Abstract. Present study was planned to clarify the effects of GH and insulin-like growth factor I (IGF-I) on testosterone secretion using premature male rats. Forty rats were divided four groups. GH, IGF-I, both of them or normal saline solution as control were subcutaneously administered to the rats of each group for seven days from 3-week to 4-week of age. After the treatment, six of each group were used to human chorionic gonadotropin (hCG) loading and four to Leydig cell preparation. Serum testosterone responses to hCG loading were significantly higher in 4-week-old rats treated with GH and/or IGF-I for 1 week than in control rats. However, the responses were similar among three treated groups (GH, IGF-I and both). After one-week treatment with GH and/or IGF-I, isolated Leydig cells were prepared from testes of 4-week-old rats and testosterone production by the stimulation of hCG was examined. Amounts of testosterone production stimulated by hCG were significantly greater in the treated rats than in control rats. These findings suggest that GH mediated by IGF-I promotes the testicular responsiveness to gonadotropin on testosterone production in premature rats.

Key words: GH, Insulin-like growth factor I, Testosterone, Leydig cell, Puberty

IT IS well-known that in patients with isolated GH deficiency, when they are not treated with GH, the onset of puberty delays 2 or 3 years (mean age of pubertal onset: 15.0 year in 42 patients) compared with normal children [1-4]. Hibi et al. reported that in boys with isolated GH deficiency, when they were treated with GH at the mean age of 10.2 year, puberty occurred at the mean age of 12.8 year [2]. Moreover, Wilson et al. reported that GH treatment hastened the onset and progression of puberty in rhesus monkeys [5]. These findings suggest that GH may be of importance in the mechanism controlling the onset of puberty and GH administration may advance the sexual maturation.

In the present study, to determine whether the administration of GH and insulin-like growth factor I (IGF-I) during prepubertal period influences the progression of gonadal maturation, we observed changes in testosterone secretion in response to short-term injection of GH and IGF-I using male premature rats.

Methods

Three-week-old male Wistar rats were used in this study. First, GH (Genotropin, Sumitomo Pharmaceutical) (1.5 U/kg/day) was administered for 2 weeks in six male rats of 3-week-old and serum testosterone levels were measured. Second, forty rats were divided four groups, and GH, IGF-I (Fujisawa Pharmaceutical), both of them or normal saline solution as control were subcutaneously administered to the rats of each group for seven days from 3-week to 4-week of age as follows. 1.5 U/kg of GH were injected at 1900 h every day in
GH group, 1.5 mg/kg of IGF-I were injected twice a day at 0800 h and 1900 h in IGF-I group, both of GH and IGF-I in GH-IGF-I group and normal saline solution at 1900 h in control group. Six rats of each group were used to human chorionic gonadotropin (hCG, Sigma) loading and four to Leydig cell preparation. HCG loading and Leydig cell preparation were done 12 h after the last injection (Leydig cell preparation was also done in non-treated 16-week-old rats). In hCG loading, 500 IU/kg of hCG were administered intraperitoneally and serum testosterone levels were determined by radioimmunoassay before and 2 h after the administration. Minimal detectable levels of testosterone in plasma were 5 ng/dl. Leydig cell preparation was performed by the method of Dufau with some modifications [6]. Leydig cells were made up to 1 x 10^6 cells/ml with the solution of Krebs Ringer Bicarbonate with 0.2% bovine serum albumin and 0.2% glucose. One milliliter of cell suspension was incubated with hCG (5 to 100 mIU) or GH (10 or 100 mIU) at 37 °C, 95% O2 and 5% CO2. Three hours after the incubation, cell suspension was centrifuged and testosterone concentrations of supernatants were measured by radioimmunoassay.

**Statistical analysis of data**

Results were presented as the mean ± SEM. One-way analysis of variance (ANOVA) or two sample t-test were used to test for differences between group means. P-values <0.05 were regarded as statistically significant.

**Results**

As shown in Fig. 1, after one-week administration of GH, serum testosterone concentrations increased to detectable levels in five of six GH-treated rats (range: 10–54 ng/dl), while those in all of non-treated rats were undetectable. Results of hCG loading were shown in Fig. 2. Serum testosterone levels after hCG loading increased in all groups and were significantly higher in GH and/or IGF-I treated groups than in control group (GH group: 148.3 ± 8.5, IGF-I group: 127.6 ± 6.4, GH/IGF-I group: 137.2 ± 9.2, control group: 92.1 ± 6.8 ng/dl, mean ± SEM, P<0.01 vs. control group). Serum testosterone levels were similar among three treated groups.

Testosterone production in isolated Leydig cells was shown in Figs. 3 and 4. In non-treated mature (16-week-old) and premature (4-week-old) rats, isolated Leydig cells produced testosterone dose-dependently in response to various concentrations of hCG (Fig. 3). Maximum testosterone production was attained by the addition of 40

![Fig. 1. Changes in serum testosterone concentrations in premature male rats (3 to 5 weeks of age) with or without GH treatment.](image)

![Fig. 2. Serum testosterone responses to hCG loading in premature male rats treated with GH and/or IGF-I. The data represent mean ± SEM (n=6). *P<0.01 vs. control.](image)
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mIU/tube of hCG. Amounts of testosterone production significantly decreased in Leydig cells prepared from premature rat testes compared with those in mature rats. The amounts of testosterone production stimulated by hCG were significantly greater in isolated Leydig cells from the groups of GH and/or IGF-I treated rats than in the cells from the group of control premature rats (maximum testosterone production; GH group: 104.9 ± 8.0, IGF-I group: 90.8 ± 3.7, GH/IGF-I group: 94.5 ± 4.7, control group: 51.5 ± 3.7 ng/10⁶ cells, mean ± SEM, P<0.01 vs. control group). There were no significant differences in testosterone production among three treated groups. The Leydig cells from the group of 16-week-old rats showed significantly higher production of testosterone (276.6 ± 8.6 ng/10⁶ cells) than those from the treated groups of 4-week-old rats (P<0.01). When isolated Leydig cells were stimulated by addition of GH (10 or 100 mIU/10⁶ cells), testosterone production did not increase (data not shown).

Discussion

Wilson and Tanner reported the effect of GH treatment on the tempo of sexual maturation in female rhesus monkeys [7]. According to recent clinical studies, GH treatment promotes the onset of puberty or accelerates the progression of pubertal maturation [8–10]. However, it is still under debate whether GH directly stimulates the gonads and promotes the gonadal maturation at prepubertal stage.

In the present study, we demonstrated that the administration of GH and IGF-I increased basal serum testosterone concentration and augmented the capacity of testosterone secretion in hCG loading in premature rats. Moreover, these results were confirmed by the changes in responsibility of isolated Leydig cells to hCG, that is, isolated Leydig cells of premature rats treated with GH and/or IGF-I produced a larger amount of testosterone than those of non-treated rats. Odell et al. reported that, in 3-week-old hypophysectomized male rat, serum testosterone response to LH was increased by GH pretreatment for 5 days similar to by FSH pretreatment [11]. This finding indicates that GH does not act through gonadotropin secretion, but directly stimulates testis. Our data demonstrated that similar response of testis to GH administration was seen by IGF-I administration as well. Whether exogenous IGF-I administration directly acts to testis or stimulates pituitary gona-
dotropin secretion was not ascertained in this study. If the exogenous IGF-I administration stimu-
lates pituitary gonadotrophs, testicular responsivity to hCG may have differed among
GH, IGF-I and both treated groups. However, there were no significant differences in serum testos-
terone responses to hCG loading and the testosterone production of isolated Leydig cells among these
three treated groups, suggesting that exogenous GH and IGF-I act through the same pathway, that
is, GH mediated by IGF-I stimulates Leydig cells. This conclusion, however, is still to be confirmed
by further experiments using hypophysectomized rats treated with IGF-I.

It has been shown that cultured rat Sertoli cells
produced IGF-I [12], and cultured pig Leydig cells
contain specific IGF-I receptor, and IGF-I enhances
not only LH receptor, but also the coupling of the
receptor to adenylate cyclase [13, 14]. No direct
effects of GH on testosterone production in isolat-
ed Leydig cells have been observed in our study.
Therefore, it appears likely that GH stimulates Ser-
toli cells, increases testicular IGF-I concentrations,
and testicular IGF-I acts on Leydig cells and in-
creases steroidogenic capacity.

In summary, present study demonstrates that GH
and IGF-I administration enhances the responsi-
ibility of Leydig cells to LH stimulation on
testosterone production in premature rats.

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