Effects of Thyroidectomy or Thiouracil Treatment on Copulatory Behavior in Adult Male Rats

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ABSTRACT. Male copulatory behavior and the function of the hypothalamo-hypophysial-gonadal axis in hypothyroid male rats were investigated in the present study. Hypothyroidism was induced by thyroidectomy or thiouracil. In male copulatory behavior test, intromission latencies in hypothyroid rats were significantly longer than those in euthyroid rats and ejaculation frequencies were reduced in hypothyroid male rats compared to control rats without reduction of plasma concentrations of testosterone. These changes in copulatory behavior in hypothyroid male rats were restored to control levels by administration of T4 (5 μg/rat). Hypothyroidism decreased adrenal weights, and basal and peak concentrations of corticosterone during diurnal variation, whereas it increased peak concentrations of ACTH in adult male rats. These results indicate that hypothyroidism causes adrenal dysfunction directly and results in hypersecretion of ACTH. The adrenal disturbance observed in hypothyroid rats may affect male copulatory behavior. — KEY WORDS: copulation, corticosterone, CRH, hypothyroidism, male.

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The effects of hypothyroidism on female gonadal functions are well established. Hypothyroidism has been known to produce an irregular menstrual or estrous cycle, amenorrhea and sterility in the female of many species [2, 24]. On the other hand, the direct effects of thyroid hormone on human and rat adult testes have never been demonstrated because thyroid hormone receptors are not present in adult human and rat testes [7–9]. However, in human clinical research, there are some reports regarding male sexual behavior in thyroid disease; an increase in libido is described in male thyrotoxicosis, while hypothyroidism is associated with diminished libido and impotence [10, 12, 31], without a concomitant decrease in plasma concentration of testosterone. Furthermore, it has been reported that myxedema can be an important cause, in up to 5% of patients, of penile erectile dysfunction [22]. The reason for these sexual symptoms in hypothyroid adult human is not clearly understood and there are few reports concerning copulatory behavior of hypothyroid males in experimental animal models.

The regulation of masculine sexual behavior is a complex process involving several neurochemicals including dopamine [30], opioid peptides [29, 30], corticotropin releasing hormone (CRH) [21], and gonadotrophin releasing hormone (GnRH) [1, 4, 15]. The medial preoptic area (MPOA) is known to be critically important for male sexual behavior in various species [3, 20], and there are numerous peptide-containing cell bodies and fibers including CRH, β-endorphin and GnRH within MPOA [3]. CRH, in addition to its stimulatory action on the pituitary-adrenal axis, exerts marked inhibitory effects on the reproductive function [17–19], and immunocytochemical evidence for a direct synaptic connection between CRH- and GnRH-containing neurons in the preoptic area has been presented [13]. CRH has also been known to produce a dose-dependent suppression of masculine sexual behavior in male rats [21].

We have previously reported that hypothyroidism causes adrenal dysfunction directly and leads to hypersecretion of adrenocorticotropic hormone (ACTH), possibly mediated by excess CRH [27]. Also preliminary findings from our laboratory have demonstrated that CRH and AVP release in the median eminence increase in hypothyroid male rats (A. Tohei, G. Watanabe, K. Taya, unpublished observations).

In this study, we investigated the effects of hypothyroidism on the copulatory behavior and the hypothalamo-hypophysial-adrenal axis in adult male hypothyroid rats.

MATERIALS AND METHODS

Animals and induction of hypothyroidism: Thirty four adult male rats (350–400 g) of Wistar-Imamichi strain were used. The animals were maintained on a 14 hr light and 10 hr dark lighting schedule (light on at 05:00 hr) at 23–26°C. They received a standard laboratory diet and water *ad libitum*. One group of 5 or 6 rats was used in each experiment. Hypothyroidism was produced by surgical thyroidectomy or the administration of 0.03% 4-methyl-2-thiouracil (Thiouracil; Wako Pure Chemical Industries Ltd., Osaka, Japan) in their drinking water for 2 or 4 weeks. Thyroid hormone replacement to thyroidectomized rats was performed daily by intraperitoneal (i.p.) injection of L-thyroxine (T4; 5 μg per injection, SIGMA CHEMICAL Co., St. Louis, U.S.A.) for the two weeks before the experiment. T4 was dissolved in 0.01N-NaOH and added to the same volume of propylene glycol to adjust the concentration of T4 solution (5 μg/0.2 m*l*).

Copulatory behavior tests: Tests of copulatory behavior were conducted between 20:00–22:00 hr under dim illumination from a 25 W red bulb, by direct observation of

males paired with proestrous female rats in semi-circular aquariums with sawdust bedding on the floor. Four group of animals (sham-operated control, thyroidectomized, Thiouracil treated and thyroidectomized+T4 treated rats) were used in this experiment. Four weeks after thyroidectomy, administration of Thiouracil, or 2 weeks after replacement of T4 to thyroidectomized rats, each male was allowed an adaptation period of 5 min prior to introduction of the stimulus female. All subjects were sexually experienced before induction of hypothyroidism. Upon introduction of the stimulus female, each male was scored for the number of mounts, intromissions and ejaculations. Subsequently, the following 6 parameters were calculated for 30 min of behavioral tests: (1) the mount latency (ML), time from introduction of female to first mount; (2) intromission latency (IL), time from induction of female to first intromission; (3) ejaculation latency (EL), time from first intromission of each ejaculatory series to ejaculation; (4) mount frequency (MF), number of mounts per each ejaculatory series; (5) intromission frequency (IF), number of intromissions per each ejaculatory series; (6) ejaculation frequency (EF), number of ejaculations during test period. The animals were killed by decapitation after the copulatory tests, and their blood was collected and centrifuged for the determination of plasma levels of tri-iodothyronine (T3), T4, thyroid stimulating hormone (TSH), corticosterone and testosterone. After the decapitation, testes and adrenal glands were removed and weighed.

Hypothalamo-hypophysial-adrenal axis in hypothyroid male rats: To investigate the effects of hypothyroidism on diurnal variation of ACTH and corticosterone, blood samples (200 μl) were drawn every 7.5 min for 4 hr, beginning at 18:00 hr (1 hr before light off) 2 weeks after administration of Thiouracil. Twenty-four hours before the experiment, a cannula (Dow Corning, Midland, MI, U.S.A.) was inserted into the right atrium via the external jugular vein in each rat under ether anesthesia for drawing blood samples. Blood (200 μl) was withdrawn through the atrial cannula into a heparinized syringe without anesthesia. 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. Basal or peak point was defined as a lowest or the highest concentration of plasma corticosterone or ACTH in each animal.

Radioimmunoassay (RIA): Concentrations of TSH were measured using NIDDK rat RIA kits for rat TSH. The hormone for iodination was rat TSH-I-9. The antiserum used was anti-rat TSH-S-5, and the results were expressed in terms of NIDDK rat TSH-RP-2. The intra- and inter-assay coefficients of variation were 6.6% and 7.9% for TSH. T3, T4 [27], ACTH [28], testosterone [26] and corticosterone [11] were measured by double-antibody RIAs using ^{125}I-labeled radioligands as described previously. Antisera to testosterone (GDN250) and corticosterone (GDN377) were kindly provided by Dr. G. D. Niswender (Colorado State University, Fort Collins, Co, U.S.A.). Antisera to T3 and T4 were kindly provided by Dr. M Suzuki (Gunma University, Gunma, Japan). The respective intra- and inter-assay coefficients of variation were 7.2% and 17.4% for T3, 9.4% and 10.9% for T4, 11.3% and 11.9% for ACTH, 9.5% and 16.4% for testosterone and 9.8% and 17.5% for corticosterone.

Statistical analyses: All results are expressed as the mean ± SEM. The data was analyzed by Student's *t*-test but when more than two means were compared, analysis of variance was carried out and the significance of the difference between the means was determined by Duncan's multiple range test [23]. The data of copulatory behavior were analyzed using Mann Whitney U tests.

RESULT

Copulatory behavior tests: Hypothyroidism induced by thyroidectomy or administration of Thiouracil caused a significant reduction in EF whereas administration of T4 in thyroidectomized rats restored it to control levels. Intromission latencies in hypothyroid rats were significantly longer than in control rats and these prolonged latencies were recovered to the control levels by administration of T4. There were no significant differences in ML, EL, MF and IF between thyroidectomized, Thiouracil-treated, thyroidectomized and T4 treated and control rats (Fig. 1).

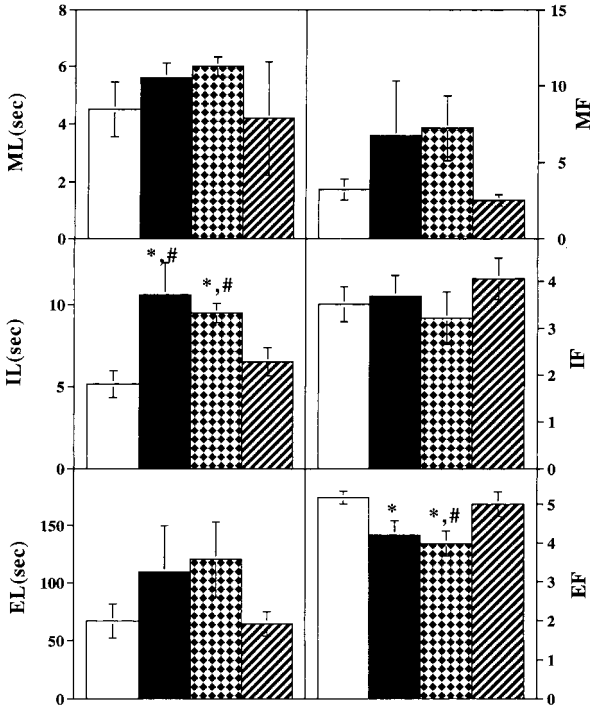

Fig. 1. Components of male copulatory behavior including (a) ML, (b) IL, (c) EL, (d) MF, (e) IF and (f) EF in control (open bars), 4 weeks thyroidectomized (solid bars), 4 weeks thiouracil treated (diamond bars) and thiouracil and T4 (2 weeks) treated (hatched bars) rats. Each bar represents the mean ± S.E.M. of five animals. Asterisks indicate $P<0.05$ compared with the value for intact control, and # indicates $P<0.05$ compared with the value for T4 treated control (Mann-whitney U tests).

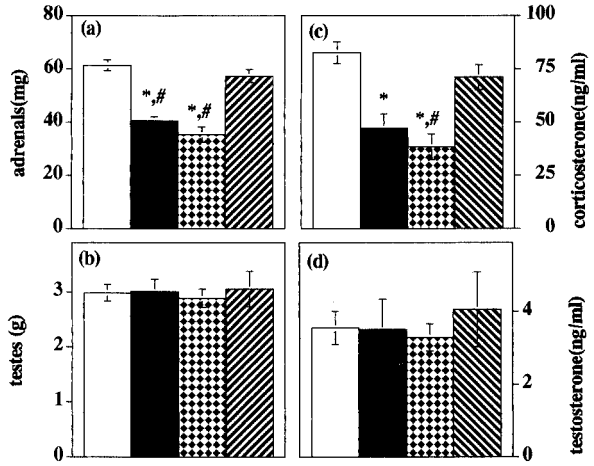

Fig. 2. Weights of adrenal glands (a) and testes (b), and plasma concentrations of corticosterone (c) and testosterone (d) in control (open bars), 4 weeks thyroidectomized (solid bars), 4 weeks thiouracil treated (diamond bars) and thiouracil and T4 (2 weeks) treated (hatched bars) rats. Each bar represents the mean ± S.E.M. of five animals. Asterisks indicate $P<0.05$ compared with the value for intact control, and # indicates $P<0.05$ compared with the value for T4 treated control (Student *t*-test).

Plasma concentrations of T3 (control 468 ± 24 *p*g/m*l*, thyroidectomy 70.45 ± 4.09 *p*g/m*l*, Thiouracil 55.4 ± 5.89 *p*g/m*l*), and T4 (control 35.8 ± 2.8 *n*g/m*l*, thyroidectomy 8.2 ± 3.09 *n*g/m*l*, Thiouracil 4.4 ± 2.85 *n*g/m*l*) were markedly suppressed in rats as a result of thyroidectomy or administration of Thiouracil for 4 weeks. On the other hand, plasma concentration of TSH (control 1.14 ± 0.24 *n*g/m*l*, thyroidectomy 20.8 ± 4.09 *n*g/m*l*, Thiouracil 23.8 ± 2.65 *n*g/m*l*) in hypothyroid rats was significantly increased. Adrenal weights and plasma concentrations of corticosterone were significantly lower in hypothyroid rats when compared with control rats (Fig. 2), though there were no significant difference of testicular weights and plasma concentrations of testosterone between hypothyroid and control rats (Fig. 2). Adrenal weights and plasma concentrations of corticosterone in hypothyroid rats recovered to control levels after administration of T4 (Fig. 2).

Hypothalamo-hypophysial-adrenal axis in hypothyroid male rats: All of the animals showed circadian variations of plasma corticosterone and ACTH. Basal concentrations of plasma corticosterone and ACTH were observed around 18:00–18:30 hr, and peak concentrations of plasma corticosterone and ACTH were observed at 1 hr (19:00–20:00 hr) after light off in the experimental periods (18:00–22:00 hr). Both basal and peak concentrations of corticosterone in the plasma were lower in hypothyroid rats induced by administration of Thiouracil for 2 weeks than in euthyroid rats. However, peak levels of plasma ACTH were higher in hypothyroid than in euthyroid rats, though there was no significant difference in basal levels of ACTH between hypothyroid and control rats (Fig. 3).

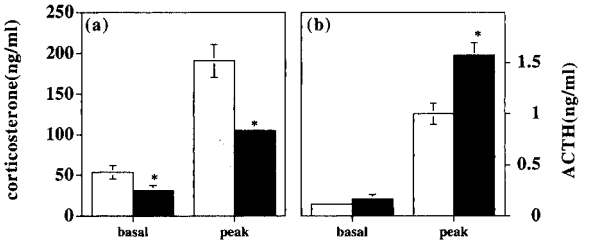

Fig. 3. Plasma concentrations of corticosterone and ACTH during evening variation (18:00 hr–22:00 hr) in control (open bars) and hypothyroid (solid bars) rats. Basal point was defined as a lowest level of plasma corticosterone (a) or ACTH (b) in each animal before light off (18:00 hr–19:00 hr). Peak point was defined as a highest concentration of plasma corticosterone (a) or ACTH (b) in each animal after light off (19:00 hr–22:00 hr). Each bar represents the mean ± S.E.M. of five animals. Asterisks indicate $P<0.05$ compared with the value for the respective control (Student *t*-test).

DISCUSSION

Plasma concentrations of ACTH and corticosterone have been known to exhibit a well characterized circadian rhythm in rats, with peak levels in the evening and lowest levels in the morning [5]. In the present study, adrenal weights and diurnal variations of plasma corticosterone were decreased in hypothyroid rats when compared with euthyroid rats. In contrast to evening variation of corticosterone, the variations of ACTH were increased in hypothyroid rats when compared with euthyroid rats, though the basal levels of plasma ACTH were not different between the two groups. We have previously reported that 1) adrenal responsiveness to ACTH decreased in hypothyroid rats, 2) pituitary responsiveness to CRH for ACTH release and pituitary contents of ACTH also increased in hypothyroid male rats and the change of pituitary responsiveness recovered to control levels after administration of T4, and 3) under the stress condition, a marked increase in plasma levels of ACTH in hypothyroid rats was observed, whereas, the increase in plasma level of corticosterone in response to immobilization stress was much smaller in hypothyroid than in control rats [27]. In the present study, we confirmed that diurnal variations of plasma corticosterone and ACTH are also disturbed in hypothyroid rats. Our observations suggest that hypothyroidism causes adrenal dysfunction directly and results in hypersecretion of ACTH, possibly mediated by CRH.

The present results indicate that hypothyroidism induced by thyroidectomy or administration of Thiouracil produces deficits in male copulatory behavior including increased IL and decreased EF without reduction of plasma concentrations of testosterone. It has been reported that hypothyroidism is associated with diminished libido and impotence in humans [10, 12, 31]. The cause of these sexual symptoms is unknown and the direct effects of thyroid

hormone on human and rat adult testes have never been demonstrated because thyroid hormone receptors are absent in testes of these species [7–9]. The regulation of masculine sexual behavior is a complex process involving several neurochemicals, and many previous reports demonstrated that GnRH, CRH and opiate peptides affect male sexual behavior in rats. Investigations into the neural control of male copulatory behavior have focused on forebrain pathways and the MPOA appears to be an integral component of this system [3]. There are numerous peptide-containing cell bodies and fibers including CRH, β-endorphin and GnRH within MPOA [20]. GnRH has been known to stimulate copulatory behavior in male rats [1, 4, 15]. It has been reported that acute microinfusions of CRH into the third cerebral ventricle of male rats produced a dose-dependent suppression of masculine sexual behavior and that a low dose of β-endorphin also inhibited masculine copulatory activity in rats when injected into the lateral ventricle [14] or MPOA [6, 30]. We have previously reported that hypothyroidism causes adrenal dysfunction and leads to hypersecretion of ACTH [27]. We have also confirmed that CRH release in ME increased more in hypothyroid male rats than in euthyroid rats (Tohei A, Watanabe G, Taya K, unpublished observation). Majority cell bodies of CRH neurons which project to ME exist in the paraventricular nucleus of the hypothalamus [25], but CRH neurons in the other nucleus of the hypothalamus, including MPOA also, may be activated in hypothyroid male rats. Considering the present data and previous observations at our laboratory, we propose that increased levels of CRH from the hypothalamus suppress copulatory behavior in hypothyroid adult male rats. CRH has been also known to increase the β-endorphin release [16] and CRH neurons have a direct synaptic connection to the GnRH neuron within MPOA [13]. β-endorphin and GnRH also may be involved in the process of suppressed copulatory behavior in hypothyroid male rats.

In the present study, hypothyroidism suppressed male copulatory behavior in rats without reducing plasma concentrations of testosterone. Hypothyroidism also causes adrenal dysfunction directly and results in hypersecretion of ACTH. The hypersecretion of ACTH is probably mediated by hypersecretion of CRH in hypothyroid rats. Deficits in male copulatory behavior in hypothyroid male rats may be at least, partly, mediated by CRH.

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