Cardiac Output and Renal Blood Flow in Glycerol-Induced Acute Renal Failure in the Rat

CHEN H. HSU, THEODORE W. KURTZ, AND THOMAS P. WALDINGER

SUMMARY Cardiac output (CO) and renal blood flow (RBF) were simultaneously evaluated by the microsphere method in water-drinking and chronic saline-drinking rats at 3, 12, and 24 hours after induction of acute renal failure by glycerol injection. Three hours after glycerol injection CO and RBF decreased to 36% and 20% of the respective controls in water-drinking rats and to 41% and 24% of the controls in saline-drinking rats. Renal vascular resistance (RVR) increased significantly in both groups at this time. Isoncotic plasma expansion (3% of body weight) restored the RBF and RVR to normal in water-drinking rats 3 hours post-glycerol injection, although CO increased to only 70% of the control. Twelve hours after glycerol injection, CO and RBF returned to normal in saline-drinking rats, whereas they remained lower than controls in water-drinking rats. Twenty-four hours post-glycerol injection, when acute renal failure was evident as indicated by blood urea nitrogen (BUN) values of 116.9 and 63.8 mg/100 ml in water- and saline-drinking rats, respectively, CO and RBF returned to normal, except that the CO of water-drinking rats was slightly higher than control. Thus, we conclude that decreased CO is an important determinant of the early decrease in renal perfusion in glycerol-induced acute renal failure. Furthermore, the observed earlier return of CO and RBF to normal in saline-drinking rats may be partly responsible for reducing the severity of acute renal failure.

RECENT STUDIES have demonstrated that renal cortical ischemia is not responsible for maintenance of impaired glomerular filtration in experimental acute renal failure.14-15 These findings are contrary to earlier reports that indicated a primary role for decreased renal perfusion in the severely decreased glomerular filtration rate (GFR) that occurs in acute renal failure.16 However, a severe reduction of renal blood flow (RBF) during the initial hours of glycerol-induced acute renal failure has been consistently demonstrated.1-4,5 and such a reduction of RBF undoubtedly contributes to the early decrease of GFR seen in this model. The mechanisms for this reduction of RBF are not entirely clear, but it has been suggested that the renin-angiotensin axis and increased intra renal vascular resistance6-8,9 may be involved. This study was designed to investigate the mechanism of decreased RBF in the early stage of glycerol-induced acute renal failure in water-drinking rats and chronic saline-drinking rats.

Methods

Experiments were performed on male Sprague-Dawley rats weighing 180-300 g. Group I contained rats main-
containing microspheres was vigorously agitated with a Vortex mixer for at least 5 minutes. After filling the syringe to a volume of 0.1 ml, the needle was removed, the syringe was capped with a hub, and radioactivity was counted for 30 seconds in a Packard gamma counter. Left ventricular injection followed quickly so that no more than 90 seconds elapsed between withdrawal of the microspheres and injection. The femoral catheter was opened upon injection of the spheres and blood flowed freely into a preweighed tube for exactly 1 minute. Approximately 0.1–0.2 ml of blood was collected from the femoral artery for each measurement as a reference blood sample. We tested the adequacy of a 1-minute collection time to assure complete removal of microspheres from the circulation and invariably found that within 30 seconds after injection all microspheres disappeared from the blood.

Following the femoral blood collection, 0.1 ml of blood was withdrawn from the carotid catheter to clear it of residual microspheres. Evaluations of carotid catheters for residual counts showed essentially no radioactivity. The blood withdrawn from the carotid catheter was then counted with the syringe, hub, and catheter needle to determine postinjection counts. This value was subtracted from the preinjection counts to yield total counts injected. Approximately 30,000 microspheres were administered in each injection. Mean arterial pressure (MAP) was measured prior to each microsphere injection using a Gilson recorder and Statham transducer connected to the femoral catheter. After the second injection of spheres, blood was drawn for urea nitrogen (BUN) determination (AutoAnalyzer) and the rats were killed. Postmortem examination verified position of the catheter in the left ventricle. The kidneys were removed, decapsulated, and counted for radioactivity. Blood samples from the $^{85}$Sr and $^{14}$Ce injections also were counted and weighed. The exact blood volume collected was calculated by dividing the weight of the blood sample by the specific gravity of rat blood previously determined to be $1.107 \pm 0.001$ g/ml. RBF, which represents the RBF of both kidneys combined, was calculated as:

$$\text{RBF} = \frac{\text{two kidneys (counts/min)}}{\text{femoral blood (counts/min)}} \times \text{femoral blood flow rate (ml/min per 100 g body wt)}.$$  

Renal vascular resistance (RVR) was calculated as:

$$\text{RVR} = \frac{\text{MAP}}{\text{RBF}}.$$  

Cardiac Output and Renal Blood Flow in Water-Drinking Rats 3 Hours after Glycerol Injection and in Dehydrated Controls

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Cardiac Output and Renal Blood Flow in Water-Drinking Rats 3 Hours after Glycerol Injection and in Dehydrated Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>CO (ml/min/100 g BW)</td>
</tr>
<tr>
<td>---------</td>
<td>----------------------</td>
</tr>
<tr>
<td>I.</td>
<td>29.9 ± 2.0</td>
</tr>
<tr>
<td>II.</td>
<td>10.6 ± 0.98</td>
</tr>
<tr>
<td>III.</td>
<td>20.9 ± 1.56</td>
</tr>
</tbody>
</table>

Probability values:

<table>
<thead>
<tr>
<th></th>
<th>&lt;0.001</th>
<th>&lt;0.001</th>
<th>&lt;0.001</th>
<th>&lt;0.001</th>
<th>&lt;0.001</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-II</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>I-III</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

CO = cardiac output; RBF = renal blood flow; MAP = mean arterial pressure; RVR = renal vascular resistance; BUN = blood urea nitrogen; BW = body weight; n = number of rats; NS = not significant ($P > 0.05$ Student's $t$-test).

Values are mean ± SEM. All CO and RBF values are averages of two determinations in each rat.

CO was calculated as:

$$\text{CO} = \frac{Q}{\text{femoral blood (counts/min)}} \times \text{femoral blood flow rate (ml/min per 100 g body wt)},$$

where $Q$ is the total amount of microspheres (counts/min) injected.

A separate group of 3-hour post-glycerol water-drinking rats (group III) received volume expansion with fresh rat plasma prior to determination of RBF and CO. These rats were surgically prepared and evaluated as described, but in addition had femoral vein catheters placed for infusion of plasma. Twenty minutes prior to the first RBF and CO measurements, isonicotinic rat plasma in a volume equivalent to 3% of body weight was infused at approximately 0.3 ml/min. The infusion rate was adjusted for each rat, so that the first determinations of RBF and CO were made as close to 3 hours after glycerol injection as possible.

Results

Mean values of CO, RBF, MAP, RVR, and BUN for water-drinking rats 3 hours after glycerol injection are presented in Table 1. These parameters for control rats dehydrated for 18 hours prior to experimentation (Table 1) were not significantly different from those of normal rats without dehydration (Table 2). Three hours after glycerol injection, CO and RBF were significantly reduced and MAP and RVR were significantly increased compared to control values. BUN was more than twice the normal value at this time. Despite the severe reduction in CO noted 3 hours post-glycerol, no deaths occurred in any group and all rats were conscious throughout the experiments.

Since a previous study had indicated that plasma volume decreased more than 2.1% of body weight within 3 hours of glycerol injection in rats, we administered fresh rat plasma in a volume equivalent to 3% of body weight to examine its effect on RBF and CO (Table 1). This maneuver significantly increased CO at 3 hours post-glycerol and restored RBF and RVR to the normal range, although MAP still was significantly elevated. RVR of plasma-expanded rats injected with glycerol was less than the RVR of 3-hour post-glycerol rats not volume-expanded ($P < 0.001$). BUN was slightly decreased after plasma infusion, presumably as a result of the dilutional effect.

Data for water-drinking rats studied 12 and 24 hours...
Table 2 Cardiac Output and Renal Blood Flow in Water-Drinking Rats 12 and 24 Hours after Glycerol Injection and in Controls

<table>
<thead>
<tr>
<th>Group</th>
<th>CO (ml/min/100 g BW)</th>
<th>RBF (ml/min/100 g BW)</th>
<th>MAP (mm Hg)</th>
<th>RVR (mm Hg/ml/min/100 g BW)</th>
<th>BUN (mg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Normal control (n = 9)</td>
<td>31.4 ± 1.85</td>
<td>4.60 ± 0.39</td>
<td>114.1 ± 2.9</td>
<td>26.1 ± 2.43</td>
<td>17.2 ± 1.21</td>
</tr>
<tr>
<td>II. 12 hr post-glycerol (n = 6)</td>
<td>24.8 ± 2.45</td>
<td>3.22 ± 0.32</td>
<td>120.1 ± 1.7</td>
<td>40.1 ± 4.0</td>
<td>75.5 ± 7.06</td>
</tr>
<tr>
<td>III. 24 hr post-glycerol (n = 7)</td>
<td>39.4 ± 2.32</td>
<td>3.55 ± 0.30</td>
<td>102.9 ± 2.7</td>
<td>30.0 ± 2.4</td>
<td>116.9 ± 4.71</td>
</tr>
</tbody>
</table>

Probability values:
- I-II: <0.05
- II-III: <0.025
- I-III: NS

Abbreviations are the same as in Table 1.

The validity of the microsphere method for measurement of CO and RBF in the rat has been documented previously. Our figures for control CO and RBF are close to these original reports. The first measurement of CO (56Cr) in normal control rats (n = 9) was 31.1 ± 2.20 ml/min per 100 g of body weight, with the second measurement (141Ce) being 31.7 ± 2.69 ml/min per 100 g of body weight. Paired statistical analysis reveals no significant difference between the two results, indicating the reproducibility of the technique. These values are similar to Goldman’s figure of 27.2 ± 0.54 ml/min per 100 g of body weight obtained in unanesthetized rats using the rubidium isotopic indicator technique.

Additional confirmation of the reliability of the microsphere technique is reflected by nearly identical percentages of microsphere uptake of the myocardium during the first and second measurements. The percentage of CO delivered to the myocardium with the first injection was 5.83 ± 0.57%. This was not significantly different from the myocardial blood flow fraction of the second injection, 5.57 ± 0.75%. These figures are practically identical to those reported by Mendell and Hollenberg and Sasaki and Wagner, using the microsphere method in the rat. MAP measurements before and after injection of microspheres were not significantly different, further indicating that the quantity of microspheres injected did not alter the hemodynamic status of the rats.

Discussion

The simultaneous measurements of RBF also were reproducible using the two different isotopes. The first mea-

Table 3 Cardiac Output and Renal Blood Flow in Chronic Saline-Drinking Rats 3 Hours after Glycerol Injection and in Dehydrated Controls

<table>
<thead>
<tr>
<th>Group</th>
<th>CO (ml/min/100 g BW)</th>
<th>RBF (ml/min/100 g BW)</th>
<th>MAP (mm Hg)</th>
<th>RVR (mm Hg/ml/min/100 g BW)</th>
<th>BUN (mg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Rats dehydrated for 18 hr (n = 6)</td>
<td>25.1 ± 1.16</td>
<td>5.62 ± 0.53</td>
<td>115.5 ± 2.9</td>
<td>20.2 ± 1.69</td>
<td>37.4 ± 3.32</td>
</tr>
<tr>
<td>II. 3 hr post-glycerol (n = 8)</td>
<td>10.4 ± 1.04</td>
<td>1.34 ± 0.16</td>
<td>134.1 ± 0.67</td>
<td>129.1 ± 33.4</td>
<td>68.7 ± 4.92</td>
</tr>
</tbody>
</table>

Probability values:
- I-II: <0.001
- II-I: <0.001

Abbreviations are the same as in Table 1.

after glycerol injection and for control normal rats without dehydration are presented in Table 2. Acute renal failure was clearly evident as the mean BUN levels were elevated to 75.5 mg/100 ml and 116.9 mg/100 ml at 12 and 24 hours post-glycerol injection, respectively. Twelve hours after glycerol injection, both CO and RBF remained significantly lower than control. Mean RVR was significantly elevated compared to the controls and CO and RBF were not different than that of nondehydrated saline-drinking rats 12 and 24 hour post-glycerol.

Twelve hours after glycerol injection in saline-drinking rats, the mean BUN values 12 and 24 hours post-glycerol injection were similar to those seen 3 hours after glycerol injection and for control normal rats without dehydration. The elevation of BUN when compared to control values. The elevation of BUN 12 hours post-glycerol was not different from that seen 24 hours after glycerol injection (P > 0.05). The protection afforded by chronic saline drinking was apparent, however, only at 24 hours when the elevation of the BUN was not as great as that seen in water-drinking rats 24 hours after glycerol injection (P < 0.01). Mean CO, RBF, MAP, and RVR were not significantly different from the normal in the saline-drinking rats 12 and 24 hour post-glycerol.
measurement (\(^{85}\)Sr) in normal control rats (n = 9) yielded a mean of 4.65 ± 0.56 ml/min per 100 g of body weight. This was not significantly different by paired statistical analysis from the second measurement (\(^{108}\)Ce) in the same group of 4.55 ± 0.42 ml/min per 100 g of body weight. These values of RBF are similar to our previously reported measurements using the microsphere method and are nearly identical to that found by Arendshorst et al.\(^\text{17}\) using an electromagnetic flow probe.

It has been established that severe cortical ischemia occurs during the initial hours of glycerol-induced acute renal failure, although the mechanisms responsible for the decreased RBF have not been defined clearly.\(^\text{1-5}\) A major role for CO in the early decrease in RBF is indicated by the finding of a 64% decrease in CO 3 hours after glycerol injection in water-drinking rats. The parallel reductions of CO and RBF were still evident 12 hours after glycerol injection. The severe volume contraction known to occur during the early phase of glycerol administration\(^\text{19}\) may induce a decreased venous return and thereby contribute to the low CO. Since plasma volume expansion did not completely restore the CO to normal, other factors, such as a direct effect of glycerol on the myocardium or increased MAP, also may be involved. The elevated CO observed 24 hours after glycerol injection could partially reflect an expansion of plasma volume due to water retention, as this stage of acute renal failure is characterized by severe oliguria.\(^\text{15}\) In addition, reduced afterload due to the slightly lower MAP may further contribute to the increased CO.

That CO can be augmented significantly by infusion of plasma in a volume equivalent to 3% of body weight in 3-hour post-glycerol rats with concomitant restoration of RBF and RVR suggests that the observed alterations in renal hemodynamics are volume-dependent. This response also belies a role for arteriolar swelling in the increased RVR\(^\text{24}\) of glycerol-induced acute renal failure. Previous attempts to restore RBF using 3% of body weight volume expansion with 0.9% NaCl were unsuccessful,\(^\text{1}\) suggesting that plasma protein is required to prevent rapid loss of fluid from the vascular compartment.

In addition to the decreased CO, it is possible that elevations of plasma renin in the early stage of glycerol-induced acute renal failure in both water-drinking and saline-drinking rats\(^\text{29}\) could further contribute to cortical ischemia. Therefore, the mechanism whereby plasma volume expansion reverses cortical ischemia could involve renin suppression as well as augmentation of CO.

The protection afforded by chronic saline loading against the development of acute renal failure\(^\text{20,21}\) is not dependent on prevention of the early decreases in CO and RBF. However, the restoration of CO and RBF which occurs by 12 hours post-glycerol in saline-drinking rats, but not in water-drinking rats, could be partly responsible for reducing the severity of acute renal failure.

### Acknowledgments

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### REFERENCES

18. Flores J, Dibbons DR, Beck CH, Leaf A: The role of cell swelling in ischemic renal damage and the protective effect of hypertonic solute. J

### TABLE 4 Cardiac Output and Renal Blood Flow in Chronic Saline-Drinking Rats 12 and 24 Hours after Glycerol Injection and in Controls

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<thead>
<tr>
<th>Group</th>
<th>CO (ml/min/100 g BW)</th>
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</tr>
</thead>
<tbody>
<tr>
<td>I. -Saline control (n = 7)</td>
<td>30.7 ± 1.55</td>
<td>4.94 ± 0.37</td>
<td>114.4 ± 2.8</td>
<td>24.1 ± 2.27</td>
<td>27.2 ± 1.12</td>
</tr>
<tr>
<td>II. 12 hr post-glycerol (n = 7)</td>
<td>29.2 ± 2.67</td>
<td>4.11 ± 0.36</td>
<td>120.4 ± 4.27</td>
<td>31.02 ± 3.42</td>
<td>76.2 ± 15.5</td>
</tr>
<tr>
<td>III. 24 hr post-glycerol (n = 7)</td>
<td>32.5 ± 2.33</td>
<td>4.05 ± 0.25</td>
<td>120.2 ± 3.9</td>
<td>30.6 ± 2.65</td>
<td>63.8 ± 10.3</td>
</tr>
</tbody>
</table>

Probability values

I-II: NS

I-III: NS

Abbreviations are the same as in Table 1.
The Distribution of Labeled Albumin across the Rabbit Thoracic Aorta in Vivo

ROBERT L. BRATZLER, GUY M. CHISOLM, CLARK K. COLTON, KENNETH A. SMITH, DONALD B. ZILVERSMIT, AND ROBERT S. LEES

SUMMARY 125I-albumin was injected intravenously into normal conscious rabbits. The rabbits were killed after 10 minutes to 24 hours, and the descending thoracic aorta was excised immediately, opened longitudinally, rinsed, and frozen. Samples of frozen aorta were sectioned parallel to the intimal surface and washed with trichloroacetic acid (TCA) prior to counting. TCA-soluble tissue radioactivity slowly increased with time, suggesting that 125I was cleaved gradually from the labeled albumin within the aortic wall. At up to 4 hours, transmural concentration profiles of TCA-precipitable radioactivity had steep gradients near the intimal surface, moderate gradients near the medial-adventitial border, and were relatively flat in the middle of the media. After 24 hours, the steep intimal gradient had disappeared. Concentrations were otherwise comparable to those at 4 hours. The rate of accumulation of TCA-precipitable radioactivity was rapid initially (measurable concentrations were found throughout the media after only 10 minutes) and decreased with time. The results are consistent with entry of 125I-albumin into the media from both the luminal and adventitial sides. Approximate calculations indicate that the albumin mass transfer resistance associated with the intimal endothelium is about 1 order of magnitude greater than that associated with the media.

THE TRANSPORT and metabolism of macromolecules in the arterial wall may be important in atherogenesis. Low density lipoproteins and fibrinogen or fibrin have been identified in atheromatous lesions by immunofluorescent and histochemical techniques. Lipoproteins have been recovered from atheromatous lesions, and the entry of radioiodinated albumin and low density lipoprotein into the human arterial wall has been demonstrated in vivo. However, the rates of macromolecular transport into and within the arterial wall, the physiological factors that influence these rates, and the mechanisms by which transport occurs have not been well defined.

We have measured the concentration profile of radioiodinated albumin across the aortic wall as a function of time following intravenous injection into conscious normal rabbits. Previous studies have provided disparate results. Duncan and colleagues measured labeled albumin uptake in the inner layer of the rabbit aortic wall. In subsequent experiments with dogs the differences between labeled albumin concentrations in the inner, middle, and outer layers were relatively small. By contrast, Adams and coworkers using autoradiography and a tissue serial sectioning technique, found higher concentrations of labeled albumin and β- and γ-globulins at the adventitial side than at the intimal side of the media of normal rabbits. The reverse was true in the presence of diet-induced fatty lesions. Bell and others sectioned frozen swine aortic tissue and found markedly higher concentrations of labeled albumin and fibrinogen near the intimal surface.

In the present study, the transmural distribution of radioactivity was determined from serial tissue sections prepared with a refrigerated microtome. Care was taken to obtain quantitatively reliable data. Excised aortas were frozen immediately after excision to prevent further diffusion, and all non-protein-bound radioactivity was removed from the tissue sections by washing with trichloroacetic acid (TCA).

Methods

MATERIALS

Rabbit serum albumin (4X crystallized, Nutritional Biochemicals) was iodinated with Na125I (17 Ci/mg in 0.1 N NaOH, New England Nuclear) by using the iodine mon-
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