Low urinary kallikrein excretion and elevated blood pressure normalized by orally applied kallikrein in essential hypertension

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Summary
1. Urinary kallikrein was measured in 67 patients with essential hypertension and 25 normotensive subjects variously on unrestricted and low sodium diet. Also, the effect of orally applied hog pancreatic kallikrein on elevated blood pressure and kallikrein excretion was evaluated.
2. Urinary kallikrein was reduced in a large subgroup of patients with sustained essential hypertension.
3. With salt restriction, urinary kallikrein rose markedly in normotensive subjects and patients with borderline hypertension but not in those with sustained hypertension.
4. Oral kallikrein normalized reduced kallikrein excretion and lowered elevated blood pressure.
5. The rise in urinary kallikrein with oral kallikrein was due to an increased formation of endogenous enzyme.
6. A defective kallikrein-kinin system may be involved in both the low urinary kallikrein excretion and the hypertension.

Key words: essential hypertension, oral kallikrein, urinary kallikrein.

Abbreviation: GFR, glomerular filtration rate.

Introduction
Previous investigations of the kallikrein-kinin system in essential hypertension have provided evidence that urinary kallikrein is excreted in smaller amounts in hypertensive than in normoten-


In the present study we reassessed the role of the kallikrein-kinin system in patients with essential hypertension by contrasting their urinary kallikrein excretions with those in normotensive subjects. Also, the effects of orally applied hog pancreatic kallikrein on urinary kallikrein activity and elevated blood pressure was evaluated.

Methods
Patients
We studied 57 patients with sustained essential hypertension, 10 patients with borderline hypertension and 25 normotensive subjects. In the patients with sustained hypertension blood pressure was greater than 155/100 mmHg and in the borderline hypertensive subjects the average pressure ranged from 140/90 to 155/95 mmHg. All groups of subjects were similar in age (25–49 years), sex and body weight. The patients had not taken antihypertensive drugs previously.

Study protocol
During the initial phase of the study, all patients were on an unrestricted salt diet. Thereafter, 10
patients with sustained hypertension, five borderline hypertensive subjects and 10 normotensive subjects were given a 10 mmol of sodium/day diet for 6 days. The effect of hog pancreatic kallikrein (Bay d 7687, Bayer AG, Leverkusen, F.R.G.) on urinary kallikrein activity and on arterial pressure was studied in 22 patients with sustained hypertension. After a 2 weeks' placebo period kallikrein in a dose of 200 biological units three times daily for 8 weeks was given. Thereafter, a second placebo period followed.

**Biochemical methods**

Urinary kallikrein activity for H-D-Val-Leu-Arg-pNA (AB Kabi, Sweden) hydrolysis was measured by a modification of the method of Amundsen (1978). Each assay sample contained 0.8 ml of Tris/HCl buffer (0.2 mol/l), pH 8-2, 0.1 ml of substrate solution (1 mmol/l) and 0.1 ml of urine. Corresponding sample blanks contained the same mixture with the exception that 7 μg of aprotinin (Bayer AG) was added. Incubation was performed at 37°C for 30 min, and then 0.1 ml of acetic acid (4 mol/l) was added to stop the reaction. Absorbance was read at 405 nm. Results are expressed in terms of enzyme units/24 h. One unit is defined as that amount of enzyme which hydrolyses 1 μmol of the substrate/min at pH 8-2 and 37°C. In patients receiving oral kallikrein, urinary kallikrein was also determined by a specific radioimmunoassay for hog pancreatic kallikrein (Fink & Güttel, 1978).

Results were analysed by Student's t-test for paired and unpaired data. Results are expressed as the mean ± SEM.

**Results**

On an unrestricted sodium diet, patients with sustained hypertension exhibited 24 h urinary kallikrein activities (0.48 ± 0.05 unit) that were significantly lower (P < 0.001) than in borderline hypertensive subjects (1.07 ± 0.1 units) or in the normotensive controls (1.26 ± 0.14 units). In the hypertensive group reduced excretion of kallikrein was not a uniform finding and apparently a subgroup of patients had normal kallikrein activities. The aforementioned differences in kallikrein excretion among the three groups were also observed when urinary kallikrein activity was standardized for creatinine clearance as an index of GFR. During the period of salt restriction, urinary kallikrein rose markedly in all of the normotensive subjects (from 1.01 ± 0.13 to 1.95 ± 0.25 units/24 h). In contrast, there was no or only a slight increase in enzyme excretion in the patients with sustained hypertension (from 0.33 ± 0.08 to 0.43 ± 0.08 unit/24 h). Borderline hypertensive subjects showed a significant (P < 0.05) although blunted response to the low sodium diet (from 0.9 ± 0.15 to 1.4 ± 0.17 units/24 h).

After oral kallikrein, mean systolic and diastolic blood pressures decreased significantly (P < 0.001) from 160/106 to 146/94 mmHg in the supine and from 156/108 to 146/98 mmHg in the standing position. The pressure drop was first seen after 2 weeks and reached its maximum after 8 weeks. Pulse rates remained unchanged. After changing again to placebo, blood pressures returned to pretreatment values after 4-8 weeks. When the patients were classified according to their urinary kallikrein activity it became evident that all
patients with markedly reduced kallikrein excretion, i.e. lower than 0.5 unit/24 h, had a fall in systolic and diastolic blood pressures as well as an increase in urinary kallikrein to normal values (Fig. 1). Conversely, small or no blood pressure responses and no changes of urinary kallikrein were observed in the patients with normal kallikrein excretion (Fig. 1). With a specific radioimmunoassay, none of the orally applied hog pancreatic kallikrein could be detected in the urine. Patients with reduced urinary kallikrein has slightly although not significantly lower GFR than patients with normal enzyme excretion. After treatment, GFR increased significantly, by a mean of 25%, in the low but not in the normal kallikrein group (Fig. 1).

Discussion

This study shows that patients with sustained essential hypertension excreted less kallikrein in the urine than did either borderline hypertensive or normotensive subjects. The defect in urinary kallikrein was most pronounced during a low salt diet. Similar results were obtained by others (Margolius et al., 1971; Levy, Frigon & Stone, 1978; Lechi et al., 1978). It has been proposed that a defect in the renal kallikrein-kinin system, possibly via an increase in renal vascular resistance (Levy et al., 1977), may contribute to the genesis of hypertension (McGiff & Nasjletti, 1976). This possibility is supported by our observation that oral kallikrein not only normalized reduced kallikrein excretion and increased GFR, but also lowered elevated blood pressure. At present, the mode of action of oral kallikrein is far from clear. It has been shown that kallikrein is reabsorbed from the gut (Moriwaki, Moriya, Yamaguchi, Kizuki & Fujimori, 1972; Fink, Dietl, Seifert & Fritz, 1978) and exerts physiological effects (Nangu, 1977; Schill, 1976) which have been attributed to the liberation of kinins (Wicklmayr & Dietze, 1977). Our data do not provide any information whether kallikrein by release of vasodilatory kinins was responsible for the fall in blood pressure. In any event, the observation that oral kallikrein normalized decreased urinary kallikrein excretion and lowered high pressure suggests that a defective kallikrein–kinin system was involved in both the low urinary kallikrein and the hypertension.

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References


