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Effects of hypothyroidism on the estrous cycle and reproductive hormones in mature female rat☆

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Abstract

The present study was undertaken to examine the systemic role of thyroid hormones by analyzing changes in reproductive functions in hypothyroid female rats. Serum concentrations of triiodo-thyronine (T_3) significantly decreased 1 week after the initiation of propyl-thiouracil treatment or thyroidectomy. The estrous cycle became irregular 3 and 2 weeks after the initiation of propyl-thiouracil treatment and thyroidectomy, respectively. Serum luteinizing hormone (LH) levels significantly reduced in both groups on the day of diestrus-1 about 1 month later. Hypothyroid rat shows the high progesterone and low testosterone levels. No significant changes in inhibin and estradiol levels were detected. The serum levels of FSH decreased in the thyroidectomy group. The irregular estrous cycle and the above changes in hormone levels were recovered by administration of T_4. Compensatory secretions of FSH and LH induced by ovariectomy were enhanced by thyroidectomy and suppressed by T_4 treatment. These results suggest that thyroid hormones play a role in the regulation of reproductive hormones secretion in the cyclic rat ovary.

Keywords: Hypothyroidism; Thyroid hormone; Thyroxine; Ovarian hormone; Gonadotropin; Estrous cycle

1. Introduction

Hypothyroidism in women is clinically associated with menstrual disorders, menstrual irregularity, sterility, decreased ability of becoming pregnant and increased frequency of spontaneous abortions (Longcope, 1991; Stradtman, 1993). In adult rats, hypothyroidism influences normal follicular maturation of the ovary (Bruni et al., 1975; Hagino, 1971; Maruo et al., 1987) and gonadotropin secretion (Bruni et al., 1975), resulting in irregular estrous cycles (Mattheij et al., 1995) and a reduction of ovarian weight. An inadequate thyroid hormone supply caused by daily administration of 6-propyl-2-thiouracil (propyl-thiouracil) disturbs folliculogenesis (Dijkstra et al., 1996). Our recent studies have shown that thyroidectomy before puberty increases the number of antral follicles (Tamura et al., 1998a), but causes the blockage of equine chorionic gonadotropin-induced first ovulation, which is mainly due to the reduction of preovulatory luteinizing hormone (LH) surge (Tamura et al., 1998b). Although some reports have shown the appearance of irregular estrous cycles in hypothyroidism, few reports have systematically described the effect of hypothyroidism on the estrous cycle and reproductive hormones. In the present study, therefore, we examined how hypothyroidism influences the secretion of ovarian hormones and gonadotropin, and the estrous cycle in order to investigate the role of thyroid hormone on the reproductive system in cyclic female rats.

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2. Materials and methods

2.1. Animals

Cyclic female rats (6 weeks old) of the Wistar-Imamichi strain (Imamichi Institute for Animal Reproduction, Ibaraki, Japan) were maintained in an air-conditioned room (temperature: 23 ± 1 °C and humidity: $55 \pm 5\%$) under controlled lighting (12-h light/day schedule), with free access to food and water. The animal care and surgery protocols were approved by the Institutional Animal Care Committees at Tokyo University of Pharmacy and Life Science, in compliance with institutional guidelines for experimental animal care. Hypothyroidism was induced by daily drinking of water containing 0.02% 6-propyl-2-thiouracil (propyl-thiouracil: Wako, Osaka, Japan) or thyroidectomy at 0800 h on the day of diestrus (D)-2 around 8 weeks old.

2.2. Experimental schedule

To confirm propyl-thiouracil- or thyroidectomy-induced hypothyroidism, the serum levels of triiodo-thyronine (T_3) were measured at 1, 2, 4 and 6 weeks after propyl-thiouracil administration or thyroidectomy. Blood (1 ml) was collected via the tail vain under ether anesthesia at 0800 h at 1, 2 and 4 weeks after each treatment. Individual 7 ml blood sample was finally collected via the abdominal aorta under ether anesthesia at 0800 h 6 weeks later. Blood was allowed to clot at 4 °C. Serum, 1.5 ml of which was usually used for assays of all hormones, was separated by centrifugation and stored frozen at -80 °C until assay for each hormone. Vaginal smears were checked daily to monitor the changes in the estrous cycle between 0730 and 0800 h. The serum levels of gonadotropins, immunoreactive inhibin and ovarian steroids were measured on the day of D-1 about 1 month after the initiation of propyl-thiouracil treatment or thyroidectomy. To determine the effect of exogenous thyroid hormone, 5 μg of thyroxine (T_4; Sigma, St. Louis, MO, USA), which can maintain physiological levels of T_3 and T_4, was injected i.p. at 0800 h once daily for about 1 month from the next day after thyroidectomy to the day before final blood collection. To examine the direct action of thyroid hormone on gonadotropin secretion, the rats were ovariectomized immediately after thyroidectomy (ovariectomy–thyroidectomy rat). Some rats received a daily administration of T_4 (5 μg, i.p.) from the second day after ovariectomy–thyroidectomy treatment until the day before blood collection. Blood samples were collected about 2 weeks after ovariectomy and thyroidectomy to measure the serum levels of follicle stimulating hormone (FSH) and LH. The same experiment were repeated three times except Fig. 1 (time-course study in T_3 levels). The levels of each hormone are shown represent single experiment.

2.3. Radioimmunoassay (RIA) of triiodothyronine (T_3), gonadotropins, immunoreactive inhibin and ovarian steroids

Serum concentrations of immunoreactive inhibin, estradiol, progesterone and testosterone were measured by RIA using ^{125}I-labeled radioligands as described previously (Tamura et al., 1997). T_3 was also measured by double-antibody RIA. ^{125}I-labelled T_3 was purchased from ICN Biomedicals, Costa Mesa, CA, USA. Antisera against estradiol (GDN#244), progesterone (GDN#377) and testosterone (GDN#250) were kindly provided by Prof. G.D. Niswender (Colorado State University, Fort Collins, CO, USA). Antiserum to T_3 was kindly provided by Dr. M Suzuki at Gunma University, Gunma, Japan. The intra- and inter-assay coefficients of variation were 8.6% and 12.2% for immunoreactive inhibin, 4.7% and 6.6% for estradiol, 5.0% and 6.0% for progesterone and 5.9% and 5.8% for testosterone, 6.5% and 6.6% for T_3, respectively. Serum concentrations of FSH and LH were measured with a NIDDK RIA kit (provided by Dr. A. F. Parlow, Director, Pituitary Program and Antisera Center, Harbor-UCLA Medical Center, Torrance, CA, USA), using antisera against FSH (S-11) and LH (S-10). The results were calculated as a comparison for a standard curve using FSH-RP-2 or LH-RP-3. The intra- and inter-assay coefficients of variation were 5.5% and 7.1% for FSH and 8.9% and 10.0% for LH, respectively.

2.4. Statistical analysis

Data are represented as means ± S.E.M. The significance of the differences was tested with an analysis of variance with the Bartlett's test followed by the Student's *t*-test or

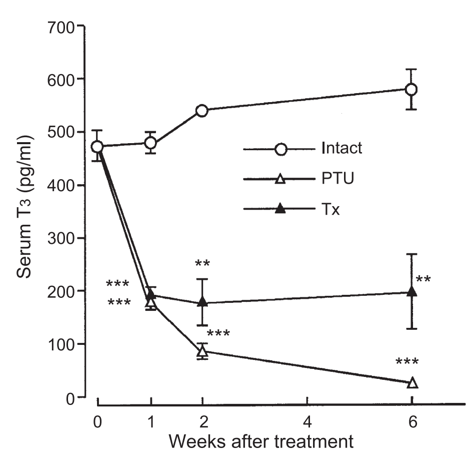

Fig. 1. Change in the serum levels of triiodothyronine (T_3) in propyl-thiouracil-administered or thyroidectomized female rats. Hypothyroidism was induced by daily drinking of water containing 0.02% propyl-thiouracil from 0800 h at diestrus-2 (D-2) or by thyroidectomy at 0800 h at D-2. Blood was collected 1, 2, and 6 weeks after these treatments. Each value shows the mean ± S.E.M. of five rats. $^{**}P<0.01$, $^{***}P<0.001$, significantly different from the intact group. PTU: propyl-thiouracil-treated rats, Tx: thyroidectomized rats.

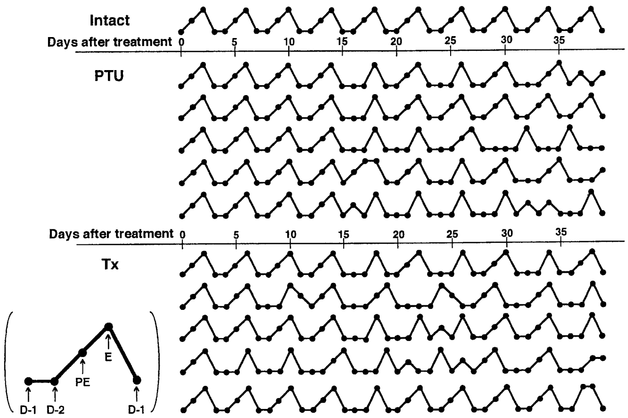

Fig. 2. Change in estrous cycle in propyl-thiouracil-administered or thyroidectomized female rats. The figure in parenthesis shows typical estrous cycle (diestrus-1: D-1, diestrus-2: D-2, proestrus: PE, estrus: E). PTU: propyl-thiouracil-treated rats, Tx: thyroidectomized rats.

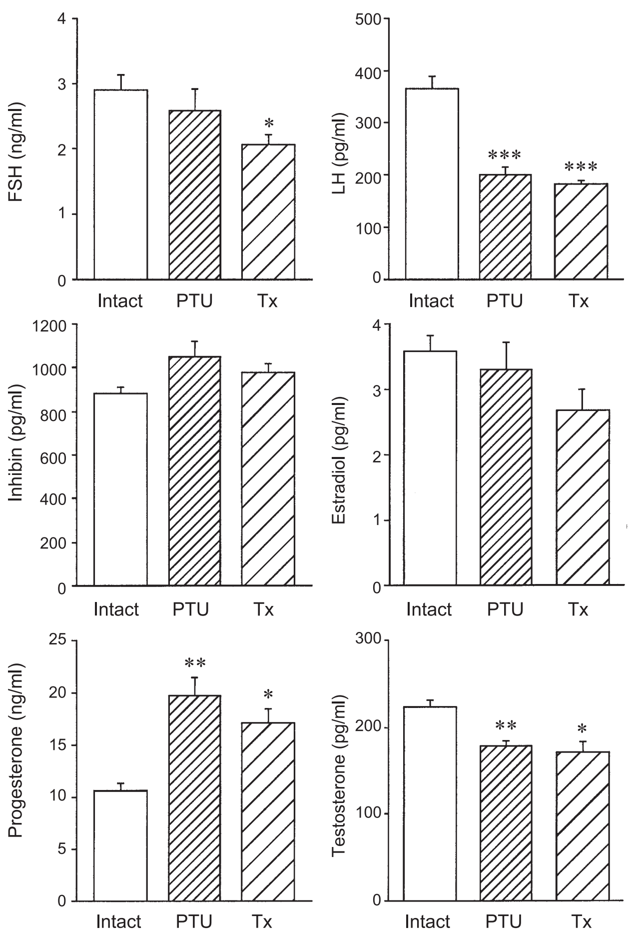

Fig. 3. Changes in the serum levels of gonadotropins, immunoreactive inhibin and ovarian steroids in propyl-thiouracil-administered or thyroidectomized female rats. Blood was collected at 0800 h on the day of diestrus-1 (D-1) about 1 month after the initiation of propyl-thiouracil treatment or thyroidectomy. Each value shows the mean ± S.E.M. of eight to nine rats. *$P<0.05$, **$P<0.01$, ***$P<0.001$, significantly different from the intact group. PTU: propyl-thiouracil-treated rats, Tx: thyroidectomized rats.

Cochran–Cox test (two-tailed). Differences were considered significant at $P<0.05$.

3. Results

Fig. 1 shows the changes in the serum levels of T_3 in propyl-thiouracil-administered or thyroidectomized female rats. There was no difference in the serum concentrations of T_3 among the intact, propyl-thiouracil and thyroidectomy groups before each treatment. In the propyl-thiouracil and thyroidectomy groups, the T_3 levels rapidly decreased 1 week after each treatment and the levels were significantly lower than those in the intact group. The serum levels of T_3 in the propyl-thiouracil group decreased to a greater extent in the period 2–6 weeks following the start of treatment, although no statistical difference was detected between the propyl-thiouracil and thyroidectomy groups. Thus, we confirmed that administration of propyl-thiouracil and thyroidectomy caused hypothyroidism in cyclic female rats. Fig. 2 shows the daily changes in the estrous cycle determined by the vaginal smear test. The estrous cycle in the intact group

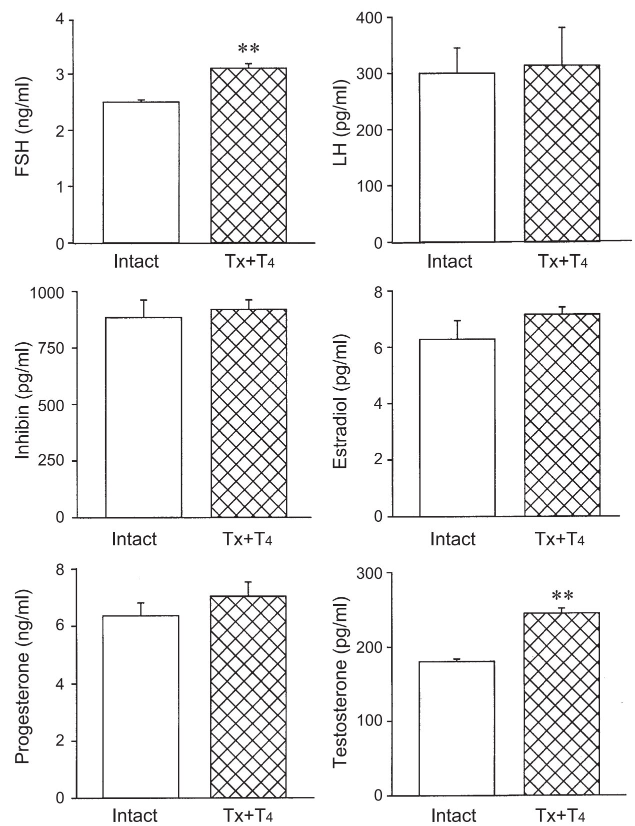

Fig. 4. Effects of thyroxine (T_4) on the serum levels of gonadotropins, immunoreactive inhibin and ovarian steroids in thyroidectomized female rats. Thyroidectomized rats were injected i.p. once daily with T_4 (5 μg) from the next day after thyroidectomy to the day before blood collection. Serum was collected at 0800 h at diestrus-1 (D-1) about 1 month after thyroidectomy. Each value shows the mean ± S.E.M. of five to eight rats. **$P<0.01$, significantly different from the intact group. Tx: thyroidectomized rats.

is shown in the figure in parenthesis as a typical pattern of estrous cycle. Intact animals had a regular cycle of 4 days. The estrous cycles in the propyl-thiouracil and thyroidectomy groups became irregular about 2–3 weeks after the initiation of propyl-thiouracil treatment and about 1–2 weeks after thyroidectomy. These irregular cycles never recovered to a normal estrous cycle until the end of this experiment.

Next we examined the effect of propyl-thiouracil or thyroidectomy treatment on the serum levels of gonadotropins and ovarian hormones. These levels were measured on the day of diestrus-1 (D-1) about a month after the initiation of propyl-thiouracil treatment or after thyroidectomy. The serum levels of FSH in the thyroidectomy group were lower than in the intact group (Fig. 3), whereas there were no differences in FSH levels between the propyl-thiouracil group and intact group. Serum LH levels were significantly reduced in both the propyl-thiouracil and thyroidectomy groups compared with the intact group. The serum levels of immunoreactive inhibin were almost the same among all groups. The serum levels of E_2 in the thyroidectomy group tended to decrease compared with those in the intact group. The serum levels of P_4 in both the propyl-thiouracil and thyroidectomy groups increased compared with those in the intact group, whereas testosterone levels in the propyl-thiouracil and thyroidectomy groups were significantly decreased.

To determine the effects of replaced-thyroid hormone on the serum levels of gonadotropin and ovarian hormones in the rats with thyroidectomy-induced hypothyroidism, thyroidectomized rats received a daily administration of T_4 (5 μg) for about 1 month from the next day after thyroidectomy to the day before blood collection. The estrous cycles in all animals that received T_4 (thyroidectomy + T_4 group) were normalized (data not shown). Marked decreases in FSH, LH and testosterone in the propyl-thiouracil or thyroidectomy group were reversed by administration of T_4, although the FSH and testosterone levels increased significantly more than the intact levels (Fig. 4). Enhanced levels of P_4 in the propyl-thiouracil and thyroidectomy groups were suppressed by T_4 treatment.

To clarify the action of thyroid hormone on the hypothalamus–pituitary axis regarding gonadotropin secretions, the rats with hypothyroidism were ovariectomized and serum FSH and LH levels were determined (Fig. 5). Both FSH and LH levels in ovariectomy rats were significantly increased compared with the intact group. Such compensatory secretions of gonadotropins were enhanced by ovariectomy–thyroidectomy, in contrast to the inhibition of LH levels seen in the animals with hypothyroidism (Fig. 3). The increased levels of FSH and LH were inhibited by T_4 treatment in the ovariectomy–thyroidectomy group, although the levels were still higher than the intact levels.

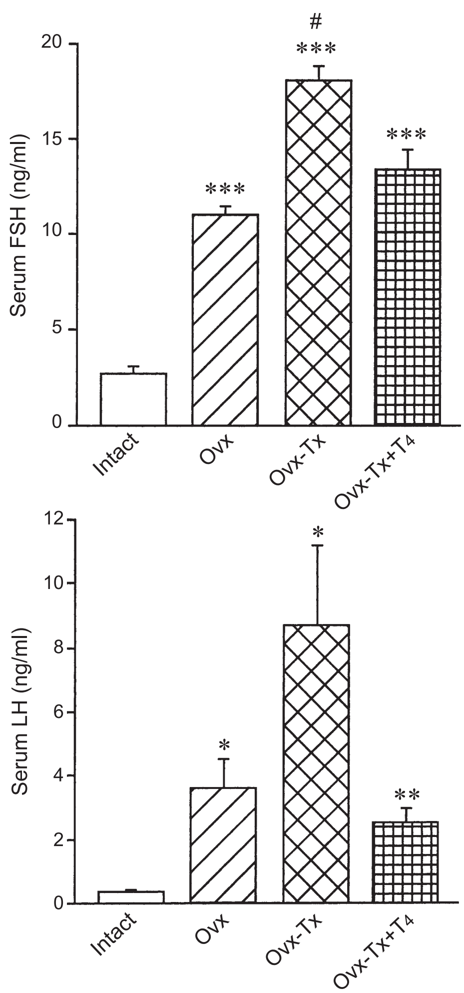

Fig. 5. Effects of thyroidectomy on the serum levels of gonadotropins in ovariectomized female rats. All animals were ovariectomized and thyroidectomy at the same time. They were injected i.p. once daily with thyroxine (T_4: 5 μg) from the next day after the operation. Blood was collected at 0800 h 2 weeks after the operation. Each value shows the mean ± S.E.M. of five to six rats. $*P<0.05$, $**P<0.01$, $***P<0.001$, significantly different from the intact group. $^{\#}P<0.05$, significantly different from the ovariectomized group. OVX: ovariectomized, Tx: thyroidectomized.

4. Discussion

In the present study, we found that hypothyroidism induced by propyl-thiouracil treatment or thyroidectomy caused irregular estrous cycles in rats. The irregular cycles were reversed by administration of T_4 for about 1 month after thyroidectomy. Thyroid hormone is probably important to maintain normal estrous cycles in rats. The serum levels of gonadotropin, particularly LH in hypothyroid rats, were lower than those in intact rats. The levels of testosterone were also significantly lower in hypothyroid rats. In contrast, the serum levels of P_4 in hypothyroid rats were higher than those in the intact group. The inhibitory effects of hypothyroid status on these hormones levels were more effective in the thyroidectomy group than the propyl-thiouracil group. This may be the reason thyroidectomy causes the irregular cycles earlier than propyl-thiouracil treatment.

In a previous report (Tamura et al., 1998b), we detected increased levels of prolactin on the day of first proestrus after thyroidectomy in immature rats. Several reports have shown that hypothyroidism induces increases in prolactin levels in experimental animals (Chen et al., 1976; Jahnke et al., 1980; Reymond et al., 1987). It has been clinically reported that many patients with hypothyroidism show hyperprolactinemia (Collu et al., 1977; Suter et al., 1978; Watanobe and Sasaki, 1995). The expression of thyrotropin-releasing hormone (TRH), which regulates both thyroid stimulating hormone (TSH) and prolactin secretions, may be influenced by thyroidectomy. The reduction of thyroid hormone in hypothyroidism probably enhances TRH secretion via negative feedback of the hypothalamus–pituitary axis. Prolactin is a luteotropic hormone (LTH) in the rodent (Astwood, 1941; Rothchild, 1981), and is well known to promote P_4 production. Furthermore, an increase in prolactin levels inhibits normal LH secretion in humans (Cheung, 1983), and reduces the pituitary response to LH releasing hormone (LHRH) in rats (Stradtman, 1993; Lu et al., 1976). The inhibition of basal LHRH secretion through the elevation of prolactin levels might have impaired LH secretion in this study. In addition to a possible direct role of prolactin on LH release at the hypothalamus–pituitary axis, our results suggested that elevation of the serum P_4 levels in hypothyroidism was caused by increases in prolactin from the pituitary. Furthermore, the serum concentration of TSH, which should be increased in hypothyroidism, may activate the LH receptor (Ryu et al., 1996) and therefore disturbance of the cycle may be created. P_4 is changed to inactive metabolites mediated via 20α-hydroxysteroid dehydrogenase (20α-HSD) (Ottenweller and Hedge, 1981). Decreases in the synthesis of 20α-HSD have also been shown in hypothyroid rats (Mattheij et al., 1995). Prolongation of the luteal phase induced by the suppression of P_4 catabolism might maintain high levels of P_4. The elevation of P_4 levels negatively affects gonadotropin secretion at the hypothalamus and pituitary levels, and it may result in decreases in basal gonadotropin levels (Taya et al., 1981).

The reduced serum LH levels probably decreased serum testosterone levels. Hypothyroidism is known to be closely associated with the inhibition of adrenal functions (Ottenweller and Hedge, 1981; Murakami et al., 1984). Daily rhythms of plasma corticosterone disappear in hypothyroid rats (Ottenweller and Hedge, 1981). Decreases in the serum levels of corticosterone increase the secretion of corticotropin-releasing hormone (CRH) in the hypothalamus. Because of the inhibitory action of CRH on LHRH secretion (Taya and Sasamoto, 1989), the basal levels of gonadotropin may decrease in hypothyroid rats. The levels of estradiol in the thyroidectomy group tended to decrease compared with those in the intact group. The reduction of estradiol levels might be due to a decrease in the responsiveness of ovarian granulosa cells to FSH (Maruo et al., 1987) or inhibition of FSH secretion induced by high levels of P_4. Such hormonal changes in hypothyroid rats were recovered to almost the same levels as those in the intact group by supplying exogenous T_4, indicating that T_4 plays a crucial role in the regulation of ovarian steroid secretion. The increased secretion of gonadotropin observed in ovariectomized animals was enhanced by thyroidectomy and then inhibited by T_4 treatment. This result indicates that thyroid hormone inhibits gonadotropin secretion directly by affecting the central nervous system. Supporting our findings, it has been reported that the pituitary response to LH release after LHRH infusion in hypothyroid rats is greater than that in normal rats (Kalland et al., 1978). The levels of LH released from the anterior pituitary in ovariectomy–thyroidectomy rats are higher than those in ovariectomy rats in vitro (Wang et al., 1980). Our data clearly demonstrated that thyroid hormones play an important physiological role in the regulations of ovarian and pituitary hormones secretion and of the estrus cycle in mature rats.

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