Nicotine Delays the Ovulatory Surge of Luteinizing Hormone in the Rat[1] (36922)

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Nicotine (1-methyl-2-(3-pyridyl) pyrrolidine), the most important pharmacological constituent of tobacco, is a potent ganglionic blocking agent which also exerts a variety of actions on the central nervous system (1). Until now, no one has investigated specifically the effects of nicotine on the neural mechanisms that initiate the ovulatory surge of luteinizing hormone (LH). This train of neuroendocrine events, normally activated during a definitive "critical period" (2), can be blocked with certain central nervous system depressant drugs such as pentobarbital (3), and a new critical period occurs 24 hr later. The ovulatory surge can be advanced a few hours by the administration of the ovarian steroids estrogen and progesterone (4). We now report that nicotine delays LH release and thereby interferes with ovulation by its action on the central nervous system.

Methods. Virgin female Sprague–Dawley rats (200–235 g) were housed under conditions of controlled temperature and lighting (14 hr of light, 10 hr of darkness). The midpoint of the light phase was designated as 1200 noon. Under this lighting schedule the critical period is defined as 1400–1600 (2). Purina laboratory chow and water were provided *ad libitum*. Daily vaginal smears were taken for at least two consecutive 4-day estrous cycles before experimentation.

A total of 2 mg of nicotine tartrate (0.65 mg of nicotine) in 0.4-ml saline was administered to rats in two subcutaneous injections 5 min apart starting at 1200, 1400, and 1600 on the afternoon of proestrus. In addition, some of these rats were given sodium pentobarbital (Nembutal, 32 mg/kg) intraperitoneally at either 1730 or 2000, and a control group was given pentobarbital alone at 1730. Ovulation was determined by examination of the uterine tubes for the presence of ova at noon the following day. Circulating levels of LH were measured in duplicate by radioimmunoassay (5) on 136 one-ml blood samples collected by heart puncture on the afternoon of proestrus in 54 rats (25 control and 29 nicotine treated). The potency of the reference preparation *NIAMD-Rat-LH-RP-1* was 0.03 × *NIH-LH-S1*. Four controls (16%) and 8 nicotine-treated rats (28%) which failed to ovulate were excluded from the LH assays in this study.

Results and Discussion. It is evident that neither injection of nicotine at 1200, 1400 and 1600 nor pentobarbital at 1730 was effective in blocking spontaneous ovulation (Table I). However, treatment with nicotine followed by pentobarbital at 1730 blocked ovulation completely in 5/7 rats and partially in one of the 2 rats that did ovulate. If the injection of pentobarbital was withheld until 2000 all of the nicotine-treated rats ovulated in full. These results indicate that insufficient LH had been released by 1730 to cause full ovulation in rats treated up to 1600 with nicotine, but by 2000 an ovulatory quota had been discharged. Ovulating rats, control and nicotine-treated, shed 12.7 ± 0.2 (mean ± SE) and 11.6 ± 0.4 ova, respectively. Al-

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TABLE I. Blockade of Ovulation by Sequential Treatment with Nicotine and Pentobarbital.

Treatment schedule on proestrus			
Nicotine tartrate 2 mg/rat injected at	Pentobarbital 32 mg/kg injected at	Proportion ovulating	Number of ova per ovulation mean ± SE
—	1730	5/7	12.4 ± 0.5
1200 1400 1600	—	6/6	11.8 ± 0.7
1200 1400 1600	1730	2/7[a]	6.5 ± 4.5
1200 1400 1600	2000	6/6	10.3 ± 1.6

[a] One of the two ovulating was incomplete with only 2 ova shed.

though full ovulation (7 or more ova) was evidenced by all rats that did ovulate in both groups, treatment with nicotine in addition to the stress of heart puncture was followed by complete failure of ovulation in 28%, as mentioned above.

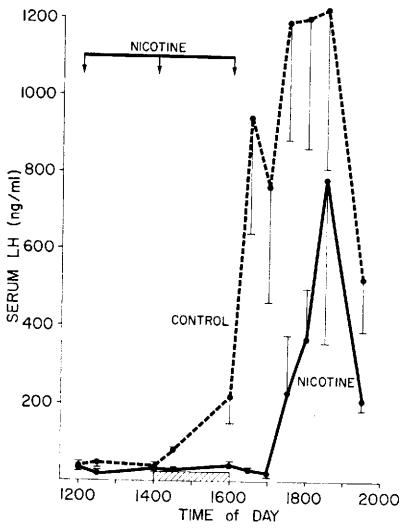

FIG. 1. Serum LH levels in control (broken line) and nicotine-treated (solid line) rats on the afternoon of proestrus. Each point represents the mean value for 4–8 animals. The vertical lines are the SE of the means. The hatched area on the abscissa represents the critical period, and the arrows mark the times of nicotine injection. The nicotine injections were given just prior to blood sampling at 1200, 1400, and 1600.

Serum LH curves are plotted in Fig. 1. The pattern observed in control rats is consistent with the numerous published reports (6). The pattern in nicotine-treated animals differs in several respects: the normal proestrous surge of LH was delayed at least 90 min and was lower in magnitude and shorter in duration than the comparable surge in control animals. In addition, the basal circulating LH values were maintained at low levels between the injections of nicotine and these levels were apparently depressed still further at 30 min after each injection of nicotine.

These data demonstrate that nicotine can delay and depress the output of a pituitary hormone necessary for ovulation. Other drugs which share this capacity in the rat have been reported to interrupt the human menstrual cycle (7). Tobacco smoking has been held responsible for a high incidence of reproductive disorders in women (8), and nicotine's interruption of brain-pituitary-ovarian neuroendocrine mechanisms may hold the key to these problems.

Summary. Nicotine tartrate administered to rats on the afternoon of vaginal proestrus delayed the ovulatory surge of luteinizing hormone (LH) output from the pituitary gland. Ovulation was completely blocked by sequential treatment with nicotine and a cen-

tral nervous depressant, pentobarbital, under conditions in which neither drug alone would prevent LH release. The principal site of nicotine's antigonadotropic action, therefore, appears to be the central nervous system.

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