Inhibition of Ovulation and Luteinizing Hormone Secretion in the Cyclic Rat by Ergotamine Tartrate[1] (37720)

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The inhibitory effects of various ergot alkaloids on pseudopregnancy (1), decidualization (2), early pregnancy (3), and lactation (4) in the rat have been well documented by several investigators. In general, these effects were attributed to a specific inhibition of prolactin secretion, as prolactin was shown to partially reverse the effects of ergocornine (1), and plasma prolactin levels decreased after treatment with the drug (5). Recently, Wuttke *et al.* (9) reported a significant decrease in the plasma LH levels in rats also treated with 50 or 200 μg of ergocornine (5). The present study was undertaken to examine the effects of one of the ergot alkaloids, ergotamine tartrate, on ovulation, estrus, and LH secretion in adult cycling rats.

Materials and Methods. Adult female Charles River rats weighing 180–210 g were used in the present experiments. The animals were housed in rooms with constant temperature and a 14-hr light (5 AM to 7 PM EST) and 10-hr darkness schedule. The estrous cycle was checked by daily vaginal smears, and only animals exhibiting two consecutive cycles of 4 days were used in the study.

Ergotamine tartrate (Sigma Chemical Co.) was administered intraperitoneally as a suspension in 0.9% saline at 12:30 PM on the day of proestrus. Control rats received saline at this time. Blood samples were obtained under light ether anesthesia between 5:30 and 6 PM on the day of proestrus, as well as at 11 AM on the day of estrus. Luteinizing hormone, when given, was injected intraperitoneally at 4 PM during proestrus. One group of treated animals was subjected to mechanical stimulation of cervix at 6:30 PM on the day of proestrus.

All the animals were autopsied between 11 AM and 12 noon on the day of estrus. The oviducts were examined for the presence of ampulla under a dissecting microscope (×10) and ova extruded by a needle puncture of this region. When an ampulla was not present, the oviducts were pressed between two slides and examined for the presence of ova. Ovum counts were performed at ×65 magnification.

Plasma samples were analyzed for LH content using a radioimmunoassay kit kindly supplied by the Endocrine Study Section of NIH, Bethesda, MD. Highly purified rat luteinizing hormone (NIAMD LH-I-1) was used for radioiodination, and all results are expressed in terms of NIAMD reference LH RP-1 preparation.

Results. At a dose level of 1 mg/100 g body wt, ergotamine tartrate inhibited ovulation totally, as compared to the vehicle controls (Table I, Group III). While no change in the ovarian weight was noticed, the ovaries from the treated animals showed ripe graafian follicles with ova *in situ*, confirming the inhibition of ovulation. A lower dose of 400 μg/100 g body wt was partially effective in inhibiting ovulation. Cervical stimulation was ineffective in reversing the effects of the drug on ovulation (Group IV). However, administration of 25 μg NIH-LH-S18 at 4 PM on the day of proestrus resulted in shedding of a normal number of ova in 100% of the rats treated with the ergot alkaloid. These results suggested a possible inhibition of the ovulatory surge of LH by the drug as its mode of action. Direct evidence for this was obtained by the measurements of plasma LH

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TABLE I. Inhibition of Ovulation by Ergotamine Tartrate and Its Reversal by LH.

Group	Treatment	N_t/N[a]	Av no. of ova/ ovulating rat	mg ovaries	mg uteri	
I	Saline control	8/8	11.4 ± 0.53[c]	72.8 ± 1.9	341.6 ± 18.2	
II	0.4 mg ergotamine tartrate[b]	3/2	11.0 ± 0.40	72.0 ± 3.0	329.3 ± 21.0	
III	1.0 mg ergotamine tartrate[b]	9/0	0	69.1 ± 3.8	452.6 ± 25.5	Fluid-filled uterus
IV	1.0 mg ergotamine tartrate[b] + cervical stimulation	5/0	0	70.6 ± 1.0	485.2 ± 14.5	Fluid-filled uterus
V	1.0 mg ergotamine tartrate[b] + 25 μg NIH LH S18 (ip)	5/5	12.4 ± 0.7	72.0 ± 3.9	338.0 ± 15.2	

[a] N_t = Total number of rats, N = Number of rats ovulating.
[b] All doses per 100 g body wt, given at 12:30 PM of proestrus.
[c] Mean ± standard error.

levels on the evening of proestrus and the morning of estrus. The plasma of control rats showed a high level (718.1 ± 100 ng/ml) at 5:30 PM of proestrus. This was suppressed totally in the treated group, which showed such low levels as found during estrus (89.3 ± 14.7). In both groups, the levels fell uniformly to low values during estrus (Fig. 1). Of the 3 rats treated with 400 μg ergotamine, two ovulated; however, one of these rats showed very low LH levels (63.0 ng/ml), while the other animal had high concentration (816.0 ng/ml). A third animal which did not ovulate had a plasma LH level of 86.3 ng/ml.

All the animals in which ovulation was inhibited had ballooned uteri filled with fluid, characteristic of continued estrogen production. This was also reflected in the significantly higher uterine weights. Vaginal smears taken on the day of expected estrus revealed a cornified smear, and all the animals exhibited a lordosis response upon stimulation.

Discussion. It is clear from the above observations that ergotamine can inhibit LH secretion and ovulation at doses ranging from 400 μg to 1 mg/100 g body wt. A number of ergot alkaloids including ergotamine have been shown to inhibit release of prolactin in the cyclic (5) as well as the lactating (4) rat. Shaar and Clemens (4) noted that the potency of the various ergot alkaloids was different, ergonovine and ergotamine being less effective than ergocornine or ergocryptine. Wuttke *et al.* observed a trend towards decreasing LH levels with as low as 5 μg ergocornine given at 1:30 PM on the day of proestrus. At a 50 or 200 μg level, the drug significantly decreased plasma LH concentrations (5). However these investigators observed tubal ova, but did not discount the possibility of fewer ova being shed. Further, Kraicer and Strauss (6) demonstrated that 1 mg ergocornine administered in dimethyl sulfoxide solution subcutaneously could suppress ovulation. It was maximally effective when given at 2 PM on the day of proestrus. However, no LH levels were measured, and, hence, the inhibition of ovulation was not related to a decrease in plasma LH levels. The present results that ergotamine in aqueous solution given intraperitoneally is also highly effective indicates that it might be a common property of this class of compounds. Differences may also

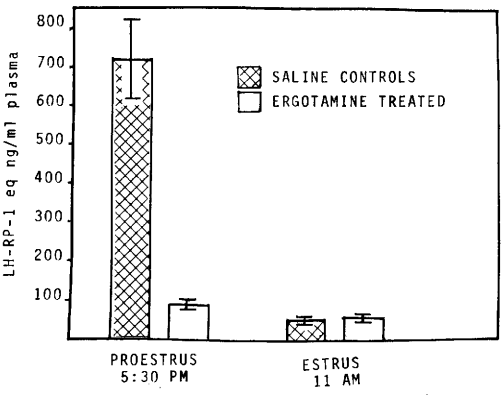

FIG. 1. Inhibition of ovulatory surge of LH by ergotamine. The values represent mean of determinations from 5 rats.

exist in potency of the various ergot alkaloids in this respect.

There seems to be considerable discrepancy in the literature with regard to the ovulation-blocking ability of ergocornine. Thus, the previous work of Kraicer and Strauss could not be confirmed by Yokohama *et al.* (7). The latter investigators injected ergocornine as a saline suspension subcutaneously and attributed the failure to inhibit ovulation to a probable delay in absorption. However, these rats were treated on the afternoon of diestrus and at 10:30 AM of proestrus as well, which discounts any effects due to delay in absorption. In the current study also, the drug was injected in aqueous medium, but intraperitoneally, and was effective in 100% of the animals. Thus, it is very likely that both ergocornine and ergotamine inhibit LH secretion in addition to their well-known effects on prolactin secretion in the rat.

The mechanism of action of ergotamine or ergocornine in blocking ovulation is not known. Based on their observations that ergocornine could inhibit ovulation when administered during diestrus, Kraicer and Strauss (6) proposed that it competes with progesterone for a CNS receptor. They postulated that such an action would interfere with the facilitatory action of progesterone on ovulation. Two lines of evidence obtained in the current study indicate that the drug inhibits the secretion of LH from the pituitary, viz. (a) plasma LH levels fail to rise during the afternoon of proestrus in the treated rats, and (b) exogenously administered LH is effective in totally reversing the blockade of ovulation. Recent evidence indicates that preovulatory estrogen secretion is a major factor leading to the LH surge on the afternoon of proestrus (10). In the present experiments, no measurements of plasma estrogen were undertaken. However, the uterine distension, cornified vaginal smears, and the onset of behavioral estrus as noted by the lordosis response suggest estrogen production in significant quantities, at least during estrus. In a previous study (7), rats treated with 1 mg ergocornine and killed at 5:30 PM on the day of proestrus also were observed to have distended uteri. Thus, ergocornine and ergotamine seem to inhibit pituitary LH release in face of the estrogen challenge.

From the foregoing discussion, it seems that the ergocornine-treated cyclic rat presents itself as an interesting model to study the temporal relation of steroids in bringing about the ovulatory surge of LH. It seems highly unlikely that the drug acts directly on the follicle (6), as exogenous LH is able to reverse its effects.

Whereas higher doses (150–500 μg) of ergocornine terminate pseudopregnancy and pregnancy in preimplantation stages (8), lower doses (5–50 μg) do not affect pseudopregnancy (9) despite an almost total suppression of prolactin. Preliminary results from this laboratory reveal that 1 mg ergotamine, capable of inhibiting ovulation, does not terminate pregnancy when given after implantation on days 8–11. These observations indicate that (a) blockade of LH, in addition to prolactin, may be an important event leading to disruption of pregnancy before implantation, and (b) nidation brings about a major shift of balance in the pituitary–gonad axis with reference to the luteotropic process. Further experiments are underway at the present time to elucidate the mechanism of action of ergot alkaloids in these systems.

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