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Direct effects of propylthiouracil on testosterone secretion in rat testicular interstitial cells

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1 The aim of this study was to investigate the mechanism by which propylthiouracil (PTU) exerts its inhibitory effects on the production of testosterone by rat testicular interstitial cells.

2 The plasma testosterone concentration was decreased 60 and 120 min after an intravenous infusion of PTU (10 or 20 mg kg^{-1}), but the concentration of plasma T_4 was unaffected by the drug treatment.

3 Exposure of anterior pituitary tissue to PTU (3–12 mM) *in vitro* did not affect either basal or gonadotropin-releasing hormone (GnRH)-stimulated luteinizing hormone (LH) release.

4 PTU (3–12 mM) inhibited both the basal and the human chorionic gonadotropin (hCG, 0.05 iu ml^{-1})-stimulated release of testosterone from rat testicular tissue *in vitro*; at the highest concentration tested (12 mM), it also inhibited the forskolin or 8-bromo-adenosine 3′:5′-cyclic monophosphate (8-Br-cyclic AMP)-stimulated release of testosterone.

5 The 25-OH-cholesterol (10^{-7}–10^{-5} M)-stimulated release of pregnenolone and testosterone by the testicular interstitial cells was inhibited by PTU (12 mM, $P<0.05$).

6 The results suggest that the inhibitory actions of PTU on testosterone secretion are exerted, at least in part, at the testicular level through a mechanism which is independent of thyroid status and which involves a reduction in P450scc activity and, hence, in the conversion of cholesterol to pregnenolone.

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Abbreviations: AP, anterior pituitary gland; 8-Br-cyclic AMP, 8-bromo-adenosine 3′:5′-cyclic monophosphate; BSA, bovine serum albumin; GnRH, gonadotropin-releasing hormone; 3β-HSD, 3β-hydroxysteroid dehydrogenase; 17β-HSD, 17β-hydroxysteroid dehydrogenase; HBSS, Hank's balanced salt solution; hCG, human chorionic gonadotropin; HEPES, N-[2-hydroxyethyl]piperazine-N′-[2-ethanesulphonic acid]; LH, luteinizing hormone; 25-OH-C, 25-hydroxy-cholesterol; 17α-OH-P, 17α-hydroxy-progesterone; P, progesterone; Δ_5P, pregnenolone; P450scc, cytochrome P450 side-chain cleavage; PTU, propylthiouracil; RBC, red blood cell; Δ_4, androstenedione.

Introduction

Propylthiouracil (PTU, MW = 170.2) is an anti-thyroid drug which inhibits both the synthesis of thyroid hormones in thyroid gland (Cooper, 1984), and the conversion of thyroxine (T_4) to its active form, triiodothyronine (T_3), in peripheral tissues (Yang & Gordon, 1997). Clinical studies have shown that the most common unwanted effect of PTU treatment in hyperthyroid patients is transient leukopenia (Cooper, 1984). In addition, the drug has been found to induce severe toxic effects on the liver (Levy, 1993; Deidiker & deMello, 1996) in a number of patients who subsequently developed jaundice, severe hepatocellular dysfunction, and hepatomegaly (Jonas & Eidson, 1988; Levy, 1993; Deidiker & deMello, 1996). However, the effects of PTU on human testicular cells are unclear.

Previous studies have shown that transient neonatal hypothyroidism, induced by treatment with PTU, increases testicular size, Sertoli cell numbers, and daily sperm production in the adult rat and mouse (Hess *et al*., 1993; Joyce *et al*., 1993), although serum testosterone is not raised (Cooke *et al*., 1991; Cooke & Meisami, 1991). Studies in rodents have suggested that PTU treatment in adults may also influence peripheral steroidogenesis. For example, Hardy *et al*. (1993) reported decreased serum testosterone levels in adult rats rendered hypothyroid with PTU, although others (Weiss & Burns, 1988) found testosterone production was unchanged by the drug treatment. Moreover, although Leydig cell activity has not been studied during thiouracil-induced hypothyroidism *in vivo*, studies in rats and rams suggest testosterone production is subnormal (Chandrasekhar *et al*., 1985; Ando *et al*., 1990).

We have previously shown that PTU decreases both the adrenocortical response to ACTH *in vivo* and the production of corticosterone by rat zona fasciculata-reticularis cells (Lo *et al*., 1998). However, the direct effects of PTU on the function of gonadal tissues are not known. In the present study, we examined the acute effects of PTU *in vitro* on (a) the production of testosterone and (b) activity of the steroidogenic enzyme in rat testicular interstitial cells.

Methods

Animals

Male Sprague-Dawley rats weighing 300–350 g were housed in a temperature controlled room (22±1°C) with 14 h of

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artificial illumination daily (0600–2000 h) and given food and water *ad libitum*.

Effects of PTU on plasma testosterone and T_4 in rats

Male rats were divided into two groups with eight rats in each group. Each animal was anaesthetized with ether and catheterized *via* the right jugular vein (Wang *et al*., 1994; Tsai *et al*., 1996). After 20 h, they were infused with 1 ml of saline or PTU (10 or 20 mg kg^{-1}, Sigma Chemical Co., St. Louis, MO, U.S.A.) *via* a peristaltic pump (Minipuls 3, Gilson, Villiers-le-Bel, France) for 30 min. Blood samples (0.3 ml each) were collected from the jugular catheter at 0, 30, 60, 120, 180 and 240 min after infusion.

Plasma was separated by centrifugation at $10{,}000 \times g$ for 1 min and stored at $-20°C$. The concentration of plasma T_4 was measured by the radioimmunoassay (RIA) kit from DiaSorin Inc. (Stillwater, MN, U.S.A.). To measure the concentrations of testosterone, 0.1 ml plasma was mixed with 0.5 ml diethyl ether, agitated for 20 min, centrifugated at $1000 \times g$ for 5 min, and then quick frozen in a mixture of acetone and dry ice. The organic phase was collected, dried, and reconstituted in a PBSG buffer solution (0.1% gelatin in phosphate-buffered saline, PBS, pH 7.5). The concentrations of testosterone in the reconstituted extracts were measured by RIA.

Effects of PTU on LH release by anterior pituitary glands

After decapitation, the rat anterior pituitary glands (APs) were excised, bisected, and preincubated with Locke's solution containing 10 mM glucose, 0.003% bacitracin, and 0.05% HEPES at 37°C for 30 min (Wang *et al*., 1994; Tsai *et al*., 1999). Each hemi-AP gland was assigned to a flask containing 1 ml medium, which was aerated with 95% O_2 and 5% CO_2. APs were then incubated with PTU (0, 3, 6 or 12 mM), gonadotropin-releasing hormone (GnRH, 10 nM, Sigma), or PTU plus GnRH for 30 min. At the end of incubation, the medium was collected and the tissues were weighed. The concentrations of LH in medium were measured by RIA.

Preparation of testicular interstitial cells

The method of collagenase dispersion of testicular interstitial cells was modified from the procedure described by Tsai *et al*. (1997). Five decapsulated testes collected postmortem were added to a 50 ml polypropylene tube containing 5 ml preincubation medium and 700 μg collagenase (Type IA, Sigma, U.S.A.). 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^{-1}, penicillin-G 100 iu ml^{-1}, streptomycin sulphate 50 μg ml^{-1}, heparin 2550 USP K units l^{-1}, pH 7.4, and aerated with 95% O_2 and 5% CO_2. The tube was placed horizontally for 15 min in a 34°C water bath and shaken continuously (100 cycles min^{-1}). The digestion was then 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, $100 \times g$ for 10 min. The cell pellets were washed with deionized water to disrupt red blood cells (RBCs) and the osmolarity immediately restored with 10 fold Hank's balanced salt solution (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^{-1}). Cell concentration (1.0×10^6 cells ml^{-1}), viability (over 97%), and the sperm cells (less than 5%) were determined by use of a hemacytometer and the trypan blue exclusion method.

Effects of PTU on testosterone release by testicular interstitial cells

Aliquots (1 ml) of cell suspensions (1.0×10^6 cells ml^{-1}) were preincubated with incubation medium in polyethylene tubes for 1 h at 34°C under a controlled atmosphere (95% O_2 and 5% CO_2), shaken at 100 cycles min^{-1}. The supernatant fluid was decanted after centrifugation of the tubes at $100 \times g$ for 10 min. The cells were then incubated with PTU (3, 6 or 12 mM), human chorionic gonadotropin (hCG, 0.05 iu ml^{-1}, Sigma), forskolin (an activator of adenylyl cyclase, 10^{-5} M, Sigma), 8-bromo-adenosine 3′:5′-cyclic monophosphate (8-Br-cyclic AMP, an analogue of cyclic AMP, 10^{-4} M, Sigma), hCG plus PTU, forskolin plus PTU, or 8-Br-cyclic AMP plus PTU in 200 μl fresh medium. Following 1 h of incubation, 2 ml ice-cold PBSG buffer solution was added to stop the incubation. The medium was centrifuged at $100 \times g$ for 10 min and the supernatant was stored at $-20°C$ until analysed for testosterone by RIA.

Effects of PTU on the steroidogenesis in testicular interstitial cells

Rat testicular interstitial cells (1.0×10^6 cells ml^{-1}) were preincubated for 1 h and then incubated for 1 h with or without PTU at 12 mM in the presence or absence of five steroidal precursors as described previously (Lin *et al*., 1998). These precursors included 25-hydroxy-cholesterol (membrane-permeable cholesterol, 25-OH-C), pregnenolone (Δ_5P), progesterone (P), 17α-hydroxy-progesterone (17α-OH-P), and androstenedione (Δ_4). At the end of incubation, 2 ml ice-cold PBSG buffer was added and the tubes centrifuged immediately at $100 \times g$ for 10 min at 4°C. The supernatant fluid was stored at $-20°C$ until analysed for testosterone by RIA.

Effects of PTU on the 25-OH-cholesterol-stimulated pregnenolone production in testicular interstitial cells

In order to explore the activity of cytochrome P450 side-chain cleavage enzyme (P450scc), cell suspensions (1.0×10^6 cells ml^{-1}) were preincubated in medium for 1 h at 34°C and then incubated for 1 h with or without PTU at 12 mM in the presence of 25-OH-cholesterol (10^{-7} or 10^{-5} M). At the end of incubation, 2 ml ice-cold PBSG buffer were added and immediately followed by centrifugation at $100 \times g$ for 10 min at 4°C. The supernatant fluid was stored at $-20°C$ until analysed for pregnenolone by RIA.

RIA of testosterone, pregnenolone and LH

The concentrations of testosterone in plasma and medium were determined by RIA as described previously (Wang *et al*., 1994; Tsai *et al*., 1996). 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 concentration of pregnenolone was determined by RIA with anti-regnenolone antiserum purchased from Biogenesis Inc (Sandown, NH, U.S.A.). The sensitivity of the pregnenolone RIA was 16 pg per assay tube. The intra- and interassay

coefficients of variation were 2.3% ($n=6$) and 3.7% ($n=4$), respectively.

The concentration of medium LH was determined by RIA as described previously with anti-LH serum PW11-2 (Wang *et al*., 1994). The rat LH-I-6 used for iodination and the rat LH-RP-3 which served as standard preparations were provided by National Hormone and Pituitary Program, National Institute of Diabetes and Digestive and Kidney Diseases, National Institute of Child Health and Human Development, and U.S. Department of Agriculture, U.S.A. The sensitivity was 0.1 ng for LH RIA. The intra- and interassay coefficients of variability were 3.8% ($n=4$), and 6.6% ($n=5$), respectively.

Materials

Bovine serum albumin (BSA), N-2-hydroxyethylpiperazine-N′-2-ethane-sulphonic acid (HEPES), Hank's balanced salt solution (HBSS), medium 199, sodium bicarbonate, penicillin-G, streptomycin, heparin, collagenase, propylthiouracil (PTU), gonadotropin-releasing hormone (GnRH), human chorionic gonadotropin (hCG), forskolin, 8-bromo-adenosine 3′:5′-cyclic monophosphate (8-Br-cyclic AMP), and testosterone were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). [^{3}H]-testosterone, and [^{125}I]-Na were obtained from Amersham International plc. (Bucks, U.K.). The doses of drugs are expressed in their final molar concentrations in the flask.

Statistical analysis

All values are given as the mean ± s.e.mean. The treatment means were tested for homogeneity by analysis of variance (ANOVA) and the difference between specific means was tested for significance by Duncan's multiple-range test (Steel & Torrie, 1960). A difference between two means was considered statistically significant when $P<0.05$.

Results

Effects of PTU on plasma testosterone and T_4 in rats

The effects of PTU infusion on plasma testosterone are shown in Figure 1. The levels of plasma testosterone were not altered by saline infusion. The plasma concentration of testosterone decreased gradually from 60–240 min after intravenous infusion of the high dose of PTU (20 mg kg^{-1} group, 60 min, 0.48 ± 0.11; 120 min, 0.34 ± 0.06; 180 min, 0.19 ± 0.05; 240 min, 0.10 ± 0.02 ng ml^{-1}, $n=8$, versus 0 min, 0.93 ± 0.23 ng ml^{-1}, $n=8$, $P<0.05$ or 0.01). The lower dose of PTU was also effective in this regard and the plasma levels of testosterone at 120, 180 and 240 min following an intravenous infusion were significantly lower in PTU-infused rats than in saline-infused animals (120 min, 10 mg kg^{-1} group, 0.41 ± 0.05 ng ml^{-1}, $n=8$, 20 mg kg^{-1} group, 0.34 ± 0.06 ng ml^{-1}, $n=8$, versus 0.79 ± 0.19 ng ml^{-1}, $n=8$, $P<0.05$; 180 min, 10 mg kg^{-1} group, 0.23 ± 0.05 ng ml^{-1}, $n=8$, 20 mg kg^{-1} group, 0.19 ± 0.05 ng ml^{-1}, $n=8$, versus 0.61 ± 0.14 ng ml^{-1}, $n=8$, $P<0.05$ or 0.01; 240 min, 10 mg kg^{-1} group, 0.14 ± 0.03 ng ml^{-1}, $n=8$, 20 mg kg^{-1} group, 0.10 ± 0.02 ng ml^{-1}, $n=8$, versus 0.59 ± 0.13 ng ml^{-1}, $n=8$, $P<0.01$).

The mean plasma levels of T_4 at all time points were 18.1 ± 3.1 ng ml^{-1} for the saline-infused animals, and 20.9 ± 2.2 ng ml^{-1} for the PTU-infused rats, respectively. The concentrations of plasma T_4 were not altered by the acute administration of PTU in rats.

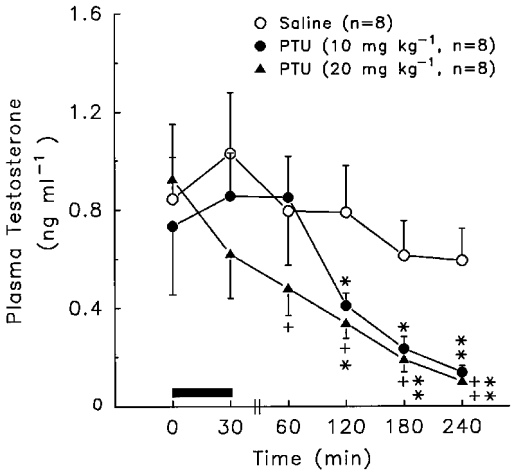

Figure 1 Plasma testosterone concentrations at various times after an intravenous infusion of saline or PTU *via* right jugular vein. The horizontal line indicates time of infusion. + $P<0.05$, + + $P<0.01$ compared to basal release. * $P<0.05$, ** $P<0.01$ compared with the saline group. Each value represents mean ± s.e.mean.

Effects of PTU on LH release by rat anterior pituitary glands

The effects of PTU on LH release by rat APs *in vitro* were examined. As compared to the control group, neither basal LH release (4.34 ± 0.36 to 5.13 ± 1.23 ng mg^{-1} 30 min^{-1}, $n=7$, versus control group 4.39 ± 0.91 ng mg^{-1} 30 min^{-1}, $n=7$) nor GnRH-stimulated LH release (9.86 ± 1.17 to 8.91 ± 1.65 ng mg^{-1} 30 min^{-1}, $n=7$, versus GnRH-treated group 11.21 ± 1.58 ng mg^{-1} 30 min^{-1}, $n=7$) by rat APs was altered by the administration of PTU (3–12 mM).

Effects of PTU on testosterone release by rat testicular interstitial cells

The effects of PTU on testosterone release by rat testicular interstitial cells *in vitro* are shown in Figure 2. Following 1 h of preincubation, testicular interstitial cells (1.0×10^6 cells) were incubated with or without human chorionic gonadotropin (hCG, 0.05 iu ml^{-1}), combined with PTU (0, 3, 6 or 12 mM) for 1 h. As compared to the control group, administration of PTU (3–12 mM) inhibited testosterone release by the testicular interstitial cells (2.39 ± 0.26 to 1.83 ± 0.34 ng 1.0×10^6 cells^{-1} h^{-1}, $n=8$, versus basal level 6.76 ± 0.88 ng 1.0×10^6 cells^{-1} h^{-1}, $n=8$, $P<0.01$). Incubation of testicular interstitial cells with hCG for 1 h increased testosterone secretion (hCG-treated group 60.0 ± 11.2 ng 1.0×10^6 cells^{-1} h^{-1}, $n=8$, versus basal group, $P<0.01$). A combination of hCG with PTU of 3–12 mM resulted in an inhibition of the hCG-stimulated release of testosterone (31.9 ± 4.9 to 12.6 ± 1.5 ng 1.0×10^6 cells^{-1} h^{-1}, $n=8$, versus hCG-treated group, $P<0.05$ or 0.01).

Figure 3 demonstrates the effects of PTU on forskolin (10^{-5} M) and 8-Br-cyclic AMP (10^{-4} M)-stimulated testosterone release by testicular interstitial cells. Treatment with hCG, forskolin, and 8-Br-cyclic AMP all produced significant ($P<0.01$) increases in testosterone release versus vehicle. PTU (3–12 mM) inhibited the basal (1.98 ± 0.43 to 1.51 ± 0.29 ng 1.0×10^6 cells^{-1} h^{-1}, $n=8$, versus basal level 3.74 ± 0.35 ng 1.0×10^6 cells^{-1} h^{-1}, $n=8$, $P<0.01$) and hCG-stimulated (37.6 ± 4.8 to 19.8 ± 2.3 ng 1.0×10^6 cells^{-1} h^{-1}, $n=8$, versus hCG-treated group 56.9 ± 8.6 ng 1.0×10^6 cells^{-1} h^{-1}, $n=8$, $P<0.05$ or 0.01) testosterone release. Administration of high

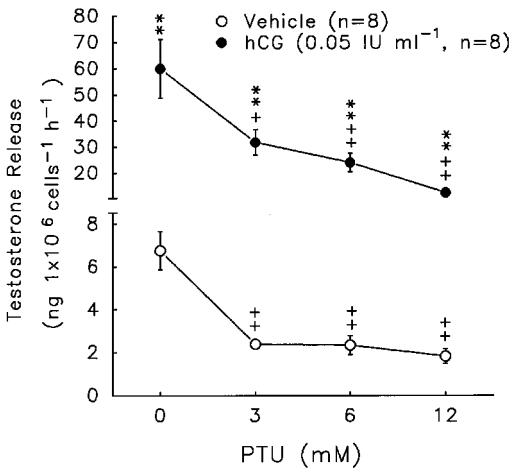

Figure 2 Effects of PTU (3–12 mM) on the release *in vitro* of testosterone by rat testicular interstitial cells in the presence or absence of hCG. $+P<0.05$ and $++P<0.01$ compared to PTU at 0 mM. ** $P<0.01$ compared with the vehicle group. Each value represents mean±s.e.mean.

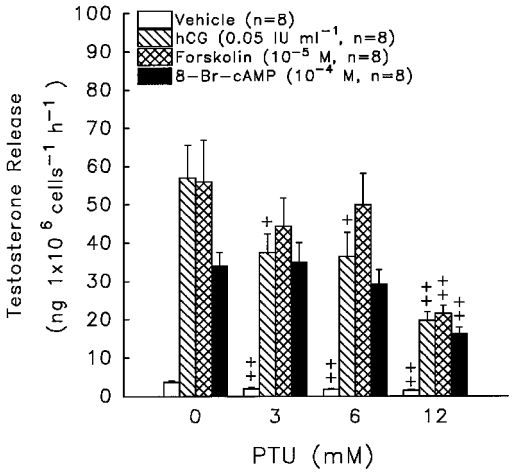

Figure 3 Effects of PTU (3–12 mM) on the release *in vitro* of testosterone by rat testicular interstitial cells after incubation with vehicle, hCG, forskolin or 8-Br-cyclic AMP. $+P<0.05$ and $++P<0.01$ compared with PTU at 0 mM. The levels of testosterone in response to hCG, forskolin, and 8-Br-cyclic AMP groups all are significantly ($P<0.01$) higher than the corresponding vehicle group. Each value represents mean±s.e. mean.

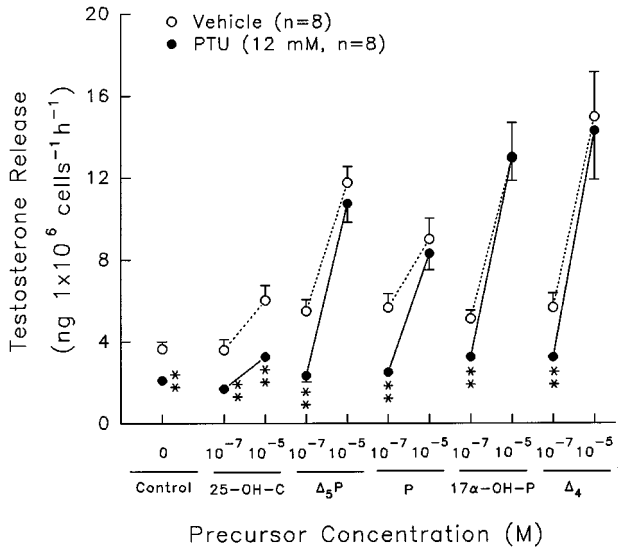

Figure 4 Effects of PTU (12 mM) on the release *in vitro* of testosterone from rat testicular interstitial cells treated with vehicle or testosterone precursors. The precursors were 25-hydroxy-cholesterol (25-OH-C), pregnenolone (Δ_5P), progesterone (P), 17α-hydroxy-progesterone (17α-OH-P) and androstenedione (Δ_4). ** $P<0.01$ compared with vehicle group. Each value represents mean±s.e. mean.

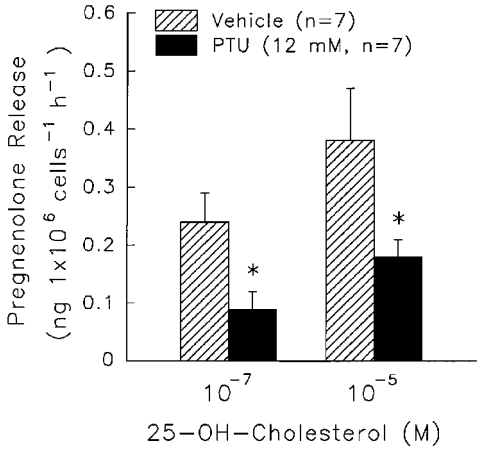

Figure 5 Effects of PTU (12 mM) on the release *in vitro* of pregnenolone after incubation of rat testicular interstitial cells with 25-OH-cholesterol. * $P<0.05$ compared with vehicle group. Each value represents mean±s.e. mean.

dose of PTU (12 mM) inhibited testosterone release in response to forskolin (21.6±2.1 ng 1.0×10^6 cells^{-1} h^{-1}, $n=8$, versus 56.0±11.0 ng 1.0×10^6 cells^{-1} h^{-1} at PTU=0 mg ml^{-1}, $n=8$, $P<0.01$) and 8-Br-cyclic AMP (16.4±1.6 ng 1.0×10^6 cells^{-1} h^{-1}, $n=8$, versus 34.2±3.4 ng 1.0×10^6 cells^{-1} h^{-1} at PTU=0 mg ml^{-1}, $n=8$, $P<0.01$).

Effects of PTU on the steroidogenesis in testicular interstitial cells

Four of the testosterone precursors tested (Δ_5P, P, 17α-OH-P and Δ_4, 10^{-5}–10^{-7} M) produced significant concentration-dependent increases in testosterone production *in vitro*. 25-OH-C also stimulated testosterone release at the higher concentration tested (10^{-5} M), but at a lower concentration (10^{-7} M), it was without effect (Figure 4). PTU (12 mM) decreased both basal testosterone release ($P<0.01$) and the production of testosterone facilitated by the low (10^{-7} M) concentration of each precursor (1.69±0.23 ng 1.0×10^6 cells^{-1} h^{-1}, $n=8$, versus 25-OH-C group, 3.59±0.51 ng 1.0×10^6 cells^{-1} h^{-1}, $n=8$, $P<0.01$; 2.33±0.31 ng 1.0×10^6 cells^{-1} h^{-1}, $n=8$, versus Δ_5P group, 5.49±0.58 ng 1.0×10^6 cells^{-1} h^{-1}, $n=8$, $P<0.01$; 2.50±0.19 ng 1.0×10^6 cells^{-1} h^{-1}, $n=8$, versus P group, 5.67±0.68 ng 1.0×10^6 cells^{-1} h^{-1}, $n=8$, $P<0.01$; 3.25±0.17 ng 1.0×10^6 cells^{-1} h^{-1}, $n=8$, versus 17α-OH-P group, 5.12±0.41 ng 1.0×10^6 cells^{-1} h^{-1}, $n=8$, $P<0.01$ and 3.23±0.16 ng 1.0×10^6 cells^{-1} h^{-1}, $n=8$, versus Δ_4 group, 5.67±0.70 ng 1.0×10^6 cells^{-1} h^{-1}, $n=8$, $P<0.01$). PTU (12 mM) also decreased the production of testosterone facilitated by the high concentration (10^{-5} M) of 25-OH-C (3.25±0.19 ng 1.0×10^6 cells^{-1} h^{-1}, $n=8$, versus 25-OH-C group, 6.03±0.73 ng 1.0×10^6 cells^{-1} h^{-1}, $n=8$, $P<0.01$). However, it did not affect the production of testosterone induced by the high concentration (10^{-5} M) of the other four precursors tested.

Effects of PTU on the 25-OH-cholesterol-stimulated pregnenolone production in testicular interstitial cells

Figure 5 demonstrates effects of PTU on 25-OH-C-stimulated pregnenolone release by testicular interstitial cells. PTU (12 mM) decreased the production of pregnenolone facilitated by the 25-OH-C (10^{-7} and 10^{-5} M, 10^{-7} M group, 0.09 ± 0.03 ng 1.0×10^6 cells^{-1} h^{-1}, $n=7$, versus vehicle group, 0.24 ± 0.05 ng 1.0×10^6 cells^{-1} h^{-1}, $n=7$, $P<0.05$ and 10^{-5} M group, 0.18 ± 0.03 ng 1.0×10^6 cells^{-1} h^{-1}, $n=7$, versus vehicle group, 0.38 ± 0.09 ng 1.0×10^6 cells^{-1} h^{-1}, $n=7$, $P<0.05$).

Discussion

The present results demonstrate that exposure of rat testicular cells to PTU *in vitro* diminishes the resting and evoked secretion of testosterone by a mechanism involving decreased activity of the P450scc enzyme. By contrast, PTU does not affect the secretion of LH by pituitary tissue *in vitro*.

Previous studies have shown that both thyroidectomy (Aruldhas *et al*., 1982; Biswas *et al*., 1994) and PTU-treatment (Hardy *et al*., 1993) decrease serum testosterone concentrations in the rat. This response has been attributed to the decreased levels of thyroid hormones in serum but the underlying mechanisms are ill-defined. Clinical studies have shown that PTU has toxic effects on the liver (Levy, 1993; Deidiker & de Mello, 1996) and the immune system (Cooper, 1984). However, the possibility that PTU exerts unwanted pharmacological effects on PTU on other tissues has scarcely been evaluated. Recently we found that PTU, given acutely to euthyroid rats decreases the rise in plasma corticosterone concentration induced by ACTH and that, *in vitro*, it decreases corticosterone production in rat zona fasciculata-reticularis cells (Lo *et al*., 1998), suggesting that PTU may directly regulate adrenal steroidogenesis. There is also evidence that neonatal administration of PTU impairs testicular steroidogenesis in the adult but this is the first study to examine the direct effects of PTU on the endocrine function of rat testicular interstitial cells.

In comparison to the doses used clinically (200–600 mg person^{-1} day^{-1}, p.o., Astwood, 1967; Gwinup 1978; McMurry *et al*., 1975; Clark *et al*., 1992), the dose of PTU used in our *in vivo* study (10–20 mg kg^{-1}, i.v. i.e. $\approx$ 0.12 mmoles kg^{-1} i.v.) was high. However it was in the region of that used previously in rodent studies to induce hypothyroidism (Hwang *et al*., 1974). The finding that this dose did not reduce the serum T_4 levels over the time course studies is not surprising as the $t_{1/2}$ of T_4 is in the region of 7 days. The concentrations of PTU used in our *in vitro* (e.g. 12 mM) studies are almost certainly lower (one tenth) than those attained in our *in vivo* model.

It is evident from the present study that PTU causes a significant decrease in the serum testosterone concentration when infused intravenously in rats. Our *in vitro* studies, which showed that PTU does not affect the basal or GnRH stimulated release of LH from rat pituitary tissue, suggest that this effect was not a consequence of a direct action of the drug on the pituitary gland and may therefore reflect an action on the testis. To investigate this possibility, we used an established *in vitro* model based on the ability of hCG to stimulate testosterone secretion by rat Leydig cells (Saez & Forest, 1979; Padron *et al*., 1980; Wang *et al*., 1994; Simpson *et al*., 1987; Nakhla *et al*., 1989; Liao *et al*., 1991) *via* a mechanism involving cyclic AMP production (Avallet *et al*., 1987; Petersson *et al*., 1988; Sakai *et al*., 1989; Wang *et al*., 1994). Our data show that PTU inhibits both the basal and the hCG-stimulated release of testosterone *in vitro*. It also decreases forskolin- and 8-Br-cyclic AMP-induced testosterone release suggesting that the drug acts directly on the rat testicular cells to regulate testosterone production at a point distal to the formation of cyclic AMP. Since these actions of PTU were observed *in vitro* in tissue removed from control rats, they were clearly independent of any effects of the drug on gonadotropin or thyroid hormone production.

We also examined the effects of PTU on the activities of steroidogenic enzymes in testicular interstitial cells by challenging the cells *in vitro* with the following testosterone precursors, including 25-OH-cholesterol (25-OH-C, substrate of P450scc), pregnenolone (Δ_5P, substrate of 3β-HSD), progesterone (P, substrate of 17α-hydroxylase), 17α-OH-progesterone (17α-OH-P, substrate of C17-20 lyase) and androstenedione (Δ_4, substrate of 17β-HSD). The results showed that PTU inhibits the increase in testosterone production induced by a low concentration (10^{-7} M) of each of these precursors. PTU also decreased testosterone production evoked by a higher concentration 25-OH-cholesterol (10^{-5} M) but failed to influence the steroidogenic responses to this concentration of pregnenolone-, progesterone-, 17α-OH-progesterone- and androstenedione. In addition, it inhibited the conversion of 25-OH-cholesterol to pregnenolone, a process which is catalyzed by P450scc, the rate limiting enzyme in gonadal steroidogenesis, and which is regulated by the gonadotropins (Iida *et al*., 1989).

Taken together these results suggest that PTU may act at the level of P450scc to inhibit testosterone production.

In conclusion, our results provide new evidence that, in high doses, PTU may act directly on the testis to repress steroidogenesis, *via* a mechanism involving up inhibition of P450scc activity. This action may explain the attenuation in serum testosterone concentration observed *in vivo* following intravenous infusion of high doses of PTU.

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