The Effect of ß-Endorphin on Sexual Receptivity of the Female Rat Intracerebrally Implanted with Estrogen

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Masafumi TORII*

Abstract

Ovariectomized rats which were intracerebrally implanted with estradiol benzoate (EB) and subcutaneously injected with progesterone were used as experimental animals to assess the effects of ß-endorphin (ß-EP) neural system on their lordosis behavior. Lordosis behavior was significantly facilitated by intraventricular injection of ß-EP given with an intracerebral implantation of crystalline EB into the septo-preoptic region. However, lordosis behavior was remarkably inhibited by ß-EP when implanted into the ventromedial hypothalamus, and in the animals implanted in the midbrain reticular formation were not affected. Accordingly, it would seem that the ß-EP neural system stimulates sexual receptivity through an action on the central nervous system in proportion to the estrogen activation. One of the sites of ß-EP facilitatory action may be estrogen receptive septo-preoptic region.

Key words: Estrogen initial action; intracerebral estrogen implantation; female sexual receptivity; lordosis reflex; septo-preoptic and hypothalamic regions; mesencephalic reticular formation.

Introduction

Our previous study (Torii and Kubo, 1991) clearly demonstrates that the female sexual receptivity has been remarkably facilitated with ß-endorphin (ß-EP), particularly in the case of the simultaneous administration at EB priming. The facilitation of the sexual behavior was completely blocked by naloxone (NLX), an opioid antagonist.

In the research field of female sexual receptivity, Sirinathsingjhi (1985) and Sirinathsingjhi *et al*. (1983a; 1983b) observed that ß-EP inhibited estrogen-activated lordosis reflex in ovariectomized rats primed with subcutaneous injections of estrogen and progesterone, and NLX blocked completely the inhibitory phenomena. These findings

*Physical Fitness Laboratory, Kyushu Institute of Technology, Kitakyushu 804 Japan

suggest that the endogenous opioid may be involved in sexual behavior. Thus, it would seem that the ß-EP neural system induces estrogen action in relation to the initial stage of estrogen feedback in the central nervous system.

In the present study, the sites and mechanisms of ß-endorphin action are explored in the estrogen-activated sexual behavior of the ovariectomized rats.

Materials and Methods

Animal

Sixty-eight intact Sprague-Dawley female rats, weighing 200-230 g, were used in these experiments. They were housed under controlled lighting (light on, 0600-1800 h) and temperature (23 ± 1.0 ℃, relative humidity, 60 %) and had *ad lib* food and water. Ovariectomy was carried out at least 2 to 3 weeks prior to the lordosis behavioral test.

Cannulae Implantation

Two to three days after overiectomy, the spayed rats were implanted with outer guide cannulae into the third ventricle and specific brain sites: the lateral septal nuclei (lat. Sept.), the medial preoptic area (MPO), the ventromedial hypothalamus (VMH) or the mesencephalic reticular formation (MRF). Under intraperitoneal injection of pentobarbital (25-30 mg/kg), the surgery to implant cannulae using a stereotaxic instrument was carried out according to the rat brain atlas of König and Klippel (1963). The outer cannlae (0.8 mm, OD) were cut to appropriate length to reach specific brain sites. The rats were also implanted with stainless steel guide cannulae [ext. tube, 0.8 mm (OD), int. tube, 0.45 mm (OD)] in the third ventricle.

Hormones and Drug Treatments, and Sexual Receptive Behavioral Test (Fig. 1)

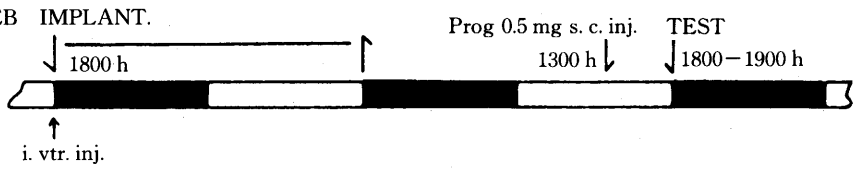


Fig. 1. Experimental procedures. Light-dark cycle (0600-1800 h light on) are shown. The ovariectomized rats were received implantation of crystalline EB (at 1800 h Day-1) into the lateral septum (lat. Sept.), diagonal band of Broca-medial preoptic area (DBB-MPO), ventromedial hypothalamus (VMH) or midbrain reticular formation (MRF) followed by subcutaneous (sc) injection of Prog (0.5 mg at 1300 h, Day-3). After Prog treatment, the behavioral testing was conducted (1800-1900 h, Day-3).

In this experiment, the spayed rats received crystalline estradiol benzoate (EB) implantation on Day 1 (1800 h) and 0.5 mg/0.1 ml progesterone (Prog) dissolved in sesame oil on Day 3 (1300 h) and intraventricular injection of 2.0 μg/1 μl ß-EP or saline

was carried out at the time of EB implantation.

Crystalline EB implantation was carried out using a stainless cannula (0.45 mm, OD). The inserted cannulae were filled by gently tapping them in a small dish of crystalline EB, and placed unilaterally in the specific brain sites while the animals were anesthetized with ether.

Beta-endorphin (ß-EB, Osaka Protein Res., Osaka) was dissolved in 0.9 % saline in concentration of 2.0 μg/1 μl and injected into the third ventricle. Each control group received an intraventricular injection of 1.0 μl saline.

After 5 h subcutaneous Prog treatment, the test for lordosis behavior was carried out in an observation room during a period of darkness lasting from 1800 h to 1900 h. Lordosis quotient (LQ) was used as an index for female sexual receptivity. In this experiment, the lordosis reflex score (LS) was also recorded and the strength was rated on a scale from zero (no response) to 3 (strongest possible reflex).

Histological Analysis

At the experimental termination, the animals were anesthetized with an overdose of Dienthyl Ether (Wako pure chemical industries, Osaka) and were immediately perfused with 10 % formalin. The brains were frozen and cut at 50 μm.

Statistical Analysis

Data were represented by mean ± SEM, and the statistically significant difference between mean values was assessed by an unpaired t-test or one-way ANOVA. The level of significance was set as $p < 0.05$.

Table 1. Effects of intraventricular injection (i. vtr. inj.) of ß-endorphin on lordosis responses in the spayed rats given implantation of crystalline EB into the various cerebrall areas

Regions *a)*	No. of Rats	i. vtr. inj. *b)* of	Lordosis Response *c)* LQ(%)	LS
lat. Sept.	(6)	saline	83.8±3.8	1.66±0.09
	(6)	ß-EP	95.0±2.6* *d)*	2.40±0.11***
DBB-MPO	(8)	saline	30.2±3.9	0.67±0.15
	(7)	ß-EP	77.9±5.0***	1.86±0.11***
rost. VMH	(13)	saline	66.9±4.3	1.03±0.13
	(15)	ß-EP	18.3±5.4***	0.24±0.35***
vent. MRF	(6)	saline	85.0±3.2	2.02±0.13
	(7)	ß-EP	92.3±2.1	2.19±0.12

a) lat. Sept, lateral septal nuclei; DBB-MPO, diagonal band of Brocamedical preoptic are; rost. VHM, rostral part of ventromedial hypothalamus; vent. MRF, ventral part of mesencephalic reticular formation.

b) At crystalline EB implanting, ß-endorphin (ß-EP, 2 μg/ μl) or saline (1 μl) was injected into the third ventricle.

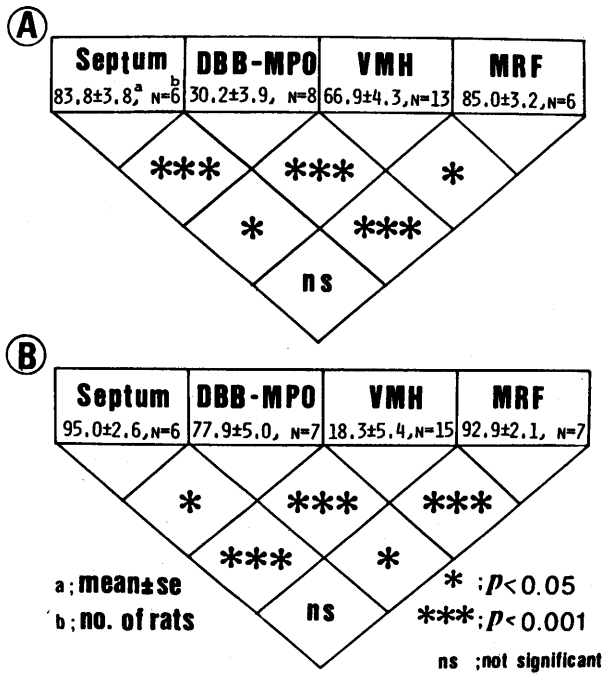
c) Lordosis response: lordosis quotient (LQ) and lordosis reflex score (LS) were measured by tests of 20 mounnt at 1800-1900 h (48-49 h after EB, or 5 h after subcutaneous injection of progesterone, 0.5 mg).

d) Data represent mean±SEM. Significantly different from each saline control group, * $P<0.05$, *** $P<0.001$.

Results

The animals implanted with crystalline EB in the lat. Sept., MPO, VMH or MRF displayed lordosis behavior. The main results are shown in Table 1. Mean LQ of the animals implanted with EB in the lat. Sept. and MRF was significantly higher than those implanted in the MPO and VMH (Table 2). Thus, estrogen sensitivity in the steroid

Table 2. Testing the difference among the specific cerebral sites affecting lordosis quotient of control (A) and experimental groups (B).



receptive region was significantly differrent (analysis of variance, $F = 35.4$, df [3, 32], $p < 0.001$).

The lordosis behavior (LQ and LS) after crystalline EB implantation into both the lat. Sept. and MPO regions was significantly facilitated when intraventricular injection of ß-EP was administered at the time of EB implantation. On the other hand, the lordosis behavior of the animals implanted in the VMH was strongly inhibited ($p < 0.001$). Mean LQ and LS after EB was implanted into the MRF were not affected by intraventricular injection of ß-EP (Table 1).

Histological analysis of individual cannula locations are schematically illustrated in Figure 2. The diagrams were taken from the frontal sections of the lat. Sept., diagonal

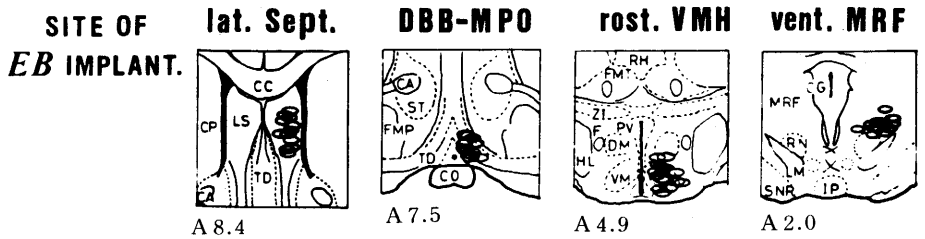


Fig 2. Histological data, the sites of implantation of crystalline EB into the lateral septum (lat. sept.), diagonal band of Brora-medial preoptic area (DBB-MPO), ventromedial hypothalamus (VMH) or midbrain reticular formation (MRF). CA, anterior commissure; CC, corpus callosum; CG, central gray; CO, optic chiasm; CP, posterior commissure; DM, dosomedial nucleus; F, fornix; FMP, fasciculus mediaiis prosencephalic; FMT, fasciculus mamillothalamicus; HL, lateral hypothalamus; IP, interpeduncular nucleus; LS, lateral septal nucleus; LM, medial lemniscus; MRF, mesencephalic reticular formation; PV, paraventricular nucleus; ST, bed nucleus of stria terminalis; TD, tractus diagonalis (Broca); VM, ventromedial nucleus; ZI, zona incerta.

band of Broca (DBB)-MPO, VMH and MRF. A part of the cannulae implanted in the MPO was present in the DBB area. As with the MPO, the DBB area is an estrogen sensitive region (Gorski, 1974). The cannulae in the MRF and VMH were implanted in the ventral and rostral parts, respectively.

Discussion

The present results show that the estrogen-sensitivity of the estrogen-receptive cerebral regions, the lat. Sept., the DBB-MPO, the VMH or the MRF was induced by local implantation of crystalline EB. However, the estrogen-sensitivity can vary strongly between the different estrogen receptive areas. In both the lat. Sept. and DBB-MPO, estrogen action-induced lordosis behavior was facilitated by ß-EP administered at time of EB implantation, whereas in case of the rats implanted with EB in the VMH, lordosis behavior was inhibited by ß-EB. In the MRF the lordosis behavior was not affected by ß-EB.

So, direct implantation of crystalline EB in the MPO (Lisk, 1962, Yanase and Gorski, 1976) or VMH (Barfield and Chen, 1977; Rubin and Barfield, 1980) could induce the lordosis behavior in the ovariectomized rats. The critical estrogen dose to induce the behavior was lower in the VMH than the MPO (Barfield and Chen, 1977). On the other hand, lesion in the region in the VMH can reduce or eliminate sexual receptivity in the ovariectomized, hormone-treated rats (Kennedy, 1964; Mathews and Edwards, 1977; Pfaff and Sakuma, 1979b, Clark *et al.*, 1981) and hamsters (Malsbury *et al.*, 1977). Electrical stimulation in the VMH (Pfaff and Sakuma, 1979a) was effective in facilitating the lordosis response in hormone-primed female rats. In contrast, it was demostrated

that lordosis behavior was remarkably reduced by electrical stimulation in the septum (Zasorin *et al.*, 1975) or MPO (Napoli *et al.*, 1972; Moss *et al.*, 1974; Pfaff and Sakuma, 1979a) in estrogen-primed famales. Bilateral septum (Nance *et al.*, 1974, 1975; McGinnis *et al.*, 1980; Gonzalka and Gray, 1980) or MPO (Powers and Valenstein, 1972) lesions markedly enhanced the lordosis behavior in the ovariectomized rats primed with estrogen.

The results of previous papers on neural deafferentation of sexual behavior have suggested that the hypothalamic cut (Malsbury and Daood, 1978; Yamanouchi and Arai, 1979) and the medial basal hypothalamus (BMH) -island (Ynmanouchi and Arai, 1979) suppresses estrogen and progesterone-induced lordosis behavior, while the anterior roof cut activates a maximal lordosis performance (Yamanouchi and Arai, 1977; Torii and Kubo, 1984).

Table 3. Summary of the roles of the lateral septal nuclei (lat. Sept.), medial preoptic area (MPO), ventromedial hypothalamus (VMH) and midbrain reticular formation (MRF) in the regulation of sexual receptivity (lordosis performance) in female rat

	Lordosis Performance			
	lat. Sept.	MPO	VHM	MRF(or CG*)
ß-EP Action on EB Impl.	↑ present study	↑ present study	↓ present study	— present study
EB Impl.	↑ present study	↑ Lisk, 1962 Yanase & Gorski, 1979 present study	↓ Barfield & Chen, 1977 Rubin & Barfield, 1980 present study	↑ present study
Lesion	↑ Nance et al., 1974; 1975 McGinnis & Gorski, 1980 Gorzalka & Gray, 1981	↑ Powers & Valenstein, 1972	↓ Kennedy, 1964 Mathews & Edwards, 1977 Pfaff & Sakuma, 1979a Clark *et al.*, 1981	↓ Sakuma & Pfaff, 1979b*
ECS	↓ Zasorin *et al.*, 1973#	↓ Napoli *et al.*, 1972 Moss *et al.*, 1972 Pfaff & Sakuma, 1979b	↑ Pfaff & Sakuma, 1979b	↑ Sakuma & Pfaff, 1979a*

↑, Facilitation; ↓, Inhibition; —, No effect; ß-EP, Intraventricular injection of ß-endorphin; EB impl., Crystalline estradiol benzoate implantation; ESC, Electrochemical stimulation. * Midbrain central gray, # Used hamster.

The above findings are summarized in Table 3. These experimental findings reported by many investigators suggest that there are the facilitatory centers in the VMH and the inhibitory centers in the MPO having the same effect as forebrain structures on rodent's sexual behavior.

On the other hand, Yanase and Gorski (1976) observed that rats that received subcutaneous injection or intracerebral implantation of EB in the DBB-MPO achieved submaximal lordosis performance, while the animals that received an intracerebral implantation into the MRF displayed enhanced lordosis behavior. However, when the

ovariectomized rats were implanted with crystalline progesterone in the MRF following EB priming, facilitatory effects on lordosis behavior resulted (Yanase and Gorski, 1976). Thus the MRF may be one of the sites of progesterone action. In the present experiment, since 0.5 mg progesterone was subcutaneously administered at 43 h after crystalline EB implanting, lordosis was facilitated. But ß-EP did not affect lordosis perfomance of the animals implanted with EB in the MRF. The effects of ß-EP may be associated with estrogen action rather than progesterone action.

It was clearly demonstrated that the localizations of ß-EP-and met-enkephalin-containing neurons in the brain was different (Watoson *et al.*, 1978; Bloom *et al.*, 1978; Barchas *et al.*, 1978). There were regional distributions of ß-EP-containing neurons in the septo-preoptic region and mid brain, *etc.*, throughout the arcuate nucleus of the hypothalamus including the cell body (Finiey, *et al.*, 1981). The present study suggests that the possible role of opioid which influences for the initial estrogen action on female sexual receptivity occurs in the forebrain or hypothalamic region.

In relation to the facilitatory effect of ß-EP neural system on lordosis behavior due to action of estrogen, adrenergic neuronal system rather than LH-RH neuronal system play an important role. The reasons for this assertion are as follows: 1) we have noticed that intraventricular injection of LH-RH at estrogen priming does not facilitate female sexual receptivity (Torii and Kubo, unpublished data) ; 2) in the previous studies, we have observed inhibitory effects of ß-EP on LH release to reflect the LH-RH neural activity (Kubo *et al.*, 1983) ; 3) as above mentioned, there are the experimental observations of Yanase (1977) and Gorski and Yanase (1981).

In conclusion, the present study suggests that the estrogen action that induces the lordosis reflex in the female rat is facilitated in the septo-preoptic region and inhibited in hypothalamic structure. Thus, the ß-endorphin neural systems may modulate the initial action of estrogen action in the central nervous system.

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