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Regulation of Thyroid Hormones on the Production of Testosterone in Rats

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Abstract The effects of a thyroidectomy and thyroxine (T_4) replacement on the spontaneous and human chorionic gonadotropin (hCG)-stimulated secretion of testosterone and the production of adenosine 3′,5′-cyclic monophosphate (cAMP) in rat testes were studied. Thyroidectomy decreased the basal levels of plasma luteinizing hormone (LH) and testosterone, which delayed the maximal response of testosterone to gonadotropin-releasing hormone (GnRH) and hCG in male rats. T_4 replacement in thyroparathyroidectomized (Tx) rats restored the concentrations of plasma LH and testosterone to euthyroid levels. Thyroidectomy decreased the basal release of hypothalamic GnRH, pituitary LH, and testicular testosterone as well as the LH response to GnRH and testosterone response to hCG in vitro. T_4 replacement in Tx rats restored the in vitro release of GnRH, GnRH-stimulated LH release as well as hCG-stimulated testosterone release. Administration of T_4 in vitro restored the release of testosterone by rat testicular interstitial cells (TICs). The increase of testosterone release in response to forskolin and androstenedione was less in TICs from Tx rats than in that from sham Tx rats. Administration of nifedipine in vitro resulted in a decrease of testosterone release by TICs from sham Tx but not from Tx rats. The basal level of cAMP in TICs was decreased by thyroidectomy. The increased accumulation of cAMP in TICs following administration of forskolin was eliminated in Tx rats. T_4 replacement in Tx restored the testosterone response to forskolin. But the testosterone response to androstenedione and the cAMP response to forskolin in TICs was not restored by T_4 in Tx rats. These results suggest that the inhibitory effect of a thyroidectomy on the production of testosterone in rat TICs is in part due to: 1) the decreased basal secretion of pituitary LH and its response to GnRH; 2) the decreased response of TICs to gonadotropin; and 3) the diminished production of cAMP, influx of calcium, and activity of 17β-HSD. T_4 may enhance testosterone production by acting directly at the testicular interstitial cells of Tx rats. J. Cell. Biochem. 73:554–562, 1999.

Key words: Tx; T_4; cAMP; T; LH; GnRH

INTRODUCTION

It has been well-established that hypothyroidism causes abnormal reproductive functions in many species [Jannini et al., 1995]. In male rats, thyroidectomy reduced the weight of the testes and accessory sex organs [Wan and Chen, 1974; Biswas et al., 1994], the number and size of gonadotropes [Amin and El-Sheikh, 1977], and the serum levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) [Valle et al., 1985; Ruiz et al., 1989; Francavilla et al., 1991; Kirby et al., 1992; Van Haaster et al., 1992; Antony et al., 1995].

Either unchanged [Cooke and Meisami, 1991; Cooke et al., 1992; Kirby et al., 1992] or decreased [Ruiz et al., 1989; Biswas et al., 1994; Antony et al., 1995] levels of serum testosterone were found in hypothyroid rats, as compared with euthyroid animals. The production of intratesticular testosterone in response to gonadotropin was either decreased [Antony et al., 1995] or unchanged [Tohei et al., 1997] by

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hypothyroidism. These conflict results reflect that the functional relationship between thyroid gland and the pituitary-testis axis is still not confirmed. Furthermore, whether thyroid hormones may alter testicular function by acting directly on the testicular interstitial cells is also not known.

In this investigation, both in vivo and in vitro effects of a thyroidectomy and thyroid hormone replacement on the secretion of LH and testosterone were studied in male rats. As for exploring the mechanism of testosterone production, the generation of adenosine 3′,5′-cyclic monophosphate (cAMP), the activation of calcium channels, and the activity of 17β-hydroxysteroid dehydrogenase (17β-HSD) were also examined. It was revealed that a thyroidectomy decreased the secretion of testosterone through the mechanisms involving the decrease of 1) spontaneous and gonadotropin-releasing hormone (GnRH)-stimulated release of LH, 2) cAMP production, and 3) the activity of 17β-HSD.

MATERIALS AND METHODS

Animals

Male rats of Sprague-Dawley strain weighing 320–370 g were housed in a temperature controlled room (22 ± 1°C) with 14 h of artificial illumination daily (0600–2000) and given food and water ad libitum.

The rats were thyroparathyroidectomized (Tx) or sham Tx under ether anesthesia, then maintained in an animal room for 2 weeks before experimentation. The Tx rats were subcutaneously injected with thyroxine (T_4, 20 μg/kg) or saline once daily for 2 weeks. The sham Tx rats were injected with saline only [Tang et al., 1986].

Plasma LH Response to GnRH

The experimental rats were catheterized via the right jugular vein [Wang et al., 1989, 1994; Hwang et al., 1990]. Twenty hours later, they were injected intravenously with GnRH (5 μg/kg). Blood samples (0.5 ml) were collected at 0, 10, 30, 60, 120, and 180 min postintravenous injection of GnRH. An equal volume of heparinized saline was injected immediately after each bleeding. Plasma was separated by centrifugation at 10,000*g* for 1 min. The concentration of LH in each plasma sample was measured by radioimmunoassay (RIA) [Hwang et al., 1990; Tsai et al., 1996].

Plasma Testosterone Response to hCG and GnRH

The experimental rats were catheterized via the right jugular vein 20 h before challenge with hCG (5 IU/kg) or GnRH (5 μg/kg) intravenously. Blood samples (0.4 ml each) were collected at -10, 0, 10, 30, 60, 120, and 180 min post-hCG challenge, or at different intervals post GnRH challenge. An equal volume of heparinized saline was injected immediately post each bleeding. Plasma was separated by centrifugation at 10,000*g* for 1 min. The concentration of testosterone in each plasma sample was measured by RIA [Wang et al., 1994; Tsai et al., 1996].

LH Release in Vitro

The experimental rats were decapitated, then the blood was collected. The concentration of thyroid stimulating hormone (TSH) in rat plasma was measured by RIA. The anterior pituitary glands (APs) were excised, bisected, preincubated, then incubated with Locke solution containing 10 mM glucose, 0.003% bacitracin, and 0.05% HEPES at 37°C for 30 min [Wang et al., 1994]. Each hemi-AP was assigned to a flask containing 1 ml medium, which was aerated with 95% O_2 and 5% CO_2. APs were then incubated either with or without 10 nM GnRH for 30 min. At the end of incubation, each medium was collected, next the tissues were weighed, then extracted by phosphate buffer saline (PBS, pH 7.5). The concentrations of LH in both medium and AP extracts were measured by RIA.

Testosterone Release In Vitro in Response to hCG

After decapitation, rat testes were decapsulated and cut into eight equal blocks [Wang et al., 1994, Tsai et al., 1996]. The testicular blocks were preincubated for 90 min with Locke's medium, then incubated for 60 min at 34°C with hCG (0, 0.5, or 5 IU/ml). Each octant piece was assigned to a flask containing 2 ml medium. Each group consisted of seven or eight testicular blocks from seven or eight different rats. The medium was aerated with 95% O_2 and 5% CO_2. At the end of the incubation, the testicular tissues were weighed. Each medium was collected then stored at −20°C until analyzed by testosterone RIA [Wang et al., 1994; Tsai et al., 1996].

Preparation of Testicular Interstitial Cells

The collagenase dispersion of testicular interstitial cells was performed by the procedure

described elsewhere [Tsai et al., 1997]. Five decapsulated testes were added to a 50 ml polypropylene tube containing 5 ml preincubation medium and 700 µg collagenase (Type IA, Sigma, St. Louis, MO). Preincubation medium was made up of 1% bovine serum albumin (BSA, Fraction V, Sigma) in Hank's balanced salt solution (HBSS, Sigma), with HEPES (25 mM), sodium bicarbonate (0.35 g/l), penicillin-G (100 IU/ml), streptomycin sulfate (50 µg/ml), heparin (2550 USP K units/l), pH 7.3, and aerated with 95% O_2 and 5% CO_2. The tube was placed horizontally in a 34°C water bath, parallel to the direction of the shaking. Fifteen min postshaking at 100 cycles/min, the digestion was stopped by adding 35 ml of cold preincubation medium and inverting the tube several times. The tube was allowed to stand for 5 min, then filtered through a four-layer fine nylon mesh. Cells were collected by centrifugation at 4°C, 100*g* for 10 min. The cell pellets were washed with deionized water for disrupting red blood cells (RBCs), then immediately, the osmolarity was recovered with 10-fold HBSS. Hypotonic shock was repeated twice for disrupting the RBCs, next, the cell pellets were resuspended in the incubation medium (substitution of HBSS in preincubation medium with Medium 199, and sodium bicarbonate, 2.2 g/l). Cell concentration (1×10^6 cells/ml), viability (over 97%), and the sperm cells (less than 5%) were determined using a hemacytometer and the trypan blue method. Total cell proteins were determined by the method of Lowry et al., [1961]. Our preparation was found to contain approximately 20% Leydig cells [Tsai et al., 1997].

Testosterone Release In Vitro in Testicular Interstitial Cells

Aliquots (1 ml) of cell suspensions (1×10^6 cells/ml) were preincubated with incubation medium in polyethylene tubes for 1 h at 34°C under a controlled atmosphere (95% O_2 and 5% CO_2), shaken at 100 cycles/min. After centrifugation, the supernatant was decanted into tubes at 100*g* for 10 min. Aliquots (1 ml) of cell suspensions (1×10^6 cells/ml) were incubated either with or without hCG (0.5 IU/ml), T_3 (10^{-7} M), T_4 (10^{-7} M), forskolin (10^{-6} M, an adenylyl cyclase activator), nifedipine (10^{-6} M, an L-type calcium channel blocker), or androstenedione (10^{-9} M, a precursor of testosterone biosynthesis) for 1 h. Then, 2 ml ice-cold PBS-gelatin buffer (0.1% gelatin in 0.01 M PBS, 0.15 M sodium chloride, pH 7.5) was added to stop the reaction. The medium was centrifuged at 100*g* and stored at -20°C until analyzed for testosterone by RIA.

Production of cAMP in Testicular Interstitial Cells

Aliquots (1 ml) of cell suspensions (1×10^6 cells/ml) were incubated with forskolin (10^{-6} M) for 1 h. After incubation, the tubes were centrifuged at 100*g*. The cell pellets were then mixed with 1 ml of 65% ice-cold ethanol, homogenized by polytron (PT3000, Kinematica Ag, Luzern, Switzerland), and centrifuged at 1,500*g* for 15 min. Both the medium and supernatants of cell extracts were lyophilized in a vacuum concentrator (Speed Vac, Savant' Holbrook, NY) and stored at −20°C until analyzed for cAMP by RIA [Chen et al., 1997].

GnRH Release In Vitro

After decapitation, the mediobasal hypothalamus (MBH) of each rat was excised, then preincubated with Locke's medium at 37°C for 90 min [Wang et al., 1987]. One MBH was assigned to a tube containing 0.5 ml medium. The MBHs were then incubated with Locke's medium for five time-intervals, 30 min for each interval. T_4 (1 µM) was added to the medium at the second interval (i.e., 30–60 min). KCl (60 mM) was added to the medium at the fifth interval (i.e., 120–150 min). At the end of incubation, the tissue was weighed, and the medium was collected, extracted by 20 µl 5 N HCl, lyophilized, and stored at −20°C until analyzed by GnRH RIA [Hwang et al., 1990].

RIA of Testosterone, LH, TSH, and GnRH

The concentration of plasma and medium testosterone was determined by RIA as described elsewhere [Wang et al., 1994]. With anti-testosterone serum No. W8, the sensitivity of testosterone RIA was 2 pg per assay tube. the intra- and interassay coefficients of variation were 4.1% (n = 6) and 4.7% (n = 10), respectively.

The concentration of LH was determined by RIA as described previously with anti-LH serum PW 11–2 [Hwang et al., 1990; Wang et al., 1994]. The rat LH-I-6 used for iodination and the rat LH-RP-3 which served as standard preparations were provided by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDKD, Bethesda, MD). The sensitivity was 0.1 ng for LH RIA. The intra- and

interassay coefficients of variation were 3.8% (n = 4), and 6.6% (n = 5), respectively.

The concentration of plasma TSH was determined by RIA as described elsewhere using the rat RIA kit provided by NIDDKD [Tang et al., 1986; Wang et al., 1989].

The concentration of GnRH in medium samples was determined by RIA using anti-GnRH serum CRR11B52 generously provided by Dr. Y. F. Chen and Dr. V. D. Ramirez [Hwang et al., 1990]. The sensitivity was 4 pg for GnRH RIA. The intra- and interassay coefficients of variation were 2.1%, and 10.5% respectively, for GnRH RIA.

Statistical Analysis

All values are given as the mean ± standard error of the mean (SEM). The treatment means were tested for homogeneity by a two-way analysis of variance, and the difference between specific means was tested for significance by Duncan's multiple-range test [Steel and Torrie, 1960]. A difference between two means was considered statistically significant when $P < 0.05$.

RESULTS

Plasma TSH, LH, and Testosterone

The concentration of plasma TSH in Tx rats was 68.4 ± 5.6 ng/ml (n = 16), which was significantly higher ($P < 0.01$) than that in sham Tx (8.9 ± 0.9 ng/ml, n = 16) and Tx + T_4 (4.3 ± 0.4 ng/ml, n = 16) rats.

The thyroidectomy in male rats resulted in a decrease ($P < 0.01$) of the basal level of plasma LH (Fig. 1) and testosterone (Figs. 2 and 3). T_4 replacement in Tx rats restored the basal levels of plasma LH and testosterone to euthyroid levels. The maximal response of plasma LH to GnRH was greater ($P < 0.01$) in the Tx rats than in the sham Tx or Tx + T_4 rats (Fig. 1). The response of plasma testosterone to GnRH in the Tx rats remained lower until 60 min post-GnRH injection, as compared with the sham Tx rats (Fig. 2). The thyroidectomy did not alter the maximal testosterone response to GnRH (Fig. 2) and hCG (Fig. 3), even the maximal response was delayed, as compared with the sham Tx group.

GnRH Release In Vitro

The thyroidectomy decreased ($P < 0.05$), but, T_4 replacement restored the basal release of GnRH in vitro (Fig. 4) in male rats. Administration of 1 μM T_4 in vitro for 30 min increased ($P < 0.05$) GnRH release in Tx + T_4 group, but not in the sham Tx nor Tx groups. Post incubation of rat MBH with T_4 for 30 min, the GnRH release in the Tx group was restored to euthyroid level. Administration of 60 mM KCl in-

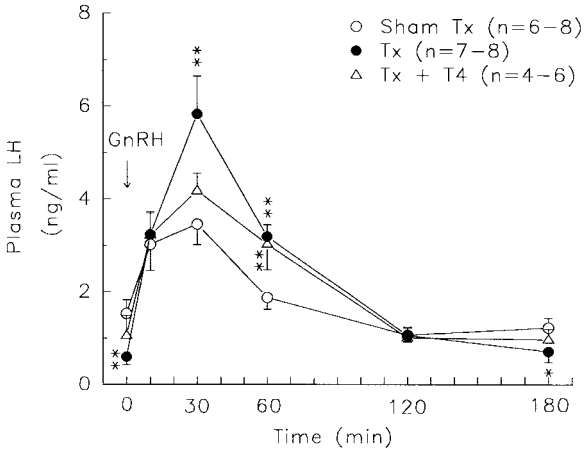

Fig. 1. Effects of the thyroidectomy and thyroxine (T_4) replacement on the concentration of LH in response to GnRH in male rats. Male rats were either thyroidectomized (Tx) or sham Tx. Tx rats were immediately injected subcutaneously with T_4 (20 μg/kg) or saline for 2 weeks. The sham Tx rats were injected with saline only. The experimental rats were catheterized via right jugular vein before challenging with GnRH (5 μg/kg). Blood samples were collected at 0, 10, 30, 60, 120, and 180 min. The concentration of LH in rat plasma was measured by radioimmunoassay. Each value represents mean ± SEM. *, **$P < 0.05$ and $P < 0.01$ as compared with the corresponding sham Tx group, respectively.

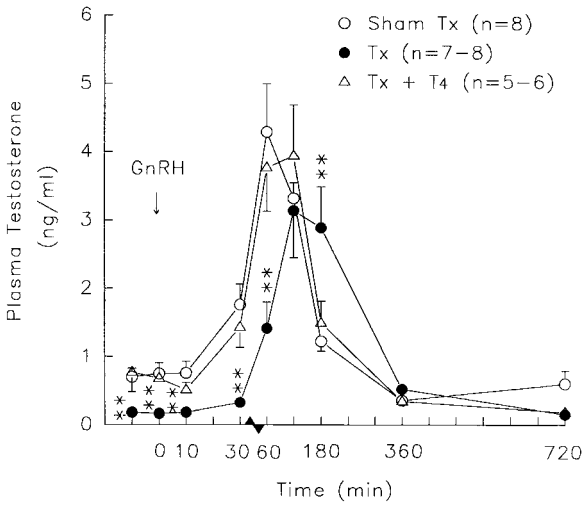

Fig. 2. Effects of the thyroidectomy and T_4 replacement on the concentration of plasma testosterone in response to GnRH in male rats. See Figure 1 legend for details.

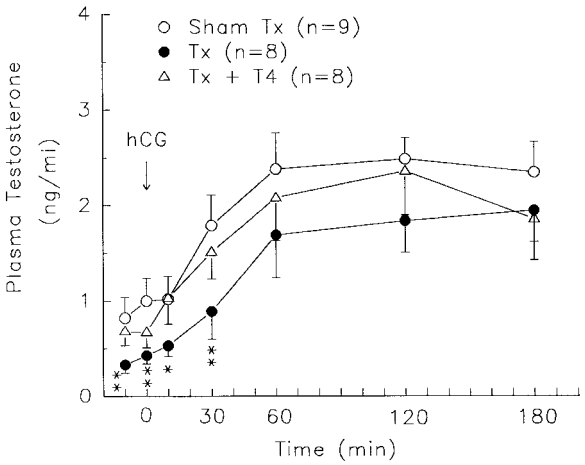

Fig. 3. Effects of the thyroidectomy and T_4 replacement on the concentration of plasma testosterone in response to hCG (5 IU/kg). See Figure 1 legend for details.

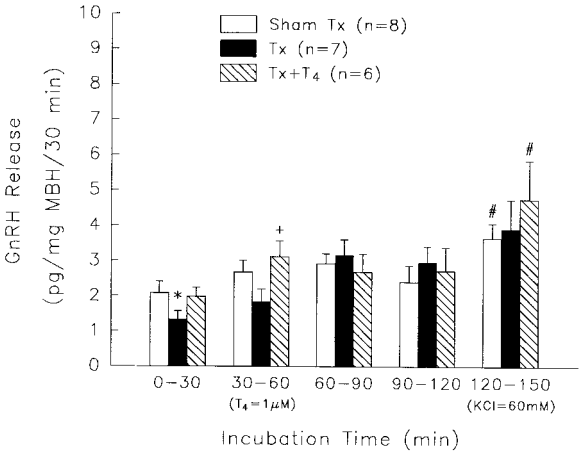

Fig. 4. Effects of the thyroidectomy and T_4 replacement on the in vitro release of GnRH by rat mediobasal hypothalamus (MBH). The MBH collected from the sham Tx, Tx and Tx + T_4 rats were preincubated, then incubated with Locke's medium at 37°C for several time-intervals; 30 min for each interval. One MBH was assigned to each incubation vial containing 0.5 ml Locke's medium. The MBHs were challenged with T_4 (1 μM) at the second interval (30–60 min) and with KCl (60 mM) at the fifth interval (120–150 min). The medium was collected at the end of each interval. Each value represents the mean ± SEM. $*P < 0.05$ as compared with the sham Tx group, $+P < 0.05$ as compared with the same group at the first interval (0–30 min), $\#P < 0.05$ as compared with the same group at the fourth interval (90–120 min).

creased GnRH release in the sham Tx and Tx + T_4 groups.

LH Release In Vitro

Administration of 10 nM GnRH in vitro increased LH release by rat APs (Fig. 5). The thyroidectomy in male rats decreased ($P < 0.01$)

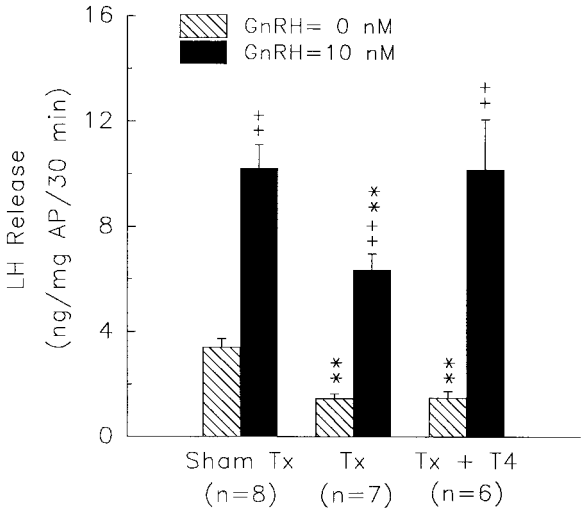

Fig. 5. Effects of the thyroidectomy and T_4 replacement on the in vitro release of LH by rat anterior pituitary gland (AP) in the presence or absence of GnRH. After decapitation, rat APs were bisected, preincubated and incubated either with or without 10 nM GnRH at 37°C for 30 min. One hemi-AP was assigned to each incubation vial containing 1 ml Locke's medium. Each value represents mean ± SEM. $**P < 0.01$ as compared with the sham Tx group. $++P < 0.01$ as compared with the group treated without GnRH.

both the basal and GnRH-stimulated release of LH. T_4 replacement in the Tx rats restored the GnRH-stimulated level of medium LH to the sham Tx level, but did not alter the basal release of LH in the Tx animals.

Post-incubation, the concentration of LH in APs was lower ($P < 0.01$) in the Tx rats as compared with the sham Tx and Tx + T_4 animals (Fig. 6).

Testosterone Release In Vitro

Administration of hCG in vitro increased ($P < 0.01$) the release of testosterone by rat testicular tissues (Fig. 7) and testicular interstitial cells (Fig. 8). The thyroidectomy in male rats decreased both basal ($P < 0.01$) and hCG-stimulated ($P < 0.05$ or $P < 0.01$) release of testosterone, whereas T_4 replacement in the Tx rats restored the levels in the testosterone release to the sham Tx (Figs. 7 and 8).

Administration of T_3 or T_4 increased the basal release of testosterone by the testicular interstitial cells of the Tx rats, but did not alter the testosterone release by the interstitial cells of the sham Tx and Tx + T_4 rats (Fig. 8, top). Administration of T_4 at 10^{-7} M in vitro increased the hCG-stimulated release of testoster-

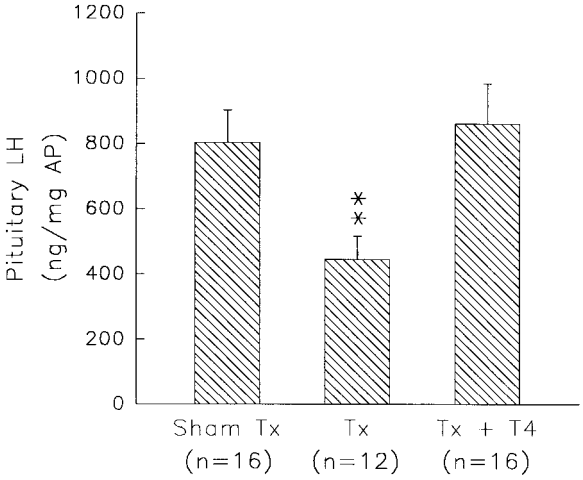

Fig. 6. Effects of the thyroidectomy and T_4 replacement on the concentration of LH in APs of male rats. Each value represents mean ± SEM. $^{**}P < 0.01$ as compared with the sham Tx group.

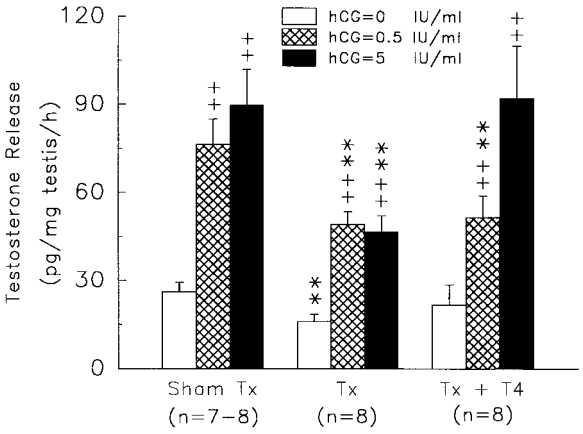

Fig. 7. Effects of the thyroidectomy and T_4 replacement on the in vitro release of testosterone by rat testicular blocks in the presence or absence of hCG. One octant testicular block was assigned to each incubation tube containing 2 ml Locke's medium. Each value represents mean ± SEM. $^{**}P < 0.01$ as compared with the sham Tx group, $++P < 0.01$ as compared with the group treated without hCG.

one by the testicular interstitial cells of the Tx rats (Fig. 8, bottom).

Signal Transduction and Steroidogenesis

The in vitro release of testosterone in testicular interstitial cells was enhanced by the administration of forskolin (10^{-6} M, $P < 0.01$) or androstenedione (10^{-9} M, $P < 0.01$), in the sham Tx, Tx, and Tx + T_4 rats (Fig. 9).

Administration of nifedipine (10^{-6} M) decreased testosterone release in vitro in the sham Tx group, but did not alter the testosterone

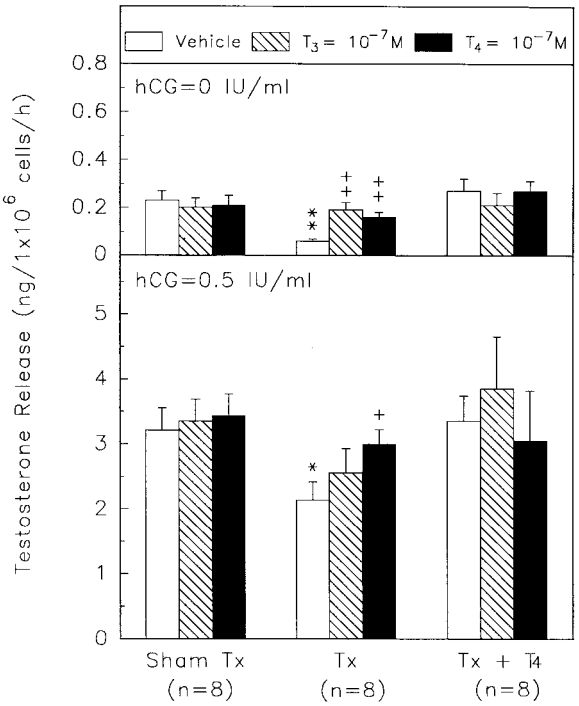

Fig. 8. Effects of the thyroidectomy and T_4 replacement on the in vitro release of testosterone in rat testicular interstitial cells in response to hCG and thyroid hormones. After collagenase digestion, the dispersed testicular interstitial cells (1 ± 10^6 cells/ml) were incubated either with or without hCG (0.5 IU/ml), T_3 (10^{-7} M) or T_4 (10^{-7} M) at 34°C for 1 h. Each value represents mean ± SEM. *, $^{**}P < 0.05$ and $P < 0.01$ as compared with the sham Tx rats, respectively. +,$++P < 0.05$ and $P < 0.01$ as compared with corresponding vehicle group, respectively.

release in the testicular interstitial cells of both the Tx and Tx + T_4 rats as compared with the vehicle control. The levels of testosterone response to forskolin, nifedipine and androstenedione were lower ($P < 0.05$ or $P < 0.01$) in the Tx group than in the sham Tx group. T_4 replacement increased the testosterone response to forskolin but did not alter that to androstenedione in the Tx rats.

Administration of forskolin in vitro increased the intracellular cAMP level in the sham Tx rats, but not in the Tx and Tx + T_4 rats (Fig. 10). The basal level of cAMP in rat testicular interstitial cells was lower in the Tx and Tx + T_4 rats than in the sham Tx animals. The intracellular cAMP level in response to forskolin was unchanged in the Tx rats, but decreased in the Tx + T_4 rats.

DISCUSSION

The results of this investigation indicate that a thyroidectomy in male rats decreased 1) the

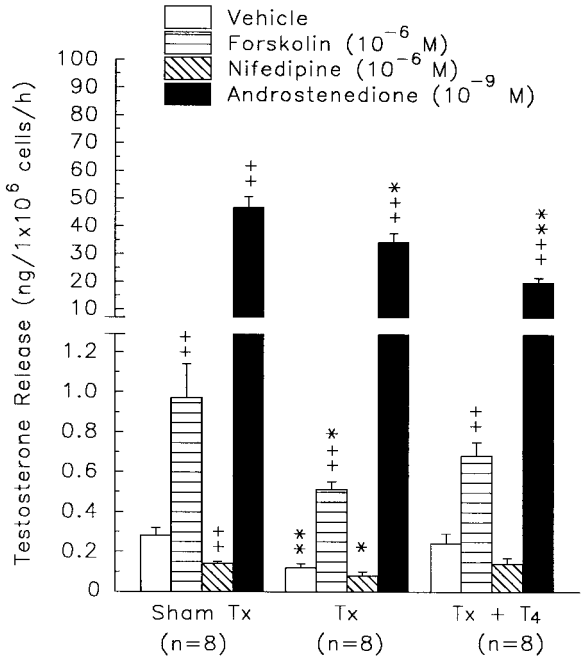

Fig. 9. Effects of the thyroidectomy and T_4 replacement on the in vitro release of testosterone in rat testicular interstitial cells in response to forskolin (10^{-6} M), nifedipine (10^{-6} M), and androstenedione (10^{-9} M). See Figure 8 legend for details. Each value represents mean ± SEM. *, **$P < 0.05$ and $P < 0.01$ as compared with the sham Tx rats, respectively. +, ++$P < 0.05$ and $P < 0.01$ as compared with corresponding vehicle group, respectively.

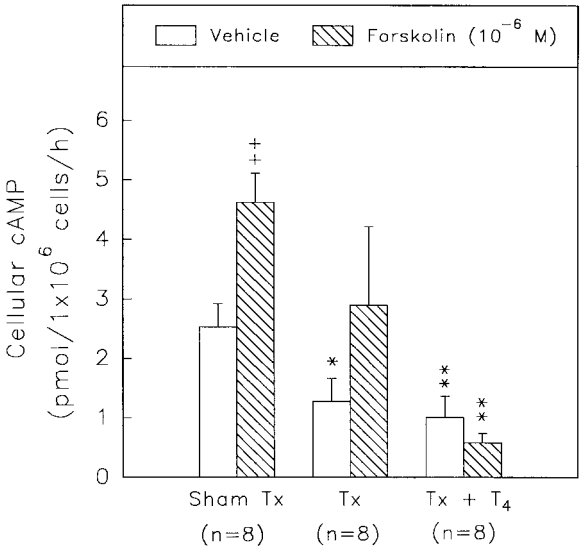

Fig. 10. Effects of the thyroidectomy and T_4 replacement on the concentration of cAMP in the cytosol samples after incubation of rat testicular interstitial cells either with or without forskolin (10^{-6} M) at 34°C for 1 h. Each value represents mean ± SEM. *, **$P < 0.05$ and $P < 0.01$ as compared with the sham Tx rats, respectively. ++$P < 0.01$ as compared with vehicle group, respectively.

secretion of GnRH, LH and testosterone, and 2) the production of cAMP, the activity of calcium channel and the activity of 17β-HSD in the testicular interstitial cells. T_4 replacement in the Tx rats restored the secretion of GnRH, LH and testosterone to euthyroid levels, but did not alter the production of cAMP , the activity of calcium channel, nor the activity of 17β-HSD in the testicular interstitial cells.

It has been well known that a thyroidectomy in male rats decreases the concentration of LH in both the anterior pituitary gland [Suzuki et al., 1978], and serum [Valle et al., 1985; Ruiz et al., 1989; Francavilla et al., 1991; Kirby et al., 1992; Van Haaster et al., 1992; Antony et al., 1995]. In this study, it was found that not only the basal level of plasma LH but also the spontaneous release of LH in vitro were lower in the Tx than in the sham Tx rats. Since the release of GnRH by the MBH and the concentration of LH in the AP were lower in the Tx than in the sham Tx rats, we submit that the reduction of basal level of plasma LH by a thyroidectomy in male rats is at least in part due to the diminished release of GnRH in MBH, and the decreased content of LH in the AP.

Our in vitro data indicates that not only the basal level but also the GnRH-stimulated release of LH was lower in the Tx than in the sham Tx rats. However, the maximal response of plasma LH to GnRH injection was higher in the Tx than in the sham Tx animals. The reason of greater response of plasma LH to GnRH in rats following a thyroidectomy is not known, but may be due to the lower metabolic clearance rate (MCR) of LH in the Tx rats as the description of lower MCR of prolactin in rats following thyroidectomy [Pu et al., 1987].

T_4 replacement in the Tx rats restored the GnRH release by MBH, the LH concentration in the AP, the basal and GnRH-stimulated level of plasma LH and in vitro release of LH in response to GnRH to euthyroid levels. However, the basal release of AP LH in vitro was not restored by T_4 replacement in vivo. It seems that the deficiency of T_4 was not the only reason for the reduction of LH release in rats following thyroidectomy.

Although unchanged [Cooke and Meisami, 1991; Cooke et al., 1992; Kirby et al., 1992] and decreased [Ruiz et al., 1989; Biswas et al., 1994; Antony et al., 1995] levels of serum testosterone have been reported in hypothyroid rats, it

was found that not only the basal level of plasma testosterone had decreased but also the response of plasma testosterone to GnRH and hCG had either decreased or was delayed in the Tx rats as compared with the sham Tx animals. T_4 replacement in the Tx rats restored both the basal and the GnRH- or hCG-stimulated levels of plasma testosterone to euthyroid levels. Inasmuch as a greater response of plasma LH at 30 and 60 min following GnRH challenge did not enhance the corresponding levels of testosterone in the Tx rats, however, other mechanisms, in addition to the deficiency of LH release are suggested to be involved in regulating testosterone secretion by thyroidectomy.

The in vitro results on this study of testosterone release in both testicular fragments and interstitial cells indicates that the thyroidectomy decreases both spontaneous and hCG-induced release of testosterone, and T_4 replacement in Tx rats restores the testosterone release to the sham Tx levels. These data confirms previous observations [Ruiz et al., 1989; Biswas et al., 1994; Antony et al., 1995]. The decreased number of LH receptors in hypothyroid rats [Hardy et al., 1993; Valle et al., 1985] may be responsible for the diminished response of testicular interstitial cells to hCG stimulation. Furthermore, the results demonstrate that administration of T_3 or T_4 at 10^{-7} M in vitro for only 1 h increased the basal release of testosterone in the testicular interstitial cells of the Tx rats, but not in the sham Tx and Tx + T_4 rats. Although it has been shown that adult rat testis is devoid of T_3 nuclear binding activity [Oppenheimer et al., 1974; Jannini et al., 1990], the present results demonstrate that thyroid hormones regulate testosterone production by acting directly on rat testicular interstitial cells in the Tx rats. These observations may also account for the restoration of plasma testosterone levels by T_4 replacement in Tx rats. It is likely that T_4 or T_3 receptors (probably membrane receptors) in the testicular interstitial cells can be stimulated by a thyroidectomy.

The reduced increase of testosterone release in response to forskolin in the testicular interstitial cells of the Tx rats reflects an inhibitory effect of the thyroidectomy on the activity of adenylyl cyclase. The basal level of cytosol cAMP was also lower in the Tx than in the sham Tx rats. Therefore, the reduction of the cAMP pathway in the testicular interstitial cells may account for the lower production of testosterone in rats following a thyroidectomy. T_4 replacement in the Tx rats was inadequate to increase the cAMP production in the testicular interstitial cells. The reason is not known, but possibly due to insufficiency of T_4 in the in vitro system.

Administration of nifedipine inhibited testosterone release by blocking the L-type calcium channels in the membrane of the testicular interstitial cells of the sham Tx rats. Therefore, the maintenance of optimal intracellular calcium concentration is involved in the production of testosterone by the testicular interstitial cells. Since the thyroidectomy caused a similar inhibitory effect on testosterone release as compared with the administration of nifedipine and no significant effect of nifedipine on testosterone release by the testicular interstitial cells in the Tx rats was found, we suggest that the inhibitory effect of the testicular interstitial cells is in part due to the inefficiency of L-type calcium channels. T_4 replacement in vivo did not alter the effect of nifedipine on testosterone release in the Tx rats.

Administration of androstenedione (10^{-9} M) increased testosterone release by testicular interstitial cells. A lesser conversion of androstenedione to testosterone in the testicular interstitial cells was found in both the Tx and Tx + T_4 rats as compared with the sham Tx rats. These data indicate that the activity of 17β-HSD in the testicular interstitial cells was inhibited by the thyroidectomy. T_4 seems not to be involved in the regulation of 17β-HSD activity in the Tx rats. The reduction of the activities of 3β-HSD and 17β-HSD in testicular interstitial cells by a thyroidectomy has been demonstrated by using different techniques [Biswas et al., 1994; Antony et al., 1995].

In summary, these results suggest that the following mechanisms are involved in the inhibitory effect by a thyroidectomy on the production of testosterone in the testicular interstitial cells: 1) the decreased secretion of GnRH and LH and LH response to GnRH; 2) the decreased response of the testicular interstitial cell to gonadotropin; and 3) the diminished production of cAMP, influx of calcium, and activity of 17β-HSD. Although T_4 did not alter the diminished cAMP pathway, calcium channel activity and the 17β-HSD activity in the testicular interstitial cells of the Tx rats, T_4 might enhance testosterone production by acting directly at the testicular interstitial cells of the Tx rats. This observation may account for the partial reversed effect of T_4 on testosterone production in the Tx rats.

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