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Differential Effects of β-Endorphin and Met- and Leu-Enkephalin on Steroid Hormone-Induced Lordosis in Ovariectomized Female Rats

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TORII, M., K. KUBO and T. SASAKI. *Differential effects of β-endorphin and Met- and Leu-enkephalin on steroid hormone-induced lordosis in ovariectomized female rats.* PHARMACOL BIOCHEM BEHAV **58**(4) 837–842, 1997.—The effect of intrathirdventricular (ITV) injections of β-endorphin, anti-β-endorphin antiserum, Met-enkephalin, Leu-enkephalin, and naloxone on the initial activation and final development of steroid hormone-mediated induction of female sexual receptivity was studied in ovariectomized female rats. The lordosis response to male mounts in ovariectomized rats after subcutaneous (SC) estradiol benzoate (EB) and progesterone (Prog) priming was facilitated by β-endorphin, and Met-enkephalin (10 $\mu g \cdot 5\ \mu l^{-1}$), but inhibited by Leu-enkephalin, when the peptides were injected into the third ventricle at the time of SC EB priming. A lower dose Met-enkephalin had no effects. Lordosis behavior in steroid hormone-primed rats was significantly facilitated when ITV injections of Met-enkephalin were given 1 h prior to behavioral testing (47 h after EB priming). At 1 h prior to behavioral testing (47 h after EB priming), ITV injection of β-endorphin significantly inhibited lordosis behavior, especially at the higher dose of β-endorphin (10 $\mu g \cdot 5\ \mu l^{-1}$). Under those conditions, Leu-enkephalin had no effect. Lordosis behavior of ovariectomized female rats receiving SC steroid hormones and ITV injection of anti-β-endorphin antiserum was significantly inhibited when anti-β-endorphin antiserum was injected at the time of EB priming. However, lordosis was significantly facilitated when anti-β-endorphin antiserum was injected 1 h prior to the behavior testing (47 h after EB priming). In contrast, ITV injection of the opioid antagonist naloxone given either at the time of EB priming or 1 h prior to behavioral testing (47 h after EB priming) decreased lordosis behavior. The present results suggest that 1) β-endorphin, Met-enkephalin, and Leu-enkephalin have differential effects in the control of lordosis behavior; 2) the opioidergic systems may modulate initial-stage and final-stage estrogen-induced lordosis behavior; and 3) the opioidergic systems could be divided into the endorphinergic modulation-type and enkephalinergic modulation-type, based on their effects on lordosis behavior. © 1997 Elsevier Science Inc.

Lordosis reflex Female sexual receptivity β-Endorphin Met-enkephalin Leu-enkephalin
Anti-β-endorphin antiserum Naloxone Multiplicity of opioid actions

INHIBITORY effects of opioid peptides on female sexual behavior in estrogen–progesterone-primed rats have been reported by many investigators [see review (14)]. For example, Sirinathsingjhi et al. (19) observed that β-endorphin inhibited estrogen-activated lordosis behavior in ovariectomized rats primed with subcutaneous (SC) injection of estrogen and progesterone, and naloxone, an opioid receptor antagonist, completely blocked β-endorphin–mediated inhibition. However, we recently reported that in estrogen–progesterone-treated ovariectomized rats, intrathirdventricular (ITV) infu-

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sion of β-endorphin at the time of estrogen priming facilitated lordosis behavior (21). Furthermore, β-endorphin-induced facilitation of lordosis behavior was observed in rats in which estrogen was implanted locally into the septal and preoptic regions but not when estrogen was given into the ventromedial hypothalamus (21). Beta-endorphin–induced facilitation of lordosis was exclusively observed within the initial 6 h of estrogen action, and after that, inhibition of lordosis occurred (22).

Because previous evidence indicated that β-endorphin administered at the time of estrogen priming facilitated lordosis, we decided to examine the effects on lordosis of two other opioid peptides, Met-enkephalin and Leu-enkephalin, administered at the time of estrogen priming. For comparison, we also examined the effects of these opioid peptides on lordosis when administered just prior to behavioral testing. Furthermore, we tested the effects of anti-β-endorphin antiserum infusion on lordosis behavior.

METHOD

Preparation

Sprague–Dawley female rats (Yenoue Labo. Animal Co. Kumamoto, Japan), weighing 200–230 g, were used in these experiments. They were housed under controlled lighting (LD, 12:12, lights on, 0600–1800 h) and temperature conditions (23 ± 1.0°C, relative humidity, 60%), and had access to food (Oriental Enzyme Co., Tokyo) and water ad lib. Ovariectomy in the experimental animals was carried out at least 2 to 3 weeks prior to the sexual receptivity test.

Two to 3 days after ovariectomy, the rats were implanted with outer guide cannulae [ext. tube, 0.8 mm (o.d.), int. tube, 0.45 mm (o.d.)] into the third ventricle. The cannulae implantation surgery was carried out under Nembutal anesthesia (25–30 mg/kg, IP) to position cannulae according to coordinates obtained from the rat brain atlas of König and Klippel (9). We confirmed cerebral spinal fluid flow from the third ventricle in all animals.

Hormone and Drug Treatments and Behavioral Testing

Two to 3 weeks after surgery, the ovariectomized rats received 8 μg or 10 μg estradiol benzoate (EB, Sigma, St. Louis, MO) via the SC route. EB was dissolved in 0.1 ml sesame oil on day 1 (1900 h), and 0.1 mg progesterone (Prog, Sigma) was dissolved in 0.1 ml sesame oil on day 3 (1400 h). Beta-endorphin, Met-enkephalin, and Leu-enkephalin (Protein Res., Osaka, Japan) were dissolved in 0.9% saline in a concentration of 1.0 μg·1.0 μl^{-1} or 2.0 μg·1.0 μl^{-1}, and naloxone (Endo Labo., New York, NY) was dissolved in 0.9% saline in a concentration of 1.0 μg·1.0 μl^{-1}. ITV injections of these drugs (1.0 μg·1.0 μl^{-1} or 10.0 μg·5.0 μl^{-1}) or saline (1.0 μl), and camel anti-β-endorphin antiserum and normal rabbit serum (NRS), were carried out at the time of SC EB-priming, or at 1 h prior to behavioral testing (47 h after EB priming). Anti-β-endorphin antiserum and NRS were diluted with artificial cerebrospinal fluid 100×, 500×, and 2500×. Five hours after SC Prog treatment, the test for lordosis behavior was conducted in an observation room during a period of darkness testing occurred between 1800 and 1900 h. Two sexually experienced males were introduced into a circular chamber (80 cm diameter, 60 cm high), and were habituated for about 30 min. Next, a female was introduced into the chamber. As previously reported in the procedural details for the sexual behavioral test, we used a total of six sexually active males. The occurrence of lordosis behavior, expressed as a lordosis quotient [LQ, number of lordosis responses divided by the number of male mounts (20 times) × 100], was used as an index for female sexual receptivity. Following the protocol of Arai et al. (2), the lordosis reflex score (LS) also was recorded and the strength was rated on a scale from zero (no response) to three (strongest possible reflex).

Statistical Analysis

The data were represented as means ± SEM, and statistically significant difference between mean values were assessed by one-way or three-way analysis of variance (ANOVA) and an unpaired *t*-test. A probability level of 0.05 or less was accepted as a significant difference.

RESULTS

Figure 1 shows the effect of ITV injection of opioid peptides on the LQ. The three-way ANOVA detected significant main effects of opioid peptide (A), $F(3, 151) = 13.47$, $p = 0.0001$, dose (B), $F(1, 151) = 4.99$, $p = 0.0269$, interaction between drug and dose (A × B), $F(3, 151) = 9.93$, $p = 0.0001$, interaction between drug and time of ITV injection (A × C), $F(3, 151) = 52.59$, $p = 0.0001$, interaction between opioid dose and time of opioid ITV injection (B × C), $F(1, 151) =$

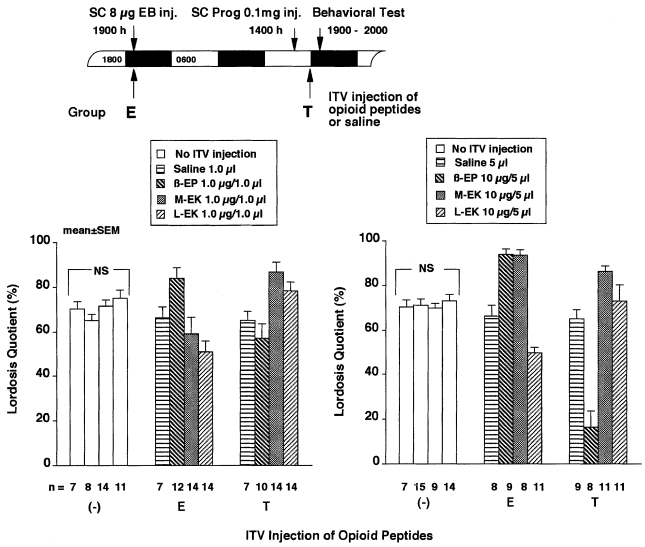

FIG. 1. Effects of various doses of β-endorphin (β-EP), Met-enkephalin (M-EK) and Leu-enkephalin (L-EK) on the lordosis quotient (LQ). Ovariectomized rats were primed with estradiol benzoate (EB, 8 μg) and progesterone (Prog, 0.1 mg) at an interval of 43 h. Lordosis behavior was tested 5 h after Prog priming. LQ signifiers number of lordosis responses/number of 20 mounts × 100 (%). (−) devotes no ITV injection. E signifies ITV injection at EB priming; T devotes ITV injection at 1 h prior to the behavioral test. Data is represented as means ± SEM. NS, not significant [by one-way ANOVA, in 1 μg group, $F(3, 37) = 1.48$, $p > 0.05$ and in 10 μg group, $F(3, 41) = 0.45$, $p > 0.05$]. Number of subjects is included at the bottom of each column. At the top, experimental procedures and light:dark cycles (LD 12:12, 0600–1800 h, lights on) are shown. The ovariectomized rats received subcutaneous (SC) injection of EB (8 μg, at 1900 h, day 1), followed by SC injection of Progesterone (Prog, 0.1 mg, at 1400 h, day 3). After Prog treatment, behavioral testing was conducted (1900–2000 h, day 3). E devotes animals receiving ITV injection at EB priming. T signifies animals receiving ITV injection 1 h prior to the behavioral test.

TABLE 1

THREE-WAY ANOVA TABLE ON THE EFFECTS OF OPIOID PEPTIDES, THEIR DOSES, AND TIME OF THEIR INJECTIONS ON FEMALE SEXUAL BEHAVIOR

Source	*df*	Sum of Squares	Mean Squares	F-Test	*p*-Value
Drug (A)	3	7001.721	2333.907	13.465	0.0001
Dose (B)	1	865.511	865.511	4.993	0.0269
AB	3	5165.252	1721.571	9.934	0.0001
Time (C)	1	272.461	272.461	1.572	0.2119
AC	3	27334.208	9111.403	52.568	0.0001
BC	1	5745.111	5745.111	33.146	0.0001
ABC	3	2141.362	713.787	4.118	0.0077
Error	151	26172.449	173.327		

33.15, $p = 0.0001$, and an interaction of opioid × dose × time (A × B × C), $F(3, 151) = 4.12$, $p = 0.0077$ (Table 1).

As shown Fig. 1, the LQ significantly increased with ITV injection of β-endorphin when SC EB was received, but significantly decreased with the ITV injection at 47 h after EB. Enkephalins, however, had different effects than β-endorphin. The larger dose of Met-enkephalin significantly increased the LQ at the time of SC EB treatment. With both doses, the LQ significantly increased when ITV injection of Met-enkephalin occurred 47 h after EB. With ITV injection of Leu-enkephalin at the time of SC EB priming, the LQ was significantly decreased. ITV injection of Leu-enkephalin at 47 h after SC EB priming had no significant effect on the LQ.

Table 2 shows the effects of ITV injection of various doses of anti-β-endorphin antiserum and NRS on lordosis responses following EB and Prog. the LQ, $F(3, 24) = 10.72$, $p = 0.0001$, and the LS, $F(3, 24) = 12.25$, $p = 0.0001$, were significantly decreased by ITV injections of anti-β-endorphin antiserum (at the 100 × and 500 × dilutions) at the time of SC EB priming. With ITV injection of anti-β-endorphin antiserum (100 × dilution), the LQ significantly increased if injection occurred before the behavioral testing, $F(1, 18) = 4.95$, $p = 0.041$.

The effects of naloxone on sexual behavior are illustrated in Fig. 2. In the case of ITV injection of naloxone, the mean LQs of two groups [E, $F(1, 23) = 21.6$, $p = 0.0001$ and T, $F(1, 18) = 23.0$, $p = 0.0001$] decreased significantly in comparison with the saline control. The main results described above are summarized in Table 3.

DISCUSSION

The present study demonstrated that β-endorphin, and Met- and Leu-enkephalin, had different effects on female sexual receptivity in ovariectomized rats treated with estrogen and progesterone. Lordosis behavior was facilitated by ITV injection of either β-endorphin, or Met-enkephalin, at the time of EB priming at the initial stage of estrogen action, but inhibited by Leu-enkephalin at the time of EB priming (Fig. 1). Furthermore, ITV injection of anti-β-endorphin serum at the initial stage of estrogen action inhibited lordosis behavior. In contrast, immunization with an anti-β-endorphin antiserum inhibited the lordosis response when given at the initial stage of estrogen action, but facilitated lordosis behavior when injected 1 h before behavioral testing (Table 2). A specific opioid antagonist, naloxone, significantly inhibited lordosis behavior at both stages (Fig. 2). Thus, both facilitation and inhibition of steroid actions on female sexual receptivity are modulated by opioidergic systems.

It has been clearly demonstrated that the localization of β-endorphin– and Met-enkephalin–containing neurons in the brain are different (1,3,6). There are regional distributions of β-endorphin–containing neurons in the septo-preoptic region and midbrain, and throughout the arcuate nucleus of the hypothalamus including arcuate nucleus cell bodies (10). The present study suggests that opioid-mediated effects on female sexual receptivity may occur in the forebrain or hypothalamic region. Thus, the functions of the opioidergic system when opioids are provided at the initial stages of estrogen action may be facilitation of lordosis behavior.

TABLE 2

EFFECTS OF ITV INJECTION OF VARIOUS DOSES OF ANTI-β-ENDORPHIN (β-EP) ANTISERUM OR NORMAL RABBIT SERUM (NRS) ON LORDOSIS RESPONSES FOLLOWING EB AND PROG

	No. of Rats	Lordosis Responses[a] (mean ± SEM)		One-Way ANOVA Test					
				df	F-Ratio	Prob.	*df*	F-Ratio	Prob.
		LQ (%)	LS		LQ			LS	
ITV injection at EB priming									
NRS[b] (100×)	(6)	75.8 ± 4.8	1.47 ± 0.14	(3,24)	10.72	$p = 0.0001$	(3,24)	12.25	$p = 0.0001$
anti-β-EP antiserum[b] (100×)	(8)	46.9 ± 6.0†[c]	0.86 ± 0.12†						
(500×)	(7)	54.0 ± 4.3†	1.08 ± 0.06*						
(2500×)	(7)	73.3 ± 2.8	1.40 ± 0.05						
ITV injection before testing									
NRS × 100	(9)	72.8 ± 4.0	1.53 ± 0.15	(1,18)	4.95	$p = 0.041$	(1,18)	2.16	$p = 0.136$
anti-β-EP antiserum × 100	(10)	85.0 ± 4.7*	1.81 ± 0.13						

Estradiol benzoate (EB, 8 μg/rat) and pregesterone (Prog, 0.1 mg/rat) were injected subcutaneously at 1900 h (on day 1) and at 1400 h (on day 3), respectively.

[a] Lordosis responses: lordosis quotient (LQ) and lordosis reflex score (LS) were measured by tests of 20 mounts at 48–49 h after EB priming.

[b] Anti-β-EP antiserum (1.0 μl) or NRS (1.0 μl) is diluted with artificial cerebrospinal fluid.

[c] Significantly different from NRS × 100, *$p < 0.05$, †$p < 0.01$ (by unpaired *t*-test).

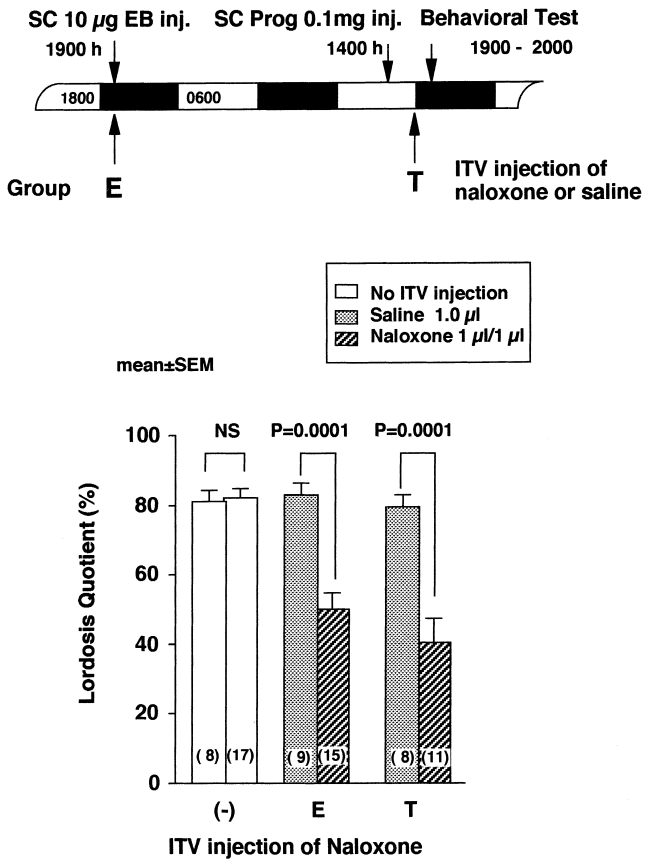

FIG. 2. Effects of ITV injection of naloxone (NLX, 1.0 μg) on lordosis quotient (LQ). The ovariectomized rats received 10 μg EB and 0.1 mg Prog. Data represent mean ± SEM. A one-way ANOVA was applied to analyze statistical significance. NS, not significant, $F(1, 24) = 0.45$, $p > 0.05$. Other legends as same as in Fig. 1.

TABLE 3

SUMMARY OF OPIOID PEPTIDE EFFECTS ON ESTROGEN-ACTIVATED SEXUAL RECEPTIVITY IN OVARIECTOMIZED RATS

	β-Endorphin	Anti-β-Endorphin Antiserum
[E]	↑	↓
[T]	↓	↑

	Met-Enkephalin	Leu-Enkephalin	Naloxone*
[E]	↑	↓	↓
[T]	↑	—	↓

Rats were primed with estradiol benzoate (SC EB, 8 μg, * 10 μg) and progesterone (0.1 mg, SC) with an interval of 43 h, and tested for lordosis reflex in response to male mount 48 h after the EB- priming. Drugs were injected into the third ventricle either at the time of EB priming [E] or at 1 h prior the behavioral test (47 h after SC EB treatment) [T].

↑, facilitation; ↓, inhibition; —, no change of lordosis response expressed in term of lordosis quotient. See Table 2 and Fig. 1.

The opioid neuronal system has received much attention and study. To date, three different opioid receptors have been identified, although the existence of others have been suggested (1,12). The receptors are pharmacologically distinct, and show different affinity for endogenous ligands. β-endorphin binds to all three types of receptors, dynorphin primarily to the κ, and enkephalin to μ and δ receptors (11). The proenkephalin A- and proenkephalin B-derived peptides may show affinity for all receptors, depending on the end-product of peptide cleavage (5,15). It has been reported that there are multiple, varied effects of opioid on female sexual behavior (13). The present data have shown that ITV injection of Leu-enkephalin significantly inhibited lordosis behavior of ovariectomized rats primed with estrogen and progesterone. Obviously, two different mechanisms are involved in the endorphinergic and enkephalinergic systems with regard to rat sexual receptivity. First, β-endorphin and Met-enkephalin in high doses at the initial stages of estrogen feedback act to facilitate estrogen-activation of lordosis behavior; in contrast, Leu-enkephalin acts to inhibit lordosis in that system. Second, β-endorphin at the final stages of estrogen feedback acts to inhibit lordosis behavior, but Met-enkephalin acts to facilitate lordosis behavior at that stage. Moreover, the results of naloxone microinfusion (Fig. 2) indicate that the Met-enkephalin neuronal system may be involved in not only the activated behavior, but also in inducing lordosis behavior. The present data suggest that there are the functional differences between endorphinergic systems and enkephalinergic systems throughout the duration of estrogen's priming effects on female sexual behavior. In addition, enkephalinergic modulation in the final stage of lordosis induction may act as an antagonist to endorphinergic systems.

It may be hypothesized that if endogenous opioid systems facilitate initial estrogen action on female sexual behavior; then naloxone, an opiate receptor antagonist, may inhibit that behavior. In general, animals receiving 8 μg EB (SC) expressed about 65–70%LQ. Thus, in the naloxone experiment, a 10 μg dose of EB was chosen. Furthermore, we expected to obtain a moderate lordosis response (submaximal lordosis performance = about 80%LQ, mean values) in experimental animals, and conducted preliminary tests of lordosis in our animals.

On the other hand, with regard to the initial activation of the effects of estrogen on sexual receptivity, Gorski and Yanase (8) have reported that when apomorphine was injected simultaneously with EB, 48 h prior to the behavioral test, 1.5 or 3.0 mg of apomorphine acutely facilitated lordosis behavior. IP injection of 50 μg epinephrine had an effect similar to that apomorphine. These findings, taken together with our own, may indicate that the endorphinergic systems interact with the adrenergic system to activate estrogen-induced sexual behavior. Resolution of this question awaits further investigation. However, we observed that ITV injection of Leu-enkephalin significantly inhibited lordosis behavior in estrogen and progesterone primed ovariectomized rats. In estrogen activated animals that received the ITV injection of Met-enkephalin, the results showed that facilitatory control by Met-enkephalin on sexual behavior was affected. Thus, enkephalinergic modulation of sexual behavior is divided into two different types: facilitatory effects at the initial stage steroid action ("methionine type"), and nonfacilitatory (or inhibitory) effects at the final stage of steroid action ("leucine type").

In a previous study (22), we reported the effects of ITV injection of β-endorphin on lordosis behavior in ovariectomized rats receiving one of two treatment: EB-EB or EB-Prog. The

lordosis behavior in rats following two injections of EB was facilitated by β-endorphin, if that opioid peptide was given exclusively during the several hours after the first EB priming. In contrast, injection of β-endorphin after Prog injection, or on the day following the second EB injection, inhibited behavioral receptivity. β-Endorphin facilitation of lordosis was exclusively observed within the initial 6 h of estrogen action, and after that, inhibition of lordosis occurred. It is impossible to create a sufficient lordosis reflex without estrogen in the CNS; for example, antiestrogen (CN-55, 945-27) effectively inhibits lordosis behavior if administered within 12 h of estrogen (7). In the present study, it was interesting to note that opioidergic modulation could cause opposite results at the initial stage and the final stage of estrogen action. With regard to the initial stage of estrogen action, we have already reported a facilitatory effect of ITV β-endorphin, injected at the time of EB implantation, was observed into medial septo-preoptic areas. With regard to the final estrogen action, Wiener and Moss (25) reported an inhibition of lordosis behavior in estrogen- and progesterone-primed ovariectomized rats when 100 ng β-endorphin was injected into the third ventricle. Thus, effects in the final stages estrogen action are quite different from those in the initial stages.

As to the control center in the brain of the lordosis reflex, Sakuma and Pfaff (16–18) have suggested that the mesencephalic central gray (MCG) plays an important role in the regulation of lordosis behavior. Thus, the MCG electrical stimulation facilitates, and the MCG lesions inhibit lordosis behavior in ovariectomized-steroid primed rats. In addition, microinfusion of luteinizing hormone-releasing hormone (LH-RH) in the MCG facilitates sexual behavior (16,20). The endorphinergic systems, in the final stages of EB action, may act on the specific mechanisms of LH-RH activation to augment the lordosis reflex at level of the midbrain, as has been suggested by Sakuma and Pfaff (16). Facilitation of lordosis behavior by naloxone injected into the MCG in estrogen-primed ovariectomized rats has also been reported (19,20). This evidence may be closely related to the inhibition of lordosis response by ITV injection of β-endorphin on the day of behavioral testing. Nonspecific behavioral inhibition is one of the physiological actions that endorphins and enkephalins exert (4). For example, β-endorphin strongly inhibited estrogen-activated behavior. We observed "no movement" in some animals following injection of 1 μg or 10 μg β-endorphin 1 h prior the behavioral test (at 60 min after ITV injection of 10 μg β-endorphin, "no movement" was observed in four animals).

Moreover, to investigate the action sites of opioidergic system in the brain, we studied the effect of ITV injection of naloxone on lordosis behavior in ovariectomized rats received with an anterior roof deafferentation (ARD), a cut between the septum and preoptic area and treated with EB and Prog (24). In sham-ARD control rats, lordosis responses significantly decreased by ITV injection of naloxone at the time of EB priming. In contrast, lordosis in the ARD-operated rats maximally facilitates, as previously reported by Yamanouchi and Arai (26). Lordosis responses in the ARD-operated rats could not decrease by ITV injection of naloxone at the time of EB priming. The data suggested that endorphinergic action to facilitate lordosis behavior was closely related to the limbic-forebrain inhibitory influences on lordosis behavior. Further studies are required to consider these matters.

In conclusion, female sexual receptivity was activated by β-endorphin and Met-enkephalin, and inhibited by Leu-enkephalin, in the initial stage of estrogen action. A facilitatory effect on lordosis behavior was seen when, at the final stage of steroid action, Met-enkephalin was infused into the third ventricle. The effect of naloxone was opposite to that of ITV Met-enkephalin in the final stage of steroid action. In contrast, no inhibitory effect on lordosis behavior was seen when, during the final stage of estrogen action, Leu-enkephalin was infused into the third ventricle. The present results suggest several conclusions. First, there are differential effects among the β-endorphin, Met-enkephalin and Leu-enkephalin peptides to control lordosis behavior. Second, the opioidergic systems may modulate initial stage and final-stage estrogen-induced lordosis behavior. Third, opioidergic actions can be divided into the "endorphinergic modulation type" and the "enkephalinergic modulation type."

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REFERENCES

1. Akil, H.; Bronstein, D.; Mansour, A.: Overview of the endogenous opioid systems: Anatomical, biochemical and functional issues. In: Rodgers, R. J.; Cooper, S. J., ed. Endorphins, opiates and behavioural processes. New York: John Wiley & Sons; 1988:1–23.
2. Arai, Y.; Yamanouchi, K.; Mastumoto, A.: Neuroendocrine regulation and sexual differentiation of lordosis behavior in rats. In: Ito, M.; Tsukahara, N.; Kubota, K.; Yagi, K., eds. Integrative control function of the brain, vol. 1. Tokyo: Kodansha/Amsterdam: Elsevier; 1978:355–369.
3. Barchas, J. D.; Akil, H.; Elliott, G. R.; Holman, R. B.; Watson, S. J.: Behavioral neurochemistry: neuroregulators and behavioral states. Science 200:964–973; 1978.
4. Bloom, F.; Segal, D.; Ling, N.; Guillemin, R.: Endorphins: Profound behavioral effects in rats suggest new etiological factors in mental illness. Science 194:630–632; 1976.
5. Corbett, A. D.; Paterson, S. J.; McKnight, A. T.; Magnan, J.; Kosterlitz, H.: Dynorphin$_{1-8}$ and dynorphin$_{1-9}$ are ligands for the κ subtype of opiate receptor. Nature 299:79–81; 1982.
6. Finley, J. C. W.; Lindström, P.; Petrusz, P.: Immunocytochemical localization of β-endorphin-containing neurons in the rat brain. Neuroendocrinology 33:28–42; 1981.
7. Gorski, R. A.: The neuroendocrine regulation of sexual behavior. In: Newton, G.; Riesen, A. H., ed.: Advances in psychobiology, vol. II. New York: John Wiley & Sons; 1974:1–58.
8. Gorski, R. A.; Yanase, M. : Estrogen facilitation of lordosis behavior in the female rat. Exp. Brain Res. Suppl. 3:222–237; 1981.
9. König, J. F. R.; Klippel, R. A.: The rat brain: A stereotaxic atlas of the forebrain and lower parts of the brain stem. Baltimore: Williams and Wilkins; 1963.
10. Krieger, M. S.; Cornard, L. C. A.; Pfaff, D. W.: An autoradiographic study of the efferent connections of the ventromedial nucleus of the hypothalamus. J. Comp. Neurol. 183:785–816; 1979.

11. Lynch, D. R.; Snyder, S. H.: Neuropeptides: Multiple molecular forms, metabolic pathways, and receptors. Annu. Rev. Biochem. 55:773–799; 1986.
12. Mansour, A.; Khachaturian, H.; Lewis, M. E.; Akil, H.; Watson, J. S.: Anatomy of CNS opioid receptors. Trends Neurosci. 11:308–314; 1988.
13. Pfaus, J. G.; Gorzalka, B. B.: Selective activation of opioid receptors differentially affects lordosis behavior in female rats. Peptides 8:309–317; 1987.
14. Pfaus, J. G.; Gorzalka, B. B.: Opioids and sexual behavior. Neurosci. Biobehav. Rev. 11:1–34; 1987.
15. Quirion, R.; Weiss, A. S.: Peptide E and other proenkephalin-derived peptides are potent κ receptor agonists. Peptides 4:445–449; 1983.
16. Sakuma, Y.; Pfaff, D. W.: LH-RH in the mesencephalic central grey can potentiate lordosis reflex of female rats. Nature 283:566–567; 1980.
17. Sakuma, Y.; Pfaff, D. W.: Facilitation of female reproductive behavior from mesencephalic central gray in the rat. Am. J. Physiol. 235:R278–R284; 1979.
18. Sakuma, Y.; Pfaff, D. W.: Mesencephalic mechanisms for integration of female reproductive behavior in the rat. Am. J. Physiol. 235:R285–R290; 1979.
19. Sirinathsinghji, D. J. S.; Whittington, P. E.; Audsley, A.; Fraser, H. M.: β-Endorphin regulates lordosis in female rats by modulating LH-RH release. Nature 301:62–64; 1983.
20. Sirinathsinghji, D. J. S.: Modulation of lordosis behaviour in the female rat by corticotropin releasing factor, β-endorphin and gonadotropin releasing hormone in the mesencephalic central gray. Brain Res. 336:45–55; 1985.
21. Torii, M.; Kubo, K.: The effects of intraventricular injection of β-endorphin on initial estrogen action to induce lordosis behavior. Physiol. Behav. 55:157–162; 1994.
22. Torii, M.; Kubo, K.; Sasaki, T.: Effect of β-endorphin on initial estrogen action to induce lordosis behavior and the activated-behavior. Proc. 9th Symp. Biol. Physiol. Eng. 1994:167–170.
23. Torii, M.; Kubo, K.; Sasaki, T.: Influence of opioid peptides on the priming action of estrogen on lordosis in ovariectomized rats. Neurosci. Lett. 212:68–70; 1996.
24. Torii, M.; Kubo, K.; Sasaki, T.: Naloxone and initial estrogen action to induce lordosis in ovariectomized rats: The effect of a cut between the septum and preoptic area. Neurosci. Lett. 195: 167–170; 1995.
25. Wiesner, J.; Moss, R.: Suppression of receptive and proceptive behavior in ovariectomized, estrogen-progesterone-primed rats by intraventricular beta-endorphin: Studies of behavioral specificity. Neuroendocrinology 43:57–62; 1986.
26. Yamanouchi, K.; Arai, Y.: Possible inhibitory role of the dorsal inputs to the preoptic area and hypothalamus in regulating female sexual behavior in the female rats. Brain Res. 127:296–301; 1977.