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ISOLATED SERTOLI CELLS FROM IMMATURE RATS PRODUCE 20α-HYDROXY-PREGN-4-EN-3-ONE FROM PROGESTERONE AND 3β,20α-DIHYDROXY-5α-PREGNANE FROM PREGNENOLONE

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SUMMARY:

Sertoli cells from 17 day old rats convert progesterone to 20α-hydroxy-pregn-4-en-3-one and pregnenolone to 3β,20α-dihydroxy-5α-pregnane after 72 hours in vitro. The metabolites were identified by several systems of thin layer and gas chromatography, derivative formation and crystallization with authentic steroids. The production of 20α-hydroxy-pregn-4-en-3-one and 3β,20α-dihydroxy-5α-pregnane amounted to 1380 and 740 pmoles/h/mg protein which can account for the total amounts of these steroids reported in the testis. It is the first direct evidence that Sertoli cells can metabolize progesterone and pregnenolone and suggests that Sertoli cells contain the major, if not the only, amounts of 20α-hydroxysteroid dehydrogenase in the immature rat testis.

INTRODUCTION:

The male gonad, in virtually all vertebrates, consists essentially of two compartments: the interstitial areas containing Leydig cells and the seminiferous tubules containing Sertoli cells and germ cells. For some time it has been suggested that steroids may be involved in inducing and/or maintaining spermatogenesis (1,2) but the cellular source of these steroids has not been established. Recently, employing a method for obtaining isolated Sertoli cells (3), we were able to show that Sertoli cells from young rats can convert androstenedione to testosterone, dihydrotestosterone and 5α-androstane-3α,17β diol (4). The conversions are stimulated by FSH but only in cells taken from rats that are between 10 and 20 days of age; in cells from older rats the FSH did not stimulate C_{19} interconversions (5). The question now considered is whether Sertoli cells also possess the capacity to utilize C_{21} steroids such as progesterone and pregnenolone as precursors for other steroids. Such a capacity might indicate that Sertoli cell steroidogenesis is not dependent on

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the ability of Leydig cells to furnish the steroid precursors and thence that the induction of gametogenesis might be independent of Leydig cell steroidogenesis.

The results presented here indicate, for the first time, that isolated Sertoli cells in the complete absence of Leydig cells, are able to metabolize progesterone and pregnenolone; the major metabolites of progesterone and pregnenolone are respectively, 20α-hydroxy-pregn-4-en-3-one (20αP)* and 3β,20α-dihydroxy-5α-pregnane (5αP-diol).

MATERIALS AND METHODS:

Sertoli cells were isolated from twenty 17 day old rats (Sprague-Dawley strain) and maintained in vitro in 18 separate 35 mm plastic culture dishes as previously described (3,4). Cells were maintained in 3 ml culture medium (3) at 32 C for 22 h, then at 40 C for 48 h and then at 32 C for 27 h. After the 97 h "preincubation", the medium was aspirated and replaced; some dishes received only 3 ml fresh medium, others received medium containing either [^{14}C]-progesterone (0.65 μCi/11.56 pmoles/dish) or [^{14}C]-pregnenolone (0.72 mCi/12.93 pmoles/dish). Incubation at 32 C was continued for 20 h and then terminated by addition of 0.1 ml 1N HCl. Contents of the dishes were then extracted 3 X with 3 vol ether/chloroform (4:1). Some control dishes had [^{14}C]-labelled progesterone or pregnenolone added just prior to the HCl addition. Extracts were spotted on 20 X 20 cm silica gel GF thin layer plates (Fisher Redi/Plate, 0.25 mm) and the plates were run 2 X in System I (chloroform/ether, 10:3), then turned 90° and run 2 X in System II (hexane/ethyl acetate, 5:2). The plates were apposed to Kodak Medical X-Ray film (X OMAT R Film) for a 7-day exposure. The Rf values of the radioactive spots were determined, then the spots were scraped from the plates and the gels extracted with ether/chloroform (4:1). The Rf values of the spots were compared to those of 55 authentic steroids chromatographed in the same systems.

To help establish the identity of the major metabolite of progesterone and of pregnenolone a Hewlett Packard 5830A gas chromatograph equipped with a 150 cm (4 mm I.D.) column packed with 3% OV-210 on Gas Chrom Q 80/100 mesh was employed. Aliquots of the cold steroids which had similar Rf values as the metabolite were chromatographed to obtain retention time (R_t). Then an aliquot of the metabolite was injected and samples were collected by means of a glass stream splitting device with ratio of approximately 1:9. The material emerging at the splitter was collected in a 24 cm snugly fitted glass tube. The radioactivity of the collected samples was determined with a Beckman LS-255 liquid scintillation counter. Tentative identities were confirmed by acyl derivative formation, or oxidation via acidic CrO_3, and by crystallization with authentic unlabelled steroids to constant specific activity.

RESULTS AND DISCUSSION:

The major metabolite of [^{14}C]-progesterone was identified as 20αhydroxy-pregn-4-en-3-one (20αP). The identification was based on the following evi-

*Abbreviations used: 20αP, 20α-hydroxy-pregn-4-en-3-one; 5αP-diol, 3β,20α-dihydroxy-5α-pregnane; 20α-HSD, 20α-hydroxysteroid dehydrogenase; R_t, retention time.

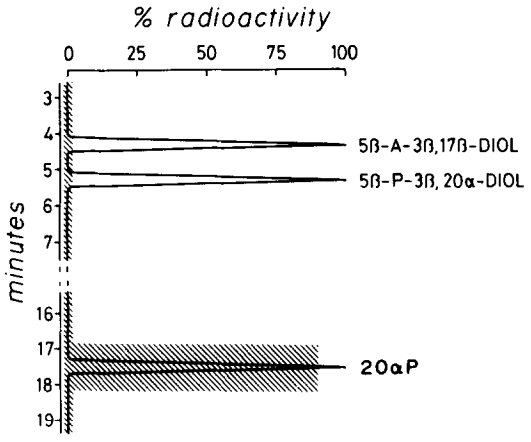

FIGURE 1. Gas chromatographic separation of the three steroids which had R_f values comparable to the major metabolite of [^{14}C]-progesterone. Aliquots (2-3 μg) of 3β,17β-dihydroxy-5β-androstane (5β-A-3β,17β-DIOL), 3β,20α-dihydroxy-5β-pregnane (5β-P-3β,20α-DIOL) and 20αP were chromatographed on a Hewlett-Packard 5830A gas chromatograph as described under Materials and Methods, at 240 C and N_2 flow rate of 35 cc/min. Aliquots (435 dpm) of the metabolites were injected and samples were collected by means of a splitting device. The radioactivity of the split off samples was determined by liquid scintillation and the percent radioactivity is indicated by the cross-hatched area. The peaks indicate the retention times of the three steroids.

dence. Out of 55 steroids chromatographed in Systems I and II, the following had R_f values comparable to those of the metabolite: 3β,17β-dihydroxy-5β-androstane, 3β,20α-dihydroxy-5β-pregnane, and 20αP. These three steroids exhibited separate retention times on the OV-210 column (Fig. 1). When an aliquot of the metabolite was injected and split off the column, together with each of the 3 steroids, over 90% of the radioactivity was recovered with 20αP (Fig. 1). Crystallization of another aliquot of the metabolite with authentic 20αP indicated constant specific activity in crystals and mother liquor from the first crystallization. (The specific activities in cpm/mg were as follows: calculated, 192; 1st crystals, 191; 1st mother liquor, 185; 2nd crystals, 186; 2nd mother liquor, 183).

The total amount of 20αP resulting from the 20-hour incubation with [^{14}C]-progesterone was 27.6 nmoles per mg of Sertoli cell protein (Table 1), or 18.3% of the total radioactivity. About 75% of all recovered radioactivity

Table 1. In vitro conversion of [^{14}C]-progesterone to 20α-hydroxy-pregn-4-en-3-one and of [^{14}C]-pregnenolone to 3α,20α-dihydroxy-5α-pregnane by Sertoli cells from 17 day old rats.

[^{14}C]-Progesterone			[^{14}C]-Pregnenolone		
	% of total radioactivity	nmoles/mg[a]		% of total radioactivity	nmoles/mg[a]
Progesterone[b]	75.7		Pregnenolone[b]	75.3	
20αP	18.3	27.6±1.2[c]	5αP-diol	10.5	14.8±2.1[c]

[a]The mean total amount of Sertoli cell protein was 39.7 µg per culture dish, equivalent to 1.82 X 10^6 Sertoli cells.

[b]Unmetabolized ^{14}C-labelled substrate.

[c]Values are mean ± S.E. in nmoles/mg total protein of three incubations and represent corrected values based on crystallization data.

was due to the unmetabolized [^{14}C]-progesterone and about 7% due to other metabolites (to be discussed in a separate publication). A separate experiment in which samples were extracted after 1, 3, 6, 20 or 48 h of incubation, showed that formation of 20αP was essentially linear between 1 and 20 hours. Thus 20αP was formed at a rate of 1380 pmoles/mg/h (or 30.09 pmole/h/10^6 Sertoli cells).

Several reports have indicated the presence of 20α-HSD activity in the rat testis and that this enzyme is present in the seminiferous tubules (6,7). The present findings show that the 20α-HSD activities previously observed in the tubules can be attributed to the Sertoli cells. The possibility that in vivo the germ cells may also contain 20α-HSD activity can not be excluded. However, the amount of 20αP produced by Sertoli cells in vitro is large enough to account for all 20α-HSD activity reported in tubules (6). The 20α-HSD activity in Sertoli cells varies with the age of the animal (Wiebe and Tilbe, unpublished) and the enzyme has been shown to increase in testes following hypophysectomy (8), to decrease after hCG treatment (8,9) and to possibly

regulate androgen formation by competing with other enzymes (10). However, further studies are required to determine the possible biological implications of large amounts of 20αP in Sertoli cells during the onset of spermatogenesis.

In order to determine the relative amounts of 20α-HSD activity in Leydig and Sertoli cells, additional incubations with [^{14}C]-progesterone were performed with preparations of which 94% of the cells were Leydig cells. These Leydig cell preparations did not produce any detectable amounts of 20αP and none of the major metabolites were the result of 20α-HSD activity. Thus it appears that in testes of young rats, the Sertoli cells possess most, if not all, of the 20α-HSD.

The major metabolite of [^{14}C]-pregnenolone was identified as 5αP-diol on the basis of the following evidence. Out of 55 known steroids chromatographed in Systems I and II, five ran with R_f values which were comparable to those of the metabolite. An aliquot of the metabolite was subjected to gas chromatography and the radioactivity was split at consecutive intervals. The R_t of the radioactivity coincided with the R_t of 3α,20β-dihydroxy-5β-pregnane, 3β,20α-dihydroxy-5β-pregnane, and 5αP-diol. Crystallization of another aliquot of the metabolite with 5αP-diol, employing, consecutively, three different solvent systems, indicated that constant specific activity was attained after the second recrystallization.

The total amount of 5αP-diol resulting from the 20-hour incubation with [^{14}C]-pregnenolone (Table 1) was 14,8 nmoles per mg of Sertoli cell protein (16.2 pmoles/h/10^6 Sertoli cells, or 740 pmoles/h/mg), which amounted to 10.5% of the total radioactivity. The *in vitro* production of 5αP-diol by seminiferous tubules has been reported (6) and the present findings show that Sertoli cells may be the source.

Sertoli cells have been implicated in the production of steroids which may be involved in gametogenesis (2). A frequent suggestion has been that the Leydig cells produce the necessary C-19 precursors which are then converted

by the Sertoli cells to active C-19 metabolites (4). The present in vitro studies show that Sertoli cells possess appreciable steroidogenic enzyme activity and can metabolize C-21 steroids. Whether Sertoli cells can produce the C-19 steroids from C-21 precursors such as progesterone and pregnenolone is currently under investigation.

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