CLINICAL STUDY

Reduced epinephrine reserve in response to insulin-induced hypoglycemia in patients with pituitary adenoma

Shinya Morita¹, Michio Otsuki¹, Maki Izumi¹, Nobuyuki Asanuma¹, Shuichi Izumoto², Youichi Saitoh², Toshiki Yoshimine² and Soji Kasayama¹

Department of ¹Medicine and ²Neurosurgery, Osaka University Graduate School of Medicine (C-4), 2-2 Yamada-oka, Suita, Osaka 565-0871, Japan
(Correspondence should be addressed to S Kasayama; Email: kasayama@imed3.med.osaka-u.ac.jp)

Abstract

Objective: Hypoglycemia induces rapid secretion of counterregulatory hormones such as catecholamine, glucagon, cortisol, and GH. Insulin-induced hypoglycemia is used for evaluating GH–IGF-I and ACTH–adrenal axes in patients with pituitary disorders. The aim of this study was to determine whether the response of catecholamine secretion to hypoglycemia is disrupted in patients with pituitary adenoma.

Methods: The study population comprised 23 patients with pituitary adenoma (non-functioning adenoma or prolactinoma). An insulin tolerance test was performed and serum catecholamines as well as plasma GH and serum cortisol were measured.

Results: The study patients showed diminished response of plasma epinephrine to insulin-induced hypoglycemia. With the cutoff level of peak epinephrine for defining severe impairment set at 400 pg/ml, more patients with secondary adrenal insufficiency showed severe impairment of the epinephrine response than did those without it. Peak epinephrine levels to insulin-induced hypoglycemia were significantly correlated with peak cortisol levels. In patients with secondary hypothyroidism, secondary hypogonadism, GH deficiency, or diabetes insipidus, the prevalence of severe impairment of the epinephrine response was similar to that in patients without these deficiencies.

Conclusions: Impaired epinephrine secretion in response to insulin-induced hypoglycemia was frequently observed in patients with pituitary adenoma. This disorder was especially severe in patients with secondary adrenal insufficiency.

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Introduction

Glucose counterregulatory mechanisms in response to hypoglycemia consist of rapid secretion of adreno-corticotropin (ACTH), growth hormone (GH), glucagon, epinephrine, and norepinephrine (1, 2). In patients with type 1 diabetes mellitus, insulin treatment increases the risk of severe hypoglycemia (3). In addition, defective glucose counterregulation is sometimes associated with diabetic patients, and becomes a cause for hypoglycemia unawareness (4), while single or recurrent episodes of hypoglycemia have been shown to impair sympatho-adrenal responses to a subsequent episode of hypoglycemia (5, 6). Moreover, patients with insulinoma often also have a lowered glycemic threshold for the activation of glucose counterregulation (7).

The brain ventromedial hypothalamus (VMH) has been shown to sense hypoglycemia and trigger the release of counterregulatory hormones (8–12). Corticotropin-releasing hormone (CRH) acting via CRH receptor 1 has been found to play an important role in the sympatho-adrenal downregulation in a rodent model of antecedent hypoglycemia (13). It has further been reported that a patient with hypothalamic sarcoidosis suffered complete

loss of the counterregulatory response to hypoglycemia as well as pituitary insufficiency and central diabetes insipidus (14). In addition, pediatric and adult patients with craniopharyngioma have been shown to have a defect in sympathoadrenal counterregulation (15, 16). However, it is not known whether the counterregulatory response of catecholamines to hypoglycemia is disrupted in patients with pituitary adenomas. Therefore, we studied the responses of catecholamine secretion in response to insulin-induced acute hypoglycemia in patients with pituitary adenoma, who had a variety of hypothalamic–pituitary hormone disorders.

Patients and methods

Patients

The study population consisted of 23 patients with diagnosed pituitary adenoma. Among 90 such patients visiting Osaka University Hospital between December 2003 and January 2007, the study patients were randomly selected after exclusion of patients with ACTH- or GH-producing adenoma. Seven patients

were males and sixteen females, their mean age at the time of this study was 55 years (range, 20–76 years), and their body mass index was $24.3 \pm 2.9 \text{ kg/m}^2$. Eighteen patients had clinically non-functioning adenomas and five patients had prolactinomas. The diagnosis of these tumors was made by enhanced magnetic resonance imaging and was confirmed by transsphenoidal surgery or craniotomy in 21 of the patients. Table 1 shows the clinical characteristics of the study population. All patients gave their informed consent, and the investigation was performed in accordance with the principles of the Declaration of Helsinki as revised in 2000.

Endocrine evaluation

All patients were hospitalized. Endocrine evaluation tests were performed between 0800 and 1000 h. All medications were allowed to be taken after the endocrine evaluation tests ended. After a 15 min rest, regular insulin (0.1 unit/kg body weight) was administered intravenously in a single injection. Blood samples were obtained for measurements of pituitary hormones, cortisol, epinephrine, and norepinephrine at the time of injection, and 15, 30, 60, 90, and 120 min after the injection. When hypoglycemia (blood glucose levels $<40 \text{ mg/dl}$ or half of basal levels) was not obtained within 30 min after the insulin injection, an additional

0.05 unit/kg body weight of insulin was injected to induce hypoglycemia. During the endocrine tests, the patients were keeping spine position.

Secondary adrenal insufficiency was diagnosed if the peak serum cortisol response was $<200 \text{ µg/l}$ during the insulin tolerance test (ITT) (17, 18) and if early morning (0800 h) serum cortisol levels were $<80 \text{ µg/l}$ and 24-h urinary-free cortisol levels were low ($<30 \text{ µg/24 h}$). Secondary hypothyroidism was indicated by low free L-thyroxine (T_4) concentrations ($<8 \text{ ng/l}$) with normal or low thyrotropin (TSH) levels (1, 2). Secondary hypogonadism was identified in premenopausal women by menstrual disturbances, low estradiol levels ($<20 \text{ pg/ml}$) with normal or low follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels, and in postmenopausal women by relatively low FSH and LH levels. In men, secondary hypogonadism was indicated by low testosterone levels ($<3 \text{ µg/l}$) with low or normal FSH and LH levels (17, 18). A diagnosis of GH deficiency was based on a peak GH response of $<5 \text{ µg/l}$ at ITT (17, 18), and a diagnosis of diabetes insipidus on the presence of a markedly large dilute urine volume ($>2.5\text{--}3 \text{ l per 24 h}$) with low urine osmolality ($<300 \text{ mmol/kg}$) (17, 18). Patients who had been diagnosed with secondary adrenal insufficiency, secondary hypothyroidism, and/or diabetes insipidus had been receiving replacement therapy with appropriate doses of hydrocortisone, T_4 , and/or desmopressin. No patient with GH deficiency had

Table 1 Type and size of pituitary adenoma, defective pituitary hormones, replaced hormones therapy, and previous surgery in the study population.

Patient no	Age/sex	Hormone produced	Size of tumor (mm)	Defective pituitary hormone(s)	Replaced hormone(s)	Duration of replacement therapy	Previous surgery
1	21/M	NF	22	ACTH, GH, TSH, LH/FSH, AVP	H, T_4 , DDAVP	60 months	TSS, craniotomy
2	31/F	NF	30	None	None		None
3	50/F	NF	23	GH, TSH, LH/FSH, AVP	DDAVP	1 month	None
4	56/F	NF	38	ACTH, GH, TSH, LH/FSH	None		TSS
5	56/F	NF	32	GH, LH/FSH	None		TSS
6	57/F	NF	12	GH	None		None
7	58/F	NF	25	GH, TSH, LH/FSH	None		None
8	58/F	NF	29	GH, TSH, LH/FSH	T_4	60 months	TSS
9	59/F	NF	26	ACTH, GH	None		None
10	62/M	NF	25	GH, LH/FSH	None		None
11	62/M	NF	24	GH	None		None
12	62/F	NF	70	GH	None		Craniotomy
13	65/F	NF	13	ACTH, GH, TSH, LH/FSH	H, T_4	1 month	None
14	71/M	NF	38	ACTH, GH, TSH	H	1 month	None
15	72/F	NF	32	LH/FSH	None		TSS
16	74/M	NF	18	GH, LH/FSH	Tes	12 months	TSS
17	75/M	NF	28	ACTH, GH, TSH, LH/FSH	None		None
18	76/F	NF	31	GH, TSH, LH/FSH, AVP	DDAVP	1 month	TSS
19	20/F	Prolactin	21	LH/FSH	None		None
20	30/F	Prolactin	13	LH/FSH	None		None
21	43/F	Prolactin	11	LH/FSH	None		None
22	53/M	Prolactin	20	LH/FSH	None		None
23	62/F	Prolactin	23	ACTH, GH, LH/FSH	None		None

NF, non-functioning; DDAVP, desmopressin; H, hydrocortisone; T_4 , L-thyroxine; Tes, testosterone; TSS, transsphenoidal surgery. The size of the tumor is shown as the maximal diameter.

received GH replacement therapy, and only one male patient with secondary hypogonadism had received testosterone replacement.

Hormone assays

All hormones were assayed in the Clinical Laboratory of Osaka University Hospital. GH, ACTH, free T₄, free triiodothyronine, and cortisol were measured by means of RIA. Prolactin, LH, and FSH were measured by means of enzyme-immunoassay, TSH by electrochemiluminescence immunoassay, epinephrine and norepinephrine by high performance liquid chromatography, and plasma glucose with the glucose oxidase method.

Statistical analysis

Peak epinephrine secretion of patients with different pituitary hormone disorders was compared using the χ^2 test. The StatView computer program (Version 5.0 for Windows; Abacus Concepts, Berkeley, CA, USA) was used for statistical analyses. $P < 0.05$ was considered statistically significant.

Results

Subjects

Table 1 shows characteristics of the tumors, defective pituitary hormones, replaced hormones therapy, and previous surgery in the study population. The maximal diameter of the tumors ranged from 11 to 70 mm. Out of the 23 patients, 22 had anterior and/or posterior pituitary hormones deficiencies; four patients showed signs of panhypopituitarism, and three of central diabetes insipidus. All five patients with prolactinoma had hypogonadism. Three patients had been receiving hydrocortisone at 10 or 20 mg daily after breakfast, while three patients had been receiving T₄ at 25 or 75 µg daily after breakfast. No patient had DHEA replacement therapy. Desmopressin had been administered to three patients with central diabetes insipidus. Eight of the study patients had previously received transsphenoidal surgery or craniotomy. There were no patients with prolactinoma who had been treated with dopamine agonist.

Catecholamines responses to insulin-induced hypoglycemia

Plasma epinephrine and norepinephrine responses to ITT are shown in Fig. 1. Baseline plasma epinephrine levels were within control ranges (< 170 pg/ml) in all patients. However, all patients showed a diminished response of plasma epinephrine to insulin-induced hypoglycemia. Baseline plasma norepinephrine levels were considerably lower in five of the patients than in

Figure 1 Epinephrine (top) and norepinephrine (bottom) responses to insulin-induced hypoglycemia in 23 patients with pituitary adenoma. The shaded area represents the mean $\pm$ s.e.m. values for normal subjects (Ref. (19)). To convert the values for epinephrine and norepinephrine to nanomoles per liter, multiply by 0.0005458 and 0.005910 respectively.

controls (150–570 pg/ml) and were within control ranges in the other 18 patients, while secretory responses of plasma norepinephrine were ambiguous.

Relationship between epinephrine responses at ITT and defective pituitary hormones of the study patients

With the cutoff level of peak epinephrine at ITT for identifying severe impairment set at 400 pg/ml, more patients with secondary adrenal insufficiency showed severe impairment of the epinephrine response than those without it (Fig. 2 and Table 2). On the other hand, patients with secondary hypothyroidism, secondary hypogonadism, GH deficiency, and/or diabetes insipidus showed a similar prevalence of severe impairment of the epinephrine response as those without these abnormalities. Peak epinephrine levels at ITT were significantly correlated with peak cortisol levels ($R = 0.506$, $P = 0.014$; Fig. 3). In contrast, there was no correlation of the peak epinephrine levels with the peak GH levels ($R = 0.072$, $P = 0.745$) and the lowest plasma glucose levels ($R = -0.147$, $P = 0.503$) at ITT.

Figure 2 Peak epinephrine levels during the insulin tolerance test in 23 patients with pituitary adenoma. The patients were divided to subgroups according to pituitary hormonal deficiencies. To convert the values for epinephrine to nanomoles per liter, multiply by 0.0005458.

Discussion

Insulin-induced acute hypoglycemia is a well-known stimulus for the secretion of GH and ACTH from the pituitary gland (17, 18). This stimulus has therefore been used for the diagnosis of GH deficiency and secondary adrenal deficiency in patients with suspected hypopituitarism (17, 18). Besides GH and ACTH/cortisol, other counterregulatory hormones such as epinephrine, norepinephrine, and glucagon, also play pivotal roles in recovery from hypoglycemia (1, 2). Glucose counterregulation deficiency has been found to be associated with diabetes mellitus (3–5), insulinoma (7), hypothalamic sarcoidosis (14), craniopharyngioma (15, 16), and psychological stress (20). Deficiencies in glucose counterregulatory hormones have been shown to vary, depending on the background disease: responses of glucagon and GH were impaired in diabetic patients (21), those of GH, cortisol, glucagon, epinephrine, and norepinephrine in a patient with hypothalamic sarcoidosis (14), and those of cortisol, epinephrine, and norepinephrine in a patient with psychological stress (20). Of the 23 patients with pituitary adenoma enrolled in our study, all showed diminished plasma epinephrine response to insulin-induced acute hypoglycemia. Reduction in the epinephrine reserve thus occurred with very high frequency in these patients. Unfortunately, neuroglycopenic symptoms during insulin-induced hypoglycemia were not accurately recorded in some patients. Thus, in the present study, we failed to reveal the relationship between these symptoms and the diminished plasma epinephrine response.

Glucocorticoids are required for the survival and maintenance of adrenomedullary chromaffin cells and their production of epinephrine (22, 23). In fact,

adrenomedullary hypofunction was observed in patients with 21-hydroxylase deficiency (24, 25) and those with isolated glucocorticoid deficiency (26). In these patients, plasma epinephrine concentrations at baseline and in response to stimuli such as exercise, upright posture and cold pressure, were reduced (24–26). Adrenomedullary dysfunction was characterized by incomplete formation of the adrenal medulla and a depletion of secretory vesicles in chromaffin cells (24). Basal and post-exercise plasma epinephrine levels were also found to be reduced in children with ACTH deficiency (27). In our study, peak epinephrine levels at ITT were correlated with peak cortisol levels, but not with peak GH levels. These results suggest that secondary adrenal insufficiency could be an important

Table 2 Prevalence of severe impairment of the epinephrine response to insulin-induced hypoglycemia.

Peak epinephrine level at ITT	< 400 (pg/ml)	≥ 400 (pg/ml)	P
Secondary adrenal insufficiency			
(–)	9 (39%)	7 (30%)	0.036
(+)	7 (30%)	0 (0%)	
Secondary hypothyroidism			
(–)	8 (35%)	6 (26%)	0.106
(+)	8 (35%)	1 (4%)	
GH deficiency			
(–)	3 (13%)	3 (13%)	0.226
(+)	13 (57%)	4 (17%)	
Secondary hypogonadism			
(–)	5 (22%)	1 (4%)	0.394
(+)	11 (48%)	6 (26%)	
Diabetes insipidus			
(–)	13 (57%)	7 (30%)	0.219
(+)	3 (13%)	0 (0%)	

ITT, insulin tolerance test.

Figure 3 Correlation of peak epinephrine levels with peak cortisol levels during the insulin tolerance test in 23 patients with pituitary adenoma. To convert for epinephrine and cortisol to nanomoles per liter, multiply by 0.0005458 and 2.759 respectively.

cause of the reduction in epinephrine reserve observed in our patients with pituitary adenoma. In our study, seven patients had secondary adrenal insufficiency, three of whom had been treated with replacement of hydrocortisone. Diminished epinephrine response was observed similarly in the patients with secondary adrenal insufficiency, irrespective of hydrocortisone replacement. Thus, glucocorticoid replacement therapy is suggested not to ameliorate the epinephrine response to insulin-induced hypoglycemia. However, all patients, only seven of whom had secondary adrenal insufficiency, showed diminished epinephrine response to the hypoglycemic stimulus, so it is unlikely that secondary adrenal insufficiency *per se* is the direct cause of the reduction in epinephrine reserve. Prolactin has been shown to stimulate catecholamine synthesis in rat hypothalamic cells (28). There were no patients with prolactin deficiency in our study, suggesting that prolactin deficiency is not involved in the decreased epinephrine reserve.

When severe impairment of the epinephrine response was defined as a peak epinephrine level of <400 pg/ml, more patients with secondary adrenal insufficiency showed severe impairment than those without it. Secondary hypothyroidism, secondary hypogonadism, GH deficiency, and diabetes insipidus, on the other hand, were not significantly associated with prevalence of severe impairment of the epinephrine response. As the order of diminishing pituitary function associated with pituitary compression is GH before the other tropic hormones, with ACTH and TSH usually being the last hormones to show functional loss (17), our results seem to indicate that a spread of deficient pituitary hormones is associated with the severity of reduction in the epinephrine reserve. The activation of glucose counter-regulation mechanisms starts with the sensing of hypoglycemia by the VMH to trigger the release of counterregulatory hormones (8–12). In rat experimental models, this counterregulation was found to be largely determined by the interaction between CRH receptor 1-mediated activation and CRH receptor

2-mediated suppression in the VMH (29). The sum total of these findings may lead the speculation that the balance between CRH receptors 1 and 2 is impaired in the VMH of most patients with pituitary adenoma and that the extent of this impairment is more severe in patients with a wider deficiency of pituitary hormones.

In contrast to plasma epinephrine, which is derived almost exclusively from the adrenal medulla, plasma norepinephrine, predominantly derived from sympathetic nerve endings acting as neurotransmitter, was within normal ranges at baseline in 18 of the 23 patients with pituitary adenoma. Norepinephrine responses to insulin-induced hypoglycemia were only marginal in these patients. Similar norepinephrine reserve to that in control subjects have been found in patients with 21-hydroxylase deficiency and/or with isolated glucocorticoid deficiency (24–26). However, increased norepinephrine secretion has been demonstrated in patients with Addison's disease (30) and in those who had undergone bilateral adrenalectomy (24), suggesting that some compensatory increases occur during sympathetic nerve activity. Similar compensation in basal sympathetic nerve activity may also occur in patients with pituitary adenoma.

In this study, we show for the first time that impaired epinephrine secretion in response to insulin-induced hypoglycemia is frequently observed in patients with pituitary adenoma. From the present study and the previous studies on patients with hypothalamic sarcoidosis or craniopharyngioma (14–16), defense mechanisms against hypoglycemia are thought to be disrupted to various extents in patients with pituitary or hypothalamic disorders. Defects in the secretion of GH and ACTH may be involved in failure to recover rapidly from hypoglycemia in patients who are deficient in these hormones. However, reductions in the epinephrine reserve may lead to defects in the defense against acute hypoglycemia in patients with pituitary and hypothalamic disorders, even though they may not show any pituitary hormone deficiency. Furthermore, the absence of the sympathoadrenal symptoms of hypoglycemia may confound a diagnosis of hypoglycemia.

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