Effect of Reserpine on Plasma LH Levels in Ovariectomized and Cycling Proestrus Rats (35984)

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In recent years, increasing attention has been paid to the influence of drugs acting on the central nervous system upon the function of the anterior pituitary (1). Special interest has been attached to the effects of reserpine upon the release of ovulating hormone. Reserpine, when injected into a proestrus female rat a few hours in advance of the "critical" period for luteinizing hormone (LH) release will prevent the ovulation that would otherwise follow (2). The drug is ineffective if injected immediately after the critical period. Meyerson and Sawyer (3) showed that the ovulatory blockade produced by reserpine could be prevented by prior treatment with either of the monoamine oxidase (MAO) inhibitors, pargyline or nialamide. Similar results have been obtained in the gonadotropin-primed immature rats (4).

The effect of reserpine upon ovulation and the ability of pretreatment with MAO inhibitors to counteract the blockade suggest that monoaminergic pathways in the hypothalamus play a significant role in controlling the secretion of LH. However, experiments attempting to correlate changes in functional levels of monoamines, such as occur after reserpine treatment, with actual levels of LH in the blood are lacking. It was the purpose of the present experiments to investigate: (a) the effects of reserpine upon blood LH levels in the ovariectomized and proestrous rat; and (b) the effect of pretreatment with pargyline on blood LH levels in the reserpine-treated proestrous rat.

Materials and Methods. Experimental animals. Female Sprague-Dawley rats (body wt, 200–250 g) were used in these studies. Unless otherwise stated, all rats were caged in an air-conditioned, temperature-controlled room with a lighting schedule of 14 hr of light and 10 hr of darkness, and supplied with Purina laboratory chow and water *ad libitum*. Under these conditions, the ovulatory surge of LH is expected to occur on the day of proestrus between 1400 and 1600 (5), the "critical" period.

Ovariectomized rats. The rats were used 3–6 weeks postovariectomy. Reserpine was administered for 3 days. On the day 4, the rats were bled; and plasma LH was measured. In order to control the effect of weight loss in the reserpine-treated animals, the saline-treated control animals were starved for 3 days prior to bleeding.

Normal rats. Vaginal smear records were taken each day and animals were used in the experiment on the day of proestrus, provided they displayed: (a) a clear 4-day cyclicity in 3 consecutive cycles; (b) a typical proestrus smear; and (c) a clearly distended uterus. The uterus was inspected on the morning of proestrus by laparotomy approached by a single lateral incision while the rat was under light ether anesthesia. Blood was collected between 1500 and 1600; and plasma LH was measured. Effects upon ovulation were determined by removing the oviducts on the following day ("estrus") and counting the ova under a microscope. Ovulation was considered complete if 6 or more ova could be counted in the 2 tubes.

LH assay. At the designated time, the rats were anesthetized and bled from the jugular vein into a heparinized syringe. Blood from 3 or more donors was pooled and the plasma was separated by centrifugation. Two milliliters of plasma were injected into suitably primed immature female rats to determine the percentage ovarian ascorbic acid de-

[1] Supported by Grant #AM 10073 from the Public Health Service and a Ford Foundation Grant.

TABLE I. Effect of Reserpine Alone and in Combination with Pargyline on Ovulation in Cycling Rats.

Treatment	Dose (mg/kg)	No. of rats treated	No. ovulating	Percentage ovulating
Control	—	8	8	100
Reserpine[a]	2.0	7	1	14
Reserpine[b]	2.0	7	6	86
Reserpine[a]	2.0	7	6	86
+ pargyline[c]	+25.0			

[a] Given at 1100 on the day of proestrus.
[b] Given at 1800 on the day of proestrus.
[c] Given 1 hr prior to reserpine.

pletion (OAAD) by a modification (6) of the method of Parlow.[2] This served as an index of plasma LH concentration. Control and treated plasma samples were always run during the same assay.

Injected materials. Reserpine (Serpasil, Ciba) and pargyline HCl (Abbot) were dissolved or diluted in 0.9% NaCl and injected subcutaneously. Control animals received the appropriate vehicle only. The actual doses and times of administration are mentioned below and in Tables I to III.

Results. Effects of reserpine alone and in combination with pargyline on ovulation in cycling rats (Table I). A single injection of reserpine (2 mg/kg) given at 1100 on the morning of proestrus clearly blocked ovulation. Ovulation failed to occur in only one animal which received reserpine after the "critical" period. Another group of rats was treated with pargyline (25 mg/kg) 1 hr prior to receiving the reserpine. Pargyline pretreatment effectively prevented the blockade of ovulation by reserpine.

Effect of reserpine on plasma LH activity in ovariectomized rats (Table II). Significant levels of LH were present in plasma from ovariectomized-starved rats. These levels are comparable to those found in ovariectomized-fed rats (7). Reserpine injections (0.5 mg/kg) for 3 days were effective in lowering plasma LH activity ($p<.01$).

Effect of reserpine alone and in combination with pargyline on plasma LH activity in cycling rats (Table III). Significant levels of LH were present in the plasma from cyclic rats on the afternoon of proestrus as previously reported (8). Plasma LH levels were significantly depressed by a single injection of reserpine (2 mg/kg), given at 1100 on the morning of proestrus.

In an attempt to determine whether pargyline pretreatment would reverse the effect of reserpine, proestrous rats were given a single injection of pargyline (25 mg/kg) 1 hr prior to the reserpine injection. Pargyline

TABLE II. Effect of Reserpine on Plasma LH Activity (% ovarian ascorbic acid depletion) in Ovariectomized Rats.

Expt. no.	Plasma LH activity (% ovarian ascorbic acid depletion)		p value
	Ovariectomized[a]	Reserpine-treated[b]	
1	17.1 ± 2.8 (5)[c]	2.8 ± 1.2 (4)	<.01
2	26.3 ± 4.1 (4)	1.0 ± 2.6 (4)	<.01
3	21.4 ± 2.2 (5)	6.5 ± 2.4 (5)	<.01

[a] Starved for 3 days.
[b] 0.5 mg/kg per day for 3 days.
[c] Mean ± SEM (no. of assay rats).

[2] Supplies of PMS and HCG were generously provided by Dr. J. B. Jewell of Ayerst Laboratories.

TABLE III. Effect of Reserpine Alone and in Combination with Pargyline on Plasma LH Activity (% ovarian ascorbic acid depletion) in Cycling Rats.

Expt. no.	Plasma LH activity (% ovarian ascorbic acid depletion)		p value
	Proestrus	Reserpine treated[a]	
1	21.9 ± 2.3 (5)[b]	5.4 ± 1.04	<.01
2	22.0 ± 3.4 (4)	4.2 ± 0.96	<.01
		Reserpine[a] and pargyline[c] treated	
3	19.2 ± 2.4 (5)	24.2 ± 3.4 (5)	NS
4	20.3 ± 4.3 (4)	17.1 ± 2.7 (4)	NS

[a] Given at 1100 on the day of proestrus, 2 mg/kg.
[b] Mean ± SEM (no. of assay rats).
[c] Given 1 hr prior to reserpine, 25.0 mg/kg.

was capable of overcoming the effect of reserpine on the proestrous rise in plasma LH levels. Plasma LH levels in rats treated with reserpine and pargyline were not significantly different from LH levels in control proestrous rats.

Discussion. The ability of reserpine to interfere with ovulation in laboratory animals is well documented. The observations by Barraclough and Sawyer (2) and Meyerson and Sawyer (3) that reserpine inhibits ovulation if injected before, but not after, the "critical" period for ovulating hormone release suggested that reserpine acted at the hypothalamo-hypophyseal level to inhibit LH release rather than directly on the ovary. However, evidence has also accumulated to suggest that this might not be the sole mechanism of reserpine action in inhibiting ovulation. A direct effect on the ovary was suggested by experiments on immature mice by Purshottam (9), and on immature rats by Hopkins and Pincus (10) and France (11) since the compound inhibited ovulation induced by a combination of PMS and HCG.

There is strong evidence that neuronal stores of monoamines (serotonin, dopamine, and norepinephrine) are depleted by reserpine, primarily via inhibition of the mechanism whereby amines are stored in granules (12–15) and via subsequent deamination by intraneuronal monoamine oxidase (16, 17). The finding that pretreatment with MAO inhibitors, which should allow the accumulation of extragranular monoamines, could overcome the inhibiting effects of reserpine on ovulation in the immature (4) and cycling rat (3) led to the assumption that the failure of discharge of ovulating hormone after reserpine treatment is related to a functional shortage of hypothalamic monoamines. The recent observations by Rubinstein and Sawyer (18) that presumably total depletion of monoamines by prolonged reserpine action blocked the triggering of ovulation by electrical stimulation of the hypothalamus, and that treatment with nialamide could restore monoamine levels to support electrically stimulated ovulation, give further support to the hypothesis that monoaminergic nervous pathways are essential in the release of ovulating hormone.

The present investigation demonstrates that plasma LH levels were indeed suppressed by prior treatment with reserpine in both the ovariectomized and cycling proestrus rat and that the effect on LH release in the cycling proestrus rat could be counteracted by pretreatment with pargyline. These findings substantially strengthen the accumulating body of evidence that intact monoaminergic mechanisms are necessary for the ovulatory discharge of LH and provide evidence that monoaminergic mechanisms may also be involved in the maintenance of high levels of LH release in the spayed female. It is assumed that the inhibition of LH release by reserpine is caused by an inhibition of hypothalamic LH-releasing factor (LRF) release. The effect of reserpine on LRF produc-

tion has not been reported as yet, but reserpine has been shown to be capable of reducing the prolactin inhibitory factor (19) and FSH-releasing factor (20) content of the hypothalamus.

Recent *in vivo* (21–24) and *in vitro* (25, 26) experiments suggest that hypothalamic dopaminergic neurons synapse with the neurosecretory neurons which secrete LRF and stimulate its release. These findings suggest that dopamine may be the synaptic transmitter for LRF. However, other studies have implicated other amines as having important roles in the control of LH release. Kordon and co-workers (27, 28) have reported that ovulation in the immature rat is blocked when hypothalamic serotonin levels are increased, suggesting an inhibitory serotoninergic component. Injection of serotonin into the third ventricle lowered LH levels in ovariectomized rats (21). Early experiments by Sawyer (29) showed the effectiveness of direct infusion of epinephrine into the third ventricle to induce ovulation in rabbits. Rubinstein and Sawyer (18) were able to characterize epinephrine as the most effective agent in triggering ovulation in the proestrus pentobarbital-blocked rat when also infused directly into the third ventricle. These latter findings suggest that the release of LRF and secondarily pituitary LH may be controlled by multiple aminergic mechanisms.

Summary. The present experiments were carried out to determine the effects of drugs which are known to alter the stores of monoamines in the hypothalamus upon the concentration of LH in the blood of ovariectomized and normal rats. LH was estimated by the ovarian ascorbic acid depletion bioassay. In agreement with earlier data, blockade of ovulation occurred when reserpine (2 mg/kg) was administered prior to the assumed "critical" period of LH release and the effect could be overcome by prior treatment with a monoamine oxidase inhibitor, pargyline (25 mg/kg). Reserpine injection for 3 days (0.5 mg/kg) was shown to be effective in lowering the elevated plasma LH levels in ovariectomized rats. A significant level of LH was present in the blood during the afternoon of proestrus in control rats; whereas the levels were depressed after a single injection of reserpine (2 mg/kg) on the morning of proestrus. The blockade of the proestrous rise in plasma LH by injection of reserpine was prevented by prior treatment with pargyline (25 mg/kg). These results provide direct support for the hypothesis that LH secretion is regulated by hypothalamic monoaminergic mechanisms.

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Received July 6, 1971. P.S.E.B.M., 1971, Vol. 138.