Pediatr. Res. 15: 912–915 (1981)

epinephrine
glucagon
glucocorticoid hormones
hypoglycemia
hypopituitarism
norepinephrine
phenyl-*n*-methyl transferase

The Adrenomedullary and Glucagon Responses of Hypopituitary Children to Insulin-Induced Hypoglycemia

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Summary

The activity of phenyl-*N*-methyl transferase (PNMT), the adrenomedullary enzyme which catalyzes the *N*-methylation of norepinephrine (NE) to epinephrine (E) is induced by endogenous glucocorticoid hormones secreted by the adrenal cortex. We quantitated the urinary output of NE and E before, during, and after insulin-induced hypoglycemia in patients with pituitary dysfunction. Plasma concentrations of cortisol, growth hormone, and glucagon were measured simultaneously. The study population was comprised of nine healthy controls (group 1), eight children with growth hormone deficiency (group 2), and eight children with combined growth hormone and cortisol deficiencies (group 3). Recovery from acute hypoglycemia was similar in all groups. Mean plasma glucagon values reached a maximum at 30 min after insulin injection, and no significant differences were observed among the groups. Plasma cortisol levels were similar in groups 1 and 2, maximum values ocurring at 45 min after insulin. Patients in group 3 did not increase their cortisol concentrations above 5.5 μg/d despite a greater than 50% drop in blood glucose. Mean urinary E output of all groups increased significantly above pretest values (groups 1 and 2, $P < 0.001$; and group 3, $P < 0.01$), whereas NE levels were unchanged. After hypoglycemia, the mean E increments in the control and cortisol-deficient groups were not significantly different.

The data can be interpreted in two ways. Endogenous cortisol production in ACTH-deficient hypopituitary children is sufficient to maintain PNMT activity at a level needed for synthesis of E from NE. Alternatively, cortisol may not be essential for E release during acute hypoglycemia because hypothalamic regulatory mechanisms supervene, and direct neural stimulation promotes PNMT activity and synthesis of E.

We conclude that patients with cortisol and growth hormone deficiencies are able to recover from acute hypoglycemia when hepatic glycogen stores are adequate because there is sufficient release of E or because other adrenergic mechanisms stimulate glucagon release and hepatic glycogenolysis.

Speculation

Although children with cortisol and growth hormone deficiencies are able to recover from acute hypoglycemia, many do not tolerate a prolonged fast because of diminished gluconeogenesis and depletion of hepatic glycogen.

Studies in animals have shown that the activity of phenylethanolamine-*N*-methyl transferase (PNMT), the adrenomedullary enzyme which catalyzes the N-methylation of norepinephrine (NE) to epinephrine (E), is induced by endogenous glucocorticoid hormones secreted by the adrenal cortex. Hypophysectomy in rats and dogs is followed by a prompt decline in PNMT activity and in E synthesis which can be restored by administration of ACTH. Treatment with large doses of glucocorticoid also restores PNMT whereas physiologic doses of dexamethasone, corticosteroid, and hydrocortisone are ineffective, probably because their intramedullary concentration is insufficient for enzyme induction (12, 22, 23).

Few investigations concerning the influence of ACTH and glucocorticoid hormones on epinephrine synthesis have been performed in man (3, 10, 13–15). On the basis of the animal experiments, we theorized that children with decreased cortisol production from ACTH deficiency secondary to hypothalamic-pituitary disorders might have impaired E synthesis and abnormalities of carbohydrate homeostasis. To test this hypothesis, we quantitated the urinary catecholamine output before, during, and after insulin induced hypoglycemia in patients with pituitary dysfunction. The plasma concentrations of the other major counterregulatory hormones were measured simultaneously. The results of these studies are described in this report.

EXPERIMENTAL SUBJECTS

Twenty-five children with short stature who were suspected of having growth hormone deficiency alone or combined with deficiencies of other anterior pituitary hormones comprised the study population. None had a history of hypoglycemia. Each child had been eating a regular diet at home, and no one received special dietary preparation before testing. All were clinically and biochemically euthyroid. Those with thyroid-stimulating hormone deficiency were receiving l-thyroxine therapy and had normal serum T_4, levels. Each was admitted to the Metabolic Unit of Buffalo Children's Hospital for evaluation of their hypothalamic-pituitary-adrenal axis according to a protocol which had been approved by the Institutional Review Board. Written informed consent was obtained from patients and/or parents before each study.

Clinical data about the participants are listed in Table 1.

MATERIALS AND METHODS

ARGININE STIMULATION TEST

After an overnight fast beginning at 2100 hr, arginine, 0.5 g/kg body weight was given intravenously over 30 min starting at 0800. Plasma samples were collected at −30, −15, 0 +15, 30, 45, 60, 120 min for growth hormone analysis.

INSULIN-INDUCED HYPOGLYCEMIA

After a similar overnight fast, a bolus injection of regular insulin, 0.05 to 0.08 units/kg body weight was given intravenously at 0800 hr. Studies were considered valid when the blood glucose level dropped to less than 50% of the baseline value. Samples of

Table 1. *Data about the study population*[1]

	Patient	Sex	Chronological Age (yr)	Height age (yr)	Bone age (yr)	Etiology	Miscellaneous
Group 1 control							
	1	M	4 11/12	3	2 1/2	G[2] and C	
	2	M	5 8/12	4 3/12	3	G and C	
	3	F	7 6/12	4	5 9/12	Cleidocranial dysostosis	
	4	M	8 3/12	4	5	G and C	
	5	M	10	6	9	IUGF	
	6	M	12 8/12	7	9	IUGF	
	7	M	12 11/12	9 9/12	11 6/12	G	
	8	M	13 10/12	9 8/12	13	G	
	9	M	14 8/12	12	12 6/12	C	
Group 2 growth hormone deficient							
	1	M	4 5/12	1 1/12	3	Idiopathic	TSH ↓
	2	M	6 2/12	3 3/12	3	Idiopathic	
	3	M	7 4/12	4	2 8/12	Idiopathic	
	4	M	8 5/12	6	7	Craniopharyngioma	TSH ↓
	5	M	9 11/12	6 6/12	7	Idiopathic	
	6	M	10 6/12	6 4/12	8	Idiopathic	
	7	M	12 1/12	7 6/12	9	Idiopathic	
	8	M	14 9/12	13 3/12	13 6/12	Idiopathic	
Group 3 growth hormone and cortisol deficient							
	1	M	6 6/12	2 10/12	2 2/12	Idiopathic	
	2	M	8 1/12	7 1/12	5	Craniopharyngioma	TSH ↓
	3	F	10 5/12	6	5	Craniopharyngioma	TSH ↓
	4	M	10 8/12	5 10/12	5	Idiopathic	TSH ↓
	5	M	12 4/12	7	11 6/12	Idiopathic	
	6	F	14 2/12	9	8 10/12	Pinealoma	TSH ↓ FSH-LH ↓
	7	M	14 4/12	10 2/12	10	Craniopharyngioma	TSH ↓ FSH-LH ↓
	8	F	17 6/12	11 10/12	12	Idiopathic	TSH ↓ FSH-LH ↓

[1] Children with thyroid stimulating hormone deficiency (TSH ↓) were euthyroid on l-thyroxine therapy. Children with gonadotropin deficiencies (FSH-LH ↓) were not receiving sex steroids.

[2] G, genetic short stature; C, constitutional delay; IUGF, intrauterine growth failure; TSH, thyroid-stimulating hormone.

plasma were obtained at −30, −15, 0, +15, 30, 45, 60, 90, and 120 min and were assayed for glucose, glucagon, growth hormone, and cortisol concentrations. Glucose levels also were determined at +20 and +25 min.

Consecutive timed urine specimens were collected before (0530 to 0800 hr), during (0800 to 1000 hr), and after (1000 to 1130 hr) acute hypoglycemia and analyzed for NE and E. This study design was chosen because measurement of urinary free E levels provides an integrated assessment of production over a specific time period and allows for comparison of baseline E excretion with the output during hypoglycemia. Recent studies in our laboratory have shown that both urinary E output and plasma E concentration increase 4 to 20 times in response to insulin hypoglycemia. Urinary free NE levels, however, may not reflect sympathetic activity because of rapid reuptake of this amine into nerve endings.

The glucose oxidase method was used for quantitation of blood glucose. Plasma growth hormone was measured by double antibody radioimmunoassay techniques (1, 7). Plasma corticoid was determined by the competitive protein binding method of Murphy (16). Antiglucagon antisera (30 K), purchased from Dr. R. Unger, was used to assay plasma glucagon according to the method of Faloona and Unger (8) except double antibody precipitation was substituted for charcoal dextran separation. Urinary free NE and E were analyzed by spectrophotofluoremetry using previously described techniques (20).

The data were analyzed using the Student *t* test.

RESULTS

CLASSIFICATION OF PATIENTS

The patients were divided into three groups based on their growth hormone and cortisol responses to arginine and to insulin-induced hypoglycemia.

Group 1: Nine children—control population
- Peak plasma growth hormone, >8 ng/ml
- Baseline plasma cortisol, > 8 μg/dl
- Peak poststimulation plasma cortisol, >16 μg/dl

Group 2: Eight children—growth hormone deficient and cortisol sufficient
- Peak plasma growth hormone, <4 ng/ml
- Baseline plasma cortisol, >8 μg/dl
- Peak poststimulation plasma cortisol, >16 μg/dl

Group 3: Eight children—growth hormone and cortisol deficient
- Peak plasma growth hormone, <4 μg/ml
- Baseline plasma cortisol, <6 μg/dl
- Peak poststimulation plasma cortisol, <6 μg/dl

BLOOD GLUCOSE

Mean fasting blood glucose levels were higher in the control children ($P < 0.03$ at −15 min). After insulin administration, there was a rapid fall in glucose levels in all patients with the nadir

Table 2. *Glucagon responses to insulin hypoglycemia (pg/ml)*

	Time								
Group	−30	0	+15	20	30	45	60	90	120
1	73 ± 13[1]	68 ± 13	92 ± 14	475 ± 52	165 ± 26	91 ± 20	87 ± 18	87 ± 13	108 ± 23
2	108 ± 30	87 ± 19	92 ± 19	125 ± 21	188 ± 23	148 ± 21	125 ± 33	77 ± 18	116 ± 27
3	105 ± 33	72 ± 17	184 ± 47	152 ± 39	248 ± 85	109 ± 20	50 ± 19	59 ± 23	74 ± 25

[1] Mean ± S.E.

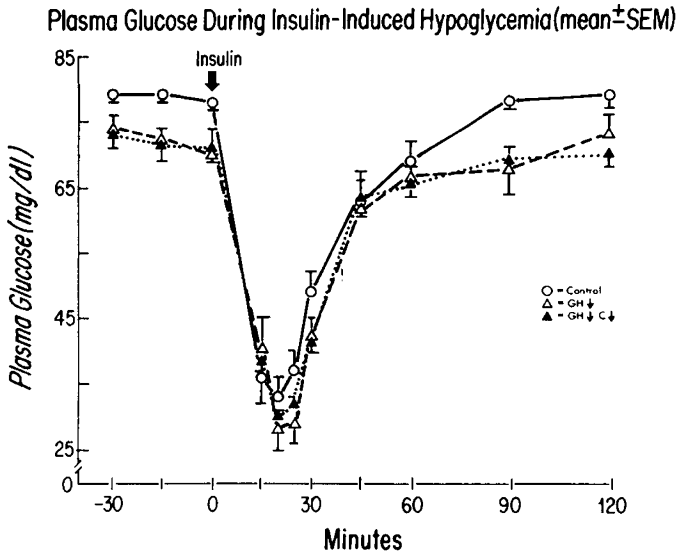

Fig. 1. Mean plasma glucose concentrations (± S.E.) of control subjects, growth hormone-deficient patients (*GH* ↓), and patients with combined growth hormone and cortisol deficiencies (*GH* ↓ *C* ↓) in response to insulin-induced hypoglycemia. Insulin given intravenously at time "0."

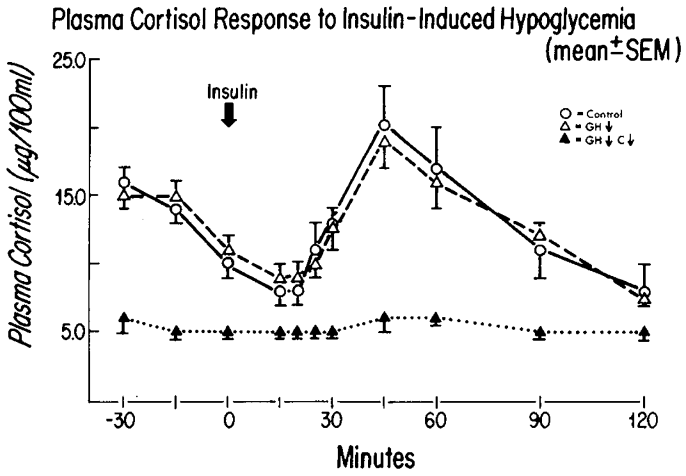

Fig. 2. Mean plasma cortisol concentrations (± S.E.) of control subjects, growth hormone-deficient patients (*GH* ↓), and patients with combined growth hormone and cortisol deficiencies (*GH* ↓ *C* ↓) in response to insulin-induced hypoglycemia. Insulin given intravenously at time "0."

usually occurring at 20 min. There were no significant differences between the groups even though patients in group 1 received 0.08 unit/kg regular insulin whereas those in group 2 and 3 were given only 0.05 unit/kg regular insulin as a bolus intravenous injection. Recovery from hypoglycemia was similar in all groups, and baseline glucose levels were reached at about 90 min after insulin administration (Fig. 1).

GLUCAGON

Mean plasma glucagon values reached a maximum at 30 min after insulin injection (Table 2) and returned to baseline concentrations by 60 to 90 min. No significant differences were observed among the groups.

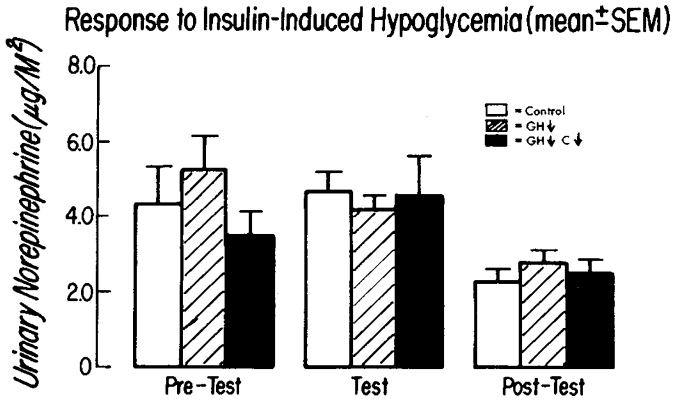

Fig. 3. Mean urinary norepinephrine levels in timed urine specimens collected before, during, and after insulin-induced hypoglycemia. Specimens were collected from control subjects, patients with growth hormone deficiency (*GH* ↓), and patients with combined growth hormone and cortisol deficiencies (*GH* ↓ *C* ↓).

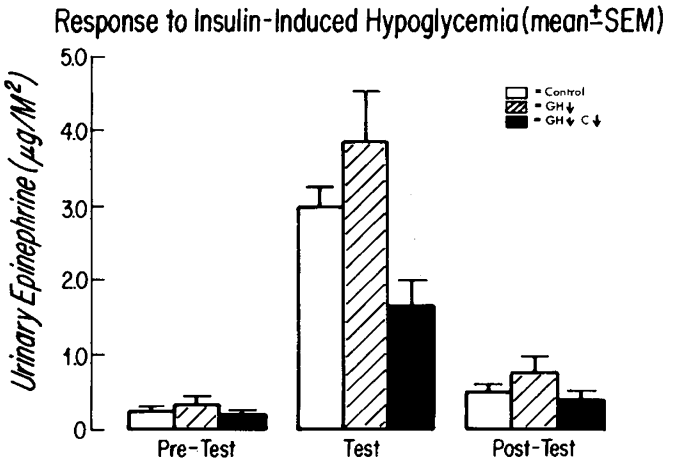

Fig. 4. Mean urinary E levels in timed urine specimens collected before, during, and after insulin-induced hypoglycemia. Specimens were collected from control subjects, patients with growth-hormone deficiency (GH ↓), and patients with combined growth hormone and cortisol deficiencies (*GH* ↓ *C* ↓).

CORTISOL

Cortisol levels were similar among members of groups 1 and 2, maximum values occurring at +45 min after insulin administration (Fig. 2). Patients in group 3 did not increase their cortisol concentrations above baseline despite a greater than 50% drop in blood glucose.

CATECHOLAMINES

Following insulin-induced hypoglycemia, the mean E output of all groups increased significantly above pretest values (groups 1 and 2, $P < 0.001$, and group 3, $P < 0.01$) whereas NE levels were unchanged (Figs. 3 and 4). Groups 1 and 2 increased their urinary

E output 12- to 13-fold as compared to an 8-fold increase in group 3. The mean E increment between the control and cortisol-deficient groups was not significantly different ($P < 0.1$).

DISCUSSION

In this study, we observed that insulin-induced hypoglycemia provoked adrenomedullary responses and rates of glucose recovery which were not significantly different in healthy subjects (group 1), in patients with isolated growth hormone deficiency (group 2), or those with combined GH-ACTH-cortisol deficiency (group 3). Urinary E output increased 8-fold in the cortisol growth hormone-deficient children compared to a 12- to 13-fold increase in the control and growth hormone-deficient groups. The increments for both groups fall within the range reported in healthy children (5- to 20-fold increase in E). (2).

We had expected that ACTH deficiency in children would result in impaired E production because ACTH and glucocorticoid regulate the adrenomedullary enzyme, PNMT, which converts NE to E. No dysfunction in this pathway was detected with the acute hypoglycemic challenge used in this study.

Two mechanisms could account for the adequate release of E in ACTH-cortisol-deficient children. First, it is possible that endogenous cortisol production was sufficient to maintain PNMT activity at the level needed for synthesis of E from NE. Little information is available concerning the quantity of glucocorticoid required for this function in man. Kitabchi and Williams (11) measured PNMT activity in human adrenals, but they could demonstrate no correlation between enzyme activity and the high concentration of ACTH or glucocorticoid which occurs in Cushing's syndrome. We have not found any published measurements of PNMT activity in glucocorticoid-deficient human adrenals. However, hypophysectomized rats have shown reduced levels of PNMT activity but still retain their ability to synthesize epinephrine, albeit, at a depressed rate (21).

Second, cortisol may not be the sole mediator of PNMT synthesis. Support for this possibility comes from the studies of Theonen *et al.* (19) which suggest that the normal level of PNMT is maintained by adrenal glucocorticoids, whereas augmented PNMT activity is dependent on splanchnic nerve stimulation. Furthermore, Himsworth (9) has reported that chemoreceptors in the hypothalamus govern the secretion of E in response to insufficient metabolizable glucose, and Stricker *et al.* (18) have shown that adrenal catecholamine secretion results from a decline in the availability of all utilizable fuels to the brain. Thus, cortisol may not be essential for E release during acute hypoglycemia because hypothalamic regulatory mechanisms supervene and direct neural stimulation provokes discharge of E.

Our study demonstrates that a rise in growth hormone and cortisol is not necessary for glucose recovery after acute hypoglycemia. Children deficient in both hormones (group 3) had glucose recovery rates comparable to the control and the growth hormone-deficient study population. Feldman *et al.* (5) also found that augmented secretion of cortisol and growth hormone is not required for restoration of normal plasma glucose concentration when they performed insulin tolerance tests on four normal volunteers whose cortisol and growth hormone secretion were reduced by cyproheptadine administration. They did not measure glucagon and catecholamines, however.

Glucagon appears to be a key hormone in restoration of normoglycemia after a sudden drop in blood glucose. This is supported in part by our studies in glucocorticoid-deficient hypopituitary children who are able to tolerate acute hypoglycemia. Normal glucagon and E production results in hepatic glycogenolysis and restoration of their glucose homeostasis provided glycogen stores are intact (6, 17). More conclusively, the importance of glucagon has been documented in adrenalectomized individuals who lack both cortisol and E and yet recover from acute glucopenia because of an appropriate alpha cell response (4).

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24. The pituitary hormones used in diagnostic testing and the growth hormone prescribed for treatment of the patients with growth hormone deficiency were provided by the National Pituitary Agency of the National Institute of Arthritis, Metabolism and Digestive Diseases.
25. The authors thank the Human Growth Foundation of Western New York, Susan T. Warren, Janet A. Ferrara, Miriam S. Link, Barbara A. Babcock, and Oleta M. Denz for their assistance with the study.

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27. This research was supported in part by BRSG Grant RR-05493 awarded by the Biomedical Research Support Grant Program, Division of Research Resources, National Institutes of Health.
28. Received for publication July 15, 1980.
29. Accepted for publication October 2, 1980.

0031-3998/81/1506-0912$02.00/0

Printed in U. S. A.