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Genetic probability related to growth proceed thyroidectomy in lab mice

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Abstract:
To examine the molecular basis for the decreased pituitary growth hormone (GH) and thyrotropin (TSH) content after thyroidectomy, we measured steady-state levels of mRNA for TSH-α TSH-β and GH in the pituitary from normal mice. Pituitary mRNA was quantified by Northern blot hybridization with cDNA probes specific for mouse TSH-α, TSH-β and GH. Although changes in the pituitary GH mRNA being obvious after or thyroidectomy, GH mRNA levels in mice (> 5%) were less in the other experimental groups ($p < 0.001$). Pituitaries from mice also contained less GH mRNA ($p < 0.05$). Thyroidectomy resulted in a marked increase in both TSH-β and TSH-α mRNAs, the changes in TSH-β mRNA being greater than those in TSH-α mRNA. These data suggest that GH, TSH-α and TSH-β gene expression are modulated by metabolic and/or endocrine changes accompanying restricted feeding, fasting and diabetes as well as thyroidectomy.

Keywords: thyroidectomy, GH, TSH, C57BL/6 mouse.

INTRODUCTION:

A genetic disorder is a case that is provoked by a break, or mutation, in an personal's DNA arrangement. It is an illness occurred mostly by alterations in DNA. These mutations events perhaps considering an failure in DNA replication or because environmental agents, such as smoking cigarette and hazard to any radiation type, which in turn lead to shifts in the DNA sequence[1].

The man's genome is being able to change. These alterations may influence the nitrogenous bases (A, C, G or T) or satisfying bigger masses of DNA or uniform chromosomes. Man DNA administers the code for producing proteins, the molecules that implement most of the tasks in our body. However, when a piece of individual DNA is corrupted in some way, the manufactured protein it codes for is also afflicted and may no longer be easy to execute its normal function. Providing where these altered mutations exist, they commit have no effect or limited, or may thoroughly shift the cell biology in a man's body, developing in a genetic defect or disorder[2].

Genetic disorders organized into three star classes according to genetics and biological scientist's recommendations in their literatures and researches[3]: Single gene disorders; Chromosome disorders and Multifactorial disorders.

Thyroidectomy is the cutout of the thyroid gland as a part or whole. Since thyroid is a butterfly-shaped structure occupying the base of the neck, it secretes a type of hormones that administer cells metabolism. Thyroidectomy is applied to recover thyroid disorders, such as cancer, noncancerous augmentation-enlargement of the thyroid (as called goiter) and high-strung thyroid (as called hyperthyroidism). How much of the thyroid gland is cut out during thyroidectomy operation depends on the reason for that. If only a chunk is detached (as called partial thyroidectomy), thyroid may be having an innate capacity for acting normally after surgery. If entire thyroid is removed (as called total thyroidectomy), subject require daily hospitalization with thyroid hormone to replace thyroid natural physiology[4].

Thyroid surgery has absolutely proceeded a interminable and arduous way through the different subsequent ages. From an operation procedure which was once regarded predestined for damn failure and even destruction death to the present times when various techniques are being applied an soon tried to make the incision or carving as small or limited as it possible, this abbreviate draft of the history of thyroid surgery explains how apparently insurmountable difficulties were bridged by brave pioneers of surgery and how they developed and advanced the surgical treatment of the thyroid from the far dark ages and guided in the modernized era[5].

C57BL/6 mouse strain was familiary used inbred for tested as a general goal strain, it was examined for the generation of congenics giving rise both spontaneous and induced genetic mutations. C57BL/6J was the DNA source for the prime extreme quality blueprint sequence of the mouse genome[6].

The major aim of this experiment is to explaining the genetic probability of thyroidectomised laboratory mice as a direct proportional relationship.

MATERIALS AND METHODS:

A total of 13 mice C57BL/6, ages 8 weeks and weighing approximately 19 g, were purchased from the Jackson laboratory. The mice were housed individually ventilated and pathogen-free conditions cage accordance with the Animal Care and Use Guidelines of the National Cancer Center, Institute, and Hospital under the protocol approved by the Institutional Animal Care and Use Committee. Animals were anesthetized by using 2% isoflurane in 100% oxygen through nose-cone masks. All animals were placed in the supine position with the neck elevated by rolled-up sterilized gauze. Operations were carried out under aseptic conditions[7].

Standard procedures were used for thyroidectomy. Briefly, a midline skin incision was made along the length of the neck. The underlying tissues were removed, and the salivary glands were retracted laterally. The two halves of the sternohyoid muscle were separated and retracted laterally. The thyroid muscle was separated from the lobes of the thyroid gland and retracted along with the sternohyoid muscle. A midline cut was made in the isthmus, and the thyroid glands were excised bilaterally. Extreme care was taken not to damage the laryngeal nerve. Sham (euthyroid/control)-operated animals underwent the same surgical procedures without removal of the thyroid gland. Mice were used for experimentation 30 and 72 days after surgery[8].

Blood samples were obtained from a separate group of adult mice by submandibular bleeding, according to a previously described technique. The volume of each sample varied between 150 µL and 200 µL. All samples were stored at -20°C until analysis. DNA from samples of blood the ultraclean DNA BloodSpin Kit (MoBio Laboratories, Carlsbad, CA, USA) was used to isolate DNA from blood. The amounts of DNA isolated from the various samples were determined by spectrophotometry with the NanoDrop ND-1000 system (NanoDrop Technologies, Inc., Wilmington, DE, USA). The purity of DNA was also determined spectrophotometrically from the ratio of absorbance at 260 and 280 nm (A_{260}/A_{280}). A ratio of approximately 1.8 was accepted as evidence of the purity of DNA. The integrity of isolated DNA was assessed by electrophoresis in 0.5 ×or 1x Tris-Borate-EDTA buffer (TBE: Gibco-Invitrogen, Grand Island, NY, USA) in an agarose gel (1%) (Pronadisa, Madrid, Spain), that contained 3 µg/mL ethidium bromide. Amplification of DNA was evaluated by PCR directed towards amplification of a mouse housekeeping gene. We focused on a 140-bp sequence of the *k-ras* gene, using the forward primer

5′CTGCCGTCCTTTACAAGCGCA-3′ and the reverse primer 5′CCTGTGGTGGTTGGAAGCTGG-3′ [9,10].

Statistical Analysis

Indices associated with thyroid's and growth hormones were compared using Student's *t*-test. *P* value less than 0.05 was assumed to be significant[11].

RESULTS:

Subtractive Hybridization

In pilot studies looking for clones of mRNAs whose expression was enriched in normal compared to hypothyroid mouse brain poly(A)+ RNA by using the method of subtractive hybridization, as implemented by Travis and Sutcliffe [12]. Two clones, F4 and E2, that, after secondary screening and Northern blot analysis proved to be decreased 2-fold in abundance in hypothyroid brain. We screened a panel of clones of known brain mRNAs to survey the extent to which thyroid hormone affects mRNA concentrations.

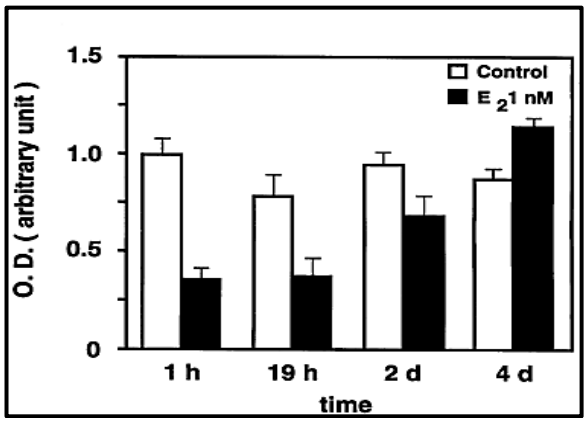

Fig.1: Time course of the effect of E2 on Nip2 expression in brain cells.

Differential Screening of Selected Brain Genes

Some of the clones tested gave a similar signal with both probes, representing mRNAs that were not dependent on the thyroidal state. Among these, NSE, cholecystokinin, somatostatin, Na+-K+-ATPase, actin, MAPs 1 and 2, neurofilament NF-68, and some of the brain-specific genes, such as pMC2A1,1A75,1B1075, 1B426B, pMC7D6 and pCD30. Other sequences gave lower signals with the hypothyroid probe, suggesting that the corresponding mRNAs were less abundant in the hypothyroidal state. These included the myelin mRNAs PLP and MAG, carbonic anhydrase type II mRNA, and the brain-specific mRNA RC3. In addition, there was a slight decrease in the mRNAs coding for the tubulins. A few mRNAs seemed to be increased in hypothyroid brains, for example *Jun*, *p53*, or pMC5B3, but they were not studied further.

Northern Blot Analysis of Brain mRNAs Using Selected cDNA Clones as Probes

To confirm and extend the above observations that neonatal hypothyroidism results in an altered expression of some brain genes, Northern blot analysis was performed on mRNA prepared from normal and hypothyroid mose brains using cDNA probes corresponding to some of the mRNAs examined above, and some additional cDNA clones. For the neuronal genes, there was no difference in the expression of NSE and Tau. There were slight decreases in NGF and MAP-2 mRNAs in the hypothyroid samples and a slight increase in N-CAM mRNA. In contrast, the expression of the neuronal mRNA RC3 was decreased 2-3 times in hypothyroid brain. For the astrocyte mRNAs there were no changes in GFAP mRNA, whereas GS mRNA increased slightly. In contrast to the effects on neuronal and astrocytic mRNAs, the expression of oligodendrocytic mRNAs for MAG, PLP, MBP, CAII, TfR, and GPDH were all decreased by a factor of 2-3.

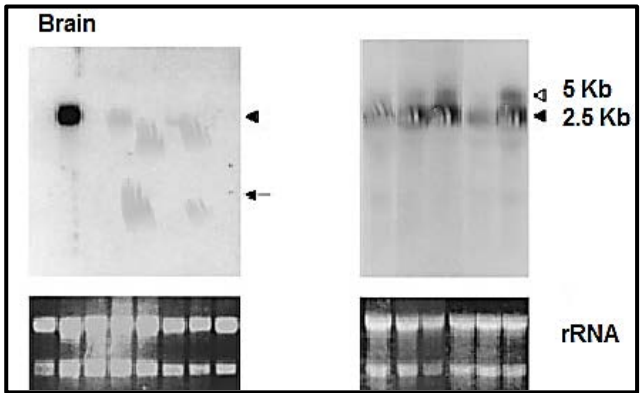

Fig-2. Northern Blot Analysis of Brain mRNAs in mice.

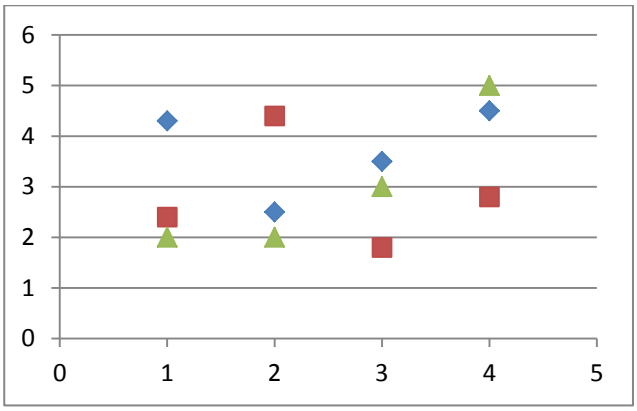

Fig. 7. Quantitative comparison of gene expression data obtained from Northern blotting analysis and microarray analysis. In the histograms, the Northern blotting data (blue) were plotted side by side with the microarray data (red) to directly compare the expression levels of the same nine significant genes. The values of the correlation coefficient (R values) for each comparison were indicated in each panel.

DISCUSSION:

Thyroid hormone deficiency is associated with a severe depletion in pituitary GH content in mice[12]. 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. Furthermore, this study showed that thyroid hormone deficiency in the mouse results in a decrease in the sensitivity (ED 50) of the somatotroph to GRH and an impaired accumulation of cAMP in response to GRH, in addition to the reduction in hypothalamic GRH content[13]. More recent 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 mouse was reported by us and others, which paralleled our results in the thyroidectomized mouse. In contrast, however, T4 replacement in hypophysectomized mice had no effect on GRH content, although the addition of GH treatment resulted in a partial restoration of GRH levels. This study 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. 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 mice, but also an increase in the number of GRH-producing neurons in the ventromedial hypothalamus. 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 mouse [14]. Lastly, GH excess has now been shown to decrease hypothalamic GRH content and secretion as well as GRH mRNA levels.

The results of the present studies indicate that the changes in GRH gene expression in the mouse 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. 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. A 5-day treatment regimen was chosen, since the pattern of spontaneous GH secretion is normalized by T4 treatment within this period [15]. However, unlike in the GH-treated group, the time required for T4 to 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. A significant increase in GRH mRNA was observed by 1 week after thyroidectomy, which was associated with a 78% depletion in pituitary GH content. Although previous data from our laboratory indicated that GRH mRNA levels were not elevated 1week after thyroidectomy, pituitary GH content showed only a 54% depletion at 1week in that study. 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 mice, it seems likely that the difference between the two experiments was related to the extent of pituitary GH depletion.

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. 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 [16]. Whether these differences in GRH secretion are related to the extent of the depletion of hypothalamic GRH content in the long term thyroidectomized mouse (45-50%) and the long term hypophysectomized mouse (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 mice suggests that the small quantity of GH remaining in these animals can still influence GRH gene expression. Moreover, in the thyroidectomized rat, GRH mRNA levels were decreased to normal by GH therapy, and GRH content was fully restored by T4 treatment. 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 [17]. 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 mice, and SRIH content and secretion are decreased in thyroidectomized mice. 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 mice 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. 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 [18].

In summary, the present studies 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.

The dependence of GH production on thyroid hormone has been previously demonstrated in the adult mouse and in experiments using mouse somatotropic cell lines [18]. More recent works stated that thyroid hormone acted directly on the thyroid hormone response element of the GH gene and increased its transcriptional activity. 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. [19] 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, [20] recently showed significant decreases of GH mRNA and GH in pituitaries of hypothyroid mice 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. [21] showed reduction in GH mRNA in food-restricted, fasting and diabetic mice, as in thyroidectomized mice. In our study, all mice had food freely available and the changes of body weight were not obvious in Tx mice. Small reductions of gain in body weight caused by the hypothyroid state indicate that the feed intake seemed to be not so restricted as to affect the level of GH mRNA. The hypothyroid state is known to be associated with a reduction of GH cell number in adult mice [22]. In this study, the number of GH cells was decreased at 15 days in the Tx mice. 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 mice were significantly decreased in all experimental periods. However, there were slight rises in serum T4 concentrations at the 15th and 20th days. Although we verified the absence of remaining thyroid glands at autopsy in Tx mice, 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 mouse are characterized by the maturation of the thyroid hormone system. Serum thyroid hormone concentrations are low at birth and increase rapidly to weaning, when they reach adult values[23].

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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