BIOLOGY OF REPRODUCTION 27, 1138–1146 (1982)

Mechanism of Glucocorticoid-Induced Suppression of Testicular Androgen Biosynthesis In Vitro[1]

THOMAS H. WELSH, JR.,[2] THOMAS H. BAMBINO
and AARON J. W. HSUEH[3]

Department of Reproductive Medicine
University of California, San Diego
La Jolla, California 92093

Downloaded from https://academic.oup.com/biolreprod/article/27/5/1138/2767403 by U.S. Department of Justice user on 17 August 2022

ABSTRACT

The mechanism whereby glucocorticoids directly inhibit gonadotropin-stimulated testosterone production was studied by using primary cultures of testicular cells from adult hypophysectomized rats. Testicular cells were maintained in serum-free media with hormone treatments administered on Day 8 and media collected 48 h later for steroid and cAMP measurement. Highly purified human chorionic gonadotropin (hCG) increased testosterone production relative to controls. Concomitant administration of either natural (cortisone > deoxycorticosterone=aldosterone) or synthetic (dexamethasone > prednisolone) corticosteroids inhibited hCG-stimulated testosterone production in a dose-dependent manner. Dexamethasone at 10^{-7} M decreased testosterone production by approximately 50–60% and this inhibitory effect was reversible upon removal of the glucocorticoid. In the presence or absence of a phosphodiesterase inhibitor, dexamethasone decreased hCG-stimulated cAMP production by approximately 60%. Dexamethasone also decreased testosterone production induced by cholera toxin and $(Bu)_2$cAMP by 43 and 63%, respectively. The dexamethasone suppression of testosterone production was accompanied by marked decreases in androstenedione (80% decrease) and 17α-hydroxyprogesterone (57%) production, with a lesser effect on progesterone production (28% decrease) and no effect on pregnenolone production. Exogenous progesterone and 17α-hydroxyprogesterone augmented hCG-stimulated testosterone production. Dexamethasone reduced the conversion of exogenous progesterone to testosterone by 33% but did not affect the conversion of 17α-hydroxyprogesterone to androstenedione and testosterone, suggesting a specific inhibition of 17α-hydroxylase. These results suggest that glucocorticoids directly suppress Leydig cell steroidogenesis by decreasing gonadotropin stimulation of cAMP production and the activity of 17α-hydroxylase.

INTRODUCTION

An inhibitory influence of the hypothalamic-hypophyseal-adrenal axis upon reproductive processes has long been suspected. In vivo evidence supportive of an adrenal-testicular endocrine interrelationship has been provided by recent investigations which evaluated the effect of hyperadrenalism or stress upon the production of luteinizing hormone (LH) and testosterone in males of several species (Desjardins et al., 1971; Gabrilove et al., 1979; Doerr and Pirke, 1976, 1979; Saez et al., 1977; Purvis and Hansson, 1978; McKenna et al., 1979; Welsh and Johnson, 1981). These studies demonstrated an association of elevated blood concentrations of either endogenous or exogenous adrenal steroids with decreased testicular testosterone production.

The glucocorticoid-induced inhibition of testicular function may occur at the hypothalamic, hypophyseal or testicular level. Support of a direct testicular effect of adrenal glucocorticoids was provided by our recent finding that dexamethasone treatment in vivo decreased testicular hCG/LH receptor content in immature, hypophysectomized rats (Bambino and Hsueh, 1981). Furthermore, dexamethasone treatment in vitro inhibited hCG-stimulated testosterone production in cultured rat testicular cells (Bambino and Hsueh, 1981). The present study further investigates the mechanism

Accepted July 6, 1982.
Received May 14, 1982.

[1] This work is supported by National Institutes of Health Research Grant HD-15667 and Program Project Grant HD-12303. A.J.W.H. is the recipient of Research Career Development Award HD-00375. Performed in partial fulfillment of the Ph.D. degree for T.H.B.

[2] T.H.W. is the recipient of a Giannini Medical Research Foundation postdoctoral fellowship.

[3] Reprint requests: Dr. A.J.W. Hsueh, Dept. of Reproductive Medicine, M-025, University of California, San Diego, La Jolla, CA 92093.

whereby adrenal glucocorticoids inhibit gonadotropin-induced androgen biosynthesis in primary cultures of adult rat testicular cells.

MATERIALS AND METHODS

Reagents and Hormones

Human chorionic gonadotropin (CR-121; 13,450 IU/mg) was provided by Dr. R. E. Canfield. Cholera toxin, $(Bu)_2$cAMP, aldosterone, androstenedione, cortisol, deoxycorticosterone, dexamethasone, prednisolone, progesterone, 17α-hydroxyprogesterone, testosterone, Hepes, bovine serum albumin (fraction V) and 1-methyl-3-isobutylxanthine (MIX) were purchased from Sigma Chemical Co. (St. Louis, MO).

McCoy's 5a medium (modified; without serum), penicillin-streptomycin solution, L-glutamine, bovine pancreatic DNAase (2100 U/mg), and trypan blue stain were obtained from Grand Island Biological Co. (Santa Clara, CA). Collagenase (144 U/mg) was purchased from Worthington Chemical Co. (Freehold, NY).

Animals

Sprague-Dawley male rats (50–60 days of age) were hypophysectomized (transsphenoidal approach) by Johnson Labs. (Bridgeview, IL) and delivered 4–5 days post-surgery. Individual rats were examined for the completeness of hypophysectomy. The rats were provided a mixture of bread, dog food, milk, tap water and physiological saline ad libitum and were maintained under a 14L:10D h regimen.

Steroid Production by Primary Cultures of Rat Testicular Cells

Testes of adult hypophysectomized rats were excised 12–15 days after hypophysectomy. Testicular cell suspensions were prepared as previously described (Hsueh, 1980; Welsh and Hsueh, 1982) by incubating decapsulated testes at 37°C for 1.5 h in an enzyme solution containing 0.4% collagenase, 10 μg/ml DNAase and 0.1% bovine serum albumin (BSA) in Hepes buffer (137 mM NaCl, 5mM KCl, 0.7mM Na_2HPO_4, 25 mM Hepes, 10 mM glucose and 360 μM $CaCl_2$, pH 7.2). Cells were washed, resuspended in culture media and cell viability quantitated by trypan blue exclusion. Cell viability averaged 90–95%. Approximately $0.9–1.1 \times 10^6$ cells/culture were maintained at 37°C under 95% air–5% CO_2 in McCoy's 5a medium supplemented with penicillin (100 U/ml), streptomycin sulfate (100 μg/ml) and L-glutamine (2 mM). Primary cultures of testicular cells are comprised of approximately 5–10% Leydig cells as determined by 3β-hydroxysteroid dehydrogenase staining (Adashi et al., 1982). Media were changed every 2 days for 8 days. Testicular cell cultures were treated between Days 8 and 10 of culture and media collected for steroid and cAMP radioimmunoassays unless otherwise indicated.

Radioimmunoassays

Medium concentrations of testosterone and androstenedione were determined by radioimmunoassays (Bambino et al., 1980) with specific antisera (<0.01% cross-reaction with progesterone and 17α-hydroxyprogesterone) provided by Dr. G. Abraham. Although the androgen antisera cross-react with DHT, the production of DHT is negligible in the present culture system. In some experiments, intermediates of the testosterone biosynthetic pathway were separated by celite column chromatography and measured by radioimmunoassays (Anderson et al., 1976) with specific antisera provided by Dr. Abraham.

Medium concentration of cAMP was determined by the radioimmunoassay procedure described by Steiner et al. (1972). Briefly, $O^{2'}$-monosuccinyl adenosine 3':5'-cyclic monophosphate tyrosyl methyl ester (Sigma Chemical Co., St. Louis, MO) was radiolabeled with ^{125}I (Radiochemical Center, Amersham-Searle, Boston, MA) according to the procedure of Hunter and Greenwood (1962). Purification of the iodinated cAMP was accomplished via thin-layer chromatography with cellulose plates (J. T. Baker Chemical Co., Phillipsburg, NJ) in a butanol:acetic acid:water (12:3:5) system. Rabbit anti-cAMP-BSA antisera #CA-1 (Miles Laboratories, Inc., Elkhart, IN) cross-reacts <0.001% with cGMP, AMP or ATP. Protein A (Pansorbin, Calbiochem, La Jolla, CA) was used to separate bound and free ^{125}I-ligand as previously described (Rebar et al., 1980).

Data Analyses

Radioimmunoassay data were analyzed with a program which utilized a weighted logit-log regression analysis (Davis et al., 1979). Experimental data are presented as the mean ± SEM of quadruplicate cultures. Comparable results were obtained in 2–4 replicates of each experiment. Treatment differences were tested by Student's *t* test (Snedecor and Cochran, 1967). Comparisons with $P>0.05$ were not considered significant.

RESULTS

Effect of Synthetic and Natural Corticosteroids on hCG-Stimulated Testosterone Production In Vitro

Primary cultures of testicular cells were maintained for 8 days with medium changes every 2 days as previously described (Hsueh, 1980). On Day 8, cultures were treated with either medium alone (control) or 10^{-6}M of dexamethasone, prednisolone, cortisone, aldosterone or deoxycorticosterone for 2 days. Some cells were also treated with hCG with or without increasing doses of various natural or synthetic corticosteroids (Fig. 1). Medium concentration of testosterone was negligible in control cultures and cultures treated with dexamethasone, prednisolone, cortisone, aldosterone or deoxycorticosterone alone (data not shown). Treatment with hCG markedly increased testosterone production relative to controls, while concomitant administration of corticosteroids decreased hCG-stimulated testosterone production in a dose-dependent manner. At 10^{-8}M,

Downloaded from https://academic.oup.com/biolreprod/article/27/5/1138/2767403 by U.S. Department of Justice user on 17 August 2022

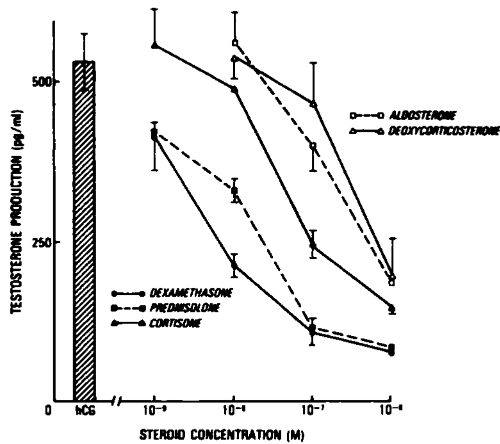

FIG. 1. *Influence of natural and synthetic corticosteroids on hCG-stimulated testosterone production by cultured testicular cells.* Testicular cell cultures were prepared as described in *Materials and Methods* and received fresh media every 2 days for 8 days. On Day 8, cultures were treated with hCG (10 ng/ml; *cross-hatched bar)* with or without increasing doses (10^{-9} -10^{-6} M) of natural (cortisone, deoxycorticosterone and aldosterone) or synthetic (dexamethasone and prednisolone) corticosteroids for 2 days. Media were collected on Day 10 of culture for testosterone radioimmunoassay. Each data point represents the mean ± SEM of quadruplicate cultures.

the synthetic steroids prednisolone and dexamethasone reduced hCG-stimulated testosterone production by 59% and 37%, respectively. The natural corticosteroids were less potent than the synthetic corticosteroids in inhibiting hCG-stimulated testosterone production (dexamethasone > prednisolone > cortisone > deoxycorticosterone=aldosterone). The natural glucocorticoid cortisone and the mineralocorticoid aldosterone were ineffective at 10^{-8}M but decreased hCG-stimulated testosterone production at 10^{-7}M by 54% and 23%, respectively. Deoxycorticosterone, a weak glucocorticoid, effectively reduced testosterone production only at 10^{-6}M. These observations are consistent with our prior demonstration that cortisol and corticosterone significantly inhibited gonadotropin-stimulated testosterone production (Bambino and Hsueh, 1981). For the following experiments, dexamethasone was utilized at 10^{-7}M.

Reversibility of Glucocorticoid-Induced Inhibition of Testosterone Production

To test if the inhibitory effect of glucocorticoids on hCG-stimulated testosterone production is reversible, testicular cell cultures were treated with hCG or hCG plus dexamethasone on Day 8 of culture. Control cultures received media alone. One day later, media were collected for testosterone measurement, cells washed and fresh media supplied. At 24-h intervals over the next 3 days hCG-treated cultures continued to receive hCG (hCG→hCG) and controls continued to receive fresh media alone (C→C). Furthermore, one-half of the cells initially treated with hCG plus dexamethasone were washed and received hCG at 24-h intervals (hCG + DEX → hCG), whereas the remaining one-half of this group continued to receive hCG plus dexamethasone (hCG + DEX → hCG + DEX) daily for 3 days.

As shown in Fig. 2, medium concentration of testosterone in control cultures (C→C) was low throughout the experimental period. Testosterone production was increased 20-fold relative to controls by 1 day after initial hCG treatment. Administration of hCG daily thereafter maintained testosterone production at a level 20- to 40-fold greater than control cultures. Concurrent administration of hCG and dexamethasone for 1 day resulted in an 80% decrease in testosterone production relative to

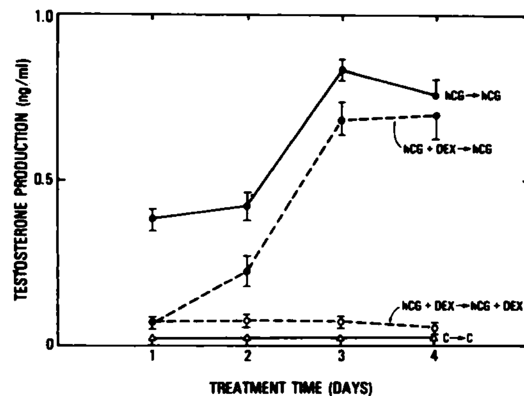

FIG. 2. *Reversibility of the inhibitory effect of dexamethasone on hCG-stimulated testosterone production.* Testicular cells were cultured as described in *Materials and Methods.* On Day 8, cultures were treated with hCG (10 ng/ml) or hCG plus dexamethasone (*DEX*; 10^{-7}M). Control cultures *(C)* received media alone. One day after treatment, media were collected for testosterone measurement (Day 1). After washing the cells, cultures received daily treatment with hCG or hCG plus dexamethasone as indicated. Media were collected daily for testosterone measurement (Days 2, 3 and 4) and fresh media containing the same hormones replaced. Each data point represents the mean ± SEM of quadruplicate cultures.

Downloaded from https://academic.oup.com/biolreprod/article/27/5/1138/2767403 by U.S. Department of Justice user on 17 August 2022

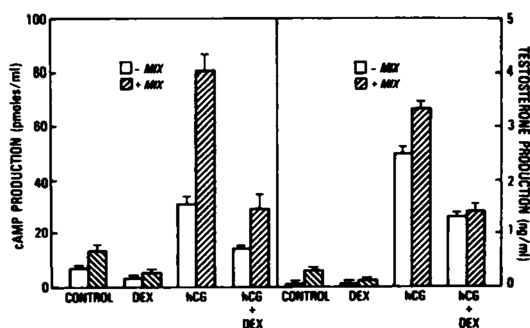

FIG. 3. *Effect of dexamethasone treatment on hCG-stimulated cAMP production.* Testicular cells were maintained as described in the legend of Fig. 1. On Day 8, cultures received fresh media with or without hCG (100 ng/ml) and/or dexamethasone (*DEX*; 10^{-7} M). Some cell cultures from each group were also treated with 0.1 mM 1-methyl-3-isobutyl xanthine (*MIX; cross-hatched bars*). Media were collected on Day 10 of culture and concentration of cAMP (*left panel*) and testosterone (*right panel*) were determined by radioimmunoassay. Each data point represents the mean ± SEM of quadruplicate cultures.

cells treated with hCG alone. This inhibitory effect of dexamethasone was reversible. When treated with hCG alone, the cells initially treated with hCG plus dexamethasone showed a gradual increase in testosterone production over the 3 daily incubations (hCG + DEX → hCG). By 48 h after cessation of dexamethasone treatment, testosterone production by this group was similar to that observed in cultures treated with hCG alone (hCG → hCG). In contrast, continuous treatment with hCG plus dexamethasone (hCG + DEX → hCG + DEX) caused persistent suppression of testosterone production. Similar results were obtained for testicular cells treated with 10^{-6}M cortisol rather than dexamethasone (data not shown).

Effect of Dexamethasone Treatment on hCG-Stimulated cAMP and Testosterone Production

Since the hCG-stimulated production of testosterone is believed to be mediated via increases in cAMP production, the influence of dexamethasone treatment on hCG-stimulated cAMP and testosterone production was examined (Fig. 3). On Day 8, cultures received fresh media with or without hCG and/or dexamethasone. Some cultures from each group were also treated with 0.1 mM of 1-methyl-3-isobutyl xanthine (MIX), a phosphodiesterase inhibitor. Media were collected 2 days later and concentrations of cAMP and testosterone were determined.

In the absence of MIX, medium concentration of cAMP was low in both control and dexamethasone-treated cells while administration of hCG increased cAMP production by 4-fold relative to controls. In contrast, concomitant administration of dexamethasone reduced hCG-stimulated cAMP production by 53%. In the presence of the phosphodiesterase inhibitor, cAMP production was increased in hCG-treated cultures; however, dexamethasone treatment reduced hCG-stimulated (but not basal; $P > 0.05$) cAMP production by 66%. In the same cultures, dexamethasone treatment also decreased hCG-stimulated testosterone production in the presence or absence of the phosphodiesterase inhibitor.

Effect of Dexamethasone on Cholera Toxin- and $(Bu)_2$cAMP-Stimulated Testosterone Production

The influence of dexamethasone upon the ability of cholera toxin (a cAMP-stimulating agent) and $(Bu)_2$cAMP to stimulate testosterone production was also evaluated (Fig. 4). Testicu-

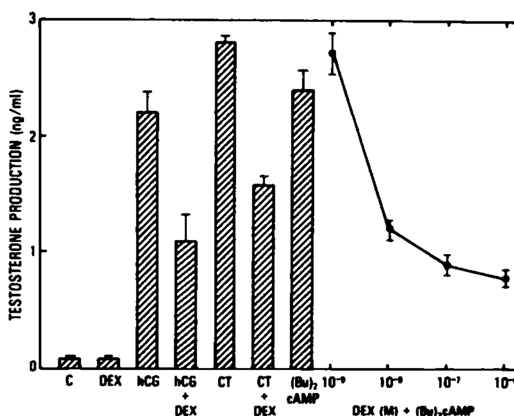

FIG. 4. *Effect of dexamethasone on cholera toxin- and $(Bu)_2$cAMP-stimulated testosterone production.* Primary cultures of testicular cells were maintained as described in the legend of Fig. 1. On Day 8, cultures were treated with hCG (10 ng/ml) or cholera toxin (*CT*; 10 ng/ml) with or without dexamethasone (*DEX*; 10^{-7}M). Some cells were treated with $(Bu)_2$cAMP (0.5 mg/ml) in the absence or presence of increasing concentrations of dexamethasone ($10^{-9}-10^{-6}$M). Control cultures (*C*) received media alone. Media were collected on Day 10 for testosterone measurement. Each data point represents the mean ± SEM of quadruplicate cultures.

Downloaded from https://academic.oup.com/biolreprod/article/27/5/1138/2767403 by U.S. Department of Justice user on 17 August 2022

lar cells were treated with cholera toxin or hCG in the presence or absence of dexamethasone. Some cells were also treated with $(Bu)_2cAMP$ in the absence or presence of increasing concentrations of dexamethasone. Media were collected 2 days later for testosterone measurement.

Administration of cholera toxin stimulated testosterone production 28-fold relative to controls. In contrast, dexamethasone treatment reduced the effect of cholera toxin by 43%. Treatment with $(Bu)_2cAMP$ also increased testosterone production 26-fold (relative to controls), whereas concomitant treatment with increasing concentrations of dexamethasone suppressed testosterone production in a dose-dependent manner with 10^{-6}M of dexamethasone decreasing testosterone production by 66% (Fig. 4).

Effect of Dexamethasone Treatment on hCG-Stimulated Production of Intermediate Steroids of the Testosterone Biosynthetic Pathway

A potential influence of dexamethasone upon steroidogenic enzymes was evaluated by measuring medium concentration of various intermediate steroids of the testosterone biosynthetic pathway following a 48-h exposure of testicular cells to either hCG or hCG plus dexamethasone (Fig. 5). The Δ^4 pathway appeared to be the preferred route of testosterone formation as the concentration of Δ^4 steroids was greater than that of Δ^5 steroids. Consistent with earlier observations, dexamethasone administration inhibited by 60% hCG-stimulated testosterone production. Furthermore, dexamethasone treatment reduced the hCG-stimulated production of androstenedione and 17α-hydroxyprogesterone by 80% and 57%, respectively. A smaller decrease (28%) in progesterone production was observed while pregnenolone production did not appear to be affected by dexamethasone treatment.

Influence of Dexamethasone on the Conversion of Exogenous Progesterone and 17α-Hydroxyprogesterone to Androgens

Whether dexamethasone may decrease the activity of the steroidogenic enzymes 17α-hydroxylase and 17,20-lyase was tested by the addition of 10^{-5}M of progesterone or 17α-hydroxyprogesterone to testicular cells treated with or without hCG and/or dexamethasone for 48 h.

In the absence of exogenous progesterone,

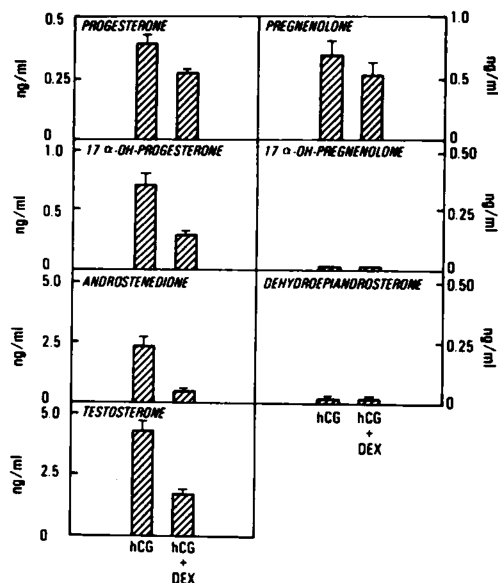

FIG. 5. *Effect of dexamethasone treatment on hCG-stimulated production of intermediate steroids of the testosterone biosynthetic pathway.* Testicular cells were maintained for 8 days as described in the legend of Fig. 1. On Day 8, cells were treated with fresh media containing either hCG (10 ng/ml) or hCG plus dexamethasone (*DEX*; 10^{-7}M). Media were collected 48 h later and intermediates of the testosterone biosynthetic pathway were separated by celite column chromatography for radioimmunoassay. Each data point represents the mean ± SEM of quadruplicate cultures. 17α-OH-progesterone: 17α-hydroxyprogesterone; 17α-OH-pregnenolone: 17α-hydroxypregnenolone.

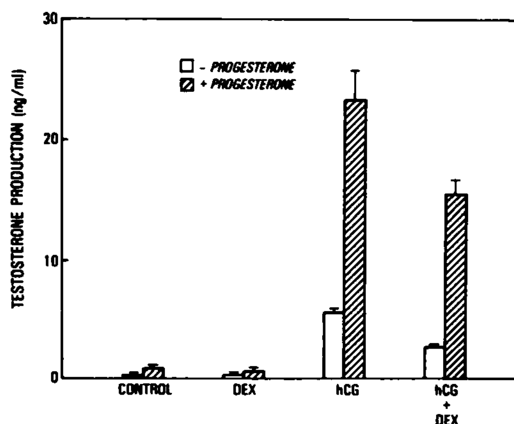

FIG. 6. *Effect of dexamethasone treatment on the conversion of exogenous progesterone to testosterone by cultured testicular cells.* Testicular cells were maintained as described in the legend of Fig. 1. On Day 8, cultures were treated with or without hCG (10 ng/ml) and/or dexamethasone (*DEX*; 10^{-7}M). Some cells from each group were also treated with 10^{-5}M progesterone (*cross-hatched bars*). Media were collected 48 h later for testosterone measurement. Each data point represents the mean ± SEM of quadruplicate cultures.

Downloaded from https://academic.oup.com/biolreprod/article/27/5/1138/2767403 by U.S. Department of Justice user on 17 August 2022

production of testosterone by control and dexamethasone-treated cells was low (Fig. 6). Addition of exogenous progesterone increased basal testosterone production. Furthermore, treatment with hCG increased testosterone production 57-fold relative to controls, while concurrent addition of progesterone further increased testosterone production by 4-fold. Concomitant administration of dexamethasone decreased hCG-stimulated testosterone production in either the absence (51% decrease) or presence (33% decrease) of exogenous progesterone. Thus, dexamethasone may decrease 17α-hydroxylase activity.

The influence of dexamethasone upon the activity of 17,20-lyase was determined by measurement of androstenedione and testosterone production in cells treated with exogenous 17α-hydroxyprogesterone (Fig. 7 A and B). In the absence of exogenous 17α-hydroxyprogesterone, control and dexamethasone-treated cells produced low quantities of androstenedione and testosterone. Addition of 17α-hydroxyprogesterone to control cultures increased the basal production of both androgens. Concomitant administration of dexamethasone did not significantly affect the conversion of 17α-hydroxyprogesterone to androstenedione and testosterone by these cultures. Treatment with hCG increased medium concentrations of androstenedione and testosterone 30- and 57-fold, respectively, while addition of 17α-hydroxyprogesterone further increased androstenedione and testosterone production by 17- and 7-fold, respectively. In the absence of 17α-hydroxyprogesterone, dexamethasone treatment reduced hCG-stimulated androstenedione and testosterone production by 61% and 51%, respectively. In contrast, when cells were treated with 17α-hydroxyprogesterone, dexamethasone did not affect the hCG-stimulated production of androstenedione and testosterone.

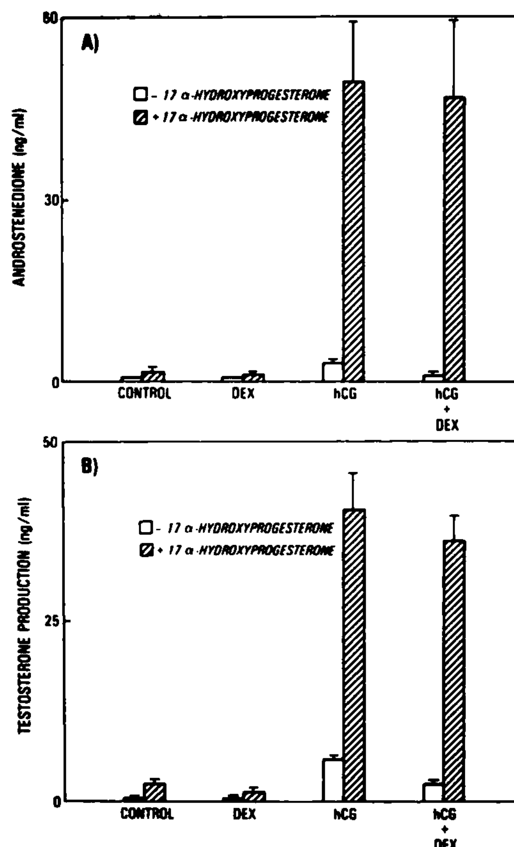

FIG. 7. ***Effect of dexamethasone treatment on the conversion of exogenous 17α-hydroxyprogesterone to androstenedione and testosterone by cultured testicular cells.*** Testicular cells were maintained as described in the legend of Fig. 1. On Day 8, cultures received fresh media with or without hCG (10 ng/ml) and/or dexamethasone (*DEX*; 10^{-7} M). Some cells from each group were treated with 10^{-6} M 17α-hydroxyprogesterone (*cross-hatched bars*). Media were collected 48 h later and concentrations of androstenedione (*A*) and testosterone (*B*) were determined by radioimmunoassays. Each data point represents the mean ± SEM of quadruplicate cultures.

DISCUSSION

The present study extends our earlier observation that glucocorticoids directly inhibit Leydig cell function in vivo and in vitro (Bambino and Hsueh, 1981). The glucocorticoid suppression of androgen biosynthesis in cultured testicular cells is associated with decreases in cAMP formation, probably through interference with the gonadotropin stimulation of adenyl cyclase activity. Furthermore, glucocorticoids also affect androgen biosynthesis distal to cAMP formation by selectively inhibiting the reaction catalyzed by 17α-hydroxylase.

The relative effectiveness (dexamethasone > prednisolone > cortisone > aldosterone= deoxycorticosterone) of these steroids in suppressing testosterone production is consistent with their potencies in binding to the glucocorticoid receptors in other tissues (Munck and Leung, 1977); the observed inhibitory action may be mediated through specific glucocorticoid receptors found in the interstitial cells of the

Downloaded from https://academic.oup.com/biolreprod/article/27/5/1138/2767403 by U.S. Department of Justice user on 17 August 2022

testis (Ballard et al., 1974; Evain et al., 1976; Ortlip et al., 1981). Since the present culture system consists of cell types other than Leydig cells, one can not rule out the possibility that the observed inhibitory effect may be mediated through other testicular cells (such as Sertoli cells).

Results of the present study must be considered as pharmacologic since the effective inhibitory dose of the more potent synthetic glucocorticoid was 10^{-8}M which is greater than circulating levels of adrenal glucocorticoids under nonstressed conditions. Nonetheless, these observations may be relevant with regard to the integrative endocrine regulation of testicular function during sustained stress, adrenal dysfunction or other pathological states associated with hyperadrenalism.

Whether the inhibitory mechanism of glucocorticoids is mediated prior or distal to cAMP formation was examined by investigation of the effect of dexamethasone on hCG-stimulated cAMP production (Fig. 3) and also on $(Bu)_2$cAMP-stimulated testosterone production (Fig. 4). Dexamethasone treatment suppressed hCG-stimulated cAMP and testosterone production in the presence or absence of an inhibitor of phosphodiesterase activity. It appears that at least a part of the inhibitory mechanism of adrenal glucocorticoids is mediated prior to cAMP formation. Since dexamethasone inhibits cAMP production in the presence of the phosphodiesterase inhibitor, our data suggests that glucocorticoids may suppress gonadotropin stimulation of adenyl cyclase activity. Direct measurement of the activities of adenyl cyclase and phosphodiesterase is needed to further elucidate this point. In addition to suppressing cAMP formation, our demonstration that dexamethasone treatment also inhibited $(Bu)_2$cAMP-stimulated testosterone production suggests an additional post-cAMP effect of glucocorticoids upon androgen biosynthesis.

We also examined the effect of dexamethasone on hCG-stimulated production of intermediate steroids of the testosterone biosynthetic pathway (Fig. 5). Dexamethasone inhibited hCG-stimulated production of 17α-hydroxyprogesterone, androstenedione and testosterone, suggesting that adrenal glucocorticoids may suppress the activity of the steroidogenic enzymes 17α-hydroxylase and 17,20-lyase. Although dexamethasone treatment only resulted in a slight inhibition of hCG-stimulated progesterone production, one also cannot rule out possible inhibition of 3β-hydroxysteroid dehydrogenase and/or enzymes prior to pregnenolone biosynthesis.

Exogenous progesterone and 17α-hydroxyprogesterone augmented hCG-stimulated androgen production. Since dexamethasone did not significantly affect the conversion of 17α-hydroxyprogesterone to androstenedione and testosterone but consistently reduced the conversion of progesterone to testosterone, the adrenal glucocorticoids may preferentially block the activity of 17α-hydroxylase (Figs. 6 and 7). Conclusive demonstration that dexamethasone can inhibit the activity of 17α-hydroxylase awaits direct measurement of the enzyme activity in a cell-free system.

The apparent effect of dexamethasone upon 17α-hydroxylase but not 17,20-lyase is of interest. Previously, LH/hCG (Dufau and Catt, 1978), estrogens (Wang et al., 1980), epidermal growth factor (Welsh and Hsueh, 1982) and arginine vasotocin (Adashi and Hsueh, 1982) have been reported to decrease testicular testosterone production via inhibition of both 17α-hydroxylase and 17,20-lyase. The basis and significance of the selective suppression by glucocorticoids of 17α-hydroxylase warrants additional investigation especially since these two enzymes were recently described as joint components of the cytochrome P-450 complex in porcine Leydig cells (Nakajin and Hall, 1981).

This study also demonstrated the reversibility of glucocorticoid-induced suppression of testosterone biosynthesis in vitro (Fig. 2). Since the inhibitory effects of dexamethasone and cortisol were reversible at 10^{-7}M, it appears that the mechanism of glucocorticoid-induced inhibition of androgen biosynthesis was not due to a cytotoxic effect of these glucocorticoids upon the testicular cells as observed for lymphocytes (Leung and Munck, 1975). Also, the inhibitory effect of dexamethasone on 17α-hydroxylase was not accompanied by decreases in another steroidogenic enzyme, 17,20-lyase. In females, glucocorticoids were demonstrated to decrease the follicle-stimulating hormone stimulation of aromatase enzymes in cultured granulosa cells (Hsueh and Erickson, 1979) but to increase the activity of enzymes involved in progesterone biosynthesis (Adashi et al., 1981).

Thus, adrenal glucocorticoids can suppress Leydig cell androgen production through pre- and post-cAMP pathways and this inhibition is

Downloaded from https://academic.oup.com/biolreprod/article/27/5/1138/2767403 by U.S. Department of Justice user on 17 August 2022

characterized by decreased 17α-hydroxylase activity. The ability of glucocorticoids to selectively inhibit the reactions mediated by adenyl cyclase and 17α-hydroxylase in the testicular Leydig cells provides an interesting model for understanding the regulation of enzymes controlled by steroids and gonadotropins in steroidogenic tissues.

ACKNOWLEDGMENTS

We thank Dr. R. E. Canfield and the Center for Population Research of the NICHHD for hCG. We thank Ms. L. Tucker and C. Fabics and Mr. B. Hopper for technical assistance and Ms. K. Watts for manuscript preparation.

REFERENCES

Adashi, E. Y. and Hsueh, A.J.W. (1982). Direct inhibition of rat testicular androgen biosynthesis by arginine vasotocin. J. Biol. Chem. 257:1301–1308.

Adashi, E. Y., Jones, P.B.C. and Hsueh, A.J.W. (1981). Synergistic effect of glucocorticoids on the stimulation of progesterone production by follicle-stimulating hormone in cultured rat granulosa cells. Endocrinology 109:1888–1894.

Adashi, E. Y., Fabics, C. and Hsueh, A.J.W. (1982). Insulin augmentation of testosterone production in a primary culture of rat testicular cells. Biol. Reprod. 26:270–280.

Anderson, D. C., Hopper, B. R., Lasley, B. L. and Yen, S.S.C. (1976). A simple assay method for the assay of eight steroids in small volumes of plasma. Steroids 28:179–195.

Ballard, P. L., Baxter, J. D., Higgins, S. J., Rousseau, G. G. and Tompkins, G. M. (1974). General presence of glucocorticoid receptors in mammalian tissues. Endocrinology 94:998–1002.

Bambino, T. H. and Hsueh, A. J. W. (1981). Direct inhibitory effect of glucocorticoids upon testicular luteinizing hormone receptor and steroidogenesis *in vivo* and *in vitro*. Endocrinology 108:2142–2148.

Bambino, T. H., Schreiber, J. R. and Hsueh, A.J.W. (1980). Gonadotropin-releasing hormone and its agonist inhibit testicular steroidogenesis in immature and adult hypophysectomized rats. Endocrinology 107:908–917.

Davis, S. G., Jaffe, M. L., Munson, P. J. and Rodbard, D. (1979). Radioimmunoassay data processing with a small programmable calculator. Technical Report, NIH, Bethesda, MD.

Desjardins, C. and Ewing, L. L. (1971). Testicular metabolism in adrenalectomized and corticosterone-treated rats. Proc. Soc. Exp. Biol. Med. 137:578–583.

Doerr, P. and Pirke, K. M. (1976). Cortisol-induced suppression of plasma testosterone in normal adult males. J. Clin. Endocrinol. Metab. 43:622–629.

Doerr, P., and Pirke, K. M. (1979). Dexamethasone-induced suppression of the circadian rhythm of plasma testosterone in normal adult males. J. Steroid Biochem. 10:81–86.

Dufau, M. L. and Catt, K. J. (1978). Gonadotropin receptors and regulation of steroidogenesis in the testis and ovary. Vitam. Horm. 36:461–592.

Evain, D., Morera, A. M. and Saez, J. M. (1976). Glucocorticoid receptors in interstitial cells of the rat testis. J. Steroid Biochem. 7:1135–1139.

Gabrilove, J. L., Nicolis, G. L. and Sohval, A. R. (1979). The testis in Cushing's syndrome. J. Urol. 112:95–99.

Hsueh, A.J.W. (1980). Gonadotropin stimulation of testosterone production in primary culture of adult rat testis cells. Biochem. Biophys. Res. Commun. 97:506–512.

Hsueh, A.J.W. and Erickson, G. F. (1978). Glucocorticoid inhibition of FSH-induced estrogen production in cultured rat granulosa cells. Steroids 32:639–648.

Hunter, W. M. and Greenwood, F. C. (1962). Preparation of iodine-131 labelled human growth hormone of high specific activity. Nature 194:495–496.

Leung, K. and Munck, A. (1975). Long-term incubation of rat thymus cells: cytolytic actions of glucocorticoid *in vitro*. Endocrinology 97:744–748.

McKenna, T. J., Lorber, D., Lacroix, A. and Rabin, D. (1979). Testicular activity in Cushing's disease. Acta Endocrinol. (Copenh) 91:501–509.

Munck, A. and Leung, K. (1977). Glucocorticoid receptors and mechanism of action. In: Receptors and Mechanisms of Action of Steroid Hormones, Part 2 (J. R. Pasqualini, ed.). Marcel Dekker Inc., New York, pp. 311–397.

Nakajin, S. and Hall, P. F. (1981). Microsomal cytochrome P-450 from neonatal pig testis: Purification and properties of a C_{21} steroid side-chain cleavage system (17α-hydroxylase-$C_{17,20}$ lyase). J. Biol. Chem. 256:3871–3876.

Ortlip, S. A., Li, S. A. and Li, J. J. (1981). Characterization of specific glucocorticoid receptor in the Syrian hamster testis. Endocrinology 109:1331–1338.

Purvis, K. and Hansson, V. (1978). Hormonal regulation of Leydig cell function. Mol. Cell. Endocrinol. 12:123–138.

Rebar, R. W., Aurand, J., Unger, M. and Hsueh, A.J.W. (1980). Protein A as an immunoadsorbant in radioimmunoassay: usefulness in a rapid assay for urinary LH. Fertil. Steril. 33:151–156.

Saez, J. M., Morera, A. M., Haour, F. and Evain, D. (1977). Effects of *in vivo* administration of dexamethasone, corticotropin and human chorionic gonadotropin on steroidogenesis and protein and DNA synthesis of testicular interstitial cells in pre-puberal rats. Endocrinology 101:1256–1263.

Snedecor, G. W. and Cochran, W. G. (1967). Statistical Methods. The Iowa State University Press, Ames, pp. 91–116.

Steiner, A. L., Parker, C. W. and Kipnis, D. M. (1972). Radioimmunoassay for cyclic nucleotides I. Preparation of antibodies and iodinated cyclic nucleotides. J. Biol. Chem. 247:1106–1113.

Wang, C., Erickson, G. F., Hopper, B. and Hsueh, A.J.W. (1980). Direct inhibitory effect of enclomiphene citrate and estradiol on testis functions in hypophysectomized rats. Biol. Reprod. 22:645–653.

Downloaded from https://academic.oup.com/biolreprod/article/27/5/1138/2767403 by U.S. Department of Justice user on 17 August 2022

Welsh, T. H. and Hsueh, A.J.W. (1982). Mechanism of the inhibitory action of epidermal growth factor on testicular androgen biosynthesis *in vitro*. Endocrinology 110:1498–1506.

Welsh, T. H., and Johnson, B. H. (1981). Stress-induced alterations in secretion of corticosteroids, progesterone, luteinizing hormone and testosterone in bulls. Endocrinology 109:185–190.

Downloaded from https://academic.oup.com/biolreprod/article/27/5/1138/2767403 by U.S. Department of Justice user on 17 August 2022