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Activation of Membrane-bound Kallikrein and
Renin in the Kidney

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ACTIVATION OF MEMBRANE-BOUND KALLIKREIN AND RENIN

IN THE KIDNEY

Key Words: (melittin/lysolecithin/aldosterone/angiotensin/

kinins)

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Abbreviations Footnote:

PM = plasma membrane   ER = endoplasmic reticulum

S-2266 = D-Val-Leu-Arg-p-nitroanilide

Ang I = angiotensin I

ABSTRACT

Rat kidney contains membrane-bound renin and kallikrein.
Kallikrein activity was measured with a spectrophotometric
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the membrane-enriched fractions, while renin activity was not
affected. It was proposed that kallikrein may be located also
on the basal membrane of tubular epithelial cells, where
aldosterone can enhance its activity.

The excretion of kallikrein in urine has been investigated in numerous laboratory and clinical conditions connected with hypertension (1-3). Although urinary kallikrein originates from the kidney (1-7), little is known about the biosynthesis, mode of activation and release of renal kallikrein. Kallikrein can be released into the urine from the distal tubules (7), but is absent from the brush border of the proximal tubules (8). Particulate fractions of the homogenized rat kidney enriched in plasma membranes (PM) contain both bound kallikrein (8-10) and renin (9, 11), and both kallikrein and renin are also found in fractions enriched in endoplasmic reticulum (ER) (8, 11). The finding that the kidney contains potent hypotensive and hypertensive enzymes bound to membranes led us to study the mode of activation of kallikrein and renin in fractions isolated from the rat kidney. We have also investigated how the sodium metabolism regulating hormone aldosterone affects the activity of kallikrein and renin in the kidney, and found that aldosterone increased membrane-bound kallikrein activity.

MATERIALS AND METHODS

Dextran T 70 was purchased from Pharmacia Inc. Piscataway,
NJ. D-Val-Leu-Arg-p-nitroanilide (S-2266) was obtained from
Kabi Diagnostica, Stockholm, Sweden. Melittin, d-aldosterone-
21 acetate and other reagents were from Sigma Chemical Co.,
St. Louis, MO. Lysolecithin was purchased from Supelco, Inc.,
Bellefonte, PA. Synthetic melittin was obtained from Professor
L. Levine of Brandeis University.

Enzyme assays

Kallikrein activity was assayed with S-2266 substrate. The hy-
drolysis of S-2266 was determined in a Cary 118 spectrophotometer,
either by continuously recording the increase in absorption due to
p-nitroaniline release at 405 nm, or by taking aliquots from the
incubation mixtures at regular time intervals (10-12). An 0.1 M
Hepes buffer, pH 9.1, was used at 37°C. With turbid solutions the
following technique was employed: the reaction was stopped with
20% (w/w) perchloric acid (300 μl) added to the supernatant fluid
(500 μl). The solution was kept at 4°C for 10 min to diazotise
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 wave length of 546 nm. Activity was expressed as nmol of
p-nitroaniline product formed per min from S-2266. Rat urinary

kallikrein activity was expressed in units (U). One U of

kallikrein activity equals 1 μmol of substrate cleaved in 1

min. Renin activity was expressed in ng or μg of angiotensin

I (Ang I) released from nephrectomized rat serum (11). If not

otherwise indicated or when no activators were used, 1% Triton

X-100 was added to both the renin and kallikrein preparations.

Protein concentration was measured by the method of Lowry et

al., with bovine serum albumin as a standard (13).

Fractionation

Male Sprague-Dawley rats (200-250 g) were killed by decapi-

tation. Twenty 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. Two ml of 0.1 M EDTA was

added and the homogenate centrifuged at 10,000 g and at 30,000 g

for 15 min. The supernatant containing microsomal particles was

centrifuged again at 100,000 g 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 hr. The microsomal

fraction was then separated into plasma membrane (PM) and endo-

plasmic reticulum (ER) enriched subfractions by layering it on

24 ml of 15% w/w dextran T 70 in 1 mM $MgSO_4$ and 1 mM Tris-HCl,
pH 8.6, and centrifuging in a SW-27 swinging bucket rotor at
25,000 rpm for 16 h(8, 14). 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 (pH 8.6) and
$MgSO_4$ and centrifuged at 100,000 g for 60 min. The pellets
were suspended in 10 mM TrisHCl (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 sam-
ples and the samples stored frozen. Freshly collected fractions
were used to assay bound renin.

Aldosterone

Sprague-Dawley rats in groups of six animals were injected s.c.
with 0.25 mg aldosterone in 0.2 ml sesame oil for six days. The
animals in the control group received only sesame oil. Urine
samples were collected for 16 hr in metabolic cages. Blood
samples were withdrawn from the tail vein. The kidneys of each
animal in the control and in the treated group were homogenized,
fractionated and assayed individually.

Activation of kallikrein

Aliquots of membrane-bound and urinary kallikrein used as
control for soluble kallikrein were routiely preincubated with
activators at $4°C$ for 30 min. In some experiments, melittin was
directly added to the cell of the spectrophotometer containing

the substrate and enzyme without preincubation.

RESULTS

Kallikrein

Rat kidneys were homogenized, and separated into PM and ER

enriched fractions by differential centrifugation as previously

described (8, 10, 11). The separation procedure included

repeated washings with hypotonic buffer. Kallikrein activity

in the PM fraction (PM-kallikrein) averaged 1.81 nmol of

S-2266 hydrolyzed per min by one mg protein prior to

activation. As initial studies indicated that detergents

(9-11), phospholipase A_2, lipids and the polypeptide

melittin (11) enhance PM-kallikrein activity, we compared

the effect of lysolecithin on PM-kallikrein with that of

melittin. Fig. 1 shows that melittin was about a thousand   Fig. 1

times more active than lysolecithin on a molar basis.

Lecithin and arachidonic acid were active only at a much

higher concentration than lysolecithin. The melittin used in

these studies was a natural product, but the synthetic peptide

gave identical results. Since melittin activity is consistent

with activation of endogenous phospholipase A_2 (15-17), we wished

to determine whether the activation of PM-kallikrein is a direct or

indirect effect of the peptide; we added an activator or an

inhibitor of phospholipase A_2 to the PM-kallikrein melittin

incubation mixture. Activation of PM-kallikrein by melittin in pre-

sence or absence of 1 mM $CaCl_2$, or 0.1 mM mepacrine or 1mM EDTA was

identical (not shown in Fig. 1). Possibly melittin has a direct
action on the membrane; alternately the phospholipase is not ac-
tivated by $CaCl_2$ or inhibited by mepacrine.

Renin

In addition to kallikrein, the PM enriched rat kidney fraction
contained low renin activity (8-30 ng Ang I/hr per mg protein).
This activity was enhanced by the activators of PM-kallikrein.
Melittin activated bound renin in presence of added 1 mM $CaCl_2$ in
the same concentration as kallikrein. However, the dose-response
curve was shifted to the right when $CaCl_2$ was deleted or EDTA was
added (Fig. 2) to the incubation mixture. For an equivalent acti-   Fig. 2
vity in absence of calcium, the concentration of melittin had to
be an order of magnitude higher.

Effect of aldosterone

Because aldosterone enhances the excretion of kallikrein in urine
(1-3, 18, 19), we studied the effect of the hormone on kallikrein
and renin in rats. In agreement with the findings of others, aldos-
terone increased the excretion of kallikrein after six days of admin-
istration from a mean of 2.4 $\pm$ 0.17 U in the urine collected in 16
hr to 4.4 $\pm$ 0.35 U (p<.001). Aldosterone also decreased the plasma
renin activity considerably from 11.1 $\pm$ 0.96 to 3.8 $\pm$ 0.37 ng Ang I/h
per ml (p<.001).

The activity of renin and kallikrein was also assayed in fractions
of the homogenized kidneys of rats treated with aldosterone and of
control animals.

Most of the renin activity in the kidney was, as expected, in
the soluble fraction, but aldosterone had no significant effect

on the renin activity in the crude homogenate or in the particu-
late fraction. The activity of renin in the crude homogenate and
in the washed particulate fraction in treated and untreated rats
was 5.1 ± 0.4, 4.9 ± 0.6, 0.4 ± 0.06 and 0.5 ± 0.07 µg of Ang I
released per hr by 1 mg protein respectively.

In contrast to renin, administration of aldosterone increased
kallikrein activity in the kidney (Table 1). The difference be-   Table 1
tween the treated animals and the control groups became highly
significant in the particulate fraction (100,000 g sediment).
After washing and resedimenting, this fraction from control
animals had an activity of 2.8 ± 0.3 nmol/min per mg while that
from treated animals cleaved 6.4 ± 0.8 nmol of substrate ($p < 0.002$).

In a second series of experiments the kidneys were further
fractionated to PM and ER enriched fractions. The activity of
kallikrein in the PM fraction was 11.2 ± 2 nmol in the control
and 23.3 ± 1.3 in the aldosterone group ($p < .001$). The correspond-
ing figures were 2.1 ± 0.7 and 5.7 ± 0.5 in the ER fractions
($p < .002$). The variations in renin activity in the PM and ER
fractions of the treated and untreated animals were within
experimental error.

DISCUSSION

The importance of the renin-angiotensin system in the
etiology of some forms of hypertension is undisputed, but
the role of kallikrein and kinins in the pathogenesis of this
disease is still being investigated. Excretion of kallikrein

in the urine is decreased in clinical and experimental hyper-
tension and is increased by aldosterone (1-3, 18, 19). The
level of this enzyme in urine is also influenced by fluid intake,
diet, diuretics, and race (1-3). The actions of kinins in the
kidney include vasodilation, increased sodium and water excre-
tion (1, 2), activation of a phospholipase and release of pros-
taglandin (20). Most of the studies on urinary kallikrein were
based on measuring its esterase activity, but the function of
kallikreins is to cleave peptide bonds.

We determined the activity of renal kallikrein with the speci-
fic peptide substrate S-2266 (12). The activity of renal kalli-
krein, as measured by radioimmunoassay of the kinin released,
correlated well with the rate of cleavage of S-2266, but not with
arginine esterase activity (10, 11). Other investigators also
concluded that changes in the urinary esterase activity do not
necessarily reflect changes in the amount of active kallikrein
excreted (21).

In the rat, the kidney contains most of the renin in the body.
Renin is concentrated in the granules of the juxtaglomerular cells,
where it is released into the circulation (22). However, renin also
has intrarenal functions (23), that may call for a localization
of the enzyme outside of the juxtaglomerular appartus. We
reported previously that renin can be induced in a particulate
(microsomal-ribosomal) fraction of the submaxillary gland of mice

(24). In addition to marker enzymes and kallikrein (8, 11), the PM and ER enriched fractions of the rat kidney also have renin activity, even after repeated washing of the fractions with hypotonic solutions. Transmission electron micrographs of the preparation revealed no trapped granules or soluble material in the isolated membrane vesicles (11).

Bound kallikrein and renin in the PM and ER enriched fractions of the kidney are activated by various agents. These include phospholipase A_2 (10, 11) and melittin. Melittin is a polypeptide with a M_r of about 2800, which contains basic and hydrophobic amino acids. It interacts with membranes (25), and its activity is consistent with activation of phospholipase at low concentration (15-17). Phospholipase A_2 of pancreas or snake venom added to the membrane fractions (10, 11) or isolated kidney cells (26) enhanced kallikrein activity. Since a chloroform extract of the incubation mixture of phospholipase A_2 and a membrane fraction also activated PM-kallikrein, we tested various lipids as activators and found lysolecithin to be more potent than prostaglandin E_2 (11), arachidonic acid or lecithin. However, melittin, on a molar basis, was about three orders of magnitude more potent than lysolecithin (Fig. 1).

Experiments with melittin indicated that PM-renin and PM-kallikrein are activated by different mechanisms. Melittin's activity was not affected by $CaCl_2$, EDTA or mepacrine. Melittin may act by

activating a membrane-bound prekallikrein prior to releasing the enzyme into solution (9), or by activating a calcium-independent phospholipase. The activation of PM-renin by melittin was greatly enhanced by calcium and was decreased by EDTA (Fig. 2). This indicates that melittin released bound renin by a calcium dependent process, possibly by activating a calcium dependent phospholipase.

The administration of aldosterone to rats for six days influenced kallikrein and renin activity differently. Aldosterone treatment decreased plasma renin activity in the rats and increased the excretion of kallikrein in the urine. The hormone did not affect activity of renin in the kidney, but increased that of kallikrein. The difference between the treated animals and the control group became more significant after the membrane fractions were separated from the crude homogenate.

The relationship of kallikrein to mineralocorticoids has been studied extensively by Margolius and his colleagues and others (1-3, 18, 19, 27-29) who reported that aldosterone increases kallikrein excretion in man and animals. Margolius et al., hypothesized that aldosterone is an inducer of renal kallikrein (30). Our experiments showed that aldosterone enhances kallikrein but not renin activity in the kidney, presumably by inducing its synthesis in the microsomal-ribosomal fraction, which leads to increase in kallikrein on the membrane of the cells of the distal tubules.

We can only guess the exact site of action of aldosterone on renal kallikrein. Over 90% of renal kallikrein is in the cortex (1), where it can be released from the distal tubules (1-3, 7, 8). It has also been shown that kallikrein is located on the luminal surface of the distal tubules (31). However, special fixation of tissues was required before kallikrein activity could be shown by immunofluorescence. This is understandable because kallikrein bound to membrane reacts much less with antibody than the soluble kallikrein (11). A localization of kallikrein on the luminal surface, however, cannot explain all the actions of kallikrein on the kidney. For example, kallikrein was detected in venous effluent of the kidney (32) and in renal lymph (33). In addition, although kallikrein substrate, kininogen, was found in the urine (34), the site of interaction of kininogen with renal kallikrein has not yet been determined.

In secretory glands kallikrein is present in cells which are rich in mitochondria and contain infoldings of basal cell membranes, a structure usually associated with water and ion transport (35). The ultrastructure of the basal part of the epithelium in distal tubules, which face the peritubular capillaries, also shows concentrated mitochondria and infolding of cell membranes (36).

In the rabbit, corticosteroids acted on the basolateral membrane of the collecting tubules and enhanced its surface area, but did not affect the luminal membrane area (37).

Using isolated tubular segments of the rabbit kidney, Gross and Kokko (38) found that aldosterone stimulated base-line potential across cortical collecting ducts in vitro. In the rat, however, aldosterone modulated in vivo Na-K-ATPase activity in all renal tubular structures studied (39). If kallikrein and prekallikrein were also located on the basal membrane of the epithelial cells of the distal tubules, the role of kallikrein in renal vascular resistance (40, 41), its release into circulation (32) and extra-cellular fluid (33), would be easier to understand than if it were located only on the luminal rim of the tubular cells. Aldosterone may also enhance renal kallikrein activity by acting on the basal membrane of tubular cells.

Fig. 1.   Activation of plasma membrane-bound kallikrein by

arachidonic acid, lecithin, lysolecithin and melittin.

Abscissa: M concentration.

Ordinate: relative rate of hydrolysis of S-2266.

Fig. 2.   Activation of plasma membrane-bound renin by

melittin in presence of 1mM CaCl$_2$ $\triangle$——$\triangle$, in absence
of added CaCl$_2$ $\square$——$\square$ and in presence of 10 mM EDTA
$\bigcirc$——$\bigcirc$.

Abscissa: M concentration.

Ordinate: ng Ang I released per hr in 1 ml.

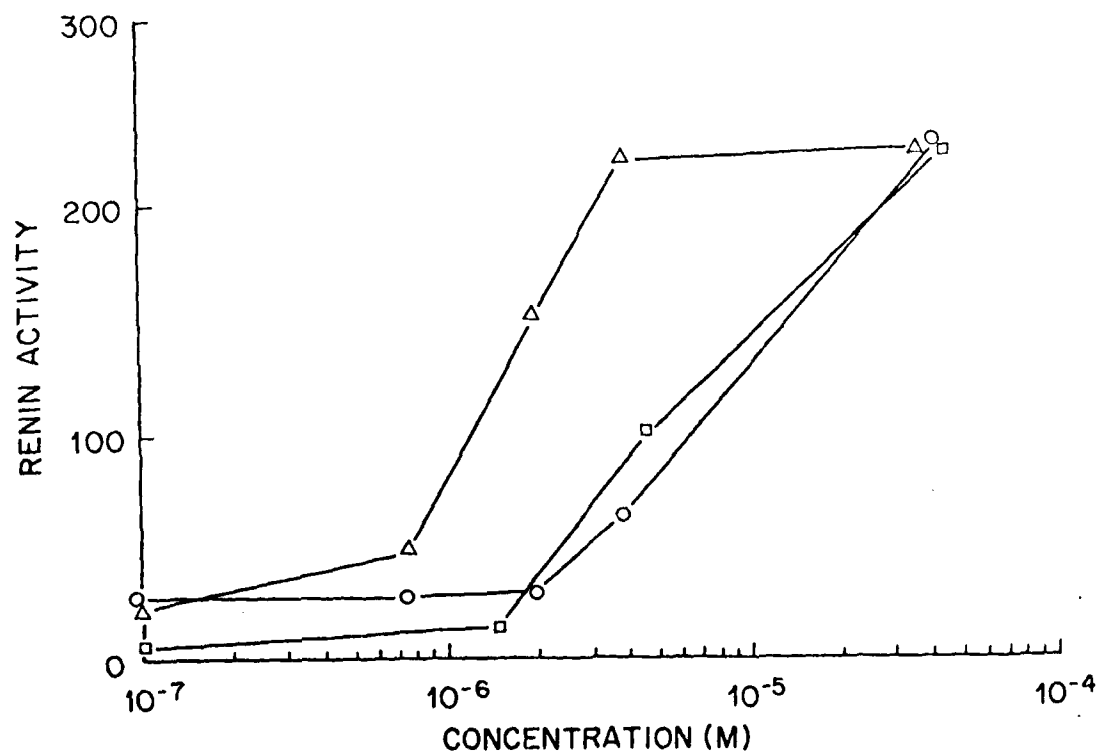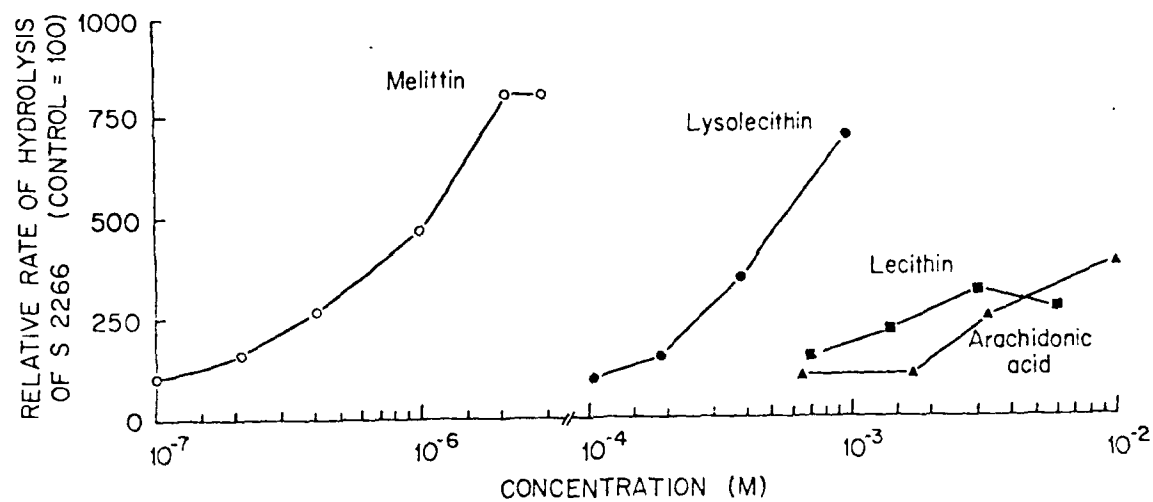ACTIVATION OF PM MEMBRANE-BOUND KALLIKREIN

Melittin

Lysolecithin

Lecithin

Arachidonic acid

RELATIVE RATE OF HYDROLYSIS OF S 2266 (CONTROL = 100)

CONCENTRATION (M)

RENIN ACTIVITY

CONCENTRATION (M)

TABLE 1

EFFECT OF ADMINISTRATION OF ALDOSTERONE

ON THE ACTIVITY OF KALLIKREIN IN RAT KIDNEY

	Control		Aldosterone		
	1	2	1	2	
Crude homogenate	0.6 ± 0.06	1	0.8 ± 0.16	1	p=.058
High speed pellet	1.92 ± 0.2	3.3	4.6 ± 0.6	5.8	p<.022
Washed particulate fraction	2.8 ± 0.3	4.3	6.4 ± 0.8	8	p<.002

1. Specific activity nmol/min/mg $\pm$ S. E.

2. Relative specific activity, enrichment factor.

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