 30 min, the absorbance was measured at the wavelength of 546 nm.[11]

Activity was expressed as cpm. per min per ml of ^{3}H-methanol product formed when ^{3}H-TAMe was used as a substrate. Activity was also expressed as nmol of p-nitroanilide product formed per min when S-2266 was hydrolyzed.

Glucose 6-phosphatase was assayed in the presence of 4 mM EDTA and 2 mM KF.[12, 13]

ATPase was determined by the method of Post and Sen.[14] Phosphate released was determined by the method of Fiske and SubbaRow.[15] Alkaline phosphatase was assayed according to Linhardt and Walter.[16]

The Km was determined by plotting 1/v against 1/s. The kallikrein activity was assayed using S-2266 as a substrate.

Protein concentration was measured by the method of Lowry et al., using bovine serum albumin as a standard.[17]

Polyacrylamide disc gel electrophoresis

Disc gel electrophoresis was performed in 7% acrylamide gel at pH 8.6 with a current of 3 mA per tube for 2 h. The gels were cut to 2 mm width segments and proteins were eluted from gels by adding 100 µl of 10 mM Tris-HCl, pH 8.6. The kallikrein activities were measured with ^{3}H-TAMe and S-2266 substrates. The recovery of the enzyme from the gel was about 70%.

Activation of kallikrein

Aliquots of PM, ER and urine were incubated with activators at 4^{o}C for 30 min. In addition, melittin (0.3 - 2µM) was also directly added to the cell of the spectrophotometer containing the substrate and enzyme without preincubation. When aliquots of PM, ER and urine were incubated with venom or pancreas phospholipase A_2, the reaction mixture contained 100 µl of sample in 10 mM Tris-HCl, pH 7.4, 0.25 M sucrose and 10 µl of either pancreatic (60 U) or venom (2 U) phospholipase A_2 preincubated for 1 h at room temperature. Venom phospholipase A_2 cleaved ^{3}H-TAMe even in the absence of kallikrein. This blank value was subtracted from the experimental data. The concentration of Triton X-100 was 1% in the incubation mixture.

Extraction of activator

ER fraction (500 µl) was incubated with venom phospholipase A_2 for 1 h at room temperature. 500 µl of chloroform was added to the

reaction mixture and shaken vigorously and centrifuged for 2 min at 700g. After the chloroform layer was evaporated by boiling, 200 µl of 50% methanol was added. The reaction mixture, 80 µl of sample and 80 µl of methanol extract was preincubated for 30 min at 4°. The control mixture contained methanol only, which had no effect on the enzyme.

Radioimmunoassay

Renin activity was assayed in 0.24 M Tris EDTA buffer at pH 7.5 according to Poulsen and Jörgensen[18] using nephrectomized rat serum as source of substrate. The kinin released by kallikrein from heated dog kininogen was determined by radioimmunoassay. The incubation mixture contained in 0.1 M Tris at pH 7.4, kininase inhibitors SQ 14225, EDTA and o-phenanthroline.[19] Kinins were adsorbed and separated from the samples on Amberlite IRC 50 resin[20] prior to the assay.

Effect of lipids

Lysolecithin, lecithin and arachidonic acid were dissolved in 50% methanol. PM and ER fractions were incubated with lysolecithin, lecithin or arachidonic acid for 30 min at 4°C and the solvent was added to the control reaction mixture.

Results

Separation of particles

The microsomal fraction of crude homogenate of rat kidney obtained in the ultracentrifuge, was further separated to PM and ER enriched fractions. The separation procedure included osmotic shock by washing

the membrane preparations twice with hypotonic solutions. Table 1 shows that the marker enzymes assayed were enriched in the ER (glucose-6-phosphatase) and in the PM (alkaline phosphatase, ATP-ases) fractions.

Transmission EM

Electron micrographs of PM enriched fraction revealed that the specimen consisted largely of round and tubular shaped vesicles. There were no identifiable ribosomes, mitochondria, dense microbodies or solid granules present. Most of the vesicles seemed to be empty. (Fig. 1)

Fractionation of kallikrein

The separation of kallikrein activity from the homogenized rat kidney is shown on Table 2. The activity of kallikrein with S-2266 and H-TAMe substrates did not increase in a parallel manner. The activity in the microsomes was enriched 2.6 fold with respect to S-2266, but only 1.3 fold when ^{3}H TAMe was the substrate. This indicates that the crude homogenate contains one or more enzymes which cleave ^{3}H-TAMe, but are not identical with kallikrein. However, when the microsomal fraction was separated to PM and ER fractions, kallikrein activity was enriched in the PM fraction 9.5 and 12.5 fold with the two substrates. In addition, kallikrein activity also increased in the ER fraction although the specific activity of kallikrein was only one-third of that in PM.

Activation of bound kallikrein

Pancreatic and venom phospholipase A_2 and the detergent Triton X-100 enhanced the kallikrein activity of the fractions. Taking the control activity as 100%, Triton increased the activity more with S-2266 than with ^{3}H-TAMe (369 v. 190%), while venom phospholipase A_2 enhanced ^{3}H-TAMe esterase activity more (550 v. 336%). Hydrolysis of S-2266 was completely inhibited by aprotinin (Trasylol; 10 U/ml), while the increased ^{3}H-TAMe hydrolysis after incubation with venom phospholipase A_2 was inhibited only 30%. This indicates that rat kidney membrane fractions contain a "silent" ^{3}H-TAMe esterase, which is unmasked during activation of bound kallikrein. In control experiments, rat urinary kallikrein was not activated by either one of the agents that enhanced activity of bound kallikrein.

To determine whether the increase in activity after incubation with phospholipase A_2 was due to direct action of the enzyme on the membrane-bound kallikrein or to a product released by the enzyme, we incubated the ER fraction with venom phospholipase A_2 and extracted the incubation mixture with chloroform. Chloroform was subsequently evaporated and the residue was dissolved in 50% methanol. An equivalent amount of methanol was used in control samples. The chloroform extract enhanced S-2266 hydrolysis to 229%, indicating that a membrane component released by phospholipase may be responsible for the activation. Following this observation we tested various lipids as activators of PM kallikrein.

Lysolecithin was the most active compound in this series; it activated PM kallikrein seven-fold at a 1 mM concentration. Arachidonic acid and lecithin were active only in a concentration of an order of magnitude higher. Prostaglandin E_2 did not activate even when used in a high concentration (100 µg/ml).

The results of the radioimmunoassay of kinin released by activated PM kallikrein resemble those obtained in experiments done with S-2266 (Table 3). Thus lysolecithin increased PM kallikrein activity seven-fold, pancreatic phospholipase A_2 was equally active, and venom phospholipase A_2 and Triton X-100 (1%) activated five-fold, as determined by radioimmunoassay.

The activity of PM kallikrein, however, was enhanced more by the peptide melittin, than by the other agents tested. Melittin is a 26 amino acid peptide from bee venom. It was active at a molar concentration three orders of magnitude lower than lysolecithin. Maximum activity (750%) was obtained at about 2 µM concentration. At 0.3 µM concentration it doubled the rate of kallikrein activity. Melittin also increased the kallikrein activity in the washed microsomal fraction. Centrifugation of the fraction at 100,000g, however, showed that melittin increased the activity in both the precipitate and in the final supernatant. (Fig. 2) This is taken as indication that melittin, in addition to solubilizing kallikrein, activates a membrane bound prekallikrein.

In control studies activators of PM-kallikrein did not affect urinary kallikrein.

Electrophoresis of kallikrein

PM kallikrein was separated to three activity peaks with Rf values of 0.3, 0.65 and 1.0 in preparative polyacrylamide gel electrophoresis at pH 8.6 (Fig. 3). However, the TAMe esterase activity in the fractions did not parallel the peptidase activity. The ratio of activities (^{3}H-TAMe v. S-2266) in the fastest migrating peak was much higher than in the two slower migrating ones. Electrophoresis of rat urinary kallikrein yielded only the two faster migrating peaks.

Reaction with antibody

Antiserum to rat urinary kallikrein inhibited PM kallikrein, ER kallikrein and rat urinary kallikrein similarly, provided that the two membrane-bound kallikreins were solubilized with Triton X-100. The antiserum reduced the activity of kallikrein by half at 1:1024 to 2048 dilution. In contrast, PM or ER kallikrein bound to membrane was inhibited by antiserum 50% only at a dilution of 1:128. (Fig. 4)

Properties of renal kallikrein

The Km of S-2266 was significantly higher with renal than with urinary kallikrein. The Km values were 70 μM (±0.9), 65 μM (±1.1) and 44 μM (±0.4) with kallikrein from the microsomal, ER and PM fractions respectively. The Km of S-2266 with urinary kallikrein was 23 μM(±0.3). The differences between the Km values of renal kallikrein and urinary kallikrein were statistically significant ($p<0.05$).

The inhibition of kallikrein in the microsomal, PM and ER fraction and in the rat urine was similar. Aprotinin (10 KIU) inhibited the enzyme completely, but soybean trypsin inhibitor (25 µg/ml) was inactive. The inhibitor of proteases, p-methylsulfonylfluoride inhibited 77-80% all kallikrein preparations at 3 mM concentration.

Activation of renin

Renin activity was present in the microsomal fraction as well as in the membrane fractions which were washed repeatedly with hypotonic buffers. The specific activity of renin was 33.5 ($\pm$2) ng of angiotensin I released in 1 h per mg protein (Table 4). The corresponding number for the ER fraction was 12.8. This low activity represented less than 3% of the total obtained after activation of the PM fraction. PM-bound renin was activated by the same agents as PM-kallikrein. Thus Triton X-100, phospholipase A_2, and lysolecithin activated about 30 to 50 fold. Melittin also enhanced renin activity 30 fold although only at a concentration 5-10 times higher than used with kallikrein. The relative rate of activation of renin in the ER fraction was similar but the specific activity of ER renin was about one third that of PM renin.

Rabbit uterus

When the source of renin was the homogenized pregnant rabbit uterus, the results were almost identical to those obtained with rat kidney. PM and ER membrane enriched fractions from the rabbit uterus contained renin. The highest activity after adding Triton X-100 to PM fraction was 1.8 µg of angiotensin I released in 1 h by 1 mg protein. As with kidney PM, renin, pancreatic and venom phospholipase A_2 also activated the enzyme.

We could not detect any kallikrein activity in the pregnant rabbit uterus.

Effect of low Na^+

A brief (five days) low salt diet doubled the kallikrein activity in the renal homogenate (Fig. 5). Low sodium diet also increased the specific activity of membrane-bound kallikrein the same way. In contrast, high sodium diet did not change kallikrein activity significantly over the control. In the same animals, the activity of renin increased significantly in the homogenized kidney of rats kept on low sodium diet (Fig. 6). The renin activity in the control was 5.12 ± 0.24 µg angiotensin I liberated in 1 hr by 1 mg protein. That increased to 8.64 ± 0.24 in the animals kept on low sodium. The activity of membrane-bound renin in the washed microsomal fraction did not increase significantly over the control; it was 1.10 ± 0.09 v. 1.27 ± 0.13 as indicated in Fig. 6.

Discussion

These experiments expand our previous studies,[1,4,5,6] which showed that rat kidney contains kallikrein and renin bound to membranes. The membrane preparations probably also contain a bound prekallikrein.

Kallikrein is also present in the ER fraction of the homogenized rat kidney as indicated in studies using several fractionation techniques. This suggests that kallikrein is synthesized in ER.[1] The experiments of Heidrich (personal communication) indicate that after its release from ER, kallikrein appears on the inside of lysosomal membranes, at least in the rabbit kidney. These lysosomes may fuse with PM to form PM kallikrein. The experiments of Chao and Margolius support this concept, since they show that kallikrein can be released from suspended kidney cells with a detergent or phospholipase.[21] Within the kidney, kallikrein was found in the distal tubules[2] and on the luminal surface of the tubules. The latter studies were done with fluorescent antibody.[3]

We found that PM kallikrein is released by detergent and phospholipase A_2. This activation of kallikrein probably does not involve prostaglandin synthesis, but rather the release of lysolecithin by phospholipase A_2, since lysolecithin increased the activity of PM-kallikrein approximately six fold. Because bradykinin is reported to activate phospholipase in the kidney,[22] renal kinins may be feedback activators of renal kallikrein. In addition, in one strain of hypertensive rats renal phospholipase A_2 and kallikrein activity are parallel.[23]

Of the activators tested, melittin is by far the most potent one. Melittin is a polypeptide of about 2,800 m.w. which contains basic and hydrophobic amino acids.[24] It interacts with membranes and activates

membrane phospholipase in low concentration.[25,26,27] Melittin releases histamine from mast cells,[24] and angiotensin I converting enzyme (kininase II) from the perfused lung.[28] Although the natural product may be contaminated with phospholipase, it was ascertained that the activity of the preparation is due to its melittin content. Synthetic melittin (kindly provided by L. Levine of Brandeis University) gave identical results as the natural product. Melittin activated PM kallikrein about 8 fold at 2 μM and about two fold at 0.3 μM concentration. As with trypsin,[5] this activation was only partially due to solubilization of kallikrein because bound kallikrein activity also increased. This indicates that melittin activates a PM bound prekallikrein. The inhibition of solubilized kallikrein and urinary kallikrein by antiserum to urinary kallikrein gave identical results. PM and ER kallikrein were inhibited only at higher concentration of the antiserum prior to adding detergent. This resistance of bound kallikrein to antibody bindings may explain some of the difficulties encountered when attempting to localize tissue kallikrein with immunofluorescence techniques.

Although the experiments suggest that PM membrane fractions contain a prekallikrein which becomes enzymatically and antigenically more active after its release from the membrane, there are some differences between rat urinary and soluble renal kallikrein since the Km of S-2266 is significantly lower with urinary kallikrein than with PM kallikrein. Thus at a low substrate concentration the apparent activity of urinary

kallikrein may be higher than that of the kidney kallikrein. In addition, in preparative electrophoresis solubilized PM-kallikrein has 3 peaks of activities while urinary kallikrein has only 2.

S-2266 seems to be a more appropriate substrate for renal kallikrein than ^{3}H-TAMe. Radioimmunoassay of kinin released by kallikrein paralleled the results obtained with S-2266. ^{3}H-TAMe, on the other hand, seemed to be cleaved by other enzymes, which are not inhibited by aprotinin. For example, after activation by phospholipase, an additional "silent" TAMe-esterase becomes apparent in the PM and ER fraction, which is not identical with kallikrein.

In addition to kallikrein, the rat kidney contained renin bound to membrane fragments. It was found in our laboratory previously that renin can be induced in the microsomal-ribosomal fraction of the submaxillary gland of the mice[29] and that kidney contains a membrane bound form of renin.[6]

These experiments show the microsomal fraction and the subsequently separated PM and ER enriched fractions contained renin. These fractions were washed repeatedly with hypotonic solution. Transmission electron micrograph revealed no granules or trapped soluble material in the membrane vesicles. Although ER represent a larger surface than PM, the specific activity of ER-renin was lower than that of PM-renin, just as we found with kallikrein. Thus a nonspecific adsorption of renin by membrane fragments during homogenization is unlikely.

The simultaneous occurrence of renin and kallikrein in the same membrane fractions is of interest, especially since kallikrein is reported to be an activator of prorenin.[30, 31, 32] Bound renin and kallikrein are activated by the same agents, including Triton X-100 phospholipase A_2, lysolecithin and melittin. However, many of the properties of bound renin and kallikrein differ. For example, PM renin has less than 3% of the activity of the fully activated preparation. There is no evidence that bound renin is prorenin. In pilot studies trypsin and plasmin did not activate PM-renin. After acid treatment PM-renin has the same molecular weight as soluble renin, about 40,000 (C. M. Wilson, to be published). Freezing releases renin but not kallikrein. Low sodium diet increases PM-kallikrein activity but not that of PM-renin. Melittin activates PM-renin at 10 times higher concentration than PM-kallikrein. Finally, PM-fractions of pregnant rabbit uterus contain renin, but we detected no kallikrein activity there.

Thus kallikrein is very likely synthetized by distal tubular epithelial cells. Prekallikrein on the plasma membrane is activated and solubilized by various stimuli. It may enter urine and maybe also the circulation. PM membrane fractions also have renin activity. This renin may be synthetized by cells outside the juxtaglomerular apparatus or may be held on the cell surface by attachment to a receptor. In contrast to the renin released in the circulation from granules, this form of renin may be a

source of the enzyme functioning in the kidney.

The experiments, where animals were kept on low sodium diet, support the concept of Margolius and others that aldosterone[33,34,35] enhances kallikrein excretion, possibly by inducing it in the kidney. In addition to the well known increase in kallikrein excretion after aldosterone administration or low sodium diet,[33,34,35] the specific activity of PM kallikrein increased in our experiments. Thus renin can control the function of PM kallikrein indirectly by the release of aldosterone.

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Legends to the Figures

Fig. 1.

Transmission electron micrograph of PM enriched fraction.

Fig. 2.

Adding melittin to the microsomal fraction increased the activity in both the final supernatant and in the particles in the precipitate. Activity shown is mean $\pm$ SE.

Fig. 3.

Preparative polyacrylamide gel electrophoresis at pH 8.6 of PM-kallikrein solubilized with Triton X-100. Solid line represents ^{3}H-TAMe hydrolysis, the shaded area, S-2266.

Fig. 4.

Inhibition of urinary, PM and ER kallikrein by antiserum to rat urinary kallikrein. When bound kallikrein was solubilized with Triton X-100 (TX-100) the inhibition was similar to that of urinary kallikrein.

However, membrane-bound kallikrein was inhibited only at a much higher concentration of the antiserum (1:128 dilution)

Fig. 5.

Low sodium diet increased kallikrein activity in the homogenized rat kidney and the activity of membrane-bound kallikrein in the washed microsomal fraction in five days. Kallikrein was solubilized with detergent.

Fig. 6.

Low sodium diet increased renin activity in the homogenized rat kidney, but not the activity of membrane-bound renin in the microsomal fraction. Renin was solubilized with detergent.

Table 1. Distribution of Marker Enzyme Activity in Membrane Fractions Isolated from Rat Kidney Cortex

Fraction	Glucose-6 Phase SA	RSA	Alk. Phosphatase SA	RSA	Total ATPase SA	RSA	Mg^{2+} ATPase SA	RSA	Na^{+} K^{+} ATPase SA	RSA
Crude Homogenate	0.07	1	.13	1	.18	1	.12	1	0.05	1
Microsomes	0.20	2.9	.48	3.7	.42	2.4	.34	2.8	0.09	1.8
Plasma Membrane	0.08	1.1	1.44	11	1.02	5.8	.87	7.3	0.19	3.8
Endoplasmic Reticulum	0.44	6.2	.54	4.1	.31	1.8	.17	1.4	0.15	3.0

SA = Specific activity, μmole/min/mg protein; n=3

RSA = Relative specific activity

Table 2. Distribution of Kallikrein Activity Among Fractions of Rat Kidney.

	Activity S-2266 (nmol/min/mg)*	RSA+	Activity ^{3}H-TAMe(cpm/min/mg)*	RSA+
Crude homogenate	0.145	1	2.3×10^3	1
Microsomal fraction	0.374	2.6	3.1×10^3	1.3
Plasma membrane Enriched fraction	1.81	12.5	21.9×10^3	9.5
Endoplasmic reticulum Enriched fraction	0.623	4.3	7.8×10^3	3.4

*Specific activity

+Relative specific activity

Table 3. Activation of PM-bound Kallikrein Determined by RIA of Kinin Released from Dog Kininogen

Activator	Activity*
Control	100
Triton X-100 (1%)	510
Pancreatic Phospholipase A_2	693
Venom Phospholipase A_2	505
Lysolecithin (1mM)	681

* n = 3

Table 4. Activation of PM- and ER-bound Renin

	Activator	Activation	Specific Activity
			(AI ng/hr/mg)
PM	Control	100	33.5 ± 2
	Triton X-100 (1%)	3,000	1,464 ± 205
	Pancreatic Phospholipase A_2 (60 U)	3,400	
	Venom Phospholipase A_2 (2 U)	800	
	Lysolecithin (0.5 mg/ml)	5,400	
	Melittin (50 µg/ml)	3,000	
ER	Control	100	12.8 ± 1.4
	Triton X-100	2,500	335 ± 81
	Pancreatic Phospholipase A_2	2,000	
	Venom Phospholipase A_2	800	

AI = angiotensin I PM = Plasma membrane ER = Endoplasmic reticulum

Fig. 1.

Higher magnification to follow.EGE.

Fig. 2.

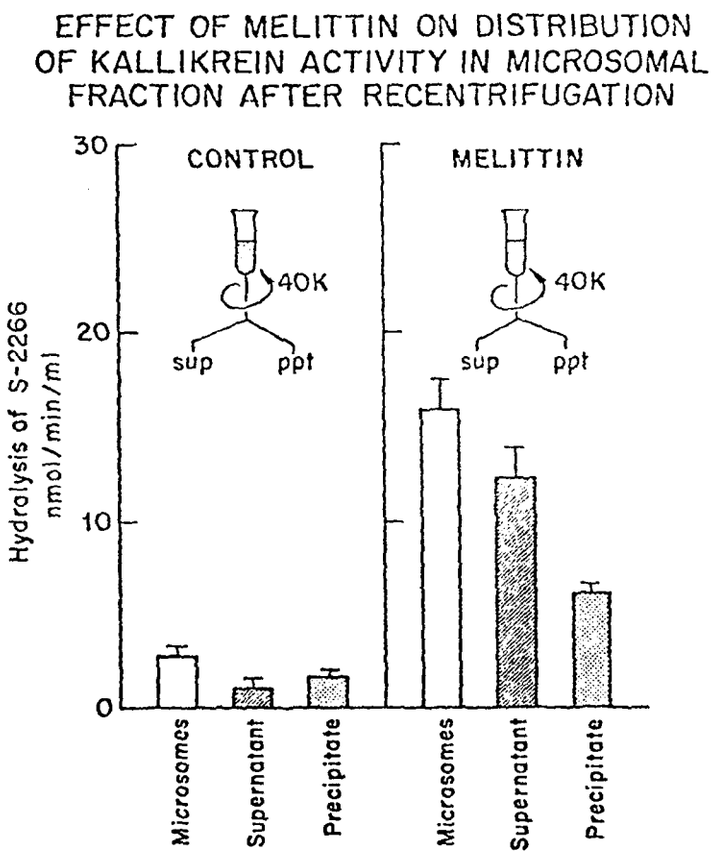

EFFECT OF MELITTIN ON DISTRIBUTION OF KALLIKREIN ACTIVITY IN MICROSOMAL FRACTION AFTER RECENTRIFUGATION
CONTROL
MELITTIN
40K
sup
ppt
40K
sup
ppt
Hydrolysis of S-2266
nmol/min/ml
30
20
10
0
Microsomes
Supernatant
Precipitate
Microsomes
Supernatant
Precipitate

Fig 3.

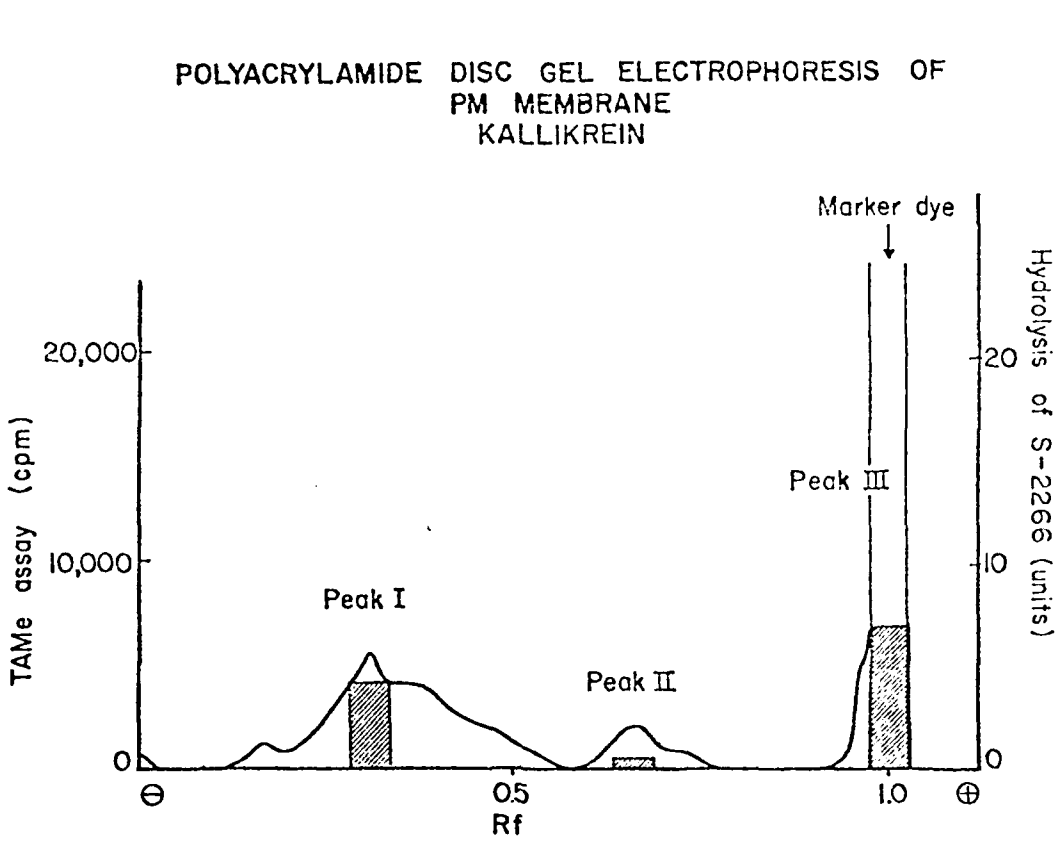

POLYACRYLAMIDE DISC GEL ELECTROPHORESIS OF
PM MEMBRANE
KALLIKREIN
Marker dye
Peak I
Peak II
Peak III
TAMe assay (cpm)
20,000
10,000
0
Hydrolysis of S-2266 (units)
20
10
0
⊖
0.5
1.0
⊕
Rf

Fig. 4.

EFFECT OF ANTISERUM TO URINARY KALLIKREIN ON THE ACTIVITY OF URINARY KALLIKREIN AND BOUND AND SOLUBILIZED RENAL KALLIKREIN

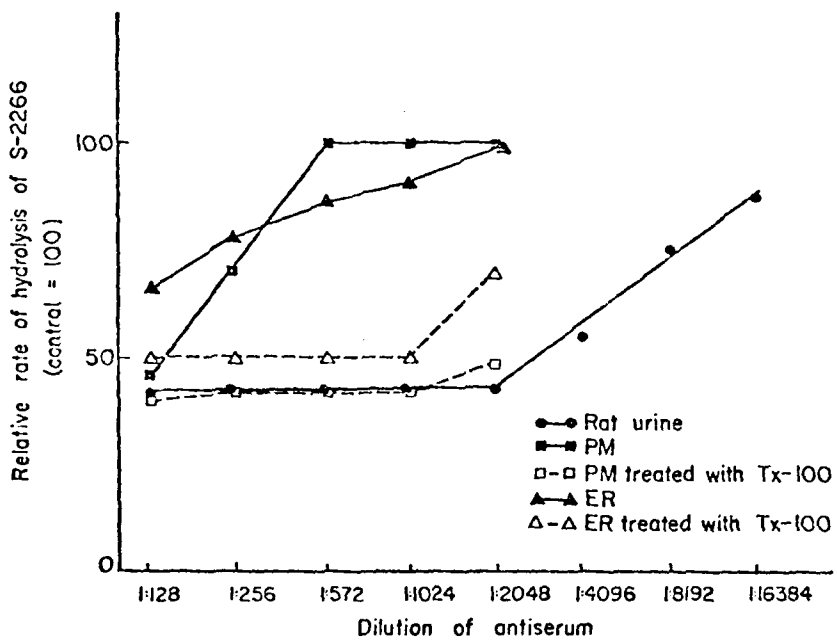


Fig. 5.

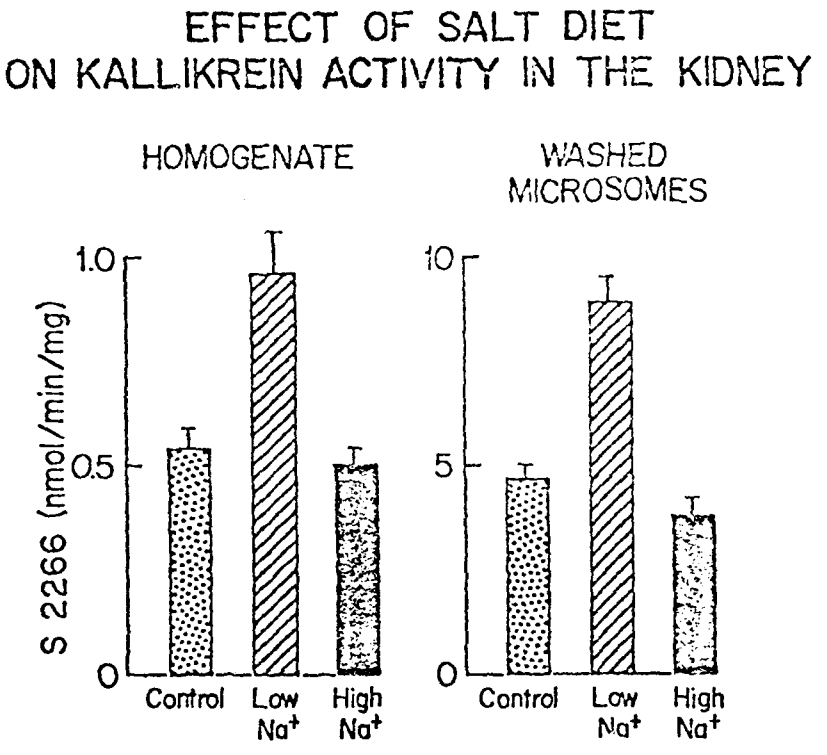
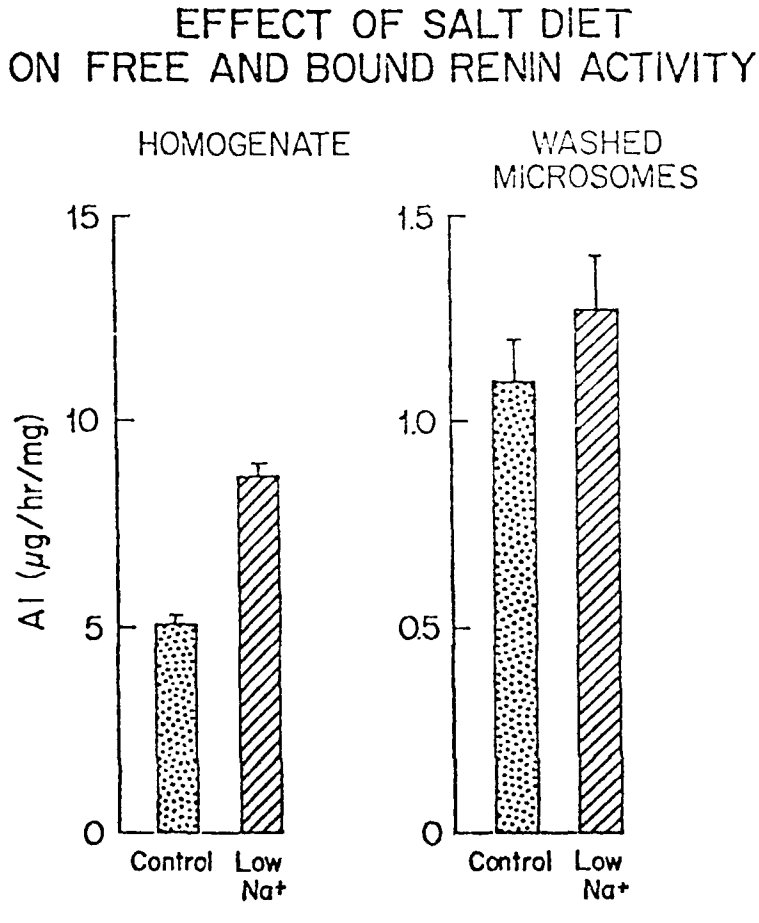

EFFECT OF SALT DIET
ON KALLIKREIN ACTIVITY IN THE KIDNEY
HOMOGENATE
WASHED MICROSOMES
S 2266 (nmol/min/mg)
1.0
0.5
0
10
5
0
Control
Low Na+
High Na+
Control
Low Na+
High Na+

Fig. 6.


EFFECT OF SALT DIET
ON FREE AND BOUND RENIN ACTIVITY
HOMOGENATE
WASHED MICROSOMES
AI (μg/hr/mg)
15
10
5
0
1.5
1.0
0.5
0
Control
Low Na+
Control
Low Na+

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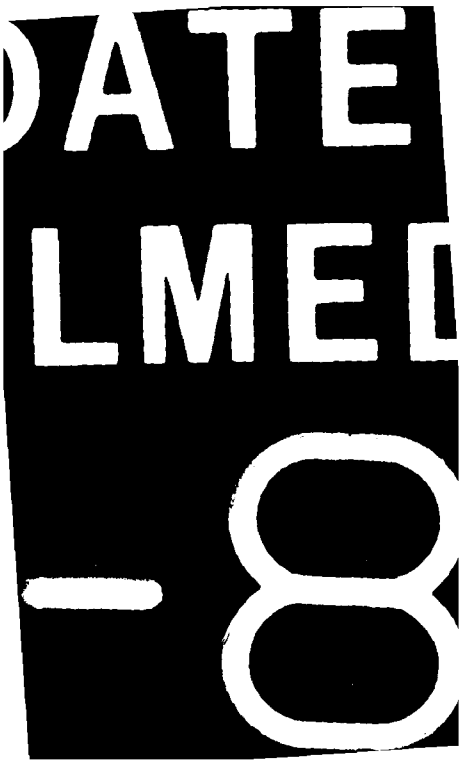
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