Inhibition of the Progesterone-Advanced LH Surge at Proestrus by Nicotine[1] (37497)

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It is well known that, whereas progesterone can suppress ovulation, it can also advance the release of luteinizing hormone (LH) under certain conditions (1, 2). Administered on the third day of diestrus in 5-day cyclic rats, the steroid advanced ovulation by 24 hr (3, 4). Injection of progesterone on the morning of proestrus resulted in LH release and ovulation in spite of barbiturate administration at the beginning of the critical period (5–7).

Nicotine, which influences central nervous mechanisms (8) and interrupts cholinergic synaptic transmission in ganglia (9), delays or reduces the "spontaneous" proestrous surge of LH (10) and may postpone ovulation 24 hr if treatment is continued for several hours during and after the critical period (11). Therefore, it was of interest to determine whether nicotine affects the mechanism of progesterone advancement of LH release at proestrus.

Materials and Methods. The experiments employed virgin female Sprague–Dawley (Simonsen) rats weighing 180–328 g. Illumination of the temperature-controlled animal room was set at 14 hr of light and 10 hr of darkness with noon at the middle of the light phase. Purina laboratory chow and water were provided *ad lib*. Daily vaginal smears were taken for at least two consecutive 4-day estrous cycles prior to experimentation. Rats showing proestrous smears in the morning were used for the experiment. The occurrence of ovulation was determined by observing the oviducts microscopically for ova at noon on the following day. If the ampullae were not swollen the oviducts were examined for ovulation 24 hr later.

Progesterone in amounts totaling 5 mg in 1 ml sesame oil/rat was injected into two subcutaneous sites. Nicotine tartrate solution was prepared at a concentration of 5 mg/ml in physiological saline and was injected every 30 min for 2.5 hr in amounts of 5 mg/kg sc. Controls received the same volume of saline. Pentobarbital sodium (Nembutal) was injected ip in amounts of 30 mg/kg.

Serum LH was measured by radioimmunoassay (RIA) with the ovine:ovine system of Niswender *et al.* (12). One milliliter of blood was taken by heart puncture immediately after pentobarbital injection. Blood was allowed to clot at room temperature before centrifuging, and serum was stored frozen at −20°. The samples were assayed in duplicate and the results were averaged. The reference preparation was NIAMD-Rat LH-RP-1 which has a biological potency equivalent to 0.03 × NIH-LH-S1.

Results. As summarized in Table I, none of 6 proestrous rats treated with progesterone at 0900 followed with pentobarbital at 1400 ovulated that night irrespective of saline or nicotine administration. Administration of progesterone at 1100 followed by six half-hourly injections of saline and finally pentobarbital at 1400 resulted in ovulation in 5 of 12 rats with an average of 6 ova/ovulating rat. When nicotine was injected instead of saline ovulation occurred in 3 of 10 rats with an average of 3 ova.

When treatment was started at 1130, pen-

[1] Supported by grants from the American Medical Association, Education and Research Foundation, The Ford Foundation and NIH (NS 01162).

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TABLE I. Effects of Progesterone–Nicotine–Pentobarbital Injections at Different Hours of Proestrus on Ovulation that Night.

No. of rats	Time of injection				Ovulation		
	Progesterone	Saline	Nicotine	Pentobarbital	No. ovulated	Ovulation (%)	Av no. of ova/ ovulated rats
3	0900	1100–1330	—	1400	0	0	0
3	0900	—	1100–1330	1400	0	0	0
12	1100	1100–1330	—	1400	5	41.7	6
10	1100	—	1100–1330	1400	3	30.0	3
5	—	1130–1400	—	1430	0	0	0
9	1130	1130–1400	—	1430	7	77.0	10
7	1130	—	1130–1400	1430	0	0	0

tobarbital at 1430 was still completely effective in blocking spontaneous ovulation in 5 saline-injected proestrous rats. Administration of progesterone at 1130 followed by six injections of saline and eventually by pentobarbital at 1430, resulted in complete ovulation (*i.e.*, 7 or more eggs) in 7 of 9 rats. When nicotine replaced saline in the protocol ovulation failed to occur. As illustrated in Fig. 1, the saline-treated controls sampled after pentobarbital at 1430 had a serum LH level of 34.5 ± 6.1 ng/ml (mean ± standard error). Progesterone at 1130 advanced LH release to the extent that the 7 rats destined to ovulate that night showed serum LH levels after pentobarbital at 1430 of 643 ± 142 ng/ml ($p < 0.01$ compared with saline controls). The two rats which failed to ovulate, in this group of nine, had a mean serum LH level of 175 ng/ml, some five times higher than that in saline controls. The six injections of nicotine completely blocked the progesterone advancement of LH release: serum LH levels averaged 24.5 ± 4.5 ng/ml, which was significantly different ($p < 0.005$) from the progesterone–saline subjects but not different from the saline controls.

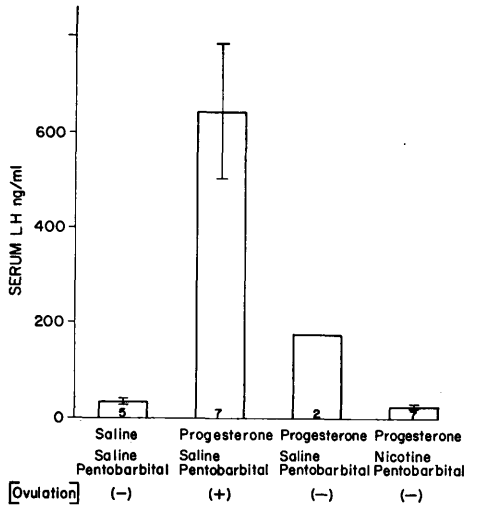

FIG. 1. Effects of nicotine on progesterone advancement of LH release in proestrous rats. Blood was taken at 1430, immediately after pentobarbital administration. Bars and their extending lines represent means ± standard errors. Number at the base of a bar indicates the number of rats used.

Control rats in which ovulation was blocked with pentobarbital at 1430 all ovulated the second night, *i.e.*, 24 hr delay. However, of 11 progesterone-treated proestrous rats which failed to ovulate the first night, only one ovulated 24 hr later.

Discussion. Earlier findings that treatment of the proestrous rat with progesterone in the morning advances the release of LH and results in ovulation that night in spite of barbiturate anesthesia during the 1400–1600 critical period (5–7) have been confirmed. The critical period in our rats appears to be somewhat later than 1400–1600 since pentobarbital blocked effectively at 1430 in the present experiments, and in unpublished experiments we have observed some blockade when starting pentobarbital treatment at 1600. In view of these observations it may

well be that the rats which ovulated in spite of nicotine and pentobarbital treatment did so from residual stimulation after the effect of the 1400 pentobarbital treatment had worn off.

The stimulatory effect of progesterone on ovulation appears to be exerted on the brain. Progesterone-induced ovulation has been blocked by anterior deafferentation of the hypothalamus (13), "roof cuts" above the hypothalamus (14), and section of the stria terminalis (13). Anterior deafferentation has also blocked the stimulatory effect of progesterone in the hamster (15). With the advent of RIA procedures it has been possible to demonstrate the release of LH following treatment with progesterone (7, 15). Starting at 1130 in the present study we have noted high serum LH levels by 3 hr after progesterone treatment whereas Brown-Grant and Naftolin (7) failed to observe a rise in LH until about 5 hr after their 0900 treatment with the steroid.

Nicotine has been shown to delay or block the proestrous surges of LH (10, 11) and prolactin (16) as well as the suckling-induced release of prolactin (17). However, the duration of action is limited. We found that during proestrus the administration of nicotine every 30 min from 1400 to 1900 failed to prevent ovulation but with two additional injections blockade was achieved (11). Our reason for giving repeated injections of the drug is that unpublished results from this laboratory have shown that nicotine-induced changes in electrical activity of subcortical regions of the rat brain last only about 30 min following a single sc injection of 5 mg/kg nicotine tartrate.

In confirmation of Redmond (6) the inhibiting effect of progesterone was not reversed by pentobarbital, *i.e.*, if progesterone-treated rats did not ovulate the first night, between proestrus and estrus, they did not ovulate the second night, and nicotine treatment on proestrus also failed to influence this effect of progesterone. This inhibitory influence of progesterone may represent a secondary effect on the brain (18) or a direct effect on the pituitary gland.

Summary. When proestrous rats were injected with progesterone at 1130, serum LH was highly elevated (643 ± 142 ng/ml) at 1430 in seven rats that ovulated that night in spite of pentobarbital administration at 1430, and somewhat elevated (175 ng/ml) in two similarly treated rats which failed to ovulate. Five saline-treated controls (no progesterone) showed a serum LH level of 34.5 ± 6.1 ng/ml at 1430. When progesterone treatment at 1130 was followed by six half-hourly injections of nicotine tartrate prior to pentobarbital treatment at 1430 none of seven rats ovulated and their serum LH level was 24.5 ± 4.5 ng/ml. Nicotine blocks the advancement of the proestrous ovulatory surge of LH by progesterone.

The authors express their appreciation to Mrs. Virginia Hoover for excellent technical assistance, Dr. C. A. Blake for help with RIA technique, Dr. Gordon D. Niswender, for the H-15 antibody, Dr. Leo Reichert, Jr., for the LER-1056-C2 LH for iodination, Mrs. Frances Smith for secretarial assistance, and Mr. Bobby McAlister for drawing of the figure.

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Received Mar. 1, 1973. P.S.E.B.M., 1973, Vol. 143.