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SHORT COMMUNICATION

THE SOLUBLE FRACTION OF THE OVARY CONTAINS AN INHIBITOR OF STEROID 17α-HYDROXYLASE

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SUMMARY

The ovarian steroid-17α-hydroxylase activity is inhibited *in vitro* by the soluble fraction (100,000 *g* supernatant) isolated from the same tissue. This inhibitor is found in the ovarian gland only. It does not display any species specificity, but is also active on the testes and the adrenal gland 17α-hydroxylase. It is heat-labile, cannot be dialyzed and is precipitated by ammonium sulphate (0–30%).

INTRODUCTION

Steroid-17α-hydroxylase catalyses the first step in the biotransformation of progesterone (or pregnenolone) into estrogens[1]. It seems likely that the 17α-hydroxylase plays an important regulatory role in the biosynthesis of ovarian hormones: (a) the relative proportion of estrogens and progesterone secreted by the ovaries varies along the menstrual cycle; (b) the plasma concentration of 17-hydroxyprogesterone reaches a peak at the time of ovulation[2].

We have recently described a new and sensitive assay to measure the activity of progesterone and pregnenolone-17α-hydroxylase based on the specific exchange of the 17α-proton by a hydroxyl group during the enzymatic oxidation[3]. Due to the hydroxylation, the 17α-tritiated progesterone (or pregnenolone) released a quantity of tritiated water directly proportional to the enzymatic activity into the medium[3].

In this communciation, we report an inhibition of the steroid-17α-hydroxylase by the 100,000 *g* supernatant of ovarian gland homogenate.

MATERIALS AND METHODS

(a) *Enzymatic preparation.* Cow, rabbit or rat ovaries were collected in ice cold isotonic KCl. The glands were first disrupted in a mixer and then homogenized in four vol. of isotonic KCl using a Potter Elvehjem–device. The homogenates were centrifuged for 10 min at 9,000 *g* and the supernatants were used directly as the enzymatic source or centrifuged for 60 min at 100,000 *g* in order to isolate the microsomes from the soluble fractions. The microsomal pellet from 2 g of wet gland was resuspended in 1 ml 0.15 M phosphate buffer, pH 7.4.

(b) *Enzymatic assay.* The assay of progesterone and pregnenolone-17α-hydroxylase[3] and the synthesis of the 17-[^{3}H]-progesterone and 17-[^{3}H]-pregnenolone have been described[4]. Cholesterol-7α-hydroxylase and progesterone-16α-hydroxylase were measured by a technique based on the same principle and described elsewhere[5, 6].

RESULTS AND DISCUSSION

The ovarian 17α-hydroxylase activity was proportional to the amount of enzyme added to the incubation medium, but the shape of the curve (Fig. 1) was quite different depending upon the nature of the enzymic preparation. With isolated microsomes (100,000 *g* pellet), the enzymic activity increased linearly up to a quantity of enzymes corresponding to 0.2 g of gland and the curves reached a plateau. The hydroxylase activity was also proportional to the amount of 9,000 *g* supernatant, but was strongly inhibited when the enzyme concentration exceeded a quantity equivalent to 0.02 g of fresh tissue.

As expected from those results, the explanation of this phenomenon is the presence of an inhibitor(s) in the 100,000 *g* supernatant fraction of the ovarian glands. Table 1 shows that the addition of 100,000 *g* supernatant to an active population of isolated microsomes was able to prevent completely the hydroxylase activity.

The testes and adrenal 17α-hydroxylase activity were not inhibited by their own 100,000 *g* supernatant. Nevertheless, it does not seem that the inhibition exhibits species specificity (Table 2).

On the other hand, 100,000 *g* supernatants prepared from testes or adrenal gland homogenates did not affect the hydroxylase activity of the ovarian gland microsomal preparation at all (Table 2).

An inhibitory effect could not only be obtained with cow ovaries, but was also demonstrated in rabbit and rat ovaries. No species specificity could be shown, 100,000 *g* supernatant prepared from ovaries of one species inhibiting

Table 1. Progesterone-17α-hydroxylase activity as a function of the quantity of ovarian microsomes and 100,000 *g* supernatant incubated

Microsomes (ml)	100,000 *g* Supernatant (ml)	Enzymatic activity (pmol/min)
0.2	—	6.9
0.1	—	3.5
0.2	0.1	0.8
0.2	0.2	0
—	0.2	0

The protein concentrations were respectively, 10 mg/ml of microsome preparation and 17 mg/ml of 100,000 *g* supernatant. The samples were incubated in a final vol. of 1 ml at 37°C with air as gas phase.

Table 2. Progesterone-17α-hydroxylase activity after incubation of microsomes from different tissues and in the presence of 100,000 *g* supernatant from adrenal, testis or ovary

Microsomes (mg of protein)		100,000 *g* Supernatant (mg of protein)		Enzymatic activity (pmol/min)
rat testis	1.5		—	30.8
	3		—	60.8
	4.5		—	103
	1.5	rat testis	1.5	30
	1.5		3	38
	1.5	cow ovary	1.5	5
	1.5		3	0
beef adrenal	1.3		—	69
	2.6		—	141
	3.9		—	175
	1.3	adrenal	1.5	68
	1.3	adrenal	3	74
	1.3	cow ovary	1.5	15
	1.3		3	0
cow ovary	1		—	3.5
	2		—	6.9
	1	adrenal	1.5	3.4
	1		3	3.6
	1	testis	1.5	3.5
	1	testis	3	3.7

with the same efficiency the 17α-hydroxylase of the other two species. The effect of the ovarian 100,000 *g* supernatant was also tested on two hepatic steroid hydroxylases, progesterone-16α-hydroxylase and cholesterol-7α-hydroxylase, but the ovarian preparation did not modify these enzymatic activities (data not shown).

Table 3 shows that the inhibitory action could be abolished by boiling the supernatant, but could not be eliminated by dialysis. The inhibitor was precipitated from the soluble fraction between 30 and 50% ammonium sulphate saturation (Table 3). After ammonium sulphate precipitation, the inhibitor(s) was unstable and Mercaptoethanol, progesterone or ascorbic acid did not stabilize the product. Using chromatography on sephadex G 100 of the ammonium sulphate precipitate, we were able to demonstrate that the inhibitor(s) was eluted with the void volume indicating a molecular weight of over 150,000.

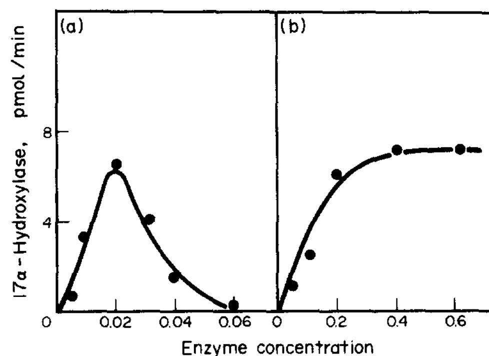

Fig. 1. Progesterone-17α-hydroxylase activity in 9000 *g* supernatant (a) and in microsomal preparation (b) of cow ovary. Enzyme concentrations are given in g of wet gland per ml of incubation.

Table 3. Inhibition of ovarian progesterone-17α-hydroxylase by the soluble fraction of cow ovary

Supernatant treatment	Enzymatic activity (pmol/min)
0.2 ml Untreated	0.8
0.2 ml Boiled	6.0
0.2 ml Dialyzed	1.0
0–30% $(NH_4)_2SO_4$ Precipitate	4.1
0–50% $(NH_4)_2SO_4$ Precipitate	0
30–50% $(NH_4)_2SO_4$ Precipitate	0
Boiled 30–50% $(NH_4)_2SO_4$ Precipitate	6.0
0–30% $(NH_4)_2SO_4$ Supernatant	2.1
30–50% $(NH_4)_2SO_4$ Supernatant	5.5

The 100,000 *g* supernatant was either boiled for 10 min, dialyzed overnight against a phosphate buffer (0.1 M, ph 7.4) or treated by ammonium sulphate. Incubation conditions are as described in Table 1.

In conclusion, the soluble fraction of the ovarian gland homogenate contains a strong inhibitor of 17α-hydroxylase. This inhibitor was only found in the ovaries of different species (rat, rabbit, cow, as well as in the only human sample we could obtain). Nature and mechanism of action of this inhibitor remains unclear and needs further experimentation. Nevertheless, it does not seem that there is a species specificity.

Concerning the role of this inhibitor, several hypotheses are obviously possible. One of those would be a control of the estrogen biosynthesis by a block at the 17α-hydroxylase level of their biosynthetic pathway. Incubation of human corpus luteum with [^{14}C]-acetate led to an important incorporation of ^{14}C into progesterone and to no detectable radioactivity incorporation into androgens and estrogens[7]. To explain a similar phenomenon in the guinea pig adrenal cortex, Strott[8] has very recently suggested that a cytoplasmic macromolecule bound the enzymatic substrate and therefore withheld it from the enzyme active site.

Further experimentation will be necessary to understand the role and the physiological importance of this inhibitor.

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