Effects of Intraventricular Injection of Delta Receptor Antagonist ICI 154, 129 on the Secretion of Luteinizing Hormone and Prolactin in the Proestrous Rat

RYUHEI HASHIMOTO AND FUKUKO KIMURA
Department of Physiology, Yokohama City University School of Medicine, Yokohama 236, Japan

Abstract. In order to ascertain the role of delta receptors in the control of gonadotropin secretion, a preferential delta receptor antagonist ICI 143,129 was microinjected into the third ventricle through chronically implanted cannulae and the effects on the serum concentration of luteinizing hormone (LH) and prolactin (PRL) were determined in female rats in proestrus. When the injection was given at 1030 h, ICI 154,129 (50 µg) exerted no significant effects on either LH or PRL. However, in the rat given a microinjection of ICI 154,129 at 1300 h, an afternoon rise in LH occurred in advance and was of greater magnitude, with the peak time more than 1 h earlier and the peak amplitude approximately 100% greater than that in the control rat, respectively. The injection also suppressed the PRL rise during the plateau phase. The results indicate that delta receptors are involved in the mediation of the inhibitory influence of endogenous opioids on the surge of LH in proestrus, and that delta receptors mediate the facilitatory influence of opioids on the PRL surge during the plateau phase.

IT IS NOW established that the major effect of opiate or opioid peptide on gonadotropin secretion which is mediated by mu receptors is an inhibition of luteinizing hormone (LH) and a facilitation of prolactin (PRL), although there an opposite effect occurs occasionally due to the dosage administered. Morphine, a preferential mu agonist, although interacting with the delta site too [1], has been shown to inhibit the secretion of LH [2–10], and to stimulate the secretion of PRL [11] in rats, either male or female and either intact or gonadectomized. Other specific mu receptor agonists, morphiceptin [12] and DAGO [13], inhibited LH secretion and facilitated PRL secretion. In contrast to this, the effect on gonadotropin which is mediated by delta receptors has been inconsistent. Delta agonists, DRP [12], and DPDPE [14], inhibited LH secretion, but DTE12 had no effect [13], and Leucine-enkephalin that has selective affinity with delta receptors did produce an increase in LH secretion [12, 15]. Further, it has been reported that DRP [12] and DPDPE [14, 16], had no effect on PRL secretion, and Leu-enkephalin had a stimulatory [17], inhibitory [12] or no [18] effect on PRL secretion.

Therefore, the purpose of the present investigation was to study the effect of a preferential delta receptor antagonist ICI 154,129 on LH and PRL secretion in rats in proestrus.

Materials and Methods

Animals
Adult female rats of Wistar strain were maintained under controlled lighting conditions (lights on 0500–1900 h), with food and water available ad libitum. Cyclicity was monitored daily by taking vaginal smears either before or after the implantation of guide cannulae into the brain.
Surgery
The guide cannulae were made of stainless steel tube with a 0.65 mm outer diameter and were stereotaxically implanted in the third cerebral ventricle, according to the atlas of Albe-Fessard et al. [19], under anesthesia with sodium pentobarbital. The cannulae were fixed to the skull with dental cement and stainless steel screws. The inner cannula with a 0.30 mm outer diameter was arranged so as to protrude approximately 0.50 mm beyond the tip of the guide cannula. The guide cannula was plugged with a dummy inner cannula until the day of the sampling experiment.

Drug injections and bleeding
The animal was implanted with an intracardiac cannula under ether anesthesia on the day of (for the afternoon experiment) or on the day before (for the morning experiment) proestrus after having exhibited 2 or more consecutive 4-day estrous cycles following implantation of the guide cannula. After surgery, the animals were kept in individual cages. In the morning experiment, blood samples (120 µl) were collected from freely moving animals at 6-min intervals for a 3-h period (1000–1300 h) through the intracardiac cannula. After 30 min of sampling, at 1030 h, ICI 154,129 (ICI Pharmaceuticals), at a dose of 50 µg, was injected for 2 min into the third cerebroventricle. The dose was the same as that used by Koenig et al. [20] who examined the effect on PRL and growth hormone in the male rat. The drug was dissolved in a volume of 2 µl sterile 0.9% NaCl (saline) and thus 2 µl saline was injected as the control. In the afternoon experiment, ICI 154,129 at a dose of 50 µg was injected at 1300 h. Blood samples (250 µl) were collected for a 9-h period (1100–2000 h) at 1-h intervals except for the period 1300–1500 h in which sampling was done at 20-min intervals in order to assess the acute effects of drugs injected at 1300 h. Samples at 1300 h were taken just prior to the injection.

Hormone assay
Serum concentrations of LH and PRL were measured by the double-antibody radioimmunoassay with materials supplied by NIDDK and are expressed in terms of rat NIH-LH-SI and NIH-PRL-RP1, respectively. Two assays were run for each of LH and PRL, and the intra- and interassay coefficient of variation (CV) estimated in 5 replicates of stock serum at the mean LH and PRL concentration of 5.5 and 36.3 ng/ml, respectively, were 5.5 and 10.7% for LH, and 7.9 and 15.2% for PRL.

Statistical analysis
For the morning experiment, the mean hormone concentration for each sampling time after drug injection was compared to the mean preinjection concentration by two-way analysis of variance and Duncan’s multiple range test. For the afternoon experiment, two-way analysis of variance and Duncan’s multiple range test were used to test the statistical significance over time in the mean hormone concentration for each sampling time, and Student’s t-test was used to test the significance of difference between the saline- and drug-injected animals.

Results
1. Morning experiment
LH: The mean serum LH concentration did not change significantly following the injection of saline into the third ventricle at 1030 h, but showed variable increases after 1200 h, suggesting that surges of LH secretion had begun in some animals (Fig. 1). ICI 154,129 at a dose of 50 µg did not induce a significant change in serum LH.

PRL: The injection of saline into the third ventricle at 1030 h did not cause a significant change in the mean serum PRL concentration, although at 1300 h the level increased slightly (Fig. 1). The ICI 154,129 injection at 1030 h did not cause any significant change in serum PRL, although the level appeared to be higher after 1200 h, corresponding to 90 min after the injection, than before in the animal injected with ICI 154,129 at 1030 h.

2. Afternoon experiment
LH: Animals injected with saline into the third ventricle at 1300 h showed an afternoon increase in serum LH (Fig. 2). Mean LH concentrations at 1400–1700 h were significantly greater (p<0.05 or 0.01) than at 1100–1300 h, peaking at 1600 h. In ICI 154,129-injected animals, an afternoon increase in LH occurred in advance and was of greater magnitude. The LH concentration at 1400–1700 h was significantly greater than at
Fig. 1. Effects of an intraventricular injection of saline (left panels) and ICI 154,129 (right panels) at 1030 h (arrow) on the serum concentration of LH (upper panels) and PRL (lower panels) in rats in proestrus. Each point and the vertical bar represent the mean and SE, respectively. The number in parenthesis shows the number of animals. *p<0.01 or 0.05 vs mean preinjection values.

Fig. 2. Effects of an intraventricular injection of saline and ICI 154,129 at 1300 h (arrow) on the serum concentration of LH in rats in proestrus. Each point and the vertical bar represent the mean and SE, respectively. The solid symbol indicates that the value was significantly greater (p<0.1 or 0.05) than at 1100-1300 h. *p<0.01 or 0.05 vs saline-injected control rats at corresponding times.
1100–1300 h, with the peak occurring at 1440 h, more than 1 h earlier than in saline-injected animals. Compared to saline-injected control animals at corresponding times, values at 1400–1500 h were significantly greater (p<0.05 or 0.01). Further the peak LH concentration at 1440 showed about a twofold increase over the peak LH at 1600 h in the control animals.

PRL: In all the animals, the mean serum PRL concentration was considerably greater at 1300 h than before, although not significantly different (Fig. 3). The injection of saline into the third ventricle at 1300 h caused the PRL concentration at 1320 h to be lower, but an afternoon increase occurred thereafter, peaking at 1400–1800 h with a significant change compared to the concentration at 1100 and 1200 h (p<0.05). ICI 154,129 injected at 1300 h did not produce a decrease in PRL at 1320 h. The afternoon rise, in which PRL concentrations at 1400–1500 h were significantly greater than at 1100 and 1200 h, peaked at 1500 h. It was noticeable that this increase in PRL secretion ceased thereafter and the PRL level promptly decreased, showing significantly smaller values at 1700 and 1800 h than in the saline-injected control animals at corresponding times.

Discussion

These studies showed that ICI 154,129 produced an advance in and augmentation of the surge of LH secretion when administered intraventricularly just before the critical period for the surge of LH secretion. This finding supports the thesis that there exists in the brain an inhibitory opioid mechanism for the LH surge whose actions are mediated by delta receptors. The inhibitory influence of opioid on the LH surge has been proven in a number of studies examining the effect of naloxone [9, 21–23]. Naloxone is reported to bind with a higher affinity to mu receptors than to other binding sites [1, 24], and it has been agreed that the mu subtype is the major receptor mediating the inhibitory influence of opioids on the LH surge. The present results advance the possibility that endogenous opioids that bind with delta receptors also contribute to
regulation of the surge of LH secretion. The effects of ICI 154,129 on the LH secretion in proestrus seemed somewhat different from those of naloxone. Naloxone was effective in eliciting LH release even in the morning of proestrus, as if it had advanced the surge of the LH secretion [9, 21, 22, 25]. However, ICI 154,129 was not; there was not even a sign of LH release after the intraventricular injection in the morning in the present study. It has been suggested that the sensitivity to naloxone of the neural mechanism controlling the LH surge becomes greater just before the critical period than in the morning, followed by a marked decrease thereafter, during the period of LH surge, based on the time-dependent changes in naloxone binding to the hypothalamic area [26] and LH secretory response to naloxone [9]. It is then probable that the sensitivity to ICI 154,129 changes more dramatically than that to naloxone, i.e., in the morning almost absent and just before the critical period extremely great. This may mean that during a limited period of proestrus, i.e., before the critical period, there exists a strong inhibitory influence mediated by delta receptors.

In the present study, the surge of PRL secretion was inhibited during the plateau phase, but not during the ascending one, by the intraventricular injection of ICI 154,129, although in the morning it again did not have a significant effect on PRL secretion. This finding is interesting in view of the recent indication that the PRL surge during the ascending phase is elicited by the PRL-releasing factor, but the plateau one probably results from an absence of dopamine input to the pituitary gland [27]. Since opioids have been shown to decrease the release and synthesis of dopamine in the tuberoinfundibular dopamine neurons [28–30], the blockade of this opioid action may evoke the inhibition of the PRL surge during the plateau phase. The peak surge will not be influenced because it occurs in spite of dopamine input to the pituitary gland [27]. In addition, the present results suggest that one of the opioids involved in the inhibition of dopamine release during the surge is that with an affinity with delta receptors. The effect of naloxone on the surge of PRL secretion has been inconsistent [4, 9]. The lack of effectiveness of ICI 154,129 in the morning of proestrus in inhibiting PRL secretion once more suggests that the activity of the delta receptor agonist is greatly increased only during the limited period of proestrus.

Acknowledgements

The authors thank NIDDK and Dr. K. Wakabayashi, Gunma University, for generously providing the radioimmunoassay materials.

References

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