Acute effects of thyroid hormones on the production of adrenal cAMP and corticosterone in male rats

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Lo, Ming-Jae, Mei-Mei Kau, Yen-Hao Chen, Shiow-Chwen Tsai, Yu-Chung Chiao, J Iann-Jong Chen, Charlie Liaw, Chien-Chen Lu, Bu-Pian Lee, Si-Chih Chen, Victor S. Fang, Low-Tone Ho, and Paulus S. Wang. Acute effects of thyroid hormones on the production of adrenal cAMP and corticosterone in male rats. Am. J. Physiol. 274 (Endocrinol. Metab. 37): E238–E245, 1998.—The acute effects of thyroid hormones on glucocorticoid secretion were studied. Venous blood samples were collected from male rats after they received intravenous 3,5,3'-triiodothyronine (T3) or thyroxine (T4). Zona fasciculata-reticularis (ZFR) cells were treated with adrenocorticotropic hormone (ACTH), T3, T4, ACTH plus T3, or ACTH plus T4 at 37°C for 2 h. Corticosterone concentrations in plasma and cell media, and also adenosine 3',5'-cyclic monophosphate (cAMP) production in ZFR cells in the presence of 3-isobutyl-1-methylxanthine, were determined. The effects of thyroid hormones on the activities of steroidogenic enzymes of ZFR cells were measured by the amounts of intermediate steroid products separated by thin-layer chromatography. Administration of T3 and T4 suppressed the basal and the ACTH-stimulated levels of plasma corticosterone. In ZFR cells, both thyroid hormones inhibited ACTH-stimulated corticosterone secretion, but the basal corticosterone was inhibited only with T3 >10^-10 M and T4 >10^-8 M. Likewise, T3 or T4 at 10^-7 M inhibited the basal- and ACTH-stimulated levels of intracellular cAMP. Physiological doses of T3 and T4 decreased the activities of 3β-hydroxysteroid dehydrogenase, 21-hydroxylase, and 11β-hydroxylase. These results suggest that thyroid hormones counteract ACTH in adrenal steroidogenesis through their inhibition of cAMP production in ZFR cells.

3,5,3'-triiodothyronine; thyroxine; zona fasciculata-reticularis cells; P450c11 activity; adenosine 3',5'-cyclic monophosphate

Physicians and physiologists have long hypothesized connections between hypothyroidism and adrenal cortical dysfunction. The interaction of pituitary-thyroid and pituitary-adrenal functions has been studied, and the influences of thyroid hormones on adrenal cortical function have been demonstrated (5, 29, 34). Chronic administration of 3,5,3'-triiodothyronine (T3) at high concentrations (40 µg; 3–36 days) in male rats increases plasma and adrenal corticosterone, as well as the induction of hypertrophy in the gland (7). Nevertheless, opposite results have been found in experiments in which administration of physiological T3 at 8 µg significantly depressed plasma and adrenal corticosterone levels during a 36-day interval (7). Plasma corticosterone and pituitary adrenocorticotropic hormone (ACTH) concentrations may remain normal in rats given T3 (15). The in vitro production of adrenal corticoids remains unchanged after thyroglobulin feeding (34). ACTH-induced increases in plasma-free corticoids are exaggerated in hyperthyroid rats (15, 34), but this may be accounted for by the reduction in volume of corticosterone distribution to peripheral tissues (34). Hypothyroid males have higher 24-h mean serum concentrations of total plasma cortisol in the normal circadian rhythmicity and cortisol production rate, with no change in serum cortisol-binding globulin concentrations compared with normal subjects (12).

It has been shown that thyroid hormones modulate adenylate cyclase activity in rat liver and heart (35), as well as human adipocytes (37) and luteinized granulosa cells (10). Rubio et al. (28) found that in brown adipose tissue the β1-adrenergic receptor number and capacity to generate adenosine 3',5'-cyclic monophosphate (cAMP) are reduced in hypothyroidism. Neri et al. (23) indicated that thyrotropin-releasing hormone markedly inhibits glucocorticoid secretion of rat adrenocortical cells, which selectively impairs the late steps of corticosterone synthesis (i.e., 11- and 18-hydroxylation).

Because of the conflicting results of previous studies regarding the role of thyroid hormones on adrenocortical function, as well as the lack of data on thyroid hormone regulation of adrenocortical function via cAMP production and steroidogenesis enzyme activity, the present study was designed to evaluate 1) the acute effects of thyroid hormones on the secretion of corticosterone both in vivo and in vitro; 2) the possible positive correlation between corticosterone and cAMP production under the influence of thyroid hormones; and 3) the possible correlation between corticosterone secretion and postpregnenolone steroid enzyme activity under the influence of thyroid hormones.

**MATERIALS AND METHODS**

Animals. Male Sprague-Dawley rats weighing 300–350 g were housed in a temperature-controlled room (22 ± 1°C) with 14 h of artificial illumination daily (0600–2000). Food and water were given ad libitum. All animal experimentation has been conducted humanely and in conformance with the policy statement of the Committee of National Yang-Ming University.
In vivo experiments: effects of a single injection of thyroid hormones. All rats were anesthetized with ether and catheterized via the right jugular vein (38). They were injected 20 h after the catheterization with saline, T3 (5 µg·ml⁻¹·kg body wt⁻¹, Sigma Chemical, St. Louis, MO), thyroxine (T4), 20 µg·ml⁻¹·kg body wt⁻¹, Sigma Chemical), ACTH (5 µg·ml⁻¹·kg body wt⁻¹), ACTH plus T3, or ACTH plus T4. Blood samples (0.3 ml each) were collected from the jugular catheter 0, 30, 60, 90, 120, and 180 min after the challenge between 0800 and 1200. The lost blood volume was replenished with heparinized saline immediately after each bleeding.

Plasma was separated by centrifugation at 10,000 g for 1 min and stored at −20°C. The concentrations of total T3 and total T4 in rat plasma were measured by radioimmunoassay (RIA). To measure corticosterone, 0.1 ml plasma was mixed with 1 ml diethyl ether (10 vol), shaken for 20 min, centrifuged at 1,000 g for 5 min, and then quickly frozen in a mixture of acetone and dry ice. The organic phase was collected, dried, and reconstituted in a buffer solution [0.1% gelatin in phosphate-buffered saline (PBS), pH 7.5] before the concentration of corticosterone was measured by RIA.

Preparation of zona fasciculata-reticularis cells for cell culture. An adenocortical preparation enriched with zona fasciculata-reticularis (ZFR) cells for culture was performed following a method described by Purdy et al. (26) in 1991 with minor modifications. Male Sprague-Dawley rats were decapitated. The adrenal glands were rapidly excised and stored in an ice-cold 0.9% NaCl solution. The adipose tissues were removed. The encapsulating glands were separated into capsule (mainly zona glomerulosa) and inner zone (mainly ZFR) fractions with forceps. The fractions of inner zone from 10–20 adrenals were incubated with collagenase (2 mg/ml, Sigma Chemical) at 37°C in a shaking water bath, 100–110 strokes/min, for 60 min. The collagenase was dissolved in 2–4 vol, shaken for 20 min, and then quickly frozen in a mixture of acetone and dry ice.öffnung

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The organic phase was collected, dried, and reconstituted in a buffer solution [0.1% gelatin in phosphate-buffered saline (PBS), pH 7.5] before the concentration of corticosterone was measured by RIA.
Activities of 3β-hydroxysteroid dehydrogenase, 21-hydroxylase, and 11β-hydroxylase ZFR cells (1 x 10^6 per tube) were preincubated for 60 min at 37°C in 95% O_2-5% CO_2 in 1 ml KRGA medium. After centrifugation at 200 g for 10 min, the supernatant was discarded, and the cells were incubated for 60 min in tubes in 0.2 ml KRGA containing pregnenolone (10^{-9} M) and [3H]pregnenolone (8,000–10,000 cpm, 4.5–5.0 pmol, NEN-Du Pont) or DOC (10^{-9} M) and [14C]DOC (18,000–20,000 cpm, 1.8–2.0 pmol, NEN-Du Pont) in the presence or absence of hormones, such as T_3 (10^{-11}–10^{-9} M) or T_4 (10^{-9}, 10^{-7} M). At the end of incubation, the medium containing radioactive products was removed from cultures by centrifugation at 200 g for 10 min. The media were extracted with 5 volumes of diethyl ether, shaken for 30 min, centrifuged at 200 g for 3 min, and then quickly frozen in a mixture of acetone and dry ice. The organic phase was collected, dried, and reconstituted in 100% ethanol. Aliquots of 50 µl of each sample and 5 µl of unlabeled carrier steroids (1 mg/ml) were spotted on silica gel G sheets containing a fluorescent indicator (Macherey-Nagel, Düren, Germany) and chromatographed in a carbon tetrachloride-acetone (4:1, vol/vol) solution. The sheets were dried, and steroid-containing spots were located under ultraviolet light. The recovery of [14C]corticosterone and [14C]DOC in the medium samples after ether extraction and thin-layer chromatography (TLC) was 60%.

The activity of 11β-hydroxylase was defined as the ratio of [14C]corticosterone and [14C]DOC in the medium samples after incubation of ZFR cells with [14C]DOC for 60 min. In the experiment of the incubation of ZFR cells with [3H]pregnenolone, the activities of 3β-hydroxysteroid dehydrogenase (3β-HSD), 21-hydroxylase, and 11β-hydroxylase were expressed as the radioactivities of [3H]progesterone, [3H]DOC, and [3H]corticosterone, respectively. The recovery of [3H]corticosterone after ether extraction and TLC was 54%.

Statistical analysis. The treatment means of both in vivo and in vitro studies were tested for homogeneity using analysis of variance (ANOVA), and the difference between specific means was tested for significance using Duncan's multiple range test (33). A difference between two means was considered statistically significant when P was < 0.05.

RESULTS

Effects of intravenous injection of T_3 and T_4 on plasma total T_3 and total T_4. A single intravenous injection of T_3 or T_4 increased plasma concentrations of T_3 (35-fold) or T_4 (9-fold) at 30 min after injection compared with the basal level in the same group (P < 0.01; Fig. 2, top and bottom). The levels of plasma T_3 or T_4 in T_3- or T_2-injected rats increased significantly from 30 to 180 min after injection compared with the saline-injected animals (P < 0.01), respectively (Fig. 2, top and bottom).

ACTH plus T_3 or ACTH plus T_4 significantly increased plasma T_3 or T_4 concentration at 30, 60, 120, and/or 180 min after injection compared with the corresponding basal levels in the same group (P < 0.01; Fig. 2, top and bottom). After injection of ACTH plus T_3 or ACTH plus T_4, plasma T_3 or T_4 concentrations from 30 to 180 min were significantly higher than those in ACTH-injected animals (P < 0.01; Fig. 2, top and bottom).

Effects of intravenous injection of T_3 and T_4 on plasma corticosterone. A single intravenous injection of T_3 significantly decreased plasma corticosterone at 30, 120, and 180 min after injection compared with the basal level in the same group (P < 0.01; Fig. 3, top).

Fig. 2. Effects (means ± SE) of a single iv injection of 3,5,3'-triiodothyronine (T_3), adrenocorticotropic hormone (ACTH), or ACTH plus T_3 on concentration of plasma total T_3 (top), and those of thyroxin (T_4), ACTH, or ACTH plus T_4 on concentration of plasma total T_4 (bottom). Male rats were iv injected with saline, T_3 (5 µg/kg), T_4 (20 µg/kg), ACTH (5 µg/kg), ACTH plus T_3, or ACTH plus T_4 via the right jugular vein. Blood samples were collected through a jugular catheter at times indicated. ***P < 0.01 vs. saline-injected rats. +, ++ P < 0.05, P < 0.01 vs. value at 0 min, respectively.
Three hours after injection of T3, plasma corticosterone diminished significantly compared with the saline-injected animals (P < 0.01; Fig. 3, top).

Thirty minutes after a single injection of ACTH, the plasma corticosterone levels responded with a 4.3-fold increase (from 20.6 ± 3.6 to 90.5 ± 8.4 ng/ml; Fig. 3, bottom). Administration of both ACTH and T3 significantly reduced (P < 0.05 or P < 0.01) the corticosterone response between 30 and 120 min after injection compared with the ACTH-stimulated group (Fig. 3, bottom).

One hundred eighty minutes after intravenous injection of T4, the plasma corticosterone levels responded with a 2.5-fold decrease (from 17 ± 1.9 to 6.7 ± 1.4 ng/ml; Fig. 4, top) compared with the basal level in the same group. A single intravenous injection of T4 significantly decreased plasma corticosterone at 120 and 180 min after injection compared with the saline-injected group (P < 0.05 or P < 0.01; Fig. 4, top). From 30 to 90 min after injection of ACTH plus T4, significantly diminished plasma corticosterone was noted compared with the level of the group treated with ACTH alone (P < 0.05 or P < 0.01; Fig. 4, top).

Effects of T3 and T4 on the release of corticosterone in vitro. ACTH stimulated the production of corticosterone for 120 min in ZFR cells in a dose-dependent manner (Fig. 5). The increase was already significant (6.6-fold) at a dose of 10^{-10} M and reached an ~15.5-fold increase at a dose of 10^{-8} M.

Incubation of either T3 (10^{-9}-10^{-7} M), T4 (10^{-7} M) alone, or T3 or T4 in combination with ACTH (10^{-8} M) significantly (P < 0.01) decreased the release of corticosterone from ZFR cells compared with the vehicle or ACTH-treated groups, respectively (Fig. 6).

Forskolin (10^{-6} M) caused a 3.5-fold rise in corticosterone production. Administration of T3 (10^{-11}-10^{-9} M) or
T4 (10⁻⁸ M, 10⁻⁷ M) significantly lowered the forskolin-stimulated production of corticosterone in ZFR cells (Fig. 7).

Administration of ZFR cells for 120 min by DOC (10⁻⁸ M) in combination with T₃ (10⁻¹₀ M) or T₄ (10⁻⁹–10⁻⁷ M) significantly (P < 0.05 or P < 0.01) decreased the release of corticosterone compared with the DOC-treated group (Fig. 7).

Effects of T₃ and T₄ on the in vitro production of cAMP in response to IBMX. The levels of extracellular (i.e., medium) and intracellular (i.e., cell) cAMP after incubation of rat ZFR cells with 0.5 mM IBMX are illustrated in Fig. 8. T₃ and T₄ did not alter the basal levels of extracellular cAMP (Fig. 8, top) and the stimulatory effect of ACTH on the levels of both extra- and intracellular cAMP. Low doses of T₃ (10⁻⁹ and 10⁻⁸ M) or T₄ (10⁻⁸ M) did not alter the basal levels but attenuated the ACTH-stimulated levels of both extra- and intracellular cAMP.

Effects of T₃ and T₄ on the activities of 3β-HSD, 21-hydroxylase, and 11β-hydroxylase. Incubation of both T₃ (10⁻¹⁰ M or 10⁻⁹ M) and T₄ (10⁻⁹–10⁻⁷ M) in combination with DOC (10⁻⁹ M) and [¹⁴C]DOC (1.8–5.0 pmol) for 60 min markedly decreased 11β-hydroxylase activity (expressed as the ratio of [¹⁴C]corticosterone/[¹⁴C]DOC) from 22 to 63% compared with the control group (Fig. 9).

Administration of ZFR cells for 60 min with T₃ (10⁻¹⁰ M) or T₄ (10⁻⁸ M) in combination with pregnenolone (10⁻⁹ M) and [³H]pregnenolone (4.5–5.0 pmol) resulted in a decline (between 49 and 66%) in 3β-HSD activity (Fig. 10, top) and 28–30% in both 3β-HSD and 21-hydroxylase activities (Fig. 10, middle).

T₃ and T₄ caused about 14% inhibition (P < 0.01) in 3β-HSD, 21-hydroxylase, and 11β-hydroxylase activities (Fig. 10, bottom).

**DISCUSSION**

It has been demonstrated that chronic administration of T₃ in low doses (8 µg) decreases the levels of plasma and adrenal corticosterone (7). Our data indicate...
These results reflect that the thyroid hormones, T₃ and T₄, exert an acute inhibitory effect on both the basal and the ACTH-stimulated secretion of corticosterone. The disagreement between past and present findings might be due to the different methods for hormone measurement (the previous fluorometric assay vs. the present RIA) and/or the duration for observations (the previous chronic vs. the present acute). Chronic administration of T₃ at high concentrations (25–40 µg) in rats induces an increase in plasma corticosterone and adrenal hypertrophy, indicating intense stimulation of adrenal cortical function in chronic, severe hyperthyroidism (7). It has been reported that the effect of in vivo T₄ on adrenocortical secretion is related to the duration of treatment (4). Thyroid hormones have also been proposed to play a role in the maintenance of biological rhythms (32). It has been found that the amplitude of the circadian rhythm of blood corticosterone levels gradually decreases with time after thyroidectomy, and daily treatment with T₃ or T₄ for 2 wk restores the amplitude of the circadian adrenocortical rhythm to prethyroidectomized levels (22). Moreover, the plasma corticosterone response to corticotropin-releasing hormone (CRH) stimulation is increased, even though the response to ACTH is decreased, in rats administered chronically with pharmacological doses of T₄ compared with euthyroid rats (14). These results reflect the fact that chronic deficiency or administration of thyroid hormones causes a complicated effect on the hypothalamus-pituitary-adrenal (HPA) axis.

It has been known that PTU-induced hypothyroidism causes a significant reduction in CRH gene transcripts in the paraventricular nucleus and reduces both anterior pituitary proopiomelanocortin expression and circulating corticosterone in the rat (30). The circulating levels of thyroid hormones have a major effect on the central regulation of the HPA axis (30). Our data indicate that acute administration of T₃ or T₄ evokes an inhibitory rather than a stimulatory effect on corticosterone secretion. In humans, hypercortisolemia in primary hypothyroidism has been attributed to the decreased metabolic clearance rate (MCR) of cortisol (12). The prolonged half-life of endogenously secreted cortisol shown in hypothyroid subjects is consistent with the decreased disappearance rates of exogenously administered labeled cortisol in hypothyroid subjects (2, 39). It has been shown that the MCR of cortisol is increased in hyperthyroid males (9) and decreased in hypothyroid males (12). Therefore, a rapid clearance rate provides one explanation for the suppression of total plasma corticosterone concentrations observed in our T₃- and T₄-injected rats.

It has been shown that feeding of thyroglobulin suppresses adrenal corticoid production in vivo in ACTH-maintained hypophysectomized rats (34). The present in vitro data provide evidence that T₃ and T₄ diminish the release of rat corticosterone by acting directly on the adrenal ZFR cells (Fig. 6). These findings are in agreement with the observations by Moore and Callas (19), who found that drastic mitochondrial alterations characterized the zona fasciculata of hyperthyroid rats, suggesting that thyroid hormones may act...
directly on adrenal fasciculata cells. Boler and Moore (4) observed that the suppression of adrenocortical steroidogenesis produced by thyroid hormone is related to a mitochondrial effect, although the mechanism is unknown. The inhibition of postpregnenolone steroidogenic enzymes in response to T_3 and T_4 (Figs. 9 and 10) reflects a strong correlation between decreased steroidogenesis (including 3β-HSD, 21-hydroxylase, and 11β-hydroxylase activities) and the inhibition of corticosterone production in ZFR cells after administration of T_3 or T_4.

It has been shown that the rat genome contains four P450c11 genes (CYP11β1, CYP11β2, CYP11β3, CYP11β4). One of these (CYP11β1) encodes P450c11β, which is the steroid 11β-hydroxylase found solely in ZFR cells and is responsible for the conversion of 11DOC to corticosterone (18). However, the regulation of P450c11β1 mRNA expression by thyroid hormones in rats is not known. Our results indicated that T_3 and T_4 inhibit the stimulatory effect of ACTH on corticosterone release (Fig. 7) and the 11β-hydroxylase activity (Figs. 9 and 10) in ZFR cells. During the last decade, the specific T_3 receptors have been identified in rat FRTL 5 thyroid follicular cells (1), in anterior pituitary GH cells (6), and in human luteinized granulosa cells (10). Although the thyroid hormone receptor has not been identified in rat adrenocortical cells, it is probable that T_3 or T_4 acts on the ZFR cells via specific thyroid hormone receptors.

Simonian demonstrated that T_3 alone had no effect on 3β-HSD activity in human fetal adrenal cell cultures for 48 h (31). However, treatment with maximal concentrations of 10 nM ACTH plus 1 nM T_3 increased the 3β-HSD activity an additional 59–115% over that for ACTH alone (31). Our results indicated that thyroid hormones alone inhibit the 3β-HSD activity of adrenocortical cells in the adult rat. The reasons for the different observations are not known at the present time, but they may be attributable to development, species, or treatment duration. It has been shown that use of adrenal homogenates or mitochondria from euthyroid animals indicates that T_4 also suppresses some phases of corticoid conversion (13) and inhibits 11β-hydroxylase (25) and the adrenal transhydrogenase enzyme (24). It has been reported that thyroidectomy for 8 wk slowed down the activity of microsomal 21-hydroxylase and mitochondrial 11β-hydroxylase by ~30% (3). The absence of thyroid hormone may decrease the transmembrane gradient of the H ions that drive ATP and NADPH synthesis, which are both coupled to electron transport chain function (8). This observation, however, was not in agreement with the results by Freedland and Murad (8), which showed a significant increase in mitochondrial malic enzyme activity after in vitro T_3 administration. The direct effect of thyroid hormones on adult rat ZFR cells and the activities of steroidogenic enzymes have not been previously investigated. We found a marked inhibitory effect of T_3 and T_4 on postpregnenolone steroidogenic enzymes, including 3β-HSD, 21-hydroxylase, and 11β-hydroxylase activities. These findings are in agreement with the results reported by Peron et al. (25).

ACTH regulates glucocorticoid production by acting on specific receptors in the adrenal cortex. The number of ACTH binding sites in adrenocortical cells is increased by exposure of these cells to the activators of the cAMP pathway, e.g., dibutyryl CAMP or forskolin (20). In the present study, we found that the stimulatory effects of ACTH on both plasma corticosterone and corticosterone production in vitro were diminished by T_3 and T_4. T_3 and T_4 attenuated the stimulatory effects of corticosterone release in ZFR cells by adenylyl cyclase agonist and forskolin and decreased the stimulatory effects of ACTH on cAMP production, indicating that cAMP mediates this regulatory mechanism. Because T_3 at 10^-9 M inhibited ACTH-induced release of corticosterone (Fig. 6) but did not alter the level of extracellular cAMP (Fig. 8), we suggest that the cAMP response element was not the only pathway of the inhibition of corticosterone production by thyroid hormones. ACTH receptor genomic DNA has been isolated in the human (21), bovine (27), and mouse (16). Whether thyroid hormones alter the gene expression of the ACTH receptor in rats is not known but is worth investigating.

In summary, these findings suggest that acute administration of thyroid hormones 1) inhibits the secretion of corticosterone, both in vivo and in vitro; 2) attenuates the stimulatory effects of ACTH on the secretion of corticosterone via a decrease of cAMP production in ZFR cells; and 3) decreases the activities of 3β-HSD, 21-hydroxylase, and 11β-hydroxylase in ZFR cells. These results may contribute to the characterization of the regulatory mechanisms of adrenocortical function by thyroid hormones. Although the in vitro effect of thyroid hormones is fast, whether the inhibition of thyroid hormones on steroidogenesis in ZFR cells is mediated by nuclear receptor mechanisms is not clear at the present time. Furthermore, the inhibitory effects of T_3 and T_4 on corticosterone secretion might be of interest in the therapy of patients with hypercortisolemia caused by primary hypothyroidism.

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