Effect of saralasin and serum in myohaemoglobinuric acute renal failure of rats

K. BAUEREISS, K. G. HOFBAUER, A. KONRADS AND F. GROSS
Department of Pharmacology, University of Heidelberg, Heidelberg, West Germany

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Summary

1. In rats deprived of food and water for 24 h acute renal failure was produced by the intramuscular injection of glycerol. Eight hours later plasma urea concentration had increased threefold despite a small rise in urine volume. Plasma concentrations of renin and renin substrate were elevated.

2. When saralasin, a competitive antagonist of angiotensin II, was infused for 8 h after glycerol injection, urine volume and plasma urea were similar to values in rats that had received an infusion of saline.

3. Administration of rat serum (4-5 ml h⁻¹ kg⁻¹) for 4 h suppressed plasma renin concentrations, but plasma urea increased to the same extent as in rats without serum.

4. When saralasin and serum were infused at the same time, urine volume, urine osmolality and solute excretion increased and the rise of plasma urea was diminished.

5. Saralasin has a protective effect against glycerol-induced acute renal failure only when volume is replaced concomitantly.

Key words: angiotensin II antagonist, glycerol-induced acute renal failure, renin–angiotensin system.

Introduction

It has been suggested that the renin–angiotensin system is significant in the development of myohaemoglobinuric acute renal failure (Brown, Brown, Gavras, Jackson, Lever, MacGregor, MacAdam & Robertson, 1972; DiBona & Sawin, 1971; McDonald, Thiel, Wilson, DiBona & Oken, 1969; Thiel, Wilson, Arce & Oken, 1967). However, most attempts to prevent the development of glycerol-induced acute renal failure or to reduce its severity by blockade of the renin–angiotensin system have failed (Baranowsky, O'Connor & Kurtzman, 1975; Klein & Greven, 1976; Matthews, Morgan & Johnston, 1974; Oken, Cotes, Flambaum, Powell-Jackson & Lever, 1975; Powell-Jackson, MacGregor, Brown, Lever & Robertson, 1973). A successful prevention by angiotensin II (All) antisera has been observed in only two studies (Powell-Jackson, Brown, Lever, MacGregor, MacAdam, Titterington, Robertson & Waite, 1972; Rauh, Oster, Dietz & Gross, 1975). The failure of All blockade might be due to limited access of antibodies or antagonists to intrarenal sites (Hofbauer, Zschiechlich & Gross, 1976b), where All might be formed in high concentrations (Thurau & Mason, 1974). However, there are other explanations of these inconsistent findings. Passive immunization with All antibodies protected against glycerol-induced acute renal failure only when relatively large amounts of antiserum were applied (Powell-Jackson et al., 1972; Rauh et al., 1975), whereas purified antibody preparations given in a small volume had no beneficial effect.
volume replacement might act synergistically with All blockade.

In the present studies saralasin, a competitive antagonist of All (Pals, Masucci, Denning, Sipos & Fessler, 1971), was infused during the initial phase of glycerol-induced acute renal failure with and without concomitant serum administration. Plasma urea concentration and urine volume were measured to assess changes in renal function.

Material and methods

General

Male Sprague–Dawley rats (SIV 50, Ivanovas, Kisslegg), weighing between 180 and 225 g, were kept in individual cages and had free access to a commercial diet (ssniff) and demineralized water. For the infusion experiments a catheter was inserted into the right jugular vein and brought to the surface at the back of the neck. The operation was performed 4–5 days before the induction of acute renal failure. The catheters were flushed daily with 0.1 ml of isotonic sodium chloride solution (154 mmol/l; saline).

Before induction of acute renal failure the rats were transferred to metabolic cages and deprived of food and water for 24 h. Subsequently glycerol (6-1 mol/l in demineralized water; 10 ml/kg) or, in control experiments, saline (10 ml/kg) was injected into the muscles of the rear limbs under light ether anaesthesia. In all experiments food and water were still with-held during the following 8 h, and for the next 16 h the rats had free access to water but not to food. The experiments were terminated either 8 or 24 h after the injection of glycerol. Under light ether anaesthesia the abdomen was opened by midline incision and after both renal pedicles had been clamped blood samples were drawn from the vena cava. Plasma urea concentration was determined by the urease method (Merckotest no. 3334). Packed cell volume was determined after centrifugation in heparinized glass capillaries. Plasma renin concentration was determined by radio-immunoassay of Al (Oster, Bauknecht & Hackenthal, 1975). Renin substrate concentration was assayed after incubation of plasma with an excess of renin. Urine was collected in graded tubes and urine volume was measured at 8 and 24 h after the injection of glycerol. Urine osmolality was determined by freezing-point depression (Knauer osmometer).

Experiments

1. Glycerol injection. In one group of rats blood was taken after 24 h of food and water deprivation. In further experiments dehydrated rats received an intramuscular injection (10 ml/kg) of either glycerol or saline (sham injection). Blood samples were drawn 8 or 24 h later.

2. Saralasin infusion. Saralasin (Norwich Pharmacal Company) at a concentration of 1.14 mmol/l in saline was infused at a rate of 0.5 ml h⁻¹ kg⁻¹ (9.5 nmol min⁻¹ kg⁻¹). The infusion was started 30 min before the injection of glycerol and was continued for 8 h thereafter. The respective control rats received 0.5 ml of saline h⁻¹ kg⁻¹. All rats were killed 24 h after glycerol injection.

3. Serum infusion. Rat serum was infused at a rate of 4.5 ml h⁻¹ kg⁻¹ during 4 h after the injection of glycerol. The serum was taken from donor rats of the same strain, which were bled immediately before the infusion. The animals were killed either 8 or 24 h after the injection of glycerol. In the group killed after 8 h urine volume was measured at 2, 4 and 8 h after the induction of acute renal failure; urine osmolality was determined in the urine voided during the total 8 h period.

4. Saralasin and serum infusion. Rats received an infusion of serum (4.5 ml h⁻¹ kg⁻¹) for 4 h after glycerol injection and also were infused with saralasin as in exp. 2. The experiments were terminated either 8 or 24 h after the induction of acute renal failure. Urine volume and osmolality were measured as in exp. 3. Blood samples were collected 30 min after the saralasin infusion had been discontinued. Expt. no. 3 and no. 4 were performed simultaneously to assure comparable experimental conditions.

Values are given as mean ± 1 SEM. Comparisons were made by unpaired t-tests.

Results

Glycerol injection

After the injection of glycerol plasma urea concentration rose to 17.7 ± 0.9 mmol/l within 8 h and to 26.2 ± 1.8 mmol/l within 24 h; in sham-injected rats the respective values were 6.0 ± 0.3 and 4.0 ± 0.2 mmol/l (P < 0.01). During the 8 h period urine volume was higher in glycerol- than in sham-injected rats (3.6 ± 0.6 vs 1.6 ± 0.2 ml/8 h; P < 0.01) but was similar in both groups from 8 to 24 h (12.6 ± 1.8 and 10.9 ± 3.2 ml/16 h). Glycerol-injected rats drank twice as much as control rats, which received saline (26.4 ± 2.2 vs
Table 1. Effects of saline, saralasin, serum and serum + saralasin on urine volume, water intake, increase in body weight, packed cell volume, kidney weight and plasma urea concentration 24 h after injection of glycerol (61 mmol/kg)

Mean values ± se are shown. n, Number of animals. 0–8 h and 8–24 h refer to the periods after glycerol injection. Significance of the difference between rats treated with saralasin + serum and with serum only: *P < 0·05; **P < 0·01.

<table>
<thead>
<tr>
<th>Infusion</th>
<th>n</th>
<th>(0–8 h) (ml/8 h)</th>
<th>(8–24 h) (ml/16 h)</th>
<th>Water intake (8–24 h) (ml/16 h)</th>
<th>Δ Body wt. (g/16 h)</th>
<th>Wt. of kidney (mg/100 g)</th>
<th>Packed cell volume (%)</th>
<th>Plasma urea (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>11</td>
<td>6-3 ± 0-8</td>
<td>10-0 ± 2-3</td>
<td>23-9 ± 2-7</td>
<td>5-9 ± 1-0</td>
<td>1082 ± 33</td>
<td>41-3 ± 0-6</td>
<td>26-8 ± 2-3</td>
</tr>
<tr>
<td>Saralasin</td>
<td>10</td>
<td>6-5 ± 0-4</td>
<td>13-7 ± 3-8</td>
<td>26-2 ± 4-2</td>
<td>4-0 ± 1-5</td>
<td>1045 ± 27</td>
<td>40-8 ± 0-8</td>
<td>22-5 ± 3-5</td>
</tr>
<tr>
<td>Serum</td>
<td>15</td>
<td>6-7 ± 1-0</td>
<td>10-2 ± 2-3</td>
<td>24-8 ± 2-3</td>
<td>7-1 ± 1-2</td>
<td>1154 ± 28</td>
<td>41-3 ± 0-5</td>
<td>25-7 ± 2-2</td>
</tr>
<tr>
<td>Serum + saralasin</td>
<td>15</td>
<td>9-8** ± 0-4</td>
<td>7-3 ± 1-9</td>
<td>20-1 ± 2-3</td>
<td>5-2 ± 1-1</td>
<td>993** ± 26</td>
<td>40-6 ± 0-6</td>
<td>19-5* ± 1-6</td>
</tr>
</tbody>
</table>

Table 2. Effects of saralasin and serum on urine volume, solute excretion (V_{osm}), kidney weight, packed cell volume and plasma urea concentration 8 h after injection of glycerol (61 mmol/kg)

Mean values ± se are shown. n, Number of animals. 0–2 h etc. refer to the periods after glycerol injection. Significance of the difference between rats treated with saralasin and the serum-infused control rats: *P < 0·05; **P < 0·02; ***P < 0·01.

<table>
<thead>
<tr>
<th>Infusion</th>
<th>n</th>
<th>(0–2 h) (ml/2 h)</th>
<th>(2–4 h) (ml/2 h)</th>
<th>(4–8 h) (ml/4 h)</th>
<th>(0–8 h) (ml/8 h)</th>
<th>V_{osm} (mosmol/8 h)</th>
<th>Wt. of kidney (mg/100 g)</th>
<th>Packed cell volume (%)</th>
<th>Plasma urea (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>13</td>
<td>1-1 ± 0-4</td>
<td>2-2 ± 0-5</td>
<td>2-3 ± 0-3</td>
<td>5-6 ± 0-9</td>
<td>3-7 ± 0-9</td>
<td>993 ± 34</td>
<td>45-3 ± 0-3</td>
<td>13-7 ± 1-5</td>
</tr>
<tr>
<td>Serum + saralasin</td>
<td>13</td>
<td>2-3* ± 0-4</td>
<td>4-4*** ± 0-2</td>
<td>2-7 ± 0-3</td>
<td>9-4*** ± 0-7</td>
<td>6-7** ± 0-6</td>
<td>880*** ± 15</td>
<td>45-3 ± 0-4</td>
<td>9-7* ± 1-0</td>
</tr>
</tbody>
</table>

11·6 ± 3·5 ml/16 h; P < 0·01). At 24 h, rats with acute renal failure had increased by 7·2 ± 0·5 g/16 h and packed cell volume fell to 42·7 ± 0·4%, whereas in sham-injected animals body weight had decreased by 7·9 ± 0·7 g/16 h (P < 0·01) and packed cell volume was 49·8 ± 0·5% (P < 0·01).

Eight hours after glycerol injection plasma renin concentration was nearly twice as high as in sham-injected animals body weight had decreased by 7·9 ± 0·7 g/16 h (P < 0·01), but was similarly reduced in both groups at 24 h (13 ± 2 and 23 ± 8 mmol of Al h^{-1} 1^{-1} respectively). Plasma renin substrate concentration did not change after saline injection (459 ± 14 mmol of Al/l at 8 h and 498 ± 12 mmol of Al/l at 24 h, as compared with 516 ± 14 mmol of Al/l in dehydrated rats), but it rose within 8 h after glycerol injection to 886 ± 45 mmol of Al/l (P < 0·01) and was still elevated at 24 h (929 ± 108 mmol of Al/l; P < 0·01).

Saralasin infusion

No significant difference in urine volume, water intake and body-weight change was found between rats treated with saralasin and the respective control animals who received a saline infusion (Table 1). Packed cell volume and kidney weight were also similar in both groups. Plasma urea concentration measured 24 h after glycerol injection was slightly lower in rats who received saralasin than in saline-infused control animals (0·1 > P > 0·05) (Table 1).

Serum infusion

Serum infusion neither enhanced urine volume nor lowered plasma urea, compared with saline infusion (Table 1). Packed cell volume and body weight changed similarly. At 8 h after glycerol injection plasma renin concentration was lower in
rabs who received serum than in animals that had no infusion (33 ± 5 vs 74 ± 8 nmol of AI h⁻¹ l⁻¹; \( P < 0.01 \)); plasma renin concentration was less increased in the former than in the latter group (667 ± 46 vs 886 ± 45 nmol of AI/l).

Saralasin and serum infusion

When saralasin was given to serum-infused rats the urine volume within 8 h exceeded by about 50\% that of rats who received only serum (Table 1 and Table 2). When urine volume was measured 2, 4 and 8 h after the injection of glycerol it was obvious that saralasin enhanced urine output mainly during the first 4 h (Table 2). Since urine osmolality was higher in saralasin-treated rats than in animals who received serum only (686 ± 18 vs 516 ± 36 mosmol/kg; \( P < 0.001 \)), solute excretion was more enhanced than water excretion (Table 2).

At 8 h after glycerol injection plasma urea concentration reached 9-7 ± 1-0 mmol/l in saralasin-treated rats, whereas it rose to 13-7 ± 1-5 mmol/l in rats infused with serum only. Thirty minutes after the saralasin infusion was stopped, plasma renin concentration (35 ± 4 nmol of AI h⁻¹ l⁻¹) and plasma renin substrate concentration (643 ± 4 nmol of AI/l) were similar to the values in those rats who only received serum.

During the period from 8 to 24 h after the induction of acute renal failure the urine volume was similar in rats who had received saralasin and in the serum-infused control rats (Table 1). The difference in mean plasma urea concentration of both groups, which was seen 24 h after the injection of glycerol (6-2 mmol/l), corresponds approximately to that observed at 8 h (4-0 mmol/l).

Discussion

The present study has confirmed that after the injection of glycerol the renin–angiotensin system is stimulated for at least 8 h (Hofbauer, Konrads, Bauereiss, Möhring, Möhring & Gross, 1977; Rauh et al., 1975). However, 24 h after the induction of acute renal failure, plasma renin was normal again, perhaps as a result of concomitant water retention. Therefore we confined the infusion of saralasin to the first 8 h after glycerol injection.

When saralasin was infused alone in doses which effectively antagonize the systemic action of angiotensin II (Pals et al., 1971) it did not improve urine excretion of rats with acute renal failure. Plasma urea concentrations measured 24 h after the injection of glycerol were only slightly lower in saralasin-treated rats than in saline-infused control animals. Our results confirm that there is no beneficial effect of All antagonists or converting-enzyme inhibitor in myohaemoglobinuric acute renal failure (Baranowsky et al., 1975; Klein & Greven, 1976; Powell-Jackson et al., 1973). The administration of rat serum during 4 h after glycerol injection also did not increase urine volume. In these volume-supplemented rats plasma urea measured 8 or 24 h after the induction of acute renal failure was similar to that in untreated animals.

Although neither saralasin nor serum alone had any beneficial effect in rats with glycerol-induced acute renal failure, their combined use significantly increased urine volume, solute excretion and urine osmolality. Accordingly, plasma urea rose less in rats that received serum and saralasin together than in animals who had serum only. It might be argued that the small amount of saline in which the saralasin was dissolved might have contributed to the beneficial effect of the All antagonist, but this is unlikely. The difference in urine volume between rats who received serum only and rats who had serum and saralasin was maximal at 4 h after glycerol injection. At that time saralasin-treated rats had received 2-25 ml of saline/kg in addition to 18 ml of serum/kg. If it is assumed that 25\% of the infused saline remained in the intravascular compartment the increase in plasma volume induced by saline amounted to only 3\% of the increase produced by serum. Recently others have also observed only a small effect from much larger volumes of saline in this model of acute renal failure (Hsu, Kurtz & Waldinger, 1977; Kurtz, Maletz & Hsu, 1976).

Our observation that saralasin improved renal excretory function only in the presence of volume supplementation could explain the discrepant results of studies with All antisera (Powell-Jackson et al., 1972; Rauh et al., 1975) and purified All antibodies (Matthews et al., 1974; Oken et al., 1975). Whereas the infusion of small volumes of saralasin might resemble the administration of small volumes of purified antibody preparations, our experiments with saralasin and serum infusion might be comparable with the administration of large volumes of All antisera. Hence, volume supplementation, although ineffective by itself (Powell-Jackson et al., 1972), might be necessary for a beneficial effect of an All blockade.

Recent studies on the pathogenesis of myo-haemoglobinuric acute renal failure (Hofbauer...
et al., 1977; Hsu et al., 1977) might explain the mechanism by which serum infusion could enhance the effect of saralasin. In the initial phase of glycerol-induced acute renal failure both renal vascular resistance (Chedru, Baethke & Oken, 1977; Hsu et al., 1977), and the cardiac output is markedly reduced (Hsu et al., 1977). After glycerol injection serum infusion raised the cardiac output, probably by an increase in venous return and reduced renal and systemic vascular resistances (Hsu et al., 1977). Since in this type of acute renal failure plasma concentrations of vasopressin are elevated, and may thus induce systemic vasoconstriction (Hofbauer et al., 1977), it could be postulated that serum replacement reduces total peripheral and renal resistance by suppressing vasopressin release.

In previous studies (Hofbauer et al., 1977) saralasin did not lower blood pressure after glycerol injection. However, it might improve renal haemodynamics; Ishikawa & Hollenberg (1976) found that saralasin increased renal blood flow in glycerol-induced acute renal failure under similar experimental conditions. Such an effect of saralasin should be more marked when plasma volume is increased by serum infusion. In addition, volume substitution (Thurau & Boylan, 1976) and AII blockade (Stowe & Scherermann, 1974) could have additive effects on the tubulo-glomerular feedback response, and thereby increase glomerular filtration rate.

The simultaneous administration of serum and saralasin induced only a transient increase in solute excretion, but did not appear to change the subsequent course of the acute renal failure. Our studies thus do not provide evidence for a major role of the renin–angiotensin system in the pathogenesis of myohaemoglobinuric acute renal failure, but suggest that AII does contribute to the reduction of renal excretory function in the initial phase of the condition induced by glycerol. However, studies with AII antagonists cannot provide a quantitative analysis of the role of the renin–angiotensin system unless their action against intrarenally formed AII can be established (Hofbauer, Baurereiss, Zschiedrich & Gross, 1976a).

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References


