Effect of neonatal thyroidectomy on growth hormone secretion in the rat

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Abstract

The influence of neonatal thyroidectomy (Tx) on GH production was investigated by means of Northern blot analysis. Tx resulted in a significant decrease in pituitary GH mRNA levels after 10, 15 and 20 days. The changes of pituitary GH mRNA were soon reflected in pituitary GH content. There was, however, no significant difference in pituitary GH mRNA levels and GH content between Tx and sham-operated rats at 5 days old. The pituitary GH cells were significantly decreased in number 15 and 20 days after Tx. These data suggest that GH mRNA is transcribed, independent of thyroid hormone, in the rat anterior pituitary gland during early neonatal life. In addition, the present study ascertained that GH dependence on thyroid hormone is acquired between the 5th and 10th day of neonatal life.

Materials and Methods

Introduction

It is known that thyroid hormone is an important regulator of growth hormone (GH) production in the rat somatotroph (Peake et al. 1973). Recent work suggests that thyroid hormone directly induces the transcription of the GH gene (Evans et al. 1982, Spindler et al. 1982, Casanova et al. 1985, Brent et al. 1991). On the other hand, previous studies demonstrated that GH dependence on thyroid hormone was not clear during the early neonatal period (Coulombe et al. 1980, Seo et al. 1981). However, the quantitative aspects of GH gene expression during neonatal hypothyroidism have not been well investigated. In the rat, serum thyroid hormone concentration is low at birth and increases to adult values in the weaning stage (Dussault & Labrie 1975). The thyroid hormone system is still immature in the neonatal period. Is thyroid hormone really essential for GH production in the neonatal rat, as in older animals? To answer this question, we investigated the influence of neonatal thyroidectomy (Tx) on GH gene expression in the rat by using Northern blot analysis. We also evaluated the amount of pituitary GH content to compare with GH mRNA levels. Additionally, we performed a morphometrical analysis of pituitary GH cells in parallel with the biochemical examinations using immunocytochemistry. Finally, we observed immunoelectron microscopic profiles of GH cells at all experimental stages in order to confirm the effects of Tx on GH cells morphologically.

RNA extraction and Northern blot analysis

Total RNA was prepared by homogenization of pituitary glands in guanidinium isothiocyanate followed by acid
phenol–chloroform extraction (Chomczynski & Sacchi 1987). RNA was ethanol precipitated, redissolved in sterile water and quantified by absorbance at 260 nm. Two µg of total RNA from one pituitary were separated on a 1% agarose gel containing formalin and transferred onto a nylon membrane (Hybond N, Amersham International plc, Amersham, Bucks, UK) by capillary action (Sambrook et al. 1989). The blot was hybridized with 32P-labeled rat GH cDNA. The rat GH cDNA insert (pRGH-1, 0.8 kb (Seeburg et al. 1977)) was excised from the pBR322/Hind III site and radiolabeled with 32P by nick translation (Rigby et al. 1977) to about 108 c.p.m./µg DNA using a commercial kit (Takara Shuzo Co. Ltd, Kyoto, Japan). The GH cDNA probe was removed, and the filters were rehybridized with human ribosomal DNA (5’ portion, pHr21Ab (Safrany et al. 1989)) to hybridize with 18S rRNA for the comparison of the amount of RNA in samples loaded on the gel between experimental groups. The human ribosomal DNA was excised from the pUC13/EcoRI site and radiolabeled as GH cDNA. Radioactivity on the filters was quantified using a Bio Imaging Analyzer BAS 2000 (Fujix, Tokyo, Japan). The ratio of the intensity of the GH mRNA band to that of the 18S rRNA band was used to express the relative amounts of mRNA present. Rat GH cDNA was provided by Dr J D Baxter of the University of California, USA. Human ribosomal DNA was provided by Health Science Research Resources Bank (Osaka, Japan).

**RIA**

The pituitary glands were homogenized in 0.05 M NaHCO3–Na2CO3 buffer (pH 10.0) and diluted with BSA-PBS. Pituitary GH content was measured by the double–antibody RIA using reagents provided by the National Hormone and Pituitary Program, Rockville, MD, USA (rat GH RIA kit, reference standard NIDDK-rGH-RP2) and Biosignal Research Center, Institute for Molecular and Cellular Regulation, Gunma University, Maebashi, Japan (second antiserum, HAC-MKA2–02GTP88). The intraassay and interassay coefficients of variation were 3.15 and 3.80% respectively. Serum thyroxine (T4) concentration was measured using a commercial kit (Eiken Co., Tokyo, Japan).

**Immunocytochemistry and morphometry**

Pituitaries were fixed in Bouin’s fluid for 24 h at 4 °C, dehydrated and embedded in paraffin. Serial frontal sections (2 µm) were mounted on glass slides and deparaffinated. They were incubated in ethanol with 3% H2O2 to inactivate endogenous peroxidase activity, and immersed in 10% normal goat serum in 0.9% NaCl–0.01 M phosphate buffer, pH 7.5, at room temperature for 30 min. Then, rabbit antibody against rat GH was applied to the sections. After overnight incubation at 37 °C, the sections were rinsed in PBS and stained by the streptavidin–biotin method using a HISTOFINE SABPO Kit (Nichirei Corp. Tokyo, Japan) with diaminobenzidine as a chromogen. Rabbit antibody against rat GH was provided by Dr K Wakabayashi of the Institute for Molecular and Cellular Regulation, Gunma University.

The sections were visualized with a microscope equipped with a CCD TV camera. Ten images were captured at random for each pituitary and analyzed with a Macintosh computer using Image 1.5 (NIH, USA; public domain). Background density points were removed by thresholding the image. The number of the cells that reacted with anti-GH antibody was counted.

For immunoelectron microscopic examination, pituitary tissues were cut into small pieces and fixed in a solution consisting of 3% paraformaldehyde and 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 2 h at room temperature and then post-fixed in 0.1% osmium tetroxide dissolved in 0.1 M cacodylate buffer for 1 h at 0 °C. After routine dehydration, the tissues were embedded in Epon–Araldite mixture. The thin sections were reacted by the protein A-gold method described previously (Bendayan & Zollinger 1983). Control immunocytochemical tests were carried out by substituting normal rabbit serum or PBS for specific antiserum. Electron microscopic observations were made with a Hitachi 7000 electron microscope.

**Data analysis**

For Northern blot analysis and RIA, experiments were reproduced six times. For immunocytochemistry and immunoelectron microscopic examination, experiments were reproduced four times. Data are expressed as mean ± s.d. Statistical analyses were performed by the Mann–Whitney U test. We considered differences significant at P<0.05.

**Results**

**Serum T4 concentrations**

Serum T4 concentrations were significantly decreased: 0.35 ± 0.11 vs 1.75 ± 0.78, 0.44 ± 0.15 vs 4.29 ± 1.99, 0.98 ± 0.49 vs 8.24 ± 2.98, 1.58 ± 0.92 vs 5.72 ± 1.24 µg/dl (n=6, P<0.005) at 5, 10, 15 and 20 days after Tx respectively.

**GH mRNA levels of Tx and sham-operated rats in the neonatal period**

In the early neonatal period, pituitary GH mRNA content was not affected by Tx. As shown in Fig. 1, no significant differences were found between Tx and sham-operated pups in the amount of pituitary GH mRNA at the 5th day.
A significant decrease was first observed at the 10th day. Tx resulted in a significant decrease of pituitary GH mRNA levels at the 10, 15 and 20th days. The amount of pituitary GH mRNA in the Tx rats decreased to levels approximately 19, 18 and 16% of the control at the 10, 15 and 20th days respectively.

**Pituitary GH mRNA and GH content**

Differences of pituitary GH content between Tx and sham-operated rats changed in the same manner when compared with pituitary GH mRNA content (Fig. 2A and B). At the 5th day, no significant differences were found between Tx and sham-operated pups in the amount of pituitary GH mRNA and GH content. However, significant decreases were observed 10, 15 and 20 days after Tx in the amount of both contents. The amount of pituitary GH mRNA contents in Tx rats decreased to levels approximately 21, 18 and 13% of the control at the 10, 15 and 20th days respectively, and the amount of pituitary GH content in Tx rats decreased to levels approximately 24, 17 and 28% of the control at the same days respectively.
Body weight

The body weights of Tx and sham-operated rats are illustrated in Fig. 3. A significant difference was first observed at the 20th day between Tx and sham-operated rats. The body weight of Tx rats decreased to a level approximately 71% of the control at the 20th day.

Morphometry

Figure 4 shows the number of pituitary GH cells in pituitary glands. At the 5th and 10th day, no significant differences were found between Tx and sham-operated pups in the number of pituitary GH cells. A significant decrease in the number of pituitary GH cells was first observed on the 15th day. The number of pituitary GH cells in the Tx rats decreased to levels approximately 60 and 61% of the control at the 15 and 20th days respectively.

Electron microscopy

The ultrastructural profiles of GH cells after Tx showed rather depressed structures. These features of GH cells became clear on the 15th day after Tx; that is the size of GH cells and the size and number of their secretory granules were reduced. The organelles of GH cells were also morphologically inactive as compared with those of sham-operated rats (Figs 5 and 6).
Discussion

The dependence of GH production on thyroid hormone has been previously demonstrated in the adult rat and in experiments using rat somatotropic cell lines (Peake et al. 1973, Hervas et al. 1975). More recent works stated that thyroid hormone acted directly on the thyroid hormone response element of the GH gene and increased its transcriptional activity (Nyborg et al. 1984, Yaffe & Samuels 1984). It is generally accepted that, in the adult rat, thyroid hormone plays an important role in the regulation of GH gene expression. However, the effect of thyroid hormone on GH in the neonatal period is controversial. Coulombe et al. (1980) and Seo et al. (1981) reported that in early neonatal life thyroid hormone deficiency did not affect the accumulation of GH when measured by RIA. Those studies suggested that the regulation of the GH gene by thyroid hormone is not established in the early neonatal period. On the other hand, Rodriguez et al. (1995a) recently showed significant decreases of GH mRNA and GH in pituitaries of hypothryroid rats at the 19th fetal day and concluded that thyroid hormone regulates GH gene at an early stage of pituitary development.

In the present study, we have demonstrated that thyroid hormone deficiency did not affect the amount of pituitary GH mRNA and pituitary GH content in early neonatal life, such as in the 5 day old. GH dependence on thyroid hormone became obvious at the 10th day. Thyroid hormone deficiency was associated with a diminution in the amount of the pituitary GH mRNA and GH content.

There may be other factors that affect GH mRNA concentrations in this study. Rodriguez et al. (1995b) showed reduction in GH mRNA in food-restricted, fasting and diabetic rats, as in thyroidectomized rats. In our study, all rats had food freely available and the changes of body weight were not obvious in Tx rats. Small reductions of gain in body weight caused by the hypothryoid state indicate that the feed intake seemed to be not so restricted as to affect the level of GH mRNA.

The hypothroid state is known to be associated with a reduction of GH cell number in adult rats (DeFesi et al. 1979). In this study, the number of GH cells was decreased at 15 days in the Tx rats. This result suggests that thyroid hormone also regulates the maturation of GH cells at the neonatal period. Changes in GH mRNA levels in the present study may be partially due to a reduction in GH cell number. However, the changes in pituitary GH mRNA and GH levels are greater than that of GH cell numbers. We also observed ultrastructural changes of the GH cells at 15 days after Tx. This morphological phenomenon indicates that Tx clearly affected cellular profiles of GH cells, and was correlated with biological results.

Serum T4 concentrations in Tx rats were significantly decreased in all experimental periods. However, there were slight rises in serum T3 concentrations at the 15th and 20th days. Although we verified the absence of remaining thyroid glands at autopsy in Tx rats, it may be possible that residual minute thyroid tissue occasionally remaining unexpectedly after the operation regenerated and produced thyroid hormone at the 15th and 20th days.

The first 3 weeks of life in the rat are characterized by the maturation of the thyroid hormone system (Walker et al. 1977). Serum thyroid hormone concentrations are low at birth and increase rapidly to weaning, when they reach adult values (Dussault & Labrie 1975). In this study thyroid hormone replacement controls were not included. Hypothyroidism during development has a number of physiological effects including the failure to thrive. In turn, this may have an effect on the GH axis. This possibility cannot be entirely excluded. Consequently the effects of the thyroid hormone on GH production were not established in the early neonatal period, such as in the 5 day old.

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Effects of single small doses of \(\text{L-thyroxine}\) and \(\text{triiodo-L-thyronine}\) on growth hormone, as studied in the rat by radioimmunoassay.


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