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 hypokalemia (KD) being 67.07 ± 12.70 μm/day, respectively. GH treatment did not accelerate growth rate. The tibial growth plate of KD rats had marked morphological alterations: lower heights of growth cartilage (228.26 ± 23.58 μm), hypertrophic zone (123.68 ± 13.49 μm), and terminal chondrocytes (20.8 ± 2.39 μm) than normokalemic CPF (264.21 ± 21.77, 153.18 ± 15.80, and 24.21 ± 5.86 μm). GH administration normalized these changes except for the distal chondrocyte height. Quantitative PCR of insulin-like growth factor I (IGF-I), IGF-I receptor, and GH receptor genes in KD growth plates showed downregulation of IGF-I and upregulation of IGF-I receptor mRNAs, without changes in their distribution as analyzed by immunohistochemistry and in situ hybridization. GH did not further modify IGF-I mRNA expression. KD rats had normal hepatic IGF-I mRNA levels and low serum IGF-I values. GH increased liver IGF-I mRNA, but circulating IGF-I levels were increased by 10.22 ± 0.33.5 on October 21, 2017 http://ajprenal.physiology.org/ Downloaded from

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, Bartter’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 53.66 ± 5.40 g); potassium depletion + GH treatment (KDBGH), potassium-deficient diet given ad libitum and treatment with GH (initial body wt 53.67 ± 5.51 g); and control pair-fed with the KD group (CPF), normal diet but in the same amount as KD (initial body wt 53.63 ± 2.23 g). The study complied with current legislation on

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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 KD group. ‡Statistically different from KD 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±10.44*</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±4.23*</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, assessed 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

Fig. 1. 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 the growth plate 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,

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.
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 contrast, 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 entirely 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 epiphysis to metaphysis and represents the production, proliferation, and hypertrophy of chondrocytes, and another one that goes from
provide valid information on the spatial distribution of a gene mechanism. In situ hybridization and immunohistochemistry regulated IGF-I mRNA expression despite simultaneous up-

this setting. As shown by qPCR, potassium depletion down-

growth plate shed some light on the mechanism of growth

expression of the GH receptor, IGF-I receptor, and IGF-I in the

longitudinal growth rate and terminal chondrocyte height (19).

in which GH treatment has been shown to increase both

phy. This differs from the effect of GH therapy on uremic rats,

emia-induced defect in the process of chondrocyte hypertro-

cytes, emphasizing the relationship of this parameter with 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 interest-
ing to note that, to a large extent, GH restored the morpho-

logical 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. How-
ever, GH did not modify the size of the most distal chon-
drocytes, emphasizing the relationship of this parameter with the

growth velocity and the failure of GH to correct the hypokal-

eaemia-induced defect in the process of chondrocyte hypertro-

phy. 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 propor-
tion 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 mat-

uration and reduced production of IGF-I by hypertrophic chon-
drocytes. 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 prolif-

erative 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

due to 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 hypothesiz-
ing on the alterations of systemic IGF-I metabolism in potas-

sium depletion. The coexistence of normal IGF-I mRNA and

low serum IGF-I concentrations points to either a posttran-
scriptional 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.

Table 5. mRNA expression of IGF-I and receptors of GH and IGF-I, assessed by quantitative PCR, in the liver of the 4 groups of animals

<table>
<thead>
<tr>
<th></th>
<th>IGF-I</th>
<th>IGF-I Receptor</th>
<th>GH Receptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.0 ± 0.08</td>
<td>1.0 ± 0.04</td>
<td>1.0 ± 0.06</td>
</tr>
<tr>
<td>KD</td>
<td>0.78 ± 0.07</td>
<td>2.59 ± 0.48*</td>
<td>0.73 ± 0.2</td>
</tr>
<tr>
<td>KDGH</td>
<td>2.1 ± 0.03*†</td>
<td>2.13 ± 0.33*†</td>
<td>0.92 ± 0.23</td>
</tr>
<tr>
<td>CPF</td>
<td>1.1 ± 0.21</td>
<td>5.08 ± 0.51*†</td>
<td>2.06 ± 0.57*†</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.

GRANTS

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REFERENCES


