Failure to induce puberty in a man with X-linked congenital adrenal hypoplasia and hypogonadotropic hypogonadism by pulsatile administration of low-dose gonadotropin-releasing hormone

Kiyoshi Kikuchi1, Masayuki Kaji1, Toru Momoi1, Haruki Mikawa1, Yosuke Shigematsu2 and Masakatsu Sudo2

Department of Paediatrics1, Faculty of Medicine, Kyoto University, and Department of Paediatrics2, Fukui Medical School, Japan

Abstract. To elucidate the mechanism of hypogonadotropic hypogonadism in a patient with X-linked congenital adrenal hypoplasia, we studied the effects on serum LH and FSH of repeated iv administration of GnRH (400 µg, over 2 h, once a day, for 14 consecutive days), pulsatile sc administration of GnRH (5 µg every 90 min during days 1–56, 10 µg every 90 min during days 57–91) and an iv bolus injection of 10 mg of naloxone. The repeated administration of GnRH restored the hyporesponsiveness of serum FSH and increased serum testosterone level from < 1.0 to 1.7 nmol/l, but the impaired LH response to the standard GnRH test was not improved. The pulsatile administration of GnRH for 91 consecutive days did not induce a clinical or a biochemical change of puberty. Serum testosterone remained undetectable < 1.0 nmol/l, the hyporesponsiveness of serum LH was not improved, but basal FSH level was significantly increased and the impaired FSH response to the standard GnRH test was slightly improved. Naloxone had no effect on serum LH or FSH before or during the pulsatile administration. We conclude that hypogonadotropic hypogonadism in our patient is due to the pituitary dysfunction and that the endogenous opioid peptides may not play a role in the mechanism of inhibited gonadotropin secretions.

X-linked congenital adrenal hypoplasia (X-linked CAH) is known to be associated with hypogonadotropic hypogonadism (Prader et al. 1975; Kelly et al. 1977; Golden et al. 1977; Zachmann et al. 1980; Hay et al. 1981; Virdis et al. 1983). Most patients with X-linked CAH have limited or absent LH and FSH responses to a single administration of the gonadotropin-releasing hormone (GnRH). However, the mechanism of hypogonadotropic hypogonadism remains unclear.

Hypothalamic hypogonadism can be distinguished from pituitary hypogonadism by the response to repeated administration of GnRH (Yoshimoto et al. 1975; Snyder et al. 1979). The pulsatile administration of GnRH can induce puberty as well as restore the hyporesponsiveness of serum LH and FSH in patients with hypothalamic hypogonadism (Valk et al. 1980; Hoffman & Crowley 1982; Skarin et al. 1982; Delemarre-van de Waal et al. 1985).

In the present paper, we describe one family with X-linked CAH. Repeated administration of GnRH was performed in one patient at the age of 16 years to elucidate whether the hypogonadotropic hypogonadism was due to hypothalamic or pituitary dysfunction. The effects of the long-term pulsatile administration of GnRH on sex maturity stage, serum LH, FSH and testosterone levels were also studied at the age of 17 years. Furthermore, we studied the effect of naloxone on serum LH and FSH to evaluate the role of the endogenous opioid peptides in the mechanism of inhibited LH and FSH secretions in the patient.
Patients and Methods

Patient 1 (proband)

He is now 17 10/12 years of age. His elder brother had generalized skin pigmentation and died at the age of one month owing to severe dehydration. Patient 2 is his younger brother by a different father, whereas two younger brothers by a different mother are healthy. He was the 3020 g product of unrelated healthy parents and a full term uncomplicated pregnancy. He was admitted on the 18th day after birth because of feeding difficulty, severe dehydration, and skin pigmentation. He had ten salt losing crises before the age of 6 years although he was treated with glucocorticoid and sodium chloride, and later with glucocorticoid and mineralocorticoid. He had delayed growth and bone maturation as well as failure of spontaneous pubertal changes (Fig. 1). The LH and FSH responses to the standard GnRH tests (100 µg, iv) measured repeatedly after the age of 11 years were consistent with gonadotropin deficiency. He had delayed GH response to insulin, normal TSH and prolactin responses to TRH, and normal testosterone response to human chorionic gonadotropin (hCG) (Table 1). Basal T₃ and T₄ levels were also normal. At the age of 16 years, serum dehydroepiandrosterone (DHEA), its sulphate (DHEA-S), and testosterone were not detectable. Between 16 1/12–16 5/12 years of age, he was given an adrenal androgen, DHEA-S (12.5 mg/day, po) (Kanebo Pharmaceuticals Ltd, Japan). Though serum DHEA, DHEA-S, androstenedione and androsterone levels were normalized and serum testosterone level rose slightly during the administration, hyporesponsiveness of serum LH and FSH was not restored during or after the administration. In addition, pubic hair and genital stages did not advance and height velocity did not increase during the treatment. At the age of 17 4/12 years his height was 152.5 cm (±3.2 sd); bone age was 12 6/12 years (Greulich & Pyle criteria); he had no signs of puberty (Tanner stage I) with testes measuring 18 × 16 mm (2 ml). Since the age of 17 5/12 years he has received treatment with hCG. This treatment induced pubic hair development and enlarged testicular size.

Patient 2

He was the 3450 g product of unrelated healthy parents and a full term uncomplicated pregnancy. He was admitted on the 19th day after birth because of feeding difficulty and generalized skin pigmentation. At the age of 4 4/12 years he had delayed GH response to insulin, absent LH and FSH responses to GnRH, subnormal TSH and exaggerated prolactin responses to TRH (Table 1). Basal T₃ and T₄ levels were normal. Now, at 8 years of age, he is being treated with glucocorticoid and

![Fig. 1.](image)

Growth pattern and bone age relative to chronological age in 2 patients with X-linked congenital adrenal hypoplasia. Normal height (mean ± 2 sd) is shown, based on the results of the survey on the physical growth and development of infants, pre-school and school of children in Japan in 1980 by the Ministry of Health and Welfare and the Ministry of Education. The ages at which patient 1 was treated with DHEA-S, pulsatile GnRH administration and hCG are indicated.
**Table 1.**

Endocrinological data.

<table>
<thead>
<tr>
<th></th>
<th>Patient 1</th>
<th>Patient 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GnRH</strong>&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td><strong>LH (IU/l), basal/peak</strong></td>
<td>13.6/25.6</td>
<td>7.6/5.5</td>
</tr>
<tr>
<td><strong>FSH (IU/l), basal/peak</strong></td>
<td>4.0/6.7</td>
<td>4.9/5.1</td>
</tr>
<tr>
<td>Testosterone (nmol/l)</td>
<td>&lt; 1.0</td>
<td>1.7</td>
</tr>
<tr>
<td><strong>hCG</strong>&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td><strong>Testosterone (nmol/l), basal/peak</strong></td>
<td>&lt; 1.0/4.6</td>
<td>&lt; 1.0/4.6</td>
</tr>
<tr>
<td><strong>TRH</strong>&lt;sup&gt;3&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td><strong>TSH (mU/l), basal/peak</strong></td>
<td>2.9/15.6</td>
<td>&lt; 0.5/4.2</td>
</tr>
<tr>
<td>Prolactin (µg/l), basal/peak</td>
<td>7.5/59</td>
<td>6.3/111</td>
</tr>
<tr>
<td><strong>Insulin</strong>&lt;sup&gt;4&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td><strong>GH (µg/l), basal/peak</strong></td>
<td>1.3/20 (at 90 min)</td>
<td>7.0/17 (at 120 min)</td>
</tr>
</tbody>
</table>

1. Patient 1, the LH and FSH responses to the standard GnRH tests (100 µg, iv) and serum testosterone levels before (day 0), during (day 8) and after (day 15) the repeated administration of GnRH for 2 weeks. Basal/peak levels, median (range), of LH and FSH in the controls were 8.9 (5.3–22.9)/55.1 (38.6–91.7) and 7.3 (4.3–13.9)/17.7 (9.5–31.3) IU/l, respectively. Controls consisted of 12 boys, aged 13–15 years, with constitutional delay and no signs of puberty (Tanner stage I) and their bone ages were between 10 and 126/12 years (Greulich & Pyle criteria). Patient 2, the LH and FSH responses to 3 µg/kg of GnRH, iv.
2. The response to 5000 U of hCG, im, for consecutive 3 days.
3. The TSH and prolactin responses to 10 µg/kg of TRH, iv.
4. The GH response to 0.1 U/kg of insulin, iv.

mineralocorticoid. Only one moderate salt losing crisis has occurred so far. He has normal growth, but delayed bone maturation (Fig. 2).

Patients 1 and 2 have neither anosmia nor a history of cryptorchism. They did not have adrenal antibody. The diagnosis of adrenal insufficiency was made on the basis of the clinical course, hyponatraemia, hyperkalaemia, low levels of urinary 17-hydroxycorticosteroids and 17-ketosteroids, abnormally low plasma steroids (deoxycorticosterone, 17α-hydroxyprogesterone, corticosterone, cortisol, aldosterone, DHEA and DHEA-S), elevated plasma ACTH, an absent serum cortisol response to ACTH and an absent plasma aldosterone response to angiotension III with high plasma renin activity.

**Test procedures**

Informed consent for all investigations was obtained from patient 1 and his parents. Because the patient sometimes forgot to take glucocorticoid, he had generalized skin pigmentation, and elevated plasma ACTH (e.g. 1500 ng/l) and β-endorphin (e.g. 130 ng/l) were often observed during the investigation.

1) The responses to repeated administration of GnRH were studied at 16 years of age according to the method of Yoshimoto et al. (1975). The repeated administration of GnRH consisted of iv infusion of GnRH (LH-RH, Tanabe Seiyaku Co Ltd, Japan) (400 µg dissolved in 250 ml of physiologic saline), over 2 h, once a day for 14 consecutive days and was started at 09.00 h except for day 8, when it was started at 13.00 h. The standard GnRH test consisted of rapid iv infusion of 100 µg of GnRH dissolved in 10 ml of physiologic saline and measurement of serum LH and FSH at 0, 30, 60, 90 and 120 min, and was performed on the day before the repeated administration of GnRH (day 0) and on days 8 and 15 of the administration period. The test was started at 09.00 h. Serum testosterone was measured just before the standard GnRH test on days 0, 8 and 15.

2) The responses to the pulsatile administration of
GnRH were studied at 17 1/12-17 4/12 years of age. The pulsatile administration of GnRH consisted of sc infusion of GnRH (5 µg = 0.10 µg/kg, every 90 min during days 1-56; 10 µg = 0.20 µg/kg, every 90 min during days 57-91) via a portable infusion pump (SP 3-1, Nipro Ltd, Osaka, Japan) for 91 consecutive days (13 weeks). The standard GnRH test was performed on the day before the pulsatile administration of GnRH (day 0) and on days 7, 21, 42, 56 and 77 of the administration period, and blood samples were taken at 0, 15, 30, 60, 90 and 120 min. The test was started at 09.00 h. The pulsatile administration was discontinued from one hour before the standard GnRH test until the end of the standard GnRH test. Serum testosterone was measured at 09.00 h on days 0, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77 and 84.

3) The effects of naloxone on serum LH and FSH were studied at 16 10/12 years of age (3a) and on day 91 during the pulsatile administration of GnRH at 17 4/12 years of age (3b) as follows: 3a) A bolus injection of naloxone, 10 mg, iv, (Endo Inc, NY, USA) was given over a period of 60 s at 09.30 h after two baseline blood samples had been taken. Blood samples were taken 30, 60, 90 and 120 min after the injection. The same measurements were made after the administration of saline within a week. 3b) Eighteen blood samples were taken every 15 min for 4 1/4 h (from 09.20 h to 13.35 h) on day 91. A bolus injection of naloxone, 10 mg, iv, and of GnRH, 10 µg, sc, were simultaneously given after 7 blood samples had been taken.

Hormone assay
Serum LH, FSH and testosterone were measured by double-antibody radioimmunoassay methods using commercial test kits (LH and FSH, Daiichi Radioisotope Labs. Ltd, Japan; testosterone, CIS Commissariat a l'Energie Atomique, Italy). The Second International Reference Preparation for Human Menopausal Gonadotropins was used as a reference standard for both LH and FSH. The intra- and inter-assay variations for LH and FSH, and testosterone were less than 10% and 15%, respectively. Statistical analysis was carried out by Student's t-test.

Results
1) The responses to repeated administration of GnRH (Table 1)
Before the repeated administration of GnRH, patient 1 had limited LH and FSH responses to the standard GnRH test and undetectable serum testosterone. After one week, the hyporesponsive-
ness of serum FSH was restored, but serum testosterone was undetectable. After two weeks, serum testosterone became detectable and an increase in the basal and peak serum FSH level after the standard GnRH test was observed although the peak FSH level was lower than the basal FSH level. No improvement in the LH response was observed.

2) The responses to the pulsatile administration of GnRH

Fig. 2 shows the LH and FSH responses to the standard GnRH test. The highest basal and peak serum LH levels were observed on day 21. The hyporesponsiveness of serum LH was not restored during the pulsatile administration. The FSH response was slightly improved on day 7. The FSH response was more improved on day 21 than on day 7. However, the FSH response on days 42, 56 and 77 was similar to that on day 21. The hyporesponsiveness of serum FSH was not restored during this treatment.

Serum testosterone was not detectable (< 1.0 nmol/l) during this treatment.

Pubic hair did not develop and testicular size did not increase during this treatment.

3) The effects of naloxone on serum LH and FSH

3a) Fig. 3 shows that no significant alterations in the levels of LH and FSH occurred after naloxone administration.

3b) No spontaneous LH or FSH surge was observed in our patient at the age of 16 10/12 years, although there was a fluctuation in serum LH level (Fig. 4A). Serum LH and FSH levels (mean ± sd) in blood samples taken every 15 min were 11.3 ± 1.9 IU/l (N = 18) and 3.6 ± 0.7 IU/l (N = 18), respectively. Fig. 4B shows that the LH and FSH surges were not induced by the pulsatile administration of GnRH on day 91, and that naloxone did not improve the LH and FSH responses to the pulsatile administration. However, a significant (P < 0.001) increase in the serum FSH level (5.7 ± 0.5 IU/l, N = 18) was observed as compared with that at the age of 16 10/12 years, whereas no significant alteration in the serum LH level (10.8 ± 1.4 IU/l, N = 18) was observed. The responses of a male control patient with pan-hypopituitarism owing to suprasellar germinoma are shown for comparison in Fig. 4C.

Discussion

The restoration of the impaired FSH response and the increase in serum testosterone by repeated iv administration of 400 µg of GnRH for

Fig. 3.
Serum LH and FSH levels after naloxone (solid line) and saline (broken line) administration in patient 1 at 169/12 years of age.
two weeks suggest that the hypogonadotropic hypogonadism in our patient might be due to hypothalamic dysfunction. However, there was a discrepancy between the restorations of the impaired serum LH and FSH responses to the standard GnRH test, which has not been observed in our other GnRH deficient patients. Although Kruse et al. (1984) and Kelch et al. (1984) reported that the hypogonadotropic hypogonadism associated with X-linked CAH may be due to hypothalamic dysfunction, their data also show that the hyporesponsiveness of serum LH was not fully restored.

We used the long-term pulsatile sc administration of GnRH to induce puberty in our patient aged 17 1/2 years. However, treatment for 13 weeks did not induce the clinical or biochemical change of puberty, although the serum FSH level slightly increased. This finding cannot be accounted for only by hypothalamic dysfunction. The impaired responses of serum LH and FSH to the standard GnRH test were restored in our other GnRH deficient patients by the end of the first week of this treatment (data not shown). Hoffman & Crowley (1982) also reported that pulsatile sc administration of GnRH induced all the clinical and biochemical changes of normal puberty in GnRH deficient patients (idiopathic hypogonadotropic hypogonadism) within three months. Thus, we conclude that the hypogonadotropic hypogonadism in our patient is due to dysfunction of the pituitary. The pituitary dysfunction in our patient is of interest, because a case of cytomegalic congenital adrenal hypoplasia was reported with similar cytomegalic changes in the pituitary (Marsden & Zakhour 1978). How-

Fig. 4.
A) Serum LH and FSH levels in patient 1 at 16 10/12 years of age (3 month before the pulsatile GnRH administration was started. B) Serum LH and FSH levels before and after naloxone administration on day 91 during the pulsatile GnRH administration in patient 1 at 17 4/12 years of age. C) Serum LH and FSH levels after the 6-week pulsatile administration of 5 µg (0.086 µg/kg) of GnRH, every 90 min, sc, in a male control patient aged 19 1/2 years with panhypopituitarism owing to suprasellar germinoma for which he received radiation therapy at 12 7/12–9/12 years of age. Serum testosterone level increased from < 1.0 to 7.1 nmol/l by the 4-week pulsatile administration.
however, we do not know whether this pituitary dysfunction results from the primary defect in the pituitary, the salt losing crises occurring until the age of 6 years, or the irregular taking of glucocorticoid.

Evidence has accumulated that β-endorphin in the brain inhibits LH and FSH secretion and naloxone increases LH and FSH levels (Kinoshita et al. 1980; Grossman et al. 1981; Lightman et al. 1981; Fraioli et al. 1982; Baranowska et al. 1984). Since ACTH and β-endorphin are formed from a large common precursor protein, they are simultaneously elevated in the plasma of the patient with glucocorticoid deficiency. Elevated plasma ACTH and β-endorphin levels were often observed in our patient. Therefore, we also studied the effect of naloxone on serum LH and FSH before and during the pulsatile administration of GnRH, although we do not know whether β-endorphin level in plasma is parallel to that in the brain or not. The finding that naloxone had no effects on serum LH and FSH may be due to the pituitary dysfunction in our patient. Further studies are necessary to elucidate whether the endogenous opioid peptides play a role in the mechanism of inhibited LH and FSH secretion in the patient with X-linked CAH or not.

An exaggerated prolactin response to TRH in patient 2 may be due to hypothalamic dysfunction. Both of our patients had delayed GH responses to insulin. Kruse et al. (1984) also reported that two patients with congenital adrenal hypoplasia had delayed GH responses to insulin. Furthermore, we had a male patient with acquired Addison's disease who had a delayed GH response to 0.1 U/kg insulin (from 3.4 to 32 μg/l at 90 min). The deficiency of glucocorticoid may modulate the GH response to insulin, although it is well known that the excess of glucocorticoid inhibits GH secretion.

References


Received April 1st, 1986.
Accepted October 13th, 1986.

Dr Kiyoshi Kikuchi,
Department of Paediatrics,
Faculty of Medicine,
Kyoto University,
54, Shogoin Kawahara-cho,
Sakyo-ku,
Kyoto 606, Japan.