Regulation of renal NaPi-2 expression and tubular phosphate reabsorption by growth hormone in the juvenile rat

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Submitted 20 February 2002; accepted in final form 29 February 2004

Growth hormone (GH) is an important factor in the development and mechanisms by which GH regulates renal Pi uptake remain unclear and are the focus of the present study. Micropuncture experiments were performed after acute thyroparathyroidectomy in the presence and absence of parathyroid hormone (PTH) in adult (14- to 17-wk old), juvenile (4-wk old), and GH-suppressed juvenile male rats. While the phosphaturic effect of PTH was blunted in the juvenile rat compared with the adult, suppression of GH in the juvenile restored fractional Pi excretion to adult levels. In the presence or absence of PTH, GH suppression in the juvenile rat caused a significant increase in the fractional Pi delivery to the late proximal convoluted (PCT) and early distal tubule, so that delivery was not different from that in adults. These data were confirmed by Pi uptake studies into brush-border membrane (BBM) vesicles. Immunofluorescence studies indicate increased BBM type IIa NaPi cotransporter (NaPi-2) expression in the juvenile compared with adult rats. While the phosphaturic effect of PTH was blunted in the juvenile rat compared with the adult, suppression of GH in the juvenile restored fractional Pi excretion to adult levels. In the presence or absence of PTH, GH suppression in the juvenile rat caused a significant increase in the fractional Pi delivery to the late proximal convoluted (PCT) and early distal tubule, so that delivery was not different from that in adults. These data were confirmed by Pi uptake studies into brush-border membrane (BBM) vesicles. Immunofluorescence studies indicate increased BBM type IIa NaPi cotransporter (NaPi-2) expression in the juvenile compared with adult rats. While the phosphaturic effect of PTH was blunted in the juvenile rat compared with the adult, suppression of GH in the juvenile restored fractional Pi excretion to adult levels. In the presence or absence of PTH, GH suppression in the juvenile rat caused a significant increase in the fractional Pi delivery to the late proximal convoluted (PCT) and early distal tubule, so that delivery was not different from that in adults. These data were confirmed by Pi uptake studies into brush-border membrane (BBM) vesicles. Immunofluorescence studies indicate increased BBM type IIa NaPi cotransporter (NaPi-2) expression in the juvenile compared with adult rats.

However, whether regulation of type IIa NaPi transporters is responsible for the increases in type IIa NaPi protein expression in the proximal tubular apical brush-border membrane (BBM) is unclear. Growth hormone (GH) plays a central role during the growth process and has been shown to be a factor that increases the renal uptake of Pi. When GH is elevated, or administered on a chronic basis to adult humans or adult animals, there is a reduction in urinary Pi excretion and elevation in plasma Pi levels. Conversely, removal of GH in the adult rat (through hypophysectomy) causes a significant decline in the maximum capacity to reabsorb Pi (TmPi) by the whole kidney and results in increased phosphaturia. This effect could not be attributed solely to GH because all pituitary hormones were removed. Furthermore, Hammerman et al. (16) reported that the effects of pharmacological doses of GH result in a selective stimulation of proximal tubular BBM NaPi transport systems.

Mulroney et al. (27, 28), using a peptic antagonist to GH-releasing factor, [N-acetyl-Tyr1-d-Arg2]-GRF-(1-29)-NH2 (GRF-AN), to suppress the pulsatile release of GH from the anterior pituitary, determined the physiological role of GH in Pi homeostasis. Administration of GRF-AN to juvenile animals for 2 days led to doubling of the urinary excretion of Pi, and attenuation of somatic body growth (19). These effects were attributed to a decrease in the TmPi (19, 27) and highlighted an important interrelationship among GH, growth, and the renal reabsorption of Pi. Interestingly, short-term GRF-AN treatment of adult rats had no effect on lowering the TmPi and increasing urinary Pi excretion (19, 27). In addition, our laboratory has demonstrated that GRF-AN treatment for 48 h is associated with a 30% reduction in the Vmax of Pi transport in proximal tubular BBM vesicles prepared from weanling rats, implicating GH.
in proximal tubule Pi uptake. However, the nephron sites of GH action and the effect of GH on type IIa NaPi transporter expression in the proximal convoluted tubule (PCT) and proximal straight segments in the juvenile rat are unknown.

The present micropuncture and renal cortical BBM Na gradient-dependent Pi uptake studies were performed to determine the nephron sites of action of GH on renal tubular Pi reabsorption seen in juvenile rats. In addition, using Western blot analysis and immunofluorescence microscopy, we assessed the role of GH in regulating the expression of proximal tubular BBM type IIa NaPi transporters in the juvenile rat.

MATERIALS AND METHODS

Animal models. To explore the role of GH on the nephron sites responsible for the increased renal phosphate reabsorption seen during growth, micropuncture studies were performed in adult (14- to 17-wk old) and juvenile (4-wk old) male Wistar rats. The rats were fed a normal-phosphate diet (0.7% P3) and allowed access to diet and water ad libitum. In juvenile rats, suppression of the pulsatile release of GH was achieved through intravenous administration of the GH-releasing hormone antagonist GRF-AN (Bachem, Torrance, CA) at a dose of 100 μg/kg twice daily. Previous studies in this laboratory have demonstrated this dosage to be effective in completely blocking pulsatile GH release (19, 27, 28). Briefly, juvenile rats were anesthetized with Nembutal (0.1 ml/100 g ip), and a Silastic catheter (inner diameter, 0.020 in.; outer diameter, 0.037 in.; Dow Corning, Medfield, MA) filled with 500 U/ml heparinized saline was placed into the left jugular vein. Patency of the catheter was maintained with 200 μl of 500 U/ml heparinized saline daily. The animals were housed in separate cages and injected twice daily at 0900 and 1300 for 2 days with saline or GRF-AN. Body weight was measured throughout the experimental protocol, and the reduced growth rate was used as an indicator of the suppression of pulsatile GH release.

The acquisition of the data for the three experimental groups was performed in parallel. The data for the adult and juvenile rats have been previously published (37). This manuscript includes parts of the previously published data as controls to contrast the effects of GH suppression and replacement on the regulation of renal tubular reabsorption of Pi in the juvenile rat.

In vivo micropuncture studies. On the day of the micropuncture experiments, the animals were anesthetized with an intraperitoneal injection of Inactin (80 mg/kg; Promonta, Hamburg, Germany) and placed on a heated table. Body temperature was maintained at 37 ± 0.5°C with a servo-controlled heat lamp (Yellow Springs Instruments, Yellow Springs, OH) and a rectal probe. The animals were acutely thyroparathyroidectomized (TPTX) using heat cautery to remove the influence of endogenous circulating PTH, and a tracheostomy was performed to allow the animals to breathe spontaneously. TPTX was considered successful when the basal urinary phosphate excretion was <1%. Polyethylene tubing was inserted into the left carotid artery (PE-50) for blood pressure measurements (Digimed BP analyzer) and arterial plasma sampling, the right jugular vein (PE-50) for infusions of insulin, and into the bladder (PE-90) for urine collections. The left kidney was prepared by making a flank incision at the left subcostal margin and dissecting the kidney free from the surrounding fat tissue without disturbing the adrenal glands. Next, the kidney was placed in a Lucite cup and fixed with cotton to prevent any movement with each breath. Warmed isotonic saline was dripped on the kidney (to prevent drying), and the animals were infused with a 2.5%ulin solution at 3% body wt (BW)/h and allowed to recover for 2 h to reach a steady state. Multiple tubular fluid samples were collected in the absence of PTH, from the last accessible site of the proximal convoluted tubule and the earliest accessible region of the distal convoluted tubule. The micropipettes were made with a sharpened tip diameter of 5–8 μm and contained light mineral oil dyed with Sudan black. Lissamine green (5%) was injected intravenously (0.1 ml) to facilitate the identification of distal convoluted segments. After proximal and distal collections, tubular fluid samples were collected in the presence of PTH. PTH (rat 1-34, Bachem, King of Prussia, PA) was administered as a bolus (45 μg/100 g BW), followed by a maintenance infusion (15 μg·100 g BW−1·h−1) as previously reported (20, 28). While intravenous infusion of PTH caused a rapid fall in mean arterial pressure (MAP), tubular collections were made after MAP returned to control levels. Urine collections were made every 30 min throughout the experiment, and a blood sample was taken at the midpoint of each clearance.

BBM isolation. Intact and GRF-AN-treated juvenile rats were anesthetized via an injection of pentothal sodium (100 mg/kg ip), and the kidneys were removed for BBM isolation. The superficial cortex (SC) and outer juxtamedullary cortex (JMC) were dissected and homogenized in 15 ml of an isolation buffer consisting of (in mM) 300 mannitol, 5 EGTA, 1 PMSF, 16 HEPES, and 10 Tris, pH 7.5. BBM from both regions were isolated from the homogenerate by Mg2+ precipitation and differential centrifugation as described previously (24, 26). The resulting BBM pellet was resuspended in a buffer of (in mM) 300 mannitol, 16 HEPES, and 10 Tris, pH 7.5, and aliquoted for NaPi transport measurement and Western blotting.

BBM phosphate transport activity measurement. BBM phosphate transport activity measurements were performed in freshly isolated SC-BBM and JMC-BBM vesicles by radiotracer uptake followed by rapid Millipore filtration. To measure Na gradient-dependent 32P uptake (NaP3, cotransport), 10 μl of BBM preloaded in an intravesicular buffer comprising (in mM) 300 mannitol, 16 HEPES, and 10 Tris, pH 7.5, were vortexed at 25°C with 40 μl of an extravesicular transport buffer consisting of (in mM) 150 NaCl, 16 HEPES, and 10 Tris, as well as 100 μM K3HPO4, pH 7.5. The final concentration of the buffer plus vesicles was (in mM) 120 NaCl, 80 P3, 12.8 HEPES, and 8 Tris, as well as 80 μM K3HPO4, pH 7.5. All BBM vesicles were handled in the same manner. Uptake after 10 s (representing the initial linear rate) was terminated by an ice-cold stop solution. All uptake measurements were performed in triplicate, and uptake was calculated on the basis of specific activity determined in each experiment and expressed as picomoles 32P per 10 seconds per milligram BBM protein.

SDS-PAGE and Western blot analysis. Samples of SC-BBM and JMC-BBM were denatured for 2 min at 95°C in 2% SDS, 10% glycerol, 0.5 mM EDTA, and 95 mM Tris·HCl, pH 6.8 (final concentrations). Ten micrograms of BBM protein/lane were separated on 9% polyacrylamide gels and electrotransferred onto nitrocellulose paper. After blockage with 5% nonfat milk powder (fat, 0.5%), the membranes were incubated with antiserum against type IIa NaPi transporter (1:20,000, previously published data as controls to contrast the effects of GH suppression and replacement on the regulation of renal tubular reabsorption of Pi in the juvenile rat.

Immunofluorescence microscopy. To assess the role of GH in the regulation of renal type IIa NaPi transporter protein, immunofluorescence microscopy was performed for adult, juvenile, and GRF-AN-treated juvenile male Wistar rats fed a normal-phosphate diet. In a separate group of chronically GH-suppressed juvenile rats, pulses of rat GH were exogenously administered at 1000, 1400, and 1800 daily to mimic three GH pulses. The animals were anesthetized using thiopental (pentothal sodium, 100 mg/kg BW ip), and a catheter (PE-190 in adults and PE-60 in juveniles) was inserted into the abdominal aorta below the renal arteries. The kidneys were fixed in vivo by perfusion with a fixative buffer in a retrograde fashion into the renal arteries. The fixative buffer consisted of 0.1% glutaraldehyde, 3% paraformaldehyde, 1% sucrose, and 0.1 M sodium phosphate, pH 7.4.
the PE tubing are flame sealed, and the sample is vigorously mixed for several minutes and then finally heated in a water bath for 2 h at 37°C to allow for the color reaction to take place. The sample is removed, injected into an injection port on the flow-through microcolorimeter, and run through the machine at a speed of 13.3 μl/min. Inside the machine is a glass cuvette with a light source on one side and a photodiode receptor and 640-nm-wavelength filter on the other. As the sample passes through the glass cuvette, deflection of the amount of light hitting the photoreceptor occurs, and this is seen as a change in voltage. The concentration of tubular fluid phosphate is assessed against a standard curve.

The glomerular filtration rate (GFR) was equated with the clearance of inulin, and the fractional delivery of Pi, {(TF/P) Pi}/[(TF/P) inulin] at the two nephron sites was assessed under the different experimental conditions.

**Statistical analysis.** Statistical comparisons between groups were made using one-way analysis of variance with Student-Newman-Keuls analysis. Results are reported as means ± SE with significance designated at *P < 0.05.*

**RESULTS**

**BBMV NaPi transport studies.** NaPi transport activity was significantly higher in BBMV prepared from SC and outer JMC of juvenile compared with adult rats. After 2-day GH suppression in the juvenile rat, there was a significant reduction in BBMV NaPi uptake at both the SC and JMC to levels observed in the adults (Fig. 1).

*In vivo experiments in the presence of exogenous PTH.*** Table 1 provides the experimental parameters in the presence of PTH. MAP was significantly higher in the adults compared with the intact- and GH-suppressed juvenile animals and was maintained throughout the study. Plasma Pi concentrations were significantly higher in juvenile animals compared with adult animals, and GRF-AN treatment reduced plasma Pi levels observed in the adults (Table 1). As expected, GFR was significantly greater in the adult rats compared with the juvenile rats. While the intact juvenile rats had a significantly lower fractional Pi excretion compared with adult rats, fractional Pi excretion was significantly elevated to levels observed in the adult rats when GH was suppressed in the juvenile animals (Table 1).

Table 2 depicts the results of micropuncture experiments from both the late proximal and early distal tubule (eDT). While there were no differences in the single-nephron GFR (SNGFR) in the juvenile groups, it was significantly lower compared with that in adult animals. However, the tubular fluid-to-plasma inulin ratio values from both the late PCT and eDT were consistent between the groups, indicating a similar fractional reabsorption of filtered water up to those puncture sites. Suppression of GH led to a significant increase in the fractional delivery of Pi to the late PCT compared with juvenile

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**Table 1. Parameters of renal function in GH-suppressed juvenile rats in the presence of PTH**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>BW, g</th>
<th>MAP, mmHg</th>
<th>Plasma Pi, mM</th>
<th>GFR, ml/min</th>
<th>FE(Pi), %</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-NPD</td>
<td>6</td>
<td>438±24</td>
<td>119±8</td>
<td>2.45±0.1</td>
<td>4.26±0.5</td>
<td>39.1±2.5</td>
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<tr>
<td>J-NPD</td>
<td>5</td>
<td>128±5*</td>
<td>93±4*</td>
<td>2.93±0.2*</td>
<td>1.26±0.1*</td>
<td>23.27±2.5*</td>
</tr>
<tr>
<td>J-NPD+GRF-AN</td>
<td>7</td>
<td>134±4*</td>
<td>98±4</td>
<td>2.04±0.1*</td>
<td>1.43±0.1*</td>
<td>40.5±4.7</td>
</tr>
</tbody>
</table>

Values are means ± SE. n, no. of rats; GH, growth hormone; PTH, parathyroid hormone; BW, body wt; MAP, mean arterial pressure; GFR, glomerular filtration rate; FE(Pi), fractional P Pi excretion; NPD, normal-P Pi diet; J-NPD and A-NPD, juvenile and adult rats on NPD, respectively; GRF-AN, [N-acetyl-Tyr'-d-Arg']-GRF-(F29)-NH2. Data for A-NPD and J-NPD were previously published in Ref. 37. *P < 0.05 vs. A-NPD. †P < 0.05 vs. J-NPD.
controls (Table 2). This indicates a role for the normal pulsatile circulating GH in the enhanced reabsorption of P<sub>i</sub> uptake in the juvenile rat. Moreover, while there was a significant decline in the delivery of P<sub>i</sub> to the eDT in the juvenile control rat, GH suppression in the juvenile rats blocked this effect. Interestingly, the delivery of P<sub>i</sub> to the eDT in the GRF-AN-treated juvenile rats was similar to that seen in the adult animals. This finding strongly supports a role for GH in the regulation of P<sub>i</sub> reabsorption along the proximal straight tubule, which contributes to the attenuation of the phosphaturic effect of PTH in the juvenile animal.

In vivo experiments in the absence of endogenous PTH. To assess the intrinsic P<sub>i</sub> transport in the GH-suppressed juvenile rat, micropuncture experiments were performed after acute TPTX. Table 3 provides values of various functional parameters in the absence of PTH. MAP was significantly higher in the adult rats compared with intact juveniles but was not significantly different vs. the GRF-AN-treated juvenile animals. Although the plasma P<sub>i</sub> concentration was significantly higher in the juvenile rats, there was no difference between the adult and GH-suppressed juvenile animals. As expected, after TPTX fractional P<sub>i</sub> excretion fell to \( \leq 1\% \) and was not significantly different between the groups.

Table 4 contains the results of micropuncture experiments from both the late PCT and eDT. Although adult rats had a significantly higher SNGFR compared with the juvenile groups, the tubular fluid-to-plasma inulin ratio values from both the PCT and eDT were consistent between the groups, indicating similar fractional water delivery. The significantly lower fractional delivery of P<sub>i</sub> to the late PCT of juvenile controls compared with adult animals was completely inhibited by GH suppression in the juvenile rats (Table 4). This provides further evidence that pulsatile GH secretion modulates P<sub>i</sub> reabsorption in the PCT. In addition, while there was a significant decline in the delivery of P<sub>i</sub> to the eDT in the juvenile rats compared with adults, there was no change in the delivery of P<sub>i</sub> between the PCT and eDT of GH-deprived juvenile rats. Again, this finding supports the role of GH in increasing P<sub>i</sub> reabsorption in the proximal straight tubules of the juvenile rat.

### DISCUSSION

The present study demonstrates that pulsatile GH release is a key factor in the enhanced proximal tubular phosphate reabsorption observed in the juvenile rat. The micropuncture studies indicate for the first time that GH is responsible, independently of PTH, for the enhanced P<sub>i</sub> uptake in both the PCT and straight tubules of juvenile rats on a normal-P<sub>i</sub> diet. In addition, circulating GH contributes significantly to the blunted phosphaturic response to PTH in the juvenile rat. The mecha-

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### Table 2. Micropuncture data obtained from the late proximal convoluted tubule and early distal tubule in GH-suppressed juvenile rats in the presence of PTH

<table>
<thead>
<tr>
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<th>Late Proximal Convoluted Tubule</th>
<th>Early Distal Convoluted Tubule</th>
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<tbody>
<tr>
<td></td>
<td>SNGFR, nl/min</td>
<td>TF/P (inulin)</td>
</tr>
<tr>
<td>A-NPD</td>
<td>50.4±5.3</td>
<td>1.9±0.2</td>
</tr>
<tr>
<td>J-NPD</td>
<td>17.8±1.9*</td>
<td>1.7±0.1</td>
</tr>
<tr>
<td>J-NPD+GRF-AN</td>
<td>20.7±1.1*</td>
<td>1.6±0.1</td>
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</table>

Values are means ± SE. SNGFR, single-nephron GFR; TF/P, tubular fluid-to-plasma ratio; FDP, fractional P<sub>i</sub> delivery. Data for A-NPD and J-NPD were previously published in Ref. 37. *P < 0.05 vs. A-NPD. †P < 0.05 vs. J-NPD.

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### Table 3. Parameters of renal function in GH-suppressed juvenile rats in the absence of PTH

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>BW, g</th>
<th>MAP, mmHg</th>
<th>Plasma P&lt;sub&gt;i&lt;/sub&gt;, mM</th>
<th>GFR, ml/min</th>
<th>FE&lt;sub&gt;i&lt;/sub&gt;, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-NPD</td>
<td>7</td>
<td>412±17</td>
<td>127±5</td>
<td>2.83±0.2</td>
<td>3.83±0.4</td>
<td>0.56±0.5</td>
</tr>
<tr>
<td>J-NPD</td>
<td>9</td>
<td>125±4*</td>
<td>98±4*</td>
<td>3.48±0.1*</td>
<td>1.24±0.1*</td>
<td>0.41±0.2</td>
</tr>
<tr>
<td>J-NPD+GRF-AN</td>
<td>9</td>
<td>131±2*</td>
<td>114±12</td>
<td>2.53±0.3†</td>
<td>1.52±0.2†</td>
<td>1.0±0.4</td>
</tr>
</tbody>
</table>

Values are means ± SE. n, No. of rats. Data for A-NPD and J-NPD were previously published in Ref. 37. *P < 0.05 vs. A-NPD. †P < 0.05 vs. J-NPD.
nism for the enhanced NaPi cotransport activity appears to be through the action of GH on the expression of proximal tubular BBM type IIa NaPi transporter protein in the juvenile rat, because suppression of the pulsatile release of GH in the juvenile rat significantly decreased BBM NaPi protein expression. The present findings indicate that GH plays a central role in the renal adaptation to reabsorb Pi seen during growth, by enhancing PCT and proximal straight tubule Pi reabsorption, significantly extending previous findings by Mulroney et al. (27, 28).

Rapidly growing neonatal and juvenile animals have enhanced proximal tubular Pi reabsorption, and it is postulated that the proximal straight tubule may contribute to the reclamation of filtered Pi (22, 27, 28). The present study confirms that Pi uptake in both the PCT and the proximal straight tubule is enhanced in the juvenile rat, and moreover, that the significant uptake in these segments is stimulated by circulating GH. This is in contrast to that observed in the adult animal, where these sites are only upregulated when Pi is conserved, such as with TPTX, dietary Pi deprivation, and respiratory alkalosis. Thus GH appears to selectively stimulate Pi reabsorption in these segments in the young. While it is clear that this effect of GH on Pi reabsorption is crucial for the proper growth and development of the young animal (19, 27, 28), the mechanism enhancing the sensitivity of the kidney to GH is unknown. GH receptor mRNA has been localized to the proximal straight tubule (7), but developmental differences in GH receptor expression have not been reported. Thus it is unclear whether

<table>
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<tbody>
<tr>
<td></td>
<td>SNGFR (nl/min)</td>
<td>TF/P (inulin)</td>
</tr>
<tr>
<td>A-NPD</td>
<td>48.7±1.9</td>
<td>1.9±0.1</td>
</tr>
<tr>
<td>J-NPD</td>
<td>13.9±1.3*</td>
<td>1.7±0.1</td>
</tr>
<tr>
<td>J-NPD + GFR-AN</td>
<td>18.8±1.2*</td>
<td>1.7±0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE. Data for A-NPD and J-NPD were previously published in Ref. 37. *P < 0.05 vs. A-NPD. †P < 0.05 vs. J-NPD.

Fig. 2. Immunofluorescence microscopy of the type IIa NaPi cotransporter in parathyroid gland intact adult, juvenile, and GH-suppressed juvenile rats. Treatment of juvenile rats with GFR-AN (J-NPD + GFR-AN) reduced the expression of BBM NaPi protein to levels observed in the adults (A-NPD). Exogenous GH pulses to GH-suppressed juvenile rats (J-NPD + GFR-AN + GH) increased the expression of NaPi-2 transporters to levels seen in the juvenile controls. A-NPD, adult; J-NPD: juvenile; J-NPD + GFR-AN: juvenile + GFR-antagonist; J-NPD + GFR-AN + GH: juvenile + GFR-antagonist + growth hormone.
an upregulation of GH receptors is responsible for the enhanced sensitivity of the juvenile kidney to the effects of GH. The age-related differences in P_i reabsorption within the proximal convoluted and proximal straight nephron segments are also consistent with previous micropuncture and BBM vesicle NaPi cotransport activity studies in our laboratory (37). In addition, the findings in the GH-suppressed juvenile rat confirm previous data showing that proximal tubular BBMV prepared from GRF-AN-treated juvenile rats had a significant reduction in V_{max} of Na-dependent P_i reabsorption compared with juvenile controls. Na-proline uptake in these studies was unaffected by GRF-AN administration, highlighting the apparent specificity to NaPi cotransporters. Because both GH and IGF-I receptor mRNA have been localized on the apical surface of proximal tubular cells, this provides evidence for a direct and/or indirect action of the GH/IGF-I axis on P_i uptake in the proximal segments (7, 15, 17, 32).

The fact that chronic suppression of pulsatile GH release in the juvenile rats significantly decreases P_i reabsorption in the proximal tubule does not address whether GH acts directly or indirectly on the tubules. It is clear that both GH and IGF-I can enhance P_i reabsorption, and it may be that GH acts both directly and indirectly through IGF-I to modulate renal P_i reabsorption in the juvenile rat. In rabbit proximal convoluted tubular segments, Quigley et al. (24) found that IGF-I, but not GH, stimulated P_i uptake. Furthermore, administration of IGF-I to the media of opposum kidney cells (2) or proximal tubular BBMV prepared from IGF-I-treated hypophysectomized rats (5) showed that this was associated with an increase in V_{max} for Na-dependent P_i transport. We have also reported that both GH and IGF-I were able to prevent the rapid decrease in TmP_i observed in GRF-AN-treated juvenile rats. The notion that suppression of GH causes a rapid (within 3 h) reduction in P_i transport suggests a direct action of GH on NaPi transporters, because an indirect route through reductions in renal IGF-I would probably take longer. Also, GH receptor mRNA has been localized to the proximal straight tubule (7), which may again point to a direct effect of GH on NaPi protein expression. The evidence from micropuncture, BBMV NaPi transport activity studies, and NaPi protein abundance clearly links circulating GH with the enhanced renal P_i reabsorption in the growing animal and indicates that NaPi may be a target for GH. However, the intracellular mechanism by which GH/IGF-I stimulates NaPi in proximal tubular cells remains unknown.

In summary, phosphate is crucial to the proper development of the rapidly growing juvenile rat. The enhanced renal P_i reabsorption seen during this state of high-P_i conservation occurs predominantly in the proximal convoluted and proximal straight tubule. Our findings indicate that pulsatile GH release plays a key role in the enhanced P_i reabsorption at these segments through modulation of BBM type IIa NaPi transporter expression.

Perspectives. It is clear that GH is a key regulator of P_i homeostasis in the juvenile animal by facilitating the avid reabsorption of P_i. This is crucial for the rapidly developing animal, because limiting P_i supply (via dietary deprivation) or transporter activity severely attenuates growth. Defining the sites of action of GH on the renal tubule is an important step in understanding the regulation of NaPi transporters and allows speculation as to the potential for GH to regulate NaPi in adults. Indeed, our findings that P_i transporters and TmP_i are dramatically reduced in the senescent animal (20, 24), coincidentally with decreases in circulating GH, suggest that one mechanism for the “anti-aging” effect of GH may be through increasing P_i transporters in the kidney and perhaps other tissues. The current findings provide strong support for future work on the effects of GH on P_i transport.

GRANTS

These studies were supported by National Science Foundation Grant IBN-95-11677, National Institute on Aging Grant NIAID-AG-18634-01, an American Heart Association Established Investigator Award (to S. E. Mulroney), and the Veterans Affairs Medical Research Service (to M. Levi).
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