Growth Hormone Deficiency Is Not Associated with Impaired Vagal Cholinergic Nervous System Activity in Children

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ABSTRACT. Central cholinergic mechanisms mediate release of growth hormone (GH) as well as peripheral secretion of pancreatic polypeptide (PP). To determine if impaired ability to secrete GH is associated with defective PP response, we studied the PP, epinephrine, and norepinephrine responses to insulin-induced hypoglycemia in 31 children evaluated for GH deficiency by insulin-arginine stimulation (IATT) and 24-h integrated concentrations of GH (IC-GH). Eleven patients had normal GH by IATT and IC-GH (controls), 10 patients had normal GH by IATT but subnormal IC-GH, 10 patients had GH deficiency by both IATT and IC-GH. PP levels peaked at the time of glucose nadir, and remained elevated for 20 min thereafter. The peak PP and incremental PP change from baseline were not significantly different among the three groups. The log peak PP response was inversely correlated with the glucose nadir ($r = -0.5$, $p < 0.005$). Peak PP levels were also significantly correlated with the peak epinephrine levels ($r = 0.6$, $p < 0.001$) but not with norepinephrine. Our findings suggest that 1) GH deficiency disorders are not associated with impaired vagal cholinergic response to hypoglycemia; 2) in children the magnitude of PP response is inversely related to the degree of hypoglycemia; and 3) the peripheral hormonal manifestation of autonomic nervous system responses to hypoglycemia as measured by PP and epinephrine levels are closely correlated. (*Pediatr Res* 24: 574-576, 1988)

Abbreviations

GH, growth hormone
PP, pancreatic polypeptide
E, epinephrine
NE, norepinephrine
IC, integrated concentration
hPP, human PP
GHND, GH neurosecretory dysfunction
GHD, GH deficiency

Central cholinergic mechanisms mediate GH release (1, 2) as well as secretion of PP (3, 4). Cholinergic agonists potentiate the secretion of both GH and PP and conversely antagonists of cholinergic muscarinic receptors such as pirenzepine and atropine blunt the secretion of these hormones (1, 5). We tested the hypothesis that children with impaired GH secretion might also have impairment of vagal cholinergic activity, manifested by

deficient PP secretion. During insulin-induced hypoglycemia which is a potent stimulant for PP and GH secretion (3, 6-8), we evaluated the responses of autonomic nervous system activity in children with GH deficiency syndromes.

SUBJECTS AND METHODS

Patients in this study were referred for evaluation of short stature. None of the patients had gastrointestinal or pancreatic disorders at the time of evaluation. All diagnostic procedures were conducted in the Endocrine Diagnostic Unit at the University of Maryland. After an overnight fast, each patient underwent an insulin tolerance test (8) followed by an arginine stimulation test for diagnosis of GH deficiency (8). During insulin-induced hypoglycemia blood glucose levels were followed in real time using a continuous glucose monitor (9, 10). Blood samples for GH, PP, E, NE, and cortisol were drawn at baseline at 20 min, at glucose nadir identified in real time, and at 10 and 20 min after the glucose nadir. A normal GH response of 10 ng/ml to paired (insulin and arginine) stimulation and a cortisol level more than 10 μ g/dl in response to hypoglycemia were considered normal. Each patient also had 24-h IC-GH and IC-cortisol assessment using constant blood withdrawal methodology (11, 12). The normal ranges of IC-GH and IC-cortisol for children at this age are 3.2-11.5 ng/ml and 2.7-9.1 μ g/dl, respectively (13, 14).

Assay techniques. Plasma hPP was measured by a double antibody radioimmunoassay as described by Gingerich *et al.* (15). Rabbit antibody to human pancreatic polypeptide, human pancreatic polypeptide standards, and labeled 125 I-bovine PP with a specific activity of approximately 400-500 UCI/ μ g were kindly donated by Dr. R. E. Chance, E. Schirmer and Lilly Research Laboratories. The lowest detection limit of the assay was 10 pg/ml. The intra- and interassay coefficients of variation were 6 and 15%, respectively.

Plasma GH was assayed using a standard double antibody technique (16). Plasma epinephrine and norepinephrine were assayed by a radioenzymatic technique (17, 18).

Statistical analysis. The effect of diagnostic grouping on PP, E, NE, glucose was evaluated by analysis of variance (19). Differences between means of diagnostic groups were then assessed by the method of Duncan (19). Logarithmic transformation of data was performed before statistical analysis when necessary to reduce heterogeneity of variances for parametric statistics. Regression analysis of peak hormone levels with glucose nadir was performed using standard methods (19). Data in Figures 1 and 2 and Tables 1 and 2 are expressed as mean $\pm$ 1 SE.

RESULTS

The 31 euthyroid patients with normal cortisol secretion were subdivided into three diagnostic groups according to the following criteria.

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Control. Eleven children had normal stimulated GH responses and normal 24-h IC-GH.

GHND. Ten children had normal responses to stimulation testing but a subnormal spontaneous 24 IC-GH (GH < 3.2 ng/ml).

GHD. Ten children had subnormal GH responses (GH < 10 ng/ml) to paired stimulation testing as well as deficient IC-GH.

The ages and body mass indices (kg body weight/height M²) and IC-cortisol for each group were similar and differences between three groups were not significant (Table 1). The differences of IC-GH among the three groups were due to selection criteria.

The effect of insulin-induced hypoglycemia on plasma PP levels in the three patient groups is shown in Figure 1. All groups had a substantial rise in plasma PP in response to hypoglycemia.

The mean PP levels became significantly higher ($p < 0.001$) than baseline at the time of the glucose nadir, and remained elevated at nadir plus 10 and nadir plus 20 min. In 21 of 31 children, the glucose nadir preceded the maximal increase in PP by 10–20 min. When glucose and PP responses to insulin were evaluated by diagnostic groups, short but otherwise healthy children without growth hormone deficiency (controls) exhibited a mean glucose nadir that was significantly higher ($p < 0.05$) than that observed in children with GH deficiency disorders (Table 2). This may be due to the fact that these non-GH-deficient patients were less sensitive to the glucose-lowering effects of insulin and thus had higher glucose nadir levels. As a consequence of higher glucose levels, controls had peak PP level and PP incremental change that were somewhat lower than the patients with GH deficiency disorders (GHND and GHD) (Table 2). However, when plasma PP levels were adjusted for glucose nadir levels there were no statistical differences between groups.

The log peak PP response was inversely correlated with the glucose nadir level ($r = -0.5$, $p < 0.005$). The relationship between the log peak PP and glucose nadir is shown in Figure 2.

Peak PP levels were also significantly correlated with the peak E levels ($r = 0.6$, $p < 0.001$). The peak NE values were not correlated with either peak PP response or the glucose nadir. The basal and peak E, NE levels were not significantly different between the groups (Table 2).

DISCUSSION

The release of GH is under dual control of two hypothalamic neurohormones, a stimulatory factor, GH releasing hormone, and an inhibitory agent, somatostatin (7). A variety of adrenergic, dopaminergic, serotonergic, and cholinergic mechanisms participate in the regulation of these two neurohormones (7). Cholinergic and cholinergic-muscarinic blocking agents blunt GH response to arginine, clonidine, exercise as well as physiologic nocturnal GH secretion (5). Central vagal cholinergic mechanisms also govern the secretion of PP. We examined the possibility that defective cholinergic release of GH could also be related to impaired PP secretion. We found no difference in the PP response to hypoglycemia between normal and GH deficiency syndrome patients. Thus the underlying defect causing isolated GH deficiency syndromes does not impair vagal cholinergic activity as manifested by PP release.

We found that the magnitude of the log PP response was inversely related to glucose nadir levels. These results are in agreement with a previous report demonstrating a proportional increase in PP response to magnitude of glucose nadir in adults (6).

In our study the peak PP response was significantly correlated with peak E. Thus, hypoglycemia activates both sympathetic and parasympathetic nervous system activity in children. Whereas sympathetoadrenal activation releases E, efferent vagal parasympathetic activity leads to PP secretion (3, 20). PP response is unaffected by adrenalectomy (21) or by section of splanchnic nerves (22) but is abolished after vagotomy (3, 19). A similar

Table 1. Physical characteristics and diagnostic classification of 31 children

	Age (yr)	BMI (kg/M ²)	IC-GH (ng/ml)	IC-F (μg/dl)
Control (n = 11)	12.6 ± 0.06	18.8 ± 1.6	5.1 ± 0.4	4.5 ± 0.4
GHND (n = 10)	10.5 ± 1.0	20.0 ± 2.4	2.2 ± 0.25	5.7 ± 0.5
GHD (n = 10)	12.0 ± 0.5	15.8 ± 0.5	1.9 ± 0.2	5.0 ± 0.56

Fig. 1. Plasma PP concentrations in response to insulin-induced hypoglycemia in three diagnostic groups. Time is expressed in min: N, time of glucose nadir, N + 10, 10 min after the glucose nadir, N + 20 = 20 min after the glucose nadir.

Table 2. Basal and stimulated PP, E, NE, and glucose responses during insulin-induced hypoglycemia in children with and without growth hormone deficiency

	Control (n = 11)	GHND (n = 10)	GHD (n = 10)
Glucose nadir (mg/dl)	46 ± 2.8	37.3 ± 2.8	34.7 ± 2*
Pancreatic polypeptide (pg/ml)			
Basal	229 ± 27	276 ± 57	190 ± 42
Peak response	747 ± 232	1110 ± 266	1042 ± 240
Increment	518 ± 220	843 ± 246	852 ± 225
E (pg/ml)			
Basal	72 ± 21	95 ± 39	50 ± 10
Peak response	639 ± 313	413 ± 115	1014 ± 233
Increment	566 ± 314	319 ± 115	933 ± 242
NE (pg/ml)			
Basal	264 ± 36	401 ± 102	199 ± 26
Peak response	373 ± 42	452 ± 94	374 ± 39
Increment	108 ± 31	51 ± 27	176 ± 31

* $p < 0.05$ compared to control patients.

correlation between PP and E responses to insulin-induced hypoglycemia has been previously reported in healthy and diabetic adult subjects (23, 24). In contrast to these studies, Polinsky *et al.* (25) failed to show similar correlation in 11 adult volunteers.

We have previously reported that the E and NE responses to insulin-induced hypoglycemia in children with isolated GH deficiency were not significantly different than the response of GH-sufficient children (26). The present study extends these obser-

Fig. 2. Relationship between peak pancreatic polypeptide and glucose nadir in individual patients according to diagnostic grouping: ●, growth hormone deficiency; Δ, growth hormone neurosecretory deficiency; □, control.

variations to the parasympathetic division of the autonomic nervous system which is not impaired in GH deficiency disorders.

We conclude that: 1) in children, hypoglycemia stimulates the peripheral release of E and PP from sympathetic and parasympathetic nervous system activation, respectively and (2) vagal cholinergic and sympathetic autonomic hormone responses to hypoglycemia are unimpaired in children with GH deficiency syndromes.

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