The effects of aldosterone and spironolactone on renal kallikrein in the rat

H. S. MARGOLIUS, JULIE CHAO AND T. KAIZU
Department of Basic and Clinical Pharmacology, Medical University of South Carolina, Charleston, South Carolina, U.S.A.

Summary
1. A technique has been developed for the measurement of kallikrein ‘production’ in rat renal cortical cells in suspension.
2. After preparative steps, column chromatography on DEAE-cellulose yielded a peak of α-N-tosyl-L-arginine methyl ester (Tos-Arg-OMe) esterase activity identical with kallikrein isolated from rat urine in respect of pH optimum, effects of inhibitors, biological activity and immunological properties.
3. The nutrient medium surrounding incubated cells contained measurable kallikrein activity, which was increased by aldosterone and decreased by spironolactone.
4. The results raised the possibility that kallikrein could be an aldosterone-induced protein.

Key words: aldosterone, hypertension, kallikrein, kidney, sodium, spironolactone.

Introduction
Urinary kallikrein (kinogenin, EC 3.4.21.8) excretion is abnormal in humans (Margolius, Geller, Pisano & Sjoerdsma, 1971) and rats (Margolius, Geller, De Jong, Pisano & Sjoerdsma, 1972) with hypertension. Although many factors may regulate kallikrein excretion, it is probable that aldosterone is a major regulatory influence. Low dietary sodium (Geller, Margolius, Pisano & Kieser, 1972; Margolius, Horwitz, Pisano & Keiser, 1974a) or high dietary potassium (Horwitz, Margolius, & Keiser, 1975) increase urinary kallikrein excretion. Administration of deoxycorticosterone to rats (Geller et al., 1972) or fludrocortisone to man (Adetuyibi & Mills, 1972; Margolius et al., 1974a) does likewise, whereas adrenalectomy (Geller et al., 1972) reduces kallikrein excretion. Kallikrein excretion is elevated in human primary aldosteronism (Margolius et al., 1971; Margolius, Horwitz, Pisano & Keiser, 1974b) and in Bartter’s syndrome (P. V. Halushka, H. J. Wohltmann, P. J. Privitera & H. S. Margolius, unpublished work). Finally, a specific aldosterone antagonist, spironolactone, decreases kallikrein excretion in normal human subjects, as well as in patients with primary aldosteronism (Margolius et al., 1974a, b). The present study was designed to determine whether changes in urinary kallikrein excretion induced by aldosterone or spironolactone are a direct reflection of events within renal cells.

Methods
Suspensions of renal cortical cells from the kidneys of male Sprague-Dawley rats were prepared according to the method of Kaizu & Margolius (1975). The percentage of viable cells was determined by the method of Hoskins, Meynell & Sanders (1956), and ranged between 85 and 95%.

Cells (>10⁶) were suspended in a sucrose-Tris buffer, pH 7.5, containing sodium deoxycholate and sodium EDTA. The cells were homogenized and the homogenate was centrifuged at 20000 g for 1 h. The supernatant was dialysed and then gel filtered on Sephadex G-25 in a centrifugal filter holder. Deoxycholate-solubilized kallikrein from homogenates of 3.5–11·0 × 10⁶ cells (112–342 mg of protein) or from the 1·0 ml of a medium (not
supplemented with foetal calf serum: Stanners, Eliceiri, & Green, 1971) surrounding the cell sus-

censions was adjusted to contain 10.0 ml of NaCl (0.12 mol/l)/sodium phosphate (0.01 mol/l), pH 7.0, and
adsorbed on to DEAE-cellulose (2.5 ml) equilibrated with the same buffer. The column was washed with
20 ml of the same buffer to remove an interfering minor esterase (Kaizu & Margolius, 1975). Kalli-
krein was eluted with 15.0 ml of NaCl (0.25 mol/l)/
sodium phosphate (0.01 mol/l), pH 7.0. Fractions with Tos-Arg-OMe esterase activity were pooled and
assayed for Tos-Arg-OMe esterase and biological activity as described below.

A modification (Margolius et al., 1974a) of the assay of Beaver, Pierce & Pisano (1971) was always
used to determine Tos-Arg-OMe esterase activity, a measure of kallikrein-like enzyme activity. Released
$[^3]$H]methanol was measured by liquid-scintillation spectrometry. One Tos-Arg-OMe esterase unit is
deﬁned as that amount of enzyme which hydrolyses 1.0 μmol of Tos-Arg-OMe/min at pH 8.0 and 30°C
in a standard titrimetric assay (Beaven et al., 1971).

Tos-Arg-OMe esterase activity from rat renal cortical cells was identiﬁed as kallikrein by com-
parison with puriﬁed rat urinary (speciﬁc activity 14.0 units/A280) and rat kidney (speciﬁc activity
2.1 units/A280) kallikrein for pH optima, the effects of aprotinin (Trasylo), soya bean, lima bean and
ovomucoid trypsin inhibitors, double-diffusion analysis (Ouchterlony, 1958) and biological activity by
bioassay with the guinea-pig ileum (Webster & Prado, 1970).

Pooled renal cortical cell suspensions of sixteen kidneys from eight rats were made. Equal numbers of
cells (6.0 × 10⁸) were incubated in 1.0 ml of α medium (Stanners et al., 1971) for either 3 or 8 h
under O₂/CO₂ (95:5) at 37°C in 5.0 ml plastic culture tubes. Kallikrein esterase activity was measured in both the cells and the supernatant medium in control suspensions and suspensions exposed to aldosterone (6.1 × 10⁻⁵–3.1 × 10⁻⁴ mol/l) or spironolactone (4.3 × 10⁻⁵–2.2 × 10⁻⁴ mol/l).

Results

The mean cell number for each renal cortex obtained from the twenty-four kidneys of twelve normal rats
was 5.7 × 10⁸ cells (range 3.0 × 10⁸ to 11.0 × 10⁸ cells/renal cortex). Of the Tos-Arg-OMe esterase activity 70% was eluted as a single peak on DEAE-
cellulose at a concentration corresponding to about 0.20 mol/l NaCl/0.01 mol/l sodium phosphate, pH
7.0. The pH optimum for Tos-Arg-OMe hydrolysis by the major renal cortical cell Tos-Arg-OMe
esterase was 9.0 and was identical with that of rat kidney kallikrein and rat urinary kallikrein. The
Tos-Arg-OMe esterase activity of the cortical cell esterase and the standard kallikrein preparations
was completely inhibited by aprotinin, strongly inhibited by soya bean trypsin inhibitor, but not
inhibited by either lima bean or ovomucoid trypsin inhibitors. Double-diffusion analysis with an un-
diluted antiserum (antibody titre approx. 2 mg/ml) (Nustad & Pierce, 1974) to rat urinary kallikrein
gave a single line of identity with rat urinary kalli-
krein and the renal cortical cell Tos-Arg-OMe esterase. Renal cortical cell Tos-Arg-OMe esterase
released kinin from heated dog plasma equivalent to 1–5 ng of bradykinin and caused a slow contrac-
tion of the guinea-pig ileum, abolished by carboxy-
peptidase B. Esterase activity ranged from 15.8 × 10⁻² to 72.1 × 10⁻² unit per kidney cortex homo-
genate in five separate experiments. The yield of rat
renal cortical cell kallikrein after DEAE-cellulose chromatography ranged from 7.1 × 10⁻² to 46.9 ×
10⁻² unit (40–110%, mean 70%). Recovery of added
known amounts of puriﬁed rat urinary kallikrein to
cell homogenates, applied to the DEAE-cellulose columns or added to the DEAE-cellulose column
fractions before assay, averaged 83, 97 and 108% re-
spectively. In nine separate kidneys, the amount of
rat renal cortical cell kallikrein after DEAE-cellulose chromatography ranged from 3.2 × 10⁻² to 46.9 ×
10⁻² unit/renal cortex (1.2–1.6 g wet weight). Bioassay of five of
these samples resulted in good agreement in the
majority of the samples. The calculated amounts of
rat renal cortical cell kallikrein esterase activity ranged from 0.6 × 10⁻¹⁰ to 4.6 × 10⁻¹⁰ unit/cell.

In contrast, in four experiments, each of which involved suspensions of renal cortical cells from the
sixteen kidneys of eight normal rats, kallikrein could
not be detected intracellularly under the present incubation conditions. However, in a representative
experiment, the medium surrounding duplicate sus-
pensions of 6.0 × 10⁸ (approximately 1.0 ml) cells
obtained from sixteen kidneys of eight normal rats,
kallikrein activity was 39.0 × 10⁻² and 72.4 × 10⁻²
unit after 3 and 8 h of incubation respectively.

Addition of aldosterone, 6.1 × 10⁻⁴ mol/l, to the
medium at the onset of the incubation period
resulted in an increase in kallikrein esterase activity
to 45.1 × 10⁻² and 91.8 × 10⁻² unit after 3 and 8 h
Kallikrein and mineralocorticoids

281s

of incubation respectively. Aldosterone, $3.1 \times 10^{-4}$ mol/l, increased kallikrein activity in the medium to $5.1 \times 10^{-2}$ and $1.616 \times 10^{-2}$ (+223%) unit after 3 and 8 h of incubation. Spironolactone, $4.3 \times 10^{-5}$ mol/l, added to the medium at the onset of the incubation period, resulted in a decrease in kallikrein activity to 19.5 $\times 10^{-2}$ and 3.18 $\times 10^{-2}$ unit after 3 and 8 h of incubation respectively. Spironolactone, 2.2 $\times 10^{-4}$ mol/l, further decreased extracellular kallikrein concentration to $1.35 \times 10^{-2}$ and 3.01 $\times 10^{-2}$ unit after 3 and 8 h of incubation.

Discussion

We have isolated and measured a renal cortical cell Tos-Arg-OMe esterase within non-incubated rat renal cortical cells and in media surrounding incubated cells in suspension. The Tos-Arg-OMe esterase appears to be identical with rat urinary or kidney kallikrein in pH optima, effects of inhibitors and the ability to release a biologically active kinin from substrate. There was also immunological identity between this esterase and a purified rat urinary kallikrein when an antiserum obtained to the latter was used. Thus this renal cortical cell Tos-Arg-OMe esterase appears to be a kallikrein within and around a heterogeneous population of viable renal cortical cells in suspension. Cells suspended in media and incubated under O$_2$/CO$_2$ for 3-8 h showed no detectable kallikrein activity intracellularly, whereas extracellular activities were measurable. Furthermore, addition of aldosterone caused an increase and spironolactone a decrease in extracellular kallikrein activity. Although the concentrations of aldosterone or spironolactone used in the present study were extremely high, these findings are consistent with the observations that aldosterone or other mineralocorticoids increase kallikrein excretion in human and rat urine, whereas spironolactone, a specific aldosterone antagonist, decreases kallikrein excretion.

These data suggest that changes in urinary kallikrein excretion caused by mineralocorticoids and an antagonist in man and animals reflect changes in renal cellular kallikrein production. Low to absent kallikrein excretion occurs in adrenalectomized rats (Geller et al., 1972) and the results of this study suggest that renal kallikrein production could be mineralocorticoid-dependent. These changes in production could represent altered release or degradation of the enzyme, but might suggest changes in kallikrein synthesis, raising the possibility that synthesis is a mineralocorticoid-dependent process.

The role of the renal kallikrein-kinin system is unclear. Fig. 1 shows one hypothesis on the role of kallikrein in the distal portion of the nephron. It is based upon the observed relationships between kallikrein and mineralocorticoids in man, animals and the data from isolated renal cortical cells reported here. Kallikrein could be an aldosterone, induced protein directly involved in the mechanism of the antinatriuretic action of aldosterone and the natriuretic actions of spironolactone. Kallikrein synthesis stimulated by aldosterone could result in the local production of a kinin, which acts either to affect membrane permeability to sodium, available energy for the sodium pump, or the sodium pump directly. Recent stop-flow studies in the dog (Carretero & Scicli, 1976) seem to show that kallikrein enters the urine in the distal nephron, and Ward, Gedney, Dowben & Erdos (1975) have shown renal kallikrein to be bound to plasma membranes that seem to be of distal tubular origin. In addition, recent studies (C. Takeguchi, G. L. Lindenmayer & H. S. Margolius, unpublished work) have failed to show any effect of kinins on renal sodium–potassium-dependent adenosine triphosphatase activity. Collectively, the data suggest that if kallikrein were an
aldosterone-induced protein, it would act upon kininogen within the cell or available in tubular fluid to generate a kinin which could then function as an ionophore or permease, increasing the influx of sodium from tubule to cell. In addition, this locally generated kinin may act to increase the activity of prostaglandin synthetase at a more distal site (McGiff, Itskovitz, Terragno & Wong, 1976) in the renal medulla resulting in the generation of prostaglandin E₂. Prostaglandin E₂ produced at that site would also function as an antinatriuretic substance. Support for this portion of our hypothesis is derived from the data of Gill, Alexander, Halushka, Pisano & Keiser (1975), who showed that inhibition of prostaglandin synthesis by indomethacin in hypophysectomized dogs resulted in inhibition of distal nephron sodium reabsorption. Similarly, either meclofenamate or the competitive prostaglandin inhibitor RO 20-5720 given to unanaesthetized dogs also resulted in a natriuresis without changes in urinary volume or potassium excretion during water diuresis, suggesting that the resultant natriuresis was due to diminished sodium reabsorption beyond the distal tubule (Kirschenbaum & Stein, 1976). These data are compatible with those of Lipson & Sharp (1971), who showed that the application of prostaglandin E₂ to the serosal side of the toad bladder increased short-circuit current and sodium transport.

Although other possible explanations exist for the gathered data, we think this hypothesis is simple and susceptible of disproof. Studies are under way to determine whether or not kallikrein is an aldosterone-induced protein. The possibility that it is raises many questions about abnormal kallikrein excretion in hypertension, especially in those patients with essential hypertension having low or absent urinary kallikrein excretion.

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References


