EFFECT OF LUTEINIZING HORMONE-RELEASING HORMONE (LH-RH) ON THE MULTIUNIT ACTIVITY OF THE ARCUATE NUCLEUS IN THE PROESTROUS RATS

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Summary: Multiple Unit Activity (MUA) of the hypothalamic arcuate nucleus as indicated by discriminated spikes with window setting between 18-30 uV, the integrated MUA and the cortical EEG of the urethane anaesthetized rats on the proestrous day was recorded with the help of a polygraph. Infusion of LH-RH in the 3rd ventricle markedly increased the arcuate nucleus MUA. The effect started within minutes of infusion, peak came after 20-30 minutes and the activity remained increased for 3-4 hours. Saline infusion in the 3rd ventricle was ineffective. Feedback action of CSF-LH-RH to the arcuate nucleus for the regulation of LH-RH is suggested.

Key words: LH-RH releasing factors arcuate nucleus LHRH feedback

INTRODUCTION

Neurones in the arcuate nucleus of the hypophysiotropic area produce LH-RH which is secreted into the proximal capillary plexus of the hypophysial portal system in the median eminence(16). Existing evidence also suggests the presence of an alternate delivery system whereby LH-RH may be secreted into the ventricular cerebrospinal fluid (6). The LH-RH in the CSF may later be delivered to the portal capillaries via the ependymal cells. It has been demonstrated that infusion of LH-RH into the third ventricle of the female rat brain stimulates the release of LH (9,14). On the other hand the LH RH in the CSF may serve to provide a system of positive feedback to the neurones of arcuate nucleus to build up the LH surge. This is supported by the evidence that iontophoretic application of LH-RH produces an increase in the unit activity of medial basal hypothalamus(5). In this paper we wish to report that the infusion of LH-RH in the third cerebral ventricle markedly increases the multiunit activity of the arcuate nucleus.

MATERIALS AND METHODS

Nine Sprague-Dawley rats, weighing 260-300 *g*, were maintained in a light controlled (14 hr light, 10 hr dark) air conditioned room, showing regular oestrous cycles were used. Under Brevital anaesthesia, stainless steel cannulae were stereotaxically implanted into the third cerebral ventricle at an angle which allowed subsequent vertical lowering of the electrode into

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the arcuate nucleus of the hypothalamus for recording its multiunit activity (MUA). After 2-3 weeks of postoperative rest, on the morning of proestrous the animal was anaesthetized with urethane (1.2 *g/kg*). This anaesthetic dose also served the purpose of blocking such hypothalamic events as lead to preovulatory LH surge (12). Epoxy-insulated stainless steel microelectrodes with 20 u tips (resistance varying between 28-35 kilo ohm) prepared from insect pins were stereotaxically advanced into the arcuate nucleus with a hydraulic micromanipulator. The extracellular potentials picked up by this electrode were fed to Tektronix 502 oscilloscope through a low gain preamplifier . From the vertical signal output of the oscilloscope the amplified potentials were fed into a window discriminator generating a standard output pulse with window setting at 18-28uV. The standard pulses of window circuitry activated a pen of the Polygraph channel at adjustable rates. The same multiunit activity after integration through a pulse integrator was fed into another channel of the polygraph.

Cortical EEG was recorded from frontal and parietal regions with stainless steel screw electrodes anchored to the corresponding regions of the skull. Respiration was recorded by a crystal phonograph cartridge. Care was taken to maintain body temperature of the animal by heating pads. After recording the basal arcuate MUA, 2 µl normal saline was slowly infused within two minutes into the third ventricle by a microdriver attached to the piston of the lambda syringe. After 1-2 hrs of saline infusion, 500 *ng* of LH-RH in 2 µl was slowly infused. MUA of arcuate was continuously recorded throughout the experiment and for about 3-4 hrs after LH-RH infusion. At the end of each experiment, the recording site in the arcuate nucleus was marked by making a small electrolytic lesion with the help of a direct anodal current (4 ua for 30 sec) through the recording electrode. The animal was then sacrificed by giving an excess dose of pentobarbitone. The brain was perfused initially with a solution of 3% potassium ferrocyanide and 3% potassium ferricyanide for the Prussian blue reaction at the site of lesion and then with 10% formalin. Placement of cannulae and electrodes was confirmed after histological processing.

RESULTS

General: The urethane-anaesthetized rat showed EEG variations typical of the sleep-wakefulness cycle which were accompanied by changes in the multiunit activity of the arcuate nucleus. The slow wave high amplitude electrocortical activity was associated with decreased number of discriminated spikes and fast wave low amplitude EEG activity with increased number of spikes (Fig. 1).

The basal activity of the arcuate nucleus as evinced in the form of discriminated spikes varied between 180-300 spikes per minute. However percentage of spike frequency in each individual rat ranged from 93-112% with the average of an hour's preinfusion record being taken as 100%. Activity during the period after infusion of normal saline in the 3rd ventricle was between 89 and 105%.

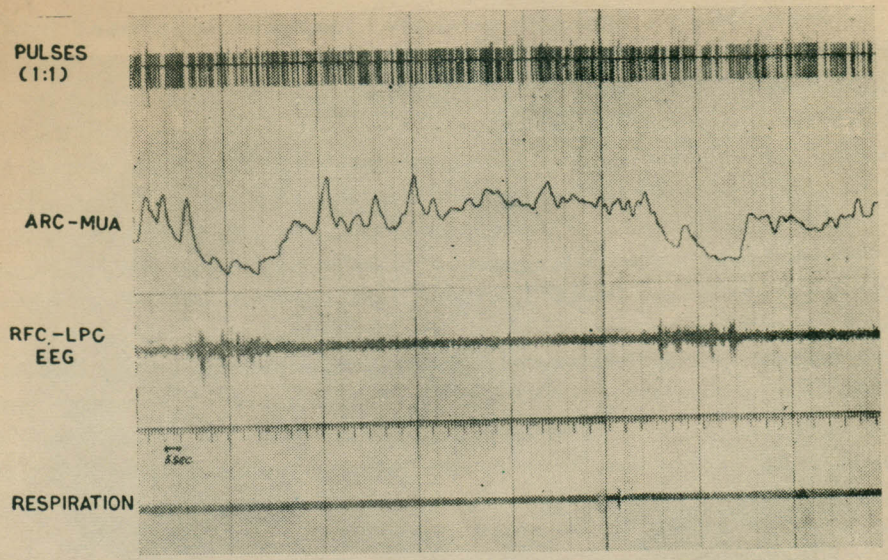

Fig. 1: Baseline polygraphic record indicating decrease in the multiunit activity of arcuate nucleus when the EEG activity gets synchronised. Tracings from above below are:

(i) uniform pulses obtained from the window discriminator (window setting between 18-28 uv), each pulse representing one spike, (ii) integrated record of the pulses (ARC-MUA), (iii) EEG activity recorded between frontal and parietal region, (iv) time mark, and (v) respiratory excursions.

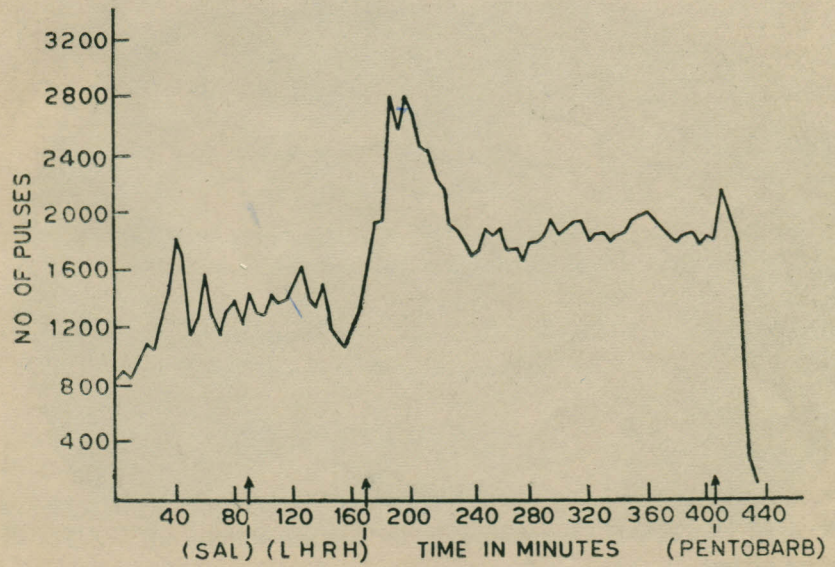

Fig. 2: Cumulative data of a typical experiment as obtained from continuous recording depicted in Fig.1. Note that infusion of saline in the 3rd ventricle does not affect the MUA. But infusion of LH-RH markedlyi ncreases the MUA. Scale of the pulse count represents MUA pulses counted per 5 min period. Pentobarbitone was injected at the end of the experiment to sacrifice the animal.

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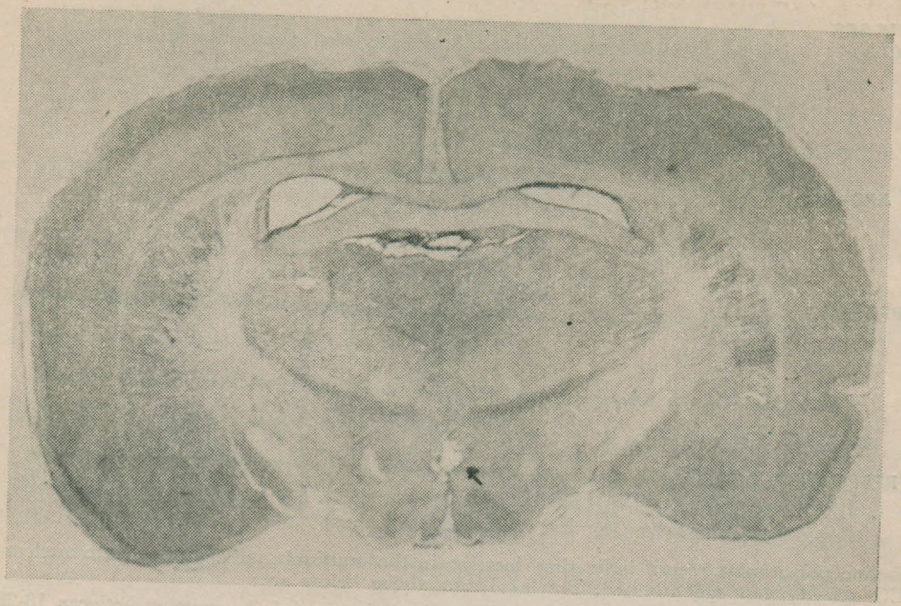
(a)



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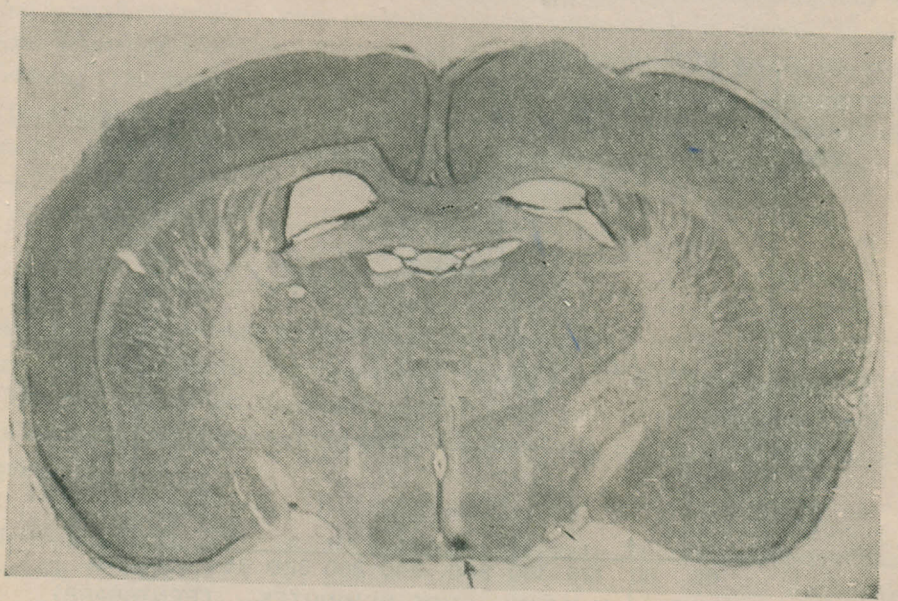

Fig. 3: Coronal sections of the rat hypothalamus localizing the tip of the infusion cannula in the III ventricle (a) and that of the recording electrode in the arcuate nucleus (b).

Third Ventricular Infusion: Cumulative data from a typical experiment is plotted in Fig. 2. It can be noted that the baseline MUA for an hour and a half after the start of recording remained around the average of about 1250 per 5 minute count. Infusion of saline in the 3rd ventricle did not affect the discharge rate but when at the 170th minute LH-RH was infused, the MUA activity rose to a peak of 3000 discriminated spikes/5 min within a period of 20-30 minutes. This pulse discharge remained fairly high (average of 2000 pulses/5 min) for another 200 minutes when the experiment was terminated by giving an excess dose of pentobarbitone. This effect of LH-RH was similar in all experiments. In 4 animals the increase in the MUA of the arcuate nucleus was in spite of the highly synchronized cortical activity during the post-infusion period. In the other 5 animals there was not much variation from the EEG of pre-infusion period. Figure 3 localizes the tip of the cannula and that of the recording electrode in the coronal sections of the brain. Such experiments were completed in a total of nine rats. From the data thus obtained the median percentage pulse frequency in each successive 5 minute MUA during the basal recording, recording after saline infusion and that after LH-RH infusion was plotted as given in Fig. 4. It can be noted that while the MUA before infusion and after

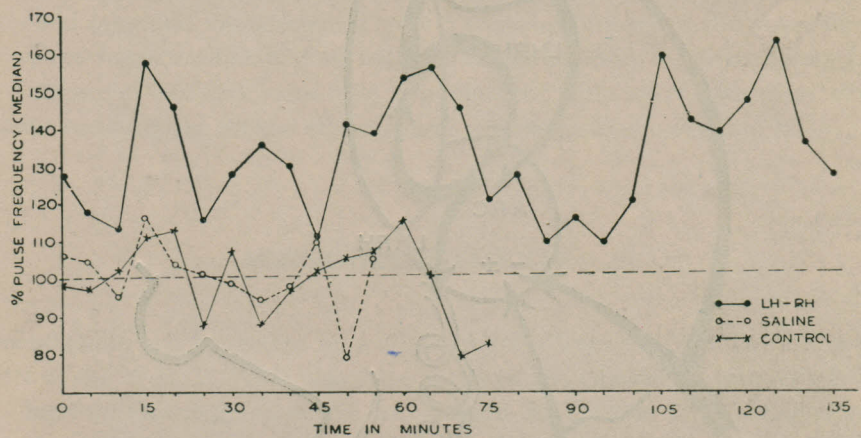

Fig. 4: Median of the % pulse frequency/5 min as obtained from the data of the nine experiments. Note the markedly increased MUA of the arcuate nucleus under the influence of LH-RH.

infusion of saline overlap each other, the MUA after the LH-RH infusion remains markedly high for as long as more than 2 hours of post-infusion period.

DISCUSSION

The present investigation clearly demonstrates that infusion of LH-RH in the 3rd ventricle increases the MUA of the arcuate nucleus. Such infusions of LH-RH have been earlier reported to increase the plasma LH concentration leading to ovulation (4,9,16) and to mating behaviour (8) which indicates that LH-RH transport from the CSF to the proximal capillary plexus in the median eminence may indeed be a normal physiological activity. Current speculations hint

that such a transport may be occuring through the tanycytes lining the 3rd ventricle. However, such transport by the ependymal cell processes to the capillaries may be quite insignificant, constituting at most 5% of the total transport (4). A recent report also indicates that LH-RH immuno-reaction localized in the ependymal cells may in reality be an artefact(11).

There is substantial evidence that a major concentration of LH-RH occurs in the axon terminals of the arcuate nucleus although some immuno-reactive LH-RH has also been localized in the more anterior preoptic part of the organum vasculosum (1,15). It is thus likely that the

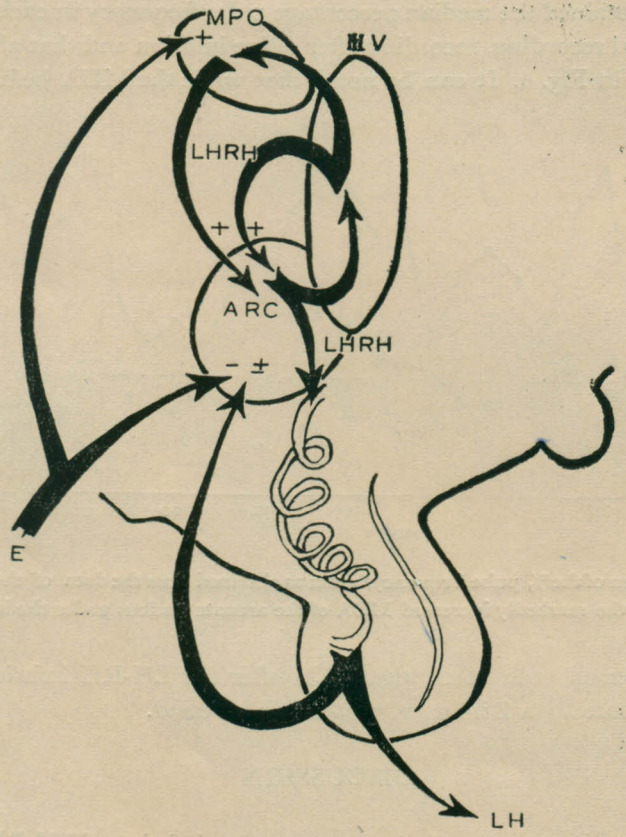

Fig. 5: Feedback mechanisms employed for the regulation of LH-RH secretion as deduced from current evidence and the present investigation. Arcuate (and medial basal) neurones secrete LH-RH under the triggering influence of medial preoptic neurones (MPO). Part of LH-RH finds entry into the CSF for the purpose of positive feedback on arcuate neurones (as reported in the present investigation) and the MPO. The remaining LH-RH finds its way into the portal system for the elaboration of LH. Increased estrogen level exercises a facilitatory influence on MPO but inhibits arcuate nucleus activity.

arcuate neurones may be a major source of LH-RH some of which when secreted is poured into the 3rd ventricle and then absorbed back to increase the activity of the same arcuate nucleus thus providing a system of positive feedback which may be requird for the build-up of LH surge. It has been speculated on the basis of morphological observations that the arcuate neurones lining the ventricle have surfaces with features which are specially suitable for pouring secretions in the ventricle as also for making contact with the ventricular liquor (13). Alternately, possibility of short processes of ependymal cells making synaptoid junctions for conveying transmitters and other substances including LH-RH cannot be excluded (10).

Although some LH-RH has been localized in the medial preoptic area, it seems that the role of this area is to provide through its axonal connections trigger to the arcuate neurones for the release of LH-RH (7). In that case the low level of immuno-reactive LH-RH may be essentially the one picked up from the CSF and not actually elaborated by the preoptic neurones themselves. LH-RH induced increase in the preoptic neurone activity has been reported in literature (5,8). Thus the positive feedback for LH-RH build-up may also be operating through the preoptic region and organum vasculosum. Figure 5 sums up in a schematic form the LH-RH positive feedback mechanism which may be required for the build-up of pre-ovulatory LH surge. However, the presence of feedback loops from hypothalamic areas other than those depicted in this figure cannot be ruled out till the experimental work actually proves it to be so.

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