Failure of Chronic Sodium Chloride Loading to Protect against Norepinephrine-Induced Acute Renal Failure in Dogs

RICHARD W. BAEHLER, THEODORE A. KOTCHEN, AND COBERN E. OTT

SUMMARY We previously have shown that chronic sodium chloride (NaCl) loading protects against HgCl₂-induced acute renal failure (ARF) in dogs. To determine whether NaCl loading protects against an ischemic model of ARF, unilateral oliguric renal failure was produced by the infusion of norepinephrine (NE) into the renal artery of both saline-expanded (SE) and water-drinking (WD) dogs (n = 7). The renal renin content (30 U/g kidney) of SE dogs was suppressed (P < 0.001) compared to that of WD dogs (132 ± 18). Forty-eight hours after infusion of NE (1.5 μg/kg per min x 100 min), inulin clearances from the infused kidney of SE (6 ml/min ± 2) and WD dogs (7 ± 2) did not differ; in both groups, respective clearances from the noninfused kidney (43 ml/min ± 3) and (36 ± 5) also did not differ from each other. The percent fall in renal blood flow to the infused kidney 48 hours after NE in SE (44%) and WD dogs (38%) did not differ. Because of failure to demonstrate protection, a lower dose of NE (0.75 μg/kg per min x 40 min) was infused into SE and WD animals (n = 6). Forty-eight hours after low dose NE, inulin clearances of the infused kidney of SE (17 ml/min ± 5) and WD dogs (17 ± 4) did not differ. Respective clearances in the noninfused kidney of SE (46 ml/min ± 6) and WD dogs (35 ± 4) did not differ. Therefore, despite suppression of renal renin content, NaCl loading failed to protect against this ischemic model of ARF. In conclusion, unlike HgCl₂-induced ARF, it is unlikely that the renin angiotensin system contributes to the pathogenesis of this ischemic model of ARF.

CHRONIC sodium chloride (NaCl) and potassium chloride loading in the rat prevents the development of renal failure induced with glycerol or mercuric chloride. Protection from renal failure has been associated with inhibition of the renin angiotensin system, and it has been suggested that protection may be related specifically to suppression of intrarenal renin content. Acute administration of deoxycorticosterone acetate (DOCA) suppresses plasma renin activity but not intrarenal renin content, and animals are not protected against glycerol-induced acute renal failure. Similarly, renin immunization does not protect and intrarenal renin content of renin-immunized rats is not suppressed.

We recently have confirmed that chronic NaCl loading also protects against the development of mercuric chloride-induced acute renal failure in the dog. To evaluate the contribution of intrarenal renin content to the pathogenesis of an ischemic model of acute renal failure, the present study was designed to determine whether chronic NaCl loading protects against renal failure due to unilateral infusion of norepinephrine into the renal artery of the dog.

Methods

Unilateral renal ischemia was produced by infusion of norepinephrine into the renal artery of chronically NaCl-loaded and water-drinking dogs. Similar to the results of Dibona et al. in a preliminary study we found that administration of DOCA in addition to substitution of normal saline for drinking water had a more profound inhibitory effect on renal renin content than saline alone. Consequently, intrarenal renin suppression was achieved by giving DOCA (15 mg/day, im) and replacing drinking water with normal saline for 10 days. Chronically NaCl-loaded (renin-suppressed) dogs were studied simultaneously with pair-fed water-drinking (normal renin) dogs.

Studies were performed on mongrel dogs weighing 14-26 kg. On the day of the experiment they were anesthetized with sodium pentobarbitol (30 mg/kg). An endotracheal tube was inserted and the dogs were ventilated with an Airco respirator (Ohio Medical Products). Cannulas were inserted in a leg vein for infusions and in the right femoral artery for blood pressure measurements and blood collection. Systolic, diastolic, and mean arterial blood pressures were recorded with a Bell & Howell transducer connected to a Mennen Greatbatch monitor (model 715-281). The left ventricle was catheterized via the aorta from the left femoral artery with a Goodale-Lubin standard wall catheter for injection of radioactive microspheres which were used to measure regional blood flow in different areas of the renal cortex as well as total renal blood flow. The nuclides used were ⁸⁵Sr and ¹⁴⁴Ce. The amount, sequence, and method of injection have been described previously. Either a high or a low dose of norepinephrine was infused into the renal artery.

High Dose Norepinephrine Studies

Seven chronically NaCl-loaded and seven water-drinking dogs were anesthetized and stabilized for 1 hour. An initial injection of microspheres then was given (period
After the initial injection, a small left flank incision was made under aseptic conditions and norepinephrine (1.5 μg/kg per min) was infused into the renal artery through a 25-gauge curved needle for 100 minutes. At the conclusion of the infusion, the needle was removed carefully and the incision closed. The arterial and left ventricular catheters were removed and the arteries were ligated. All animals tolerated the procedure well and were returned to their cages. The dogs consumed their daily allotment of food and either water or saline (including DOCA) in the 48-hour interval between study periods.

Forty-eight hours later, the dogs again were lightly anesthetized with pentobarbital (30 mg/kg) and subsequently given small maintenance doses as necessary. The arterial and left ventricular catheters were reinserted proximal to the previous insertion and both ureters were cannulated with PE 190 tubing. After stabilization, a second injection of microspheres was given (period 2). After the second microsphere injection, an infusate was given (50 mg/kg) followed by a maintenance infusion at 1 ml/min to maintain the plasma level constant between 0.2 and 0.3 mg/ml. Forty-five minutes later, three 10-minute clearance periods were obtained to determine single whole kidney glomerular filtration rates. The dogs then were given Ringer’s solution at a rate of 1 ml/kg per min for 40 minutes to exclude volume depletion as an additional factor resulting in low glomerular filtration rates. Following the maintenance infusion, three additional 10-minute clearances were obtained. Because inulin clearances did not differ before and after Ringer’s loading, the data were combined and are presented as the mean ± SEM for each dog.

At the end of the experiments, both kidneys were removed and weighed. Determination of regional blood flow was carried out by the method described by Stein et al.15 The cortex was divided into four equal zones, with zone 1 the outermost cortical zone and zone 4 the innermost cortical zone. The method of sectioning, counting, and calculating the corrected percent distribution of blood flow to cortical zones 1-4 and the method for determining total renal blood flow have been described in detail.

**Low Dose Norepinephrine Studies**

In six separate paired studies a lower dose of norepinephrine (0.75 μg/kg per min) was infused into the renal artery for 40 minutes. The dietary regimen and all experimental procedures were the same with some minor modifications. Microspheres were not given in these studies. Instead, after the inulin clearance measurements the non-infused kidneys were obtained for determination of renal renin content to confirm that 10 days of DOCA and saline did suppress renal renin content. In five additional dogs, peripheral venous plasma renin activity (PRA) was measured before and 10 days after DOCA and saline; the dogs were not anesthetized at the time of venipuncture.

In four additional studies, two NaCl-loaded dogs and two water-drinking dogs were used to demonstrate stability of the preparation and to show that changes in total renal blood flow, fractional distribution of blood flow, and glomerular filtration rate were not related to surgical preparation or the experimental protocol. An initial injection of microspheres (period 1) was given followed by the infusion of saline into the renal artery in lieu of norepinephrine. Forty-eight hours later, a second injection of microspheres (period 2) was given. Following the second injection of microspheres, inulin was infused as described above, and 45 minutes later, three 10-minute clearance periods were carried out.

Plasma renin activity was measured by the radioimmunoassay procedure of Haber et al.11 Renal renin content was measured by a procedure previously described for the rat.12 13 A minor modification of this procedure has been validated by Haas13 for the dog. Briefly, renin was extracted from individual kidneys by an ammonium sulfate precipitation method. A sample of each extract was incubated at 37°C with excess sheep renin substrate for 15 minutes, and the concentration of angiotensin I generated was measured by radioimmunoassay. Angiotensin I was not detectable in incubations containing enzyme alone or substrate alone. One unit of renin is arbitrarily defined as that concentration of renin that generates angiotensin I at the rate of 100 ng/ml per hour.

Plasma and urine inulin concentrations were determined by the anthrone method.14 Results are recorded as mean ± SEM. Statistical difference was determined by the paired or unpaired t-test as appropriate. Wilcoxon’s rank-sum test was used for comparing the percent fall in renal blood flow between the two groups of dogs. A P value of <0.05 was considered significant.

**Results**

The intrarenal renin content of dogs given DOCA plus saline (30 ± 3 U/g kidney) was significantly lower (P < 0.001) than that of water-drinking dogs (132 ± 18). Similarly, the PRA after 10 days of DOCA plus saline (0.8 ± 0.1 ng/ml per hour) was significantly lower (P < 0.05) than the PRA before DOCA plus saline (1.3 ± 0.2).

**High Dose Norepinephrine Study**

Forty-eight hours after infusion of high dose norepinephrine (1.5 μg/kg per min × 100 min), inulin clearances of the infused kidneys were significantly lower than clearances of the non-infused kidneys in both NaCl-loaded and water-drinking dogs (Table 1). Clearances of the infused kidneys from NaCl-loaded dogs did not differ significantly from the infused kidneys of water-drinking dogs, nor did respective clearances from the noninfused kidneys of both groups (Table 1).

Blood flow to the infused kidneys of both NaCl-loaded and water-drinking dogs fell significantly 48 hours after norepinephrine infusion (Table 2). The 44% fall in renal blood flow to the infused kidneys of NaCl-loaded dogs was not significantly different from the 38% fall in blood flow to the infused kidneys in water-drinking dogs. Total renal blood flow to the non-infused kidneys in both groups of animals 48 hours after norepinephrine was not significantly different from flow measured prior to infusion.

In NaCl-loaded and water-drinking dogs there was a
### Table 1. Glomerular Filtration Rate 48 Hours after Unilateral Norepinephrine Infusion

<table>
<thead>
<tr>
<th>Glomerular filtration rate (ml/min)</th>
<th>NaCl-loaded dogs</th>
<th>Water-drinking dogs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infused kidney</td>
<td>6 ± 2*</td>
<td>7 ± 2*</td>
</tr>
<tr>
<td>Non-infused kidney</td>
<td>43 ± 3</td>
<td>36 ± 5</td>
</tr>
</tbody>
</table>

**High Dose Studies**

- Infused kidney: 7 ± 2*
- Non-infused kidney: 43 ± 3

**Low Dose Studies**

- Infused kidney: 6 ± 5
- Non-infused kidney: 46 ± 6

Results are expressed as mean ± SE. Values in NaCI-loaded and water-drinking dogs did not differ significantly.

*P < 0.01 when comparing the infused vs. non-infused kidney in the same dog.

### Table 2. Summary of Total Renal Blood Flow Measurements

<table>
<thead>
<tr>
<th>Renal blood flow (ml/min)</th>
<th>NaCl-loaded dogs</th>
<th>Water-drinking dogs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infused kidney</td>
<td>189 ± 18</td>
<td>117 ± 15†</td>
</tr>
<tr>
<td>Non-infused kidney</td>
<td>186 ± 12</td>
<td>220 ± 20</td>
</tr>
<tr>
<td>Infused kidney</td>
<td>169 ± 19</td>
<td>95 ± 16†</td>
</tr>
<tr>
<td>Non-infused kidney</td>
<td>157 ± 16</td>
<td>209 ± 34</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SE.

*NE = norepinephrine.
†P < 0.01 compared to value before NE.

### Discussion

Cox et al. have shown that unilateral oliguria is consistently produced at 48 hours by infusing norepinephrine (0.75 μg/kg per min × 40 min) into the renal artery of the dog. This model allows the contralateral kidney to serve as an internal control, and renal ischemia can be produced without the complicating biochemical alterations of uremia. The present study was designed to determine the effect of chronic NaCl loading on the course of acute renal failure following norepinephrine infusion. In contrast to our previous study with mercuric chloride, salt loading did not protect against the reduction of glomerular filtration rate and renal blood flow following norepinephrine infusion, despite suppression of intrarenal renin. In our previous study with mercuric chloride, renin suppression was achieved with chronic NaCl loading but not DOCA, and functional protection occurred with a 57% decrease in intrarenal renin. However, in the present study, no functional protection was observed after norepinephrine infusion.

### Table 3. Effect of Unilateral Norepinephrine Infusion on Percent Distribution of Blood Flow in Renal Cortex

<table>
<thead>
<tr>
<th>Zone 1</th>
<th>Zone 2</th>
<th>Zone 3</th>
<th>Zone 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

**NaCl-loaded dogs**

- Infused kidney: 7 41 ± 3†, 38 ± 4, 36 ± 3, 34 ± 3, 17 ± 1, 16 ± 1, 6 ± 1, 11 ± 1†
- Non-infused kidney: 7 42 ± 3, 38 ± 3, 34 ± 2, 34 ± 2, 18 ± 1, 19 ± 2, 6 ± 1, 9 ± 1†

**Water-drinking dogs**

- Infused kidney: 7 48 ± 3, 44 ± 5, 35 ± 2, 34 ± 3, 13 ± 1, 16 ± 2‡, 4 ± 1, 5 ± 1
- Non-infused kidney: 7 47 ± 4, 37 ± 3§, 32 ± 2, 37 ± 1‡, 15 ± 2, 19 ± 2‡, 6 ± 1, 7 ± 1

* I = control period; II = 48 hours after norepinephrine infusion. Mean ± SEM for four or more sections in the same kidney.
†P < 0.01.
‡P < 0.02.
§P < 0.05.
inephrine infusion despite even greater suppression of intrarenal renin (77%) by the addition of DOCA. Consequently, it is unlikely that lack of protection in this ischemic model of acute renal failure is related to inadequate suppression of intrarenal renin.

Furthermore, we do not believe our inability to show functional protection in the chronically NaCl-loaded dogs was related to an acute increase in intrarenal renin content of the norepinephrine-infused kidney. Variations in the renin content of the kidney occur slowly and it takes several days or even weeks before they become apparent and reach their maximum. Acute renal ischemia, which is provoked by a reduction in renal blood flow, is not accompanied by an immediate increase in renin concentration of renal tissue. Rather, it takes 48 to 72 hours before the renin content begins to rise. In addition, although norepinephrine stimulates renin release in normal dogs, this has not been our experience in chronically NaCl-loaded dogs. In three dogs, after 10 days of DOCA plus saline, renin secretion rates were determined by measuring arterial-venous differences of PRA and renal blood flow (electromagnetic flow probe). Renin secretion did not increase in any of the three dogs during norepinephrine infusion.

The decrease in total renal blood flow 48 hours after high dose norepinephrine infusion in the present study was not significantly different in NaCl-loaded and water-drinking dogs and was similar to that found by Cox et al. The mechanism responsible for the increase in renal resistance of the infused kidney in both groups is unknown. It is known, however, that this increase in renal resistance can be reversed with saline or acetylcholine and therefore appears to be an active vasoconstriction rather than a passive process, such as arterial obstruction by intraluminal thrombus. The increase in total renal blood flow to the non-infused kidney of both groups was not significant. Also, we found a small but significant redistribution of renal blood flow from outer cortex (zones 1 and 2) to inner cortex (zones 3 and 4) 48 hours after norepinephrine in both groups of dogs.

Several factors, including tubular obstruction and a decrease in glomerular capillary permeability, have been implicated in the pathogenesis and maintenance of norepinephrine-induced acute renal failure. Whether or not increased solute excretion is associated with functional protection is controversial. Although Patak et al. have recently shown that renal vasodilation prior to the initiation of intrarenal norepinephrine attenuates the renal functional impairment in this model, increasing renal blood flow to supranormal values by volume expansion 48 hours after norepinephrine does not correct the filtration defect. Similarly, we have recently demonstrated that the decrease in filtration after mercuric chloride also persists following volume expansion and supranormal renal blood flow 48 hours after mercuric chloride.

It may be that the mechanisms responsible for the initiation of renal failure with mercuric chloride and norepinephrine are fundamentally different, since chronic saline loading in the dog protects against the development of acute renal failure after mercuric chloride but not after norepinephrine. However, the following hypothesis could explain the reduction in glomerular filtration rate seen in both models. A decrease in the glomerular ultrafiltration coefficient ($K_f$) initially may be caused by afferent arteriolar vasoconstriction, and this $K_f$ defect may persist 48 hours irrespective of renal blood flow. Following mercuric chloride, intrarenal renin and angiotensin may be necessary for the initial vasoconstriction, and it is this initial vasoconstriction which then produces a decrease in $K_f$. With norepinephrine, the renin angiotensin system is not necessary, as norepinephrine itself produces the initial vasoconstriction which again leads to a decrease in $K_f$. It is thus possible that a decrease in $K_f$ is a common mechanism responsible for the diminished glomerular filtration rate found in both the mercuric chloride and norepinephrine models of acute renal failure.

References

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The Role of Canine Superficial Ventricular Muscle Fibers in Endocardial Impulse Distribution

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SUMMARY Thin sections of canine right and left ventricular endocardium and myocardium were studied in a tissue bath to compare conduction properties of intraventricular specialized conducting tissue [Purkinje fibers (PF)], the superficial layers of subendocardial ventricular muscle (SVM), and the deeper ventricular muscle (DVM) below this level. The study was carried out because of observations that some areas of the endocardium, which are devoid of either specialized conducting tissue or PF-VM junctions between specialized conducting tissue and ventricular muscle, conduct relatively rapidly, favoring specific orientations of propagation. Preparations containing PF, SVM, and DVM were studied electrophysiologically and histologically. A technique of stripping limited areas of endocardium was used to expose DVM in order to determine its intrinsic calculated conduction velocity. In 12 preparations, the average calculated conduction velocity in PF was 1.62 m/sec, and the average in DVM was 0.26 m/sec. The SVM conduction velocity was intermediate between the two, averaging 0.98 m/sec when propagation was parallel to SVM fiber orientation. Conduction velocity transverse to SVM fiber orientation was not significantly different from DVM conduction velocity. Histologically, the most superficial layers of VM were oriented uniformly in the direction of rapid subendocardial conduction, in contrast to DVM fibers in which orientation varied. It is concluded that the geometric arrangement of SVM fibers may provide a means for rapid subendocardial conduction and that the geometric arrangement of SVM fibers may provide a means for rapid subendocardial conduction and impulse distribution at a conduction velocity intermediate between PF and DVM in areas devoid of specialized conducting tissue.

RECENT observations in this laboratory\textsuperscript{1-5} have suggested the possibility of preferential orientation of impulse spread through endocardial muscle in regions of the ventricles devoid of specialized conducting tissue or functional junctions between specialized conducting tissue and surrounding muscle. The present investigation was carried out: (1) to determine the relationship between the defined role of the specialized conducting tissue and that of subendocardial muscle in impulse distribution through ventricular endocardium, (2) to determine whether the most superficial endocardial muscle plays a unique role in impulse distribution to intramural ventricular muscle, and (3) to determine whether there is an anatomic basis for such a relationship as might be inferred from the earlier studies of Pruitt et al.\textsuperscript{6}

Methods

Studies were performed on thin preparations of endocardium and myocardium dissected from the hearts of adult mongrel dogs weighing 5-25 kg. The dogs were anesthetized with sodium pentobarbital, 30 mg/kg, intravenously, and the hearts were removed rapidly through a high gain differential amplifier (Tektronix, model 3A3) and were displayed on oscilloscopes for

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