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STUDIES ON RAT RENAL CORTICAL CELL KALLIKREIN

II. IDENTIFICATION OF KALLIKREIN AS AN ECTO-ENZYME

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Summary

Suspensions of viable renal cortical cells hydrolyzed a synthetic ester substrate (α-*N*-tosyl-L-argnine methyl ester, Tos-Arg-OMe) and generated kinins from a kininogen substrate. This kallikrein-like esterase activity increased linearly with cell number, or time of exposure to substrate. No radiolabelled substrate or product was found within the cells. Most of the activity appeared to be on cell surfaces as supernatant media had less than 20% of the Tos-Arg-OMe esterase activity of the cell suspensions. Cell surface Tos-Arg-OMe esterase activity was inhibited by aprotinin, benzamidine, pentamidine, and a tris-amidine derivative (α,α′,α″-tris(3-amidinophenoxy)mesitylene). Preincubation of cells with phospholipase A_2 increased renal cell surface esterase activity up to 76% while only slightly increasing supernatant activity. In contrast, preincubation with deoxycholate caused clearing of suspensions and a marked increase in supernatant esterase activity. Renal cell kininogenase (EC 3.4.21.8) activity was inhibited by preincubation with aprotinin, the tris-amidine derivative, or anti-rat urinary kallikrein antibody. Kallikrein elaborated by renal cells formed a single precipitin line with an antibody to rat urinary kallikrein but the two enzymes were not immunologically identical. We conclude that kallikrein's active sites are facing the external environment of renal cortical cells in suspension with access to substrates, inhibitors, and antibody.

Abbreviations: Tos-Arg-OMe, α-*N*-tosyl-L-arginine methyl ester; IRT-63, α,α′,α″-tris(3-amidinophenoxy)-mesitylene.

Introduction

Renal and urinary kallikreins (EC 3.4.21.8) are serine proteinases which act preferentially upon low molecular weight kininogen substrates to liberate kallidin (lysyl-bradykinin), a decapeptide with a broad spectrum of biological effects. Kallikrein has been detected in microsomal fractions of homogenized kidney [1,2] and more recently reported to be enriched in plasma membrane fractions, possibly of distal nephron origin [3,4]. Immunocytochemical studies have localized kallikrein either upon or just below the luminal membrane of epithelial cells of the distal tubule [5,6]. It has been shown that aldosterone and other sodium-retaining steroids increase renal and urinary kallikrein activity in a variety of clinical and experimental circumstances, whereas spironolactone reduces kallikrein activity [7—12]. It has been suggested that renal kallikrein and its product kinin may have some role in membrane transport events or actions of aldosterone [12]. The orientation of renal kallikrein upon or within cell surface membranes could be crucial to the interaction of this enzyme with either urine or cellular constituents, such as substrates or inhibitors. The present study identifies the active sites of kallikrein on the external surfaces of renal cells in suspension.

Materials and Methods

Biochemical materials. The following were obtained from commercial sources: α-*N*-Tosyl-L-arginine methyl ester (Tos-Arg-OMe), bradykinin (Schwartz-Mann, West Nyack, NY); Tos-Arg-O[^{3}H]Me (specific activity, 200 Ci/mol, Biochemical and Nuclear Corp., Burbank, CA); L-[U-^{14}C]leucine (specific activity, 333 Ci/mol (New England Nuclear, Boston, MA); aprotinin (identical to Kunitz basic pancreatic inhibitor), trypsin (from bovine pancreas, Type IV, twice crystallized), phospholipase A_2 (from bee venom), sodium deoxycholate, and benzamidine (Sigma Chemical Corp., St. Louis, MO); trypsin inhibitors from soybean, lima bean, ovomucoid and carboxypeptidase B (Worthington Biochemical Corp., Freehold, NJ); Nitex (Tetko, Elmsford, NY); α-medium (Grand Island Biological Co., Grand Island, NY). Pentamidine and α,α′,α″-tris(3-amidinophenoxy)mesitylene, a tris-amidine derivative (IRT-63), were gifts of Drs. J.D. Geratz and R.R. Tidwell, University of North Carolina. Purified human urinary kallikrein was a gift from Dr. J.V. Pierce, National Institutes of Health, SQ 20881 (teprotide, <Glu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro) was a gift from Dr. Z. Horowitz of the Squibb Institute for Medical Research, New Brunswick, NJ.

Preparation of renal cortical cell suspensions. Renal cortical cell suspensions were prepared according to the procedures of Kaizu and Margolius [13] with some notable modifications. All glassware and media were sterile. Male Sprague-Dawley rats weighing 200—300 g were anesthetized with ether; a middle abdominal incision was made and the kidneys were removed under aseptic conditions. Each kidney was perfused via the renal artery over a 5 min period with room temperature Dulbecco's phosphate-buffered saline, pH 7.3 (Ca^{2+}, Mg^{2+} free) containing sodium EDTA 0.02%, delivered by a 10 ml syringe and 26 gauge needle. The cortex was separated, weighed, finely minced with

dissecting scissors, and suspended in 50 ml of the buffer at 37°C. After slow magnetic stirring for 5 min, the minced tissue was allowed to settle and medium was decanted. The minced tissue was resuspended and the procedure was repeated. 50 ml of the buffer containing trypsin 0.1%, was added to the minced tissue and the mixture was stirred at 37°C. Stirring was continued for intervals of 30 min. At the end of each run, the stirring was stopped for 1—2 min to allow non-dispersed fragments to settle, and the supernatant cells in suspension were decanted. These runs were repeated until all of the tissue appeared dispersed. After each run, the supernatant cell suspensions were filtered through several layers of gauze and then through a single layer of Nitex (100 μm pore size). The cell suspensions were then centrifuged for 10 min at 90 × *g* at room temperature. Immediately after centrifugation, the supernatant was removed by suction, the cell pellet was resuspended in 20 ml of the buffer containing lima bean trypsin inhibitor (0.1%) and soybean trypsin inhibitor (0.1%) added to inhibit trypsin and incubated at 37°C with gentle shaking for 10 min. The cell suspensions were then centrifuged at 90 × *g* for 10 min and the procedure was repeated. The cells were then washed three to five times with phosphate-buffered saline containing 2 mM $CaCl_2$. The washed cells were suspended in the same buffer and counted using a hemocytometer. The percentage of harvested, viable cells was determined in the buffer containing 2 mM $CaCl_2$ or in α-medium [14] using the trypan blue exclusion method of Hoskins et al. [15]. Protein concentration of these renal cortical cells was determined by the method of Lowry et al. [16], using bovine serum albumin as the standard.

Ammonium sulfate fractionation of rat urinary kallikrein antiserum. The production of a sheep antiserum against purified rat urinary kallikrein B has been reported previously [17,18]. The kallikrein antiserum was diluted 1 : 5 with the buffer. Ammonium sulfate (final concentration, 45% of saturation) was added and the pH was adjusted to 7.0 with 1 M NaOH. After stirring at room temperature for 1 h, the mixture was centrifuged at 12 000 × *g* for 20 min at 4°C and the precipitate was washed twice in 45% ammonium sulfate, pH 7.0. The washed precipitate was dissolved in distilled water and dialyzed against three changes of 4 l of 0.03 M phosphate buffer/0.15 M NaCl (pH 7.5) during 24 h at 4°C.

Radiochemical Tos-Arg-OMe esterase assay. A modification [19] of the assay of Beaven et al. [20] was used routinely to determine Tos-Arg-OMe esterase activity, a measure of kallikrein-like enzyme activity. The incubation mixture contained 0.047 μCi of Tos-Arg-O[^{3}H]Me (10 μl, approximate specific activity 200 Ci/mol), 20 μl of the enzyme solution or cells in suspension, and 30 μl of 0.2 M Tris-HCl, pH 8.0. [^{3}H]Methanol released was measured in a Beckman LS-355 liquid scintillation spectrometer (counting efficiency 40%). One Tos-Arg-OMe esterase unit (E.U.) is defined as that amount of enzyme which hydrolyzes 1.0 μmol of Tos-Arg-OMe/min at pH 8.0 and 30°C using a human urinary kallikrein as a standard in a titrimetric assay [17,20].

Kallikrein biological assay. The biological activity of kallikrein was determined by incubating 0.3 ml of 0.2 M Tris-HCl buffer, pH 8.0, 0.2 ml of renal cells in suspension ($1.5 \cdot 10^7$ cells/ml) in the buffer containing 2 mM $CaCl_2$, 0.1 ml of dog plasma kininogen (dog plasma heated to 60°C, 30 min and acidified

to pH 2.0 with 0.1 N HCl, incubated at 37°C for 10 min and neutralized to pH 7.3) and 0.02 ml of SQ 20881, an inhibitor of kininase II, (6 mg/ml) for 30 min at 37°C. Renal cells were removed by centrifugation at 1000 × *g* for 10 min and aliquots of the supernatant were assayed for biological activity. The kinin released was measured on the isolated guinea pig ileum [21] using bradykinin as a standard.

Immunodiffusion in agarose. Double-diffusion analysis [22] was done at room temperature overnight in 1.0% agarose in 0.1 M phosphate buffered saline, pH 7.5, containing 0.05% NaN_3.

Results

Renal cortical cell integrity

The mean cell number/pair of renal cortices obtained from ten normal rats was $8.9 \cdot 10^7$ cells (range $6.3 \cdot 10^7$—$12 \cdot 10^7$ cells). The percentage of viable cells determined by trypan blue exclusion ranged between 90 and 80% for up to 5 h of incubation in α-medium. The integrity of these cells was further determined by the linear incorporation of L-[U-^{14}C]leucine into newly synthesized proteins for at least 5 h. Cycloheximide (10 μg/ml) blocked protein synthesis completely (Fig. 1).

Alkaline esterase activity on external cell surfaces

The hydrolysis of Tos-Arg-O[^{3}H]Me by freshly prepared intact renal cells was measured at pH 8.0. About 10—20% of the substrate was normally hydrolyzed during the 30 min assay incubation period. Fig. 2 shows that this alkaline esterase activity was dependent upon cell number and that the radioactive-labelled substrate was not found within or upon the cells. The lack of substrate penetration into cells was also tested in the following ways. After incubation of the same numbers of renal cells (as in Fig. 2) with substrate under the assay conditions, the cells were trapped on Millipore filters and washed with the buffer; the filters were dried under a heat lamp and counted in a toluene-based scintillator. No significant radioactivity was found upon the filters (less than 50 cpm/10^5 cells). Another method of testing substrate penetration was by incubation of renal cells ($5 \cdot 10^5$—$2 \cdot 10^6$ cells) with the radiolabelled substrate for 30 min, centrifugation at 1000 × *g* for 10 min, washing once with the buffer, and resuspension in 0.1 ml of H_2O. The the cells were dissolved and fully lysed in 10 ml of water-soluble counting cocktail (Aquasol, Beckman) and counted. Again, no significant cellular radioactivity was found (less than 70 cpm/10^6 cells). Approximately 90% of cells excluded trypan blue before and after the 30 min assay incubation period. The measured esterase activity of renal cells in suspension was linear with time up to 60 min (data not shown). This suggests that cell breakage during the course of incubation was not adding appreciable esterase activity, and that enzyme activity in the medium or on the cells was not increasing within this time period [23]. Therefore, possible artifacts related to increased breakage of cells under the assay conditions were ruled out. Most of the measured esterase activity appeared to be on the cell surface since suspension supernatants obtained after 30 min of incubation by centrifugation at 1000 × *g* for 10 min contained from

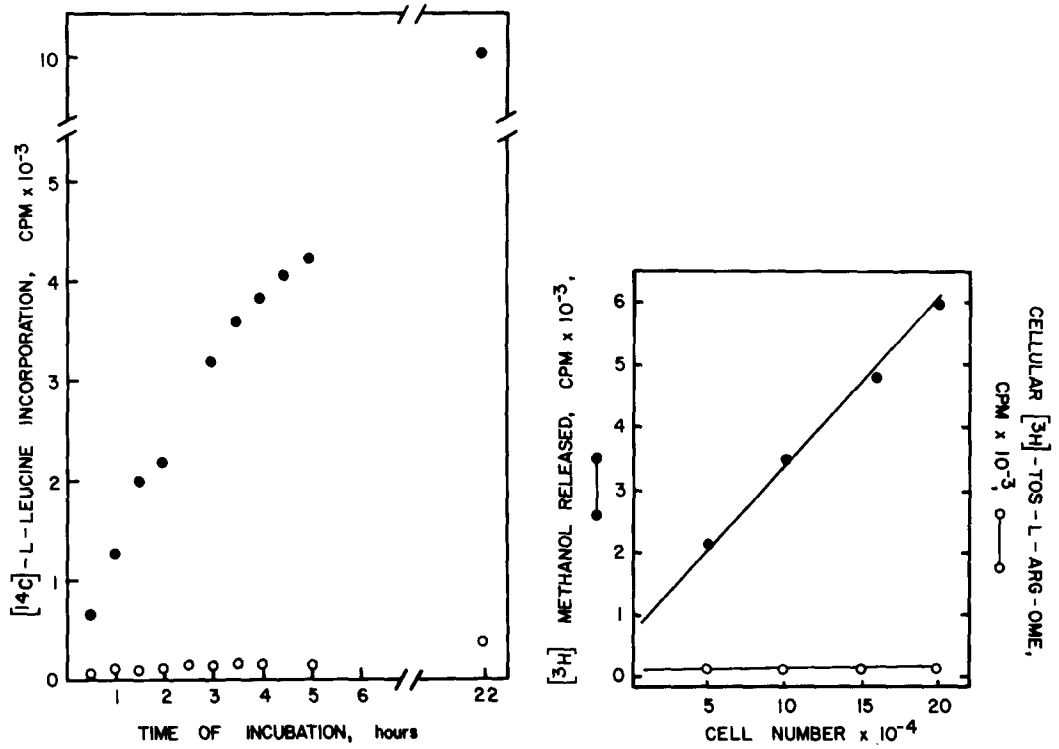

Fig. 1. L-[U-^{14}C]Leucine incorporation in renal cortical cell suspensions in the presence and absence of cycloheximide (10 μg/ml). 1 ml cell suspensions (4.3 · 10^6 cells/ml) were incubated in α-medium without L-leucine in 25-ml Erlenmeyer flasks at 25°C under 95% O_2/5% CO_2, with shaking. L-[U-^{14}C]Leucine, 5 μCi/ml, was added at time zero. 25 μl aliquots were withdrawn at the times indicated. 5 ml of 5% trichloroacetic acid containing 5 mM L-leucine was added to the aliquots, which were boiled for 5 min, cooled and then filtered through glass-fiber filter papers (Gelman). The filters were washed twice with 3 ml each of H_2O and then twice with 3 ml each of 95% alcohol. They were then dried under a heat lamp and counted in a toluene-based scintillator. ●———●, no added cycloheximide; ○———○, cycloheximide.

Fig. 2. Esterase activity of freshly prepared intact renal cortical cells. Assays were done at room temperature in 1.5 ml polypropylene tubes. Renal cells (5 · 10^6/ml) in phosphate-buffered saline containing 2 mM Ca^{2+} (cell numbers as indicated in the figure) and 0.2 M Tris-HCl buffer, pH 8.0, were mixed to a total volume of 50 μl. Tos-Arg-O[^{3}H]Me (3.0 · 10^4 cpm, 10 μl) was added, mixed and allowed to incubate for 30 min. The reaction was stopped and [^{3}H]methanol released measured as described previously [19]. The ability of Tos-Arg-O[^{3}H]Me to penetrate renal cortical cells was determined using the same incubation conditions. After 30 min incubation time, the mixture was diluted in 2 ml of the buffer, immediately vacuum filtered through Millipore membrane filters (0.45 μm pore size, prewashed with boiled and then cold water) and washed twice with the same buffer, 2 ml each. The cells were trapped on the filters. Cellular radioactivity was determined by counting the filters in a Triton X-100-based aqueous scintillator. ●———●, renal cell esterase activity; ○———○, cellular Tos-Arg-O[^{3}H]Me content.

14.0 to 18.8% (mean 16.5%, n = 5) of whole cell suspension activity. After cells (5 · 10^6—1.5 · 10^7/ml) were incubated in nutrient media under 95% O_2/5% CO_2 for up to 16 h at 25°C, at least 65% of total suspension esterase activity was still associated with the cells.

Cell surface esterase activity was inhibited by glandular kallikrein inhibitors including aprotinin and several amidine derivatives (Table I). Ovomucoid trypsin inhibitor did not inhibit cells surface esterase activity in concentrations of up to 1.7 mg/ml.

TABLE I

INHIBITION OF RENAL CELL SURFACE ESTERASE ACTIVITY

IC_{50} is the inhibitor concentration giving 50% inhibition. 20 μl of renal cells ($5 \cdot 10^6$/ml), 10 μl of inhibitor (at various concentrations) and 20 μl of 0.2 M Tris-HCl buffer, pH 8.0, were mixed and allowed to stand for 20 min. Measurement of Tos-Arg-O-[^{3}H]Me hydrolysis was as described in the legend for Fig. 2. Each value represents the average of three experiments in duplicate.

Inhibitor	IC_{50}
Aprotinin	6.5 units/ml
Benzamidine	$4 \cdot 10^{-4}$ M
Pentamidine	$3 \cdot 10^{-6}$ M
IRT-63	$9 \cdot 10^{-7}$ M

Cell surface kinin-generating activity

Intact renal cells released kinin from a kininogen substrate in the presence of SQ 20881 (200 μg/ml) and caused a slow contraction of the guinea pig ileum (Fig. 3). The addition of carboxypeptidase B to the tissue bath abolished the effect. Renal cells or kininogen alone caused no contractile response. When the glandular kallikrein inhibitors, aprotinin [24] or a substituted tris-amidine (IRT-63) [25,26] were preincubated with renal cells, the contractile responses were markedly inhibited. Preincubation of renal cells with the kallikrein anti-

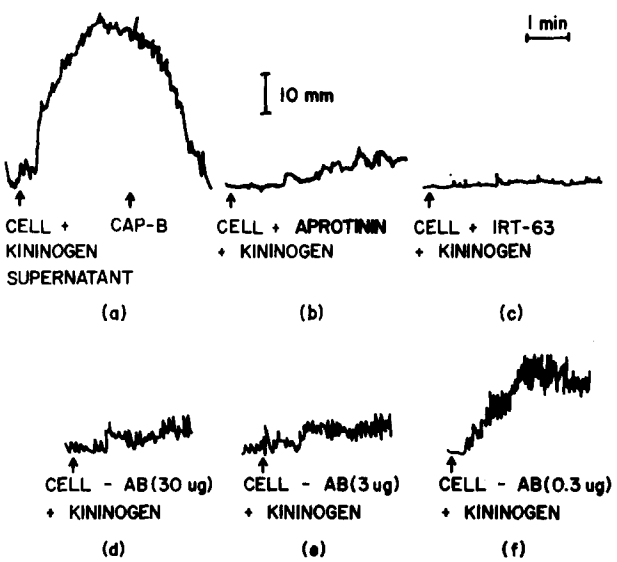

Fig. 3. Bioassay of renal cell surface kallikrein activity using the isolated guinea pig ileum. 0.1 ml of kininogen substrate (prepared as described in Materials and Methods) was incubated with 0.2 ml of renal cells ($1.5 \cdot 10^7$/ml) in the buffer containing 2 mM Ca^{2+}, 0.02 ml of SQ 20881 (6 mg/ml) and 0.3 ml of 0.2 M Tris-HCl buffer, pH 8.0, for 30 min at 37°C. Renal cells were removed by centrifugation and aliquots of the supernatant were assayed for kinin-releasing activity. A 0.2 ml aliquot produced a slow contractile response equivalent to that seen with 20 ng of synthetic bradykinin. Addition of carboxypeptidase B (2 units/ml) to the 10 ml tissue bath abolished the contractile response (a). Preincubation of renal cells with aprotinin (100 units) before the addition of kininogen inhibited the contractile response (b). Preincubation of renal cells with IRT-63 (10^{-5} M) (c), or with urinary kallikrein-antibody, 30 μg (d), 3.0 μg (e) and 0.3 μg (f), also abolished or attenuated the response.

body (fractionated from 45% ammonium sulfate) for 30 min inhibited the cell surface kinin-generating activity (Fig. 3), but not cell surface esterase activity. Kinin-forming activity of renal cells was not inhibited by the serum obtained from the sheep prior to immunization with kallikrein. This indicates that kinin destruction by any serum kininase activity was not responsible for the observed inhibition.

Other properties of cell surface kallikrein

Double -immunodiffusion analysis of kallikrein elaborated by intact renal cells showed a precipitin line with the urinary kallikrein antibody which was not identical with that seen with purified rat urinary kallikrein (Fig. 4). Rat spleen cells showed no cross-reactivity with the antibody. No precipitin line appeared between renal cells and normal sheep serum (data not shown).

The pH profile of purified rat urinary or solubilized renal kallikrein is known to follow a Gaussian distribution with an optimum of 9.0 [13,18], whereas the pH profile of renal cell surface kallikrein esterase increased almost linearly as the alkalinity of the buffers increased (Fig. 5). This increase in activity was not due to 'leakage' of the enzyme to the medium at higher pH, since preincuba-

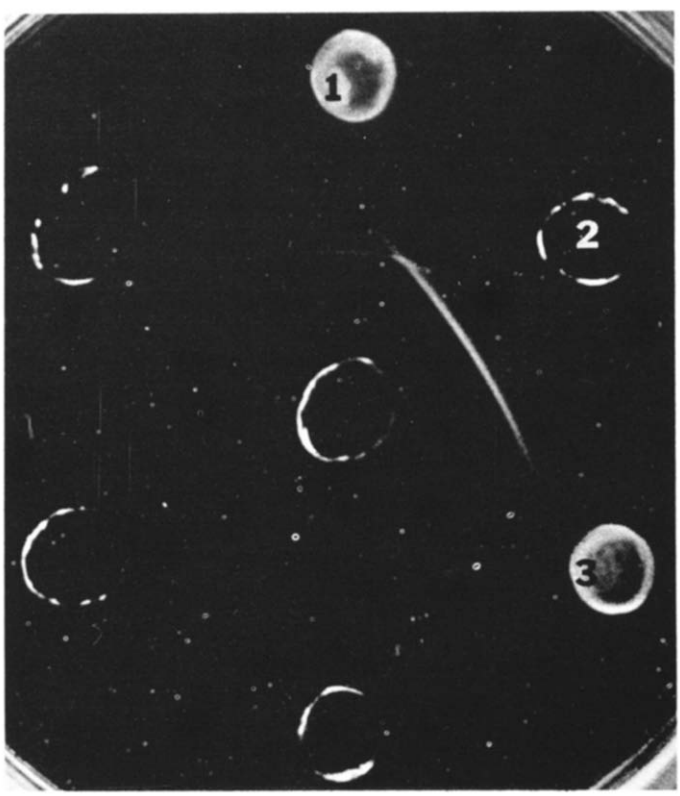

Fig. 4. Double-diffusion analysis of intact renal cells. Center well: Rat urinary kallikrein antibody (10 mg/ml X 5); peripheral wells: (1) renal cortical cells ($1.5 \cdot 10^7$/ml X 5); (2) purified rat urinary kallikrein (28 E.U./ml X 5); and (3) rat spleen cells ($4 \cdot 10^7$/ml X 5).

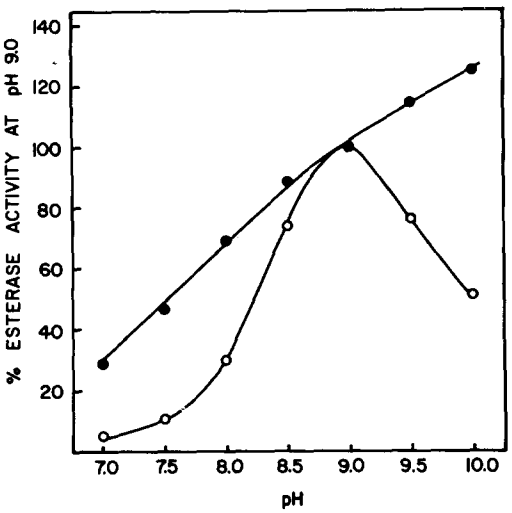

Fig. 5. Effect of pH on the Tos-Arg-O[^{3}H]Me esterase activity of renal cell surface kallikrein and rat urinary kallikrein. 20 μl of renal cells ($5 \cdot 10^6$/ml) in the buffer containing 2 mM Ca^{2+} or urinary kallikrein ($1.5 \cdot 10^{-2}$ E.U./ml) was added to 30 μl of 0.2 M Tris-HCl buffer (up to pH 9.0) or 0.2 M glycine/NaOH buffer (beyond pH 9.0) and incubated for 20 min. Tos-Arg-O[^{3}H]Me hydrolysis was determined as described in the legend for Fig. 2. ●———●, renal cells; ○———○, rat urinary kallikrein.

tion of renal cells at pH values up to 10.0 did not increase the amount of enzyme activity more than 10% in supernatants.

Preincubation of renal cell suspension for 30 min with phospholipase A_2 (0.02—0.2 U/ml) was found to increase total suspension esterase activity more than 100%. These low concentrations of phospholipase A_2 had no effect on the esterase activity of purified rat urinary kallikrein. Table II shows that phospholipase A_2 predominantly increased cells surface esterase activity (76%) rather than releasing it into the medium. In contrast, preincubation with deoxycholate (0.1%) induced cell lysis and the release of activity to the supernatant (Table II).

TABLE II

EFFECTS OF PHOSPHOLIPASE A_2 OR DEOXYCHOLATE PREINCUBATION ON RENAL CELL SURFACE AND SUPERNATANT ESTERASE ACTIVITY

0.3 ml of renal cells ($1 \cdot 10^7$/ml) in the buffer containing 2 mM Ca^{2+}, 0.2 ml of 0.2 M Tris-HCl buffer, pH 8.0, were incubated with 0.1 ml of phospholipase A_2 or deoxycholate at the indicated concentration at room temperature for 30 min. Cells were then centrifuged at 1000 × *g* for 10 min and resuspended in the same buffer. 20 μl of cells or supernatant were assayed for Tos-Arg-O[^{3}H]Me esterase activity as described in the legend for Fig. 2. The enzyme activity is expressed as the percentage of total control (supernatant plus cell) activity (mean ± S.E.).

Treatment	Concentration	% of total control activity	
		Cell surface	Supernatant
Control (n = 5)	—	83.6 ± 1.2	16.4 ± 1.2
Phospholipase A_2 (n = 5)	0.1 U/ml	147.4 ± 10.5	23.5 ± 4.0
Deoxycholate (n = 4)	0.1%	11.5 ± 3.4	83.3 ± 15.5

Discussion

The results show that the active sites for Tos-Arg-OMe esterase and kinin-generating activity are apparent on the surface of viable renal cortical cells. The cell surface Tos-Arg-OMe esterase activity can be ascribed to kallikrein for the following reasons. First, both Tos-Arg-OMe esterase and kinin-generating activities are inhibited by aprotinin and IRT-63. Second, like purified urinary kallikrein [18], or renal cortical homogenate kallikrein [13], the cell surface esterase activity is not inhibited by ovomucoid trypsin inhibitor. This also rules out the presence of trace amounts of trypsin, which would be inhibited by this high concentration of trypsin inhibitor. Third, it has been shown that DEAE-cellulose column chromatography of solubilized renal cortical homogenates reveals a single peak of Tos-Arg-OMe esterase and kinin-generating activity [13]. Fourth, Nustad et al. [27] and Nustad and Pierce [17] have shown that alkaline esterase activity upon various substrates including Tos-Arg-OMe is always associated with kinin-generating activity in studies of renal slice kallikrein synthesis, and of purified urinary kallikreins. Finally, Ward et al. [3] have shown that Tos-Arg-OMe esterase and kinin-generating activity increased in parallel as renal plasma membrane fractions were purified. These findings suggest that the cell surface esterase and kinin-generating activity can be ascribed to the same enzyme, kallikrein. However, cell surface esterase activity was not inhibited by antibody against rat urinary kallikrein which does inhibit kinin-generating activity of the cells. Nustad and Pierce [17] also found that the esterase activity of purified urinary kallikreins were not inhibited by an antibody which clearly inhibited their kinin-generating action. α_2-Macroglobulin inhibits kinin-releasing but only slightly inhibits the esterolytic activity of plasma kallikrein [28]. A kallikrein inhibitor found in rat renal preparations inhibited kinin-forming, but not esterolytic activity of porcine pancreatic or rat urinary kallikrein [29]. Collectively, the data suggest that higher molecular weight inhibitors or antibody block the hydrolytic center(s) of kallikrein by some steric effect which does not prevent access to these sites by smaller substrates to such as Tos-Arg-OMe.

Substrate hydrolysis by intact cells may occur either outside the cell where products remain, or inside the cell with products subsequently transported to the exterior [23]. It seems unlikely that either Tos-Arg-O[^{3}H]Me or kininogen penetrate the cells. First, no radioactivity was found within cells after incubation with Tos-Arg-O[^{3}H]Me under our standard conditions (Fig. 2). In addition, Millipore filter-trapped cells, or cells centrifuged, washed and dissolved in water-soluble counting cocktail after incubation with substrate contained no significant radioactivity. Incubating the intact cells with kininogen substrate produced biological activity (smooth muscle contraction) which was blocked by anti-urinary kallikrein antibody. Dog plasma kininogens (α-globulins, molecular weight range 57 000—197 000) are unlikely to penetrate intact cells [30]. Collectively, these data support the notion that kallikrein's active sites are on the cell surface.

Double-immunodiffusion analysis showed that the antibody formed a single precipitin line with enzyme which diffused from intact cortical cells placed in an Ouchterlony plate. However, the precipitin line was non-identical with that

produced by purified rat urinary kallikrein. This suggests that kallikrein released from the cells is antigenically dissimilar to purified urinary enzyme or to solubilized renal kallikrein [13,18].

The increase in cell surface kallikrein esterase activity with increasing pH suggests increased binding of positively charged Tos-Arg-OMe to the membrane-bound enzyme or increased access of the substrate to additional enzyme. Goldstein et al. [31] have shown that the pH optimum of soluble trypsin is displaced towards more alkaline values, using α-*N*-benzoyl-L-arginine ethyl ester as the substrate, when the enzyme was covalently bonded to negatively charged maleic anhydride-ethylene copolymers. One attractive possibility is that membrane negative charges, which generate a surface potential, attract the positively charged substrate towards the membrane such that the substrate concentration in the region of membrane-bound kallikrein is higher than the bulk concentration in the medium bathing the membrane (Bartschat, D.K., personal communication). Whether the present results indicate that the renal cellular kallikrein could be closely associated with negatively charged components of the cell membrane remains to be determined.

The finding that phospholipase A_2 stimulates renal cell surface esterase activity is consistent with an hypothesis that membrane structural changes induced by phospholipase A_2 affect the activity of membrane-bound kallikrein. However, increased membrane-bound kininogenase activity after phospholipase A_2 has not yet been demonstrated, although others [32] have confirmed our finding of activation of membrane-bound 'kallilkrein-like' esterase activity by phospholipase A_2.

In summary, the data show that membrane-bound kallikrein associated with rat renal cortical cells in suspension is accessible to substrates, inhibitors, and antibody. It is likely that ecto-kallikrein at the renal distal tubular cell surface is appropriately oriented for action upon a kininogen within tubular fluid and possibly present in urine [33,34] to produce kinins. These products, known to enter urine along the distal nephron [35] may then influence membrane permeability and transport processes directly, or via their previously observed stimulation of prostaglandin production [36].

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