Effect of Electrical Stimulation of the Medial Preoptic Area on Hypothalamic Multiple Unit Activity in Relation to LH Release

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Synopsis

The effect of electrical stimulation of the medial preoptic area (MPO) on integrated multiple unit activity (MUA) in the arcuate nucleus (ARC) during the estrous cycle of the Wistar rat was examined with reference to the release of "the ovulating hormone" from the anterior pituitary gland. The electrical stimulation (0.5 ms, 200 Hz, SW, 100–500 μA) was applied for 30 min between 14:00 and 16:00 under pentobarbital anesthesia. 1) When the stimulus induced ovulation on the following day, MUA changes in the ARC were observed during the electrical stimulation on the day of proestrus, in contrast to their appearance after the electrical stimulation on the day of diestrus II. 2) The electrical stimulation facilitated the release of "the ovulating hormone" on the day of proestrus, while on the day of diestrus II it increased pituitary potency to induce ovulation as well, tested by an injection of a crude extract from the anterior pituitary of the experimental animals into recipients of diestrus II. 3) The circulatory level of the luteinizing hormone (LH) elevated immediately after the electrical stimulation, reached the maximum level 30 min later and returned to the control level after 60 min on the day of proestrus. However, on the remaining days of the cycle it elevated immediately, reached the maximum level at 60 min, but did not return to the control level even 120 min after the electrical stimulation.

Therefore, that the electrical stimulation of the MPO induces neural excitation of the ARC and increase of LH content in the circulation with a discrepancy in the timing throughout the cycle, indicates that the stimulation induces both synthesis and release of LH in the pituitary gland.

Although the "critical period" occurs only for 2 hr on the day of proestrus when "the ovulating hormone" is released, it may be possible that daily neurogenic stimulus on the pituitary ovarian axis exists in female rats with normal cycles. Everett and Sawyer demonstrated a daily 14:00 to 16:00 neural facilitatory period for release of the ovulatory surge of LH (1949; 1950). Even in immature rats, the LH release appears to occur only during the "critical period" (14:00 to 16:00) (McCormack and Meyer, 1962; Zarrow et al., 1969).

In a previous study we observed that the episodes of the characteristic pattern of multiple unit activity (MUA) in the basal hypothalamus and the medial preoptic septal complex appear daily between 13:00 and 17:00 (Kawakami et al., 1970). Furthermore, induction of ovulation by electrical stimulation in the medial preoptic area (MPO) on the day of diestrus II was most effective between 14:00 and 16:00 (Kawakami and Terasawa, 1970). However, comparing diestrus II and proestrus, a great difference has been observed in the content of "the ovulating hormone" in anterior pituitary gland (APG), in the condition of the ovary, and in the circulatory level.
of hormones. Therefore, it would be of interest to examine the role of the brain in inducing the release of "the ovulating hormone" on these 2 days.

The experiment was, first of all, to examine the effect of the electrical stimulation on the elevation of MUA in the arcuate nucleus (ARC), when an ovulatory amount of pituitary gonadotropin was released both on the day of proestrus and the day of diestrus II. This elevation had previously been observed in rats to which electrochemical stimulation was applied during the critical period, activity of the brain being blocked by pentobarbital anesthesia (Terasawa and Sawyer, 1969).

Secondly, experiments were designed to test whether, using the electrical stimulation of the MPO, any difference in the pituitary potency to induce ovulation and in the luteinizing hormone content of the pituitary and the peripheral circulation could be observed on these 2 days.

**Materials and Methods**

Three hundred and twenty eight Wistar female rats weighing 180 to 220 g were maintained in a room illuminated from 5:00 to 19:00. By vaginal sears taken every morning, at least 2 consecutive 4-day cycles were determined prior to the commencement of the experiment. Sodium pentobarbital (Abbott Co. 30 mg/kg body weight) was regularly injected into all animals 15 min before 14:00.

*The electrical stimulation:* A monophasic square wave of a pulse duration of 0.5 ms with a frequency of 200 Hz, was applied to the left side of the medial preoptic region (MPO) through concentric bipolar electrodes for 30 min between 14:00 and 16:00 at 30 sec periods alternating with 30 sec rest periods. The current was monitored by an oscilloscope and a threshold current which would induce ovulation was chosen; that is, 100 μA was used for stimulus on the day of proestrus and 500 μA was used for the other 3 days of the cycle (Kawakami and Terasawa, 1970).

Pituitary potency to induce ovulation was determined by checking for ovulation in rats of diestrus II which were injected with a crude extract from the APG of the experimental rats. There were 5 groups of donar animals. The control group was killed for taking extracts without electrical stimulation. The 4 other groups were killed 1) immediately after stimulation, 2) 30 min after, 3) 60 min after, and 4) 120 min after it. The APG was quickly dissected, weighed and refrigerated by aceton-dry-ice. Four APG were homogenized together in 0.5 ml of normal saline and washed out with 1.0 ml of the saline. The homogenate was centrifuged at 3000 rpm for 20 min at 0°C. The supernate was diluted with the saline up to 2.0 ml and injected into the recipients by the femoral vein between 14:00 and 16:00. Ovulation was determined and the number of ova was counted the following day.

Radioimmunoassay was used for determination of LH in the serum and APG. Blood samples were obtained by heart puncture, and APG samples by homogenation with 2.0 ml of phosphate buffer saline. All samples were stored at −20°C until the assay was started. The technique of LH radioimmunoassay described by Niswender et al. (1968), the so-called 0-0-rat RIA system, was employed. LER 1056-C2 (biological potency was 1.73 × NIH-LH-S1) was used for radioiodination of LH,* standard preparation of the rat pituitary extract (biological potency was 0.135 × NIH-LH-S1) was used for the standard.** Anti-ovine LH serum (W15) was used for the first antibody.***

Recording of MUA was obtained under pentobarbital anesthesia from the left side of the ARC, the suprachiasmatic nucleus (SC) and other related nuclei in the basal hypothalamus by the techniques used by Sawyer et al. (1968) and Kawakami et al. (1970). The coordinate used for orientation when inserting electrodes was taken from the atlas of Albe-Fessard et al. (1966). Recording was started at 14:00 and stopped at 19:40 (the electrical stimulation was performed between 15:10 and 15:40).

Ovulation was determined microscopically by the presence of ova in oviducts the morning following treatment (on the day of estrus in the animals used for recording purposes and on the day of proestrus in the rats which were examined for pituitary potency). Histological sections were made to examine the sites of electrodes used in stimulation and recording.

**Results**

1. **Changes in MUA of the ARC induced by**

   *supplied by Dr. L. E. Reichert, Jr. (Emory University, Atlanta, Georgia).

   **supplied by Dr. J. C. Porter (University of Texas Southwestern Medical School, Dallas, Texas).

   ***supplied by Dr. A. R. Midgley, Jr. and G. D. Niswender (University of Michigan, Ann Arbor, Michigan).
Fig. 1. Effect of electrical stimulation of the medial preoptic area (MPO) on multiple unit activity (MUA) in the arcuate nucleus (ARC) and suprachiasmatic nucleus (SC) on the day of diestrus II (A and B) and on the day of proestrus (C). The rats used for A and C ovulated the following morning, while those for B did not.

The electrical stimulation of the MPO

The day of proestrus stimulation: Changes of MUA in the ARC were observed, when rats ovulated on the morning following electrical stimulation of the MPO, MUA in the ARC elevated during the stimulation for 30 min in 7 rats. That is, the number and size of MUA as well as the level of integration increased from the first stimulation and reached the maximum level at the 10th to 20th stimulation, then returned to a slightly higher level than pre-stimulation before the end of stimulation. In
3 out of 7 rats, this higher level lasted for 30 min to 1 hr after the end of stimulation, and made a secondary mild peak during the next 2 hr. In the remaining 4 rats, MUA decreased to the level prior to the stimulation for 20 to 30 min immediately after stimulation, then it increased again during the next 2 to 2.5 hr (Fig. 1C). The changes in MUA of the SC were almost the same as observed in the ARC. In the case of stimulation by subthreshold current (i.e. less than the current needed to induce ovulatory response), MUA increased slightly at the beginning of stimulation and thereafter returned to the same or a slightly lower level than that observed prior to the stimulation.

The day of diestrus II stimulation: A slight elevation of integrated MUA in the ARC during the electrical stimulation was also observed in rats which, being stimulated on the day of diestrus II, ovulated the following morning. The highest level of MUA during the stimulation occurred between the 10th and the 20th stimulation. The elevation in MUA on the day of diestrus continued for 1.5 to 2.5 hrs after stimulation. The maximum value was observed 30 to 40 min after the end of stimulation in contrast to the changes of proestrus when the maximum value occurred during the stimulation (Fig. 1A). Very little change was observed during and after the stimulation in the case when no ovulation was induced by the electrical stimulation (Fig. 1B).

2. Pituitary potency on ovulation

In the control groups prior to the MPO stimulation the ovulatory potency of the crude extract from the APG was higher in the proestrus than that in the second diestrus as shown in Figure 2. Thirty min after the end of electrical stimulation of the MPO in proestrus rats, the potency decreased to the same level as found in the diestrus control. However, pituitary potency in the diestrus rats increased to the level of the proestrus control group immediately after the electrical stimulation. Thirty min after the stimulation, this high level in diestrus rats had already decreased to the control level. Therefore, while the electrical stimulation of MPO induced release of the ovulatory hormone from the APG during the stimulation on the day of proestrus, on the day of diestrus II the stimulation increased pituitary potency after it and then release within 30 min thereafter.

3. LH content in serum

LH content in the 1.0 ml serum on the afternoon of proestrus before electrical stimulation was significantly higher than that on the afternoon of the remaining days (Fig. 3). The electrical stimulation of MPO did significantly increase the serum level of LH immediately after the application in the rats of diestrus I, diestrus II and estrus as well as in the rats of proestrus. However, the time when the maximum value appeared differed each day. That is, the highest value was recorded 30 min after the stimulation on the day of proestrus and 60 min after it on the day of diestrus II and estrus.

In other words, on the day of proestrus, serum content of LH increased immediately after the stimulation and reached the maximum value 30 min after it. On the other hand, on the day of diestrus II, LH content, while significantly higher than control immediately after stimulation, reached its peak later than in the former case, and did not return to the control level even 120 min after the stimulation. In the case of diestrus I and estrus as well, the level of LH did not return to the pre-stimulation level after 120 min.

4. LH content in the pituitary gland

The content of LH in the APG before and after electrical stimulation of the MPO is shown in Table 1. For the control rats the LH content on the proestrus day was significantly higher than that during the remaining days. This was true in both total content and LH/mg. The LH content in the proestrus rats increased 30 min after the stimulation and returned to the control level or sub-control level 120 min after it. The electrical stimulation
induced an increase of LH in the pituitary gland (LH/mg) immediately after the stimulation in the rats of diestrus II and an increased level returned to the control level 120 min after stimulation. In the rat of estrus and diestrus I the electrical stimulation of MPO induced elevation of pituitary LH 60 min after the stimulation.

**Discussion**

This experiment confirmed that, on all 4 days of the cycle, neural stimuli induced the release of LH from the APG and, on the day of diestrus II as well as proestrus, MPO stimulation caused "the ovulating hormone" to be liberated, supporting the concept of a daily neurogenic surge for LH release during the estrous cycle. Injection of pentobarbital just before the critical period delayed ovulation by 24 hr and, when it was given the following day delayed ovulation by another full day (Everett and Sawyer, 1949). Progesterone administration on the day of diestrus III to 5-day cyclic rats advanced ovulation by 24 hr (Everett, 1948; Zeilmaker and Moll, 1967), progesterone administration on the day of diestrus II to 4-day cyclic rats delayed ovulation by 24 hr (Zeilmaker, 1966).
Fig. 3. Effect of electrical stimulation of the MPO on the LH content in 1.0 ml of serum during estrous cycle. Abscissa indicates the experimental groups and ordinate indicates serum content of LH. Numbers on the bar indicate the total number of animals tested.

Table 1. Effects of electrical stimulation of the medial preoptic area on LH content in the pituitary gland and in the circulation

<table>
<thead>
<tr>
<th>The day of</th>
<th>After the electrical stimulation</th>
<th>Numbers</th>
<th>Pituitary weight mean ± SE</th>
<th>Total LH in APG mean ± SE</th>
<th>LH/mg in APG mean ± SE</th>
<th>LH in 1.0 ml of serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>8</td>
<td>7.5 ± 0.5 mg</td>
<td>2154 ± 244 ng</td>
<td>313 ± 46 ng</td>
<td>a</td>
<td>0.16 ± 0.17 ng</td>
</tr>
<tr>
<td>0 min</td>
<td>8</td>
<td>6.8 ± 0.5</td>
<td>1951 ± 224</td>
<td>336 ± 50</td>
<td>b</td>
<td>1.41 ± 0.25</td>
</tr>
<tr>
<td>30</td>
<td>10</td>
<td>8.3 ± 0.5</td>
<td>2380 ± 160</td>
<td>300 ± 27</td>
<td>c</td>
<td>4.28 ± 1.31</td>
</tr>
<tr>
<td>60</td>
<td>10</td>
<td>9.1 ± 0.5</td>
<td>4047 ± 377</td>
<td>468 ± 60</td>
<td>d</td>
<td>3.82 ± 0.18</td>
</tr>
<tr>
<td>120</td>
<td>8</td>
<td>9.1 ± 0.7</td>
<td>3291 ± 411</td>
<td>373 ± 53</td>
<td>e</td>
<td>2.86 ± 1.23</td>
</tr>
<tr>
<td>Diestrus I</td>
<td>12</td>
<td>7.7 ± 0.4</td>
<td>2067 ± 683</td>
<td>260 ± 37</td>
<td>f</td>
<td>0.51 ± 0.16</td>
</tr>
<tr>
<td>0 min</td>
<td>9</td>
<td>7.5 ± 0.4</td>
<td>2911 ± 302</td>
<td>398 ± 44</td>
<td>g</td>
<td>1.44 ± 0.29</td>
</tr>
<tr>
<td>30</td>
<td>12</td>
<td>7.3 ± 0.3</td>
<td>2213 ± 213</td>
<td>313 ± 41</td>
<td>h</td>
<td>4.44 ± 1.17</td>
</tr>
<tr>
<td>60</td>
<td>9</td>
<td>7.5 ± 0.4</td>
<td>2360 ± 655</td>
<td>333 ± 96</td>
<td>i</td>
<td>9.22 ± 2.07</td>
</tr>
<tr>
<td>120</td>
<td>8</td>
<td>7.8 ± 0.6</td>
<td>2408 ± 174</td>
<td>283 ± 26</td>
<td>j</td>
<td>6.22 ± 1.11</td>
</tr>
<tr>
<td>Diestrus II</td>
<td>10</td>
<td>7.8 ± 0.5</td>
<td>3725 ± 324</td>
<td>489 ± 38</td>
<td>k</td>
<td>1.13 ± 0.21</td>
</tr>
<tr>
<td>0 min</td>
<td>9</td>
<td>7.8 ± 0.5</td>
<td>4127 ± 456</td>
<td>530 ± 44</td>
<td>l</td>
<td>4.19 ± 1.31</td>
</tr>
<tr>
<td>30</td>
<td>10</td>
<td>7.8 ± 0.7</td>
<td>5745 ± 524</td>
<td>793 ± 101</td>
<td>m</td>
<td>15.07 ± 4.54</td>
</tr>
<tr>
<td>60</td>
<td>10</td>
<td>8.1 ± 0.5</td>
<td>5497 ± 531</td>
<td>703 ± 84</td>
<td>n</td>
<td>2.50 ± 1.02</td>
</tr>
<tr>
<td>120</td>
<td>10</td>
<td>8.7 ± 0.6</td>
<td>2932 ± 389</td>
<td>335 ± 34</td>
<td>o</td>
<td>1.44 ± 0.74</td>
</tr>
<tr>
<td>Proestrus</td>
<td>9</td>
<td>7.6 ± 0.5</td>
<td>1600 ± 296</td>
<td>219 ± 43</td>
<td>p</td>
<td>0.18 ± 0.05</td>
</tr>
<tr>
<td>0 min</td>
<td>10</td>
<td>7.7 ± 0.7</td>
<td>2479 ± 541</td>
<td>311 ± 66</td>
<td>q</td>
<td>2.16 ± 1.07</td>
</tr>
<tr>
<td>30</td>
<td>12</td>
<td>7.8 ± 0.5</td>
<td>2034 ± 245</td>
<td>268 ± 34</td>
<td>r</td>
<td>2.30 ± 1.13</td>
</tr>
<tr>
<td>60</td>
<td>8</td>
<td>9.9 ± 0.5</td>
<td>3742 ± 330</td>
<td>379 ± 34</td>
<td>s</td>
<td>3.12 ± 0.66</td>
</tr>
<tr>
<td>120</td>
<td>8</td>
<td>9.5 ± 0.6</td>
<td>3576 ± 465</td>
<td>364 ± 44</td>
<td>t</td>
<td>1.87 ± 0.56</td>
</tr>
</tbody>
</table>

a ≤ b, a < d, a ≡ k, a ≤ c, c ≤ b, f < g, f ≡ k, h ≤ g, j < g, k < m, n ≤ m, o < m, p ≡ k, p ≤ g, p ≡ s, p < t, a' < b', a' < c', a' ≡ d', a' ≤ e', a' ≡ k', c' ≥ e', f' < g', f' ≡ h', f' ≡ i', f' < j', f' < k', j' ≤ i', k' < l', k' ≤ m', k' ≤ n', k' ≤ o', p' < k', p' < q', p' < r', p' ≡ s', p' ≡ t', t' ≤ s'.

a: p < 0.01, b: p < 0.05, c: n.s.
Thus, it is possible that neural stimulus could occur every afternoon. Kobayashi’s observation (1970) and related studies performed by us indicate that the APG has potency to induce ovulation at the proestrous ovary throughout the cycle. However, according to van Rees et al. (1968), the ovary was not in situ for ovulation on the days of estrus and diestrus I to respond to pregnant mare serum (PMS). Therefore, the final responsibility for ovulation exists in the condition of ovary rather than in the pituitary gland or in the brain.

The timing of LH accumulation in the pituitary, of LH content in the circulation and of the changes of MUA in the basal hypothalamus, induced by electrical stimulation of the MPO evidences a great difference between proestrous rats and the rats during the remaining days of cycle. In other words, the changes of MUA occurred in the basal hypothalamus during the stimulation in the rats of proestrus, while in the rats of diestrus II they occurred after the stimulation. The high level of pituitary potency in the proestrous rats, observed prior to the electrical stimulation, decreased right after the stimulation, while in the diestrous II rats pituitary potency increased immediately after the stimulation to the level of proestrus and then declined to the control level. The highest value of the LH concentration in the circulation was observed at 30 min post-stimulation in the rats of proestrus, in contrast to 60 min post-stimulation in the rats of diestrus II and estrus. The increased level of circulatory LH took only 60 min to return to the control level in the proestrus rats, while it took more than 120 min in the rats of diestrus I, diestrus II and estrus. These results suggest that the brain influenced by estrogen and progesterone may be responsible for the release of LH to be faster on the day of proestrus than on the remaining days of the cycle. It has been well evidenced that estrogen and progesterone concentration in the ovarian blood and ovary is highest on the day of proestrus (Eto et al., 1962; Feder et al., 1967; Hori et al., 1968; Uchida et al., 1969; Yoshinaga et al., 1969). Our previous study showed that the threshold current needed to induce ovulation was lowest on the day of proestrus (Kawakami and Terasawa, 1970), due to the effect of estrogen and progesterone upon the excitation of the brain. These steroids synergically lowered the arousal threshold recorded in the neocortex by the stimulation of the midbrain reticular formation (Kawakami and Sawyer, 1959), and elevated the MUA in ARC and MPO (Kawakami et al., 1971). Furthermore, because estrogen was already higher on the day of diestrus II than diestrus I and estrus, more LH was released into the circulation from the APG by the electrical stimulation on this day (than on the days of diestrus I and estrus) (see Fig. 3).

The rise in MUA recorded in the arcuate nucleus and median eminence following the application of electrical stimulation to the MPO seems to relate to the release of “the ovulating hormone” from the pituitary gland. On the day of proestrus, this change occurred during the stimulation with a latency of 10 min from the start of stimulation and a secondary rise with a latency of 20 min after the stimulation. The elevation in integrated MUA during the stimulation, which was observed only when the stimulus led to ovulation, seems similar in latency, time course and process to a rise of MUA in the ARC after electrochemical stimulation (Terasawa and Sawyer, 1969). The secondary rise is probably not directly due to electrical stimulation but rather due to the effects of pituitary and ovarian hormones which had been released by electrical stimulation. It has been well substantiated that these hormones are liberated when a neural surge occurs. And after intravenous injections of LH (Terasawa et al., 1969), FSH (Kawakami and Sawyer, 1969) and subcutaneous injection of progesterone under the estrogen base (Terasawa and Sawyer, 1970; Kawakami et al., 1971) increases of MUA with latency of 15 to 45 min lasting 2 to 3 hr were observed.

A gradual rise of MUA on the day of
diestrus II may also be correlated to the release of “the ovulating hormone,” because facilitation of ovulation by 24 hrs on the day of diestrus II was observed when MUA in the ARC elevated after the electrical stimulation of the MPO. Thus, stimulation to the MPO induces different timing effects in the MUA changes of ARC.

A prolonged time course in the elevation of MUA in the ARC after electrical stimulation was also observed in androgenized animals (Terasawa and Kawakami, 1971). These sterilized rats could ovulate by the electrochemical stimulation of the MPO even though their pituitary LH was lower than normal cycling rats (Terasawa et al., 1969).

Electrical stimulation of the MPO seems to induce both increases in pituitary potency and LH content as well as release of “the ovulating hormone” (and LH) from the pituitary gland as shown in Figures 2 and 3 and Table I. Comparison of the results observed in Figure 2 and Table I shows a slight discrepancy concerning the accumulation of hormones in pituitary gland on the day of proestrus. We would expect the accumulation of both the ovulating hormone and LH to show a similar behavior upon electrical stimulation, as it does in the case of diestrus II. While as yet unclear, this discrepancy may be related to 1) “the ovulating hormone consisting not only of LH but of other hormones (including FSH), and 2) the radioimmunological activity of LH not being exactly parallel to its biological activity which was employed in this experiment.

Nevertheless, this experiment does clearly indicate that the electrical stimulation of the MPO induces both synthesis and release of the ovulating hormone in the pituitary gland. Recent research has tended to view a factor in the hypothalamus as responsible for the release of pituitary hormones (the “releasing factor”). However, our results showed that electrical stimulation also caused a release of “the ovulating hormone” as well as synthesis in the pituitary, therefore, raising several questions about the relationship between these two stimuli. Is electrical stimulation equivalent to some chemical substances in the hypothalamus? If not, does electrical stimulation cause the production of these chemical substances (the “releasing factor”) which in turn act upon the pituitary gland? Furthermore, the fact that two operations (i.e. synthesis and release of the ovulating hormone) were influenced by electrical stimulation suggests either that the chemical substance heretofore termed the “releasing factor” causes both release and synthesis or that there exists a separate “synthesizing factor” which acts to increase pituitary potency. We do not have adequate answers to these questions. Further experiments need to be designed.

Acknowledgments

The authors wish to thank Dr. B. Tamaoki (National Institute of Radiological Sciences) for his great help in measuring LH by means of radioimmunoassay and to thank Mrs. M. Fukasawa for her assistance. We would like to appreciate National Institute of Arthritis and Metabolic Diseases, National Institutes of Health for supplying the materials for radioimmunoassay.

This investigation was supported by grants from the Ministry of Education, Japan.

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