Direct Action of Kallikrein and Other Proteases on the Renin-Angiotensin System

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SUMMARY Kallikrein is present in the renal tubule near the macula densa, and it has recently been shown to actuate inactive renin in human plasma. We recently showed that kallikrein was a potent stimulus of renin release and increased renin secretion in a dose-dependent fashion. To study its effect on renal renin release, we superfused rat renal cortical slices with purified rat urinary kallikrein. Kallikrein-stimulated renin release was completely abolished by trasylol and by amiloride, but was not affected by soybean trypsin inhibitor. Indomethacin did not block kallikrein action, indicating that kallikrein's effect is not mediated via kinin generation and prostaglandins. Kallikrein-stimulated renin release was not blocked by propranolol, trasyrol did not block isoproterenol, and dibutyryl cyclic AMP stimulated renin release, indicating that kallikrein may not play a role in the β-adrenergic mechanism of renin release. There was no demonstrable acid-actiratable or kallikrein activatable renin in the superfusate, suggesting that all of the renin released was in the active form. Cathepsin D and plasmin also stimulated renin release from kidney slices in pH 6.0 buffer, whereas tryspin and pepsin did not. Our results support the hypothesis that kallikrein may play a role in the secretion of renin by the kidney. Other proteases can also release renin from the kidney. (Hypertension 3 (supp I): I-13-I-17, 1981)

KEY WORDS • rat urinary kallikrein • inactive renin • bradykinin • trasylol • tryspin, cathepsin D • plasmin • pepsin

THE renin-angiotensin system, the kalli- kreira-kinin system, and the prostaglandin system have relationships with each other and play important roles in renal function and sodium and water balance. It is considered that renal kallikrein may stimulate the production of prostaglandin via kinin generation. Prostaglandins are known to be potent stimulators of renin release.

Sealey et al. and Derkx et al. reported that kallikrein converts human plasma inactive renin to active renin. Since renal kallikrein is produced in the renal tubules, especially near the macula densa, it has been proposed that kallikrein may be the endogenous renin activator in the kidney. Recently we reported that rat urinary kallikrein is a potent stimulus of renin release at doses between 70 and 140 mEU/ml. The present study was carried out to further evaluate the role of renal kallikrein on renin release. The effects of other proteolytic enzymes were also evaluated and their actions on renin release compared to those of kallikrein.

Materials and Methods

Isolation of Rat Urinary Kallikrein

Rat urinary kallikrein (RUK) was isolated from a urine pool of Sprague-Dawley rats using the Nustad and Pierce method. Esterolytic activity of the kallikrein fraction was measured using the proteinase substrate, α-N-p-tosyl-L-arginine methyl ester HCl (TAME HCl). The unhydrolyzed ester was measured by the Roberts colorimetric method.

Superfusion of Rat Renal Cortical Slices

Sprague-Dawley female rats weighing 200–250 g were used for the experiments. The rats were maintained on a regular Purina Chow diet and killed by decapitation. The kidneys were rapidly removed and sliced using Stadie-Riggs microtome. Renal cortical tissue, 60 to 80 mg, was placed in the tissue holder and superfused at 37°C with Krebs-Ringer-bicarbonate-glucose buffer (KRBG) pH 7.4 by the technique previously described by us. Renin concentration was measured by radioimmunoassay of angiotensin I (A1) (Becton-Dickinson kit). The plasma of rats nephrectomized 24 hours prior to bleeding was used as a source of renin substrate. Superfusate samples (100 µl each) were incubated for 3 hours at 37°C with 150 µl of

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rat renin substrate, 180 μl of 0.1 M Tris acetate lysozyme buffer at pH 7.4, 50 μl of 4% EDTA-2Na, 10 μl of 0.34 M 8-hydroxyquinoline, and 10 μl of 2% dimer caprol. To rule out the presence of other serine proteases active at neutral pH, the same kallikrein-stimulated samples and control samples were assayed in the presence and absence of 5 μl of the serine protease inhibitor, phenylmethyl sulfonylfluoride (5% in ethanol). There was no significant difference in A1 generation by the addition of phenylmethyl sulfonylfluoride (r = 0.98, p < 0.01, n = 10). Therefore, we did not use phenylmethyl sulfonylfluoride in the experiments.

The interassay coefficient of variation using superfusate samples was 11.5% (n = 10), and the intraassay coefficient of variation was 10.5% (n = 10). The recovery of A1 added to superfusate was 85% ± 2% (n = 10).

Acid Activation of Renin

One ml samples of superfusate or rat plasma were dialyzed for 24 hours at 4°C against 0.15 M glycine-HCl buffer at pH 3.3, then dialed against 0.18M sodium phosphate buffer at pH 7.4 for a further 24 hours. Renin concentration was then measured as described in the preceding section.

Tetradecapeptide Experiments

Tetradecapeptide obtained from Boehringer Mannheim Biochemicals (Indianapolis, Indiana), 100 ng/ml, was incubated with RUK, trypsin, cathepsin D, plasmin, and pepsin at 37°C for 1 hour. Trypsin (from bovine pancreas), cathepsin D (from bovine spleen), plasmin (from porcine blood), and pepsin (from porcine stomach mucosa) were obtained from Sigma Chemical Company (St. Louis, Missouri). The pH of the incubation medium of these proteases was adjusted to 5.0, 6.0, and 7.4. Aliquots of 50 μl were used for radioimunoassay of A1 with the Becton-Dickinson kit, and results were expressed as ng/ml/hr.

Rat Nephrectomized Plasma Experiments

Rat nephrectomized plasma, 150 μl, was incubated with various proteolytic enzymes at 37°C for 1 hour. The pH and measurement of AI were the same as for the tetradecapeptide experiments.

Unit definition of kallikrein, trypsin, cathepsin D, plasmin, and pepsin has been done as follows. Kalli krein and trypsin: one esterase unit will hydrolyze 1.0 mole/min of α-N-p-tosyl-L-arginine methyl ester HCl (TAME) at pH 8.6 at 37°C. Cathepsin D: one unit will produce an increase in A280 of 1.0 mole/min/ml at pH 3.0 at 37°C measured as TCA-soluble products using hemoglobin as substrate. Plasmin: one unit will release 1.0 mole/min of tyrosine at pH 7.5 at 37°C, using α-casein as substrate. Pepsin: one unit will produce a ΔA280 of 0.001/min at pH 2.0 at 37°C, measured as TCA-soluble products using hemoglobin as substrate.

Prostaglandin E2 was obtained from Upjohn Company (Kalamazoo, Michigan), and isoproterenol, dibutyryl cyclic AMP, d-1 propranolol, indomethacin, ascorbic acid, trasylool, and soybean trypsin inhibitor were obtained from Sigma Chemicals (St Louis, Missouri). Amiloride was obtained from Merck Sharp and Dohme Research Laboratory (West Point, Pennsylvania). Ascorbic acid, 6 × 10⁴ M, was used to prevent the oxidation of isoproterenol. Statistical analysis was performed by paired t test with log transformation, and p < 0.05 was considered significant.

Results

The effects of trasylool and soybean trypsin inhibitor (SBTI) on kallikrein-stimulated renin release are shown in figure 1. Rat urinary kallikrein, 120 mEU/ml, increased renin release significantly. This increase was completely abolished by 472 kallikrein inhibiting units/ml of trasylool; however, it was not affected by 10⁻⁴ M of SBTI. Furthermore, amiloride, 10⁻⁴ M, blocked this kallikrein action (fig. 2).

Since kallikrein can generate bradykinin and bradykinin stimulates prostaglandin production, which in turn can stimulate renin release, the effect of indomethacin on renin release from rat renal cortical slices was evaluated. We had shown that doses of bradykinin from 10⁻⁷ to 10⁻⁴ M did not stimulate renin release. Indomethacin, 10⁻⁴ M, which blocked the effect of 10⁻⁴ M of arachidonic acid on renin release,
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FIGURE 2. Effect of amiloride. RUK 120 mEU/ml stimulated renin release significantly and amiloride 10^-4 M completely abolished the action of 120 mEU/ml of RUK.

FIGURE 3. Effect of indomethacin. Indomethacin 10^-4 M, completely abolished the action of arachidonic acid, 10^-4 M. However, indomethacin, 10^-4 M, did not affect the action of 120 mEU/ml of RUK.

FIGURE 4. Effect of propranolol. Isoproterenol, 8 X 10^-7 M, stimulated renin release significantly, and propranolol, 2 X 10^-4 M, completely abolished the action of 8 X 10^-7 M of isoproterenol. However, propranolol, 2 X 10^-3 M, did not affect the action of 120 mEU/ml of RUK.

did not block kallikrein action (fig. 3), indicating that kallikrein's effect is not mediated via prostaglandin generation.

To test the possibility that kallikrein acts in the test tube instead of renal tissue, the effects of adding RUK to control superfusion samples were evaluated. The direct addition of kallikrein at doses of 3.5, 35, and 350 mEU/ml to control superfusate samples did not cause any significant increase of renin concentration as compared with control superfusate.

To investigate the role of kallikrein in the β-adrenergic mechanism of renin release, the effect of propranolol on kallikrein-stimulated renin release was evaluated (fig. 4). Propranolol, 2 X 10^-4 M, which completely abolished the renin-stimulating effect of isoproterenol (fig. 4, upper graph), did not affect kallikrein-stimulated renin release. Furthermore, trasylol (472 kallikrein-inhibiting units/ml), which completely abolished the renin-stimulating effect of kallikrein, did not affect the renin-stimulating effects of isoproterenol (fig. 5, upper graph) and dibutyryl cyclic AMP (fig. 5, lower graph).

The effect of other proteases on renin release from kidney slices is shown in figure 6. Cathepsin D in amounts of 10 to 100 mU/ml and plasmin from 1 to 10 mU/ml also stimulated renin release in pH 6.0. However, in pH 7.4, 10 mU/ml of cathepsin D and 1 to 10 mU/ml of plasmin did not stimulate renin release. Trypsin and pepsin did not affect renin release.

Table 1 shows the AI-generating activity of the proteases. Rat nephrectomized plasma and synthetic
tetradecapeptide were used as renin substrate. These proteases did not produce AI from rat nephrectomized plasma, whereas cathepsin D and pepsin produced AI from tetradecapeptide. Therefore, the proteases were not converting an inactive renin to active renin in the substrate nor generating AI directly from natural renin substrate.

**Discussion**

Recent reports suggest that renal renin is largely in the form of a prorenin or inactive form. In human plasma, both active and inactive renin have been found. Various proteolytic enzymes, including trypsin, pepsin, cathepsin D, plasmin, and kallikrein have been reported to be able to activate inactive human renin. However, it is still not clear whether these proteolytic enzymes play a physiological role in vivo. In view of the fact that kallikrein is made in the renal cortex, especially near the juxtaglomerular apparatus, the suggestion has been made that renal kallikrein may convert the inactive prorenin to active renin intrarenally.

Our studies provide support for this hypothesis. Kallikrein was a potent stimulator of renin release from kidney slices, and this release was blocked by the kallikrein inhibitors, trasylol, and amiloride. This release due to kallikrein did not appear to be due merely to tissue damage and leakage of renin from the renal slice, since repeated doses of kallikrein showed similar stimulation, and the baseline returned to control levels after stimulation with kallikrein. Furthermore, we had shown that the concentration of the tissue enzyme glutamic oxaloacetic transaminase and γ-glutamyl transpeptidase was not significantly different in the control and kallikrein-stimulated samples. Since soybean trypsin inhibitor did not inhibit kallikrein stimulation of renin release, this kallikrein action may be specific. This kallikrein action may not be via bradykinin generation since bradykinin did not stimulate renin release. Furthermore, the prostaglandin synthesis inhibitor, indomethacin, did not block the action of kallikrein, indicating that this kallikrein action may not be via prostaglandin generation. The β-adrenergic mech-
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suggest that kallikrein may play a role in the activation and release of renin by the kidney. Another possibility is that kallikrein alters the plasma membrane of the juxtaglomerular cells, leading to renin release. However, our preliminary data show no effect of kallikrein on red cell Na/K ATPase and Ca/Mg ATPase.

References


TABLE 1. Angiotensin I-Generating Activity of Proteases

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<th>Protease group</th>
<th>Control A</th>
<th>RNP</th>
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<td>Control B</td>
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Rat nephrectomized plasma (RNP) 150 µl and tetradecapeptide (TDP), 100 ng/ml, were used as renin substrate. Control A and Control B were Tris-acetate-lysozyme buffer, and total incubation volume was 1.0 ml (0.5 ml of protease solution and 0.5 ml of renin substrate). Final incubation pH was adjusted just before incubation. Values represent angiotensin I generation in ng/ml; nd = not detectable.

anism is also not involved in the action of kallikrein since propranolol did not block its action. The addition of kallikrein to control superfusate, that is, after the superfusate had perfused the kidney, did not increase renin activity, ruling out the possibility that kallikrein merely converted prorenin to active renin while in contact with the perfusate. Since acid activation did not increase renin concentration, kallikrein releases only active renin from the rat kidney.

We previously showed that Sephacryl S-200 column chromatography of the superfusate from basal and kallikrein-stimulated samples revealed only one peak of active renin of approximately 40,000 mw.12 Furthermore Inagami et al.3 reported that rat kidneys contain high molecular weight renin that is readily converted to the low molecular weight renin by renal proteases. In the isolated perfused kidney, Nakane et al.24 showed that the rat kidney only secretes active renin, and our superfusion data support their findings. In view of the latter finding and the fact that kallikrein can convert inactive to active renin in vitro, we
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