Acute Effect of Hypothalamic Deafferentation on Progesterone-Induced Ovulating Hormone Release in the Rats

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Synopsis

A study was made to analyze the facilitatory effect of progesterone on ovulating hormone (OH) release in proestrous rats. Neural connections to the medial basal hypothalamus (MBH) or to the preoptic-suprachiasmatic (POA-SC) region of the hypothalamus were transected on the morning of proestrus with a curved knife or with an L-shaped knife. After the recovery from the surgical anesthesia, the rats were injected subcutaneously with progesterone, 1 mg per rat, and anesthetized again with pentobarbital during the time corresponding to the critical period for OH release in the intact rat. Facilitation by progesterone of OH release was confirmed in the next morning by counting the ova in the oviducts. Posterior deafferentation of the hypothalamus at the level of the mamillary body did not block the progesterone-induced ovulation. Anterior deafferentation at the retrochiasmatic level completely blocked the ovulation. Progesterone-induced ovulation was also blocked by more rostral or broad dorsal deafferentation with a large curved or an L-shaped knife, the operation of which did not provide any damage on the structures of the POA-SC region. It is concluded that the facilitation of OH release by exogenous progesterone is due to the increase in the responsiveness of MBH to the ovulatory stimulus coming from the higher CNS via the POA-SC region, rather than due to the direct stimulation of the hypothalamus to secrete releasing factor(s).

It has been demonstrated that progesterone has an activity to facilitate ovulating hormone release in normal 5-day cyclic rats (Everett, 1948; Brown-Grant, 1967), in ovariectomized rats primed with either estradiol benzoate or testosterone propionate (Caligaris et al., 1968; Taleisnik et al., 1969), in normal proestrous rats (Zeilmaker, 1966; Redmond, 1968) and in prepuberal rats given pregnant mare serum gonadotropin or follicle stimulating hormone (Meyer and McCormack, 1967). In the previous study, we elucidated the facilitation of ovulating hormone release in 4-day cyclic rats either by injecting progesterone subcutaneously or by implanting the steroid into the median eminence-arcuate region of the hypothalamus on the morning or on the early afternoon of proestrus (Kobayashi et al., 1970). Uchida et al. (1969 and 1970) of our laboratory also demonstrated the facilitation of preovulatory progesterone secretion, which might reflect the early secretion of luteinizing hormone (LH), when the rats were primed with exogenous progesterone on the morning of proestrus. In spite of these many evidences indicating the stimulatory action of progesterone on ovulating hormone release, the site and mechanism of action of the steroid in the CNS-hypothalamo-pituitary axis are still unknown. Recently, Taleisnik et al. (1970) have shown that the preoptic area (POA) is involved in the sites of stimulatory action of progesterone, the result of which is similar to that initially reported by Bar-
raclough et al. (1964). We also attempted to analyze the stimulatory action of progesterone on the mechanism of ovulating hormone release by interrupting acutely the neural afferents to the hypothalamus.

**Materials and Methods**

Adult female wistar rats weighing approximately 200 g at operation were housed in an air-conditioned room (25 ± 1°C; 50–60 % humidity) illuminated 12 hr a day from 08:00 to 20:00. Vaginal smearing was carried out daily around at 10:00 and only the rats showing at least 3 regular 4-day estrous cycles were employed for the operation described below. The operation procedures were performed under Na-methylhexabital (125 mg/kg, ip) or Na-methohexital (50 mg/kg, ip) anesthesia before 10:30 on the morning of proestrus. In order to facilitate the timing of ovulating hormone release, which normally occurs after 17:00 proestrus under our lighting schedule (Kobayashi et al., 1968), progesterone, 1 mg per rat, dissolved in sesame oil was injected subcutaneously at 13:00 proestrus as described in the previous report (Kobayashi et al., 1970). Nearly all the operated rats were anesthetized again with Na-pentobarbital (Nembutal, 35 mg/kg, ip) at 16:15 on the same afternoon to prevent later release of ovulating hormone. The facilitation of ovulating hormone release was confirmed by ovulation which was identified the next morning by counting ova expelled from the dilated ampullae of the oviducts as described previously (Kobayashi and Miyake, 1960). The brains of all animals were carefully removed out from the skull and the site of deafferentation was verified by the frontal or sagittal cutting with a razor under the dissecting microscope. Histological examination on the serial sections of the brain was not carried out in this study. Although the body weights of all operated rats decreased markedly, no animals died during the period between the operation at proestrus and the autopsy on the morning of estrus.

 Interruption of the neural fibers was made by the deafferentation technique of Halász and Pupp (1965) with the aid of stereotaxic instrument. The following types of hypothalamic deafferentation were performed with either a curved knife of bayonet shape or an L-shaped knife.

**Anterior deafferentation**

Anterior interruption of the neural afferents to the hypothalamus was performed at 4 rostral levels with a small (horizontal, 1.0 mm; height, 1.0 mm) knife and a large (horizontal, 2.0 mm; height, 2.0 mm) curved one to make half-dome shaped cuts. The knife was lowered through the sagittal sinus until the tip attained the cranial floor and then rotated 90° at each side of the midline. Anterior-I cut (Fig. 1) with a small knife was placed just caudal to the suprachiasmatic nucleus (SC), which interrupted the neural connections of the medial basal hypothalamus (MBH) with POA and extended dorsally up to the ventral region of the paraventricular nucleus. Anterior-II cut (Fig. 1) with the same knife was located rostral to the SC. For the Anterior-III and IV cuts, a large curved knife was used. Anterior-III cut (Fig. 1) was located rostral to the optic chiasma and extended dorsally up to the anterior commissure. This cut interrupted the anterior, lateral and dorsal connections of the POA region with the rest of the brain but its connection with the MBH remained intact. Anterior-IV cut (Fig. 1) was placed much more rostral than Anterior-III cut.

**Posterior deafferentation**

A half-dome shaped cut (Fig. 1, posterior) with a small curved knife was placed caudal to the medial mammillary nucleus.

**Roof-cut**

To interrupt completely the dorsal connections of the hypothalamus with the limbic system and the thalamus, a horizontal cut (Roof-cut, Fig. 2) was performed with an L-shaped knife having a horizontal blade of 2.5 mm in length. The shaft of the knife was placed caudal to the anterior commissure and its horizontal blade was lowered to the ventral margin of this structure, where the knife was rotated 360° to make circular transection of neural fibers.

**Sham deafferentation (Control)**

In all the control experiments, a small curved knife was lowered at the Anterior-I location through the sagittal sinus to the base of the brain and removed without rotation.

**Results**

The results are summarized in Table 1. The stereotaxic and surgical procedures of the sham operation performed on the morning of proestrus did not inhibit the spontaneous ovulation (Group 1). When the sham-operated rats were anesthetized with Nembutal during the critical period for ovulating hormone
Table 1. Effect of hypothalamic deafferentation on progesterone-induced advancement of ovulating hormone release in proestrous rat

<table>
<thead>
<tr>
<th>Group</th>
<th>Operation and Injection</th>
<th>Progesterone (1 mg/rat, sc. 13:00)</th>
<th>Nembutal (35 mg/kg, ip. 16:15)</th>
<th>No. of rats ovulated</th>
<th>No. of ova per ovulating rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sham (control)²</td>
<td>-</td>
<td>-</td>
<td>6/6</td>
<td>15.2 ± 0.7*</td>
</tr>
<tr>
<td>2</td>
<td>Sham (control)²</td>
<td>-</td>
<td>+</td>
<td>0/6</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>Sham (control)²</td>
<td>+</td>
<td>+</td>
<td>5/6</td>
<td>9.4 ± 1.7</td>
</tr>
<tr>
<td>4</td>
<td>Anterior-I</td>
<td>+</td>
<td>+</td>
<td>0/5</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>Anterior-II</td>
<td>+</td>
<td>+</td>
<td>2/6</td>
<td>6.0, 14.0</td>
</tr>
<tr>
<td>6</td>
<td>Posterior</td>
<td>+</td>
<td>+</td>
<td>4/6</td>
<td>9.3 ± 0.9</td>
</tr>
<tr>
<td>7</td>
<td>Anterior-III</td>
<td>+</td>
<td>+</td>
<td>0/8</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>Anterior-IV</td>
<td>+</td>
<td>+</td>
<td>1/6</td>
<td>12.0</td>
</tr>
<tr>
<td>9</td>
<td>Roof-cut</td>
<td>-</td>
<td>-</td>
<td>0/5</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>Roof-cut + e</td>
<td>+</td>
<td>+</td>
<td>0/7</td>
<td>0</td>
</tr>
</tbody>
</table>

a) A small curved knife (horizontal, 1.0 mm; height, 1.0 mm) was used for the groups 1 to 6, while a large curved knife (horizontal, 2.0 mm; height, 2.0 mm) was used for the groups 7 and 8. For the Roof-cut, L-shaped knife (horizontal, 2.5 mm) was used.

b) A knife was lowered at the anterior-I region of the hypothalamus through the sagittal sinus to the base of the brain and removed without rotation.

c) Progesterone was injected at 12:00 noon into this group.

* Mean ± SE  
+: injected  
-: not injected

release (Group 2), no ovulation occurred in the next morning, suggesting that the sham-operation procedures did not act themselves as the stimuli for ovulating hormone release. However, when progesterone was injected subcutaneously at 13:00 proestrus into the sham-operated rats, ovulation was manifested in 5 out of 6 rats in spite of the administration of Nembutal (Group 3). This also indicates that the sham-operation procedures did not affect the CNS-hypothalano-pituitary responsiveness to the facilitatory action of progesterone.

As well as the sham-operated controls, the posterior-deafferented rats showed the facilitation by progesterone of ovulating hormone release in 4 out of 6 rats (Group 6). On the contrary, when anterior deafferentation of the hypothalamus was performed on the morning of proestrus with either a small or a large curved knife, ovulation failed to occur in nearly all the rats in spite of progesterone administration. When the rats were deafferented with a small knife at the retrochiasmatic region (Group 4, Anterior-I in Fig. 1), ovulation did not occur at all in the next morning. In the rats given more rostral deafferentation at POA-SC region with a small knife (Group 5, Anterior-II in Fig. 1), ovulation was observed in the next morning in only 2 out of 6 animals. In these ovulated rats, however, the knife shaft was slightly dislocated from the midline to the left side, indicating the incomplete deafferentation of the nerve fibers at the right side of the hypothalamus.

Similar results were observed in the rats placed anterior deafferentation with a large curved knife (Groups 7 and 8, Anterior-III and IV in Fig. 1). In these two groups, ovulation did not occur regardless of progesterone administration except only 1 rat, which received the most rostral deafferentation. In the rats given this type of deafferentation, the neural afferents to the hypothalamus were interrupted at far rostral and dorsal levels without any surgical trauma in the structures of the POA-SC region.

The failure of facilitation by progesterone of
ovulating hormone release was also confirmed by the Roof-cut deafferentation of the hypothalamus (Group 10). The rats given this type of deafferentation but neither progesterone nor Nembutal (Group 9) also failed to ovulate in the next morning.

Discussion

The results of the present study demonstrate that the facilitation by progesterone of ovulating hormone release is blocked in proestrous rats when the anterior neural afferents to the MBH are disconnected by the cutting with a small curved knife placed at the retrochiasmatic or POA-SC region of the hypothalamus. This is similar to the results reported by Barraclough et al. (1964), who failed to induce ovulation by progesterone in POA-SC lesioned rats, and to the recent works of Taleisnik et al. (1970) and Terasawa and Sawyer (1970), who demonstrated that anterior deafferentation of the hypothalamus blocked the increase by progesterone of plasma LH level and modified the changes of hypothalamic multiunit activity in this process, respectively. All of these results suggest that the POA-SC region of the hypothalamus or the extrahypothalamic location in the higher CNS may be responsible for the induction of ovulating hormone release by progesterone.

It is well established that the cyclic ovulatory stimulus reaches the MBH from the higher CNS via the anterior hypothalamic region (Halász and Pupp, 1965; Halász and
Gorski, 1967; Szentágothai et al., 1968; Halász, 1969). Taleisnik et al. (1970) also demonstrated that progesterone injection induced an increase of plasma LH level in ovariectomized estrogen-primed rats, in which the neural connection to the POA region from the limbic system had been chronically interrupted with an L-shaped knife, and suggested that the active site of progesterone to stimulate ovulating hormone release is located in the POA-SC region of the hypothalamus. If so, the following assumptions are presented as a possible explanation for the participation of progesterone to stimulate ovulatory hormone release: (A) progesterone directly activates the specific nuclei or neurons located in the POA-SC region, which regulates the cyclic release of ovulating hormone, or (B) progesterone alters the physiological function of afferent neural fibers crossing the POA-SC region to increase or decrease the sensitivity of neurons to the afferent impulses coming from the higher CNS. However, the present study clearly demonstrates that the effect of progesterone to induce ovulating hormone release does not take place after acute dorsal and rostral interruptions of neural afferents to the POA-SC region by using a large curved knife or an L-shaped one as well as in the animals with retrochiasmatic deafferentation. The neural structures in the ventral and caudal region of the deafferentation with these knives are not given direct surgical damage in these experiments, although the operation may produce the cytological disfunction caused by surgical damage or edema in the histological structures along the plane of knife cutting. In addition, facilitation of ovulating hormone release does not occur in the rats having progesterone crystals implanted in the POA-
SC region of the hypothalamus on the morning of proestrus as described in the previous report (Kobayashi et al., 1970). These results are not able to support the above assumptions and also deny a possibility that the active site of progesterone to stimulate ovulating hormone release is located in the POA-SC region of the hypothalamus.

The rats given acute deafferentation procedures on the morning of proestrus did not ovulate at all in response to progesterone administration in the present experiment. This suggests that the failure of progesterone-induced ovulation in proestrous rats after an acute interruption of the rostral and dorsal afferents to the hypothalamus reflects the surgical blockade of ovulatory stimulus coming from the higher CNS. However, a possibility must be taken in account in the acute experiment that the operation may temporally diminish the excitability of neural structures ventral or caudal to the cutting by the surgical trauma or edema. Recently, Halász (1969) and Köves and Halász (1970) demonstrated that nearly normal estrous cycles and spontaneous ovulation occur in the rats given chronic interruption of the bilateral, anterior and superior connections to the POA-SC region of the hypothalamus, and suggested that the intrinsic neurogenic stimulus that triggered ovulation arose, at least in part, from the POA-SC. A similar result was reported by Kaasjager et al. (1971). In these chronic studies as well as the experiment of Taleisnik et al. (1970) mentioned above, there is, however, a possibility that the ovulatory stimulus arising in the higher CNS reaches the anterior hypothalamic region via the bypassing neural afferents.

In our previous study (Kobayashi et al., 1970), progesterone implanted into the median eminence-arcuate region of the hypothalamus induced advancement of ovulating hormone release when given on the morning of proestrus. A similar result was reported by Döcke and Dörner (1969). These results suggest that the stimulatory feedback effect of progesterone is exerted at the MBH. However, a possibility is denied that progesterone directly induces the secretion of hypothalamic neurohumor(s) such as releasing factor(s) from the MBH into the portal vessels and thus causes ovulating hormone release from the pituitary, because the facilitation by progesterone of ovulating hormone release does not take place when the neural afferents to the hypothalamus are interrupted at the retrochiasmatic or much rostral levels without surgical damage in the structures of MBH. It is assumed from the present and the previous experiments (Kobayashi et al., 1970) that the action mechanism of progesterone to facilitate ovulating hormone release in proestrous rats may be due to the increase of neural sensitivity to the ovulatory stimulus coming from the higher CNS to the MBH via the POA-SC region of the hypothalamus.

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References