SERTOLI CELLS FROM IMMATURE RATS : IN VITRO STIMULATION OF STEROID METABOLISM BY FSH

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SUMMARY:

Sertoli cells from 10 day old rats convert androstenedione to testosterone and 5α-androstane-3α,17β-diol, testosterone to 17β-hydroxy-5α-androstan-3-one and 5α-androstane-3α,17β-diol, and 17β-hydroxy-5α-androstan-3-one to 5α-androstane-3α,17β-diol after 72 hours in vitro. Conversions of androstenedione to testosterone and 5α-androstane-3α,17β-diol, and testosterone to 5α-androstane-3α,17β-diol were 2 to 3 times greater in FSH treated cultures. Steroid conversion was not stimulated significantly by LH or TSH. The results are interpreted as evidence that in young rats Sertoli steroid metabolism is stimulated by FSH, that Sertoli cells are an androgen target and that FSH may induce or facilitate Sertoli androgen responsiveness.

INTRODUCTION:

Among the aspects of testicular endocrinology which have stimulated research in recent years are the site of action of FSH[2] and the possible involvement of the Sertoli cells in steroidogenesis. Although it has often been suggested that the actions of FSH may be specificially on the Sertoli cells (1-6), most of the inferences are based on studies employing either seminiferous tubules or preparations at best containing also peritubular and germ cells. Similarly, the suggestions that Sertoli cells are capable of steroid interconversions have been based on studies employing normal (7,8) or germ cell depleted seminiferous tubules (7,9) or Sertoli cell enriched cell cultures (8). In the present study Sertoli cell cultures which are virtually free of any other viable cells (10) were employed and we here report the in vitro stimulation by FSH of Sertoli cell 5α-reductase and 3α- and 17β-oxidoreductase activities.

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[2]The following abbreviations have been used: FSH, follicle-stimulating hormone; LH, luteinizing hormone; TSH, thyroid stimulating hormone; A, androstenedione; T, testosterone; DHT, 17β-hydroxy-5α-androstan-3-one; DIOL, 5α-androstane-3α,17β-diol.

MATERIALS AND METHODS:

Sertoli cells were prepared by our method (10) using testes from 10 day old rats. The culture medium of our previous report (10) and containing 2% fetal calf serum (Gibco) was employed in all experiments; 3 ml of the medium were used in plastic petri dishes (Corning) of 35 mm diameter. Each culture contained from 0.3 to 0.7 mg protein and consisted of 94% to 98% Sertoli cells; the contaminating cells were identified cytologically as degenerating spermatogonia. Control Sertoli cells were maintained in culture medium without hormones; treated cells were maintained throughout in a medium containing 5 µg/ml of either FSH (NIH-FSH-S-10), LH (NIH-LH-S16) or TSH (NIH-TSH-B5). After 72 hours *in vitro*, culture medium was replaced with fresh medium containing the same concentrations of hormone as before plus tritium labelled steroids: 2.7×10^{-7}M [^{3}H]-A (1 µCi/0.54 nmoles) or 2.3×10^{-7}M [^{3}H]-DHT (1 µCi/0.92 nmoles) or [^{3}H]-T (1 µCi/0.47 nmoles). Each culture was maintained for 6 hr at 32C in the presence of the labelled steroids and then terminated with 2 drops of 1 N HCl. Cells and media were transferred to glass tubes and stored at -20C. The treatments and results of 5 separate experiments are indicated in Fig. 1.

For extraction of steroids, samples were thawed and the pH adjusted to 9-10 with 1 N NaOH. Unlabelled steroid carriers were added (30-40 µg) as well as about 12,000 DPM [^{14}C]-A, [^{14}C]-T, or [^{14}C]-DHT to serve as internal standards for calculation of recovery efficiency. Samples were extracted twice with four volumes of diethyl ether. Samples were dried, spotted on silica gel G thin layer plates and chromatographed in solvent systems C and L of Lisboa (11) to separate products. Possible metabolites were initially characterized by their mobilities in solvent systems A, C, D, K, and L of Lisboa (11,12). Tentative identities of products were confirmed by recrystallization with authentic unlabelled steroids to constant specific activity or to constant [^{3}H]/[^{14}C] ratio, when [^{14}C] labelled authentic compounds were available. Thin layer chromatography of acyl derivatives and gas chromatography and recrystallization of derivatives further confirmed product identity. Scintillation spectrometry was performed on a Beckman LS-255 liquid scintillation counter and steroid mass was determined with a Hewlett-Packard 5830A gas chromatograph.

Protein was estimated by the method of Lowry *et al* (13). Results are expressed as ng of product formed per hr per mg protein (ng/hr/mg $\pm$ S.E.) corrected for losses in extraction and chromatography. Data were tested for statistical significance by analysis of variance; experiment 2 was also tested by an *a posteriori* multiple range test, the Student-Newman-Kuels test (14).

RESULTS AND DISCUSSION:

Sertoli cell cultures converted A to T and DHT to DIOL at rates ranging between 24.3 and 84.0 ng/hr/mg (Fig. 1a) and A to DIOL and T to DIOL at rates between 2.2 and 3.2 ng/hr/mg (Fig. 1b). In all the conversions examined, FSH treatment resulted in significant increases: 2.5 fold for A to T, 3 fold for A to DIOL, 2 fold for T to DIOL and 1.2 fold for DHT to DIOL. Specificity of the hormone response can be seen in the results of expt. 2 which show that neither TSH nor LH significantly stimulated conversions of A to T and DIOL. The slight increase evident for the LH treated cells (Fig. 1, expt 2) could

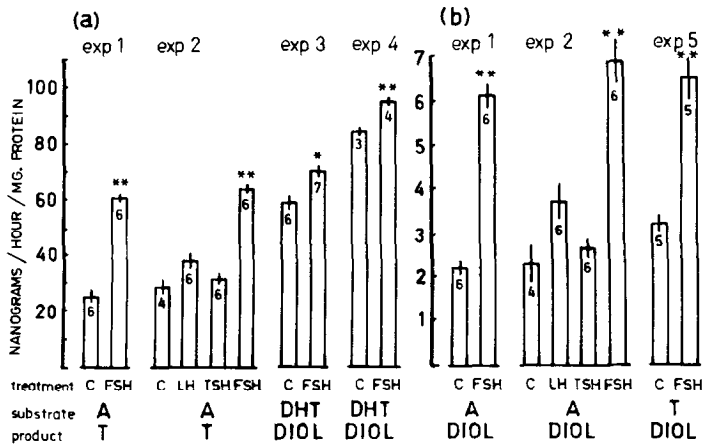

Figure 1: The effect of trophic hormones on the in vitro conversion of steroid substrates (A, DHT or T) to products (T or DIOL) by rat Sertoli cells. Cells were derived from a total of 74 rats at 10 days of age and were cultured as described in Materials and Methods. Bars and lines represent mean conversion rates ± S.E. for the number of replicate determinations indicated on each bar. Note differences of ordinate scales in (a) and (b). Virtually all added labelled steroid could be accounted for as A, T, DHT, or DIOL. While estradiol-17β was detected by recrystallization after TLC, the amount was at the limit of detectability.

* Significantly different from controls (C) at $p < 0.01$.

** Significantly different from controls (C) at $p < 0.001$.

be the result of FSH contamination of the LH preparation (which for NIH-LH-S16 may be somewhat less than 5% on a per unit weight basis). The rates reported here for C_{19} steroid metabolism are 55 to 2400 times greater than recently reported for FSH-stimulated estradiol synthesis from T by Sertoli-enriched preparations (15).

The FSH effect on Sertoli cell steroidogenic enzyme activities appears to be selective rather than a general stimulatory effect. While FSH increased the activity of 17β-oxidoreductase (conversion of A to T) by 126% and 146% in the two experiments (Fig. 1a; expts 1 and 2) and doubled the 5α-reductase activity (expt 5), the hormone only increased the 3α-oxidoreductase (conversion of DHT to DIOL) activity by 13% and 20% (expts 3 and 4). And, although a stimulatory effect of FSH on the dinucleotide cofactors may also exist, the difference in stimulation between 17β- and 3α-oxidoreductase and 5α-reductase

can not be adequately explained by this mode of action since all three enzyme activities are known to employ NADPH (16). Thus, as with other metabolic pathways only certain rate-limiting steps in the pathway may be under regulatory control and FSH may be the regulatory hormone in Sertoli cells as LH appears to be in Leydig cell steroidogenesis (17).

FSH stimulates the production of DIOL from either A or T. The pathway of DIOL formation is via the intermediate DHT but, as shown in expts 3 and 4, the rate of conversion of DHT to DIOL is very high. Thus, very little DHT would be detected. The rate of formation of DIOL therefore appears to be a reasonable measure of the activity of 5α-reductase.

That FSH doubles 5α-reductase activity may be of special significance. One of the characteristics which has been suggested for androgen responsive tissues is that the target tissues possess 5α-reductase and receptors for 5α-reduced androgens (16,18). Thus, the FSH stimulation of 5α-reductase in immature rat Sertoli cells, could be evidence of another action of FSH i.e. the induction or augmentation of androgen responsiveness. In this regard it is of interest that from Means' lab has recently come evidence for a Sertoli cell androgen receptor, distinct from androgen binding protein, found in both the cytoplasm and nucleus (5).

The present findings provide the first direct evidence of a link between FSH and Sertoli cell androgen metabolism. The steroidogenic capacity of Sertoli cells and the stimulatory effect of FSH may represent hormonal mechanisms involved in initiation and control of spermatogenesis. It is generally recognized that androgens are required for vertebrate spermatogenesis (19,20) and that FSH also plays a role in the process (2,19,20). The suggestion has been made that the effect of FSH on germ cells is mediated by the Sertoli cells (1,2,7,8), possibly via the production, in the Sertoli cells, of specific steroids (7). Such an hypothesis is not discordant with the recent observations of Fawcett (21) who suggests that if communication does occur between Sertoli cells and germ cells, the most likely vehicle would be a small, lipid

soluble molecule. Our observations of FSH stimulation of C_{19} steroid metabolism by Sertoli cells of prepubertal rats could be an indication of a possible mechanism of gametogenetic control by Sertoli cells steroids. That the metabolism of steroids by Sertoli cells may be of significance in relation to the initiation of spermatogenesis is also supported by other results from our laboratory. Recent findings (in preparation) show elevated activities of 17β- and 3α oxidoreductases in Sertoli cells in vitro derived from rats of the age when meiosis first becomes established. There is also a very marked increase in activity of certain oxidoreductases which interconvert saturated C_{19} steroids in Leydig cells at about the same time (22) and in response to LH (17). It may be that saturated steroids from Leydig cells are used by Sertoli cells as precursors for an active gametogenetic steroid.

We suggest that in the immature rat the Sertoli cell produces locally high concentrations of androgen(s), possibly via steroids (saturated and unsaturated) derived from Leydig cells (17); the particular steroid(s) may then effect the spermatogonia to replicate and enter meiosis. Additionally, the Sertoli cells may maintain a high androgen titre in the tubules and epididymis by production of androgen binding protein in response to FSH and androgen (5). We therefore suggest that in addition to being a target of FSH, Sertoli cells are also an androgen target, possessing 5α-reductase activity, and that their responsiveness to androgens is initially induced or facilitated by FSH.

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