Luteolytic Role of Prolactin During the Estrous Cycle of the Rat (35711)

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During each estrous cycle of the rat, luteolysis of the corpora lutea (CL) formed during the previous cycle occurs (1, 2). The mechanism responsible for luteolysis of each successive crop of CL is unknown, but LH has been suggested to be the responsible agent (3). Prolactin administration has been demonstrated to exert a luteolytic action on the CL under some conditions, particularly when they are nonfunctional as in hypophysectomized rats (4–6). Since serum prolactin concentrations during each cycle are highest on the days of proestrus and estrus, reaching peak levels on the afternoon of proestrus (7–9), it is possible that prolactin is responsible for luteolysis of the earlier crop of CL formed during each cycle.

We recently observed that a single injection of the drug, ergocornine (EC) at 1:30 p.m. on the day of proestrus, completely blocks the normal rise in serum prolactin on the late afternoon without interfering with ovulation on the subsequent day (10). Daily injections of EC or implantation of a small amount of EC in the median eminence inhibit prolactin release throughout the estrous cycle, but do not interfere with regularity of the cycle (10). Administration of EC to cycling rats for 15 days resulted in significant enlargement of the ovaries (11), due to the accumulation of many old CL. Heuson *et al* (12) reported that injections into rats of ergocryptine, a drug related to EC, also induced enlargement of the ovaries with numerous CL. In view of the above observations, it was of interest to determine whether administration of EC could prevent luteolysis of the CL during the cycle, and whether administration of prolactin during proestrus or proestrus and estrus could produce luteolysis in EC-treated rats.

Materials and Methods. Mature 3–4-month-old female Sprague-Dawley rats were obtained from Spartan Research Animals, Haslett, Mich. The rats were housed in a temperature controlled (75 ± 1°F) and artificially illuminated (lights on from 5 a.m. to 7 p.m.) room, and given Wayne Lab Blox pellets and water *ad libitum*. Estrous cycles were followed by taking daily vaginal smears for 2 weeks prior to the beginning of the experiments, and only rats undergoing regular 4- or 5-day cycles were used.

EC-methanesulfonate[3] (base) was first dissolved in 70% ethanol and then in 0.9% saline to give a final volume of 4% ethanol. Ovine prolactin (NIH-P-S8, 28 IU/mg) was dissolved in 0.9% saline.

1. Effects of EC with or without prolactin on the ovaries during a single estrous cycle. A total of 26 rats were each injected ip with 50 μg of EC/100 g of body wt at 9 a.m. and 6 p.m. daily, beginning on the last p.m. of diestrus prior to the expected day of proestrus and continuing for 3 days thereafter. This dose of EC was previously shown to completely block any rise in serum prolactin during the estrous cycle and not to interfere with the p.m. rise in serum LH on the day of proestrus (10). At 2 p.m. on the day of proestrus, 14 of the 26 rats were each injected subcutaneously with a single dose of 1 mg of prolactin. A group of 13 control cycling rats were each injected ip daily with the saline–ethanol solution during the same period as the above rats. At the end of treatment,

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TABLE I. Effects of Ergocornine (EC) and Prolactin During One Estrous Cycle on Ovaries.

Treatment and no. of rats	Av Body wt (g)	Av Ovarian wt (mg)	Av No. of corpora lutea[a]
Saline (13)	253.3 ± 3.8	64.3 ± 2.3	6.5 ± 0.4
EC (12)	238.8 ± 5.0	78.4 ± 2.7[b]	9.3 ± 0.0[c]
EC + prolactin (14)	230.7 ± 3.8	68.4 ± 2.2	6.9 ± 0.5

[a] Represents single cross-section count.
[b] EC vs saline, and EC vs EC + prolactin: $p < 0.001$; [c] $p < 0.001$.

during diestrus, the rats were killed; and the ovaries were removed, cleaned, weighed, fixed in Bouin's fluid, and stained with hematoxylin and eosin for microscopic examination. The number of CL were counted from a single section taken from the longest axis of individual ovaries from all rats of each group.

2. Effects of EC with or without prolactin on the ovaries during 3 estrous cycles. A total of 24 rats were each injected with 50 μg of EC at 9 a.m. and 6 p.m. daily, beginning on the evening prior to the expected day of proestrus, and continuing for 13–16 days (3 cycles). During each cycle, at 2 p.m. on the day of proestrus and again at 9 a.m. on the first day of estrus, 11 of the 24 rats were each injected with 1 mg of prolactin. A group of 12 control rats were injected daily for 3 cycles with the saline–ethanol vehicle. All rats were killed during the diestrus after the third cycle, and the ovaries were removed and treated as in the first experiment.

Results. 1. Effects of EC with or without prolactin on the ovaries during a single estrous cycle. Injections with EC resulted in a significant increase in ovarian weight as compared with controls (Table I). On the other hand, the ovaries of rats injected with EC and prolactin weighed about the same as in control rats. The number of CL per ovary in the EC-treated rats was significantly greater

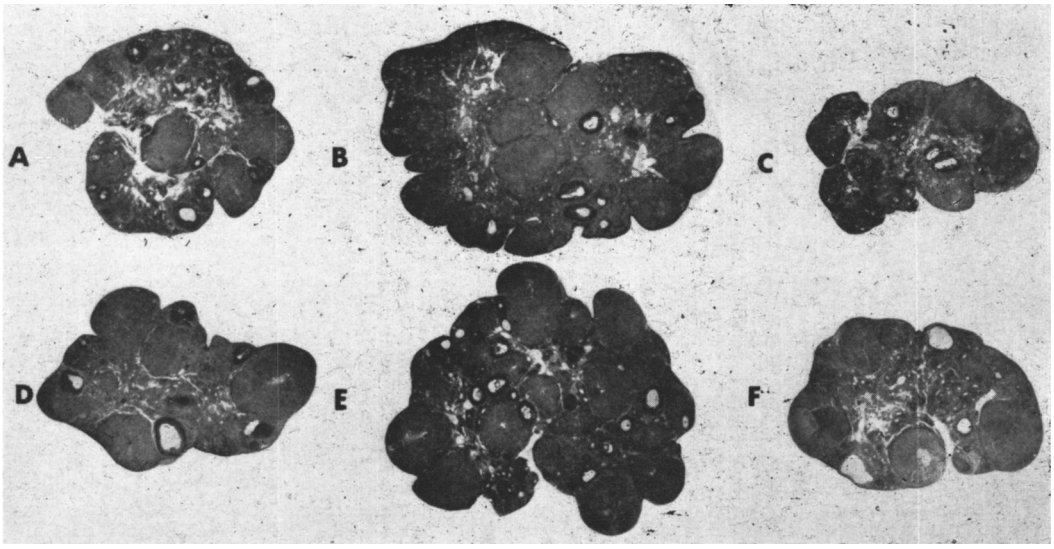

FIG. 1A–F. Representative ovary from: (A) control rat, single cycle; (B) EC-treated rat, single cycle; (C) EC and prolactin, single cycle; (D) control rat. 3 cycles; (E) EC-treated rat, 3 cycles; (F) EC and prolactin, 3 cycles. Note increase in number of CL in ovaries of EC-treated rats, and decrease in CL to about the same number as in controls in ovaries from rats given EC and prolactin.

than the CL per ovary in the controls or rats given both EC and prolactin. Figure 1A–C show representative ovaries from each of the 3 groups. It can be seen that in the controls (Fig. 1A) the most recent crop of large CL are predominant although some small atretic CL can also be distinguished together with developing follicles. The ovaries of the EC-treated rats contain significantly more CL than the control ovaries and presumably include both the most recent as well as the older crop of CL (Fig. 1B). It is difficult to distinguish between the older and newer CL in these ovaries, since they all are relatively large and the lutein cells appear to be full of lipid material. A single injection of prolactin into EC-treated rats resulted in ovaries similar to those of the control rats, with fewer large CL (Fig. 1C). Body weight was slightly reduced in the EC-injected rats as compared to body weight in the control rats.

2. Effects of EC with or without prolactin on the ovaries during 3 estrous cycles. EC given for 3 cycles increased the weight of the ovaries even more than when given for 1 cycle (Table II). Prolactin injections during each of the 3 cycles into EC-treated rats completely prevented any increase in ovarian weight. The ovaries of the EC-injected rats contained more CL (Fig. 1E) than the ovaries of the control rats (Fig. 1D). This appears to be due to retention of the earlier as well as the most recent crop of CL. No readily distinguishable differences were observed between the older and newer CL. Injections of prolactin on the days of proestrus and estrus of each cycle reduced the number of CL (Fig. 1F) to about the same number as in the controls. There were no effects of any of the treatments on body weight.

Discussion. These results demonstrate that injections of EC during 1 or 3 cycles result in enlargement of the ovaries and accumulation of CL. EC prevented luteolysis of the older CL, presumably by inhibiting release of prolactin. Although a direct action of EC on the CL is possible, the counteraction of its effects on the CL by prolactin indicates that it acts by inhibiting prolactin release. The increase in size of the ovaries previously reported by this laboratory (11) after injecting EC for 15 days was much greater than observed in the present study, but the dose of EC used was about 12 times as great as in the present experiments. Injection of prolactin into EC-treated rats on the day of proestrus during a single cycle, or on the days of proestrus and estrus during each cycle of a 3-cycle period, completely prevented any significant increase in ovarian weight and induced atrophy of many CL. The number of CL remaining were decreased to about the same number as in control rats. This strongly suggests that the elevated serum prolactin levels on the day of proestrus and perhaps also during estrus induce luteolysis of the previous crop of CL.

Rothchild (3) ascribed luteolysis of the older CL during each cycle in the rat to the release of LH by the pituitary. This appears to be unlikely, since the dose of EC (50 mg) used in the present study was previously shown not to interfere with the normal rise of LH on the afternoon of proestrus (10). This dose of EC also does not inhibit the normal elevation of FSH on the day of proestrus (unpublished). The observation that daily injections of EC do not interfere with the regularity of the estrous cycles also indicates that release of LH and FSH are not signifi-

TABLE II. Effects of Ergocornine (EC) and Prolactin During Three Cycles on Ovaries.

Treatment and no. of rats	Av. Body wt (g)	Av. Ovarian wt (mg)	No. of corpora lutea[a]
Saline (12)	283.3 ± 6.6	63.2 ± 3.2	6.0 ± 0.3
EC (13)	278.5 ± 4.6	89.4 ± 3.5[b]	13.4 ± 0.9[b]
EC + prolactin (11)	286.4 ± 6.7	70.0 ± 3.2	5.8 ± 0.4

[a] Represents single cross-section count.

[b] EC vs saline, and EC vs EC + prolactin, $p < 0.001$.

cantly inhibited. We have reported that much larger doses of EC can reduce serum LH levels without preventing ovulation (10). It appears doubtful therefore, that the increases of serum LH or FSH on the day of proestrus contribute to luteolysis of CL during the cycle.

Whether prolactin exerts a luteotropic action during the estrous cycle of the rat is unknown. LH is believed to be responsible for the rise in blood progestins during proestrus (13–15). It appears therefore that the major effect of the rise in serum prolactin on the ovaries during the estrous cycle of rats is to induce luteolysis of the previous crop of CL.

Summary. Ergocornine (EC), an inhibitor of prolactin release, was injected daily into normal cycling rats for 1 or 3 estrous cycles. EC produced significant increases in weight of the ovaries and in number of corpora lutea (CL) as compared to the ovaries of control rats. These effects were more marked in rats injected for 3 cycles than in rats injected for 1 cycle. Injection of 1 mg of ovine prolactin in EC-treated rats on the day of proestrus or on the days of proestrus and estrus completely prevented any increases in ovarian weight and number of CL. It is suggested that the normal rise in blood prolactin on the day of proestrus and continuing into estrus serves to induce luteolysis of the previous crop of CL formed during each cycle.

1. Greenwald, G. S., and Rothchild, I., J. Anim. Sci. **27**, 139, Suppl. 1 (1968).

2. Schwartz, N. B., and Waltz, P., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **29**, 1907 (1970).

3. Rothchild, I., Vitam. Horm. (New York) **5**, 209 (1965).

4. Malven, P. V., Anat. Rec. **151**, 381 (1965).

5. Piacsek, B. E., and Meites, J., Neuroendocrinology **2**, 129 (1967).

6. Saito, M., Arimura, A., Sawano, J., and Schally, A. V., Endokrinologie **56**, 129 (1970).

7. Niswender, G. D., Chen, C. L., Midgley, A. R., Meites, J., and Ellis, S., Proc. Soc. Exp. Biol. Med. **130**, 793 (1969).

8. Gay, V. L., Midgley, A. R., and Niswender, G. D., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **29**, 1880 (1970).

9. Wuttke, W., and Meites, J., Proc. Soc. Exp. Biol. Med. **135**, 648 (1970).

10. Wuttke, W., Cassell, E., and Meites, J., Endocrinology **88**, 737 (1971).

11. Nagasawa, H., and Meites, J., Proc. Soc. Exp. Biol. Med. **135**, 469 (1970).

12. Heuson, J. C., Waelbroeck-Van Gover, C., and Legros, M., Eur. J. Cancer **6**, 353 (1970).

13. Miyake, T., Integrative Mech. Neuroendocrine Syst. **1**, 139 (1968).

14. Goldman, B. D., Kamberi, A. I., Siiteri, P. K., and Porter, J. C., Endocrinology **85**, 1137 (1969).

15. Schneider, T., Piacsek, B., and Gay, V., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **29**, 1101 (1970).

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