INTRATESTICULAR SITE OF AROMATASE ACTIVITY AND POSSIBLE FUNCTION OF TESTICULAR ESTRADIOL

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INTRODUCTION

Estrogen biosynthesis in testes has been known for many years. The first demonstration of testicular aromatization of testosterone to estradiol was reported from studies with homogenates of stallion testes (1). In more recent studies, Kelch and associates demonstrated the secretion of estradiol by human, simian, canine, and rat testes (2). The site and regulation of testicular aromatization has been studied by numerous investigators during the past decade. As early as 1974, in vitro studies by de Jong and associates were interpreted as indicating that aromatization occurred in seminiferous tubules rather than interstitial tissue (3). Dorrington, Fritz, and Armstrong reported that Sertoli cells obtained from 20-day-old or younger rats, and maintained in culture, had the capacity for estradiol synthesis in the presence of added testosterone and follicle-stimulating hormone (FSH) (4). In contrast, earlier studies by Pierrepoint et al. indicated that Leydig cells are the source of testicular estrogens in man (5). Studies from our laboratory on separated seminiferous tubules and intact testicular tissue from human testes were consistent with interstitial or Leydig cells as the major site of aromatization in human testes (6). In vivo treatment with luteinizing hormone (LH), human chorionic gonadotropin (hCG), or FSH to adult (7) or immature rats (8) demonstrated that hCG/LH but not FSH could stimulate

testicular aromatase activity. Studies by Pomerantz on the effects of *in vivo* treatment of immature rats with FSH and LH suggested that in the very young rat, before 18 days of age, aromatization is a function of both Sertoli and Leydig cells (9,10) and that after 18 days of age, Sertoli cells no longer have the capacity for FSH-stimulated aromatization.

In addition to the confusion as to the intratesticular site of aromatase, considerable controversy exists as to the function of testicular estrogen biosynthesis. In this review, studies will be presented on the intratesticular site and hormonal regulation of estradiol synthesis, and a possible function of testicular estradiol will be proposed.

INTRATESTICULAR LOCALIZATION AND HORMONAL REGULATION OF AROMATASE ACTIVITY

To establish whether aromatase activity was present in Leydig cells and to examine if this activity can be stimulated *in vitro* by gonadotropin, Metrizamide gradient-purified Leydig cells obtained from mature rats were studied (11). These cells were incubated for 4 h with [^{3}H]testosterone in the presence or absence of hCG, and the amount of [^{3}H]estradiol produced was taken as an index of Leydig cell aromatase activity. We determined that in the absence of hCG, maximal aromatization was observed at a concentration of 0.6 μM testosterone. This concentration of testosterone is similar to the concentration of testosterone found in rat interstitial tissue, 0.8 μM (3). The effect of increasing concentrations of hCG on estradiol biosynthesis from testosterone is shown in Figure 1. Approximately a 6-fold increase in aromatase activity was observed at a concentration of 10 pM hCG. The data presented in Figure 2 illustrate that this hCG stimulation of

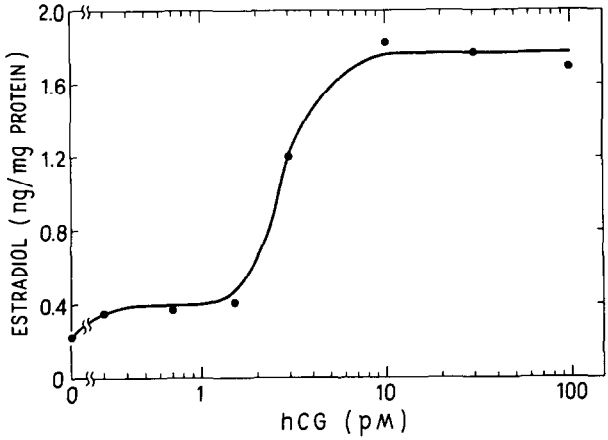

Figure 1. Effect of increasing concentration of hCG on in vitro aromatization in purified Leydig cells from 70-day-old rats. From Valladares and Payne (11).

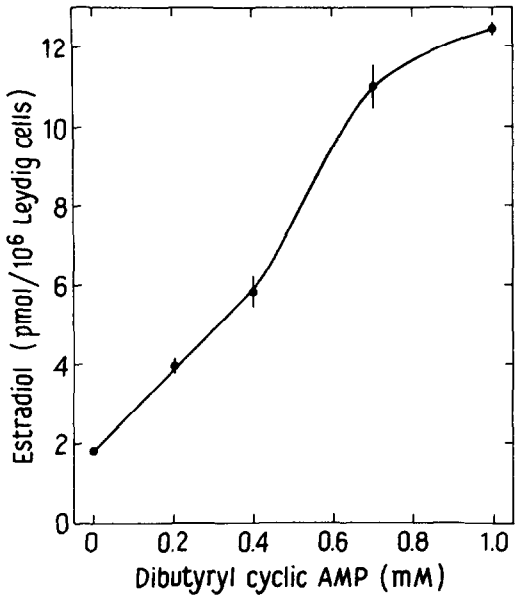

Figure 2. Effect of increasing concentrations of dibutyryl cAMP on aromatization in purified Leydig cells from approximately 70-day-old rats. From Valladares and Payne (12).

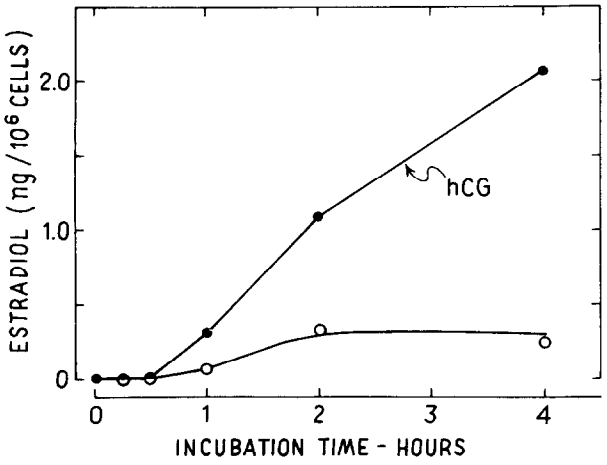

Figure 3. Time course of in vitro hCG stimulation of aromatization in purified Leydig cells. From Valladares and Payne (11).

aromatase activity can be mimicked by incubation of purified Leydig cells with dibutyryl cyclic AMP (cAMP). hCG-stimulated aromatase activity was completely inhibited by including a competitive inhibitor of aromatase 7α[4'-amino]phenylthioandrost-4-ene-3,17-dione, in the incubation medium (12). The time course of in vitro hCG stimulation of aromatase activity in purified Leydig cells is shown in Figure 3. In contrast to acute hCG-stimulated increases in testosterone production, which can be seen as early as 15 min, a 30-min lag period is observed before a time-dependent increase in hCG-stimulated aromatization. These data demonstrate that hCG acting via cAMP has the capacity to stimulate Leydig cell aromatase activity during short-term incubations. Since the Leydig cells were incubated with a saturating concentration of testosterone, the effect of hCG or cAMP on aromatase activity is independent of acute stimulation of testosterone biosynthesis by hCG or cAMP.

Since in vivo studies by Pomerantz (9,10) indicated that, in rats younger than 18 days of age, FSH administration increased

testicular estradiol concentration whereas hCG administration increased estrogen formation in 18-day-old rats, we studied the in vitro effect of hCG and cAMP on purified Leydig cells from 15- and 25-day-old rats. Purified Leydig cells from these younger rats were incubated for 4 h in the presence of either hCG (30 pM) or dibutryl cAMP (1 mM). Although Leydig cells from 15-day-old rats in the absence of hCG or cAMP exhibited approximately 2-fold greater aromatase activity compared with basal aromatase activity in Leydig cells from 70-day-old rats, no increase in activity by either hCG or cAMP was demonstrable in the younger animals (Figure 4). Leydig cells from 15-day-old rats also did not show an increase in testosterone production in response to hCG or cAMP (12). Figure 4 illustrates that

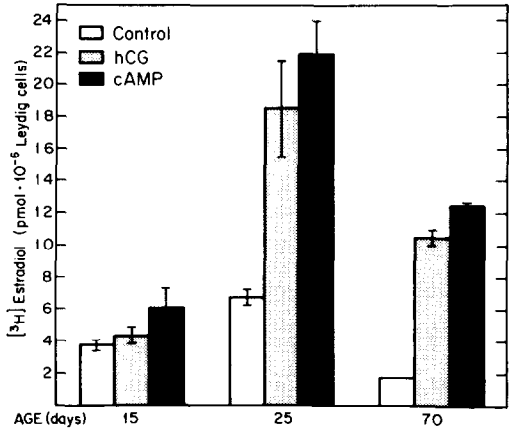

Figure 4. hCG- or cAMP-stimulated aromatase activity in Leydig cells of rats of different ages. Purified Leydig cells obtained from rats at the indicated age were incubated in vitro for 4 h with 0.6 μM [^{3}H]-testosterone in the absence or presence of 30 pM hCG or 1 mM dibutyryl cAMP. The amount of [^{3}H]estradiol synthesized was determined. Data taken from Valladares and Payne (12).

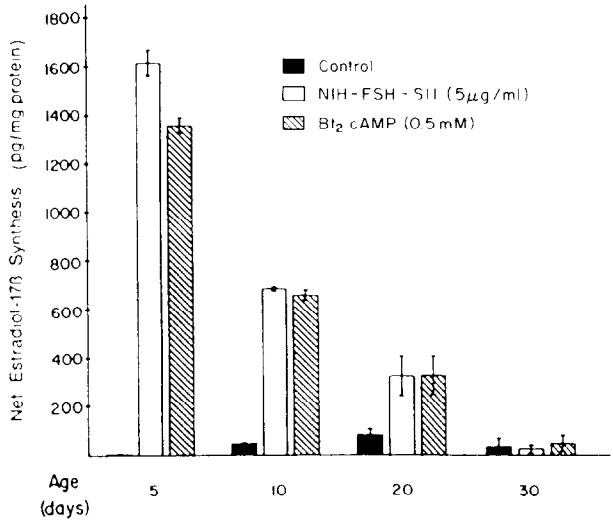

Figure 5. Estradiol synthesis by Sertoli cells from rats of different ages, cultured for 24 h in the presence of 0.5 μM testosterone. From Dorrington, Fritz, and Armstrong (4).

Leydig cells from 25-day-old rats had the highest capacity for hormonal stimulation of aromatase activity, which decreased by 50% in Leydig cells from 70-day-old rats. These data suggest that maximal aromatase activity in Leydig cells changes with age. In vitro studies on FSH-stimulated aromatization in cultures of Sertoli cells exhibit a change relative to age in the capacity for aromatization of these cells with maximal activity observed in cells obtained from 5-day-old rats and the absence of activity in cells from 30-day-old rats (Fig. 5) (4). These in vitro studies in Leydig and Sertoli cells from different ages suggest that Sertoli cells may be the major site of aromatization in neonatal rats, whereas Leydig cells are the major or only site of aromatization during pubertal development. No data are available about the site or presence of aromatase in Leydig cells of rats after 70 days of age.

FUNCTION OF TESTICULAR ESTRADIOL

The function of estradiol produced in Leydig or Sertoli cells remains to be established. Studies by Dufau and colleagues (13-15) suggest that the increased production of estradiol after administration of a high dose of hCG to rats leads to a decrease in the activities of the microsomal P-450 enzymes, 17α-hydroxylase/C_{17-20} lyase (see Fig. 6). Their studies indicate that this estrogen-induced decrease in the microsomal P-450 enzymes is the cause for the diminished capacity for Leydig cell testosterone production in response to subsequent acute stimulation with trophic hormone or cAMP (steroidogenic desensitization). Evidence was presented that the effects of hCG on 17α-hydroxylase/C_{17-20} lyase activities and on testosterone biosynthesis could be prevented by a simultaneous administration of the estrogen receptor antagonist, tamoxifen (16). Additional reports from the same laboratory indicate that the hCG-induced decrease in testosterone synthesis could be mimicked by the addition of estradiol

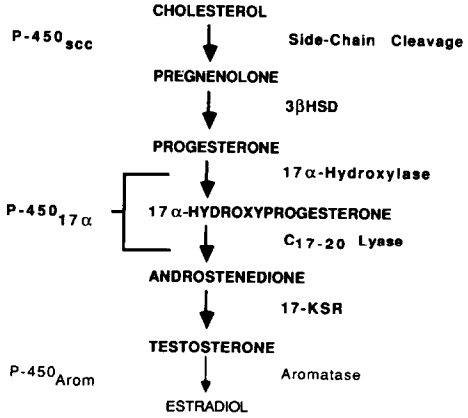

Figure 6. Pathway of cholesterol to estradiol in rodent Leydig cells.

to cultures of testicular cells (13,14). Other investigators, however (17-20), have been unable to demonstrate effects of estradiol and/or tamoxifen similar to those described by Nozu and colleagues (14,15).

To investigate the role of estradiol as the possible cause of steroidogenic desensitization as well as to evaluate the general mechanism by which microsomal P-450 activities are decreased, Quinn and Payne (21,22) developed a primary culture system of purified mouse Leydig cells from mature mice. In these studies, it was demonstrated that treatment of Leydig cells in culture with cAMP for 48 h decreased microsomal P-450 activities, 17α-hydroxylase/C_{17-20} lyase in an oxygen-

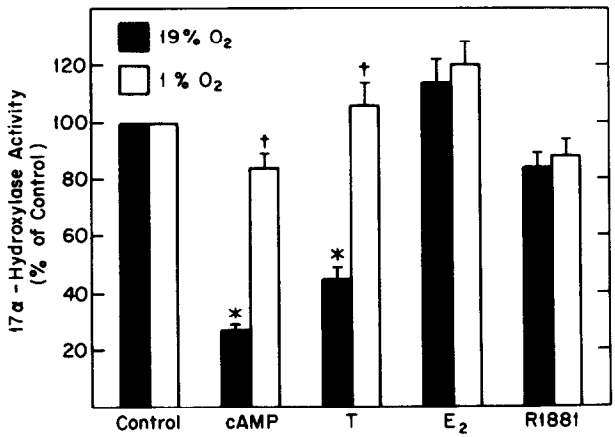

Figure 7. Effect of cAMP and steroids on 17α-hydroxylase activity of mouse Leydig cells in culture. Leydig cell cultures were maintained at 19% O_2 (95% air, 5% CO_2) or 1% O_2 (1% O_2, 5% CO_2, 94% N_2) and treated for 48 h with no additions (control) or 1 mM 8-Br-cAMP, 2 μM testosterone (T), 2 μM estradiol (E_2), or 2 μM methyltrienolone (R1881). Cultures were washed at the end of 48 h to remove treatment agents, and 17α-hydroxylase activity was determined by incubating 10 μM [^{3}H]progesterone for 1 h and isolating the ^{3}H-labeled products. * $p < 0.05$ versus control. † $p < 0.05$ versus 19% O_2. From Quinn and Payne (22).

dependent manner and resulted in desensitization of the steroidogenic response. Reduction of the oxygen tension from 19 to 1% prevented the decrease in P-450 activities, but not the reduction in steroidogenic capacity. The decrease in enzyme activity could also be prevented by blocking steroid synthesis with aminoglutethimide, thus demonstrating that the decrease in enzyme activities after cAMP stimulation was not due to a direct effect of the cAMP, but was mediated by increased steroid production. To determine whether the presence of increased concentrations of steroid products could decrease the microsomal P-450 activities in an oxygen-dependent manner, the effect of addition of steroids on the Leydig cell cultures was investigated. The data presented in Figure 7 demonstrate that the addition of testosterone at a concentration of 2 μM, which is equivalent to the concentration of testosterone present in the medium of cultures after stimulation with LH or cAMP for 24 h, caused a decrease in 17α-hydroxylase activity similar to that observed with cAMP treatment (22). This testosterone-induced decrease in P-450 activity was prevented by reduction of the oxygen tension to 1%. Estradiol or the androgen agonist methyltrienolone (R1881) had no effect on 17α-hydroxylase activity. Similar studies were carried out using mature rat Leydig cells in culture. As can be seen in Figure 8, the addition of a high concentration of estradiol (1 μM) had no effect on C_{17-20} lyase activity of rat Leydig cells. Furthermore, the presence of an estradiol receptor antagonist (LY 156758) did not prevent the decrease in C_{17-20} lyase activity in cAMP-treated cultures. If the increased production of estradiol were the cause for the decrease in the microsomal P-450 activities, as suggested by Nozu et al. (13,14), the

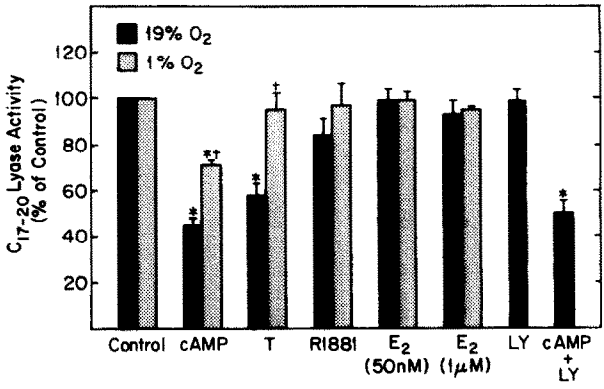

Figure 8. Effect of cAMP, steroid products, androgen receptor agonist, and estradiol receptor antagonist on C_{17-20} lyase activity of rat Leydig cells in culture. Leydig cells were maintained in culture and treated for 48 h as described for Figure 7 and treated with no additions (control), 1 mM 8-Br-cAMP (cAMP), 10 μM testosterone (T), 2 μM methyltrienolone (R1881), 50 nM or 1 μM estradiol (E_2), 100 nM LY 156758 (LY), or cAMP plus LY. Cultures were washed at the end of 48 h to remove treatment agents, and C_{17-20} lyase activity was determined by incubating for 1 h, 10 μM 17α-hydroxy-[^{3}H]progesterone and isolating ^{3}H-labeled products. * $p < 0.05$ versus control. † $p < 0.05$ versus 19% O_2. From Georgiou et al. (24)

addition of an estradiol receptor antagonist should have prevented the loss in enzyme activity. These data demonstrate that estradiol is not involved in the loss of microsomal P-450 activities in either rat or mouse Leydig cells and that the mechanism of loss of these activities is similar in rat and mouse Leydig cells.

To examine if the observed oxygen-dependent, testosterone-induced decrease in microsomal P-450 activities is due to damage to and concomitant loss of the enzyme protein, mouse Leydig cell cultures were treated for 2 days with various steroids and the amount of immunoreactive P-$450_{17\alpha}$ was determined by Western blotting. The data presented in Figure 9 demonstrate that of the compounds tested, only testosterone and its 17α-epimer, epitestosterone, caused a decrease in

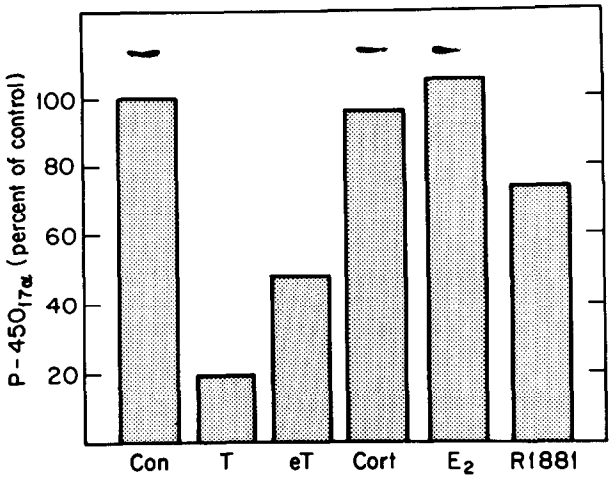

Figure 9. Effect of steroids and an androgen agonist on the amount of immunoreactive P-450$_{17\alpha}$ in mouse Leydig cells in culture. Incubation conditions were as described in Figure 7. Lysates were prepared from treated Leydig cells, and immunoreactive P-450$_{17\alpha}$ was determined by immunoblotting. Specifically bound antibody was detected by ^{125}I-protein A. Each lane in the autoradiogram was scanned by laser densitometry. The areas of each peak were determined and are represented with control as 100%.

the amount of immunoreactive P-450$_{17\alpha}$. These decreases in the amount of the enzyme, specifically caused by testosterone or epitestosterone and not by estradiol, cortisol, or the androgen agonist, are similar to observed decreases in 17α-hydroxylase activity (22). Taken together, these various studies indicate that the enhanced loss of Leydig cell microsomal cytochrome P-450 activities in cAMP-treated cultures is caused by the increased production of testosterone. This product of the enzyme-catalyzed reaction probably acts as a pseudosubstrate for the P-450 by binding to the enzyme (23) and triggers the release of reactive oxygen species which either directly or indirectly, via lipid peroxidation, damage the P-450, leading to increased degradation of enzyme. The results do not support any role

Table 1. Mitogenic Activity Versus Hormone-stimulated Aromatase Activity of Sertoli and Leydig Cells from Rats of Different Ages

	HORMONE-STIMULATED MITOTIC ACTIVITY	AROMATASE ACTIVITY
SERTOLI	Proliferate until 15 days	Highest at 5 days, absent after 20 days
LEYDIG	Atrophy in neonate	Low or absent
	Proliferate at early puberty	Highest at ∿25 days

Hypothesis: Estradiol functions as a mitogen for Sertoli and Leydig cells.

for an estrogen receptor-mediated action in the decrease of microsomal P-450 activities during steroidogenic desensitization. Thus, the physiologic significance of estradiol production in testes remains to be established.

CONCLUSION

It is well known that estradiol stimulates cell division in several tissues. We therefore propose the following hypothesis: estradiol functions as a mitogen for Sertoli and Leydig cells. Table 1 compares mitotic activity with hormone-stimulated aromatase activity in Sertoli and Leydig cells of the rat at different ages during development. From this table it can be seen that during development, the time of highest aromatase activity coincides with the time of greatest mitotic activity in Sertoli or Leydig cells. Whether high estradiol production is responsible for the high mitotic rate needs to be examined.

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NOTE

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