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# Urinary kallikrein in normotensive subjects and in patients with essential hypertension

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## Summary

1. Excretion of urinary kallikrein was normal in 13 out of 15 patients with uncomplicated essential hypertension.

2. Frusemide increased urinary kallikrein excretion in normotensive subjects and in patients with essential hypertension. The stimulating effect of frusemide on urinary kallikrein was significantly diminished in patients with essential hypertension.

3. No correlations of urinary kallikrein with sodium, potassium, and aldosterone excretion were found.

4. The results do not support the idea that urinary kallikrein plays a primary role in the pathogenesis of essential hypertension.

Key words: essential hypertension, frusemide, kallikrein.

Abbreviation: PRA, plasma renin activity.

## Introduction

There is evidence that urinary kallikrein is formed in and secreted by the kidney (Nustad, 1970). The role of the intrarenal kallikrein–kinin system in renal handling of water and electrolytes and its interaction with other renal endocrine systems has been studied extensively within the last few years (Obika, 1978). Subnormal urinary kallikrein excretion in essential hypertension was first reported by Elliot & Nuzum (1934) and confirmed by Margolius, Geller, Pisano & Sjoerdsma (1971), and this suggested a possible role in the pathogenesis of high blood pressure.

The aim of this study was to examine urinary kallikrein excretion in respect of its relationship to water and electrolyte excretion, and to the renin–angiotensin–aldosterone system in normotensive subjects and in patients with essential hypertension.

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## Subjects and methods

Fourteen male normotensive subjects (mean age 31 ± 2·4 years) and 15 male patients with mild (WHO I; $n = 10$) or moderate essential hypertension (WHO II; $n = 5$) (mean age 36 ± 2·7 years) were included in the study. Average blood pressure in the normal subjects was 117 ± 2·4/77 ± 1·6 mmHg and in the hypertensive patients was 163 ± 6·4/105 ± 2·8 mmHg. Antihypertensive treatment had been withdrawn for at least 2 weeks. Diuretic agents had been withdrawn for at least 4 weeks before the study.

Studies were performed under ambulatory conditions without dietary restrictions. Urine was collected for 24 h in 8 h periods (period 1: 07.00–15.00 hours; period 2: 15.00–23.00 hours; period 3: 23.00–07.00 hours). On the following morning, after a 30 min resting period, blood samples were taken for estimation of plasma renin activity (PRA) and plasma creatinine. Thereafter 40 mg of frusemide (Lasix) was given intravenously and urine was collected for another 30 min period. At the end of the collection period, blood was again taken for estimation of PRA.

In each urine sample creatinine, sodium, potassium, and aldosterone were estimated.

Urinary kallikrein was estimated by four different methods.

(1) Amidolytic activity of urinary kallikrein was measured with a chromogenic tripeptide substrate (S 2266; KABI, Stockholm) in non-dialysed as well as in dialysed urines.

(2) Esterolytic activity of urinary kallikrein was estimated with acetyl-L-phenylalanyl-L-arginine-ethylester (APAE) as substrate in dialysed urines only (Fiedler, Geiger, Hirschauer & Leysath, 1978).

Values for urinary kallikrein activity from assays (1) and (2) are expressed in units of substrate-splitting activity (units/l = $\mu$mol $min^{-1}$ $l^{-1}$).

(3) Bioassay utilized the vasodepressor effect of urinary kallikrein, tested in anaesthetized dogs (Nembutal, 40 mg/kg) after intravenous injections of dialysed urine.

(4) Urinary kallikrein was also estimated by a direct radioimmunoassay in non-dialysed as well as in dialysed urine samples (Mann, Geiger, Göring, Lipp, Fink, Keipert & Karl, 1979).

Bioassay and radioimmunoassay values were standardized by using a purified human urinary kallikrein preparation (Geiger, Stuckstedte, Förg-Brey & Fink, 1979), and are expressed as ng/ml.

Urine samples were dialysed against distilled water for 24 h at +4°C. The non-dialysed urine samples (assay 1 and 4) for comparison were stored for 24 h at +4°C without any pretreatment.

Sodium, potassium and creatinine were measured by standard methods. Aldosterone excretion was measured by radioimmunoassay, PRA was estimated as previously described (Distler, Keim, Cordes, Philipp & Wolff, 1978).

The data were analysed by the Student's *t*-test. Linear dependencies were examined by regression analysis. Data are given as mean values ± SEM.

## Results

The amidolytic activity of urinary kallikrein is shown in Table 1. There were no significant differences in activities between the periods 1, 2 and 3, both in normal subjects and in patients with hypertension. Individual values varied considerably. In normal subjects the range was between 347 and 1658 units/24 h; in the hypertensive patients the range was between 175 and 2089 units/24 h (non-dialysed urine). Urinary kallikrein in no more than two patients was found to be diminished in comparison with the values for normal subjects. Dialysis of urine led to a consistent increment of the amidolytic activity in both groups by about 25%.

Urinary kallikrein increased after frusemide in both groups (normal, $P < 0.001$; hypertensive, $P < 0.05$); mean activity after frusemide, however, was significantly less ($P < 0.05$) in hypertensive patients.

PRA did not differ in recumbency, whereas frusemide-stimulated PRA was lower in hypertensive patients ($P < 0.05$). No difference could be demonstrated in urinary volume and the excretion of sodium, potassium and aldosterone, or in the creatinine clearance in both groups, neither under basal conditions nor after frusemide administration.

No correlation was found between urinary kallikrein and the excretion of sodium, potassium and aldosterone, the creatinine clearance and resting and stimulated PRA. Kallikrein excretion per 24 h, however, correlated significantly with urinary volume in the normal subjects only (non-dialysed urine: $r = 0.65$, $P < 0.05$).

The following correlations were obtained with the four methods for estimation of urinary kallikrein: (1) APAE ($y$) vs S 2266 ($x$) in dialysed urine ($n = 113$): $y = 56.40x + 1.80$; $r = 0.97$; (2) bioassay ($y$) vs S 2266 ($x$) in dialysed urine ($n = 71$): $y = 0.44x + 0.76$; $r = 0.80$; (3) radioimmunoassay ($y$) vs S 2266 ($x$) in non-dialysed urine ($n = 22$): $y = 1.18x - 2.25$; $r = 0.95$; (4) radioimmunoassay ($y$) in non-dialysed vs radioimmunoassay ($x$) in dialysed urine ($n = 22$); $y = 0.96x + 3.17$; $r = 0.95$.

## Discussion

We did not find a significant difference in urinary kallikrein excretion between patients with uncomplicated essential hypertension and age-matched normotensive control subjects. A normal excretion was also reported by Lawton & Fitz (1977), who compared young male patients with mild essential hypertension with age-matched control subjects. Other authors reported a normal kallikrein excretion in labile essential hypertension, but a decreased kallikrein excretion in the established condition (Nekrasova, Lantsberg, Chernova & Khukharev, 1970; Overlack, Stumpe, Zywzok, Ressel & Krück, 1978). These findings suggest that subnormal kallikrein excretion is secondary to the hypertensive process.

Frusemide stimulates kallikrein excretion in man (Seino, Abe, Irokawa, Ito, Yasujima, Sakurai, Chiba, Saito, Ritz, Kusaka, Miyazaki & Yoshinaga, 1978), as was observed also in this study. However, in hypertensive patients the effect

TABLE 1. *Urinary kallikrein, volume, sodium, potassium and aldosterone, creatinine clearance, and plasma renin activity in normotensive subjects (NS) and patients with essential hypertension (EH) under basal conditions (NS, n = 14; EH, n = 15) and after intravenous frusemide (40 mg) (NS, n = 11; EH, n = 15)*

PRA, Plasma renin activity; $C_{Cr}$, creatinine clearance; control, periods 1 + 2 + 3 (see text). Mean values ± SEM are shown. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

| Period | | Kallikrein (S 2266) (m units/period) | | PRA (ng $h^{-1}$ $ml^{-1}$) | Volume (ml/period) | Sodium (mmol/period) | Potassium (mmol/period) | $C_{Cr}$ (ml $min^{-1}$ 1·73 $m^{-2}$) | Aldosterone (μg/period) |
|---|---|---|---|---|---|---|---|---|---|
| | | Non-dialysed urine | Dialysed urine | | | | | | |
| 07.00–15.00 hours | NS | 281 ± 39 | 377 ± 40* | | 437 ± 58 | 60 ± 9 | 35·0 ± 3·2 | 115 ± 10 | 5·9 ± 1·1 |
| | EH | 317 ± 55 | 401 ± 60*** | | 466 ± 42 | 66 ± 6 | 31·5 ± 2·0 | 120 ± 9 | 6·2 ± 0·7 |
| 15.00–23.00 hours | NS | 270 ± 31 | 335 ± 37 | | 534 ± 75 | 76 ± 7 | 29·9 ± 3·7 | 106 ± 7 | 3·6 ± 0·5 |
| | EH | 271 ± 44 | 329 ± 50*** | | 522 ± 68 | 80 ± 9 | 29·4 ± 3·1 | 119 ± 7 | 5·3 ± 0·6 |
| 23.00–07.00 hours | NS | 252 ± 40 | 359 ± 49* | | 377 ± 41 | 51 ± 6 | 15·1 ± 1·9 | 97 ± 4 | 3·0 ± 0·5 |
| | EH | 264 ± 38 | 347 ± 44*** | | 433 ± 55 | 65 ± 9 | 15·3 ± 1·2 | 102 ± 5 | 3·6 ± 0·5 |
| 07.00–07.00 hours | NS | 808 ± 102 | 1066 ± 117* | | 1348 ± 151 | 184 ± 17 | 80·0 ± 7·3 | 106 ± 5 | 12·5 ± 2·0 |
| | EH | 850 ± 129 | 1072 ± 146*** | | 1421 ± 138 | 211 ± 20 | 76·2 ± 4·5 | 113 ± 5 | 15·1 ± 1·5 |
| Control (30 min) | NS | | 20·7 ± 3·0 | 2·4 ± 0·3 | 29 ± 4 | 3·7 ± 0·4 | 1·6 ± 0·2 | 105 ± 5 | |
| | EH | | 22·4 ± 3·0 | 2·1 ± 0·3 | 30 ± 3 | 4·4 ± 0·4 | 1·6 ± 0·1 | 113 ± 5 | |
| Frusemide (30 min) | NS | | 48·6 ± 7·5 | 7·0 ± 0·8 | 521 ± 18 | 66·6 ± 2·5 | 8·3 ± 0·4 | 106 ± 5 | |
| | EH | | 32·6 ± 4·6 | 4·4 ± 0·7 | 503 ± 24 | 66·1 ± 2·9 | 7·9 ± 0·4 | 99 ± 6 | |

was less pronounced. According to the findings of Croxatto, Albertini, Corthorn & Rosas (1977) increments of kallikrein excretion in rats are caused by an increased synthesis in the kidney. Therefore our findings could indicate a reduced synthesis of glandular kallikrein after stimulation in hypertensive patients.

Interassay correlations showed that the synthetic substrates used are appropriate for the estimation of urinary kallikrein. Dialysis of urine samples leads to an increase of the amidolytic activity. However, the amounts of kallikrein measured in non-dialysed and dialysed urine samples by radioimmunoassay did not differ. It seems possible that a kallikrein inhibitor as was found in renal tubules in rats (Geiger & Mann, 1976) is excreted in urine and removed by dialysis.

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