J. steroid Biochem. Vol. 22, No. 4, pp. 495–499, 1985
Printed in Great Britain.

0022-4731/85 $3.00 + 0.00

HORMONAL REGULATION OF ACTIVITIES OF 4-ENE-5β- AND 5α-REDUCTASES AND 17β-HYDROXY-DEHYDROGENASE IN IMMATURE GOLDEN HAMSTER TESTIS

H. YABUMOTO*, F. IKOMA*, M. TAKEYAMA†, M. TSUJI† and K. MATSUMOTO†[1]

*Department of Urology, Hyogo College of Medicine, Nishinomiya, Hyogo 663 and
†Department of Pathology, Osaka University Medical School, Kita-ku, Osaka 530, Japan

(*Received* 16 *August* 1984)

Summary—We have reported [1–3] in immature golden hamster testis that 5β-reductase is localized in the tubular nongerm cells, while 5α-reductase is present in the interstitial tissue and that the 17β-hydroxy-dehydrogenase activity is found predominantly in the tubular nongerm cells. Hormonal regulation of these enzyme activities was examined in the present study. Male golden hamsters were hypophysectomized on day 22 after birth. The hypophysectomized hamsters in groups of 3–8 were injected daily with 10 μg NIH-LH-S19, 50 μg NIAMD-Rat-FSH-B-1, 8 or 16 μg NIAMD-oFSH-13, 8 μg NIAMD-oFSH-13 plus 5 or 10 μg NIH-LH-S19, 1 mg testosterone propionate or saline for 5 days starting from day 23. Testicular homogenates of the treated hamsters and intact hamsters on day 28 were incubated with [^{14}C]4-androstene-3,17-dione and NADPH, and enzyme activity (nmol/testes/h) was estimated. The activities of 5β- and 5α-reductases and 17β-hydroxy-dehydrogenase decreased significantly 6 days after hypophysectomy. In the hypophysectomized hamster testis, a distinct response to FSH but not to LH in the activities of 5β-reductase and 17β-hydroxy-dehydrogenase was found. The injection of LH in addition to FSH showed no significant additive effects on these enzyme activities. The 5α-reductase activity was stimulated significantly by LH plus FSH but not by LH alone, FSH alone or androgen. These results show that 5β-reduction of 4-ene-3-ketosteroids takes place in the Sertoli cells under the influence of FSH while 5α-reduction occurs in the interstitial cells under the influence of LH and FSH in immature hamster testis.

INTRODUCTION

Previous studies have shown that testes from neonatal and adult rodents such as golden hamsters, rats and mice can rapidly convert progesterone to testosterone but not to 5α-C_{19}-steroids whereas testes from immature rodents produce 5α-C_{19}-steroids, such as androsterone and 5α-androstane-3α,17β-diol, as major products from progesterone [1, 4–8]. Activities of 5α-reductase have been found to be much higher in immature than adult rodent testes [2–8]. In immature rat testes, 5α-reductase is localized primarily in the interstitial cells [9–11] and is regulated by LH [12, 13]. Although 5α-reductase in immature hamster testes was present largely in the interstitial cells [2], the regulation of its activity is not known.

The formation of 5β-C_{19}-steroids from progesterone and 5β-reductase activity were much greater in immature than adult golden hamster testes and the enzyme activity was largely localized in the tubular nongerm cells [1–3]. However, the regulation of 5β-reductase activity in the testis has not been reported. In the present paper, we demonstrate hormonal regulation of 5α- and 5β-reductase activities in the testis of immature golden hamsters.

[1]To whom correspondence should be addressed.

EXPERIMENTAL

Animals

Immature male golden hamsters purchased from Awazu Laboratory for Experimental Animals (Osaka, Japan) were used. The hamsters were hypophysectomized on day 22 after birth. Starting from day 23, the hypophysectomized hamsters in groups of 3–8 were subcutaneously injected daily with 10 μg NIH-LH-S19, 50 μg NIAMD-Rat-FSH-B-1, 8 or 16 μg NIAMD-oFSH-13 or 8 μg NIAMD-oFSH-13 plus 5 or 10 μg NIH-LH-S19 dissolved in 0.5 ml saline, 0.5 ml saline or 1 mg testosterone propionate in 0.1 ml vehicle (0.9% NaCl, 0.4% Polysorbate 80, 0.5% carboxymethylcellulose and 0.9% benzyl alcohol) for 5 days. The hamsters were killed 20 h after the end of the treatment period (on day 28) and testicular enzyme activities were measured. The complete removal of the hypophysis was confirmed. Intact hamsters were injected daily with 0.5 ml saline.

Histology

Testes were fixed in 10% formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

Chemicals

[^{14}C]4-Androstene-3,17-dione (52 mCi/mmol) ob-

Table 1. Formation of [^{14}C]steroids from [^{14}C]4-androstene-3,17-dione by 28-day old hamster testes, expressed as % of substrate

	Intact	Hyp	Hyp + LH 10 μg	Hyp + oFSH 8 μg	Hyp + oFSH + LH 10 μg	Hyp + TP 1 mg	Buffer
4-Androstene-3,17-dione	71.2	67.2	62.3	63.7	63.9	81.7	98.1
Testosterone	7.8	13.6	19.8	21.5	19.0	9.8	0.0
5α-Androstane-3,17-dione	0.6	0.4	0.3	0.2	0.7	0.2	<0.1
17β-Hydroxy-5α-androstan-3-one	0.1	0.1	0.1	<0.1	0.1	<0.1	<0.1
Androsterone	3.1	2.8	1.9	0.9	3.9	0.9	0.0
3β-Hydroxy-5α-androstan-17-one	0.2	0.2	0.2	0.1	0.3	0.1	0.0
5α-Androstane-3α,17β-diol	0.0	0.1	0.5	0.0	0.1	0.0	0.0
5α-Androstane-3β,17β-diol	0.0	0.0	0.1	0.0	0.0	0.0	0.0
5β-Androstane-3,17-dione	0.5	0.3	0.4	0.5	0.5	0.4	<0.1
3α-Hydroxy-5β-androstan-17-one	10.2	4.4	7.8	9.3	7.2	4.3	<0.1
17β-Hydroxy-5β-androstan-3-one	0.4	<0.1	0.2	0.3	0.3	0.1	0.0
5β-Androstane-3α,17β-diol	0.1	0.1	0.8	0.6	0.2	0.1	0.0

Treatments are shown in Table 2. Testicular homogenates (20 mg wet weight) were incubated with [^{14}C]4-androstene-3,17-dione (7.7 nmol/0.4 μCi/tube) and NADPH at 34°C for 30 min in 0.8 ml incubation mixture. Values were obtained after recrystallization to constant specific activity. Percentage formation of 3β-hydroxy-5β-androstan-17-one was <0.2% in all testes. Mean value of two estimations under the same conditions are shown, since the two estimations showed similar values.

tained from New England Nuclear Corporation, Boston, U.S.A. were purified by paper chromatography using the hexane–formamide system [14] just before use. The purified radioactive substrate contained very small amounts (<0.1%) of contaminating radioactive steroids (see Table 1). Non-radioactive steroids were obtained from Steraloids, Inc., Wilton, NH, U.S.A. and Ikapharm, Israel. Other reagents were of analytical grade.

Incubation procedure

Testicular tissues were homogenized, and the homogenates (20–60 mg tissue) were incubated with purified [^{14}C]4-androstene-3,17-dione (7.7 nmol; 0.4 μCi/tube) and NADPH in air at 34°C for 30 min in 0.8 ml incubation mixture, as previously described [2, 15]. The incubation mixture consisted of 0.15 M potassium phosphate buffer (pH 7.4), 0.13 M sucrose, 0.03 M nicotinamide, 1 mM $MgCl_2$, 0.5 mM EDTA and 1.5 mM NADPH.

Estimation of activities of 5β- and 5α-reductases and 17β-hydroxy-dehydrogenase

The method for estimation of the activities of 5β- and 5α-reductases and 17β-hydroxy-dehydrogenase was previously described [2, 15]. The activities were expressed as the sum of all 5β-reduced steroids (five 5β-steroids shown in Table 1), the sum of all 5α-reduced steroids (six 5α-steroids shown in Table 1) and the sum of all 17β-hydroxy-steroids (six 17β-hydroxy-steroids shown in Table 1) formed from 4-androstene-3,17-dione. These assays were quantitative when at least 60% of the substrate remained and the incubation time did not exceed 30 min [3, 15]. Under the assay conditions used, the rate of production of 5β-, 5α- or 17β-hydroxy-products was proportional to the amount of tissue and the incubation time, when a sufficient amount of NADPH was added. The enzyme activities were expressed as nmol of steroids formed per both testes/h. Because the concentration of substrate (7.7 nmol/20–60 mg tissue) was supraphysiological, the assumption was made that endogenous levels of the steroid intermediates can be disregarded when estimating enzyme activities (for example, endogenous testosterone was found to be less than 0.02 nmol/10 mg testicular tissue).

RESULTS

Weight and histological findings of testes and seminal vesicles

The mean body, testis and seminal vesicle weights of 28-day old hamsters used in the present studies are

Table 2. Mean weights of body, testes and seminal vesicles of 28-day old golden hamsters

	Number of animals	Body Mean ± SD (g)	Testes Mean ± SD (mg)	Seminal vesicles Mean ± SD (mg)
Hyp	8	34 ± 7	76 ± 19	6.8 ± 2.1
Hyp + LH 10 μg	5	38 ± 5	74 ± 15	8.2 ± 1.3
Hyp + rFSH 50 μg	3	29 ± 3	154 ± 17**	5.0 ± 1.0
Hyp + oFSH 8 μg	4	33 ± 3	211 ± 32**	7.0 ± 1.4
Hyp + oFSH 16 μg	4	38 ± 5	442 ± 110**	10.3 ± 4.1
Hyp + oFSH 8 μg + LH 5 μg	4	38 ± 4	245 ± 46**	13.3 ± 3.3*
Hyp + oFSH 8 μg + LH 10 μg	4	35 ± 1	230 ± 41**	14.0 ± 2.2**
Hyp + TP 1 mg	4	28 ± 3	171 ± 21**	44.0 ± 3.2**
Intact	9	42 ± 3*	408 ± 94**	18.9 ± 4.9**

Hamsters were hypophysectomized (Hyp) on day 22 after birth. The hypophysectomized hamsters in groups of 3–8 were injected daily with 10 μg NIH-LH-S19 (LH), 50 μg NIAMD-Rat-FSH-B-1 (rFSH), 8 or 16 μg NIAMD-oFSH-13 (oFSH) or 8 μg oFSH + 5 or 10 μg LH dissolved in 0.5 ml saline, 0.5 ml saline or 1 mg testosterone propionate (TP) in 0.1 ml vehicle for 5 days starting from day 23. Intact animals were injected daily with 0.5 ml saline. The hamsters were killed on day 28. Differences from Hyp (P): * < 0.05, ** < 0.01 (t-test).

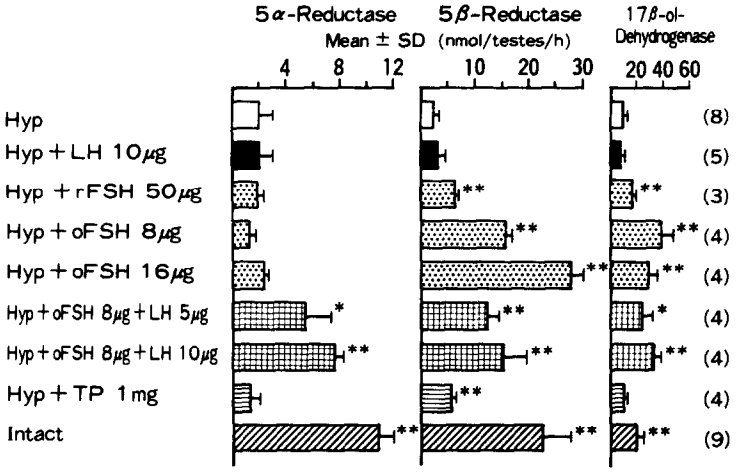

Fig. 1. Effect of treatment with LH, FSH, LH + FSH or androgen on activities of 5α- and 5β-reductases and 17β-hydroxy-dehydrogenase (17β-ol-dehydrogenase) in testes of immature hypophysectomized hamsters. Treatments are shown in Table 2. For estimation of enzyme activities, 20–60 mg homogenates were incubated with [^{14}C]4-androstene-3,17-dione (7.7 nmol; 0.4 μCi/tube) and NADPH at 34°C for 30 min in 0.8 ml incubation mixture. Parentheses: No. of estimations examined. Differences from Hyp (P): * < 0.05, ** < 0.01 (t-test).

shown in Table 2. Hypophysectomy caused a significant decrease in the weights of body, testes and seminal vesicles. The weight of testes increased significantly from the hypophysectomized control by treatment of FSH, FSH plus LH or androgen but not by LH alone. The weight of seminal vesicles increased significantly from the hypophysectomized control by treatment of LH plus FSH or androgen, but treatment by LH alone or FSH alone had no significant effect.

In the intact testes of 28-day old hamsters, germ cells including immature spermatids occupied the major part of the seminiferous tubules. In the testes of hypophysectomized 28-day old hamsters, marked atrophy of the seminiferous tubules including a decrease in number of germ cells and atrophy of interstitial cell clusters were seen. The injection of FSH, FSH plus LH or androgen in hypophysectomized animals produced an increase in size and number of germ cells of the seminiferous tubules but the injection of LH had no significant effects. An increase in size of interstitial cell clusters was induced only by the injection of LH plus FSH.

Hormonal regulation of activities of 5α- and 5β-reductases and 17β-hydroxy-dehydrogenase in immature hamster testis

Effects of treatment with LH, FSH, LH + FSH or androgen on the activities of 5α- and 5β-reductases and 17β-hydroxy-dehydrogenase in the testes of 28-day old hypophysectomized golden hamsters are shown in Fig. 1.

The activities of 5α- and 5β-reductases and 17β-hydroxy-dehydrogenase expressed as nmol/testes/h decreased significantly 6 days after hypophysectomy. A distinct response to rat and ovine FSH in the 5β-reductase activity in the testis of hypophysectomized immature hamster was found. The activity increased from 2.6 nmol/testes/h in the hypophysectomized control to the level of 28 nmol/testes/h, using doses up to 16 μg/day of NIAMD-oFSH-13. In contrast, LH was not effective on the 5β-reductase activity. Similarly, the activity of 17β-hydroxy-dehydrogenase in the testis of hypophysectomized hamster was stimulated by injection of rat and ovine FSH but not by LH injection. Although preparations of FSH may be contaminated by LH (for example, 50 μg NIAMD-Rat-FSH-B-1 may contain LH activity found in 0.2 μg NIH-LH-S19), the present results clearly show that the stimulative effect by FSH preparations on activities of 5β-reductase and 17β-hydroxy-dehydrogenase does not arise by contamination. The injection of either LH or FSH, however, had no significant effects on the 5α-reductase activity in the testis of hypophysectomized immature hamster (Fig. 1).

It was reported in the testis of immature rat [9–13] that 5α-reductase is localized in the interstitial cells and is regulated by LH. No effects of LH injection on the 5α-reductase activity in the testis of hypophysectomized immature hamster seemed to be unexpected, since the 5α-reductase activity in the immature hamster testis was also found to be localized in the interstitial cells [2]. A possibility that ovine LH (10 μg/day of NIH-LH-S19) injected was ineffective in the hamster seemed to be ruled out, because the 5α-reductase activity in the ovary of hypophysectomized immature hamster was stimulated significantly by injection of 2.3 μg/day of NIH-LH-S19 [16]. Since FSH induced responsiveness to LH in the interstitial cells of immature rat testes [17], we examined the effect of treatment with LH plus FSH on testicular 5α-reductase activity in the hypophysectomized immature hamsters. The 5α-reductase ac-

tivity was stimulated evidently by the injection of 8 μg NIAMD-oFSH-13 plus 5 or 10 μg of NIH-LH-S19, though the injection of FSH alone or LH alone failed to increase the 5α-reductase activity. The injection of LH in addition to FSH, however, showed no significant additive effects on the 5β-reductase activity or 17β-hydroxy-dehydrogenase activity in the same animals (Fig. 1). Although no stimulation of the 5α-reductase activity or 17β-hydroxy-dehydrogenase activity was involved by daily injections of a large amount of androgen (1 mg testosterone propionate/day) in the hypophysectomized immature hamster, 5β-reductase activity was stimulated slightly by the injection of androgen (Fig. 1).

Table 1 shows representative metabolic patterns of [^{14}C]4-androstene-3,17-dione used for the estimation of enzyme activities shown in Fig. 1. Androsterone was obtained as the major 5α-product. Testosterone and 3α-hydroxy-5β-androstan-17-one were obtained as the major 17β-hydroxy- and 5β-products, respectively.

DISCUSSION

The present results demonstrate that the 5β-reductase activity is regulated by FSH but not by LH treatment in the testis of immature hamster. Since the 5β-reductase activity has been found to be localized in the tubular nongerm cells [2, 3], the 5β-reductase appears to be confined to the Sertoli cells in the immature hamster testis. Although a large amount of testosterone propionate stimulated slightly the activity of 5β-reductase in the testis of hypophysectomized immature hamster, various doses of rat and ovine FSH, all of which failed to increase the weight of the seminal vesicles (Table 2), stimulated the 5β-reductase activity, suggesting that the effect of FSH on the 5β-reductase activity is not generally mediated by androgen. The 5β-reductase activity in the ovary of hamster was found to be regulated predominantly by FSH [16]. The present results on the regulation of 5β-reductase by FSH are consistent with the previous findings in the hamster ovary [16]. The present and previous [2, 3] results on 5β-reductase demonstrate, for the first time, the regulation (by FSH) and localization (in Sertoli cell) of this enzyme in the testis.

The present findings demonstrate that the 5α-reductase activity, which is localized in the interstitial tissue of the immature hamster testis [2], is enhanced by LH in the presence of FSH. No stimulation of the 5α-reductase activity was involved by androgen treatment in the testis of hypophysectomized immature hamster, showing that the effect of gonadotrophins on the 5α-reductase activity is not mediated by androgen. FSH appeared to induce responsiveness to LH in the interstitial cells of the immature rat testis [17]. The same effect of FSH was also clearly demonstrated by the present findings in the testis of immature hamster. Although relatively slight but significant increases in testosterone secretion [17] and testicular 5α-reductase activity [12, 13] were induced by treatment with LH alone in hypophysectomized immature rats, the injection of LH alone failed to increase the seminal vesicle weight and testicular 5α-reductase activity in hypophysectomized immature hamsters, suggesting that the induction by FSH of responsiveness to LH is more evident in immature hamster testes than in immature rat testes. However, the 5α-reductase activity in the ovary of immature hamster has been found to be stimulated markedly by treatment with LH alone [16]. The present results on the induction and regulation of 5α-reductase in the testis of immature hamster extend previous observations by others [12, 13, 17] on those in the immature rat testis.

The activity of 17β-hydroxy-dehydrogenase was stimulated by FSH but not by LH treatment in the testis of immature hamster. No stimulation of the 17β-hydroxy-dehydrogenase was involved by daily injections of androgen, showing that the effect by FSH was not mediated by androgen. The specific activity (nmol/mg protein/h) of 17β-hydroxy-dehydrogenase was almost equal in the seminiferous tubules and the interstitial tissue of immature hamster testis [2, 3], indicating that the total 17β-hydroxy-dehydrogenase activity (nmol/testes/h) is much greater in the tubules than in the interstitial tissue. These previous findings are not inconsistent with the present findings that the injection of LH in addition to FSH showed no significant additive effect on the total activity of 17β-hydroxy-dehydrogenase in the testis of hypophysectomized immature hamster. It is possible to speculate that additive effect induced by stimulative effect of LH on the interstitial tissue may be too small to be demonstrated by the present method used. The present and previous findings [2, 3] seem to indicate that the 17β-hydroxy-dehydrogenase activity, which is present predominantly in the Sertoli cells of hamster testis is regulated by FSH but not by LH or androgen. The 17β-hydroxy-dehydrogenase activity in the ovary of golden hamster was found to be stimulated by FSH but not by LH or estrogen [16]. The previous findings in the ovary do not conflict with the present findings on hormonal regulation of 17β-hydroxy-dehydrogenase.

In the testis of immature golden hamster, 5β-reduction of testosterone and 4-androstene-3,17-dione secreted from the interstitial cells takes place in the Sertoli cells under the influence of FSH, while 5α-reduction occurs in the interstitial cells under the influence of LH and FSH.

Acknowledgements—This work was supported in part by Grant from the Japanese Ministry of Education, Science and Culture. The authors are grateful to the National Institute of Arthritis, Metabolism and Digestive Disease, U.S.A. for their generous supply of NIH-LH-S19, NIAMD-Rat-FSH-B-1 and NIAMD-oFSH-13.

REFERENCES

1. Terada N., Sato B. and Matsumoto K.: Formation of 5β- and 5α-products as major C_{19}-steroids from progesterone *in vitro* in immature golden hamster testes. *Endocrinology* **106** (1980) 1554–1561.
2. Furubayashi Y., Terada N., Sato B. and Matsumoto K.: Localization of Δ^4-5β- and 5α-reductases and 17β-ol-dehydrogenase in immature golden hamster testes. *Endocrinology* **111** (1982) 269–272.
3. Yabumoto H., Ikoma F., Terada N., Takeyama M., Tsuji M. and Matsumoto K.: Intratubular localization of 4-ene-5β-reductase and 17β-hydroxy-dehydrogenase in immature golden hamster testis. *J. steroid Biochem.* **19** (1983) 1623–1626.
4. Ficher M. and Steinberger E.: *In vitro* progesterone metabolism by rat testicular tissue at different stages of development. *Acta endocr., Copenh.* **68** (1971) 285–292.
5. Coffey J. C., French F. S. and Nayfeh S. N.: Metabolism of progesterone by rat testicular homogenates. IV. Further studies of testosterone formation in immature testis *in vitro*. *Endocrinology* **89** (1971) 865–872.
6. Inano H., Hori Y. and Tamaoki B.: Effect of age on testicular enzymes related to steroid bioconversion. *Colloq. Endocr.* **16** (1967) 105–119.
7. Moger W. H.: Serum 5α-androstane-3α,17β-diol, androsterone, and testosterone concentrations in the male rat. Influence of age and gonadotropin stimulation. *Endocrinology* **100** (1977) 1027–1032.
8. Tsujimura T. and Matsumoto K.: Progesterone metabolism *in vitro* by mouse testes at different stages of development. *Endocrinology* **94** (1974) 288–290.
9. Folman Y., Ahmad N., Sowell J. G. and Eik-Nes K. B.: Formation *in vitro* of 5α-dihydrotestosterone and other 5α-reduced metabolites of [^{3}H]testosterone by the seminiferous tubules and interstitial tissue from immature and mature rat testes. *Endocrinology* **92** (1973) 41–47.
10. Wiebe J. P.: Steroidogenesis in rat Leydig cells: Changes in activity of 5-ane- and 5-ene-3β-hydroxysteroid dehydrogenases during sexual maturation. *Endocrinology* **98** (1976) 505–513.
11. Yoshizaki K., Matsumoto K. and Samuels L. T.: Localization of Δ^4-5α-reductase in immature rat testes. *Endocrinology* **102** (1978) 918–925.
12. Nayfeh S. N., Coffey J. C., Hansson V. and French F. S.: Maturational changes in testicular steroidogenesis: Hormonal regulation of 5α-reductase. *J. steroid Biochem.* **6** (1975) 329–335.
13. Nayfeh S. N., Coffey J. C., Kotite N. J. and French F. S.: Gonadotrophin regulation of 5α-reductase activity in the immature rat testis. *Res. Reprod.* **8** (1976) 4.
14. Zaffaroni A. and Burton R. B.: Identification of corticosteroids of beef adrenal extract by paper chromatography. *J. biol. Chem.* **193** (1951) 749–767.
15. Tsuji M., Terada N., Sato B. and Matsumoto K.: 5β- and 5α-reductases for 4-ene-3-ketosteroids in golden hamster ovaries at different stages of development. *J. steroid Biochem.* **16** (1982) 207–213.
16. Tsuji M., Terada N., Yabumoto H., Takeyama M. and Matsumoto K.: Hormonal regulation of activities of 4-ene-5β- and 5α-reductases and 17β-ol-dehydrogenase in immature golden hamster ovary. *J. steroid Biochem.* **18** (1983) 777–781.
17. Odell W. D. and Swerdloff R. S.: The role of testicular sensitivity to gonadotropins in sexual maturation in the male rat. *J. steroid Biochem.* **6** (1975) 853–857.