Effects of growth hormone therapy on the developmental changes of follicle stimulating hormone and insulin-like growth factor-I serum concentrations in Turner's syndrome

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Summary

OBJECTIVE The aim was to investigate whether, in the absence of gonads, GH could bring forward the age of neuroendocrine activation resulting in onset of puberty.
DESIGN In girls with Turner's syndrome, we evaluated the effects of GH therapy on developmental changes in FSH serum concentrations used as an indicator of neuroendocrine maturation in the absence of gonads.
PATIENTS Thirty-nine girls with Turner's syndrome aged 4·0–17·1 years were treated using GH (25 IU/m² week) for 1 year.
MEASUREMENTS Serum levels of FSH and IGF-I were measured before initiation of GH therapy and 12 months later, after interruption of GH treatment for 2 days.
RESULTS Pretreatment FSH levels were low between 6 and 10 years and increased markedly at 10–11 years of age. This pattern was unchanged after 1 year of GH therapy. Pretreatment IGF-I levels were positively correlated with age and they were uniformly increased after 1 year of GH therapy.
CONCLUSIONS Our data suggest that GH and its effector, IGF-I, do not influence the timing of the onset of puberty through an effect on its neuroendocrine control.

In patients with isolated GH deficiency, puberty was shown to occur at late chronological ages (Tanner & Whitehouse, 1975; Bourguignon et al., 1986) and GH therapy could result in accelerated rate of pubertal development (Van der Werff ten Bosch & Bot, 1990). Recently, puberty was shown to be shortened in GH-treated patients, this being possibly related

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to GH therapy (Darendeliler et al., 1990) though we concluded earlier to an effect of late onset of puberty independent of GH (Bourguignon, 1988). In some non-GH-deficient short children treated with GH, early onset of puberty was also reported suggesting a priming effect of GH on the hypothalamo–pituitary–gonadal axis (Wit et al., 1989; Albertsson-Wikland & Karlberg, 1991).

While these data provided some evidence of a possible GH effect on timing of puberty, it was not possible to determine whether such effects of GH resulted from hypothalamo–pituitary activation or direct gonadal action. Agonadal patients could provide a model to address that question. These patients were shown to exhibit developmental changes in serum gonadotrophin levels reflecting gonadal-independent neuroendocrine maturation (Conte et al., 1975) and GH therapy was proposed to promote growth of Turner patients (Raiti et al., 1986; Ross et al., 1986; Rosenfeld et al., 1986; Vanderschueren-Lodeweyckx et al., 1990). Therefore, we evaluated the age-related changes in serum concentrations of follicle stimulating hormone (FSH) and insulin-like growth factor-I (IGF-I) before and after a year of GH therapy in patients with Turner's syndrome.

Patients

This study was performed on 39 girls with Turner's syndrome treated using GH (Norditropin) at a weekly dosage of 25 IU/m². Twenty patients received GH as a single daily s.c. injection in the evening while 19 received a similar dose divided into two daily injections. The effects of GH on growth, bone maturation and some metabolic parameters have previously been reported (De Schepper et al., 1991). Karyotype was XO in 24 patients while mosaicism or other alterations were found in 15. All the patients were prepubertal at initiation of GH therapy. We excluded from this study one patient who developed spontaneous puberty during GH therapy. Chronological age at onset of GH therapy varied between 4·0 and 17·1 years. Bone age was assessed by a single radiologist according to Tanner et al. (1983) based on radius, ulna and short bones (TW2 RUS method). Bone age at onset of GH therapy varied between 3·0 and 13·1 years. This study was approved by the Ethical Committee of the University of Liège and written informed consent was obtained from the patients or their parents.

Methods

Blood samples were obtained at the beginning of GH therapy and one year later, after withdrawal of GH injections for 2 days. Serum was kept frozen until assayed. Using a highly sensitive enzyme immunoassay for FSH (kindly supplied by Medgenix Belgium), 0·05 ml serum samples were run in duplicate within a single assay. The detection limit was 0·15 IU/l (Reference preparation: 2nd IRP-78/549). The intra and inter-assay coefficients of variation were 4 and 9%, respectively. Cross-reactivity of luteinizing hormone (LH), human chorionic gonadotrophin, thyroid stimulating hormone (TSH) and α-subunit in the assay was less than 0·1%.

IGF-I was measured within a single assay using a radioimmunoassay developed in our laboratory. Recombinant IGF-I (generously provided by Kabi Pharmacia, Stockholm, Sweden) was labelled by ^{125}I using chloramine-T (Greenwood *et al.*, 1963). The specific activity of the tracer was 160 Ci/g. A polyclonal rabbit antiserum was raised against recombinant IGF-I (Kabi Pharmacia) coupled to ovalbumin. This antiserum was used at a final dilution of 1/48000. No significant cross-reactivity (<0·1%) was observed in the presence of IGF-II, insulin, GH, FSH, LH, TSH, glucagon, vasoactive intestinal peptide, β-endorphin and Leu- and Met- enkephalins. Fragments 13–20, 21–32, 33–36, 52–70 and 54–58 of IGF-I (Kabi Pharmacia) did not result in any significant cross-reaction. Acid–ethanol extracts of 0·05 ml serum (Daughaday *et al.*, 1980) were preincubated with antiserum for 4 hours at room temperature, radioiodinated IGF-I being then added for a 20-hour incubation, at 4°C. Precipitation of bound radioactivity was obtained by addition of anti-rabbit-gammaglobulin antiserum raised in sheep (1/200) and enriched with polyethyleneglycol 6000, 60 g/l. The limit of detection of this assay was 0·05 ng/tube. Intra and inter-assay coefficients of variation were 5 and 8%, respectively.

Statistical analysis

The significance of differences between pretreatment hormone levels and concentrations achieved after 1 year of GH therapy was calculated using the two-tailed paired Student's *t*-test. The significance of differences in hormone concentrations related to age and number of daily injections of GH was determined using ANOVA with three-factor factorial, all results being considered to be significant at the 5% level.

Results

Pretreatment serum FSH concentrations varied markedly with age ($P<0·001$), the lowest values being observed between 6 and 10 years while elevated values were observed
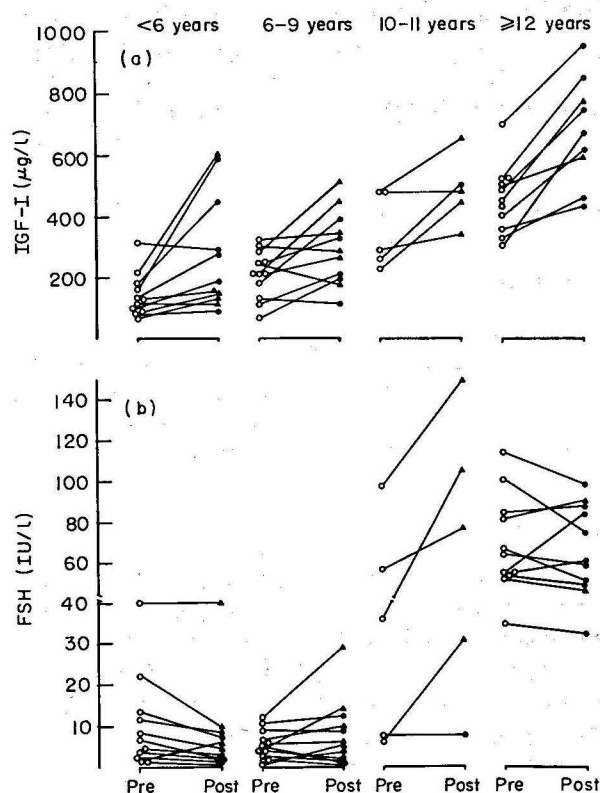
Fig. 1 Age-related changes in serum concentrations of a, IGF-I and b, FSH in 39 patients with Turner's syndrome studied before (Pre) and after (Post) 1 year of GH therapy (25 IU/m² wk) given as ●, one or ▲, two daily injections. Patients were divided into four age groups. Post-treatment data were obtained 2 days after the last injection of GH. In three patients, post-treatment IGF-I data were omitted because blood was sampled 12–24 hours after the last injection.

in some patients before 6 years and in all patients after 12 years. On that basis, the data were analysed in four age groups (Fig. 1). GH therapy resulted in slight but not significant changes in FSH concentrations except in four out of five patients aged 10–11 years. Among the five patients, three had shown increased FSH levels before GH therapy. Among 12 patients aged between 6·1 and 9·5 years at onset of GH therapy, 10 showed low and unchanged levels of FSH after GH therapy (Fig. 1b). In one patient who showed increased serum FSH (29 IU/l) after therapy, FSH was already elevated (13·3 IU/l) before GH treatment. However, bone age was not advanced in this patient (7·4 years).

Pretreatment serum concentrations of IGF-I were positively correlated with age ($r=0·795$, $P<0·001$). After 1 year of GH therapy, the vast majority of patients showed increased concentrations of IGF-I when compared to pretreatment levels (Fig. 1a). This increase was significant

($P < 0.05$) in the four age groups studied. The increments in serum IGF-I concentrations after 1 year of GH therapy were 3–7 times greater than the annual increment (28 μg/l) calculated from the age-related linear correlation obtained using pretreatment data. Comparison between patients treated using one and two daily injections of GH did not show any significant difference, either for IGF-I, or for FSH.

Discussion

A facilitatory role of GH or signals under its control in the onset of puberty was suggested by observations in patients with isolated GH deficiency (Van der Werff ten Bosch & Bot, 1990) or normal variant short stature (Wit et al., 1989; Albertsson-Wikland & Karlberg, 1991). In this paper, we have investigated whether activation of somatotrophic signals could be involved in the neuroendocrine control of the time when puberty is initiated. This question was difficult to address on account of the multiple interactions between GH, IGF-I and the hypothalamo–pituitary–gonadal axis as well as the multiple anatomical sites possibly involved (Bourguignon, 1988). Evidence was accumulating that GH and GH-dependent IGF-I secretion had direct gonadal effects in humans (Kulin et al., 1981; Homburg et al., 1988; Mason et al., 1990). In contrast, there were no clinical studies on the central effects of GH/IGF-I on the pituitary–gonadal axis. In vitro, a stimulatory effect of IGF-I on GnRH secretion from rat hypothalamic explants was reported recently (Hiney et al., 1991). In vivo, Wilson et al. (1989) evaluated the effects of administration of GH for 12 months in intact or castrated and oestrogen-treated female monkeys. Premature initial ovulation was seen in three out of five treated intact animals but age at menarche was not affected. In post-menarcheal animals treated with GH, oestradiol secretion was increased. In ovariectomized animals, the developmental elevation in LH was not influenced by GH administration. It was concluded that GH was not involved in the initiation of LH secretion at puberty whereas GH played a facilitatory role in the ovarian response to the gonadotrophins. Such a conclusion was in agreement with our findings in Turner patients. Using weekly doses of GH relatively similar to that used by Wilson et al. in monkeys, the developmental changes of FSH secretion in Turner patients were not affected. Indirectly, our data suggested that GH effects, if any, were more likely to occur at the gonadal level than at the hypothalamo–pituitary level.

In contrast to the experimental study in castrated monkeys, we were unable to provide data of a control group of untreated patients followed in parallel with the patients receiving GH. Since growth in untreated Turner patients was extensively documented, such a group could not be justified

for ethical reasons. It is unlikely that insufficient GH stimulation would account for absence of effects on FSH secretion in our patients. GH therapy resulted in significant growth response (De Schepper et al., 1991). The increase in serum levels of IGF-I provided additional evidence of GH endocrine effects in our patients. We confirmed the age-related increase in serum levels of IGF-I and its increase in response to GH therapy (Raiti et al., 1986; Rosenfeld et al., 1986; Ranke et al., 1987; Bergmann et al., 1990). The 1·4 to 1·9-fold increase in serum IGF-I levels that we saw 2 days after the last GH injection was less than the three to four-fold increase reported within 24 hours after the last administration of GH (Rosenfeld et al., 1986; Bergmann et al., 1990). It is noteworthy that the GH-dependent changes in binding proteins of IGF-I (Schalch et al., 1982) should not account for the observed changes in IGF-I concentrations since the possible interaction of binding proteins in the assay of IGF-I was prevented by an appropriate extraction method (Daughaday et al., 1980). Though our observations do not support an endocrine effect of GH/IGF-I on GnRH and gonadotrophin secretion, we cannot rule out a paracrine or local effect involving short feedback action of GH on the hypothalamus and IGF-I synthesized in the central nervous system.

The suitability of patients with Turner's syndrome as a model for the study of the mechanism of puberty could be controversial. In the absence of clinical manifestations of puberty in these patients, only endocrine manifestations can be studied. In untreated patients, our data confirmed previous observations on changes in serum FSH levels with age (Conte et al., 1975; Ross et al., 1983; Hosoda et al., 1991). Serum FSH was low between 6 and 10 years and increased markedly between 9 and 11 years of age. This relatively early time with respect to onset of normal female puberty (Tanner, 1962) might reflect latency between endocrine and clinical manifestations of puberty. On account of absence of negative feedback control by the gonads, the increase in FSH secretion was more marked than that of LH. This was the reason why we elected to study FSH levels. An additional limitation of Turner's syndrome as a model of neuroendocrine changes at puberty is that frequency of pulsatile LH secretion is already increased at prepubertal ages on account of absent gonadal negative feedback effect. At the time of rise in gonadotrophin secretion, these patients do not show any further increase in LH pulse frequency (Ross et al., 1983; Hosoda et al., 1991). This is in contrast with the increase in LH pulse frequency described at onset of normal puberty (Wu et al., 1990).

In summary, the developmental changes in FSH secretion used as an index of neuroendocrine maturation in Turner's syndrome were not significantly affected by GH therapy. These data provide evidence against a role of GH and IGF-I

as a somatotrophic signal modulating the central control of onset of puberty. Indirectly, the role of possible gonadal effects of GH/IGF-I is emphasized.

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