Influence of Thiouracil-induced Hypothyroidism on Adrenal and Gonadal Functions in Adult Female Rats

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(Received 6 August 1997/Accepted 1 December 1997)

ABSTRACT. The effect of hypothyroidism on adrenals and gonads in adult female rats was investigated throughout the estrous cycle. Hypothyroidism was induced by administration of 4-Methyl-2-Thiouracil (Thiouracil) in the drinking water. The weight of ovaries and adrenals, and the plasma levels of corticosterone decreased in hypothyroid rats as compared with euthyroid rats during the estrous cycle. Hypothyroidism resulted in decreased concentrations of plasma LH on the day of estrus and proestrus, whereas the plasma concentrations of prolactin and progesterone increased as compared with euthyroid rats. The weight of uteri and plasma concentrations of estradiol decreased during the day of estrus and proestrus in hypothyroid rats as compared with euthyroid rats. To further clarify the dysfunction of hypothalamic-hypophysial-adrenal axis in hypothyroid rats, animals were stressed by immobilization for 3 hr. In hypothyroid rats, a marked increase in plasma levels of ACTH in response to immobilization stress was observed compared to euthyroid control, whereas increases in plasma concentrations of corticosterone were much smaller in hypothyroid than euthyroid rats. These results clearly indicate that hypothyroidism causes both gonadal and adrenal disturbances in adult female rats. The increased concentrations of plasma prolactin may be due to hyposecretion of estrogen during the day of proestrus and estrus, which in turn result in disruption of the estrous cycle. — KEY WORDS: adrenal, gonad, hyperprolactinemia, hypothyroidism.

The lack of thyroid hormone has been known to produce reproductive abnormalities, including irregular menstrual or estrous cycle, amenorrhea and sterility in many species of female mammals [1, 9, 10, 19, 24, 31]. Thyroidectomy has been reported to cause decreased basal plasma levels of luteinizing hormone (LH) and follicle stimulating hormone (FSH), and a smaller LH surge on the day of proestrus [2, 4, 29]. Besides the reproductive disturbances, hypothyroidism results in decreases in adrenal weights and plasma concentrations of corticosterone [11, 21]. Although it has been reported that thyroid hormone affects both hypothalamic-hypophysial-gonadal and hypothalamic-hypophysial-adrenal axes [34, 35], the mechanism of relationship between these two axes and thyroid status in adult cyclic female rats is poorly understood. Most of the previous reports regarding the relationship between thyroid status and gonadal function have only mentioned the direct effects of hypothyroidism on the pituitary-gonadal axis in female animals. We have previously reported that hypothyroidism causes adrenal dysfunction directly and results in hyposecretion of ACTH from pituitary gland in adult male rats [35]. The adrenal dysfunction, may contribute to the inhibition of luteinizing hormone releasing hormone (LH-RH) secretion from hypothalamus, possibly mediated by excess corticotropin-releasing hormone (CRH) [35]. A recent study from our laboratory demonstrated that CRH and AVP release in median eminence increase in hypothyroid male rats [36]. We hypothesize that the hypothalamic-hypophysial-adrenal axis plays an important role in the gonadal dysfunction in hypothyroid adult not only male, but also female rats.

In the present study, we investigated the effects of hypothyroidism on these two axes throughout the estrous cycle in adult female rats.

MATERIALS AND METHODS

Animals and treatments: Adult female Wistar-Imamichi rats (The Imamichi Institute for Animal Reproduction, Ibaraki, Japan) were used throughout the present study. Animals were maintained on a 14 hr light and 10 hr dark lighting schedule (light on at 05:00 hr). The room was kept at 23–26°C. They received a standard laboratory diet and water ad libitum. Hypothyroidism was induced by administration of 0.03% 4-Methyl-2-Thiouracil (Thiouracil; Wako Pure Chemical Industries, Ltd. Osaka, Japan) in the drinking water. We have confirmed the effectiveness of Thiouracil in our previous study [35]. Each rat of all experimental group was confirmed vaginal smear every day during administration of Thiouracil (until 32 days after administration of Thiouracil for first group (20 rats) and 16 days after treatment for second group (160 rats). At the 4th estrous cycle (16 days after the initiation of Thiouracil treatment), 5 animals of each group (total 160 adult female rats) were sacrificed by decapitation every 6 hr throughout 4-day estrous cycle. Trunk blood was collected and centrifuged immediately and plasma was separated and stored at −20°C until assayed tri-iodothyronine (T3), thyroidine (T4), thyroid stimulating hormone (TSH), LH, FSH, prolactin (PRL), estradiol, progesterone, adrenocorticotropic hormone (ACTH) and corticosterone. After the decapitation, ovaries, uteri and adrenals were removed and weighed.

Effects of stress on hypothalamic-hypophysial-adrenal
axis in female hypothyroid rats: To investigate the effects of hypothyroidism on the response of pituitary and adrenal cortex to acute stress, animals were stressed by immobilization in a small disposable restrainer (Decapitcone BRAINTREE SCI ENTIFICINC., MA, U.S.A.) for 3 hr on the day of diestrus at 16 days after administration of Triouaricil. Twenty-four hours before stress experiment, a cannula (Dow Corning, Midland, MI, U.S.A.) was inserted into the right atrium via the external jugular vein in each rat for drawing blood samples. Blood (0.8 ml) was taken before the stress, 0.25, 0.5, 1, 2 and 3 hr after the restraint stress and 2 hr after release from stress. After separation of the plasma by centrifugation at 4°C, the red blood cells were resuspended in the same volume of 0.85% (W/V) NaCl solution (saline) and returned to the animal through the cannula.

Follicular maturation in hypothyroid female rats: To investigate the effects of hypothyroidism on follicular maturation, 10 i.u. human chorionic gonadotrophin (hCG, 2,200 IU/mg; Sankyo Zoki Co., Tokyo, Japan) dissolved in 0.2 ml 0.85% (W/V) NaCl were injected intravenously under ether anaesthesia at 17:00 hr on the day of metestrus, diestrus and proestrus. Animals were killed at 11:00 hr on next day by decapitation and the oviducts were examined for oocytes. hCG injection induces ovulation of only healthy mature follicles and the number of oocytes ovulated by hCG injection reflect the number of healthy mature follicles in the ovary.

Radioimmunoassy (RIA): Concentrations of TSH, LH, FSH and PRL in plasma were measured using NIDDK rat RIA kits for rat TSH, LH, FSH, and PRL. Hormones for iodination were rat TSH-I-9, rat LH-I-7, rat FSH-I-7 and rat PRL-I-5. The antisera used were anti-rat TSH-S-5, anti-rat LH-S-10, anti-rat FSH-S-11 and anti-PRL-S-6. Results were expressed in terms of NIDDK rat TSH-RP-2, LH-RP-2, FSH-RP-2 and PRL-RP-2. The intra- and inter-assay coefficients of variation were 6.6 and 7.9% for TSH, 5.5 and 13.9% for LH, 7.9 and 17.5% for FSH, 9.8 and 17.9% for PRL respectively.

ACTH [37], estradiol, progesterone, testosterone [33], and corticosterone [13] were measured by double-antibody RIAs using 125I-labelled radioligands as described previously. Antiser to testosterone and corticosterone were kindly provided by Dr. G. D. Niswender (Colorado State University, Fort Collins, CO, U.S.A.). The intra- and inter-assay coefficients of variation were 11.3 and 11.9% for ACTH, 7.8 and 10.3% for estradiol, 6.9 and 11.2% for progesterone, 9.5 and 16.4% for testosterone and 9.8 and 17.5% for corticosterone, respectively.

T3 and T4 were also measured by double-antibody RIAs as described previously [35]. The standard buffers for RIAs were 0.2 M glycine (pH 8.6) containing 0.128 M sodium acetate, 0.4% (W/V) gelatin and 1% (W/V) sodium salicylate for T3 or 2% (W/V) sodium salicylate for T4 (working buffer). Antiser to T3 and T4 were kindly provided by Dr. M. Suzuki (Gunma University, Maebashi, Japan). 125I-labelled T3 or T4 was purchased from ICN Biomedicals Inc., CA, U.S.A. The intra- and inter-assay coefficients of variation were 7.2 and 14.4% for T3 and 9.4 and 10.9% for T4, respectively. Sensitivities of these assays (defined as the amount of hormone that reduced binding to 85% of that occurring in the absence of unlabelled hormone) were 6.25 pg/tube for T3 and 2.5 pg/tube for T4.

Statistical analysis: All results are expressed as the mean ± S.E.M. The significance of the difference between two means was tested by Student's t-test but when more than two means were compared an analysis of variance was carried out and significance of the difference between means was determined by Duncan's multiple range test; a value of P<0.05 was considered significant. The effects of stress was analyzed using one-way analysis of variance (ANOVA), followed by tukey test; a value of P<0.05 was considered significant.

RESULTS

Effects of Triouaricil on thyroid function (Fig. 1): Plasma concentrations of T3 and T4 were obviously suppressed in adult female rats throughout the estrous cycle as a result of administration of Triouaricil for 16 days. On the other hand, plasma concentration of TSH was increased in female rats throughout the estrous cycle (Fig. 1).

Organ weights (Fig. 2): In hypothyroid female rats, the weights of adrenals significantly decreased as compared with euthyroid rats throughout the estrous cycle (Fig. 2-a). The weights of ovaries (Fig. 2-b) significantly decreased on the day of diestrus and the weights of uterus (Fig. 2-c) decreased between 17:00 hr on the day of diestrus and 17:00 hr on the day of proestrus in hypothyroid rats as compared with euthyroid rats.

Effects of Triouaricil on gonadal function (Fig. 3): Forty percent of hypothyroid rats (4 out of 10) showed irregular estrous cycles by 20 days (the 5th estrous cycle) after administration of Triouaricil and most of animals (90%) showed prolonged diestrus pattern by the 8th estrous cycle in the first group. All of hypothyroid rats showed regular estrous cycles until 16 days after administration of Triouaricil in the second group (n=160). Plasma concentrations of estradiol and testosterone decreased in hypothyroid rats between 17:00 hr on the day of diestrus and 17:00 hr on the day of proestrus as compared with euthyroid rats (Fig. 3-b,c). In hypothyroid female rats, the basal concentrations of plasma LH decreased on the day of diestrus and the day of proestrus (Fig. 3-a), whereas the plasma concentrations of prolactin and progesterone increased as compared with euthyroid rats (Fig. 3-d,e).

Effects of Triouaricil on adrenal function (Fig. 4): The plasma concentrations of corticosterone decreased in hypothyroid female rats throughout the estrous cycle as compared with euthyroid rats (Fig. 4-b). On the other hand, the plasma concentrations of ACTH were not different between hypothyroid and euthyroid rats (Fig. 4-a).

Follicular maturation in hypothyroid female rats (Table 1): The number of oocytes after hCG challenge was
Fig. 1. Plasma concentrations of TSH (a), T3 (b) and T4 (c) in control female rats (△) and hypothyroid rats (○) induced by administration of Thiouacil for 16 days. Values are means ± S.E.M. for 5 observations. P: Proestrus, E: Estrus, M: Metestrus and D: Diestrus. *P<0.05 (Student t-test) compared to control group.

Fig. 2. Weights of adrenal glands (a), ovaries (b) and uterus (c) in control female rats (△) and hypothyroid rats (○) induced by administration of Thiouacil for 16 days. Values are means ± S.E.M. for 5 observations. P: Proestrus, E: Estrus, M: Metestrus and D: Diestrus. *P<0.05 (Student t-test) compared to control group.

Effects of stress on hypothalamo-hypophysial-adrenal
Fig. 3. Plasma concentrations of LH (a), testosterone (b), estradiol (c), prolactin (d) and progesterone (e) in control female rats (△) and hypothyroid rats (●) induced by administration of Thioracil for 16 days. Values are means ± S.E.M. for 5 observations. *P<0.05 (Student t-test).

axis in female hypothyroid rats (Fig. 5): A marked increase in plasma concentrations of ACTH was observed in hypothyroid rats subjected to restraint stress (Fig. 5-a). whereas plasma concentrations of corticosterone were significantly lower in hypothyroid rats as compared with euthyroid controls (Fig. 5-b).
ADRENALS AND GONADS IN HYPOTHYROID RATS

DISCUSSION

Hypothyroidism caused irregular estrous cycles marked by prolonged diestrous periods in female rats. One reason for excessive diestrous smears in hypothyroid female rats is the hypersecretion of progesterone considered to secrete from ovary. Taya et al. [32] have reported that an injection of exogenous progesterone postponed the time of the preovulatory LH surge and ovulation by 24 hr. It has been also shown that the concentrations of plasma PRL from 15:00 hr through 23:00 hr on the day of proestrus remained consistently higher in 5-day cycling rats than in 4-day cycling rats [10] and progesterone secretion remained significantly higher during the day of diestrous in 5-day cycling rats than in 4-day cycling rats. Injection of a sufficient amount of antiprogestrone serum at 23:00 hr on the day of metestrus in 5-day cycling rats advanced

Time and stage of the estrous cycle

Fig. 4. Plasma concentrations of ACTH (a) and corticosterone (b) in control female rats (△) and hypothyroid rats (●) induced by administration of Thyroid for 16 days. Values are means ± S.E.M. for 5 observations. *P<0.05 (Student t-test).

Table 1. Induction of ovulation by 10 i.u. human chorionic gonadotrophin (hCG) given i.v. at 17:00 hr on metestrus, diestrus and proestrus during the estrous cycle of rat

<table>
<thead>
<tr>
<th>Stage of estrous cycle</th>
<th>No. of oocytes/ ovulation rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>hypothyroid</td>
</tr>
<tr>
<td>Metestrus</td>
<td>5.2 ± 0.8</td>
</tr>
<tr>
<td>Diestrus</td>
<td>12.6 ± 0.68</td>
</tr>
<tr>
<td>Proestrus</td>
<td>13.3 ± 1.00</td>
</tr>
</tbody>
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Hypothyroidism was induced by administration of Thyroid for 16 days. Animals were killed at 11:00 hr on next day and oviducts were examined for oocytes. Values are means ± S.E.M. for 5 observations. *P<0.05 (Student t-test) compared to control group.
ovulation and completion of the cycle by 1 day in the majority of animals [12]. In the present study, the plasma concentrations of PRL and progesterone increased in hypothyroid rats as compared with euthyroid rats. The increased plasma concentrations of progesterone is probably due to hypersecretion of PRL during the day of proestrus and estrus. Baksi [1] and Mattheiji et al. [19] also have shown that hypothyroidism caused irregular estrous cycles in adult female rats, though they have not mentioned the relationship among hyperprolactinemia, hypersecretion of progesterone and irregular estrous cycles in adult female hypothyroid rats. Previous reports regarding the effects of hypothyroidism on PRL secretion in female rats are conflicting and no study regarding to PRL secretion in hypothyroid adult female rats throughout estrous cycle. Wang et al. [39] have reported that T4 greatly strengthened the stimulatory effect of estradiol benzoate on PRL secretion. On the other hand, increased plasma concentrations of PRL in ovariectomized and thyroidectomized rats with estrogen treatment also has been shown previously [23, 41]. It is possible that such a discrepancy was mainly due to the difference of the estrogen treatment. Pan and Chen [23] used a long-acting estrogen polyestradiol phosphate in their study. Furthermore, it has been reported that hypothyroidism results in increases in both peptides and mRNAs levels of vasopressive intestinal polypeptides (VIP), as a PRL releasing factor in the anterior pituitary [16, 30]. We have also confirmed that pituitary content of VIP increased in hypothyroid adult female rats (A. Tohei & J. L. Voogt, unpublished data). This increased levels of pituitary VIP may act as a paracrine regulator of PRL release. In the present study, we clearly demonstrated that hypothyroidism caused hyperprolactinemia in adult female rats without estrogen treatment.

We confirmed that hypothyroidism results in a decrease in adrenal weights and the plasma concentrations of corticosterone. These results agree with the previous observations [20, 21, 35]. In the stress experiment, we observed a marked increase in plasma concentrations of ACTH in hypothyroid rats, whereas, in contrast to the ACTH response, the increase in plasma concentrations of corticosterone in response to immobilization stress was much smaller in hypothyroid than in control rats. These findings clearly indicate that hypothyroidism causes adrenal dysfunction directly, which contributes to the hypersecretion of ACTH. It is possible to assume that the hypersecretion of ACTH in hypothyroid rats is due to the hypersecretion of CRH by reduction of the negative feedback effect of glucocorticoid from adrenal cortex. We recently demonstrated that CRH and AVP release as estimated with push-pull perfusion in median eminence increase in hypothyroid male rats [36]. Recent studies have reported that hyperprolactinemia also caused hypersecretion of CRH in vivo [15] and in vitro [40]. Murakami et al. [21] have shown results opposite to the present result regarding ACTH secretion in hypothyroid female rats. They have reported that thyroidectomy resulted in decreases in plasma and pituitary concentrations of ACTH. On the other hand, Kamilaris et al. [11] reported that pituitary responses to exogenous ovine CRH for ACTH release were exaggerated in thyroidectomized male rats. Our present results were supported by Kamilaris's results. The reason for the discrepancy between our results and those of Murakami's result is not clear at the present time.

The basal concentrations of plasma LH decreased between 17:00 hr on the day of diestrus and 05:00 hr on the day of proestrus in this study. A previous report has also shown that hypothyroidism was associated with decreased basal concentrations of plasma LH in female rats [2]. Elevated concentrations of plasma progesterone is known to inhibit the secretion of gonadotrophin releasing hormone (GnRH) and gonadotrophin [5, 32]. Therefore, as long as the plasma concentrations of progesterone remain elevated, follicular growth will be retarded and small LH surges will occur. It also has been reported that hyperprolactinemia can reduce the secretions of LH [8, 14] and GnRH [3, 14, 38]. Recent reports have suggested that CRH is probably involved in the suppressed secretion of LH during hyperprolactinemia [7, 15]. Considering that hypothyroidism causes hypersecretion of PRL, decreases plasma concentrations of corticosterone, and results in hypersecretion of ACTH, the secretion of CRH is probably increased in hypothyroid female rats. One of the main factors recognized to exert a potent inhibitory influence on GnRH secretion is CRH [25-28]. It has been shown that CRH inhibits the in vitro release of GnRH at the level of neurosecretory terminals in the median eminence [6]. There is also an immunocytochemical evidence for direct synaptic connections between CRH and GnRH containing neurons [17]. Considering the present data in conjunction with the previous reports, we assume that increased CRH secretion is probably one of the reasons for the inhibition of LH secretion observed in hypothyroid female rats.

We found that hypothyroidism resulted in a decrease in ovarian weight on the day of diestrus. The number of oocyte after hCG challenges was significantly lower on the day of diestrus in hypothyroid rats as compared to euthyroid rats, though there is no difference the number of oocyte on the day of proestrus between hypothyroid and control rats. The concentrations of plasma estradiol and testosterone, a precursor of estradiol were also suppressed in hypothyroid rats between 17:00 hr on the day of diestrus and 17:00 hr on the day of proestrus as compared with euthyroid rats. These findings suggest that hypothyroidism interferes with follicular maturation and results in a decrease in plasma concentrations of estradiol. Previous reports also have shown that hypothyroidism caused a decrease in ovarian weight [1, 22, 24] and inhibited follicular maturation [22, 24]. Hypothyroidism is also known to inhibit FSH-induced aromatase activity of cultured porcine granulosa cells [18]. It is likely that the decrease in uterine weight in hypothyroid female rats was a result of decreased levels of plasma concentrations of estradiol.

In conclusion, hypothyroidism causes gonadal and adrenal
disturbances in adult female rats. The increased level of plasma concentrations of progesterone is probably due to hypersecretion of prolactin during the day of proestrus and estrus, which in turn causes prolonged periods of diestrus and suppression of LH secretion. The adrenal dysfunction probably leads to hypersecretion of CRH from hypothalamus and results in stimulation of ACTH secretion from the anterior pituitary gland. Therefore, suppression of LH secretion in hypothyroid female rats may be also mediated by the inhibitory effects of increased CRH secretion.

ACKNOWLEDGEMENTS. We wish to express our gratitude to Dr. J. L. Voogt, Department of Physiology, University of Kansas Medical Center, KS, U.S.A. for reading the original manuscript and his valuable suggestions. We are grateful to the Rat Pituitary Hormone Distribution Program, NIDDK, NIH, Bethesda, MD, U.S.A. for providing RIA materials of rat LH, FSH, TSH and prolactin; Dr. G. D. Niswender, Animal Reproduction and Biotechnology Laboratory, Colorado State University, Fort Collins, CO, U.S.A. for providing antisera to corticosterone (GDN 377), progesterone (GDN 337), oestradiol (GDN 244) and testosterone (GDN 250); Dr. M. Suzuki, Gunma University, Maebashi, Japan for antisera to T3 and T4; Dr R. Hokao, The Imamichi Institute for Animal Reproduction, Ibaraki, Japan for Wistar rats. This work was supported by a grant-in-aid for Scientific Research from the Ministry of Education of Japan No. 0660375. A. Tohei received a fellowship from the Japan Society for the Promotion of Science (JSPS).

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