SUPPLEMENT

(AUTHOR'S PUBLICATIONS)
Thyroid dysfunction status with special reference to polymorphism of thyroid peroxidase (TPO) gene

AUTHOR'S PUBLICATION


Thyroid dysfunction and its effect on testis in rat

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Thyroid dysfunction may have important role in testicular activity in mammals. We aimed to understand the role of thyroid hormones in sperm maturation in rat. Methimazole induced hypothyroid rat exhibits significant changes in testis. Thyroid hormones modulate the sperm production and maturation leading perhaps to infertility in mammal.

Key words: Thyroid, methimazole, sperm maturation, testis, rat

Introduction

The thyroid gland secretes sufficient amount of thyroid hormones, primarily 3,5,3'5'-l-tetraiodothyronine (T4), and a lesser quantity of 3,5,3'-l-triiodothyronine (T3). These hormones promote normal growth and development and regulate a number of homeostatic functions in the body (Fujita, 1988). Iodine deficiency has been found to enhance the conditions like hypothyroidism, which in turn results in a generalized slowing down of metabolic processes associated with abortion, stillbirth, higher infant mortality, congenital anomalies, retarded growth, endemic goitre and endemic cretinism (Lal et al., 1996).

In recent times, thyroid diseases are being diagnosed very common and their relation with reproductive failure has been the topic of intense investigation. Ovulation may be impaired in both hypo- and hyperthyroidism (Armada-Dias et al., 2001; Davis, 1999). Some studies revealed that neonatal onset of hypothyroidism adversely affect Leydig cell proliferation and regeneration along with impaired steroidogenesis (Ariyaratne et al., 2000). A significant decrease in sperm motility also observed in hypothyroid men (Hudson and Edward, 1992). The proposed work is aimed to understand the histological changes of testis in rat under experimentally induced hypo-and hyperthyroid condition. Therefore, the work will help to diagnose those human reproductive disorders, which may have relation with thyroid dysfunction directly or indirectly.

Materials and methods

The laboratory experiments were performed using rat (Rattus rattus) as
Figure 1. Plasma concentrations (ng/dL) of $T_3$ (A) and $T_4$ (B) in hypothyroid and hyperthyroid rats. Values are expressed as Mean ± SEM.
mammalian model. Our experiment included oral feeding of rat with Methimazole for induction of hypothyroidism and oral feeding of Thyroxine (Thyroxine sodium) for hyperthyroidism. Normal young adult rat aged 8-10 weeks and weight 100-110 g were housed in polypropylene cages and were acclimatized in laboratory condition for a week with natural light and dark schedules prior to experimentation. The animals were fed standard rodent diet and water was provided ad libitum. They were divided into hypothyroid, hyperthyroid and their respective control groups. Group I animals were treated with Methimazole 20 mg/kg body weight/day for 14 days. Group II animals were treated with thyroxine 600 mg/kg body weight/day for 14 days. Before autopsy, Rats were anesthetized with chloroform. Blood was drawn directly from heart for serum T3, T4 hormones. Histological analysis of thyroid and testis were also done for comparison. T3 or T4 concentrations in rat serum were determined by radioimmunoassay (RIA). 125I-labeled thyroid hormone (either T3 or T4) competes with thyroid hormone in the serum sample and for antibody sites on the tube, in the presence of blocking agents for thyroid hormone binding proteins.

Results

In the present study the rats were induced experimentally to be hypo- and hyperthyroid. The results showed significant changes in T3, T4 (Figures 1A, B) hormones and histology of testis (Figures 2A, B). The serum T3 and T4 level was significantly reduced in hypothyroid rat whereas in contrast the level significantly increased in hyperthyroid rat as expected and confirming that they were indeed in a hypo- and hyperthyroid state. Significant changes were observed in the cytology of hypothyroid testis group when compared with the control group (Figures 2A, B). General morphology of the seminiferous tubules was altered in hypothyroid rat. The tubules were shrunken, reduced in size with very less number of mature sperm in the reduced lumen. The normal process of spermatogenesis seemed to be entirely disrupted. Sertoli cells were immature and highly compressed. Leydig cells were atrophied and delimited from the seminiferous tubules. The individual boundary of the spermatogonial cells was not maintained. Sertoli cells also lost their normal morphology and position. Furthermore, these cells demonstrated late development. But no significant changes were observed in the hyperthyroid induced rats.

Discussion

In the present investigation on Methimazole treatment forwarding hypothyroid and thyroxine to induce hyperthyroid like conditions are reflected in the serum T3, T4 levels (Pantos et al., 2005; Grofte et al., 1997). Methimazole induced hypothyroidism adversely affects the normal process of spermatogenesis and inhibits the maturational events like seminiferous tubule canalization (Holsberger et al., 2005). Hypothyroidism significantly reduced seminiferous tubule and lumen diameter. Control rats showed active spermatogenesis whereas in hypothyroid rats, the proliferation
Figure 2A. Normal Rat testis x400

Figure 2B. Methimazole treated Rat testis x 400
and differentiation of germ cells were arrested and their number was decreased. The present study clearly indicates that hypothyroidism adversely affects spermatogenesis. Their effect may be either direct or indirect.

In the present experiment, size, shape, maturation, and localization of Sertoli cells were significantly changed. Previous research had revealed that thyroid hormone exerts its effect on Sertoli cell maturation via thyroid hormone receptor (TR) (Holsberger and Cooke, 2005; Maran, 2003). The changes of Sertoli cells in hypothyroid groups of rats might be due to unavailability of thyroid hormones.

The hypothyroid condition significantly affects the Leydig cells. The results also indicate that thyroid hormones may have direct regulation on Leydig cell functions. It may be concluded that hypothyroidism is deleterious for testicular growth and spermatogenesis. Sperm production and maturation is directly or indirectly related to the thyroid function possibly leading to infertility in male rats. The observations from the model animal will help us to understand those human diseases having direct or indirect relation with thyroid dysfunction.

Literature Cited


Thyroid dysfunction modulates glucoregulatory mechanism in rat

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The role of the thyroid gland in glucose homeostasis remains incompletely understood. To get a better insight hypothyroid and hyperthyroid conditions were experimentally induced in rat and found severe defects in glucose homeostasis. While blood glucose level returned to normal level after 2.5 hr of oral glucose challenge in control rats the blood glucose level remained high even after 24 hr of glucose load in both hypo- and hyperthyroid rats. These experimentally manipulated rats displayed higher levels of liver glycogen (10.45-22.8-fold) and serum glutamic pyruvic transaminase (1.48-9.8-fold). Liver histology of hyperthyroid treated rats revealed hepatotoxicity. From the results it can be concluded that thyroid gland plays an important role in glucose homeostasis.

Keywords: Blood glucose, Glycogen, Hyperthyroid, Hypothyroid Liver

Thyroid hormones (primarily 3,5,3'-5'-l-tetraiodothyronine (T4), and to a lesser extent 3,5,3'-l-triiodothyronine (T3)) regulate a variety of biochemical reactions in virtually all tissues. These hormones are known as important factors in gene regulation in tissues such as brain, liver, muscles and adipose tissue. They are involved in the control of resting metabolism. Thyroid hormone status is also important for glucose homeostasis. In thyrotoxic subjects, glucose turnover and oxidation rates are increased, whereas non-oxidative glucose turnover is unchanged or decreased or increased. In contrast, glucose production is decreased in hypothyroidism. Impaired glucose tolerance is a frequent complication of hyperthyroidism. This alteration changes both insulin secretion and degradation in humans. Impairment in the insulin-induced suppression of glucose production in hyperthyroid patients has been reported. At low insulin levels, insulin-stimulated glucose disposal is usually unaffected, whereas it has been reported to be decreased, unchanged or even increased at high insulin levels. In addition, thyroid hormones also blunt the insulin-induced increase in the total distribution volume of the exchanging pool of glucose, possibly by accelerating intracellular glucose degradation.

However, the role of thyroid gland in glucose homeostasis has remained elusive. Therefore, to uncover this complex interaction an attempt has been made by generating hypothyroidism (by treatment with methimazole) and hyperthyroidism (by treatment with thyroxine) in rats to study the effects on glucose levels during oral glucose tolerance test and changes in serum glutamic pyruvic transaminase (SGPT) have been examined. In addition, morphological and biochemical changes in liver have been observed. Higher glucose levels were noted in both hypo- and hyperthyroid rats in response to oral glucose tolerance test, higher liver glycogen content, and elevated levels of SGPT. In addition, profound alteration in liver histology were observed. These findings may help to better understand the complex relationship between thyroid dysfunction and glucose homeostasis.

Materials and Methods
Young adult rats, Rattus rattus (8-10 weeks: 70-80 g) were housed in polypropylene cages and were acclimatized in the laboratory condition for a week with standard food and water in natural light and dark schedules. Rats were divided into following 4 groups: group I (for hypothyroid studies), group II (for hyperthyroid studies) and their respective control groups. While Group I animals were treated with Methimazole (20 mg/kg body weight in 1 ml water/day) for 14 days, Group II animals received thyroxine (600 μg/kg body weight in 1 ml of water/day) for 14 days. Control rats received 1 ml of water only. Rats were anesthetized with chloroform...
and blood was collected directly from the heart for serum T3 and T4 hormone level. T3 or T4 concentrations in rat serum were determined by radioimmunoassay (RIA). $^{125}$I-labeled thyroid hormone (either T3 or T4) competes with thyroid hormone in the serum sample and for antibody sites on the tube, in the presence of blocking agents for thyroid hormone binding proteins. Thyroid gland and liver were dissected out, fixed in Bouin’s fixative, and processed for routine histology. Liver glycogen was determined by the colorimetric method by digesting 1 g fresh liver in 30% KOH and treatment with the anthrone reagent. Serum SGPT was measured colorimetrically using the method of Reitman. For oral glucose tolerance test (OGTT) blood was collected first from the tail veins of control and treated (hypothryoid and hyperthyroid) rats after 18 hr of fasting followed by challenge with glucose (25 mg glucose/100 g body weight) and at the following time point after oral glucose infusion: 0.5, 2.5 and 24 hr. Blood glucose was measured estimated using a blood glucose monitoring system (ACON Laboratories, Inc. San Diego, USA).

Results

**T3 and T4 levels, body weight and liver glycogen**

Rats with hypothyroidism showed lower levels of thyroid hormones T3 and T4 and with higher levels in hyperthyroid rats as compared to control rats (Fig. 1). Rats with hypothyroidism also showed increase in body weight (~18%) while those with hyperthyroidism exhibited a decrease in body weight (~18%). Liver glycogen increased dramatically in both hypothyroid (by ~22.8-fold) and hyperthyroid (by ~10.45-fold) (Fig. 2).

**Oral glucose tolerance test**— While hypothyroid rats displayed low glucose level (by 18%) no change in glucose level was seen for hyperthyroid rats (Fig. 3). In the control rats the blood glucose level returned to the normal level after 2.5 hr of glucose feeding. Like control rats, in hypothyroid rats glucose level increased by 90% after 0.5 hr of glucose challenge but the elevated glucose didn’t return to control level even after 24 hr of glucose challenge (Fig. 3). Unlike control and hypothyroid rats, the increments in glucose level after 0.5 hr of glucose challenge was modest (by ~22%) in hyperthyroid rats. However, like hypothyroid rats, the glucose remained elevated even after 24 hr of glucose feeding (Fig. 3).

Serum SGPT — Hyperthyroid rats showed dramatic increments in SGPT level (by ~9.8-fold) as compared to a modest (by ~0.5 fold) increase in hypothyroid rats (Fig. 4).

Liver histology — Hyperthyroid rats exhibited marked changes in the general cytomorphology of
hepatocytes as evidenced by the enlargement of central terminal hepatic venule and disorientation of the nuclei and the loss of the individual boundary. Small numbers of hepatocytes were found to be picnotic. Few uniform sized cell bodies stained in eosin were found in the periphery of central terminal hepatic venule (Fig. 5). No discernible change in liver histology was detected in hypothyroid rats (data not shown).

Discussion

The T3 and T4 levels are low in hypothyroid and high in hyperthyroid rats (Fig. 1). A complex relationship exists between thyroid disease, body weight and metabolism. It is well known that hyperthyroidism causes extensive weight loss despite normal or increased calorie intake\textsuperscript{19,20}. The weight loss is related to the severity of the overactive thyroid. In congruence with these findings 18% loss of body weight was observed in hyperthyroid rate. Weight loss reflects not only a depletion of body adipose tissue stores but also a loss of muscle mass caused by accelerated catabolism and heat elimination. Because of low BMR hypothyroidism is generally associated with some weight gain\textsuperscript{30}. There was an 18% increase in body weight in the present experimental hypothyroid which is in line with reported observation.

Hypothyroidism is associated with decreased gluconeogenesis and glycogenolysis resulting in increment in glycogen in liver. In addition,
hypothyroidism lowers the glycogen phosphorylase activity in the liver. Consistent with these findings profound increments in liver glycogen in hypothyroid rats were observed. In contrast, the hyperthyroid state is associated with low hepatic glycogen levels, but paradoxically with a high activity of glycogen synthase and low activity of glycogen phosphorylase. In hyperthyroidism, insulin-stimulated rates of glucose utilization in muscle to form lactate are increased mainly because of a decrease in glycogen synthesis. In the present study, elevated glycogen content in the liver of hyperthyroid rats is paradoxical and may be due to the high activity of glycogen synthase.

In hyperthyroid humans as well as in experimental thyrotoxicosis in animals, glucose turnover and hepatic glucose production are increased due to increased metabolic rate and peripheral glucose utilization. Experimental as well as spontaneous hyperthyroidism in humans cause increased glucose production and impaired suppression of glucose production by insulin. Consistent with these previous reports increments in blood glucose levels were observed after OGGT and the hyperglycemia persisted even 24 h after glucose load. It is believed that insulin response to a glucose load is relatively decreased in hyperthyroidism, and that an inability to increase their insulin response further impairs glucose tolerance in low β-cell responders. It is possible that T4 causes deleterious effects on β-cell function thereby impairing insulin secretion after an oral glucose load. In line with this thought, Lenzen reported that injections of T4 (50-2000 μg/kg/day for 5 days) dose-dependently inhibited glucose-induced insulin secretion in the isolated pancreas. It has also been reported that hyperthyroid patients with younger age (<30 years) showed an increased secretion of insulin to compensate hyperglycemia after glucose load, but older patients (>31 years), who may have a low β-cell reserve, showed a blunted response of insulin. In addition, insulin sensitivity is altered in hyperthyroid patients. In the present study, the reason behind impaired glucose tolerance in response to glucose overload in hyperthyroid rats is not known and further experiments need to be performed.

Liver maintains a stable blood glucose level by taking up and storing glucose as glycogen, breaking this down to glucose when needed and forming glucose from non-carbohydrate sources such as amino acids. Because of the impairment of glucose tolerance in both hypo- and hyperthyroid rats we found dramatic changes in SGPT enzyme levels in these rats. It is believed that abnormal liver enzyme levels may cause liver damage. Profound alteration in liver histology in hyperthyroid rats is a reflection of changes in SGPT level in the liver an observation that is unique to this study.

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Correction Lists

1. Abbreviations ‘g’ for gram, kDa for kilo Dalton, kg for kilogram μg/dl for microgram/deciliter, ml for milliliter, mM for milli mole, ng/dl for nanogram/deciliter, rpm for revolutions per minute and U/l for unit/liter

2. Here, I mentioned the results obtained by the earlier worker (Dimitriadis et al., 2006)- Page 18

3. Incorporated complete sentence -page 20

4. Alcohol terminology has been replaced by ethanol as suggested by examiner - Page 22

5. Total magnification = Magnification of eyepiece x Magnification of objective lens Page 28

6. Ketoglutaric acid may refer to either of two chemical compounds:
   • α-Ketoglutaric acid
   • β-Ketoglutaric acid (acetonedicarboxylic acid)- page 36

7. p value calculated by students ‘t’ test table, less than 0.05 considered significant. -Page 38

8. The experiment was to induce hypothyroid and hyperthyroid. As like previous chapter we found lower T3 and T4 in hypothyroid and higher in hyperthyroid.-Page 47

9. Hyperthyroid induced rat not exhibited any significant histological changes under light microscope. As there were no changes I could not incorporated the histological pictures in my thesis. –Page 48

10. Gel picture marked on page 61

11. Reference correction was performed.