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A COMPARISON OF METABOLISM OF PROGESTERONE TO 5α-STEROIDS IN MONKEY, MOUSE AND RAT OVARIES

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SUMMARY

Ovarian homogenates from suckling, immature and adult rhesus monkeys and mice and also those from immature rats were incubated with [^{3}H]-progesterone and NADPH. After incubation, the radioactive products were separated and identified by column and paper chromatography with derivative formation and recrystallization to constant specific activity. All ovaries from monkeys and mice of different ages converted 10–80% of progesterone to 17-hydroxy-4-pregnene-3,20-dione, androstenedion, testosterone, 20α-hydroxy-4-pregnen-3-one and/or 16α-hydroxy-4-pregnene-3,20-dione. In monkeys, however, all ovaries of different ages tested in the present study formed no 5α-reduced metabolites of these 4-ene-3-ketosteroids. In mice, ovaries at 2 and 10 weeks of age were unable to form 5α-reduced C_{21}-17-OH- and C_{19}-steroids, whereas those at 3 and 4 weeks of age formed significant amounts of these 5α-products. In contrast to immature rats, immature ovaries of monkeys do not form significant amounts of 5α-reduced C_{19}-steroids. Although immature ovaries of mice synthesize 5α-reduced C_{19}-steroids from progesterone, this age dependent formation of 5α-steroids is less active than that in rats.

INTRODUCTION

Previous studies *in vitro* have demonstrated that ovarian homogenates from immature rats form large amounts of 5α-reduced C_{19}-steroids from progesterone, whereas adult rat ovaries are unable to form significant amounts of 5α-reduced C_{19}-steroids [1, 2]. Recently, we [2] and Lerner and Eckstein[3] found that progesterone was converted to these 5α-reduced C_{19}-steroids primarily by a pathway through 5α-reduced C_{21}-steroids in immature 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 immature rats but these products then disappeared after the onset of puberty [4]. 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 [5, 6] and induce precocious ovulation in rats [4, 6], the formation of 5α-reduced C_{19}-steroids in the immature rat ovary seems to have a biological significance in immature female rats.

The formation of androstenedione, testosterone and estrogens has been reported in ovaries of adult humans [7, 8], adult rhesus monkeys [9] and mice [10]. However, very little or no data on the age dependent pattern of 5α-androgen formation in ovaries of these species have been shown. Because sampling of fresh ovaries from healthy humans of different ages was impossible, we are reporting on the *in vitro* metabolism of progesterone by ovaries of rhesus monkeys and mice at different stages of development.

MATERIALS AND METHODS

Animals. Rhesus monkeys (*Macaca irus*) of different ages, 1.3, 1.5, 3.0, 3.1, 4 and 5 years, were born and raised in the Fujisawa Research Laboratory, Osaka, Japan. Ovaries were removed under Nembutal anesthesia, immediately frozen and stored at −75°C for 2–4 weeks until used for incubations. Weights of ovaries were 120, 140, 350, 240, 500 and 460 mg, respectively. The ovaries from 4- and 5-year-old monkeys contained corpora lutea, while corpora lutea were not formed in the ovaries of 1.3-, 1.5-, 3.0- and 3.1-year-old monkeys which consisted of only follicles and interstitial cells. Since the first ovulation of rhesus monkeys at the Fujisawa Research Laboratory generally begins shortly after 3 years of age, ovaries from 3.0- and 3.1-year-old monkeys used were just before the first ovulation. Ovaries were also removed from 28-day-old rats of the Wistar strain, immediately frozen and stored at −75°C for 4 weeks until used for incubations. Albino mice of the d.d. strain (2, 3, 4 and 10 weeks of age) were also used. Ovaries were removed from 10–30 mice in each age group, weighed and immediately used for incubations. Weights of ovaries were 1.7, 5.0, 8.7 and 18.7 mg, respectively. The ovaries from 10-week-old mice contained corpora lutea, while corpora lutea were not formed in the ovaries of 2-, 3- and 4-week-old mice which consisted

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Table 1. Percentage formation of [^{3}H]-steroids from [^{3}H]-progesterone by 30 mg ovarian homogenates from rhesus monkeys and rats

Age (yr)	1.3	1.5	3.0	3.1	4-L*	4-F**	5-L*	5-F**	Rat (28 days)
Progesterone (unchanged)	71.0	60.2	32.0	12.6	76.1	93.3	78.7	83.2	2.8
16α-Hydroxy-4-pregnene-3,20-dione	2.1	3.0	7.0	10.3	1.7	0.4	—	—	—
20α-Hydroxy-4-pregnen-3-one	3.9	3.8	3.3	2.2	2.4	3.4	1.5	2.2	0.0
5α-Pregnane-3,20-dione	<0.1	<0.2	<0.1	0.0	<0.1	<0.1	<0.1	<0.1	1.9
3β-Hydroxy-5α-pregnan-20-one	<0.1	0.0	<0.1	0.0	<0.2	<0.1	0.0	0.0	10.2
17-Hydroxy-4-pregnene-3,20-dione	13.8	22.4	44.3	56.5	1.4	2.5	1.8	3.4	<0.1
17-Hydroxy-5α-pregnane-3,20-dione	<0.1	0.0	<0.1	0.0	<0.1	<0.1	<0.2	<0.2	1.1
3α,17-Dihydroxy-5α-pregnan-20-one	<0.1	0.0	<0.1	<0.2	<0.1	<0.1	<0.1	<0.1	14.8
3β,17-Dihydroxy-5α-pregnan-20-one	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6
Androstenedione	0.1	0.1	0.5	0.8	<0.2	<0.1	<0.1	<0.1	<0.1
Androsterone	0.0	<0.1	0.0	0.0	0.0	0.0	0.0	0.0	25.5
3β-Hydroxy-5α-androstan-17-one	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.8
17β-Hydroxy-5α-androstan-3-one	<0.2	<0.1	<0.1	0.0	<0.1	<0.1	<0.2	<0.1	2.0
5α-Androstane-3α,17β-diol	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	13.3
5α-Androstane-3β,17β-diol	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6

Ovarian homogenates were incubated with [^{3}H]-progesterone (1 nmol: 1 μCi per tube) and NADPH at 37°C for 2 h in 1 ml. Values were obtained after recrystallization to constant specific activity except for 16α-hydroxy-4-pregnene-3,20-dione. The percentage formation of testosterone and 5α-androstane-3,17-dione was <0.2% in all ovaries. * Corpora lutea. ** Follicles and interstitial cells. —: Not determined.

of only follicles and interstitial cells. In the present study, the ovaries of monkeys and rats were stored at −75°C for 2–4 weeks while the mouse ovaries were used immediately. Since the monkey ovaries of different ages were generously supplied from the Fujisawa Research Laboratory, it was difficult to obtain fresh monkey ovaries of different ages at the same time. By preliminary experiments in monkey and rat ovaries, the effect of storage at −75°C for 4 weeks on the conversion patterns of progesterone was insignificant.

Chemicals. [1,2-^{3}H]-Progesterone (45 Ci/mmol) obtained from Daiichi Pure Chemical Co., Ltd., Japan was purified by paper chromatography using the hexane-formamide system [11] just before use. Nonradioactive steroids were obtained from Steraloids, Inc., Wilton, N.H., and Ikapharm, Ramat-Gan, Israel. Other reagents were of analytical grade.

Incubation procedure. Ovaries from monkeys, rats and mice were homogenized in 0.25 M sucrose containing 1 mM EDTA. The purified [^{3}H]-progesterone (1 or 2 nmol: 1 μCi per tube) was introduced into 2 × 10 cm tubes and dissolved in 0.02 ml ethanol. One-half ml of buffer–cofactor solution was added to each tube. The buffer–cofactor solution consisted of 0.3 M potassium phosphate buffer, pH 7.4, 0.06 M nicotinamide, 2 mM $MgCl_2$ and 3 mM NADPH. One-half ml of the tissue homogenate containing 30–90 mg tissue was then introduced to make the total volume of the incubation mixture 1 ml. The samples were incubated in a shaking water bath in air at 37°C for 1 or 2 h. 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.

Analysis and identification of steroids. Two to fifty micrograms of 17 steroids shown in Table 1 were added as non-radioactive carriers to the incubation mixtures. The extraction of steroids, analysis of these steroids by paper [11] and column [12] chromatography with acetylation of steroids, identification or tentative identification of metabolites separated by recrystallization to constant specific activity and calculation of metabolite found in each steroid fraction were the same as previously described [13–15]. Since the radioactivity in the phenolic fraction was proved to be less than 0.3% of that in the corresponding extracts, no further analysis of it was attempted. The present procedure for calculating the rate of formation of metabolites can permit an approximate estimation of the percent conversion of radioactive precursors, which seems to be satisfactory for the present purpose.

RESULTS

1. *Metabolism of progesterone by monkey ovaries of different ages*

The percentage formation of [^{3}H]-steroids from [^{3}H]-progesterone by ovarian homogenates from monkeys of different ages is shown in Tables 1 and 2. In incubations of 30 and 90 mg tissues with 1 nmol of [H^3]-progesterone for 2 h, ovaries from 1.3 to 5-year-old monkeys converted 1–60% of progesterone to 17-hydroxy-4-pregnene-3,20-dione and <0.1–2.5% to androstenedione. In follicles and interstitial cells of different ages, the rate of production of 4-ene-C_{19}-steroids and/or 4-ene-C_{19}-steroids plus 17-hydroxy-4-pregnene-3,20-dione was roughly proportional to the weight of tissue used. In corpora lutea, greater amounts of radioactive products were formed in incubations of 30 mg tissue. However, no 5α-reduced

Table 2. Percentage formation of [^{3}H]-steroids from [^{3}H]-progesterone by 90 mg ovarian homogenates from rhesus monkeys

Age (yr)	1.5	3.0	3.1	4-L*	4-F**	5-L*	5-F**	No tissue
Progesterone (unchanged)	26.0	21.1	5.1	75.7	75.5	88.9	73.0	95.1
16α-Hydroxy-4-pregnene-3,20-dione	4.6	8.5	12.1	1.3	0.6	—	—	0.0
20α-Hydroxy-4-pregnen-3-one	3.8	3.0	1.9	1.9	3.4	0.5	1.9	<0.2
17-Hydroxy-4-pregnene-3,20-dione	54.7	51.7	62.2	0.5	9.3	0.5	13.0	0.0
Androstenedione	0.4	1.8	2.5	<0.2	0.2	<0.1	0.3	<0.1

Ovarian homogenates were incubated with [^{3}H]-progesterone (1 nmol: 1 μCi per tube) and NADPH at 37 C for 2 h in 1 ml. The percentage formation of testosterone, 5α-pregnane-3,20-dione, 3β-hydroxy-5α-pregnan-20-one, 17-hydroxy-5α-pregnane-3,20-dione, 3α,17-dihydroxy-5α-pregnan-20-one, 3β,17-dihydroxy-5α-pregnan-20-one, 5α-androstane-3,17-dione, androsterone, 3β-hydroxy-5α-androstan-17-one, 17β-hydroxy-5α-androstan-3-one, 5α-androstane-3α,17β-diol and 5α-androstane-3β,17β-diol was <0.2% in all ovaries. * Corpora lutea. ** Follicles and interstitial cells. —: Not determined.

metabolites of these 4-ene-3-ketosteroids were formed in all monkey ovaries of different ages examined. On the other hand, ovaries from 28-day-old rats converted 75% of progesterone to 5α-reduced steroids such as 3α,17-dihydroxy-5α-pregnan-20-one, androsterone and 5α-androstane-3α,17β-diol under the same incubation conditions. In these immature rat ovaries, accumulation of 17α-hydroxy-4-pregnene-3,20-dione, androstenedione and testosterone was less than 0.1%. Control incubations without tissue were included in the present investigation. There was no or very little transformation of [^{3}H]-progesterone as shown in Tables 2 and 3.

2. Metabolism of progesterone by mouse ovaries of different ages

The percentage formation of [^{3}H]-steroids from [^{3}H]-progesterone by ovarian homogenates from mice of different ages is shown in Table 3. In incubations of 30 and 50 mg tissues with 2 nmol of [^{3}H]-progesterone for 1 h, ovaries from 2 to 10-week-old mice converted 0.1–1% of progesterone to 17-hydroxy-4-pregnene-3,20-dione and 0.3–4% to androstenedione and testosterone. Significant amounts (0.3–1.5%) of 5α-reduced C_{21}-17-OH- and C_{19}-steroids were shown to be formed in ovaries from 3- and 4-week-old mice, whereas ovaries from suckling and adult mice were unable to form these 5α-reduced steroids. Major metabolites of progesterone were 20α-hydroxy-4-pregnen-3-one and 3β-hydroxy-5α-pregnan-20-one in ovaries from 3- and 4-week-old mice, whereas 20α-hydroxy-4-pregnen-3-one was the major metabolite in ovaries from suckling and adult mice. However, the rate of formation of 5α-reduced C_{19}-steroids by immature mouse ovaries was less than that by immature rat ovaries (Table 1).

DISCUSSION

The present investigation demonstrated that significant amounts of progesterone were metabolized to 17-hydroxy-4-pregnene-3,20-dione, androstenedione and testosterone *in vitro* in all ovaries from rhesus monkeys and mice of different ages. It was also demonstrated that significant amounts of 5α-reduced C_{21}-17-OH- and C_{19}-steroids were formed only by immature mouse ovaries but not by ovaries from suckling mice, adult mice and all monkeys tested (Tables 1–3). The concentrations of endogenous progesterone in ovaries of 1.3 to 3.1-year-old monkeys, follicles and interstitial cells of 4- and 5-year-old monkeys, corpora lutea of 4- and 5-year-old monkeys, ovaries of 2 to 4-week-old mice and ovaries of 10-week-old mice, estimated by the competitive pro-

Table 3. Percentage formation of [^{3}H]-steroids from [^{3}H]-progesterone by mouse ovaries

Age (weeks)	Experiment 1 (50 mg) 2	3	4	10	No tissue	Experiment 2 (30 mg) 2	3	4	10
Progesterone (unchanged)	24.2	34.5	25.5	20.1	95.3	40.2	47.7	37.9	25.1
20α-Hydroxy-4-pregnen-3-one	54.2	25.2	23.1	54.7	<0.3	30.4	19.5	19.5	50.7
5α-Pregnane-3,20-dione	<0.1	0.9	1.6	<0.2	<0.1	<0.1	0.5	1.0	<0.1
3β-Hydroxy-5α-pregnan-20-one	2.7	9.1	16.8	3.2	<0.2	2.0	8.0	11.2	2.1
5α-Pregnane-3α,20α-diol	0.6	2.2	5.2	<0.2	0.0	0.4	1.7	3.8	0.2
17-Hydroxy-4-pregnene-3,20-dione	0.6	0.9	0.9	0.1	0.0	0.4	0.4	0.4	0.1
3α,17-Dihydroxy-5α-pregnan-20-one	<0.1	0.6	0.8	<0.1	<0.1	<0.1	0.3	0.4	<0.1
Androstenedione	1.6	2.9	2.7	0.5	<0.1	1.0	1.6	1.8	0.4
Testosterone	<0.1	0.8	1.2	0.1	0.0	0.0	0.2	0.2	<0.1
Androsterone	0.0	0.5	1.2	0.0	0.0	0.0	0.3	0.7	0.0
5α-Androstane-3α,17β-diol	<0.2	<0.1	0.2	0.0	0.0	<0.2	<0.2	<0.2	<0.1

Ovarian homogenates were incubated with [^{3}H]-progesterone (2 nmol: 1 μCi per tube) and NADPH at 37 C for 1 h in 1 ml. Values were obtained after recrystallization to constant specific activity. The percentage formation of 17-hydroxy-5α-pregnane-3,20-dione, 3β,17-dihydroxy-5α-pregnan-20-one, 5α-androstane-3,17-dione, 3β-hydroxy-5α-androstan-17-one, 17β-hydroxy-5α-androstan-3-one and 5α-androstane-3β,17β-diol was <0.2% in all ovaries.

tein-binding method using pregnant guinea pig serum were 4–5, 3–5, 120–150, 2–10 and 120 ng per 10 mg tissue, respectively. The concentrations of endogenous testosterone in ovaries of monkeys and mice of different ages estimated by radioimmunoassay were 0.2–0.5 ng per 10 mg tissue. Since 310 or 620 ng of [^{3}H]-progesterone was added to 30–90 mg tissue homogenates, the effect of dilution by endogenous steroids on the metabolism of radioactive progesterone in ovaries without corpora lutea seems to be limited. In corpora lutea from adult monkey and mouse ovaries, however, the formation of [^{3}H]-steroids from [^{3}H]progesterone must be underestimated due to dilution by endogenous precursor pools present in the corpora lutea. In fact, the percentage formation of [^{3}H]-17-hydroxy-4-pregnene-3,20-dione from [^{3}H]-progesterone by corpora lutea was higher in 30 mg tissue homogenate than in 90 mg tissue homogenate (Tables 1 and 2). However, it seems that an approximate estimation of *in vitro* formation of 5α-reduced C_{21}-17-OH- and C_{19}-steroids from progesterone compared with that of C_{21}-17-OH- and C_{19}-4-ene-3-ketosteroids can be made from the results shown in Tables 1–3. These observations suggest that efficient formation of 5α-reduced C_{21}-17-OH- and C_{19}-steroids already demonstrated in immature rat ovaries [1–4] is not present in immature monkey ovaries but is present weakly in immature mouse ovaries.

In rats, large amounts of 5α-reduced C_{21}-17-OH- and C_{19}-steroids have been shown to be formed from progesterone by immature testes but not by testes from suckling and adult animals [14, 16–18]. In mice, similar but weak age dependent change of testicular 5α-androgen synthesis was found [19]. However, in humans [15, 20] and rhesus monkeys [15], no or very small amounts of 5α-reduced C_{21}-17-OH- and C_{19}-steroids were formed by immature and adult testes. These findings, and previous [1–4] and present (Tables 1–3) results seem to show that in each species, testes and ovaries of different ages show a similar age-dependent pattern of 5α-androgen synthesis and that significant formation of 5α-reduced C_{19}-steroids may not be present in ovaries of immature humans. Prepubertal changes of gonadal 5α-androgen biosynthesis seem to be variable in different species of animals.

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