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Inhibitory Effect of Dorsal Cut of Hypothalamus on Ovulation Delayed by Pentobarbital in Female Rats

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Abstract. To clarify the role of the extrahypothalamic signals to the hypothalamus in regulating ovulation, the effect of interruption of the dorsal inputs of the hypothalamus on delayed ovulation induced by the treatment with pentobarbital (PB) was examined in female rats. A posterior half-circle horizontal cut at the dorsal of the hypothalamus (PDC) was made and spontaneous ovulation or ovulation delayed by PB were observed. When PDC was performed at 10:00–11:30 h on the day of proestrus, ovulation occurred normally the next morning in all rats. Treatment with PB at 16:00h on the day of proestrus, but not at 13:00 h, induced one-day-delayed ovulation in most rats without brain surgery. In contrast, ovulation was seen normally on estrus in 60.0% of PDC females, when treated with PB at 16:00 h. However when PB was injected at 13:00 h, ovulation was delayed one day in 88.9% of PDC females. Twenty days after making PDC, delayed ovulation was not induced by PB at 16:00 h in 54.5% of rats. These results suggest the possibility that PDC advances the effective period in which PB can cause delayed ovulation. Furthermore, the effect of the PDC continues for at least 20 days after the operation. Thus, dorsal inputs to the hypothalamus may be concerned with a control mechanism of the critical period for triggering of ovulation in female rats.

Key words: Delayed ovulation, Pentobarbital, Extrahypothalamic influence, Critical period, Cuts, Rat (J. Reprod. Dev. 47: 253–258, 2001)

Ovulation is known to be delayed one day by anaesthetizing the brain, by injection with pentobarbital (PB) for example, in female rats [1]. The period during which PB is effective at delaying ovulation is the early evening of proestrus, because injection of PB in this period delays ovulation but when PB is injected in the morning or late evening of proestrus there is no delay [2]. In this critical period, an ovulation-triggering mechanism in the brain is thought to start operating [3]. Following this period, a GnRH-surge occurs. Since, increase of GnRH mRNA-expressing cells in the preoptic area (POA) on the evening of the proestrus is

blocked by PB [4], and electrical stimulation of this nucleus recovered ovulation suppressed by PB [5], it is assumed that a GnRH releasing signal is released from the cyclic center of ovulation in the POA in the critical period.

The critical period is not rigid, because change of daily illumination time can move it. Under daily illumination from 5:00 to 19:00, the critical period of rats is 14:00–16:00 [1], and LH-surge is seen from 17:00 to 19:00 [6], but under illumination from 7:00 to 21:00, the LH-surge occurs from 19:00 to 21:00 [7]. A constant light condition induces anovulatory syndrome [8]. Thus, the critical period for ovulation is dependent on the length of illumination time. The suprachiasmatic nucleus,

which receives fibers from the retina and controls circadian rhythm, is involved in the mechanisms which transduce light signals to the ovulation regulating system, because restricted lesions of this nucleus suppress ovulation in female rats [9].

The limbic area also exerts important influences on regulation of ovulation. The amygdala [11–13] and the hippocampus [14] play roles in modification of the ovulatory cycle. In the finding of Schuiling and Van Rees [15], dorsal inputs of the hypothalamus modified the critical period for ovulation. In this experiment, to confirm their results and to investigate further the role of dorsal inputs of the hypothalamus in regulating ovulation, a posterior half-circle horizontal cut was made at the dorsal of the hypothalamus and delayed ovulation induced by PB was examined in female rats.

Materials and Methods

Animals

Female Wistar rats were housed under controlled a photoperiod (14L:10D, lights on from 07:00 to 21:00 h) and temperature (23–25 °C). The rats received food and water ad libitum. Vaginal smears were taken throughout the experimental period and only rats exhibiting 2 or more consecutive regular 4-day estrous cycle were used. This experiment was done according to the Guideline for the Care and Use of Laboratory Animals in the Human Science Department of Waseda University.

Ova counting

The ovulatory condition was assessed by counting ova in unilateral oviducts during the estrus (E) and diestrus I (DI) in the same animal [see 16]. On the morning of E, the left oviduct was dissected under ether anesthesia and ova in the oviduct were counted under a microscope. Next morning on DI, animals were sacrificed by overexposure to ether and ova in the remaining oviduct (right side) were checked.

Brain surgery

To investigate the role of the dorsal afferents of the POA-medial basal hypothalamus passing posterior to the anterior commissure in the ovulation delaying mechanism activated by PB, a

posterior half-circle horizontal cut (PDC) at the dorsal of the hypothalamus (Fig. 1) was made on the proestrous (P) morning (10:00–11:30 h).

In the brain surgery under ether anesthesia, 39 rats were fixed to a stereotaxic instrument with an incisor bar set at 5 mm below the interaural line. A L-shaped Halász knife with a horizontal blade of 2 mm directed posteriorly was lowered 7.0 mm from the bregma level to a point where the blade tip was 2.4 mm posterior to the bregma on the midline and was rotated posteriorly 90° to the left and right. In 9 females, the sham operated group, the knife was lowered to the same level as the PDC group without rotation. Twenty-four females without brain surgery were prepared as intact or control groups.

PB injection

In order to investigate the effect of PDC on spontaneous ovulation, ovulation was checked by the methods described above after PDC with no treatment in 9 females (PDC group). As an intact control, ovulation was observed on E and DI in 8 females without brain surgery (intact group).

PB (30 mg/kg B.W., Nembutal, DAINABOT) was injected intraperitoneally at 16:00 h or 13:00 h on P on the same day as brain surgery, because a preliminary experiment showed that delayed ovulation occurred when PB is injected at 16:00 h but not at 15:00 h under our light regime. Nine and 7 females with no brain surgery were injected with PB at 16:00 h and 13:00 h respectively. In 10 and 9 PDC females, PB was injected at 16:00 h and 13:00 h, respectively (PDC+PB group).

In addition, PB was injected at 16:00h on P in 11 PDC females to investigate the chronic effect of PDC on ovulation delayed by PB, after the reappearance of 5 consecutive normal 4-day vaginal estrous cycles (about 20 days).

Ova in the unilateral oviducts were counted on E and DI by the methods described above. All animals were sacrificed on the second day of ova counting by overdose of ether anesthesia.

Histological examination

To determine the precise localization of the cut, a histological examination of the brain was performed. Each brain was removed and fixed in 10% formalin solution and 100- μ m frozen sections were made and stained with cresyl fast violet. The location of the cut was determined according to

Fig. 1. Sagittal section of the brain showing the line of PDC in the PDC+PB (16:00) (A), cPDC+PB (B) and PDC+PB (13:00) (C) groups. AC: anterior commissure; AH: anterior hypothalamic nucleus; ARC: arcuate hypothalamic nucleus; AVPO: anteroventral preoptic nucleus; CC: corpus callosum; FX: fornix; MPA: medial preoptic area; MPO: medial preoptic nucleus; SCN: suprachiasmatic nucleus; VMH: ventromedial hypothalamic nucleus.

Table 1. Effects of posterodorsal horizontal cut (PDC) of the hypothalamus on spontaneous or ovulation delayed by PB

Groups	No. of Rats	Day 1 (expected Estrus)				Day 2 (expected Diestrus I)				Body Weight (g)
		No. of Rats ovulating	%	No. of ova (Mean±S.E.)	Incidence of Vaginal Estrus	No. of Rats ovulating	%	No. of ova (Mean±S.E.)	Incidence of Vaginal Estrus	
Intact	8	8/ 8	(100)	5.8±0.6	8/ 8	0/ 8	(0)	–	0/ 8	291 ±10
PDC	9	8/ 9	(88.9)	6.6±0.5	9/ 9	1/ 9	(11.1)	7	1/ 9	257 ±10
PB administration at 16:00										
Control	9	0/ 9	(0)	–	9/ 9	9/ 9	(100)	5.8±0.7	9/ 9	256 ± 8
Sham	9	1/ 9	(11.1)	4	9/ 9	6/ 9	(66.7)	6.2±1.2	6/ 9	276 ± 5
PDC	10	6/10*	(60.0)	7.3±1.2	10/10	3/10*	(30.0)	6.7±1.7	4/10	248 ± 8
cPDC	11	6/11*	(54.5)	6.2±1.1	11/11	5/11*	(45.5)	6.6±0.5	5/11	306 ± 9
PB administration at 13:00										
Control	7	6/ 7	(85.7)	7.5±0.7	7/ 7	1/7	(14.3)	11	1/ 7	266 ± 7
PDC	9	1/ 9**	(11.1)	3	9/ 9	8/9**	(88.9)	9.4±1.4	8/ 9	253 ± 9

*P<0.01 vs Control (PB at 16:00), **P<0.01 vs Control (PB at 13:00).

Paxinos and Watson [17].

The data regarding the incidence of ovulation was evaluated by means of the χ^2 -test and the number of ova in each group was analyzed by unpaired Student's t-test.

Results

In the intact group, ova were found in all animals on E and the mean number of ova was 5.8 ± 0.6 (Table 1). As well as the intact group, most PDC rats without PB showed ovulation on E and the mean number of ova was almost the same as that in

the intact group.

When PB was injected at 16:00 h, ovulation did not occur on E but ova were found on DI in all control rats. In the sham group, all rats with PB except 1 showed no ovulation on E, but 6 out of 9 rats ovulated on DI. Two sham rats had no ovulation during E and DI. All rats that ovulated on DI showed vaginal cornification in 2 consecutive days in the control and sham groups. In contrast, when PB was injected at 16:00 h, 6 out of 10 PDC rats ovulated on the E and the mean number of ova was comparable to those of the control and sham groups. Three PDC+PB rats ovulated on DI, and no ovulation was observed in one PDC rat. In the

chronic PDC+PB group, 6 out of 11 rats also ovulated on E and 5 rats showed ovulation on DI. When PB was injected at 13:00 h, 6 out of 7 control rats ovulated normally on E. In contrast, in the PDC+PB group, ovulation was observed in only 1 rat on E and the remaining 8 rats ovulated on DI. Vaginal cornification was observed 2 consecutive days in the rats ovulating on the DI. In rats ovulating on E, vaginal smear showed a diestrous condition in DI.

In the histological examination, PDC was located at the level of the anterior commissure (Fig. 1). The cuts were extended from the posterior end of the anterior commissure to the area including the ventral part of the nucleus reuniens thalami, dorsal to the paraventricular hypothalamus nucleus. In the surface of the cortex and in the triangular septal nucleus, traces of the lowering knife were seen. Differences in the location of the cut between animals which showed delayed ovulation and animals with no delayed ovulation after PB were not detected clearly.

Discussion

In the present experiment, ovulation occurred normally on E, even when PDC was performed on the morning of P in 4-day estrous cycle rats. This indicates that PDC in the morning of the day before ovulation has no effect on induction of ovulation. When PB was injected at 16:00 h, ovulation was delayed one day in all control rats. This is consistent with many reports that PB injection before the critical period for ovulation causes one-day-delayed ovulation [1]. In contrast, 60% of PDC females showed normal ovulation on E. This result agreed with a report that PDC cancels the effect of PB on ovulation [15]. However, in 40% of animals, PDC did not cancel the PB effect. Although the reason for the absence of PDC effect in these animals is not known, a possible explanation is that the quantity of fibers controlling ovulation injured by the half-circle cut of PDC was not enough. Furthermore, no ovulation in 3 rats of the sham and PDC groups may have been caused by the stress of acute brain surgery. In the case of PB injection at 13:00 h, control females showed normal ovulation on the morning of E but ovulation in 89% of PDC females was delayed by one day. This suggests that PB at 13:00 h is effective at inducing delayed

ovulation when PDC is performed. Because anesthetization of the brain during the critical period causes delayed ovulation [3], it can be said that PDC advances the critical period. Furthermore, the advancement of the critical period by PDC continued for more than 20 days. Thus, the neuronal fibers, which were cut by PDC, may play an important role in determining the time schedule of the critical period for ovulation in female rats. In our laboratory, a preovulatory LH-surge was determined to occur from 19:00 to 21:00 [7]. Since there is a possibility that this time also advances in PDC females, a hormonal assay is necessary to clarify this point.

It can be assumed that limbic projections are involved in the critical period regulating mechanisms. Hippocampal efferents have been shown to leave the ventral subiculum, presubiculum and parasubiculum and project to the POA, arcuate nucleus, ventromedial nucleus, dorsomedial nucleus and ventral premammillary nucleus via the fornix and medial corticohypothalamic tract [18]. Electrical or electrochemical stimulation of the dorsal and ventral hippocampus on P inhibits the increase of LH and FSH release and ovulation [14]. The blockade of ovulation by electrical stimulation of the ventral hippocampus was overcome by the transection of the medial corticohypothalamic tract [19]. The multiple unit activity (MUA) of the arcuate nucleus was increased by stimulation of the ventral hippocampus and this increase was also prevented by transection of the medial corticohypothalamic tract [20]. When the fornix was transected on DII, ovulation was advanced one day in rats with 5-day estrous cycles [21]. The MUA of the hippocampus and fornix started to decrease on the day of DII corresponding with the increase of estrogen secretion from the ovary [22]. These facts indicate that the hippocampus plays an inhibitory role in regulation of ovulation. Thus, it can be speculated that PDC transected the fibers from the hippocampus resulting in disruption of ovulation delayed by PB.

On the other hand, electrical or electrochemical stimulation of the lateral amygdala on P inhibits the increase of LH and FSH release and ovulation [11, 13]. The MUA of the lateral amygdala started to decrease on DII and continued into the afternoon of P [21]. The lateral amygdala contains large amounts of estrogen receptors [23, 24]. These

reports indicate that the lateral amygdala exerts an inhibitory influence on the regulation of ovulation. As well as the hippocampus, the amygdala may also have contributed to the shifting of the effective period of PB in PDC in this experiment.

The condition of illumination is an important factor in maintaining normal cyclic ovulation, because a persistent light condition induces anovulation in female rats [8]. The suprachiasmatic nucleus (SCN) is well known to produce signals for 24-h rhythm in the brain [25], and destruction of the SCN disturbs ovulation [9, 26]. There is a neural connection between the limbic area and the SCN [27]. Based on the report showing that PB blocks both light-induced phase shifts and photic induction of Fos in the suprachiasmatic nucleus [28], we propose that PB inhibits the transmission of photic information before it reaches the circadian oscillator within the suprachiasmatic nucleus. It is possible to say that mechanisms for ovulation delayed by PB involve the SCN connecting with the limbic mechanism.

PB enhances activity of the GABA neuronal system [29, 30]. The GABA neuronal system is thought to be involved in the regulatory system for ovarian cyclicity and LH secretion [6, 31]. GABA receptors are found in the neurons of the POA [32]. The SCN also contains a GABA receptor system [33]. Infusion of GABA_A receptor antagonist, bicucullin, has been reported to advance the timing

of the LH surge [31]. Pulsatile secretion of GnRH is thought to be an important factor in the precise control of LH secretion and the generator exists in the medial basal hypothalamus [34]. It has been reported that PB stimulates the activity of GnRH pulsatile secretion in proestrous [35]. However, endogenous GABA receptor activity has also been suggested as having a small contribution to the control of the pulse generator [36]. Thus, rather than the pulsatile secretion system, LH-surge control system in the POA including the suprachiasmatic nucleus involves the GABAergic system. The limbic area contains GABA neurons [37]. Although there is no direct evidence, the possibility can not be excluded that PDC disturbs GABAergic neural inputs to the LH-cyclic secretion center in the POA. Further studies are needed to elucidate this.

Acknowledgement

We wish to express our hearty thanks to Dr. S. Shibata of Waseda University for his valuable suggestions. This study was supported by a Grant-in-Aid from the JSPS (11640669), grants for special research projects (2001A-611) and grants from AR Center for Human Sciences of Waseda University and the Promotion and Mutual Aid Corporation for the Private Schools of Japan to K.Y.

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