Modulation by ß-Endorphin of Lordosis Performance in the Ovariectomized Rat

(Received November 14, 1990)

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Abstract

The effect of intraventicular injection of ß-endorphin (ß-EP) on female sexual behavior (lordosis reflex) was studied in the ovariectomized rats primed with estradiol benzoate (EB) and progesterone. Intraventricular injection of ß-EP, when it was given at EB priming, siginificantly facilitated lordosis behavior. Naloxone, a specific antagonist, given at EB priming inhibited the behavior. The present results suggest that ß-EP may stimulate the female sexual receptivity through an action on the central nervous system in the initial stages of estrogen activation of lordosis reflex.

Key Words: Estrogen Action; ß-Endorphin; Naloxone; Lordosis reflex; Female sexual receptivity; Central nervous system.

The concept that in the central nervous system (CNS) opioid peptides such as ß-endorphin and met-enkephalin modulate brain function has been well accepted (See review, Barchas *et al.* 1978). The effects of these peptides on various behavioral responses (Bloom *et al.*, 1976; De Caro *et al.*, 1979) have been extensively documented.

On the other hand, lordosis response is typical sexual receptive behavior in the female rodents (Fig. 1). This behavior is dependent on the ovarian steroid hormone level in the blood, and especially the concentration of estrogen within this (See review, Gorski 1974; Gorski *et al.*, 1975). With regard to female sexual receptive behavior, LH-RH (Moss and MacCann, 1973; Pfaff, 1973), α-MSH (Thody *et al.*, 1979) and ACTH (Feber and Ruf, 1969) have been shown to facilitate lordosis behavior in the ovariectomized rats.

Recently, Sirinathsinghji *et al.* (1983a; 1983b) have reported that through ß-endorphin infusion into the midbrain central gray, the effects of inhibitory action of opioid on lordosis behavior, and in naloxone infusion, have been blocked. However, there is as yet no evidence of a role for the endogenous opioid in the regulation of female sexual behavior, especially in relation to the

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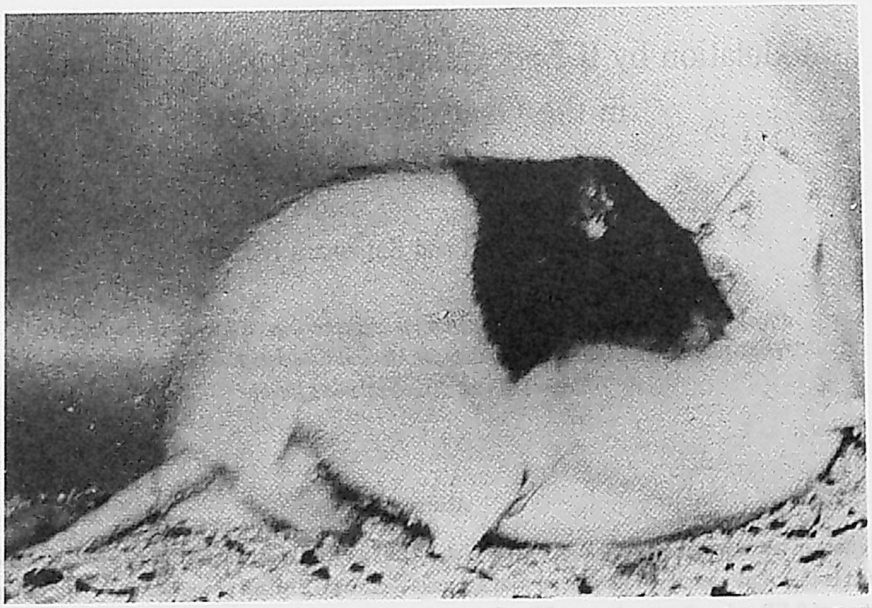

Fig. 1 An example of lordosis reflex exhibited by the receptive female rat to the mounting activity of the male (redrawn from Gorski *et al.*, 1975). The behavioral receptivity in the rat is another rhythmic neural process that is dependent upon gonadal hormones such as estrogen and progesterone. The ovariectomized rat will not display sexual behavior, as defined by the lordosis reflex.

initial activation of estrogen in the CNS.

Therefore, the purpose of the present study is to investigate the role of ß-endorphin neural system in controlling estrogen-activated lordosis behavior. Some preliminary results have been reported elsewhere in abstract form (Kubo and Torii, 1984; Torii and Kubo, 1984).

Experiments

Preparation

Sprague-Dawley female rats, weighing 200–230 g, were used in the experiments. They were housed under controlled lighting (light on, 0600–1800 h) and temperature (23°C) and had *ad lib* food and water. Ovariectomy was carried out at least two to three weeks prior to the lordosis behavioral test.

Two to three days after ovariectomy, the rats were implanted with stainless steel guide cannulae [ext. tube, 0.8 mm (OD), int. tube, 0.45 mm (OD)] into the third ventricle. Under intraperioneal injection of pentbalbital (25–30 mg/kg BW), the surgery to implant the cannulae using a stereotaxic instrument was carried out according to the rat brain atlas (Köing and Klippel, 1963).

Hormone and Drug Treatments

Before the behavioral test, the ovariectomized rats were received subcutaneously with estradiol benzoate (EB, Sigma, St. Louis) dissolved in 0.1 ml sesame oil on Day 1 (1900 h) and 0.1 mg progesterone (Prog, Sigma) in oil on Day 3 (1400 h), the day of the test. The dose of EB used was 8–10 μg according to the experimental design. Beta-endorphin (ß-EP, Protein Res., Osaka) was dissolved in 0.9% saline in concentration of 0.1, 1.0 or 2.0 μg/1 μl and naloxone (NLX, Endo Labor., New York) dissolved in 0.9% saline in concentration of 1.0 μg/1 μl. These drugs were injected into the third ventricle at various times.

Behavioral Test on Female Sexual Receptivity

After 5 h Prog treatment, the test for lordosis behavior was carried out in an observation room during a period of darkness lasting from 1900 h to 2000 h. The sexually experimenced males were introduced into a circular chamber (80 cm×60 cm) to adapt for about 30 min, and then a female was introduced into the chamber. Lordosis was measured as lordosis quotient (LQ), calculated thus: number of lordosis responses divided by the number of mounts, time 20 ×100 (%). This was used as an index for female sexual receptivity.

Experiment 1

Method: In this experiment the spayed rats received 8 μg EB on Day 1 and 0.1 mg Prog on Day 3 and either 0.1 μg/1.0 μl, 1.0 μg/1.0 μl or 10.0 μg/5 μl ß-EP. The administration of ß-EP into the third ventricle was carried out at EB priming [E], at Prog priming [P] or 1 h prior test [T].

Results: As shown in Table 1, In intraventricular injection of 1.0 μg or 10.0 μg ß-EP the mean LQ in E group was significantly higher than control group [C] ($p<0.001$). On intraventricular injection of 1.0 μg ß-EP, mean LQ of P or T group was not significantly different from control group ($p>0.05$). In contrast, on injection of 10.0 μg ß-EP, the mean LQ of T group was significantly more decreased than that of the control group ($p<0.001$). At 0.1 μg ß-EP, LQs of the experimental groups were not affected. There was a dose-response relationship, dose of ß-EP *vs* LQ ($p<0.05$) (Fig 2).

Experiment 2

Method: In this experiment the spayed rats received 10 μg EB on Day 1 and 0.1 mg Prog on Day 3 and 1.0 μg/1 μl NLX. The administration of NLX into the third ventricle was carried out at EB treatment time, Prog treatment time or 1 h prior to the test.

Results: In case of intraventricular injection of NLX, mean LQs of three groups [E, P and T] decreased significantly in comparison with the control [C] (Table 1).

Experiment 3

Method: In this experiment the spayed rats were 8 μg EB on Day 1 and 0.1 mg Prog on Day

Table. 1 Effects of intraventricular injection of ß-endorphin and naloxone at various times on mean lordosis quotient (LQ) in spayed rats following treatment of gonadal steroids.

Group	No. of rats	Treatment[a] at Indicated Hour			20 Mount LQ[b] (mean±SEM)
		0	43	47	
C	(10)	EB	Prog		70.3± 3.3
E	(7)	EB+SAL	Prog		66.4± 4.5
P	(8)	EB	Prog+SAL		70.0± 4.7
T	(9)	EB	Prog	SAL	65.0± 4.0
C	(8)	EB	Prog		65.0± 3.0
E	(12)	EB+ß-EP	Prog		83.8± 3.0***[c]
P	(8)	EB	Prog+ß-EP		75.0± 5.9
T	(10)	EB	Prog	ß-EP	57.0± 6.4
C	(15)	EB	Prog		71.5± 2.9
E	(9)	EB+ß-EP§	Prog		93.9± 2.5***
P	(12)	EB	Prog+ß-EP§		47.9±10.9*
T	(7)	EB	Prog	ß-EP§	16.7± 7.3***
C	(17)	EB†	Prog		81.8± 2.7
E	(15)	EB†+NLX	Prog		50.0± 5.0***
P	(10)	EB†	Prog+NLX		56.9± 6.4**
T	(11)	EB†	Prog	NLX	40.5± 6.7***

a) EB, subcutaneous (sc) injection of estradiol benzoate, 8 μg/rat, †10 μg/rat; Prog, sc injection of progesterone, 0.1 mg/rat; ß-EP, intraventricular injection of ß-endorphin, 1 μg/1 μl, § 10 μg/5 μl; NLX, intraventricular injection of naloxone, 1 μg/1 μl, SAL, intraventricular injection of saline, 1 μl. *b*) Measured 48–49 h after EB-priming. *c*) Significant difference from each Group C, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ (by unpaired *t*-test).

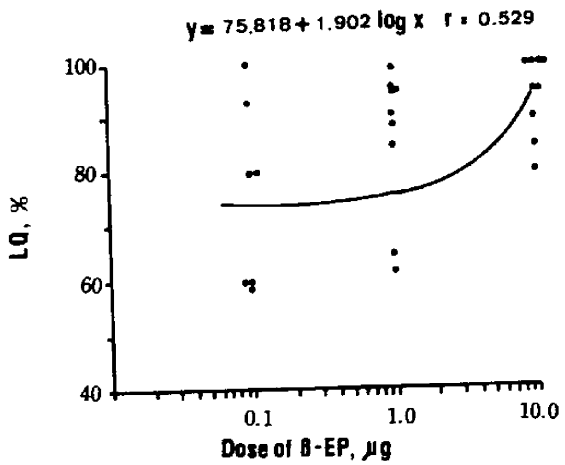

Fig. 2 Effects of various doses (0.1, 1.0 and 10.0 μg) of ß-endorphin on lordosis quotient (LQ).

3 and either 1.0 μl saline (SAL). The intraventricular injection of SAL was carried out at EB priming, at Prog priming or 1 h prior to test.

Results: In intraventricular injection of SAL the mean LQ between the three groups [E, P or T] and control [C] was not significantly different ($p>0.05$) (Table 1).

Discussion

The data obtained from the present experiment clearly demonstrate that female sexual receptivity is remarkably facilitated with ß-EP, especially when simultaneously administered at EB priming. The facilitation of sexual behavior is completely blocked by NLX, an opioid antagonist. Few results have been reported from experiment concerning the relation between initial estrogen action induced-sexual behavior and the endogenous opioid system.

Recently, Sirinathsingji *et al.* (1983a; b) have observed that ß-EP inhibited estrogen-activated lordosis reflex in ovariectomized rats primed with s.c. injection of estrogen and progesterone, and that NLX blocked completely the inhibitory phenomena. These findings suggest that the endogenous opioid may be involved in the sexual behavior as well as body shaking behavior (Bloom *et al.*, 1976). Thus, it would seem that ß-EP neural system induces estrogen action in relation to the initial stages of estrogen feedback in the CNS.

As to initial activation of estrogen in sexual receptivity, Yanase (1977) and Gorski and Yanase (1981) have reported that when apomorphin was injected simultaneously with EB, that was 48 h prior to the behavioral test, 1.5 or 3.0 mg of this drug acutely facilitated lordosis behavior and i.p. injection of 50 μg epinephrine was similarly effective. The above findings may indicate that the ß-EP systems is associated with a biogenic amine system of estrogen-induced sexual behavior. The biochemical role of possible neurotransmitters in the reproductive physiology has recently been reported. Several drugs have been described that can stimulate sexual behavior by direct interaction with cental nervous neurotransmitters; *i.e.*, sexual behavior appears to be not only under the control of the sex hormones but of neurotransmitters as well (See review, Arai and Yamanouchi, 1981).

It was clearly demonstrated that the localizations of ß-EP- and met-enkephalin-containing neurons in the brain were different (Bloom *et al.*, 1978). There were regional distributions of ß-EP-containing neuron in the septal-preoptic region and mind brain, etc., throughout the arcuate nucleus of the hypothalamus including the cell body (Finley, *et al.*, 1981). Thus, in the present study, the possible critical role of opioid which influences for the initial activation of estrogen action in female sexual receptivity is suggested to exist in the forebrain or hypothalamic region.

The estrogen-activated behavior was inhibited significantly by microinjection of ß-EP into the midbrain central gray (MCG) (Sirinathsingi *et al.*, 1983a). The behavior after LH-RH (Sakuma and Pfaff, 1980; Sirinathsingji *et al.*, 1983a; 1983b) or NLX (Sirinathsingji *et al.*, 1983a) infusion into the MCG occurred immediately a maximum response. These findings may

implicate that 1) LH-RH activity inhibited by endogenous ß-EP system, 2) LH-RH system involve to regulate the female sexual behavior. The MCG is profuse both ß-EP-immunoreactivity (Watson *et al.*, 1978; Bloom *et al*, 1978) and opioid receptors, and contains heavy descending hypothalamus projection including LH-RH axons (Krieger *et al.*, 1979).

We have reported that intraventricular injection of met- and leu-enkephalin inhibited significantly lordosis behavior in estrogen and progesterone priming with ovariectomized rats and but in the estrogen activated animals which received with the intraventricular injection of met-enkephalin, the behavior has been observed facilitatory effect Torii and Kubo, in submission). Moreover, as assumed from the results of NLX infusion (Table 1), the met-enkephalin neuronal system may involve the activated behavior rather than the initial action of estrogen to induce lordosis behavior (Kubo and Torii 1984).

In conclusion, the present study suggests that the ß-EP system may be contribute the facilitatory control in the initial stages of estrogen action to induce the female sexual receptivity.

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