Hypokalemia induces renal injury and alterations in vasoactive mediators that favor salt sensitivity

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Suga, Shin-Ichi, M. Ian Phillips, Patricio E. Ray, James A. Raleigh, Carlos P. Vio, Yoon-Goo Kim, Marilda Mazzali, Katherine L. Gordon, Jeremy Hughes, and Richard J. Johnson. Hypokalemia induces renal injury and alterations in vasoactive mediators that favor salt sensitivity. Am J Physiol Renal Physiol 281: F620–F629, 2001.—We investigated the hypothesis that hypokalemia might induce renal injury via a mechanism that involves subtle renal injury and alterations in local vasoactive mediators that would favor sodium retention. To test this hypothesis, we conducted studies in rats with diet-induced K⁺ deficiency. We also determined whether rats with hypokalemic nephropathy show salt sensitivity. Twelve weeks of hypokalemia resulted in a decrease in creatinine clearance, tubulointerstitial injury with macrophage infiltration, interstitial collagen type III deposition, and an increase in osteopontin expression (a tubular marker of injury). The renal injury was greatest in the outer medulla with radiation into the cortex, suggestive of an ischemic etiology. Consistent with this hypothesis, we found an increased uptake of a hypoxia marker, pimonidazole, in the cortex. The intrarenal injury was associated with increased cortical angiotensin-converting enzyme (ACE) expression and continued cortical angiotensin II generation despite systemic suppression of the renin-angiotensin system, an increase in renal endothelin-1, a decrease in renal kallikrein, and a decrease in urinary nitrate/nitrates and prostaglandin E₂ excretion. At 12 wk, hypokalemic rats were placed on a normal-K⁺ diet with either high (4%) or low (0.01%)-NaCl content. Despite correction of hypokalemia and normalization of renal function, previously hypokalemic rats showed an elevated blood pressure in response to a high-salt diet compared with normokalemic controls. Hypokalemia is associated with alterations in vasoactive mediators that favor intrarenal vasoconstriction and an ischemic pattern of renal injury. These alterations may predispose the animals to salt-sensitive hypertension that manifests despite normalization of the serum K⁺.

angiotensin II; endothelin; kallikrein; hypertension

WE HAVE BEEN INTERESTED IN the role of subtle renal injury in the pathogenesis of some forms of salt-sensitive hypertension. Several years ago, we proposed that microvascular and tubulointerstitial injury might predispose an animal to a salt-sensitive state, in part mediated by intrarenal ischemia and local alterations in vasoactive mediators that favor Na⁺ retention (17). In support of this hypothesis, we have found that transient administration of angiotensin II (24), catecholamines (16), cyclosporin (18), or nitro-L-arginine methyl ester (L-NAME) (31) all result in renal microvascular and tubulointerstitial injury that then predisposes the animal to develop an increased blood pressure (BP) in response to a high-Na⁺ diet.

Low-K⁺ diets are also associated with salt-sensitive hypertension (reviewed in Ref. 19). Several studies have suggested that acute K⁺ restriction can result in Na⁺ retention and an increase in BP (10, 20, 21). The pathogenesis has generally been ascribed to acute effects of K⁺ restriction to increase plasma renin (26), stimulate the sympathetic nervous system (22), and alter nitric oxide metabolism (47).

An additional mechanism by which a low-K⁺ diet may induce salt sensitivity could be via the ability to induce subtle renal injury. Hypokalemia is known to induce renal structural changes, consisting of renal hypertrophy and tubulointerstitial injury (29, 33). Therefore, on the basis of our hypothesis that tubulointerstitial injury may create a predisposition to salt sensitivity by causing local alteration in vasoactive mediators that favor intrarenal vasoconstriction (17), we examined whether hypokalemic renal injury could alter local vasodilators and vasoconstrictors in the kidney. We also determined whether the development of the renal injury would result in salt sensitivity that would manifest even after the K⁺ deficit was corrected.

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METHODS

Experimental Protocol

Male Sprague-Dawley rats (230–270 g, n = 18, Simonsen Labs, Gilroy, CA) were fed a K\(^+\)-deficient diet (0.01% K\(^+\), 0.26% NaCl, Zeigler Bros., Gardners, PA) for 12 wk. Control rats (n = 18) were placed on a diet with normal-K\(^+\) content (0.36% K\(^+\), 0.26% NaCl). This resulted in marked hypokalemia within 2 wk, and K\(^+\) levels averaged 2.0 meq/l at the end of the 12 wk vs. 4.2 meq/l in the controls (Table 1). At the end of week 12, six rats from each group were killed. Kidneys were excised, and the left kidney was used for histological studies. The right renal cortex and medulla were surgically separated, immediately frozen in liquid nitrogen, and stored at −70°C. To determine whether the chronic hypokalemia predisposed the animals to salt sensitivity, the remaining rats in each group were placed on either a high-Na\(^+\), normal-K\(^+\) (4% NaCl, 0.36% K\(^+\)), or low-Na\(^+\), normal-K\(^+\) diet (0.01% NaCl, 0.36% K\(^+\)) for an additional 5 wk (n = 4–6). The diets during the period contained normal K\(^+\) content to determine whether the alteration in sodium sensitivity could be shown after correction of the serum K\(^+\).

At day 2 and week 4 after the switch to the high- or low-salt diet, animals were housed separately in metabolic cages and urine was collected for 16 h. Serum was collected at weeks 2 and 4 on the subsequent high- or low-salt diet.

A separate set of male rats (230–270 g body wt) was studied at week 2 after placement on a K\(^+\)-deficient (n = 3) or a normal-K\(^+\) diet (n = 3) to determine whether there was increased renal hypoxia present, as measured by a pimonidazole assay. Nitroimidazole compounds, which are known to bind to cellular macromolecules at low-oxygen concentration, have been used to detect hypoxia in a variety of tissues (3, 27). In the present study, pimonidazole hydrochloride, a 2-nitroimidazole, was used to detect hypoxia in the kidney as previously reported (50). To investigate the existence of renal hypoxia by the K\(^+\)-deficient diet, pimonidazole hydrochloride (75 mg/kg) was injected from the tail vein after 2 wk of the K\(^+\)-deficient diet. Two hours after administration of pimonidazole, kidneys were excised and fixed for subsequent histological studies. Pimonidazole binding was detected by immunohistochemistry with a specific antibody (50).

Renal Histological Studies

Methyl Carnoy-fixed tissue was processed and paraffin embedded, and 4-μm sections were stained with the periodic acid-Schiff reagent (PAS). An indirect immunoperoxidase method was used to identify the following antigens (24, 39): osteopontin with OP 199, a goat anti-mouse osteopontin antibody (gift of C. Giachelli, Univ. of Washington, Seattle); macrophages with ED-1, a monoclonal IgG1 to rat macrophages (Harlan Bioproducts, Indianapolis, IN); type III collagen with a goat anti-human type III collagen antibody (Southern Biotechnology Associates, Birmingham, AL); renin with F37.2D12, an antibody to human renin (gift of M. Laprade, Sanofi Recherche, Montpellier, France); angiotensin-converting enzyme (ACE) with a rabbit anti-ACE antibody directed against the intracellular domain (45); kallikrein with a rabbit anti-kallikrein antibody (37); and pimonidazole with a mouse anti-pimonidazole monoclonal antibody (50).

Tubulointerstitial injury was graded (0–5+) in a blinded manner on the basis of the presence of tubular cellularity, basement membrane thickening, dilation, atrophy, sloughing, or interstitial widening as follows (39): 0, no changes present; grade 1, <10% tubulointerstitial changes present; grade 2, 10–25% tubulointerstitial involvement; grade 3, 25–50%; grade 4, 50–75%; and grade 5, 75–100%. For each biopsy, the entire cortical and outer medullary regions were evaluated (16–30 fields of 1 mm²), and a mean score per biopsy was calculated. A second measurement of tubulointerstitial injury was based on observations that osteopontin expression by injured tubules is a sensitive marker of tubulointerstitial injury (45). Utilizing computer-assisted image-analysis software (Optimas, v. 6.2, Media Cybernetics, Silver Spring, MD) and digitized images, the percent area occupied by osteopontin-positive tubules (including the entire cortical and outer medullary regions, exclusive of glomeruli) was measured per field (4 mm²) at ×50, and the mean percent area was calculated for each biopsy. The number of macrophages (ED-1-positive cells/mm²) in the cortex and medulla was also quantified. In addition, the percentage of glomeruli with juxtaglomerular renin immunostaining was determined in each biopsy. Previous studies from our group have shown that this measurement semiquantitatively correlates with tissue renin content (8).

Glomerular injury was graded for the percentage of sclerotic glomeruli showing focal or global obliteration of capillary loops with syncytium formation by PAS staining. For each biopsy, all glomeruli (n > 110) were examined.

Determinations of Plasma and Renal Angiotensin II Concentrations

Blood was drawn from the lower portion of the inferior vena cava at the time of death. Blood samples were transferred to chilled tubes containing EDTA and 1,10-phenanthroline, then immediately placed on ice and promptly centri-
fuged for 10 min at 4°C. An aliquot of plasma was frozen at −70°C. Extraction of angiotensin II from plasma was performed using reverse-phase phenylsilica extraction cartridges as previously described (48).

Renal cortex and medulla were boiled in 10 vol of 1 M acetic acid for 7 min and homogenized with a polytron homogenizer (Omni International, Waterbury, CT). The homogenate was centrifuged at 10,000 g for 30 min at 4°C. The supernatants were further purified on Sep-Pak C18 cartridges (Waters, Milford, MA). Measurement of tissue and plasma angiotensin II concentration was performed using RIA for angiotensin II as reported earlier (30).

**Determination of Renal Endothelin Concentration**

Extraction of endothelin (ET) from renal cortex and medulla utilized the same method as that for angiotensin II (described above). Measurement of tissue ET concentration was performed using RIA for ET-1 (Phoenix Pharmaceuticals, Mountain View, CA). The cross-reactivity to ET-2, ET-3, Big ET-1, Big ET-2, and Big ET-3 was 3.5, 28, 50, 70, and 0.2%, on a molar basis, respectively, according to the manufacturer’s instructions.

**Urinary Nitrite/Nitrate Assay**

Urine was collected in metabolic cages, and the concentrations of nitrate and nitrite, which are stable end products of nitric oxide (NO), were measured as previously reported (42). In brief, urine samples were first incubated with nitrate reductase (Sigma, St. Louis, MO) in the presence of NADPH for 10 min at 37°C. After the incubation, the total nitrite content was measured using the Griess reagent (Clontech, Palo Alto, CA).

**Enzyme Immunoassay for PGE2**

The urinary concentration of PGE2 was measured using a commercial enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI). The cross-reactivity to PGF2α, PGE1, and 6-keto PGF1α was 43, 19, and 1%, on a molar basis, respectively.

**Additional Measurements**

Serum and urinary creatinine and Na+ and K+ concentrations were measured by a Cobas autoanalyzer.

**Statistical Methods**

Values are expressed as means ± SE. A comparison among groups was made by unpaired Student’s t-test or ANOVA with Fisher’s protected least significant difference test for multiple comparisons.

**RESULTS**

**General Features**

Hypokalemia was induced in rats within 2 wk by the K+-deficient diet and was maintained until the end of the study period (Table 1). Although hypokalemic rats gained weight less rapidly than controls, kidney weights were greater than those of controls, as previously reported (29). Renal function, as assessed by creatinine clearance per kidney weight, was significantly decreased in the hypokalemic rats at week 12 (Table 1).

**Renal Histology**

Consistent with prior reports (29, 33), hypokalemic rats had tubulointerstitial injury with preservation of glomeruli (Table 2). In the cortex, there was focal atrophy or dilatation of tubules with mild interstitial accumulation of mononuclear cells and interstitial expansion (Fig. 1A). The most prominent injury was observed in the medulla, where there was diffuse swelling and hyperplasia of collecting duct epithelial cells with interstitial widening and mononuclear cell infiltration (Fig. 1B). An accumulation of eosinophilic granules in collecting duct epithelial cells was observed in the inner medulla.

Immunohistological studies documented an increased expression of osteopontin by both proximal and distal tubules in hypokalemic rats (Fig. 1C and Table 2). Osteopontin expression was most prominent in distal tubules and was also observed in collecting ducts of the outer medulla (Fig. 1D). In the cortex, the osteopontin expression was distributed in a striped pattern radiating into the cortex from the outer medulla. The number of macrophages (ED-1-positive cells) was markedly increased in the cortex and medulla (Fig. 1, E and F, and Table 2). There was also an increase in type III collagen deposition (Fig. 1, G and H) in both the cortical and medullary interstitium of hypokalemic kidneys.

**Renal Hypoxia is Increased in Hypokalemic Rats**

The striped distribution pattern of tubulointerstitial injury in the cortex, in concert with the known effect of hypokalemia to cause renal vasoconstriction, raised the possibility that intrarenal ischemia and/or hypoxia...
could be involved in hypokalemic renal injury. To test this hypothesis, rats on a K⁺-deficient diet were injected with pimonidazole 2 wk after placement on the diet. Pimonidazole is a nitroimidazole that is taken up by markedly hypoxic cells \( (P_{O_2} < 10 \text{ Torr}) \) \( (3, 27, 50) \). As previously reported \( (50) \), control rats showed pimonidazole uptake in tubules of the outer medulla and medullary rays, which are normally borderline hypoxic \( (9) \). In contrast, in hypokalemic rats the pimonidazole accumulation extended into the cortex \( (Fig. 2B) \).

**Hypokalemic Nephropathy is Associated With an Alteration in Vasoactive Mediators That Favors Vasoconstriction**

**Renin-angiotensin system.** Plasma angiotensin II was suppressed by \( \approx 50\% \) in hypokalemic rats, and this was paralleled by a similar reduction in medullary angiotensin II content \( (Table 3) \). In contrast, cortical angiotensin II levels were not different from controls, and the cortical-to-plasma angiotensin II ratio was actually higher in hypokalemic rats \( (5.0 \pm 0.7 \text{ vs. } 2.70 \pm 0.4, P < 0.05) \), indicative of dissociation between systemic and cortical angiotensin II generation. Although the percentage of glomeruli with juxtaglomerular renin staining was not different between hypokalemic and control animals \( (Table 3) \), there was a substantial increase in cortical ACE immunostaining in hypokalemic animals. ACE was weakly expressed in the brush borders of proximal tubules in control animals but was markedly increased in hypokalemic rats in both proximal tubular brush borders and cells within the interstitial lesions \( (Fig. 3) \).

**ET.** Using a RIA for ET-1, a 2-fold increase in cortical ET-1 and \( >10\)-fold increase in medullary ET-1 were documented in kidneys of hypokalemic animals at week 12 \( (Table 3) \).

**NO.** Urinary nitrite/nitrate excretion was reduced by \( 70\% \) in hypokalemic rats at week 12 \( (Table 3) \).

**Urinary PGE₂.** We also measured urinary excretion of PGE₂, a potent vasodilator produced in the outer medulla \( (35) \). We observed the reduction of urinary
PGE2 (Table 3) at week 12, consistent with previous reports of chronic hypokalemia (4, 6).

Renal kallikrein. In control rats, kallikrein was present in the connecting tubule as previously reported (5). In contrast, hypokalemic rats had significantly reduced kallikrein immunostaining in biopsies obtained at week 12 (Fig. 4).

Rats With Hypokalemic Nephropathy Show Salt Sensitivity Despite Normal Renal Function and Correction of Serum K^+

We next determined whether hypokalemic nephropathy could predispose animals to salt sensitivity. Rats with hypokalemic nephropathy were placed on a normal-K^+ diet to correct the K^+ deficit and were matched to either a low- or high-Na^+ diet. As shown in Table 4, urinary Na^+ excretion 2 days after the diet was switched tended to be lower in hypokalemic rats (groups I and II) compared with normokalemic rats (groups III and IV), although the difference was not statistically significant. Hypokalemic rats gained more body weight than control rats regardless of Na^+ intake, despite almost the same amount of food intake (data not shown). There was a similar degree of weight gain in hypokalemic rats (groups I and II) during the 5 wk, which may reflect catch-up growth after K^+ repletion. During the first week, however, the increase in body weight was greater in hypokalemic rats on a high-NaCl diet (group I) than those on a low-NaCl diet (group II) (Table 4), which suggests that hypokalemic rats were retaining sodium.

Serum K^+ completely normalized within 2 wk of placement of animals on the normal-K^+ diet (Table 4), and, similarly, renal function (creatinine clearances) at 4 wk returned to normal in rats with previously established hypokalemia (Table 4). Despite the normalization of serum K^+ and renal function, rats with underlying hypokalemic nephropathy showed a gradual increase in BP in response to the high-salt diet compared with control rats placed on a high-salt diet (Fig. 5A and Table 5). The normalization of serum K^+ preceded the elevation of BP. Hypokalemic rats switched to a low-NaCl diet (group II) did not show the increase in BP, thereby documenting that the increase in BP was dependent on Na^+ intake. The difference in mean BP between hypokalemic rats on a high-NaCl diet and on a low-

<table>
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<th>Table 3. Hypokalemia-induced alterations in vasoactive mediators</th>
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<tr>
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<th>Control (n = 18)</th>
<th>Hypokalemic (n = 18)</th>
<th>P Value</th>
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<tr>
<td>%Glomeruli with juxtaglomerular renin</td>
<td>33 ± 4</td>
<td>29 ± 5</td>
<td>NS</td>
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<tr>
<td>Plasma ANG II, pg/ml</td>
<td>119 ± 45</td>
<td>58 ± 19</td>
<td>P = 0.061</td>
</tr>
<tr>
<td>Cortical ANG II, pg/g tissue</td>
<td>284 ± 31</td>
<td>267 ± 34</td>
<td>NS</td>
</tr>
<tr>
<td>Medullary ANG II, pg/g tissue</td>
<td>171 ± 12</td>
<td>118 ± 33</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Cortical ANG II-to-plasma ANG II ratio</td>
<td>2.7 ± 0.8</td>
<td>5.0 ± 1.3</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Medullary ANG II-to-plasma ANG II ratio</td>
<td>1.6 ± 0.6</td>
<td>2.1 ± 0.3</td>
<td>NS</td>
</tr>
<tr>
<td>Cortical endothelin-1, pg/g tissue</td>
<td>194 ± 25</td>
<td>386 ± 29</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Medullary endothelin-1, pg/g tissue</td>
<td>383 ± 40</td>
<td>4,010 ± 830</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Urinary nitrate/nitrate, nmol/day</td>
<td>1,480 ± 570</td>
<td>476 ± 356</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Urinary prostaglandin E2, ng/day</td>
<td>44.2 ± 7.3</td>
<td>21.1 ± 5.2</td>
<td>P &lt; 0.05</td>
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</tbody>
</table>

Values are means ± SE. n, no. of rats.
NaCl diet was 20 mmHg at week 4 and 32 mmHg at week 5. In contrast, BP increased slightly in normokalemic rats on a high-NaCl diet (group III) (Fig. 5B and Table 5), and the difference of mean BP between normokalemic rats on a high-NaCl diet and on a low-NaCl diet (group IV) was 5 mmHg at weeks 4 and 5.

**DISCUSSION**

In this study, we investigated whether hypokalemia might lead to alterations in renal structure and function that would favor the development of salt sensitivity. Specifically, we hypothesized that hypokalemia might induce tubulointerstitial injury that would result in alterations in local vasoactive mediators that would favor Na⁺ retention. This hypothesis was based on previous studies that have documented that subtle tubulointerstitial injury induced by transient exposure to angiotensin II (24) or phenylephrine (16) can result in salt sensitivity.

**Hypokalemic Nephropathy: An Ischemic Nephropathy?**

Hypokalemic nephropathy was induced by the placement of rats on a K⁺-deficient diet for 3 mo. These rats developed the characteristic renal hypertrophy with tubular hyperplasia, particularly in outer regions of the renal medulla, in association with tubular injury and early interstitial fibrosis, as reflected by the expression of osteopontin by tubules, an infiltration of macrophages, and type III collagen deposition. The mechanism responsible for the renal injury has been postulated to be due to intrarenal complement activation secondary to amidation of C3 from the increased ammonia generated with hypokalemia (40).

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**Fig. 3.** Increased angiotensin-converting enzyme (ACE) expression in hypokalemic nephropathy. In control rats, ACE is detected in small amounts in the brush border of the S3 segment of the proximal tubule (A). In contrast, ACE expression is increased in the S3 segment of hypokalemic rats, as well as in capillary endothelium and in infiltrating interstitial cells (B; ×100).

**Fig. 4.** Renal kallikrein is reduced in chronic hypokalemia. In normal rats, kallikrein is present in the connecting tubules (A); however, staining was markedly less in the connecting tubules of hypokalemic rats (B; ×100).
rent circulation and high metabolic demands of the kidney have shown basal uptake of pimonidazole in the outer medulla and medullary rays (50), which are known to be borderline hypoxic due to the countercurvatures of intrarenal ischaemia. First, hypokalemia is known to cause renal vasoconstriction (23, 46), and decreased renal blood flow could theoretically impair oxygen delivery especially to the vulnerable tubules in the outer medulla that are normally borderline hypoxic (9). Consistent with this observation, we found that the fibrosis was most severe in the outer medulla. Furthermore, the fibrosis often radiated into the cortex in a “striped” pattern, similar to that observed in other models of vasoconstrictive injury, such as with administration of cyclosporin (36), angiotensin II (24), and catecholamines (16), or with chronic inhibition of nitric oxide synthases (49). Finally, we were able to demonstrate that hypokalemic animals had an increased cortical uptake of pimonidazole (3, 27, 50). The uptake of nitroimidazole compounds, such as pimonidazole, into tissues is followed by their reduction under low PO2. The reduced form may then be detected using a specific antibody (3, 27, 50). Previous studies in the normal rat kidney have shown basal uptake of pimonidazole in the outer medulla and medullary rays (50), which are known to be borderline hypoxic due to the countercurvature circulation and high metabolic demands of the medullary thick ascending limb (9). In hypokalemic rats, the pimonidazole uptake extended from the medullary rays into the cortex, which suggests that the renal injury induced by hypokalemia is associated with intrarenal hypoxia. A cautionary comment, however, is that, although studies suggest that the rate at which reduction in pimonidazole occurs is determined by the oxygen concentration independently of the redox state (2), verification of intrarenal hypoxia must await studies using intrarenal oxygen electrode measurements.

Table 4. General features of rats after the switch to a high-NaCl diet

<table>
<thead>
<tr>
<th>Group</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
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<tr>
<td>Serum K⁺ at week 12, meq/l</td>
<td>1.9 ± 0.1*</td>
<td>1.7 ± 0.1*</td>
<td>4.0 ± 0.1</td>
</tr>
<tr>
<td>Serum K⁺ at week 14 (2 wk on a high-NaCl diet), meq/l</td>
<td>4.6 ± 0.1</td>
<td>4.2 ± 0.2</td>
<td>4.5 ± 0.1</td>
</tr>
<tr>
<td>Body weight at week 12, g</td>
<td>312 ± 10*</td>
<td>304 ± 4*</td>
<td>424 ± 8</td>
</tr>
<tr>
<td>Body weight gain between weeks 12 and 13, g/wk</td>
<td>41 ± 6*‡</td>
<td>25 ± 10</td>
<td>0 ± 1</td>
</tr>
<tr>
<td>Body weight at the end of experiment, g</td>
<td>402 ± 8†</td>
<td>390 ± 8†</td>
<td>452 ± 8</td>
</tr>
<tr>
<td>Urinary Na⁺ excretion at week 12 (2 days on a high-NaCl diet), meq/day</td>
<td>0.6 ± 1.4</td>
<td>0.07 ± 0.03</td>
<td>2.3 ± 0.3</td>
</tr>
<tr>
<td>Creatinine clearance at week 16 (4 wk on a high-NaCl diet), ml/min</td>
<td>0.87 ± 0.11</td>
<td>0.99 ± 0.14</td>
<td>0.94 ± 0.12</td>
</tr>
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</table>

Values are means ± SE. n = 5, 4, 5, and 6 rats in groups I, II, III, and IV, respectively. *P < 0.001 vs. groups III and IV. †P < 0.05 vs. groups III and IV. ‡P < 0.05 vs. group II.

However, several lines of evidence suggested that the renal injury may result, at least in part, from intrarenal ischemia. First, hypokalemia is known to imbalance in local vasoactive mediators favoring vasoconstriction.

Hypokalemic Nephropathy is Associated With an Imbalance in Local Vasoactive Mediators Favoring Vasoconstriction.

We have previously hypothesized that intrarenal ischemia associated with microvascular and tubulointerstitial injury may lead to local alterations in vasoactive mediators that would favor vasoconstriction and Na⁺ retention (17). We therefore examined the effect of chronic hypokalemic nephropathy on various intrarenal vasoactive mediators.

Hypokalemia is known to stimulate renin release, but this effect was no longer observed at 12 wk in our rats (Table 2), presumably because of the counter-regulatory effect of hypokalemia to induce Na⁺ retention and plasma volume expansion (10, 19–21, 26).
Indeed, at week 12, rats with marked hypokalemia had a reduction in plasma and medullary angiotensin II levels. Despite evidence for systemic suppression of the renin-angiotensin axis, a new finding was the evidence for continued angiotensin II generation in the renal cortex of hypokalemic rats. Hypokalemic rats exhibited increased expression of ACE by interstitial cells at sites of tubulointerstitial damage, increased ACE expression in proximal tubular brush borders, and nonsuppressed cortical angiotensin II levels. Interestingly, local expression of ACE with continued expression of angiotensin II has also been recently observed at sites of renal injury after angiotensin II administration (25, 34).

A second new finding was the marked increase in intrarenal ET-1 levels. Hypokalemic rats exhibited a 2-fold increase in ET-1 in the cortex and a 10-fold increase in the medulla, where renal injury was most marked. ET is a very important vasoconstrictor, and ET-1 transgenic mice develop tubulointerstitial injury and renal fibrosis (13). These studies suggest that ET-1 should be investigated as a potential mechanism for hypokalemic vasoconstriction and renal fibrosis.

A third new finding was a marked decrease in renal kallikrein that was observed with hypokalemic injury. Kallikrein is known to be natriuretic (44) and to be upregulated by a high-K⁺ diet (15, 43), but the effect of a low-K⁺ diet on kallikrein has not been reported. Whether the decrease in kallikrein is secondary to the hypokalemia or to the renal damage is not known. Regardless of the mechanism underlying the decreased kallikrein, an impaired kallikrein system could contribute to salt sensitivity. Thus kininogen-deficient rats (Brown Norway Katholie) (15, 43), bradykinin B₂ receptor-null mice (1), and the rat strain inbred for low urinary kallikrein excretion (28) are salt sensitive and develop hypertension on a high-NaCl diet.

We also found that hypokalemic rats had a decrease in urinary nitrite/nitrate excretion. Although care must be taken in an interpretation of urinary nitrite/nitrate excretion as a reflection of systemic or renal nitric oxide production (38), these results suggest that there is a general imbalance in intrarenal vasoactive mediators favoring vasoconstriction. In addition to hemodynamic effects, alterations in these mediators may also favor the development of structural changes.

In summary, chronic hypokalemic nephropathy resulted in a marked alteration in the local vasoconstrictor and vasodilator balance. There was evidence for continued cortical angiotensin II generation and ET-1 generation, despite systemic suppression of the renin-angiotensin system and a decrease in renal kallikrein and urinary nitrites and prostaglandins. These alter-

### Table 5. Blood pressure profiles of individual rats after switch to a high-NaCl diet

<table>
<thead>
<tr>
<th>Group/Rat No.</th>
<th>Week No.</th>
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<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td><strong>Group I (K⁺-deficient, high-NaCl)</strong></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>95</td>
</tr>
<tr>
<td>2</td>
<td>110</td>
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<tr>
<td>3</td>
<td>96</td>
</tr>
<tr>
<td>4</td>
<td>86</td>
</tr>
<tr>
<td>5</td>
<td>99</td>
</tr>
<tr>
<td><strong>Group II (K⁺-deficient, low-NaCl)</strong></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>99</td>
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<td><strong>Group III (normal K⁺, high NaCl)</strong></td>
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<td><strong>Group IV (normal K⁺, low NaCl)</strong></td>
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Rats were administered a K⁺-deficient diet for 12 wk and switched to either a high (4%)- or low (0.01%)-NaCl diet with normal K⁺ content. Each blood pressure measurement (mmHg) represents the mean of 3 readings. Values at week 0 represent blood pressure before switch to the high- or low-NaCl diet.
ations would be expected to result in increased salt sensitivity (17).

Effects of Hypokalemic Nephropathy on Salt Sensitivity

$K^+$-restricted diets can induce $Na^+$ retention and an increase in BP within several days (10, 20, 21). Although this clearly documents the ability of low-$K^+$ diets to increase BP, few studies have examined whether the salt sensitivity persists after correction of the $K^+$ deficit. Recently, we found that hypokalemic renal injury in very young rats results in persistent salt sensitivity, and the studies suggested that this was due to continued renin stimulation in concert with substantial renal damage (32). In the present studies, we also found $Na^+$-dependent increases in BP that occurred after normalization of the serum $K^+$. Whether this is due to persistent renal injury and alterations in vasoactive mediators is not known. This may be a reasonable hypothesis to test in the future, as the presence of renal fibrosis itself may create a predisposition to continued ischemia by impairing the diffusion of oxygen from the peritubular capillaries to the tubules that could induce alterations in vasoactive mediators favoring $Na^+$ retention (17).

In conclusion, $K^+$ deficiency results in tubulointerstitial injury that is greatest in the outer medulla and is consistent, at least in part, with a vasoconstrictive and ischemic-type of injury. In concert, there is a remarkable alteration in intrarenal vasoactive mediators that favors vasoconstriction and which may contribute to the hemodynamic and structural changes. These changes predispose the rat to an increased BP in response to a high-salt diet that occurs despite correction of the $K^+$ deficit.

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