Expression of insulin-like growth factor-I and transforming growth factor-β in hypokalemic nephropathy in the rat

TANNY TSAO, JANET FAWCETT, FERNANDO C. FERVENZA, FAY W. HSU, PHILLIP HUIE, RICHARD K. SIBLEY, and RALPH RABKIN

Research Service Veterans Affairs Palo Alto Health Care System and Departments of Medicine and Pathology, Stanford University, Palo Alto, California, USA

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Background. Potassium deficiency (KD) in the rat retards body growth but stimulates renal enlargement caused by cellular hypertrophy and hyperplasia, which is most marked in the outer medulla. If hypokalemia persists, interstitial infiltrates appear and eventually fibrosis. Since early in KD insulin-like growth factor-I (IGF-I) levels in the kidney are elevated, suggesting that it may be an early mediator of the exaggerated renal growth, and as transforming growth factor-β (TGF-β) promotes cellular hypertrophy and fibrosis, we examined the renal expression of these growth factors in prolonged KD.

Methods. Rats were given a K-deficient diet or were pair fed or ad libitum fed a K-replete diet for 21 days. Growth factor mRNA levels were measured in whole kidney and protein expression localized by immunohistochemistry.

Results. KD rats weighed less than pair-fed controls, while the kidneys were 49% larger. Their serum IGF-I and kidney IGF-I protein levels were depressed, as were their IGF-I mRNA levels in liver, kidney, and muscle. These changes can largely be attributed to decreased food intake. In contrast, kidney IGF binding protein-1 (IGFBP-1) mRNA and TGF-β mRNA levels were increased significantly. Histology of outer medulla revealed marked hypertrophy and adenomatous hyperplasia of the collecting ducts and hypertrophy of the thick ascending limbs of Henle with cellular infiltrates in the interstitium. Both nephron segments immunostained strongly for IGF-I and IGFBP-1, but only the nonhyperplastic enlarged thick ascending Henle limb cells immunostained for TGF-β, which was strongly positive. Prominent interstitial infiltrates with ED1 immunostained macrophages were present.

Conclusions. These findings are consistent with a sustained role for IGF-I in promoting the exaggerated renal growth of KD and appear to be mediated through local trapping of IGF-I by the overexpressed IGFBP-1, which together with IGF-I can promote renal growth. The selective localization of TGF-β to hypertrophied nonhyperplastic nephron segments containing IGF-I raises the possibility that TGF-β may be serving to convert the mitogenic action of IGF-I into a hypertrophic response in these segments. It is also conceivable that TGF-β may be a cause of the tubulointerstitial infiltrate. Finally, the low circulating IGF-I levels likely contribute to the impaired body growth.

Potassium deficiency (KD) has remarkable and opposite effects on kidney and body growth. When rats are placed on a K-deficient diet, renal growth accelerates, and within eight days, there is a 25% increase in kidney mass [1]. In contrast, body growth slows down [1, 2]. The renal enlargement is the result of expansion of all the regions of the kidney and occurs through both tubule cell hypertrophy and hyperplasia. These changes are most prominent in the outer medulla, especially the inner stripe [3, 4]. In this region of the kidney, the hyperplastic enlarged collecting duct (CD) cells form cellular outgrowths that project into the lumen causing partial obstruction. In contrast to the increase in size of the renal tubules, glomerular volume remains essentially unchanged [4]. If the K-deficient state persists, then cellular infiltrates appear in the renal interstitial compartment, and eventually, tubulointerstitial fibrosis develops [5]. In humans with long-standing KD, there is a propensity to form renal cysts and to develop tubulointerstitial fibrosis with loss of renal function [6, 7].

Studies from this and other laboratories have identified insulin-like growth factor-I (IGF-I) as a potential mediator of the early renal hypertrophy [1, 8, 9]. Indeed, kidney IGF-I levels are elevated even before the kidneys enlarge [9]. The increase in IGF-I occurs despite low kidney IGF-I mRNA and serum IGF-I protein levels and has been attributed to local trapping of IGF-I by IGFBP-1, a member of a family of IGFBP-1 [10]. In KD animals, IGFBP-1 is produced in increased amounts in the kidney [1, 8], and after seven days of KD, there is immunohistochemical evidence of an increase in both IGFBP-1 and IGF-I in the hyperplastic distal nephron segments [8]. Paradoxically, alterations of the growth hormone (GH)/IGF-I axis have

Key words: potassium deficiency, cathepsin, kidney disease, fibrosis, renal growth, hypertrophy.

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also been implicated in the impaired body growth. It has been suggested that diminished GH secretion, low serum and tissue IGF-I levels, and resistance to GH and IGF-I may all contribute to the growth retardation [1, 2].

In this study, we set out to examine the role of the renal GH/IGF-I axis in the progressive renal enlargement and tubulointerstitial disease of chronic KD. In addition, since transforming growth factor-β (TGF-β) has been identified as a major mediator of tubular cell hypertrophy and interstitial fibrosis [11, 12], we also examined the expression of this growth factor to see whether it might be playing a role in hypokalemic nephropathy. In the anatomical component of this study, we focused on the outer renal medulla since the morphologic changes are most severe in this region of the kidney [3, 4].

METHODS

Animal and experimental design

Male Sprague-Dawley rats weighing approximately 170 g were placed on a 1% K diet, and after a run-in period of five days, they were separated into three groups comprising six rats per group. Group 1 consisted of K-deficient rats fed on an ad libitum 0.01% K diet. This diet was otherwise identical to the 1% diet (Purina, Richmond, IN, USA). Group 2 consisted of rats kept on a 1% K diet and pair fed with the group 1 rats. Group 3 consisted of control rats maintained on an ad libitum 1% K diet. During the study, rats were weighed daily, and their food consumption was measured. After 21 days of study, the rats were sacrificed. The kidneys, liver, and muscle were removed and weighed. One kidney and the other tissues were frozen in liquid nitrogen. The remaining kidney was bisected, fixed in 4% paraformaldehyde, and paraffin embedded.

Northern blot analysis

Total kidney RNA was isolated by an acid guanidinium thiocyanate-phenol chloroform single-step method and was size separated by agarose gel electrophoresis as before [13]. In short, 20 μg of total RNA were separated by electrophoresis through a denaturing agarose gel containing 0.55 mol/L formaldehyde. RNA was transferred to nitrocellulose filters and photographed under ultraviolet light. Filters were then prehybridized in a solution containing 50% formamide, 2.5 × SSPE, 2.5 × Denhart’s solution, 50 μg/mL salmon sperm DNA, 0.2% sodium dodecyl sulfate (SDS) at 42°C for four hours. The mRNA levels of the GH receptor, IGFBP-1, TGF-β, and cathepsins B and L were detected by hybridizing radiolabeled cDNA probes to the mRNA at 42°C overnight. Washed filters were then exposed to x-ray films, and the mRNA was quantitated by laser densitometry (GelscanXL; Pharmacia LKB, Alameda, CA, USA). Alternatively, the filters were exposed and the signals measured in a phosphor-imager (Molecular Dynamics, Sunnyvale, CA, USA). The abundance of each mRNA was adjusted for the levels of the 18S rRNA readings obtained from photographic negatives of the nitrocellulose filters.

The GH receptor probe was prepared from the 2.2 kb full-length mouse cDNA (Dr. F. Talamantes, University of California, Santa Cruz, CA, USA). This cDNA encodes the extra (GH receptor binding protein) through intracellular domain of the receptor [14]. The cDNA probe for IGFBP-1 was prepared from rat cDNA (Dr. S. Shimasaki, Whittier Institute, La Jolla, CA, USA), which consisted of 407 bp corresponding to the coding region spanning nucleotide position 486 to 892 in the rat IGFBP-1 cDNA sequence [15]. The cDNA probe for TGF-β was prepared from mouse cDNA (Dr. D.E. Miller, University of Utah School of Medicine, Salt Lake City, UT, USA) and was size separated by agarose gel electrophoresis as before [13]. In short, total RNA (20 μg) was hybridized with α-32P-CTP labeled antisense IGF-I or IGF-I receptor and 18S rRNA probes overnight. The mixture was then incubated with an RNase digestion buffer followed by the addition of proteinase K. Ethanol-precipitated protected hybrids were separated on a 5% polyacrylamide/8 mol/L urea denaturing gel. Autoradiographs or phosphorimages were obtained, and the density of the protected bands was measured. Only the Ea IGF-I mRNA transcripts were quantitated since it accounted for >95% of the IGF-I mRNA signal in the kidney and muscle. The IGF-I riboprobe, a 376 bp Sau3A-EcoRI fragment, was designed to detect both the Ea and Eb IGF-I mRNAs [18]. The IGF-I receptor riboprobe contained 280 bases complementary to the IGF-I receptor mRNA. The 18S riboprobe contained 80 complementary bases of the human 18S rRNA [19].

Kidney plasma membrane IGF-I receptor binding assay

Crude plasma membranes were isolated from the kidney as before [13]. The kidney was homogenized in 8% buffered sucrose, pH 7.0, followed by differential centrifugation and collection of a 47,000 × g pellet in calcium-
free Krebs-Ringer HEPES buffer (KRH; pH 7.4). Membrane receptor binding was determined as before with minor modifications [13]. In brief, 100 μg of membrane protein were incubated at 4°C overnight with 10 to 11 mol/L 

Radioimmunoassay of serum and kidney IGF-I peptide

Insulin-like growth factor-I was measured by radioimmunoassay (RIA) in acid-ethanol extracts of serum and acetic acid extracts of kidney with a commercial RIA kit (Nichols Institute, San Juan Capistrano, CA, USA) as before [13]. Recovery of IGF-I added to homogenate of kidneys from ad libitum control and K-depleted rats averaged $78 \pm 1.2$ and $81 \pm 1.8\%$, respectively. Recovery from serum averaged $96 \pm 23.9$ and $95 \pm 3.4\%$, respectively. Adjustment for serum IGF-I contamination of kidney samples was not made because serum trapped in kidney forms less than 2% of the organ volume, and it has been estimated that the IGF-I in serum accounts for approximately 6% of kidney tissue IGF-I content [1].

Immunohistochemistry

Insulin-like growth factor-I was detected with a rabbit polyclonal anti-human antibody (Drs. L.E. Underwood and J.J. Van Wyk, National Hormone and Pituitary Program, Bethesda, MD, USA), IGFBP-1 with a rabbit polyclonal anti-human antibody (Upstate Biotechnology, Lake Placid, NY, USA), TGF-β1 with a rabbit polyclonal anti-human antibody selectively directed against TGF-β1 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and ED1 with a mouse anti-rat ED1 monoclonal antibody (Serotec Ltd., Oxford, UK). Tamm-Horsfall protein was detected with a mouse antihuman monoclonal antibody (Accurate Chemical & Scientific Corp., Westbury, NY, USA) and aquaporin 2 with a rabbit polyclonal antibody provided by Dr. Alan Verkmman (University of California, CA, USA). Anti-IGF-I and IGFBP-1 antibodies were used in a 1:400 dilution, anti-TGF-β antibody in a 1:50 dilution and the anti-ED1 antibody in a 1:100 dilution. Paraffin-embedded tissue was cut in 4 μm sections and dewaxed with xylene and hydrated with ethanol in decreasing concentrations. Sections were then incubated in 3% hydrogen peroxide for five minutes to quench endogenous peroxidase activity and rinsed in distilled water. To retrieve antigens, the sections were heated in a microwave in the presence of a solution of saturated lead thiocyanate and 1% zinc sulfate in distilled water, as described by Shi, Key, and Kalra [20]. After washing in phosphate-buffered saline (PBS) containing Tween-20, sections were incubated with 10% normal goat serum in 0.1 mol/L Tris with 0.05% Tween-20 at room temperature for 30 minutes to suppress nonspecific binding. Sections were briefly rinsed with PBS with Tween-20, and the specific primary antibody was applied for two hours at room temperature. To demonstrate specificity of binding, a second set of sections was incubated with an equal concentration of normal rabbit serum or nonimmune mouse IgG diluted in 0.1 mol/L Tris with Tween-20 instead of specific primary antibody. Sections were rinsed in PBS with Tween-20 and a secondary antibody (biotinylated goat anti-rabbit or anti-mouse IgG; Vector Laboratories, Burlingame, CA, USA) was applied for 30 minutes. Sections were washed in PBS for 20 minutes and incubated with streptavidin-horseradish peroxidase (Vector Laboratories) for 30 minutes. After a rinse and wash in PBS with Tween-20 for 20 minutes, diaminobenzidine (Sigma Chemical, St. Louis, MO, USA) was applied for three minutes. Sections were washed in distilled water and were counterstained with hematoxylin. Coverslips were applied after dehydration with ethanol and clearing in xylene.

Biochemical measurements

Serum K was determined in a IL943 flame photometer (Instrumentation Labs, Lexington, VA, USA) and tissue protein by the BioRad method (BioRad Laboratories, Richmond, CA, USA).

Data analysis

 Autoradiographic and phosphorimager readings of the mRNAs of interest were adjusted for the corresponding 18S rRNA reading. Corrected values are expressed relative to the average of the control group. For comparison between the three study groups, the data were analyzed with an analysis of variance followed by Student–Newman–Keuls t test. A P value of <0.05 was taken as significant. Results are expressed as mean ± SEM and are from five to six rats per group.

RESULTS

Serum potassium levels, body and kidney weights, and food conversion efficiency

The data from the three groups of animals studied are given in Table 1. At the end of the 21-day study period, serum K level (2.0 ± 0.26 mEq/dL) and body weight were all significantly lower in the K-restricted rats compared with either the pair-fed or ad libitum fed controls on a 1% K-replete diet ($P < 0.05$). Food intake fell when the rats were placed on the 0.01% K diet and averaged 13 g/day. This was considerably lower than the average
intake of 18 g/day by the ad libitum fed control rats. Food conversion efficiency in the K-depleted rats was one third that observed in the pair-fed controls (10.2 ± 1.0 vs. 27.6 ± 0.8%, P > 0.01). Thus, despite the equal quantity of food ingested, the K-deficient rats gained significantly less weight than the pair-fed controls over the three-week period (27 ± 2.3 vs. 78 ± 6.2 g, P > 0.01). The largest weight gain occurred in the ad libitum fed controls (142 ± 6 g). In contrast to the lower body weight, left kidney weight was 49% greater in K-depleted rats than in the pair-fed controls (1.21 ± 0.05 vs. 0.81 ± 0.01 g, respectively, P < 0.01) and 14% greater than in the ad libitum controls (1.06 ± 0.04 g, P < 0.05). When kidney weight was expressed as a percentage of body weight, the differences between the K-deficient rats and controls were even more striking for the value in the K-deficient group was twofold that of the controls (Table 1).

### Insulin-like growth factor-I mRNA levels

These results are summarized in Table 2 and are illustrated in Figure 1. In the K-deficient state, there was a 51% decrease in kidney IGF-I mRNA levels when compared with the ad libitum control values (P < 0.05). However, there was a similar 53% decrease in the IGF-I mRNA level in the pair-fed control rats, indicating that the fall in IGF-I gene expression can be attributed to attenuated food intake. In liver, the IGF-I mRNA levels were reduced by 54% in the K-deficient rats and by 38% in the pair-fed rats compared with the ad libitum fed controls (P < 0.05). Although the levels were on average lower in the K-deficient group, they did not differ significantly from the pair-fed control values. In muscle, the IGF-I mRNA levels were reduced by 45% in the K-deficient rats (P < 0.05) and by 20% in the pair fed rats (P = NS) compared with the ad libitum fed controls. The levels in K-deficient and pair-fed groups differed significantly, indicating that KD has a direct effect on IGF-I gene expression in muscle.

### Insulin-like growth factor-I receptor mRNA levels

These results are summarized in Table 2 and are illustrated in Figure 1. Kidney IGF-I receptor mRNA levels

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### Table 1. Serum body and kidney weight/body weight, food intake and food conversion efficiency

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Serum K (mEq/L) Day 0</th>
<th>Body weight g Day 0</th>
<th>Kidney weight g Day 21</th>
<th>Kidney weight/ body weight %</th>
<th>Cumulative food intake g</th>
<th>Food conversion efficiency %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ad libitum</td>
<td>6</td>
<td>4.3 ± 0.17</td>
<td>168 ± 3.1</td>
<td>310 ± 4.7</td>
<td>1.06 ± 0.04</td>
<td>0.34 ± 0.02</td>
<td>380 ± 7.1</td>
</tr>
<tr>
<td>Pair-fed control</td>
<td>6</td>
<td>3.7 ± 0.13</td>
<td>172 ± 2.4</td>
<td>250 ± 7.9</td>
<td>0.81 ± 0.06</td>
<td>0.32 ± 0.01</td>
<td>272 ± 10.6</td>
</tr>
<tr>
<td>Low K</td>
<td>6</td>
<td>2.0 ± 0.26ab</td>
<td>171 ± 3.3</td>
<td>198 ± 3.9</td>
<td>1.21 ± 0.05ab</td>
<td>0.61 ± 0.03b</td>
<td>269 ± 12.2</td>
</tr>
</tbody>
</table>

Ad libitum and pair-fed control rats were fed a 1% K diet. Low K rats were fed a 0.01% K diet. Food conversion efficiency = body weight gain divided by food intake × 100.

*a P < 0.05 vs. ad libitum control

*b P < 0.05 vs. pair-fed control

### Table 2. Relative insulin-like growth factor (IGF-I), IGF-I receptor and growth hormone (GH) receptor mRNA levels in kidney, muscle and liver

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Ad libitum</th>
<th>Pair-fed</th>
<th>Low K</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGF-I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>100 ± 18</td>
<td>47 ± 6.9</td>
<td>49 ± 7.6</td>
</tr>
<tr>
<td>Muscle</td>
<td>100 ± 5</td>
<td>80 ± 7.2</td>
<td>55 ± 7.5</td>
</tr>
<tr>
<td>Liver</td>
<td>100 ± 8</td>
<td>62 ± 6.1</td>
<td>46 ± 3.4</td>
</tr>
<tr>
<td>IGF-I receptor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>100 ± 25</td>
<td>176 ± 26</td>
<td>171 ± 9</td>
</tr>
<tr>
<td>Muscle</td>
<td>100 ± 6</td>
<td>102 ± 8</td>
<td>103 ± 9</td>
</tr>
<tr>
<td>Liver</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>GH receptor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>100 ± 15</td>
<td>79 ± 10</td>
<td>61 ± 3</td>
</tr>
<tr>
<td>Muscle</td>
<td>100 ± 6</td>
<td>88 ± 6</td>
<td>71 ± 8</td>
</tr>
<tr>
<td>Liver</td>
<td>100 ± 9</td>
<td>69 ± 8</td>
<td>63 ± 5</td>
</tr>
</tbody>
</table>

IGF-I and IGF-I R mRNA levels were determined simultaneously in a solution hybridization ribonuclease protection assay. These values were corrected for 18S rRNA levels measured in the same assays. Corrected values are expressed relative to the average value of the control group, assigned a value of 100. Results are mean ± SEM, 5 to 6 rats/group. ND is not detectable.

*a P < 0.05 vs. ad libitum control

*b P < 0.05 vs. pair-fed control
were elevated near twofold in both the K-deficient and pair-fed rats compared with the ad libitum controls \( (P > 0.05) \). This suggests that the increase in kidney IGF-I receptor mRNA levels is mainly due to the reduced food intake. In muscle, the IGF-I receptor mRNA levels were similar in the three groups.

### Growth hormone receptor mRNA levels

As shown in Table 2 and Figure 2, kidney GH receptor mRNA levels were reduced by 44% in the K-deficient rats compared with the ad libitum fed controls \( (P < 0.05) \). The pair-fed control values were modestly reduced, but did not differ from the other groups. A similar response was seen in liver and muscle. Thus, in all three tissues, the fall in GH receptor expression appears to be largely a direct consequence of KD and in part due to the reduced food intake.

### IGFBP-1 mRNA levels

The IGFBP-1 mRNA levels in kidneys from ad libitum and pair-fed controls and the K-deficient rats are depicted in Figure 2. While the IGFBP-1 mRNA levels in the pair-fed and ad libitum-fed control groups did not differ significantly, the values in the K-deficient group were significantly higher than in both the control groups \( (P < 0.05) \). Indeed, the average IGFBP-1 mRNA level in the K-deficient group was 11-fold greater than in the ad libitum controls \( (1085 \pm 115 \text{ vs. } 100 \pm 14 \text{ arbitrary units}) \). In liver, there was only a modest increase in IGFBP-1 mRNA levels in the K-deficient rats compared with the ad libitum controls \( (150 \pm 12 \text{ vs. } 100 \pm 10 \text{ arbitrary units}, P < 0.05) \). This increase appears to be partly by the reduced food intake, since the levels in the K-deficient state did not differ significantly from the pair-fed controls \( (119 \pm 13 \text{ arbitrary units}) \), which in turn did not differ from the ad libitum controls. IGFBP-1 mRNA was not detected in muscle.

### Transforming growth factor-\( \beta \) mRNA levels

As shown in Figure 2, kidney TGF-\( \beta \) mRNA levels were similar in the pair-fed and ad libitum fed control groups \( (104 \pm 5 \text{ vs. } 100 \pm 5 \text{ arbitrary units}, \text{respectively}) \). In contrast, there was a significant 68% increase in the K-deficient group \( (168 \pm 12 \text{ arbitrary units}, P < 0.05) \).

### Cathepsin B and cathepsin L mRNA levels

In another state of renal hypertrophy associated with increased renal ammoniagenesis, namely acidosis, we have shown that the increase in cell protein mass is caused in part by depressed cathepsin B and cathepsin L gene expression and activity \([21, 22]\). Accordingly, we measured cathepsin B and cathepsin L mRNA levels in the present study. An entirely different response occurred in the K-deficient state. Depletion of K was associated with a threefold increase in kidney cathepsin L mRNA levels compared with pair-fed and ad libitum control rats \( (305 \pm 14 \text{ vs. } 112 \pm 9 \text{ and } 100 \pm 6 \text{ arbitrary units}, \text{respectively}, P < 0.01) \). In contrast, cathepsin B mRNA levels did not differ (data not shown) among the three groups.

### IGF-I peptide levels in serum and tissues

These results are summarized in Table 3. The serum IGF-I levels were reduced significantly in the K-deficient and pair-fed groups \( (P < 0.05) \) compared with the ad libitum controls. The lowest values were seen in the K-deficient group \( (P < 0.05) \) and indicate that the low serum IGF-I levels in KD can only partly be explained by the reduced food intake. The IGF-I levels in the whole kidney were significantly lower in the K-deficient group compared with the ad libitum controls \( (342 \pm 24 \text{ vs. } 537 \pm 16 \text{ ng/g}) \), while the levels in the pair-fed rat kidneys were intermediate \( (442 \pm 42 \text{ ng/g}) \) and did not differ significantly from the other groups. Since the IGF-I mRNA levels in the K-deficient and pair-fed kidneys were depressed to a similar extent and as kidney IGF-I peptide is in part serum derived, it is likely that the lower IGF-I peptide levels in the K-deficient rat kidneys were elevated near twofold in both the K-deficient and pair-fed rats compared with the ad libitum controls \( (P > 0.05) \). This suggests that the increase in kidney IGF-I receptor mRNA levels is mainly due to the reduced food intake. In muscle, the IGF-I receptor mRNA levels were similar in the three groups.

### Table 3. Immunoreactive IGF-I concentrations in serum and kidney

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Serum ng/mL</th>
<th>Kidney ng/g tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ad libitum</td>
<td>5/6</td>
<td>1283 ± 112</td>
<td>537 ± 16</td>
</tr>
<tr>
<td>Pair-fed</td>
<td>6</td>
<td>855 ± 68</td>
<td>442 ± 42</td>
</tr>
<tr>
<td>Low K</td>
<td>6</td>
<td>570 ± 71*</td>
<td>342 ± 24*</td>
</tr>
</tbody>
</table>

* \( P < 0.05 \) vs. ad libitum control

\* \( P < 0.05 \) vs. pair-fed control
Kidney plasma membrane IGF-I receptor binding

Specific binding of radiolabeled IGF-I to isolated kidney plasma membranes was increased to a similar extent in the K-deficient and pair-fed groups compared with ad libitum fed group. The values were $14.0 \pm 1.0$, $13.1 \pm 0.6$, and $9.7 \pm 0.3\%$ of $100 \mu g$/membrane protein, respectively ($P < 0.05$). The increase in binding likely reflects receptor up-regulation in response to reduced food intake [18].

Immunohistochemistry

As previously reported by others, there was marked hypertrophy and adenomatous hyperplasia of the collecting ducts (CD) cells in the outer medulla, especially in the inner stripe [3, 4]. Several of the larger nephron segments had a microcystic appearance. Cellular interstitial infiltrates were also present with widening of the interstitium [5]. All of these changes are evident in Figure 3, which depicts the inner stripe of the outer medulla from pair-fed control (A) and K-deficient (B) animals. The CDs and thick ascending limbs of Henle (TALHs) of all of the animals displayed IGF-I immunoreactivity, which appears to be increased in the K-deficient kidney (Fig. 3 A, B). It is noteworthy that in the K-deficient animals, both the hyperplastic- and nonhyperplastic-enlarged nephron segments were positively stained. A similar pattern of immunoreactivity was detected with the IGF-BP-1 antibody in the K-deficient animals (Fig. 3 C, D); both the hyperplastic and nonhyperplastic distal nephron segments showed increased immunostaining. TGF-β immunoreactivity was only faintly positive over the distal nephron segments in control kidneys (Fig. 3E). In contrast, the kidneys of the K-deficient animals displayed strong immunoreactivity over the hypertrophied nonhyperplastic distal nephron segments, while the hyperplastic tubules were devoid of immunoreactivity (Fig. 3F). This difference in the distribution of TGF-β immunoreactivity compared with that of IGF-I and IGFBP-1 is particularly noteworthy. Interstitial infiltrates were absent in the control kidneys, while infiltrates present in the K-deficient kidneys displayed ED-1 immunoreactivity consistent with infiltrating macrophages/monocytes (Fig. 3 G, H). To identify the enlarged medullary nephron segments, sections were immunostained with an antibody against aquaporin-2, which is expressed in the CD, or an antibody against Tamm-Horsfall protein, which is expressed in the TALH. As depicted in Figure 4A, the hyperplastic tubules immunostained for aquaporin-2, and the hypertrophic nonhyperplastic tubules immunostained for Tamm-Horsfall protein (Fig. 4B). This pattern of immunostaining identifies these nephron segments as being the CD and TALH, respectively.

DISCUSSION

In this study, we have evaluated the role of the renal IGF-I axis and of TGF-β in the renal hypertrophy and tubulointerstitial disease of sustained KD. After three weeks on a K-depleted diet, food intake decreased, and weight gain was severely attenuated. The latter was in part due to diminished food utilization for the hypokalemic rats gained less weight than their pair-fed controls. In contrast to the lack of body growth, the kidneys enlarged and, at the end of the 21-day study, were 49% heavier than the kidneys of pair-fed controls. This increase is double that which we previously observed after eight days of KD [1]. Thus, in KD, the exaggerated renal growth continues for a prolonged period of time. The anatomical changes observed are of particular interest and were most striking in the outer medulla, especially the inner stripe. Tubular epithelial cells were enlarged, and there was exuberant proliferation of some medullary CD cells with the formation of papillary outgrowths that projected into the dilated tubular lumina, findings consistent with earlier reports [3, 4]. The enlarged medullary CD had a microcystic appearance, and these changes in the rat kidney may serve as a model for the cysts that develop in chronically hypokalemic patients [6]. Interstitial infiltrates were also present in the KD rat kidneys and contained ED1-positive cells consistent with monocytes/macrophages.

Serum and kidney IGF-I peptide levels were reduced significantly in the KD rats compared with the ad libitum- and pair-fed controls, while values in the pair-fed group were intermediate. These changes in IGF-I peptide levels could be attributed to depressed IGF-I gene expression. IGF-I mRNA levels were reduced in kidney, liver, and muscle, and this appears to be largely, although not entirely, caused by decreased food intake. The observation that whole kidney IGF-I peptide levels are low after three weeks of K restriction differs from the situation in early KD [1, 8, 9]. After eight days of K restriction, kidney IGF-I peptide levels increase more than twofold, even though the IGF-I mRNA levels are depressed [1]. This paradoxical elevation in kidney IGF-I levels appears to be a result of several processes, including increased delivery of IGF-I bound to low-molecular-weight IGFBPs to the kidney, diminished renal IGF-I degradation and trapping by IGFBP-I, which is overexpressed in the kidney of K-deficient rats [1, 8, 9]. In the present study, we attribute the low whole kidney IGF-I peptide levels, present despite an increase in kidney IGFBP-1 expression, to the sustained depression of IGF-I production. GH receptor mRNA levels were reduced significantly in kidney, liver, and muscle of the KD animals compared with ad libitum fed controls and, while lower than in the pair-fed controls, were not significantly different. The reduction in GH receptor gene
expression, which is coexpressed with IGF-I in the TALH the major site of IGF-I gene expression, may partly account for the reduced whole kidney IGF-I expression. Reduced growth hormone receptor gene expression is consistent with the report that hepatic GH receptor binding is depressed in KD rats [21].

Initially, these findings imply that IGF-I does not play a role in the progressive renal enlargement of KD. However, review of the immunohistochemical findings leads us to a different conclusion. In the inner stripe of the outer medulla, where kidney growth is most exaggerated, there was strong staining for IGFBP-1 and IGF-I, which colocalized over the CD and TALH. Cells of the latter nephron segment were enlarged, while those of the CD were both enlarged and hyperplastic. Since the whole kidney IGF-I peptide levels were depressed, these observations suggest that IGF-I is being sequestered in these nephron segments by locally overexpressed IGFBP-1. It may well be that this trapped IGF-I, possibly together with the binding protein, serves to stimulate the cellular proliferation and hypertrophy. Indeed, Van Buul-Offers et al recently reported that infusions of IGFBP-1 alone or together with IGF-I stimulate kidney, but not body, growth in Snell dwarf mice [22]. After five days of IGFBP treatment, kidney weight increased approximately 50%, and when given together with IGF-I, the weight increased even more, by approximately 70%. Another possible explanation for the local increase in IGF-I is selective up-regulation of IGF-I gene expression in these nephron segments, but reduced expression in others. However, since the TALH is the major site of IGF-I gene expression, the fall in whole kidney IGF-I gene expression likely reflects a corresponding change in expression in this nephron segment [10]. Since the morphologic findings are consistent with that observed after a week of KD [8], it suggests that IGF-I may play a continuous role in mediating the exaggerated renal growth caused by KD. It is also conceivable that IGF-I may play a role in the expansion of the interstitial infiltrates by stimulating macrophage proliferation [23].

Of particular interest are the changes in TGF-β expression in the kidney of the K-restricted rats. Whole kidney TGF-β mRNA levels were increased, and immunohistochemical staining for TGF-β was greatly enhanced after three weeks of KD. In the inner stripe of the outer medulla of the kidneys of normokalemic con-

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**Fig. 3.** Localization of IGF-I, IGFBP-1, TGF-β, and ED1 immunoreactivity in the inner stripe of outer medulla of kidneys of hypokalemic rats on a K-deficient diet for 21 days (B, D, F, and H) and their pair-fed controls (A, C, E, and G). Magnification ×250. Counterstain hematoxylin. (A) Control kidney, IGF-I immunoreactivity. Note the light immunoperoxidase staining of tubules. (B) K-deficient, IGF-I immunoreactivity. Note the marked hyperplasia of some tubules, identified as collecting ducts (CDs) in Fig. 4A, with formation of papillary projections (*). Other tubules, identified as thick ascending limb of Henle (TALH) in Fig. 4B, are enlarged but are not hyperplastic (arrows). Both the hyperplastic and nonhyperplastic tubules show increased IGF-I immunoreactivity. Prominent interstitial infiltrates are present. (C) Control kidney, IGFBP-1 immunoreactivity. Tubules are lightly immunostained. (D) K-deficient animals, IGFBP-1 immunoreactivity. Immunostaining for IGFBP-1 is intense and has the same anatomical distribution as IGF-I. Some of the enlarged tubules have an early microcystic appearance. (E) Control kidney, TGF-β1 immunoreactivity. Tubules are lightly immunostained. (F) K-deficient animal, TGF-β1 immunoreactivity. TGF-β1 immunostaining is intense and confined to the nonhyperplastic nephron segments. (G) Control kidney, ED-1 immunoreactivity. No immunostaining is evident. (H) K-deficient animal, ED-1 immunoreactivity. ED1-positive cells (monocytes/macrophages) are present in the interstitial infiltrate.
trol rats, TGF-β immunostaining was very faint, while in the KD rats, staining was prominent over some, but not all, of the distal nephron segments. The hypertrophied TALH were strongly TGF-β positive, while in contrast, the hyperplastic CD were TGF-β negative. This differs from the distribution of IGF-I and IGFBP-1, as described previously in this article, and leads us to suggest for consideration that it is the absence of TGF-β in the CD that allows IGF-I and presumably other growth factors to stimulate cellular proliferation. In contrast, we suggest that the presence of TGF-β in the hypertrophied TALH may serve to convert the mitogenic stimulus of IGF-I into a hypertrophic response. This is consistent with the kidney cell culture finding that TGF-β blocked the mitogenic action of insulin and together with insulin induced cellular hypertrophy [24]. We also suggest that TGF-β may be playing a role in the formation of the prominent interstitial infiltrate in the KD state, since TGF-β also functions as a chemoattractant for monocytes and macrophages from the circulation [25].

It has been proposed that some of the pathologic changes in the kidney of KD animals may be initiated by the high levels of ammonia generated in KD and mediated through the activation of the alternate complement pathway [5]. In support of this thesis is the finding that bicarbonate supplementation sufficient to suppress renal ammoniagenesis attenuates the renal enlargement of KD [3]. In support of this thesis is the finding that bicarbonate supplementation sufficient to suppress renal ammoniagenesis attenuates the renal enlargement of Veterans Affairs and the American Heart Association Western States Affiliates. Dr. F. Fervenza was supported by funds from the Satellite Dialysis Fund of Northern California.

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