Changes in renal and urinary kallikrein activity by mannitol-induced osmotic diuresis

G. BÖNNER, M. MARIN-GREZ, D. BECK, M. DEEG AND F. GROSS

Department of Pharmacology, University of Heidelberg, Federal Republic of Germany

(Received 15 August/29 December 1980; accepted 30 January 1981)

Summary

1. Osmotic diuresis was induced in male Sprague-Dawley rats by a 30% (w/v) mannitol solution injected three times at 15-min intervals. Kallikrein excretion increased for a short period after the first two injections, but, despite marked diuresis, the increment of kallikrein excretion after the second injection was less marked than after the first and no enhanced kallikrein excretion was observed after the third injection of mannitol.

2. Urinary kallikrein excretion correlated only with urinary potassium excretion. No correlation was found with either urine volume or urinary sodium excretion.

3. At the end of the osmotic diuresis kallikrein activity was significantly reduced both in the urine and in the renal cortex. At that time plasma aldosterone concentration was slightly greater in the mannitol-treated than that in the control group, but the difference did not reach statistical significance.

4. In this experiment no relationship was observed between the activity of the renal kallikrein-kinin system and the plasma aldosterone concentration.

5. The transient increase in urinary kallikrein excretion is interpreted as a wash-out effect of renal kallikrein, which is followed by a diminished kallikrein activity in urine and in renal cortex.

Key words: aldosterone, glandular kallikrein, kallikrein-kinin system, mannitol, osmotic diuresis, renal kallikrein activity.

Correspondence: Dr G. Bönner, Department of Internal Medicine, Merheim Hospital, University of Cologne, Ostmerheimerstr. 200, D-5000 Köln 91, FRG.

Abbreviation: KIU, kallikrein inhibiting units.

Introduction

The effect of enhanced diuresis on the activity of the renal kallikrein-kinin system has been studied in different experimental models. Acute diuretic responses have been provoked by acute water-loading [1, 2], by intragastric or intravenous administration of isotonic saline solution [3-5] or by the administration of various diuretics such as frusemid [6, 7], acetazolamide [6], bumetanide or Bendroflumethiazide [8]. In all these investigations, diuresis was accompanied by an elevated excretion of renal kallikrein. Conversely, potassium-sparing diuretics, such as spironolactone [9], reduced the activity of renal kallikrein. Since the influence of an acute osmotic diuresis on the renal kallikrein-kinin system has not been reported so far, we studied the effects of repeated injections of mannitol on urinary and renal kallikrein activity.

Materials and methods

Twelve male Sprague-Dawley rats weighing 223.3 ± 2.6 g were kept in individual cages and fed with a standard rat chow (100 mmol of Na/kg and 200 mmol of K/kg; Sniff). After 3 days the rats were divided into two groups of six rats each. All animals were anaesthetized with pentobarbital sodium (Nembutal; 30 mg/kg intraperitoneally). A catheter (PP 50) was placed in the femoral vein for the injection of mannitol or NaCl solution (0-9 g/l saline) respectively. Urine was collected through a catheter placed in the bladder; to avoid urine losses the urethra was ligated.
Fifteen minutes after surgery urine collection was started. After three control periods of 10 min each, 0.1 ml of a 30% mannitol solution (w/v in saline) was injected intravenously in the six rats of group one (M). Injections of mannitol were repeated after 15 min (0.1 ml) and after 30 min (0.05 ml) respectively. This method of mannitol injection was chosen to obtain an increasing and prolonged diuresis rather than a single diuretic peak. The control group (C) received an equal volume of saline at the same time intervals. Urine was collected for 5 min immediately after the first and second injection of mannitol; all other collection periods lasted 10 min.

Urinary kallikrein activity was measured by means of a bradykinin radioimmunoassay as described previously [10, 11]. Urine was incubated for 20 min at 37°C and pH 8.5 with an excess of partially purified dog kinogen dissolved in Tris/HCl buffer (0.1 mol/l); 1,10-phenanthroline and disodium EDTA were added as kininase inhibitors. The reaction was stopped by the addition of 96% (v/v) ethanol, which also precipitated the remaining kinogen. After centrifugation and evaporation of the sample by addition of aprotinin (500 KIU/ml of buffer) to the incubation medium, the remaining chromophore p-nitroaniline was cleaved by addition of 250 kallikrein inhibiting units (KIU) of aprotinin (Trasylol, Bayer) in 0.1 ml of buffer solution. To exclude a non-specific splitting of the substrate, a control was made for each sample by addition of aprotinin (500 KIU/ml of buffer) to the incubation medium. Absorbance at 280 nm was determined photometrically (spectrophotometer 550 A, Perkin–Elmer). Kallikrein activity was calculated as described by Amundsen, Putter, Friberger, Knoes, Larsbraten & Claesson [12] and expressed as m-units/g of tissue. Since the tripeptide substrate has a much lower sensitivity for plasma kallikrein than for glandular kallikreins [13], no difference in kallikrein activity was observed between tissue samples (1:100 diluted taken from perfused (saline) and those from non-perfused kidneys. The values of kallikrein activity determined by this assay correlated highly with the values obtained by the aforementioned bradykinin radioimmunoassay (r = 0.9698, n = 37, P < 0.001). Hence, the activity of kallikrein can be determined in kidneys from which blood has not been removed completely, as performed in this experiment.

To measure aldosterone, blood was collected at the end of the experiment after rats were decapitated and calcium heparin (Calcieparin, Nattermann) was added. After centrifugation, aldosterone was determined in the plasma by radioimmunoassay as previously described [14].

Sodium and potassium in the urine were measured by means of a flame photometer with an internal standard (Klina, Beckman). Total protein in the renal cortex was determined by the method of Lowry, Rosebrough, Farr & Randall [15] with bovine serum albumin as a standard.

All values are given as means ± SEM. The statistical significance of differences between means was calculated by Student’s t-test. Significance of correlations was tested against zero.

Results
The first injection of mannitol caused a significant increase in both urine volume and urinary kallikrein excretion (Table 1), kallikrein activity remaining unchanged during the first 5 min; afterwards, it decreased continuously (Fig. 1). The second injection of mannitol was followed by a more marked diuresis, but the increase in kallikrein excretion was less pronounced than after the first injection. After the third injection a prolonged diuresis was obtained, but urinary kallikrein excretion decreased gradually and, towards the end of the experiment, it was significantly lower than in the control rats (Table 1). Kallikrein activity in urine was still diminished when urine volume had almost returned to control values (Fig. 1).
Mannitol and renal kallikrein

TABLE 1. Urine volume and urinary kallikrein excretion after intravenous injection of 30% (w/w) mannitol solution (n = 6) or 0.9% (w/v) NaCl solution (n = 6) as control

Values are calculated for 1 min and expressed as means ± SEM. Significance of differences: * P < 0.05; ** P < 0.02; *** P < 0.005; **** P < 0.001.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Control</th>
<th>Injection 1</th>
<th>Injection 2</th>
<th>Injection 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Manitol</td>
<td>30%</td>
<td>5%</td>
<td>0.2%</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0.9%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>4.25</td>
<td>7.86</td>
<td>13.80</td>
<td>11.57</td>
</tr>
<tr>
<td>± 0.74</td>
<td>± 0.56</td>
<td>± 0.41</td>
<td>± 0.70</td>
<td>± 0.34</td>
</tr>
<tr>
<td>35</td>
<td>5.40</td>
<td>9.25</td>
<td>17.30</td>
<td>15.25</td>
</tr>
<tr>
<td>± 0.75</td>
<td>± 0.84</td>
<td>± 0.85</td>
<td>± 1.04</td>
<td>± 0.90</td>
</tr>
<tr>
<td>45</td>
<td>6.50</td>
<td>12.60</td>
<td>22.30</td>
<td>19.75</td>
</tr>
<tr>
<td>± 0.85</td>
<td>± 1.00</td>
<td>± 1.10</td>
<td>± 1.34</td>
<td>± 1.00</td>
</tr>
<tr>
<td>50</td>
<td>7.60</td>
<td>14.30</td>
<td>24.50</td>
<td>21.50</td>
</tr>
<tr>
<td>± 0.90</td>
<td>± 1.10</td>
<td>± 1.20</td>
<td>± 1.55</td>
<td>± 1.20</td>
</tr>
<tr>
<td>60</td>
<td>8.70</td>
<td>16.50</td>
<td>27.70</td>
<td>24.75</td>
</tr>
<tr>
<td>± 1.00</td>
<td>± 1.20</td>
<td>± 1.30</td>
<td>± 1.60</td>
<td>± 1.30</td>
</tr>
<tr>
<td>70</td>
<td>9.80</td>
<td>18.60</td>
<td>30.90</td>
<td>27.75</td>
</tr>
<tr>
<td>± 1.20</td>
<td>± 1.30</td>
<td>± 1.40</td>
<td>± 1.70</td>
<td>± 1.40</td>
</tr>
<tr>
<td>80</td>
<td>10.90</td>
<td>20.70</td>
<td>34.10</td>
<td>30.75</td>
</tr>
<tr>
<td>± 1.30</td>
<td>± 1.40</td>
<td>± 1.50</td>
<td>± 1.80</td>
<td>± 1.50</td>
</tr>
<tr>
<td>90</td>
<td>12.00</td>
<td>22.80</td>
<td>37.30</td>
<td>33.75</td>
</tr>
<tr>
<td>± 1.40</td>
<td>± 1.50</td>
<td>± 1.60</td>
<td>± 1.90</td>
<td>± 1.60</td>
</tr>
</tbody>
</table>

Fig. 1. (a) Course of urine volume and (b) kallikrein activity in urine (U_kall) after three intravenous injections of 30% (w/v) mannitol solution in male Sprague-Dawley rats (O—O, n = 6). Control rats (O—O, n = 6) received the same volume of saline. Mean values ± SEM are shown.

After both the first and the second mannitol injection sodium and potassium excretion rose for a short period only and, at the end of the experiment, the electrolyte excretion of rats injected with mannitol did not differ from that of the controls (Na+: M, 0.31 ± 0.09 vs C, 0.34 ± 0.04; K+: M, 0.91 ± 0.19 vs C, 1.09 ± 0.11 μmol/min). There was no correlation between urinary kallikrein excretion and either urine volume or urinary sodium excretion, whereas potassium excretion correlated with the kallikrein excretion (r = 0.7366, P < 0.05).

During the experiment, body weight fell about 1 g in all rats; packed cell volume was the same in both groups at the end of the experiment (M, 43.4 ± 0.3; C, 43.5 ± 0.3%). Plasma aldosterone concentration was slightly higher in the rats injected with mannitol than in the controls, but did not reach the statistical significance level (M, 5.77 ± 1.69; C, 3.42 ± 0.74 ng/100 ml; Fig. 2).

Kallikrein activity in the renal cortex calculated per gram of tissue was at the end of the experiment significantly lower in the rats injected with mannitol (Fig. 2) than in the control rats injected with saline. Since the protein content of the cortical tissue was unchanged, the specific kallikrein activity calculated per milligram of protein was also diminished (M, 0.22 ± 0.02 vs C, 0.36 ± 0.06 m-unit/mg of protein; P < 0.05).

Discussion

Osmotic diuresis of about 1 h duration, produced by repeated injections of mannitol, caused an initial and transient increase in kallikrein excretion in urine. Measurement of urinary kallikrein excretion at various time intervals after mannitol indicated that the kallikrein present in
FIG. 2. Effect of mannitol-induced diuresis on (a) the concentration of aldosterone in plasma, (b) kallikrein activity in renal cortex \( R_{\text{KAL}} \) and (c) urinary kallikrein excretion per minute. Measurements were made at the end of the experiment, 1 h after the first mannitol injection. Mean means ± SEM are shown. * \( P < 0.05 \); ** \( P < 0.02 \). \( \square \) Control rats \( (n = 6) \); \( \square \), mannitol-treated rats \( (n = 6) \).

The tubular system is washed out by the enhanced urine flow. The course of excretion does not suggest that the production or secretion of kallikrein is stimulated by this type of diuresis. On the contrary, since the activity of kallikrein in renal cortical tissue is diminished at the end of the diuresis period, it may be presumed that the stores of the enzyme within the kidney have been depleted. Possibly the ‘wash-out’ is a physiological and not a biochemical phenomenon induced by mannitol, since similar findings have been obtained after the administration of frusemide or bumetanide, which also cause only a transient increase in kallikrein excretion, with a subsequent reduction of kallikrein activity in the urine (G. Bönner, Th. Unger, G. Speck, D. Ganten & F. Gross, unpublished work) and in the kidney (G. Bönner, M. Deeg, D. Beck, M. Marin-Grez & F. Gross, unpublished work; [16]).

From these findings it is obvious that the effect of diuresis on the renal kallikrein–kinin system can only be assessed if it is followed up over a sufficiently long period. An initial elevation of kallikrein excretion in urine does not permit conclusions as to what may happen within the kidney, since it may simply be an accompanying effect elicited by a sudden enhancement of urine flow. Probably, the biphasic course of kallikrein excretion occurs in all types of diuresis and the intensity of the initial increase depends on the degree to which urine flow is augmented and on the amount of kallikrein present in the renal tubular system.

Since, in our experiments, the duration of the diuresis was short and of moderate degree, no major changes in the intravascular volume occurred at the end of the experiment. Although plasma concentration of aldosterone rose only slightly, this tendency contrasts in some respect with the decrease in the activity of the renal kallikrein–kinin system, which is usually stimulated by mineralocorticoids [9, 17–20]. Such a dissociation between plasma aldosterone concentration and the activity of the renal kallikrein–kinin system has also been described in rats with renal hypertension [21]. However, the acute changes we observed cannot be compared with chronic influences on the activity of the renal kallikrein–kinin system both in the urine and in the renal cortex. The short-lasting effects caused by an acute diuresis in anaesthetized rats do not permit any conclusion as to what may happen during prolonged studies in conscious rats. It may, however, be stated that acute diuretic responses, independent of the mechanism by which they are induced, do not stimulate the renal kallikrein–kinin system, but cause only a transient ‘passive’ increase in kallikrein excretion, with a subsequent depletion of the enzyme measured in the renal cortex.

Acknowledgments

This study was supported by the German Research Foundation (GRF) within the SFB 90. G.B. is a fellow of GRF; M. M.-G. is a fellow of the Alexander von Humboldt Foundation. Aprotinin was kindly supplied by Bayer AG, Leverkusen, and calcium heparin by Nattermann Cie., Köln, FRG. We are grateful to Professor P. Vecsei, who performed the measurements of plasma aldosterone.
References


