The Roles of Extrahypothalamic and Intrahypothalamic Impulses to the Medial Basal Hypothalamus in the Control of the Pituitary-Gonadal-Axis

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Synopsis

Adult female New Zealand white rabbits with chronically implanted electrodes were used. Potentials (EVPs) were consistently evoked in the medial basal hypothalamus (MBH) including the median eminence and the arcuate nucleus by stimulation of the dorsal hippocampus (HPC), medial amygdala (AMYG) and preoptic area (POA). These EVPs were variously influenced under endogenous or exogenous hormonal condition such as during estrus, after copulation and after hCG administration.

The MBH-EVP by HPC stimulation was depressed during estrus and was much more depressed after copulation than during estrus except the phasic facilitation at ten hours after copulation. The MBH-EVP by AMYG stimulation was significantly facilitated after copulation, nevertheless it was not so affected during estrus. The MBH-EVP by POA stimulation showed a reciprocal changes between during estrus and after copulation, that is, the facilitation in the former and the depression in the latter.

Namely, the excitability of the HPC-MBH neuronal system may be inhibited, that of the AMYG-MBH neuronal system may be facilitated and that of the POA-MBH neuronal system may be facilitated and successively inhibited from the beginning of estrus to the accomplishment of the copulatory ovulation.

Finally, it was discussed that hCG directly might affect and depress the EVP representative of the neuronal pathways mentioned above.

It has been well-known that the limbic structures and the hypothalamus are closely related to the neural regulation of the pituitary-gonadal axis. According to the experiments by Kawakami and Sawyer (1959), Kawakami (1960), Kawakami et al. (1966), Kawakami and Terasawa (1967), Kawakami and Saito (1967), Endrözi et al. (1968), Kawakami et al., (1969) and Kimura (1969), alterations of gonadotropin and ovarian hormone concentration in the systemic circulation in cats, rabbits and rats exert remarkable influences upon the excitability of neural pathways in the central nervous system.

In the present experiment, the potentials in the medial basal hypothalamus including the median eminence and the arcuate nucleus evoked by stimulation of the limbic structure and the preoptic area were studied and how they might be influenced by endogenous or exogenous hormonal alterations in the rabbit was discussed.

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Materials and Methods

Adult female New Zealand white rabbits, weighing between 2.5 and 3.5 kg, were used. These rabbits were given on chronic preparations at least ten days after surgical procedures. Coaxial bipolar electrodes insulated with the epoxy resin, excluding their tips, were used. The distance between bared portion of tip and end of barrel of electrodes was 0.5 to 0.8 mm. Electrodes were implanted stereotaxically according to the atlas of the rabbit brain (Sawyer et al., 1954). A silver ball electrode was placed on the dura of the frontal cortex for recording surface cortical electroencephalogram (EEG). The rabbit was free to move around on a table in the shielded, sound proof recording chamber, to eat, drink and sleep. Bipolar electrodes were used monopolarly for recording evoked potentials (EVPs) through a dual-beam cathode ray oscilloscope (Nihon Kohden VC-7) and a continuous recording camera (Nihon Kohden PC-1B). Simultaneous EEG recording from the intracerebral electrodes and surface cortical electrode was monitored through an inkwriter oscillograph (Nihon Kohden WI-180). This was done because it was a rule to record EVPs while the animal was at rest and its cortical EEG showed an arousal pattern or while the desynchronized phase appeared after disappearance of EEG spindle burst. Stimulation composed of monophasic square-wave pulse was delivered with an electronic stimulator (Nihon Kohden MSE-3). Pulse duration was between 0.01 and 0.05 msec, and voltage did not exceed over 18 volts.

In the present experiment, the following gonadotropin and ovarian hormone were used; human chorionic gonadotropin (hCG, Teikoku-Zoki), estradiol benzoate (dissolved in sesame oil, Teikoku-Zoki), progesterone propionate (dissolved in sesame oil, Teikoku-Zoki). For each experiment, three to five rabbits were used. At the end of experiments, localization of the electrode tip was verified histologically.

Results

The sites of stimulating electrodes were as follows; the medial amygdala (AMYG), dorsal hippocampus (HPC), and preoptic area (POA). The recording electrode was implanted only in the medial basal hypothalamus (MBH) which was restricted to the median eminence and the arcuate nucleus (Fig. 1). The neural connections from AMYG, HPC and POA to the MBH have been known (Koikegami, 1968). Also in the present experiment, EVPs were elicited in the latter by stimulation of the former. The changes of those EVPs during estrus, after copulation and after hCG administration were investigated. Ovariectomized rabbits were induced to be behaviorally estrous after treatment with estradiol benzoate (0.1 mg/day) for two days (EE) and with 2 mg of progesterone propionate on the third day (EEP), as reported by Sawyer and Everett (1959). Non-castrated female rabbits, which were used for copulation experiment, were treated with estradiol benzoate (0.1 mg/day) for two days to induce behavioral estrus. HCG (80 I.U. iv injection) was administered to castrated estrous (treated with estradiol benzoate 0.1 mg/day for two days) or castrated anestrus rabbits.

Electrical single stimulus was given ten times every 10 to 30 sec at the fixed time; the number of times of stimulus to elicit EVPs was limited within ten times not to disturb the internal endocrine circumstances by over-repetition.

Observation was focussed particularly on the amplitude and peak-latency of EVPs. Changes of EVPs in each experiment were indicated by superimposing of five to ten EVPs that were recorded. The significance of such changes was examined statistically by t-test.

1. The EVPs in the MBH elicited by stimulation of the HPC (hippocampally EVP) or the AMYG (amygdaloidly EVP). The hippocampally EVP was also consistently observed and it was usually composed of short-latency negative deflection with 4 to 6 msec peak-latency and positive deflection with 8 to 14 msec peak-latency. Occasionally much shorter-latency (about 2 msec) deflection was observed. Amygdaloidly EVP was consistently observed in all of the cases and it usually
Consisted of large negative deflection with 8 to 11.5 msec peak-latency and large, broad positive deflection with 17 to 20 msec latency. In some cases, short-latency (less than 4.5 msec) deflection was occasionally observed.

**Estrus**: The negative deflection of the hippocampally EVP started to decrease in amplitude within one hour after progesterone injection followed by EE treatment and maintained the decreased level for two to three hours after the progesterone treatment. The positive deflection with 8 to 14 msec peak-latency did not show any remarkable changes except within one hour. The peak-latency of deflections did not show any marked changes (Fig. 2).

Either the negative deflection or the positive deflection with 17 to 20 msec peak-
latency of the amygdaloidly EVP showed a slightly increasing tendency without any significant changes in amplitude and in peak-latency (Fig. 3).

**Copulation**: The hippocampally EVP increased phasically in amplitude of the negative deflection six to ten hours after copulation. The positive deflection with 17 to 20 msec peak-latency showed remarkable decrease one to five hours after copulation and slightly decreased afterwards for more than twenty hours. The peak-latency was scarcely influenced (Fig. 4).

The amygdaloidly EVP started to increase in amplitude both in the negative and the positive deflection with 17 to 20 msec peak-latency about two hours after copulation. This change was marked especially in the positive deflection and lasted for more than twenty hours. The peak-latency did not show any marked changes (Fig. 5).

**Single administration of hCG to estrous or anestrous rabbits**: The negative deflection of the hippocampally EVP did not show any remarkable changes in amplitude. Positive deflection with 8 to 14 msec peak-latency decreased temporarily within one hour after administration of hCG (Fig. 6).

The amygdaloidly EVP slightly decreased in amplitude of both deflections and maintained the level for one to three hours, and the positive deflection with 17 to 20 msec peak-latency had marked changes about four to six hours after the administration (Fig. 7). The peak-latency of deflections either of the amygdaloidly EVP or of the hippocampally EVP did not show any remarkable changes.

**The EVPs in the MBH elicited by stimulation of the POA**.

The EVP elicited by stimulation of the POA was usually composed of the negative deflection with 1.5 to 2.5 msec peak-latency
and the positive deflection with 9 to 10 msec peak-latency. Occasionally, EVP elicited by stimulation of the POA had positive or negative deflection with shorter-latency (0.5–1.0 msec).

**Estrus:** Either the negative deflection with 1.5 to 2.5 msec peak-latency or the positive deflection did not show any remarkable changes in amplitude until four to five hours after progesterone injection following EE

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**Fig. 6.** Changes of evoked potential in the MBH elicited by hippocampal stimulation after hCG injection. Only temporary decrease is seen in the amplitude of positive deflection within one hour after injection.

**Fig. 7.** Changes of evoked potential in the MBH elicited by amygdaloid stimulation after hCG injection. a: control. b: about thirty minutes after injection. c: about one and a half hours after injection. d: about six hours after injection.

**Fig. 8.** Changes of evoked potential in the MBH elicited by preoptic stimulation following EEP. a: control. b: about six hours after progesterone (P) injection. c: about eight hours after injection.
treatment and then increased for about three hours in amplitude of the positive deflection, but the negative deflection increased markedly in amplitude about six hours after the progesterone treatment. The peak-latency was scarcely influenced (Fig. 8).

Copulation: The EVP elicited by stimulation of the POA decreased gradually in amplitude of both deflections one to three hours after copulation and lasted for more than four hours. The peak-latency was scarcely influenced (Fig. 9).

Single administration of hCG to estrous or anestrous rabbit:

Administration of hCG caused an increasing tendency in amplitude of positive deflection, but this increase was not statistically significant. The negative deflection showed a decreasing tendency and a remarkable drop one to three hours and eight to nine hours after administration. The peak-latency did not show any marked changes (Fig. 10).

Discussion

It may be said that the synaptic delay in the neuronal pathway between the stimulating site and the recording site is the principal factor in the change of peak-latency of the EVPs, whereas an increase or the decrease in amplitude of the EVPs suggests the involvement of numbers of neurons in the response. Commonly, an increase or a decrease in amplitude of EVPs may be considered to reflect the changes in excitability of the neuronal pathways associated with the response. In the present experiment, attention was focussed on the amplitude and the peak-latency, but the latter was hardly affected even under various changes of hormonal circumstances.

Data of this study are summarized in Table 1 (A, B, C). Table 1 (D) is the data of another experiment (Ishida et al., 1977), which was undertaken to estimate whether each deflection of EVPs was concerned with the direct or pauci-neuronal pathways or the multi-neuronal pathways, based on the findings of French et al. (1953) and Bayer et al. (1962). The deflection with 2 msec peak-latency of the hippocampally EVP, the deflection with less than 4.5 msec peak-latency of the amygdaloidly EVP, and the
Table 1. Summary of changes of EVPs in the MBH elicited by stimulation of the HPC, AMYG and POA

<table>
<thead>
<tr>
<th>Stimulating site</th>
<th>latency of deflection</th>
<th>(A) Estrus</th>
<th>(B) Copulation</th>
<th>(C) hCG</th>
<th>(D) Pentobarbital</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPC → MBH</td>
<td>2 m sec (*)</td>
<td>↓</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td>4-6</td>
<td>↓</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td></td>
<td>8-14</td>
<td>←</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>AMYG → MBH</td>
<td>4.5 m sec (*)</td>
<td>●</td>
<td>●</td>
<td>↓</td>
<td>●</td>
</tr>
<tr>
<td></td>
<td>8-11.5</td>
<td>●</td>
<td>●</td>
<td>↓</td>
<td>●</td>
</tr>
<tr>
<td></td>
<td>17-20</td>
<td>●</td>
<td>●</td>
<td>↓</td>
<td>●</td>
</tr>
<tr>
<td>POA → MBH</td>
<td>1.5-2.5 m sec (*)</td>
<td>↑</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td></td>
<td>9-10</td>
<td>↑</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
</tbody>
</table>

●: no changes ↑: significant increase ↓: significant decrease *: not consistently recorded

deflection with 1.5 to 2.5 msec peak-latency of the EVP in response to stimulation of the POA were unaffected by pentobarbital. Therefore, these responses may be mediated through the direct or the pauci-neuronal pathways (discussed in the paper of Ishida et al., 1977).

Kawakami and Terasawa (1967) and Kimura (1969) reported that the amplitude of the hippocampally EVP in the periventricular arcuate nucleus decreased during estrus in the rabbit and that at the same time the amplitude of the amygdaloidly EVP increased. Electroencephalographically, the HPC had a suppressed activity and the AMYG had an enhanced activity in estrous rabbit (Kawakami et al., 1966). The multi-unit activity (MUA) of the HPC decreased and that of the AMYG was elevated with the advance of estrous state of the rat (Manaka, 1976). These data suggest that the HPC is inhibited and the AMYG is facilitated during estrus of the rabbit and rat. The unit activity in the periventricular arcuate nucleus tended to be facilitated by hippocampal stimulation and inhibited by amygdaloid stimulation in estrous rats treated with estrogen (Kawakami and Kubo, 1971). This experiment was done under the acute procedure and the result was on the contrary to the above. However, it is interesting that these reports are in accordance with the reciprocal change of electrical activity between the HPC and the AMYG. Although the clear reciprocality was not observed in the present experiment, the response with 4 to 6 msec peak-latency and the response with 8 to 14 msec peak-latency of the hippocampally EVP which may originate in the multi-neuronal pathway was depressed, and the change of deflections of the amygdaloidly EVP showed a slightly increasing tendency without any statistically significant changes during estrus. This result did not exactly agree with the changes of EVPs previously reported by Kawakami and Terasawa (1967), which may be due to the difference in the experimental procedure and the electrode localization because the EVPs reported in both experiments were very dissimilar in the peak-latency.

Electroencephalographically, the activity of the POA was reported to be depressed during estrus in the rabbit (Kawakami et al., 1966). However, Manaka (1976) reported that the MUA of the POA-MBH system was elevated with the advance of estrous state of rat. Also in the present experiment, the potential evoked by stimulation of the POA increased after EEP. Thus, the POA-MBH neuronal system was suggested to be facilitated during estrus. It is noticeable that the negative deflection of EVP elicited by POA stimulation increased dramatically in ampli-
tude around six hours after EEP, which is supposed to be the phasic response of the POA-MBH pauci-neuronal pathway and may be related to the phasic change of internal hormonal circumstances in estrus.

The hippocampally EVP, which was depressed in estrus, was phasically enhanced in amplitude of the negative deflection after copulation while the positive deflection was continuously suppressed. This result suggests that excitability of the one multi-neuronal pathway from the HPC to the MBH rebounded temporarily after the estrous rabbit accepted copulation, but the excitability of the other multi-neuronal pathway seems to be much more depressed after copulation than in estrus. On the other hand, both multi-neuronal pathways of 8 to 11.5 msec and 17 to 20 msec peak-latency from the AMYG to the MBH seems to be much more enhanced in its excitability after copulation than in estrus. These data differ from the results of Kimura’s report (1969). In this experiment, the site of AMYG-stimulating electrode was much more medially located and the site of HPC-stimulating electrode was located in the alvenus, while in the Kimura’s report, the AMYG is the medial or intermediate nucleus and the HPC is the cornus ammonis. Concerning excitability of the neuronal pathway from the POA to the MBH, it was much depressed after copulation in opposition to the changes after EEP. Hilliard et al. (1964) have reported that progestin (20α-hydroxyprogesterone) secretion by the rabbit ovary increased rapidly postcoitally and remained elevated for about eight hours. Since non-ovariectomized rabbits were used in the present experiment on copulation, the postcoital changes of EVPs might have been caused by the feedback action of the postcoitally increased progestin and/or ovulatory hormone in the systematic circulation. The efferent multi-neuronal pathways from the HPC, AMYG and POA, which could be concerned with reflex ovulation except the pathway from the HPC (Ishida et al., 1977), are supposed to be exerted by postcoital changes of pituitary-gonadal axis; the HPC-MBH and POA-MBH neuronal system is subjected to negative feedback action and the AMYG-MBH neuronal system subjected to positive feedback action. Another multi-neuronal pathway from the HPC to the MBH indicative of the negative deflection with 4 to 6 msec phasically and dramatically enhanced around ten hours after copulation when the reflex ovulation has been said to be induced in the postcoital rabbit (Everett, 1961).

This phasic change may be also related to the feedback action of ovarian steroid hormone.

An internal feedback action of gonadotropin on the central nervous system has been suggested by Kawakami and Sawyer (1959). In the present experiment, both the multi-neuronal pathway from the HPC and the AMYG to the MBH and the pauci- or multi-neuronal pathway from the POA to the MBH were depressed in their excitability soon after administration of hCG. Since ovariectomized rabbits were used in the present experiment on hCG treatment, the changes of EVPs may be due to the direct action of hCG upon the neuronal pathways.

In conclusion, the HPC-MBH multi-neuronal system may be continuously depressed in the estrous rabbit and transiently facilitated at the just time when the reflex ovulation may be provoked. On the contrary, the AMYG-MBH multi-neuronal system may not be significantly concerned with keeping or progressing the estrus, but may be dramatically facilitatory for copulatory ovulation. The POA-MBH neuronal system may positively exert to keep and progress the estrous state, and on the contrary may be continuously inhibitory for copulatory ovulation.
Acknowledgments

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References
