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Urinary Kallikrein Excretion in a Spontaneously Hypertensive Strain of Rats (38939)

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The consistent alterations in diverse parameters of the kallikrein–kinin systems as recorded in hypertensive rats, suggest that kallikrein may be involved in the mechanism of hypertension. Not only urinary kallikrein (1, 2) but also kininogenase activity in the kidney are strikingly decreased either in Grollman or Goldblatt experimental models (3), while blood kininogen is increased (4).

These findings do not provide definite evidence as to the role of the kallikrein–kinin system on the antihypertensive mechanism of the kidney but they reflect at least a reduced capacity of the kidney to produce or release kallikrein.

The key role of the kidney on arterial hypertension is well known. It has been shown by performing renal transplants on rat strains with opposite genetic propensity for hypertension, that the kidney has genetic determinants on blood pressure (5, 12). Using the technique of selective inbreeding, starting from a single Wistar strain, Bianchi (6) obtained two separate lines: spontaneously hypertensive rats (SHR) and normotensive rats (NR), thus providing a unique possibility to study the changes occurring in genetic hypertension. Normotensive rats deriving from common ancestors were used as controls.

We report here the levels of kallikrein activity found in these two lines of rats which are genetically related. Results show that urinary kallikrein is significantly decreased in hypertensive rats as compared to controls.

Material and Methods. The technique used to obtain the hypertensive and normotensive strains was described elsewhere (6). A selective inbreeding was started in 1964 from an original Wistar strain (different from Okamoto strain) to obtain a line of rats with high blood pressure. Later, and from the same strain, another line of rats with normal blood pressure was developed (6).

In the present experiments, 38 2-mo-old rats were used (body wt 150–265 g) with blood pressures as follows: males, 10 hypertensive, 160–180 mmHg; 9 normal, 120–150 mmHg. Females, 10 hypertensive, 160–180 mmHg; 9 normal, 120–135 mmHg. Hypertensive rats were from the 25th generation and normotensive from the 24th generation.

Blood pressure was measured at the tail by the method of Friebel and Vreden (7) after having preheated the animals in a warming box (8). Animals were fed with a normal salt diet (1% NaCl). On the day of the experiment they were placed into individual metabolic cages, in order to collect the urine for 24 hr. An adequate device prevented urine contamination either with feces or food. 2 ml of toluene was introduced in each collecting recipient and at the end of the collection period the urine was measured and immediately frozen and kept at $-20°$ until the kallikrein determinations.

At weaning (21–25 days of age) the blood pressure of both NR and SHR strains does not differ. Later, the blood pressure of SHR increases more rapidly and a statistically significant difference ($P < 0.01$) between both strains appears at 35 days of age.

Kallikrein determinations. Kallikrein activity was determined in each sample by chemical and biological methods: (a) the esterase activity method was applied according to Porcelli (9). Benzoylarginine-ethylester (BAEE) was used as substrate, following the hydrolysis rate in the Beckman, model Acta III C spectrophotometer. For measurements, 0.2 ml urine was diluted up to a volume of 5 ml, adding. 1 *M* Tris–HCl buffer pH 8. 0.2 ml of this mixture was introduced in the cuvette containing 2.5 ml of the buffer solution plus .5 ml of the BAEE solution (1 mg × ml) warmed up to 25°.

Purified rat urinary kallikrein prepared by chromatographic affinity containing 1030

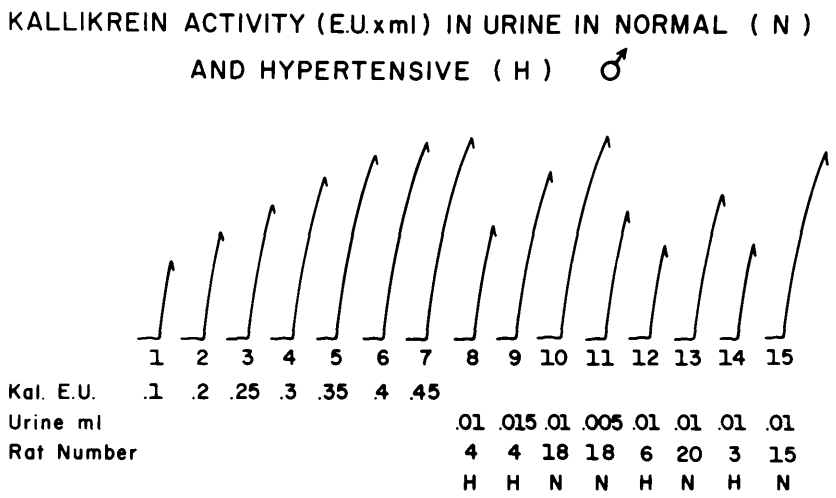

FIG. 1. Effects upon isolated rat uterus of several doses of standard rat urinary kallikrein (kal. E.U.) and of urine samples of three hypertensive male rats (4, 6, and 3) and of three samples of normotensive male rats (18, 20, 15). The amounts of urine introduced in the uterine bath are indicated in milliliters.

esterase units/mg (9) was used as the standard. It was established that 1 E.U. corresponds to the amount of the enzyme which hydrolyzes .05 μM BAEE and elicits and OD_{253}-variation of .018 in the spectrophotometer; (b) the bioassay was performed by measuring the direct oxytocic effect of the urine upon an isolated rat uterus horn immersed in Tyrode solution according the method already described (10). The same purified rat urinary kallikrein employed in the chemical method was used as standard; with the adequate dilution it contained 7.2 E.U./ml. Furthermore the specificity of the biological effect was checked by testing the kininogenase activity of the urine samples with the indirect method (10). For this purpose subthreshold oxytocic doses of urine were incubated at pH 7.4 for 2 min, with rat plasma kininogen II, prepared according to the technique of Jacobson and Kris (11). The liberated kinins set free in 2 min of incubation, where evaluated on isolated rat uterus by comparison to that produced by different doses of the standard kallikrein (10).

The chemical and biological methods were performed in the urine samples, before and after dialysist against distilled water, in the cold room. No significant differences were found between dialyzed and nondialyzed urine. The kallikrein activity of the urine measured by either method was expressed in esterase units (E.U.) per milliliter and 24 hr excretion.

Results. Figure 1 exemplifies some of the results obtained by testing the oxytocic activity in urine samples of both groups of rats.

Figure 2 summarizes some data including mean blood pressure and mean kallikrein activity found with both methods in the 24-hr vol of urine from NR and SHR strains. The figures show that there is a significant decrease in the total amount (24 hr) of kallikrein in the urine of SHR males and females.

Mean values and SD of E.U. found in urinary kallikrein excreted in 24 hr measured by the chemical method were: 225.7 $\pm$ 77.58 in NR males; 155 $\pm$ 32 in SHR males; 297 $\pm$ 64 in NR females and 176.2 $\pm$ 34.4 in SHR females. The differences between NR and SHR rats of each group were significant at the level of $P < 0.001$. Significant differences were also found in kallikrein concentration. The following figures in E.U. of kallikrein activity per milliliter of urine, obtained with the chemical method, were: 29.85 $\pm$ 8.6 in NR females and 20.67 $\pm$ 6 in SHR females ($P < .05$). 34.84 $\pm$ 8.2 in NR males, and 17.9 $\pm$ 2.9 in SHR males ($P < .01$). A good correlation was found between the E.U. data obtained with both chemical and biological methods ($r = .955$). Line

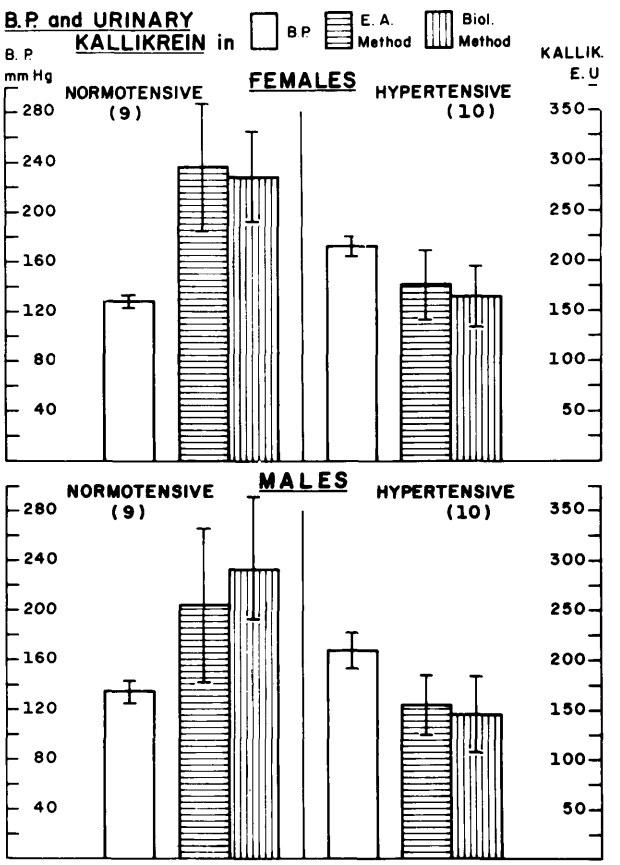

FIG. 2. The columns provide average values and SD of blood pressure and of urinary kallikrein, excreted in 24 hr measured by both chemical and biological methods. Kallikrein activity is expressed in E.U.

regression was given by $y = 1.858 \pm 1.038 x$ ($P < .05$).

There was also a good correlation between results recorded by measuring the oxytocic direct effect and the oxytocic indirect activity, evaluating the amount of kinins released when the urine was incubated with kininogen II.

Discussion. The results clearly demonstrate that kallikrein is significantly diminished in the urine of rats which develop hypertension of hereditary nature when compared to the rats of the normotensive line descending from common ancestors.

This difference appears particularly relevant to the possible causative role of the kidney in this hereditary type of hypertension. Previous studies (6) had shown that no difference was demonstrable between both strain (NR and SHR) in regard to kidney and adrenal histological appearance. However, results obtained with cross-kidney transplantation between SHR and NR suggested a pressor role of the kidney in SHR (12), "Normotensive" kidneys reduced the blood pressure of hypertensive recipients, but the hypertensive had no such effect. These results indicate that the kidneys of adult hypertensive rats are involved in the maintenance of hypertension. The lower level of kallikrein in the urine of these rats might be an indicator of a genetical biochemical abnormality in the kidney of SHR. In contrast with our result, higher levels of kallikrein excretion were found in spontaneously hypertensive rats NIH F21–23 generation as compared to normotensive age-matched group NIH Wistar rats (13). It can be assumed that NIH Wistar rats belonging to a quite different strain were not an adequate

control for the spontaneously hypertensive rats.

It is interesting to emphasize that kallikrein activity in the urine of this hereditary type of hypertension does not fall to the low levels found in renal hypertensive rats (Grollman type). It is a common observation that in the latter the excretory rate of kallikrein is $\frac{1}{10}$ or $\frac{1}{20}$ the amount exhibited by normotensive ones.

Since the renin angiotensin system is rather depressed and, on the other hand, there is a different pattern of sodium and water handling by the kidney of SHR as compared to NR (14) the question arises whether renal kallikrein which has been considered a "natriuretic hormone" (14) would be involved in the basic water and electrolyte disturbance observed in SHR (15). There is an increasing evidence that urinary kallikrein is closely related to kidney kallikrein (16, 17) and therefore one might think that variations in kallikrein concentration could reflect the capacity of the kidney to secrete that enzyme.

It is still a matter of speculation to ascribe a physiological role to renal kallikrein but, considering the striking effects of kinins on sodium excretion and on intrarenal circulation (18) it is not hazardous to assume that renal kallikrein can play the role of a renin antagonist.

Summary. Concentration and 24-hr excretion of urinary kallikrein in spontaneous hypertensive Wistar strain rats of both sexes obtained by selected inbreeding (25th generation) are significantly decreased as compared with the excretion in normotensive inbred rats (24th generation) descending from common ancestors. Apparently in these hypertensive rats there is an abnormal capacity of the kidneys to produce or release kallikrein, but more studies will be necessary to correlate this findings with blood pressure increase.

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