Kallikrein–kinin system in one- and two-kidney Goldblatt hypertensive rats

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(Received 4 May 1978; accepted 2 October 1978)

Summary

1. Urinary kallikrein, renal tissue kininogenase activity, and plasma kininogen were measured during the development of hypertension in rats, either with left renal artery clamped and contralateral kidney intact (Goldblatt 2-kidney) or removed (Goldblatt 1-kidney). Plasma urea, plasma volume and blood volume were also measured.

2. In Goldblatt 2-kidney rats, urinary kallikrein was not significantly different from that of sham-operated rats up to the 5th post-operative week even though at this time the Goldblatt 2-kidney animals were hypertensive. Urinary kallikrein started to decrease at week 8. Plasma kininogen was significantly elevated 5 and 10 weeks after surgery and returned to normal by week 15. No changes in renal tissue kininogenase activity were observed. Plasma urea and plasma and blood volumes were normal 5 weeks after, but significantly increased at 10 and 15 weeks after surgery.

3. In Goldblatt 1-kidney rats from the fifth week urinary kallikrein and renal kininogenase activity were significantly decreased compared with unilaterally nephrectomized rats, whereas plasma kininogen, plasma urea and plasma and blood volumes were significantly elevated.

4. These results show important differences in the kallikrein system between the Goldblatt 1-kidney and 2-kidney models of renovascular hypertension in rats.

Key words: blood volume, plasma kininogen, plasma uréa, plasma volume, renal tissue kininogenase activity, renal hypertension, urinary kallikrein.

Abbreviations: G-2 rats, Goldblatt 2-kidney rats; G-1 rats, Goldblatt 1-kidney rats.

Introduction

Clamping one renal artery induces hypertension in rats whether or not the contralateral kidney is present. Renin concentration in plasma increases when the unclamped kidney is left in situ although it remains within normal values when the intact kidney is removed (Hutchinson, Mathews, Dax & Johnston, 1975). It has been suggested that two different mechanisms may be involved in the development and maintenance of these two types of renal hypertension and that the renin–angiotensin system plays an important role only in the first type (Swales, Thurston, Queiroz, Medina & Holland, 1971; Brunner, Kirshman, Sealey & Laragh, 1971). As an alternative hypothesis it has been proposed that blood pressure elevation results from the loss of a renal antihypertensive factor. Thus, there is evidence that renal prostaglandins (Lee, Covino, Takman & Smith, 1965), renomedullary neutral lipid (Muirhead, Brooks, Kosinski, Daniels & Hinman, 1966) and renal kallikrein (Croxatto, Roblero, Albertini, Corthorn & San Martin, 1974a) could be effective humoral vasodepressor factors. The renal kallikrein system has been implicated in the development of hypertension on
several groups: (a) the decrease of urinary kallikrein excretion in different types of clinical, spontaneous and experimental hypertension (Margolius, Harwitz, Pisano & Keiser, 1974; Porcelli, Bianchi & Crozatto, 1975; Crozatto & San Martin, 1970); (b) the significant fall of kidney kallikrein in experimental hypertension associated with normal or low plasma renins (Crozatto, Albertini, Roblero & Corthorn, 1974b); and (c) the increase in plasma kininogen in experimental hypertension (Albertini, Roblero, Corthorn & Crozatto, 1974). Further, the natriuretic-diuretic actions of kinins (Carretero & Oza, 1973; Mills & Ward, 1975), and the rapid response of renal kallikrein under conditions requiring acceleration of water and electrolyte excretion (Crozatto, Huidobro, Rojas, Roblero & Albertini, 1976a) provide further experimental support for the concept that renal kallikrein may be an important antihypertensive factor.

The purpose of the present study is to characterize changes in the kallikrein–kinin system during the development of renovascular hypertension. Thus the amount of kallikrein excreted, the kininogenase capacity of renal tissue and the total amount of plasma substrate in Goldblatt 1-kidney and 2-kidney hypertensive rats were examined. The relationship between the kallikrein–kinin system and kidney excretory function was also studied by measuring urinary volume, sodium (UNa+) and potassium (UK+) excretion, plasma urea, plasma volume and blood volume.

**Method**

Hypertension was induced in male Sprague–Dawley rats, weighing between 90 and 100 g, by placing a silver clamp (internal gap 0.23 mm) around the left renal artery: the contralateral kidney was left intact (Goldblatt 2-kidney, G-2 rats) or removed a week later (Goldblatt 1-kidney, G-1 rats). Sham-clamped two-kidney and one-kidney hypertensive rats were examined. The surgical procedures were performed under tribromoethanol (Avertin, Winthrop) given intraperitoneally (20 mg/100 g body wt.).

Tail blood pressure was measured weekly, under light ether anaesthesia at room temperature. The pressure on the tail cuff and the tail artery pulse were recorded simultaneously on a Grass pulse detector. The G-2 rats and the two-kidney control rats were killed at 35, 74 and 115 days after clamping. The G-1 rats and the one-kidney control rats were killed at 35 and 74 days after clamping.

The day before the final experiment, urine was collected and blood pressure was assessed in hypertensive and control groups. Some G-2 rats and two-kidney control rats, selected at random, were used for urine collection every week, starting at the first week after clamping.

Urine was collected by placing the rats in individual metabolic cages for 8 h, without food and with tap water ad libitum. Toluene (1 ml) as antiseptic was added to each container. Urine volume was measured and urine samples were kept frozen at −20°C for subsequent determination of kallikrein activity and electrolytes.

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Urinary kallikrein was measured in most of the rats by either the direct oxytocic activity on rat uterus (Beraldo, Rocival & Mares Guia, 1966; Crozatto & Noé, 1972) or by kinin generation (Crozatto & Noé, 1972; Crozatto et al., 1972). Because there was good correlation between values by both methods (r = 0.83) only the data derived by the direct oxytocic effect of the urine are reported in this study. Before the determination, urine samples were dialysed against 0.9% NaCl solution at 4°C for 24 h. A freshly prepared bradykinin solution was used as standard, and kallikrein activity was expressed as µg of bradykinin in the total volume of urine collected during an 8 h period (after dialysis). Appropriate corrections were made for dilution.

UNa+ and UK+ were determined by using an Eppendorf flame photometer.

Plasma volume was measured by a dilution-technique method. Between 11.00 hours and 15.00 hours, one control rat and one hypertensive rat were simultaneously anaesthetized with tribromoethanol and injected with 5 µCi (0.2 ml) of 131I-labelled human serum albumin, via a PE-50 polyethylene catheter inserted into the jugular vein, and completely bled 10 min later. Blood was collected in a plastic container through a heparinized PE-90 polyethylene catheter, inserted into one carotid artery, and centrifuged at 2500 g for 20 min. A 1 ml sample of plasma was taken for the radioactivity determination against a standard in a gamma counter. Blood volume was calculated from plasma volume and packed cell volume and expressed in ml/100 g body wt. Urea was measured in plasma using the Skeggs (1957) micromethod adapted to a Technicon Autoanalyzer, and was expressed in mmol/l of plasma. Immediately after bleeding, the kidneys were removed, weighed and frozen. Kallikrein was extracted 24 h later and the capacity of each extract to liberate kinines was tested on longitudinal cat jejunum strips, bathed in...
Kallikrein in renovascular hypertension

Five weeks after clamping, urinary kallikrein of G-2 rats was not significantly different from that of control sham-operated rats, despite the fact that blood pressure was significantly elevated \((P < 0.001)\). However, at weeks 10 and 15, a significant decrease was observed in urinary kallikrein concentrations \((P < 0.001)\) (Fig. 1a). In G-1 rats, urinary kallikrein was markedly decreased at weeks 5 and 10 \((P < 0.001)\) (Fig. 1b). Further, the values were significantly lower than those found during the same period in the urine of the G-2 rats \((P < 0.01)\). Urinary kallikrein of unilaterally nephrectomized control rats was not different from that of two-kidney sham-operated rats (Figs. 1a and 1b). Blood pressure of G-2 rats increased significantly from week 2 after the clamping (Fig. 2). Urinary kallikrein concentration during the first 6 weeks was only slightly lower than that of the sham-operated rats. From week 8 a significant drop in urinary kallikrein concentrations was observed, continuing to fall in the subsequent weeks (Fig. 2).

During the first 4 weeks, urine volume of the hypertensive rats was slightly lower, but this difference was not significant \((P < 0.20)\). From weeks 5 on, the hypertensive rats showed an increase in urine volume, which was significantly different from control rats at weeks 5, 10 and 12 (Fig. 2). The UNa⁺ and UK⁺ of G-2 rats and control rats showed parallel changes all during the experiment except at week 10, when G-2 rats excreted more Na⁺ \((P < 0.05)\) (Fig. 2).

### Results

Blood pressure, body weight and kidney weight are summarized in Table 1. In G-2 rats, in spite of the small size of the clamped kidney, the total weight of renal tissue was not significantly different from that of two-kidney control rats. In G-1 rats, the clamped kidney showed a small, but significant weight loss in comparison with the kidney of the unilaterally nephrectomized control rats \((P < 0.05)\) 10 weeks after surgery.

### Table 1. Blood pressure, body and kidney weight of Goldblatt two-kidney (G-2) and one-kidney (G-1) hypertensive rats

Values are mean ± SEM. Results of statistical analysis between control and experimental groups are: *\(P < 0.05\); **\(P < 0.01\); ***\(P < 0.001\). N.S. = not significant. (*) or (N.S.) refers to the statistical analysis between clamped and unclamped kidneys. C-2, Two-kidney control rats; C-1, one-kidney control rats.

<table>
<thead>
<tr>
<th>Rats</th>
<th>Time (weeks)</th>
<th>n</th>
<th>Blood pressure (mmHg)</th>
<th>Body weight (g)</th>
<th>Kidney weight (g)</th>
<th>Both kidneys</th>
<th>Unclamped kidney</th>
<th>Clamped kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-2</td>
<td>5</td>
<td>13</td>
<td>112 ± 2***</td>
<td>277 ± 6**</td>
<td>1-87 ± 0-04 N.S.</td>
<td>1.89 ± 0.08</td>
<td>1.17 ± 0.04</td>
<td>0.72 ± 0.04</td>
</tr>
<tr>
<td>G-2</td>
<td>5</td>
<td>14</td>
<td>186 ± 4</td>
<td>240 ± 11</td>
<td>1-31 ± 0-02 N.S.</td>
<td>2.34 ± 0.10</td>
<td>1.53 ± 0.08</td>
<td>0.81 ± 0.05</td>
</tr>
<tr>
<td>C-2</td>
<td>10</td>
<td>9</td>
<td>117 ± 2***</td>
<td>374 ± 7***</td>
<td>2-10 ± 0-04 N.S.</td>
<td>2.47 ± 0.20</td>
<td>1.53 ± 0.08</td>
<td>0.81 ± 0.08***</td>
</tr>
<tr>
<td>G-2</td>
<td>10</td>
<td>13</td>
<td>188 ± 7</td>
<td>297 ± 20</td>
<td>1-67 ± 0-16 N.S.</td>
<td>2.47 ± 0.20</td>
<td>1.53 ± 0.08</td>
<td>0.81 ± 0.08***</td>
</tr>
<tr>
<td>C-1</td>
<td>5</td>
<td>6</td>
<td>119 ± 4**</td>
<td>213 ± 7*</td>
<td>0-97 ± 0-02</td>
<td>1-00 ± 0-12 N.S.</td>
<td>0-97 ± 0-02</td>
<td>1-00 ± 0-12 N.S.</td>
</tr>
<tr>
<td>G-1</td>
<td>10</td>
<td>6</td>
<td>109 ± 6**</td>
<td>273 ± 13*</td>
<td>1-46 ± 0-08</td>
<td>1-46 ± 0-08</td>
<td>1-46 ± 0-08</td>
<td>1-46 ± 0-08</td>
</tr>
</tbody>
</table>

Both kidneys

Unclamped kidney

Clamped kidney

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Aprotinin (Trasylol, Bayer) was used as an inhibitor of kininogenase activity and the activity of the extracts was expressed as \(\mu g\) of bradykinin generated per g and per total mass of wet tissue.

Duplicate 0-2 ml samples of plasma were taken for kininogen determination. To estimate plasma kallikrein, trypsin was used by the method of Fasciolo, Espada & Carretero (1963). Instead of the hind-leg perfusion bioassay, the isolated cat jejunum was used to test kinins (Ferreira & Vane, 1967). Kininogen was expressed as \(\mu g\) of bradykinin generated/ml of plasma.

All results were expressed as means ± SEM. The differences in mean values were determined by Student's t-test and \(P < 0.05\) was considered statistically significant.

A Krebs solution. The methods of extracting kallikrein and testing the kinin-liberation capacity of kidney extracts have been previously described (Croatto et al., 1974b).

Urinary kallikrein, urine volume, sodium and potassium excretion

Aprotinin (Trasylol, Bayer) was used as an inhibitor of kininogenase activity and the activity of the extracts was expressed as \(\mu g\) of bradykinin generated/ml of plasma. The methods of extracting kalli-

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Blood pressure, body weight and kidney weight are summarized in Table 1. In G-2 rats, in spite of the small size of the clamped kidney, the total weight of renal tissue was not significantly different from that of two-kidney control rats. In G-1 rats, the clamped kidney showed a small, but significant weight loss in comparison with the kidney of the unilaterally nephrectomized control rats \((P < 0.05)\) 10 weeks after surgery.
Fig. 1. Urinary kallikrein activity in Goldblatt two-kidney (a) and Goldblatt one-kidney (b) hypertensive rats (hatched columns) and their respective control groups (open columns). The activity is expressed as the equivalent effect of bradykinin for the total volume of 8 h urine collection. The values in the columns refer to the number of rats. *** P < 0.001. Mean values ± SEM are shown.

Fig. 2. Blood pressure (B.P.), urinary kallikrein, urine volume, Na⁺ and K⁺ excretion at different times after clamping or sham clamping in Goldblatt two-kidney hypertensive rats (●) and sham-operated control rats (○). Numbers of animals are indicated by numbers above ● symbols and beneath ○ symbols in the upper part of the Figure. All values in the curves are mean ± SEM. * P < 0.05; *** P < 0.001.

Fig. 3. Renal kininogenase activity in total tissue (top) and per g of wet tissue (bottom) in Goldblatt two-kidney (a) and one-kidney (b) hypertensive rats (hatched columns) and their control groups (open columns). The activity is expressed as the effect of bradykinin generated by 2 min incubation with an excess of substrate. Numbers in the columns of the bottom set are the numbers of rats. *** P < 0.001.

Kininogenase activity in renal tissue

When G-2 rats were compared with sham-operated rats, there was no significant difference in the total kininogenase activity or in the activity/g of wet tissue, in any of the experimental periods (Fig. 3a).

In G-1 rats, the total kininogenase activity at weeks 5 and 10 was significantly lower than in the...
unilaterally nephrectomized control rats. The activity/g was also significantly reduced (P < 0.001) (Fig. 3b). In the unilaterally nephrectomized control group, the total kininogenase activity was not different from the activity found in the sham-operated two-kidney group, but was significantly higher/g of wet tissue in each experimental period (P < 0.001 at week 5 and P < 0.05 at week 10) (Figs. 3a and 3b). Aprotinin (200 units) completely abolished the kininogenase activity of the kidney extracts.

No significant differences in kininogenase activity were found between the clamped kidney and the intact kidney in G-2 rats. The amounts of kinin liberated were 2.48 ± 0.24 in the intact kidney against 1.99 ± 0.12 μg of bradykinin/g in the clamped kidney, 1.86 ± 0.17 against 1.73 ± 0.08 μg of bradykinin/g and 1.77 ± 0.14 against 1.73 ± 0.20 μg of bradykinin/g at weeks 5, 10 and 15 respectively.

**Plasma kininogen, plasma urea, plasma and blood volumes**

Plasma substrate was significantly increased in both types of hypertensive rats at weeks 5 and 10, compared with the control groups. Nevertheless, in the G-2 rats, plasma kininogen concentrations diminished gradually with time and reached control values by week 15 (Table 2).

A similar pattern was observed in plasma urea, plasma volume and blood volume in G-2 rats. At week 5, there were no significant differences, but by week 10 plasma urea, plasma volume and blood volume increased compared with controls. This increment persisted until the end of the experiment. All three were increased in G-1 rats by week 5 and remained high at week 10 (Table 2).

**Discussion**

Our results show important differences between G-1 and G-2 hypertensive rats, with regard to the time-course and patterns of changes in urinary and renal kallikrein concentrations, plasma kininogen concentrations, and plasma and blood volumes. Urinary kallikrein falls rapidly in both Wistar and Sprague-Dawley rats, made hypertensive by placing a figure eight ligature on one kidney followed by contralateral nephrectomy (Croxatto & San Martin, 1970). Margolius, Geller, Pisano & Sjoerdsma (1971) confirmed the fall of urinary kallikrein concentration in rats made hypertensive by placing a silver clamp on one renal artery while leaving the contralateral kidney intact (G-2). They measured urinary kallikrein only at week 16 after clamping and did not follow its time course during the onset and development of hypertension. Johnston, Matthews & Dax (1976) reported normal kallikrein excretion by G-1 rats and increased kallikrein excretion by G-2 rats, at least until the 28th post-operative day. In the present study, as early as 5 weeks after clamping, in G-1 rats a large fall in urinary kallikrein concentration was observed; in contrast, at this time G-2 rats had values similar to the sham-operated control. We cannot explain the discrepancy between our findings and those of Johnston _et al._ (1976).

The concept that the renal kallikrein–kinin system participates in the regulation of extracellular fluid volume by control of water excretion has been suggested by Mills & Ward (1975). We have

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**Table 2. Plasma kininogen, plasma urea, plasma and blood volumes, in Goldblatt two-kidney (G-2) and one-kidney (G-1) hypertensive rats**

Values are mean ± SEM. Results of statistical analysis between control and experimental groups are: * P < 0.05; ** P < 0.01; *** P < 0.001. N.S. = not significant. C-2, Two-kidney control rats; C-1, one-kidney control rats.

<table>
<thead>
<tr>
<th>Time (weeks)</th>
<th>n</th>
<th>Plasma kininogen (µg of bradykinin/ml)</th>
<th>Plasma urea (mmol/l)</th>
<th>Plasma volume (ml/100 g body wt.)</th>
<th>Blood volume (ml/100 g body wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-2</td>
<td>5</td>
<td>13</td>
<td>2.60 ± 0.17***</td>
<td>410 ± 19 N.S.</td>
<td>3.58 ± 0.10 N.S.</td>
</tr>
<tr>
<td>G-2</td>
<td>5</td>
<td>14</td>
<td>3.85 ± 0.15</td>
<td>490 ± 61</td>
<td>3.57 ± 0.14</td>
</tr>
<tr>
<td>C-2</td>
<td>10</td>
<td>9</td>
<td>2.15 ± 0.09***</td>
<td>390 ± 9***</td>
<td>3.68 ± 0.20**</td>
</tr>
<tr>
<td>G-2</td>
<td>10</td>
<td>13</td>
<td>3.14 ± 0.18</td>
<td>778 ± 57</td>
<td>5.62 ± 0.46</td>
</tr>
<tr>
<td>C-2</td>
<td>15</td>
<td>17</td>
<td>2.10 ± 0.00 N.S.</td>
<td>332 ± 15***</td>
<td>3.53 ± 0.13**</td>
</tr>
<tr>
<td>G-2</td>
<td>15</td>
<td>14</td>
<td>2.42 ± 0.23</td>
<td>577 ± 66</td>
<td>4.79 ± 0.29</td>
</tr>
<tr>
<td>C-1</td>
<td>5</td>
<td>6</td>
<td>2.13 ± 0.10***</td>
<td>480 ± 13***</td>
<td>3.03 ± 0.22**</td>
</tr>
<tr>
<td>G-1</td>
<td>5</td>
<td>6</td>
<td>2.32 ± 0.17</td>
<td>730 ± 60</td>
<td>3.82 ± 0.10</td>
</tr>
<tr>
<td>C-1</td>
<td>10</td>
<td>6</td>
<td>2.05 ± 0.28***</td>
<td>427 ± 28**</td>
<td>3.40 ± 0.10**</td>
</tr>
<tr>
<td>G-1</td>
<td>10</td>
<td>6</td>
<td>3.23 ± 0.34</td>
<td>597 ± 32</td>
<td>5.15 ± 0.15</td>
</tr>
</tbody>
</table>

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**Kallikrein in renovascular hypertension** 231
previously showed that water-loading, as well as sodium chloride loading (Croxatto et al., 1976a) and administration of diuretics (Croxatto, Roblero, Garcia, Corthorn & San Martin, 1973) produce increased kallikrein excretion in normal rats. In G-1 rats, which show reduced urinary kallikrein, only a moderate increase in the urinary excretion of this enzyme is observed when diuresis is stimulated by water (Croxatto, Albertini, Arriagada, Roblero, Rojas & Rosas, 1976b). These observations support the concept that the stimuli which increase diuresis and natriuresis promote an activation of the renal kallikrein–kinin system. They also support the idea that this system is markedly impaired in G-1 rats. This impairment is also observed in G-2 rats, but only after long-term hypertension, apparently when plasma and blood volume are increased. In addition, in unilaterally nephrectomized control rats, the decrease in kidney mass promoted an increase in the renal kininogenase activity/g of wet tissue. This compensatory mechanism is not present in G-1 rats, and may be related to the increase in plasma volume found in the early phase of this model of experimental hypertension.

The increase in plasma kininogen has been utilized as evidence for the involvement of the kallikrein–kinin system in hypertension (Croxatto, Corthorn, Roblero, Garcia & Albertini, 1974c). The persistent high concentration of plasma kininogen and the low kallikrein activity found in renal tissue and urine, support the idea that the substrate concentration in G-1 rats hypertension might reflect enzymic activity. However, the high concentration of plasma kininogen with normal kininogenase activity in renal tissue and urine in G-2 rats is not consistent with this hypothesis. Wong, Talamo, Williams & Colman (1975) demonstrated that in normal subjects plasma angiotensin II and bradykinin exhibit parallel changes, when saline is infused or body position modified. The response to an angiotensin II antagonist suggests that in G-2 rats the renin–angiotensin system is hyperactive (Brunner et al., 1971). Therefore, the components of the kallikrein–kinin system could also be stimulated.

The decrease of renal kininogenase activity in G-1 rats agrees with previous reports (Croxatto et al., 1974b) but our findings are in contrast with those of Carretero, Oza, Scicli & Schork (1974), who described a fall in renal-cortex kallikrein in G-2 rats 36 days after renal clamping. Only one rat at the 5th week, two rats at the 10th week and two rats at the 15th week in our experimental series showed lower renal kininogenase activity than the mean values found in sham-operated rats. The findings by Carretero et al. (1974) that both clamped and unclamped kidneys in G-2 rats have similar kallikrein concentrations were confirmed.

Our results, which provide data only about kininogen in blood, kallikrein in urine and kininogenase activity of the kidneys, are not sufficient to assign to the kallikrein–kinin system a causative role in hypertension. Before ruling out the involvement of this system in the etiology of hypertension, including essential hypertension, it would be necessary to assess whether renal kallikrein is contributing to kinin formation in the blood or participating, through kinin formation, in renal function.

Acknowledgments

This study was supported by Grants from the Catholic University of Chile (no. 33/74) and from PNUD/UNESCO-RLA 75/047 no. 4. We thank Miss Patricia Oliveri and Mr. Joaquin Eyzaguirre for technical assistance.

References


Kallikrein in renovascular hypertension


