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Potentiative Action of α- and β-Adrenergic Receptor Stimulation in Inducing Lordosis Behavior

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FERNÁNDEZ-GUASTI, A., K. LARSSON AND C. BEYER. *Potentiative action of α- and β-andrenergic receptor stimulation in inducing lordosis behavior.* PHARMACOL BIOCHEM BEHAV 22(4) 613–617, 1985.—Ovariectomized (OVX) and ovariectomized-adrenalectomized (OVX-ADX) rats were injected with estradiol benzoate (EB, 4.0 μg/rat) and received 44 hr by infusion into the ventromedial hypothalamic area one of the following treatments: norepinephrine (NE), clonidine (an α-agonist), isoproterenol (a β-agonist) or a combination of clonidine and isoproterenol. Infusion of NE (200 ng/rat) induced lordosis in both OVX and OVX-ADX rats 15 minutes after its administration. NE-induced lordosis was blocked by systemic treatment with either the α-antagonist, prazosin (1.0 mg/kg), or the β-antagonist, propranolol (4.0 mg/kg). Intrahypothalamic infusion of clonidine (200 ng/rat) or isoproterenol (200 ng/rat) induced lordosis behavior in OVX, but not in OVX-ADX rats, suggesting the involvement of adrenal secretions in this response. Combined administration of clonidine (100 ng/rat) and isoproterenol (100 ng/rat) induced lordosis behavior 15 minutes after its intrahypothalamic infusion in OVX-ADX animals. Results are discussed in relation to a model proposed for the induction of lordosis behavior involving steroid-NE interactions.

Norepinephrine Clonidine Isoproterenol Propranolol Prazosin Molecular regulation of lordosis

IT has been proposed that the central adrenergic transmission is involved in the steroid induction of lordosis behavior in rodents. Thus, administration of norepinephrine (NE), epinephrine (E), or adrenergic agonists may substitute for progesterone in inducing lordosis in estrogen-primed rodents [4,10]. Moreover, treatment with compounds that block NE synthesis [7,20] or adrenergic antagonists [3,9] prevents the expression of estrogen-progesterone induced lordosis in rodents.

The ventromedial hypothalamus (VMH) is probably a site for catecholamine-steroid interaction for inducing lordosis behavior. The following data support this assumption: (a) implantation of estrogen and progesterone in the VMH induces lordosis [23,24], (b) infusion of NE or E in the VMH induces lordosis in estrogen-primed rats [10], (c) although free of NE itself, the VMH receives a dense adrenergic innervation from cell bodies in mesencephalon [5,17], (d) administration of adrenergic antagonists in the VMH blocks the effect of progesterone (Collado and Beyer, unpublished data) or of estrogen [11] on the adenylate cyclase cAMP system.

The molecular events underlying the steroid-catecholamine interaction for the induction of lordosis are poorly understood. Recently we have proposed a model for the steroid induction of lordosis according to which progesterone stimulates an adenylate cyclase-cAMP system by triggering adrenergic transmission [9]. *In vitro* studies by Palmer *et al.* [21] and Daly *et al.* [6] demonstrate that NE or adrenergic agonists stimulate an adenylate cyclase-cAMP system in brain. The hypothalamus is unique in being the only brain region in which potentiative interactions between α- and β-adrenergic receptor activation occur to increase cAMP levels [6] and blockage of either of these adrenergic receptors prevents the NE stimulatory effect on cAMP [21].

This study was aimed at providing further support for the idea that adrenergic stimulation facilitates the expression of lordosis behavior through an adenylate cyclase cAMP system. Because of the hypothalamic peculiarity of the adenylate cyclase cAMP system to respond to adrenergic stimulation, we hypothesize that lordosis can only be induced in estrogen primed rats by combined administration of α-and β-adrenergic agonists, and that the NE effect on this behavior can be prevented by treatment with either an α- or a β-blocking agent. To rule out the possibility that adrenal secretions mediate the effect of the adrenergic agents on lordosis behavior, the effect of these drugs was studied in both ovariectomized and ovariectomized-adrenalectomized estrogen-primed rats.

METHOD

Experiments were conducted with sexually inexperienced

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adult Wistar female rats (250–300 g body wt). Rats were housed in single cages and maintained in a temperature controlled room (23°C) with an inverted light:dark cycle (lights on at 00:00 hr). Rat chow and water (saline for adrenalectomized animals) were available ad lib. All rats were ovariectomized, under ether anesthesia, three weeks before treatment. Fifteen days before drug administration the skull was exposed and two cannulae (23 gauge) were aimed at the VMH nuclei with a guide of a stereotaxic atlas in reference to bregma [16]. Coordinates for cannulae placement were 1.0 mm posterior to bregma and 0.5 mm lateral to both sides of the midline, imbedded 9.5 mm beneath the skull surface with the incisor bar elevated 5.0 mm above the interaural plane. Cannulae were fixed to the skull with acrylic dental cement. At least seven days of recovery were allowed before experimentation. Adrenalectomy was performed ten days before treatment. All rats received a subcutaneous injection of estradiol benzoate (EB) (4.0 μg/rat, dissolved in 0.2 ml sesame oil), followed 44 hr later by one of the following treatments: Group 1. 1.0 μl saline, 12 OVX rats; Group 2. 200 ng clonidine, 10 OVX rats; Group 3. 200 ng isoproterenol, 10 OVX rats; Group 4. 200 ng NE, 10 OVX rats; Group 5. 1.0 μl saline, 9 OVX-ADX rats; Group 6. 200 ng clonidine, 8 OVX-ADX rats; Group 7. 200 ng isoproterenol, 9 OVX-ADX rats; Group 8. 100 ng clonidine + 100 ng isoproterenol, 11 OVX-ADX rats; Group 9. 200 ng NE, 10 OVX-ADX rats; Group 10. 200 ng NE + 4.0 mg/kg propranolol, 9 OVX-ADX rats; Group 11. 200 ng NE + 1.0 mg/kg prazosin, 9 OVX-ADX rats.

EB, propranolol and isoproterenol were purchased from Sigma Chemicals. NE (Arterenol) was purchased from 20 Century Chemicals. Clonidine and prazosin were kind gifts from Boehringer and Pfizer Laboratories respectively. Compounds administered by hypothalamic infusion were dissolved in saline and infused in 1.0 μl. Propranolol was dissolved in saline and injected IP 15 minutes before NE administration. Prazosin was dissolved in 100% acetic acid and 5% glucose, buffered to a pH of 7.4 with a solution of 1 M NaOH and injected IP 15 minutes before NE infusion.

Tests for lordosis behavior were conducted in a circular Plexiglas arena 15 minutes and 3 hr after intrahypothalamic saline or drug infusion. Sires were sexually vigorous experienced males. During a test, each female received a total of 10 mounts, receptivity was then quantified by a lordosis quotient (LQ=number of lordosis/10 mounts × 100). After observation, animals were killed, perfused through the heart with 10% formalin and their brains removed for histological analysis of cannulae placement. Brains were embedded in paraffin, cut every 200 μm, and stained with hematoxyline-eosine. Only animals showing cannulae placement in the VMH were included in the experimental analysis.

RESULTS

Results of the different treatments are shown in Tables 1 and 2. Following treatment with EB alone, ovariectomized (OVX), or ovariectomized-adrenalectomized (OVX-ADX) rats (Groups 1 and 5) did not display lordosis behavior. Intrahypothalamic infusion of NE (200 ng) in EB-primed rats, resulted in the display of lordosis behavior 15 minutes after its administration in both OVX and OVX-ADX rats (Groups 4 and 9). This effect was effectively prevented by systemic treatment with either propranolol (4.0 mg/kg, IP) or prazosin (1.0 mg/kg, IP) (Groups 10 and 11). Results also show that intrahypothalamic infusion of clonidine (200 ng) or isoproterenol (200 ng) induced lordosis behavior in OVX EB-primed rats (Groups 2 and 3). However, this effect was not observed until 3 hr after the infusion and was reduced to non-significant levels in OVX-ADX rats (Groups 6 and 7). The combined administration of clonidine (100 ng) and isoproterenol (100 ng) induced lordosis behavior 15 minutes after its intrahypothalamic infusion in OVX-ADX rats (Group 8).

Proceptive behavior (darting, hopping and ear wiggling) was observed in 50% of the OVX and OVX-ADX animals 15 minutes after the infusion of NE. Motor disturbances or other behavioral alterations were not observed after the administration of any drug.

DISCUSSION

Confirming previous findings of Foreman and Moss [10] and Crowley *et al.* [4], we have found that administration of NE or the adrenergic agonists clonidine and isoproterenol may induce lordosis in estrogen-primed rodents. However, according to present findings, the effect of NE and that of either clonidine or isoproterenol seem to be mediated by different mechanisms. When either clonidine or isoproterenol were infused into the hypothalamus, the ovariectomized rats showed lordosis with a latency of 3 hours. These drugs were ineffective in inducing lordosis in ovariectomized-adrenalectomized animals, suggesting that the effect of these agonists was mediated through adrenal secretions.

By contrast, infusion of NE, a compound stimulating both α- and β-adrenergic receptors, or the combination of clonidine and isoproterenol, induced lordosis within 15 minutes in ovariectomized-adrenalectomized rats indicating that this response occurs independently of adrenal secretions. It should be noted that in the study of Foreman and Moss [10], the lordosis response induced by hypothalamic infusion of NE or isoproterenol was assessed 1.75 hours after the administration of these agents. Present findings emphasize the necessity to rule out a possible participation of the pituitary-adrenal axis in studying central catecholamine-steroid interactions in lordosis behavior.

Present results show that systemic treatment with either an α- or a β-adrenergic antagonist, block the induction of lordosis behavior induced by hypothalamic NE infusion. It has been suggested that adrenergic transmission is involved in the sensory pathway for lordosis display [13], and therefore it could be argued that the α- and β-antagonists used interfere with NE-induced lordosis behavior through blocking sensory and/or motor pathways involved in the display of lordosis. However, we recently demonstrated that neither prazosin nor propranolol, in the doses used, interfered with the display of lordosis behavior induced by estrogen alone, indicating that these agents do not act at synapses of the reflex arc for lordosis [9].

On the molecular level, the mechanism of action of adrenergic agents in the hypothalamus has been analysed by Palmer *et al.* [21] and Daly *et al.* [6]. They found that stimulation of α- and β-receptors causes a synergistic increase of hypothalamic cAMP levels and that either α- or β-antagonists prevent NE stimulation on cAMP levels. Our finding that combined α- and β-adrenergic stimulation by clonidine and isoproterenol, was required for elicitation of lordosis behavior, and that treatment with either an α- or a β-adrenergic antagonist prevented NE-induced lordosis behavior, support the idea that an adenylate cyclase-cAMP system is involved in the adrenergic induction of lordosis. Clonidine may act both at the pre- and post-synaptic level

TABLE 1

EFFECT OF INTRAHYPOTHALAMIC INFUSION OF SALINE, CLONIDINE, ISOPROTERENOL OR NOREPINEPHRINE ON LORDOSIS BEHAVIOR IN OVX EB-PRIMED RATS (4.0 μg/RAT, 44 HR BEFORE TREATMENT)

Group	n	Treatment	Median Lordosis Quotient and Percentage of Responding Rats at: 15 min	3 hours
1	12	1.0 μl saline	00	00
			0%	17%
2	10	200 ng/rat clonidine	00	65*
			30%	70%†
3	10	200 ng/rat isoproterenol	00	90‡
			30%	100%‡
4	10	200 ng/rat norepinephrine	40‡	00
			90%‡	40%

Tests for lordosis behavior were performed 15 min and 3 hours after the intrahypothalamic infusion. Statistical comparisons were made between Groups 2 to 4 and Group 1. Median lordosis quotient was compared using Mann Whitney U test. Percentage of responding rats was compared using Fisher F test [27]. *$p<0.05$, †$p<0.025$, ‡$p<0.005$.

TABLE 2

EFFECT OF VARIOUS ADRENERGIC AGENTS ON THE LORDOSIS BEHAVIOR OF OVX-ADX EB-PRIMED RATS (4.0 μg/RAT, 44 HR BEFORE TREATMENT)

Group	n	Treatment	Median Lordosis Quotient and Percentage of Responding Rats at: 15 min	3 hours
5	9	1.0 μl saline	00	00
			22%	33%
6	8	200 ng/rat clonidine	00	00
			25%	38%
7	9	200 ng/rat isoproterenol	00	20
			11%	67%
8	11	100 ng/rat clonidine + 100 ng/rat isoproterenol	30*	00
			72%*	36%
9	10	200 ng/rat norepinephrine	55†	00
			90%†	40%
10	9	200 ng/rat norepinephrine +4.0 mg/kg propranolol	00*	—
			44%*	
11	9	200 ng/rat norepinephrine + 1.0 mg/kg prazosin	00†	—
			0%†	

Tests for lordosis behavior were performed 15 min and 3 hours after the intrahypothalamic infusion. All compounds, but prazosin and propranolol IP, were infused into the ventromedial hypothalamic nuclei. Statistical comparisons were made between Groups 6 to 9 and Group 5. Groups 10 and 11 were compared with Group 9. Median lordosis quotient was compared using Mann Whitney U test. Percentage of responding rats was compared using Fisher F test [27]. *$p<0.05$, †$p<0.025$.

[28]. However, according to Skolnick and Daly [28], the rise of cAMP levels produced by combined administration of clonidine and isoproterenol are due to post-synaptic adrenergic stimulation.

Epinephrine and NE may act on brain tissue through occupation of several types of receptors [22], each specific adrenergic receptor interaction leading to a particular cellular event [8,22]. Besides activation of an adenylate cyclase-cAMP system [6,28], stimulation of α-receptors may induce inhibition of this system with a consequent decrease in cAMP levels [15]. It may also cause intraneuronal changes in cGMP [12], Ca^{++} [26] or adenosine [25] levels. Beta receptor stimulation is generally considered to act exclusively by stimulating adenylate cyclase-cAMP systems [22]. Therefore, it should not be excluded that the adrenergic agents stimulate lordosis behavior through a mechanism not involving the activation of an adenylate cyclase-cAMP system.

Nock and Feder [19] have proposed a model for the interaction of steroids and NE in inducing lordosis in guinea pigs. According to this model, estrogen induces the synthesis of progestin receptors which are activated by progesterone. Adrenergic transmission is involved in the synthesis of progestin receptors. In support of this hypothesis, they found that administration of compounds that block the synthesis of NE or adrenergic antagonists diminish the amount of cytosolic progestin receptors in the hypothalamus and abolish lordosis in estrogen-progesterone treated guinea pigs [3, 19, 20]. While this model also may apply to estrogen-progesterone induced lordosis in rats, it does not explain how adrenergic stimulation activates lordosis in the absence of progesterone. According to our model, estrogen induces the synthesis of proteins which are activated by a cAMP dependent phosphorylation process [1,2]. Because NE may stimulate the adenylate cyclase-cAMP system in the hypothalamus [6,21] and progesterone influences NE release [14,18], we propose that NE mediates the induction of lordosis behavior by progesterone. Further studies, involving a cellular level of analysis, should be undertaken to reveal the precise mechanism of steroid-catecholamine interactions for inducing lordosis behavior.

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