Ergocornine Blockade of the Surge of Prolactin at Proestrus Failed to Block Ovulation in Cycling Rats (36418)

A. YOKOYAMA, H. TOMOGANE, AND K. ÔTA
(Introduced by C. H. Sawyer)
Faculty of Agriculture, Nagoya University, Chikusa, Nagoya, 464 Japan

We reported previously (1) that a decrease in pituitary content and concentration of prolactin occurred on the afternoon of proestrus around the 'critical period' for LH surge in the cycling rat. These findings are in good agreement with the changes in the plasma concentration of prolactin recently reported by several workers with the use of radioimmunoassay (2–6). We also demonstrated the possibility that estrogen secreted between the afternoon of day 2 of diestrus [D_2, day before proestrus (PE)] and the morning of PE could trigger the surge of prolactin (7).

In order to elucidate the physiological role of prolactin release during the critical period, it would be useful to be able to block the surge of prolactin without affecting the surge of LH. Since ergocornine methanesulfonate has been reported to inhibit the secretion of prolactin (6–12), the effect of ergocornine treatment on the prolactin content and concentration in the pituitary gland was reexamined while the effect of the drug on the ovulation was investigated.

Materials and Methods. Virgin female Wistar–Imamichi rats weighing between 245 and 290 g were used. They were kept in a temperature (24 ± 2°) and light (on at 05.00 hr and off at 19.00 hr) controlled animal room. Animals were maintained on a stock diet (CA-1, Nihon CLEA Ltd., Tokyo) and water *ad libitum*. Vaginal smears were taken every morning by lavage between 09.30 hr and 10.00 hr. Only animals showing at least 2 consecutive regular 4-day cycles were used.

One milligram of ergocornine methanesulfonate (ECO, Sandoz Ltd., Basel)[1] suspended in 0.1 ml of physiological saline was injected sc on the afternoon (16.30 hr) of D_2 and the morning (10.30 hr) of PE. Animals injected with 0.1 ml of saline sc served as controls. Half of the animals injected were killed on the afternoon (17.30 hr) on PE, and the remainder on the morning of estrus by decapitation under ether anesthesia.

Anterior pituitaries were dissected free from the posterior pituitary, weighed and frozen at −20° until the time of assay. Prolactin content of the pituitary gland was determined by disc electrophoresis on polyacrylamide gel and measured by a microdensitometer (Chromoscan, MK II, Joyce Loeble Co. Ltd., England). The results are expressed as micrograms of NIH-P-B-3.[1]

Ovaries and uterus were removed and weighed. Ovulation was judged from the presence of ova in the oviducts in animals killed on the morning of estrus. The oviducts were dissected free from the ovary and placed between two glass slides, and the number of ova shed was counted under a microscope. Results were subjected to Duncan's multiple range test modified by Kramer (13).

Results and Discussion. Vaginal cycles were not interrupted by ECO treatment. This observation agrees well with that of Nagasawa and Yanai (14) and Wuttke *et al.* (6).

In control animals killed on the afternoon of PE, prolactin content and concentration of the pituitary gland were as low as those reported previously in intact cycling rats killed on the same stage of the estrous cycle (1). Prolactin content and concentration on the afternoon of PE in the animals treated with ergocornine were about 3 times higher

[1] We wish to thank Sandoz Ltd., Basel, Switzerland for erogocornine methanesulfonate and Dr. R. W. Bates, NIAMD, for NIH Prolactin B-3.

TABLE I. Prolactin Content and Concentration in the Anterior Pituitary of Rats Treated with ECO.

Grp.	Treatment[a]	Time of autopsy	No. of rats	Anterior pituitary (AP) wt mg	Prolactin content[b] (μg/AP)	Prolactin concentration[b] (μg/mg AP)
A	ECO	Estrus 10.30 hr	6	$10.15 \pm 0.71^{c,d}$	130.10 ± 20.70^{e}	12.55 ± 1.43^{g}
B	Saline	Estrus 10.30 hr	5	10.32 ± 0.84^{d}	117.32 ± 16.85^{e}	11.17 ± 0.90^{g}
C	ECO	Proestrus 17.30 hr	6	9.70 ± 0.56^{d}	128.25 ± 10.81^{e}	13.39 ± 1.17^{g}
D	Saline	Proestrus 17.30 hr	6	10.13 ± 0.45^{d}	38.94 ± 4.27^{f}	3.81 ± 0.36^{h}

[a] Ergocornine methanesulfonate (1 mg in 0.1 ml saline) or saline (0.1 ml) was injected subcutaneously on the afternoon of D_2 and on the morning of proestrus.

[b] Prolactin content and concentration are expressed as μg of NIH-P-B-3.

[c] Mean $\pm$ SE.

[d-h] Means which have the same superscripts are not significantly different from each other. ($p < .05$).

than those obtained in the control animals (Table I). These results seem to indicate that ECO blocked the release of prolactin which has been reported to occur on the PE afternoon. These results also coincide well with the findings of Wuttke *et al.* (6) that the elevation of plasma prolactin level on the day of PE is blocked by ECO treatment.

The level of prolactin content and concentration in the pituitary gland of animals treated with ECO or saline and killed on the morning of estrus was very high. No significant difference in the level was found between the treaments, and the level was very similar to that obtained in animals that were treated with ECO and killed on the afternoon of PE.

The facts that high prolactin content and concentration were obtained in animals treated with ECO are inconsistent with those reported by Nagasawa and Meites (10) and Nagasawa and Yanai (11) in rats and mice. The discrepancy between the present results and others may be due to the length of the ECO treatment. Treatment of ECO for two days used in the present experiment may block the release of prolactin from the pituitary gland without affecting the synthetic activity of the gland, whereas a long-term treatment (15–23 days) used by other workers seems to block not only release but also synthesis of prolactin in the pituitary gland.

No significant differences in weights of the anterior pituitary gland were observed among groups.

Ovulation occurred in 5 out of 6 animals treated with ECO and in all animals treated with saline. Wuttke *et al.* (6) suggested the possibility that the number of ova ovulated was fewer in the ECO treated animals than in normal cycling animals, since the treatment significantly depressed the increase in plasma LH levels which was observed in the afternoon of PE. However, the average number of ova shed in ECO treated animals in the present experiment was not significantly different from that obtained in saline treated control animals (Table II), although the number of ova is slightly less in the former animals than in the latter. There is no explanation, at present, for the fact that ovulation did not occur in one animal in the ECO treated group, since neither weights of uterus and ovaries nor the content and concentration of prolactin in the pituitary gland of this animal were significantly different from other animals in the same group. Therefore, the results in Table I and II include the data of this particular animal. Kraicer and Strauss (15) reported that the single administration of ECO either on D_2 or PE inhibited ovulation. The discrepancy between the present results and their results is unexplained at the present time. However, this discrepancy may

TABLE II. Effects of ECO Treatment on Ovulation, Number of Ova, and on Weight of Ovaries and Uterus.

Group[a]	No. of rats	Body weight (g)	Ovarian weight (mg)	No. of rats ovulating	Avg. no. of ova shed	Uterine weight (mg)
A	6	253.80 ± 4.53[b,d]	84.90 ± 5.55[e]	5/6	13.8 ± 1.0[f]	376.35 ± 15.99[g]
B	5	261.88 ± 8.55[d]	77.76 ± 4.55[e]	5/5	14.6 ± 0.7[f]	356.20 ± 18.52[g]
C	6	261.67 ± 6.56[d]	76.85 ± 4.43[e]	—		423.05 ± 28.65[g,h]
D	6	260.58 ± 3.88[d]	78.58 ± 3.12[e]	—		449.73 ± 24.75[h]

[a] Detail for the groups, see Table I.

[b] Mean ± SE.

[c] One rat showing no ovulation was excluded for calculation of the mean.

[d–h] Means which have the same superscripts are not significantly different from each other. ($p < .05$).

be due to the rapid absorption of the ECO in their solvent (dimethylsulfoxide) and the slower uptake of the drug in saline used in the present study.

There were no significant differences in ovarian and uterine weights between ECO treated animals and saline-treated animals killed at the same stage of the cycle (Table II).

These results were essentially the same as those reported by Wuttke *et al.* (6) and indicate that blockade of prolactin resulting from the treatment with ECO, at the dosage used in the present experiment, did not affect either ovulation itself or number of ova shed in the rat. Therefore, prolactin released on the afternoon of PE seems to play a role or roles in mechanisms other than ovulation during the estrous cycle in the rat.

Summary. A study was made of the effects of ergocornine methanesulfonate (ECO) administered to normal cycling rats on the afternoon (16.30 hr) of D_2 (day before PE) and on the morning (10.30 hr) of PE on prolactin content and concentration of the pituitary gland and on ovulation.

Pituitary prolactin content and concentration on the afternoon of PE in the animals treated with ECO were about 3 times higher than those obtained in the saline-treated control animals. When animals treated with ECO were killed on the morning of estrus, the level of prolactin content and concentration was very high and the level was not significantly different from that obtained in the saline-treated control animals killed at the same stages of the estrous cycle.

Ovulation occurred in 5 out of 6 animals treated with ECO. No significant difference was obtained in the average number of ova shed between the ECO treated animals and the saline-treated controls.

A possible physiological role of prolactin release around the critical period was discussed.

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Received Sept. 30, 1971. P.S.E.B.M., 1972, Vol. 140.