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NOTE

Nicotine Given Intracerebroventricularly Does Not Inhibit the Preovulatory Surge of LH and PRL Secretion in Female Rats

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Abstract. To determine the effect of nicotine on LH and PRL secretion, nicotine bitartrate (nicotine) dissolved in saline was administered at 1400 h, just before the critical period for the preovulatory surge of LH and PRL secretion, either intracerebroventricularly (icv) or intravenously (iv) in female rats in proestrus. Nicotine neither at a dose of 5 μg nor at a dose of 10 μg injected icv at 1400 h caused significant changes in the surge of LH and PRL secretion. When nicotine was given iv at a dose of 100 μg, a significant decrease in LH and PRL concentrations occurred immediately, lasting for 2 h. After 1700 h, LH and PRL concentrations as high as that observed after 1700 h in saline-injected control rats were recovered, just as if nicotine caused a transient deficit of the surge secretion of these hormones. The results indicate that nicotine does not inhibit the preovulatory surge of LH and PRL secretion by acting at the hypothalamic level accessible via the third ventricle, but inhibits it by acting at certain other site(s).

Key words: Nicotine, Intracerebroventricular injection, Intravenous injection, Proestrous rats, LH surge, PRL surge

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IT WAS early found that, in female rats, nicotine injected subcutaneously (sc), i.e., systemically, delayed the preovulatory surge of both LH and PRL secretion on the day of proestrus [1–4], although tobacco smoke delayed only the LH surge [5]. The LH surge advanced by progesterone injection in the morning of proestrus was also inhibited by nicotine injected intraperitonearly (ip) [6].

The site of this inhibitory action of nicotine on the preovulatory LH and PRL surge has not yet been determined, but, the same inhibitory action on the pulsatile LH secretion has been suspected to occur at the mediobasal hypothalamus [7]. We also have data showing that the electrical activity of the GnRH pulse generator is inhibited by nicotine, suggesting nicotine action at the mediobasal hypothalamus [Kimura and Sano, unpublished observation].

Recently we have hypothesized that there are two separate mechanisms in the brain for the control of gonadotropin secretion: the gonadotropin-releasing hormone (GnRH) pulse generator and the GnRH surge generator [8]. These two generators respond differently to drugs such as pentobarbital, naloxone, bicuculline and insulin [9–13, Kawaguchi, Funabashi and Kimura, unpublished observation]. It is then possible that the GnRH surge generator in the preoptic-mediobasal

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hypothalamus, which we hypothesized as responsible for the surge of LH secretion, has a nicotine response different from that of the pulse generator responsible for pulsatile LH secretion. In the present study, we therefore examined whether the surge generator will still be inhibited by nicotine administered intra-cerebroventricularly (icv) which will more directly reach the medial preoptic area-hypothalamus than that administered sc.

Materials and Methods

Animals

Female Wistar-Imamichi rats were obtained from the Animal Research Center (Oomiya, Japan) at 7-weeks old and kept in controlled lighting conditions (lights on 0500–1900 h), with food and water available *ad libitum*. All animal housing and surgical procedures were carried out according to the Guidelines laid down by the Institutional Animal Care and Use Committee of the Yokohama City University School of Medicine.

Surgery

A stainless steel guide cannula (0.75 mm in outer diameter, 13 mm in length) was placed stereotaxically into the third ventricle according to the atlas of Albe-Fessard *et al.* [14] (stereotaxic coordinates: A=6.0, V=2.0 and L=0.0) under sodium pentobarbital (31.5 mg/kg bw) anesthesia. The guide cannula was fixed to the skull by small stainless steel screws and dental cement, and was plugged with an inner cannula (0.35 mm in diameter). Placement of the canula in the third ventricle was confirmed by welling up of cerebrospinal fluid.

Estrous cyclicity was monitored by checking the vaginal smear every morning at 0900–1000 h. Rats having at least two regular consecutive 4-day estrous cycles were randomly assigned to different experimental groups.

An intraatrial silicone cannula (0.3 mm in inner diameter) was placed under ether anesthesia through the jugular vein into the right atria, 24 h prior to the experiment i.e., on the day of diestrus 2.

Injection and blood sampling

Nicotine bitartrate (Wako, Japan) was dissolved in saline to make 5 $\mu g/2\ \mu l$ and 10 $\mu g/2\ \mu l$ solutions for icv injections, and 100 $\mu g/100\ \mu l$ for iv injections. Saline (2 μl or 100 μl) was injected as the control. Serial blood samples (250 μl) were drawn from 1200 h to 2000 h at hourly intervals and an equal volume of heparinized saline was replaced after each sampling. The injection was given just after the sampling at 1400 h, after which samples were drawn at 1415, 1430, 1500, 1530 and 1600 h to see the immediate effects, followed by sampling at hourly intervals till 2000 h.

Hormone assay

Serum LH and PRL concentrations were measured by RIA with materials supplied by NIDDK. The reference standard was NIDDK rat LH-RP-3 and PRL-RP-3, but the amounts of LH and PRL are expressed as NIH-LH-Sl and NIH-PRL-Sl, respectively. The mean of the minimal detectable amount of LH in two assays was 0.17 ng/m*l* and that of PRL 0.31 ng/m*l*. The intra- and interassay coefficients of variation (CVs), estimated at the LH level of 2.02 ng/m*l* were 8.3% and 6.3%, respectively. CVs estimated at the PRL level of 5.9 ng/m*l* were 13.2% and 11.6%, respectively.

Statistical analysis

Significant fluctuations in LH or PRL concentrations were determined by one way ANOVA repeated measures in the saline-injected or nicotine injected group of rats. To determine the effect of nicotine injection on LH and PRL concentrations, raw data were analyzed by two way ANOVA where variables were time and treatments. If a significant interaction was observed, Scheffe's post hoc analysis was followed. Significance was attained at $P<0.05$. For the figures, means (± SEM) were obtained with respect to the time of sampling for each of the saline- and nicotine-injected groups.

Results

Effects of iv injection of nicotine on LH and PRL surge (Fig. 1)

In the group injected iv with saline at 1400 h, LH concentrations fluctuated significantly, showing the peak of the LH surge at 1530 h. The iv injection of nicotine at 1400 h at a dose of 100 μg caused an acute decrease in LH levels, making a trough 15 min after the injection. The difference was significant at 1415 h, 1430 h, 1500 h, 1530 h and 1600 h compared to serum LH in the corresponding saline-injected control group. Thereafter, LH concentrations increased rapidly and at 1700 h reached levels as high as those in the saline-injected control group, after which they declined along with the latter group. This change suggests that the iv nicotine caused only a transient deficit of the LH surge.

The same was exactly true for PRL secretion. In the saline-injected group, PRL concentrations showed a surge of secretion peaking at 1600 h, but in the nicotine-injected group, there was a transient deficit of the surge. The decrease in PRL concentrations was significant between 1415 h and 1600 h, just as in the case of LH. After 1700 h, PRL concenrations recovered the levels in the saline-injected control group.

Effects of icv injection of nicotine on LH and PRL surge (Fig. 2)

In the saline-injected group, LH concentrations fluctuated significantly, showing a surge of LH secretion which peaked at 1700 h. In both groups of rats injected with nicotine at doses of 5 μg and 10 μg, the surge of LH secretion occurred similarly to that in the saline-injected group. In addition, no significant effects of nicotine, either at a dose of 5 or 10 μg, on LH concentrations were observed, compared to LH concentrations in the saline-injected group. The icv injection of nicotine therefore had no significant effects on the surge of LH secretion in the afternoon of proestrus.

PRL concentrations fluctuated significantly, showing a surge of PRL secretion peaking at 1800 h. In the rat injected with nicotine, either at a dose of 5 μg or 10 μg, PRL concentrations also showed

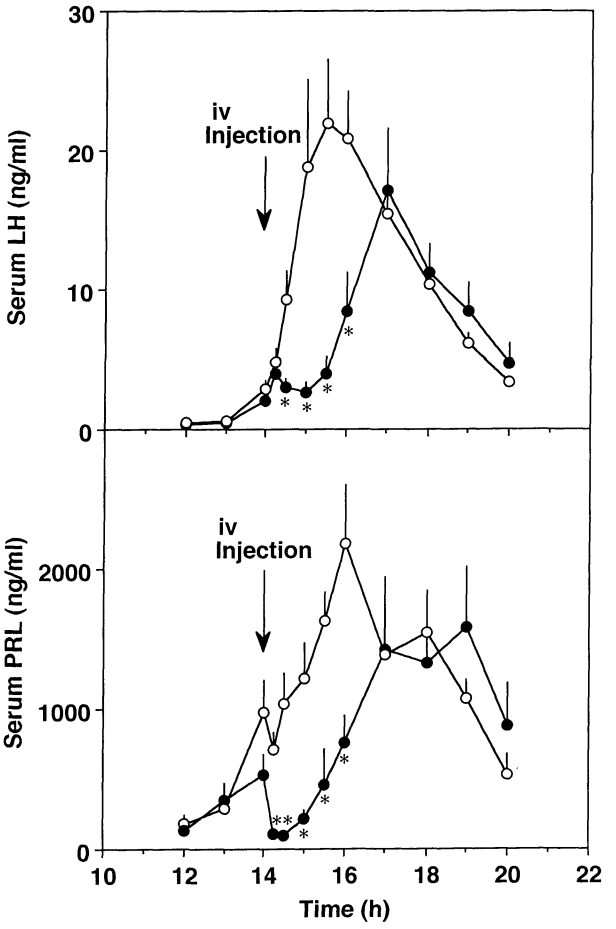

Fig. 1. Serum concentrations of LH (upper) and PRL (lower) in proestrous rats injected iv with saline (○, n=11) or nicotine (100 μg, ●, n=9). Each point and vertical line represent the mean and SEM, respectively. *shows significant differences from saline-injected controls (*post-hoc*, $P<0.05$).

significant fluctuations, with peaks of the surges at 1700–1800 h. There was also no significant difference between PRL concentrations in saline- and nicotine-injected groups, indicating that icv nicotine had no significant effects on the surge of PRL secretion in the afternoon of proestrus.

Discussion

The results of the present study showed that nicotine administered icv had no effect on the surge of LH and PRL secretion in the afternoon of proestrus. It was reported that icv administered

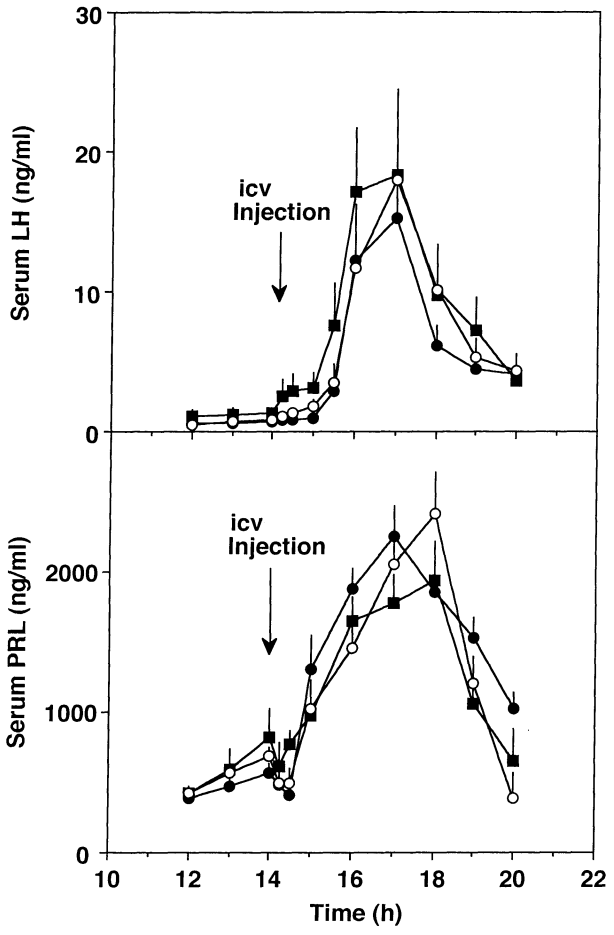

Fig. 2. Serum concentrations of LH (upper) and PRL (lower) in proestrous rats injected icv with saline (○, n=5) or nicotine (5 μg, ●, n=5 and 10 μg, ■, n=5). See Fig. 1 for further details.

nicotine at a dose of 0.125–2.5 μg was effective in stimulating PRL secretion in male rats [15, 16], and at a dose of 1–5 μg dose-dependently stimulated noradrenaline secretion in the paraventricular nucleus of the hypothalamus [17]. The present nicotine dose, 5 or 10 μg, would therefore not be too small to induce neuronal changes in the hypothalamus.

This was an expected result as mentioned in the introduction. Together with our unpublished observation that nicotine, although administered iv, inhibited electrical activity of the GnRH pulse generator in the mediobasal hypothalamus, it seems likely that the surge and pulse generator for GnRH secretion respond differently to nicotine. The present results provide further evidence to support our hypothesis that two subgroups of GnRH neurons act in the control of gonadotropin secretion [8].

In contrast to the icv injection, systemic injection of nicotine caused a transient reduction in the surge secretion of both LH and PRL, after which the surge continued as usual. This finding is the same as those in previous reports showing that a single nicotine iv or sc injection does not totally abolish the LH and PRL surge but causes an acute reduction in both LH and PRL concentrations after the injecton [1–4]. Taking the present finding that icv nicotine injection did not cause any changes in the surge secretion of these hormones, all of these studies seem to suggest that nicotine injected systemically causes the effects by acting on certain sites(s) other than the central nervous system, in other words the site of action of nicotine is not on the GnRH surge generator, which is presumably in the preoptic area.

We could not determine the precise sites of action of nicotine administered iv, but the most probable sites are the superior and inferior hypophysial arteries that give rise to the primary plexus of the portohypophysial system, which also is in close proximity to the axon terminals of the GnRH neurons. These vessels are innervated by post ganglionic sympathetic nerve fibers [18] from the superior cervical ganglion (SCG) and their smooth muscles are also exposed to locally secreted catecholamines, dopamine and other vasoactive peptides. It was reported that after nicotine sc injections, more than 80% of the neuronal nuclei in the SCG showed fos-like immunoreactivity [19]. It has also been shown that iv nicotine causes peripheral vasoconstriction in dogs [20] and cerebral and umbilical vasoconstriction in ovine fetuses [21]. It is therefore possible that iv nicotine causes vasoconstriction of the hypophysial arteries and transiently delays GnRH and PRF in reaching the anterior pituitary, resulting in acute reduction in LH and PRL secretion after which the proestrous surge occurs. But to confirm this, assessment of the effect of iv nicotine on other anterior pituitary hormones would be neccessary.

Another possible site of nicotine action could be the anterior pituitary gonadotrophs and lactotrophs. Supporting this hypothesis, previous reports showed that nicotine acted directly on pituitary GH3 cells to inhibit transcription directed by PRL

promoter [22]. Also in GH3 pituitary cell homogenates, acetylcholine decreased cyclic AMP content and reduced PRL release [23]. An extensive review of the novel aspects of GnRH receptor and intracellular signaling in the pituitary gonadotrophs showed that inositoltriphosphate (IP3) and diacylglycerolphosphate (DAG) are involved in LH secretion [24]. It is likely therefore that nicotine acts through the IP3 and DAG pathways to inhibit LH release, as shown in growth hormone release in anterior pituitary cells [25].

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