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SHORT COMMUNICATION

PROGESTERONE METABOLISM *IN VITRO* BY OVARIES FROM ANDROGEN-STERILIZED RATS OF DIFFERENT AGES

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Summary—Metabolism of [^{3}H]-progesterone by ovarian homogenates from androgen-sterilized rats of different ages was examined. Ovaries of 26-day-old rats synthesized a significant quantity of 5α-reduced C_{21}-17-OH- and C_{19}-steroids. In contrast, ovaries of 40- and 55-day-old rats formed very little or no 5α-reduced C_{21}-17-OH- and C_{19}-steroids while significant amounts of 17α-hydroxy-4-pregnene-3,20-dione, 4-androstene-3,17-dione and testosterone were shown to be produced.

Previous studies have demonstrated that immature rat testes synthesize large amounts 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 [1–3]. Although the formation of a significant quantity of 5α-reduced C_{19}-steroids by immature rat ovaries has also been reported [4, 5], age dependent pattern of 5α-steroid formation from progesterone by follicles and interstitial tissue of rat ovary has never been demonstrated. This is because the ovary of adult rat contains corpora lutea which can not be completely separated from follicles and interstitial tissue. Furthermore, corpora lutea contain large amounts of endogenous progesterone and its 5α-products which interfere in the metabolism of radioactive progesterone by the ovary of adult rat. Female rats given testosterone in neonatal life develop, after sexual maturation, a syndrome characterized by ovulatory failure and persistent vaginal cornification [6, 7]. The present study reports on age dependent pattern of progesterone conversion to C_{19} steroids by ovarian follicles and interstitial tissue of the androgenized rat.

Female rats of the Sprague–Dawley Strain were used. A single injection of 250 μg testosterone propionate in 0.025 ml sesame oil was given subcutaneously at 5 days of age. The androgenized rats in groups of 5 were killed at 26, 40 and 55 days of age. Uninjected normal rats in groups of 5 were killed at 26 days of age and 55 days of age in estrus. Ovaries from 5 rats of different ages were homogenized in 0.25 M sucrose containing 1 mM EDTA. [1,2-^{3}H]-progesterone (5 nmol: 0.5 μCi per tube), purified by paper chromatography just before use, was introduced into tubes and dissolved in 0.02 ml ethanol. To each tube, 0.5 ml of buffer co-factor solution was added. The buffer co-factor solution consisted of 0.3 M potassium phosphate buffer pH 7.4, 0.06 M nicotinamide, 2 mM $MgCl_2$ and 2.5 mM NADPH. One-half ml of the tissue homogenate was then introduced. The samples were incubated in air at 37°C for 30 min. After incubation, radioactive metabolites in the incubation mixtures were extracted, separated, identified and measured by column and paper chromatography, with derivative formation and repeated crystallizations to constant specific activity, as previously described [8, 9]. Concentrations of ovarian progesterone were estimated by the competitive protein binding method using pregnant guinea pig serum.

Ovarian weights of the androgenized rats at 55 days of age were found to be approximately one-half of the normal controls, whereas weights of ovaries at 26 days of age, uterus and body of the androgenized rats did not differ significantly from the control weights. All ovaries examined contained follicles at various stages of development and interfollicular interstitial tissue. Corpora lutea were found to be formed only in ovaries of 55-day-old normal rats.

As shown in Table 1, immature ovaries of the androgenized rats synthesized a significant quantity of 5α-reduced C_{21}-17-OH- and C_{19}-steroids. The ratio of the sum of 5α-reduced C_{21}-17-OH-steroids to total C_{21}-17-OH-steroids and that of the sum of 5α-reduced C_{19}-steroids to total C_{19}-steroids were higher in 40 mg of ovarian homogenates than in 10 mg of ovarian homogenates from the 26-day-old androgenized rats. This seems to be due mainly to the difference of the amount of tissue used for the same amount of substrate. In contrast, ovaries of 40- and 55-day-old androgenized rats formed very little or no 5α-reduced C_{21}-17-OH- and C_{19}-steroids while significant amounts of 17α-hydroxy-4-pregnene-3,20-dione, 4-androstene-3,17-dione and testosterone were shown to be produced. This age dependent pattern of progesterone metabolism by the ovary of the androgenized rat is similar to that by rat testes reported previously [1–3]. There was a greater over-all metabolism of progesterone by the ovary of the 26-day-old androgenized rat than by the ovary of the mature androgenized rat. Concentrations of progesterone in the androgenized ovaries at 26, 40 and 55 days of age and in the normal ovaries at 26 and 55 days of age were 2, 10, 11, 3 and 156 ng per 10 mg tissue, respectively. Since 1550 ng of [^{3}H]-progesterone was added to 10 or 40 mg tissue homogenate, the effect of dilutions by endogenous progestins on the metabolism of [^{3}H]-progesterone in the androgenized ovaries seems to be insignificant. When metabolism of progesterone by the androgenized ovaries is compared with that by the normal ovaries, metabolic pattern is similar at 26 days of age but is different at 55 days of age (Table 1). The ovary of the mature normal rat reduced progesterone primarily to 20α-hydroxy-4-pregnen-3-one while the ovary of the mature androgenized rat produced essentially no 20α-hydroxy-4-pregnen-3-one with a less over all conversion of progesterone. A greater conversion of progesterone to 17α-hydroxy-4-pregnene-3,20-dione, 4-androstene-3,17-dione and testosterone by the mature androgenized ovary than by the mature normal ovary (Table 1) may simply represent the greater availability of substrate in view of the inactivation of the

Table 1. Percentage formation of [^{3}H]-steroids from [^{3}H]-progesterone by rat ovaries

	Normal		Androgenized			Androgenized			
Days after birth:	26	55	26	40	55	26	40	55	No
Amounts of tissue (mg)*:	40	40	40	40	40	10	10	10	tissue
Progesterone (unchanged)	9.7	12.4	16.6	78.6	80.5	67.8	87.5	89.6	94.1
20α-Hydroxy-4-pregnen-3-one	<0.3	51.1	<0.2	<0.3	<0.4	<0.1	<0.1	<0.1	<0.2
5α-Pregnane-3,20-dione	1.5	1.4	3.0	0.6	0.3	2.5	0.7	0.1	0.3
3-Hydroxy-5α-pregnan-20-one	25.8	10.4	33.3	4.8	3.3	10.2	1.2	1.1	0.1
17α-Hydroxy-4-pregnene-3,20-dione	7.8	0.2	1.8	2.9	2.6	3.3	1.1	1.5	0.0
17α-Hydroxy-5α-pregnane-3,20-dione	<0.2	<0.2	<0.1	<0.1	<0.3	<0.1	0.0	0.0	<0.1
3α,17α-Dihydroxy-5α-pregnan-20-one	10.6	<0.1	10.5	0.4	<0.3	0.8	<0.2	<0.1	<0.1
3β,17α-Dihydroxy-5α-pregnan-20-one	10.8	<0.1	1.2	0.0	0.0	0.4	0.0	0.0	0.0
4-Androstene-3,17-dione	15.9	0.5	8.3	3.1	2.8	3.5	0.8	0.7	0.1
Testosterone	1.4	<0.1	1.8	0.4	0.2	0.2	<0.1	<0.1	0.0
5α-Androstane-3,17-dione	0.4	0.0	<0.2	<0.1	<0.2	0.0	0.0	0.0	<0.1
17β-Hydroxy-5α-androstan-3-one	<0.2	0.0	<0.3	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
Androsterone	2.0	<0.2	1.6	<0.1	<0.1	0.3	<0.1	<0.1	<0.1
3β-Hydroxy-5α-androstan-17-one	2.4	<0.1	1.2	<0.1	<0.1	0.2	<0.1	<0.1	0.0
5α-Androstane-3α,17β-diol	0.2	0.0	0.3	<0.1	0.0	<0.1	0.0	0.0	0.0
5α-Androstane-3β,17β-diol	0.6	<0.1	0.6	0.0	0.0	<0.1	0.0	0.0	0.0
(Sum of 5α-reduced C_{19}-steroids)	(5.6)	(0.0)	(3.7)	(0.0)	(0.0)	(0.5)	(0.0)	(0.0)	(0.0)

Ovarian homogenates were incubated with [^{3}H]-progesterone (5 nmol: 0.5 μCi per tube) and NADPH at 37°C for 30 min in 1 ml. Values were obtained after repeated crystallizations to constant specific activity.* Amount of tissue per incubation.

20α-reductase. The difference at 55 days of age seems to be due mainly to the presence of corpora lutea in the normal ovary.

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