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Involvement of Prolactin-Releasing Peptide in the Preovulatory Luteinizing Hormone and Prolactin Surges in the Rat

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Prolactin (PRL)-releasing peptide (PrRP) is a novel hypothalamic peptide reported as a potent and specific stimulator of PRL secretion. In this study, we examined a possible role of PrRP in the ovarian steroid-induced PRL surge in the rat, simultaneously observing the change in luteinizing hormone (LH) surge. Experiments were performed on both normally-fed and three-day-fasted rats, which were ovariectomized and primed with estradiol and progesterone. From 11:00 to 18:00 h, blood was collected every 30 min to measure LH and PRL. All the following substances were given intracerebroventricularly at 11:00 h. Compared to control serum, anti-rat PrRP31 serum caused a significant reduction of the LH and PRL surges. The antiserum also delayed the onset of PRL surge. Fasted rats were devoid of significant surges of the hormones, while 3.0, but not 0.5 nmol of rat PrRP31 given to these animals produced a significant recovery of PRL surge. Although LH surge was not reinstated, basal LH secretion was transiently stimulated by 3.0 nmol of PrRP31. These results demonstrate for the first time a significant participation of PrRP in the preovulatory LH and PRL surges in the rat. Possible indirect pathways mediating this effect of PrRP were discussed, in view of the unique anatomical distribution of PrRP in the hypothalamus.

Key Words: **prolactin-releasing peptide; luteinizing hormone; prolactin; surge; normal feeding; fasting; rat.**

PrRP is a recently isolated peptide that acts as the endogenous ligand for the hypothalamic orphan receptor, hGR3 (1). *In vitro* the peptide was reported to specifically stimulate the release of PRL, but not of any other anterior pituitary hormone from rat anterior pituitary cells (1). The hormone was therefore named PrRP. The preproprotein encoded by the PrRP cDNA generates at least two peptides, i.e., PrRP31 and PrRP20 (1). It was reported that PrRP mRNA is most abundantly expressed in the medulla oblongata (1–6). Immunoreactive PrRP can be detected in rat hypothalamus in very high concentrations (7), and a high density of PrRP receptor mRNA was detected in the rat anterior pituitary (1, 2, 5). Although these biochemical characteristics of PrRP may suggest the neuropeptide as a good candidate for a physiological PRL-releasing factor, accumulating data reported to date do not necessarily support this possibility. Although iv administration of PrRP has been reported to stimulate PRL secretion in both male and female rats, in males a pharmacologically high dose of the peptide was required to induce the hormonal response (8). Other researchers also reported similar data that the PRL-releasing potency of PrRP31 *in vivo* was much weaker than that of thyrotropin-releasing hormone (9), and another study *in vivo* even concluded that PrRP is unable to stimulate PRL secretion (10). In addition, by using anterior pituitary cell culture *in vitro,* Samson *et al.* (11) reported that the PRL-releasing activities of both PrRP31 and PrRP20 were not demonstrable in male rats, and very weak even in female rats. Furthermore, we also reported recently that PrRP may not play a significant role, or at least play a much weaker role than VIP, in mediating PRL secretion induced by ether stress and suckling in the rat (12).

Abbreviations used: PrRP, prolactin-releasing peptide; PRL, prolactin; iv, intravenous(-ly); VIP, vasoactive intestinal peptide; LH, luteinizing hormone; OVX, ovariectomy(-ized); icv, intracerebroventricular(-ly); aCSF, artificial cerebrospinal fluid; NRS, normal rabbit serum; LHRH, luteinizing hormone-releasing hormone.

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However, as far as we know, there is no previous study examining a possible involvement of PrRP in the preovulatory PRL surge in the rat. This PRL surge occurs concomitantly with LH surge in the afternoon of proestrus of the estrous cycle, and the surge-like secretion of both hormones can be simulated by priming OVX rats with estrogen and progesterone. Therefore, in this study we tested the effects of icv administration of neutralizing anti-PrRP serum on the ovarian steroid-induced LH and PRL surges in OVX rats. We also examined a possible effect of PrRP given icv on the hormonal surges in fasted female rats, which is a model deprived of inherent surges of LH and PRL.

MATERIALS AND METHODS

All the following procedures for experimentation were approved by the Hirosaki University Ethical Committee for Animal Experiments. Animals were maintained in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Female rats (250–270 g) of the Wistar strain were used. They were housed in an air-conditioned room with controlled lighting (light 08:00–20:00 h), and were given free access to laboratory chow and tap water. Animals were OVX under light ether anesthesia about two weeks before experimentation. Seven to ten days before experimentation, animals were anesthetized with sodium pentobarbital (40 mg/kg body weight, intraperitoneally), and a guide cannula (22 gauge) with a removable inner stylet was stereotaxically implanted towards a lateral cerebroventricle. Coordinates for placement of the cannula were taken from the atlas of Pellegrino *et al.* (13) (0 mm anterior to and 1.5 mm lateral to the bregma, and 2.8 mm ventral from the dura). The cannula was fixed onto the skull with anchor screws and dental cement. Two days prior to the experiment, under light ether anesthesia, the animals were implanted with a jugular vein catheter filled with heparin solution, and also implanted subcutaneously with a single Silastic capsule containing 300 μg/ml of estradiol-17β (Sigma Chemical Company, St. Louis, MO) in the same manner as in our previous reports (14–16).

Experiments were performed on both normally-fed and three-day-fasted rats. At about 08:00 h on the day of the experiment, the jugular vein catheter was exteriorized for frequent blood sampling. The inner stylet of the guide cannula was removed and replaced with a 30-gauge injection needle connected to Teflon tubing. At 09:00 h, 5 mg per rat of progesterone (Mochida Pharmaceutical Co., Ltd., Tokyo, Japan) was injected intramuscularly. At 11:00 h, normally-fed rats received an icv injection of aCSF, anti-rat PrRP31 serum (Phoenix Pharmaceuticals, Inc., Mountain View, CA), or NRS (Zymed Laboratories, Inc., San Francisco, CA). The anti-PrRP31 serum and NRS were used as neat serum. Fasted rats received an icv administration of 0.5 or 3.0 nmol of rat PrRP31 (Peptide Institute, Inc., Osaka, Japan) dissolved in aCSF, or aCSF only at 11:00 h. The constituents of aCSF were the same as in our previous studies (14, 15). Every icv injection was done in a volume of 5 μl over 2–3 min. Blood samples (200 μl) were collected every 30 min over a total period of 420 min (11:00–18:00 h). To prevent the loss of circulating plasma volume, 200 μl of 0.9% NaCl was injected immediately after each blood collection. The blood was collected in EDTA-2Na (2.5 mg/ml)-containing tubes, centrifuged, and the plasma was stored at −70°C until assayed for LH and PRL. Within 30 min of the experiment, 15–20 μl of 0.1% ethylene blue solution was injected icv, and then the animals were killed by decapitation. The brains were removed, and we checked whether the median eminence was stained with the dye. Only such animals whose median eminence was dyed were considered to have undergone a successful icv injection, and allowed to contribute to the data given in the Results.

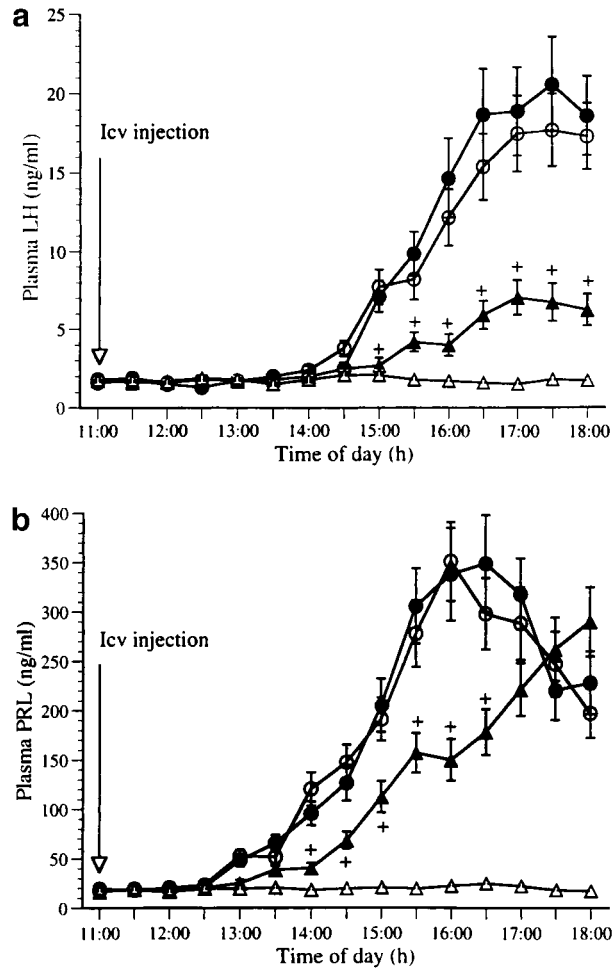

FIG. 1. Effects of icv administration of anti-PrRP31 serum on steroid-induced LH and PRL surges in normally-fed OVX rats. The number of rats examined was 6–8 per group. ●, normally-fed + aCSF; ○, normally-fed + NRS; ▲, normally-fed + anti-PrRP serum; △, fasted + aCSF. +, significantly different vs normally-fed + aCSF and normally-fed + NRS groups. In this figure and in Fig. 2, where standard errors are not shown, they were smaller than the symbols. For further details see text.

Plasma LH and PRL levels were determined by RIA using the reagents kindly donated by Dr. A. F. Parlow (NIDDK). Rat LH-RP-3 and PRL-RP-3 were used as the standards. Sensitivity of the LH assay was 0.2 ng/ml, and that of PRL assay was 0.8 ng/ml. For both hormones, samples from individual rats were analyzed within the same assay. Both the intra- and interassay coefficients of variation were less than 10% in the two assays.

Results were expressed as the mean ± SEM. One-way or two-way ANOVA followed by Scheffe's post-hoc test was used to analyze the data. Differences were considered significant if P was smaller than 0.05.

RESULTS

Figure 1 shows the temporal profiles of plasma LH and PRL in the three groups of normally-fed rats and also the fasted + aCSF group. With respect to LH levels (Fig. 1a), the normally-fed + aCSF group showed

significantly higher levels of the hormone (LH surge) during the period of 14:30–18:00 h as compared to their 11:00-h value. The temporal pattern and magnitude of LH surge in the normally-fed + NRS group was statistically indistinguishable from those of the normally-fed + aCSF group, which indicates that NRS did not exert nonspecific effects. In agreement with our previous studies (14–16), the fasted + aCSF group did not exhibit a significant surge of LH. The administration of anti-PrRP serum to normally-fed animals significantly depressed the magnitude of LH surge during the period of 15:00–18:00 h, even though plasma LH levels of this group were still significantly higher than those of the fasted + aCSF group between 15:30–18:00 h. A similar finding was also observed for plasma PRL levels (Fig. 1b). Significantly higher levels of PRL than 11:00-h values (PRL surge) were observed in both normally-fed + aCSF and normally-fed + NRS groups between 13:00–18:00 h. The PRL levels of these two groups were statistically the same throughout the entire period of observation. As in the case of LH surge (Fig. 1a), the fasted + aCSF group did not have a significant PRL surge. Although the normally-fed + anti-PrRP serum group also showed a significant surge of PRL, the magnitude of the hormonal surge was significantly smaller than that of the normally-fed + NRS group during the period of 14:00–16:30 h. In addition, it is interesting to note that the onset of PRL surge in the normally-fed + anti-PrRP serum group occurred later than that in the normally-fed + NRS group. In the normally-fed + anti-PrRP serum group, the first significant rise in plasma PRL over the 11:00-h value occurred at 14:30 h (1.5-h behind the normally-fed + NRS group), and peak PRL levels were observed at the final time point of sampling (18:00 h).

Figure 2 shows plasma LH and PRL levels of the three fasted groups. The data of the normally-fed + aCSF group are shown again for comparison. As in the case of the fasted + aCSF group, the fasted + PrRP (0.5 nmol) group did not show a significant surge of either LH or PRL. By contrast, 3.0 nmol of PrRP given to fasted animals stimulated the secretion of both hormones. Plasma LH levels of the fasted + PrRP (3.0 nmol) group started to rise at 11:30 h, reached a peak 30 min later (i.e., 60 min after injecting the PrRP), and gradually declined thereafter. The 12:00 and 12:30-h levels of LH in the fasted + PrRP (3.0 nmol) group were significantly higher than those in the remaining three groups, and also significantly exceeded its own 11:00-h value. However, during the hours of the day when LH surge occurs normally, the fasted + PrRP (3.0 nmol) group did not have higher concentrations of plasma LH than the fasted + aCSF group. By contrast, plasma PRL levels of the fasted + PrRP (3.0 nmol) group formed almost a normal pattern of PRL surge, although the surge magnitude was significantly smaller than that of the normally-fed + aCSF group between 14:30–17:00 h.

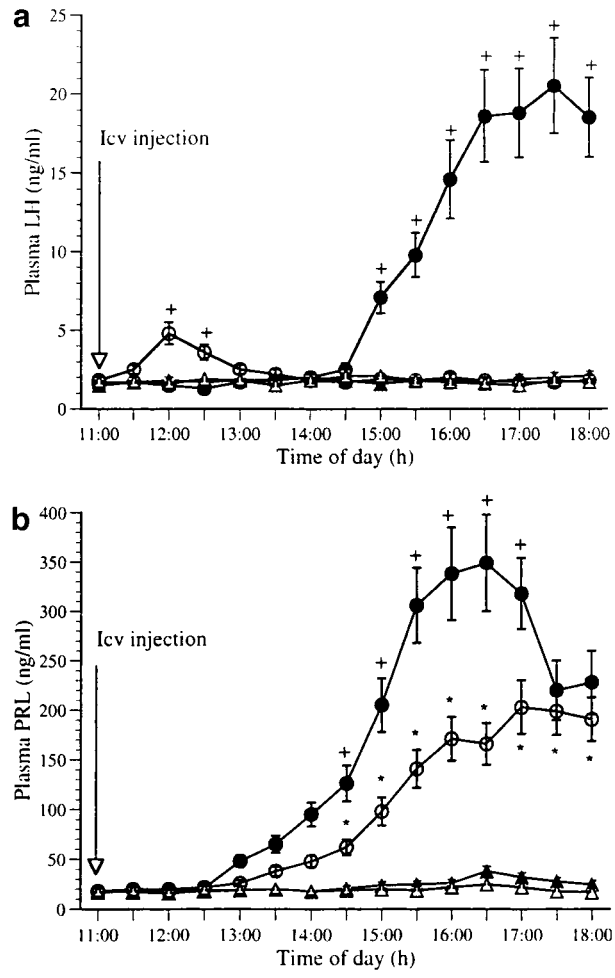

FIG. 2. Effects of icv administration of rat PrRP31 on steroid-induced LH and PRL surges in three-day-fasted OVX rats. The number of rats examined was 7–9 per group. ●, normally-fed + aCSF; ▲, fasted + PrRP (0.5 nmol); ○, fasted + PrRP (3.0 nmol); △, fasted + aCSF. +, significantly different vs the remaining three groups. *, significantly different vs fasted + aCSF group. For further details see text.

DISCUSSION

There are several previous studies which reported an excitatory action of PrRP on PRL release *in vivo* (8, 9) and *in vitro* (1, 17), although conflicting reports also exist (10, 11). However, to the best of our knowledge, the present study is the first to have examined a possible role for PrRP in the generation of ovarian steroid-induced LH and PRL surges in the rat.

With respect to the involvement of PrRP in PRL surge, it is interesting to note that immunoneutralization of brain PrRP resulted in significant reduction and delay of PRL surge, and also that the icv administration of PrRP significantly reinstated the hormonal

surge. These results suggest that PrRP may play a significant role in the generation and maintenance of the preovulatory PRL surge. We recently reported that PrRP may not be a significant mediator of PRL secretion induced by ether stress and suckling in the rat (12). In this sense, the present study seems to be the first to demonstrate a significant participation of PrRP in a specific physiological event, which is normally associated with a massive discharge of PRL from the pituitary.

It is an unexpected finding that PrRP antiserum also caused a significant attenuation of LH surge. Although this observation might indicate a significant participation of PrRP in the preovulatory LH surge, the icv administration of PrRP to fasted rats did not lead to a significant recovery of LH surge, differing from its effect on PRL surge. Even so, the PrRP injection caused a short-lived stimulation of LH secretion, with its peak level occurring 60 min postadministration. This excitatory action of PrRP on LH seems to agree with the very recent report of Seal *et al.* (18) that icv administration of PrRP produced a significant elevation of plasma LH and follicle-stimulating hormone in the rat. Since our data may suggest it unlikely that PrRP subserves a significant role in the generation and maintenance of the preovulatory LH surge, the significant reduction of LH surge by anti-PrRP serum may probably have occurred via its indirect action involving other hypothalamic factors, as discussed below.

An important question which reasonably emerges from the present data is the neuroendocrine mechanism whereby PrRP affects the LH and PRL surges. Several previous studies agreed that the anterior pituitary contains the greatest concentration of PrRP receptor (1, 2, 5). However, this receptor does not seem to function to receive PrRP transported from the hypothalamus via hypophyseal portal vessels, because no (19) or only scarce (20) PrRP-immunopositive fibers exist in the external layer of the median eminence. Therefore, it is very likely that at least part of the effects of PrRP on pituitary hormone secretion occur indirectly via other hypothalamic factors. In this context, the above-mentioned study of Seal *et al.* (18) reported additional interesting data that PrRP31 was able to stimulate the release of LHRH, VIP, and galanin from rat hypothalamic explants *in vitro.* A recent *in situ* hybridization study reported that PrRP receptor mRNA is expressed in moderate levels in the medial preoptic area of the hypothalamus (5), which is the primary anatomical structure where LHRH neuronal cell bodies are localized. This anatomical characteristic allows for the assumption that PrRP may affect gonadotropin secretion via modulating LHRH release. The present finding that LH surge was significantly suppressed by anti-PrRP serum suggests that PrRP may exert a tonic excitatory input on LHRH neurons.

With respect to the demonstrated involvement of PrRP in PRL surge, VIP and galanin are candidates for hypothalamic peptides which may mediate the action of PrRP. This is because both VIP and galanin are such peptides that can be released by PrRP *in vitro* (18), and also are known to exert stimulatory effects on both LH and PRL secretion (21–23). Especially regarding VIP, there is a recent study which directly tested the effect of icv treatment of VIP antiserum on the preovulatory LH and PRL surges in the rat (24). This study reported a significant delay and reduction of both LH and PRL surges after the VIP immunoneutralization, and thus indicated a significant stimulatory role of VIP in the generation of the hormonal surges. Although whether PrRP affects the hypothalamic dopaminergic system has yet to be examined to date, it is also possible that PrRP influences the preovulatory PRL surge via modulating the release of dopamine, the most important PRL-inhibiting factor (25). Further detailed studies are required to disclose the neuroendocrine circuitry which PrRP exploits to modulate the preovulatory LH and PRL surges.

In summary, in this study we demonstrated that PrRP plays an important role in regulating the preovulatory LH and PRL surges in the rat. Although it is very likely that these effects of PrRP are indirect via modulating other hypothalamic factors, the present results are the first to demonstrate a significant participation of PrRP in a specific physiological event which is normally associated with altered secretion of LH and PRL.

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