Vasopressin and Renin in Glycerol-Induced Acute Renal Failure in the Rat

KARL G. HOFBAUER, ARNO KONRADS, KURT BAUEREISS, BÄRBEL MÖHRING, JAN MÖHRING, AND FRANZ GROSS

SUMMARY Acute renal failure was induced in dehydrated rats by intramuscular injection of glycerol (50%, 10 ml/kg). The total period of observation after glycerol injection was 8 hours. Hematocrit and plasma osmolality increased transiently and reached maximum values between 1 and 2 hours; plasma urea concentration rose progressively during the 8-hour period. Plasma levels of arginine-vasopressin had increased 40-fold by 2 hours after glycerol injection, whereas plasma renin concentrations were only 2–3 times higher than in controls. Plasma renin substrate concentrations had doubled by 8 hours. In rats with acute renal failure, blood pressure was higher than in controls injected with saline. Injection of vasopressin antiserum lowered blood pressure by 10 ± 2 (SE) mm Hg, while a competitive antagonist of angiotensin II, saralasin, had no effect. It is concluded that after glycerol injection the increased plasma concentrations of vasopressin induced systemic vasoconstriction. The renin-angiotensin system does not significantly contribute to the rise in systemic vascular resistance.

IN ACUTE RENAL failure induced by the injection of glycerol, renal blood flow is markedly reduced.1-4 In addition to intrarenal mechanisms,5,6 cardiovascular changes also might be responsible for the impairment of renal hemodynamics and excretory function. Blood pressure is not lowered in this experimental model,6 despite a decrease in plasma volume7 and cardiac output;8 the mechanisms underlying these changes in the systemic circulation have not yet been investigated.

In the present study we have analyzed the early phase of glycerol-induced acute renal failure. Since in former investigations the renin-angiotensin system has been considered to be of pathogenetic significance for the increase in intrarenal resistance,9-12 the plasma concentrations of renin and its substrate were measured. The accumulating evidence for a vasoconstrictor role of antidiuretic hormone in shock and hypovolemia13-15 prompted us to determine the plasma levels of arginine-vasopressin. By means of specific blockade, attempts were made to establish the role of angiotensin II and arginine-vasopressin in the maintenance of blood pressure during the development of glycerol-induced acute renal failure.

Methods

GENERAL EXPERIMENTAL PROTOCOL

Male Sprague-Dawley rats (SIV-50 strain, Ivanovas) weighing 180–240 g were kept at constant temperature (23 ± 1°C) and humidity (60 ± 3%) in a room lighted automatically from 6 a.m. to 6 p.m. They were given a commercial diet (ssniff) and demineralized water ad libitum.

Prior to the experimental period, the rats were placed in individual cages and deprived of food and water for 24 hours. Subsequently, either glycerol (50% wt/wt in demineralized water, 10 ml/kg) or 0.9% saline (10 ml/kg) was injected under light ether anesthesia into the muscles of the hind limbs. During the following 8-hour period, food and water still were withheld.

SERIES 1

After the injection of glycerol, blood samples were taken at 0, 0.5, 1, 2, 4, or 8 hours and after the injection of saline at 0, 0.5, 4, or 8 hours. At each time interval, six to eight rats were used. Under light ether anesthesia, the abdomen was opened by a midline incision and both renal pedicles were clamped; from the vena cava, 1 ml of blood was taken into a tube containing 50 μl of a 3.8% ethylenediaminetetraacetate (EDTA) solution, and another 1 ml was collected into a heparinized tube. Blood sampling was performed between 2 and 5 p.m. Measurements of hematocrit, plasma urea concentration (Merckotest), plasma osmolality (Knauer osmometer), and plasma renin and renin substrate concentrations16 were made on each set of samples.

In a parallel study, groups of six to eight control rats and groups of seven to 11 rats with acute renal failure were studied at corresponding time intervals. Blood was obtained by decapitation between 2 and 4 p.m. Concentrations of arginine-vasopressin (AVP) in the plasma samples were determined by radioimmunoassay.17

In addition, plasma samples were pooled from two groups each containing eight rats. These rats had received glycerol 2 or 8 hours earlier. Plasma vasopressin concentrations were measured in serial dilutions of these two plasma pools. There was a linear relation between the amount of AVP measured and the volume of the sample added; similar curves were obtained in previous experiments with two plasma pools taken from untreated rats17 (Fig. 1).
SERIES 2

In 15 rats, a polyethylene catheter was placed in the femoral artery under light ether anesthesia. Glycerol was injected into eight of these rats, whereas the other seven received the same amount of saline. Subsequently, blood pressure was continuously recorded during 8 hours from the conscious, unrestrained rats.

SERIES 3

In an initial study, catheters were placed in the femoral artery and vein, and blood pressure was continuously measured. Two hours after the administration of glycerol, 0.4 ml of an AVP antiserum from a rabbit was injected, iv, in six rats, whereas eight other rats with acute renal failure received 0.4 ml of a normal rabbit serum. The characteristics of the AVP antiserum and its blood pressure-lowering effect in rats infused with AVP have been described previously.\textsuperscript{18}

In a second study, saralasin (Norwich), a competitive antagonist of angiotensin II, was given 2 hours after the injection of either glycerol or saline (10 and six rats, respectively). Saralasin was infused into the femoral vein at increasing doses (2, 10, and 50 \(\mu\)g/kg per min) for 20 minutes each, and blood pressure was continuously recorded.

STATISTICS

All values given in the text, table, and figures are mean \(\pm\) SEM. For the calculation of statistical significance of differences, Student's \(t\)-test, or, when appropriate, \(t\)-test for paired data was used.

Results

PLASMA UREA, HEMATOCRIT, AND PLASMA OSMOLALITY

After the injection of glycerol, plasma urea concentration rose progressively and was 3-fold higher than in control rats by 8 hours (Fig. 2). After a small initial fall, hematocrit increased and was significantly higher than in control rats by 1, 2, and 4 hours after the onset of acute renal failure \((P < 0.01)\). Thereafter, hematocrit fell and was in the control range by 8 hours (Fig. 2). The plasma samples obtained 0.5, 1, 2, and 4 hours after glycerol injection showed a marked hemolysis, whereas, in samples taken after 8 hours, hemolysis occurred only occasionally. Plasma osmolality sharply increased and reached peak values by 1 and 2 hours. Eight hours later, osmolality was still higher than in control rats (Fig. 2).

PLASMA RENIN, RENIN SUBSTRATE, AND VASOPRESSIN CONCENTRATIONS

Mean plasma renin concentration increased 2- to 3-fold in rats with acute renal failure as compared with saline-injected controls (Fig. 3). Plasma concentrations of renin substrate rose progressively and, by 8 hours, reached values that were almost twice as high as in controls (Fig. 3). Plasma levels of AVP increased markedly after the induction of acute renal failure and, after 2 hours, reached a maximum that was 40-fold above control levels (Fig. 3).

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Serial dilutions of plasma pools obtained from rats in acute renal failure 2 hours (\(\blacktriangle\)) and 8 hours (\(\blacktriangleup\)) after the injection of glycerol, and from normal rats (\(\bullet\), \(\circ\)). \(\text{Values are not corrected for the recovery rate of 60\%.}^{17}\) \(\text{These data are taken from Mohring and Mohring}^{11}.\) \(\text{Values are not corrected for the recovery rate of 60\%.}^{17}\) \(\text{Values are not corrected for the recovery rate of 60\%.}^{17}\) \(\text{Values are not corrected for the recovery rate of 60\%.}^{17}\) \(\text{Values are not corrected for the recovery rate of 60\%.}^{17}\)}
\end{figure}

\begin{figure}
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\includegraphics[width=\textwidth]{figure2.png}
\caption{Plasma urea concentration, hematocrit, and plasma osmolality in rats sacrificed at various time intervals after the injection of either glycerol (\(\bullet\)) or saline (\(\circ\)) (means \(\pm\) SEM).}
\end{figure}

\(\text{These data are taken from Mohring and Mohring}^{11}.\) \(\text{Values are not corrected for the recovery rate of 60\%.}^{17}\)
conscious, unrestrained control rats (Fig. 3). After glycerol injection, blood pressure was 123 ± 4 and 120 ± 3 mm Hg at 2 and 4 hours, respectively (Fig. 3), but fell thereafter to 111 ± 3 mm Hg at 8 hours.

EFFECTS OF AVP ANTISERUM AND SARALASIN ON BLOOD PRESSURE

AVP antiserum given 2 hours after glycerol injection induced a transient fall of blood pressure in five of the six rats studied (mean change, -10 ± 2 mm Hg; n = 5; range, 5–15 mm Hg; P < 0.01). Within the first 2 minutes after the start of the injection, blood pressure slightly but consistently increased (up to 4 mm Hg); thereafter it fell and reached a minimum within 5–12 minutes. Between 20 and 30 minutes after injection of antiserum, it rose again toward preinjection levels. Blood pressure of the five responsive rats ranged from 120 to 138 mm Hg before antiserum injection, whereas it was only 110 mm Hg in the one rat, which showed no fall in blood pressure.

When an injection of 0.4 ml of a normal rabbit serum was given 2 hours after glycerol injection, blood pressure rose slightly (mean, 4 ± 1 mm Hg). Blood pressure of these rats ranged from 116 to 136 mm Hg before the injection of control serum. Results of typical experiments are shown in Fig. 4.

Twenty minutes after the start of the saralasin infusion, mean blood pressure was essentially unchanged at each dose level, compared with the preceding control values (Table 1).

Discussion

The present studies have confirmed that, in myoglobinuric acute renal failure of rats, plasma urea concentrations rise progressively after the injection of glycerol and hematocrit increases subsequent to the development of hypovolemia.7 Plasma osmolality increased sharply after glycerol injection; this may be attributed to the influx of glycerol from its injection site into the blood. The subsequent decline in plasma osmolality might reflect mainly the metabolism of glycerol19 and, to a minor extent, its renal excretion.

Blood pressure rose in conscious, glycerol-injected rats despite hypovolemia, whereas it remained unchanged in saline-treated controls. In view of the recent observations made by Kurtz et al.8 and by R. Dietz (personal communication), that cardiac output is reduced to approximately 1/3 of control values during the initial phase of this type of acute renal failure, the rise in blood pressure indicates a marked elevation of total peripheral resistance subsequent to glycerol injection. An increase in blood pressure has also been observed in patients with crush syndrome. This may be considered a clinical counterpart of the experimental model of glycerol-induced acute renal failure,10,20 which probably also reflects systemic vasoconstriction.20

Blood pressure changed in parallel to plasma vasopressin concentrations. Within 2 hours after glycerol injection, blood pressure rose to its maximal values and plasma

<table>
<thead>
<tr>
<th>Infusion</th>
<th>2 μg/kg per min</th>
<th>10 μg/kg per min</th>
<th>50 μg/kg per min</th>
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<tbody>
<tr>
<td>Saline</td>
<td>1 ± 1</td>
<td>0 ± 2</td>
<td>0 ± 2</td>
</tr>
<tr>
<td>Glycerol</td>
<td>1 ± 1</td>
<td>1 ± 2</td>
<td>-2 ± 2</td>
</tr>
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Mean ± SEM; saline: n = 7; glycerol: n = 10.

* Increasing doses were given for 20 minutes each to Sprague-Dawley rats 2 hours after the injection of either saline or glycerol.
vasopressin levels increased steeply, most likely as the consequence of hyperosmolality and hypovolemia. Subsequently, plasma vasopressin concentrations fell and blood pressure declined, but values were still elevated 8 hours after the onset of acute renal failure.

Mean plasma renin concentration also increased after the injection of glycerol but did not correspond as clearly as vasopressin to the concomitant changes in blood pressure. Plasma renin substrate levels progressively rose in rats with acute renal failure; this is comparable to clinical observations in man. Maximal concentrations of plasma renin substrate were reached at a time when blood pressure showed a tendency to decrease again.

In order to demonstrate a possible vasopressor role of AVP in acute renal failure, we used an AVP antiserum that lowered blood pressure transiently in five of the six rats studied. If the slight blood pressure elevation after injection of inactive control serum is taken into account, the blood pressure increase of glycerol-treated rats was reduced by about 1/3 after the administration of AVP antiserum. These findings indicate that AVP contributed to systemic vasocostriction in this type of acute renal failure. Such a conclusion is further supported by our observation that, in rats with hereditary hypophyseal diabetes insipidus, which cannot produce any AVP, blood pressure did not rise after the injection of glycerol.

The effects of angiotensin II on blood pressure in this experimental model were analyzed by means of a competitive antagonist of angiotensin II. Saralasin did not reduce blood pressure in any of the doses used. Since the antagonist has been shown to block almost completely the vasopressor effects of exogenous angiotensin II in the dose range used in our experiments (unpublished observations), these data do not support a significant action of angiotensin II on systemic vascular resistance in myoglobinuric acute renal failure.

The finding of a systemic vasocostrictor effect of AVP in glycerol-induced acute renal failure in rats raises the question, of whether or not vasopressin could also be involved in causing the increased renal vascular resistance in this pathophysiological model. Various studies suggest that vasopressin might increase renal vascular resistance and reduce renal blood flow, and it has been shown that experimental acute renal failure is aggravated by the injection of vasopressin. However, the marked differences in the function of single nephrons in glycerol-induced acute renal failure6 argue against a contribution of systemic vasoconstrictors such as vasopressin in the pathogenesis of this disorder. Wilson et al.20 have shown that the course of renal insufficiency after glycerol injection is comparable in rats with diabetes insipidus and in normal Sprague-Dawley rats. In recent experiments we have observed that, during the initial phase of glycerol-induced acute renal failure, the reduction in urine flow and the increase in plasma urea concentration are even more pronounced in rats with diabetes insipidus than in normal rats. For these reasons, vasopressin does not appear to be a major pathogenetic factor in the development of renal insufficiency after the injection of glycerol.

The fact that it was not possible to demonstrate a contribution of the renin-angiotensin system to the systemic vasocostriction of rats with acute renal failure does not necessarily imply that renin is not involved in the pathogenesis of renal lesions in this syndrome. Angiotensin formed within the kidney, rather than circulating angiotensin II, might be involved in the increase in renal vascular resistance after the injection of glycerol, and the production of angiotensin within the kidney might be much more enhanced than is estimated from the rise of plasma renin concentration. Recently we observed that saralasin administered together with the rat, did not significantly improve the renal excretory capacity in this model of acute renal failure. This finding, together with the lack of an effect of saralasin on blood pressure in the present study, suggests an intrarenal rather than a systemic role of the renin-angiotensin system in the pathogenesis of myoglobinuric acute renal failure in the rat.

Acknowledgments

We thank the Norwich Pharmacal Company for the gift of saralasin and the Merck Company for the urea reagents. We are indebted to Prof. Dr. E. Hackenthal for the determination of renin and renin substrate concentrations. The valuable technical assistance of C. Kahl, D. Mayer, and U. Werner is gratefully acknowledged.

References

5-Hydroxytryptamine and Neurotransmitter Release in Canine Blood Vessels

Inhibition by Low and Augmentation by High Concentrations

MICHAEL A. McGrath

SUMMARY Experiments were designed to determine the effect of 5-hydroxytryptamine on adrenergic neurotransmission in blood vessels. Strips from canine saphenous veins and tibial arteries were incubated in norepinephrine\[^{3H}\] and mounted for superfusion and isometric tension recording. The superfusate was collected for estimation of total radioactivity and for column chromatographic separation of norepinephrine\[^{3H}\] and its metabolites. 5-Hydroxytryptamine (5-HT) \(10^{-8}\) M and \(10^{-7}\) M inhibited the increase in smooth muscle tension and the release of norepinephrine\[^{3H}\] caused by transmural electric stimulation of the sympathetic nerve endings. By contrast, the increase in tension caused by direct stimulation of the smooth muscle with norepinephrine was either unchanged or augmented. In addition, 5-HT augmented the increase in tension with tyramine but did not affect the release of radiolabeled compounds by this substance. In unstimulated preparations low concentrations of 5-HT \(10^{-8}\) M caused contraction but did not affect the release of norepinephrine\[^{3H}\]. With a higher concentration \(10^{-7}\) M the release of neurotransmitter was markedly increased. This response was inhibited by cocaine. Whereas 5-HT-induced contractions were inhibited by phentolamine and methysergide, these antagonists had no effect on its inhibitory action at the sympathetic nerve ending. Hence, low concentrations of 5-HT depressed sympathetic tone by inhibiting the release of transmitter during nerve depolarization. At higher concentrations 5-HT has a direct excitatory effect on vascular smooth muscle together with an indirect effect which involves the uptake of 5-HT into the sympathetic nerve ending via the cocaine-sensitive mechanism and the release of norepinephrine.

IN HUMANS the intra-arterial infusion of 5-hydroxytryptamine (5-HT) consistently causes a decrease in skin blood flow.\(^1\)\(^-\)\(^3\) However, its effect on total limb and skeletal muscle blood flow is more complex. For example, in small doses it causes an increase in forearm and calf blood flows but when large doses are infused the increase in flow usually is followed by a dose-dependent decrease.\(^1\)\(^-\)\(^3\) Studies on isolated blood vessels support the conclusion that the vasoconstriction is predominantly a direct effect of 5-HT on the vascular smooth muscle.\(^5\)\(^-\)\(^9\) In several tissues, for example, rabbit heart,\(^10\) guinea pig vas deferens,\(^11\) and cat spleen capsule,\(^12\) 5-HT causes the release of norepinephrine from the sympathetic nerve endings; however, it is not known whether an indirect sympathomimetic action contributes to the vasoconstriction induced by this substance.

Although the mechanism of the increase in limb blood flow with 5-HT is unknown, there are several observations which suggest that this may result, at least in part, from the inhibition of neurogenic vasoconstriction. For example, it has been demonstrated in animal experiments that the
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