Recovery from acute renal failure predisposes hypertension and secondary renal disease in response to elevated sodium

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Spurgeon-Pechman KR, Donohoe DL, Mattson DL, Lund H, James L, Basile DP. Recovery from acute renal failure predisposes hypertension and secondary renal disease in response to elevated sodium. Am J Physiol Renal Physiol 293: F269–F278, 2007. First published May 16, 2007; doi:10.1152/ajprenal.00279.2006.—Recovery of renal function is a well-characterized feature of models of acute renal failure; however, more recent studies have reported a predisposition to chronic renal disease. This study sought to determine the susceptibility to sodium-dependent hypertension following recovery from ischemic acute renal failure. Following ischemia-reperfusion (I/R) injury, rats were allowed to recover for 35 days on a 0.4% salt diet, then were switched to 4.0% salt diet for an additional 28 days. Blood pressure was significantly increased in postischemic rats switched to high-sodium diet at day 35 (19 ± 9 mmHg) compared with postischemic rats maintained on low-sodium diet. Plasma renin activity and creatinine clearance were not affected by I/R injury. The ischemic injury combined with transfer to 4.0% salt diet resulted in marked renal hypertrophy characterized by interstitial cellular deposition, tubular dilation, and enhanced rates of albumin excretion. Glomerular structure was altered in post-I/R rats switched to high-sodium diet but not in those maintained on low-sodium diets. When rats were acclimated to high-sodium diet before I/R injury, the early injury was similar to that observed in animals acclimated to low-sodium diet, and these animals progressed rapidly toward chronic kidney disease, as evidenced by advancement of albuminuria. These data suggest that the recovery from acute I/R injury is not complete, compromises Na homeostasis, and predisposes hypertension and secondary renal disease.

Ischemia; blood pressure; sodium sensitivity

ACUTE RENAL FAILURE (ARF) is defined as a rapid decline of renal function and retention of nitrogenous waste, and it is often associated with a mortality rate of 50%. A primary cause of ARF is injury to the kidney secondary to ischemia or hypoxia (19). Despite the high mortality rate, ARF is considered reversible in surviving patients. The injury and recovery process has been studied in several rodent models of ARF, including that induced by ischemia-reperfusion (I/R). In this model, renal morphology is significantly compromised and damage is most severe in the proximal tubules of the outer medulla. The early course of injury is characterized by disruption of the actin cytoskeleton, loss of polarity of tubules, and redistribution of the Na⁺/K⁺-ATPase (30). Recovery of renal function is characterized by a restoration of the glomerular filtration rate. Recovery of the proximal tubule following ischemic injury is characterized by proliferation, migration, cellular hypertrophy, and differentiation of new functional proximal tubule cells and a remodeling of the basement membrane. These events are thought to promote restoration of renal structure and function following recovery from ARF (12, 19).

Alterations in renal vascular structure and function are well-described in the setting of renal I/R injury. For example, endothelial injury has been described as lost barrier function and increased adhesion to inflammatory cells (42, 43, 46). In addition, early reductions in blood flow that occur within minutes of reperfusion can be ameliorated by supplementation with transplanted endothelial cells (3, 46). We have reported that there is a loss of peritubular capillaries and a decrease in oxygen delivery in the kidney following the initial recovery response from ARF. These observations on long-term blood vessel density emanated from our studies (5, 8), and those of other investigators (10, 15, 17, 18, 34, 35), that renal function does not recover completely following I/R. For example, postischemic rats develop a urinary concentrating defect and develop secondary chronic and progressive renal disease characterized by progressive proteinuria and interstitial fibrosis. These observations, made in animal models, may have relevance to clinical studies that have reported an incomplete recovery following ARF and a secondary decline in renal function (11, 14, 23). The development of chronic and progressive renal dysfunction following severe ARF has been reported by several investigators (5, 11, 14, 23). As such, several clinical studies report that the complete normalization of renal function may never be achieved, and other studies report that a small percentage of patients will show a progressive loss of renal function over time (5, 11, 14, 23). This may be particularly prominent in the pediatric population; in a recent study, >50% of pediatric patients showed indications of progressive renal disease and hypertension within 3–5 years of the initial episode (4). As a corollary to ARF, delayed graft function following renal transplantation is associated with an increased risk for renal nephropathy and hypertension (29, 31, 32). These studies suggest an association of acute kidney injury and the development of chronic renal disease. However, the nature of altered function is not well explored. One hypothesis is that reductions in peritubular capillary density may predispose chronic renal failure, perhaps due to hypoxia. Interestingly, there are a number of models characterized by renal interstitial damage that also show reductions in peritubular capillary density; these include those induced by cyclosporine, radiation, aging, and catecholamine infusion (20, 24). More-
Table 1. Effect of sodium on recovery from renal I/R injury for 9 wk after surgery; serum creatinine and kidney weight

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>PCr, mg/dl 24 h After Surgery</th>
<th>PCr, mg/dl 7 Days After Surgery</th>
<th>Kidney wt, g</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Protocol I</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>7</td>
<td>0.45±0.06</td>
<td>0.45±0.03</td>
<td>1.55±0.09</td>
</tr>
<tr>
<td>Sham UNx</td>
<td>3</td>
<td>0.57±0.04</td>
<td>0.51±0.01</td>
<td>2.64±0.30*</td>
</tr>
<tr>
<td>ARF L-L</td>
<td>3</td>
<td>3.00±1.54</td>
<td>0.95±0.80</td>
<td>1.75±0.18</td>
</tr>
<tr>
<td>ARF L-H</td>
<td>7</td>
<td>3.47±0.24</td>
<td>1.04±0.13</td>
<td>1.83±0.19</td>
</tr>
<tr>
<td><strong>Protocol II</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham L-L</td>
<td>11</td>
<td>0.36±0.07</td>
<td>0.29±0.08</td>
<td>1.20±0.05</td>
</tr>
<tr>
<td>Sham L-H</td>
<td>12</td>
<td>0.38±0.05</td>
<td>0.61±0.05</td>
<td>1.24±0.05</td>
</tr>
<tr>
<td>ARF L-L</td>
<td>11</td>
<td>2.36±0.48</td>
<td>0.76±0.12</td>
<td>1.36±0.09*</td>
</tr>
<tr>
<td>ARF L-H</td>
<td>10</td>
<td>2.85±0.38</td>
<td>0.61±0.26</td>
<td>1.61±0.18*</td>
</tr>
<tr>
<td><strong>Protocol III</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham L-H</td>
<td>6</td>
<td>0.68±0.09</td>
<td>0.48±0.02</td>
<td>1.67±0.02</td>
</tr>
<tr>
<td>Sham UNx</td>
<td>4</td>
<td>0.81±0.22</td>
<td>0.75±0.04</td>
<td>2.15±0.13*</td>
</tr>
<tr>
<td>ARF L-L</td>
<td>9</td>
<td>3.90±0.32*</td>
<td>0.85±0.19</td>
<td>1.74±0.09*</td>
</tr>
<tr>
<td>ARF L-H</td>
<td>9</td>
<td>3.25±0.50*</td>
<td>0.75±0.09</td>
<td>2.04±0.13*</td>
</tr>
</tbody>
</table>

Data are means ± SE. I/R, ischemia-reperfusion; PCr, creatinine; UNx, nephrectomized animals; L, low-sodium; H, high sodium. *P < 0.05 vs. sham-operated control by 2-way ANOVA.

Moreover, these models have also been characterized by the predisposition of sodium-dependent hypertension. Therefore, because recovery from ARF has features in common with many of these models, we sought to determine if posts ischemic animals would develop sodium-dependent hypertension.

METHODS

Animals. Care of the rats before and during the experimental procedures was conducted in accordance with the policies of the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All protocols had received prior approval by the Institutional Animal Care and Use Committees at the Medical College of Wisconsin and the Indiana University School of Medicine.

Male Sprague-Dawley rats weighing ~250 g were housed in pairs in standard shoebox cages on a 12/12-h light/dark cycle. Animals were given standard laboratory rat chow (AIN76A; Dyets, Bethlehem, PA) with a defined 0.4% or 4.0% salt content, as described below; food and water was available ad libitum.

Protocol I was designed to determine the effects of I/R injury on the development of sodium-sensitive hypertension. In these studies, rats were acclimated to 0.4% salt diet. Before ischemia, rats were instrumented with chronic blood pressure transducers; rats were anesthetized with ketamine HCl (60 mg/kg ip), xylazine (6 mg/kg ip), and acepromazine maleate (0.9 mg/kg ip). The catheter tip of the telemetric blood pressure transducer (TA11PFA-C40; Data Science) was implanted into the right femoral artery, and the body of the pressure transducer was implanted subcutaneously. Rats were allowed to recover for 14 days, during which baseline blood pressure measurements were taken at 7 days before ARF induction. To induce ARF, rats were anesthetized with ketamine (100 mg/kg ip) and pentobarbital sodium (25 mg/kg ip) and were placed on a heated surgical table. A midline incision was made. Blood supply to the kidneys was interrupted by applying microvascular clamps on the renal pedicles of both kidneys. The clamps were then released, and reperfusion was visualized. Additional rats were subjected to sham surgery where the kidneys were exposed but not touched.

All animals were allowed to recover for 35 days (i.e., 5 wk after I/R or sham surgery) on a 0.4% salt diet and were then allowed to recover for an additional 28 days (total of 63 days after surgery) on either low- or high-sodium diet. One group of posts ischemic animals was switched at day 35 to a diet containing a high sodium content (4.0% NaCl; Dyets) for an additional 28 days; this group is referred to as I/R L-H, indicating their switch from low-sodium to high-sodium diet at day 35 (n = 7). As a control for the effects of sodium, another group of posts ischemic animals was maintained on 0.4% salt diet for the entire study; this group is referred to as I/R L-L, indicating that they were maintained on 0.4% salt diet after day 35 (n = 3). In the sham-operated control group, rats were switched to 4.0% salt chow at day 35 after surgery, and this group is referred to as sham L-H (n = 7). Finally, to determine the effect of reduced renal mass, some rats were subjected to right unilateral nephrectomy and sham surgery. This

Fig. 1. Effect of acute renal failure (ARF) and Na level in diet on blood pressure. Blood pressure was measured via telemetry at indicated times relative to time of ischemia-reperfusion (I/R) or sham surgeries in rats. Baseline values were obtained from average measurements at 14, 21, and 28 days after surgery just before challenge with high-Na diet. After 35 days of recovery (dashed line), 3 groups were placed on a 4.0% salt diet (H), and 1 group of post-I/R rats were maintained on 0.4% salt diet (L). UNx, rats subjected to right unilateral nephrectomy and sham surgery. This group was also switched to a 4.0% salt diet at day 35. Data are expressed as change in mean arterial pressure from baseline ± SE. *P < 0.05, sham-operated control vs. day 28 values by 2-way ANOVA.
group was also switched to a 4.0% salt diet at day 35 and is referred to as UNx-sham L-H \((n = 3)\). Blood pressures were measured via telemetry as described below. Animals were killed at day 63 after surgery, and kidneys were obtained for histological analysis. Protocol II was designed similarly to protocol I and allowed for conscious creatinine measurements and analysis of plasma renin levels. Two groups of rats were subjected to sham surgery and were maintained on 0.4% salt diet \((\text{L})\); \(n = 11\) for the entirety of the study or switched to 4.0% salt diet on days 35–63 \((\text{L-H})\; n = 12\). In addition, two groups of rats subjected to bilateral I/R were also maintained on 0.4% salt diet \((\text{I/R L-L}; n = 11)\) or switched to a 4.0% salt diet at day 35 \((\text{I/R L-H}; n = 10)\). At 49 days \((\text{i.e.}, 7\text{ wk after ischemia or sham surgery})\), rats were anesthetized with ketamine HCl \((60\text{ mg/kg ip})\), xylazine \((6\text{ mg/kg ip})\), and acepromazine maleate \((0.9\text{ mg/kg ip})\). Polyvinyl catheters were implanted in the femoral artery, tunneled subcutaneously, and exteriorized at the scapula. The catheters were placed inside a spring that was secured onto the rat. After recovery, rats were housed in individual stainless-steel cages. The rats were allowed to recover for an additional 1 wk after surgery. Beginning at day 56 \((\text{i.e.}, 7\text{ wk after ischemia and sham surgery})\), blood pressure was measured in conscious rats via femoral catheter from 0900 to 1200. After blood pressure measurements, plasma samples were obtained in these conscious rats through the arterial catheter for measurement of plasma creatinine, sodium, and renin activity. Urine was collected overnight for measurement of urinary sodium, creatinine, and albumin excretion rates. Protocol III was similar to protocol I but was designed to more thoroughly assess the effects of sodium on renal morphology and the development of proteinuria without the use of blood pressure measurements. Urine was collected for 24 h in metabolic cages at days 1, 28, 35, 42, 56, and 63 after surgery. Animals were killed at day 63 after surgery after the last urine collection, and tissues were processed for histological analysis (see below).

Protocol IV was designed to determine the effects of acclimation of animals to 4.0% salt diet before the initiation of I/R injury on the extent of renal damage and the development of secondary complications. Rats were acclimated on 4.0% salt for a minimum of 7 days and were subjected to I/R surgery and reperfusion. These animals were maintained on 4.0% salt diet with urine collections occurring weekly. At 49 days \((\text{i.e.}, 7\text{ wk post ischemia})\), telemetric blood pressure transducers were implanted into the right femoral artery as in protocol I for acquisition of blood pressure between days 56 and 63. Postschismic animals in this group did not tolerate this procedure well (see RESULTS).

**Measurement of renal function.** For measurement of serum creatinine, tail blood samples \((0.5\text{ ml})\) were collected into heparinized tubes and plasma was obtained by centrifugation. Serum and urine creatinine were determined by using standard assays (Sigma creatinine kit 555A). Urine volume from metabolic cages was determined gravimetrically. Urine was collected for 24 h in metabolic cages at indicated times. Rats were maintained for following ARF. Urine collections \((24)\) were obtained from rats in metabolic cages at indicated times. Rats were maintained for initial 5 wk on 0.4% salt \((\text{L})\) and were changed to 4.0% salt \((\text{H})\) or remained on 0.4% for 4 wk, as described in Fig. 1. Albumin excretion was expressed as milligrams output/day \pm SE. *\(P < 0.05\) I/R L-H vs. I/R L-L and sham L-H by 2-way ANOVA.

**Table 2. Effect of sodium and I/R on recovery from renal I/R injury**

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean Arterial Pressure, mmHg</th>
<th>Heart Rate, bpm</th>
<th>PRA, ANG I (\mu\text{g}\cdot\text{ml}^{-1} \cdot \text{h}^{-1})</th>
<th>Creatinine Clearance ((\text{ml} \cdot \text{min}^{-1} \cdot \text{100 g body wt}^{-1}))</th>
<th>Na Excretion ((\mu\text{Eq} \cdot \text{min}^{-1} \cdot \text{100 g body wt}^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham L-L</td>
<td>124 ± 2</td>
<td>369 ± 8</td>
<td>3.25 ± 0.92</td>
<td>0.35 ± 0.08</td>
<td>0.08 ± 0.02</td>
</tr>
<tr>
<td>Sham L-H</td>
<td>125 ± 1</td>
<td>360 ± 7</td>
<td>0.89 ± 0.29</td>
<td>0.44 ± 0.60</td>
<td>1.99 ± 0.36</td>
</tr>
<tr>
<td>ARF L-L</td>
<td>119 ± 2</td>
<td>380 ± 8</td>
<td>2.72 ± 0.70</td>
<td>0.25 ± 0.06</td>
<td>0.06 ± 0.02</td>
</tr>
<tr>
<td>ARF L-H</td>
<td>142 ± 8*</td>
<td>387 ± 9</td>
<td>0.88 ± 0.28</td>
<td>0.40 ± 0.06</td>
<td>1.71 ± 0.24</td>
</tr>
</tbody>
</table>

Data are means ± SE. Rats treated with sham surgery or I/R (acute renal failure) recovered for the first 35 days on standard 0.4% salt diet \((\text{L})\) and day 35–63 on 0.4% \((\text{L})\) or 4.0% \((\text{H})\) salt diet. PRA, plasma renin activity. *\(P < 0.05\) vs. sham-operated control by 2-way ANOVA.

**Fig. 2. Rapid development of proteinuria on high-Na diet following ARF.** Urine collections \((24)\) were obtained from rats in metabolic cages at indicated times. Rats were maintained for initial 5 wk on 0.4% salt \((\text{L})\) and were changed to 4.0% salt \((\text{H})\) or remained on 0.4% for 4 wk, as described in Fig. 1. Albumin excretion is expressed as milligrams output/day ± SE. *\(P < 0.05\) I/R L-H vs. I/R L-L and sham L-H by 2-way ANOVA.
Measurement of blood pressure. For protocols I and IV, blood pressure was measured telemetrically from 0900 to 1200 by using DSI Dataquest ART acquisition software. Blood pressure in rats was measured weekly during recovery on low-sodium diet through day 35. After the switch to high-sodium diet on day 35, blood pressure was measured twice weekly until day 63 after ischemia. Mean arterial blood pressures were established for each animal at each collection time by averaging blood pressure values over the 3-h collection period. For protocol I. Baseline blood pressures for each animal were established by averaging blood pressure values obtained at days 14, 21, and 28, which correspond to the three blood pressure measurements just before changing dietary sodium level. Blood pressure is expressed as a change in baseline measurements to account for differences in baseline blood pressure between rats.

For protocol II, blood pressure was measured through the exteriorized catheters and was measured between 0900 and 1200 on days 56–60. Measurements were made online by using a solid-state pressure transducer (Argon Medical Technologies, Athens, TX). Signals were amplified and acquired online by custom-designed data-acquisition software (Department of Physiology, Medical College of Wisconsin); pulsatile blood pressure signals were reduced to periodic (1-min) averages of mean arterial pressure. Data are expressed as mean arterial pressure averaged over the course of the 3-h time period (28).

Assessment of interstitial cellularity. At 63 days after ischemia, rats were anesthetized with ketamine HCl (60 mg/kg), xylazine (6 mg/kg), and acepromazine maleate (0.9 mg/kg). The kidneys were quickly removed and cut longitudinally. Half of each kidney was immersed in 10% formalin and was processed for histological analysis. For morphometric measurements, tissues were combined from protocols I and III. Determination of the degree of interstitial cellularity was carried out on photomicrographs of hematoxylin and eosi-n-stained kidney sections as previously described (41). A minimum of five representative micrographs were taken from each zone of the kidney of each animal. Computer-aided image analysis (MetaMorph) was used to apply an arbitrary array of points over the photomicrographs, and points were counted that lay over structures that corresponded to acellular, tubular lumen, glomerular, tubular epithelium, or interstitial cell types. Data are presented as the average number of points overlaying each structure.

Immunohistochemistry. Localization of ED-1 was performed on 3-μm formalin-fixed paraffin sections. Sections were stained by using a robotic Dako autostainer (S3400). Following deparaffinization and rehydration, tissue was prepared as follows: 1) antigen retrieval was performed by incubating in proteinase K; 2) endogenous peroxidase activity was blocked by incubation in 3% H2O2; 3) endogenous biotin was blocked with sequential incubations with avidin and biotin (avidin-biotin blocking kit; Zymed); and 4) nonspecific sites were blocked by incubation in 0.01 M PBS containing 0.3% Triton X-100, 10% goat serum, and 0.3% BSA. ED-1 antibody was obtained from Serotec (MCA341R; 1 μg/ml) and localized with a Vectastain ABC kit (Vector Laboratory) using diaminobenzidine tetrahydrochloride as a substrate.

Localization of fibroblasts and myofibroblasts was carried out by staining with antibodies to S100A4 and smooth muscle actin. S100A4 staining was carried out by using a primary antibody obtained from Dako as described previously (9) and by using AEC as a substrate; colocalization with α-smooth muscle actin was performed by using an antibody from Zymed as described previously (7) and by using alkaline phosphatase/NTB as a method for detection.

Assessment of glomerular damage. Sections of fixed, paraffin-embedded kidneys were stained with Masson’s trichrome and were visualized by using a Nikon Eclipse E400 microscope. Glomeruli (30–40 per rat) were scored by using a semiquantitative index developed by Raji et al. (37) in which a glomerular score of 0 is considered normal and in which a score of 4 is considered most severely damaged, indicated by occlusion of capillaries with matrix.

Fig. 3. Effect of I/R and high-Na diet on renal morphology. Shown are sections through renal outer medulla obtained from rats following sham surgery (A), unilateral nephrectomy (B), or I/R of animals maintained on 0.4% salt diet for 35 days and switched to elevated, 4.0% salt diet for an additional 4 wk (C, referred to as L-H). D shows kidneys from animals maintained on 0.4% salt diet for the duration of the 9-wk protocol (referred to as L-L). Note substantial interstitial cells present in postischemic kidneys (arrows) and extent of tubular dilation. E and F: morphometric analysis of representative sections from cortex and outer stripe of medulla of sham-operated, UNx, and post-I/R animals maintained on high-Na diet from wk 5 to 9. Data were collected by counting the number of points of any given structure type over an arbitrary array of points overlaying the image. E: data corresponding to interstitial (Int), tubular lumen (TL), acellular (Ace), and glomerular compartments. F: data corresponding to tubular epithelial area. Data expressed are mean number of points that lie over each compartment from a minimum of 5 micrographs from both cortex and outer medulla of each animal. Note significant increase in tubulointerstitial cell volume and substantial increase in luminal volume (E) and relative reduction in tubular compartment in animals placed on 4.0% salt (HS). Data are means ± SE. *P < 0.05 by Student’s t-test in post-I/R group vs. sham-operated control.
RESULTS

We addressed the effects of high-sodium diet on renal structure and function following recovery from I/R. Rats on 0.4% salt chow were subjected to bilateral I/R injury for 45 min and were allowed to recover for 35 days before animals were switched to a 4.0% salt chow or maintained on the 0.4% salt diet. This time point was chosen because tubular morphology is largely normal, but there is a permanent loss of peritubular capillaries and a modest increase in interstitial cellularity (8). I/R injury resulted in a transient decrease in renal function, indicated by serum creatinine values of 3.5 ± 0.22 mg/dl (Table 1) at 24 h after surgery. Recovery was indicated by a trend toward returning to sham-operated values by day 7. There was no difference in serum creatinine levels at 28 and 63 days after surgery in all groups of rats.

Blood pressure was measured before I/R injury and up to 63 days following recovery. Blood pressure, measured telemetri-
cally in protocol I, was transiently elevated in all groups immediately following surgery but resolved within 7–14 days, as shown in Fig. 1. Blood pressure was not directly affected by I/R injury itself and was similar to presurgery values between 14 and 35 days after injury, consistent with previous results from our laboratory (6, 8) demonstrating that I/R has little or no effect on mean arterial pressure in the absence of added stimulation.

In contrast, when animals were challenged with 4.0% salt diet, blood pressure was significantly increased between days 35 and 63 in post-I/R rats to 19 ± 2 mmHg above baseline. This increase in blood pressure was not observed in post-I/R rats that were maintained on the 0.4% salt diet for the entire study, nor was it increased in sham-operated controls or UNx shams challenged with high sodium.

We confirmed the effect of 4.0% salt and I/R injury on mean arterial pressure in second series of studies (protocol II). In these studies, blood pressure of postischemic rats switched to 4.0% salt diet measured between days 56 and 60 was 142 ± 7 mmHg and was significantly greater than values measured in sham-operated controls placed on 4.0% salt diet (125 ± 1 mmHg). This value was not different from values obtained from either sham-operated or postischemic animals maintained on 0.4% salt diets (Table 2).

In addition, we examined the effects of I/R injury and dietary salt on plasma renin activity. Plasma renin activity of sham-operated control rats maintained on a 0.4% salt diet was 3.25 ± 0.92 ng ANG I·ml⁻¹·h⁻¹; these values were not different from values measured in postischemic rats maintained on 0.4% salt diet (Table 2). Plasma renin activity was significantly decreased in both sham and postischemic animals placed on a 4.0% salt diet, and it was not different between sham-operated and ischemic animals. Therefore, I/R injury does not interfere with the suppression of plasma renin activity induced by increased sodium intake. In addition, there was no difference in conscious creatinine clearance between any groups (Table 2).

Animals from protocols I and III were used to obtain frequent urine samples to determine the onset of albuminuria following I/R injury. In agreement with our previous studies, albumin excretion was modest in postischemic animals maintained on a 0.4% salt chow for the entire 63 days of recovery (6, 8). In contrast, when postischemic rats were switched to a 4.0% salt diet, albuminuria developed rapidly. This was not observed in sham-operated controls on a 4.0% salt diet (Fig. 2). Similar results were obtained when albumin excretion was determined in protocol II at day 56 after ischemia (28 mg/day for ARF L-H vs. 3 mg/day for ARF L-L; P < 0.05 by Student’s t-test).

Effects on renal morphology. Renal morphology was also severely affected by the combination of 4.0% salt diet and I/R injury. Kidney wet weight was significantly higher after ischemic injury than in sham-operated control animals (1.74 ± 0.09 vs. 1.67 ± 0.02 g); this hypertrophy was enhanced in postischemic animals switched to a 4.0% salt diet (2.04 ± 0.13 g; Table 1). Histological analysis of postischemic rats switched to a 4.0% salt diet for 4 wk demonstrated evidence of profound secondary renal damage characterized by the increased deposition of interstitial cells and dilatation of the tubular lumen (Fig. 3). Morphometric analysis of kidney from postischemic rats revealed that the most dramatic alteration in post-I/R animals exposed to elevated sodium was the increase in the interstitial compartment (Fig. 3E). There was no apparent increase in the glomerular compartment when analyzed in this

![Image](http://ajprenal.physiology.org/)

**Fig. 4.** Identification of multiple interstitial cell types in kidney following I/R and high-Na diet. Shown are sections through renal cortex (A and B) or outer medulla (C and D) of kidneys at 63 days after ischemia that were maintained on 0.4% salt diet for duration of recovery period (A and C) or changed to 4.0% salt diet from days 35 to 63 (B and D). Immunohistochemical localization of ED-1 (A and B), with an obvious increase in ED-1-positive cells after I/R when animals are switched to high Na. Similarly, S100A4, a marker of fibroblasts, is shown in C and D (red staining) with a relative increase in post-I/R rats switched to higher Na. Interstitial α-smooth muscle actin staining (blue, arrow) is moderate in post-I/R rats on 0.4% (C) and is more prominent after transfer to 4.0% salt diet (D). Representative of 4–5 samples/group.
fashion (Fig. 3E), whereas the tubular epithelial component was reduced in the presence of the expanded interstitial and luminal area (Fig. 3F). In contrast, the kidneys of postischemic animals maintained on 0.4% salt showed only moderate damage and little evidence of interstitial expansion. There was a significant increase in the number of interstitial cells after ischemia, particularly in the outer medulla, compared with sham-operated controls, but this expansion of the interstitial compartment was strongly enhanced by 4.0% salt diet (Fig. 3).

To characterize the nature of interstitial cells, we carried out immunohistochemical analysis of markers for macrophages and fibroblasts. Immunohistochemical staining for ED-1 was not prominent in kidneys of sham-operated control rats at 63 days after surgery on 0.4% or 4.0% NaCl diets. I/R injury resulted in the presence of several detectable ED-1-positive cells in the interstitium of postischemic animals. ED-1-positive cells were profoundly increased when animals were switched to a high-sodium diet (Fig. 4, A vs. B). Similarly, S100A4, a marker of fibroblasts, was present in postischemic kidneys of animals on 0.4% salt but was markedly elevated in animals switched to 4.0% salt (red staining, Fig. 4, C vs. D). Myofibroblasts identified by α-smooth muscle actin (blue staining) were typically observed in vascular bundles and only mildly in the interstitial space in postischemic animals maintained on low-sodium diet, whereas interstitial smooth muscle actin staining was more prominent when postischemic animals were switched to the 4.0% salt diet (Fig. 4, C vs. D).

To determine whether or not the albuminuria correlated to damage in glomerular structure, glomeruli of sham-operated or postischemic animals were analyzed according to commonly used criteria. Recovery from bilateral I/R injury had no detectable effect on glomerular structure when animals were allowed to recover on 0.4% salt diet for the entire 63 days relative to sham-operated controls. However, the combination of I/R injury and 4.0% salt from days 35 to 63 resulted in moderate alterations in glomerular morphology, indicated by a tendency to increase matrix deposition and by reduced patent capillary loops (Fig. 5).

**Effect of acclimation to 4.0% salt diet on initiation and progression of injury.** It is possible that 0.4% salt diet may exacerbate elements of the initial renal injury and therefore influence the subsequent development of chronic kidney disease. To evaluate this possibility, rats were acclimated to 4.0% salt diet 1 wk before I/R or sham surgery and were maintained on this diet for the duration of the 9-wk recovery period (protocol IV). Initial injury levels measured at 24 h were similar to those obtained in animals initially acclimated with 0.4% salt diets (Fig. 6). Following implantation at wk 7 with blood pressure recording instrumentation, all rats recovered for at least 12 h, but 6 of 9 postischemic animals died before 24 h.
DISCUSSION

The long-term effects of ARF are not well explored; however, recent studies suggest that renal structure and function do not faithfully recover to their pristine preinjury state. Among the chronic alterations that have been reported, ARF results in a permanent alteration in urinary concentrating defect and susceptibility to proteinuria and interstitial fibrosis. In addition, there is a loss of peritubular capillaries and the deposition of interstitial cells such as fibroblasts and/or macrophages (5, 26, 41). Therefore, it is becoming clear that acute renal injury is not completely reversible and that there may develop a predisposition to chronic progressive kidney disease.

Similarly, studies using other models have shown that transient or subtle injuries may predispose the kidney to future complications. Johnson and colleagues (21) have shown that subtle renal injury induced by ANG II predisposes the development of sodium-dependent hypertension. In this model, ANG II (435 ng·kg⁻¹·min⁻¹) was infused with subcutaneous osmotic minipumps for 2 wk, resulting in decreased peritubular capillaries and tubulointerstitial damage (1, 24). Similarly, treating rats with cyclosporine for 4 wk (8 mg·kg⁻¹·day⁻¹) resulted in tubulointerstitial damage. In both studies, these treatments resulted in the development of sodium-dependent hypertension, and the tubulointerstitial damage correlated with the increase in blood pressure. The alteration in blood pressure may be associated with a decrease in medullary blood flow as suggested by Cowley and colleagues (16). In view of our previous studies on the effects of ARF on capillary density, these observations led us to hypothesize that post-ARF animals would develop sodium-dependent hypertension.

In this study, we examined the influence of sodium on long-term postischemic function and morphology in rats. The protocols used allowed animals to recover on 0.4% salt diet, enabling recovery without overt evidence of renal disease. However, because animals on 0.4% salt have higher plasma renin activity than animals on 4.0% salt, additional studies were carried out to demonstrate that the reported effects were not due to sensitization by ANG II at the time of renal injury. The data presented in this report are consistent with our previous observation (6, 8) that ARF, in and of itself, is not sufficient to increase blood pressure. Nevertheless, our data clearly demonstrate that, when challenged with a high-sodium diet, postischemic animals do develop increased blood pressure over time. The mechanism by which blood pressure is increased in postischemic animals following I/R is not yet known; however, these data are consistent with the suggestion that I/R injury compromises the normal sodium-handling capabilities of the kidney even after the apparent recovery from the initiating insult.

With regard to possible causes of sodium-dependent hypertension following recovery from I/R injury, it is possible that incomplete tubular repair may lead to a dropout of functioning nephrons, resulting in hyperfiltration and susceptibility to sodium-dependent hypertension (13, 35). Although reduction of functioning nephrons by unilateral nephrectomy did not result in an elevation in blood pressure in animals changed to a high-sodium diet, the potential that hyperfiltration is present in the urinary albumin excretion of animals that died following instrumentation surgery was dramatically elevated relative to sham-operated controls, and of the three postischemic animals that survived this surgery, there was no difference in the urinary albuminuria measured at that time. The level of albuminuria measured at day 49 died within 1 day of surgery; these postischemic animals are referred to as “I/R nonsurvival,” whereas those that survived are referred to as “I/R survival.” The urinary albumin excretion of animals that died following instrumentation surgery was significantly different from I/R survival group after day 14.

![Graph showing the effect of acclimation on urinary albumin excretion](http://ajprenal.physiology.org/graphics/graph.png)

**Fig. 6.** Effect of acclimation to 4.0% salt diet on early and chronic manifestations of I/R injury. Rats were acclimated to 4.0% salt diet for 1 wk before I/R or sham surgery and were allowed to recover for 7 wk before a second surgery to implant telemetric blood pressure transducers. Of postischemic animals, 6 of 9 died within 1 day of surgery; these postischemic animals are referred to as “I/R nonsurvival,” whereas those that survived are referred to as “I/R survival.” Top: serum creatinine values. *P < 0.05 vs. sham-operated control values. Albuminuria values were significantly different from I/R survival group after day 14.

The urinary albumin excretion of animals that died following instrumentation surgery was dramatically elevated relative to sham-operated controls, and of the three postischemic animals that survived this surgery, there was no difference in the urinary albumin between sham-operated controls and the postischemic animals. The level of albuminuria measured at day 49 in those animals that died was ~100 mg/day, which is substantially higher than levels measured in rats changed to 4.0% salt at 5 wk (compare with Fig. 2). These observations suggest that acclimation to 4.0% salt before initiation of I/R does not interfere with early or progressive manifestations of renal I/R. These data also highlight the heterogeneity of the secondary progression of injury following I/R. Indeed, initiating serum creatinine values measured 24 h following I/R between rats that survived and those that perished at the time of implantation surgery were not different (Fig. 6, top), although both albuminuria and serum creatinine values at later time points were different in these groups. The reason for this heterogeneity is unclear.

postischemic animals following bilateral I/R has not been addressed, and its potential role in sodium sensitivity remains an issue to be clarified. Importantly, we must point out that although creatinine clearance values were not different in postischemic vs. sham-operated animals, we did not carry out formalized analysis of glomerular filtration rate (GFR) at recovery by inulin clearance in the current series of studies. A more formal evaluation of GFR and renal hemodynamics in the recovery state will be required to gain a clear understanding of the mechanism of altered Na sensitivity in this model.

Alternatively, Johnson and Schereiner (22) suggested that alterations in the tubulointerstitium (including peritubular capillary loss, fibrosis, and hypoxia, features that are all present in this model) lead to sodium retention. These features are consistent with our previous characterization of I/R-induced injury. We have suggested that the loss of peritubular capillaries in response to I/R compromises the oxygen delivery to renal parenchyma and may contribute to secondary damage in the kidney (7). Chronic renal hypoxia is been associated with increased glomerular hypertrophy, arteriolar thickening, tubulointerstitial inflammation, and fibrosis (28). It has been suggested that alterations in the renal interstitium may affect sodium by altering normal sodium-handling mechanisms such as the tubuloglomerular feedback mechanism or by affecting interstitial pressures that regulate sodium diffusion across tubular epithelia (22).

Such effects may be mediated by alterations in intrinsic renal vasoactive factors. It is also possible that ARF contributes to an imbalance of vasoactive factors, resulting in sodium-sensitive hypertension. For example, we have previously found (9) that I/R injury results in a substantial reduction in the renal expression of kallikrein. Kallikrein stimulates the production of bradykinin- and NO-dependent vasodilatory factors. Reduction in kallikrein levels has been implicated in sodium-sensitive hypertension, blood pressure regulation, and sodium excretion (25). Infusion of subdepressor doses of kallikrein attenuates hypertension in Dahl salt-sensitive rats. In humans, administered kallikrein normalizes blood pressure in hypertensive patients (2).

Data from the current study suggest that the alterations in sodium sensitivity are not attributable to inappropriate regulation of renin, and, in a recent study (6), we also found no evidence that ARF chronically affects circulating ANG II concentration. Nevertheless, ANG II responsiveness may be increased in postischemic rats (6, 8). In the current study, we did not evaluate intrarenal ANG II, and we cannot rule out that intrarenal ANG II may participate in the sodium sensitivity described in this study.

In addition to the hypertension, sodium dramatically hastens the secondary renal damage characterized by tubulointerstitial damage and albuminuria after ischemia. The hypertrophy in postischemic rat is consistent with our previous studies. However, the addition of sodium to animals recovered from I/R had a striking effect on the degree of hypertrophy, deposition of interstitial cells, and renal scarring seen in post I/R kidneys. How sodium intake exacerbates secondary renal disease following I/R injury is not yet clear. However, it is possible that increased blood pressure in response to high-sodium diet exacerbates the injury process or conversely that it fuels proliferation of fibroblastic and inflammatory cell types that deposited in the interstitium following the initial recovery response.

In the current study, transfer of animals to a 4.0% salt diet exacerbated fibrosis that was characterized by an increase in the number of in macrophages and fibroblasts. How dietary sodium would affect these pathways directly is unclear, although a number of profibrotic factors such as TGF-β may be regulated by sodium in the diet (47). We suggest that alterations of such factors in the setting of recovery from I/R results in a greater degree of tissue damage than would be manifested in the setting of elevated sodium by itself. In addition, other models of renal injury are characterized by infiltration of T cells and monocytes in the renal interstitium. The T cells produce ANG II and reactive oxygen species (36, 38, 39). By blocking the inflammatory response with mycophenolate mofetil, the leukocyte accumulation was blocked and animals did not develop sodium-sensitive hypertension.

In summary, data from this study support the view that elements of renal function are not reversible in the setting of ARF induced by I/R injury. Despite the initial recovery response of GFR and tubular regeneration, postischemic animals have a defect in their normal ability to handle sodium. One element of this is the predisposition to develop hypertension and hasten the progression of secondary renal disease when sodium intake is elevated. These results may be due to alterations in the vasculature of the kidney, as described previously (7, 8). However, the specific role of capillary dropout, interstitial cell deposition, or other elements of altered renal structure or function that may be affected chronically by I/R on sodium sensitivity have not yet been evaluated.

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REFERENCES


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