Effects of Growth Hormone and Insulin-like Growth Factor I on Rabbit Proximal Convoluted Tubule Transport

Raymond Quigley and Michel Baum
Departments of Pediatrics and Medicine, The University of Texas Southwestern Medical Center at Dallas, Dallas, Texas 75235-9063

Abstract

This in vitro microperfusion study examined the effects of growth hormone and insulin-like growth factor I (IGF-I) on proximal convoluted tubule (PCT) transport. Tubules were perfused with an ultrafiltrate-like solution and bathed in a serum-like albumin solution. Neither a physiologic $(5 \times 10^{-10})$ M nor a pharmacologic $(5 \times 10^{-8})$ M dose of growth hormone had an effect on PCT phosphate or bicarbonate transport, or volume absorption. Addition of $5 \times 10^{-9}$ M and $5 \times 10^{-8}$ M IGF-I, but not $5 \times 10^{-10}$ M IGF-I, to the bathing solution resulted in an increase (12-15%) in phosphate transport, but no change in volume absorption or bicarbonate transport. Addition of IGF-I to the luminal perfusate also stimulated phosphate transport. The effect was noted at a concentration of $5 \times 10^{-11}$ M IGF-I (27% stimulation) and was maximal at a concentration of $5 \times 10^{-9}$ M IGF-I (46% stimulation). There was no effect of luminal IGF-I on volume absorption or bicarbonate transport. These data indicate that growth hormone has no direct effect on PCT transport. In the PCT, IGF-I stimulates phosphate transport specifically and acts via both basolateral and apical membranes. However, the magnitude of the maximal response to the luminal addition of IGF-I was threefold greater than that measured upon addition of the hormone to the bath, and the stimulation occurred at a 100-fold lower concentration. These data are consistent with IGF-I mediating the in vivo stimulation of phosphate transport by growth hormone. (J. Clin. Invest. 1991. 88:368-374.) Key words: in vitro microperfusion • phosphate transport • bicarbonate transport • volume absorption

Introduction

There is substantial evidence that growth hormone affects renal function (1-13). Growth hormone excess, whether from endogenous hypersecretion or chronic exogenous administration, has been associated with an increase in renal blood flow, glomerular filtration rate (1-6), and increased levels of serum phosphate (7-9, 13-15). Administration of growth hormone to dogs increased glomerular filtration rate, serum phosphate, and the maximal rate of tubular phosphate reabsorption, effects independent of parathyroid hormone (8). Brush border membrane vesicles prepared from the renal cortex of dogs treated with growth hormone for 3 d had an increase in sodium-dependent phosphate uptake (16). Likewise, hypophysectomized animals had a reduction in renal blood flow and glomerular filtration rate, effects reversed by growth hormone administration (1, 10, 11). Selective growth hormone deficiency, induced by administration of a synthetic peptide antagonist to growth hormone releasing factor, resulted in a reduction in glomerular filtration rate and increased the fractional excretion of phosphate (12).

While chronic growth hormone administration produces renal hemodynamic changes and modulates phosphate transport, acute administration of growth hormone does not produce these changes (5, 6, 17, 18). Renal blood flow, glomerular filtration rate, and tubular reabsorption of phosphate remained stable 2 h after growth hormone administration in normal and thyroparathyroidectomized dogs (17). Similarly, renal blood flow and glomerular filtration rate remained stable for 6 h in man after growth hormone administration (5, 6, 18). However, on the second and third day after a single injection of growth hormone, there was a rise in renal blood flow and glomerular filtration rate (5, 6). The increase in these parameters paralleled a rise in serum insulin-like growth factor I (IGF-I)$^1$ levels, and occurred at a time when growth hormone levels had returned to normal. In normal human subjects, infusion of IGF-I increased creatinine clearance and decreased plasma creatinine and urea levels (19). IGF-I increased renal plasma flow and glomerular filtration rate in fasted rats within 40 min of infusion (20). Thus, these studies suggest that the hemodynamic and tubular effects of growth hormone may be mediated by IGF-I.

The proximal tubule has both growth hormone and IGF-I receptors (21-25). It has been demonstrated recently that growth hormone has direct effects on this epithelium in the absence of IGF-I (22). Growth hormone produced an increase in phospholipase C activity when added to proximal tubular basolateral membranes. Addition of growth hormone to proximal tubule suspensions stimulated gluconeogenesis (26, 27) and ammoniagenesis (27). Whether growth hormone directly affects proximal tubule transport or acts indirectly via IGF-I is not known. The purpose of this in vitro microperfusion study was to determine if growth hormone and IGF-I can directly modulate transport in the proximal tubule.

Methods

Isolated segments of proximal convoluted tubules (PCT) were perfused as previously described (28, 29). Briefly, kidneys from female New

1. Abbreviations used in this paper: IGF-I, insulin-like growth factor I; $J_{\text{Na}}$ phosphate transport; $J_{\text{CO}_2}$ bicarbonate transport; PCT, proximal convoluted tubules; PD, potential difference.
Zealand white rabbits were sliced in coronal sections. Individual PCT segments were dissected in a cooled (4°C) ultrafiltrate-like solution which contained in mM: 115 NaCl, 25 NaHCO3, 4 Na2HPO4, 10 Na acetate, 1.8 CaCl2, 1 MgSO4, 5 KCl, 8.3 glucose, and 5 alanine. Tubules were then transferred to a 1.2 ml-temperature-controlled bath chamber and perfused with concentric glass pipettes.

Tubules were perfused with the above ultrafiltrate-like solution and bathed in a similar serum-like solution containing 6 g/dl albumin. The osmolality of the bath and perfusate was adjusted to 295 mOsm/kg by adding either water or NaCl. The bath was exchanged at a rate of 0.5 ml/min to maintain a constant pH and osmolality. The bath temperature was 37°C–38°C. After a 60-min equilibration period, the control period was begun. Subsequent periods were separated by 30 min of equilibration. To ensure that the albumin containing bathing solution had no growth hormone or IGF-I contaminants, samples of bath solution were sent to two independent laboratories (Nichols Institute, Irving, TX, and Endocrine Sciences, Tarzana, CA) for determination of growth hormone and IGF-I content. Both of these substances were found to be below the detectable limits of the assay (< 2.7 x 10^-11 M growth hormone and < 1.3 x 10^-10 M IGF-I).

Volume absorption (in nanoliters per minute per millimeter) was calculated as the difference between perfusion (Vw) and collection (Vs) rates, and normalized per millimeter of tubule length (I). Exhaustively dialyzed [methoxy-3H]insulin was added to the perfusate at a concentration of 50 μCi/ml so that the perfusion rate could be determined. The collection rate was measured by timed collections using a constant volume pipette. The length (in millimeters) was measured using an eyepiece micrometer.

Bicarbonate transport (JCO2, picomoles per minute per millimeter) was calculated using the equation: JCO2 = (VwC0 - VsC0)/L, where C0 and Cs represent the bicarbonate concentrations in the perfusate and collected fluid, respectively. Bicarbonate was measured using microcalorimetry (pica-Newton, model GV1; World Precision Instruments, Inc., New Haven, CT) (30).

Phosphate transport (Jp, picomoles per minute per millimeter) was measured using perfusate 32PO4 (50 μCi/ml). Phosphate transport was then calculated using the equation: Jp = [(VwCp - VsCp)/L] (P0/Cp), where P0 is the phosphate concentration in the perfusate and Cs and Cp are the concentrations of 32PO4 in the perfusate and collected fluid, respectively, in counts per minute per nanoliter. The transepithelial potential difference (PD, millivolts) was measured by using the perfusion pipette as the bridge into the tubular lumen. The perfusion and bathing solutions were connected to the recording and reference calomel half-cells using a bridge containing the perfusate in series with a 3.6 M KCl/0.9 M KNO3 agarose bridge. This arrangement avoided direct contact between the KCl/KNO3 agarose bridge and the solution bathing the tubule. The recording and reference calomel half-cells were connected to the high and low impedance sides, respectively, of an electrometer (model 602; Keithley Instruments, Inc., Cleveland, OH).

To determine if growth hormone (Eli Lilly, Indianapolis, IN) directly affects PCT transport, tubules were perfused with an ultrafiltrate-like solution and bathed in a serum-like albumin solution. During the experimental period either 0, 5 x 10^-10, or 5 x 10^-8 M growth hormone was added to the bath. 5 x 10^-8 M growth hormone was added to the luminal perfusate in additional studies to determine if growth hormone had a direct effect on the apical membrane.

To determine if bath IGF-I affects PCT transport, tubules were again perfused with an ultrafiltrate-like solution and bathed in a serum-like albumin solution. During the experimental period either 0, 5 x 10^-10, 5 x 10^-9, or 5 x 10^-8 M IGF-I (Amgen Biologicals, Thousand Oaks, CA) was added to the bathing solution. Because IGF-I is known to direct phospho-phorylation of its own receptor and subsequent phosphorylation of other intracellular proteins, an apparent increase in Jp could be due to an increase in cellular uptake and utilization of phosphate, and not to an increase in the transepithelial transport of phosphate. To examine this possibility, 32PO4 accumulation in the bath was measured in a series of tubules incubated with 5 x 10^-8 M bath IGF-I.

### Table 1: Effect of Both and Luminal Growth Hormone on Proximal Convoluted Tubule Transport

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Bath</th>
<th>Control</th>
<th>Experimental</th>
<th>PD</th>
<th>Experimental</th>
<th>Control</th>
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<td>Time control</td>
<td>5 x 10^-8 M Bath</td>
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Values are mean ± SEM. * P < 0.05.

Insulin-like Growth Factor I Stimulates Phosphate Transport
Three 1-ml samples of bath from both the control and experimental periods were counted for $^{32}$PO$_4$ and then averaged for each experiment. The lumen to bath flux of phosphate was then calculated from the rate of appearance of $^{32}$PO$_4$ in the bath. In the last series of experiments IGF-I was added to the luminal perfusate to determine if IGF-I had an effect on transport via the apical membrane. During the experimental period either $0, 5 \times 10^{-12}, 5 \times 10^{-11}, 5 \times 10^{-10}$, or $5 \times 10^{-8}$ M IGF-I was added to the luminal perfusate.

There were four to six measurements of PD, volume absorption, bicarbonate transport, and phosphate transport in each period in all tubes. The mean value for individual periods in a given tubule was used to calculate the mean for that period. Data are expressed as mean±SEM. The $t$ test for paired data was used to determine significance at the 0.05 level.

**Results**

**Effect of lumen and bath growth hormone on PCT transport.** The first series of experiments examined whether growth hormone has a direct effect on PCT transport. These results are shown in Table I. The mean tubular length for these experiments was 1.5±0.1 mm. Time controls were done to ensure the stability of our preparation. In these experiments vehicle, rather than hormone, was added during the experimental period. There was a small but statistically significant increase in the PD with time, but volume absorption, phosphate and bicarbonate transport remained constant. An increase in PD with time has been found previously in the in vitro PCT (31, 32). A physiologic concentration of bath growth hormone ($5 \times 10^{-10}$ M) had no effect on volume absorption, bicarbonate or phosphate transport, but caused a small significant increase in PD, comparable to that observed in the time control studies. The concentration of growth hormone used was similar to peak plasma levels in humans during puberty (33), and to the concentration that caused half-maximal stimulation of phospholipase C activity in canine proximal tubular basolateral membranes (22). A pharmacologic concentration of growth hormone ($5 \times 10^{-8}$ M) also had no effect on PCT volume absorption, or bicarbonate or phosphate transport when added to the bath.

Because growth hormone is filtered, the effect of luminal growth hormone was also examined (34, 35). Addition of $5 \times 10^{-8}$ M growth hormone to the luminal perfusate had no effect on PCT transport (Table I). Time controls with luminal perfusate exchanges, done to ensure that the luminal exchange did not affect transport, demonstrated no significant effect on PCT transport. Thus, neither physiologic nor pharmacologic doses of growth hormone had an effect on PCT volume absorption, or the transport of bicarbonate and phosphate.

**Effect of lumen and bath IGF-I on PCT transport.** The next series of experiments examined the effect of bath IGF-I on PCT transport. During the experimental period, IGF-I was added to the bath in concentrations of $5 \times 10^{-10}$ M, $5 \times 10^{-9}$ M, or $5 \times 10^{-8}$ M. This range includes the concentration that results in half-maximal occupancy of IGF-I receptors in the proximal tubule (23–25). The results are shown in Table II and Fig. 1. The mean tubular length in these studies was 1.4±0.1 mm. When $5 \times 10^{-9}$ M IGF-I was added to the bath, there was a small but statistically significant increase in PD, comparable to that seen in the time control experiments. There was also a small but statistically significant increase in volume absorption during the studies examining the effects of $5 \times 10^{-9}$ M bath.
IGF-I, but there was no effect on volume absorption at bath IGF-I concentrations of 5 × 10^{-10} M and 5 × 10^{-8} M. There was no change in bicarbonate transport at any bath IGF-I concentration. However, 5 × 10^{-9} M and 5 × 10^{-8} M IGF-I stimulated phosphate transport by 15% and 12%, respectively. Thus, bath IGF-I causes a significant increase in phosphate transport, but no significant change in bicarbonate absorption.

To determine if the increase in phosphate transport was due to an increase in uptake and metabolism of phosphate, or to a true increase in transepithelial transport, the bathing solution was analyzed for 32P in three of the experiments examining the effect of 5 × 10^{-8} M bath IGF-I on proximal tubule transport. These results are shown in Table III. The lumen to bath flux of phosphate was calculated from the appearance rate of 32P in the bath. These data are in close agreement to the fluxes calculated from the perfused and collected fluid phosphate concentrations, confirming that there was a significant increase in transepithelial phosphate transport in the presence of bath IGF-I.

Table III. Effect of Bath IGF-I on 32P Counts Appearing in the Bath

<table>
<thead>
<tr>
<th>Experimental protocol</th>
<th>n</th>
<th>32P counts mean±SEM</th>
<th>J_{max}*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3</td>
<td>160.3±67.1</td>
<td>9.0±2.1</td>
</tr>
<tr>
<td>5 × 10^{-8} M bath IGF-I</td>
<td>3</td>
<td>177.6±70.7*</td>
<td>10.2±2.5*</td>
</tr>
</tbody>
</table>

* J_{max} calculated from rate of appearance of 32P in the bath. \(^* P < 0.01; \dagger P < 0.05.\)

Discussion

This in vitro microperfusion study examined the effects of growth hormone and IGF-I on proximal convoluted tubule transport. Growth hormone had no direct effect on PCT volume absorption, phosphate or bicarbonate transport at either physiologic (5 × 10^{-10} M) or pharmacologic (5 × 10^{-8} M) concentrations. However, addition of IGF-I to the bathing solution resulted in a dose-dependent increase in phosphate transport, with no change in volume absorption or bicarbonate transport. Addition of IGF-I to the luminal perfusate resulted in a dose-dependent stimulation of PCT phosphate transport with no change in volume absorption or bicarbonate transport. 5 × 10^{-12} M IGF-I in the luminal perfusate had no effect on phosphate transport, but there was a stimulation of phosphate transport at higher concentrations of IGF-I. Maximal stimulation of phosphate transport occurred at 5 × 10^{-10} M luminal IGF-I. These data demonstrate that growth hormone has no direct effect on PCT transport. However, IGF-I directly stimulates PCT phosphate transport, an effect which can be mediated from both the basolateral and apical membranes.

Receptors for growth hormone are present on the proximal tubule (21, 22). Growth hormone has been shown to bind to receptors present in the basolateral membrane vesicles isolated from canine renal cortex and stimulate phospholipase C activity (22). Half-maximal stimulation of phospholipase C activity occurred at 5 × 10^{-10} M growth hormone and was noted within 15 s of incubation with growth hormone. Growth hormone has been shown to stimulate gluconeogenesis in rat kidney cortical slices (36), and in suspensions of canine proximal tubules (26, 27). In these latter studies 10^{-6} M growth hormone increased glucose production by 55% after 120 min of incubation. The half-maximal response occurred between 10^{-9} M and 10^{-8} M growth hormone (26, 27). Growth hormone also increased ammoniagenesis, where half-maximal stimulation occurred at a concentration of 10^{-10} M and 10^{-9} M (27).

The acute effect of growth hormone on tubular phosphate reabsorption has been examined in dogs (17). Dogs received a growth hormone infusion for 2 h, then inulin and para-aminohippurate clearances and phosphate reabsorption were measured over the subsequent 2 h. There was no significant change in renal blood flow, glomerular filtration rate, or tubular reabsorption of phosphate in these dogs, results which agree with our findings. In this study, the concentrations of growth hormone used are well within the range of those that have been previously reported to stimulate phospholipase C activity, gluconeogenesis, and ammoniagenesis in the proximal tubule (22, 26, 27, 36). The total time of incubation with growth hormone during the experimental period was 90–120 min. This is comparable to the time of incubation where growth hormone has been shown to increase ammoniagenesis and gluconeogenesis (26, 27, 36).

Serum IGF-I levels increase after growth hormone administration and could mediate the effects of growth hormone on phosphate transport. Hypophysectomized rats given IGF-I via osmotic minipump for 6 d had an elevated serum phosphate and an increased maximal rate of tubular reabsorption of phosphate (37). Brush border membrane vesicles isolated from the renal cortex of these animals had a higher Na-dependent Pi transport, compared to vehicle treated controls. Sodium-dependent glucose and alanine uptake were not affected. The stimulatory effect of IGF-I on maximal rate of tubular reb-
sorption of phosphate was also observed in thyroparathyroid-ectomized animals indicating that IGF-I's action was independent of parathyroid hormone.

Serum 1,25-dihydroxyvitamin D₃ levels have correlated well with serum IGF-I levels under a number of experimental conditions (38, 39). Infusion of IGF-I in hypophysectomized rats increased serum 1,25-dihydroxyvitamin D₃ levels (37). Since 1,25-dihydroxyvitamin D₃ stimulates phosphate transport (40, 41), the effect of IGF-I in vivo could have been mediated by 1-25-dihydroxyvitamin D₃. Our study, however, demonstrates a stimulation of phosphate transport by IGF-I in the absence of 1,25-dihydroxyvitamin D₃.

The acute effect of IGF-I on phosphate transport has been studied in a cell culture line derived from opossum kidneys (42). These cells have sodium-dependent phosphate transport that is inhibited by parathyroid hormone (43, 44). IGF-I stimulated sodium-dependent phosphate uptake within 15 min of incubation (42). Half-maximal stimulation occurred at a dose of 10⁻⁷ M IGF-I, and maximal stimulation was found at 10⁻⁶ M IGF-I. Kinetic analysis demonstrated an increase in Vmax with no change in the Kmax for phosphate transport. It was also noted that the effect was specific for phosphate transport since the transport of alanine was not affected.

These studies examined the effect of IGF-I on proximal tubule transport. In vitro microperfusion of individual PCT demonstrated that both IGF-I stimulated phosphate transport, but did not change volume absorption or bicarbonate transport. The concentration of bath IGF-I that increased phosphate transport (5 × 10⁻⁹ M) was similar to that found in the cell culture studies (42). This concentration of IGF-I was that required for half-maximal binding to IGF-I receptors on basolateral membranes (23–25), and the concentration that caused autophosphorylation of the β-subunit of the IGF-I receptor (45).

Receptors for IGF-I are present on both the basolateral and apical membranes of the proximal tubule with the density of receptors greater on the basolateral membrane (24). Incubation of basolateral membrane vesicles (but not brush border membrane vesicles) with IGF-I resulted in phosphorylation of the receptor indicating an intracellular signalling process linked to IGF-I (24). These data indicate that, while IGF-I receptors are present on the apical membrane, they may not function by the same intracellular signal transduction mechanism as those on the basolateral membrane. The present study demonstrates that IGF-I does have a direct effect to stimulate phosphate transport when presented to the apical membrane.

The significance of the IGF-I effect on the apical membrane is not clear. IGF-I has a mol wt of ~ 7,500 and thus could be filtered by the glomerulus. However, most of the IGF-I in the serum is tightly bound to carrier proteins (46, 47). The unbound fraction of IGF-I has been estimated to be 0.1–0.8% of the total (46, 47). The circulating concentration of IGF-I is ~ 10⁻⁷ M. Thus, if only 0.1% of IGF-I is free, 10⁻¹⁰ M IGF-I could be in the glomerular ultrafiltrate. This concentration is within the range that stimulated phosphate transport in this study. Recently, studies have found that mesangial cells are capable of synthesizing IGF-I (48). This raises the possibility that mesangial cells could play a role in the regulation of proximal tubule phosphate transport.

IGF-I is produced by several organs in the body including the liver, lungs, heart, and kidneys (49). The tissue concentrations of IGF-I in these organs decreases with hypophysectomy.
and increases when the animal is treated with growth hormone. Recent evidence has shown that rat collecting ducts produce IGF-I in direct response to growth hormone (50). The significance of this finding is not clear. The major site of phosphate transport is the proximal tubule. It has been postulated that the IGF-I from the collecting duct cells may traverse the interstitium or travel via the vasa rectae to capillaries surrounding the proximal tubule (21). In this manner IGF-I synthesized by the distal nephron may influence phosphate transport by the proximal tubule.

This in vitro microperfusion study directly addressed the issue of whether growth hormone or IGF-I affects proximal tubular transport. These studies were performed in the rabbit PCT and species differences are possible. However, our study demonstrates that while growth hormone had no effect on PCT transport, IGF-I stimulated directly phosphate transport in the rabbit PCT. This stimulation is specific for phosphate as volume absorption and bicarbonate transport remained unchanged. The stimulation in transport by IGF-I is much greater when presented to the apical membrane and suggests that apical receptors may play an important role in the regulation of phosphate transport in the proximal tubule.

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References


