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HORMONAL REGULATION OF 4-ENE-5α-REDUCTASE ACTIVITY IN PREPUBERTAL RAT OVARIES

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SUMMARY

Female rats were hypophysectomized at 21 days of age, and after a lapse of 3 days the hypophysectomized rats in groups of 4–13 were injected daily with NIH-LH-S19, NIAMD-Rat-FSH-B-I, 5α-androstane-3α,17β-diol, testosterone, oestradiol-17β, saline or sesame oil for 3 days. Ovarian homogenates from these rats at 27 days of age were incubated with [^{14}C]-testosterone and 5α-reductase activities were estimated.

The 5α-reductase activity (nmol/g tissue/h or nmol/both ovaries/h) decreased significantly 6 days following hypophysectomy. A distinct response to LH in 5α-reductase activity of the hypophysectomized rat ovaries was found, with a dose-response in activity from 260 ± 94 (S.D.) in the hypophysectomized control to the maximum level of 1960 ± 177 (S.D.) nmol/g/h, using doses from 3 to 90 μg per day. In contrast, FSH was not effective, for it had little effect on 5α-reductase activity even when 10 or 50 μg was given each day. No stimulation of 5α-reductase activity by injection of androgens and oestradiol-17β in large doses was involved, showing that the effect of LH is not mediated by sex steroids. These results show that 5α-reductase activity in prepubertal rat ovaries is regulated by LH.

INTRODUCTION

Previous studies *in vitro* demonstrated that the ovary of prepubertal rat contains high 5α-reductase activity [1] and that ovarian homogenates from prepubertal rats form large amounts of 5α-reduced C_{19}-steroids from pregnenolone and progesterone while adult rat ovaries are unable to form a significant amount of 5α-reduced C_{19}-steroids [2, 3]. Recently, we [3] and Lerner and Eckstein[4] found that progesterone is converted to these 5α-reduced C_{19}-steroids primarily by a pathway through 3α,17α-dihydroxy-5α-pregnan-20-one in prepubertal rat ovaries *in vitro*. Furthermore, high levels of 5α-androstane-3α,17β-diol and its 3β epimer (100–200 ng/ml) were found in the peripheral circulation of prepubertal female rats but they disappeared after the onset of puberty [5, 6]. Since these 5α-reduced C_{19}-steroids which are of no use for estrogen biosynthesis, have been shown to exert a negative feedback on gonadotrophin release [7, 8] and induce precocious ovulation in rats [5, 6, 8, 9], the formation of 5α-reduced C_{19}-steroids by 5α-reductase in prepubertal rat ovary seem to have a biological significance in immature female rats. However, the regulation of 5α-reductase activity in prepubertal rat ovary has not been clarified. In the present paper, we show that this is regulated by LH.

The following trivial names are used in this paper: androstenedione = 4-androstene-3,17-dione; 5α-dihydrotestosterone = 17β-hydroxy-5α-androstan-3-one; epiandrosterone = 3β-hydroxy-5α-androstan-17-one; pregnenolone = 3β-hydroxy-5-pregnen-20-one.

MATERIALS AND METHODS

Animals. Prepubertal female rats of the Sprague–Dawley Strain were used. Rats were hypophysectomized at 21 days of age, and treatment was started 3 days later. Rats in groups of 4–13 were injected daily with 1–90 μg of NIH-LH-S19 dissolved in 0.5 ml of saline, 10 or 50 μg of NIAMD-Rat-FSH-B-I in 0.5 ml of saline, 0.5 ml of saline, 1 mg of 5α-androstane-3α,17β-diol in 0.2 ml of sesame oil, 1 mg of testosterone in 0.2 ml of sesame oil, 20 μg of oestradiol-17β in 0.2 ml of sesame oil or 0.2 ml of sesame oil for 3 days. Intact rats were treated daily with 0.5 ml of saline for 3 days, starting from 24 days after birth. The rats were killed at 27 days of age and complete removal of the hypophyses was confirmed in the hypophysectomized rats. The onset of puberty in the Sprague–Dawley rats is at 35–38 days of age.

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

Chemicals. [4-^{14}C]-Testosterone (3.6 nmol/0.2 μCi), obtained from Daiichi Pure Chemical Co., Ltd., Japan was purified by paper chromatography using the hexane–benzene (1:1, v/v)-formamide system [10] just before use. The purified radioactive substrate behaved as a single compound on paper [10] and column [11] chromatography and no, or very little, metabolic contamination corresponding to any of the compounds tested was observed in control vessels. Non-radioactive steroids were obtained from Steraloids, Inc., Wilton, N.H., and Ikapharm, Ramat-Gan, Israel. Other reagents were of analytical grade.

Estimation of 5α-reductase activity. Both ovaries from each rat were weighed and were homogenized in 0.25 M sucrose containing 1 mM EDTA. The purified substrate, [^{14}C]-testosterone (3.6 nmol:0.2 μCi per tube) was introduced into 2 × 10 cm tubes and dissolved in 0.02 ml ethanol. To each tube, 0.5 ml of buffer–cofactor solution was added. The buffer–cofactor solution consisted of 0.3 M potassium phosphate buffer, pH 7.4, 0.06 M nicotinamide, 2 mM $MgCl_2$ and 0.8 mg of NADPH per 0.5 ml. One-half ml of the tissue homogenate containing 1.2, 2.5 or 5.0 mg of tissue was then introduced to make the total vol. of the incubation mixture 1 ml. The samples were incubated in a shaking water bath in air at 37°C for 30 min. At the end of incubation, the mixtures were immediately acidified with 0.1 ml 1 N HCl and mixed with ether–chloroform (4:1, v/v) to stop the reaction.

To the incubation mixtures, 10–50 μg quantities of androstenedione, testosterone, 5α-androstane-3,17-dione, androsterone, epiandrosterone, 5α-dihydrotestosterone, 5α-androstane-3α,17β-diol and 5α-androstane-3β,17β-diol were added. The extraction and analysis of the 8 C_{19}-steroids by paper [10] and elution [11] chromatography, with acetylation of steroids were the same as previously described [12]. 5α-Reductase activity was expressed as the sum of all 5α-reduced C_{19}-steroids formed from testosterone. The enzyme activity was estimated as nmol 5α-reduced steroids formed per g tissue or both ovaries per h. In some estimations, radioactive 5α-products were recrystallized with 15 mg of nonradioactive steroids to constant S.A. in order to identify the steroids formed. The [^{14}C]-testosterone was used in appropriate amounts to ensure a linear relationship under various conditions between the amount of enzyme and the product formed in the incubation time used. Cofactor was added at saturating level.

Estimation of testosterone and progesterone in ovaries. In the testosterone radioimmunoassay, the antibody, raised against testosterone-3-carboxymethyloxime-BSA, was supplied by Dr. H. Imura and the radioactive steroid was [1,2-^{3}H]-testosterone (Daiichi Pure Chemical), 40 Ci/mmol. This antibody cross-reacts with 5α-reduced androstans-5α-dihydrotestosterone (65%), 5α-androstane-3α,17β-diol (17%) and 5α-androstane-3β,17β-diol (7%), but not with oestradiol-17β, progesterone, cortisol or corticosterone. Since ovarian testosterone was extracted with distilled methylene chloride and measured by the radioimmunoassay without chromatographic purification, concentrations of testosterone shown in Tables 2 and 3 should include some of 5α-reduced C_{19}-steroids. The intra- and interassay coefficient of variation in male serum range obtained from 10 assays were found to be 8.2 and 9.9%, respectively.

The competitive protein binding assay of progesterone was carried out utilizing the pregnant guinea pig serum described by Milgrom *et al.*[13] and [1,2-^{3}H]-progesterone (Daiichi Pure Chemical), 45 Ci/mmol. Ovarian progesterone was extracted with distilled petroleum ether. The intra- and interassay coefficient of variation in nonpregnant female serum range obtained from 8 assays were 6.1 and 8.3%, respectively.

RESULTS

1. *Ovaries and uterus*

The weights (mean ± S.D.) of body, both ovaries

Table 1. Mean weight of body, ovaries and uterus of 27-day-old rats

	Number of rats	Body Mean ± S.D. (g)	Ovaries Mean ± S.D. (mg)	Uterus Mean ± S.D. (mg)
Experiment 1				
Intact	13	97.3 ± 11.5**	29.2 ± 6.7**	105.5 ± 37.1**
Hypox control	13	71.2 ± 13.2	13.9 ± 4.2	33.6 ± 9.4
LH 1 μg	9	63.3 ± 4.9	15.0 ± 2.3	37.2 ± 11.6
LH 3 μg	4	65.3 ± 10.2	10.8 ± 1.1	29.5 ± 6.7
LH 10 μg	12	67.6 ± 10.8	14.6 ± 3.8	36.9 ± 8.2
LH 30 μg	4	69.8 ± 7.1	13.0 ± 0.7	35.8 ± 4.6
LH 90 μg	4	72.8 ± 6.8	12.8 ± 1.8	39.5 ± 3.0
FSH 10 μg	9	71.3 ± 7.5	17.2 ± 1.5*	37.0 ± 13.0
FSH 50 μg	8	78.0 ± 8.6	34.8 ± 8.2**	76.6 ± 36.7**
Experiment 2				
Hypox control	5	65.8 ± 8.6	10.8 ± 2.3	43.0 ± 10.3
Androstane-3α-17β-diol	5	69.8 ± 8.0	10.2 ± 1.0	111.8 ± 30.7**
Testosterone	5	62.8 ± 9.6	9.6 ± 1.7	40.0 ± 10.2
Oestradiol-17β	4	64.8 ± 12.9	12.3 ± 1.5	151.0 ± 24.9**
LH 10 μg	4	57.8 ± 13.7	9.5 ± 1.5	23.8 ± 3.8*

Rats were hypophysectomized at 21 days of age, and treatment was started 3 days later. Rats in groups of 4–13 in experiment 1 were injected daily with 1–90 μg of NIH-LH-S19, 10 or 50 μg of NIAMD-Rat-FSH-B-1 or 0.5 ml of saline for 3 days. Intact rats were treated with saline for 3 days. Rats in groups of 4 or 5 in experiment 2 were injected daily with 1 mg of 5α-androstane-3α,17β-diol (androstane-3α,17β-diol), 1 mg of testosterone, 20 μg of oestradiol-17β, 10 μg of NIH-LH-S19 or 0.2 ml of sesame oil for 3 days. The rats were killed at 27 days of age.

Differences from Hypox (hypophysectomized) control (P): * < 0.05, ** < 0.01 (A t-test was used).

Table 2. Effect of LH and FSH treatment on 5α-reductase activity in ovaries of prepubertal hypophysectomized rats

		5α-Reductase: Mean ± S.D.			Testosterone	Progesterone
		Estimations	(nmol/g/h)	(nmol/both ovaries/h)	Estimations	Mean ± S.D. (ng/10 mg ovaries)
Intact		10	421 ± 209*	11.3 ± 5.4**	5	0.4 ± 0.2	4.5 ± 3.3
Hypox	control	10	256 ± 94	3.2 ± 1.4	5	0.2 ± 0.2	1.6 ± 1.6
LH	1 μg	6	281 ± 59	4.0 ± 0.8	5	0.2 ± 0.2	1.6 ± 1.6
LH	3 μg	4	590 ± 36**	6.3 ± 0.3**	1	0.5	1.5
LH	10 μg	9	777 ± 209**	10.1 ± 1.9**	5	0.4 ± 0.2	7.3 ± 11.6
LH	30 μg	4	1860 ± 156**	24.2 ± 2.5**	1	0.3	7.5
LH	90 μg	4	1960 ± 177**	25.2 ± 5.2**	1	0.5	5.0
FSH	10 μg	6	261 ± 95	4.4 ± 1.6	5	0.3 ± 0.3	2.3 ± 1.4
FSH	50 μg	5	104 ± 32**	3.6 ± 0.7	4	0.4 ± 0.1	3.5 ± 3.7

Rats were hypophysectomized at 21 days of age, and treatment was started 3 days later. Hormonal treatments are described under Table 1. In each estimation of 5α-reductase activity, both ovaries were homogenized and 1.2–5 mg of homogenates were incubated with [^{14}C]-testosterone (3.6 nmol: 0.2 μCi per tube) and NADPH at 37°C for 30 min in 1 ml. Concentrations of testosterone and progesterone were estimated in each homogenate or combination of homogenates.

Differences from Hypox control (P): * < 0.05, ** < 0.01 (A t-test was used).

A portion of rats in some groups shown in Table 1 was not used for the estimation of 5α-reductase activity.

and uterus of 27-day-old rats used in the present incubation studies are shown in Table 1. Hypophysectomy at 21 days of age, caused a significant decrease in the weight of body, ovaries and uterus. In the hypophysectomized rats, LH, FSH, androgens or oestradiol-17β were injected for 3 days, starting from 24 days after birth. No significant increase in body weight was found in any of the hypophysectomized rats treated with LH, FSH or sex steroids. The weight of ovaries increased significantly from the hypophysectomized control by injection of FSH but not by LH, androgens or oestradiol-17β. The weight of uterus increased significantly from the hypophysectomized control by injection of FSH, 5α-androstane-3α,17β-diol or oestradiol-17β but not by LH or testosterone. This increase in the weight of the uterus by 5α-androstane-3α,17β-diol was smaller than that produced by a much smaller amount (1/50) of oestradiol-17β.

In the ovaries of intact 27-day-old rats, primary and developing follicles and the interfollicular interstitial cell islands occupied most parts of the ovaries, while corpora lutea were not formed. In the ovaries of hypophysectomized 27-day-old rats, atrophic changes of the follicles and interstitial cells were seen. In the ovaries of hypophysectomized 27-day-old rats treated with hormones, an increase in size of interstitial cell islands in LH-injected rats which was found to be a dose dependent change, stimulation of follicular growth in FSH-injected rats and no significant changes in steroid-injected rats were found. Histological examination and estimation of the ovarian concentration of progesterone (Tables 2 and 3) showed that corpora lutea were not formed in any ovaries treated with LH, FSH or sex steroids. However, there appeared to be some rise in progesterone concentration with LH, which reached the level of the intact controls.

2. *Effect of hormones on 5α-reductase activity in ovaries of prepubertal hypophysectomized rats*

Activities of testosterone 5α-reductase (nmol/g tissue/h or nmol/both ovaries/h) in ovaries of intact, hypophysectomized and hormone-injected hypophy-

Table 3. Effect of sex steroids and LH on 5α-reductase activity in ovaries of prepubertal hypophysectomized rats

	Estimations	5α-Reductase	Testosterone	Progesterone
		Mean ± S.D. (nmol/g/h)	Mean (ng/10 mg ovaries)	
Hypox control	4	264 ± 95	0.3	2.1
Androstane-3α,17β-diol	4	356 ± 13	0.5	2.0
Testosterone	4	170 ± 16	1.2	6.9
Oestradiol-17β	4	336 ± 50	0.3	3.0
LH 10 μg	4	1110 ± 169**	0.5	9.0

Treatment is described under Table 1. In each estimation, both ovaries were homogenized and 2.5–5 mg of homogenates were incubated with [^{14}C]-testosterone (3.6 nmol: 0.2 μCi per tube) and NADPH at 37°C for 30 min in 1 ml. Concentrations of testosterone and progesterone are the mean of 2 estimations.

Differences from Hypox control (P): ** < 0.01 (A t-test was used).

sectomized rats at 27 days of age are shown in Tables 2 and 3. When [^{14}C]-testosterone was incubated with ovarian homogenates, radioactive 5α-androstane-3α,17β-diol, 5α-androstane-3β,17β-diol and androsterone were obtained as the main products. Relative amounts of these products and other products were 10, 3–5, 1–2 and less than 1, respectively which were not altered significantly by various treatment tested.

5α-Reductase activity decreased significantly 6 days following hypophysectomy (Table 2). This prompted an investigation of the role of gonadotrophins in the regulation of 5α-reductase in prepubertal rat ovaries. Hypophysectomized rats in groups of 4–13 were injected daily with various hormones for 3 days starting from 24 days after birth, and 5α-reductase activity in the ovaries was measured 20 h after the end of the treatment period. A distinct response to LH in 5α-reductase activity in the hypophysectomized rat ovaries was found. The activity increased from 260 in the hypophysectomized control to the maximum level of 1900 nmol/g/h, using doses of up to 30 and 90 μg/day. In contrast, FSH was not effective, for it failed to stimulate 5α-reductase activity even when 10 or 50 μg was given each day (Table 2). Although preparation of LH may be contaminated (less than 2%) by FSH, the present results clearly show that this stimulative effect by LH preparation does not arise by FSH contamination. No stimulation of 5α-reductase activity was involved by daily injection of 5α-androstane-3α,17β-diol, testosterone or oestradiol-17β in large doses, showing that the effect of LH is not mediated by sex steroids (Table 3). The concentration of endogenous testosterone in ovaries was less than 0.01 nmol per 10 mg tissue even in the ovaries from testosterone-injected rats. Since 3.6 nmol of [^{14}-C]-testosterone was added to 1.2–5 mg ovarian homogenates, the effect of dilution by endogenous androgens on the estimation of 5α-reductase activity seems to be insignificant.

DISCUSSION

The present results shown in Tables 2 and 3 demonstrate for the first time that 5α-reductase activity in the prepubertal rat ovary is regulated by LH but not by FSH or sex steroids. This LH stimulation of ovarian 5α-reductase is in contrast to the androgen-dependent 5α-reductase in androgen target tissues, such as prostate and epididymis[14]. Amounts of FSH, androgens and oestradiol-17β used are shown to be biologically effective doses, since the weight of ovaries and/or uterus increased significantly following the injection (Table 1). In the ovaries of hypophysectomized prepubertal rats, a gradual increase in size of the interstitial cell islands was found following LH injection in doses from 1 to 90 μg per day while the administration of FSH produced marked follicular growth. Since corpora lutea were not formed in these ovaries, no changes of ovarian components were found in any ovaries treated with various amounts of LH or FSH. From these observations, there seems to be a possibility that in the prepubertal rat ovary, 5α-reductase is largely localized in the interstitial cells.

Previous studies have demonstrated that prepubertal rat testes synthesize a significant quantity of 5α-reduced C_{19}-steroids while adult rat testes which secrete testosterone as a major C_{19}-steroid, form little or no 5α-reduced C_{19}-steroids [15–18]. We [19, 20] found that progesterone is converted to these 5α-reduced C_{19}-steroids primarily by a pathway through 5α-reduced C_{21}-steroids in prepubertal rat testes. Hormonal regulation of 5α-reductase activity in the prepubertal rat testis was investigated by Nayfeh *et al.*[21, 22] who found that this activity is regulated by LH. It is suggested that efficient formation of 5α-reduced C_{19}-steroids and the presence of high 5α-reductase activity in the gonads which are shown to be regulated by LH (Tables 2 and 3 and [21, 22]) may be of biological significance in prepubertal male and female rats.

Odell and Swerdloff[23] reported the results of administering various doses of LH to 21-day-old female rats beginning 5 days after hypophysectomy. LH administration in very high doses of 400 μg/100 g body weight per day was ineffective in inducing an increase in uterus weight in immature animals. FSH given to the prepubertal female rats 5 days after hypophysectomy was effective in stimulating an increase in gonadal and uterus weight. These observations are in agreement with the present results shown in Table 1. It seems that the nonresponse of the uterus of prepubertal rats to the effect of LH administered after hypophysectomy may be explained in part by the stimulative effect of LH on 5α-reductase activity in the ovaries. The high 5α-reductase activity stimulated by LH administration may serve to decrease the formation of estrogens in these prepubertal rat ovaries.

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