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Inhibitory Effect of Renin Extracts Upon Urinary Kallikrein Excretion[1] (40170)

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Evidence has been provided showing that the kallikrein-kinin system is altered in renal hypertension. In hypertensive rats, not only is kallikrein considerably decreased in the urine (1, 2) but it is also significantly diminished in the kidney (3). Furthermore blood kininogen is abnormally elevated (4). It has been postulated that the kallikrein enzymatic system could be involved in the mechanism of renal hypertension and could act as an antagonist of the reninangiotensin system (1, 5). In this connection it seemed of interest to investigate whether renin was able to change the excretory rate of urinary kallikrein. The present results show that the administration of purified renin extracts to hyperhydrated rats significantly inhibits kallikrein excretion in the urine.

Methods. Adults Sprague–Dawley rats of both sexes weighing 200–220 g were employed. To collect the urine they were placed in metabolic cages under similar experimental conditions to those described earlier (6).

Food was withdrawn the night before the experiment but the animals had free access to tap water. In each experiment 12 rats were used.

Hyperhydration was performed with distilled water, except when indicated, 5% body weight through a stomach tube. Immediately after gavage six control rats were injected intraperitoneally with 0.4 ml of 0.9% NaCl solution, and the experimental rats with the same volume of this solvent containing renin. Six experiments with hog kidney renin and five with rat kidney renin were performed. In the three experiments, using rat renin some modifications in the experimental design were introduced: in one experiment hyperhydration was carried out 60 min, and in an another 105 min after renin injection; in the third experiment 0.4% NaCl solution was employed instead of distilled water for gavage and renin was given simultaneously with fluid overloading. In all the experiments (11) urine collection started immediately after hyperhydration. For 2 hr the urine was measured every 15–30 min.

At the end of this period a second fluid overloading was performed with the same fluid volume as in the first one, but no injections were administered. Again for 2 hr urine was collected and measured every 30 min. Sodium and kallikrein in the urine samples corresponding to these two periods were measured.

Kallikrein activity was determined as described elsewhere (7). In this method the oxytocic activity of urine samples was measured using rat uterus immersed in Tyrode solution. Bradykinin solution prepared immediately before starting the bioassay was employed as the standard.

Kallikrein activity of urine was expressed in the equivalent of ng of bradykinin, according to its direct oxytocic effect. Preliminary experiments showed no significant differences in kallikrein activity between dialyzed and non dialyzed samples of urine. However, dialysis against 5% NaCl solution of every sample was performed before biological testing.

To confirm that the oxytocic effect of urine was due to kallikrein action, the effect of inhibitors such as aprotinin (Trasylol, Bayer) or DFP was determined as previously described (7). An excellent correlation was found between kininogenase activity and oxytocic effects in the urine. Kininogen II obtained from rat plasma (8) was used as substrate. For this method small amounts of urine are required (005–02 ml) to release measurable amount of kinins by incubating the urine with the substrate for 2 min. Such

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small amount offers the advantage to prevent the interference of eventual oxytocic substances, other than kallikrein, which can occur in the urine.

Sodium was determined with an Eppendorf flame photometer. The excretory rate was expressed in μEq per rat. Water diuresis was expressed as the porcentage of water volume administered to each rat after the first and second hyperhydrations. Two renin preparations were used: (a) Purified pig kidney (Nutritional Biochemical Lab., Cleveland).

The crystalline material was dissolved in 4 ml sterile NaCl 9% and stored frozen. One ml of the solution contained 25 I.U. (equivalent to 4.2 Goldblatt Units) of renin. Doses of 5, 2.5, and 1.25 of renin in a volume of 0.4 ml per rat were assayed in three different groups of six rats each. Simultaneously, control rats under similar conditions were injected with saline intraperitoneally.

(b) Rat kidney renin. To obtain purified rat renin the method of Corthorn *et al.* 1973 (9) was used. Rat kidneys (1Kg) free of adipose tissue were cut into four pieces each. Four volumes of cold acetone were added (−10°). The mixture was then stirred and stored in a cold room for 24 hr. The acetone was then removed and another similar volume of cold acetone was added in order to entirely dehydrate the tissue. Twenty-four hours later the acetone was removed and the remaining solids were washed with one volume of cold ether. This solvent was left in contact with the tissue for 4 hr in the cold room. Then the dehydrated kidneys, free of solvent, were ground into a very fine powder. The powder (about 170 g) was then introduced into several centrifuge tubes, along with 4 ml/g of powder of a solution containing 2 g NaCl and 10 mg EDTA/100 ml of water.

The mixture was then agitated, left for 24 h and centrifuged (13,000 g).

The supernatant was separated and the residue was agitated for 2 h with a new volume of the NaCl—EDTA solution and centrifuged once more. The supernatant which contained more than 90% of the renin and kallikrein were pooled and acidified to pH 4.5 with 1 *N* HCL, and rapidly centrifuged. The inactive proteins were discarded and the supernatant immediately neutralized (NaOH, 1 *N*). To separate renin from kallikrein the following procedure was used: the extract was passed two times through a column containing Sephadex, G-100. In the effluent kallikrein and renin were found in one single peak. The solution was introduced in a CM-52 column equilibrated with 0.01 *M* acetic acid solution pH 3.6.

Kallikrein was eluted with ammonium acetate 0.18 M, pH 4.6. The renin that remained attached to the resin at pH 4.6 was then eluted by a 0.05 *M* ammonium acetate solution pH 6.8. The solutions containing either kallikrein or renin activity were separately rechromatographied in the CM-52 column. This procedure furnished a kidney kallikrein free of renin when tested with the method of Nasjletti and Masson, 1970 (10) but even after the second CM-52 chromatography the renin still contained traces of kininogenase activity. The amount of Kallikrein in 1 I.U. of rat renin preparation was equivalent to 5.2 ng of bradykinin which is lower than the activity usually found in 0.02 ml of rat urine. From 1 kg of kidney a final renin extract (25 ml) was obtained which contained 8 mg of protein per ml and whose pressor effect was equivalent to 2.2 I.U. of renin per mg.

Blood pressure control. Experiments were carried out to test the pressor activity of the renal extracts using rats under sodium pentobarbital anesthesia (4 mg/100 g bw). Some of these animals served to express the renin activity of the extracts in I.U. and others to investigate the blood pressure changes when renin was injected by intraperitoneal route. The carotid artery was cannulated and the blood pressure recorded continuously for 150–180 min after a single intraperitoneal injection of one of the same renin doses used in the rats placed in the metabolic cages.

Results. The renin extracts in different doses given simultaneously with hyperhydration induce a striking decrease of kallikrein activity in the urine collected 60–120 min after the first fluid overloading (Table I, Figs. 1, 2.). The duration of the inhibition of kallikrein excretion is directly related to the dose. With renin higher doses, practically all the samples collected in the first 45–90 min showed no oxytocic activity in the urine (Table I).

The smallest doses 1 and 1.25 I.U. induced the shortest lasting effect: kallikrein did not appear in the first half hour and during the second half hour it was only 8–16% of the control's activity.

The inhibitory excretion upon kallikrein was also observed when renin injection preceded overhydration by 60 min (Table I) but not when it was injected 105 min before (Table I). The inhibitory effect of renin is quite reversible, kallikrein tended to return to normal levels in the urine excreted after the second hyperhydration (Figs. 1, 2).

In two experiments, 2 hr after the second hyperhydration, kallikrein excretion was greater in the experimental group than in controls.

When the rats were gavaged with NaCl solution the inhibitory effect of renin upon kallikrein excretion was similar: kallikrein activity disappeared from the urine collected during the first 90 min.

In this period sodium excretion reached its highest values (Table I). After the second hydration there was an increase in kallikrein excretion but it amounted to only 50% that of the control.

Confirming previous observations (6) the administration of renin in rats by intraperitoneal route elicited in all experiments a considerable natriuresis: five to eightfold that of the control level. Natriuresis reached its peak 30–75 min after renin injection in the rats overhydrated with distilled water. After the second hydration, sodium excretion tended to

EXCRETORY RATE PER RAT AT 60 (A) ON 120 (B) MINUTES AFTER HYPERHYDRATION IN RENIN INJECTED AND CONTROL RATS

RENIN DOSE I.U.	RENIN GROUPS KALLIKREIN A	KALLIKREIN B	Na μEq A	Na μEq B	%WATER EX A	%WATER EX B	CONTROL GROUPS KALLIKREIN A	KALLIKREIN B	Na μEq A	Na μEq B	%WATER EX A	%WATER EX B
PIG RENIN (6)												
● 1.25	25	73	370	405	78	101	305	531	65	73	38	71
● 2.5	0	19	295	391	43	74	295	391	46	72	17	53
● 5.0	0	12	426	563	54	83	96	145	42	74	46	91
RAT RENIN (3)												
● 1.0	29	54	270	580	51	102	230	390	22	62	24	68
● 2.2	0	20	-	750	46	83	290	351	-	25	26	48
□ 2.2	0	10	619	920	37	78	178	210	52	182	17	34
+ 2.2	0	15	169	303	46	58	66	125	50	79	16	50
++2.2	-	130	-	152	80	104	-	86	-	75	49	78

Excretory rate of kallikrein (expressed in ng Bradykinin, equivalent to the oxytocic effect), on sodium (in μEq) and water (% of fluid given by gavage) in the urine of treated and control rats. Figures are cumulative at 60 min (A) or 120 min (B) of excretion after hyperhydration (5% bw). The column on the left indicates the dose in I.U. of pig renin and of rat renin extracts. (⊗): Injections given simultaneously with fluid overloading; (□): 0.4% NaCl solution given by gavage instead of distilled water; (+): hyperhydration performed 60 min after injections, and (++): hyperhydration 105 min after injections.

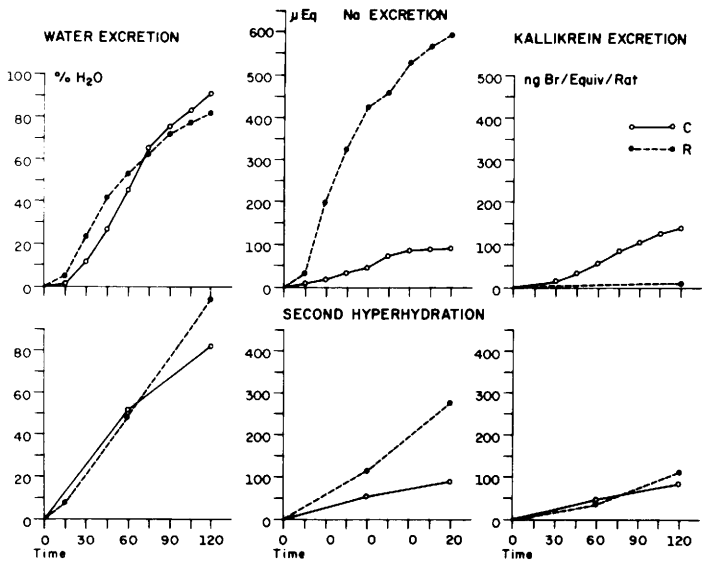

FIG. 1. Upper part: curves showing the excretion in the urine of water (as % of administered water) sodium in μEq, and kallikrein (expressed in ng Br. eq.), every 15 min during 120 min after the first hyperhydration. Six animals (R) were given 5 I.U. of hog renin, ip simultaneously with wateroverloading; C: Six control rats injected with saline.
Lower part: Curves showing the same parameters after a second hyperhydration with distilled water.

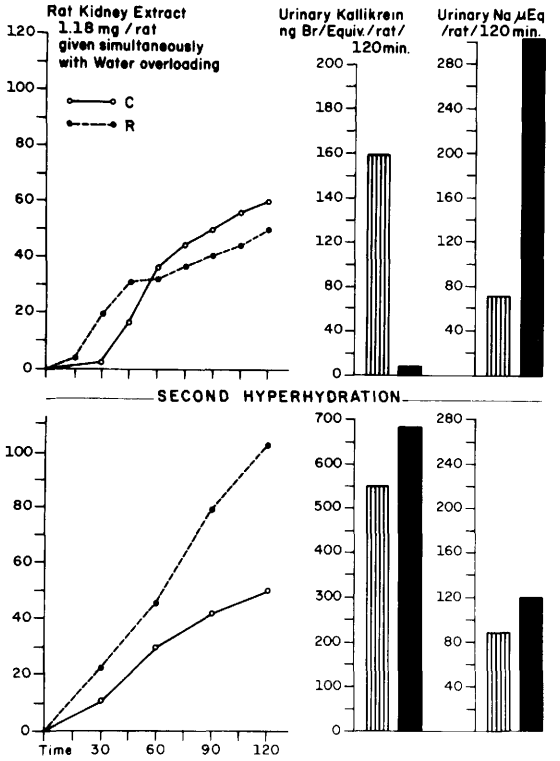

FIG. 2. Excretion of water, kallikrein and Na after the first (above) and second (below) hyperhydration, in six renin injected rats (R) and six control rats (C). The excretion of water (cummulative values) is indicated in the curves every 15 min for 120 min. Columns on the right: Total amount of kallikrein (in ng Br. eq) and sodium (in μEq) excreted in 120 min after hyperhydration. Black columns: Rats injected with renin; hatched columns: Control rats.

diminish in the renin group as compared to controls. In 9 out of 11 experiments rats responded to renin with an accelerated excretion of water, particularly notorious in the first hour after hyperhydration, which is also in keeping with results already published (6).

Renin effect upon blood pressure, when given by intraperitoneal route (1–1.5, 2.5 and 5 I.U.) in contrast with the effect of intravenous injections, induced none or moderate rise of blood pressure, depending on the dose. The highest increase 17 mm Hg for 42 min was obtained with 5 I.U. of renin, but doses of 1 to 3 I.U. did not change significantly blood pressure level (Fig. 3).

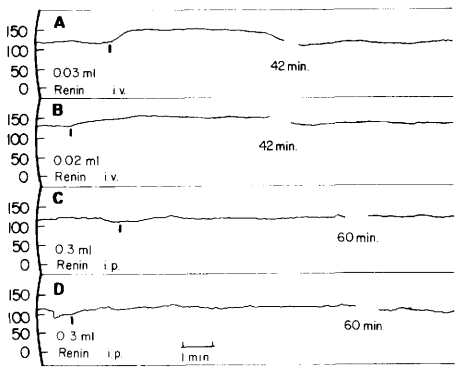

FIG. 3. Effects on mean arterial blood pressure (mmHg) in four anesthetized rats. In A and B, 0, 03 and 0, 02 I.U. of renin, respectively, were injected intravenously. In C and D, 3 I.U. were given intraperitoneally. Time in min.

Discussion. The transient but conspicuous decrease in kallikrein activity observed in the urine of hyperhydrated rats after the administration of purified renin extracts shows the complexity of renin action upon kidney excretory functions.

Renin not only elicites water and sodium diuresis (11) but also strikingly decreases urinary kallikrein. This enzyme, measured either by the oxytocic or the kininogenase activity of the urine disappears for 45–60 min after intraperitoneal injection of 1.25–5 I.U. of

renin. Considering previous findings, such as the natriuretic effect of kinins (12, 13) the dependence of urinary kallikrein on Na excretion requirements (14) and the statement that kallikrein is the renal natriuretic hormone (15) the coincidence of decreasing kallikrein excretion with rising natriuresis appears to be a paradoxical issue.

However this result does not disprove the assumption that in normal rats sodium intake can account for kallikrein excretion. It is conceivable that as long as excessive natriuresis occurs, a compensatory blockade of any other natriuretic mechanism would take place in the kidney and the stimulatory effect of renin extracts on sodium excretion could induce a lowering in plasma sodium level which can act as a negative signal for kallikrein production.

It is still questionable if the natriuretic effect of purified renin extracts from different species is due to renin itself or to some unknown natriuretic factor. Evidence has been provided that a factor non identifiable with renin is responsible for natriuresis by renal extracts (16).

Apparently blood pressure change induced by renin, is not a necessary factor for the inhibition of kallikrein excretion, because the smaller doses of renin, when injected intraperitoneally did not modify blood pressure but were still able to lower significantly kallikrein excretion.

The strong inhibitory effect of renin upon kallikrein excretion in the urine favors the idea that renin and kallikrein enzymatic system are interrelated.

Summary. Intraperitoneal injections of 1–5 I.U. of renin purified extracts, obtained either from hog or rat kidneys, in hyperhydrated rats receiving distilled water or 0.4% NaCl (5% body weight) produce not only a striking increase in the sodium excretion rate but a very significant decrease in kallikrein excretion as well. In the urine excreted in the first hour after renin administration kallikrein practically disappeared in the urine; with higher doses the inhibitory effect was very marked and lasted up to 120 minutes. In the same rats under a second hyperhydration, nonassociated with renin injection, kallikrein tends to return to control levels.

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