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Growth hormone secretion from pituitary cells in chronic renal insufficiency

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Growth hormone secretion from pituitary cells in chronic renal insufficiency. To examine whether growth hormone (GH) secretion is adversely affected by chronic renal insufficiency (CRI), the GH secretory response of dispersed anterior pituitary cells perifused with GH-releasing hormone (GHRH) was investigated in 5/6 nephrectomized (CRI, $N = 18$) and sham-operated ($N = 18$) rats. Two weeks after nephrectomy, during a period of stable uremia, CRI rats had significantly higher serum concentrations (mean ± SEM) of urea nitrogen and creatinine than sham rats, 16.8 ± 1.4 mmol/liter (47 ± 4 mg/dl) and 79.6 ± 0.0 μmol/liter (0.9 ± 0.0 mg/dl) versus 6.1 ± 0.4 mmol/liter (17 ± 1 mg/dl) and 35.4 ± 0.0 μmol/liter (0.4 ± 0.0 mg/dl), respectively ($P < 0.0001$). Incremental gains in body weight and nose to tail-tip length of CRI rats over two weeks were also significantly depressed, 53.3 ± 5.38 g (CRI) versus 87.0 ± 3.78 g (sham; $P < 0.0001$) and 3.2 ± 0.2 cm (CRI) versus 3.6 ± 0.1 cm (sham; $P < 0.05$). The cumulative food intake as well as food efficiency (g food consumed/g weight gain) were also adversely influenced by the uremic state: food intake 304 ± 1 g (CRI) versus 397 ± 6 g (sham; $P < 0.0001$) and food efficiency 0.173 ± 0.013 g/g of weight gain (CRI) versus 0.219 ± 0.008 g/g of weight gain (sham). No significant difference in GH secretory rate (ng/min/10^7 cells) was found between the uremic and sham animals under basal conditions, 65.2 ± 2.1 (CRI) and 67.9 ± 2.2 (sham) or in response to GH-releasing hormone, 282.8 ± 42.4 (CRI) versus 306.2 ± 42.6 (sham). The secretory curves representing concentration-GH response were similar in both groups of animals. This study provides direct evidence that the response of pituitary cells to GHRH is preserved in moderate CRI and suggests that, at this degree of renal function reduction, any disturbance of GH secretion must be due to dysfunctions other than the secretory capacity of the pituitary gland itself.

Growth impairment is a typical feature of CRI in children [1, 2]. Multiple factors including sustained metabolic acidosis, mineral disorders, hormonal disturbances, and malnutrition are involved in its pathogenesis [3, 4]. It is believed that low caloric intake and abnormalities in growth hormone (GH) metabolism play a major pathogenic role [5–8]. Improvement of the growth rate has been obtained in children and animals with CRF from the employment of caloric supplementation [9–11] and, more recently, through the use of GH therapy [12–18].

Normal or high serum concentrations of GH [19, 20] and low insulin-like growth factor I [21, 22] have been found in uremic individuals. The decreased serum somatomedin activity seen in patients with CRI may possibly be due to circulating inhibitors related to the uremic state [23]. In addition, several reports have suggested that GH secretion might be altered in humans [24–28] and rats [26] in uremia. Enhanced [24] and depressed [27] GH response following different stimuli, such as L-dopa, hypoglycemia or GH-releasing hormone (GHRH), has been demonstrated in CRF patients, but the influence of the uremic state on pituitary GH secretion is still undefined.

The present study was designed to evaluate GH secretion in CRF by using perifusion of dispersed anterior pituitary cells with GHRH in 5/6 nephrectomized and control rats. This represents the first direct examination of the effect of uremia from CRI on the GH secretory capacity of pituitary cells.

Methods

Animals

Male Sprague-Dawley rats weighing 120 ± 5 g were obtained from Charles River Laboratories (Wilmington, Massachusetts, USA) and maintained in individual cages in an environmentally controlled animal facility (12-hour light-darkness cycle, temperature between 21 and 23°C). Animals were fed standard 23.4% protein rat chow (Purina 5001, St. Louis, Missouri, USA) in a powdered form. Both food and tap water were available *ad libitum*. After their arrival and before surgery, rats were acclimated to the new environment for four days.

Experimental design

The experimental protocol was approved by Virginia Commonwealth University's Institutional Animal Care and Use Committee.

After a four-day acclimation period, rats underwent either two-stage 5/6 nephrectomy (CRF rats, $N = 18$) or sham surgery (sham rats, $N = 18$). Nephrectomy was performed in two stages [29, 30]. During the first stage (day 0), the left kidney was exposed, decapsulated, and the upper and lower thirds of the kidney were removed. The second stage was performed one week later (day 7). The right kidney was exposed, decapsulated, renal pedicle was ligated, and the kidney was excised. Sham animals were treated in a similar way, including bilateral

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renal decapsulation, but no kidney tissue was removed. Surgical procedures were performed under anesthesia with pentobarbital (1.25 mg/100 g body weight, intraperitoneal) and ketamine (4 mg/100 g body weight, intramuscular).

Fourteen days after either the second-stage nephrectomy or second sham operation, the animals were lightly anesthetized with methoxyflurane and sacrificed by decapitation (day 21).

Rats were weighed using an electronic balance on days 0, 7, 10, 14, 18 and 21. Rats' snout to tail-tip lengths were measured on days 0, 7, and 21, while the animals were anesthetized. Food intake was measured from days 7 to 21. Food efficiency was calculated by dividing the weight gained (g) and the food ingested (g) during the period of time the animals were in stable CRI (days 7 to 21). At the time of sacrifice (day 21), blood was collected and adenohypophysis, anterior tibial muscle, and the liver were excised. Muscle and liver were weighed before and after desiccation for 16 hours at 120°C to determine their water content.

Serum urea nitrogen and glucose were measured by electrode using an auto analyzer (Astra, Beckman Instruments, Inc., Brea, California, USA). Creatinine was determined by means of a Beckman autoanalyzer (Beckman Instruments, Inc.). Cholesterol and triglycerides were measured by enzymatic methods using a bioanalyzer (Cobas, Roche Laboratories, Nutley, New Jersey, USA). Albumin was determined by photometric techniques with an Albumin Test Kit (DuPont Co., Wilmington, Delaware, USA) and a Serometer Model 370 filter photometer.

Six repetitions were carried out in each of the two groups of animals. The adenohypophysis of three rats were used for each group in each repetition for a total of 36 rats. The continuous perifusion of anterior pituitary cells was specifically performed [31] in the present experiments as follows. After rapid decapitation, the posterior lobe of the pituitary gland was gently removed and the anterior pituitary was submersed in Earle's Balanced Salt Solution (EBSS; Gibco, Grand Island, New York, USA), and then diced. Fragments were incubated for 20 minutes in 10 ml EBSS with 0.2% (wt/vol) trypsin and 0.15 mg DNAse (deoxyribonuclease-I, Sigma Chemical Co., St. Louis, Missouri, USA) at 37°C. Fragments were washed with calcium- and magnesium-free EBSS. Cell dispersion was completed by gentle trituration with a 1.0 ml pipette (Pipetman, Rainin Instrument Co., Woburn, Massachusetts, USA). Stranded DNA was removed, and an aliquot of cells was taken to determine the total number of viable cells by the trypan blue exclusion test, using a standard hemocytometer. Cells were then gently mixed with Bio-Gel P2 (200 to 400 mesh, Bio-Rad Laboratories, Richmond, California, USA), which had been preswollen overnight in normal saline. The pituitary cell-Bio-Gel mixture was packed into 2.0 ml plastic syringes (Sabre International Products Ltd., Reading, Berkshire, England, UK), which served as the perifusion chambers. The chambers were submersed in 37°C water bath, and perifusion was performed with Medium 199 (Gibco), containing 0.25% BSA, 10 U/ml penicillin, 0.5 μg/ml streptomycin, 187.5 ng/ml amphotericin B, and 5 μg/ml gentamicin at a mean flow rate of approximately 0.43 ml/min. Cells were allowed to equilibrate to perifusion conditions for four hours, at which time GHRH was administered in 2.5 minute pulses at 30 minute intervals. The concentrations of GHRH (human; 1-40; Bachem, Inc., Torrance, California, USA), were 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10, 30, and 100 nM, and were applied in a random order. Eluate was collected as five minute fractions and stored at −20°C until measurement of GH.

Concentrations of GH in the eluate fractions were measured by radioimmunoassay with reagents supplied by Dr. A. F. Parlow and the National Pituitary Hormone Program of the NIDDK. Anti-rat GH serum S-4 and GH reference preparation RP-2 were used. All standards were assayed in triplicate. Intra-assay coefficients of variation were 5.8% and 6.5% at 0.25 and 1.0 ng/tube, respectively, and the interassay coefficient of variation was 8.3% at 0.6 ng/tube.

Data analysis

Data, expressed as mean ± SEM, on weight, length, food intake, and food efficiency were compared by analysis of variance. Statistical significance was considered at a P value less than 0.05. GH response to GHRH was determined taking into account the differences in flow rate and the number of cells per column. Thus, the GH concentration in μg/liter (ng/ml) measured in each fraction was converted to a secretory rate in ng/min/10^7 cells. The secretory response to each concentration of GHRH was calculated as the secretory rate above baseline during the 30 minute period following administration of the pulse GHRH. The baseline was determined using trough GH levels between GHRH-stimulated secretory episodes in conjunction with the first preinfusion concentration and last postinfusion concentrations of GH. The amount of GH secreted above the baseline was calculated as the difference between the absolute GH value after stimulation and the paired baseline value. The sum of resulting differences (quantitative GH secretion) was divided by the number of fractions (six 5-min fractions collected over a 30 min interval following each GHRH pulse) to obtain the final value for the GH secretory response. The sequence of responses was then rearranged in concert with the ascending order of administered GHRH concentration. The concentration-response relationship between the GH secretory rate and the log of the GHRH concentration was evaluated by regression analysis.

Results

Serum biochemical data are summarized in Table 1. All nephrectomized animals had reduced renal function, as revealed by the serum urea nitrogen and creatinine concentrations. Compared with the sham rats, CRI rats were hypercholesterolemic and had significantly lower concentrations of serum glucose.

CRI severely impaired the growth of the nephrectomized rats. The weight of each group of rats at the time of the first stage nephrectomy was 164.8 ± 2.5 and 168.3 ± 2.5 g for CRF and sham groups, respectively. During the two week period following the second stage nephrectomy, weight gain of stable CRF rats was significantly lower than that of sham animals (53.3 ± 4.5 vs. 87.0 ± 3.7 g; $P < 0.0001$). The gain in nose to tail tip length was also lower in the CRF group (3.2 ± 0.2 vs. 3.6 ± 0.1 cm; $P < 0.05$). Food intake of CRF animals was approximately 75% of that of sham rats (304 ± 1 vs. 397 ± 6 g; $P < 0.0001$). Food efficiency of CRI rats was significantly ($P < 0.005$) lower in the CRI group (0.173 ± 0.013 vs. 0.219 ± 0.008). CRI and sham rats did not differ in their water content of muscle (75.8 ± 0.1 vs. 75.9 ± 0.2%) and liver (71.5 ± 0.3 vs. 71.2 ± 0.2%).

Table 1. Serum concentrations of urea nitrogen (S_{UN}), creatinine, cholesterol, triglycerides, albumin and glucose in 5/6 nephrectomized (CRI, $N = 18$) and sham-operated (sham, $N = 18$) rats

Rat group		S_{UN} *mmol/liter* (*mg/dl*)	Creatinine *μmol/liter* (*mg/dl*)	Cholesterol	Triglycerides *mmol/liter* (*mg/dl*)	Glucose	Albumin *g/liter* (*mg/dl*)
CRF:	Mean	16.8	79.6	2.02	0.58	9.1	9
		(47)	(0.9)	(78)	(51)	(163)	(0.9)
	SEM	1.4	8.8	.08	0.07	0.1	0
		(4)	(0.1)	(3)	(6)	(2)	(0.0)
Sham:	Mean	6.1	35.4	1.42	0.75	9.8	9
		(17)	(0.4)	(55)	(66)	(176)	(0.9)
	SEM	0.4	0.0	.05	0.07	0.3	0
		(1)	(0.0)	(2)	(6)	(5)	(0.0)
P value < 0.0001			<0.0001	<0.0001	0.10	<0.025	>0.25

Table 2. Concentration-response relationships between growth hormone releasing hormone (GHRH) and growth hormone (GH) release for continuously perifused pituitary cells

Group	Slope *ng/min/10⁷cells per nM GHRH*	Intercept *ng/min/10⁷ cells*	r^2	*P*
Sham	88.2 ± 7.7	308.5 ± 23.0	0.71	0.0001
CRF	81.9 ± 8.9	284.7 ± 26.3	0.62	0.0001

CRI means chronic renal insufficiency. Values for slope and intercept are expressed as mean ± SEM. *P* values reflect the presence of linear concentration-response relationships.

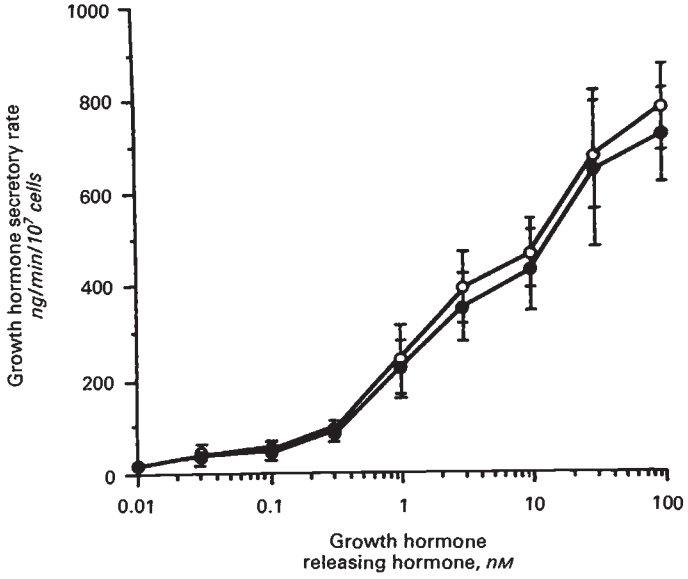

Fig. 1. *In vitro growth hormone (GH) secretory response of dispersed pituitary cells from sham and chronic renal insufficiency (CRI) rats to nine different concentrations (nM) of GH-releasing hormone (GHRH).* GH release (mean ± SEM) is expressed in ng/min/10^7 cells.

Analysis of the perifusion study data demonstrated a similar response in the pituitary cells from CRI and sham rats. In both groups, the mean percentage of viable cells exceeded 95%. Basal GH secretory rates were 65.2 ± 2.1 and 67.9 ± 2.2 ng/min/10^7 cells for CRI and sham rats, respectively. Likewise, there was no significant difference in the GHRH-stimulated GH secretory rates. Both groups of animals showed a linear dose-dependent concentration response (Table 2) as well as a similar overall mean GHRH-stimulated GH secretory rate, calculated as the sum of the responses to serial increments in GHRH concentrations (282.8 ± 42.4 and 306.2 ± 42.6 ng/min/10^7 cells for CRF and sham groups, respectively). Figure 1 illustrates the two study groups' similarity in the GH secretory responses to the ascending concentrations of GHRH doses.

Discussion

Renal insufficiency was produced in the 5/6 nephrectomized animals, reflected by the mean serum urea nitrogen being almost three times higher than that of sham rats. As a result of CRI and diminished food intake, the nephrectomized rats had hypercholesterolemia and a reduction in serum glucose concentrations compared to the sham animals. More importantly, the degree of reduction of renal function in our nephrectomized rats resulted in severe growth retardation and depressed food intake. The CRI rats not only ate less than sham animals, but, moreover, had less efficient utilization of the food consumption. Accordingly, incremental gains in weight and length were significantly lower in the CRI group.

It is well established that circulating concentrations of GH are frequently high in individuals with CRI [19, 20]. The typical pulsatile pattern of GH secretion makes the interpretation of this finding difficult. Thus, despite decreased renal clearance of GH in patients with low glomerular filtration rate [32], elevated plasma GH concentrations in these CRI patients have usually been attributed to abnormal regulation of pituitary GH secretion rather than reduced renal elimination [25, 33]. However, studies on GH secretion in CRI have been inconclusive and have often led to contradictory results.

Clinical investigations to assess pituitary GH secretion in CRI patients in response to conventional stimuli have yielded no uniform results, with supra and subnormal responses being reported [24, 27, 28]. Studies on pulsatile GH secretion have suggested that GH secretion might be depressed in renal failure [34], and a decreased pituitary content of GH has been reported in uremic rats [26]. This might be explained by the presence of high plasma concentrations of somatostatin, a potent inhibitor of GH secretion, which has been found in patients with CRI [35].

On the other hand, Phillips et al [23] initially reported the presence of a circulating peptide of low molecular weight that decreased somatomedin activity as assessed by stimulation of

sulfate uptake by hypophysectomized rat costal cartilage in vitro. Andress, Howard and Birnbaum [36] confirmed the presence of a bone cell mitogenic inhibitor in uremic plasma, and agree with a recent report indicating that it might actually not be a peptide [37]. The presence of high circulating levels of a somatomedin inhibitory factor in uremia might influence GH secretion by interfering with the well-known negative feedback action of somatomedin on GH secretion [34]. In this regard, the potential for increased GH secretion might be related to the increase in a somatomedin inhibitory factor. On the other hand, high plasma concentrations of somatostatin, a potent inhibitor of GH secretion, have been found in patients with CRI [35].

Our results are the first to present evidence on the ability of pituitary cells in stable CRI to secrete GH at the basal state as well as in response to incremental GHRH stimulation. Perifusion of pituitary cells with GHRH is a reliable method of assessing GH secretion [31, 38]. Therefore, although these in vitro findings do not necessarily imply the same response of pituitary cells in the in vivo uremic state, our results strongly suggest that the GH response to GHRH is not disturbed in CRI. It should be pointed out that the GH secretory rates of the pituitary cells from CRI animals were slightly, although not significantly, lower than that of sham rats for all concentrations of GHRH (Fig. 1). Accordingly, it cannot be ruled out that a greater degree of uremia than obtained in our animals may still impair pituitary GH secretion. However, it is also important to note that, in the presence of normal GH secretory response, the moderate CRI induced in the nephrectomized rats in our study resulted in severe growth retardation, as assessed by inadequate weight and length gains.

CRI rats consumed 25% less food than sham rats. Therefore, interpretation of our results must require consideration of the role played by malnutrition on pituitary GH secretion. Clinical [39] and experimental [40] studies have shown impaired GH release in malnourished individuals [39], and failure of serum somatomedins to rise in response to GH injections has been reported in nutritionally deprived rats [41] and humans [42]. Preliminary data from our laboratory indicate that there is no difference in the GH secretion of perifused pituitary cells of 5/6 nephrectomized rats and pair-fed sham animals (unpublished observations). Nevertheless, the interaction of malnutrition and CRI on GH secretion deserves further studies.

In conclusion, our study shows for the first time that pituitary GH secreting capacity is preserved in rats with moderate CRI, and suggests that peripheral circulating somatomedin inhibitors and/or nutritional factors, rather than depressed responsiveness of GHRH are responsible for the stunted growth of these animals.

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