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Letter to the Editor

Effect of Oophorectomy on Progesterone Metabolism in DMBA-Induced Mammary Tumours of the Rat*

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IN THIS Journal, we recently reported that the 5α-reductase activity in pregnancy-dependent mammary tumours of mice (GRS/A strain) was significantly reduced by oophorectomy, but increased when the animals were treated with oestradiol-17β [1]. In this regard, we present our current experimental results which indicate that oophorectomy causes a significant decrease in the specific activity of the 5α-reductase in the DMBA (9, 12-dimethyl-1, 2-benzanthracene)-induced mammary tumour of rats.

Mammary tumours were induced in female Sprague–Dawley rats by the oral administration of DMBA (20 mg dissolved in 1 ml olive oil per rat) at 55–60 days of age. In a few months, the tumour became palpable and was used for the present experiments. Approximately one half of each individual tumour was extirpated under ether anaesthesia, and simultaneously the host animals were oophorectomized. The residual portion of each tumour, left *in situ*, was extirpated 1 week later, after the operation. The change in size of the tumour due to the oophorectomy was examined by measuring it with calipers across the two major rectangular diameters. The two portions of the tumours thus obtained before and after oophorectomy were washed with ice-cold 0.25 M sucrose solution (pH 7.4) and homogenized separately using a Polytron 20 OD (Kinematica, Switzerland) in this cold isotonic solution (5 ml/g wet tissue). The homogenates were centrifuged at 800 ***g*** for 20 min and the supernatant fractions were used as the cell-free homogenates for incubation studies. [4-^{14}C]Progesterone (7.6×10^4 counts/min, 840 pmole) and [^{14}C]5α-pregnane-3, 20-dione (1.3×10^4 counts/min, 15.9 nmole) were aerobically incubated at 37°C for 1 hr with the cell-free homogenates (3.4–18.4 mg protein for the incubation of progesterone and 0.85–4.6 mg protein for that of 5α-pregnane-3, 20-dione) in the presence of 1.34 μmole NADPH in 10 ml Tris–HCl buffer (pH 7.4) containing 0.25 M sucrose and 1 mM $MgCl_2$ (final concentrations). Total incubation volume was adjusted to 5 ml. At the end of incubation, the radioactive steroid was extracted three times with 15 ml of methylene chloride. The extracted steroids were separated by thin-layer chromatography as described previously [2]. Protein contents in the homogenates were measured by the Folin–copper method [3].

Metabolites of [4-^{14}C]progesterone, namely: 5α-regnane-3, 20-dione; 3α-hydroxy-5α-pregnan-20-one; 20α-hydroxy-5α-pregnan-3-one; 5α-pregnane-3α, 20α-diol and 20α-hydroxy-4-pregnen-3-one were identified by the methods previously described [2]. When 5α-pregnane-3, 20-dione was employed as the substrate, 3α-hydroxy-5α-pregnan-20-one, 20α-hydroxy-5α-pregnan-3-one, and 5α-pregnane-3α, 20α-diol were isolated. The activities of the enzymes related to the metabolism were calculated from the total amounts of the relevant metabolites formed, and were expressed as pmole of products per mg protein

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Table 1. Effect of oophorectomy on size and 5α-reductase activity in the DMBA-induced mammary tumour of rats

Tumour No.	Tumour size (cm^2)			5α-Reductase activity (pmole products/mg protein/hr)		
	Intact	Oophorectomized	Change (%)	Intact	Oophorectomized	Change (%)
1	1.56	2.10	+35	6.7	2.4	−64
2	2.88	1.10	−62	23.5	7.0	−70
3	2.60	0.66	−75	34.5	10.8	−69
4	2.40	1.10	−54	29.7	20.5	−31
5	2.88	2.52	−12	25.4	10.9	−57
6	2.85	2.08	−27	8.7	9.0	+3
7	0.60	0.72	+20	23.2	6.9	−70
8	1.08	0.42	−61	22.1	22.0	0
9	2.72	1.43	−47	15.2	8.5	−44
10	2.10	2.52	+20	14.1	9.4	−33
Average ± S.E.	2.17 ± 0.26	1.47 ± 0.25*		20.3 ± 2.8	10.7 ± 1.9†	

*$P < 0.05$ per pair by t-test.
†$P < 0.01$ per pair by t-test.

per hr. Table 1 shows the changes in size of each tumour and in 5α-reductase activity after oophorectomy. Regression in the sizes of tumours and decrease in 5α-reductase activity were found statistically significant. However, within individual tumours, the change in 5α-reductase activity seemed to be uncorrelated with the degree of the regression in the tumour size. Neither the activity of 20α-hydroxysteroid dehydrogenase which was expressed as the relevant metabolites of progesterone or 5α-pregnane-3, 20-dione, nor the activity of 3α-hydroxysteroid dehydrogenase which was estimated from the 3α-reduced metabolites obtained from 5α-pregnane-3, 20-dione showed any systematic changes after oophorectomy (data not shown).

Among the enzymes related to the progesterone metabolism, the only activity significantly decreased by the oophorectomy was 5α-reductase. This is in agreement with the results previously reported on the progesterone metabolism by the mammary tumour of GRS/A strain mouse, which was dependent upon the pregnancy [1]. In contrast to the present results, Miller [4] reported that oophorectomy caused an increase in the 5α-reduction of testosterone in DMBA-induced mammary tumours of rats. Since the 5α-reductase activities toward testosterone and progesterone among individual tumours have been shown to be very closely correlated [2], a single species of 5α-reductase seems to be involved in the reduction of these two steroids. If so, the discrepancy between ours and Miller's results is difficult to explain. Apart from the use of different substrates, and the different expressions of 5α-reductase activity, the tumour slices in Miller's experiments were incubated in the presence of $NADP^+$, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase. On the other hand, we employed cell-free homogenates of the tumours and incubated them in the presence of excess NADPH.

Our previous studies indicated that 4-pregnene-3α,20α-diol, which was obtained as a unique metabolite of progesterone in normal mammary glands of mice and rats, was diminshed in the DMBA-induced mammary tumour due to the enhanced activity of 5α-reductase [2, 5]. In addition our present study suggested that the 5α-reductase activity in the tumours was dependent upon the ovarian function.

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