Plasma Growth Hormone and Plasma 11-OHCS in Corticosteroid Treated Patients

Reiko Demura, Hiroshi Demura, Masanori Iino, Takashi Nunokawa
and Kiyoshi Miura

*Department of Internal Medicine (Prof. T. Torikai),
Tohoku University School of Medicine, Sendai*

Plasma growth hormone and 11-OHCS in response to insulin hypoglycemia were determined simultaneously in corticosteroid treated patients. Long-term daily administration of corticosteroids, more than 800 tablets and longer than 6 months, had a marked inhibitory effect on GH secretion. Lower doses or shorter term administration on daily basis resulted in less suppression. Intermittent administration with various schedules usually had a less inhibitory effect than daily administration. In intermittently administered patients, better responses were observed in a group tested on off-day than in a group tested on on-day of corticosteroids. A single administration of 1-8 mg of dexamethasone to normal subjects at a midnight before a test had a variable effect on GH secretion. Plasma GH responses to arginine infusion were also suppressed by long-term and high-dose administration of corticosteroids. The suppression of GH seemed to go parallel with that of pituitary-adrenal axis determined by plasma 11-OHCS in corticosteroid treated patients.

A suppressive effect of corticosteroids upon GH secretion has long been suspected from clinical observations such as reduced statural growth and delayed skeletal maturation in corticosteroid treated children. In 1964, Frantz and Rabkin,¹ and Hartog *et al.*² reported a suppressive effect of corticosteroid on GH release in response to insulin hypoglycemia for the first time. The reports thereafter were not quite consistent and the mechanism remains controversial. The present study was attempted to study the effect of corticosteroids upon GH secretion in connection with their suppressive effect upon a hypothalamo-pituitary axis.

MATERIALS AND METHODS

Twenty-one corticosteroid treated patients with various diseases as listed in Table 1 were divided into 3 groups according to the mode of administration of corticosteroids, and an insulin tolerance test was performed on each group. Group 1 was a daily administered group. Group 2 was an intermittently administered group with various schedules such as alternate day therapy or 3 or 4 consecutive days of therapy for every week and a test was performed on on-day of corticosteroids. Group 3 was also an intermittently administered group, but a test was performed on off-day, at least 24 hours after the last dose of corticosteroids.

Received for publication, September 18, 1969.

TABLE 1. *Insulin tolerance test performed in*

Case No.	Name	Age	Sex	Diagnosis
<i>1. Daily administered group</i>				
1	T S	18	m	Weber-Christian's disease
2	T K	14	m	Schönlein-Henoch purpura
3	S S	23	m	Nephrotic syndrome
4	A Y	13	m	Acute myeloid leukemia
5	T S	64	m	Nephrotic syndrome
6	T Y	21	m	Chr. glomerulonephritis
7	Y N	40	f	Myelo-optico-neuritis
8	T B	31	f	Bell's palsy
9	H H	13	f	Chr. glomerulonephritis
<i>2. Intermittently administered group, test performed on on-day</i>				
1	T O	57	m	Bronchial asthma
2	T O	22	m	Chr. glomerulonephritis
3	N N	15	m	Chr. glomerulonephritis
4	F I	19	f	Nephrotic syndrome
5	M Y	17	f	Nephrotic syndrome
<i>3. Intermittently administered group, test performed on off-day</i>				
1	K K	55	f	Bronchial asthma
2	S S	11	m	Nephrotic syndrome
3	K E	30	m	Chr. glomerulonephritis
4	M T	40	m	Chr. glomerulonephritis
5	M H	24	m	Chr. glomerulonephritis
6	F S	19	f	Chr. glomerulonephritis
7	H S	17	f	Chr. glomerulonephritis

TABLE 2. *Insulin tolerance test in normal subjects given single dose of dexamethasone*

Case No.	Name	Age	Sex	Dose of dex. (mg)	Plasma 11-OHCS ($\mu\text{g/dl}$)	
					Before insulin	60 min after insulin
1	E O	25	m	1	3.0	4.0
2	I O	25	m	4	3.0	4.0
3	S T	37	f	4	4.0	5.0
4	S S	17	m	8	3.0	4.0
5	K C	33	m	8	3.0	3.0

Five normal adults were subjected to an acute study. They were given 1-8 mg of dexamethasone orally as a single dose on midnight before an insulin tolerance test (Table 2).

Seven patients who were given corticosteroids daily for various period of time were subjected to an arginine infusion test (Table 3).

Plasma GH was measured by coated charcoal radioimmunoassay. ^{125}I -labeled HGH was prepared by the method of Greenwood *et al.*³ The labeled material was purified using a Sephadex G-75 column. Antibody made in a rabbit was used at a final dilution

corticosteroid treated patients

Total doses	Duration	Daily dose at test (mg)	Kind of CS*	Plasma 11-OHCS	
				Before insulin	60 min after insulin
10,000 tab.	5 y	2.5	Pa.	3.0	2.0
1,140	8 m	20	P.	2.0	3.0
840	6 m	40	P.		
840	6 m	30	P.	2.0	4.0
800	3 m	40	P.		
700	3 m	30	P.	5.5	28.5
418	3 m	15	P.	4.0	5.5
42	1 w	30	P.	4.0	4.0
28	1 w	2	D.	2.0	2.0
350+x	6 m+x	1.5, 3d/w, B.		6.0	4.0
1,190	18 m	25, 3d/w, P.		6.0	15.0
820	8 m	25, 3d/w, P.		2.0	11.0
270	3 m	40, alternate, P.			
155	3 m	15, alternate, P.		6.0	12.0
2,000	5 y	2, 3d/w, B.		3.0	5.0
1,005	19 m	1, alternate, D.		1.0	2.0
1,560	9 m	40, alternate, P.		11.0	19.0
1,020	7 m	40, 4d/w, P.		12.0	21.0
420	7 m	20, 3d/w, P.		14.0	19.0
64	2 w	30, 4d/w, P.		18.0	22.0
48	2 w	30, alternate, P.		12.0	24.0

TABLE 3. *Arginine infusion test performed in corticosteroid treated patients*

Case No.	Name	Age	Sex	Diagnosis	Total dose	Duration	Daily dose at test (mg)	Kind of CS*
1	T S	19	m	Weber-Christian's disease	10,000 tab.	5 y	2.0	Pa.
2	H A	39	m	Behcet's syndrome	1,440	6 m	20	P.
3	M K	35	m	Chr. glomerulonephritis	1,200	5 m	40	P.
4	Y S	19	f	Chr. glomerulonephritis	500	9 w	40	P.
5	S N	28	f	S L E	450	8 w	40	P.
6	Y S	14	f	Nephrotic syndrome	224	4 w	40	P.
7	S S	12	m	Nephrotic syndrome	200	4 w	40	P.

* Pa.: paramethasone. P.: prednisolone. B.: betamethasone. D.: dexamethasone.

of 1: 375,000. After 4 days of incubation, bound and free GHG were separated with dextran coated charcoal, which was made by mixing an equal amount of 5% charcoal suspension (5 g of bone charcoal (Allied Chem. Co.) in 100 ml of water) and a 0.5% dextran solution (0.5 g of dextran (Mann Co.) in 100 ml of water). One ml of 2/3 diluted dextran coated charcoal was added to 1 ml of incubation mixture. After standing for 30 min at room temperature, samples were centrifuged and both supernatants and precipitates were counted. The most sensitive and consistent assay with paperchromato-electrophoresis was obtained at 2/3 dilution of dextran coated charcoal, and approximately 85% of free

HGH- ^{131}I was adsorbed to charcoal at this dilution. HGH supplied by Dr. Wilhelmi was used as a standard.

Plasma 11-OHCS was measured by a fluorometric technique with DeMoor's method.⁴ Blood glucose levels were determined using a glucose oxidase method. Insulin tolerance test was done during morning time after an overnight fast and rest. Regular insulin, 0.1 U/kg, was injected intravenously and blood samples were drawn before and 20, 30, 45, 60, 90, and 120 min after the injection. Arginine infusion test was performed after overnight fast and rest. L-Arginine-HCl, 0.5 g/kg, dissolved as a 10% solution in saline (Morishita Pharm. Co.) was infused intravenously for 30 min. Plasma samples were drawn before and 30, 60, 90 and 120 min after the beginning of the infusion.

RESULTS

Insulin induced hypoglycemia

1) Daily administered group (Fig. 1, Table 1).

Blood sugar levels fell by 32% of the resting levels in an average. In 5 of 9 patients, both resting levels and responses of GH to insulin hypoglycemia were completely suppressed. Plasma GH was mostly undetectable throughout the test comparing with normal resting levels of 0–6 m $\mu\text{g/ml}$ and normal responses of 12–54 m $\mu\text{g/ml}$ at 45–60 min after the injection (normal ranges were shown as shaded areas in figures). They were all given more than 800 tablets of various kinds of corticosteroids, 4,000 mg of prednisolone equivalent, for longer than 6 months (average daily dose of 25 mg) except for Case 5 (TS) who had been given 800 tablets of prednisolone in 3 months. As to the remaining cases with lower doses or shorter term administration, both resting levels and responses of GH were low or in low normal ranges. Two cases, Case 8 (TB) and Case 9 (HH), both treated for only 1 week with a daily dose of 30 and 20 mg, respectively, also showed deficient responses.

Fig. 1. Plasma GH responses to insulin induced hypoglycemia in daily administered group.

Plasma 11-OHCS levels, both the basal and after insulin injection, were completely suppressed to less than $5 \mu\text{g}/\text{dl}$ in all cases except for Case 6 (TY). The normal basal levels of plasma 11-OHCS ranged from 8 to $18 \mu\text{g}/\text{dl}$ with a mean of $11.6 \mu\text{g}/\text{dl}$ and rose up to $25\text{--}30 \mu\text{g}/\text{dl}$ in response to insulin hypoglycemia mostly 60 min after the injection. Case 6 (TY) who had been treated for 6 months with 40 mg of prednisolone daily and had a suppressed basal level of plasma 11-OHCS showed a normal rise of plasma 11-OHCS in response to insulin hypoglycemia. He also had a most striking response of GH in the group.

2) Intermittently administered group tested on on-day of corticosteroids (Fig. 2, Table 1).

Blood sugar levels fell by 35% of the resting levels. Only 1 patient with bronchial asthma, who had been treated for 6 months with 1.5 mg of betamethasone for 3 consecutive days a week, showed a complete inhibition of GH responses. He had had corticosteroids almost daily for previous 10 years. The resting levels of GH were somewhat suppressed but almost normal responses with an average peak value of $20.2 \text{ m}\mu\text{g}/\text{ml}$ were observed in all cases including those who had been given more than 800 tablets of corticosteroids.

Fig. 2. Plasma GH responses to insulin induced hypoglycemia in intermittently administered group. Test was performed on on-day of corticosteroids.

The resting levels of plasma 11-OHCS were suppressed to less than $6 \mu\text{g}/\text{dl}$ in all cases, but slight responses to more than $10 \mu\text{g}/\text{dl}$ were observed in most of the cases except for Case 1 (TO), whose plasma 11-OHCS remained unchanged.

3) Intermittently administered group tested on off-day of corticosteroids (Fig. 3, Table 1).

Blood sugar levels fell by 40% of the resting levels. GH responses were insufficient in 3 cases of 5 years, 10 months and 9 months of therapy, respectively,

Fig. 3. Plasma GH responses to insulin-induced hypoglycemia in intermittently administered group. Test was performed on off-day of corticosteroids.

all of whom received more than 1,000 tablets of corticosteroids. The rest of the cases had normal resting levels and normal maximal responses of $42 \mu\text{g/ml}$ in an average. An average maximal response of GH after insulin in this group was significantly greater than that of the group 2.

Both basal levels and responses of plasma 11-OHCS were suppressed in 2 cases, Case 1 (KK) and Case 2 (SS), whose responses of GH were also impaired. All other cases had normal basal levels of plasma 11-OHCS and subnormal responses, less than $24 \mu\text{g/dl}$.

Normal subjects given a single dose of dexamethasone (Fig. 4)

The effects of a single dose of dexamethasone on GH secretion were variable. Two normal subjects, Case 3 (ST), with 4 mg and Case 5 (KC) with 8 mg, showed definitely impaired responses of GH and Case 2 (IO) with 4 mg showed subnormal response, while the remaining 2 cases, Case 1 (EO) with 1 mg and Case 4 (SS) with 8 mg, showed normal responses. No relationship was observed between a dose of dexamethasone and a suppressibility of GH. In contrast, both basal levels and responses of plasma 11-OHCS to insulin were completely suppressed to less than $5 \mu\text{g/dl}$ in all cases.

Arginine infusion test in corticosteroid treated patients (Fig. 5)

Out of 7 patients who had been given corticosteroids daily for various periods of time, 3 cases, Case 1 (TS), Case 2 (HA) and Case 3 (MK), who had been treated for more than 5 months, and 2 other cases, Case 5 (SN) and Case 7 (SS), with shorter duration of 8 and 4 weeks, respectively, showed impaired responses of GH to arginine. The remaining 2 cases, Case 4 (YS) and Case 6 (YS), of 9 and 4 weeks, respectively, showed normal responses.

Fig. 4. Plasma GH responses to insulin induced hypoglycemia in normal subjects after a single dose administration of 1-8 mg of dexamethasone.

Fig. 5. Plasma GH responses to arginine infusion test in daily administered patients.

DISCUSSION

Some investigators^{1,2,5} have reported suppressive effects of corticosteroids on GH secretion to insulin hypoglycemia, while Morris *et al.*^{6,7} recently reported that no differences were seen in GH levels, both basal and in response to insulin hypoglycemia, between normal and corticosteroid treated children. Martin *et al.*⁸ reported that GH responses were somewhat lower in subjects given corticosteroid intermittently than normal but the differences were not significant. Nakagawa *et al.*⁵ showed definite suppressive effects even with 9 mg of dexametha-

sone for 2 days. Moses *et al.*⁹ reported that overnight steroid treatment did not affect the rise of plasma GH to insulin hypoglycemia. As to the effect of corticosteroids on GH secretion by arginine infusion, Martin *et al.*⁸ reported almost normal responses in patients with intermittent therapy, and Nakagawa *et al.*⁵ reported no influences of short-term administration of dexamethasone, 9 mg for 2 days. The present results clearly demonstrated a suppressive effect of corticosteroids on GH secretion to insulin hypoglycemia and also to arginine infusion. The study with insulin hypoglycemia showed that the responses of GH were different in 3 groups. A standard amount of 0.1 U/kg of regular insulin was used for every test and a degree of hypoglycemia attained was not significantly different in 3 groups and comparable to normal subjects. It is not likely that a suppressive effect was exerted mediating a reduction of the hypoglycemic effect. Each group mostly consisted of patients with renal diseases and a variety of chronic afebrile diseases. The kinds of corticosteroids used were mostly prednisolone and dexamethasone and distributed equally in each group. So the differences in the responses of GH in 3 groups could be attributable to the differences in the mode of administration of corticosteroids and in the timing of the tests performed. It could be generally said that the secretion of GH were inhibited more easily in a daily administered group than in intermittently administered groups. A large amount of corticosteroids, 800 tablets or 4000 mg of prednisolone equivalent, which were given on daily basis with usual therapeutic daily dose in 6 months or so, resulted in a complete suppression of GH, both basal levels and responses to insulin hypoglycemia, without an exception but the suppression was less when the same amount of corticosteroids were given intermittently. A larger amount, 1000 tablets or 5000 mg of prednisolone equivalent, seemed to bring about a definite suppressive effect on GH responses for the first time in intermittently administered group. With less than 4000 mg, the suppression was less, variable and not necessarily related to the dose even in daily administered group and mostly normal responses were seen in intermittently treated group. A difference in a magnitude of the responses in 2 groups of intermittently treated group might be explained by a suppressive effect of the last dose of corticosteroids given within 24 hours on the secretion of GH. A single dose experiment makes the above explanation probable. A suppressive effect of a single dose of dexamethasone upon GH secretion was not uniform and not related to the dose, but a definite suppressive effect was seen in some of the cases. A variability in a suppressibility by a single dose of dexamethasone may be due to a difference in an individual sensitivity. An inconsistency in the reports which have appeared hitherto might be partly due to the differences in the dose, duration and a mode of administration of corticosteroids. Frantz and Rabkin¹ pointed out a dose dependency of a suppressive effect of corticosteroids.

It is worthy of note that in the present study the responses of GH secretion were parallel to that of 11-OHCS to insulin induced hypoglycemia in corticosteroid treated patients. Fig. 6 summarized the responses of both GH and plasma

11-OHCS in 3 groups in a comparative way. The suppression of 11-OHCS, both basal levels and responses, was almost complete in daily administered group, moderate in intermittently treated group on on-day of corticosteroids and slight in intermittently treated group on off-day. As to the adrenocortical suppression by corticosteroids, it has been generally observed that a complete suppression of adrenocortical function occurred within a month with an usual therapeutic dose when corticosteroids were given continuously, but the suppression was much less when they were given intermittently even for a prolonged period of time.^{10,11,12} Our results were quite consistent with the above, and furthermore the responses of GH seemed to go parallel to that of 11-OHCS according to the mode of administration of corticosteroids.

Fig. 6. Responses of plasma GH and 11-OHCS to insulin induced hypoglycemia in 3 groups of patients treated with corticosteroids in different ways.

The suppression of 11-OHCS was always higher in grade and less in variation than that of GH in each group. This difference might express the difference in the grade of suppressibility of both systems, but it could be partly explained by the differences in the levels of parameters, that is GH is a direct reflection of pituitary function while 11-OHCS is an expression at adrenals, a target organ level. So, suppressed 11-OHCS represents a decreased capacity of pituitary superimposed by a suppressed adrenocortical reactivity. GH should be compared directly with plasma ACTH for a further discussion.

A similarity in the suppressibility of GH and 11-OHCS by corticosteroids in response to insulin hypoglycemia suggests that insulin hypoglycemia stimulates the secretion of both GH and ACTH through a similar fashion mediating central nervous system and corticosteroids may exert its inhibitory effect of GH secretion on hypothalamo-pituitary segments of GH secreting pathways as their suppressive effect of ACTH secreting mechanism. Some animal experiments showed that corticosteroids depleted the hypothalamus of somatotropin releasing factor in rats,¹³ and some showed a direct suppressive effect of corticosteroid on a rat pituitary.¹⁴ In the present series, probably both release and synthesis of GH will be inhibited by long-term administration of corticosteroids and only a release of GH will be suppressed by a single dose or short-term administration.

It seems that a parallelism of GH and 11-OHCS to insulin hypoglycemia does not necessarily mean the existence of a common pathway for both GH and ACTH secretion or GH secretion being accompanied with ACTH secretion as a result of stress. On the other hand, the responses of GH and ACTH were known to be separable to a fair number of stimuli.^{15,16} As we observed in normal subjects and others reported, arginine is not an effective stimulus for ACTH but it is effective, as well as insulin induced hypoglycemia for GH secretion. It is difficult to point out the site of corticosteroid suppression on GH secretion by arginine, as the secretory mechanism of GH by arginine is obscure. But it is likely that a suppression is exerted through central nervous system rather than mediating metabolic changes, as Nakagawa *et al.*⁵ reported no significant changes in plasma free fatty acid and blood glucose after corticosteroids administration. Though we have a limited number of cases with arginine infusion test, the grade of suppression seemed almost as equal as seen in insulin induced hypoglycemia. The pituitary, a terminal site of GH secreting pathway by arginine, might be a major site for a corticosteroid suppression.

References

- 1) Frantz, A.G. & Rabkin, M.T. **Human growth hormone**. Clinical measurement, response to hypoglycemia and suppression by corticosteroids. *New Engl. J. Med.*, 1964, **271**, 1375-1381.
- 2) Hartog, M., Gaafar, M.A. & Fraser, R. Effect of corticosteroids on serum **growth hormone**. *Lancet*, 1964, **2**, 376-378.
- 3) Greenwood, F.C., Hunter, W.H. & Glover, J.S. The preparation of ¹²⁵I-labelled human growth hormone of high specific radioactivity. *Biochem. J.*, 1963, **89**, 114-123.
- 4) DeMoor, P., Steeno, O., Raskin, M. & Hendrikx, A. Fluorimetric determination of free plasma 11-hydroxycorticosteroids in man. *Acta endocr.*, 1960, **33**, 297-307.
- 5) Nakagawa, K., Horiuchi, Y. & Mashimo, K. Responses of plasma growth hormone and corticosteroids to insulin and arginine with or without prior administration of dexamethasone. *J. clin. Endocr.*, 1969, **25**, 35-40.
- 6) Morris, H.G., Jorgensen, J.R. & Jenkins, S.A. Plasma growth hormone concentration in corticosteroid-treated children. *J. clin. Invest.*, 1968, **47**, 427-435.
- 7) Morris, H.G., Jorgensen, J.R., Elrick, H. & Goldsmith, R.E. Metabolic effects of human growth hormone in corticosteroid-treated children. *J. clin. Invest.*, 1968, **47**, 436-451.

- 8) Martin, M.M., Gaboardi, F., Podolsky, S., Raiti, S. & Calcagno, P.L. Intermittent steroid therapy. Its effect on hypothalamic-pituitary-adrenal function and the response of plasma growth hormone and insulin to stimulation. *New Engl. J. Med.*, 1968, **279**, 273-278.
 - 9) Moses, A.M. & Miller, M. Assessment of pituitary reserve in subjects pretreated with dexamethasone. *Metabolism*, 1969, **18**, 376-386.
 - 10) Miura, K. Clinical and experimental studies on some new synthetic corticoids. *Tohoku Igaku Zasshi* (Jap.), 1960, **63**, 541-565.
 - 11) Bierick, J.R., Kersten, I. & Maruektad, S. Plasma corticosteroids and their responsiveness to corticotrophin after longterm therapy with corticosteroids and corticotrophin. *Acta endocr.*, 1959, **31**, 40-48.
 - 12) Ackerman, G.L. & Nolan, C.M. Adrenocortical responsiveness after alternate-day corticosteroid therapy. *New Engl. J. Med.*, 1968, **278**, 405-409.
 - 13) Pecile, A. & Müller, E. Suppressiv action of corticosteroids on the secretion of growth hormone. *J. Endocr.*, 1966, **36**, 401-408.
 - 14) Birge, C.A., Peake, G.T., Marix, I.K. & Daughaday, W.H. Effects of cortisol and diethylstilbestrol on growth hormone release by rat pituitary *in vitro*. *Proc. Soc. exp. Biol. Med.*, 1967, **126**, 342-345.
 - 15) Berson, S.A. Radioimmunoassay of peptide hormones. 49th Meeting of Japanese Endocrine Society, Gumma, Japan, 1969.
 - 16) Müller, E.E., Saito, T., Arimura, A. & Schally, A.V. Hypoglycemia, stress and growth hormone release: Blockade of growth hormone release by drugs acting on the central nervous system. *Endocrinology*, 1967, **80**, 109-117.
-