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[^{3}H]OESTRADIOL METABOLISM BY 18-DAY RAT INTERSTITIAL CELLS IN CULTURE AND THE EFFECT OF FSH: PRESENCE OF 16α-HYDROXYLASE

R. K. TCHOLAKIAN*, A. S. BERKOWITZ and S. N. NEWAZ
Department of Obstetrics, Gynecology and Reproductive Sciences, University of Texas Medical School at Houston, P.O. Box 20708, Houston, TX 77025, U.S.A.

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Summary—The metabolism of [^{3}H]oestradiol by interstitial cells in culture prepared from 18-day old rat testes was investigated. Interstitial cells were able to convert [^{3}H]oestradiol to [^{3}H]oestriol as confirmed by recrystallization of oestriol to constant specific activity from samples containing cells and not from controls. This demonstrated for the first time the presence of 16α-hydroxylase in rat testicular interstitial cells. The effect of *in vitro* FSH treatment on the cells in culture was also investigated. FSH failed to affect 16α-hydroxylase activity since we could not demonstrate a significant difference between treated and untreated preparations.

The 16α-hydroxylation of phenolic steroids is widely regarded as the major pathway of oestrogen metabolism in mammals. This metabolic step significantly reduces the biological activity of oestradiol. The presence of 16α-hydroxylase in interstitial cells suggests that it may play a role in inactivating the oestradiol that is produced in the Leydig cells and thus prevent its intracellular accumulation. Such activity may conceivably play a role in the overall local "fine tuning" of androgen biosynthesis.

INTRODUCTION

Interest in the cellular site of testicular steroid production has led several investigators to study the ability of various cellular compartments of the testes to metabolize selected steroid substrates. Interstitial cells and seminiferous tubules were able to convert progesterone to testosterone [1,2], although the former is considered the main site of steroid synthesis that maintains circulating androgen levels in the male. Interstitial cell preparations have been estimated to contain between 10–40% Leydig cells and steroid metabolism by this compartment has been attributed to these cells [3,4]. More direct evidence of the cell type involved in this metabolism was provided when purified Leydig cell preparations metabolized C-21 pregnenes to C-19 androstenes [5].

Recently we have demonstrated that interstitial cells from 18d rat testes can metabolize [^{3}H]testosterone to [^{3}H]oestradiol [6]. This is in agreement with the work of others regarding the production of oestradiol by Leydig cells [7]. Canick *et al.* [8] found that HCG, but not FSH, stimulated testicular aromatase in immature rats and localized this activity in the interstitial tissue. Later Valladaras and Payne [7] were able to demonstrate aromatization of [^{3}H]testosterone to [^{3}H]oestradiol in Leydig cells prepared from rats of all ages.

We have recently reported on the metabolism of [^{3}H]oestradiol to [^{3}H]oestriol by cultured Sertoli cells from immature rats and established the presence of 16α-hydroxylase in these cell preparations [9]. Due to the deleterious effects of oestradiol on testicular androgen production [10–12] we were interested in understanding how interstitial cells would metabolize the locally produced oestradiol. To do this, cultures of interstitial cells were prepared from 18-day rat testes and incubated in the presence of [^{3}H]oestradiol; since FSH was shown to affect the activity of some testicular steroidogenic enzymes, its effect on oestradiol metabolism was also investigated.

EXPERIMENTAL

Interstitial cell cultures

Testes were aseptically removed from 18-day old Sprague–Dawley rats, decapsulated, and the interstitial cells dissociated from the seminiferous tubules by incubation in 0.05% collagenase [Worthington Diagnostics; 206 units/mg] at a volume of 2 ml/testis. Testes were incubated for 15 min at 34°C in a shaking water bath [120 strokes/min]. The incubation mixture was filtered through a 106 μ grid, using two washes of Minimum Essential Media [MEM, Gibco] at a volume at 5 ml/testis. The filtered interstitial cells were pelleted, resuspended in MEM, and plated at a concentration of approx 0.75 testis/2 ml/60 mm culture plate. The cultures were incubated for 72 h at 34°C in 95% air and 5% CO_2; the media was then changed and 16 culture plates were used for the experiments. Each culture dish received 2 μCi [2,4,6,7,16,17-^{3}H]oestradiol [130 μCi/mmol, NEN] as

*To whom correspondence should be addressed.

substrate. The substrate was delivered into each dish in 4 μl 95% ethanol using a 10 μl Hamilton syringe. Four plates with cells received 2 ml MEM only and six plates with cells received 2 ml MEM + 5 μg/ml FSH [NIH-FSH-S10]. Three dishes were prepared as controls [media and substrate only] while three additional control dishes were prepared receiving media, substrate + 5 μg/ml FSH. None of these six controls contained interstitial cells. All the plates were incubated for an additional 72 h at 34°C in 95% air and 5% CO_2.

Extraction, separation and purification of radiolabeled metabolites

At the termination of incubation, 0.2 ml 1N HCl was added to all incubates. The content of each dish was transferred to a 50 ml conical tube, each dish was washed 3 times with 2 ml distilled water, and each wash was transferred to its respective tube. Each plate then received 15 μg of oestradiol and oestriol in 2 ml of methanol as carrier steroids and left to equilibrate for 30 min with the remaining radiolabeled steroids on the plate surface. The contents of each dish were then repeatedly washed with methanol and dicholoromethane until background counts were reached. Each wash was transferred to its respective tube, the methanol fraction in each samples was evaporated under nitrogen and the remaining water–dichloromethane phase was repeatedly extracted to background with dicholoromethane.

The extracts were chromatographed on freshly prepared silica gel thin-layer plates and developed in benzene–ethanol [9:1, v/v]. Radioactivity chromatographing like authentic oestradiol [$R_f = 0.5$] and oestriol [$R_f = 0.2$] were detected by scanning the plates on a radiochromatogram scanner. Radioactive peaks behaving like authentic steroids were scraped and eluted with dichloromethane.

Radioactivity in controls and samples behaving like authentic oestriol was eluted and a known mass of unlabeled oestriol was added. The samples were filtered through medium porosity fritted funnels and the mixtures were recrystallized to constant specific activity from ethanol–isooctane, ethanol–water, and ethanol–heptane.

Calculations

Losses were estimated by accounting for all the radioactivity after each step in the procedure. The percent substrate converted was derived as previously described [13] using the purity of the isolated compound and multiplying the constant specific activity with the total authentic steroid added. This resulted in total counts of pure steroid in each sample which was used to determine the percentage of substrate metabolized to the specific steroid.

RESULTS

Untreated samples

[^{3}H]Oestriol was isolated and recrystallized with authentic oestriol to constant specific activity from samples 1–4 consisting of interstitial cells and media only. The mean specific activity of the samples are shown in Table 1. All three control samples composed of media only did not contain any [^{3}H]oestriol by failure of radioactivity chromatographing like oestriol to recrystallize to constant specific activity. The data illustrate the presence of 16α-hydroxylase in the cultured interstitial cells of 18-day rat testes.

FSH treated samples

[^{3}H]Oestriol was isolated and recrystallized with authentic oestriol to constant specific activity from samples 5–10 consisting of media, FSH and interstitial cells. The mean specific activity of the samples are shown in Table 1. Radioactivity chromatographing like oestriol failed to recrystallize to constant specific activity from controls consisting of media and FSH without cells. This further confirmed the presence of 16α-hydroxylase in interstitial cells of 18-day rat testes.

A summary of the recrystallization data of [^{3}H]oestriol from non-treated and FSH-treated cultures is shown in Table 1. Non-treated cultures

Table 1. Summary of recrystallization data of [^{3}H]oestriol produced by interstitial cells in culture prepared from 18-day old rats in presence and absence of FSH

Sample no.	Treatment	Mean specific activity dpm/mg	Pure* oestriol dpm	Oestriol as % conversion
1	None	7050	56,400	1.29
2	None	2650	26,450	0.62
3	None	7010	70,070	1.62
4	None	5850	58,480	1.93
		Mean	52,850	1.37 ± 0.3†
5	FSH	6920	34,575	1.21
6	FSH	3180	31,800	0.73
7	FSH	7210	72,050	1.64
8	FSH	5130	51,290	1.12
9	FSH	6440	64,370	1.44
10	FSH	9690	96,910	2.25
		Mean	58,500	1.40 ± 0.2†

*Total dpm in each sample calculated to be pure oestriol based on mean specific activity and 10 mg mass added per sample for recrystallization [except for samples 1 and 5 which received 8 mg mass].

†Mean ± standard error of the mean.

converted a mean of 52,850 dpm of substrate to oestriol with a range of 26,450–70,070. The mean (±SE) as percent of substrate converted was 1.37 ± 0.3. Oestriol from FSH treated cultures had a mean of 58,500 dpm with a range of 31,800–96,910 dpm. The mean (±SE) of oestriol in treated cultures was 1.4 ± 0.2%. Comparing means and standard errors, there was no significant difference between cultures with or without FSH indicating that FSH had no effect on altering the 16α-hydroxylase activity involved in the conversion of oestradiol to oestriol.

DISCUSSION

The results of our study demonstrate the ability of interstitial cells to metabolize oestradiol to oestriol, thus establishing the presence of 16α-hydroxylase in this compartment of the testis. This represents the first report of active 16α-hydroxylase in rat interstitial cells and along with our previous report on the presence of this enzyme in Sertoli cells [9] indicates the ability of the two major testicular compartments to metabolize the biologically potent oestradiol to the relatively inactive 16α-hydroxylated form, oestriol.

The percent conversion of oestradiol to oestriol was similar in non-treated [1.37 ± 0.3] and FSH-treated [1.4 ± 0.2] cultured interstitial cells. This data along with our previous findings [9] indicate that 16α-hydroxylase interaction with phenolic steroid substrates in the whole testes of immature rats is not under the influence of FSH.

The major physiological significance of testicular oestradiol synthesis may be a local effect of the oestrogen aimed at modulating Leydig cell steroid production. The ability of Leydig cells to metabolize testosterone to oestradiol and the presence of oestrogen receptors within the same cells [14,15] suggests that oestradiol may modulate androgen production locally at the site of synthesis within the Leydig cell. This concept is possible when one considers that locally produced testosterone may play a role in regulating Leydig cell steroidogenesis, since infusion of testosterone into the testicular artery of the isolated perfused rat testis does rapidly inhibit LH-stimulated testosterone secretion [16].

16α-Hydroxylation of phenolic steroids is widely regarded as the major pathway of oestrogen metabolism in mammals. This conversion step is significant in that it substantially alters the biological activity of oestradiol [17]. The presence of 16α-hydroxylase is usually demonstrated in oestradiol target tissues. The presence of aromatase and oestrogen receptors in interstitial cells suggests that the locally produced oestradiol may play a role in modulating androgen synthesis in the Leydig cell. The role of 16α-hydroxylase in Leydig cell function could be to alter the activity of all intratesticular oestradiol, regardless of its origin, thus preventing its intracellular accumulation and thereby forming an extremely sensitive local regulatory mechanism, one that can "fine tune" androgen biosynthesis.

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