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The effect of thyroid activity on adult rat spermatogenesis

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Summary

The influence of hypo- and hyper-thyroidism on spermatogenesis was studied in 60-day-old adult male Wistar rats. To confirm hypo- and hyper-thyroidism, the concentration of plasma thyroid hormones were assayed by radioimmunoassay. The hypothyroid state, induced by administration of 25 mg/kg/day methimazole for 5 successive days, resulted in significant decrease in the number of Sertoli cells, sperm count, Leydig cells and the diameter of seminiferous tubules. The hyperthyroid state, induced by administration of 1 mg/kg/day L-thyroxine for 10 successive days, increased the number of Sertoli cells, sperm count, Leydig cells and the diameter of seminiferous tubules. Serum levels of FSH and LH and testosterone were also evaluated. Hypo- and hyper-thyroidism had no effects on the concentrations of FSH and LH, while the concentration of testosterone was significantly increased in hyperthyroid state; it decreased in hypothyroid state in comparison with the control euthyroid rats. In conclusion, our data indicated that hypo- and hyper-thyroidism affect spermatogenesis through their effects on germinal, interstitial and Sertoli cells but not through the pituitary-gonadal axis.

Key words: Hypothyroidism, Hyperthyroidism, Spermatogenesis, Rat

Introduction

Thyroid hormone receptors are exist in sperm, developed germ cells, Sertoli, Leydig, and peritubular cells in neonatal, prepubertal and adult rats (Buzzard *et al*., 2000). Recent evidences support the concept of a critical role for thyroid hormones in testicular development during perinatal period (Meisami *et al*., 1994; Maran, 2003; Umezu *et al*., 2004). The presence of thyroid hormone receptors in the adult rat testis indicates that the adult rat testis may be responsive to thyroid hormone. However, the effects of altered thyroid hormone status on the adult testis are unclear, and contradictory results were reported not only in different species but also in the same animal model (Jannini *et al*., 1995). In the adult male rat, hypothyroidism induced by thyroidectomy or goitrogen treatment was found not to affect the testicular size or the seminiferous tubule morphology (Vilchez-Martinez, 1973; Weiss and Burns, 1988). Based on reports, the hyperthyroid state results in acceleration of growth, a comparative increase in the number of spermatogonia, interstitial cells, and Sertoli cells (Amin and El-sheikh, 1977) while hypothyroid state results in depression of growth rate, destructive changes of the spermatogenic and interstitial cells and adverse effects on the lumen of the seminiferous tubules (Amin and El-sheikh, 1977; Tahmaz *et al*., 2000).

Thyroid hormones regulate Sertoli and Leydig cells function (Maran, 2003). The presence of functional Leydig cells are essential for the well-being of the adult mammalian males at all stages of life, because they are the primary source of testosterone and testosterone is vital for function of the male reproductive system (Mann and Fraser, 1996). Thyroid hormones

increase the number of Leydig cell luteinizing hormone (LH) receptors, stimulate Leydig cell testosterone production and secretion. LH is necessary for cellular maintenance of Leydig cells (Maran *et al*., 2000; Maran, 2003). The number of Sertoli cells is the major determinant of the magnitude of sperm production (Orth *et al*., 1988). Sertoli cells are capable of supporting only a fixed number of germ cells. Therefore, the final number of these cells set the upper limit for testicular sperm production and influences the levels of male fertility (Walker, 2003). Thyroid hormones affect the energy metabolism of Sertoli cells and stimulate Sertoli cell lactate and IGF-I secretion (Palmero *et al*., 1994).

The trophic factors traditionally associated with the growth and maturation of the testis and its constituent cell types are follicle stimulating hormone (FSH) and LH (Sharpe, 1994). LH triggers differentiation of Leydig cells and stimulates testosterone production and secretion by Leydig cells (Mann and Fraser, 1996). FSH is the major endocrine factor regulating mitogenesis and differentiation of Sertoli cells and the onset of secretary activity (Sharpe, 1994).

The objective of the present study was to investigate the effects of hypo- and hyper-thyroidism on seminiferous tubules morphology, number of spermatogenic, Leydig, Sertoli and sperm cells in the adult male rats.

Materials and Methods

Animals

Sixty-day-old Wistar rats weighing 250 ± 20 g, housed under controlled temperature (22–25°C) and lighting (12 L: 12 D) conditions. The rats were fed with standard dry pellets and drank tap water ad libitum.

Treatments

Three groups of hypo-, hyper- and eu-thyroid (control) rats (n = 20 per group) were used. Hypothyroidism was induced by daily oral administration of 25 mg/kg methimazole for five successive days (Francavilla *et al*., 1991). Hyperthyroidism was induced by administering 1 mg/kg body weight levothyroxine for 10 successive days (Omarani *et al*., 1992). Rats in the control group were received the same volume of normal saline for the same period of time.

Blood sampling, testes collection and hormone assay

The rats in the hypothyroid and their control group were sacrificed on day 5, and hyperthyroid rats and their control group were sacrificed on day 10 after treatments. Blood was collected from the heart of each rat after deep inhalation of ether. Serum was taken and stored at -80°C until hormonal assay by radioimmunoassay (RIA). One testis from each rat in each of the three groups was removed, fixed in neutralized formalin solution and used for paraffin embedding.

Morphology and morphometry

Serial 5-µm thick sections were cut from each testis tissue and stained with haematoxylin and eosin. The sections of the testes were compared among three groups. Different cell types in the testis tissue were identified by their morphological characteristics.

RIA for hormones

Testosterone, T_3 and T_4 concentrations in the serum were quantified by commercial available RIA kits (Immunotech, France). The serum levels of FSH, LH were measured by rat specific FSH and LH RIA kits (DRG international, Germany).

Statistical analysis

All data were expressed as mean±SD. The difference in serum concentration of FSH, LH, testosterone, T_3 and T_4 between hypo-, hyper- and eu-thyroid control rats was evaluated by one-way ANOVA. Differences were considered to be significant at $p<0.05$.

Results

Qualitative morphology

The microscopic appearance (×200) of the size of seminiferous tubules in sections differed among the three studied groups. In the hypothyroid rats, the diameter of the

seminiferous tubules was lesser and the distance between the tubules was significantly more than that of the control group. While in the hyperthyroid rats, the diameter of the seminiferous tubules was more and the distance between the tubules was lesser than the control rats (Fig. 1). Spermatogonia cells in the control group were in their normal shape and were located on the basement membrane in a chain form, while in the hypothyroid group, their number was lesser and they were irregularly positioned; in the hyperthyroid rats, the number of spermatogonia cells was more than the control group (Fig. 2).

Morphometry

There was a few number of spermatozoa cells and high secretions in the lumen of seminiferous tubules in the hypothyroid rats. The number of spermatozoa was much higher in the hyperthyroid group in comparison with the control group (Fig. 3 and Table 1). In the hypothyroid rats, the number of Sertoli cells was decreased; the nuclei of these cells were denser and appeared flat. In the hyperthyroid rats, the number of Sertoli cells was increased and their nuclei were more voluminous, columnar in shape and the nucleolus was acentric, which showed high activity of these cells, compared to the control group. The number of Leydig cells was more in the hyperthyroid rats; in hypothyroid rats, it was decreased when compared to the control group (Table 1).

Hormone assay

T_3 and T_4 levels in hypothyroid and hyperthyroid rats were lower and higher than those in the control rats, respectively ($P<0.05$) (Table 2). The serum concentrations of FSH and LH were not significantly different among hypo-, hyper- and eu-thyroid rats (Table 2). The serum concentrations of testosterone was higher in hyperthyroid and lower in hypothyroid rats in comparison with the control group ($P<0.05$) (Table 2).

Body weights

In both euthyroid and hypothyroid rats, body weight was increased while in hyperthyroid rats, the body weight was decreased during the experiment (Table 2).

Discussion

To explore the effects of thyroid hormones in vivo on spermatogenesis in adult rats the hyper- and hypo-thyroid rat models were established by administration of levothyroxine and methimazole, respectively. Serum concentrations of T_3 and T_4 in hyper- and hypo-thyroid rats in the present investigation were in agreement with their hyper- and hypo-thyroid conditions. Hair and weight loss in hyperthyroidism animals and weight increase in

Table 1: The number of seminiferous tubules per testis and the number of Leydig, Sertoli, spermatogonia and spermatozoa cells in the cross section of each seminiferous tubule

Group	n	Seminiferous tubules	Spermatogonia	Primary spermatocyte	Spermatozoid	Leydig cells	Sertoli cells
Control	20	40.86±0.44	41.53±0.42^a	81.88±0.43^a	264.66±1.48^a	17.93±0.23^a	30.53±0.32^a
Hypothyroid	20	41.00±0.62	31.33±0.39^b	67.17±0.66^b	207.40±1.12^b	9.81±0.22^b	20.66±0.43^b
Hyperthyroid	20	42.00±0.71	55.00±0.54^c	97.52±0.60^c	329.46±0.76^c	29.00±0.41^c	40.26±0.94^c

$^{a, b, c}$ different superscripts within columns are significantly different ($P<0.05$). Values are mean ± SD

Table 2: Changes in body weight and serum concentrations of hormones in control, hypothyroid and hyperthyroid rats

Group	n	Weight (%changes)	T_3 (ng/100 mL)	T_4 (µg/100 mL)	LH (mIu/mL)	FSH (mIu/mL)	Testosterone (ng/mL)
Control	20	+5.2±0.23	155.70±7.28^a	4.96±0.19^a	4.80±0.17	2.83±0.17	4.17±0.14^a
Hypothyroid	20	+6.4±0.36	84.30±1.67^b	2.61±0.11^b	4.97±0.23	3.10±0.19	2.94±0.12^b
Hyperthyroid	20	-8.3±0.41	364.20±4.90^c	12.57±0.37^c	5.04±0.22	3.14±0.29	5.81±0.23^c

$^{a, b, c}$ different superscripts within columns are significantly different ($P<0.05$). Values are mean ± SD

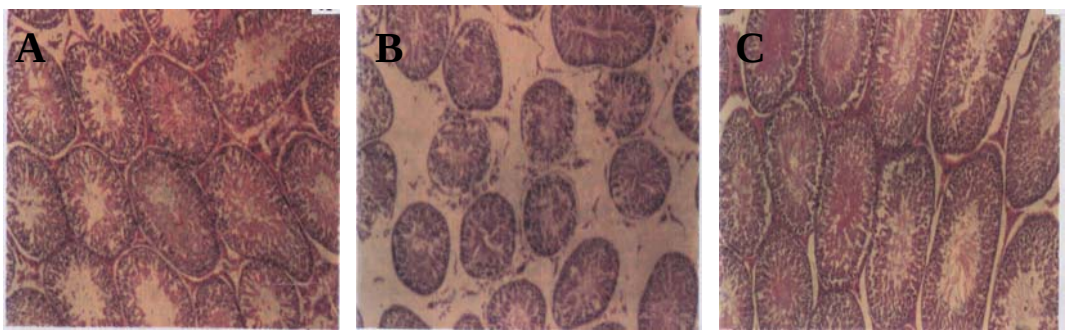

Fig. 1: Representative light micrographs of testes taken from euthyroid (A), hypothyroid (B) and hyperthyroid (C) rats. Seminiferous tubule diameter is much reduced in the hypothyroid rat and increased in hyperthyroid rat. (×40)

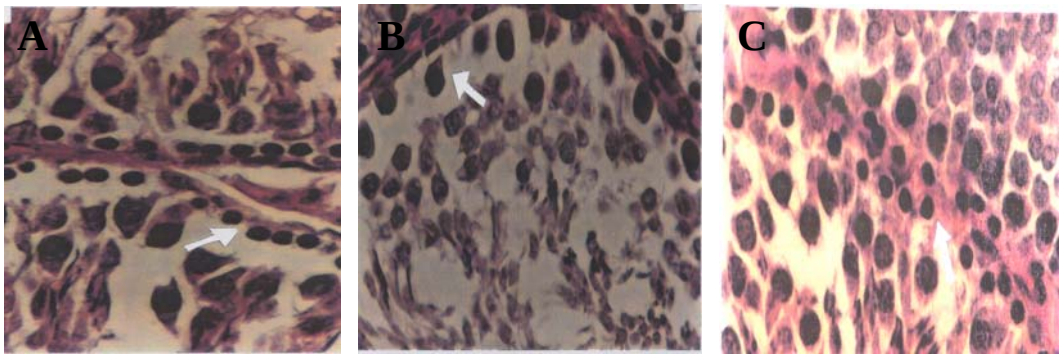

Fig. 2: Representative light micrographs of basal membrane of seminiferous tubule from control (A), hypothyroid (B) and hyperthyroid (C) rats. Arrows show spermatogonia. (×1000)

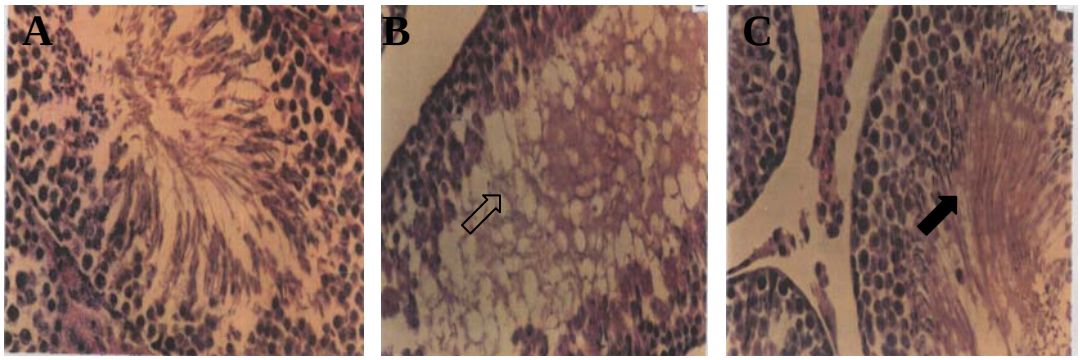

Fig. 3: Representative light micrographs of the lumen of seminiferous tubule from control (A), hypothyroid (B) and hyperthyroid (C) rats. A lot of sperm (arrow) were present in the lumen of seminiferous tubule in hyperthyroid rats but in the hypothyroid rats much secretion and low sperm were present (blank arrow) (×400)

hypothyroidism animals were other signs of hyper- and hypo-thyroidism.

Histologic analysis of testes in the hypothyroid rats revealed smaller-size seminiferous tubules and a significant reduction in the number of spermatogonia and primary spermatocyte within seminiferous tubules compared to the control group. These results are consistent with other reports (Francavilla *et al*., 1991; Tahmaz *et al*., 2000). The decrease in diameter of seminiferous tubules may be related to increased degeneration or arrested proliferation of germ cells in hypothyroid state. By contrast, hyperthyroid rats contained significantly higher number of spermatogonia and primary spermatocyte per tubule and greater diameter of seminiferous tubules in comparison with control group. These results were comparable with the findings of the previously-published studies (Amin and El-

sheikh, 1977). In addition, the number of sperm in the lumen of seminiferous tubules was decreased significantly in hypothyroid rats and increased in hyperthyroid rats in comparison with the control group rats. It was shown that thyroid hormone receptor expresses in the germ cells from spermatogonia to primary spermatocytes (Buzzard *et al*., 2000). It suggests a possible direct effect of thyroid hormone in proliferation and maintenance of germ cells. Alternatively, these effects can be attributed to the effects of thyroid hormone on the number of Sertoli and Leydig cells. Sertoli cells are the major determinants of the magnitude of sperm production (Orth *et al*., 1988). Sertoli cells express thyroid hormone receptor and thyroid hormone control proliferation and maturation of Sertoli cells (Cooke *et al*., 1994). Consistent with another report (Tahmaz *et al*., 2000), findings of the present study showed a significant reduction in the number of Sertoli cells in the hypothyroid rats. We also observed a significant increase in the number of Sertoli cells in hyperthyroid rats. The increased number of Sertoli cells could be responsible for the secondary changes such as the increased number of spermatogonia, and sperm production in hyperthyroid rats.

FSH has been demonstrated to be a major endocrine factor regulating mitogenesis and differentiation of Sertoli cells and the onset of their secretary activity (Sharpe, 1994). Serum FSH concentrations were not different between the hypo-, hyper- and eu-thyroid rats, although the values were somewhat variable. In agreement with an in vitro study (Cooke *et al*., 1994), the current results indicated that the majority of effects of hypothyroidism and hyperthyroidism on the Sertoli cells may be its direct effect on these cells.

Adult's Leydig cells have utmost importance for the mammalian male due to many functions associated with reproduction (Mann and Fraser, 1996). Leydig cells express thyroid hormone receptors and thyroid hormones control their proliferation and differentiation (Mendis-Handagama and Ariyaratne, 2004). Our results showed a reduction in the number of Leydig cells in the hypothyroid rats and an increase in the number of Leydig cells in the hyperthyroid rats. Serum concentrations of testosterone in both hypo- and hyper-thyroid rats in the present investigation closely reflected the cellular changes taken place in the testis. The decreased serum testosterone concentrations in hypothyroid rats and the increased serum testosterone in hyperthyroid rats compared with the control group rats were attributed to the decreased and increased number of Leydig cells in hypo- and hyper-thyroid-states, respectively, as compared to the control rats. Furthermore, the changes in serum concentration of testosterone in the hypo- and hyper-thyroid rats could be due to the effect of thyroid hormones on steroidogenic function of Leydig cells. In keeping with our results, Antony *et al*. (1995) showed a decrease in serum testosterone and a reduction in specific activity of Leydig cell β-hydroxyl steroid dehydrogenase in hypothyroid rats. LH has been suggested as a possible candidate for triggering proliferation and differentiation of Leydig cells and testosterone production by Leydig cells (Mendis-Handagama and Ariyaratne, 2004). Serum LH concentrations were not different between hypo- and hyper-thyroid rats. This confirmed the finding of previous reports in which hypothyroidism did not change serum LH concentrations (Armada *et al*., 2001).

In conclusion, morphometric and hormonal findings of the present study revealed that spermatogenesis in adult rats testes were decreased by hypothyroidism and increased by hyperthyroidism. Decreased and increased number of Leydig cells, Sertoli cells, in hypo- and hyper-thyroid rats and decreased and increased serum testosterone concentrations in hypo- and hyper-thyroid rats, respectively, beside the lack of difference in concentrations of LH and FSH in hypo- and hyper-thyroid rats suggest that the effects of hypo- and hyper-thyroidism on testis may be due to direct effects of thyroid hormones on testis and not due to their effects on the pituitary-gonadal axis.

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