Extrahypothalamic Control of Daily Surges of Prolactin Secretion in Pseudopregnant Rat

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Abstract

The role of the limbic forebrain structures in controlling twice daily surges of prolactin (PRL) induced by cervical stimulation was investigated after acute or chronic deafferentation of the limbic forebrain afferents to the hypothalamus in rats. The preoptic area-roof section (POA-RS), which interrupted the rostral limbic afferents at the dorsal level of the anterior commissure, induced pseudopregnancy (PSP) and initiated the same nocturnal PRL surges as those initiated by the cervical stimulation. Diurnal PRL surges, however, did not occur following this procedure. The nocturnal PRL surge by POA-RS also occurred in ovariectomized rats. Deafferentation between the diagonal band of Broca and the medial preoptic area (F_2 -cut) initiated PSP in 37 % of the rats and induced an apparent but small nocturnal PRL surge. The rats with POA-RS or F_2 -cut showed restoration of their regular estrous cyclicities. Cervical stimulation after POA-RS did not affect the initiation of nocturnal PRL surge induced by POA-RS alone. POA-RS after cervical stimulation also did not affect the initiation of nocturnal PRL surge induced by cervical stimulation, though a diurnal PRL surge was initiated in these rats. The cut made just before the diagonal band of Broca after cervical stimulation did not inhibit the occurrence of either surge. Nocturnal and diurnal PRL surges were manifested after cervical stimulation in the rats with chronic POA-RS or F_2 -cut and their vaginal cyclicities were resumed. These results suggest that the limbic forebrain structures are not indispensable for the initiation of nocturnal PRL surges induced by cervical stimulation but may modify the hypothalamic mechanism(s) initiating a nocturnal PRL surge through the rostral part of the hypothalamus.

The neural connections that arrive rostrally at the medial basal hypothalamus are necessary for the induction and maintenance of rat pseudopregnancy (PSP), which is characterized by twice daily surges of prolactin (PRL), since a retrochiasmatic cut in the hypothalamus results in the disappearance of the daily PRL surges (Freeman *et al.*, 1974) and interruption of PSP (Arai,

1969; Carrer & Taleisnik, 1970; Velasco *et al.*, 1974). Also, restricted lesion of the suprachiasmatic nucleus (SCN) abolishes the cervically stimulated PRL surges (Betha & Neill, 1980), whereas lesion of the medial preoptic area (MPOA) initiates nocturnal PRL surges (Freeman & Banks, 1980) and produces repetition of PSP without any cervical stimulation (Clemens *et al.*, 1976). These results suggest that a hypothalamic site controlling the twice daily surges of PRL may be located both in the SCN, which promotes the PRL surges, and in the MPOA

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which suppresses the surges. In addition, the interruption of neural connections between the limbic forebrain structures and the hypothalamus, *e.g.*, by a horizontal cut at the level of the anterior commissure (Velasco & Taleisnik, 1971), ablation of the septal area and hippocampus-fornix system (Terasawa & Kawakami, 1973), interruption of the stria terminalis, or lesion of the fimbria (Brown-Grant & Raisman, 1972), is known to prolong vaginal cyclicity and then induce PSP in cyclic rats. Electrical stimulation of the amygdala or hippocampus also increases the incidence of PSP in cyclic rats (Peters & Gala, 1975a), but inhibits the induction of PSP by the cervical stimulation (Peters & Gala, 1975b). These results suggest that neural influences coming from the extra-hypothalamic area may play an important role in the induction and maintenance of twice daily surges of PRL induced by the cervical stimulation, but the details remain unknown.

The present study was designed to clarify the pattern of PRL secretion in PSP rats induced by preoptic area-roof section (POA-RS) or frontal deafferentation and to elucidate the role of limbic forebrain structures in initiating and maintaining PRL surges induced by cervical stimulation in rats.

Materials and Methods

Animals

Female rats of the Sprague-Dawley strain (Shizuoka Agricultural Cooperative Association for Laboratory Animals), weighing 160–200 g, were housed under controlled conditions of temperature, humidity, and illumination (light on 08:00–20:00 h) and maintained with ordinary laboratory chow and water *ad libitum*. Vaginal smears were taken each morning and the rats showing at least two consecutive 4-day estrous cycles were used for the experiments. Cervical stimulation was performed at 10:00h on either estrus (Day 0 of PSP) or diestrus 1 by mechanical stimulation with a vibrator.

Deafferentation of the limbic afferents to the hypothalamus

Fig. 1. Schematic drawing of the deafferentation area. A, POA-RS; B, F₁-CUT; C, F₂-CUT.

The effects of acute or chronic deafferentation were investigated after operations with three types of knives originally proposed by Hálasz and Pupp (1965) and modified by Taleisnik *et al.* (1970). Three types of deafferentation were performed to interrupt the neural afferents coming from the amygdala and hippocampus to the anterior basal hypothalamus under sodium pentobarbital anesthesia (40 mg/kg).

a) POA-RS

Using a L-shaped knife with a horizontal blade 2.2 mm long, neural afferents coming from the amygdala and hippocampus through the stria terminalis and fornix system were interrupted at the dorsal level of the anterior commissure (Fig. 1).

The knife was lowered into the brain, with the aid of a stereotaxic apparatus, to the dorsal level of the anterior commissure and rotated 360°. This interrupted dorsal connections of the MPOA and the anterior hypothalamus.

b) *Frontal cut*

Two types of frontal cuts of a half-dome shape were made by lowering a bayonet-shaped knife into the base of the brain and rotating the blade 90° to each side of the mid-line (Fig. 1). In one group, the cut was made just before the diagonal band of Broca (DBB) with a knife having a blade horizontally 2.0 mm long and vertically 2.0 mm long (F_1 -cut). In the other group, the operation was made just before the MPOA with a knife having a blade horizontally 2.0 mm long and vertically 2.2 mm long (F_2 -cut). The F_2 -cut interrupted the neural afferents coming from the limbic system through the DBB to the MPOA.

Animal experiments

a) *POA-RS*

To analyze whether the twice daily surges of PRL are induced by the acute POA-RS alone or in combination with cervical stimulation, 4-day cyclic rats were subjected to POA-RS operation on the day of estrus. In the experiment producing cervical stimulation, one group of rats received cervical stimulation at 10:00 h on Day 1, 24 h after POA-RS, and the others received the stimulation at 10:00 h on Day 0 followed by POA-RS in the afternoon of the same day. Control rats received sham POA-RS procedures in which the knife blade was inserted into the brain but not rotated. Blood samples were collected by decapitation at 15:00, 19:00, 23:00, 07:00 or 13:00 h on Day 2, Day 4 and Day 5 after the POS-RS operation.

The second experiment was performed to investigate the effect of ovariectomy on the surges of PRL induced by POA-RS. On the day of estrus, one group of 4-day cyclic rats were ovariectomized just after POA-RS operation, and the other group additionally received cervical stimulation at 10:00 h on Day 1 after POA-RS and ovariectomy operations given a day before. Blood samples were collected by decapitation at 19:00 h on Day 4 and at 07:00 and 13:00 h on Day 5. Control rats were ovariectomized after sham POA-RS operation on the day of estrus and given cervical stimulation at 10:00 h on the following day.

In the next experiment, the patterns of the twice daily surges of PRL initiated by cervical stimulation were investigated in the rats given chronic POA-RS. The rats given POA-RS operation on diestrus 1 of the estrus cycle showed typical diestrous smears lasting for 6 to 22 days. Sham-operated rats showed regular estrous cycles. Following this prolonged diestrous period, the rats having chronic POA-RS

resumed regular 4- or 5-day estrous cycle. The rats regularly showing two or three consecutive estrous cycles were cervically stimulated at 10:00 h on estrus (Day 0) and then decapitated at 07:00, 13:00 or 19:00 h on Day 2 to collect blood samples.

b) *Frontal cut*

F_1 -cuts were performed on diestrus 1 to the rats given cervical stimulation the day before at 10:00 h on estrus (Day 0). Blood samples were collected at 19:00 on day 4 and at 07:00 h on Day 5. F_2 -cuts were also performed on estrus of the normal cyclic rats and blood samples were collected on Day 2. No cervical stimulation was given to these rats. The vaginal cyclicities of a group of rats given F_2 -cut on estrus were followed after the operation. After irregular vaginal cyclicities for various period, they showed regular estrous cycles. Cervical stimulation was given to the rats at 10:00 h on estrus (Day 0) and blood samples were collected on Day 2. Control rats in these experiments received sham operations.

After the blood samples had been collected, the brains were fixed in buffered formalin and the precise location of each deafferentation was histologically confirmed by staining with thionin.

Assay of plasma PRL

Plasma PRL was measured by radioimmunoassay (RIA) using a rat PRL RIA kit supplied by Dr. S. Raiti through NIAMDD. The amounts of assayed PRL were expressed in terms of the rat PRL standard, NIAMDD-rat PRL-RP-1. The data were analyzed by Student's *t*-test.

Results

Acute experiments

POA-RS performed on estrus (Day 0) in normal cyclic rats produced significant elevation of plasma PRL levels at 07:00 h on Day 2 and 5 (Fig. 2). However, no diurnal PRL surges were observed at 19:00 h on Day 2 and 4 in the rats given this operation. Sham POA-RS rats resumed vaginal estrus by Day 4 or 5. Their plasma PRL levels did not rise at 07:00 h on Day 2 and 5, but a proestrous surge of PRL was apparent at 19:00 h on Day 4.

Cervical stimulation given on Day 1 induced nocturnal PRL surges at 07:00 h on Day 5 in sham POA-RS rats as well as in those given POA-RS operation (Fig. 3).

Fig. 2. Plasma PRL levels in rats given acute POA-RS or SHAM POA-RS on estrus (Day 0). The number on each bar is the number of animals per group, and the vertical lines represent the SEM.

Fig. 3. Plasma PRL levels in rats given POA-RS or SHAM POA-RS on estrus (Day 0) and also cervical stimulation (CS) at 10:00 h on Day 1. The number on each bar is the number of animals per group, and the vertical lines represent the SEM.

There were no statistical differences in these PRL levels between sham POA-RS and POA-RS rats. With cervical stimulation, diurnal PRL surge, but not proestrous PRL surge, was apparent in sham POA-RS rats at 19:00 h on Day 4. Also, diurnal PRL surge was not observed in POA-RS rats. However, the rats given POA-RS operation after cervical stimulation on Day 0 clearly manifested nocturnal and diurnal surges of PRL on Day 4 and 5, whereas the sham

POA-RS rats showed no diurnal PRL surges (Fig. 4).

Although ovariectomy in POA-RS rats decreased PRL levels, nocturnal PRL surge was apparent at 07:00 h on Day 5, but not in ovariectomized rats with sham POA-RS. No diurnal surge of PRL was observed in either POA-RS or sham POA-RS rats (Fig. 5-A). In the ovariectomized rats given cervical stimulation before POA-RS operation, plasma PRL levels at 19:00 h on Day 4 and

Fig. 4. Plasma PRL levels in rats given cervical stimulation (CS) at 10:00 h on estrus (Day 0) and then POA-RS or SHAM POA-RS operation in the afternoon of the same day. The number on each bar is the number of animals per group, and the vertical lines represent the SEM.

Fig. 5. (A). Plasma PRL levels in rats given ovariectomy (OVX) and POA-RS or SHAM POA-RS on estrus (Day 0). (B). Plasma PRL levels in OVX rats given cervical stimulation (CS) at 10:00 h and then POA-RS or SHAM POA-RS operation in the afternoon of estrus (Day 0). The number on each bar is the number of animals per group, and the vertical lines represent the SEM.

Fig. 6. (A). Plasma PRL levels in rats given F₂-CUT or SHAM F₂-CUT on the day of estrus (Day 0). (B). Plasma PRL levels in rats given F₁-CUT or SHAM F₁-CUT in the afternoon of the day of cervical stimulation (CS) (Day 0). The number on each bar is the number of animals per group, and the vertical lines represent the SEM.

at 07:00 h on Day 5 were not significantly different from those in sham POA-RS rats. The PRL level at 07:00 h was significantly ($p < 0.05$) higher than that at 13:00 h (Fig. 5-B).

Although plasma PRL levels were significantly lower than those in cervically stimulated rats, F₂-cut alone induced nocturnal, but not diurnal, PRL surges. Sham F₂-cut failed to induce either PRL surge. F₁-cut given to the rats after cervical stimulation did not affect either diurnal or nocturnal PRL surges on Day 4 and 5 (Fig. 6-B).

Chronic experiments

Normal cyclic rats immediately shifted to PSP after the POA-RS operation but resumed regular vaginal cyclicities after an appropriate period. Sham POA-RS induced no PSP. Plasma PRL levels in the rats with chronic POA-RS which returned to normal estrous cycles were not elevated at 07:00 h

on Day 2 (diestrus 2) (Fig. 7). In these rats with chronic POA-RS, cervical stimulation initiated diurnal and nocturnal PRL surges as observed in sham-operated rats. The levels of PRL surge in POA-RS rats, however, were slightly lower than those of sham POA-RS rats.

Immediate shift to PSP from normal estrous cycle was observed in 9 out of 24 rats after the F₂-cut. The remaining rats did not show PSP. The PSP rats resumed their 4- or 5-day estrous cyclicities after an appropriate period. In sham F₂-cut rats, 4-day vaginal cyclicities continued except for a one-day delay of vaginal cornification in the first cycle immediately after the operation. Cervical stimulation initiated the diurnal and nocturnal PRL surges in chronic F₂-cut rats as well as in sham F₂-cut rats and the magnitudes of these surges were the same (Fig. 8).

Fig. 7. Plasma PRL levels in chronic POA-RS rats. Cervical stimulation (CS) was given at 10:00 h on estrus (Day 0) after resumption of vaginal cyclicity. The number on each bar is the number of animals per group, and the vertical lines represent the SEM.

Discussion

The present study demonstrated that deafferentation of the limbic neurons with POA-RS induces the same nocturnal PRL surges as induced by cervical stimulation in normal PSP rats, but fails to induce diurnal PRL surges. This agrees with previous findings that acute and restricted lesions of the brain such as in the stria terminalis, the medial cortico-hypothalamic tract and the fimbria, result in continuous vaginal diestrus for 10 to 15 days and PSP in rats (Velasco & Taleisnik, 1971; Brown-Gant and Raisman,

Fig. 8. Plasma PRL levels in chronic F₂-CUT rats. Cervical stimulation (CS) was given at 10:00 h on estrus (Day 0) after resumption of vaginal cyclicity. The number on each bar is the number of animals per group, and the vertical lines represent the SEM.

1972; Terasawa & Kawakami, 1973). These results suggest that the rostro-dorsal afferents coming from the extrahypothalamic areas through the preoptico-anterior hypothalamus (Heimer and Nauta, 1969; Raisman *et al.*, 1966) participate in the mechanism(s) of PRL surges to initiate and maintain PSP. F₂-cut, which was positioned just before the MPOA and interrupted the limbic neural

afferents coming through the DBB to the MPOA, also induced nocturnal PRL surges, although their magnitude and the incidence of PSP in these rats were low. This F₂-cut interrupts the neural afferents between the DBB and MPOA to the base of the brain but leaves almost intact the afferents passing through the region above the MPOA and the anterior hypothalamic area. These results indicate that the limbic neural afferents responsible for the occurrence of complete PRL surges may pass through the region above the MPOA and the anterior hypothalamic area rather than the basal part of the DBB.

In contrast to the nocturnal PRL surges, the rats given POA-RS, F₂-cut, or POA-RS followed by cervical stimulation did not show any diurnal surges of PRL. Restricted lesion of the MPOA also induces the disappearance of diurnal PRL surges but results in the repetition of PSP and nocturnal PRL surges (Clemens *et al.*, 1976; Freeman & Banks, 1980). Since diurnal surges of PRL are known to be easily abolished by various nonspecific factors such as surgical stress (Freeman *et al.*, 1974), the failure of diurnal PRL surges in the rats given neural deafferentation may be due to the nonspecific effect of the surgery. In the present study, diurnal PRL surges were only observed in the rats given cervical stimulation before deafferentation surgery or in the rats given sham operations followed by cervical stimulation. These results suggest that the neural mechanism(s) regulating diurnal and nocturnal PRL surges may differ from each other in details. From these results, we also speculated that the neural mechanism(s) regulating diurnal PRL surges may exist in the hypothalamus and, once the mechanism(s) is activated such as by cervical stimulation, the neural influences coming from the extra-hypothalamic areas cannot modify the activity. However, the hypothalamic neural mechanism(s) regulating diurnal PRL surges and its activating system by stimulation of the uterine cervix are still unknown and

remain for future elucidation.

In the present study, normal cyclic rats immediately shifted to PSP by POA-RS or F₂-cut but resumed regular vaginal cyclicities after an appropriate period of diestrus. Similar results have been reported for rats with chronic deafferentation of the stria terminalis and the medial cortico-hypothalamic tract and with chronic lesion of the fimbria and the medial amygdala (Velasco & Taleisnik, 1971; Brown-Grant & Raisman, 1972). The rats which resumed their vaginal cyclicities after these operations exhibit twice daily surges of PRL and PSP when given the cervical stimulation. However, restricted lesion of the MPOA produces repetition of PSP without any cervical stimulation (Clemens *et al.*, 1976). These results suggest that complete destruction of a special nucleus within the hypothalamus induces permanent changes in the mechanism(s) regulating PRL surges but interruption of limbic neural afferents at the retro-dorsal region of the preoptico-anterior hypothalamus induces only temporary changes. The fact that a single PSP followed by regular vaginal cyclicities was induced by the deafferentation of POA-RS or F₂-cut in the present study suggests that the response may be the result of surgical stimulation rather than the chronic effect of surgery. However, a retrochiasmatic cut fails to initiate PRL surges (Freeman *et al.*, 1974) and electrochemical stimulation of the amygdala and hippocampus decreases the concentration of serum PRL (Kawakami *et al.*, 1973) and the incidence of PSP in rats (Peters & Gala, 1975b). From these results, we assumed that nocturnal PRL surges observed after POA-RS or F₂-cut may be not initiated only by nonspecific surgical stimulation but be controlled by the limbic neural influences through the rostro-dorsal region of the preoptico-anterior hypothalamus. The rats with chronic POA-RS or F₂-cut exhibited twice daily surges of PRL when they were given the cervical stimulation after resuming their vaginal cycli-

cities. This suggests that the neural stimulus coming from the uterine cervix to the hypothalamus does not always pass through the POA-RS area and another neural mechanism(s) responsible for the stimulation of the uterine cervix may exist in the central nervous system (CNS). Recently, Bethea & Neill (1980) and Kawakami & Arita (1981) demonstrated that complete lesion of the SCN abolishes PRL surges induced by cervical stimulation. Either restricted lesion of the MPOA or electric stimulation of the dorso- and ventromedial areas of the hypothalamus induces nocturnal PRL surges (Freeman & Banks, 1980). These results suggest that a neural mechanism(s) responsible for the occurrence of PRL surges is present in the hypothalamus itself. Thus, the limbic forebrain structures probably do not exert the main influence on the PRL surge mechanism(s) induced by cervical stimulation, but may modify the hypothalamic control of the PRL surges.

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