

EFFECT OF THIRD VENTRICULAR INJECTION OF β -ENDORPHIN ON THE ELECTROPHYSIOLOGICAL RESPONSES OF SOME REGIONS OF ENDOCRINE HYPOTHALAMUS

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(Received on March 15, 1985)

Summary : Multiunit activity (MUA) of arcuate nucleus and cortical EEG were recorded in the regularly cycling female rats on the day of proestrous under urethane anaesthesia. The MUA was compared before and after injection of β -endorphin in third ventricle. In some animals MUA increased after 30-40 min and persisted for 3-4 hr, in others MUA got inhibited within 5-10 min of injection of β -Endorphin and effect lasted for 5-6 hr. There was no change in frontoparietal EEG activity. In another group of animals medial pre-optic responses (MPO) to stimulation of medial amygdala were tested before and after ventricular infusion of β -endorphin. Most of the facilitatory MPO responses got blocked. These observations suggest the involvement of opioid receptors in the mediation of neuroendocrinal control of preovulatory events through the amygdalo-preoptico-medial basal hypothalamic axis. There seems to be heterogeneity of β endorphin receptors in the arcuate nucleus.

Key words : MUA (multiunit activity)
third cerebral ventricle

arcuate nucleus
 β -endorphin

MPO (medial pre-optic)
MAMY (medial Amygdala)

INTRODUCTION

Discovery of opiate receptors and of endogenous opioid peptides in the central nervous system has aroused considerable interest in assigning them various functions like analgesia, control of sexual and neuroendocrinal activity and involvement in learning and memory. As early as 1955, Barraclough and Sawyer, (1) and recently Pang *et al.* (16) reported that morphine blocked pre-ovulatory LH surge and other workers confirmed this by saying that the morphine antagonist naloxone reversed it (6, 14, 15). Subsequently inhibitory effect of Met-enkephalin on LH release was demonstrated in rats (2, 13). Involvement of β -endorphin in causing inhibition of LH surge was reported by Schultz *et al.* (18). Injection of β -endorphin in third ventricle has shown that its suppressive effect was on LH-RH neurone system in the medial pre-optic tuberal and medial basal hypothalamic circuits, (4,11,17,22). Local implants of naloxone (7) or anti β -Endorphin antibodies

into the arcuate nucleus increased LH release but injection into other regions has no effect (18). Therefore, it was of an interest to conduct an electrophysiological study to see how arcuate neurones behave in terms of their excitability, firing pattern and other characteristics in presence of β -endorphin being administered into third ventricle and also whether β -endorphin so administered had effect on limbic inputs to MPO area in proestrous animals?

MATERIAL AND METHODS

The regularly cycling Wister rats kept under controlled environmental conditions (12 hr light, 12 hr darkness at ambient temperature of 24°C, water and food *ad libitum*) were used for the study. Under short acting Brevital anesthesia, stainless steel cannulae with stilette inside were stereotaxically lowered into the third ventricle, as described earlier (9, 10). After 3 to 4 weeks of post operative recovery the animals resuming regular estrous cycle were anesthetised with Urethane (1.2 g/kg, bw) on the day of proestrous. In order to record surface EEG, stainless steel screws were anchored on to the frontal and contralateral parietal regions of the skull. Insect pin with 12-20 μ tip diameter and of 40-50 K Ohms impedance, insulated with epoxy except at the tip was stereotaxically lowered into the arcuate nucleus for recording MUA. These electrodes were fed to pre-amplifier and signals recorded through oscilloscope on the polygraph channels. The protocol and the circuit diagram of the parameters recorded is shown in Fig. 1, MUA was recorded before and after third ventricular injection of saline, and β -endorphin. Saline and β -endorphin were infused slowly @

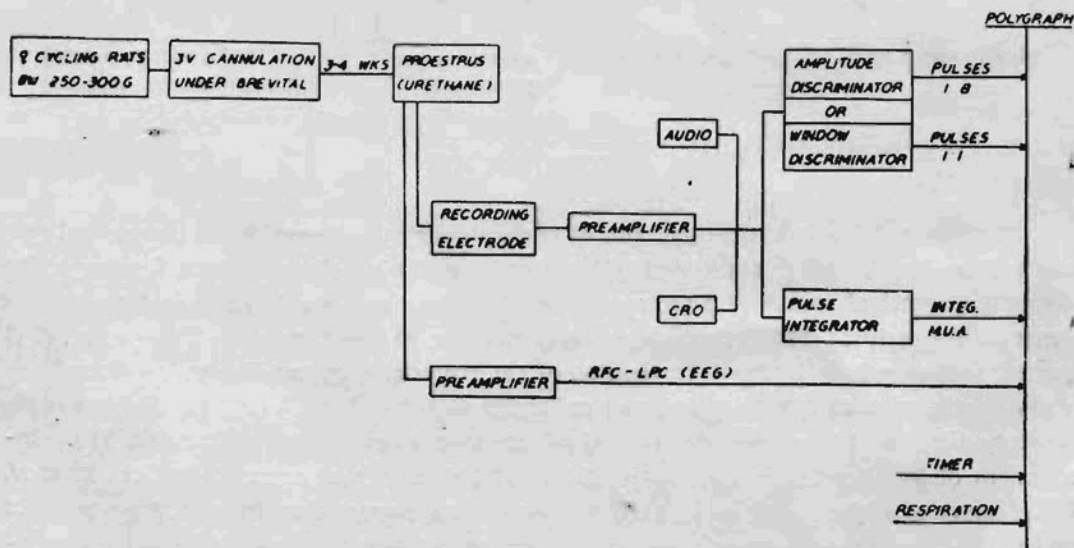


Fig. 1 : Schematic drawing of the protocol and the experimental set up. Amplitude discriminated (window setting 20-30 μ v) MUA was recorded on first two channels of polygraph as 1:1 and 1 for 8 pulses.

$\mu\text{g}/0.5 \mu\text{l}$ in 30 secs. A total dose of 2 μg was used in different animals. At the end of each experiment, anodal current of 4 μA was passed through recording electrode for making a small electrolytic lesion at the recording site. In another group of animals, concentric bipolar, stainless steel electrodes insulated with Epoxy except at the tips were oriented to the medial amygdala and a glass microelectrode lowered in the MPO as per stereotaxic co-ordinates. Medial amygdala (MAMY) was stimulated in order to record MPO post stimulatory histograms as per parameters given by Tandon *et al.* (20) in proestrous animals. These responses were compared before and after β -endorphin injection in third ventricle. The animal was then perfused with a solution of 3% potassium ferro and ferricyanide in 10% formalin solution. Position of recording electrodes and cannulae was confirmed on histology.

RESULTS

The basal MUA (window setting between 20-30 μV) showed firing frequency ranging from 80-120 spikes/min and it was not affected by saline injection. The basal

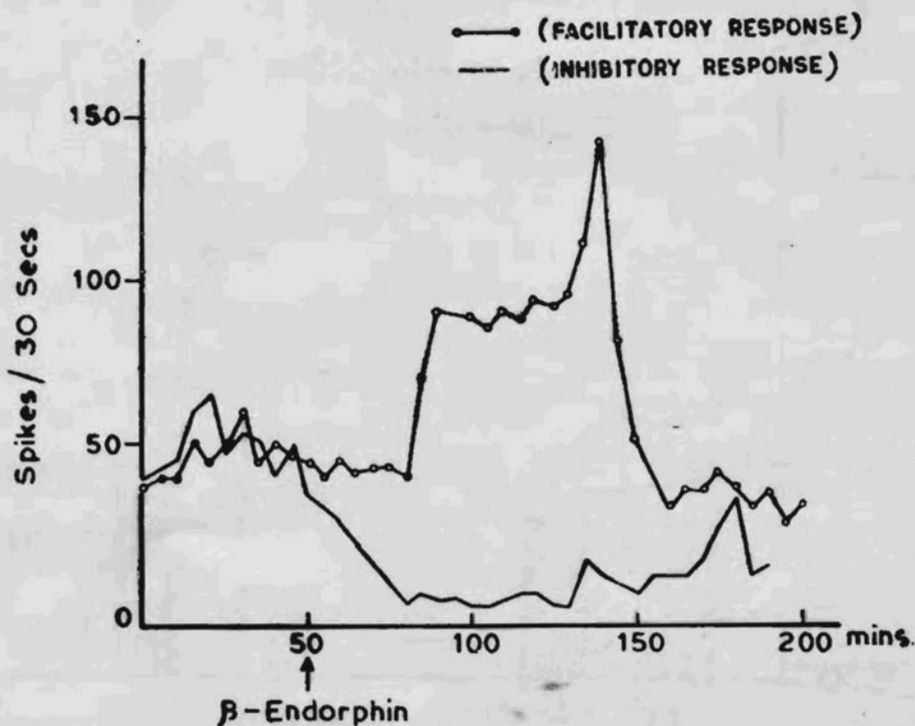


Fig. 2 : Graph showing number of MUA pulses/30 sec. picked up from arcuate nucleus before and after β -endorphin injection in the third cerebral ventricle. Two types of responses are obtained : facilitatory with a latency of 30 min and immediate long lasting inhibitory response.

electrical activity pattern coincided with cortical EEG, during spindling, MUA frequency was 80-85 spikes/min it increased to 115-120 spikes/min during desynchronised EEG activity. This correlation between cortical EEG and MUA is well documented by Tandon and Manchanda (19) in animals under urethane anaesthesia, indicating optimal state of excitability of CNS and that the preparation is good for electro-physiological experiments.

In a group of ten animals used, five showed inhibitory responses to β -endorphin injection. The responses started within minutes of injection and maximum inhibition was seen after 25-30 mins, effect lasted for more than 3-4 hr of recording session. A representative response is shown in Fig. 2. On histology, the tips of recording electrodes were seen in the arcuate nucleus.

Three animals showed facilitatory responses on β -endorphin injection. The firing increased after latency of 30 min the maximum rate of firing was seen after 90 min and the effect lasted for 120 mins. The pattern of facilitatory response is also shown in Fig. 2.

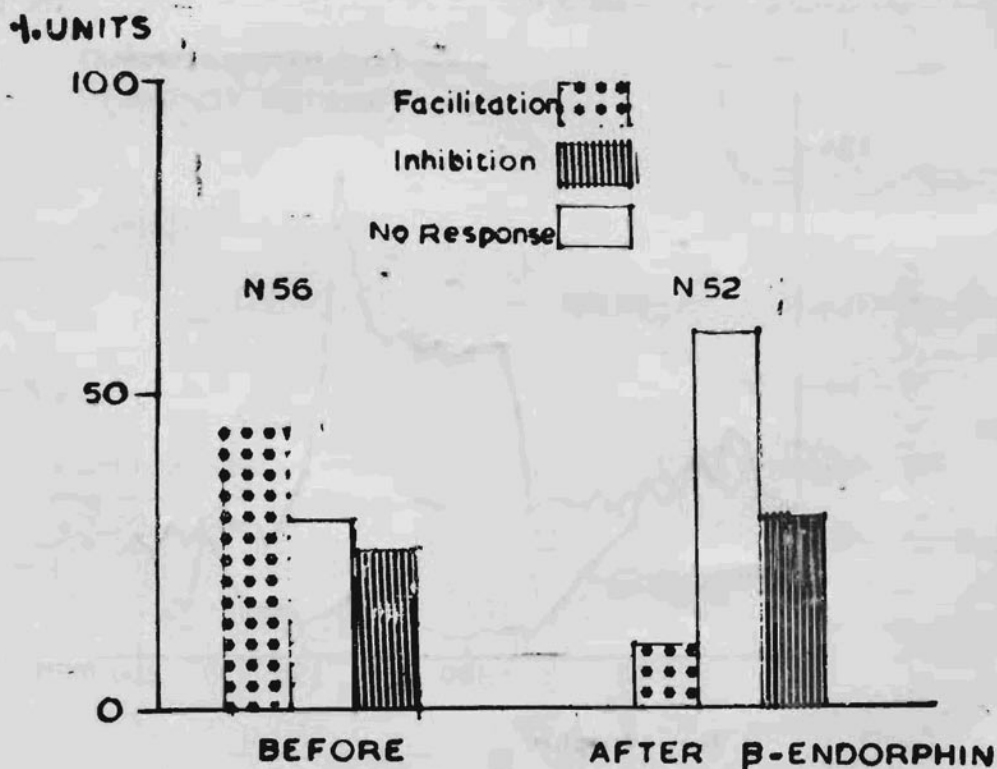


Fig. 3 : MPO unit response histograms to MAMY stimulation in proestrous rats showing % of facilitatory, inhibitory and non-responsive units before and after injection of β -endorphin. Most of the facilitatory units became non-responsive after β -endorphin administration.

On histology the tips of the recording electrodes were found in the anterolateral border of the arcuate nucleus, between it and the VMH but well within arcuate nuclear territory. In the remaining two animals no specific response pattern was seen on β -endorphin injection. They were excluded from this study as the placement of electrodes was not in arcuate nucleus. In the second group of 5 animals MPO responses to stimulation of MAMY were observed. Of the total 56 MPO responses picked up before β -endorphin injection, 45% were facilitatory, 25% inhibitory and remaining 30% did not show any response. After β -endorphin injection most of the facilitatory responses (about 80%) were blocked; Out of total 52 responses recorded, only 10% were facilitatory, 30% inhibitory and 60% showed no response (Fig. 3). Fig. 4. shows classical blocking effect of β -endorphin on one of the MPO facilitatory responses. The inhibitory MPO responses to MAMY stimulation so obtained did not show any change on β -endorphin injection (Fig. 4).

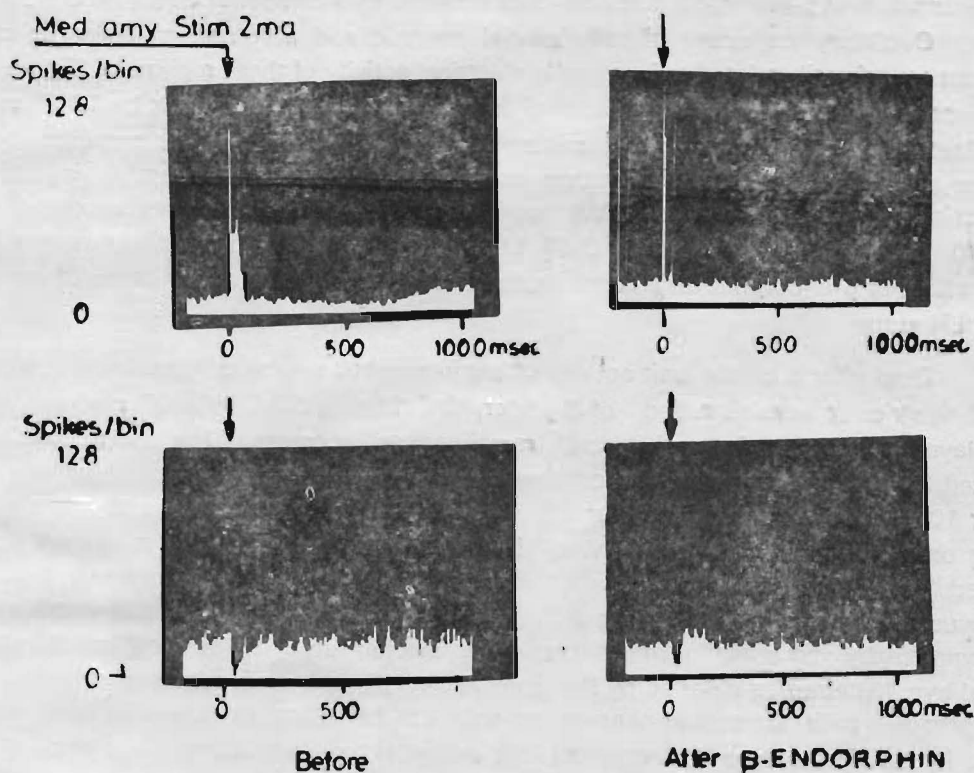


Fig. 4 : A facilitatory MPO unit response to M-AMY stimulation got blocked (upper panel), whereas an inhibitory response showed no change after β -endorphin injection in the third ventricle (lower panel).

DISCUSSION

Mediobasal hypothalamus forms the major part of LH-RH neuronal system. Recent studies have shown that opioid peptides particularly β -endorphins control the pre-ovulatory gonadotrophin surges from this region of the hypothalamus (11). The present study has shown that third ventricular injection of β -endorphin appears to have both suppressive and facilitatory effect on the firing of arcuate neurones. These dual effects are not only qualitatively opposite but also quantitatively different. The suppressive effect (Fig. 2) starts without any latency and is a prolonged one, but the maximum effect is seen after about 25 minutes. Such time course response is expected to last for few hours only as it is reported that β -endorphin in CSF is degraded rapidly (12). This suppressive effect might be related to inhibition of LH release as reported by other workers where they have injected β -endorphin or its antibodies in the medial basal hypothalamus or arcuate nucleus and seen the requisite response (4,17,18,22). Inhibition of LH release on third ventricular administration of β -endorphin has also been reported by Kineshita *et al.* (8) and Kubo *et al.* (11). Ovulatory responses of the medial preoptic and also median eminence-arcuate regions are primarily related to increase in electrical activity of these regions (3,21) in which the increase in multiunit activity started immediately, reached a peak by 19-25 minutes and lasted from 40-60 minutes to sometimes 2-4 hr (3). The inhibitory response observed in the present study, bears similar time course relationship to β -endorphin injection, in the third ventricle, the arcuate MUA got inhibited immediately, reached maximum within 25-30 min and effect lasted for 2-2½ hr. This further confirms the notion that inhibitory effect of β -endorphin on arcuate multiunit activity is related to inhibition of preovulatory LH surge

Dual effects on the unit activity of septo preoptic neurones, have been observed on third ventricular administration of β -endorphin (11). About 75% of responding units displayed a decrease in activity and remaining an increase. The decreased activity started 20 min after injection and continued for 3-5 hr whereas increased activity started after 10-30 min and continued for 50-70 min. The present study has shown similar results on the arcuate multiunit activity. Both facilitatory and inhibitory responses thus obtained (Fig. 2) suggest existence of opioid receptors and their heterogeneity in the arcuate nucleus. However, these findings do not rule out the possibility of β -endorphin acting presynaptically and inhibiting the dopaminergic mechanism of LH-RH release from mediobasal hypothalamus as reported by Rotsztein *et al.* (17) in their *in vitro* experiments. Opioid receptor and their functional connections with LH-RH neurones have also been shown in extra hypothalamic areas like amygdala, the medial septum and dorsal band of Broca, controlling LH release which gets blocked by third ventricular β -endorphin injection (5).

The present findings in no way rule out the indirect effect of β -endorphin on arcuate nucleus through extrahypothalamic functional connections. In order to investigate this

aspect of functional modulation of M-AMY-MPO connections by β -endorphin, in a group of animals MPO units in response to stimulation of M-AMY were picked up in form of histograms. Most of the responses, 45% were facilitatory in proestrous animals as compared to 25% inhibitory (Fig. 3). After third ventricular injection of β -endorphin most of these units became non-responsive (Fig. 3). Fig. 4 shows the MPO unit which was showing facilitatory response on M-AMY stimulation, got blocked after ventricular injection of β -endorphin. These observations suggest that β -endorphin might be acting as neuro-modulator in the limbic inputs to hypothalamus. The findings reported in the present study indicate that β -endorphin affects the electrical activity of arcuate directly and/or indirectly through the circuitry between amygdala-preoptic-arcuate regions involving β -endorphin modulation. Parallel observations have also been made by Kubo *et al.* (11), where they have generally observed ovulation blocking effect of ICV administration of β -endorphin. Electrochemical stimulation (ECS) of AMY in such animals did not produce normal ovulatory responses, whereas normal ovulation was seen on stimulation of MPO or ME. Third ventricular administration of β -endorphin also blocked estrogen induced LH-surges. They concluded that β -endorphin system might be involved in inhibitory control of preovulatory LH release not only by acting directly on the pre-optico tuberal LH-RH neuronal system but also by affecting extra hypothalamic inputs initiating and developing feed back of estrogen to medial basal hypothalamus.

The question that arises is whether both these facilitory and inhibitory arcuate responses to β -endorphin play role in controlling gonadotrophin surges from hypothalamus or they are involved in other endorphin mediated behavioural responses? Immunocytochemical studies have shown two groups of β -endorphin reactive neurones in the basal hypothalamus one within arcuate nucleus and the other anterolateral to it. There seems to be connections between these and those β -endorphin reactive neurones in the diencephalon and peri-aqueductal gray of midbrain, which are involved in central analgesic mechanism. In the present study, inhibiting responses were obtained from sites within the arcuate nucleus and facilitatory from the loci anterolateral to this. Could both these responses be related to hypothalamically mediated analgesia and or LH surges through endorphin mechanism?

Evidence so far reported suggest that inhibitory responses could be correlated to inhibition of gonadotrophin surges. Since there are opioid receptor present in the regions anterolateral to arcuate and the β -endorphinergic connection between these and central aqueductal gray of midbrain, these facilitatory responses obtained might be involved in central mechanism of analgesia. However, more elaborate and precise experiments need to be conducted to prove this.

ACKNOWLEDGEMENTS

The authors wish to thank Miss Lalita for secretarial help and Mr. K. Sharma of UCMS for photography work.

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