Original

Impaired Development of Somatotropes, Lactotropes and Thyrotropes in Growth-Retarded (grt) Mice

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Abstract: Congenitally primary hypothyroid growth-retarded (grt) mice exhibit a characteristic growth pause followed by delayed onset of pubertal growth. We characterized the developmental pattern of somatotropes, lactotropes and thyrotropes in the anterior pituitary, as well as plasma levels of their secretory hormones, in grt mice. Compared with normal mice, the weight of grt pituitary gland was similar at 8 weeks of age but significantly heavier after 12 weeks of age. Compared with normal mice, there were significantly fewer somatotropes in the grt pituitary until 8 weeks of age, but the number gradually increased up to 48 weeks. The number of lactotropes in grt mice was consistently lower than that in normal mice from 2 through 48 weeks, whereas the number of thyrotropes in the grt pituitary was consistently higher than in the normal pituitary. Thyrotropes in the grt pituitary exhibited hypertrophy and hyperplasia with less intensive thyroid-stimulating hormone (TSH) immunoreactivity than normal thyrotropes. In normal mice, the sum of the relative proportions of these cells plateaued at 8 weeks, where it remained up to 48 weeks of age. In grt mice, these proportions almost reached normal levels at 12 weeks of age but gradually declined after 24 weeks. Plasma growth hormone concentrations did not differ between grt and normal mice until 24 weeks of age. Compared with normal mice, grt mice exhibited significantly lower plasma prolactin and thyroxine levels but higher TSH levels. These findings indicate that development of somatotropes, lactotropes and thyrotropes in grt mice is impaired, being followed by altered hormone secretion. (J Toxicol Pathol 2009; 22: 187–194)

Key words: grt mouse, anterior pituitary, somatotrope, lactotrope, thyrotrope

Introduction

The anterior pituitary gland synthesizes and releases several types of tropic hormones that are classified as five major hormones, namely, growth hormone (GH), prolactin (PRL), thyroid-stimulating hormone (TSH), adrenocorticotropic hormone (ACTH) and gonadotropin hormone (GTH). These hormones regulate thyroidal, adrenal and gonadal function in addition to lactation, bodily growth and somatic development1.

Several spontaneous mutant dwarf animals have been used to gain insight into genes involved in dwarfism. Strains of mice that exhibit autosomally inherited dwarfism include Snell dwarf (dw)2 and Ames dwarf (df)3, both of which are forms of primary hypopituitarism. The growth-retarded (grt) mouse is a mutant with congenital primary hypothyroidism that shows a characteristic growth pause followed by delayed onset of pubertal growth16. Plasma concentrations of thyroxine (T4) are significantly lower in grt mice compared with controls16, whereas TSH concentrations are greatly elevated17,18. The unresponsive nature of TSH receptors to TSH is considered to be attributable to dysfunction of the grt thyroid gland19.

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Sasaki et al. reported that a missense mutation of tyrosylprotein sulfotransferase 2 (Tpt2) is responsible for this phenotype and proposed that tyrosine sulfation of TSH receptors by TPST2 is crucial for TSH signaling and thyroid gland function. Severe postnatal growth retardation and hormonal abnormalities are observed in grt mice. In male grt mice, delayed testicular development is associated with reduced fertility. Ovarian and uterine development is defective in female grt mice, which results in infertility. The density of immunoreactive GH and PRL cells is lower in grt mice than in normal mice at 12–13 months of age. However, the developmental pattern of these cells in the anterior pituitary of grt mice has not been described, nor has the endocrine status of these hormone levels in the plasma. Thus, we examined the developmental pattern of somatotropes, lactotropes (PRL cells) and thyrotropes (TSH cells), as well as developmental changes in plasma hormone concentrations, in normal and grt mice.

Materials and Methods

Animals

Phenotypically male normal (+/+ or grt/+) and growth-retarded (grt/grt) mice were obtained as described previously. The mice were maintained under controlled temperature (23 ± 1°C), relative humidity (60 ± 5%) and lighting (08:00–20:00) conditions. Experimental procedures were performed in accordance with the “Institutional Guidelines for Animal Care and Use” of Saitama University (Saitama, Japan).

Hormones and antisera

Mouse PRL (AFP-6476C), mouse GH (AFP-10783B), rabbit anti-mouse PRL serum (AFP-131078) and monkey anti-rat GH serum (NIDDK-anti-rGH-S-5) were supplied by the Pituitary Hormone & Antisera Center, Harbor-UCLA Medical Center, Los Angeles, CA, USA. Goat anti-rabbit IgG serum (HAC-RBA2-05GTP91), goat anti-tyrosylprotein sulfotransferase 2 (HAC-MKA2-02GTP88), rabbit anti-rat GH (HAC-RT25-02RTB85), rabbit anti-rat PRL (HAC-RT26-02RPB85) and rabbit anti-rat TSH (HAC-RT29-01RPB85) were used as antigens and reference standard, respectively. Goat anti-rabbit IgG serum (1:4000), goat anti-tyrosylprotein sulfotransferase 2 serum (1:4000) or anti-TSH serum (1:6000) overnight at 4°C, followed by swine anti-rabbit IgG (1:50; Dako) for 1 h at room temperature and then rabbit peroxidase anti-tyrosylprotein sulfotransferase 2 complex (1:200, Dako) for 1 h at room temperature. The sections were rinsed in PBS, and the peroxidase activity was visualized with 0.1% diaminobenzidine (Wako Pure Chemical Industries, Osaka, Japan) in 0.05 M Tris-HCl (pH 7.4) containing 0.005% H2O2. After washing in distilled water, the sections were counterstained with hematoxylin.

Counting of immunoreactive cells

The pituitaries from 3 mice in each group were used. Each sample was sectioned sagittally through the pituitary, and then the approximately one-quarter (1/4), half (midsagittal position) (1/2) and three-quarters (3/4) positions were selected for analysis. The number of immunoreactive cells as well as the number of total pituitary cells on the three sections was counted under a light microscope. The ratio of the number of immunoreactive cells to the number of total pituitary cells was calculated and expressed as a percentage.

Hormone determinations

Blood samples were collected from 4–17 mice in each experimental group (except for normal mice at 48 weeks of age; n=2) between 14:00 and 18:00 h by orbital puncture. Plasma samples were obtained by centrifugation at 4°C and stored at −30°C for analysis. Radioimmunoassays for plasma GH and PRL were performed according to the method described previously. Purified mouse GH and mouse PRL were used as antigens and reference standard, respectively. Plasma T4 and TSH levels were assayed using radioimmunoassays for T4 (Ortho-Clinical Diagnostics, Raritan, NJ, USA) and rat-TSH (GE Healthcare Bio-Sciences Corp., Piscataway, NJ, USA), respectively, according to the manufacturer’s instructions.

Statistical analysis

Values were expressed as the mean ± SEM. The statistical significance (P<0.05) of differences was determined using Student’s or Welch’s t-test.
Results

Pituitary growth

The weights of male normal (1.42 ± 0.12 mg) and grt (1.53 ± 0.09 mg) pituitaries were comparable at 8 weeks of age. The pituitaries of male grt mice (1.58 ± 0.05 mg) were significantly heavier than those of normal mice (1.18 ± 0.03 mg) at 12 weeks of age and were approximately 5.4-fold heavier than those of normal mice at 48 weeks of age (5.92 ± 1.09 vs. 1.50 ± 0.05 mg, respectively; Fig. 1).

Immunohistochemistry of GH, PRL and TSH cells

Representative photomicrographs of GH, PRL and TSH cells in sagittal sections of anterior pituitary glands from 5-week-old male grt and normal mice are shown in Fig. 2. In the normal mouse pituitary, GH and PRL cells were uniformly widespread (Fig. 2, A and C). In grt mice, there were fewer GH and PRL cells than in normal mice (Fig. 2, B and D). The number of TSH cells was greater in grt mice than in normal mice (Fig. 2, E and F). TSH cells in the grt pituitary exhibited hypertrophy and hyperplasia, and their immunoreactivity for TSH was weak compared with that of the thyrotropes in the normal pituitary.

Developmental changes of GH, PRL and TSH cells in anterior-lobe cells GH cells

The number of GH cells was significantly lower in grt mice than in normal mice from 2 weeks of age (713 ± 23 and 1772 ± 119, respectively) to 8 weeks of age. However, the numbers of GH cells gradually increased with age in grt and normal mice from 12 weeks to 48 weeks of age (4638 ± 555 and 5371 ± 636, respectively; Fig. 3A).

PRL cells

At 2 weeks of age, the number of PRL cells was significantly lower in grt mice than in normal mice (3.7 ± 2.7 and 68 ± 51, respectively). Normal mice had a pubertal rise in the number of PRL cells at 8 weeks of age. However, the rise was considerably delayed in grt mice, and the number of PRL cells was significantly lower than in normal mice up to 48 weeks of age (1058 ± 153 and 2626 ± 522, respectively; Fig. 3B).

TSH cells

There were significantly more TSH cells in grt mice than in normal mice at 2 weeks of age (581 ± 38 and 299 ± 59, respectively). This increase of TSH cells in the grt pituitary remained significant up to 48 weeks of age (1490 ± 163 and 436 ± 11, respectively; Fig. 3C).

Total pituitary cells

There was no significant difference in the total number of anterior pituitary cells between grt and normal mice from 2 weeks of age (4508 ± 145 and 4845 ± 252, respectively) to 24 weeks of age. However, the value for grt mice became significantly higher than that for normal mice at 48 weeks of age (19095 ± 753 and 12405 ± 971, respectively; Fig. 3D).

Percentages of GH, PRL and TSH cells

The percentage of GH cells per section was significantly lower in grt mice than in normal mice at 2 weeks of age (15.9 ± 1.0% and 36.5 ± 0.7%, respectively) and then gradually increased up to 12 weeks of age. Thereafter, the percentage of GH cells in grt mice gradually decreased; the values were significantly lower in grt mice compared with normal mice at 24 and 48 weeks of age (Fig. 4). The percentage of PRL cells in grt and normal mice was comparable at 2 weeks of age (0.08 ± 0.06% and 1.5 ± 1.2%, respectively), but the percentage in grt mice was significantly lower than in normal mice from 5 through 48 weeks of age (Fig. 4). In contrast, the percentage of TSH cells was significantly greater in grt mice than in normal mice from 2 weeks (13.0 ± 1.2% and 6.2 ± 1.1%, respectively) through 48 weeks of age (7.8 ± 0.6% and 3.6 ± 0.4%, respectively; Fig. 4). In normal mice, the sum of the relative proportions of these cells plateaued at 8 weeks, where it remained up to 48 weeks. In grt mice, these proportions almost reached normal levels at 12 weeks of age but gradually declined after 24 weeks (Fig. 4).

Plasma hormone concentrations

In normal mice, plasma GH levels rose at 5 weeks of age and then peaked with a value of 26 ng/ml at 8 weeks of age. There was no pubertal rise in plasma GH in grt mice at this time, although small and transient rises of GH levels were observed after 5 weeks of age. Plasma GH levels did not differ significantly between grt and normal mice up to 24 weeks of age. At 48 weeks, however, GH levels in grt mice were significantly higher than in normal mice (Fig. 5A). Plasma PRL levels in normal mice had a transient pubertal peak at 5 weeks of age (63 ng/ml) and then gradually decreased to adult levels where they remained up to 48 weeks of age. In grt mice, PRL levels lacked a pubertal rise and were significantly lower than those of normal mice from 5 weeks through 48 weeks of age (31.1 ± 2.2 and 47.6 ± 2.9
Plasma TSH levels in normal mice remained between 25 and 40 ng/ml at all ages examined. In grt mice, plasma TSH levels were dramatically higher than those in normal mice from 2 through 48 weeks of age (465.5 ± 107.7 and 36.3 ± 6.4 ng/ml, respectively; Fig. 5C), whereas plasma T₄ levels in grt mice were invariably lower than those in normal mice throughout the period from 2 to 48 weeks of age (13.8 ± 1.6 and 24.3 ± 0.7 ng/ml, respectively; Fig. 5D).

Discussion

The present study was conducted to evaluate the developmental pattern of GH, PRL and TSH cells, as well as circulating plasma hormone levels, in a congenital hypothyroid mouse, grt. The pituitary growth and developmental composition ratios of these cells were altered drastically in grt mice (Figs. 1–4). In contrast to GH and PRL cells, TSH cells in the grt mouse pituitary are increased in number, although their immunoreactivities were relatively weak, presumably because they were in a state of hypersecretion.

In rats treated with the reversible goitrogen propylthiouracil, the percentage of TSH cells in the pituitary increases and that of GH cells decreases²⁴,²⁵. In neonatally thyroidectomized rats, PRL mRNA levels, PRL contents in the pituitary and serum PRL concentrations are decreased²⁶. Propylthiouracil treatment leads to a marked increase in circulating TSH²⁷. These phenomena are in accordance with our results in grt mice, all coming from the deceased levels of plasma thyroid hormone.

The grt mice used in the present experiment, which have...
characteristics of congenital hypothyroidism, are derived from phenotypically normal DW/J mice by autosomal recessive inheritance. The growth pattern of grt mice differs from that of dw mice\textsuperscript{16}, which have been used as a rodent model of panhypopituitarism\textsuperscript{2}. The dw phenotype is caused by a point mutation in the gene encoding pituitary transcription factor-1 (Pit-1), which is an essential factor for the development and proliferation of GH cells, PRL cells and TSH cells. In the case of dw mice, the pituitary lacks GH, PRL and TSH cells, as demonstrated by immunocytochemically using both light microscopy\textsuperscript{29} and electron microscopy\textsuperscript{30}. In association with this, immunoassayable GH and PRL levels

Fig. 3. Developmental changes in the numbers of GH- (A), PRL- (B), TSH-immunoreactive cells (C) and total pituitary cells (D) in normal and grt male mice. Each point represents the mean ± SEM of 3 mice. *Significantly different from age-matched normal mice (P<0.05).

Fig. 4. Mean percentages of GH, PRL and TSH cells in the pituitaries of developing normal and grt male mice.
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are extremely low\textsuperscript{31}. Both hyt mice and rdw rats are also other dwarf rodent models with congenital primary hypothyroidism. There are fewer GH cells in hyt mice than in wild type\textsuperscript{10}. In rdw rats, GH and PRL cells in the anterior lobe are fewer than in normal rats\textsuperscript{7}, and again both plasma and mRNA levels of GH and PRL expression in the pituitary are reduced, whereas plasma and mRNA levels of TSH are elevated\textsuperscript{13–15}.

In the present experiment, a delay of GH cell development was observed in grt mice during the first 8 weeks after birth (Fig. 3). According to Yoshida et al.\textsuperscript{16}, GH content in the grt pituitary gradually increases with age but remains below normal until about 1 year of age. A delay of body weight increase in grt mice compared with normal mice has also been noted. The difference in body weight between grt and normal mice becomes apparent by 3 weeks of age and most evident (about half of the value for normal mice) at about 1 month of age. However, grt mice gradually catch up with normal mice in terms of body weight, and no difference in weight is noted between grt and normal mice at about 1 year of age. Collectively the results from the present study and the study by Yoshida et al.\textsuperscript{16} indicate the importance of pituitary GH in the rapid somatic growth and metabolic change at puberty. In contrast to the development changes in the grt pituitary cells, plasma GH levels did not differ between grt and normal mice except at 48 weeks (Fig. 5A). At present, it is difficult to explain the discrepancy between the number of pituitary GH cells and circulating GH levels at each developmental stage. GH is known to be released in a pulsatile manner, particularly in young animals\textsuperscript{32}, and this might be one of the reasons for the disaccord between the plasma GH levels and GH cell population.

PRL plays a pivotal role in reproduction and increases the number of luteinizing hormone receptors and responsiveness to luteinizing hormone in mouse testis\textsuperscript{33}. Fertility is reduced in male grt mice and is associated with delayed testicular development\textsuperscript{20}. In the present study, grt mice possessed fewer PRL cells and exhibited lower plasma PRL levels than normal mice (Figs. 2–5). Targeted disruption of the PRL gene in mice partially affects reproductive development and function; PRL deficiency reduces fertility along with delayed testicular development\textsuperscript{34}. Thus, reduced fertility in grt mice at young ages, but not in subsequent stages, is considered to be due in part to insufficient PRL production.

Fig. 5. Developmental changes in the plasma GH (A), PRL (B), TSH (C) and T\textsubscript{4} (D) levels in normal and grt male mice. Data points represent the mean ± SEM of 4 to 17 mice. *Significantly different from age-matched normal mice (P<0.05).
TSH stimulates thyroid hormone synthesis and release from the thyroid gland and induces thyroidal folliculogenesis. TSH secretion is under the dual control by hypothalamic thyrotropin-releasing hormone (TRH) and thyroid hormones. An experimental study on hypothyroid animal indicates that thyroid hormones not only regulate GH synthesis and secretion of GH but also affect differentiation and proliferation of GH cells. The reduction in GH mRNA during the hypothyroidal state is accompanied by a decrease in serum GH and pituitary GH content. In grt mice, the number of PRL cells was reduced and plasma PRL levels remained low as compared with normal mice (Figs. 2 and 3). A transgene ablation experiment revealed that most PRL cells are derived from GH cells. Thus, the reduction of PRL cells in grt mice is likely to be a consequence of reduced numbers of GH cells.

It is known that TSH, PRL and GH cells are derived from a common undifferentiated pituitary transcription factor-1 (Pit-1) precursor cell. Accordingly, another explanation for the underdevelopment of GH and PRL cells in grt mice may be that the overproduction of TSH cells from Pit-1 precursor cells reduces the potency of generation of GH and PRL cells from the precursor cells. A study using thyroid hormone-deficient mice showed that thyroid hormone replacement rescues the dwarfism, prevents and reverses the hypertrophy and hyperplasia of TSH cells and normalizes the population of PRL cells and GH cells.

The anterior pituitaries of grt mice contain various cells as observed in that of normal mice, namely, PRL cells, GH cells, TSH cells, ACTH cells, GTH cells and chromophobic cells. According to Hibasami et al., chromophobic cells in grt anterior lobes show conspicuous proliferation, resulting in distinct hyperplasia. They concluded that the proliferation of these cells is secondary to the hypothyroidism and is a likely cause of the enlarged anterior lobes that are observed in grt mice. In the present experiment, we also noticed that the grt pituitary became significantly heavier than the normal pituitary (Fig. 1), and the total number of anterior pituitary cells in grt mice was significantly greater than that in normal mice (Fig. 3D). Accordingly, the reduction in the percentages of GH, PRL and TSH cells as observed after 24 weeks of age is considered to be a consequence of increased numbers of chromophobic cells.

The gene responsible for the grt mutation is Tpt2, which plays a crucial role in tyrosine sulfation of TSH receptor. Impaired tyrosine sulfation of the TSH receptor molecules may reduce responsiveness to TSH and eventually cause thyroidal hypofunction in grt mice. T3 replacement therapy restores growth retardation and serum TSH levels, indicating the importance of thyroid hormone for normalization of grt mice. The present study indicates that early-onset of growth retardation and the subsequent pubertal delay in grt mice might be associated with, at least in part, the impaired development of GH, PRL and TSH cells that accompanies primary congenital hypothyroidism.

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