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Growth Hormone Secretory Capacity of Individual Somatotropes in Rats with Chronic Renal Insufficiency

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ABSTRACT. Growth failure is a common consequence of chronic renal insufficiency (CRI) in children and may be due to a number of factors. With regard to growth hormone (GH), regulation is often abnormal in CRI patients. The present study investigated the effect of CRI on the GH secretory responsiveness to GH-releasing hormone in individual rat pituitary somatotropes. Male Sprague-Dawley rats underwent a ⅝ nephrectomy to produce CRI. Control rats (SHAM) received sham operations, which included kidney decapsulation but not removal. Two wk later, during a period of stable uremia, serum creatinine [CRI: 1.1 ± 0.08 mg/dL (97 ± 7 μmol/L); SHAM: 0.4 ± 0.04 mg/dL (35 ± 4 μmol/L)] and serum urea nitrogen [CRI: 60.7 ± 8.3 mg/dL (21.7 ± 3.0 mmol/L); SHAM: 15.8 ± 1.2 mg/dL (5.6 ± 0.4 mmol/L)] were significantly elevated in the CRI rats ($p < 0.0005$). Weight gain ($p < 0.0005$), length gain ($p < 0.0005$), food intake ($p < 0.0005$), and food efficiency ($p < 0.005$) were all significantly lower in the CRI rats. The GH secretory capacity of individual somatotropes was determined using the reverse hemolytic plaque assay technique. Plaque areas were measured to assess relative amounts of GH secreted. The total number of pituitary cells per rat, the percentage of somatotropes, and the mean plaque areas were similar for the two groups. These findings compare favorably with our *in vitro* study of GH responsiveness in perifused rat pituitary cells under conditions of mild uremia. We conclude that the secretory responsiveness of individual somatotropes is not significantly changed under conditions of moderate CRI, and that potential abnormalities in GH secretion are due to factors other than an alteration in the capability of somatotropes to respond to GH-releasing hormone. (*Pediatr Res* 31: 528–531, 1992)

Abbreviations

CRI, chronic renal insufficiency
GH, growth hormone
GHRH, growth hormone-releasing hormone

CRI in children is frequently complicated by growth failure (1–5). The etiology of this growth failure is complex, and nutritional, metabolic, and hormonal disturbances have been implicated (3, 5–7).

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The role played by the GH axis is unclear. Circulating concentrations of GH have been found to be elevated in patients with CRI (8–12) and in partially nephrectomized animals (13). This may be due in part to inadequate excretion of GH, inasmuch as the kidney is the major site for GH excretion (14). However, some evidence suggests that the elevated concentrations are mainly due to increased GH secretion (12). In addition to high circulating levels of GH, the GH response to various stimuli (glucose, hypoglycemia, thyrotropin releasing hormone) is abnormal among patients with CRI. Abnormalities include a paradoxical rise in GH with glucose infusion (8–11, 15, 16) and a lack of GH release with hypoglycemia (16). In one study, a decreased pituitary GH content was observed in uremic rats (17). The involvement of the GH axis in the etiology of growth failure in CRI may be the basis of improved growth seen in uremic rats (13, 18, 19) and children (20–22) given high doses of GH.

The current study was undertaken to gain further understanding of the effects of CRI on GH secretion. Using the reverse hemolytic plaque assay technique, the secretory capacity of individual pituitary somatotropes in response to varying concentrations of GHRH was determined in nephrectomized and sham-operated rats.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats were obtained from Charles River Breeding Laboratories (Wilmington, MA) and maintained in individual cages in an environmentally controlled animal facility with a 12-h light, 12-h dark schedule. The temperature was maintained at 21–23°C. Standard rodent laboratory Chow (Ralson-Purina Co., St. Louis, MO) in powdered form and water were available *ad libitum*.

Nephrectomy. The experimental protocol was approved by Virginia Commonwealth University's Institutional Animal Care and Use Committee. To produce CRI, the animals ($n = 6$) underwent a two-stage nephrectomy. In the first stage, the right kidney was decapsulated and the upper and lower thirds were removed. In the second stage 1 wk later, the entire left kidney was excised. Sham-operated control rats underwent identical operative procedures up to and including decapsulation of the kidneys. Surgical procedures were performed on rats anesthetized with pentobarbital (1.25 mg/100 g body weight intraperitoneally) and ketamine (4.0 mg/100 g body weight intramuscularly).

Measurements. Linear measurements (nose to tail tip) were carried out using a ruler (19). The weights of the rats and the amount of food consumed were determined using an electronic balance (19). Food intake was quantitated every other day as the weight difference between food containers when full and just before refill. Food efficiency was calculated by dividing the weight gained during the 2-wk period after nephrectomy by the total food intake during the same 2-wk period (19).

Blood was collected after rapid decapitation under anesthesia. Serum creatinine concentrations were determined using a Beckman Creatinine Analyzer II (Beckman Instruments, Inc., Brea, CA). Serum urea nitrogen was determined by electrode using an autoanalyzer (Astra; Beckman Instruments, Inc.).

Reverse hemolytic plaque assay. The reverse hemolytic plaque assay for individual pituitary somatotropes was performed as previously described (23). Briefly, the pituitary glands were removed immediately after decapitation. After separating and discarding the neurointermediate lobe, the anterior pituitary was diced and dispersed using 0.25% (0.0025) trypsin in 10 mL minimum essential medium (SMEM, no. 410-1400; Gibco, Grand Island, NY). Dispersion took place during gentle stirring in a siliconized spinner flask (Bellco Glass, Inc., Vineland, NJ) partially submerged in a 37°C water bath for 60 min followed by gentle trituration using a 1.0-mL pipetter (Pipetman; Rainin Instruments, Woburn, MA). The cells were washed and resuspended in SMEM containing 0.1% (0.001) BSA. A total cell count was performed using a standard hemocytometer. The cell suspension was adjusted to a density of 4×10^5 cells/mL, and 0.5 mL of pituitary cells were combined with 0.5 mL of a 12% (0.12) suspension of ox erythrocytes (Colorado Serum Co., Denver, CO) that had been previously coated with protein-A (*Staphylococcus aureus*; no. P8143, Sigma Chemical Co., St. Louis, MO). The cell mixture was infused into a culture chamber similar to that described by Cunningham and Szenberg (24) constructed on a glass microscope slide previously coated with fresh poly-l-lysine (0.2 g/L; 30-min coat time) to enhance cell adhesion. After a 60-min incubation at 37°C to allow cell attachment, the culture medium within the chambers was replaced with Dulbecco's Modified Eagle's Medium (DMEM, no. 430–1600; GIBCO Laboratories, St. Lawrence, MA) and all cells that had not attached to the slide were washed out. After another 30-min incubation, each chamber was infused with DMEM containing monkey antimurine GH antiserum in a 1:200 dilution and one of the following concentrations of GHRH: 0, 0.01, 0.03, 0.1, 0.3, or 1.0 nM. After a 90-min incubation in the presence of the antiserum and GHRH, hemolytic plaques were developed over 35 min by addition of guinea pig complement (no. CS1250; Colorado Serum Co.) at a 1:25 dilution. The cells were then fixed with 1% (0.01) gluteraldehyde, refrigerated in a beaker of distilled water overnight, stained with methyl green pyronin, and permanently mounted with Permount (Fisher Scientific Co., Fair Lawn, NJ).

Each slide was analyzed with respect to the percentage of plaque-forming cells and the mean plaque area. The percentage of plaque-forming cells was determined by microscopic quantification with respect to nonplaque-forming cells in a total population of 500 cells per slide. Occasional polymorphonuclear leukocytes and lymphocytes were excluded based on their characteristic morphology. This procedure was facilitated by the use of methyl green pyronin, which stains only nucleated cells. A hemolytic plaque was defined by the presence of a clear zone of red blood cell hemolysis that was at least as wide as the diameter of the pituitary cell and completely surrounded it. Any pituitary cell with a hemolytic plaque was considered to be a somatotrope. The relative amount of GH secreted was evaluated using a Videoplan image analysis system (Carl Zeiss, Kontron, Munich, Germany), as described previously (25). For each slide, the mean area of 100 plaques was determined, and statistical analyses of differences between plaque areas in the control and nephrectomized (CRI) groups were determined using analysis of variance followed by *t* test. All mean plaque areas are expressed as the mean ± SEM.

RESULTS

Effects of 5/6 nephrectomy on serum, growth, and food utilization. Table 1 presents the results of serum, growth, and food use measurements for the CRI and sham-operated groups. The serum urea nitrogen and creatinine concentrations were significantly elevated in the CRI group; growth in the CRI group, measured by length and weight gain over the 2-wk period, was significantly impaired in the CRI group and both food intake and food efficiency were significantly lower for the CRI group. It was noted that the mean growth rate of the uremic rats was only 1.1 ± 0.9 g/d over the first 3 d postnephrectomy, compared with 4.5 ± 0.8 g/d during d 3 to 14. For the sham rats, the mean growth rates for the 0- to 3-d period and the 3- to 14-d period were 6.1 ± 0.4 and 8.2 ± 0.3 g/d, respectively. This shows that the growth rate of the uremic rats declined dramatically during the first few days postnephrectomy and then increased but never reached that of the sham rats.

Number of pituitary cells and percentage of somatotropes. Typical hemolytic plaques for GH-secreting pituitary cells treated with 0 and 1.0 nM GHRH concentrations are shown in Figure 1. Table 2 presents data regarding the number of anterior pituitary cells, the percentage of somatotropes, and the absolute number of somatotropes. The mean total number of cells of the CRI group was not significantly different from that of the sham group. The percentage of the total pituitary cells that were capable of secreting GH was not significantly different in either group, nor was the absolute number of somatotropes ($p > 0.10$).

Plaque areas. An illustration of the concentration-response relationships between GHRH and mean plaque area for the CRI and sham groups is presented in Figure 2. The relationships for both the CRI and sham groups are similar, and no significant difference in mean plaque area was noted at any of the GHRH concentrations.

DISCUSSION

Elevated serum GH concentrations have been associated with CRI (8–13). Interpretation of these data is difficult because of the typical pulsatile pattern of GH secretion. Also, the elevated serum concentration may be due in part to inadequate excretion, because the kidney is a major site for GH excretion (14). However, a prompt decrease in GH concentration upon somatostatin administration suggests that the elevated concentration is mainly due to increased GH secretion (12).

Altered hypothalamic-pituitary regulation of GH secretion in the uremic environment is also suggested by observed abnormal GH responses to various stimuli in patients with CRI. This includes a paradoxical rise in GH upon glucose administration (8–11), a rise in GH after thyrotropin-releasing hormone administration (15, 16), and a lack of GH release with hypoglycemia (16). With regard to serum IGF-I concentration in uremia, no significant difference has been observed between uremic and control individuals when RIA is performed after removal of interfering proteins (20, 26). Somatomedin activity, as measured by incorporation of radiolabeled sodium sulfate into cartilage, has been shown to be decreased (27), perhaps due to circulating inhibitors (28) in the uremic state. It is possible that this decreased somatomedin bioactivity is responsible for increased GH release, because there is evidence that IGF-I exerts negative feedback control on both the hypothalamus and the pituitary gland (29).

In this current study, a 5/6 nephrectomy was used to mimic the uremic environment in rats. As illustrated by the elevated serum urea nitrogen and creatinine levels (Table 1), the 5/6 nephrectomy produced a moderate degree of renal insufficiency in these animals. This degree of renal insufficiency resulted in marked growth failure, reduced food intake, and lower food efficiency (Table 1).

Using the reverse hemolytic plaque assay technique described above, the ability of pituitary somatotropes to secrete GH in response to varying GHRH challenges was determined, and no difference in responsiveness was found between the nephrectomized and sham-operated groups. These findings compare favorably with our *in vitro* study of GH responsiveness in perifused pituitary cells under conditions of mild uremia (30). These results

Table 1. *Serum urea nitrogen (SUN), serum creatinine, weight gain, length gain, food intake, and food efficiency for 5/6 nephrectomized (n = 6) and sham-operated (n = 6) rats (mean ± SEM)*

Rat group	SUN (mg/dL)	Creatinine (mg/dL)	Weight gain (g)	Length gain (cm)	Food intake (g)	Food efficiency (g wt gain/g food)
5/6 Nephrectomy	60.7 ± 8.3 (21.7 ± 3.0)*	1.1 ± 0.08 (97 ± 7)†	53.2 ± 6.6	3.6 ± 0.2	281.6 ± 22.2	0.19 ± 0.01
Sham	15.8 ± 1.2 (5.6 ± 0.4)*	0.4 ± 0.04 (35 ± 4)†	108.2 ± 3.0	5.0 ± 0.1	442.8 ± 24.2	0.25 ± 0.01
p value	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.005

* Value in mmol/L.

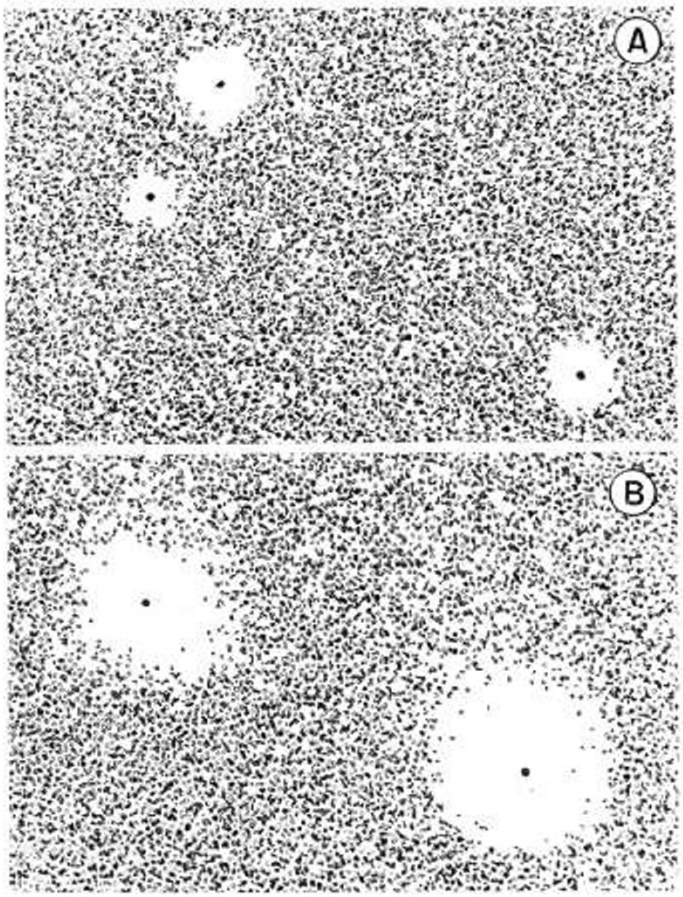

Fig. 1. Photomicrographs of plaques formed by rat pituitary cells using the reverse hemolytic plaque assay. *A* shows typical plaques formed by cells without the addition of GHRH. *B* shows typical plaques formed by cells treated with 1.0 nM GHRH.

Table 2. *Total number of pituitary cells, percentage of GH-secreting cells, and absolute number of somatotropes for 5/6 nephrectomized (n = 6) and sham rats (n = 6) (mean ± SEM)*

Rat group	Total number of pituitary cells (millions)	% GH-secreting cells	Absolute number of somatotropes (millions)
5/6 Nephrectomy	3.5 ± 0.6	40.5 ± 1.8	1.45 ± 0.29
Sham	4.5 ± 0.6	38.6 ± 0.5	1.75 ± 0.21

suggest that abnormal GH secretion observed in the CRI environment is due to something other than a change in somatotrope sensitivity to GHRH.

This experiment was designed to investigate GH secretion under conditions of sustained uremia. We noted that the growth rate dropped dramatically over the first 3 d after nephrectomy. The growth rate in the uremic rats increased after this initial

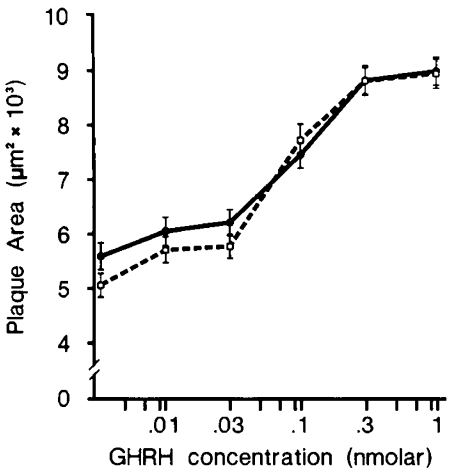

Fig. 2. GH secretory capacity of somatotropes at varying concentrations of GHRH for 5/6 nephrectomized (*dotted line*) and sham rats (*solid line*). Secretory capacity is expressed as mean plaque area ± SEM in μm^2. GHRH concentration ranged from 0 to 1.0 nM.

period, but not to that of the sham rats. Thus, this experiment does not rule out the possibility of a change in somatotrope secretory responsiveness under conditions of acute renal failure and severe growth rate impairment that occurs during the first few days after nephrectomy.

It should be noted that the current study was carried out *in vitro* and does not necessarily correspond to the *in vivo* uremic state. One concern is that any inhibitors or enhancers of GH secretion that could be present in uremic serum may have been washed away from somatotropes during the *in vitro* dispersion and incubation. Also, the food intake of the CRI rats was significantly lower than that of the control group, and this lower nutrition intake may affect GH regulation (31). Finally, many of the studies cited above that observed abnormal GH responses to various stimuli were carried out on human patients, and the rat model may not accurately correlate to those findings.

In conclusion, this study provides evidence that the GH secretory responsiveness of rat somatotropes is unchanged in a moderately uremic environment and that any abnormalities in GH regulation would be due to something other than altered responsiveness to GHRH.

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