Effects of Thyroid Hormone (thyroxine) and Testosterone on Hepatic 11β-Hydroxysteroid Dehydrogenase mRNA and Activity in Pubertal Hypothyroid Male Rats

Yan-Jun Liu, Yuichi Nakagawa, Kazuhiko Toya, Hirokazu Saegusa, Kaoru Nasuda, Akira Endoh, and Takehiko Ohzeki

To investigate the effects of thyroid hormone and testosterone on 11β-hydroxysteroid dehydrogenase type 1 (11 β-HSD1), we measured changes in hepatic 11β-dehydrogenase activity and its mRNA levels in pubertal methimazole (MMI)-induced hypothyroid male rats following treatment with thyroxine ([T_4] 50 μg/kg/d) or testosterone (250 μg/d) for 14 days. Hypothyroidism in male rats markedly reduced hepatic 11β-HSD1 mRNA levels and serum testosterone concentrations ($P < .01$). Subcutaneous injection of T_4 in the hypothyroid rats significantly ($P < .01$) increased hepatic 11β-HSD1 mRNA to approximately normal levels and simultaneously increased serum testosterone levels. However, the same daily dose of T_4 administered to castrated male hypothyroid rats for 14 days did not elevate hepatic 11β-HSD1 activity. Treatment with testosterone for 14 days in castrated hypothyroid male rats and rats without gonadectomy significantly ($P < .01$) increased the enzyme activity without administration of T_4. Variations in hepatic 11β-HSD1 activity were demonstrated to be accompanied by changes in serum testosterone levels in the rats following alteration of the thyroid hormone state. These results suggest that the effect of T_4 in increasing the subnormal 11β-HSD1 gene expression in hypothyroid male rats is mediated by its ability to increase testosterone production in these rats, because in castrated hypothyroid rats, T_4 does not elevate 11β-HSD1 gene expression.

THE 11β-HYDROXYSTEROID dehydrogenase (11β-HSD) is a membrane-bound pyridine nucleotide–dependent microsomal enzyme complex found in many glucocorticoid target tissues.[1] This enzyme is responsible for the interconversion of active steroids (corticosterone and cortisol) to inactive steroids (11-dehydrocorticosterone and cortisone), thus regulating the level of intracellular bioactive steroids and their physiological activity.[2] Previous studies have indicated that regulation of this enzyme activity must involve not only the hypophysis and gonads, but also the thyroid and adrenal glands.[3-6] Hellman et al[7] and Zumoff et al[8] have suggested that a high level of circulating thyroid hormone appears to stimulate 11β-HSD activity, as evidenced by a decrease in the ratio of urinary excretion tetrahydrocortisol to tetrahydrocortisone in hyperthyroid patients. However, the regulatory mechanism of 11β-HSD1 in the thyroidal disorder is still unclear. Recently, cDNA encoding the 11β-HSD1 gene was cloned.[9,10] Analysis of 11β-HSD1 mRNA expression and enzyme activity in different tissues demonstrates that expression of the 11β-HSD1 gene is regulated in a species- and tissue-specific manner.[11] Therefore, we studied the effects of thyroid hormone on hepatic 11β-HSD mRNA in pubertal hypothyroid male rats.

It has been reported that T_3 receptors are located in prepubertal Sertoli cells of the testes.[12,13] Several studies have demonstrated that thyroid hormone plays a critical role in the maintenance and regulation of circulating androgen concentrations in rats.[14-16] It can be speculated that thyroid hormone plays an important role in the regulation of hepatic steroid enzyme activity by influencing the level of circulating androgens. However, little is known as to whether a thyroid hormone–associated alteration in androgen concentrations contributes to the effects of thyroid hormone on hepatic 11β-HSD activity in rats.

MATERIALS AND METHODS

Animals

Pregnant Sprague-Dawley rats were obtained from Japan SLC (Hamamatsu, Japan). The animals were housed under normal laboratory-controlled conditions (22° to 26°C and lights on for 12 hours per day), and rat chow and water were available ad libitum. Hypothyroidism was induced in newborn rats by adding 0.05% (wt/vol) methimazole (MMI) to the drinking water of the lactating mothers every day starting from the day of birth. After weaning, MMI was added to the drinking water of the weaned rats.[17,18] Euthyroid animals received drinking water without MMI and were used as controls. The degree of hypothyroidism was checked by evaluation of serum levels of L-thyroxine (T_4) and triiodothyronine (T_3) as previously reported.[19,20] The animals were divided into control, hypothyroid, hypothyroid + L-T_4, and hypothyroid + testosterone groups. Administration of T_4 50 μg/kg body weight/d or testosterone 250 μg/d-(single daily subcutaneous injection) to hypothyroid rats was begun on day 35 after birth and continued for 14 days. Male hypothyroid rats aged 35 days were castrated under ether anesthesia and administered T_4 or testosterone (250 μg/d) by subcutaneous injection for 14 days, while sham-operated hypothyroid rats received daily injections of vehicle alone or sesame oil. The dosage of T_4 and the treatment regimen were similar to others previously reported.[21,22] All animals were killed on the morning of day 49.

Hormone Assays

Serum T_4 and testosterone concentrations were determined using commercially available radioimmunoassay kits. The detection limits of T_4 and testosterone were 0.01 ng/mL and 1 pg/mL, respectively.

11β-HSD Activity Assay

11β-HSD activity was determined by measuring the rate of conversion of corticosterone to 11-dehydrocorticosterone, as we previously

From the Department of Pediatrics, Hamamatsu University School of Medicine, Hamamatsu, Japan.

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Address reprint requests to Yan-Jun Liu, MD, Department of Pediatrics, Hamamatsu University School of Medicine, 3600 Handa-Cho, Hamamatsu 431-31, Shizuoka, Japan.

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reported.[20,23] Briefly, liver tissues were homogenized in Krebs-Ringer bicarbonate (KRB) buffer. After yields of consistent protein concentrations were obtained using the Bradford method, the liver homogenates (250 μL) were incubated with 2 μmol/L [^{3}H]corticosterone, 3.4 mmol/L NADP, and KRB (containing 2% glucose and bovine serum albumin, pH 7.4) at 37°C for 12 minutes. Preliminary studies established that the protein concentration was adjusted to ensure the linearity of product formation over the 12-minute incubation. The steroids were extracted with ethyl acetate and separated by thin-layer chromatography in a chloroform:ethanol (9:1) system.

RNA Preparation and Northern Blot Assay

Total RNA was isolated from the rat livers using a single-step guanidinium isothiocyanate–acid phenol chloroform extraction procedure. For Northern blotting, aliquots of RNA were electrophoresed and transferred to Hybound N^+ membranes (RPN.3050N; Amersham International, Bucks, UK) as previously described by our group.[20,24] To assist in the quantification of mRNA levels, cDNA-encoded rat liver 11β-HSD1 (1,265 base pairs; kindly provided by Dr P. White[10]) was labeled with [^{32}P]adenasine triphosphate (Amersham International; specific activity, 6,000 Ci/mmol) using nick translation (Nick Translation System; BRL Life Technologies, Bethesda, MD). Autoradiographs were obtained by exposure of the membranes to x-ray film with an intensifying screen at −70°C for up to 7 days. The relative abundance of each RNA species hybridized with each of the radiolabeled probes was determined by densitometric scanning of the lanes.

Statistical Analysis

The data are expressed as the mean ± SEM. Comparisons among the different experimental groups were made by unpaired Student's *t* test and ANOVA. A *P* level less than .05 was considered statistically significant.

RESULTS

Basal Levels

Hypothyroidism was confirmed by markedly ($P < .01$) lower circulating T_3 and T_4 levels in the pubertal 35- and 49-day-old rats treated with MMI from birth. Treatment of the newborn male rats with MMI resulted in a significant reduction in serum testosterone levels determined at ages 35 and 49 days ($P < .01$). T_4 replacement in MMI-treated 35-day-old pubertal rats for 14 days normalized serum concentrations of T_3, T_4, and testosterone (Table 1).

Table 1. Serum Levels of Total T_3, T_4, and Testosterone in Pubertal Hypothyroid Male Rats

Treatment	T_3 (ng/dL)	T_4 (μg/dL)	Testosterone (ng/mL)
35 d postnatal			
Control	83.3 ± 5.63	5.22 ± 0.01	6.53 ± 0.02
MMI	11.2 ± 2.31*	<1	1.83 ± 0.01*
49 d postnatal			
Control	81.2 ± 7.69	5.50 ± 0.02	10.3 ± 1.26
MMI	10.9 ± 2.43*	<1	2.5 ± 0.21*
MMI + L-T_4	86.5 ± 7.32†	5.66 ± 0.02	9.7 ± 1.6†

NOTE. Values are the mean ± SEM.
*$P < .01$ *v* control of the same age (Student's unpaired *t* test).
†$P < .01$ *v* MMI-treated.

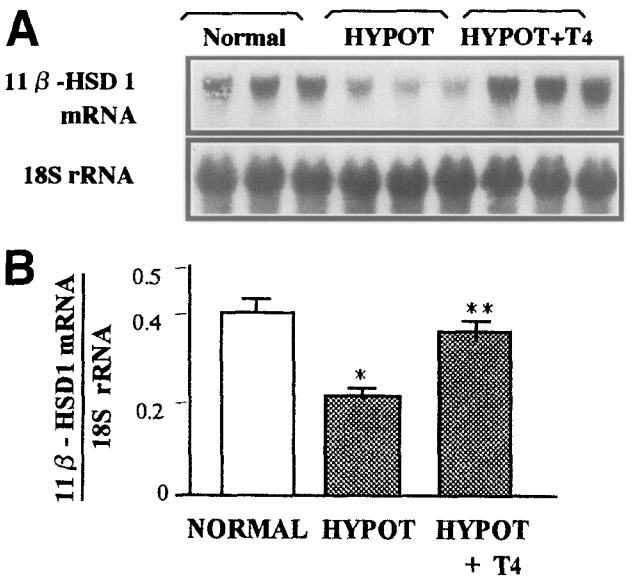

Fig 1. Northern blot hybridization of 11β-HSD1 cDNA and 18S rDNA probes showing mRNA expression (A) and relative quantification of hepatic 11β-HSD1 mRNA levels (B) in normal male rats, hypothyroid (HYPOT) male rats, and hypothyroid male rats treated with T4 (HYPOT + T4) Values are the mean ± SEM for the ratio of 11β-HSD1 mRNA to 18S rRNA. *$P < .01$ *v* normal; **$P < .01$ *v* HYPOT.

Effects of Thyroid Hormone and Testosterone on Hepatic 11β-HSD1 in Both Intact and Castrated Pubertal Male Rats With Hypothyroidism

Hepatic 11β-HSD1 mRNA levels were significantly reduced by 45% in pubertal 35-day-old hypothyroid male rats compared with the age-matched normal males ($P < .01$; Fig 1). In parallel, hepatic 11β-HSD1 activity was significantly ($P < .01$) lower in these MMI-treated male rats than in the normal males (Fig 2). Subcutaneous injection of T_4 in the pubertal 35-day-old hypothyroid male rats for 14 days restored hepatic 11β-HSD1 gene expression to the age-matched normal level (Fig 1). Fourteen days of T_4 replacement resulted in a significant ($P < .01$) increase in hepatic 11β-HSD1 activity in 35-day-old hypothyroid male rats compared with untreated controls (Fig 2). Moreover, treatment of hypothyroid rats with testosterone for 14 days significantly induced both the enzyme activity and gene expression in comparison to untreated controls ($P < .01$).

Castrated hypothyroid rats treated with L-T_4 showed no increase in 11β-HSD1 in contrast to intact hypothyroid rats, in which low 11β-HSD1 levels normalized in response to L-T_4. Administration of testosterone to castrated hypothyroid rats normalized the low 11β-HSD levels without administration of L-T_4 (Fig 3).

DISCUSSION

It is well known that 11β-HSD is under complex hormonal regulation. Previous studies have shown the effects of thyroid hormone on the metabolism of cortisol in man.[7,8] It has been suggested that the conversion of cortisol to cortisone is increased in hyperthyroidism and decreased in hypothyroidism. Similarly, thyroidectomy has been shown to cause a reduction in the ratio of the conversion of cortisol to cortisone in pubertal

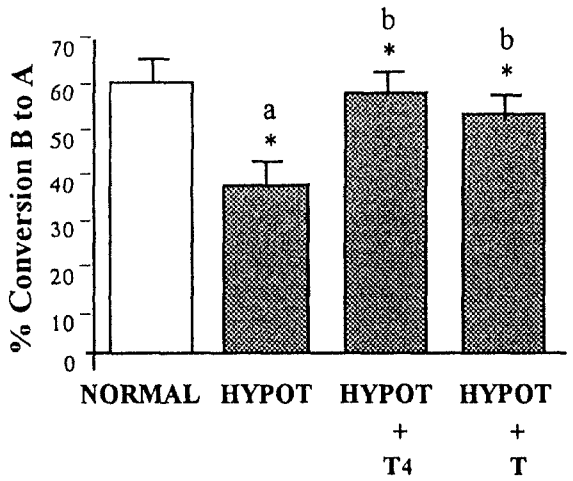

Fig 2. Hepatic 11β-HSD1 activity in normal, hypothyroid (HYPOT), and hypothyroid male rats treated with T_4 (HYPOT + T_4) or testosterone (HYPOT + T). Enzyme activity is expressed as the percent conversion of [^{3}H]corticosterone (B) to [^{3}H]11-dehydrocorticosterone (A). Values are the mean ± SEM. $^{*a}P < .01$ *v* normal; $^{*b}P < .01$ *v* HYPOT.

male rats.[6] However, the regulation mechanism of thyroid hormone on hepatic 11β-HSD1 activity remains unknown. A close correlation exists between tissue levels of 11β-HSD1 mRNA and its activity, suggesting a pretranslational regulation of expression of this enzyme.[1] Our data demonstrate that hypothyroidism markedly reduced hepatic 11β-HSD1 mRNA levels and activity. Administration of T_4 to hypothyroid male rats significantly increased the enzyme activity and gene expression, suggesting that circulating levels of thyroid hormone do indeed exert an effect on 11β-HSD1 gene expression, which in turn regulates 11β-HSD1 activity in pubertal rats. Recently, it was reported that the thyroid hormone response

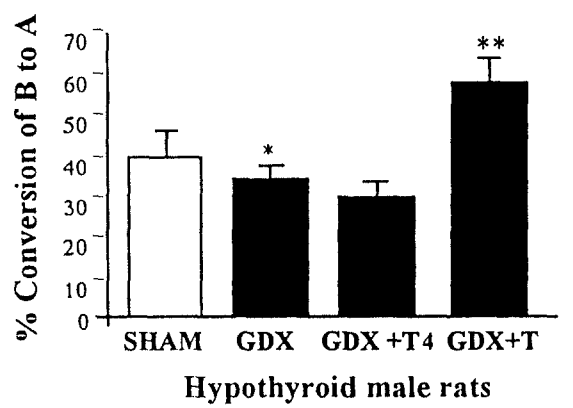

Fig 3. Effects of T_4 and testosterone on hepatic 11β-HSD1 activity in sham-operated male hypothyroid rats (SHAM), gonadectomized hypothyroid male rats (GDX), GDX + T_4, and GDX + testosterone (GDX + T). Data are expressed as the percent conversion of [^{3}H]corticosterone (B) to [^{3}H]11-dehydrocorticosterone (A). Values are the mean ± SEM. $^{*}P < .05$ *v* SHAM; $^{**}P < .01$ *v* GDX.

elements located in the 5′-flanking region of rat 11β-HSD genes and the expression of male-specific hepatic p450 2c genes were increased by thyroid hormone in hypothyroid male rats,[25,26] consistent with the possible regulation of 11β-HSD1 by T_4 at the level of transcription.

It is now well known that optimal thyroid hormone stimulation is essential to sustain normal growth and maturation of the rat testes.[14-16] Several studies on pubertal hypothyroid male rats pointed to decreased serum testosterone.[21,27,28] Recently, it has been reported that thyroid hormone supplementation in hypothyroid male rats increased the concentration of testosterone and maintained the normal response of Leydig cells to produce testosterone to luteinizing hormone stimulation in vitro.[21,22] These studies indicate that thyroid hormone plays a critical role in the maintenance and regulation of circulating androgen concentrations, especially in pubertal male rats. The data in this study show that hypothyroidism reduced serum testosterone levels and simultaneously decreased the hepatic 11β-HSD1 mRNA level and activity in pubertal male rats. It is noteworthy that treatment of hypothyroid male rats with T_4 restored hepatic 11β-HSD1 activity and simultaneously normalized the low serum testosterone levels. These results indicate that T_4 modifies hepatic 11β-HSD1 activity, and its gene expression may be associated with serum testosterone levels in pubertal hypothyroid male rats following treatment with T_4. However, whether these results have a bearing on human physiology remains to be determined.

It has been reported that gonadectomy decreased hepatic 11β-HSD activity in male rats, and administration of testosterone to the gonadectomized males resulted in a marked increase in the enzymatic activity.[5] In the present study, our results indicate that treatment of castrated male hypothyroid rats with testosterone significantly increased hepatic 11β-HSD1 activity. However, administration of T_4 to castrated hypothyroid male rats did not increase the enzyme activity. The inducing effects of thyroid hormone on the gene expression of 11β-HSD1 in the liver of intact male rats and the lack of a similar effect in castrated animals treated with thyroid hormone suggests that thyroid hormone induction of hepatic 11β-HSD1 gene expression is indirect and may be dependent on the presence of the testes. Similarly, Lax et al[6] found that the activities of three established sex steroid–dependent enzymes (3α-, 3β-, and 11β-HSD) act uniformly insofar as they respond to thyroidectomy in male rats with a decrease, which parallels the response seen after gonadectomy. However, the mechanism of the testosterone-induced activity of hepatic 11β-HSD1 remains unknown. Further studies are needed to clarify the active sites of testosterone.

Our results demonstrate that low circulating levels of thyroid hormone in male rats indeed exert a suppressive effect on 11β-HSD1 mRNA levels, which regulate 11β-HSD1 activity. We also reported that the regulatory effect of thyroid hormone on hepatic 11β-HSD1 is associated with changes in serum testosterone levels induced by T_4, which influences testosterone production by the testes in pubertal male rats.

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