Chinese Journal of Physiology 43(2): 55-59, 2000

Direct Effects of Propylthiouracil on Testosterone Production in Monkeys

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Abstract

Propylthiouracil (PTU) is an anti-thyroid drug. However, the direct effects of PTU on the endocrine functions of non-thyroid glands are unclear. In the present study, we examined the acute effects of PTU on testosterone secretion in monkeys. Male monkeys were infused intravenously with PTU for 30 min. Blood samples were collected at several time intervals. Monkey testicular interstitial cells were cultured with PTU, human chorionic gonadotropin (hCG), or forskolin, at 34 °C for 1 h. In another study, steroidogenesis in monkey testicular interstitial cells were examined. PTU decreased plasma testosterone but not plasma thyroxine (T_4) and luteinizing hormone (LH) levels in monkeys. Administration of PTU resulted in a dose-dependent inhibition of basal and hCG-, as well as forskolin-stimulated testosterone release by monkey testicular interstitial cells. PTU also diminished the stimulatory effects induced by androstenedione. These results suggest that PTU inhibits testosterone secretion *via* a mechanism independent of the secretion of T_4 and LH in primates. The inhibitory mechanism of PTU on testosterone production involves a decreased activity of 17β-hydroxysteroid dehydrogenase (17β-HSD) and post-cAMP pathways.

Key Words: propylthiouracil (PTU), testosterone (T), cAMP, steroidogenesis, 17β-HSD, monkey

Introduction

Propylthiouracil (PTU) inhibits the synthesis of thyroid hormones in thyroid gland (7) and the deiodination of thyroxine (T_4) in peripheral tissues (19). Although being a well-known anti-thyroid drug, PTU treatment still induces several side effects in hyperthyroid patient. The most common side effects are transient leukopenia (7), jaundice, hepatocellular dysfunction, and hepatomegaly (8, 11, 14).

Although several previous studies on PTU-induced hypothyroid rats indicate a decreased serum testosterone (10), whether the inhibition was due to PTU or hypothyroidism was not known. It has been well known that PTU-induced transient neonatal hypothyroidism produces [1] a sustained increase in the testis weight and sperm production of adult rats but does not increase testosterone levels (5, 6), and [2] a significant reduction in gonadotropin-releasing hormone (GnRH)-stimulated luteinizing hormone (LH) production in adult male rats (13). Although the studies in rats and rams suggest a subnormal testosterone production caused by the administration of anti-thyroid drugs (e.g. methimazole or methylthiouracil) in the testicular tissues (1, 4), Leydig cell activity under PTU-induced hypothyroidism has not been explored.

We have investigated the direct effects of PTU on corticosterone secretion both *in vivo* and *in vitro*. It has been shown that PTU decreases not only the rat plasma corticosterone response to ACTH but also the corticosterone production in rat zona fasciculata-reticularis cells (16). However, the direct effects of PTU on the endocrine functions of gonads are not known. In the present study, we examine the acute effects of PTU on the secretion of testosterone in monkeys. The effects of PTU on the activity of 17β-hydroxysteroid dehydrogenase (17β-HSD)

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Received April 10, 2000; Revised April 28, 2000; Accepted May 1, 2000.

in monkey testicular interstitial cells were also evaluated.

Materials and Methods

Animals

Male monkeys (*Macaca cyclopis*, weighing 8-12 kg) were housed respectively in the temperature controlled rooms (22±1 °C) with 14 h of artificial illumination daily (0600-2000). Purina monkey chow was fed twice daily, fresh fruit was supplied once daily, and tap water was provided *ad libitum*.

Effects of PTU on Plasma Testosterone, T_4 and LH in Monkeys

Three male monkeys were infused intravenously with PTU (10 mg/kg, 10 ml, respectively) *via* catheter in tibial vein for 30 min. Blood samples (3 ml each) were collected at 0, 30, 60, and 120 min post intravenous infusion of PTU. An equal volume of saline was injected immediately after each bleeding.

Plasma was separated by centrifugation at 10000 × g for 1 min and stored at -20 °C. Plasma LH was measured by radioimmunoassay (RIA). To measure the concentrations of testosterone, 0.1 ml plasma was extracted with 0.5 ml diethyl ether. The concentrations of testosterone in the reconstituted extracts were measured by RIA. The concentration of plasma T_4 was measured by the RIA kit provided from DiaSorin Inc. (Stillwater, MN, USA).

Preparation of Monkey Testicular Interstitial Cells

The method of collagenase dispersion of testicular interstitial cells was modified from the procedure described by Lin *et al.* (15). Monkeys were weighed, anesthetized with ketamine (10 mg/kg BW), and hemicastrated under surgical conditions. Neither their health nor their reproductive capacity were affected. The testes were decapsulated and cut into small fractions (1 mm^3). Each monkey testis was added to a 50 ml polypropylene tube containing 5 ml preincubation medium and 700 μg collagenase (Type IA, Sigma). Preincubation medium was made up of 1% bovine serum albumin (BSA, Fraction V, Sigma, U.S.A.) in Hank's balanced salt solution (HBSS), with HEPES 25 mM, sodium bicarbonate 0.35 g/l, penicillin-G 100 IU/ml, streptomycin sulfate 50 μg/ml, heparin 2550 USP K units/l, pH 7.4, and aerated with 95% O_2 and 5% CO_2. The tube was placed horizontally in a 34 °C water bath, parallel to the direction of the shaking. Fifteen min after shaking at 100 cycles/min, the digestion was stopped by adding 35 ml of cold preincubation medium and inverting the tube several times. The tube was allowed to stand for 5 min and then filtered through a four-layer nylon mesh. Cells were collected by centrifugation at 4 °C, 100 × g for 10 min. The cell pellets were washed with deionized water to disrupt red blood cells and the osmolarity immediately restored with 10-fold HBSS. Hypotonic shock was repeated twice for RBC disruption and cell pellets resuspended in incubation medium (substitution of HBSS in preincubation medium with Medium 199, and sodium bicarbonate 2.2 g/l).

Effects of PTU on Testosterone Release by Monkey Testicular Interstitial Cells

Monkey testicular interstitial cell (2×10^6 cells/ml) were preincubated at 34 °C for 1 h under a controlled atmosphere (95% O_2 and 5% CO_2), shaken at 100 cycles/min. The supernatant fluid was decanted after centrifugation of the tubes at 100 × g for 10 min. The cells were then incubated with PTU (0, 3, 6, or 12 mM) in the presence of human chorionic gonadotropin (hCG, 0.05 IU/ml) or forskolin (an adenylyl cyclase activator, 10^{-5} M) in 200 μl fresh medium. Following 1 h of incubation, 2 ml ice-cold PBSG buffer was added to stop the incubation. The medium was centrifuged at 100 × g for 10 min at 4 °C and the supernatant was stored at -20 °C until analyzed for testosterone by RIA.

Effects of PTU on the Activity of 17β-Hydroxysteroid Dehydrogenase (17β-HSD) in Monkey Testicular Interstitial Cells

Monkey testicular interstitial cells (2×10^6 cells/ml) were preincubated for 1 h and then were incubated for 1 h with or without PTU at 12 mM in the presence or absence of androstenedione (Δ_4, 10^{-5} M). At the end of incubation, 2 ml ice-cold PBSG buffer were added and immediately followed by centrifugation at 100 × g for 10 min at 4°C. The supernatant fluid was stored at -20°C until analyzed for testosterone by RIA.

Hormone RIAs

The concentrations of testosterone in plasma and media were determined by RIA as described previously (15, 18). With anti-testosterone serum no. W8, the sensitivity of testosterone RIA was 2 pg per assay tube. The intra- and interassay coefficients of variation (CV) were 4.1% (n = 6) and 4.7% (n = 10), respectively.

The monkey LH-I-1 used for iodination and the monkey LH-RP-1 which served as standard preparations were provided by the National Institute

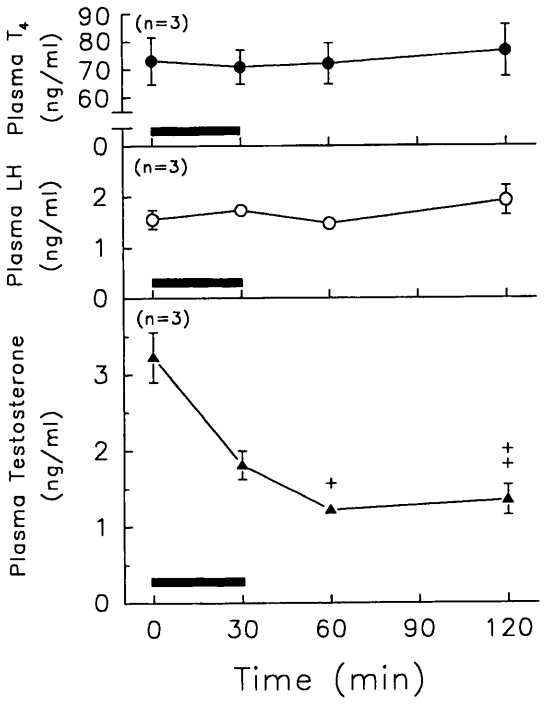

Fig. 1. Effects of PTU on levels of plasma T_4, LH and testosterone in monkeys. Three monkeys were given an intravenous infusion of PTU (10 mg/kg, 10 ml, 30 min) *via* tibial vein. The concentrations of plasma T_4 (●, n=3), LH (○, n=3), and testosterone (▲, n=3) were measured by RIA. The horizontal line indicates time of infusion. $+P<0.05$, $++P<0.01$ compared to basal release (time at 0 min), respectively, by student's paried *t*-test. Each value represents mean±SEM.

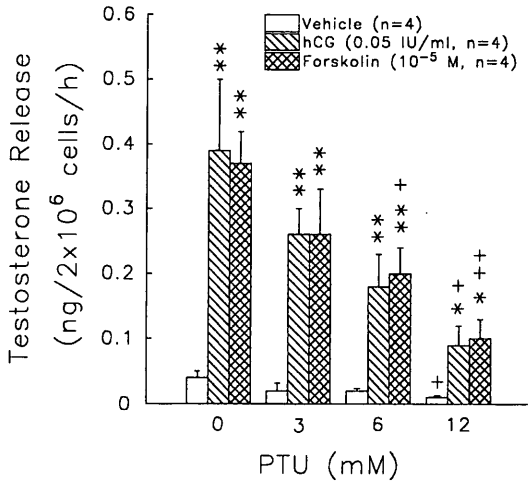

Fig. 2. Inhibitory effects of different doses of PTU on the *in vitro* release of testosterone in response to hCG (0.05 IU/ml, n=4) or forskolin (10^{-5} M, n=4) by monkey testicular interstitial cells. $+P<0.05$, $++P<0.01$ compared to PTU at 0 mM, $*P<0.05$, $**P<0.01$ compared to vehicle group, respectively. Each value represents mean±SEM.

of Diabetes and Digestive and Kidney Diseases, USA. The sensitivity was 0.3 ng for monkey LH RIA. The intra- and interassay coefficients of variation were 5.7% (n=4), and 10.3% (n=4), respectively.

The concentration of total T_4 in monkey plasma was measured by the RIA kit provided from DiaSorin Inc. (Stillwater, MN, USA).

Statistical Analysis

All values are given as mean ± standard error of mean. The treatment means were tested for homogeneity by a two-way analysis of variance (ANOVA) and the difference between specific means was tested for significance by Duncan's multiple-range test (17). The concentrations of plasma hormones was analyzed by Student's paired *t*-test (17). A difference between two means was considered statistically significant when $P<0.05$.

Results

Effects of PTU on Plasma T_4, Testosterone and LH in Monkeys

After 30 min of intravenous infusion (10 mg/kg, respectively), the effects of PTU on monkey plasma T_4, LH and testosterone are shown in Fig. 1. The concentration of plasma testosterone decreased at 60 and 120 min after PTU infusion (1.22±0.06 and 1.35±0.20 ng/ml *vs.* 0 min, 3.23±0.33 ng/ml, $P<0.05$ or 0.01, n=3). Plasma T_4 were between 70.8±6.1 and 76.6±9.3 ng/ml. Plasma LH were between 1.48±0.12 and 1.93±0.29 ng/ml. Neither T_4 nor LH concentration in monkey plasma was altered by the acute administration of PTU. Saline-infusion did not affect concentration of plasma testosterone (30 - 120 min, 2.34±0.01 - 2.22±0.08 ng/ml vs. 0 min, 2.61±0.07 ng/ml, n=3, data not shown).

Effects of PTU on Testosterone Release by Monkey Testicular Interstitial Cells

The effects of PTU on testosterone release by monkey testicular interstitial cells in vitro were studied. Following 1 h of preincubation, testicular interstitial cells (2×10^6 cells) were incubated with vehicle, human chorionic gonadotropin (hCG, 0.05 IU/ml) or forskolin (10^{-5} M), combined with PTU (0, 3, 6, or 12 mM) for 1 h (Fig. 2). Incubation of testicular interstitial cells with hCG or forskolin for 1 h increased testosterone secretion (0.39±0.11 and 0.37±0.05 ng/2×10^6 cells/h vs. 0.04±0.01 ng/2×10^6 cells/h, $P<0.01$, n=4). The basal release of testosterone in testicular interstitial cells was decreased by 12 mM PTU ($P<0.05$). A combination of hCG with PTU of 12 mM resulted in an inhibition of the hCG-stimulated release of testosterone (0.09±0.03 ng/2×10^6 cells/h *vs.* 0.39±0.11 ng/2×10^6 cells/h, $P<0.05$, n=4). PTU

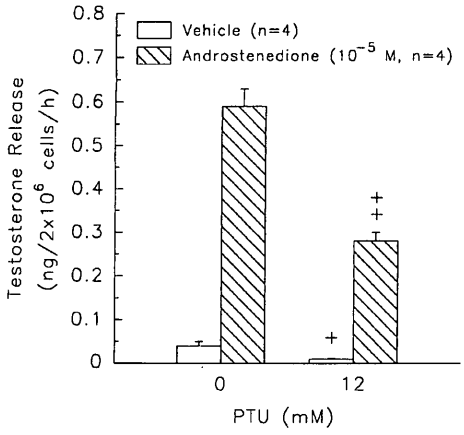

Fig. 3. Effects of PTU on the release of testosterone after incubation of monkey testicular interstitial cells with androstenedione (10^{-5} M, n=4). +P<0.05, ++P<0.01 compared to PTU at 0 mM. Each value represents mean±SEM.

(6 and 12 mM) also inhibited the forskolin-stimulated release of testosterone (0.20±0.04 and 0.10±0.03 ng/2×10^6 cells/h *vs.* 0.37±0.05 ng/2×10^6 cells/h, P< 0.05 or 0.01, n=4).

Effects of PTU on the Activity of 17β-HSD in Monkey Testicular Interstitial Cells

The effects of PTU on the activity of 17β-HSD in monkey testicular interstitial cells were studied. At the concentration of 10^{-5} M, androstenedione increased the production of testosterone by rat testicular interstitial cells (0.59±0.04 ng/2×10^6 cells/h vs. 0.04±0.01 ng/2×10^6 cells/h, P<0.01, n=4) (Fig. 3). PTU at 12 mM decreased not only the basal but also the androstenedione-stimulated release of testosterone by monkey testicular interstitial cells (0.01±0.00 ng/2×10^6 cells/h *vs.* 0.04±0.01 ng/2×10^6 cells/h, 0.28±0.02 ng/2×10^6 cells/h *vs.* 0.59±0.04 ng/2×10^6 cells/h, P<0.01, n=4).

Discussion

The present results demonstrate that administration of PTU in monkeys diminishes the secretion of testosterone via a mechanism independent of the action of gonadotropin and thyroid hormones.

Previous studies on both thyroidectomy- (2, 3) and PTU-induced hypothyroid (10) rats demonstrate a decreased level of serum testosterone. The decrease of testosterone production in response to hypothyroidism was attributed to decreased levels of thyroid hormones in serum. However, the direct pharmacological effects of PTU on the gonads have not been investigated. Recently we found that PTU decreases plasma corticosterone response to ACTH in rats and directly decreases corticosterone production in rat zona fasciculata-reticularis cells (16). The results indicate that PTU may directly regulate steroidogenesis in rodents. The present study was the first investigation to examine the direct effects of PTU on the endocrine functions of monkey testicular interstitial cells.

For controlling the dosage of PTU, we treated animals with PTU by intravenous infusion rather than single injection. The dose of PTU employed in our *in vitro* study was in the range of that used previously in rat studies to induce hypothyroidism, although it was higher than clinically dose. However, the concentration of PTU used in our *in vitro* studies are lower (one tenth) than those employed in our *in vivo* model. In our rat studies, PTU did not affect LH release by rat anterior pituitary glands *in vitro* (data not shown). It indicated that PTU inhibits testosterone release by acting directly on testis rather than nonspecific toxicity. The *in vivo* results indicated that PTU infusion acutely decreased plasma testosterone but not T_4. Since the half-life of T_4 is approximately 7 days, it is obviously that plasma T_4 would not be changed within 120 min. Because the levels of plasma T_4 were not altered, the diminishment of plasma testosterone would be due to the direct effects of PTU rather than the effects of hypothyroidism on gonads. Since the acute infusion of PTU markedly decreased the level of plasma testosterone without altering the levels of plasma T_4 and LH, the inhibition of PTU on testosterone secretion was independent of the actions of gonadotropins and thyroid hormones. Therefore, it indicated that PTU possesses toxic effects on testosterone production in primates. Furthermore, our *in vitro* results of the inhibition of PTU on testosterone production by monkey testicular interstitial cells confirmed our *in vivo* observations.

It has been well established that hCG stimulates testosterone secretion both *in vivo* and *in vitro*, and increases testicular cAMP production. In the present study, we found that PTU inhibits both the basal and the hCG-stimulated release of testosterone *in vitro*. It also decreased forskolin-induced testosterone release in monkeys, suggesting that PTU acts directly on the testicular interstitial cells to regulate testosterone production at a point associated with the generation of cAMP. Since the actions of PTU were evident *in vitro* in tissue removed from intact monkeys, they were clearly independent of the actions of gonadotropins and thyroid hormones.

The biosynthesis of testosterone by testicular cells is constituted with sequential reactions that convert cholesterol to testosterone. The conversion of androstenedione to testosterone is catalyzed by the 17β-HSD. This step is reversible (9) and requires the

contribution of the glycolytic pathway to meet ATP (12). In monkey testicular interstitial cells, androstenedione-stimulated testosterone release was inhibited by PTU indicating that PTU reduced the activity of 17β-HSD in primates.

In summary, the present investigation demonstrated that [1] PTU possesses toxic effects on testosterone production in primates, and [2] PTU decreased the secretion of testosterone by acting directly on monkey testicular interstitial cells *via* the mechanisms in part involving an inhibition on the activity of the steroidogenic enzyme 17β-HSD.

Acknowledgments

This study was supported by the Grant NSC88-2314-B-010-076 from the National Science Council, and awards from the Medical Research and Advancement Foundation in memory of Dr. Chi-Shuen Tsou, to P. S. Wang.

Monkey LH RIA kits were kindly supplied by the National Hormone and Pituitary Program, National Institute of Diabetes and Digestive and Kidney Diseases, National Institute of Child Health and Human Development, and US Department of Agriculture, USA.

The technical assistance provided by Dr. William J. S. Huang, Mr. Ho Lin, and Mr. Jiann-Jong Chen, is appreciated.

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