Studies on the Functional Relationship between Thyroid, Adrenal and Gonadal Hormones

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Abstract. In order to clarify the functional relationship between thyroid, adrenal and gonadal hormones, hypothyroidism was induced by administration of thiouacil in adult male and female rats, and the effects of hypothyroidism on the adrenal and the gonadal axes were investigated in the present study. 1. The functional relationship between thyroid and adrenal hormones: Adrenal weights and corticosterone were lowered, whereas the secretion of ACTH, corticotrophin-releasing hormone (CRH) and arginine vasopressin (AVP) increased in hypothyroid rats compared to euthyroid rats. These results indicate that hypothyroidism causes adrenal dysfunction directly and results in hypersecretion of CRH and AVP from the hypothalamus. 2. The functional relationship between thyroid and gonadal hormones: The pituitary response to LHRH was lowered, whereas the testicular response to hCG was not changed in hypothyroid rats. Hypothyroidism suppressed copulatory behavior in male rats. These results suggest that hypothyroidism probably causes dysfunction in gonadal axis at the hypothalamic-pituitary level in male rats. In adult female rats, hypothyroidism inhibited the follicular development accompanied estradiol secretion, whereas plasma concentrations of progesterone and prolactin (PRL) increased in hypothyroid female rats. Hypothyroidism significantly increased the pituitary content of vasoactive intestinal peptide (VIP) though it did not affect dopamine synthesis. These results suggest that hypothyroidism increases pituitary content of VIP and this increased level of VIP likely affects PRL secretion in a paracrine or autocrine manner. In female rats, inhibition of gonadal function in hypothyroid rats mediated by hyperprolactinemia in addition to hypersecretion of endogenous CRH.

Key words: Hypothyroidism, Adrenal, Gonad, Corticotrophin-releasing hormone (CRH), Prolactin (PRL)

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hypothyroid rats.

**Induction of Hypothyroidism and the Effects of Hypothyroidism on Adrenal, Testes and Pituitary**

There are several chemical compounds called "goitrogen", including methimazole and propylthiouracil or methylthiouracil. In the present study, 4-Methyl-2-Thiouracil (thiouracil : Wako Pure Chemical Industries, LTD. Osaka, Japan) was chosen for the induction of hypothyroidism. It has been reported that thiouracil showed the antithyroid activity resulting from its interference with the iodination of thyroxine precursors and inhibited the conversion of thyroxine (T4) to tri-iodothyronine (T3) in extrathyroidal tissue [5] in addition to its direct action on the thyroid gland. To confirm the effects of thiouracil on thyroid function, plasma concentration of T3, T4 and thyroid stimulating hormone (TSH) were examined in adult male rats. Adult male (350–400 g) rats of Wistar strain were used and thiouracil (0.03%) was added in the drinking water.

As a result of administration of thiouracil, plasma concentrations of T3 and T4 were markedly suppressed in male rats by 2 weeks (Fig. 1). On the other hand, plasma concentration of TSH in hypothyroid rats was significantly increased by 1 week after administration of it and continued to increase depending on the duration of its administration (Fig. 1). From these data, the duration of thiouracil administration in male rats was determined for 2 weeks. In hypothyroid male rats, adrenal weight and the plasma concentration of corticosterone were significantly lower in hypothyroid rats compared to intact rats (Fig. 2). On the other hand, there was no significant difference of testicular weights and the plasma concentration of testosterone between hypothyroid and control rats (Fig. 2). Adrenal weights in hypothyroid rats recovered to control levels by administration of T4 (Fig. 2). Pituitary contents of LH and PRL decreased, whereas pituitary content of ACTH increased in hypothyroid rats. These changes were recovered to control levels by administration of T4. Pituitary contents of FSH showed a similar pattern to that of LH and prolactin, though there was no significant difference between hypothyroid and euthyroid rats (Fig. 3).

These results imply that hypothyroidism can produce adrenal and gonadal dysfunction. The site of dysfunction in pituitary-adrenal axis may be at the adrenal level in hypothyroid rats since the high level of ACTH in pituitary was observed. On the other hand, the site of dysfunction in gonadal axis may be at the hypothalamus-pituitary level in hypothyroid rats. In the consecutive experiments, the effects of hypothyroidism on the hypothalamic-pituitary-adrenal (HPA) and hypothalamic-pituitary-gonadal (HPG) axes in rats were investigated in detail.
Functional Relationship between Thyroid Gland and HPA Axis in Adult Male Rats

Hypothyroidism has been reported to reduce the weight of adrenals [1, 2] and the plasma concentration of corticosterone, and to affect circadian adrenocortical rhythm [1]. In contrast, excess amount of thyroid hormone enlarges the weight of adrenals [4]. It has been reported that thyroidectomy decreased plasma and pituitary adrenocorticotropin hormone (ACTH) levels [1], and caused a reduction in corticotropin releasing hormone (CRH) gene transcription [6] in the hypothalamic paraventricular nucleus (PVN). On the other hand, a recent report shows that ACTH responses to exogenous CRH are exaggerated in hypothyroid rats [2]. The increased level of ACTH content in pituitary was confirmed in the present study [7]. Although it is obvious that thyroid hormone affects the HPA axis, the site of action of thyroid hormone on this axis is not clearly understood. In order to clarify the HPA axis activity in hypothyroid rats, the adrenal response to ACTH for corticosterone release and the pituitary response to CRH or arginine vasopressin (AVP) for ACTH release were examined in the present study. CRH and AVP release in median eminence (ME) were also measured directly using a push-pull perfusion method in adult male hypothyroid rats.

As the result of ACTH challenge test, the adrenal response to ACTH for corticosterone release was lower in hypothyroid than euthyroid rats (Fig. 4). On the other hand, the pituitary response for ACTH release was higher in hypothyroid than euthyroid (Fig. 5). In vivo releases of CRH and AVP in median eminence by push-pull perfusion significantly increased in hypothyroid rats compared to euthyroid rats (Fig. 6). There were no significant differences in hypothalamic concentrations of CRH and AVP. These results clearly suggest that hypothyroidism causes adrenal
dysfunction directly and the hypersecretion of CRH and AVP is due to reduction of the negative feedback effect of glucocorticoid. In contrast to the results from the present study, a previous report by Shi et al. [6] has shown that hypothyroidism causes a reduction in CRH gene transcripts in PVN of male rats, though the detection of the peptide was not reported [6]. It is difficult to explain the differences between the results and those of Shi at the present time, but push-pull perfusion technique provides direct evidences that CRH and AVP releases are increased from at least median eminence in hypothyroid rats.

Functional Relationship between Thyroid Gland and HPG Axis in Adult male Rats

It has been reported that hypothyroidism can produce menstrual disorders, amenorrhea and/or sterility in women [4]. 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 few present in adult human and rat testes [8–10]. Human and rat testes have high concentration of thyroid hormone receptors before puberty [10], but the number of the receptor decreases in testes after puberty [10]. 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 [11–13] without a concomitant decrease in plasma concentration of testosterone. Furthermore, it has been reported that hypothyroidism decreased the
plasma concentrations of LH and FSH [14, 15] and reduced the number and size of gonadotropes [16]. It is clear that hypothyroidism is associated with gonadal dysfunctions, but the mechanisms for these changes are not clear. In the present study, to clarify the action site of hypothyroidism on gonadal axis, the pituitary response to luteinizing hormone releasing hormone (LHRH) and the testicular response to LH were examined in adult male rats treated with thiouracil. The effect of hypothyroidism on the copulatory behavior was also examined in adult male rats.

As a result of LHRH challenge test, pituitary response to LHRH was significantly lower in hypothyroid rats than in control rats (Fig. 7), although the basal level of plasma LH was not different between hypothyroid and euthyroid rats. The testicular response to hCG for testosterone release was not different between euthyroid and hypothyroid animals (Fig. 7). Hypothyroidism caused a significant reduction in the number of ejaculation within 60 min. Intromission latencies were significantly longer in hypothyroid rats than in control rats, and these prolonged latencies were recovered to the control levels by administration of T4.

The present results indicate that hypothyroidism causes dysfunction in HPG axis at the hypothalamic-pituitary level. The neuropeptide that regulates gonadal function at the hypothalamic-pituitary level is LHRH [17–20]. There are several factors which inhibit LHRH secretion, and CRH is one of major factors for inhibiting it [21–23]. In the present study, hypersecretion of CRH [24] was confirmed in hypothyroid rats. Deficits in gonadal function at the hypothalamic-pituitary levels in hypothyroid rats may be mediated at least, partly, by CRH.
It is well recognized that functional relationship exists between adrenal and gonad [22]. Stress is accompanied by both an increase in the activity of the HPA axis and a decrease in the activity of the HPG axis [22, 25]. Stress-related hormones can influence reproductive functions [22, 25] and one of main factors known to have an inhibitory influence on LHRH secretion is CRH [22, 23, 25–27]. There is immunocytochemical evidence for direct synaptic connection between CRH and LHRH containing neurons [23], suggesting one of the action sites of CRH on LHRH neurons is probably medial preoptic area (MPOA). On the other hand, the physiological significance of the effects of CRH on LHRH secretion at the ME which has high concentrations of both CRH [28] and CRH receptor [29], is not definitive. To clarify the effects of CRH at the ME, two animal models (adrenalectomized and restraint stressed rats) for hypersecretion of CRH were used and the effect of iv administration of CRH antiserum on LH secretion was examined in the present study.

As a result of adrenalectomy for 2 weeks, plasma concentrations of ACTH in adult male rats were clearly increased (Fig. 8). On the other hand, plasma concentrations of LH were significantly decreased in adrenalectomized rats compared to control rats (Fig. 8). The iv injection of CRH antiserum decreased plasma concentrations of ACTH (Fig. 8) and increased plasma concentrations of LH in adrenalectomized rats (Fig. 8). In response to restraint stress, plasma concentrations of LH were decreased in vehicle-treated rats. On the other hand, the iv injection of CRH antiserum blocked the suppression of LH secretion induced by stress.

These results suggest that immunoneutralization of endogenous CRH at the ME increases LH secretion probably mediated by LHRH release and the ME is probably one of the action site of CRH on LHRH secretion in addition to MPOA. In contrast to the present results, a previous report by Walker et al. (1993) has shown that the iv injection of CRH antiserum did not alter the decrease in the secretion of LH by suckling [30] and concluded that the changes in LH secretion observed in lactating animals probably were not mediated by the effect of CRH at the level of LHRH release from nerve terminals in the ME [30]. The reason why the differences between the present result and those of Walker et al. are not clear at the present time, but it may be due to the difference of CRH antiserum or physiological conditions in experimental animals. The other reports have shown that CRH inhibits the in vitro release of LHRH at the ME [21], and inhibit LH secretion mediated by opioid peptides at the level of the anterior pituitary [31]. These reports support our results and suggest that the ME or the pituitary may be one of the action site of CRH on LHRH or LH secretion.

**Functional Relationship between Adrenal and Gonadal Glands in Adult Male Rats**

Hypothyroidism has been known to produce reproductive abnormalities, including irregular menstrual or estrous cycle, amenorrhea and sterility in many species of female mammals [4].
Most of the previous reports regarding the relationship between thyroid status and gonadal function only mentioned the direct effects of hypothyroidism on the HPG axis in female animals [4, 32–34]. The results from the present study suggest that hypothyroidism causes adrenal dysfunction directly and results in hypersecretion of ACTH in adult male rats [7, 24]. The adrenal dysfunction, may contribute to the inhibition of LHRH secretion from hypothalamus, possibly mediated by excess CRH [7, 24]. It was hypothesized that the HPA axis plays an important role in the gonadal dysfunction in hypothyroid adult female rats in addition to male rats, and the effects of hypothyroidism on these two axes in adult female rats were investigated in the present study.

As a result of administration of thiouracil, forty percent of hypothyroid rats (4 out of 10) showed irregular estrous cycles by 20 days (the 5th estrous cycle) after its administration and most of animals (90%) showed prolonged diestrous pattern by the 8th estrous cycle after the treatment. All of hypothyroid rats showed regular estrous cycles until 16 days after administration of thiouracil, and blood samples were collected by decapitation at 16 days after its administration. Plasma concentrations of estradiol and testosterone decreased in hypothyroid rats compared to euthyroid rats between 17:00 h on the day of diestrus and 17:00 h on the day of proestrus (Fig. 9). In hypothyroid female rats, the basal concentrations of plasma LH decreased on the day of diestrus and the day of proestrus (Fig. 9), whereas the plasma concentrations of prolactin and progesterone increased compared to euthyroid rats (Fig. 9). Plasma concentrations of corticosterone decreased in hypothyroid female rats throughout the estrous cycle as compared with euthyroid rats. On the other hand, the plasma concentrations of ACTH were not different between hypothyroid and euthyroid rats. In the restraint stress experiment, the increase in plasma concentrations of ACTH induced by stress was much higher in hypothyroid rats than in euthyroid rats, whereas plasma concentrations of corticosterone were significantly lower in hypothyroid rats compared to euthyroid controls. The number of oocytes after hCG challenge was significantly lowered on the day of diestrus in hypothyroid rats compared to euthyroid rats, though there is no difference on the day of proestrus between hypothyroid and control rats.

The present results show that hypothyroidism causes dysfunction in both the HPA and HPG axes in female rats in addition to male rats. In the present study, one likely reason for irregular estrous cycle in hypothyroid female rats is the hypersecretion of progesterone. The increased level of progesterone is probably due to hypersecretion of PRL during the day of proestrus and estrus. Taya et al. [35] have reported that an injection of exogenous progesterone postponed the time of the preovulatory LH surge and ovulation by 24 h. It also has been shown that the injection of antiprogestosterone serum on the day of metestrus in 5-day cycling rats advanced ovulation by 1 day [36]. Elevated concentrations of plasma progesterone is known to inhibit the secretion of LHRH and gonadotrophin [35, 37]. 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
Hyperprolactinemia in Hypothyroid Adult Female Rats

Hypothyroidism has been known to produce reproductive abnormalities accompanied with hyperprolactinemia in women [4]. Studies using male rats [7, 43], ovariectomized [44] or immature female rats [45], on the other hand, reported that hypothyroidism resulted in decreased levels of plasma and pituitary PRL. Pan et al. have reported that ovariectomized, thyroidectomized rats treated with estrogen show increased levels of plasma PRL, suggesting that estrogen plays significant role in the induction of hyperprolactinemia in hypothyroidism [44, 46]. The present study also demonstrated that plasma concentration of PRL increased on proestrus and estrus in thiouracil-induced hypothyroid adult female rats [47]. In this study, the regulation of PRL secretion was investigated in hypothyroid female rats. It is well established that the secretion of PRL is under tonic inhibitory hypothalamic control exerted by dopamine [48, 49]. In addition to the inhibitory dopaminergic tone, it is generally accepted that PRL secretion is influenced by thyrotropin releasing hormone (TRH), vasoactive intestinal peptide (VIP) and other neuropeptides acting as PRL releasing factors [48, 49]. Recent studies demonstrated that VIP is also synthesized within the rat anterior pituitary gland [50] and has an autocrine or paracrine role in basal PRL release as shown both in vivo and in vitro [51–53].

Hypothyroidism has been shown to increase VIP content [45], its release [54] and its gene expression [55] in anterior pituitary and to increase the immunoreactivity and the gene expression in the PVN [56]. The present study was designed to explore PRL regulation during hypothyroidism. Specifically, the activity of the rate limiting enzyme for dopamine synthesis, tyrosine hydroxylase (TH) in the ME and VIP concentrations in the anterior pituitary, ME and PVN were examined in hypothyroid adult female rats. The intromission threshold for induction of pseudopregnancy also was examined to evaluate the effect of elevated PRL on the response to coital stimulation in hypothyroid rats.

As a result of administration of thiouracil for 16 days, plasma concentrations of PRL increased on
proestrus in adult female rats (Fig. 10). Hypothyroidism did not affect tuberoinfundibular dopamine neuronal activity as measured by TH activity (DOPA accumulation 30 min after administration of m-hydroxybenzyl hydrozine dihydrochloride: NSD 1015, an L-aromatic amino acid decarboxylase inhibitor; 100 mg/kg, ip) in the ME compared to euthyroid rats (Fig. 10), whereas pituitary concentration of VIP was dramatically increased (Fig. 10). The proportion of female rats exhibiting pseudopregnancy was higher in hypothyroid animals (100%) receiving seven intromissions than in euthyroid animals (43%). Administration of T4 in hypothyroid rats decreased in the proportion of pseudopregnancy (40%) to the level of euthyroid animals (Fig. 11).

The present results confirmed that hypothyroidism induced hyperprolactinemia in intact adult female rats without additional estrogen treatment. The increased levels of PRL in hypothyroid rats suggests that the PRL response to coital stimulation may be activated in hypothyroid female rats and it accounts for the higher incidence of pseudopregnancy. No studies have been reported showing a concomitant increase of pituitary VIP and plasma PRL in hypothyroid adult female rats. In the present study, a concomitant increase of pituitary VIP and PRL secretion was observed in hypothyroid female rats whereas TH activity in the ME was not different between both groups. These results suggest that hypothyroidism-induced increase in PRL may be due to an increase in pituitary VIP, which can affect PRL secretion by acting as a paracrine or autocrine regulator. VIP receptors have been shown to exist in anterior pituitary [57] and it also has been reported that anti-VIP serum decreases basal PRL secretion from cultured hypothyroid pituitary cell [54].

In conclusion, hypothyroidism did not affect TH activity in the ME, whereas it dramatically increased pituitary concentration of VIP in adult female rats. This increased level of VIP likely affected PRL secretion in a paracrine or autocrine manner, inducing hyperprolactinemia in hypothyroid female rats. A decrease in the intromission threshold for induction of pseudopregnancy is likely due to this increased level of PRL in hypothyroid female rats.

In summary, hypothyroidism causes adrenal dysfunction and hyposecretion of corticosterone by the adrenal gland directly and results in the hypersecretion of CRH and AVP [7, 24]. The site of dysfunction in gonadal axis is mainly at the hypothalamus-pituitary level [7, 58] in hypothyroid male rats, and the dysfunction is probably mediated by endogenous CRH [7, 24, 59]. In female rats, hypothyroidism causes hyperprolactinemia, and result in prolonged diestrous period [47]. Pituitary concentrations of VIP increased in hypothyroid female rats. This increased level of VIP likely affects PRL secretion in a paracrine or autocrine manner, inducing hyperprolactinemia in hypothyroid female rats [60]. In female rat, thus, inhibition of gonadal function in hypothyroid rats mediated by hyperprolactinemia in addition to hypersecretion of endogenous CRH.

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