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Salt Loading Effect on Renal Kallikrein Activity in Rat (40738)

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It has been shown that angiotensins promote sodium retention through aldosterone, whereas kinins acting on the kidney accelerate sodium loss (1). Although the rate of kallikrein excretion in the urine under several experimental conditions in the dog (2), in the rat (3), and man (4) appears to be directly related to the rate of sodium excretion, other data suggest that mineralocorticoids are the factors regulating kallikrein excretion (5, 6). Acute NaCl loading induces in the rat a rapid and considerable increase in the urinary kallikrein excretory rate, suggesting that the release of renal kallikrein is rapidly stimulated, by a still unknown mechanism (7). This result suggests that other factors are equally important and thus may support one of two possibilities: the existance of a sodium receptor in the gastrointestinal tract or that a rapid change following the sodium absorption is able to act upon the renal kallikrein system. In order to investigate how rapidly the kallikrein–kinin system in the kidney can respond to sodium, renal kallikrein activity was measured in rats at different intervals (5, 10, 20, 30, and 60 min), after NaCl loading by gavage.

In addition the renal kallikrein activity was tested in rats loaded with distilled water at the same time intervals.

Methods. Male Sprague–Dawley rats, 200–300 g, fed *ad libitum* were used. All rats (except for one set of control rats), were submitted to loading via stomach tube, according to the procedure described by Barnafi *et al.* (8).

Two groups of rats were submitted to the following treatment: One was loaded with distilled water and the other received a sodium chloride solution (0.342 mol/liter). In either case the amount of load was equal to 5% of body weight. Two types of nonloaded control rats were used: (a) nontreated rats and (b) rats submitted to sham-gavage procedure: the tube was introduced in the stomach but no solution was administered. Unanesthetized rats at a given time (5 to 60 min) after treatment were decapitated, and the kidney quickly removed, decapsulated, weighed, and minced with scissors. The minced tissue was suspended in 4 vol of 0.250 M sucrose solution containing 0.010 M Hepes, pH 7.4, and homogenized with three full strokes at 1000 rpm using a 50-ml Potter–Elvehjem type homogenizer (inside diameter of the glass vessel, 1.000 in.) and Teflon pestle machined to a diameter of 0.988 in. The homogenate was filtered through a 110 mesh nylon monofilament bolting cloth, diluted with 4 vol of cold distilled water and adjusted to pH 5.0 with 0.200 M sodium acetate buffer, pH 4.5, incubated for 2 hr at 37°C (9), and was then adjusted to pH 7.4 with dilute NaOH. Control rats submitted to the sham gavage were decapitated 5 min after treatment.

Protein in the renal homogenate was estimated by the Lowry *et al.* (10) procedure, using crystalline bovine serum albumin as standard. In most of the experiments kallikrein activity (k.a.) in the homogenate was measured by its stimulating effect on uterine contractility (11) expressed as nanograms of bradykinin equivalents per milligram of protein.

Aprotinin was used as a specific inhibitor of kallikrein activity. The addition of aprotinin as described in (11) produced a complete inhibition of the effect of both control and experimental renal homogenates upon isolated rat uterus.

In order to correlate the data obtained by this method with the data of other methods currently used to determine k.a., we prepared three pools of kidney homogenates of five rats each: (1) nonloaded rats, (2) loaded with distilled water 5% body weight, and (3) loaded with 0.342 M NaCl solution 5% body

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weight. The rats of the last two groups were decapitated 5 min after treatment. The kidneys of the different groups were homogenized and submitted to the same procedure. In each of these three kidney homogenates kallikrein activity was measured: (a) testing the stimulating effect on uterine contractility (11), (b) by kininogenase activity (11), and (c) measuring the esterase activity (9). The results of these procedures are shown in Table I.

When kallikrein activity was measured by its effect upon the isolated rat uterus, it was expressed in nanograms of bradykinin equivalents. For this purpose a freshly prepared solution of bradykinin was used as standard. The measurement of kallikrein activity by its kininogenase effect was carried out using human kininogen II prepared by the method of Jacobsen (12). The substrate and renal extract were incubated for 2 min at 37°C and pH 7.4. The amount of bradykinin formed after the incubation was tested by using cat jejunum. Kallikrein activity was expressed in nanograms of bradykinin per minute per milligram of protein of the kidney extract.

Benzoyl-L-arginine-ethyl/ester (BAEE) esterase activity was determined spectrophotometrically according to the enzymatic assay described by Carvalho and Diniz (9), buffered at pH 6.5, following the color reaction by the method of Brown (13). Activity is expressed in nanomoles per minute and milligrams of protein (nmol/min/mg protein).

Results. Kallikrein activities in the kidneys of control rats distilled water-loaded and NaCl-loaded rats, removed at different times after gavage, are shown in Fig. 1. Measured by bioassay, the mean k.a. of kidney homogenates from nontreated control rats was 1.11 ± 0.09 ng of bradykinin equivalent/mg of protein. An approximately sixfold increase was observed in the NaCl-loaded rats, 6.71 ± 0.42 ng of bradykinin equivalent/mg protein ($P < 0.001$) in the kidney removed 5 min after gavage. After this initial peak the mean k.a. decreased considerably. At 10 min after gavage k.a. was 1.95 ± 0.23 ng of bradykinin equivalent/mg protein about one-third of the maximum activity, but still higher

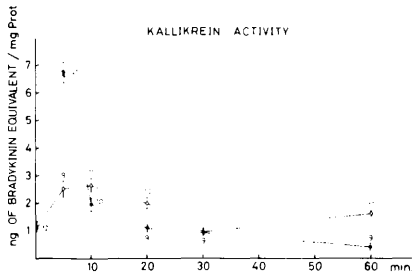

FIG. 1. Kallikrein activity of renal homogenates after NaCl loading (solid circles) and distilled water loading (open circles). Number of animals tested is shown in parentheses; results are shown as mean values ± SE. The lines between the points are for ease of visualization, and are not meant to imply the shape of the curve. The values at zero time were obtained from nontreated rats.

than the control ($P < 0.01$). At 20 and 30 min k.a. was not significantly different from the control, but 60 min after gavage we found the value to be significantly lower than the control ($P < 0.001$). Water loading produced a slight but significant increase ($P < 0.001$) for about half an hour. At 5, 10, and 20 min after gavage values were 2.52 ± 0.30, 2.65 ± 0.26, and 1.99 ± 0.20 ng of bradykinin equivalent, respectively.

The mean value of 1.68 ± 0.20 ng of bradykinin equivalent found in the nine rats submitted to sham gavage and sacrificed 5 min later, was somewhat higher than the value obtained in nontreated rats ($P < 0.02$). Nevertheless in water-loaded rats k.a. at 5 min was 50% higher than the sham value ($P < 0.001$); the highest activity in the water-loaded group was found at 10 min and was nearly 60% higher than the same sham rats ($P < 0.001$).

Table I shows the k.a. measured by three different methods in pools of kidney homogenates corresponding to three groups of rats: control, water loaded, and NaCl loaded.

Discussion. The transient and rapid change in k.a. are very striking. The greatest value was found 5 min after NaCl loading, but it is possible that earlier changes may occur in the mechanism which synthesizes or activates renal kallikrein.

It is likely that the rapid increase in NaCl in the extracellular fluid or in the glomerular filtrate can be directly sensored by the

TABLE I. RENAL KALLIKREIN LEVELS ESTIMATED BY THREE DIFFERENT METHODS[a]

	Uterine contractility effect[b]	Kininogenase activity[c]	Esterase Activity[d]
Control, nonhydrated	1.03	8.7	41
Water-loaded	2.85	17.8	57
NaCl-loaded	7.39	27.7	172

[a] Water-loaded and NaCl-loaded values were obtained after 5 min of treatment. Values from pools of five animals for each of the three methods tested.
[b] Results expressed in ng bradykinin/mg protein.
[c] Results expressed in ng bradykinin/min/mg protein.
[d] Results expressed in nmol/min/mg protein.

cells in the distal tubule which synthesize or activate kallikrein. However, one can not preclude an indirect action by other humoral or nervous factors triggered by NaCl either in the gastrointestinal tract or elsewhere. Lennane *et al.* (14, 15) demonstrated that man as well as rabbits receiving oral doses of sodium chloride respond with a much greater natriuresis than when the doses were given intravenously. These results suggest the presence of a sodium sensor in the gut or portal circulation. Alternative explanations for the fast k.a. response to NaCl include: activation of a preenzyme, decreased catabolism, or inactivation of an endogenous kallikrein inhibitor. In addition acute NaCl administration induces also an accelerated excretion of kallikrein in urine (3). So the stimulating effect of NaCl upon the kidney may be more prolonged than the 5 min found in our experiment as the time of maximum activity.

The value found in rats submitted to sham gavage and sacrified 5 min later was only 0.57 ng of bradykinin equivalent/mg of protein higher than the control value found in the nontreated rats. This information coupled with the significant increases over the sham-gavage value produced by saline loading (300%) and distilled water loading (52%) demonstrates that the increases observed at 5 min are not the consequence of a nonspecific response to the handling or the stress induced by the gavage.

The data shown in Table I provide additional evidence that the stimulating effect on uterine contractility of the homogenates not only correlates with kininogenase activity and esterase activity, but also that 5 min after NaCl loading there is a significant increase in the activities as measured by all three methods.

Unfortunately, our experiments supply no evidence about the triggering factor which in the renal cell may induce this rapid and considerable increase of k.a.

Summary. The level of renal kallikrein activity in the rat has been determined at different times after a single NaCl loading by gavage.

The renal kallikrein activity increased rapidly, reaching its maximum level of 6.71 ng of bradykinin equivalents/mg of protein about 5 min after treatment and returning to control level, 1.11 ng of bradykinin equivalents/mg of protein, 20 min after gavage.

The levels of renal kallikrein activity found in rats submitted to sham gavage demonstrates that the increase is not the consequence of a nonspecific response to the handling or the stress induced by the gavage.

The results suggest that NaCl ingestion promotes very rapid increase in the level of renal kallikrein activity through some unknown cellular mechanism.

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