The Roles of Endogenous Opioids in the Inhibitory Action of the Hippocampus on Preovulatory of Luteinizing Hormone in Rats

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Abstract

Whether endogenous opioid peptides were involved in the inhibitory action of the hippocampus (HPC) on luteinizing hormone (LH) release was studied examining the effect of naloxone, an opioid antagonist, on the inhibitory action of electrical stimulation of the HPC and also by examining the effect of met-enkephalin administration in the HPC, on preovulatory release of LH in proestrous rats. In rats treated with saline at 13:00 h, either sham stimulation or electrical stimulation of the dorsal HPC, which was performed acutely under ether anesthesia, significantly inhibited the afternoon rise in serum LH. In animals treated with naloxone at a dose of 2.0 mg/kg body weight, the afternoon rise in LH appeared smaller than that in the control group. However, statistical analysis showed no significant difference in LH values compared to the control group. Direct administration of met-enkephalin at a dose of 10 μ g at 13:00 h through a chronically implanted cannula in the HPC did not induce any significant change in the afternoon rise in LH, regardless of whether it induced behavioral seizures or not. The results suggest that opioid peptides in the HPC do not play a significant role in the inhibitory action of HPC on LH release. Opioids existing in areas other than the HPC may play a certain, but small role.

Electrical stimulation of the hippocampus (HPC) has been shown to reduce the preovulatory release of luteinizing hormone (LH) and the incidence of spontaneous ovulation in proestrous rats (Velasco & Taleisnik, 1969, Gallo *et al.*, 1971,

Kawakami *et al.*, 1972). Similar stimulation of the HPC also prevented ovulation normally observed after stimulation of the medial preoptic area in persistent estrous rats in continuous illumination and in ovulation-blocked proestrous rats with pentobarbital sodium (Velasco & Taleisnik, 1969, Kawakami *et al.*, 1973).

There is an indication that the inhibitory action of the HPC stimulation is related to the HPC seizure discharges. The mere insertion of an electrode in the HPC

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produced an EEG seizure pattern in this area, spreading to the neocortex and the amygdala (Kawakami *et al.*, 1972), suggesting that the inhibitory action of the HPC stimulation which was reported from the acute experiments was correlated with HPC seizures. Further, stimulation of the HPC with chronically implanted electrodes did not inhibit ovulation when a sub-threshold current to induce HPC seizure discharges was used as the stimulation intensity (Kawakami *et al.*, 1972), but inhibited it when a threshold current was used to induce seizure discharge (Kawakami & Terasawa, 1974).

On the other hand, opioids have been suggested as playing an important role in the regulation of the activity of the HPC. Electrophysiological studies have demonstrated excitatory effects of opioids on the HPC neurons (Nicoll *et al.*, 1977, Zieglgänsberger *et al.*, 1979, Haas & Ryall, 1980, Dunwiddie *et al.*, 1980, Gahwiler, 1980, Henriksen *et al.*, 1982). Intracerebroventricular administration of opioids has been shown to induce EEG seizure discharges of the HPC (Henriksen *et al.*, 1978, Snead & Bearden, 1982), and direct administration of opioid or opioid agonist into the HPC caused convulsions (Cain & Corcoran, 1984, 1985, Lee *et al.*, 1988). Further, these opioid effects were antagonized by opioid antagonists as naloxone (Henriksen *et al.*, 1978, Cain & Corcoran 1984, Snead & Bearden, 1982, Lee *et al.*, 1988).

The purpose of this study was to determine whether endogenous opioid peptides are involved in the inhibitory action of the HPC on LH release. This issue was approached by examining whether naloxone will interfere with the inhibitory effect of the HPC stimulation on the preovulatory surge of LH secretion and whether direct injection of opioid will affect the LH surge.

Materials and Methods

Animals

Female Wistar-Imamichi rats obtained from Animal Reproduction Research Co., Urawa, at 7–8 weeks of age were housed four to five per cage under controlled lighting (lights on from 5:00 to 19:00 h) and temperature (24°C) and allowed free access to food and water. Vaginal smears from each rat were obtained daily in the morning.

Electrical Stimulation Experiment

Animals in proestrus after having exhibited 2 or more consecutive 4-day estrous cycles were implanted with an intraatrial catheter of silicone tubing (Dow Corning) through the jugular vein, and then were fixed to the stereotaxic apparatus, received an incision of the head skin and had a hole drilled in the skull. These procedures were performed under ether anesthesia between 12:00 and 13:00 h. The first blood sample (300 μ l) was collected and replaced with an equal volume of heparinized saline (saline-treated) or of heparinized saline containing naloxone (Sigma Chemical Co.) (naloxone-treated) at a dose of 2.0 mg/kg body weight. Immediately after the first blood sampling, a stimulating electrode was inserted into the dorsal HPC, according to the atlas of Albe-Fessard *et al.* (1966), followed by electrical stimulation in a group of animals (stimulation group), but not in another group (sham stimulation group). A third group of animals did not receive even the insertion of an electrode (control group). All three groups of animals remained in the stereotaxic apparatus under ether anesthesia for another 30 min which was the period for electrical stimulation. The second blood sampling was performed at the end of this 30-min period and the electrode withdrawn. Blood sampling at 1-hour intervals was started at 14:00 h and continued until 20:00 h.

The stimulating electrode used in this series of experiments was a concentric bipolar electrode consisting of a wire insulated except for the 0.1 mm tip (0.13 mm in diameter) which served as the cathode and an outer barrel (0.15 mm exposed surface) which served as the anode. Electrical stimulation consisting of a monophasic square wave at a pulse duration of 0.1 ms and a frequency of 100 Hz was applied for 30 min

in 30 sec periods alternating with 30 sec rest periods. A 100 μ A current was employed.

Met-Enkephalin Administration Experiment

The guide cannula was made of stainless steel tube with a 0.65 mm outer diameter and was stereotactically implanted unilaterally into the dorsal HPC under sodium pentobarbital anesthesia. The cannula was 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 0.5-mm beyond the tip of the guide cannula. The guide cannula was plugged with a dummy inner cannula until the day of the experiment.

The animals in proestrus, after having exhibited 2 or more consecutive estrous cycles following implantation of the guide cannula, were implanted with an intraatrial cannula under ether anesthesia in the morning. The first blood sample (300 μ l) was collected at 12:00 h and met-enkephalin (Peptide Institute, Minoo) at a dose of 10 μ g dissolved in a volume of 1 μ l sterile 0.9% saline was injected at 13:00 h immediately after the second sampling. The blood sampling was performed at 1-h intervals afterwards until 20:00 h

Hormone Assay

Serum concentrations of LH were measured by the double-antibody radioimmunoassay using materials supplied by the NIDDK. The refer-

ence standard was NIDDK rat LH-RP-1, but the amounts of LH were expressed in terms of NIH-LH-S1. The within- and between-assay variances for LH assays, which were calculated from 5 replicate samples of a standard pooled serum with a mean LH concentration of 5.81 ng/ml in each assay, were 5.1% (mean for 6 assays) and 13.8%, respectively.

Histology

After the final bleeding at 20:00 h, the rats were anesthetized with ether and perfused with 10% buffered formalin. The site of the electrode was microscopically identified in frozen sections of the brain.

Statistical Analysis

Two-way analysis of variance and Duncan's multiple range test were used to test the statistical significance of fluctuations over time in the mean hormone values for each sampling time. Student's *t*-test was used to test the significance of difference between the treatment groups.

Results

1. Electrical Stimulation Experiment

This sites of electrical stimulation in the HPC are shown schematically in

Fig. 1. Schematic illustrations showing the sites of electrical stimulation, as indicated by the tip of the electrode in the hippocampus. The left panel shows the sites for saline-treated rats and the right shows those for naloxone-treated rats.

Figure 1, and the mean ($\pm$ SE) of serum concentrations of LH for each sampling time is shown diagrammatically in Figure 2 for saline-treated and in Figure 3 for naloxone-treated rats.

Control group: In animals in the control group, treated with either saline or naloxone, there was a significant increase in LH in the afternoon. Mean LH peaked at 18:00 or 17:00 h in saline ($p < 0.001$)- and naloxone ($p < 0.05$)- treated animals, respectively, with significant increase compared to the values at 13:00–16:00 h. The peak LH time was apparently delayed by 1 or 2 hours compared to that in the met-enkephalin administration experiment described later. The major factor responsible for this delay was probably anesthesia with ether (Blake, 1974). There was no significant differences in the mean LH in naloxone-treated animals compared to the value in saline-treated control animals at corresponding times.

Sham stimulation group: Saline-treated animals in the sham stimulation group showed a significant afternoon rise in LH, peaking at 18:00 and 19:00 h with significantly greater values than those at 13:00–17:00 h ($p < 0.01$ or 0.05 for the value at 18:00 h) and at 13:00–16:00 h ($p < 0.01$ for the value 19:00 h). The mean LH values at 18:00 and 19:00 h were significantly smaller than those in the control group ($p < 0.05$). In naloxone-treated animals, the sham stimulation group showed a significant afternoon increase, peaking at 17:00 and 18:00 h with significantly greater values than those at 13:00–15:00 h ($p < 0.05$ for the value at 17:00 h) and at 13:00–16:00 h ($p < 0.01$ or 0.05 for the value at 18:00 h). The values in the sham stimulation group were apparently smaller than those in the control group treated with naloxone, but no values were significantly different from those in the control group.

Fig. 2. Time course of serum concentrations of LH in saline-treated rats. The points and the vertical lines represent the mean and SE. Asterisks indicate significant difference from rats in the control group at corresponding times; * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

Stimulation group: The stimulation group, either saline- or naloxone-treated animals, showed a small but significant afternoon rise. In saline-treated animals, the mean LH value peaked at 18:00 and 19:00 h with a significant difference compared to values at 13:00–17:00 h ($p < 0.01$ or 0.05 for the value at 18:00 h and $p < 0.001$ or 0.01 for the value at 19:00 h). The values at 18:00 and 19:00 h were significantly ($p < 0.001$ and 0.01 , respectively) smaller than those in the control group at corresponding times, but did not differ from those in the sham group. Naloxone-treated animals showed peak values at 18:00 and 19:00 h with significant differences compared to values at 13:00–16:00 h ($p < 0.05$). The values in the stimulation group appeared markedly smaller than the values in either the control or sham stimulation group, as in the saline-treated animals. However, no values were significantly smaller than those in the control or the sham group, except

the value at 19:00 h that was significantly smaller than the value in the control group ($p < 0.05$).

2. Met-Enkephalin Administration Experiment

Injection of met-enkephalin into the dorsal HPC evoked wet-dog shakes in 7 out of 20 rats, and in one rat out of 7, resulted in bilaterally generalized convulsions. The sites of met-enkephalin injection the HPC are shown schematically in Figure 4 with respect to the behavioral effect. The mean ($\pm$ SE) of serum concentrations of LH for each sampling time is shown diagrammatically in Figure 5 with respect to the behavioral effect, too.

There was a significant afternoon increase in LH in the saline-injected animals. LH peaked at 16:00 or 17:00 h, with significant differences from values at 12:00–15:00 h. Met-enkephalin-injected animal behavioral seizures showed a significant afternoon increase in LH, peaking at 16:00

Fig. 3. Time course of serum concentrations of LH in naloxone-treated rats. For further details, see Fig. 2.

and 17:00 h with significant differences from values at 12:00–15:00 h. There were no significant differences in the values in the met-enkephalin treated animals, either with or without behavioral seizures compared to values at corresponding times in saline-treated animals.

Discussion

The present study supports the earlier finding that electrical stimulation of the HPC reduces the preovulatory release of LH in rats. LH release in saline-treated animals was significantly reduced either by sham stimulation or electrical stimulation of the dorsal HPC.

In naloxone-treated animals, neither sham stimulation nor electrical stimulation was effective in reducing LH release, although there was an apparent tendency

toward reduction. This decrease in the effect of both sham and electrical stimulation of the HPC could have been interpreted in two ways. First, it was possible that naloxone reduced the opioid activity in the HPC and thus the excitability of the HPC was decreased. The second possibility was that naloxone reduced the opioid activity in the preoptic-hypothalamic area and thus the inhibitory action of the HPC on LH release was not able to occur.

The first possibility was likely in the light of the distribution of opioid immunoreactivity and opioid receptor subtypes in the HPC (Meibach & Haayani, 1980, Crain *et al.*, 1986, McLean *et al.*, 1987). Further, electrophysiological studies have demonstrated a role of μ -receptor in evoking seizure activities (Henriksen *et al.*, 1978, Snead & Bearden 1982, Cain & Corcoran, 1984, Lee *et al.*, 1988) and in increasing excitability of the HPC (Nicoll *et al.*, 1977, Zieglgänsberger *et al.*, 1979, Gahwiler, 1980, Haas & Ryall, 1980, Dunwiddie *et al.*, 1980, Henriksen *et al.*, 1982). Opioid was then injected into the HPC to determine whether the opioid activity in the HPC was related to the HPC action in inhibiting LH release. If the inhibition of LH release occurred in response to the injection of met-enkephalin into the HPC, the first possibility concerning the stimulation experiment is more likely. Met-enkephalin injection at a dose of 10 μ g evoked behavioral seizures in about 30% of the animals as described previously for met-enkephalin (Cain and Corcoran, 1984) and μ -receptor agonist (Lee *et al.*, 1988). However, no change was seen in the preovulatory secretion of LH in these animals. Since these behavioral seizures have been closely correlated with EEG seizure discharges in the HPC (Henriksen *et al.*, 1978, Snead & Bearden, 1982, Cain & Corcoran, 1984, Lee *et al.*, 1988), the results imply that the HPC seizure discharges are not profoundly involved in the mechanism of the inhibitory

Fig. 4. Schematic illustrations showing the sites of met-enkephalin injection, as indicated by the tip of the cannula in the hippocampus.

effect of HPC stimulation. Further, the present finding that the LH secretion in animals that did not show signs of behavioral seizures after met-enkephalin injection was not changed significantly suggests a negligible role of opioids existing in the HPC in the inhibitory action of HPC. Therefore, the first possibility we suggested can be reasonably denied.

On the other hand, considerable concentrations of beta-endorphin and enkephalin, and their receptors exist in the septal-preoptic-hypothalamic region (Uhl *et al.*, 1978, Woodhams *et al.*, 1983, Cuello, 1983) and have been believed to mediate various kinds of inhibition of LH release, such as gonadal steroid hormones (see review by Kalra, 1986) and stress (Nikolarakis *et al.*, 1988). This region receives projections from the HPC through two distinct pathways: the descending column of the fornix and the medial corticohypothalamic tract (Meibach & Siegel, 1977). Both of these pathways

have been shown to be involved in transmitting the inhibitory influence of the HPC on LH release (Velasco & Taleisnik, 1969, Kawakami & Kimura, 1975). It is therefore possible that endogenous opioids distributed in the septal-preoptic-hypothalamic region somehow mediate this HPC influence on LH release. Decreases in the opioid activity in these areas due to naloxone would have been related with the reduction of the inhibition.

However, the apparent tendency toward inhibition which was seen in both the sham and electrical stimulation groups of naloxone-treated animals suggests the possibility that naloxone actually does not affect the inhibitory action of the HPC. It is probable that large variations in the LH value observed in naloxone-treated animals account for the statistical insignificance of differences between control and stimulation groups. In addition, it was noticeable that electrical stimulation of the HPC produced

Fig. 5. Time course of serum concentrations of LH in rats injected with met-enkephalin. For further details, see Fig. 2.

almost the same LH values in both the saline- and naloxone-treated animals: i. e., no LH values in animals in the stimulation group were significantly different in the two groups, although not described in Results.

In view of the results of one of our earlier studies, we suggested a significant role of cholinergic transmission in the expression of the HPC inhibition of preovulatory LH release (Kawakami *et al.*, 1976). Atropine treatment at a dose of 200 mg/kg body weight, which was subthreshold to block ovulation, abolished the inhibitory effect of electrical stimulation of the HPC on preovulatory LH release. On the basis of rather classical studies attempting to map the cholinergic neurons in the rat brain (Lewis & Shute, 1967), recent studies have disclosed a septo-hippocampal and a diagonal band-HPC projection (see review by Bjorklund *et al.*, 1984). In addition, the HPC is characterized by a high concentration of putative muscarinic receptors. Therefore, it is very likely that the most important neural transmission in maintaining the HPC activity related to the inhibition of LH is cholinergic afferents, but not intrinsic opioidergic neurons.

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