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THE EFFECTS OF OVARIECTOMY AND ADRENALECTOMY ON THE 5α-REDUCTION OF TESTOSTERONE AND CORTICOSTERONE BY FEMALE RAT TISSUES *IN VITRO*

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SUMMARY

The effects of ovariectomy, adrenalectomy and ovariectomy–adrenalectomy on 5α-reduction of testosterone and corticosterone by female rat tissues have been studied. The results suggest that separate enzymes are responsible for 5α-reduction of testosterone and corticosterone. Removal of ovaries or adrenal glands or both had no effect on 5α-reduction of corticosterone by the liver or by pituitary and adrenal glands *in vitro*. Reduction of testosterone, however, was affected by ovariectomy in all tissues examined and by adrenalectomy in liver tissue. Ovariectomy resulted in an increase in testosterone 5α-reduction by the liver and by pituitary and adrenal glands. Adrenalectomy and ovariectomy–adrenalectomy produced an increase in testosterone 5α-reduction per unit of liver weight, but did not change the capacity of the liver to degrade testosterone, because of the decrease in liver weight caused by adrenalectomy. Adrenalectomy had no effect on testosterone-5α-reduction by the pituitary and ovariectomy–adrenalectomy caused the same changes in enzyme activity as ovariectomy alone.

Most of the androgenic effects of testosterone in target tissues are thought to be mediated by its 5α-reduced derivative, dihydrotestosterone (DHT) (17β-hydroxy-5α-androstan-3-one). DHT binds to receptors in the cytoplasm and nucleus [1–3] and thereby affects the mechanism of protein synthesis in target tissues. Testosterone is not only a substrate for 5α-reductase, but it also significantly modifies the activity of this enzyme in various tissues [4–7], either by its direct action or *via* the pituitary hormones.

The liver, adrenal gland and pituitary gland reduce corticosterone to 11β,21-dihydroxy-5α-pregnane-3,20-dione (dihydrocorticosterone, DHC). The purpose of this reduction is still an open question, because the unreduced form of corticosterone is the active hormone [8, 9], *i.e.* this form is bound to the receptors, *e.g.* to liver cells and lymphocytes.

It has been demonstrated that testosterone and corticosterone are reduced by different 5α-reductases in the liver [10–12] and adrenal glands [13]. This paper presents data on the effects of ovariectomy, adrenalectomy and ovariectomy–adrenalectomy on testosterone and corticosterone 5α-reduction by the liver, pituitary and adrenal gland tissue of adult female rats.

MATERIALS AND METHODS

Female rats of the Fischer strain, aged 3 to 4 months, were maintained in 12 h light and 12 h dark environment, in temperature and humidity controlled rooms, and on *ad libitum* food and water. Ovariectomy, adrenalectomy and sham operations were performed by dorsal approach under ether anesthesia. The adrenalectomized rats were given saline in place of tap water. The animals were killed 7 or 14 days after the operation. Liver, pituitary and adrenal gland tissue samples were taken immediately after decapitation, cut in pieces weighing about 2–5 mg, and incubated in 2 ml Krebs–Ringer solution at 37°C for 3 h in an atmosphere of 95:5, O_2–CO_2 with 200 ng of ^{14}C-labelled substrate. The weights of tissue samples used for incubation were as follows: liver approximately 25 mg, adrenal gland 20 mg (one gland) and pituitary approximately 9 mg (a whole pituitary).

[4-^{14}C]-Testosterone (59 mCi/mmol) and [4-^{14}C]-corticosterone (61 mCi/mmol) were obtained from the Radiochemical Centre, Amersham. [4-^{14}C]-DHT was obtained by biological conversion of labelled testosterone with rat prostate tissue slices and [4-^{14}C]-DHC by conversion of [4-^{14}C]-corticosterone with rat liver tissue as described previously [14]. All labelled hormones were tested for purity before use by thin-layer chromatography (t.l.c.).

Steroids were extracted from aqueous media with chloroform–methanol (2:1 v/v), and separated by t.l.c. on silica gel G (Merck, Darmstadt, GFR). The plates were first developed in n-heptane to remove impurities. Testosterone metabolites were separated by using the solvent system chloroform–acetone–n-heptane (4:1:3, by vol.). T.l.c. of corticosterone metabolites was carried out with benzene–acetone (9:5 v/v) on plates sprayed with ascorbic acid [14]. Autoradiography was performed by covering the plate with an X-ray film (Sanix, Fotokemika, Yugoslavia) for 7 days.

Table 1. 5α-reductase activity of liver tissue in adult female rats 7 and 14 days after ovariectomy, adrenalectomy and ovariectomy adrenalectomy

Treatment	Body weight (g)	Liver weight (g)	5α-reduced testosterone metabolites (dpm/mg)	5α-reduced corticosterone metabolites (dpm/mg)
Sham ovariectomy and adrenalectomy	158 ± 2.0	5.2 ± 0.19	1739 ± 98.3 (8)	748 ± 64.7 (6)
Ovariectomy				
- 7 days	149 ± 2.5	5.3 ± 0.17	2015 ± 138.5 (4)	893 ± 68.8 (4)
- 14 days	168 ± 9.8	5.9 ± 0.30	2274 ± 155.9 (4)[b]	644 ± 44.7 (4)
Adrenalectomy				
- 7 days	148 ± 2.2	5.0 ± 0.76	2071 ± 153.2 (4)	754 ± 45.2 (4)
- 14 days	134 ± 2.0	3.4 ± 0.91	2436 ± 175.4 (4)[a]	761 ± 73.9 (4)
Ovariectomy-adrenalectomy				
- 7 days	136 ± 1.1	4.2 ± 0.17	2110 ± 123.8 (4)[c]	872 ± 76.0 (4)
- 14 days	140 ± 5.3	4.0 ± 0.35	2361 ± 126.8 (4)[a]	632 ± 66.9 (4)

Mean ± SE; Student's t test was used to calculate the significance of differences among mean values; ( ) = number of observations;
a = significant difference compared to sham ovariectomized and adrenalectomized animals, $p < 0.01$
b = significant difference compared to sham ovariectomized, and adrenalectomized animals, $p < 0.02$
c = significant difference compared to sham ovariectomized and adrenalectomized animals, $p < 0.05$.

The activity zones, corresponding to shadows on the film were scraped off and counted in a Nuclear Chicago Mark II liquid scintillation counter using Permablend ™III scintillant (Packard, U.S.A.) with an average efficiency of 92% for ^{14}C.

The activity of 5α-reductase was expressed as the radioactivity of 5α-reduced products formed during a 3-h incubation per mg of wet tissue weight (d.p.m./mg).

To detect all the 5α-reduced products formed during incubation of a given tissue with testosterone the following procedure was used: two tissue samples of the same organ of an animal were incubated simultaneously under identical conditions. In one incubation labelled testosterone was used as substrate and in the other DHT of the same S.A. Since 5α-reduction is considered to be the first in a series of catabolic reactions, and it is, at the same time, irreversible [11], all products resulting from incubation with DHT remain reduced in the 5α-position. By a comparison of the two autoradiograms it was possible to discern the 5α-reduced products resulting from incubation with testosterone. The same procedure was used to detect 5α-derivatives of corticosterone by comparing the products resulting from incubation of corticosterone with derivatives from incubation of DHC. In this way the 5α-reduced metabolites in all the tissues incubated with testosterone and corticosterone as substrates were determined.

The results obtained were statistically evaluated by analysis of variance and the test of homogeneity of variance. Student's *t* test or Kramer's multiple range test were used to calculate the significance of differences among the mean values compared.

RESULTS

Effects of ovariectomy and/or adrenalectomy on 5α-reduction of liver tissue

In incubations of testosterone with liver tissue 58–81% of the substrate was converted to 5α-derivatives.

Ovariectomy caused an increase in liver 5α-reductase activity when testosterone was used as substrate (Table 1). On the 14th day after the operation the increase amounted to 31%. Adrenalectomy resulted in a 40% increase per mg of wet wt. 14 days after the operation and a similar increase (36%) was also obtained after the combined intervention.

Table 2. Liver capacity for conversion of testosterone and corticosterone in adult female rats following ovariectomy, adrenalectomy and ovariectomy–adrenalectomy

Treatment	Relative liver weight (g/100 g body weight)	5α-reduced testosterone metabolites dpm/100 g $\cdot 10^3$	5α-reduced corticosterone metabolites dpm/100 g $\cdot 10^3$
Sham ovariectomy and adrenalectomy	3.3 ± 0.13 (7)	5698 ± 425 (8)	2576 ± 192 (6)
Ovariectomy			
- 7 days	3.6 ± 0.09 (4)	7173 ± 443 (4)	3206 ± 313 (4)
-14 days	3.6 ± 0.09 (4)	7999 ± 513 (4)*	2282 ± 202 (4)
Adrenalectomy			
- 7 days	3.4 ± 0.05 (4)	6958 ± 541 (4)	2531 ± 141 (4)
-14 days	2.6 ± 0.10 (4)*	6175 ± 363 (4)	1905 ± 164 (4)
Ovariectomy-adrenalectomy			
- 7 days	3.1 ± 0.13 (4)	6535 ± 513 (4)	2719 ± 333 (4)
-14 days	2.9 ± 0.26 (4)**	6685 ± 459 (4)	1776 ± 141 (4)

Mean ± SE; Kramer's multiple range test was used to calculate the significance of differences among mean values; ( ) = number of observations;
* = significant difference compared to sham ovariectomized and adrenalectomized animals, $p < 0.05$
** = significant difference compared to animals ovariectomized for 14 days, $p < 0.05$.

Table 3. Pituitary 5α-reductase activity in adult female rats 7 and 14 days after ovariectomy, adrenalectomy and ovariectomy–adrenalectomy

Treatment	Body weight (g)	Pituitary weight (mg)	5α-reduced testosterone metabolites (dpm/mg)	5α-reduced corticosterone metabolites (dpm/mg)
Sham ovariectomy and adrenalectomy	157 ± 2.0	8.7 ± 0.51	373 ± 27.4 (7)	98 ± 41.8 (6)
Ovariectomy				
- 7 days	153 ± 1.9	8.3 ± 0.20	971 ± 56.8 (9)*	206 ± 51.8 (6)
- 14 days	167 ± 5.2	9.3 ± 0.34	1445 ± 149.9 (5)*	141 ± 21.6 (4)
Adrenalectomy				
- 7 days	150 ± 1.5	9.7 ± 0.48	421 ± 26.0 (4)	174 ± 44.4 (5)
-14 days	145 ± 6.0	9.2 ± 0.55	571 ± 54.3 (3)	47 ± 26.6 (4)
Ovariectomy–adrenalectomy				
- 7 days	141 ± 2.5	8.2 ± 0.25	1011 ± 142.9 (5)*	287 ± 36.4 (5)
- 14 days	147 ± 5.9	8.5 ± 0.36	1381 ± 101.3 (3)*	227 ± 93.7 (3)

Mean ± SE; Kramer's multiple range test was used to calculate the significance of differences among mean values; ( ) = number of observations;
* = significant difference in comparison to sham ovariectomized and adrenalectomized animals, $p < 0.01$.

Incubation of corticosterone with liver tissue yielded 21 to 30% conversion of the substrate to 5α-derivatives.

When corticosterone was used as substrate no change in liver 5α-reductase activity was noted 7 and 14 days after ovariectomy and/or adrenalectomy.

Adrenalectomy and ovariectomy–adrenalectomy resulted in a decrease in relative liver weight (Table 2). An increase in the capacity of the liver to degrade testosterone was found only 14 days after ovariectomy (43%). After adrenalectomy and ovariectomy–adrenalectomy no change in total liver capacity for degradation of testosterone occurred despite an increase in enzyme activity per unit of weight, because of the simultaneous liver weight decrease which follows adrenalectomy.

Ovariectomy and/or adrenalectomy induced no change in the capacity of the liver to degrade corticosterone.

Effects of ovariectomy and/or adrenalectomy on 5α-reduction in pituitary tissues

After incubation of testosterone with pituitary tissue the conversion of substrate to 5α-derivatives amounted to 4–18%.

As early as 7 days following ovariectomy pituitary 5α-reductase activity increased by 160% when testosterone was used as substrate (Table 3) and 14 days after the operation the increase amounted to 287%. Adrenalectomy had no effect on pituitary 5α-reductase activity. The increase in 5α-reductase activity, using testosterone as substrate, amounted to 169 and 270%, respectively, 7 and 14 days after ovariectomy–adrenalectomy.

In incubations of corticosterone with pituitary tissue the conversions of the substrate to 5α-derivatives amounted to 1–3%. Following ovariectomy and/or adrenalectomy 5α-reductase activity in pituitary tissue ranged from 47 to 287 d.p.m./mg when corticosterone was used as substrate, but the values found did not significantly differ from those obtained in the animals from the sham operated group.

Effect of ovariectomy on 5α-reduction in adrenal gland tissue

When testosterone was used as substrate (43–55% conversion rate to 5α-derivatives) adrenal tissue 5α-reductase activity increased by 35% between the measurements made 7 and 14 days after ovariectomy (Table 4).

Ovariectomy of 7 or 14 days had no effect on 5α-reductase activity when corticosterone was used as substrate and the conversion of the substrate amounted to 9–10%.

DISCUSSION

Since we performed incubation of tissue slices without addition of cofactors to the incubation medium,

Table 4. 5α-reductase activity in the adrenal glands of female rats 7 and 14 days after ovariectomy

Treatment	Body weight (g)	Adrenal gland weight (mg)	5α-reduced testosterone metabolites (dpm/mg)	5α-reduced corticosterone metabolites (dpm/mg)
Sham ovariectomy and adrenalectomy	157 ± 1.9	40.4 ± 1.55	1949 ± 154.2 (4)	341 ± 69.6 (7)
Ovariectomy				
- 7 days	150 ± 2.4	40.2 ± 1.26	1610 ± 49.7 (4)	363 ± 43.6 (4)
- 14 days	168 ± 9.8	37.8 ± 2.58	2178 ± 85.7 (4)*	344 ± 18.6 (4)

Mean ± SE; Kramer's multiple range test was used to calculate the significance of differences among mean values; ( ) = number of observations;
* = significant difference compared to the group of animals ovariectomized for 7 days, $p = 0.05$.

the values called "enzyme activity" denote the total capacity of a tissue to carry out 5α-reduction using the enzyme and coenzyme at its disposal. We believe that the data obtained in this way more closely reflect the true enzymatic changes in the tissues than would incubation with the addition of cofactors in excess.

The observation that the reduction of testosterone, but not of corticosterone, was affected by organ ablation suggests the existence of separate enzymes for the reduction of these two substrates.

In addition to earlier published evidence on the existence of several 5α-reductases in the liver [10–12], some recent reports have presented data on separate 5α-reductases for testosterone and corticosterone in the adrenal glands [13]. So far no data on the number and type of 5α-reductases in the pituitary have been published. However, Kniewald *et al.*[4] noted that addition of corticosterone to the incubation medium does not decrease testosterone reduction by pituitary tissue, while progesterone elicits a significant decrease in testosterone reduction by competing for the same enzyme. These findings support our idea of the existence of separate mechanisms for the 5α-reduction of testosterone and corticosterone in all the tissues examined.

The increase in 5α-reductase activity following ovariectomy could have been caused in several ways. It is possible that it was due to a direct effect of the ovarian hormones, not only estrogen and progesterone but also the androgenic hormones produced by the ovaries. According to Hilliard *et al.*[15] testosterone is the principal steroid in the rabbit ovarian venous effluent. The increase in pituitary testosterone-5α-reductase activity following ovariectomy could be reduced only by testosterone [5]. The ovarian hormones could act also indirectly by modification of pituitary gonadotrophic hormone secretion. In *in vitro* experiments FSH increases testosterone-5α-reductase activity in the pituitary [4]. It is possible that ovariectomy modifies the secretion of some other pituitary trophic hormones, which in turn become capable of influencing testosterone-5α-reductase either directly or via their secondary endocrine glands. According to some recent publications the pituitary hormones are responsible for the regulation of testosterone [16] and corticosterone [6, 17] metabolism in the liver during the neonatal period of life and in maturity.

An increase in testosterone-5α-reductase activity after ovariectomy has been observed also by other authors in the pituitary [5, 18] and adrenal glands [19]. Gustafsson and Stenberg[20] detected a decrease in testosterone-5α-reduction in the liver after long-term ovariectomy and we have found an increase in testosterone-5α-reductase activity 7 and 14 days after ovariectomy. Their and our results on liver testosterone-5α-reductase activity could be explained by the changes in FSH secretion after ovariectomy [21]. Increased FSH secretion persists until the 15th day post ovariectomy, when it is maximal. Our results refer to this time interval. After the 15th day post ovariectomy FSH secretion decreases, by the 30th day becomes lower than in intact animals and thereafter it persists at this low level. The low level of FSH secretion was accompanied by a decreased testosterone-5α-reductase activity in the liver found by Gustafsson and Stenberg[20] following long-term ovariectomy.

Whatever the mechanism for regulating 5α-reduction of testosterone and corticosterone is, our data indicate that these enzymes are not regulated by a common mechanism, but that there are two separate different mechanisms governing their activities.

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