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Parallelism and Divergence in Luteinizing Hormone and Follicle-Stimulating Hormone Release in Nicotine-Treated Rats[1] (37881)

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It has generally been considered that a single hypothalamic hormone was responsible for release of a single anterior pituitary hormone. This concept has been challenged with the isolation and synthesis of TRH and the decapeptide LHRH. TRH does release pituitary prolactin in addition to TSH in humans (1), and Schally *et al.* have claimed that LHRH is also FSHRH (2). There is little doubt that the decapeptide releases rat pituitary LH and FSH *in vitro* (3, 4). However, *in vivo* LHRH does not increase rat plasma FSH concentration unless a large dosage of the releasing hormone is administered or unless the decapeptide is infused over a period of hours (3, 5). It is difficult to explain the wealth of physiological data in which circulating levels of LH and FSH do not parallel each other on the basis of a single hypothalmic releasing hormone.

It has recently been reported that nicotine has the property of temporarily delaying the ovulatory surge of LH on the afternoon of proestrus in cycling rats (6). This study investigates the effects of nicotine and LHRH on LH and FSH release in individual proestrous and ovariectomized (OVX) rats. Data are described which support the view that LHRH is the natural regulator of LH release and that FSH release under physiological conditions is not easily explained solely on the basis of LHRH release.

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Methods and Materials. Female Simonsen Sprague–Dawley rats (200–220 g) were kept on a controlled lighting schedule with the lights on from 0500 to 1900 hr. Under this lighting schedule, the critical period is defined as 1400–1600 (7). Only virgins showing at least two regular 4-day estrous cycles were used in experiments on cycling rats while either 4- or 5-day cyclers were ovariectomized 7–8 weeks prior to experimentation on OVX animals.

In cycling rats, 0.2 ml saline, 1.0 mg of tartaric acid in 0.2 ml saline, or 1.0 mg of nicotine tartrate (N) in 0.2 ml saline (0.32 mg free nicotine; K and K Lab., Inc.) was injected subcutaneously at 1400, 1500, 1530 and 1600 hr on the afternoon of proestrus. The animals had received atrial cannulas between 1100 and 1330 hr that day and 0.7 ml of blood was withdrawn through each cannula with a heparinized syringe at hourly intervals from 1600 to 2000. Cannulas were flushed with an equal amount of heparinized (10 U/ml) saline after each bleeding. The blood was centrifuged at 4° to separate plasma from cells, and plasma was stored frozen at −25°. The oviducts were examined for the presence of ova between 1200 and 1400 hr the following day.

Pituitary responsiveness to exogenous LHRH was tested in similarly cannulated (1030–1130 hr) proestrous rats. After a 0.6-ml blood sample was withdrawn at 1230 ± 0015, rats received a total of 2 mg of tartaric acid or 2 mg of N in 0.4 ml saline in two sc injections administered 5 min apart. Ten minutes after the second in-

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jection, a second blood sample was withdrawn, and the animals were injected with 100 ng of synthetic LHRH (Beckman) through the cannula. Blood was collected at 2, 10, and 20 min after injection of LHRH and also at 1700 hr.

In OVX rats, an atrial cannula was introduced 3 days prior to the sequential injections of 1.0 mg of N in 0.2 ml saline at 30-min intervals. Blood (0.3 ml) was collected at −10 min, immediately before the first injection of N at time O, and every 40 min thereafter for 280 min. In other OVX animals, the same experiment was conducted except that 0.4-ml blood samples were withdrawn and injection of N was curtailed 120 min after the first injection, i.e., a total of 5 injections.

Pituitary responsiveness to synthetic

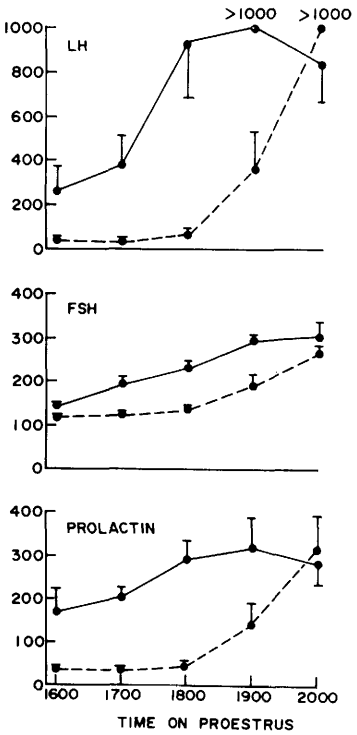

FIG. 1. Plasma LH, FSH, and prolactin concentrations (ng/ml) are plotted as mean ± SE from 1600 to 2000 hr on the afternoon of proestrus. Controls are represented by a solid line and nicotine-treated rats by a dashed line. Each point represents the mean of 10 rats. All three hormones were measured on each blood sample, and all 20 rats ovulated that night.

LHRH was also tested in OVX animals. Blood collection and treatment with N were as described for proestrous rats except that saline not containing tartaric acid was used as the control. Twenty nanograms of LHRH was injected through each atrial cannula implanted 3 days prior to experimentation.

Radioimmunoassay methods for measuring LH have been described previously (8). FSH, prolactin, and TSH were measured with the NIAMD kits. Values are expressed in terms of the NIAMD standards for these hormones. The standard for LH has a biological potency equivalent to 0.03 × NIH-S1 while that of FSH, prolactin, and TSH are 2.1 × NIH-S1, 11 IU/mg, and 0.22 USP (Bovine) TSH U/mg, respectively. Student's *t* test was used to compare the data for significance.

Results and Discussion. Five saline-treated and five tartaric acid-treated rats had similar plasma concentrations of gonadotropic hormones on the afternoon of proestrus. These values were combined into one control group (Fig. 1), and the rats ovulated with 10.6 ± 1.2 ova (M ± SE). In 10 additional rats, measurement of LH, FSH, and prolactin in blood samples withdrawn sequentially on the afternoon of proestrus revealed that four injections of N temporarily blocked the spontaneous rise in plasma concentration of all these hormones for approximately 2 hr. After recovery from N, all three gonadotropins appeared to be released at the same time, and by 2000 hr, the plasma concentration of these hormones in N-treated rats had risen to peak values recorded in control animals. These 10 rats ovulated with 9.6 ± 2.2 ova. Three other rats treated with N did not ovulate, gonadotropin levels did not rise, and these values are not included in Fig. 1. The susceptibility of gonadotropin hormone release to the temporary inhibitory action of N is of great value in pointing out the similarities in the neural train of events responsible for the proestrous surges of LH, FSH, and prolactin.

Nicotine was effective in inhibiting LH release in OVX rats. A rapid and sustained

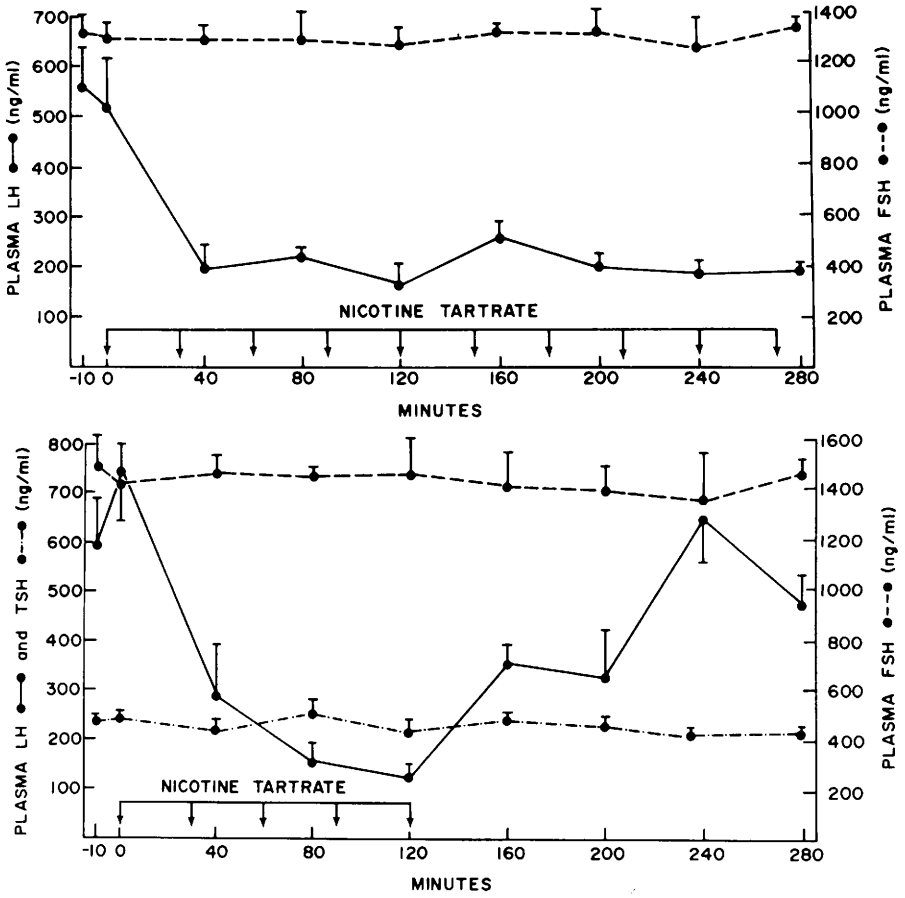

FIG. 2. Plasma LH (solid line), FSH (dashed line), and TSH (dashed-dot line) are plotted as mean ± SE before and after sc injection of 1.0 mg nicotine tartrate in 0.2 ml saline at each of the times indicated by the arrows. Each point represents the mean of eight OVX rats.

decrease in plasma LH concentration was observed after repeated injections of nicotine (Fig. 2). Plasma FSH concentration showed minor fluctuations in individual rats, but nicotine did not significantly reduce these levels over a 280-min period. Similar results were seen after only five injections of nicotine except that plasma LH concentration rose sharply to preinjection values after recovery from nicotine's inhibitory action (Fig. 2). Plasma FSH concentration was again unaltered by the alkaloid and did not rise along with the increase in plasma LH after recovery from nicotine.

The present study adds further support to the view (6) that N is exerting an effect, at least on LH, within the central nervous system. The drug did not suppress pituitary LH release in response to exogenous LHRH in both proestrous and OVX rats. Plasma LH was significantly ($P < 0.001$) elevated in control and N-treated proestrous rats at 2, 10, and 20 min after LHRH, but plasma FSH was not significantly altered (Table I). Administration of N and LHRH early on the afternoon of proestrus did not appear to affect the spontaneous surge of LH and FSH. Plasma LH and FSH concentrations at 1700 hr were significantly ($P < 0.05$) elevated when compared with the 10- or 20-min post-LHRH samples, and all of the rats ovulated that night with eight or more ova. Administration of LHRH to OVX rats pretreated with N

TABLE I. Effects of Nicotine on Pituitary Responsiveness to LHRH in Proestrous Rats.

Group[a]	Time on proestrus—plasma LH (ng/ml)					
	1230	1245[b]	1247	1255	1305	1700
Tartaric acid	30 ± 1[c]	32 ± 3	98 ± 5	283 ± 23	235 ± 28	747 ± 110
Nicotine tartrate	32 ± 3	39 ± 5	94 ± 4	271 ± 17	268 ± 21	671 ± 134
	Time on proestrus—plasma FSH (ng/ml)					
Tartaric Acid	167 ± 12	163 ± 11	152 ± 3	159 ± 8	157 ± 10	275 ± 21
Nicotine tartrate	148 ± 8	148 ± 7	140 ± 15	156 ± 12	176 ± 18	261 ± 15

[a] A total of 2 mg of tartaric acid or nicotine tartrate in 0.4 ml saline was administered by two sc injections at 1230 and 1235 hr.

[b] One-hundred nanograms of synthetic LHRH was injected at 1245 hr through an atrial cannula implanted between 1030 and 1130 hr that morning.

[c] Mean ± SE for 4 rats in each group each bled at all of the indicated times.

caused plasma LH concentration to rise rapidly to levels not significantly different from values recorded in OVX animals pretreated with saline and given LHRH (Table II). Neither LHRH nor N altered plasma FSH or TSH concentration. If N was inhibiting LH release by interfering with blood flow to the adenohypophysis via the portal vessels, one would expect to see alterations in plasma concentrations of other anterior pituitary hormones. In the present study, N did not alter plasma FSH or TSH concentration in OVX rats. In another study, plasma TSH concentration did not rise on the afternoon of proestrus in control or N-treated rats (9).

After release from nicotine's inhibitory action in proestrous rats, plasma FSH concentration rises proportionately as rapidly as plasma LH. However, plasma FSH concentration did not rise along with plasma LH after administration of exogenous LHRH on the afternoon of proestrus at a time when the hypothalamic–hypophyseal axis is "primed" to initiate spontaneous release of FSH. These data cast doubt on the hypothesis that LHRH is solely responsible for the rise in plasma FSH concentration on the afternoon of proestrus. Results obtained after administration of N and/or LHRH to OVX rats support this conclusion. In long-term OVX rats, plasma LH and FSH concentrations are highly elevated. Nicotine treatment dramatically reduces plasma LH concentration in OVX animals and does not inhibit pituitary LH

TABLE II. Effects of Nicotine on Pituitary Responsiveness to LHRH in Ovariectomized Rats.

Group[a]	Plasma LH (ng/ml)				
	−15 min	Time 0[b]	2 min	10 min	20 min
Saline	470 ± 40[c]	410 ± 58	960 ± 32	1072 ± 74	807 ± 86
Nicotine tartrate	454 ± 37	352 ± 49	862 ± 61	1040 ± 22	656 ± 116
	Plasma FSH (ng/ml)				
Saline	1400 ± 62	1358 ± 79	1321 ± 65	1418 ± 24	1399 ± 31
Nicotine tartrate	1381 ± 56	1398 ± 31	1347 ± 49	1364 ± 47	1348 ± 64
	Plasma TSH (ng/ml)				
Saline	205 ± 8	192 ± 14	202 ± 6	202 ± 7	215 ± 16
Nicotine tartrate	212 ± 20	217 ± 21	200 ± 11	190 ± 14	202 ± 7

[a] A total of 0.4 ml saline or 2 mg nicotine tartrate in 0.4 ml saline was administered by sc injections at −15 and −10 min.

[b] Twenty nanograms of synthetic LHRH was injected at time 0 through an atrial cannula implanted 3 days previously.

[c] Mean ± SE for 6 rats in each group each bled at all of the indicated times.

release in response to exogenous LHRH. However, plasma FSH concentration is not reduced in OVX rats after treatment with N, plasma FSH levels do not rise as those of LH after recovery from N, and exogenous LHRH does not release significant amounts of FSH. In conclusion, under different background hormonal conditions in which both LH and FSH release occur, LHRH by itself does not appear to be responsible for FSH release.

Summary. In rats, sc injections of nicotine tartrate during the critical period on the afternoon of vaginal proestrus delayed the spontaneous release of pituitary LH, FSH, and prolactin for approximately 2 hr. In ovariectomized rats, nicotine inhibited LH release but had no effect on plasma FSH or TSH concentration. Nicotine did not inhibit LH release in response to LHRH, and LHRH did not release FSH in proestrous or ovariectomized rats. The results support the view that two releasing hormones are involved in LH and FSH release.

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