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HORMONAL REGULATION OF ACTIVITIES OF 17β-OL-DEHYDROGENASES, AROMATASE AND 4-ENE-5α-REDUCTASE IN IMMATURE RAT OVARIES

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SUMMARY

Female rats were hypophysectomized at 21 days of age, and after 3 days, the hypophysectomized rats in groups of 3–20 were injected daily with 10 μg of NIH-LH-S19, 10–100 μg of NIAMD-Rat-FSH-B-1, 20 μg of oestradiol-17β or saline for 3 days. Ovarian homogenates from these rats and intact rats at 27 days of age were incubated with [^{14}C]-4-androstene-3,17-dione, [^{14}C]-oestrone, [7-^{3}H]-4-androstene-3,17-dione or [7-^{3}H]-progesterone and enzyme activities and metabolism of progesterone were estimated. The activities of 5α-reductase, testosterone and oestradiol 17β-ol-dehydrogenases and aromatase decreased significantly 6 days following hypophysectomy. A distinct response to LH but not to FSH in the 5α-reductase activity in the hypophysectomized rat ovary was found. On the other hand, the activities of 17β-ol-dehydrogenases and aromatase in the hypophysectomized rat ovary were stimulated (10 to 200 times) by FSH but not by LH. No stimulation of these enzyme activities by oestradiol-17β was involved. The formation of oestradiol-17β from progesterone could be demonstrated only in the FSH-injected rat ovary.

These results show that the 5α-reductase activity is regulated by LH and the activities of 17β-ol-dehydrogenases and aromatase are regulated by FSH in immature rat ovaries. It is also suggested that nonresponse to LH and response to FSH of the uterus of hypophysectomized immature rats can be explained in part by the present results.

INTRODUCTION

Odell and Swerdloff[1] reported the results of administering various doses of LH and FSH to immature female rats beginning 5 days after hypophysectomy. LH administration in very high doses was ineffective but FSH was effective in inducing an increase in uterus weight in the immature animals. On the contrary, LH administration was effective in inducing an increase in uterus weight in hypophysectomized adult rats [1]. However, these studies failed to explain the insensitivity to LH and sensitivity to FSH in the immature hypophysectomized rats. On one hand, previous studies by us and other investigators have demonstrated that immature rat ovaries form large amounts of 5α-reduced C_{19}-steroids primarily by a pathway through 5α-reduced C_{21}-steroids while adult rat ovaries are unable to form a significant amount of 5α-reduced C_{19}-steroids [2–7]. Recently, we found that the 5α-reductase activity in the immature rat ovary is regulated by LH but not by FSH [8].

Recently, the two cell-two gonadotropin theory for follicular oestradiol production has been fairly established [9–15]. The theca cells under the influence of LH produce androgen, which is aromatized by granulosa cells to oestradiol under the influence of FSH [9–15]. It seems that the non-response to LH in the hypophysectomized immature rats can be explained in part by the stimulative effect of LH on 5α-reductase activity in the ovary [8], since 5α-C_{19}-steroids accumulated can not be aromatized by granulosa cells to oestrogen. In order to extend our studies on the immature rat ovary, effects of FSH and LH treatment on the activities of ovarian 17β-ol-dehydrogenases and aromatase, which are involved in a final step of oestradiol-17β synthesis, are investigated in hypophysectomized immature rats.

MATERIALS AND METHODS

Animals. 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 3–20 were injected daily with 10 μg of NIH-LH-S19, 10–100 μg of NIAMD-Rat-FSH-B-I in 0.5 ml of saline, 20 μg of oestradiol-17β in 0.2 ml of sesame oil or 0.5 ml of saline 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 20 h after the end of the treatment period (at 27 days of age) and ovarian enzyme activities were measured. The complete removal of hypophyses was confirmed. The onset of puberty in the Sprague–Dawley rats is at 35–38 days of age.

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

Chemicals. [4-^{14}C]-4-androstene-3,17-dione (5.2 nmol/0.3 μCi) and [4-^{14}C]-oestrone (5.2 nmol/0.3 μCi), obtained from the New England Nuclear Corporation and [7-^{3}H]-4-androstene-3,17-dione (0.7 nmol/10 μCi) and [7-^{3}H]-progesterone (1.3 nmol/10 μCi), obtained from the Radiochemical Centre, Amersham were purified by paper chromatography using the hexane-formamide or banzene-formamide system [16] just before use. The purified radioactive substrates behaved as a single compound on paper [16] and column [17] 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 Sterar-oids, Inc., U.S.A. and Ikapharm, Israel. Other re-agents were of analytical grade.

Incubation procedure. Ovaries were weighed and were homogenized in cooled 0.25 M sucrose contain-ing 1 mM EDTA with a Teflon pestle by hand. The purified substrates, [^{14}C]-4-androstene-3,17-dione, [^{14}C]-oestrone, [7-^{3}H]-4-androstene-3,17-dione or [7-^{3}H]-progesterone were introduced into 1.3 × 10 cm tubes, dissolved in 0.01 or 0.02 ml ethanol. To each tube, 0.2 or 0.5 ml 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 1 mg NADPH per 0.5 ml. Homogenate (0.2 or 0.5 ml) containing 1.2–40 mg tissue was then introduced to make the total volume of the incubation mixture 0.4 or 1 ml. The final substrate concentrations of [^{14}C]-4-andros-tene-3,17-dione, [^{14}C]-oestrone, [7-^{3}H]-4-andros-tene-3,17-dione and [7-^{3}H]-progesterone used for the greater part of incubations were 13 μM, 13 μM, 0.7 μM and 1.3 μM, respectively. The samples were incubated in a shaking water bath in air at 37°C for 30 or 60 min. After the end of incubation, the mix-tures were immediately acidified with 0.1 ml 1 N HCl and mixed with 2 ml of ether-chloroform (4:1, V/V) to stop the reaction.

Estimation of enzyme activities. For the estimation of 5α-reductase and testosterone 17β-ol-dehydro-genase activities, 0.4 ml incubation mixture with [^{14}C]-4-androstene-3,17-dione was extracted three times with 10 ml of ether–chloroform (4:1, V/V). To the extract, 5–50 μg quantities of the 7 C_{19}-steroids shown in Table 6 were added as nonradioactive car-riers. The analysis of the 7 C_{19}-steroids by paper [16] and elution [17] chromatography, with acetylation of steroids was the same as previously described [18].

For the estimation of oestradiol 17β-ol-dehydro-genase activity, 0.4 ml incubation mixture with [^{14}C]-oestrone was extracted three times with 10 ml of ether-chloroform (4:1, V/V). The extract was separ-ated into oestrone and oestradiol-17β by paper chromatography in the benzene-formamide system.

For the estimation of aromatase activity, a reduced amount of 4-androstene-3,17-dione with high specific activity (0.7 nmol:10 μCi per tube) was employed and the amount of tissue was increased (20–40 mg per tube), since the yield of oestrogen formation was limited. In this particular experiment, 30 nmol of non-radioactive progesterone dissolved in 0.02 ml ethanol was added to each incubation mixture to protect the substrate 4-ene-3-keto-C_{19}-steroid from consumption by 5α-reductase [19]. One ml incubation mixture with [7-^{3}H]-4-androstene-3,17-dione was extracted and the extract was separated into the phenolic and neutral fractions. The phenolic fraction was separated into oestrone and oestradiol-17β as described above. In parallel incubations with [7-^{3}H]-4-androstene-3,17-dione, 0.01 μCi of [^{14}C]-oestrone was added to each tube after incubations. Recoveries of extraction and extraction plus separation procedures (isolation of phenolic fraction and paper chromatography) calcu-lated by [^{14}C] in 4 parallel incubations were 89–96% and 67–77%, respectively. For the estimation of oes-trone and oestradiol-17β formation, corrections using mean values of the four values were made for pro-cedural losses. The neutral fraction added with the seven nonradioactive C_{19}-steroids shown in Table 6 was separated into the 7 C_{19}-steroids as described above. Under these incubation conditions, more than 50% of substrate radioactivity was recovered in 4-androstene-3,17-dione and testosterone fractions for all incubations.

The enzyme activities were expressed as follows: the activities of 5α-reductase and testosterone 17β-ol-dehydrogenase were expressed as the sum of all 5α-reduced C_{19}-steroids and the sum of all 17β-hydroxy C_{19}-steroids formed from 4-andros-tene-3,17-dione, respectively. The oestradiol 17β-ol-dehydrogenase activity was expressed as oestra-diol-17β formed from oestrone, inasmuch as no sig-nificant 17β-hydroxysteroids other than oestra-diol-17β were formed under the incubation conditions used. The data in Tables 1 and 2 demonstrates that these assays meet quantitative criteria when at least 50% of substrates remained and the incubation time did not exceed 60 min. The enzyme activities were expressed as nmol of steroids formed/g wet weight/h or /both ovaries/h. Because concentrations of sub-strates (5.2 nmol/1.2–5 mg tissue) used were much higher than the physiological levels, the assumption was made that endogenous levels of the steroid inter-mediates can be neglected for the estimation of enzyme activities (concentrations of endogenous testosterone estimated by the radioimmunoassay in these immature rat ovaries were less than 0.003 nmol/10 mg tissue). The aromatase activity was expressed as oestrone plus oestradiol-17β formed from 4-androstene-3,17-dione. The [7-^{3}H]-4-andros-tene-3,17-dione was used in appropriate amount to ensure a linear relationship between the amount of tissue and the product formed in the incubation time used. For example, total oestrogens formed per 10, 20, 40 and 80 mg ovaries from FSH-injected rats per h were 0.56, 1.4, 2.2 and 4.0 pmol, respectively. The enzyme activity was expressed as pmol of oestrogens formed/g wet weight/h or /both ovaries/h. In some

Table 1. Total 17β-hydroxy and 5α-reduced C_{19}-steroids formed from 4-androstene-3,17-dione by varying amounts of ovarian tissues from immature rats and NADPH at different incubation times

Amount of tissue (mg)	Incubation time (min)	NADPH (mg/0.4 ml)	17β-OH-products formed		5α-Products formed	
			nmol	nmol/g*/h	nmol	nmol/g*/h
1.25	30	0.4	0.05	80	0.40	640
2.5	30	0.4	0.12	96	0.80	640
5.0	30	0.4	0.25	100	1.39	556
2.5	15	0.4	0.06	96	0.40	640
2.5	30	0.4	0.11	88	0.70	560
2.5	60	0.4	0.23	92	1.39	556
2.5	30	0.1	0.10	80	0.77	616
2.5	30	0.0	0.00	0	0.01	8

Ovarian homogenates from 27-day-old rats were incubated with [^{14}C]-4-androstene-3,17-dione (5.2 nmol: 0.3 μCi per tube) at 37°C in 0.4 ml.
* g wet weight.

estimations including all estimations for aromatase, radioactive products were recrystallized with 15 mg nonradioactive steroids to constant specific activity in order to identify the steroids formed. The decreases of specific radioactivity of each steroid on 4 repeated recrystallizations were 30% to 100% (no formation of estradiol-17β in some incubations as shown in Table 5) in estimations of the aromatase activity but were generally less than 15% in estimations of the activities of 5α-reductase and 17β-ol-dehydrogenases.

Metabolism of progesterone. One ml incubation mixture with [7-^{3}H]-progesterone (1.3 nmol/5 or 15 mg) was extracted and separated into the neutral and phenolic fractions. To the fractions, 5–50 μg quantities of the 18 steroids shown in Table 6 were added as nonradioactive carriers. The analyses of the neutral and phenolic fractions into these 18 steroids by paper [16] and elution [17] chromatography with acetylation of steroids, identification or tentative identification of metabolites by recrystallizations, and calculation of metabolites found in each steroid fraction were the same as previously described [20, 21]. Since the concentrations of endogenous progesterone estimated by the competitive protein binding assay in these immature ovaries were less than 0.03 nmol/10 mg tissue, the effect of dilution by endogenous steroids on the metabolism of radioactive progesterone seems to be limited.

Statistics. Students unpaired *t*-test was used.

RESULTS

1. *Weight and histological finding of ovaries and uterus*

The weights of body, both ovaries and uterus of immature rats used in the present incubation studies are shown in Table 3. Hypophysectomy caused a significant decrease in the weights of body, ovaries and uterus. The weight of ovaries increased significantly from the hypophysectomized control by injection of 25–100 μg of FSH but not by LH or oestradiol-17β. The weight of uterus increased significantly from the hypophysectomized control by injection of 50–100 μg of FSH or oestradiol-17β but not by LH.

In the ovaries of intact 27-day-old rats, primary and developing follicles and the interstitial cells occupied most parts of the ovaries. 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, an increase in size of follicules in FSH (25–100 μg)-injected rats and no signifi-

Table 2. Total 17β-hydroxy and 5α-reduced C_{19}-steroids formed from varying concentrations of 4-androstene-3,17-dione by ovaries from 27-day-old hypophysectomized rats

Non-treated				FSH (50 μg/day) injected			
Amount of tissue (mg)	Substrate (nmol/ 0.4 ml)	17β-OH-products (nmol/g*/h)	5α-Products (nmol/g*/h)	Amount of tissue (mg)	Substrate (nmol/ 0.4 ml)	17β-OH-products (nmol/g*/h)	5α-Products (nmol/g*/h)
5.0	0.7	27	195	2.5	0.7	592	157
5.0	1.4	39	267	2.5	1.4	1020	274
5.0	2.8	45	355	2.5	2.8	1490	269
5.0	5.6	62	392	2.5	5.6	1750	319
5.0	11.2	65	431	2.5	11.2	1820	336

Ovarian homogenates were incubated with [^{14}C]-4-androstene-3,17-dione and NADPH (0.4 mg/0.4 ml) at 37°C for 30 min in 0.4 ml. Treatments of rats are described under Table 3.
* g wet weight.

Table 3. Mean weight of body, ovaries and uterus of 27-day-old rats

	Number of rats	Body Mean ± SD (g)	Ovaries Mean ± SD (mg)	Uterus Mean ± SD (mg)
Intact	12	100 ± 11***	25.9 ± 5.2***	102.7 ± 21.5***
Hypox control	20	73 ± 8	13.3 ± 2.3	42.6 ± 10.6
LH 10 μg	15	70 ± 9	14.6 ± 2.0	36.9 ± 9.0
FSH 10 μg	8	72 ± 4	14.5 ± 1.1	45.3 ± 7.6
FSH 25 μg	4	83 ± 12	23.3 ± 5.7***	67.5 ± 39.3
FSH 50 μg	10	72 ± 6	32.2 ± 4.7***	88.2 ± 12.8***
FSH 100 μg	3	65 ± 10	42.3 ± 5.4***	108.3 ± 13.1***
Oestradiol-17β	5	64 ± 13	13.6 ± 3.3	138.8 ± 14.1***

Rats were hypophysectomized at 21 days of age, and treatment was started 3 days later. Rats in groups of 3–20 were injected daily with 10 μg of NIH-LH-S19, 10–100 μg of NIAMD-Rat-FSH-B-I in 0.5 ml of saline, 20 μg of oestradiol-17β in 0.2 ml of sesame oil or 0.5 ml of saline for 3 days. The rats were killed at 27 days of age.

Differences from Hypox (hypophysectomized) control (P): *** < 0.001 (a t-test was used).

cant changes in estrogen-injected rats were found. No corpora lutea were formed in all immature ovaries examined. The present morphological findings were obtained by examinations of a part of 21 ovaries in eight groups.

2. *Effect of LH, FSH and oestradiol-17β treatment on enzyme activities in ovaries of immature hypophysectomized rats*

Activities of 5α-reductase, 17β-ol-dehydrogenases and aromatase decreased significantly 6 days following hypophysectomy (Tables 4 and 5, Fig. 1). A distinct response to LH but not to FSH in the 5α-reductase activity in the hypophysectomized rat ovaries was found. Although the 5α-reductase activity expressed as nmol/g/h decreased significantly from the hypophysectomized control by injection of 50–100 μg of FSH (Table 4), the activity expressed as nmol/both ovaries/h increased by 100 μg of FSH (Fig. 1). On the other hand, the activities of testosterone and oestradiol 17β-ol-dehydrogenases and aromatase in the hypophysectomized rat ovaries were stimulated markedly (10 to 200 times) by injection of 25–100 μg of FSH. Furthermore, the results shown in Table 2 clearly indicate that a marked increase in formation of the sum of 17β-hydroxy C_{19}-steroids from 4-androstene-3,17-dione in the FSH-injected ovary was due to increase in 17β-ol-dehydrogenase activity but not to increase in affinity of substrate for 17β-ol-dehydrogenase (apparent K_m in non-treated and FSH-injected ovaries were 3×10^{-6} M and 4×10^{-6} M, respectively). LH was not effective, for it failed to stimulate the activities of 17β-ol-dehydrogenases and aromatase even when 10 μg was given each day. Although preparations of FSH and LH may be contaminated by LH (less than 1%) and FSH (less than 2%), respectively, the present results clearly show that the stimulative effect on 17β-ol-dehydrogenase and aromatase activities by FSH preparation and the stimulative effect on 5α-reductase activity by LH preparation do not arise by contaminations. No stimulation of these enzyme activities was involved by daily injection of oestradiol-17β in the large doses (Table 4 and Fig. 1), showing that the effects of FSH and LH are not mediated by oestrogens.

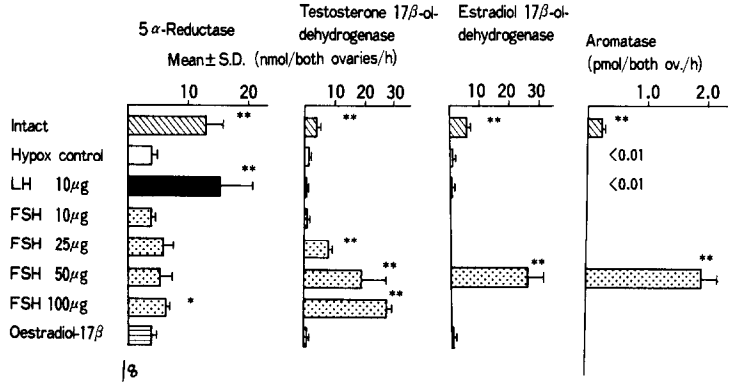

Fig. 1. Effect of LH, FSH and oestradiol-17β treatment on enzyme activities in ovaries of immature hypophysectomized rats. Rats were hypophysectomized at 21 days of age, and treatment was started 3 days later. Hormonal treatments, the number of experiments and conditions for incubations are described in and under Tables 3–5. The enzyme activities (mean ± SD) are expressed as nmol or pmol of steroids formed/both ovaries/h. Differences from Hypox control (P) * < 0.05, ** < 0.01 (a t-test was used).

Table 4. Effect of LH, FSH and oestradiol-17β treatment on enzyme activities (nmol/g wet weight/h) in ovaries of immature hypophysectomized rats

	Estimations	5α-Reductase Mean ± SD	T 17β-ol-dehydrogenase Mean ± SD	Estimations	E_2 17β-ol-dehydrogenase Mean ± SD
Intact	6	509 ± 115**	159 ± 71**	3	220 ± 16***
Hypox control	6	307 ± 41	45 ± 18	4	39 ± 8
LH 10 μg	4	1060 ± 409**	34 ± 31	3	27 ± 6
FSH 10 μg	4	266 ± 21	41 ± 8	—	—
FSH 25 μg	3	286 ± 27	339 ± 3***	—	—
FSH 50 μg	6	176 ± 68**	606 ± 244***	4	799 ± 189***
FSH 100 μg	3	153 ± 2***	685 ± 22***	—	—
Oestradiol-17β	4	274 ± 89	65 ± 7	3	54 ± 18

Treatments of rats are described under Table 3. In each estimation of enzyme activities, a portion of both ovaries was homogenized and 1.2–5 mg of homogenates were incubated with [^{14}C]-4-androstene-3,17-dione (5.2 nmol: 0.3 μCi per tube) or [^{14}C]-oestrone (5.2 nmol: 0.3 μCi per tube) and NADPH (0.4 mg/0.4 ml) at 37°C for 30 min in 0.4 ml.
Differences from Hypox control (P): ** < 0.01, *** < 0.001 (a t-test was used).
T: testosterone; E_2: oestradiol; —: not estimated.

3. *Effect of LH and FSH treatment on metabolism of progesterone in ovaries of immature hypophysectomized rats*

In incubations, results of which are shown in Table 6, two different amounts of ovarian tissues obtained from intact, hypophysectomized and LH or FSH-injected hypophysectomized immature rats were incubated with [^{3}H]-progesterone. The results are given as percentage of total steroid introduced and are not representative of percentage of the activity of different enzymes. Under the incubation conditions used, the rate of production of the sum of 5α-reduced C_{19}-steroids, 17β-hydroxy C_{19}-steroids and/or oestrogen was roughly proportional to the weight of tissue used.

Formation of the sum of C_{19}-steroids from progesterone decreased evidently 6 days following hypophysectomy. The formation of 17α-hydroxylated steroids plus total C_{19}-steroids and total C_{19}-steroids increased distinctly from the hypophysectomized control by injection of LH but not by FSH indicating that the activities of 17α-hydroxylase and 17,20-desmolase were stimulated by LH but not by FSH. Major C_{19}-metabolites formed from progesterone were 4-androstene-3,17-dione in the hypophysectomized immature rat ovary, androsterone and 3β-hydroxy-5α-androstan-17-one in the LH-injected hypophysectomized rat ovary and testosterone in the FSH-injected hypophysectomized rat ovary. As shown under Table 6, the formation of 17β-hydroxy-5α-androstan-3-one could not be demonstrated in all immature rat ovaries examined. The formation of oestradiol-17β from progesterone could be demonstrated only in the FSH-injected rat ovary. These metabolic patterns seem to be produced by changes of the enzyme activities following LH and FSH injections shown in Tables 4 and 5.

DISCUSSION

The present results demonstrate that in immature rat ovaries, the 5α-reductase activity and the formation of C_{19}-steroids from progesterone are enhanced by LH but not by FSH injection and that the activities of 17β-ol-dehydrogenases and aromatase are stimulated by FSH but not by LH injection. We already reported the stimulative effect of LH treatment on the 5α-reductase activity in immature rat ovaries [8]. Other investigators also reported that FSH stimulates the aromatase activity in rat ovaries [11, 12, 15, 19], as well as in cultured rat

Table 5. Effect of LH and FSH treatment on aromatase activity in ovaries of immature hypophysectomized rats

	Estimations	Oestrone formed	Oestradiol-17β formed	Aromatase activity
		M ± SD (pmol/g wet weight/h)		
Intact	4	3.7 ± 0.6***	4.9 ± 0.8***	8.6 ± 1.2***
Hypox control	4	<0.4	0.0	<0.4
LH 10 μg	4	<0.2	0.0	<0.2
FSH 50 μg	4	12.8 ± 2.8***	48.1 ± 4.2***	60.9 ± 6.3***

Treatments of rats are described under Table 3. Ovarian homogenates (20–40 mg) were incubated with [7-^{3}H]-4-androstene-3,17-dione (0.7 nmol: 10 μCi per tube), progesterone (30 nmol per tube) and NADPH (0.4 mg/0.4 ml) for 60 min in 1 ml. Aromatase activity is expressed as oestrone plus oestradiol-17β formed from 4-androstene-3,17-dione. Values were obtained after recrystallizations to constant specific activity.
Differences from Hypox control (P): *** < 0.001 (a t-test was used).

Table 6. Percentage formation of [^{3}H]-steroids from [^{3}H]-progesterone by 27-day-old rat ovaries

Group	Intact		Hypox control		LH 10 μg		FSH 50 μg	
Amount of tissue (mg)	5	15	5	15	5	15	5	15
Progesterone (unchanged)	7.7	5.0	64.4	33.1	19.3	4.4	63.6	30.1
5α-Pregnane-3,20-dione	2.2	1.5	1.9	2.3	3.3	2.6	2.0	3.5
3β-Hydroxy-5α-pregnan-20-one	45.0	37.4	15.9	37.7	27.2	26.7	20.4	41.5
17-Hydroxy-4-pregnene-3,20-dione	1.5	0.2	3.2	2.9	5.4	0.2	1.5	1.1
3α,17-Dihydroxy-5α-pregnan-20-one	14.9	22.0	0.8	4.4	11.4	22.8	0.5	2.8
3β,17-Dihydroxy-5α-pregnan-20-one	2.7	2.8	0.4	1.4	3.0	3.3	0.2	0.5
4-Androstene-3,17-dione	2.1	2.1	1.5	3.7	6.8	7.1	0.5	0.7
Testosterone	0.9	1.5	0.1	0.4	<0.1	0.1	0.5	1.7
Androsterone	2.4	4.6	0.1	0.4	2.1	11.6	<0.1	0.1
3β-Hhydroxy-5α-androstan-17-one	0.8	1.7	<0.1	<0.1	1.4	2.3	0.0	0.0
5α-Androstane-3α,17β-diol	0.5	4.1	0.0	0.1	0.0	<0.2	0.1	0.3
5α-Androstane-3β,17β-diol	0.3	1.7	0.0	0.1	0.0	0.1	0.0	0.2
(Total C_{19}-steroids)	(7.0)	(15.7)	(1.7)	(4.7)	(10.4)	(21.2)	(1.1)	(3.0)
Oestradiol-17β	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	0.03

Treatments of rats are described under Table 3. Ovarian homogenates were incubated with [7-^{3}H]-progesterone (1.3 nmol: 10 μCi per tube) and NADPH at 37°C for 60 min in 1 ml. Percentage formations of 20α-hydroxy-4-pregnen-3-one, 17-hydroxy-5α-pregnane-3,20-dione, 5α-androstane-3,17-dione, 17β-hydroxy-5α-androstan-3-one and oestrone were <0.5%, <0.6%, <0.3%, <0.3% and <0.01% in all ovaries, respectively. Values were obtained after recrystallizations to constant specific activity. Results are mean of duplicate determinations.

granulosa cells [9, 14, 15]. The present results on the activities of 5α-reductase and aromatase confirm and extend these previous observations. The present results shown in Table 4 and Fig. 1 demonstrate for the first time that the activities of 17β-ol-dehydrogenases in immature rat ovaries are stimulated evidently by FSH but not by LH, though suggestive data were reported by Kraiem and Samuels in immature mouse ovaries [22].

In the ovaries of hypophysectomized immature rats, a gradual increase in size of the follicles was found following FSH injection in doses from 25–100 μg per day while the injection of LH produced proliferation of interstitial cells. No corpora lutea were formed in all these ovaries. These observations seem to suggest that 5α-reductase is largely localized in the interstitial cells and 17β-ol-dehydrogenases and aromatase are largely localized in the follicles in the immature rat ovary.

The previous [1, 8] and the present results (Table 3) demonstrate the non-response to LH and response to FSH of the uterus of hypophysectomized immature rats. The non-response of the uterus to LH can be explained in part by the stimulative effect of LH on 5α-reductase activity and the non-stimulative effect on 17β-ol-dehydrogenase and aromatase activities in the ovary (Tables 4 and 5), since such changes serve to decrease in the formation of oestradiol-17β. In fact, no oestradiol-17β was formed from progesterone in the LH-injected hypophysectomized immature rat ovary, even though the formation of the sum of C_{19}-steroids increased evidently (Table 6). The response of the uterus to FSH can be explained in part by the stimulative effect of FSH on 17β-ol-dehydrogenase and aromatase activities and the non-stimulative effect on 5α-reductase activity (Tables 4 and 5), since these changes serve to an increase in the production of oestradiol-17β as shown in Table 6.

If the two-cell gonadotropin theory [9–15] and the present results are combined, a following working hypothesis seems to be formulated. Not only theca cells but also interstitial cells under the influence of LH produce androgens, which are aromatized by granulosa cells to oestradiol under the influence of FSH. In the ovaries of hypophysectomized LH-injected immature rats, large amounts of androgens are formed by the theca and interstitial cells but the androgens formed consist largely of 5α-C_{19}-steroids. Remaining aromatizable C_{19}-steroids can be poorly metabolized by granulosa cells to oestradiol-17β because of low activities of aromatase and 17β-ol-dehydrogenases. In those of FSH injected rats, only small amounts of androgens are formed by the theca and interstitial cells but the androgens consist largely of 4-ene-3-ketosteroids. The aromatizable C_{19}-steroids can be effectively metabolized by granulosa cells to oestradiol-17β because of high activities of aromatase and 17β-ol-dehydrogenases.

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REFERENCES

1. Odell W. D. and Swerdloff R. S.: Etiologies of sexual maturation: a model system based on the sexually maturing rat. *Recent Prog. horm. Res.* **32** (1976) 245–277.
2. Mason N. R.: Steroid A-ring reduction by rat ovaries. *Endocrinology* **87** (1970) 350–355.
3. Springer C. and Eckstein B.: Regulation of production *in vitro* of 5α-androstane-3α,17β-diol in the immature rat ovary. *J. Endocr.* **50** (1971) 431–439.
4. Eckstein B. and Ravid R.: On the mechanism of the onset of puberty: identification and pattern of 5α-androstane-3β,17β-diol and its 3α epimer in peripheral blood of immature female rats. *Endocrinology* **94** (1974) 224–229.
5. Karakawa T., Kurachi K., Aono T. and Matsumoto K.: Formation of 5α-reduced C_{19}-steroids from pro-

gesterone *in vitro* by a pathway through 5α-reduced C_{21}-steroids in ovaries of late prepubertal rats. *Endocrinology* **98** (1976) 571–579.
6. Lerner N. and Eckstein B.: Identification of two 5α-reduced pregnanes as major metabolites of progesterone in immature rat ovaries (1000 *g* supernatant) *in vitro*. *Endocrinology* **98** (1976) 179–188.
7. Inaba T., Imori T. and Matsumoto K.: Formation of 5α-reduced C_{19}-steroids from progesterone *in vivo* by 5α-reduced pathway in immature rat ovaries. *J. steroid Biochem.* **9** (1978) 1105–1110.
8. Terakawa N., Kondo K., Aono T., Kurachi K. and Matsumoto K.: Hormonal regulation of 4-ene-5α-reductase activity in prepubertal rat ovaries. *J. steroid Biochem.* **9** (1978) 307–311.
9. Dorrington J. H., Moon Y. S. and Armstrong D. T.: Estradiol-17β biosynthesis in cultured granulosa cells from hypophysectomized immature rats: stimulation by follicle stimulating hormone. *Endocrinology* **97** (1975) 1328–1331.
10. Makris A. and Ryan K. J.: Progesterone, androstenedione, testosterone, estrone and estradiol synthesis in hamster ovarian follicle cells. *Endocrinology* **96** (1975) 694–701.
11. Moon Y. S., Dorrington J. H. and Armstrong D. T.: Stimulatory action of follicle-stimulating hormone on estradiol-17β secretion by hypophysectomized rat ovaries in organ culture. *Endocrinology* **97** (1975) 244–247.
12. Armstrong D. T. and Papkoff H.: Stimulation of aromatization of exogenous and endogenous androgens in ovaries of hypophysectomized rats *in vivo* by follicle-stimulating hormone. *Endocrinology* **99** (1976) 1144–1151.
13. Moor R. M.: Sites of steroid production in ovine graafian follicles in culture. *J. Endocr.* **73**, (1977) 143–150.
14. Fortune J. E. and Armstrong D. T.: Hormonal control of 17β-estradiol biosynthesis in proestrus rat follicles: estradiol production by isolated theca versus granulosa. *Endocrinology* **102** (1978) 227–235.
15. Erickson G. F. and Hsueh A. J. W.: Stimulation of aromatase activity by follicle stimulating hormone in rat granulosa cells *in vivo* and *in vitro*. *Endocrinology* **102** (1978) 1275–1282.
16. Zaffaroni A. and Burton R. B.: Identification of corticosteroids of beef adrenal extract by paper chromatography. *J. biol. Chem.* **193** (1951) 749–767.
17. Seki T. and Matsumoto K.: Chromatographic separation of 17-hydroxycorticosteroids and 17-ketosteroids. *J. Chromatogr.* **27** (1967) 423–430.
18. Yamada M., Yasue S. and Matsumoto K.: Formation of 5α-reduced products from testosterone *in vitro* by germ cells from immature rats. *Acta endocr., Copenh.* **71** (1972) 393–408.
19. Suzuki K., Kawakura K. and Tamaoki B.: Effect of pregnant mare's serum gonadotrophin on the activities of 4-ene-5α-reductase, aromatase, and other enzymes in the ovaries of immature rats. *Endocrinology* **102** (1978) 1595–1605.
20. Yamada M. Yasue S. and Matsumoto K.: Formation of C_{21}-17-hydroxysteroids and C_{19}-steroids from 3β-hydroxypregn-5-en-20-one and progesterone *in vitro* by germ cells from immature rat testes. *Endocrinology* **93** (1973) 81–87.
21. Yamada M. and Matsumoto K.: Pathway from progesterone to 5α-reduced C_{19}-steroids not involving androstenedione and testosterone in immature rat testes *in vitro*. *Endocrinology* **94** (1974) 777–784.
22. Kraiem Z. and Samuels L. T.: The influence of FSH and FSH + LH on steroidogenic enzymes in the immature mouse ovary. *Endocrinology* **95** (1974) 660–668.