Growth Hormone and Renal Glutamine and Glutamate Handling

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ABSTRACT

Growth hormone administration effects a positive nitrogen balance in part by recycling glutamine nitrogen as glutamate at the expense of ureagenesis. The study presented here focuses on the response of the isolated perfused hypophysectomized rat kidney to acute growth hormone administration during infusions of either glutamine or glutamate. Growth hormone at 50 nM acutely decreases the renal utilization of both glutamine and glutamate while enhancing reabsorption of the latter. During glutamine infusions of either 1,000 or 500 nmol/min, growth hormone markedly reduced net glutamine utilization by 55% at the high loads and reversed utilization to release at the lower load; associated with decreased glutamine utilization was reduced ammonium production and increased glutamate release. Although glutamate reabsorption was unchanged, glutamate reabsorption increased and NH₄⁺ excretion decreased. During glutamate infusion of 180 nmol/min, growth hormone reduced glutamate utilization 66%, the residual utilization matching increased glutamate production. Growth hormone-enhanced glutamate reabsorption was associated with enhanced bicarbonate reabsorption and a redistribution of NH₄⁺ release into the urine; all three responses were eliminated by amiloride. These responses to growth hormone are consonant with reduced glutamate oxidation underlying decreased glutamate utilization and accelerated luminal Na⁺-H⁺ exchange mediating luminal transport, events that are conceivably interrelated.

Key Words: Growth hormone, kidney function, glutamate handling

Growth hormone administration results in a positive nitrogen balance and accelerated growth, reflecting the redistribution of nitrogen from ureagenesis into protein (1,2). Because interorgan glutamine fluxes constitute the major form of nitrogen transfer (3), attention was directed to the hepatic utilization of glutamine and conversion to urea. Surprisingly, growth hormone reduced ureagenesis not by decreasing hepatic glutamine extraction, but rather by reversing glutamate utilization to release (4). The fate of nitrogen recycled as glutamate has not been elucidated, although hindquarter utilization has been shown to increase (5). As a consequence, organs such as the kidneys are presented with a large glutamine and, more importantly, glutamate load which raises a question as to renal handling. To this end, kidneys from hypophysectomized rats were isolated and perfused with an artificial plasma solution delivering glutamine and glutamate at the estimated in vivo rate. The handling of both nitrogen carriers was then compared with and without the addition of growth hormone. The results to follow show a profound effect of growth hormone on both glutamine and glutamate handling, with the primary action apparently on glutamate transport and metabolism influencing, in turn, glutamine utilization.

METHODS

Kidneys were obtained from hypophysectomized male Sprague-Dawley rats weighing between 230 and 270 g. Hypophysectomy was performed by the supplier (Harlan, Indianapolis, IN) via the pharyngeal approach and was checked for completeness by monitoring body and kidney wet weight; animals that gained weight or exhibited kidney weight greater than 0.49% of body weight were rejected. All experiments were carried out within 3 wk of hypophysec-
tomy with the animals maintained ad lib on rat chow and 5% dextrose as the drinking solution.

Kidneys were isolated as previously described (6) and were perfused with an artificial plasma solution containing (in mM): NaCl, 120; NaHCO₃, 18; KCl, 5; NaHPO₄, 2; CaCl₂, 1.2; MgSO₄, 1; and dextrose, 5; plus either an initial glutamine or glutamate concentration of 0.5 or 0.1 mM, respectively; the perfusate also contained dialyzed albumin (5% g%) and tracer amounts of radiolabeled inulin (¹⁴C methoxy labeled; DuPont, NEN Research Products, Boston, MA). Just before perfusion the perfusate was filtered, aerated with (O₂: CO₂, 95:5%), and adjusted to pH 7.40. Kidneys were perfused with 75 mL of perfusate for 90 min. After an initial 15-min equilibration period, five sequential 15-min clearance periods were observed with perfusate samples drawn at the midpoints; urine samples were collected in tared microfuge tubes at 15-min intervals. Glutamine (1,000 or 500 nmol/min) or glutamate (180 nmol/min) was infused into the perfusate reservoir throughout the perfusion time course. After 0.5 h, either 50 nM bovine growth hormone (Genentech, Inc., South San Francisco, CA) or 0.5 mL of vehicle (perfusion media) was added to the reservoir; when used, amiloride (0.75 mM) was added to the perfusate at 1 h. At the perfusion termination, the kidneys were clamped between blocks of dry ice and were promptly processed for analysis by HPLC (7). Perfusate and urine samples were analyzed for glutamate and glutamine by HPLC. NH₄⁺ was analyzed by enzymatic or colorimetric analysis (6), and total CO₂ was analyzed with a microgasometer (7). Radioactivity in the perfusate and urine was monitored by liquid scintillation spectrometry, and the GFR was calculated from the standard inulin clearance formula. Reabsorption rates for glutamate, glutamine, and bicarbonate were obtained from the filtered minus excretion rates; fractional excretion or release of glutamine, glutamate, and ammonium was estimated from the rate of rise in the perfusate minus the sum of the excreted and infused rates over the 30- to 60-min perfusion time course. Kidney glutamate content was expressed per gram wet weight based on the wet weight of the left nonperfused kidney (0.48 ± 0.02 g). Differences between renal handling of glutamine and glutamate were assessed from the average of the two 15-min periods over the 30- to 60-min interval for vehicle- and growth hormone-treated groups and from the 75- to 90-min interval for the growth hormone versus growth hormone plus amiloride-treated groups. The in vivo effect of growth hormone on glutamate reabsorption was studied as previously described (7); comparisons were made between time controls and growth hormone-administered animals over a 30-min clearance period. Differences between time control and growth hormone-treated kidneys were judged significant by t test at P < 0.05.

RESULTS

The effect of growth hormone administration on renal glutamate handling is presented in Table 1. The response appears qualitatively similar at both the high and low loads (1,000 and 500 nmol/min, respectively); glutamate utilization falls to only 45% of the time control at high load (Table 1A) and at the low load (Table 1B) reverses from net utilization to release. Accompanying this effect is an increase in glutamate appearance in the perfusate and a drop in ammonium production (Table 1). Renal glutamate levels were increased by growth hormone from 4,970 ± 210 to 7,225 ± 190 nmol/g wet wt (P < 0.025; N = 5) at 60 min in kidneys perfused with the low load. Thus, the effect of growth hormone is to acutely decrease glutamine utilization and ammonium production while increasing cellular glutamate levels and release into the perfusate.

The effect of growth hormone on glutamine, glu-

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**TABLE 1. Effect of growth hormone on glutamine utilization**

<table>
<thead>
<tr>
<th></th>
<th>Glutamine Infused</th>
<th>Removed</th>
<th>Glutamate Released</th>
<th>NH₄⁺ Released</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1,000</td>
<td>1,180 ± 162</td>
<td>58 ± 38</td>
<td>859 ± 31</td>
</tr>
<tr>
<td>Growth Hormone</td>
<td>1,000</td>
<td>534 ± 14°</td>
<td>123 ± 36</td>
<td>534 ± 14°</td>
</tr>
<tr>
<td><strong>B</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>500</td>
<td>522 ± 78</td>
<td>28 ± 12</td>
<td>494 ± 20</td>
</tr>
<tr>
<td>Growth Hormone</td>
<td>500</td>
<td>-100 ± 38°</td>
<td>108 ± 16°</td>
<td>206 ± 48°</td>
</tr>
</tbody>
</table>

* Results are means ± SE in nanomoles per minute from six kidneys per group with measurements taken over the 30-60 min time course.

* Growth hormone was added to an estimated 50 nM concentration.

* Different from time controls, P < 0.01. Minus sign signifies net release.

* Different from time controls, P < 0.05.
Growth hormone, and \( \text{NH}_4^+ \) excretion is depicted in Figure 1. Fractional excretion of glutamine was unaffected by growth hormone at both glutamine loads, reabsorption accounting for more than 90% of the filtered loads. In contrast, the effect on glutamate reabsorption was striking; fractional excretion rates were 27 ± 7 and 25 ± 3% at the high and low loads and fell to 12 ± 4 and 11 ± 3%, respectively \((P < 0.05)\). Growth hormone also increased ammonium excretion despite the fall in overall production because of a large shift in \( \text{NH}_4^+ \) release into the urine similar to that described in a previous report \((8)\); this effect was more striking at the lower load, the percent released into the urine rising from 49 ± 6 to 96 ± 6% \((P < 0.01)\). Amiloride eliminated the actions of growth hormone, with fractional excretion of glutamate and ammonium increasing and decreasing to 73 ± 6 and 38 ± 9% respectively; in contrast, glutamine fractional excretion remained unchanged at 9 ± 3%. Thus, growth hormone effects diametrically opposite shifts in glutamate and \( \text{NH}_4^+ \) distribution between the perfusate and urine.

Because growth hormone increased glutamate release while decreasing glutamine utilization and ammonium production, the effect on glutamate handling itself was studied during the infusion of glutamate (180 nmol/min; Table 2). Growth hormone reduced net glutamate utilization from 163 ± 14 to 58 ± 20 nmol/min \((P < 0.01)\) while increasing glutamine release from 34 ± 4 to 54 ± 5 nmol/min \((P < 0.05)\); as with glutamine, growth hormone markedly reduces ammonium production (from 207 ± 30 to only 29 ± 18 nmol/min; \(P < 0.01)\).

The influence of growth hormone on urinary excretion is presented in Figure 2 and in more detail for glutamate in Table 3. Fractional glutamate excretion was 29 ± 5% of the filtered load (Figure 2) and falls to 14 ± 3% with growth hormone, similar to the glutamine infusion studies. Noteworthy is the fact that administering amiloride virtually abolished the increase in glutamate reabsorption after growth hormone administration as it did for glutamine infusions; fractional excretion increased to 67 ± 9% (Figure 2), and reabsorption dropped from 96 ± 12 to 36 ± 8 nmol/min \((P < 0.01)\). Growth hormone also increased ammonium excretion from 120 ± 12 to 202 ± 17 nmol/min \((P < 0.01)\) as the percentage of total \( \text{NH}_4^+ \) released rose from 57 ± 8 to more than 100% (uptake occurring from the perfusate). Thus, despite enhancing luminal glutamate reabsorption, the overall effect of growth hormone is to reduce glutamate utilization, specifically that coupled to ammoniagenesis.

Besides the effect of growth hormone of enhancing glutamate reabsorption, it also increases both bicarbonate reabsorption \((6)\) and \( \text{NH}_4^+ \) excretion (Figures

![Figure 1. Growth hormone affects glutamate (GLU) and \( \text{NH}_4^+ \) but not glutamine (GLN) fractional excretion. Glutamine infusion. Fractional excretion of glutamine, glutamate, and ammonium was measured over the 0.5- to 1-h perfusion period during infusion of 500 or 1,000 nmol of glutamine per min, growth hormone, 50 nM, or vehicle administered at 0.5 h. Bars are means ± SE of the average for the 2- to 15-min period per kidney \((N = 6)\); asterisks indicate significantly different from control at \( P < 0.05 \).](image)

### TABLE 2. Effect of growth hormone on glutamate utilization

<table>
<thead>
<tr>
<th>Glutamate Infused</th>
<th>Removed</th>
<th>Glutamine Released</th>
<th>( \text{NH}_4^+ ) Produced</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>180</td>
<td>163 ± 14</td>
<td>34 ± 4</td>
</tr>
<tr>
<td>Growth Hormone(^a)</td>
<td>180</td>
<td>58 ± 20(^a)</td>
<td>54 ± 5(^a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>29 ± 18(^a)</td>
</tr>
</tbody>
</table>

\(^a\) Results are means ± SE in nanomoles per minute from eight kidneys per group with measurements taken over the 30-60-min time course.

\(^a\) Growth hormone added to an estimated 50 nM concentration.

\(^a\) Different from time controls, \( P < 0.01 \).

\(^a\) Different from time controls, \( P < 0.05 \).
reabsorption and glutamate reabsorption normalized for glomerular filtration. Over the response range spanning growth hormone and amiloride treatment, approximately 1 nmol of glutamate was reabsorbed per 50 nmol of bicarbonate reabsorbed, consistent with indirect coupling secondary to H* secretion such that some 59% of the glutamate reabsorption rate (177 - 73 or 104 nmol/mL) appears dependent upon a proton gradient.

To assess the relevance of the isolated kidney results, experiments were performed on kidneys in vivo (Table 4). Compared with the isolated preparation, which excretes 28 ± 5% of the filtered glutamate, the in vivo kidney excretes only 3.9 ± 0.4%. Nevertheless, the hypophysectomized rat kidney excretion rate observed in vivo is still eightfold higher than that exhibited by normal rat kidneys (9). Fractional excretion of glutamine, on the other hand, was 0.64 ± 0.24% in the hypophysectomized rat in vivo, not significantly different from 0.5% in the normal rat kidney (9). This demonstrates a specific glutamate reabsorption defect observable in vivo as well as in vitro. Administering growth hormone increases glutamate reabsorption with fractional excretion falling to 1.1 ± 0.5% (P < 0.01 versus time controls; the fractional excretion rate of glutamine, on the other hand, was unchanged at 0.54 ± 0.18%. Associated with enhanced reabsorption was a significant drop in urine pH from 6.58 ± 0.09 to 6.29 ± 0.10 U (P < 0.05). These results are therefore consistent with the expected action of growth hormone based on the response of the isolated kidney.

**DISCUSSION**

Growth hormone contributes to a positive nitrogen balance in part by shunting glutamine nitrogen from hepatic ureagenesis into glutamate destined for peripheral growth sites (5). Depending on ill-defined events at these sites, glutamate and, to a lesser extent, glutamine levels in arterial blood increase (4), constituting an increased load delivered to the kid-

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**TABLE 3. Effect of growth hormone on glutamate reabsorption**

<table>
<thead>
<tr>
<th></th>
<th>GFR (mL/min)</th>
<th>[Glutamate•]o (nmol/mL)</th>
<th>Filtered (nmol/min)</th>
<th>Excreted (nmol/min)</th>
<th>Reabsorbed (nmol/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.45 ± 0.05</td>
<td>162 ± 19</td>
<td>74 ± 17</td>
<td>19 ± 4</td>
<td>54 ± 14</td>
</tr>
<tr>
<td>Growth Hormone</td>
<td>0.54 ± 0.05</td>
<td>198 ± 15</td>
<td>108 ± 11</td>
<td>11 ± 4b</td>
<td>96 ± 12b</td>
</tr>
<tr>
<td>Growth Hormone plus Amiloride</td>
<td>0.50 ± 0.03</td>
<td>270 ± 32b</td>
<td>122 ± 17</td>
<td>85 ± 10c</td>
<td>36 ± 8c</td>
</tr>
</tbody>
</table>

* Results are means ± SE of the averaged 30-60 min clearance periods for control and growth hormone treated and for the 75-90 min clearance period with growth hormone plus amiloride. Growth hormone was added to a perfusate concentration of 50 nM; amiloride was added to a perfusate concentration of 0.75 mM. Six individual kidneys were run for the control and growth hormone data. Four kidneys were used for the growth hormone plus amiloride groups. [Glutamate•]o plasma glutamate concentration.

b Significantly different from control, P < 0.05.

c Significantly different from growth hormone treated, P < 0.01.
neys for reabsorption. In addition, the kidney, like the liver, is a target organ for growth hormone, exhibiting hormone-initiated metabolic events (10,11) as well as insulin-like growth factor (IGF)-1 release (12,13). Consequently, growth hormone influences on renal glutamine and, particularly, glutamate and glutamine utilization (Tables 1 and 2). The observed fall in ammoniagenesis and accelerated glutamine production are entirely consistent with displacement of glutamate from the oxidative pathway. Thus, like the liver (4), the renal response to growth hormone administration is to decrease the nitrogen end product produced—in this case, ammonium—with a shift to glutamate release. Unlike the liver, the kidney response entailed a decrease in net glutamine utilization. The most plausible explanation for this is enhanced apical glutamate transport coupled with glutamine production. Although both organs contain glutamine synthetase at downstream sites (14,15), the renal enzyme receives substrate across both the apical as well as basolateral surfaces. Thus, the shift from oxidative disposal into anabolic pathways may be an early conditioning event supporting interorgan fluxes as well as a prerequisite for local growth processes.

The renal reabsorption of filtered glutamate was markedly increased by growth hormone both in vitro and in vivo (Figures 1–3; Tables 2 and 4). In these studies, growth hormone at concentrations found at the upper range of episodic surges (6) promptly decreased glutamate excretion as a consequence of increased reabsorption: luminal transport increased some twofold (Tables 3 and 4), in turn reducing the fractional excretion rate some 60%. In contrast, glutamine’s fractional excretion, initially much lower than glutamate, remained unchanged. Associated with enhanced glutamate reabsorption was increased urinary acidification, bicarbonate reabsorption, and ammonium excretion, suggesting dependency upon accelerated Na⁺-H⁺ exchanger activity. Supporting this inference was the ability of amiloride to virtually eliminate the growth hormone-enhanced glutamate, but not glutamine, reabsorption (Figure 2). Noall et al. (16) originally demonstrated an effect of growth hormone on amino acid transport using α-aminoisobutyrate, a nonmetabolized amino acid whose transport has recently been shown to be H⁺ gradient dependent (17). Mechanistically, brush border vesicle preparations demonstrate a proton-driven glutamate uptake even in the absence of any sodium gradient (18), in contrast to neutral amino acid uptake (9). The perfused kidney is advantageous for

![Graph](image-url)

**Figure 3.** Relationship between glutamate and bicarbonate reabsorption per milliliter of glomerular filtrate (GF) in the hypophysectomized rat kidney. The datum points are means ± SE for growth hormone (GH) plus amiloride (AM), vehicle (V), and growth hormone-administered kidneys (left to right).

<table>
<thead>
<tr>
<th>TABLE 4. Effect of growth hormone on glutamate reabsorption in vivo⁸</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFR (mL/min)</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Hypox</td>
</tr>
<tr>
<td>Hypox plus growth hormone⁸</td>
</tr>
</tbody>
</table>

⁸ Results are means ± SE from four rats per group. Experiments were performed as referred to in the Methods section.

⁹ Growth hormone (50 μg) was administered via the jugular vein in 0.15 M NaCl.

⁸ Significantly different from control, P < 0.05.
distinguishing contributions from this auxiliary system because sodium gradient systems are operating less than optimally, especially in kidneys with limited glucocorticoid availability (7). Indeed, activating this auxiliary system probably explains why glutamine reabsorption was not increased because apical uptake is exclusively sodium gradient dependent (9). Substituting choline for sodium eliminated glutamate, but not glutamate, reabsorption, and residual sodium <15 mM was apparently sufficient to drive urinary acidification (7); on the other hand, ouabain eliminated the uptake of both glutamine and glutamate (18), demonstrating the ultimate dependence on the sodium gradient (9). In the study presented here, both growth hormone and amiloride had major effects on glutamate transport with little, if any, influence on the reabsorption of glutamine.

Growth hormone sparing of both glutamine and glutamate nitrogen does not appear to be mediated by IGF-I for several reasons. First, the response was rapid, occurring within 30 min, and growth hormone does not appear to elevate IGF-I levels within this time frame (19). Indeed, we were unable to detect an increase in either perfusate or urine-free IGF-I levels during this time period (data not shown). However, it is possible that undetected changes occurred in the levels of bound IGF-I or local IGF-I levels within the kidney (13). Further, based on estimated cellular pH, no effect of IGF-I on Na"-H" exchanger activity could be detected (20), whereas the study presented here suggests the growth hormone response should lead to a transient alkalosis. Finally, the effect of IGF-I on amino acid metabolism is more likely to be increased utilization (21) rather than the decrease observed for glutamine and glutamate in this study. Therefore, a direct effect of growth hormone likely mediated by the Na"-H" exchanger, rather than secondary to IGF-I, appears to initiate these responses.

How growth hormone might affect accelerated Na"-H" exchanger activity may be inferred from the studies of Hammerman and Rogers (11,22); these investigations demonstrated the presence of growth hormone receptors on basolateral membranes of proximal tubules. Application of growth hormone elicits a "second messenger" response capable of accelerating the Na"-H" exchanger (22) and thus of providing a cellular pathway by which growth hormone itself could acutely increase glutamate reabsorption secondary to proton secretion. Noteworthy is the fact that administration of growth hormone in vivo results in increased glutamate reabsorption (Table 4) and elevated kidney glutamate concentration (23) as well as in decreased ammonium levels (23)—results consistent with and predictable from the conclusions of the study presented here. Finally, it is unclear what role this response to growth hormone plays in the overall growth response although kidneys grow at a disproportionate rate in hypophysectomized rats involving both hyperplasia and hypertrophy (11) and glutamate promotes cellular differentiation and protein synthesis in proximal tubule-like LLC-PK cells (unpublished observation). In this regard, the Na"-H" exchanger has been implicated in these processes (24), whereas amiloride blocks growth associated with compensatory hypertrophy (25). Further investigations are therefore warranted to elucidate the relationships between growth hormone-accelerated acid extrusion, glutamate uptake, and IGF-I activity in the renal growth response.

ACKNOWLEDGMENTS

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REFERENCES


