Lack of effect of intracerebroventricular infusion of H1 or H2 histamine receptor antagonists on luteinizing hormone secretion in fed or fasted rats

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Abstract. The effects of intracerebroventricular infusion of histamine receptor antagonists on pulsatile secretion of luteinizing hormone (LH) were examined in ovariectomized, estradiol-primed rats under conditions of normal feeding or fasting for 48 h. Blood samples for determination of the plasma LH concentration by radioimmunoassay were collected every 6 min for 3 h through an atrial indwelling catheter. Ten microliters of either the H1 receptor antagonist chlorpheniramine (3 mM), the H2 receptor antagonist ranitidine (3 mM), or artificial cerebrospinal fluid (vehicle) were infused into the third ventricle during the second 1-h period of blood sampling. The infusion of either receptor antagonist failed to restore the pattern of pulsatile LH release in fasted animals to that observed in fed rats. The plasma LH concentration as well as the pulse frequency and pulse amplitude of LH secretion in fed rats were also not affected by either receptor antagonist. These results indicate that H1 or H2 receptor antagonists did not affect on tonic LH secretion in fed or fasted rats.

Key words: chlorpheniramine, fasting, LH pulse, ranitidine, rat.

We previously showed that intracerebroventricular (ICV) infusion of histamine (HA) inhibited the pulsatile secretion of luteinizing hormone (LH) in normally fed rats but not in fasted rats (Komatsu et al. 2000b). However, no marked effect of fasting on HA immunoreactivity in hypothalamic fibers or cell bodies was apparent (Komatsu et al. 2000b). These observations thus suggested that HA might contribute to the regulation of LH secretion in normally fed rats, but it remained unclear whether endogenous HA is involved in the suppression of pulsatile LH secretion apparent in fasted animals.

H1, H2, and H3 subtypes of HA receptors have been identified in the mammalian hypothalamus (Palacios et al. 1981; Bouthenet et al. 1988; Ruat et al. 1990; Pallard et al. 1993). Both H1 and H2 subtypes are postsynaptic receptors, whereas H3 is a presynaptic receptor that inhibits both the synthesis and release of HA (Ito et al. 1994).

Histaminergic postsynaptic events modulate both H1 and H2 receptors. The H1 receptor, but not the H2 receptor, has been implicated in the secretion of LH and of gonadotropin-releasing hormone (GnRH) in vitro (Miyake et al. 1987; Otsuka et al. 1989; Noris et al. 1995). ICV infusion of HA was shown to increase LH secretion in rats in proestrus (Donoso 1978) as well as in ovariectomized (OVX), estradiol (E2)–primed (Libertun and McCann 1976) and OVX, E2– and progesterone-primed (Donoso et al. 1976) rats, with this effect being mediated by the H1 receptor (Fekete et al. 1999). These observations thus suggest that the role of HA in LH secretion may depend on sex steroid status.

To provide further insight into the possible role of HA in the fasting-induced suppression of LH secretion, we have now examined the effects of ICV infusion of H1 and H2 receptor antagonists on pulsatile LH release in OVX, E2–primed rats under both normal feeding and fasting conditions.

Materials and methods

Animals

All experiments were performed in compliance with
the guidelines for the use and care of laboratory animals set by the Committee for the Regulation of Animal Experiments of the Faculty of Agriculture, Shinshu University. Virgin female Wistar rats (Charles River Japan, Yokohama, Japan) with body masses of 230 to 270 g that had exhibited two successive 4-day estrous cycles were studied. Animals were maintained in a temperature (24 ± 2°C)- and lighting (L/D:14/10, lights on at 0500 hour)-controlled room and given food (commercial pellet, CE-2 CLEA, Nihon CLEA Co., Ltd., Tokyo, Japan) and water ad libitum.

**Surgery**

On the day of estrus a stainless steel guide cannula (22G, Plastic One Co., Roanoke, VA, USA) was stereotaxically implanted into the third ventricle (midline, 0.8 mm posterior and 8.0 mm ventral to bregma (Paxinos and Watson 1986) under light ether anesthesia. Dummy cannula (28G, Plastic One Co.) was inserted to the guide cannula to prevent outflow of cerebrospinal fluid until the time of drug infusion.

Two days after the brain cannulation, ovaries were removed bilaterally under light ether anesthesia, and a silicone tubing (i.d. 2.0 mm, o.d. 3.0 mm, 25 mm in length; Silascon® No. 2, Kaneka-Medics Co., Tokyo, Japan) filled with peanut oil solution of estradiol-17β (20 μg/ml, E₂; Sigma Chemical Co., St. Louis, MO, USA) was subcutaneously implanted at the same time. An atrial indwelling catheter of silicone tubing (i.d. 0.5 mm, o.d. 1.0 mm, 50 cm in length; Silascon® No. 00) was attached via right jugular vein two days before the blood sampling (Komatsu et al. 2000a). Distal end of the indwelling catheter was coiled and stored in a small plastic case sutured on the neck of animal until just before the blood sampling.

**Experimental protocols**

The H1 receptor antagonist (±) chlorpheniramine maleate (Research Biochemicals, Natick, MA, UA) and the H2 receptor antagonist ranitidine hydrochloride (Research Biochemicals) were dissolved at a concentration of 3 mM and a pH of 7.6 in sterile artificial cerebrospinal fluid (aCSF: 145 mM NaCl, 3.5 mM KCl, 1.0 mM MgCl₂, and 1.0 mM CaCl₂). The solutions were stored at −30°C until use.

Five days after the ovariectomy and E₂ tube implantation, fasting was started at 1200 h. From 48 hours of fasting, blood samples of 120 μl were withdrawn every 6 min for three hours. At one hour of the blood sampling, an injection cannula (26G; Plastic One Co.), connected with polyethylene tubing to a 10 μl Hamilton microsyringe attached to a microsyringe pump, was inserted into the guide cannula, and H1 or H2 receptor antagonist solution or aCSF was infused into the third ventricle via this injection cannula at a rate of 10 μl/hour. The infusion was lasted for one hour and blood collection was continued during this and one hour post-infusion periods. Similar treatments were also done in normally fed rats as unfasted controls. Blood samples were centrifuged immediately at 4°C and plasma obtained was stored at −30°C until the use for LH assay. At the end of blood sampling, animals were deeply anesthetized with pentobarbital sodium (0.05 mg/kg, Nembutal®, Abbot Lab., North Chicago, IL, USA), and 2 μl of 0.01% brilliant blue solution in acetate buffer (pH 4.6) was infused into brain via the injection cannula. Then, animals were perfused transcardially with heparin (10 IU/ml; Novo Nordisk A/G, Denmark)-saline at first and then with 10% formalin-0.1 M phosphate buffer (pH 7.4). At the initial step of experiment, tissue sections of brain were prepared for the histological check of the position of cannula tip, but visual confirmation of the third ventricle filled with the dye solution in the razor cut brain sections was mostly adopted in the later step. In either case, only the data of animals in which correct insertion of injection cannula into the third ventricle was verified were included in the results.

**Radioimmunoassay (RIA) and statistical analysis**

Plasma LH concentration was measured with a double-antibody RIA kit for rat LH (National Hormone and Pituitary Program, Baltimore, MD, USA). The NIDDK antiserum S11 to rat LH and reference standard, respectively. The lowest detectable concentration of LH in plasma was 0.16 ng/ml, and the intra-assay variation was 9.5%.

Plasma LH profiles were analyzed with the Pulsar program (Merriam and Wachter 1982). Assay variations were accommodated by the equation $y = 9.25x^2 - 14.08x + 13.44$, and cutoff criteria were $G(1) = 2.5$, $G(2) = 2.1$, $G(3) = 1.85$, $G(4) = 1.52$, and $G(5) = 1.52$. Plasma LH concentration as well as the pulse frequency and pulse amplitude of LH secretion were calculated separately in each animal before infusion (preinfusion, 1200 to 1300 hours) and after infusion (infusion, 1300 to 1400 hours; postinfusion, 1400 to 1500 hours). Data were expressed as the percentage change after infusion versus the...
preinfusion value for each treatment. The statistical significance of differences was determined by two-way analysis of variance (ANOVA) followed by the Bonferroni-Dunn test; a $P$ value of $<0.05$ was considered significant.

**Results**

The time courses of plasma LH concentration during ICV infusion of aCSF or H1 or H2 receptor antagonists under the normal feeding and fasting conditions are shown in Fig. 1. LH pulses were detected in animals of all groups both before and after treatment. Figure 2 shows the three LH secretion parameters after infusion (infusion and postinfusion period). Plasma LH concentration in rats treated with the H2 receptor antagonist as well as LH pulse frequency and amplitude in rats treated with the H1 receptor antagonist were significantly lower in the fasted state than in the fed state. However, infusion of neither the H1 nor the H2 receptor antagonist significantly affected any of the three LH secretion parameters in either the fed or fasted conditions, and no interaction was seen between the drug treatments and nutritional states (two-way ANOVA).

**Discussion**

The pulsatile secretion of LH was inhibited by fasting and restored by refeeding. A main trigger of the suppression of the hormone secretion had been considered the neural input emitted from the empty stomach in fasted animals (Cagampang et al. 1992). However, we observed the chronological change in LH secretion during fasting for 48 h and succeeding refeeding for 24 h in rats (Komatsu et al. 2000a). The suppression of LH secretion first appeared from 6 h after fasting, corresponding to the onset of dark period, i.e., that of the period of intensive feeding in normally fed animals, and the secretion promptly restored by refeeding and also on the morning of the next day. The above observation is not explained simply by the gastric factor, daily cycle of feeding and LH secretion. It is possible that leptin is participated in the change in LH secretion due to feeding manipulation (Kalra et al. 1998; Gonzalez et al. 1999).

The observation that brain HA neurons are activated in the early phase of fasting (Sakata et al. 1994) suggested that the resulting increase in HA release might contribute to the inhibition of LH secretion apparent in fasted rats.
Prevention of HA action by HA receptor antagonists might therefore be expected to relieve the fasting-induced suppression of LH secretion. However, we have now shown that ICV administration of either the H1 receptor antagonist chlorpheniramine or the H2 receptor antagonist ranitidine at a concentration of 3 mM had no effect on pulsatile LH secretion in OVX, E$_2$-primed rats in either the fasted or fed state. This concentration of chlorpheniramine corresponds to >10,000 times those shown to be effective in eliciting feeding in rats (Sakata et al. 1988) or inducing GnRH release from GT1-1 cells in vitro (Noris et al. 1995). This concentration of ranitidine is 15,000 times that shown to be effective in inhibition of insulin-induced adrenocorticotropic hormone release in rats (Kjær et al. 1993).

We previously showed that HA inhibited LH secretion in normally fed rats, although no difference in HA immunoreactivity in any area of the hypothalamus was apparent between fed and fasted animals (Komatsu et al. 2000b). Our present results now suggest that the HA system in the brain does not participate in the suppression of LH secretion by fasting, at least during the first 48 h of food deprivation. It is possible that the fasting-induced inhibition of LH secretion results from the activation of neurons that contribute to the release of corticotropin-releasing hormone (CRH) or of other neurotransmitters that modulate GnRH release. CRH has been shown to markedly suppress pulsatile LH secretion of the 48-h fasting rat (Maeda et al. 1994). It also remains possible that the infusion of HA receptor antagonists indeed inhibits a negative action of endogenous HA on LH release in fasted animals, but that this effect is masked by a more pronounced negative action of CRH. It remains to be examined an optimal dose for the effect of HA antagonist on LH secretion.

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