Screening and DNA Methylation of Growth Hormone in Thyroidectomies Rats

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Abstract

The goal of this research concerns with the methylation role for DNA of thyroidectomized lab rat. Thyroidectomy operation was applies on 40 lab rats. After 60 days of operation, these rats were injected with single dose/day of both GHL-T (2.5 mg/100 g Sc) and monkey anti-rGH serum (0.3 ml,ip). Deoxyribonucleic acid methylation of hypothalamic growth hormone gene expression was applied for lab animals subsequently by using northern blot analysis technique. The results of this experiment revealed significant correlation between thyroidectomy and decline in both thyroxine and thyroidiodotherionine values ($p< 0.05$) that's affect GH value. Conclusion of this study proves that deoxyribonucleic acid methylation suppressing the feedback of growth hormone releasing hormone GRH to prevent physiological stress in thyroidectomized rats.

Keywords: GH, thyroidectomy, DNA methylation, Growth hormone receptors, GHR gene, GH rats.

تقصي ومثيلة الحمض النووي الرايبي منقوص الاوكسجين لهرمون النمو في الفئران التي أزيلت منها الغدة الدرقية

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الخلاصة

الهدف من هذا البحث يتعلق بدور مثيلة الحمض النووي للجرذان المختبرية مستأصلة الغدة الدرقية. حيث تم تطبيق عملية استئصال الغدة الدرقية على 40 جرذا مختبريا. وبعد 60 يوما من العملية، تم حقن هذه الجرذان بجرعة واحدة/ يوم من كل من هرمون النمو طويل الامد (2.5 ملغ/ 100 غرام، تحت الجلد) ومصل مضادات هرمون النمو الطبقي القردي (0.3 مل، تحت البريتون). تم تطبيق مثيلة حمض دنا من التعبير الجيني لهرمون النمو تحت المهاد لدى الحيوانات المختبرية في وقت لاحق باستخدام تقنية تحليل اللطخة الشمالية. كشفت نتائج هذه التجربة عن ارتباط كبير بين استئصال الغدة الدرقية والانخفاض في كل من الغدة الدرقية وقيم الغدة الدرقية (احتمالية< 0.05) التي تؤثر على قيمة هرمون النمو.

استنتج في هذه الدراسة أن مثيلة حمض دنا كبح ردود الفعل من هرمون النمو لأجل اطلاق هذا الهرمون منعا للإجهاد الفسيولوجي في الجرذان مستأصلة الغدة الدرقية.

الكلمات المفتاحية: هرمون النمو، إقتطاع الغدة الدرقية، مثيلة دنا، مستقبلات هرمون النمو، مورثة هرمون النمو، هرمون نمو الجرذان.

Introduction

A thyroidectomy in rats is a surgical operation that includes removal of all or part of the thyroid gland. Thyroidectomised rats showed a marked elevation in both GH and TSH mRNAs. Results suggest that GH and TSH gene expression are modulated by metabolic and/or endocrine changes accompanying stressful events [1, 2]. Researcheres explain that at both pathological and physiological concentrations in serum, thyroid hormone acts as an inhibitory modulator of GRF secretion, probably not involving a feedback mechanism through GH [3, 4]. This biphasic effect of thyroid hormone on pituitary GH values seems to derive from the contrast in primary select tissues of hyper- and hypothyroidism, the hypothalamus and the pituitary, per capita. It establish that elevated GRH mRNA levels, elevated GRH secretion, and declined GRH levels after thyroidectomy are provoked by the GH deficiency put on by thyroid hormone depletion, rather than a lead effect of thyroid hormone on the hypothalamus [5]. These alterations are stable with those looked in other models of GH deficiency and further support the role of GH as a normal negative feedback coordinator in vivo of GRH gene expression [6,7].

In different thyroidectomy cases, some gene promoter's islands acquire abnormal methylation, which results in transcriptional silencing. Alterations of DNA methylation have been recognized as an important component after thyroidectomy surgery [8, 9,10].

Hypomethylation, in general, arises earlier and is linked to chromosomal instability and loss of imprinting, whereas hypermethylation is associated with promoters and can arise secondary to gene (oncogene suppressor) silencing [11,12].

Aim of this research paper is that in the rat, GH gene expression is highly dependent on thyroid hormones. Because of the rapidity of the response, the effect is probably mediated by a transcriptional mechanism.

Materials and Methods

A total of 40 Sprague Dawley rat, ages 10 weeks and weighing approximately 100 g, were purchased from the Charles River Laboratories. The rats were housed in good conditions cage accordance with the Animal Care and Use Guidelines of the Institutional Animal Care and Use Committee. Animals were anesthetized and placed in the supine position, then operations were carried out under aseptic conditions.

Standard procedures were used for thyroidectomy, sham (euthyroid/control)-operated animals underwent the same surgical procedures without removal of the thyroid gland. Rats were used for experimentation 60 days after surgery. Rats were injected once daily with rat GH L-T< (2.5 Mg/100 g, sc), monkey anti-rGH serum (0.3 ml, ip).

Tissue Extraction and RIA were applied after removing anterior pituitary glands and hypothalami and extraction done immediately. The extracts were assayed for GRH content by RIA, as previously described [14], and the results were expressed as nanograms of GRH per hypothalamus.

RNA Extraction and Northern Blot Hybridization: total RNA was isolated from individual hypothalami by homogenization in 0.8 ml guanidinium-phenol solution followed by extraction with 0.1 vol chloroform and precipitation of the aqueous phase with an equal volume of isopropanol. Electrophoresis was performed where RNA isolated from a single hypothalamus, this RNA was transferred to a Nytran membrane (Schleicher and Schuell, Keene, NH) and hybridized with a 510-basepair 32P-labeled rat GRH cDNA probe (0.6 ng/ml; SA, 109 cpm//ig) for 24-48 h at 62 C, as previously described [4,7]. After autoradiography at -70 C for 24-72 h, quantification of the

hybridization signal was performed on a scanning densitometer (model EC910, E-C Apparatus Corp., St. Petersburg, FL).

Statistical Analysis for indices associated with parameters determined by single factor analysis of variance and Duncan's new multiple range test [13].

Results

The time course of the effect of thyroidectomy on hypothalamic GRH content and GRH mRNA levels is shown in Figure 1. Thyroidectomy resulted in a progressive decrease in GRH content (Figure 2), which was significant ($P < 0.05$) by 2 weeks.

The autoradiogram of the hypothalamic GRH mRNA hybridization signal from one of the thyroidectomy time studies (Figure 1) is shown only a single band of approximately 750 bases was generally detected with the rGRH cDNA probe, which is consistent with the reported size of the final GRH gene transcript. Occasionally, in hypothalamic RNA extracts from thyroidectomized rats, several larger forms of RNA also hybridized to the rat GRH cDNA probe. Whether these bands represent incompletely processed GRH mRNA transcripts or nonspecific hybridization is unknown. The additional treatment of 6-week thyroidectomized rats with antirat GH (anti-rGH) serum for 3 days resulted in a further elevation of GRH mRNA levels ($P < 0.01$ vs. 6-week thyroidectomized rats alone) despite the prior state of severe GH deficiency.

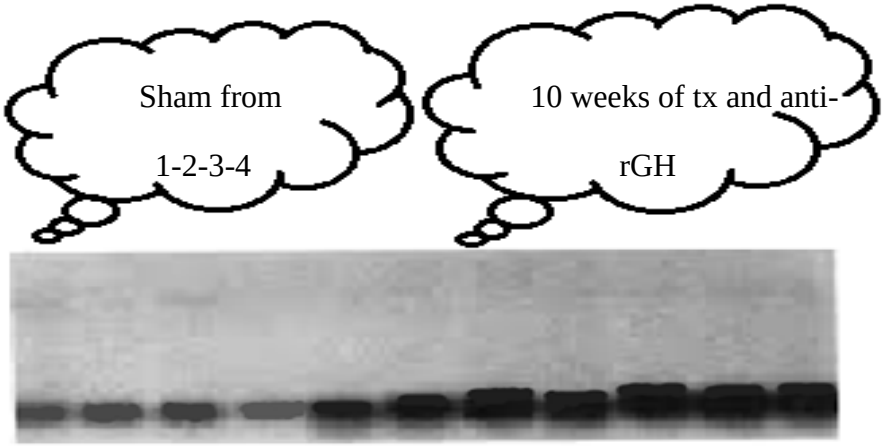

Figure (1): Effect of Thyroidectomy on Time of Hypothalamic GR mRNA

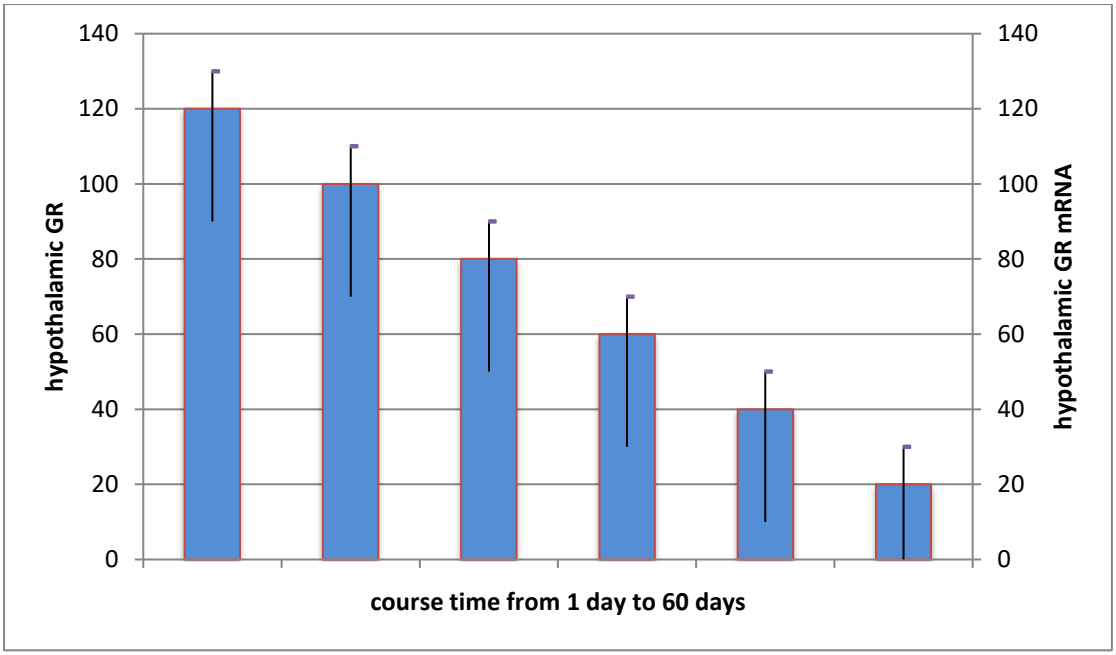

Figure (2): Thyroidectomy Effect on Hypothalamic GR Content and Hypothalamic GR mRNA Levels by Course Time

Discussion

Thyroid hormone deficiency is associated with a marked impairment of spontaneous GH secretion, GH responses to various stimuli and growth in man and rodents. In the rat, these changes are coupled with a severe depletion in pituitary GH content. Thyroid hormone replacement restores GH content and secretion, primarily through a direct action of thyroid hormone to stimulate *GH gene* expression at the transcriptional level [13,14] Furthermore, we have previously shown that thyroid hormone deficiency in the rat results in a decrease in the sensitivity (ED50) of the somatotroph to GRH and an impaired accumulation of cAMP in response to GRH, in addition to the reduction in hypothalamic GRH content. Treatment of hypothyroid animals with T4 completely normalized hypothalamic GRH content and the previously absent spontaneous GH secretory bursts despite a pituitary GH content only one third that of controls [15, 16]. We interpreted these results as suggesting that thyroid hormone, in addition to stimulating GH synthesis, was acting at the hypothalamus to regulate GRH synthesis and secretion, leading to restoration of normal GH secretion [17, 18].

More previous data, however, indicate that GH exerts the primary feedback control in the regulation of GRH gene expression. Initially, a dramatic decrease in hypothalamic GRH content after hypophysectomy in the rat was reported by us and others, which paralleled our results in the thyroidectomized rat [19,20]. In contrast, however, T4 replacement in hypophysectomized rats had no effect on GRH content, although the addition of GH treatment resulted in a partial restoration of GRH levels. We subsequently showed that the decrease in GRH content after hypophysectomy was coupled with a reciprocal time-dependent increase in hypothalamic GRH mRNA levels and a transient increase in GRH secretion [21]. These changes were partially reversed or prevented by GH treatment alone, while the addition of T4, glucocorticoid, and gonadal steroid therapy had no further influence on the restoration of GRH gene expression induced by GH. Other recent evidence using *in situ* hybridization techniques has revealed not only an increased quantity of GRH mRNA per cell in the hypothalamic arcuate nucleus of hypophysectomized rats, but also an increase in the number of GRH-producing neurons in the ventromedial hypothalamus [22,23]. Both changes were partially reversed by GH treatment. Furthermore, increased levels of hypothalamic GRH mRNA and decreased GRH content have been observed in two dwarf animal models with isolated GH deficiency of different etiologies: the lit/lit mouse and the dw/dw rat. Lastly, GH excess has now been shown to decrease hypothalamic GRH content and secretion as well as GRH mRNA levels [24, 25].

The results of the present studies indicate that the changes in GRH gene expression in the rat after thyroidectomy paralleled those observed after hypophysectomy and were not a primary consequence of thyroid hormone deficiency, but were due to the GH deficiency that occurred as a result of thyroidectomy [26]. The increased levels of GRH mRNA after thyroidectomy were restored to normal by GH treatment alone despite persistent thyroid hormone deficiency. The apparent increase in the effectiveness of this reversal by GH compared to the results of T4 treatment was probably due to the length of the hormone replacement period [27]. A 5-day treatment regimen was chosen, since the pattern of spontaneous GH secretion is normalized by T4 treatment within this period. However, unlike in the GH-treated group, the time required for T4to stimulate GH synthesis would delay the effects on GRH gene expression in the T4-treated group. Based on previously published data, approximately

12-24 h would be required after T4 injection before a significant increase in plasma GH could be detected with the dose of T4 used in our study [28].

A significant increase in GRH mRNA was observed by 1 week after thy roidectomy, which was associated with an obvious depletion in pituitary GH content. Although previous data from our laboratory indicated that GRH mRNA levels were not elevated at 1 week after thyroidectomy, pituitary GH content showed only a slight depletion at 1 week in this study [29]. Coupled with the sensitivity of the GH feedback regulatory mechanisms, as demonstrated by the effectiveness of anti rGH serum in further elevating GRH mRNA levels in 6-week thyroidectomized rats, it seems likely that the difference between the two experiments was related to the extent of pituitary GH depletion [30].

Taken together, the results of these experiments and others have provided evidence that GH exerts a negative feedback control on hypothalamic GRH gene expression, primarily at the level of transcript accumulation. However, although classic alterations have been demonstrated after GH excess (decreases in GRH mRNA, GRH content, and GRH secretion), the changes in GRH gene expression in states of GH deficiency have been less predictable. Increased levels of GRH mRNA are associated with reduced GRH content, which, although seemingly inconsistent with the concept of negative feedback control, appears to be a consequence of an increase in GRH secretion [31,32]. The release of GRH from incubated hypothalami was clearly elevated by 2 weeks after thyroidectomy. In the hypophysectomized rat, GRH secretion was initially augmented, but decreased below control values by 2 weeks. Whether these differences in GRH secretion are related to the extent of the depletion of hypothalamic GRH content in the long term thyroidectomized rat (45-50%) and the long term hypophysectomized rat (70-75%) or to other factors is not known. However, the ability of anti-GH serum to further increase GRH mRNA levels in long term thyroidectomized rats suggests that the small quantity of GH remaining in these animals can still influence GRH gene expression [33]. Moreover, in the thyroidectomized rat, GRH mRNA levels were decreased to normal by GH therapy, and GRH content was fully restored by T4 treatment. Neither of these parameters of GRH gene expression returned to normal in the hypophysectomized rat, however, suggesting that other pituitary or pituitary-dependent factor(s) may be required together with GH for efficient processing of GRH at a posttranscriptional step [34].

Whether the effects of GH on GRH gene expression are regulated by a direct action of GH itself on GRH producing perikarya, by an effect of GH on an intermediary hormone(s) such as somatostatin (SRIH) or insulin-like growth factor-l (IGF-I), or by a combination of these events is unknown. Both peripheral and intracerebroventricular injections of GH block normal spontaneous GH secretion and appear to involve not only an inhibition of GRH secretion, but an increase in SRIH release as well [35]. GH has been shown to stimulate the synthesis and secretion of SRIH in several different systems. Moreover, hypothalamic SRIH mRNA levels, SRIH content, and SRIH release are reduced in hypophysectomized rats, and SRIH content and secretion are decreased in thyroidectomized rats. The reduction in SRIH mRNA and content is confined to SRIH-producing regions of the hypothalamus involved in the regulation of GH secretion in hypophysectomized rats and coincides with increases in hypothalamic GRH gene expression. Destruction of SRIH-producing perikarya in the medial preoptic area of the hypothalamus or passive neutralization of SRIH with anti-SRIH serum also results in an elevation of GH and GRH secretion [36,37]. Furthermore, intracerebroventricular injection of IGF-I inhibits spontaneous GH secretion, and IGF-I has been shown to increase SRIH secretion and decrease GRH release in vitro [38, 39].

In summary, the present study demonstrate that the increased GRH mRNA levels, increased GRH secretion, and decreased GRH content after thyroidectomy are caused by the GH deficiency produced by thyroid hormone depletion, rather than a direct effect of thyroid hormone on the hypothalamus. These changes are consistent with those observed in other models of GH deficiency and further support the role of GH as a physiological negative feedback regulator in vivo of GRH gene expression.

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