Effect of Methimazole-induced Hypothyroidism on Adrenal and Gonadal Functions in Male Japanese Quail (Coturnix japonica)

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Abstract. To investigate the effect of hypothyroidism on gonadal and adrenal functions in male Japanese quail (Coturnix japonica), hypothyroidism was induced in male adult Japanese quail by daily administration of 2-Mercapto-1-methylimidazole (methimazole) in their drinking water. Four weeks after methimazole treatment, the Japanese quail were scarificed, and the plasma concentrations of free triiodothyronine (FT3), free thyroxine (FT4), total T3 (TT3), total T4 (TT4), corticosterone, testosterone, LH and immunoreactive (ir) inhibins were measured by radioimmunoassy, the testes and adrenal glands were removed and weighed and the thyroid glands and testes were fixed in 4% paraformaldehyde for histological observation. The results showed that the hypothyroidism induced by methimazole caused a significant decrease in body and testes weight; the plasma levels of FT3, FT4 and TT4 significantly decreased, and the hypothroid quail possessed a greater number of small follicles and more follicular epithelial cells in the thyroid gland. In addition, hypothyroidism resulted in a significant decrease in the plasma concentrations of corticosterone, LH, testosterone and ir-inhibin. Furthermore, no spermatogenesis was found in the seminiferous tubules of the methimazole treatment groups. These results clearly demonstrate that hypothyroidism caused both gonadal and adrenal disturbances in the adult male Japanese quail.

Key words: Adrenal, Gonad, Hypothyroidism, Japanese quail (Coturnix japonica), Methimazole (J. Reprod. Dev. 53: 1335–1341, 2007)

Thyroid hormones, such as triiodothyronine (T3) and thyroxine (T4), are important for the growth, development and metabolism of humans and animals. In addition to these effects, they have also been shown to play an important role in adre-
differentiation into adult Leydig cells in neonatal prepubertal rats [4, 5]. In addition, daily injections of T3 to neonatal prepubertal rats accelerates the differentiation of Leydig cells [5]. It is clear that hypothyroidism is associated with dysfunction of the adrenal and gonadal glands in mammals, although the mechanisms for these changes are not clear [7]. 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 animals. We have previously reported that hypothyroidism causes adrenal dysfunction directly and results in hypersecretion of ACTH from the pituitary gland in adult male rats [8]. Adrenal dysfunction may contribute to the inhibition of luteinizing hormone releasing hormone (LH-RH) secretion from the hypothalamus, possibly mediated by excess corticotrophin-releasing hormone (CRH) [8]. A previous study from our laboratory demonstrated that hypothyroidism causes gonadal and adrenal disturbances in adult female rats [9].

Kirby et al. [10] showed that transient hypothyroidism during reproductive development in the male chick results in permanent alteration of adult reproductive characteristics. Other studies in birds support the hypothesis that the thyroid axis plays a permissive role in the reproduction of seasonally breeding species [11–13]. Therefore, the aim of this study was to investigate the effects of hypothyroidism on the adrenal and gonadal functions in adult male Japanese quail.

**Materials and Methods**

*Animals and treatments*

Japanese quail were obtained from low antibody response (L) selected lines, in which chicks hatch after 17 days of incubation and the quail reach sexual maturity in 6 weeks. The birds were provided with food (Kanematsu quail diet; Kanematsu Agri-tech Corporation, Ibaraki, Japan) and water and were allowed to feed *ad libitum*. Male quails (9 weeks old) were housed in metal cages in a controlled environment (light on, 0500–1900 h; temperature, 23 ± 2 C; humidity, 50 ± 10%; air exchanged 20 times hourly). This study was conducted in accordance with the Guiding Principles in the Use of Animals in Toxicology and was approved by the Animal Care and Use Committee of the Japanese National Institute for Environmental Studies.

*Experimental design*

The adult male Japanese quail were divided into 2 groups, the control and treatment groups. Hypothyroidism was induced in the quail of the treatment group by daily administration of 2-mercapto-1-methylimidazole (methimazole; Sigma-Aldrich Chemical, St. Louis, MO, USA) to their drinking water (500 mg/l) for 4 weeks, whereas the quail in the control group were provided with normal drinking water. After a study period of 4 weeks, the quail in both the control and treatment groups were decapitated, and blood samples were collected into heparinized plastic tubes. The blood samples were centrifuged at 1,700 g for 15 min at 4 C, and the separated plasma samples were immediately stored at –20 C until they were assayed for free triiodothyronine (FT3), free thyroxine (FT4), LH, testosterone, immunoreactive (ir) inhibins and corticosterone. Body weight was also measured on that day. The adrenal glands and testes were excised, and their organ wet weights were weighted. The thyroid and testes tissues were fixed in 4% paraformaldehyde for histological observation.

*Histology*

The samples of thyroid and testes obtained were dehydrated in an ethanol series and embedded in paraffin wax. Serial sections (4 µm) were mounted on slides coated with poly-L-lysine (Sigma). Some sections were stained with hematoxylin and eosin (HE) for observations of general histology.

*Radioimmunoassay (RIA)*

The plasma concentrations of corticosterone [14] and testosterone [15] were measured by double-antibody RIA systems using 125I-labeled radioligands as described previously. The intra- and interassay coefficients of variation were 9.8 and 17.5% for corticosterone and 6.3 and 7.2% for testosterone. The plasma concentrations of ir-inhibins were measured using a rabbit antiserum against purified bovine inhibin (TNDH 1) and 125I-labeled 32-kDa bovine inhibin, as described previously [16]. The results were expressed in terms of 32-kDa bovine inhibin. The intra- and interassay coefficients of variation were 8.0 and 16.2%. The LH
concentrations were measured with a USDA-ARS RIA kit (Beltsville, MD, USA) for chicken LH. The antiserum used was anti-avian LH (HAC-CH27-01 RBP75). The hormone for iodination was chicken USDA-cLH-I-3. The results are expressed in terms of USDA-cLH-K-3. The intra- and interassay coefficients of variation were 5.2 and 11.2%, respectively. The plasma concentrations of free and total T3 and free and total T4 were measured using AMERLEX-MAB T3 and T4 kits (Daiichi Radioisotope Laboratories, Tokyo, Japan). The intra- and interassay coefficients of variation were 7.2 and 14.4% for free T3, 3.7 and 4.5% for total T3, 9.4 and 10.9% for free T4 and 2.7 and 3.6% for total T4, respectively.

Statistical analysis
All data were expressed as means ± SEM. The data were analyzed by independent sample t-tests. Results with P<0.05 were considered to be statistically significant.

Results

Body and organ weights
The body and testicular weights of the hypothyroid Japanese quail significantly decreased compared with the control group 4 weeks after methimazole treatment (Fig. 1a and c). On the other hand, there were no significantly differences in the weights of the adrenals between the methimazole treatment and control groups (Fig. 1b).

Histology
There were no significant inflammatory, degenerative or necrotic changes in the thyroid tissues of the control group (Fig. 2a). However, dwindled follicles and increased numbers of follicular epithelial cells were found in the methimazole treatment group (Fig. 2b). Testicular morphology was normal in the control group, showing compartmentalization of germ cells in the seminiferous tubules and visible spermatozoa in a normal-sized lumen (Fig. 2c). In contrast, the methimazole treatment groups showed no compartmentalization of germ cells or spermatozoa and had highly atrophic seminiferous tubules that were devoid of all cells except spermatogonia (Fig. 2d).

Circulating hormones
The plasma concentrations of FT4, TT4 and FT3 were significantly decreased by 4 weeks after methimazole treatment (Fig. 3a, b and d). However, the plasma concentration of TT3 showed no significant changes compared with the control group (Fig. 3c). Moreover, the plasma concentrations of corticosterone, LH, testosterone and inhibin decreased significantly in the hypothyroid quail compared with the control quail (Fig. 4a, b, c and d).
Discussion

The present study demonstrated that methimazole-induced hypothyroidism caused remarkable decreases in body and testes weights and in the plasma levels of LH, testosterone and ir-inhibin.
and that the plasma concentration of corticosterone was significantly lower in the hypothyroid quail compared with the control quail. In addition, our histological results demonstrated that there was no spermatogenesis in the seminiferous tubules of the hypothyroid quail compared with the control quail. These results suggest that hypothyroidism is associated with dysfunction of the adrenal and gonadal glands in adult male Japanese quail, although the mechanisms for these changes are not clear.

The therapeutic effects of the anti-thyroid drug methimazole have been ascribed to its ability to decrease thyroid hormone production [17]. In this study, the plasma concentrations of T3 and T4 were obviously suppressed by 4 weeks after methimazole treatment, and dwindled follicles and increased numbers of follicular epithelial cells in the thyroid glands were found in the treatment group. These results are in agreement with previous reports showing that hypothyroidism induced with methimazole might occur not only in mammals but also in avian species [18–20]. In mammals, previous studies have reported that hypothyroidism results in a decrease in the adrenal weights and plasma concentrations of corticosterone in rats [7–9]. In hypothyroid male rats, adrenal weight and the plasma concentration of corticosterone are significantly lower in hypothyroid rats compared with intact rats [7]. In addition, the adrenal weights of hypothyroid rats recover to control levels as a result of administration of T4 [7]. A previous study that subjected rats to a stress experiment showed a marked increase in the plasma concentrations of ACTH in hypothyroid rats, whereas, the increase in the plasma concentrations of corticosterone was much lower in the hypothyroid rats compared with the controls [9]. These results clearly indicate that hypothyroidism causes adrenal dysfunction directly and that it contributes to hypersecretion of ACTH. In birds, the thyroid gland partially controls avian growth, and artificial changes in thyroid hormone levels do not always change growth predictably [20]. Previous studies showed that hypothyroidism produced by treatment with an anti-thyroid drug, propylthiouracil (PTU), causes a significant dose-related reduction in body weight and in relative lymphoid organ weights in chickens [19]. The present results clearly showed that hypothyroidism caused adrenal disturbances in the adult male Japanese quail.

Thyroid hormones appear to play a permissive role in the annual reproductive transition of seasonal breeders in mammal and avian species [21]. In birds which are long day breeders, thyroidectomy prevents refractoriness developing, and this results in the birds remaining in a breeding condition indefinitely under long days [22–24]. In rats, previous studies have shown that hypothyroidism decreases the plasma concentrations of LH and FSH [25, 26] and reduces the number and size of gonadotropes [27], suggesting that hypothyroidism is associated with gonadal dysfunctions. A previous study has also reported that exposure to a short-day photoperiod (SD) for 15 weeks significantly decreased the weights of the testes and epididymis and the plasma concentrations of testosterone, LH, ir-inhibin, inhibin B and inhibin pro-αC in adult male hamsters [28].

The direct action of thyroid hormones on the hypothalamic-pituitary-gonadal (HPG) axis in adult male quail is unknown. However, in the present study, hypothyroidism resulted in decreased plasma concentrations of LH, testosterone and ir-inhibin in the treatment group, implying that hypothyroidism probably causes dysfunction at the hypothalamus and pituitary levels in adult male Japanese quail. The present results concerning avian testes clearly demonstrated no spermatogenesis in seminiferous tubules in the treatment group compared with the control group. This finding is similar to that in other animals, such as in male rats [29, 30], male guinea pigs [31] and male hamsters [32–34]. In rats, previous reports have demonstrated that hypothyroidism inhibits steroidogenesis [29, 30]. It has been reported that cessation of spermatogenesis and dysfunction of the reproduction system are observed concomitant with the reduction in the plasma concentrations of FT3 and FT4 in male hamsters maintained under a short day cycle or treated with melatonin [32, 33]. A previous study indicated that fertility increases as a result of T4 administration and decreases as a result of thyroxectomy in male guinea pigs [31]. Therefore, the present results are in agreement with previous studies using mammals that show that gonadal function is associated with thyroid function and suggest that hypothyroidism might cause dysfunction of gonadal function; the influence of hypothyroidism on gonadal function is very severe in Japanese quail compared with mammals. The present study clearly showed that hypothyroidism caused severe dysfunction in both gonadal and
adrenal function in the male Japanese quail.

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References

GONADS AND ADRENALS IN HYPOTHYROID QUAIL


