Growth hormone regulates ammoniagenesis in canine renal proximal tubule segments

MICHAEL C. CHOBANIAN, CAROL M. JULIN, KEVIN H. MOLTENI, AND PETER C. BRAZY
Division of Nephrology, Departments of Pediatrics and Medicine, University of Wisconsin School of Medicine, Madison 53792; and The William S. Middleton Memorial Veterans Affairs Medical Center, Madison, Wisconsin 53705

Chobanian, Michael C., Carol M. Julin, Kevin H. Molteni, and Peter C. Brazy. Growth hormone regulates ammoniagenesis in canine renal proximal tubule segments. *Am. J. Physiol.* 262 (*Renal Fluid Electrolyte Physiol.* 31): F878–F884, 1992.—To determine whether growth hormone (GH) directly affects ammoniagenesis in the renal proximal tubule, ammonia production was measured in suspensions of isolated canine renal proximal tubule segments (IPTs) incubated with 2.5 mM L-glutamine and varying concentrations of human growth hormone (hGH). Ammonia production from IPTs significantly increased by nearly threefold in the presence of hGH (10^{-6} M) at 60 min. This increase was dose dependent, with as little as 10^{-9} M hGH significantly stimulating ammonia production. In addition, hGH enhanced glucose production when lactate, alanine, and succinate replaced L-glutamine as substrate. hGH significantly stimulated ammonia production when IPTs were incubated at alkalotic and neutral pH. The effect of hGH was lost at acidic pH. When hGH was added to IPTs incubated under Na^+-equilibrated conditions, ammonia production was not different from control. hGH stimulated ouabain-sensitive Na^+-K^+-adenosinetriphosphatase (ATPase) activity by 8.1 ± 1.1% in basolateral membranes isolated from IPTs. hGH stimulation of proximal tubule ammonia production from L-glutamine occurs at physiological concentrations of hGH and when the extracellular-to-intracellular Na^+ gradient favors L-glutamine transport. This effect is associated with an increase in basolateral Na^+-K^+-ATPase activity. The data suggest a role for hGH in the regulation of renal acid-base metabolism under physiological conditions in which increased net acid excretion is important.

renal acid-base metabolism; gluconeogenesis; glutamine

HORMONAL REGULATION of renal metabolic processes has been well described in virtually all nephron segments. In particular, recent and remote evidence has demonstrated a key role for hormonal regulation of proximal tubule ammonia (2, 4, 5) and glucose metabolism (28). Previous studies of polypeptide hormone action on proximal tubule metabolism have revealed both concurrent (5) and divergent (2) effects. For example, insulin stimulates renal proximal tubule ammoniagenesis but inhibits gluconeogenesis. Insulin-like growth factor (IGF)-I has no affect on ammonia production (2) but stimulates glucose production (27). Because of the divergent metabolic effects of glucoregulatory hormones and somatomedins (i.e., IGFs), the effect of other hormones on renal proximal tubule metabolism is unpredictable. Human growth hormone (hGH) is considered an essential glucoregulatory hormone (6) the actions of which may be either direct or indirect via the somatomedins or via IGF-I or -II (13). A direct effect of bovine GH on renal glucose metabolism has been recently shown by Rogers et al. (27). Specifically, bovine GH stimulated glucose production when incubated directly with suspensions of canine renal proximal tubules (27). Furthermore, the concomitant production of IGF-I was not observed, so the effect of GH on the renal proximal tubule appeared direct. With the discovery of hGH receptors on basolateral membranes of renal proximal tubule cells and the discovery that hGH activates phospholipase C, the likelihood of a direct action of hGH on renal metabolism has been confirmed (26).

Growth itself is an acid-forming physiological process and is dependent on GH. Linear growth or skeletal growth is particularly acidifying in young individuals and is associated with enhancement of net acid excretion (20). GH release can occur from ingestion of a protein meal and in response to an exogenous acid load, arginine hydrochloride (6, 10). Therefore, during periods of rapid growth and exogenous acid loads, hGH may have an integral role in modifying renal acid-base metabolism.

Because ammonia metabolism is linked to the kidney's ability to excrete acid and adapts to states of endogenous or exogenous acid production, we investigated the role of hGH in modifying renal proximal tubule ammonia metabolism. Our results show that hGH stimulates ammonia production in a concentration-dependent manner in canine renal proximal tubule segments. This direct action of hGH is dependent on an extracellular-to-intracellular Na^+ gradient and is abolished by cellular acidosis. A positive effect of hGH on Na^+-K^+-adenosinetriphosphatase (ATPase) activity supports this Na^+-gradient dependency. The data suggest that hGH may enhance proximal tubule ammonia production by directly stimulating glutamine uptake or cellular glutamine utilization. GH may serve to augment proximal tubule ammoniagenesis physiologically when increased ammonia production is needed to enhance net acid excretion, e.g., during chronic acid-producing states, such as growth. Furthermore, GH may serve to regulate renal metabolism by modifying the renal response to diminished function (1) or to an abnormal metabolic environment by contributing to systemic glucose and acid-base homeostasis (32).

METHODS

Preparation of proximal tubule segments. Suspensions of proximal tubule segments from canine kidney were prepared by Percoll-density centrifugation of collagenase-digested renal cortical slices as before (2, 5).

Measurements of ammonia and glucose production in proximal tubule segments. Ammoniagenesis and gluconeogenesis in suspensions of proximal tubule segments were quantitated by measuring ammonia (NH_3) and glucose production from (in mM) 2.5 L-glutamine or 10 lactate, 1.0 alanine, and 1.0 succinate as described previously (2, 5). Proximal tubule segments were suspended in Krebs-Henseleit bicarbonate (KHB) buffer,

pH 7.4, composed of (in meq/l) 145 Na^+, 129 Cl^-, 6 K^+, 1.0 $H_2PO_4^-$, 25 HCO_3^-, 1.2 SO_4^{2-}, 2 Ca^{2+}, and 1.2 Mg^{2+}. Before use, all solutions were gassed for at least 30 min with 95% O_2-5% CO_2. When pH was modified, appropriate changes in Na^+, Cl^-, and HCO_3^- were made to assure the osmolality and pH of the media. Extracellular pH has been previously shown to be constant at all three pH values evaluated (5). Under Na^+ reequilibrated conditions, choline replaced Na^+ in the KHB, as before (2, 5). All flasks contained 5 ml of proximal tubule suspensions containing 20–30 mg of protein capped under an atmosphere of 95% O_2-5% CO_2. All suspensions were incubated in a shaking-water bath at 37°C for 60 min. hGH was added to the flasks after the 15-min reequilibration period at 37°C. Sample aliquots were removed at specific times during the incubation and mixed with equal volumes of 6% perchloric acid/1.0 mM EDTA. After centrifugation, supernatants were removed and stored at −70°C until measurements of ammonia and glucose were made, as before (2, 5).

Preparation of basolateral membranes from isolated proximal tubule. Renal cortical basolateral membranes were isolated using the methods of Windus and Hammerman (33) and as before (4). Briefly, proximal tubule segments were isolated, and 40–50 ml of 10 mg protein/ml tubules were incubated with 10 mM L-glutamine and KHB at pH 7.4 in the presence or absence of 10^{-6} M hGH. After a 30-min incubation in a shaking-water bath under an atmosphere of 95% O_2-5% CO_2, the flask contents were spun at 31,000 *g* for 20 min at 4°C in a Beckman JA-20 rotor. The pellets were resuspended in 10 ml sucrose homogenizing media [(in M) 0.25 sucrose; 0.1 phenylmethylsulfonyl fluoride; 0.01 tris(hydroxymethyl)aminomethane (Tris), pH 7.5] and polytron-agitated gently for three 30-s periods. After two centrifugation steps, the crude membrane fraction was centrifuged in a Percoll density gradient. Purified basolatral membranes (BLM) were removed and washed in spin media [(in M) 0.25 sucrose; 0.05 Tris, pH 7.5; 0.10 NaCl] in two subsequent centrifugations. BLM were resuspended in spin medium at concentrations of 1–4 mg protein/ml, and Na^+-K^+-ATPase enzyme activity was assayed. Enrichment of Na^+-K^+-ATPase activity in BLM compared with crude tubule homogenate was used as an indicator of purity and averaged 8.24 ± 0.25 (n = 6). Contamination of BLM by brush-border membrane was assessed by the presence of the brush-border enzyme, maltase. The enrichment factor was 0.92 ± 0.31 (n = 6) for maltase. Contamination with other subcellular organelles (determined by specific enzymic activity) were all insignificant as before (4, 33).

Na^+-K^+-ATPase activity. BLM ouabain-sensitive Na^+-K^+-ATPase activity (measured as inorganic phosphate resulting from the hydrolysis of ATP) was determined by the method of Jørgensen and Skou (18). BLMs were incubated at 37°C for 15 min with (in mM) 110.0 NaCl, 15.0 KCl, 30.0 imidazole, 5.0 NaN_3, 0.5 ethylene glycol-bis(β-aminoethylether)-*N,N,N',N'*-tetraacetic acid (EGTA), 4.0 $MgCl_2$, and 4.4 ATP in the presence or absence of 1.0 mM ouabain at a final vol of 1.0 ml. The reaction was stopped with 0.05 ml 100% trichloroacetic acid, and inorganic phosphate was determined colorimetrically (740 nm) with the addition of a modified Fiske-SubbaRow reagent (1.0 ml), 1% ammonium molybdate, and 5% $FeSO_4$ in 1.0 N H_2SO_4 (11). Results were calculated as micromoles P_i per milligram protein per minute and expressed as percent change from control.

Protein assay. All protein concentrations were measured using the bicinchoninic acid (BCA) assay (Pierce Chemical) of Smith et al. (30).

ΔpH measurements. Changes in intracellular pH (pHi) were measured fluorometrically by the methods of Deamer et al. (7) using the fluorescent dye 9-aminoacridine (9-AA). Prior to the addition of IPTs, 9-AA (15 μl total vol, 4 μM final concn) was injected via a microsyringe into a continuously agitated, thermostated (37°C) cuvette containing Krebs-Henseleit saline (KHS)/25 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), pH 7.4, previously gassed with 95% O_2-5% CO_2 (final vol 2.85 ml). Fluorescence was continuously monitored (λ excitation 400 nm; λ emission 450 nm) during the entire experiment using an Aminco-Bowman single-wavelength spectrophotofluorometer and photomultiplier microphotometer (model J10-222A; Silver Spring, MD). A stable signal was obtained within 5 min of adding 9-AA to the cuvette. IPTs were preincubated for 20 min in KHS/25 mM HEPES at 37°C and then added to the cuvette (150 μl; 3 mg/ml protein, final vol 3.0 ml). Fluorescence decreases acutely after adding IPTs, as 9-AA enters the cells and becomes fully quenched (7). The fluorescence signal reaches a constant value when 9-AA achieves a steady-state distribution between buffer and proximal tubule cells. Attainment of equilibrium between intra- and extracellular 9-AA signal never exceeded 5 min. Once a constant signal was achieved, NH_4Cl (50 μl, 50 mM final concn) or hGH (13 μl, 10^{-6} M final concn) was added via microsyringe into the cuvette. The change in transmembrane pH gradient was calculated as described (7) from the distribution of 9-AA between the proximal tubule cells and buffer. The change in transmembrane pH gradient attributable to the addition of NH_4Cl or hGH can be expressed as

$$\delta(\Delta pH) = \log\left[\frac{[A]_T - [AH^+]_{out}}{[AH^+]_{out}} \times \frac{[AH^+]_{out}}{[A]_T - [AH^+]_{out}}\right]$$

after before

where $[A]_T$ and $[AH^+]_{out}$ are proportional to the observed fluorescence in the absence or presence of IPTs, respectively. At this concentration, 9-AA had no effect on IPT cellular respiration, ammoniagenesis, gluconeogenesis, or cellular volume (data not shown). Over the physiological pH range, 9-AA was fully protonated and exhibited constant fluorescence in a cell-free medium. The calculated value of $\delta(\Delta pH)$ is proportional, but not equal, to the actual change in transmembrane pH gradient (16). Each curve was obtained by discharging the transcellular pH gradient using 2.0 μM nigericin in KHS buffer of varying pH under conditions in which no K^+ gradient existed between the buffer and IPTs (31).

Statistics. Dunnett's multiple-comparison procedure was used for statistical analysis of multiple comparisons (9). Student's *t* test was used as indicated for nonmultiple comparisons. Significance was determined at $P < 0.05$. As before (2–5) interexperimental variability occurred, and thus statistical analysis was applied to data generated under paired experimental conditions.

Materials. Recombinant hGH was a kind gift from A. Johanson, Genentech (South San Francisco, CA). 9-AA was a gift from N. Hadjokas, and nigericin was purchased from Sigma. All other chemicals were of the highest purity available from commercial sources.

RESULTS

To determine the time course of ammonia and glucose production in proximal tubule segments suspended in KHB, pH 7.4, ammonia and glucose concentrations were measured as functions of time in the presence or absence of 10^{-6} M hGH. As illustrated in Fig. 1, NH_3 production was linear from 0 to 30 min, but by 60 min the rate of NH_3 production significantly accelerated in the presence of hGH, compared with control ($P < 0.05$, rate of ammonia production 0–30 min vs. 30–60 min, paired Student's *t* test, n = 12 experiments). Rates of NH_3 production between 0–30 min and 30–60 min were 1.27 ± 0.17 and

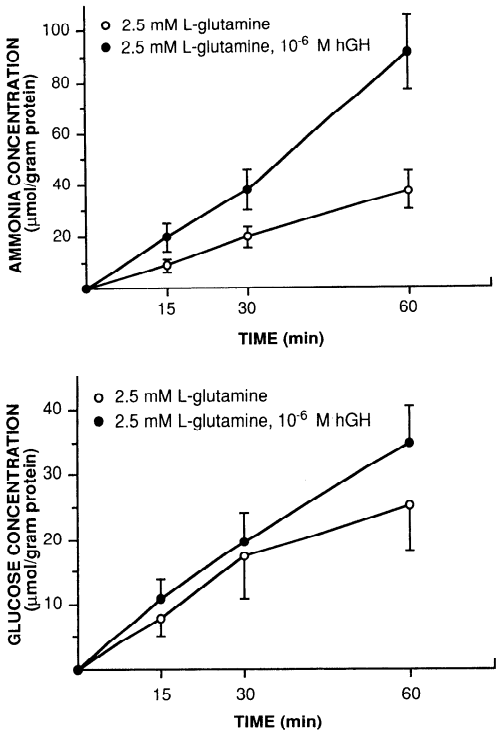

Fig. 1. Ammonia and glucose concentration in suspensions of proximal tubule segments as a function of time of incubation at pH 7.4. Human growth hormone (hGH) (10^{-6} M) was added at *time 0* after a 15-min equilibration period using 2.5 mM L-glutamine as the sole substrate. Data are expressed as mean values ± SE of 12 paired experiments. Both absolute concentrations at each time point and rates of ammonia and glucose production were significantly different ($P < 0.05$) for ammonia but not glucose.

1.78 ± 0.47 μmol·g protein^{-1}·min^{-1}, respectively. Comparisons of absolute ammonia production at specific time points, as well as those of production rates between each time point, were all significantly enhanced in the presence vs. the absence of hGH ($P < 0.05$, paired Student's t test, $n = 12$ experiments). Ammonia concentration at 60 min was 91.3 ± 14.1 μmol/g protein in the presence of hGH and 37.6 ± 7.7 μmol/g protein in the absence of hGH. Glucose concentration measured in the same suspensions was not significantly different in the presence or absence of hGH when L-glutamine was the only substrate. At 60 min, glucose concentration was 35 ± 5.5 and 25.3 ± 7.0 μmol/g protein in the presence and absence of hGH, respectively [P = not significant (NS), paired Student's t test, $n = 12$ experiments]. Calculated rates of glucose production were not significantly different in either the presence or absence of hGH. When lactate, L-alanine, and succinate replaced L-glutamine as metabolic substrate, glucose production was significantly enhanced by the presence of hGH (Fig. 2). Rates of glucose production were 2.72 ± 0.33 vs. 2.29 ± 0.17 μmol·g protein^{-1}·min^{-1} ($P < 0.05$, paired Student's t test, $n = 10$) in the presence and absence of hGH, respectively. These data are comparable to those reported by Joseph and Subrahmanyam (19) in which glucose production from succinate or pyruvate, but not glutamine, was enhanced in cortical slices from GH-treated rats.

To determine the concentration dependence of hGH-stimulated ammonia production, proximal tubule segments were incubated with varying concentrations of hGH at pH 7.4 for 60 min using 2.5 mM L-glutamine as the sole substrate. Figure 3 illustrates ammonia production expressed as micromole per gram protein per hour, with varying concentrations of hGH. Ammonia production was significantly enhanced over control [from 10^{-6} to 10^{-9} M hGH, but not 10^{-10} M hGH ($P < 0.05$, two-tailed Dunnett's multiple-comparison procedure, $n = 5$)]. An approximation of half-maximal stimulation occurred at 10^{-9} M hGH. This value corresponds to the value of plasma GH in normal dogs after stimulation by arginine hydrochloride infusion (10). However, no plateau was achieved with increasing hGH concentrations during this short incubation period, which is similar to the findings of Rogers et al. (27).

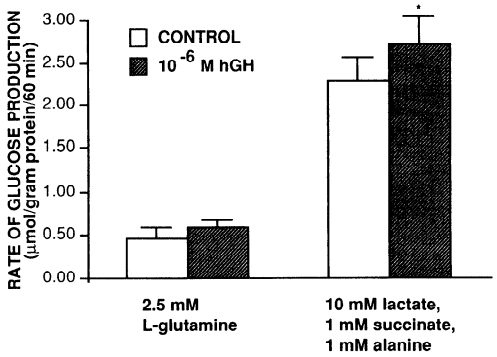

Fig. 2. Rate of glucose production in proximal tubules measured between 0 and 60 min incubation at pH 7.4 with (in mM) 2.5 L-glutamine or 10 lactate, 1.0 alanine, and 1.0 succinate as substrates. Each combination of substrates was added at *time 0* after a 15-min equilibration period. Data are expressed as rates of glucose production per 60 min, mean ± SE, of 10 paired experiments (* $P < 0.05$).

Lowering the pH of the proximal tubule suspension medium from 7.4 to 7.1 results in enhanced ammonia and glucose production from L-glutamine over time (2, 5). To determine whether hGH-stimulated ammonia production is dependent on the pH of the medium, rates of ammonia production from 2.5 mM L-glutamine in suspensions of IPTs were measured with and without 10^{-6} M hGH at pH 7.7, 7.4, or 7.1. As shown in Fig. 4, the ability of hGH to significantly stimulate the rate of ammonia production in IPTs at pH 7.4 is also observed at pH 7.7 but not at pH

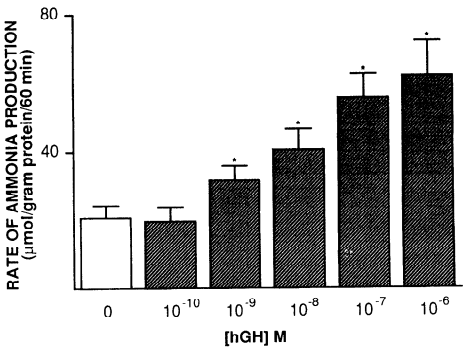

Fig. 3. Ammonia production in proximal tubule segments measured at 60 min of incubation at pH 7.4 with 2.5 mM L-glutamine as sole substrate. Varying concentrations of hGH were added at *time 0* after a 15-min equilibration period. Data are expressed as mean ± SE of absolute production at 60 min of 5 paired experiments (* $P < 0.05$). Ammonia production averaged 20.3 ± 3.7 μmol·g protein^{-1}·60 min^{-1} in the absence of hGH.

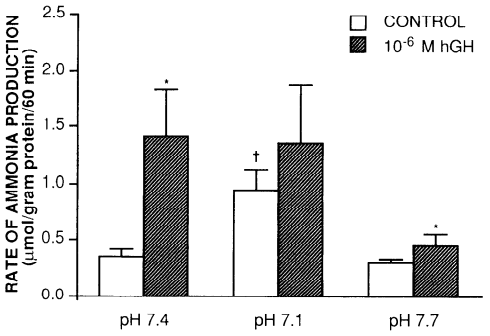

Fig. 4. Rate of ammonia production in proximal tubules between 0 and 60 min from 2.5 mM L-glutamine at pH 7.4, 7.1, and 7.7. hGH (10^{-6} M) was added at *time 0* after a 15-min equilibration period. Data are expressed as mean production rates between 0 and 60 min (±SE) of 5 paired experiments in presence of hGH at varying pH (* $P < 0.05$). Rates of ammonia production in absence of hGH at pH 7.1 compared with pH 7.4 or pH 7.7 were significantly different, whereas pH 7.4 vs. 7.7 was not († $P < 0.05$).

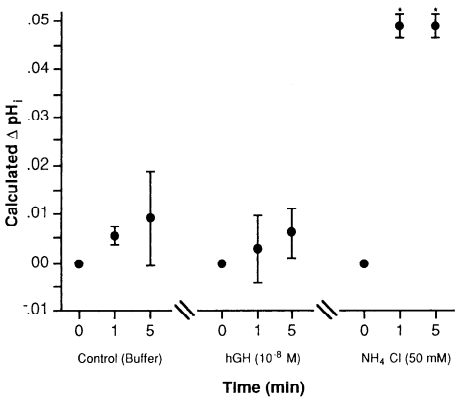

Fig. 5. Relative change in ΔpH_i of proximal tubules using fluorescent dye 9-aminoacridine (9-AA). hGH (10^{-8} M) or NH_4Cl (50 mM) was added at *time 0* after a 20-min equilibration period using 4.0 μM 9-AA. Data are expressed as calculated ΔpH_i relative to the change in fluorescence in the absence or presence of 9-AA and hGH or NH_4Cl of $n = 5$ paired experiments (* $P < 0.05$).

7.1 ($P < 0.05$ for pH 7.4 and 7.7; P = NS for pH 7.1, in the presence vs. absence of hGH, $n = 5$, two-tailed Dunnett's multiple-comparison procedure). Rates of ammonia production in the presence or absence of hGH at pH 7.4, 7.7, and 7.1 were 1.42 ± 0.41 vs. 0.36 ± 0.06, 0.45 ± 0.11 vs. 0.31 ± 0.04, and 1.36 ± 0.52 vs. 0.95 ± 0.18 $\mu mol \cdot mg\ protein^{-1} \cdot min^{-1}$, respectively. Comparisons made between the different pH values in the absence of hGH confirm the positive affect of acidic pH on proximal tubule ammonia production previously described (2, 5) (pH 7.4 vs. pH 7.7, P = NS; pH 7.1 vs. pH 7.4 or pH 7.7, $P < 0.05$, $n = 5$, two-tailed Dunnett's multiple-comparison procedure). Thus both acidic pH and hGH at neutral and alkalotic extracellular pH increased ammoniagenesis. There was no additive effect of extracellular acidosis and hGH on ammonia production, yet the maximal effects of both occurred after 30 min of incubation (Fig. 1 and previously described, 2, 4). These findings are consistent with utilization of a common pathway to stimulate ammoniagenesis. Although statistical significance was not achieved when comparing ammonia production at acidic pH in the presence or absence of hGH, a large standard error may prevent detection of a stimulatory effect by hGH.

GH possesses certain "insulinomimetic" characteristics, and recently insulin has been shown to affect cellular pH in rat IPTs (12) by alkalinizing the cytosol and activating the luminal Na^+-H^+ exchanger. To determine whether hGH, like insulin, affects IPT cellular pH directly and thereby affects ammonia production, we measured relative change in IPTs cellular pH (ΔpH_i) using the fluorescent dye 9-AA. As illustrated in Fig. 5, 10^{-6} M hGH had no effect on ΔpH_i (*middle*) from 0–5 min (P = NS, hGH vs. control, Student's t test, $n = 5$). Control buffer also had no effect on ΔpH_i, whereas 50 mM NH_4Cl (a weak base known to initially alkalinize this cell, Ref. 22) caused a significant positive deflection (alkalinization) in ΔpH_i. Because hGH failed to change ΔpH_i, it appears the mechanism by which hGH stimulates ammonia production is not via an acute shift in cellular pH.

L-Glutamine transport depends on the chemical outward-to-inward gradient for Na^+ across the plasma membrane of proximal tubule cells (33). Previously, we determined an Na^+ gradient dependency of insulin to enhance ammoniagenesis (2), and insulin has been shown to enhance Na^+-coupled transport of amino acids into a variety of cells (23). Like insulin, hGH-stimulated ammonia production may be mediated by enhancement of Na^+-dependent L-glutamine transport. To test this hypothesis, IPTs were suspended in KHB buffer or buffer in which Na^+ was replaced by choline before incubation with hGH, as before (2, 5). Intracellular [Na^+] and extracellular [Na^+] equilibrate at 25 mM, without significantly affecting cellular pH or ATP levels under these conditions (2, 5, 22). Reduction of extracellular Na^+ per se inhibited ammonia production from 2.5 mM L-glutamine as before (2, 5), shown in Fig. 6. Rates of ammonia production in the presence or absence of hGH at 145 mM Na^+ gradient (extra- to intracellular) were 1.84 ± 0.25 vs. 0.91 ± 0.08 and at 25 mM Na^+-equilibrated conditions were 0.70 ± 0.14 vs. 0.59 ± 0.15, respectively. The stimulatory effect of hGH on ammonia production was lost under Na^+-equilibrium conditions (P = NS, Dunnett's multiple-comparison procedure, $n = 5$, extracellular fluid Na^+ 25 mM in presence vs. absence of hGH). These

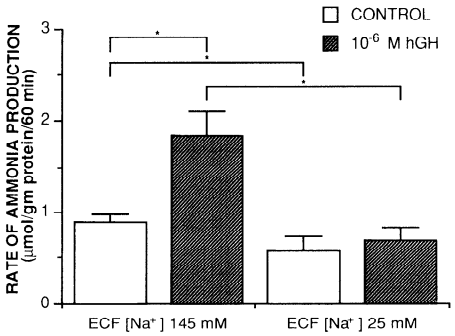

Fig. 6. Rate of ammonia production in proximal tubule segments incubated at pH 7.4 in presence of 145 mM extracellular [Na^+] or no extracellular [Na^+], using 2.5 mM L-glutamine as sole substrate. hGH was added at *time 0* after a 15-min equilibration period. Data are expressed as mean rates (±SE) of ammonia production from 0 to 60 min of 5 paired experiments (* $P < 0.05$).

findings provide evidence for a link between hGH-stimulated ammonia production and the presence of a Na^+ gradient and perhaps for Na^+-dependent glutamine transport.

Substrate availability may also play an equally important role in the transporting of L-glutamine into the mitochondrial ammoniagenic pathway. Experiments designed to determine the dependence of ammonia metabolism on the concentration of extracellular L-glutamine in IPT at neutral pH demonstrate saturation at an approximate concentration of 10 mM [rates of NH_3 production at 120 min ($\mu mol \cdot g\ protein^{-1} \cdot min^{-1}$) at various concentrations of L-glutamine were 4.57 ± 0.22 at 15 mM, 4.22 ± 0.44 at 10 mM, 2.50 ± 0.13 at 5.0 mM, 1.44 ± 0.10 at 2.0 mM, 0.66 ± 0.07 at 1.0 mM, and 0.10 ± 0.06 at 0 mM L-glutamine, $P < 0.05$ two-tailed Dunnett's multiple-comparison procedure, $n = 5$]. Thus availability of L-glutamine for ammonia production in IPT saturates at supraphysiological levels and can be rate limiting. Ammonia production was not enhanced in the presence or absence of hGH under conditions in which 2.5 mM L-glutamine was the sole substrate and the Na^+ gradient was abolished (Fig. 6). These findings would suggest the action of hGH on ammonia metabolism affects mitochondrial glutamine availability and is dependent on an outward-to-inward Na^+ gradient.

The driving force for Na^+-glutamine cotransport is the extracellular-to-intracellular Na^+ gradient (33). The Na^+ gradient of the renal proximal tubule is generated by basolateral Na^+-K^+-ATPase activity. To investigate whether hGH affects Na^+-K^+-ATPase activity directly (and thus Na^+-glutamine transport indirectly), basolateral membranes isolated from IPTs that had been incubated in the presence or absence of 10^{-6} M hGH were assayed for ouabain-sensitive Na^+-K^+-ATPase activity. Significant enhancement of ouabain-sensitive Na^+-K^+-ATPase activity in the presence (0.608 ± 0.03 $\mu mol\ P_i \cdot mg^{-1} \cdot min^{-1}$) vs. the absence (0.558 ± 0.02 $\mu mol\ P_i \cdot mg^{-1} \cdot min^{-1}$) of 10^{-6} M hGH was demonstrated ($P < 0.05$, paired Student's t test, 6 determinations in $n = 2$ preparations). These data provide evidence for the ammoniagenic action of hGH being dependent in part on the enhancement of Na^+-dependent L-glutamine transport into proximal tubule cells.

DISCUSSION

The data presented in this study demonstrate that hGH, like insulin, directly stimulates ammonia production from L-glutamine in canine isolated proximal tubules. Classically, GH effects on metabolism are thought to be direct, whereas those involving somatic changes and growth are thought to be indirect (15). Those indirect effects of hGH are mediated by somatomedins or insulin-like growth factors. A role for IGF-I mediation of hGH-stimulated ammoniagenesis is unlikely, as we have previously shown that IGF-I does not affect ammonia production in canine IPTs (2).

The stimulatory effect of hGH on renal ammoniagenesis is similar to that of insulin. However, the metabolic effects of hGH on glucose homeostasis include both insulinomimetic and insulin-antagonist action (17). GH, when incubated with renal proximal tubules, stimulates gluconeogenesis (26), whereas insulin inhibits this metabolic pathway (2). The inability of hGH to enhance glucose production from the gluconeogenic substrate L-glutamine is a well-documented observation in canine kidney (2). The movement of the carbon skeleton of L-glutamine into the Krebs cycle is the preferred metabolic pathway in canines, whereas in the rat, the carbon skeleton is gluconeogenic. A role for direct activation of hGH on ammoniagenic enzymes has not been investigated here, but seems consistent with the late and somewhat exaggerated ammonia stimulation after 30 min (Fig. 1 and RESULTS) without a complementary increase in glucose production. Furthermore, the lack of additive hGH and acidic pH effects (Fig. 4) also suggest a common mechanism of action. In rats, systemic acidosis results in activation of both phosphate-dependent glutaminase (PDG; 34) and glutamate dehydrogenase (GDH; 35). Under those conditions, whereby hGH or acute acidic conditions stimulate ammonia production, enzymic activation may indeed contribute to that enhancement.

Recent data reported by Rogers and Hammerman (27) have demonstrated direct activation of phospholipase C by GH in canine renal basolateral membranes. We previously have demonstrated inhibition of ammoniagenesis by tumor-promoting phorbol esters (3). Activation of phospholipase C results in the formation of diacylglycerol and inositol phosphates. Diacylglycerol, in turn, activates protein kinase C. Tumor-promoting phorbol esters are thought to directly activate protein kinase C. Protein kinase C activation has been shown to activate the apical Na^+-H^+ exchanger, resulting in cellular alkalinization in canine proximal tubule cells (22). Our findings failed to demonstrate an alkalinization of the proximal tubule cell (Fig. 5). Therefore, we speculate that the signal transduction mechanism by which hGH stimulates ammonia production involves at least the hydrolysis of inositol phosphates and/or the release of cellular Ca^{2+}, and not protein kinase C.

Patients with chronic growth-hormone excess, or acromegaly, are known to be volume overloaded due to Na^+ retention (8). This chronic Na^+ overload is thought to be secondary to increased Na^+ transport mediated by hGH or IGFs. In vivo data suggests that GH or IGFs may indeed enhance Na^+ reabsorption by stimulating the basolateral Na^+-K^+-ATPase activity (29). Shimomura et al. (29) demonstrated a 212% increase in Na^+-K^+-ATPase activity in homogenized rat kidneys from GH-treated hypophysectomized rats. Although the authors did not demonstrate a direct effect of GH on Na^+-K^+ ATPase, the marked enhancement of enzyme activity after 7 days would provide a logical explanation for the positive Na^+ balance seen in patients with acromegaly. Recently, Quigley and Baum (25) failed to demonstrate an acute effect of either GH or IGF-I on rabbit proximal convoluted tubule volume absorption (J_v) or bicarbonate transport. However, both GH and IGF-I affected the basolateral potential difference (PD). No investigations evaluating J_v and the effects of chronic administration of GH or IGF-I in normal animals have been undertaken. Our data demonstrate a direct action of hGH on ammonia metabolism that is Na^+ gradient

dependent (Fig. 6). hGH also directly stimulated Na^+-K^+-ATPase activity in basolateral membranes from IPTs by ~8%, which is identical to the PD change observed by Quigley and Baum (25). However, the small increase (~8%) in ouabain-sensitive Na^+-K^+-ATPase activity or that of the change in PD would not entirely account for the nearly threefold increase in IPT ammonia production by hGH.

Enhancement of the extracellular-to-intracellular Na^+ gradient results in increased L-glutamine transport (33). L-Glutamine loading, per se, increased basal rates of ammonia production both in canines in vivo (14) and in IPT in vitro (2 and RESULTS). Conversely, in the absence of a Na^+ gradient, hGH had no affect on L-glutamine transport or NH_3 production. Thus, part of the hGH effect on IPT ammonia production appears to involve increased L-glutamine availability. These findings support the Na^+ dependency of the hGH effect on NH_3 production presumably by enhancing substrate availability.

Ammonia production is known to increase in response to an acid load in vivo, such as ingesting a protein meal (21), or in vitro, as demonstrated in Fig. 4. GH release from the pituitary is similarly enhanced by ingestion of a protein meal (6). Under otherwise neutral conditions, increased levels of GH could enhance ammonia production after eating a protein meal. In a preliminary communication, we demonstrated enhanced net acid excretion in both normal and chronically renal insufficient growing and adults rats receiving exogenous GH (1). Further support of a role for GH enhancing net acid excretion has been recently demonstrated by Welbourne and Cronin (32), in which a direct enhancement of ammonium excretion was observed in isolated perfused kidneys from normal and hypophysectomized rats treated with GH and after direct GH infusion.

Growth itself has been shown to be an acidifying process (20). Skeletal and linear growth (including soft tissue) can add nearly 33% more acid burden on the kidney during early rapid human neonatal growth (20). This acid burden coincidental with a known exogenous acid load (e.g., ingestion of protein) by growing individuals mandates extrarenal and renal adaptations. Either of these mechanisms may be operative in a coordinated response to stimulate GH release, resulting in increased renal ammoniagenesis.

In conclusion, GH directly stimulates ammoniagenesis via a receptor-mediated event in proximal tubule segments. This action of hGH displays Na^+ dependency, increased substrate availability, and affects Na^+-K^+-ATPase activity. However, hGH also appears to have an action on IPT ammonia production similar to that of acidosis. This mimicry would suggest that IPT enzymic processes are being affected by hGH. The ability of GH to enhance ammonia production and excretion (1, 32) in the proximal tubule provides a logical physiological response to enhance net acid excretion. This action of hGH may have important implications in pathophysiological states in which diminished net acid excretion plays a significant part in morbidity of the whole animal or when physiological acid-producing processes necessitate an enhanced acid excretion response.

We thank B. Imm for expert technical assistance, T. Goodfriend for the use of the single-wavelength spectrophotofluorometer, and N. Hadjokis for help in determining the ΔpH_i (all at Univ. of Wisconsin). We acknowledge the advice of M. R. Hammerman (Washington University, St. Louis, MO). We also appreciate the typing and graphic computer skills of Emilie Ebel and Audrey Williamson.

M. C. Chobanian was supported by grants from the National Kidney Foundation of Wisconsin and the Graduate School of the University of Wisconsin. This project also received support from a research grant to the University of Wisconsin School of Medicine from the National Institutes of Health, Division of Research Facilities and Resources. K. H. Molteni was a recipient of a National Kidney Foundation fellowship. P. C. Brazy was supported by a Merit Award through the Department of Veterans Affairs.

Parts of this work have previously been presented to the 22nd Annual American Society of Nephrology Meeting, December 1989, and have appeared in abstract form in *Kidney Int.* 37: 526, 1990.

Received 26 August 1991; accepted in final form 16 December 1991.

REFERENCES

1. **Allen, D. B., R. El-Hayek, A. L. Friedman, and M. C. Chobanian.** Growth hormone stimulates urinary ammonia excretion in normal and 75% nephrectomized rats (Abstract). *Pediatr. Res.* 29: 336A, 1991.
2. **Chobanian, M. C., and M. R. Hammerman.** Insulin stimulates ammoniagenesis in canine renal proximal tubular segments. *Am. J. Physiol.* 253 (*Renal Fluid Electrolyte Physiol.* 22): F1171–F1177, 1987.
3. **Chobanian, M. C., and M. R. Hammerman.** Phorbol esters inhibit ammoniagenesis and gluconeogenesis in proximal tubular segments. *Am. J. Physiol.* 252 (*Renal Fluid Electrolyte Physiol.* 21): F1073–1079, 1987.
4. **Chobanian, M. C., and M. R. Hammerman.** Parathyroid hormone stimulates ammoniagenesis in canine renal proximal tubular segments (Abstract). *Kidney Int.* 33: 389, 1988.
5. **Chobanian, M. C., and C. M. Julin.** Angiotensin II stimulates ammoniagenesis in canine renal proximal tubule segments. *Am. J. Physiol.* 260 (*Renal Fluid Electrolyte Physiol.* 29): F19–F26, 1991.
6. **Daughaday, W. H.** Growth hormone: normal synthesis, secretion, control, and mechanisms of action. In: *Endocrinology,* edited by L. J. DeGroot. Philadelphia, PA: Saunders, 1989, vol. 1, p. 318–329.
7. **Deamer, D. W., R. C. Prince, and A. R. Crofts.** The response of fluorescent amines to pH gradients across liposome membranes. *Biochim. Biophys. Acta* 274: 323–335, 1972.
8. **Deray, G., M. Rieu, M. A. Devynck, M. G. Pernollet, P. Chanson, J. P. Luton, and P. Meyer.** Evidence of an endogenous digitalis-like factor in the plasma of patients with acromegaly. *N. Engl. J. Med.* 316: 575–580, 1987.
9. **Dunnett, C. W.** A multiple comparison procedure for comparing several treatments with a control. *J. Am. Stat. Assoc.* 50: 1096–1121, 1955.
10. **Eigenmann, J. E., and R. Y. Eigenmann.** Radioimmunoassay of canine growth hormone. *Acta Endocrinol.* 98: 514–520, 1981.
11. **Fiske, C. H., and Y. SubbaRow.** The colorimetric determination of phosphorus. *J. Biol. Chem.* 66: 375–400, 1925.
12. **Gesek, F. A., and A. C. Schoolwerth.** Insulin increases Na^+-H^+ exchange activity in proximal tubules from normotensive and hypertensive rats. *Am. J. Physiol.* 260 (*Renal Fluid Electrolyte Physiol.* 29): F695–F703, 1991.
13. **Guler, H.-P., C. Schmid, J. Zapf, and E. R. Froesch.** Effects of recombinant insulin-like growth factor I on insulin secretion and renal function in normal human subjects. *Proc. Natl. Acad. Sci. USA* 86: 2868–2872, 1989.
14. **Halperin, M. L., and C. Bun-Chen.** Influence of acute hyponatremia on renal ammoniagenesis in dogs with chronic metabolic acidosis. *Am. J. Physiol.* 258 (*Renal Fluid Electrolyte Physiol.* 27): F328–F332, 1990.
15. **Hammerman, M. R.** The growth hormone-insulin-like growth factor axis in kidney. *Am. J. Physiol.* 257 (*Renal Fluid Electrolyte Physiol.* 26): F503–F514, 1989.

16. **Horne, W. C., N. E. Norman, D. B. Schwartz, and E. R. Simons.** Changes in cytoplasmic pH and in membrane potential in thrombin-stimulated human platelets. *Eur. J. Biochem.* 120: 295–302, 1981.
17. **Isaksson, O. G., P. S. Eden, and J.-O. Jansson.** Mode of action of pituitary growth hormone on target cells. *Annu. Rev. Physiol.* 47: 483–499, 1985.
18. **Jørgensen, P. L., and J. C. Skou.** Preparation of highly active Na^+-K^+ ATPase from outer medulla of rabbit kidney. *Biochem. Biophys. Res. Commun.* 37: 39–46, 1969.
19. **Joseph, P. K., and K. Subrahmanyam.** Effect of growth hormone, insulin, thyroxine, and cortisone on renal gluconeogenesis. *Arch. Biochem. Biophys.* 127: 288–291, 1968.
20. **Kildeberg, P., K. Engel, and R. W. Winters.** Balance of net acid in growing infants. *Acta Pediatr. Scand.* 58: 321–329, 1969.
21. **Koeppen, B. M., and G. Giebisch.** Segmental hydrogen ion transport. In: *The Regulation of Acid-Base Balance,* edited by D. W. Seldin and G. Giebisch. New York: Raven, 1989, p. 139–167.
22. **Mellas, J., and M. R. Hammerman.** Phorbol ester-induced alkalinization of canine renal proximal tubular cells. *Am. J. Physiol.* 250 (*Renal Fluid Electrolyte Physiol.* 19): F451–F459, 1986.
23. **Moore, R. D.** Effects of insulin upon ion transport. *Biochim. Biophys. Acta* 737: 1–49, 1983.
24. **Noël, J., A. Tejedor, P. Vinay, and R. Laprade.** Fluorescence measurement of intracellular pH on proximal tubule suspensions. *Renal Physiol. Biochem.* 12: 371–387, 1989.
25. **Quigley, R., and M. Baum.** Effects of growth hormone and insulin-like growth factor I on rabbit proximal convoluted tubule transport. *J. Clin. Invest.* 88: 368–374, 1991.
26. **Rogers, S. A., I. E. Karl, and M. R. Hammerman.** Growth hormone directly stimulates gluconeogenesis in canine renal proximal tubule. *Am. J. Physiol.* 257 (*Endocrinol. Metab.* 20): E751–E756, 1989.
27. **Rogers, S. A., and M. R. Hammerman.** Growth hormone activates phospholipase C in proximal tubular basolateral membranes from canine kidney. *Proc. Natl. Acad. Sci. USA* 86: 6363–6366, 1989.
28. **Schoolwerth, A. C., and R. M. Culpepper.** Renal gluconeogenesis. *Miner. Electrolyte Metab.* 14: 347–361, 1988.
29. **Shimomura, Y., M. Lee, J. Oku, G. A. Bray, and Z. Glick.** Sodium potassium dependent ATPase in hypophysectomized rats: Response to growth hormone, triiodothyronine, and cortisone. *Metabolism* 31: 213–216, 1982.
30. **Smith, P. K., R. I. Krohn, G. T. Herman, A. K. Mallia, F. H. Gartner, M. D. Provenzano, E. K. Fujimoto, N. M. Goeke, B. J. Olsen, and D. C. Klenk.** Measurement of protein using bicinchoninic acid. *Anal. Biochem.* 150: 76–85, 1985.
31. **Thomas, J. A., R. N. Buchsbaum, A. Zimniak, and E. Racker.** Intracellular pH measurements in Erlich ascites tumor cells utilizing spectroscopic probes generated in situ. *Biochemistry* 18: 2210–2218, 1979.
32. **Welbourne, T. C., and M. J. Cronin.** Growth hormone accelerates tubular acid secretion. *Am. J. Physiol.* 260 (*Regulatory Integrative Comp. Physiol.* 29): R1036–R1042, 1991.
33. **Windus, D. W., D. E. Cohn, S. Klahr, and M. R. Hammerman.** Glutamine transport in renal basolateral vesicles from dogs with metabolic acidosis. *Am. J. Physiol.* 246 (*Renal Fluid Electrolyte Physiol.* 15): F78–F86, 1984.
34. **Wright, P. A., and M. A. Knepper.** Phosphate-dependent glutaminase activity in rat renal cortical and medullary tubule segments. *Am. J. Physiol.* 259 (*Renal Fluid Electrolyte Physiol.* 28): F961–F970, 1990.
35. **Wright, P. A., and M. A. Knepper.** Glutamate dehydrogenase activities in microdissected rat nephron segments: effects of acid-base loading. *Am. J. Physiol.* 259 (*Renal Fluid Electrolyte Physiol.* 28): F53–F59, 1990.