Alterations of growth plate and abnormal insulin-like growth factor I metabolism in growth-retarded hypokalemic rats: effect of growth hormone treatment

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Growth retardation is frequently found in primary hypokalemic tubular disorders (32). Potential pathogenic factors of growth impairment in these tubulopathies include maintained metabolic acidosis, polyuria with decreased food intake and repeated dehydration episodes, disturbed bone mineralization, sodium deficit, and potassium depletion. In children with renal tubular acidosis, growth retardation is common and is ameliorated or reversed after sustained correction of metabolic acidosis (29). Metabolic acidosis disturbs normal growth through various mechanisms such as the stimulation of protein catabolism (18), interference with growth hormone (GH) action (21), and the alteration of the structure and dynamics of growth cartilage (5). In hypokalemic tubulopathies associated with alkalosis, Barter’s or Gitelman’s syndromes, the adverse effect on growth is not well documented and the response of growth to treatment is rather unpredictable. Growth impairment is frequently described in Barter’s syndrome (22). Gitelman’s syndrome, traditionally considered as asymptomatic or responsible for mild clinical manifestations (9), has been reported to be accompanied by short stature in over 30% of children (8). Although clinical cases of isolated GH deficiency as well as improvement of growth rate following administration of indomethacin have been reported in patients with Gitelman’s syndrome, the pathogenesis of stunted growth in these hypokalemic disorders is unclear (3, 17, 31). Few studies have used an experimental rat model of chronic potassium depletion to know the effect of potassium deficiency on the tubular sodium and potassium transport, acid-base balance control, salt sensitivity, and kidney growth (1, 10–13, 15, 16, 20, 22–26, 33, 34, 36–38). Some of these studies have found that potassium deficiency leads to renal hypertrophy and inhibits body growth markedly (36–38).

As the growth plate of long bones is where longitudinal growth takes place, the study presented here was carried out to investigate the effect of chronic potassium depletion and subsequent GH treatment on growth cartilage metabolism. We hypothesized that retarded growth caused by chronic potassium deficiency should be accompanied by alterations in growth plate morphology and in the processes of chondrocyte proliferation and maturation as well as by local and/or systemic disturbances in the GH-insulin-like growth factor I (IGF-I) axis. Treatment with GH might improve growth retardation and correct the metabolic and growth plate alterations.

MATERIALS AND METHODS

Animals. Twenty-one day-old weaning female Sprague-Dawley rats (Harlan Iberica, Barcelona, Spain) were lodged in the animal facility building of our institution and kept under controlled conditions of light (12:12-h light-dark cycle) and temperature (21–23°C) in individual cages. Rats received standard chow and water ad libitum and, after 3 days of acclimation, were divided into four groups of similar weight (n = 10): control, normal diet (17.7% protein, 3,700 kcal/kg) containing 0.36% of potassium (Harlan TD 88239) ad libitum (initial body wt 53.29 ± 5.36 g); potassium depletion (KD), potassium-deficient diet (Harlan TD 88239) with 0.01% potassium content and the same content of other nutrients as the normal diet ad libitum (initial body wt 53.63 ± 5.23 g). The study complied with current legislation on...
animal experiments by the European Union and was approved by the Ethical Committee on Animal Research of our institution.

Experimental protocol. The experimental protocol lasted 14 days. The study was approved by the Animal Investigation Committee of our institution and complied with the current Spanish legislation on animal protection for scientific purposes. The KDGH group received recombinant human GH (provided by NovoNordisk Pharma, Madrid, Spain) at a daily dose of 3.3 mg/kg given in two intraperitoneal injections (9 a.m. and 5 p.m.) for 1 wk (days 7–13). Untreated groups received solvent injections. Animals were killed under anesthesia on day 14 of the protocol. Three days before, the rats received 30 mg/kg of calcein (Sigma, St. Louis, MO) by an intraperitoneal route. Brodoxoridazine (BrDu; 100 mg/kg; Sigma-Aldrich. Madrid, Spain) was injected intraperitoneally 1, 9, and 17 h before death. At death, blood, liver samples, and both tibias were obtained. Liver samples and the proximal end of right tibias were immediately frozen in liquid nitrogen for mRNA expression studies. Left tibial proximal ends were embedded in methyl-metacrilate as previously described (2, 19). Samples from one-half the animals were fixed in 4% paraformaldehyde (PFA; Sigma-Aldrich) and used for histomorphometry, immunohistochemistry, and in situ hybridization. Frontal sections of 5 μm were used except for calcein labeling, which was performed on 10-μm-thick sections.

Blood biochemistry. Potassium and acid-base balance were measured with an IRMA TRUpoint blood-analysis system from ITC (Biomed, Madrid, Spain) in 1 ml of blood obtained under anaerobic conditions. The remaining blood was centrifuged immediately, and the resulting serum was stored in aliquots of 500 μl at −20°C. IGF-I serum concentrations were measured by ELISA with a commercial kit (Immunodiagnostic Systems, Boldon, UK). Each sample was measured twice. Inter- and intra-assay variation coefficients were <7%.

Growth and nutrition. Body weight and food intake were daily measured with a balance Ohaus GT 2001 (Ohaus, Pine Brook, NJ). Food efficiency was calculated as the ratio of weight gained to food consumed (g/g) during the week of GH treatment. Snout-to-tail-tip length of rats was measured at death. The longitudinal bone growth rate (μm/day) during the 3 days before death was calculated means of calcein labeling as previously described (2, 19).

Growth cartilage analysis. The heights of the growth plate, the hypertrophic zone, and the three most distal chondrocytes were measured in tibial sections stained by alcian-blue safranin (Merck, Madrid, Spain). Osteoclasts were identified by tartrate-resistant acid phosphatase (TRAP) reaction and quantified, as TRAP-positive cells/100 terminal chondrocytes, in an area of the primary spongiosa extending 50 μm beyond the distal end of the growth cartilage (2). BrdU-labeled chondrocytes were identified by immunocytochemical procedures as described elsewhere (2) using a monoclonal antibody to BrdU (1:20, Dako Diagnosticos, Barcelona, Spain). Proliferative activity was expressed as the number of BrdU-positive cells/100 cells in the proliferative zone. mRNA was extracted from the growth cartilage of frozen right tibial samples using the Chomczynski method (6). Samples were diluted to a 1 μg/μl concentration before retrotranscription was performed with a commercial kit (Qiagen Iberia, Madrid, Spain). IGF-I as well as GH and IGF-I receptor mRNA expression was quantified by PCR (qPCR), using GAPDH as a housekeeping gene for normalization, on a Sequence Detection System Step One (Applied Biosystems. Foster City, CA) with SYBR Green (ABgene Products, Thermo Fisher Scientific, Rockford, IL) as a fluorophore. Primer sequences for qPCR were designed with Prime Express software (PerkinElmer Applied Biosystems) and synthesized by Invitrogen (Fisher-Invitrogen, Barcelona, Spain). The primer sequences were as follows: IGF-I: forward TACCAAGTCGCCACACGAC, reverse GTGGCTTGGTTAAGAATGAGC, control GCTGTGCGCTGCGGT GCCGTACAGTTGGGCAGGTACACGGCACGGGGCAC, IGF-IR: forward GCACCACACACACATGTGGTGCCCTCCGAATGCTGGAGCCATAGCC; IGF-IR: forward AACATCTTCGTTGGTTGGCCAC, reverse TGTGGTGCCCTCCGAATGCTGGAGCCATAGCC; IGF-IR: forward GCTGTGCGCTGCGGT GCCGTACAGTTGGGCAGGTACACGGCACGGGGCAC, reverse GAAACGACACTTGGTGAATCG. The primers were designed to amplify the common part of the various transcripts. The primer for the housekeeping gene, GAPDH, was synthesized and supplied by Qiagen (Qiagen Iberia). Results were normalized to the control group by the C(T) values method. In situ hybridization was used to define the mRNA distribution pattern of IGF-I and receptors of IGF-I and GH in the growth cartilage. RNA probes (IGF-I: ATCCACAATGTGGGTGTTGTGCTCCTCGCAATGCTGGAGCCATAGCC; IGF-IR: GTACAGTCTTCGTTGGTTGGCCAC, IGF-IR: forward AAATACAGCTGTCTTGGAGAGC, reverse GTGGCTTGGTTAAGAATGAGC; GHR: forward AATTTGCGTTGGCCAC, reverse GTGGCTTGGTTAAGAATGAGC) were supplied by GeneDetect (Auckland, New Zealand). The procedure was as already described (19) but with adjustments of prehybridization and hybridization temperatures for each probe to that of annealing temperature. Two parallel sections served as negative controls. One section was hybridized with a labeled sense riboprobe, and a second section was incubated adding a negative control probe to the hybridization mixture. For immunohistochemical staining of IGF-I and the IGF-I receptor, rabbit polyclonal anti-rat-specific antibodies (Santa Cruz Biotechnology, Heidelberg, Germany) were used. The immunohistochemical staining followed the procedure described in other studies (2, 19) with minor modifications. Incubation (30 min at 45°C in a pepsin solution (5 mg/ml for IGF-I and 10 mg/ml for IGF-I receptor) was used for antigen retrieval. Nonspecific binding was avoided by incubation with 20% normal goat serum in phosphate-buffered saline for 75 min at room temperature. Primary antibodies were used at a 1:10 dilution and incubated for 48 h at 4°C. The anti-rabbit secondary conjugated antibody (EnVision+, Dako

Table 1. Blood biochemistry, weight, and length in the 4 groups of animals

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>Bicarbonate, meq/l</th>
<th>Potassium, meq/l</th>
<th>IGF-I, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.41±0.03</td>
<td>24.48±1.95</td>
<td>3.81±0.41</td>
<td>196.0±81.2</td>
</tr>
<tr>
<td>KD</td>
<td>7.43±0.04*</td>
<td>31.19±3.09*</td>
<td>1.78±0.18</td>
<td>119.5±37.7*</td>
</tr>
<tr>
<td>KDGH</td>
<td>7.45±0.04*</td>
<td>35.08±4.13*</td>
<td>1.60±0.23*</td>
<td>112.3±34.8*</td>
</tr>
<tr>
<td>CPF</td>
<td>7.40±0.04†‡</td>
<td>23.83±2.24‡</td>
<td>3.73±0.33‡</td>
<td>85.2±20.4*</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 10 animals/group for blood analysis and 5 animals/group for serum analysis. KD, potassium depletion; KDGH, KD + growth hormone treatment; CPF, control pair-fed with KD; IGF-I, insulin like growth factor I. *Statistically different from control group. †Statistically different from KD group. ‡Statistically different from KDGH group. All P < 0.05.

Table 2. Growth and nutrition in the 4 groups of rats

<table>
<thead>
<tr>
<th></th>
<th>Body Weight Gain, g</th>
<th>Cumulative Food Intake, g</th>
<th>Food Efficiency, g</th>
<th>Body Length, cm</th>
<th>Osseous Front Advance, μm/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13.95±4.47</td>
<td>60.63±5.21</td>
<td>0.22±0.07</td>
<td>26.57±1.15</td>
<td>146.29±19.6*</td>
</tr>
<tr>
<td>KD</td>
<td>5.78±2.02*</td>
<td>47.90±4.22*</td>
<td>0.12±0.03*</td>
<td>23.64±1.14*</td>
<td>67.07±10.4*</td>
</tr>
<tr>
<td>KDGH</td>
<td>10.56±1.78*</td>
<td>48.24±4.23*</td>
<td>0.22±0.04*</td>
<td>23.41±0.67*</td>
<td>77.72±9.80*</td>
</tr>
<tr>
<td>CPF</td>
<td>6.23±3.12‡</td>
<td>47.78±2.43*</td>
<td>0.14±0.06‡</td>
<td>24.76±0.66*</td>
<td>81.56±12.70*</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 10 animals/group. Weight gain, cumulative food intake, and food efficiency are given for the growth hormone (GH) treatment period (days 7–13). Body length and longitudinal growth rate, assayed by the osseous front advance over the last 3 days of the protocol, were measured at death. *Statistically different from control group. †Statistically different from KD group. ‡Statistically different from KDGH group. All P < 0.05.
Diagnostics, Barcelona, Spain) was incubated for 30 min at room temperature. The final reaction product was revealed with 3–3’-diaminobenzidine in 50 ml of 0.05 M Tris-hydrochloric buffer plus 50 µl of 30% hydrogen peroxide. Sections were counterstained with methyl green.

Liver mRNA. Liver mRNA was extracted as previously described (6), and the hepatic mRNA expression of IGF-I, GH, and IGF-I receptor genes was measured by qPCR as described above.

Statistical analysis. The results of each group are given as means ± SD. Differences between groups were assessed by ANOVA followed by the Student-Newman-Keuls method. For two-group comparisons, Student’s t-test was used. Statistical analysis of real-time PCR results was performed by ANOVA followed by the Student-Newman-Keuls test for comparison of each group with the control group and with DK group. A P value ≤0.05 was considered indicative of statistical significance.

RESULTS

Blood biochemistry. As shown in Table 1, the groups receiving a low-potassium diet (KD) and low-potassium diet with GH treatment (KDGH) were markedly hypokalemic and had metabolic alkalosis. No differences in the degree of hypokalemia or in the severity of alkalosis were found between KD

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A: osseous front advance in the proximal tibias of the 4 groups of animals. The distance between white lines (arrows), which indicate the metaphysial end of growth cartilage and the proximal end of calcein label, represent the osseous front advance over a 3-day interval. B: heights of growth cartilage and its hypertrophic stratum in the proximal tibias of the 4 groups of animals. White lines delineate both the proximal and distal ends of growth cartilage, as well as the transition between proliferative and hypertrophic zones (middle line). C: proliferative activity in the growth cartilage of the proximal tibias of the 4 groups of animals, as assessed by bromodeoxyuridine labeling (arrow). D: osteoclastic activity in the primary spongiosa of the proximal tibiae of the 4 groups of animals, as assessed by the tartrate-resistant acid phosphatase technique (arrow). Images in the same column belong to the same experimental group. KD, potassium depletion; KDGH, KD + growth hormone (GH) treatment; CPF, control pair-fed with KD.
and KDGH rats. Decreased concentrations of IGF-I were found in KD and KDGH rats and in normokalemic animals pair-fed with the KD group (CPF).

**Growth and nutrition.** Data on growth and nutrition of the four groups of animals are shown in Table 2. The KD animals were growth retarded as demonstrated by lower weight gain, body length, and longitudinal growth rate than those of the control group. KD animals ate less than controls fed ad libitum, and the anabolic use of consumed food was also lower. With respect to the CPF group, weight gain, food efficiency, and body length of the KD group tended to be lower, but the differences did not reach statistical significance. The osseous front advance of KD animals was lower than that of CPF rats (Fig. 1A). GH treatment exerted a beneficial effect on the nutrition of KDGH animals, as shown by greater weight gain and food efficiency than those of KD group, but did not improve longitudinal growth (Fig. 1A).

**Growth plate cell proliferation and histomorphometry.** Growth retardation of KD rats was associated with reduced heights of growth cartilage, hypertrophic stratum, and terminal chondrocytes in the proximal tibia growth plate compared with control and CPF normokalemic groups (Table 3). The percentage of proliferating chondrocytes and relative number of osteoclasts in the primary spongiosa were also depressed in KD rats. GH treatment normalized the height of growth cartilage, the height of the hypertrophic zones, the chondrocyte proliferation rate, and the relative number of osteoclasts but not the height of terminal chondrocytes (Fig. 1B–D).

**mRNA expression of IGF-I, the IGF-I receptor, and GH receptor in growth cartilage as analyzed by qPCR.** As shown in Table 4, potassium depletion downregulated IGF-I mRNA expression, while resulted in upregulation, over the control, of mRNA levels of the IGF-I receptor. Upregulation of GH receptor and IGF-I mRNAs was found in food-restricted normokalemic rats (CPF group). Modifications in the mRNA expression of IGF-I induced by potassium depletion was not further changed by GH treatment, but GH therapy induced greater upregulation of the IGF-I receptor.

**In situ hybridization of IGF-I, IGF-I receptor and GH receptor in growth cartilage.** In situ hybridization studies disclosed the patterns of mRNA distribution for the three analyzed genes in the four groups of animals (Fig. 2, A–C). IGF-I mRNA was located all over the growth cartilage, with a particularly intense signal in the proliferative and adjacent hypertrophic stratum. This pattern of distribution remained essentially unchanged in the four groups of rats. However, the proportion of labeled cells was apparently lower in the potassium-depleted groups (KD and KDGH). IGF-I receptor mRNA was mostly confined to the hypertrophic stratum in the four groups of rats. In agreement with the PCR results, the signal looked more intense in the KD and KDGH groups than in samples from normokalemic animals. The signal for GH receptor mRNA, in samples from the control, KD, and KDGH groups, was rather uniform in the hypertrophic area with isolated positive cells in the proliferative zone, whereas in CPF rats the signal spread throughout the whole cartilage.

**Immunohistochemistry of IGF-I and the IGF-I receptor in growth cartilage.** Immunohistochemistry for IGF-I and the IGF-I receptor in growth cartilage. Immunohistochemistry for IGF-I and the IGF-I receptor in growth cartilage revealed a similar distribution pattern for both proteins. Both of them were found mostly confined to the hypertrophic zone in the four groups of rats. (Fig. 2, D and E).

mRNA expression of IGF-I, the IGF-I receptor, and GH receptor in the liver as analyzed by qPCR. As shown in Table 5, in the KD group the levels of GH receptor and IGF-I mRNA expression did not change with respect to control, whereas the IGF-I receptor was upregulated. In CPF rats, GH receptor and IGF-I receptor mRNA levels were increased but the mRNA expression of IGF-I was not modified. Treatment with GH increased the mRNA expression of IGF-I in KDGH rats, but it did not further stimulate the IGF-I receptor and did not change GH receptor mRNA levels.

**DISCUSSION.** This study discloses the alterations in the structure and dynamics of the long-bone growth plate in an experimental model of potassium depletion and provides novel information on the mRNA expression of genes of the GH-IGF-I axis in the growth cartilage of hypokalemic animals. Furthermore, the study offers some insight into the molecular mechanism of the resistance to GH treatment in hypokalemia.

As shown in former experimental studies, sustained reduction in potassium intake causes hypokalemia and metabolic alkalosis and leads to growth retardation, as confirmed in our study by the low weight gain, length, and osseous front advance of KD animals compared with control rats (27, 30, 37, 40).

Table 3. Histological analysis of growth plate in the 4 groups of rats

<table>
<thead>
<tr>
<th>Cartilage Height, μm</th>
<th>Hypertrophic Zone Height, μm</th>
<th>Chondrocyte Height μm</th>
<th>BrdU Labeling, %</th>
<th>TRAP+Cells/100 Terminal Chondrocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>291.83±28.65</td>
<td>157.22±22.97</td>
<td>23.49±4.85</td>
<td>31.94±4.24</td>
</tr>
<tr>
<td>KD</td>
<td>228.26±23.58*</td>
<td>123.68±13.49</td>
<td>20.80±2.39</td>
<td>26.47±4.82*</td>
</tr>
<tr>
<td>KDGH</td>
<td>264.91±17.33†</td>
<td>154.61±12.53†</td>
<td>21.56±2.62*</td>
<td>33.15±3.21†</td>
</tr>
<tr>
<td>CPF</td>
<td>264.21±21.77†</td>
<td>153.18±15.80†</td>
<td>24.21±5.86†</td>
<td>22.19±4.42†</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 10 animals/group; histomorphometry, chondrocyte proliferation, and osteoclastic activity. BrdU, bromodeoxyuridine; TRAP, tartrate-resistant acid phosphatase. *Statistically different from control group. †Statistically different from KD group. ‡Statistically different from KDGH group. All P < 0.05.

Table 4. mRNA expression of IGF-I and receptors of GH and IGF-I, assessed by quantitative PCR, in the growth plate of the 4 groups of animals

<table>
<thead>
<tr>
<th></th>
<th>IGF-I (Fold Change)</th>
<th>IGF-I Receptor (Fold Change)</th>
<th>GH Receptor (Fold Change)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1±0.02</td>
<td>1±0.03</td>
<td>1±0.20</td>
</tr>
<tr>
<td>KD</td>
<td>0.59±0.09*</td>
<td>2.18±0.07*</td>
<td>1.53±0.14</td>
</tr>
<tr>
<td>KDGH</td>
<td>0.59±0.07*</td>
<td>3.15±0.13†</td>
<td>1.79±0.15*</td>
</tr>
<tr>
<td>CPF</td>
<td>1.94±0.07†</td>
<td>1.00±0.33†</td>
<td>2.75±0.08†</td>
</tr>
</tbody>
</table>

Values are means ± SD of gene fold-change in 3 groups relative to the control animals normalized by the GAPDH housekeeping gene expression levels; n = 5 animals/group. *Statistically different from control group. †Statistically different from KD group. All P < 0.05.

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38). The KD rats ate less food than controls fed ad libitum. 
Comparison of KD rats with the normokalemic CPF group 
showed that the reduction in weight was mostly due to the low 
food consumption because weight gain and food efficiency 
were not significantly different between both groups. By con-
trast, potassium depletion decreased the osseous front advance 
more in KD than in CPF rats, showing that potassium depletion 
adversely impairs longitudinal growth by mechanisms not 
totally dependent on food intake reduction. The finding that 
the body length of hypokalemic animals at the time of death 
was not significantly lower than that of CPF animals does not 
contradict the previous statement since a longer period of 
potassium depletion had likely led to significant differences.

In agreement with the specific effect of potassium depletion 
on longitudinal growth, the growth plate of KD animals had 
structural abnormalities not found in the CPF group. Thus 
potassium deficiency reduced growth cartilage height as a 
result of a shorter hypertrophic stratum, but not of a decreased 
chondrocyte proliferation. However, modifications in the size 
of growth cartilage have a limited pathophysiological value 
because variable chondrocyte column heights can be found 
with different longitudinal growth velocities (7). The growth 
cartilage size depends on the balance between two virtual 
vectors with antagonistic sense: one that goes from epiphysis to 
metaphysis and represents the production, proliferation, and 
hypertrophy of chondrocytes, and another one that goes from
metaphysis to epiphysis and represents the vascular invasion phenomenon and cartilage replacement by new bone (28). This apposition bone vector reflects the longitudinal growth rate, and the association of reduced growth velocity and diminished cartilage height, as happened in the KD group, suggests that the process of progression and hypertrophy of chondrocytes became more damaged than the rate of new bone formation. It is also of note that the height of chondrocytes adjacent to the metaphyseal bone was markedly decreased in the KD rats, confirming the profound interference of potassium depletion with the normal process of chondrocyte maturation and hypertrophy. A positive correlation between longitudinal growth velocity and terminal chondrocyte height has formerly been shown (4). In agreement with the reduction of growth rate, the osteoclastic activity, as assessed by the number of TRAP-positive cells in the primary spongiosa, was markedly diminished in the KD animals. By contrast, the proliferative activity of chondrocytes was similarly decreased in KD and CPF animals, indicating that it was not specifically affected by the potassium deficiency.

Our study confirms previous findings of Schaefer et al. (30) on the dissociation of the effect of GH treatment on weight and length in potassium-depleted rats. GH treatment resulted in a significant anabolic effect demonstrated by increased weight and food efficiency, but did not accelerate longitudinal growth rate of KDGH rats compared with the KD group. It is interesting to note that, to a large extent, GH restored the morphological aspect of the growth plate because the height of the growth cartilage and that of the hypertrophic zone, as well as the osteoclastic and proliferative activities, were not different from controls in the GH-treated, potassium-depleted rats. However, GH did not modify the size of the most distal chondrocytes, emphasizing the relationship of this parameter with the growth velocity and the failure of GH to correct the hypokalemia-induced defect in the process of chondrocyte hypertrophy. This differs from the effect of GH therapy on uremic rats, in which GH treatment has been shown to increase both longitudinal growth rate and terminal chondrocyte height (19).

The findings of the study presented here on the mRNA expression of the GH receptor, IGF-I receptor, and IGF-I in the growth plate shed some light on the mechanism of growth retardation in potassium depletion and the lack of GH effect in this setting. As shown by qPCR, potassium depletion down-regulated IGF-I mRNA expression despite simultaneous up-regulation of the IGF-I receptor, likely due to a compensatory mechanism. In situ hybridization and immunohistochemistry provide valid information on the spatial distribution of a gene but are not sensitive techniques for assessing quantitative changes in tissue gene mRNA expression. Our results indicate that IGF-I was mainly expressed in the early hypertrophic zone in the four groups of rats, although the signal and the proportion of positive cells were visually lower in the potassium-depleted animals. The histomorphometric and gene expression studies suggest that potassium depletion leads to profound disturbances in the process of growth plate chondrocyte maturation and reduced production of IGF-I by hypertrophic chondrocytes. These alterations likely play a key role in the growth retardation of hypokalemic disorders. The administration of GH normalized the height of the growth plate and the proliferative activity of chondrocytes but did not accelerate the longitudinal growth rate, did not restore to normal the size of the terminal chondrocytes, and did not increase the local expression of IGF-I mRNA. We did not investigate whether this resistance to GH treatment was mediated by alterations in the GH-activated signaling pathway as it has been found in the liver of potassium-depleted rats (30). According to Schaefer et al. (30), the resistance to GH in potassium depletion cannot be accounted for by alterations in the Janus-associated kinase (JAK), signal transducers and activators of transcription (STAT) transduction pathway and presumably arises either because of a distal defect to the binding of STAT to DNA or, alternatively, because of a defect in a STAT-independent GH-activated signaling pathway.

The findings of our study on serum IGF-I and the hepatic expression of IGF-I mRNA and its receptor allow hypothesizing on the alterations of systemic IGF-I metabolism in potassium depletion. The coexistence of normal IGF-I mRNA and low serum IGF-I concentrations points to either a posttranscriptional defect in the synthesis of IGF-I or an accelerated catabolism of circulating IGF-I, although a transient increase in serum IGF-I not detected in our experiment, in which serum for IGF-I measurement was drawn 15 h after the last GH dose, cannot be ruled out. In addition, an increase in the low-molecular fractions of IGF binding proteins, such as described in potassium-depleted rats (14), might accelerate the transport of IGF-I out of the vascular bed. However, the upregulation of the IGF-I receptor is consistent with the already described stimulatory action of malnutrition and low circulating IGF-I on the mRNA expression of this receptor (39). Moreover, our results also show that GH treatment stimulated the expression of liver IGF-I mRNA but did not subsequently modify serum IGF-I levels, suggesting a mechanism for GH resistance distal to the activation of the IGF-I gene.

In summary, in an experimental model of growth retardation secondary to potassium depletion, our study demonstrates alterations in the structure and dynamics of the growth plate and marked changes in systemic and growth cartilage GH and IGF-I metabolism. It also shows that GH treatment does not increase longitudinal growth rate or serum IGF-I. In the growth plate, this lack of response to GH administration is associated with the persistence of the disturbed process of chondrocyte hypertrophy and depressed mRNA expression of local IGF-I.

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