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Effects of Hypophysectomy on the Functions of Ovarian Mitochondria of Mature Rats[1] (39930)

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Ovarian mitochondria of immature rats showed increases in specific cytochrome oxidase activity and decreases in the ADP:O ratio and in the succinate oxidation rate after gonadotropic treatment (1). Although these changes in ovarian mitochondria were thought to be associated with their ability to convert cholesterol to pregnenolone, it was not determined if these mitochondrial parameters would change after the removal of gonadotropic stimulation or after a decrease in steroidogenic activity. To answer this question, functional differences in mitochondria prepared from ovaries of intact, mature rats, hypophysectomized mature rats and PMSG-treated, hypophysectomized mature rats were determined.[2]

Materials and methods. Intact and hypophysectomized Sprague-Dawley rats (175–200 g) were purchased from Hormone Assay Co., Chicago, Ill. and were maintained in our animal care facilities for at least 3 days prior to their use in experiments. The stage of the estrous cycle of intact rats was not considered in these studies because of the necessity of pooling the ovaries of several rats for each experiment. Hypophysectomized rats were given 5% glucose solution for drinking water, and serum GH levels of several rats were measured by the method of Garay *et al.* (2) as a means of validating the thoroughness of the operation. One group of hypophysectomized rats was untreated, while another group was injected sc with 40 IU of PMSG and killed 54 hr later. All hypophysectomized rats were used approximately 2–4 weeks after their operation.

Ovaries of 8 to 12 rats were pooled and homogenized in 250 m*M* sucrose solution, pH 7.4, containing 20 m*M* KCl and 1 m*M* EDTA, and were prepared as previously reported (1). Oxygen consumption was measured polarographically with a Clark Model 5331 electrode (Yellow Springs Instruments) using a modification of the method of Estabrook (1, 3). The reaction mixture of 1.1-ml vol, pH 7.4, 25°, contained 225 m*M* mannitol, 70 m*M* sucrose, 1 m*M* EDTA, 10 m*M* potassium phosphate, and 7.6 m*M* succinate or glutamate. State III respiration (mitochondrial oxygen uptake rate in the presence of exogenous ADP), state IV respiration (the rate after phosphorylation of the exogenous ADP), respiratory control (the ratio of state III to state IV respiration), and the ADP:O ratio (moles of exogenous ADP used per atom of oxygen) were measured by adding 25 μl of 4 m*M* ADP to the reaction mixture. The ADP concentration was checked weekly by the method of Adam (4).

Mitochondrial cholesterol oxygenase activity was determined by measuring the percentage of [4-^{14}C]cholesterol converted to [4-^{14}C]pregnenolone and [4-^{14}C]progesterone. The assay used for these studies was that of Robinson and Stevenson (5) as previously modified by our laboratory (1). Briefly, 1 mg of mitochondrial protein was incubated with 20,000 cpm of [4-^{14}C]cholesterol (50–60 Ci/mmole; New England Nuclear) and 0.76 m*M* succinate for 2 hr at 37°. The steroids were extracted, and [4-^{14}C]cholesterol, [4-^{14}C]pregnenolone, and [4-^{14}C]progesterone were separated by thin-layer chromatography and counted.

Protein concentration was assayed by the method of Lowry *et al.* (6) and cytochrome oxidase activity was measured as the rate of oxidation of reduced cytochrome *c* (7). Total serum estrogen and serum progesterone were determined by radioimmunoassays (1) to characterize the steroidogenic activity of the three experimental groups.

Results. Total serum estrogen levels were

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[2] PMSG (pregnant mare serum gonadotropin) was a gift of the National Institute of Arthritis, Metabolism and Digestive Diseases.

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0.103 ± 0.009 ng/ml for intact mature rats, 0.029 ± 0.005 ng/ml for hypophysectomized rats, and 0.33 ± 0.020 ng/ml for PMSG-treated, hypophysectomized rats. Serum progesterone levels were 27.2 ± 4.1, 3.8 ± 0.4, and 22.4 ± 3.8 ng/ml for intact, rats, hypophysectomized rats and PMSG-treated, hypophysectomized rats, respectively.

There were no differences in specific cytochrome oxidase activities (activity per milligram of mitochondrial protein) in mitochondria of the three experimental groups (Table I). However, the percentage of [4-^{14}C]cholesterol conversion was significantly lower in mitochondrial preparations of ovaries of hypophysectomized rats than in those of intact rats. Mitochondria from hypophysectomized rats treated with PMSG showed significant increases in the percentage of [4-^{14}C]cholesterol conversion as compared to mitochondria of the other two experimental groups.

Using glutamate as substrate, no differences were found in states III and IV oxygen utilization rates or respiratory control of mitochondria of the three experimental groups (Table II). However, the ADP:O ratio of mitochondria of hypophysectomized rats was significantly greater than that of mitochondria of intact rats. PMSG treatment of hypophysectomized rats returned the ADP:O ratio to the level found in mitochondria of intact mature rats.

TABLE I. CYTOCHROME OXIDASE AND STEROIDOGENIC ACTIVITIES OF OVARIAN MITOCHONDRIA OF INTACT, HYPOPHYSECTOMIZED AND PMSG-TREATED, HYPOPHYSECTOMIZED MATURE RATS.[a]

	Cytochrome oxidase activity ($k \times 10^{-2}$/mg of mitochondrial protein)	Percentage of [4-^{14}C]cholesterol conversion
Intact (5)[b]	6.11 ± 0.71	2.9 ± 0.4
Hypophysectomized (5)[c]	6.46 ± 0.58	n.d.[e]
PMSG-treated, hypophysectomized (4)[d]	7.01 ± 0.69	4.7 ± 0.6[e]

[a] Values represent means ± SE. Numbers of experiments for each group are given in parentheses.
[b] Intact: ovaries of sexually mature rats (175–200 g).
[c] Hypophysectomized: ovaries of mature rats hypophysectomized 2–4 weeks prior to experiment.
[d] PMSG-treated, hypophysectomized; ovaries of hypophysectomized mature rats that received 40 IU of PMSG 54 hr prior to experiment.
[e] $P < 0.01$, experimental vs intact.

With succinate as substrate, states III and IV oxygen uptake rates and the ADP:O ratio were significantly greater in ovarian mitochondria of hypophysectomized rats than in mitochondria of intact rats (Table III). Gonadotropic treatment of hypophysectomized rats decreased oxygen uptake rates and the ADP:O ratio to levels which were comparable to those of preparations of intact rats. There were no differences in respiratory control among the three experimental groups.

Discussion. The development and maintenance of the ovarian mitochondrial cholesterol oxygenase system is dependent upon gonadotropic stimulation. This conclusion is supported by results showing that the cholesterol conversion rate disappears in ovarian mitochondria of hypophysectomized rats and reappears in hypophysectomized rats after PMSG treatment. On the other hand, specific cytochrome oxidase activity of mitochondria did not differ among the three experimental groups, indicating that at least this enzyme complex is not dependent upon gonadotropic stimulation. This finding is in contrast to previous studies showing that specific cytochrome oxidase is lower in mitochondria of immature rats than in those of gonadotropic-treated rats (1). It can be hypothesized that there is a maturation of the cytochrome oxidase complex, and possibly of other parts of the respiratory chain, which is independent of gonadotropins for its maintenance. However, the increase in cytochrome oxidase activity in mitochondria of immature rats after PMSG treatment suggests that gonadotropic stimulation might initiate and/or accelerate maturation of the cytochrome oxidase complex, and possibly of the entire respiratory chain, in ovarian mitochondria of immature rats.

Hypophysectomy increased the ADP:O ratio and the succinate oxidation rates in ovarian mitochondria, while PMSG treatment of hypophysectomized rats returned these oxidative phosphorylation parameters to the values of intact mature rats. While these present studies were not concerned with the reason for lower ADP:O ratios in mitochondria from steroidogenically active ovaries, previous experiments using inhibi-

TABLE II. OXIDATIVE PHOSPHORYLATION BY OVARIAN MITOCHONDRIA OF INTACT, HYPOPHYSECTOMIZED, AND PMSG-TREATED, HYPOPHYSECTOMIZED MATURE RATS WITH GLUTAMATE AS SUBSTRATE.[a]

	Oxygen uptake (ng atoms of O/min/mg of protein)		Respiratory control	ADP:O
	State III	State IV		
Intact (5)	29.9 ± 2.2	13.1 ± 1.1	2.30 ± 0.21	2.31 ± 0.10
Hypophysectomized (5)	26.2 ± 2.6	11.0 ± 0.8	2.54 ± 0.16	2.82 ± 0.06[b]
PMSG-treated, hypophysectomized (4)	29.2 ± 2.8	12.7 ± 0.9	2.29 ± 0.27	2.39 ± 0.11

[a] Values represent means ± SE. Numbers of experiments for each group are given in parentheses. Experimental groups are the same as those given in Table I.
[b] $p < 0.01$, hypophysectomized vs. intact.

TABLE III. OXIDATIVE PHOSPHORYLATION BY OVARIAN MITOCHONDRIA OF INTACT, HYPOPHYSECTOMIZED, AND PMSG-TREATED, HYPOPHYSECTOMIZED MATURE RATS WITH SUCCINATE AS SUBSTRATE.[a]

	Oxygen uptake (ng atoms of O/min/mg of protein)		Respiratory control	ADP:O
	State III	State IV		
Intact (6)	87.3 ± 4.9	43.6 ± 2.5	2.10 ± 0.18	1.25 ± 0.07
Hypophysectomized (5)	114.3 ± 2.6[b]	55.1 ± 1.4[b]	2.11 ± 0.05	1.58 ± 0.08[b]
PMSG-treated, hypophysectomized (4)	93.1 ± 4.1	39.9 ± 3.9	2.23 ± 0.20	1.31 ± 0.07

[a] Values represent means ± SE. Numbers of experiments for each group are given in parentheses. Experimental groups are the same as those given in Table I.
[b] $p < 0.01$, hypophysectomized vs intact.

tors suggest that there is both an increase in oxygen consumption and a decrease in ATP synthesis associated with mitochondrial steroidogenesis (1). The lower succinate oxidation rates in steroidogenic mitochondria, while difficult to explain, nevertheless shows another parameter of mitochondrial function which is influenced by gonadotropic stimulation.

In summary, these data show that ovarian mitochondria of mature rats are dependent upon gonadotropic stimulation for their steroidogenic activity, but not for their cytochrome oxidase activity. Furthermore, oxidative phosphorylation in ovarian mitochondria of mature rats may be influenced by cholesterol oxygenase activity.

Summary. Steroidogenic activity and oxidative phosphorylation were measured in ovarian mitochondria of intact mature rats, hypophysectomized mature rats and gonadotropic-treated, hypophysectomized mature rats to determine the dependence of these parameters on gonadotropic stimulation. Mitochondrial cholesterol oxygenase activity was not detectable in ovarian preparations of hypophysectomized rats, but increased dramatically 54 hr after treatment with 40 IU of pregnant mare serum gonadotropin (PMSG). In contrast, specific cytochrome oxidase activities were similar in mitochondria of all three experimental groups. With both glutamate and succinate as substrates, the ADP:O ratio of ovarian mitochondria of hypophysectomized rats increased significantly over that of mitochondria of intact rats. PMSG treatment of hypophysectomized rats returned the ADP:O ratio to the value observed in intact rats. The succinate oxidation rate of ovarian mitochondria of hypophysectomized rats was significantly greater than those of preparations from intact and from PMSG-treated, hypophysectomized rats. These results indicate that steroidogenic activity in ovarian mitochondria is dependent upon gonadotropic stimulation for its initiation and maintenance, but that cytochrome oxidase activity is not. Also, differences in oxidative phosphorylation between mitochondria of steroidogenically active and inactive ovaries may be related to their cholesterol oxygenase activity.

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