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Ovarian Dehydrogenase Activities: Suggested Adrenal Involvement in Luteolysis[1] (39226)

V. DANIEL CASTRACANE AND JAMES H. LEATHEM[2]

Bureau of Biological Research, Rutgers University, New Brunswick, New Jersey 08903

McKerns has suggested that dehydrogenase activity, particularly linked to NADP, in both adrenal (1) and ovary (2) may serve as a direct source of reducing potential for steroidogenic reactions. Attention was directed to glucose-6-phosphate dehydrogenase (G6PD) as an essential event following trophic hormone stimulation. Recent evidence suggests that cytoplasmic reducing potential may become effective in intramitochondrial reactions only in very special situations (3, 4), and that more often, some form of intramitochondrial generation of reducing potential for steroidogenesis occurs (4, 5). Even though the role of cytoplasmic NADP-dehydrogenases is in question (6), these enzymes, particularly G6PD, have been shown to increase dramatically after trophic hormone stimulation, suggesting some role, perhaps metabolic, in the stimulated cell. To examine this question several ovarian dehydrogenases were studied in the PMS-treated rat, either with or without adrenalectomy and during a period of corpus luteum involution after cessation of PMS administration.

Materials and methods. Immature female rats from our own Long-Evans colony were fed Purina Laboratory Chow until they attained a body weight of 60-65 g, after which they were fed a semipurified diet containing 20% casein and 25% fat (7). Rats were treated with 20 IU PMS (Equinex, Ayerst), sc daily for 10 days, with or without adrenalectomy on Day 6, and were autopsied 10 days after the last PMS injection. Following PMS withdrawal, adrenalectomized rats were either untreated or given corticosterone (0.5 mg) or progesterone (4 mg) daily. Intact animals, with and without PMS treatment, served as controls. Animals were autopsied after an ip injection of sodium pentobarbital (Nembutal), 50 mg/kg of body weight. Tissue samples for enzyme assay were rapidly removed, trimmed of adherent tissue, weighed, and either placed in ice-cold homogenizers for immediate homogenization and assay or were stored on Dry Ice until assayed within several days. The ovaries were analyzed for glucose-6-phosphate dehydrogenase (G6PD), 6-phosphogluconate dehydrogenase (6PGD) (8, 9), NADP-linked isocitrate dehydrogenase (ICDH) (10), NADP-linked malate dehydrogenase (MDH) (11), and lactic dehydrogenase (LDH) activities (12). The activity of 20α-hydroxysteroid dehydrogenase (20α-OHSD) was also measured relative to the reduction of progesterone (13). In all cases, enzyme activity was presented as per milligram of protein (14). Statistical comparisons were made using the Student's t test.

Results. PMS treatment for 20 days caused a significant increase in ovarian glucose-6-phosphate, 6-phosphogluconate, malate, and isocitrate dehydrogenase activity. Furthermore, ovarian enzyme activity exceeded that of the controls when PMS treatment was limited to 10 days followed by a 10-day post-treatment period (G6PD excepted). Conversely the NAD-linked dehydrogenase LDH was not increased (Table I).

Gonadotrophin treatment for 10 days with adrenalectomy on Day 6, followed by a 10-day post-treatment period, resulted in a significantly greater G6PD activity than in similarly treated intact rats. On the other hand, malate dehydrogenase activity was less in the ovaries of adrenalectomized rats, but still significantly greater than untreated controls. Ovarian ICDH and LDH activities were not influenced by adrenalectomy.

Administration of corticosterone (0.5 mg)

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[2] Author from whom to request reprints: Bureau of Biological Research, Rutgers University, New Brunswick, New Jersey 08903.

TABLE I. EFFECT OF PMS AND ADRENALECTOMY ON OVARIAN DEHYDROGENASE ACTIVITIES.[a]

Treatment and days	G6PD[b]	6PGD[b]	MDH[b]	ICDH[b]	LDH[b]	20α-OHSD[c]
None × 20	11.2 ± 1.1	3.6 ± 0.2	4.2 ± 0.4	21.6 ± 2.0	40.0 ± 1.1	–
PMS × 20	19.3 ± 0.7	9.1 ± 0.4	28.8 ± 2.3	40.7 ± 1.4	34.8 ± 2.5	–
PMS × 10	12.7 ± 1.0	5.3 ± 0.3	15.0 ± 1.1	32.6 ± 1.3	43.0 ± 1.7	25.1 ± 2.7
None × 10						
PMS × 5-ADX - PMS × 5-None × 10	23.3 ± 1.8	5.2 ± 0.3	10.5 ± 1.2	36.6 ± 1.4	44.2 ± 2.7	91.1 ± 3.7
PMS × 5-ADX - PMS × 5-Corticosterone × 10	12.2 ± 1.6	5.4 ± 0.2	12.4 ± 1.2	–	–	53.3
PMS × 5-ADX - PMS × 5-Progesterone × 10	15.4 ± 1.4	6.1 ± 0.3	11.9 ± 1.2	–	–	70.7 ± 8.7

[a] ± = Standard error of mean.
[b] Micromoles of NADP or NAD reduced per minute per milligram of protein.
[c] Nanomoles of NADPH formed per minute per milligram of protein. Eight to thirteen rats per group.

for 10 days following PMS withdrawal in adrenalectomized rats completely prevented the anticipated increase in ovarian G6PD. Progesterone (4 mg) was equally effective. On the other hand, steroid treatment had no significant effect on malate dehydrogenase activity in similarly treated rats.

Ovarian 20α-OHSD activity is very low or not detectable in the immature rat ovary, but was sharply elevated in rats 10 days after the last of 10 daily injections of PMS. However, if the rat was adrenalectomized on Day 6 of the PMS treatment regime, then an even greater increase in 20α-OHSD activity occurred (25.1 vs 91.1 nmole NADPH/min/mg of protein). Treatment of the adrenalectomized rats with corticosterone or progesterone during the 10-day PMS withdrawal period moderated the increase in enzyme activity (53.4 and 70.7 nmole NADPH/min/mg of protein).

Discussion. An adrenal–ovarian interrelationship is well known, and an adrenomimetic ovarian function has been suggested for the luteinized ovary induced by PMS as survival of adrenalectomized rats is prolonged (15). Furthermore, withdrawal of PMS is followed by ovarian regression and biochemical evidence of adrenal insufficiency (16). Associated with this decline in ovarian weight was a sharp rise in 20α-OHSD activity. Since an increase in the activity of 20α-OHSD has been observed in the rat during luteolysis (17), adrenalectomy would appear as enhancing the luteolytic process as has been suggested previously (18). Both corticosterone and progesterone proved partially protective as replacement treatment. However, corticoids may also induce luteolysis with a rise in 20α-OHSD when given to pregnant animals (19).

Ovarian G6PD increases at the termination of pregnancy in a pattern similar to 20α-OHSD (20). Current studies reveal that adrenalectomized–gonadotrophin-treated rats exhibit a significant rise in ovarian G6PD following PMS withdrawal. Furthermore, corticosterone or progesterone can prevent this suggested luteolytic activity.

Malate dehydrogenase activity tends to decline at the time of luteolysis during pregnancy in a pattern essentially opposite to that of 20α-OHSD (20). A similar pattern of MAD activity was noted in the current studies in which enzyme activity declined after PMS withdrawal, and the decrease was enhanced by adrenalectomy.

Ovarian ICDH activity which does not change at the termination of pregnancy (20) seemingly does not reflect the luteolytic changes which follow adrenalectomy.

Thus, on the basis of ovarian enzyme activity, adrenalectomy appears to enhance luteolysis and may relate to a decreased response to gonadotrophins (21), a modified gonadotrophin environment (22), or a direct or indirect effect of elevated ACTH (23).

Summary. Immature rat ovarian dehydrogenase activity was studied during corpus luteum regression following withdrawal of prior pregnant mare serum gonadotrophin. Glucose-6-phosphate dehydrogenase activity declined to nontreatment levels whereas

6-phosphogluconate, malate, and isocitrate dehydrogenase dehydrogenases exhibited a partial return to normal. Adrenalectomy prior to PMS withdrawal enhanced the decline in MAD while sharply elevating G6PD and 20α-hydroxysteroid dehydrogenase. Corticosterone and progesterone prevented the G6PD changes induced by adrenalectomy and moderated the rise in 20α-OHSD. Adrenalectomy appears to enhance the process of luteolysis.

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