Renal kallikrein activity and urinary kallikrein excretion in rats with experimental renal hypertension

M. MARIN-GREZ, G. SCHAECHTELIN, G. BÖNNER, G. SPECK, D. GANTEN AND F. GROSS
Department of Pharmacology, University of Heidelberg, Federal Republic of Germany

(Received 5 August 1981/5 April 1982; accepted 4 May 1982)

Summary

1. Rats were made hypertensive by ligating the aorta between the origins of both renal arteries. Sham-operated animals served as controls. Urinary and renal kallikrein activities, as well as plasma and renal renin activities, were measured 8 and 90 days after surgery.

2. Blood pressure was 155 ± 6 mmHg on day 8 after aortic ligation and 142 ± 6 mmHg on day 90; in controls pressures were 107 ± 3 and 110 ± 5 mmHg respectively.

3. Eight days after aortic ligation, kallikrein activity in the ischaemic kidneys was about 6.5 times, and in the non-ischaemic kidneys almost 2 times, that in controls. After 90 days the kallikrein activity was reduced to one-half of that in the controls in the ischaemic kidneys and it was normal in the contralateral.

4. The urinary kallikrein excretion of hypertensive rats was about one-third of that of the controls at both 8 and 90 days after aortic ligation.

5. The plasma renin activity in hypertensive rats was approximately seven times that in control animals 8 days after aortic ligation and did not differ from the control value after 90 days. Renin activity in the kidneys showed the same pattern as in other models of renovascular hypertension: elevation in the ischaemic kidney and reduction in the non-ischaemic one.

Key words: experimental hypertension, kallikrein–kinin system, plasma renin activity, renal kallikrein, renal renin.

Introduction

Exogenously administered kinins cause natriuresis and arteriolar vasodilatation [1], hence it is conceivable that variations in the endogenous release of these peptides affect sodium excretion and peripheral vascular resistance. Sodium retention and/or arteriolar vasoconstriction could result if the activity of the kallikrein–kinin system is reduced. Subnormal production of kinins is thought to be involved in the pathogenesis of renal hypertension. This assumption is based on the observation that the urinary kallikrein excretion is diminished in established renal experimental hypertension and in clinical renovascular hypertension [2–5]. Kallikrein excretion could be viewed as an index of a (as yet hypothetical) secretion of kallikrein into the blood stream.

To investigate whether this system is already affected during the initial stage of renovascular hypertension, we assayed the urinary kallikrein excretion 8 days after inducing a unilateral renal ischaemia by total occlusion of the aorta above the origin of the left renal artery in rats. The probable involvement of kallikrein in the late stage of this type of high blood pressure was investigated by measuring the kallikrein excretion 3 months after induction of the left renal ischaemia.

Variations in the intrarenal kallikrein activity, irrespective of a release to the circulation, might also affect blood pressure. An alteration in the intrarenal vascular resistance and/or in the water and electrolyte balance could be the responsible mechanism. For this reason, the kininogenase activity (i.e. kinin-generating capacity) in the renal cortex was also measured. To obtain information about the relationship between the renal kallikrein–kinin system and the renin–angiotensin system, plasma renin activity and the
renin activity in the renal cortex were also determined.

Materials and methods

Male Wistar rats weighing 200–300 g were used. Under ether anaesthesia, the aorta was ligated between the origins of the renal arteries [6]. Sham-operated rats were used as controls. Until the metabolic studies were performed, the animals were kept in a room lighted from 06.00 to 18.00 hours, under constant temperature and humidity (21°C, 50%). The rats were then placed in stainless-steel metabolic cages, at least 3 days before urine collection. Eight and 90 days after ligature of the aorta (AL) or sham operation (C), urine was collected for 24 h in plastic containers at room temperature. Forty-two animals were operated on for the experiment lasting 8 days (AL: 22 rats; C: 20 rats) and 25 (AL: 13 rats; C: 12 rats) for the experiment of 90 days.

At the end of the urine collection the animals were anaesthetized with sodium pentobarbital (30 mg/kg, intraperitoneally). The left carotid artery was cannulated with a polyethylene tube for measuring blood pressure and taking blood samples to determine packed cell volume as well as plasma sodium, potassium and renin activity. By means of a catheter introduced through the left ventricle into the ascending aorta, the kidneys were perfused with sodium chloride solution (150 mmol/l) at a hydrostatic pressure of 100 cm until they were macroscopically free of blood. Subsequently, the kidneys were excised and weighed. The renal cortex was homogenized and incubated for 20 min at 4°C with 0.4% sodium deoxycholate to solubilize the kallikrein. After centrifugation (50 000 g, 30 min, 4°C), the kininogenase activity was measured in the supernatant by incubation with partially purified dog kininogen [7]. The kinins released were estimated by radioimmunoassay with synthetic bradykinin used as standard [8]. The kallikrein activity in the renal cortex was calculated per minute of incubation and milligram of solubilized cortical protein.

Kallikrein in the diluted urine (1:50–1:100) was also measured by incubation with dog substrate and radioimmunoassay of kinins [9]. The kallikrein activity in the urine was calculated per minute of incubation and millilitre of urine and the kallikrein excretion by multiplying this activity by the 24 h urine volume.

Urinary and plasma sodium and potassium were measured by internal standard flame photometry.

Renin was measured (without acid activation) in the renal cortex and in plasma by incubation with partially purified rat angiotensinogen. The released angiotensin I was determined by means of a specific radioimmunoassay [10].

Protein concentration in the supernatant of the homogenates of the renal cortex was measured by the method of Lowry et al. [11].

Results

The body weight of rats with total occlusion of the aorta, which before the ligature did not differ from that of controls (AL: 259 ± 9 g, n = 17; C: 238 ± 8, n = 16), was reduced 8 days after surgery (AL: 202 ± 8 g, n = 17; C: 255 ± 9, n = 16; P < 0.001). The blood pressure at this time was higher in AL rats (155 ± 6 mmHg, n = 17) than in sham-operated rats (107 ± 3 mmHg, n = 13; P < 0.001). The plasma renin activity was also significantly increased (AL: 65.5 ± 14.8 pmol of angiotensin I h⁻¹ ml⁻¹; n = 7; C: 9.3 ± 2.0, n = 6; P < 0.01). A reduced plasma potassium concentration (AL: 3.8 ± 0.1 mmol/l, n = 4; C: 4.8 ± 1.0, n = 8; P < 0.001) and an increased packed cell volume (AL: 50 ± 2%, n = 16; C: 46 ± 1, n = 16; P < 0.05) were found in the hypertensive animals. No differences were detected in the plasma sodium concentration. The food intake of AL rats was lower (9 ± 1 g/day, n = 17) than that of controls (21 ± 1 g/day, n = 13; P < 0.001), but the water intake did not differ (AL: 37 ± 5 ml/day, n = 17; C: 34 ± 2, n = 13).

In spite of a reduced kallikrein excretion, the kallikrein activity in the renal cortex of AL rats was markedly elevated (P < 0.001) in the ischaemic (left) kidney and slightly but also significantly enhanced (P < 0.02) in the non-ischaemic one (Fig. 1). The renal renin activity of the ischaemic kidney was increased and that of the contralateral reduced (Table 1). The mean weight of the ischaemic kidney of AL rats was lower than that of the same side from sham-operated animals (Table 1). The urine volume of AL rats was increased and the sodium and potassium excretion was diminished (Table 1).

The body weight of rats with complete occlusion of the abdominal aorta increased from 227 ± 2 g (n = 11) at the time of ligature to 466 ± 11 g (n = 11) 90 days thereafter. Similarly, in sham-operated rats the body weight increased from 227 ± 2 to 475 ± 14 g (n = 11). Three months after aortic ligature, the arterial blood pressure was still elevated (AL: 142 ± 6 mmHg, n = 11; C: 110 ± 5, n = 11; P < 0.001). Plasma
TABLE 1. Urinary and renal measurements made 8 days and 90 days after sham operation (C) or ligature of the aorta between the origin of the renal arteries (AL) in rats

N.S.: not significant; ANG I: angiotensin I.

<table>
<thead>
<tr>
<th></th>
<th>8 days</th>
<th>90 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>AL</td>
</tr>
<tr>
<td></td>
<td>8 days</td>
<td>90 days</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>AL</td>
</tr>
<tr>
<td>Urine volume (ml/24 h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium excretion (mmol/24 h)</td>
<td>3.8 ± 0.6 (13)</td>
<td>2.1 ± 0.4 (17)</td>
</tr>
<tr>
<td>Potassium excretion (mmol/24 h)</td>
<td>4.1 ± 0.2 (13)</td>
<td>1.9 ± 0.2 (17)</td>
</tr>
<tr>
<td>Right kidney wt. (mg)</td>
<td>1093 ± 48 (16)</td>
<td>1171 ± 35 (17)</td>
</tr>
<tr>
<td>Left kidney wt. (mg)</td>
<td>1042 ± 38 (16)</td>
<td>543 ± 23 (17)</td>
</tr>
<tr>
<td>Right renal cortex protein (mg/g wet wt.)</td>
<td>131 ± 9 (14)</td>
<td>134 ± 5 (14)</td>
</tr>
<tr>
<td>Left renal cortex protein (mg/g wet wt.)</td>
<td>134 ± 6 (14)</td>
<td>124 ± 6 (12)</td>
</tr>
<tr>
<td>Right renal cortex renin (µg of ANG I h⁻¹ g⁻¹)</td>
<td>38.9 ± 7.0 (4)</td>
<td>24 ± 0.6 (8)</td>
</tr>
<tr>
<td>Left renal cortex renin (µg of ANG I h⁻¹ g⁻¹)</td>
<td>37.3 ± 3.9 (4)</td>
<td>162.6 ± 21.0 (8)</td>
</tr>
</tbody>
</table>

FIG. 1. Renal (a) and urinary (b) kallikrein measured 8 days after ligation of the aorta above the left renal artery (AL, □) or after a sham operation (C, ◯) in rats. The kallikrein activity in renal cortex and in urine was measured by incubation with dog kininogen and radioimmunoassay of the kinins released. Significance of difference from control: * P < 0.02; ** P < 0.001.

renin activity from AL rats did not differ from that of controls (AL: 2.3 ± 0.4 pmol of angiotensin I h⁻¹ ml⁻¹, n = 11; C: 3.2 ± 0.6, n =

FIG. 2. Renal (a) and urinary (b) kallikrein measured 90 days after aortic ligature (AL, □) or sham operation (C, ◯) in rats. Kallikrein activity was assayed with a kininogenase method and radioimmunoassay of the kinins released. Significance of differences from control: ** P < 0.005; *** P < 0.001.

11). The urinary excretion of kallikrein was reduced (P < 0.001) and the activity of the enzyme in the ischaemic kidney was slightly lower (P < 0.005) than that in sham-operated normotensive rats (Fig. 2). The renin activity was
higher than the control value in the ischaemic kidney, and lower in the contralateral one (Table 1). Urine volume, as well as the sodium and potassium excretion, were similar in AL and in control animals (Table 1). Also food and water intake did not differ significantly (AL: 30 ± 3 g/day and 75 ± 9 ml/day, n = 11; C: 27 ± 1 g/day and 57 ± 5 ml/day, n = 11, respectively).

### Discussion

The urinary excretion of kallikrein is reduced in patients with hypertension due to a renal artery stenosis [4], and also in rats with experimental renal hypertension caused by placing a clip on a renal artery or by a ligature in figure-of-eight on the kidney [2–4]. It was assumed that the decreased kallikrein excretion reflects a diminished activity of renal kallikrein and a lowered production of vasodilatory kinins. Consequently, it was suggested that a reduced activity of the renal kallikrein–kinin system might be involved in the development of hypertension. However, our previous finding that the blood pressure of rats with renal hypertension (two kidneys, one clip) rises before the excretion of kallikrein is lowered, does not support this interpretation [12].

In the experiment described in this report, the kallikrein excretion was already reduced 1 week after induction of renal ischaemia. It is unclear whether this reduction is related to the hypertensive process or merely reflects a condition in which only one kidney adds kallikrein to the urine. Unilateral nephrectomy also markedly reduces the kallikrein excretion [12]. The elevated urine volume, not accompanied by a corresponding increase in water intake, could have resulted in a dehydration of the AL rats (8 days experiment). The higher packed cell volume found in these rats supports this assumption. We have previously shown that dehydration reduces the kallikrein excretion [13]. Thus the lower kallikrein excretion of AL rats could be due to a combination of several factors: (a) reduction or absence of urine production and therefore minimal addition of kallikrein to the final urine by the ischaemic kidney; (b) loss of kallikrein-producing cells; (c) dehydration.

The elevated kallikrein activity found in the ischaemic kidney 8 days after ligature may be the consequence of an accumulation of kallikrein secondary to a marked reduction of the urine flow. We have previously reported that a rapid enhancement of urine flow, brought about by injection of diuretics, leads to a transient rise of kallikrein excretion with a concomitant reduction of kallikrein activity in the renal cortex (washout effect) [14] (G. Bömer, M. Marin-Grez, U. Schwertschlag, M. Deeg, D. Beck & F. Gross, unpublished work). The reverse might occur when the urine flow is impaired. Alternatively, the intrarenal blood pressure might affect the activity of kallikrein in the ischaemic kidney. It has been reported that kallikrein excretion decreases after acute constriction of the renal artery [15–17]. But since we have shown that the vasodilator hydralazine does not alter the excretion of the enzyme [18], it appears that blood pressure influences kallikrein excretion indirectly (as diuretics do), through changes in urine flow [14]. Thus an inverse (though non-specific) relationship might exist between blood pressure and kallikrein activity in the kidney.

The kallikrein activity in the renal tissue might depend not only on the rate of excretion of the enzyme and on its presumable release into lymph [19] and blood, but also on the rate of enzyme synthesis, as well as on its renal inactivation [20]. The synthesis of kallikrein might be influenced by renal renin activity. A rise of angiotensin formation could stimulate the release of prostaglandins. Both angiotensin and prostaglandins increase kallikrein excretion [21–23], but since this rise has been shown to accompany parallel changes of urine flow, nothing can be said about its specificity nor about its part in the enhancement of kallikrein activity observed in the ischaemic kidney 8 days after aortic ligature.

The rise of kallikrein activity in the non-ischaemic kidney 8 days after aortic ligature could be due to a greater release of aldosterone by the stimulated renin–angiotensin system. This would correspond to the stimulation of renal kallikrein activity by exogenous mineralocorticoids [24, 25]. Elevated systemic blood pressure might also be responsible for such a stimulation if, as has been shown to occur after an acute elevation of blood pressure, sustained hypertension could increase the kallikrein excreted by that kidney. However, the possibility that the changes of kallikrein excretion reported in acute experiments are due to a non-specific wash-out and do not reflect the activity in the kidney was already pointed out.

During the early stage (8 days after AL) the hypertension is probably related to the increased plasma renin activity, since administration of antibodies against angiotensin II reduces blood pressure [26].

It has been reported that the kallikrein activity of both the ischaemic and the non-ischaemic kidney is reduced 5 weeks after unilateral constriction of a renal artery in rats [27] (two
Renal kallikrein activity in hypertension

kidneys, one-clip model). This finding has not been confirmed by others, who observed unchanged renal kallikrein activity in both kidneys [28]. In our experiment, 90 days after induction of hypertension the activity of kallikrein was lower than that of sham-operated controls in the ischaemic kidney only. It is unlikely that this reduction is due to a decrease of renal blood flow beyond the autoregulatory range, as might be presumed from the lower kallikrein excretion observed after pronounced reduction of renal blood flow in acute bleeding experiments [29], since the kallikrein activity of the left kidney 8 days after AL was increased in spite of the ischaemia. The lower activity of the enzyme is probably due to an atrophy of the kallikrein-producing cells.

Although there is as yet no published support for the hypothetical role of renal kallikrein in the control of blood pressure, the possibility that lower kallikrein activity in the ischaemic kidney contributes to the hypertension cannot be ruled out. Either a local (reduced natriuresis, increased renal vascular resistance) or a systemic involvement of kallikrein (reduced release of the enzyme into the blood stream) is in theory possible. However, it should be pointed out that renal ischaemia may reduce kallikrein excretion without altering blood pressure [4, 30].

Ninety days after AL the plasma renin activity had returned to values similar to that of control animals. This confirms observations of other investigators [31], and suggests that the renin–angiotensin system is not responsible for the late stage of high blood pressure in this experimental model.

It has been suggested that the activation of prorenin to renin would be accomplished by the proteolytic activity of kallikrein [32, 33]. Our experiments indicate that renal kallikrein is not a major determinant of renal renin activity, since kallikrein was increased and renin decreased in the non-ischaemic kidney 8 days after AL, and the reverse was found in the ischaemic kidney 90 days after AL.

Impairment of a natriuretic role of kallikrein (either by decreased intrarenal formation of kallidin or prostaglandins) could contribute to the hypertension [7]. The early stages of high blood pressure in the model under discussion could not be explained by such a mechanism, since kallikrein activity in the kidney was increased. However, in the later stages of the hypertension (90 days after AL) an intrarenal role of kallikrein cannot be excluded.

The possibility that the lower kallikrein excretion reflects a reduction of the ‘usual’ release of kallikrein into the blood stream cannot be disregarded. Since the kidney seems to clear (rather than to secrete) kallikrein from the circulation [20, 34], this possibility appears remote. The enhancement of blood pressure does not seem to be due to a deficit of circulating renal kallikrein.

Our data indicate that in experiments of short duration the excretion of kallikrein in the urine does not necessarily reflect the activity of the kallikrein–kinin system in the kidney.

Acknowledgments

We are indebted to Mrs G. Breypohl and Ms J. Kopatsch for excellent technical assistance. M.M.-G. was a fellow of the Alexander von Humboldt Foundation. This work was supported by the SFB 90 of the Deutsche Forschungsgemeinschaft.

References

The dependence of urinary kallikrein excretion on renal artery pressure.


