Renin–angiotensin and kallikrein–kinin systems in sodium homeostasis and hypertension in rats

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Summary

1. Urinary kallikrein excretion was measured in rats by an enzyme kinetic method employing radioimmunoassay of generated bradykinin.

2. Rats given a sodium load (NaCl solution, 20 g/l, to drink) for 28 days showed acute and prolonged significant falls in urinary kallikrein excretion associated with suppression of plasma renin and angiotensin.

3. Conversely sodium-depleted rats showed increases in urinary kallikrein excretion, associated with rises in plasma renin and angiotensin.

4. A close and significant direct relation between plasma renin activity and urinary kallikrein excretion was demonstrated.

5. The diuresis and natriuresis induced by frusemide in rats was associated with increased urinary kallikrein excretion and acute rises in plasma renin.

6. In chronic renal hypertensive rats urinary kallikrein excretion was increased only in the animals with two-kidney Goldblatt hypertension. This group was also the only group that demonstrated a significant rise in plasma renin activity.

Key words: bradykinin radioimmunoassay, diuretics, hypertension, kallikrein–kinin, renin–angiotensin, sodium balance.

Introduction

Changes in the kallikrein–kinin system have been reported in association with alterations in sodium balance (Adetuyibi & Mills, 1972; Carretero & Oza, 1973; Geller, Margolius, Pisano & Keiser, 1972), in both experimental and human hypertension (Croxatto & San Martin, 1970; Margolius, Horwitz, Pisano & Keiser, 1974) and with changes in the renin–angiotensin system (Wong, Talamo, Williams & Colman, 1975). We have therefore investigated the parallel changes that occur in renal sodium excretion, urinary kallikrein excretion, plasma renin and angiotensin and blood pressure in rats during changes in dietary sodium intake, after administration of a diuretic and during the development of experimental renal hypertension. Urinary kallikrein was measured by a newly developed enzyme kinetic method employing radioimmunoassay of generated bradykinin.

Methods

Groups of eight Sprague–Dawley rats were fed a normal diet for 5 days, followed by either the same diet with NaCl solution, 20 g/l, in place of distilled water for drinking for 28 days or a low-sodium diet (0.05 mmol per day) for 28 days. For the first 10 days and on days 26, 27 and 28 they were placed in individual metabolic cages and their food and fluid intake measured and their urine was collected for measurement of volume, sodium, potassium and kallikrein content.

Ten other rats were placed in metabolic cages and the urine volume, sodium, potassium and kallikrein excretion measured over 4 h. After this five rats received 2 mg of frusemide (Hoechst) and five control rats received 0.1 ml of glucose solution intraperitoneally and the same variables were measured for a further 4 h.

Groups of ten rats underwent unilateral nephrectomy, or had a renal clip (0.2 mm internal diameter) placed on the left renal artery (two-kidney Goldblatt rats) or had the left renal artery constricted with contralateral nephrectomy (one-kidney Goldblatt
rats). Twenty-eight days later five two-kidney and five one-kidney Goldblatt rats that had developed stable severe hypertension, together with rats with unilateral nephrectomy, were placed in individual metabolic cages and their urinary volume, sodium, potassium and kallikrein excretion measured over 3 days.

Plasma renin and angiotensin was measured on peri-orbital venous sinus blood from each rat. Plasma renin activity was measured by a competitive binding radioimmunoassay (Johnston, Mendelsohn & Casley, 1969; Johnston, Matthews, Davis & Morgan, 1975) and plasma angiotensin by radioimmunoassay (Johnston, Mendelsohn & Doyle, 1972).

Urinary kallikrein was measured by an enzyme kinetic method, by quantifying the bradykinin generated on incubation by radioimmunoassay. Urine was diluted 1:50 and incubated at pH 8-4 at 37°C for 30 min with exogenous kininogen prepared from nephrectomized dog plasma. The bradykinin generated was measured in a radioimmunoassay, specific antibodies raised in rabbits by repeated injections of synthetic bradykinin (Sandoz) coupled to human serum albumin being used with [Lzsl]tyr-lys-bradykinin and synthetic bradykinin as standards (Johnston, Matthews & Dax, 1976).

Results

Urinary kallikrein and bradykinin radioimmunoassay

The bradykinin radioimmunoassay showed cross-reactivity to kallidin (lysyl bradykinin) and methionyl-lysyl bradykinin, but did not cross-react with angiotensin I or II, vasopressin or oxytocin. The sensitivity was 50 pg of bradykinin; the intra-assay variability was 4% and the inter-assay variability was 10-2%.

The kininogen substrate was free of bradykininases and free of endogenous kallikrein activity. Increasing the volume of rat urine in the incubation mixture or the time of incubation up to 120 min with dog kininogen produced linear proportional increases in the quantities of bradykinin generated. All values are mean ± SEM.

Sodium loading and depletion

With sodium loading there was an increase in urinary sodium excretion from 0.48 (SEM 0.05) to 27.3 (SEM 2.9) mmol/day, a fall in PRA<sup>(1)</sup> from 22.9 to 2.6 (SEM 0.6) pmol of AI h<sup>-1</sup> ml<sup>-1</sup> (t = 8.41, P < 0.001, n = 16) and a fall in PAII from 70 (SEM 18.0) to 19.5 pmol/ml (t = 8.57, P < 0.001, n = 16) after 5 days. Urinary kallikrein excretion fell markedly by the first day, and at 28 days was still significantly reduced, from 89.2 (SEM 8.9) to 54.7 (SEM 14.3) µg of bradykinin/h per day (t = 5.68, P < 0.005, n = 6) together with continued suppression of PRA and PAII.

During sodium depletion there was a fall in urinary sodium to 0.07 (SEM 0.07) mmol/day, a rise in PRA to 46.5 (SEM 3.7) pmol of AI h<sup>-1</sup> ml<sup>-1</sup> (t = 8.4, P < 0.001, n = 16) and PAII to 171.2 (SEM 23.2) pmol/ml (t = 4.6, P < 0.05, n = 16), with little alteration in urinary kallikrein excretion by 5 days. However, after 28 days of sodium depletion urinary kallikrein excretion had risen significantly to 171.3 (SEM 25.8) µg of bradykinin/h per day (t = 4.55, P < 0.005, n = 6) together with sustained rises in PRA and PAII.

Urinary kallikrein excretion was not correlated with urinary volume or potassium. However, it bore an inverse hyperbolic relationship to urinary sodium excretion similar to the relationship between urinary sodium and plasma renin. Furthermore urinary kallikrein concentration bore a direct linear relationship to PRA (r = 0.950, n = 5) and PAII (r = 0.989, n = 5) and similarly urinary kallikrein excretion bore a direct relationship to PRA (r = 0.76, n = 13).

Effect of frusemide

Frusemide (2 mg) intraperitoneally was associated with an increase in urinary sodium excretion from 0.24 (SEM 0.007) to 0.606 (SEM 0.025) mmol/h together with an increase in urinary kallikrein excretion from 2.30 (SEM 0.33) to 18.4 (SEM 4.6) µg of bradykinin/h for 4 h (t = 5.89, P < 0.005, n = 6) and a significant rise in PRA from 17.42 (SEM 4.7) to 37.94 (SEM 9.96) pmol of AI h<sup>-1</sup> ml<sup>-1</sup> (t = 6.42, P < 0.005, n = 8).

Chronic renal hypertension

The changes in blood pressure, urinary sodium and kallikrein excretion and PRA in normal, unilateral nephrectomized rats, two-kidney Goldblatt and one-kidney Goldblatt hypertensive rats are shown in Table 1. Two-kidney Goldblatt rats had significant increases in their urinary kallikrein excretion (t = 3.12, P < 0.005, n = 14) and their

<sup>(1)</sup> Abbreviation: PRA, plasma renin activity; AI, angiotensin I; PAII, plasma angiotensin II.
Changes in blood pressure, urinary sodium and kallikrein excretion, and plasma renin activity are shown as mean values±SEM for normal rats, unilaterally nephrectomized rats, rats with unilateral renal artery stenosis (two-kidney Goldblatt) and rats with unilateral renal artery stenosis and contralateral nephrectomy (one-kidney Goldblatt) at 28 days. * P<0.005.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Blood pressure (mmHg)</th>
<th>Urinary sodium excretion (mmol/day)</th>
<th>Urinary kallikrein excretion (μg of BK h⁻¹ day⁻¹)</th>
<th>Plasma renin activity (pmol of AⅠ h⁻¹ ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>10</td>
<td>128±5</td>
<td>0.62±0.05</td>
<td>89.2±8.9</td>
<td>22.9±1.7</td>
</tr>
<tr>
<td>Unilateral nephrectomy</td>
<td>5</td>
<td>123±4</td>
<td>0.75±0.07</td>
<td>120.1±8.1</td>
<td>17.0±4.1</td>
</tr>
<tr>
<td>Two-kidney Goldblatt</td>
<td>5</td>
<td>191±9</td>
<td>0.75±0.09</td>
<td>215.2±24.2*</td>
<td>36.3±6.1*</td>
</tr>
<tr>
<td>One-kidney Goldblatt</td>
<td>5</td>
<td>196±10</td>
<td>0.87±0.07</td>
<td>154.3±31.8</td>
<td>9.7±2.4</td>
</tr>
</tbody>
</table>

The relation between kallikrein and hypertension is more complex. Urinary kallikrein excretion has been reported to be low in essential hypertension and renal hypertensive rats (Geller et al., 1972; Croxatto et al., 1970). However, it is high in spontaneously and deoxycorticosterone hypertensive rats. In this study urinary kallikrein excretion was only increased in two-kidney Goldblatt hypertensive animals. This is interesting, as this hypertensive model, as shown here and previously (Hutchinson, Matthews, Dax & Johnston, 1975), is associated with elevated plasma renin, angiotensin and aldosterone, again demonstrating the close relationship that appears to exist between renin and kallikrein. It is possible that the diuretic, vasodilator properties of the kallikrein–kinin system act to homeostatically counter the antidiuretic, vasoconstrictor properties of the renin–angiotensin system particularly within the kidney.

Discussion
Both the renin–angiotensin system and kallikrein–kinin systems appear to be involved in sodium homeostasis and blood pressure regulation. During sodium loading both plasma renin and urinary kallikrein are depressed while during sodium depletion both renin and kallikrein excretion are stimulated. These findings are similar to those reported by Geller et al. (1972) and Margolius et al. (1974), who found that urinary kallikrein increased in rats and man when sodium depleted.

The relationship between urinary sodium and kallikrein excretion is an inverse hyperbolic one, similar to the relationship between urinary sodium and plasma renin. However, urinary sodium excretion can be dissociated from urinary kallikrein in acute situations. Thus the diuresis and natriuresis caused by administration of a diuretic was associated with a rise in urinary kallikrein excretion together with a rise in plasma renin. It is interesting to speculate whether the increase in kallikrein–kinin associated with diuretic therapy is related to their vasodilator and hypotensive effects.

In these studies we were able to demonstrate a significant direct relation between plasma renin and urinary kallikrein. These two hormonal enzyme systems showed a close parallel under all conditions studied. A similar direct relation between plasma angiotensin and plasma bradykinin has been recently reported in man by Wong et al. (1975).

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References


