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MATURATIONAL CHANGES IN TESTICULAR STEROIDOGENESIS: HORMONAL REGULATION OF 5α-REDUCTASE

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SUMMARY

Studies were undertaken to compare the capacity of the human and rat testis to convert progesterone-^{3}H (^{3}H-P) both to testosterone (T) and 5α-androstane-3α,17β-diol (5α-diol) at various stages of development, and to determine the possible mechanisms regulating the maturational changes in 5α-reductase activity in the rat testis. Testes from 1- and 13-yr-old males converted ^{3}H-P to T (7–13%) and 17-hydroxyprogesterone (3–20%) and several unidentified metabolites. A similar metabolic pattern was obtained when ^{3}H-P was incubated with testicular homogenate of 20-yr-old subjects, with the exception that the formation of T (27%) was higher than in 13-yr-old testis. Very little 5α-reduced androstane products, however, accumulated in these three incubations. Under the same incubation conditions, 90-day-old rat testis converted ^{3}H-P to T, while 28-day-old testis converted ^{3}H-P mainly to 5α-diol (58%). To determine whether 5α-reductase activity is dependent on gonadotropic stimulation, 5α-reductase was measured in seminiferous tubules and interstitial cells following various periods of hypophysectomy. In both testicular compartments, 5α-reductase activity decreased rapidly, reaching a minimal level 7 days following hypophysectomy. Treatment with either FSH or LH (3 days) 2 days following hypophysectomy increased significantly 5α-reductase activity in whole testis. Large doses of testosterone propionate, however, did not appear to increase the activity above the level of the untreated hypophysectomized control, indicating that this effect of gonadotropins is not mediated through androgens. These studies clearly demonstrated that neither the immature nor the mature human testis possesses a high level of 5α-reductase activity as compared with the rat testis, and that 5α-reductase activity in the rat testis is dependent on gonadotropic stimulation, in contrast to the androgen-dependent 5α-reductase in other target tissues.

INTRODUCTION

It is well established that, in the testis, the interstitial cells of Leydig are the principal source of testosterone and that testosterone production is stimulated by the trophic hormones, interstitial cell stimulating hormone (ICSH) (LH) and human chorionic gonadotropin (HCG). In all the species studied so far, testosterone is formed from cholesterol by way of two major pathways. In the so-called Δ^4-pathway, cholesterol is converted to pregnenolone → progesterone → 17-hydroxyprogesterone† → androstenedione → testosterone and in the Δ^5-pathway, pregnenolone → 3β,17α-dihydroxy-5-pregnen-20-one → 3β-hydroxy-5-pregn-17-one → Δ^5-androstene-3α,17β-diol or androstenedione → testosterone. While one or the other pathway is dominant in testis of some species, in others both pathways may be active and contribute equally to testosterone production.

In the rat testis, the Δ^4-pathway is dominant, and the enzymes which catalyze the conversion of progesterone to testosterone appear to be present at all stages of development from fetal life to sexual maturity. Under conditions in which neither the substrate concentration nor NADPH is limiting, progesterone is rapidly and equally metabolized in rat testicular homogenates of various ages, though testosterone accumulation varies significantly with the age of the rat. The biosynthetic pathway of testosterone formation from progesterone has been well established in several species. However, the mechanisms which regulate the maturational changes in testosterone accumulation are not yet fully understood. In this presentation, we will limit our discussion to [1] the capacity of the human and rat testis to convert progesterone both to testosterone and 5α-androstanediol at various stages of development and [2] the possible mechanisms regulating the maturational changes in testosterone accumulation in the rat testis.

Testosterone and 5α-androstanediol formation at various ages

In earlier studies, Stylianou *et al.* [1] and Nayfeh and Baggett [2] demonstrated the formation of 5α-androstanediol and several other 5α-reduced products from testosterone and progesterone in rat testicular homogenates. Baggett and Nayfeh [3], Nayfeh *et al.* [4] and Strickland *et al.* [5] demonstrated that the activity of 5α-reductase in the immature rat limits the accumulation of testosterone in rat testis homogenates incubated with labeled progesterone or cholesterol. They observed that testosterone is rapidly

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† The following abbreviations and trivial names were used: 17-hydroxyprogesterone = 17α-hydroxy-4-pregnene-3,20-dione; 5α-androstanediol = 5α-androstane-3α,17β-diol; androstenedione = 4-androstene-3,20-dione; 5α-dihydrotestosterone (DHT) = 17β-hydroxy-5α-androstan-3-one; 20-dihydroprogesterone = 20α-hydroxy-4-pregnen-3-one.

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formed in these *in vitro* systems but accumulates in inverse proportion to the rate of formation of 5α-androstanediol. Furthermore, the activity of testicular 5α-reductase was noted to undergo a striking change with age [4–7]. In the immature rat, the formation of 5α-androstanediol was extremely rapid and little testosterone accumulated. At about the age of puberty, however, testosterone became the major product and the formation of 5α-androstanediol was greatly reduced. Coffey *et al.* [8] later extended these studies to earlier ages of the rat to determine whether testosterone formation could be demonstrated at all stages of immaturity. Labeled progesterone was incubated with testicular homogenates from rats 7, 15, 20, 30, and 90 days of age. The accumulation of testosterone and 5α-androstanediol was measured. The labeled progesterone was completely metabolized in all incubations. Radioactive testosterone accumulated only in incubations of 7 and 90 day testis, while 5α-androstanediol was the major end product in 15, 20, and 30 day incubations. By using very short incubation times (15 min) and low levels of substrate, and by adding unlabeled testosterone to the medium to prevent metabolism of newly formed radioactive testosterone, it was possible to demonstrate that a major portion of the labeled progesterone was converted to testosterone in the immature (15–30 day old) rat testis [8]. In spite of the high concentration of added unlabeled testosterone, the accumulation of radioactive testosterone decreased during longer incubation periods, and 5α-androstanediol accumulated in proportion to the decrease in testosterone. Using heparin to inhibit the conversion of testosterone to 5α-androstanediol, Steinberger and Ficher [9] demonstrated similar testosterone accumulation in the immature rat testis.

Effect of NADPH on testosterone accumulation in minced testis

Since the enzymatic conversion of progesterone to 5α-androstanediol is dependent on the co-factor NADPH, experiments were carried out to determine the extent to which these reactions are influenced by the concentration of NADPH *in vitro*. Experiments were first carried out using minced 20 day old testis in the absence of an NADPH generating system, and it was found that testosterone and androstenedione accumulated as major products (Fig. 1). Addition of increasing amounts of NADP with fixed amounts of glucose-6-phosphate and glucose-6-phosphate dehydrogenase resulted in a diminution in androstenedione and testosterone accumulation associated with a concomitant increase in the formation of androstanediol. These results indicated that end-product accumulation *in vitro* is greatly influenced by the availability of co-factor.

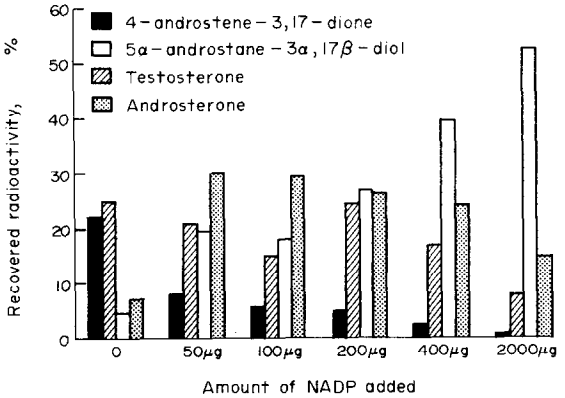

Fig. 1. Minced testes (100 mg) were incubated for 15 min with 0·1 μM progesterone-1,2-^{3}H and either none, 50 μg, 100 μg, 200 μg, 400 μg, or 2000 μg of NADP. The accumulation of labeled products is expressed as a per cent of the total radioactivity from the chromatoplates.

5α-androstanediol formation in vivo

To determine whether 5α-androstanediol is a major product of progesterone metabolism *in vivo*, ^{3}H-progesterone was injected into 28 day old rats and the labeled metabolites were extracted from the testis and identified (Fig. 2). ^{3}H-progesterone was rapidly metabolized and the major product was 5α-androstanediol. A similar result was obtained when ^{3}H-androstenedione was injected. Podesta and Rivarola have measured the endogenous levels of androstanediol, testosterone and 5α-dihydrotestosterone (DHT) in plasma and testicular tissues at various ages of the rat [10] and have shown plasma 5α-androstanediol levels to be increased between the ages of 20–35 days, at which time plasma testosterone levels were minimal.

Accumulation of 5α-dihydrotestosterone from progesterone and 17-hydroxyprogesterone

Lee and Baggett [11] have previously demonstrated that 5α-dihydrotestosterone (DHT) is an intermediate

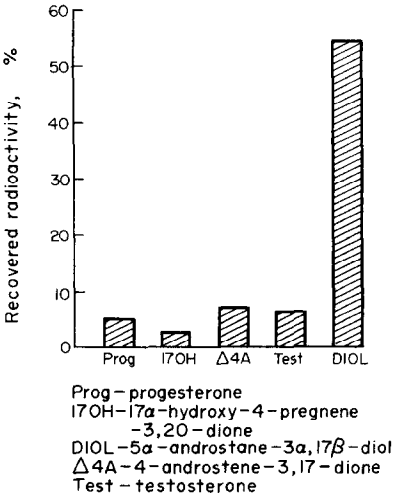

Fig. 2. After functional hepatectomy and evisceration, 28-day-old rats were injected intravenously with 50 μCi of [1,2-^{3}H]-progesterone (40 Ci/mmol) and the testes were removed one hour later. The tissue was extracted with chloroform–methanol (2:1), and the radioactive metabolites separated by thin-layer chromatography on silica gel plates as described previously [2].

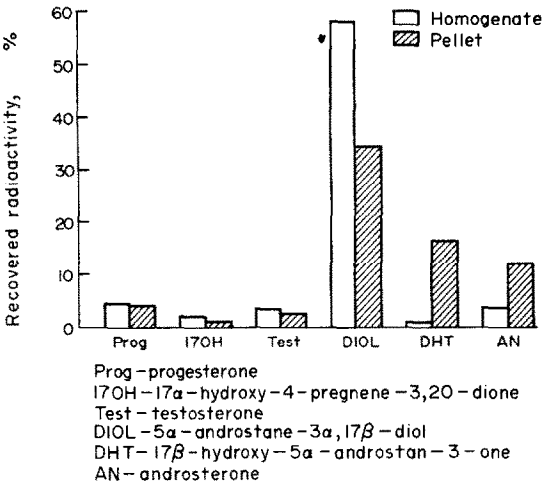

Fig. 3. [1,2-^{3}H]-Progesterone (0·1 μM) was incubated with testicular homogenates or 105,000 *g* pellet, and the radioactive metabolites separated as described previously [2].

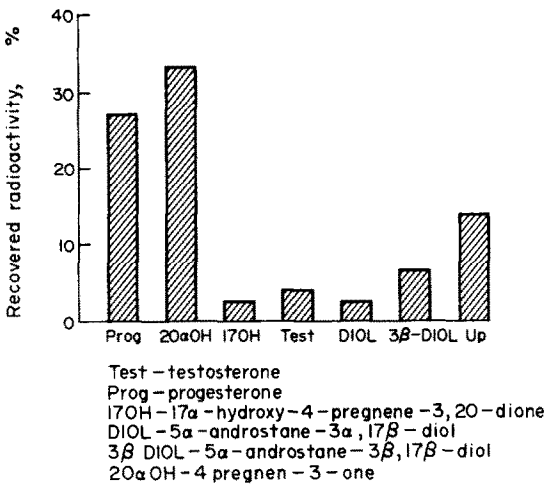

Fig. 4. [1,2-^{3}H]-Progesterone (0·1 μM) was incubated with testicular homogenate (100 mg testicular tissue) for 3 h at 37°, and the radioactive metabolites separated.

in the conversion of testosterone to 5α-androstanediol in the immature rat testis. To ascertain that DHT is also an intermediate in the conversion of progesterone to androstanediol, we next looked for accumulation of DHT after incubations of 30-day-old rat testis with tritium labeled progesterone and 17-hydroxyprogesterone. The labeled substrate was incubated with the testicular homogenate and with the resuspended 105,000 *g* pellet to remove most of the 5α-androstane-3α(β)-hydroxysteroid dehydrogenase activity. As shown in Fig. 3, radioactive DHT accumulated to a much greater extent in the 105,000 *g* pellet than in the whole homogenate. Closely correlated with the accumulation of DHT was a proportional decrease in the formation of 5α-androstanediol, suggesting that DHT is an intermediate. Similar results were obtained with 17-hydroxyprogesterone.

Metabolism of progesterone in human testicular homogenates at various stages of development

It is difficult, of course, to study changes in steroidogenesis with age in the human testis because of the lack of availability of normal tissues. However, some information has been obtained from *in vitro* studies on surgical specimens and from measurements of steroid secretion *in vivo*. It appears that the human testis may not undergo changes similar to the rat. Using the same *in vitro* incubation conditions in which testosterone and 5α-androstanediol are formed from ^{3}H-progesterone by rat testis, human testis from a 1-year-old produced a different pattern of metabolites (Fig. 4). Neither testosterone nor 5α-androstanediol was formed in large amounts, though 70% of the incubated progesterone was metabolized, and 20-dihydroprogesterone (35%) and an unknown metabolite (UP), tentatively identified as 16α-hydroxyprogesterone (12%) were the major products. Progesterone metabolism in testicular homogenate from a 13-year-old subject differed from the 1 year old (Fig. 5). 20-dihydroprogesterone did not accumulate in a major yield, and testosterone (13%), 17-hydroxyprogesterone (22%) and UP (47%) were formed. Very little 5α-reduced androstane products accumulated in this preparation. A similar metabolic pattern was obtained when progesterone was incubated with testicular homogenates from a 20-year-old subject with testicular feminization, with the exception that the radioactivity accumulating in testosterone was higher (27%) while that in 17-hydroxyprogesterone (8%) and UP (41%) were lower in comparison with testis from the 13 year old subject (Fig. 6). The failure of the testis from the 1 year old subject to convert progesterone to testosterone is interesting since 3 months earlier the same child responded to HCG with a marked elevation in plasma testosterone. HCG, 1000 I.U./day i.m. given for 4 days, increased plasma testosterone from less than 100 to 1200 ng%. Recently, Fan *et al.* [12] suggested that the enzyme 20α-hydroxysteroid

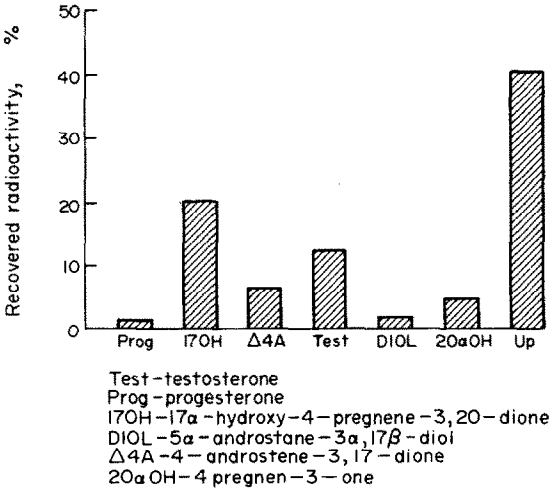

Fig. 5. [1,2-^{3}H]-Progesterone (0·1 nm) was incubated with testicular homogenate (100 mg) for 3 h at 37°.

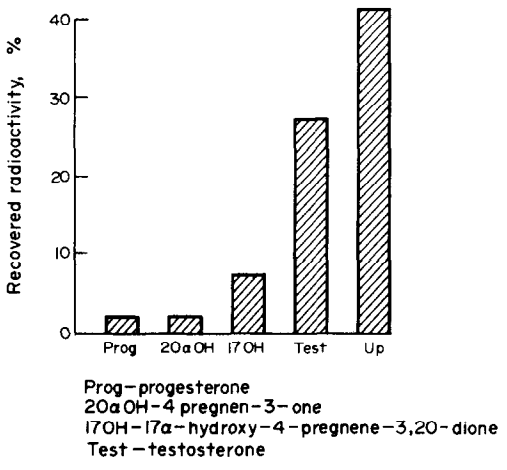

Fig. 6. [1,2-^{3}H]-Progesterone (0·1 nm) was incubated with testicular homogenate (100 mg) for 3 h at 37°.

dehydrogenase may regulate androgen formation in human testis by competition with 17-hydroxylase for progesterone. The reaction products of 20α-hydroxysteroid dehydrogenase, 20-dihydroprogesterone or 17α and 20-dihydroxy-4-pregnen-3-one, might also be inhibitors of 17α-hydroxylase. In the present studies, the accumulation of 20-dihydroprogesterone in big yields in the 1 year old testis is suggestive of this competition. It is possible that ICSH (or HCG) inhibits 20α-hydroxylase, thus allowing more progesterone to be utilized by the 17α-hydroxylase.

From the above studies, it appears that the human and rat testis may differ in three ways: Immature human testis contains a highly active 20-hydroxysteroid dehydrogenase which is almost nonexistent in testis from the intact rat. It is interesting, however, that the steroidogenic activity of immature human testis is similar to that of testis from hypophysectomized rats. Steinberger and Ficher [13] studied the effect of hypophysectomy on the metabolism of progesterone in adult rat testis. Two weeks after hypophysectomy, the formation of testosterone diminished and 20-dihydroprogesterone accumulated in large amounts. Although the effect of 20-dihydroprogesterone on 17α-hydroxylase was not studied by these investigators, it is possible that the lack of progesterone conversion to testosterone by hypophysectomized rat testis resulted from competition between 17α-hydroxylase and 20α-hydroxysteroid dehydrogenase for progesterone. In either case, this finding suggests that this rate limiting step is under the control of pituitary gonadotropins. The observed differences between the immature rat and human testis may indeed result from a higher level of gonadotropin stimulation in the immature rat than in the human. A second difference between the human and rat testis is in 5α-androstanediol formation. In the human testis, 5α-androstane-3β,17β-diol is the major 5α-androstanediol formed, while in the rat 5α-androstane-3α,17β-diol is the major diol [2]. Finally, a third difference between immature testis of the two species is in the testicular 5α-reductase activity which catalyzes the conversion of testosterone to 5α-androstanediol *via* 5α-dihydrotestosterone. While the number of incubation studies carried out with human testis by ourselves and other investigators [14,15] is still very limited, the evidence available indicates that 5α-androstanediol is a minor product of progesterone metabolism in immature human testis even in the presence of saturating concentrations of NADPH. Since neither ^{3}H-androstenedione nor ^{3}H-testosterone were converted to 5α-androstanediol by human testis, the absence of progesterone conversion to 5α-androstanediol was not due to a lack of available substrate for the 5α-reductase but more likely to a low level of enzyme activity.

Our studies on the androgen receptor in immature rat testis [16] have shown that testosterone and DHT are both bound selectively by cytoplasmic and nuclear receptors following the intravenous injection of ^{3}H-testosterone. This finding suggests that testosterone and DHT are both active androgens in the testis. Nevertheless, it is possible that DHT alone might have actions on one or more cell types which mature during the peak of testicular 5α-reductase activity.

Recently, Dorrington and Fritz [17] showed that testosterone is converted mainly to DHT in suspensions of spermatocytes from rat testis, suggesting that DHT might account for a major portion of androgen activity in these cells.

Regulation of 5α-reductase activity in the rat testis

The mechanisms regulating testicular 5α-reductase activity during various stages of development of the rat are not yet known. The changes in testicular 5α-reductase at different ages of the rat appear to correlate with maturational changes controlled by pituitary gonadotropins and androgens in the testis. We next carried out experiments to determine whether 5α-reductase activity is dependent on gonadotropic stimulation.

Effect of testis homogenate from adult rats on 5α-reductase activity in testis homogenate from immature rats

Since the decrease in 5α-reductase activity in the mature testis could result from the formation of a protein inhibitor in the mature testis or the absence of essential co-factors, we measured 5α-reductase in homogenates prepared by combining equal weights of testis from 30- and 110-day-old rats. As shown in Table 1, neither the addition of whole testis homogenate or testicular 105,000 *g* supernatant nor the addition of serum from 110-day-old rat significantly influenced the 5α-reductase activity in 30-day-old testis, thus excluding the presence of 5α-reductase inhibitor in either testis or serum of the mature rat. On the other hand, 5α-reductase activity in the 110-day-old mature testis was not increased by the addition of 105,000 *g* supernatant from 30-day-old testis, indicating that the mature rat testis is not lacking a necessary soluble co-factor which is present in the immature testis.

Effect of hypophysectomy on 5α-reductase of the immature testis

Metabolism of testosterone has recently been examined separately in interstitial cell and seminiferous tubular compartments. Rivarola *et al.* [18] showed that seminiferous tubules of 20 and 45-day-old rats produce more androstanediol from testosterone than do interstitial cells. Formation of androstanediol was especially high in tubules at 26 days when meiotic divisions are first observed [18]. However, in sexually mature animals, 5α-reductase activity decreased markedly in the tubules and remained low in interstitial tissue. Somewhat different results were obtained by Folman *et al.* [19] and Matsumoto and Yamada [20] who observed 5α-reductase activity to be much higher in the interstitial tissue than in seminiferous tubules of immature rats. However, in mature rats, 5α-reductase activity in the interstitial tissue decreased dramatically to a level equal to that in the tubular compartment. To explain this discrepancy between their findings and those of Rivarola and Podesta, Matsumoto and Yamada suggested that the low 5α-reductase activity in the interstitial tissue may be due to a deficiency of co-factors. Furthermore, they found that the preparation of separate tubular and interstitial cell fractions caused a decrease in 5α-reductase activity in the interstitial tissue but had little effect on activity in the seminiferous tubules. The presence of higher 5α-reductase activity in the interstitial tissue of the immature rat testis supports the concept that 5α-reductase may serve to counteract the accumulation and secretion of testosterone in the immature rat testis [4].

To determine whether 5α-reductase is regulated by gonadotropins, we next measured testicular 5α-reductase activity in partially purified seminiferous tubules and interstitial cells under various conditions of hormonal stimulation. 5α-reductase activity was measured first at various intervals following hypophysectomy and was expressed as pmol/g tissue/min in isolated seminiferous tubules and as pmol/mg protein/min in interstitial cells. In both testicular compartments, 5α-reductase activity decreased rapidly, reaching a minimal level 7 days following hypophysectomy (Fig. 7).

Effect of hormones on 5α-reductase of the hypophysectomized rat testis

The decrease in 5α-reductase activity after hypophysectomy prompted an investigation of the role of gonadotropins in the regulation of 5α-reductase. Hypophysectomized immature rats (28-day-old) in groups of 4–6 were injected daily with various hormone preparations for 4 days, and 5α-reductase activity was measured at the end of the treatment period.

Table 1. 5α-Reductase activity in 30 and 110-day-old rat testis preparations*

Type of testis preparation	Per cent of recovered radioactivity 5α-androstane-3α,17β-diol plus 5α-dihydrotestosterone	Testosterone	Polar
30 day homogenate	80·2	17·4	2·4
110 day homogenate	5·2	91·9	3·0
30 day homogenate + 110 day homogenate	73·4	23·8	2·8
30 day homogenate + heated 110 day homogenate	64·7	32·7	2·6
110 day homogenate + 30 day supernatant	6·7	90·1	3·2
30 day homogenate + 30 day serum	77·3	20·0	2·7
30 day homogenate + 110 day serum	80·7	17·2	2·1
110 day supernatant	8·5	89·5	2·0
110 day pellet + 110 day supernatant	3·4	93·5	3·1
30 day pellet + 110 day supernatant	64·9	32·9	2·2
110 day pellet	4·8	92·0	3·2

* Homogenates were prepared from testes of 30- or 110-day-old rats. Where indicated, homogenates were centrifuged at 105,000 *g* for 1 h. Aliquots of the supernatant were mixed with equal volume of homogenate and incubated. In some cases, the 105,000 *g* pellet was resuspended in 0·154 M KCl and combined with supernatant for incubation. In some incubations, serum (20%) was combined with homogenate. All incubations were for 1 h with 0·1 μM testosterone-1,2-^{3}H.

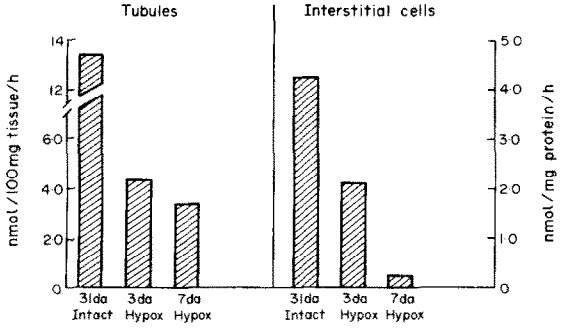

Fig. 7. Rats were hypophysectomized at 28 days of age, and the reductase activity was assayed at various intervals following hypophysectomy. Seminiferous tubules and interstitial cells from each testis were prepared by teasing and washing with 200 ml of 0·154 M KCl. The washed tubules and interstitial cells were homogenized in a mixture of 0·154 M KCl and co-factors as described previously [2]. 1·2 ml of both homogenates were separately incubated with 20·8 nmol ^{3}H-T (S. A. 0·29 μCi/mmol) at 37° for 1 h. 5α-Reductase was quantitated by measuring the total radioactivity accumulating in androstanediol, dihydrotestosterone and androsterone. Protein in interstitial cell preparation was determined by the Lowry method.

5α-Reductase activity in whole testis was increased by FSH and by LH (Fig. 8). Testosterone propionate in large doses, however, did not appear to increase the activity above the level of the untreated hypophysectomized rat. Endogenous testosterone was measured in the testosterone-treated rats by competitive protein binding after purification of the tissue extract on Sephadex LH-20 columns. The concentration of endogenous testosterone was only 2% of the labeled testosterone added. Thus gonadotropin stimulation of 5α-reductase is not likely mediated through androgens. This is in contrast to the androgen-dependent 5α-reductases in other androgen target tissues, such as prostate and epididymis [21, 22].

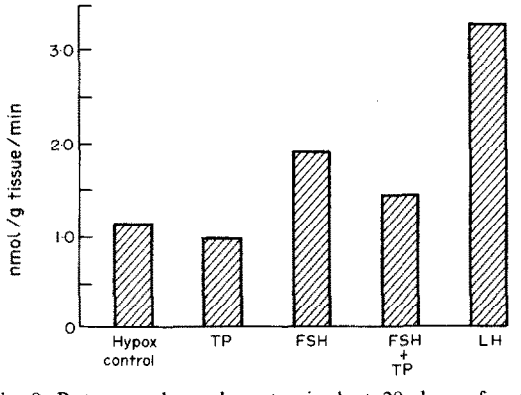

Fig. 8. Rats were hypophysectomized at 28 days of age, and treatment was started 2 days later. Rats in groups of 4 were injected daily either with 500 μg of NIH-FSH-S_{10}/day, or 300 μg of NIH-LH-S_{18} for 4 days. Parallel groups were first treated for 3 days with 2 mg of testosterone propionate (TP)/day followed by either 2 mg TP + 500 μg FSH or 2 mg TP alone daily for 4 additional days. 100 mg of testicular tissue was homogenized in a mixture of 0·5 ml 0·145 M KCl and 0·7 ml of cofactors. 1·2 ml of homogenate was incubated and 5α-reductase activity quantitated as described in the legend to Fig. 7.

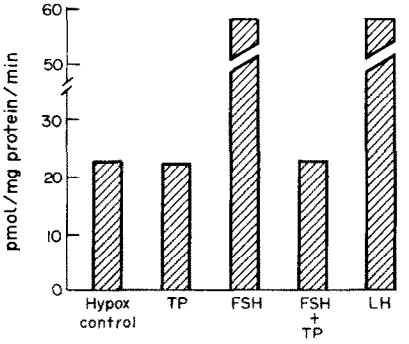

Fig. 9. Interstitial cells were prepared from duplicate testes described under Fig. 8. Homogenization and 5α-reductase quantitation were carried out as described under Fig. 7.

We next carried out experiments to determine whether FSH also acts on 5α-reductase in isolated interstitial cells. As shown in Fig. 9, both FSH and LH increased 5α-reductase activity to the same level above that of the hypophysectomized control.

Since the Sertoli cell is the primary target for FSH action in the testis [23, 24], we next measured 5α-reductase activity in testis from the Sertoli cell-only rats (SCO). It has previously been shown that the seminiferous tubules from these rats are completely devoid of germ cells [24]. The 5α-reductase activity was measured in whole testis and in interstitial cells following various hormonal treatments (Figs. 10, 11). Despite an increased number of Sertoli cells per mg protein in the SCO testis, hormonal stimulation of 5α-reductase activity was not significantly greater than in the nonirradiated testis.

We have recently studied the effects of gonadotropins and testosterone propionate *in vivo* on 5α-reductase and 3α-hydroxysteroid dehydrogenase activities in testis from 50-day-old rats 5 days following hypophysectomy. Preliminary results show that 5α-reductase and 3α-hydroxysteroid dehydrogenase activities

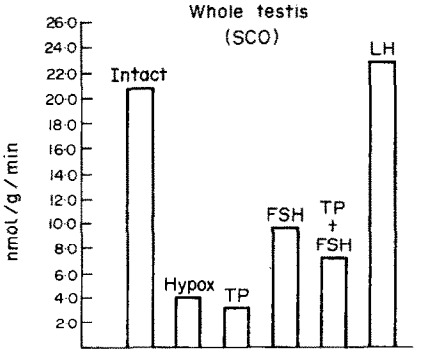

Fig. 10. *Effect of hormonal treatment on 5α-reductase in testicular homogenates from the Sertoli-cell only rats.* Sertoli-cell only rats were obtained by exposing the pregnant mothers to x-irradiation (150 Rad) on the 19th day of gestation. Rats were hypophysectomized on the 24th day of age, and treatment started 7 days later. Hormonal treatment was similar to the experiment described under Fig. 8, with the exception that the LH dose was only 150 μg/day.

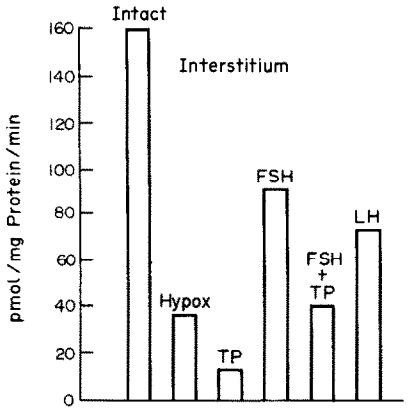

Fig. 11. *Effect of hormonal treatment on 5α-reductase in interstitial cells from the Sertoli-cell only rats.*
Interstitial cells were prepared and 5α-reductase measured as described under Fig. 9.

in whole testis homogenates are increased by LH treatment but not by FSH. Furthermore, 5α-reductase activity was increased by LH in highly purified seminiferous tubules, while FSH and TP had no effect. In contrast, 5α-reductase activity in interstitial cells was not stimulated by any of the three hormones. This finding suggests a possible direct tubular effect of LH in the mature rat.

CONCLUSION

In conclusion, the present studies demonstrated the following:

(1) In contrast to the rat, the human immature testis does not form large amounts of 5α-androstanediol. This might reflect a different mechanism controlling testosterone accumulation in the prepubertal human testis.

(2) 5α-Reductase activity decreases rapidly following hypophysectomy in both mature and immature rat testis. Gonadotropin treatment reverses the effect of hypophysectomy and increases the level of 5α-reductase activity in the whole testis. Both FSH and LH stimulate 5α-reductase activity in the immature testis, while only LH is effective in the mature rat testis. The effect of LH on 5α-reductase does not appear to be mediated by androgen.

(3) Although FSH and LH both appeared to stimulate 5α-reductase in interstitial cells of immature rat testis and LH alone appeared to stimulate 5α-reductase in seminiferous tubules of mature testis, it cannot be concluded from these *in vivo* experiments that the effects of FSH on interstitial cells and LH on tubules resulted from direct actions of the hormones.

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