Effect of Variation in Dietary NaCl Intake on Total and Fractional Renal Blood Flow in the Normal and Mercury-Intoxicated Rat

NORBERT LAMEIRE, M.D., SEVERIN RINGOIR, M.D., AND ISIDOOR LEUSEN, M.D.

SUMMARY We studied the effect of different chronic (3-4 weeks) dietary salt intakes on intrarenal hemodynamics of normal and mercury-intoxicated rats. Cardiac output (CO), total renal blood flow (RBF), and the zonal perfusion rate in the outer cortex (OC) and inner cortex (IC) were measured by the radioactive microsphere method. The distribution of cortical blood flow was calculated as the distribution index (DI), which reflects the ratio OC/IC. Rats were placed on a high salt diet (group I), intermediate salt diet (group II), or low salt diet (group III). For each group control rats (subgroup A) and mercury-intoxicated rats (subgroup B) were studied. No effect of the different salt intakes on the DI could be detected. The DI in group I was 2.35 ± 0.14; in II, 2.40 ± 0.16; and in III, 2.38 ± 0.09 (P > 0.05). After mercury injection RBF changed from 5.32 ± 0.36 ml/g min⁻¹ (II A) to 5.31 ± 0.20 ml/g min⁻¹ (II B), and from 4.32 ± 0.11 ml/g min⁻¹ (III A) to 1.98 ± 0.10 ml/g min⁻¹ (III B) (P < 0.01). The DI was lowered to 1.53 ± 0.06 (II A) (P < 0.05) and to 1.16 ± 0.10 (III A) (P < 0.01). In both II B and III B a marked elevation of the blood urea was noted (II B = 79 ± 8 mg/100 ml and III B = 182 ± 25 mg/100 ml). In group I, no effect on RBF, OC, IC, or DI could be observed (for all values, P > 0.05) despite similar histological renal lesions. Group I rats also had normal blood urea levels (31 ± 6 mg/100 ml; P > 0.05). We conclude (1) that variations in dietary salt intake appear to have no detectable effect on the intracortical blood flow distribution; and furthermore (2) that the mercury-induced acute renal failure (ARF) is characterized hemodynamically by a total renal and preferential outer cortical ischemia and that chronic salt loading prevents the ARF while preserving normal renal perfusion.

THE PATHOPHYSIOLOGY of acute renal failure (ARF) remains unclear. Several mechanisms have been postulated to participate in the renal functional impairment in the various nephrotoxic models: renal vasoconstriction, tubular obstruction, back diffusion of filtrate through damaged tubular epithelium, and a decrease in glomerular capillary permeability. Although an increase in renal vascular resistance does not seem to be necessary to maintain nephrotoxic ARF, renal vasoconstriction is usually present during its initiation. Several studies have suggested that the renal vasocostriction is related to stimulation of the renin-angiotensin system. In support of this view are studies which demonstrate that there is protection against the development of numerous forms of ARF when intrarenal renin is depleted by chronic salt loading.

Alterations in the intrarenal distribution of blood flow also have been reported in nephrotoxic models of ARF. Flamenbaum and associates reported a redistribution of flow to inner cortical nephrons in the first 6 hours after administration of uranyl nitrate. Since intrarenal renin is preferentially located in the outer cortical nephrons and the plasma renin is also markedly increased during the initial phase after uranyl nitrate administration, it was suggested that renin release led to preferential vasoconstriction in outer cortical nephrons in this model. However, studies by Stein and associates, using this same uranyl nitrate model in the dog, have not confirmed this alteration in intrarenal distribution of flow in the first 6 hours after uranyl nitrate administration.

From the Nephrological Division, Department of Medicine, and the Department of Physiology and Pathological Physiology, University Hospital, Ghent, Belgium.

Address for reprints: Dr. Norbert Lameire, Nephrological Division, Department of Medicine, University Hospital, De Pintelaan 135, B-9000, Ghent, Belgium.

Received February 3, 1976; accepted for publication June 8, 1976.

It was the purpose of our study to further evaluate the intrarenal distribution of blood flow in a model of nephrotoxic ARF. If an augmentation of intrarenal renin release occurs during ARF and is responsible for the development of the renal hemodynamic alterations, suppression of renin release by chronic salt loading might modify the pattern of renal vascular resistance and intrarenal blood flow distribution. In keeping with this view, the present studies indicate that chronic salt loading prevents azotemia, renal vasoconstriction, and redistribution of blood flow to inner cortical nephrons after mercurial ingestion by rats.

Methods

Experiments were performed on 57 white male inbred rats weighing 280-325 g. The rats were anesthetized with pentobarbital (50 mg/kg, ip). A tracheostomy was performed and PE 50 catheters were introduced into the tail and the right carotid arteries. The latter catheter was then introduced through the aortic valve into the left ventricular outflow tract with the pressure tracing used to determine proper placement. Radioactive microspheres (3M Co.) 15 ± 5 μm in diameter were used to measure cardiac output (CO), total renal blood flow (RBF), and cortical blood flow distribution. The nuclides used were 85Sr and 103Ce. Fifteen microliters of a microsphere suspension (approximately 40,000 spheres) were drawn into a short piece of PE 50 tubing. The total radioactivity of the injected dose was measured. This injection lasted 4-5 seconds. For the measurements of CO and RBF a reference flow rate is required. The reference sample was collected from the tail artery at a constant rate of 0.393 ml/min with a Harvard withdrawal pump. The withdrawal was started 5 seconds before the injection. Sampling did not affect heart rate or arterial blood pressure, and the arterial microsphere concentration was zero at the end of the collection time. The reference
The tissue sections were counted with a well scintillation detector using a thallium-activated sodium iodide crystal, 7.5 × 7.5 cm (Baird Atomic) and a 256-channel height analyzer (Nuclear Data series 1100 analyzer). Cross-over corrections were performed according to the method of Rudolph and Heymann.28 The blood urea nitrogen concentration was measured with a Technicon AutoAnalyzer. Renal histological sections, obtained from at least three rats in each group, were fixed in formalin and processed for examination under the light microscope. The sections were reviewed without knowledge of the salt intake or renal functional status of the rat under study.

**Calculations**

\[
\text{CO (ml/min)} = \frac{\text{total counts/min injected} - \text{blood withdrawal rate (ml/min)}}{\text{total counts/min in reference sample}}
\]

RBF was calculated by:

\[
\frac{\text{RBF (ml/min)}}{\text{total counts/min in kidney}} = \frac{\text{blood withdrawal rate (ml/min)}}{\text{total counts/min in reference sample}}
\]

Zonal perfusion rate of cortical blood flow per gram was calculated by:

\[
\frac{\text{RBF (ml/min) counts/min in tissue slice/g}}{\text{total counts/min in kidney}} = \frac{\text{RBF (ml/min)}}{\text{OC perfusion (ml/g.min}^{-1})} - \frac{\text{IC perfusion (ml/g.min}^{-1})}{\text{Cortical distribution index (DI) was calculated by:}}
\]

**GROUP I (HIGH SALT RATS)**

These results are summarized in Table 1. After 3 or 4 weeks on a high salt diet, CO was 71 ± 2.6 ml/min, systolic blood pressure was 135 ± 7.0 mm Hg, and RBF was 5.9 ± 0.15 ml/min g⁻¹. Only the latter value was significantly greater than values for rats on a normal sodium intake (P < 0.05). In addition, there was a parallel increase in the zonal perfusion rate of both the OC and IC, as compared to groups II and III (P < 0.05). In the high salt rats given mercaptomerin, there was no change in CO, blood pressure, RBF, or intrarenal distribution of blood flow. As is shown in Table 1, there was no significant increase in the blood urea nitrogen concentration in the group given mercury, the means being 25 ± 6 and 31 ± 6 mg/100 ml in the control rats and mercury-treated rats on a high salt diet (P > 0.1), respectively.

**GROUP II (INTERMEDIATE SALT RATS)**

The results of these studies are summarized in Table 1. In the control animals (group IIa) CO was 75 ± 5.0 ml/min, blood pressure was 135 ± 1.0 mm Hg, RBF was 5.32 ± 0.36 ml/min g⁻¹. After mercurial injection there was a significant fall in both the OC and IC perfusion rates, from 8.65 ± 0.10 to 4.38 ± 0.19 ml/min g⁻¹ (P < 0.01) and from 3.60 ± 0.18 to 2.86 ± 0.10 ml/min g⁻¹ (P < 0.01), respectively. The decrease in the zonal perfusion rate, however, was much greater in the OC, leading to a decreased cortical DI of 1.53 ± 0.06 (P < 0.01) in comparison to the control (group IIa) value of 2.40 ± 0.16. The blood urea was significantly increased after mercaptomerin administration, 30 ± 6 mg/100 ml vs. 97 ± 9 mg/100 ml (P < 0.01).

**GROUP III (LOW SALT RATS)**

In the rats on the low salt diet there was a decrease in both CO and systolic blood pressure in comparison to values for the control rats in either group I or IIa (Table 1) (P < 0.05). In addition, RBF was significantly decreased to 4.32 ± 0.11 ml/min g⁻¹ (P < 0.05) in comparison to groups Ia and IIa. Both OC and IC perfusion rates also were
HISTOLOGICAL STUDIES

Examination with the light microscope revealed that all three groups of rats had severe histological lesions similar to those previously described in mercurial intoxication. No distinction among the three groups could be made. Examples of the group I and group III histological lesions are shown in Figures 1 and 2, respectively. As can be noted, there was severe tubular necrosis of a fairly generalized nature, with swelling, necrosis, and shedding of epithelial cell cytoplasm into the tubular lumen. Intertubular edema of modest degree was present. The blood vessels and glomeruli were unremarkable. It should be noted therefore that no distinction could be made on a histological basis between the group I and III B rats, although in the former the blood urea nitrogen concentration averaged 31 mg/100 ml, whereas in the low salt group III B rats the mean blood urea nitrogen concentration was 182 mg/100 ml.

Discussion

The present study was designed mainly to evaluate the role of cortical blood flow distribution in the daily regulation of the sodium balance and in the pathogenesis of mercury-induced ARF. Renal hemodynamics were measured with the radioactive microsphere method, the validity of which has been tested in studies from this laboratory and other laboratories. Although in our work the regional blood flow distribution was compared in control and experimental rats, since only one microsphere injection per rat was used the results in a given control group were highly reproducible. These results also are in agreement with two other recently published studies providing microsphere data for the same species. On the basis of studies with the inert gas washout method in both humans and animals, it has been suggested that changes in the distribution of intrarenal blood flow could play a role in the regulation of the sodium balance. However, many important theoretical and practical objections to the inert gas method have been formulated, and in recent experiments using radioactive microspheres the distribution of RBF could not be related to the state of sodium balance.

Only two studies on the distribution of cortical blood flow have been performed after chronic (2–4 weeks) salt loading. Using the xenon washout method in the rat, Truong et al. found a net redistribution of the cortical blood flow favoring the outer cortical nephrons over the juxtamedullary ones after chronic sodium loading, with a reverse pattern after chronic sodium restriction. These different results can be attributed to the questionable value of the xenon washout method for the measurement of intracortical blood flow distribution. In the second study, Blantz et al., using a glomerular basement membrane antibody technique in the unanesthetized rat, could detect no effect of different salt intakes on the distribution of the intrarenal plasma flow. Although their results are in agreement with ours, this method also has recently been criticized.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Cardiac Output (CO), Systemic Systolic Blood Pressure (BP), Total Renal Blood Flow (RBF), Outer Cortical Perfusion Rate (OC), Inner Cortical Perfusion Rate (IC), Distribution Index (DI), and Blood Urea in the Different Groups of Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td>CO (ml/min)</td>
</tr>
<tr>
<td>---------</td>
<td>-------------</td>
</tr>
<tr>
<td>I A (8)</td>
<td>71 ± 2.6</td>
</tr>
<tr>
<td>I B (9)</td>
<td>70 ± 6.8</td>
</tr>
<tr>
<td>II A (12)</td>
<td>75 ± 5.0</td>
</tr>
<tr>
<td>II B (13)</td>
<td>66 ± 2.2</td>
</tr>
<tr>
<td>III A (14)</td>
<td>58 ± 2.7</td>
</tr>
<tr>
<td>III B (9)</td>
<td>54 ± 1.8</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM.

Groups I, II, and III are the rats on the high, normal, and low salt intakes, respectively. Subgroups A and B are normal and the mercury-treated rats, respectively. The values are given in parentheses.
Our results clearly demonstrate that chronic changes in sodium intake have no effect on the distribution of intracortical blood flow as measured by radioactive microspheres in the adult rat. With respect to the results obtained for the rat with the same method after acute sodium loading, where a preferential increase in the outer cortical blood flow was observed, the difference probably can be explained by the only minor degree of renal vasodilation that results from the chronic salt loading. It seems therefore that no change in the intrarenal blood flow distribution can be detected among rats on markedly different chronic sodium intakes.

An increased renal vascular resistance has been suggested to play an important role in the initiation of ARF. In many of the previously reported studies, however, it was not clear whether the observed renal ischemia was selective, or whether the impaired RBF was due to more general hemodynamic disturbances such as a change in cardiac output. Particularly in the HgCl₂ model there is severe cardiotoxicity with a fall in cardiac output. This cardiotoxic effect was avoided in our study by the use of an organomercurial compound. From the constant cardiac output and blood pressure, it can be calculated that the observed renal ischemia was due to a selective increase in renal vascular resistance. This augmented resistance was preferentially localized to the outer cortical zones (Table 1). This redistribution of cortical blood flow is in agreement with the microsphere results of Flumenbaum et al., obtained 6 hours and 48 hours after usanyl nitrate injection in the dog, and partly in agreement with the results of Stein et al., who observed the same redistribution pattern only 48 hours after administration of uranyl nitrate. Recently, a relative increase in the medullary blood flow, as measured by a radioactive albumin accumulation method, has been found in mercury-induced ARF. This finding is consistent with the relatively well preserved inner cortical blood flow in our present study. The cortical ischemia that we observed in this study was less severe than in our previous study; here a very marked decrease in cortical perfusion after mercury intoxication was found. However, a 3- to 4-fold greater dose of mercury was injected to induce ARF in the previous study. The significance of the total and preferential outer cortical ischemia 24 hours after mercury injection is not clear. The redistribution cannot be related to an increased renal vascular resistance per se, because the same pattern can be observed with either a rise or fall in renal vascular resistance.

It also is difficult to explain the oliguria of the mercury-intoxicated rats on the basis of this cortical ischemia. It has been shown by Cox et al. that marked oliguria could persist with preserved RBF and normal fractional distribution after a high dose of norepinephrine. Although their study suggests that renal ischemia is not necessary to maintain oliguria, it does not exclude a possible important role of intense cortical vasoconstriction in the initiation of the ARF. In this regard it is important to note that oliguria or permanent renal functional impairment do not occur in the salt-loaded rats (group 1B) when normal renal perfusion is maintained.

The mechanism responsible for the initiation or mainte-
nance of the renal ischemia in ARF is obscure and controversial, although there is some evidence that the intrarenal renin-angiotensin system is involved. In favor of this view is the finding that the chronic administration of saline, which diminishes but does not completely deplete the renin stores of the kidney, has a protective effect against nephrotoxic acute renal failure. Our results confirm and extend these findings by demonstrating a preserved total RBF together with an unchanged cortical blood flow distribution in the salt-loaded rats. Despite severe histological lesions of acute tubular necrosis, normal renal function was maintained in these rats. On the other hand, salt depletion (group IIIa) provoked a much more severe renal insufficiency, together with a greater fall in RBF, than was observed with a normal salt intake (group IIa) but with qualitatively the same degree of histological injury to the tubules.

Although we did not measure intrarenal renin in this study, it has been shown repeatedly that chronic salt loading, as in the present study, results in a decrease in intrarenal renin content and that chronic salt restriction has the opposite effect. It is also important to note that in the rat the outer cortical nephrons have the greatest renin content, and that this renin gradient remains unchanged after both uranyl nitrate and mercury intoxication. However, chronic salt loading resulted in an increased RBF even before the mercury was administered. Therefore, the possibility that the protection against the nephrotoxicity was mediated by this previous renal vasodilation also must be considered.

In conclusion, the present results indicate that mercury-induced ARF is characterized hemodynamically by an important total and preferential outer cortical ischemia and that chronic salt loading prevents the ARF while preserving normal renal perfusion.

References

7. Steinhausen M, Eisenbach GM, Helmsdorfer V Concentration of lissamine green in proximal tubules of anuric and mercury poisoned rats and the permeability of these tubules. Pfluegers Arch 311: 1-15, 1969
8. Stein JH, Gottschall J, Osgood RW, Ferris TF: Pathophysiology of a
19. Lameire N, Ringoir S: Comparison of the xenon wash-out method, the electromagnetic flowmeter and radioactive microspheres for the measurement of renal blood flow (abstr). Kidney Int 7: 363, 1975

PENTOBARBITAL AND CARDIOVASCULAR FUNCTION/Manders and Vantr

Effect of variation in dietary NaCl intake on total and fractional renal blood flow in the normal and mercury-intoxicated rat.
N Lameire, S Ringoir and I Leusen

Circ Res. 1976;39:506-511
doi: 10.1161/01.RES.39.4.506

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/39/4/506