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Lordosis Behavior and GABAergic Neurotransmission

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FERNÁNDEZ-GUASTI, A , K LARSSON AND C BEYER *Lordosis behavior and GABAergic neurotransmission* PHARMACOL BIOCHEM BEHAV 24(3)673–676, 1986 —γ-Aminobutyric acid (GABA) (1 0 μg/cannula) or muscimol (50 ng/cannula) was injected into the ventromedial hypothalamus or the lateral septi nuclei of ovariectomized rats brought to sexual receptivity by combined treatment of estrogen and progesterone No inhibitory effects of GABA or muscimol were observed on the lordosis behavior Furthermore, systemic (1 0 mg/kg) or intrahypothalamic (50 ng/cannula) picrotoxin administration was followed by a statistically significant increase in lordosis behavior in ovariectomized, estrogen-primed rats No such effect was observed in ovariectomized-adrenalectomized animals, indicating its dependence on adrenal secretions Present results do not support the hypothesis of a GABAergic mechanism in the hormonal control of lordosis behavior

Muscimol Picrotoxin GABA Ventromedial hypothalamus Lateral septi nuclei Lordosis behavior

LORDOSIS is a component of the reproductive behavior pattern of the female rat, normally occurring in response to the combined action of ovarian estrogen and progesterone GABA, a recognized inhibitory neurotransmitter in the central nervous system (see [14] for references), participates in the neural regulation of pituitary hormone secretion [3], and GABAergic transmission might be involved in the control of the lordosis behavior [1,11] Large concentrations of GABA and its synthetic enzyme, glutamic acid decarboxylase (GAD), occur in the hypothalamus, a structure of critical importance for the neuroendocrine control of lordosis behavior [2,10] Moreover, Wallis and Luttge [15] reported that hypothalamic GAD activity increases after ovariectomy (OVX), and this increase can be counteracted by administration of either estrogen or progesterone They also found that combined treatment with estrogen and progesterone did not reduce GAD activity in any brain region except the septum, and suggested that the septum is the only brain area where estrogen and progesterone interact to modify GABA levels Interestingly, septal lesions facilitate the expression of lordosis in estrogen-primed rats [7, 11, 12] Al Satli *et al* [1] reported that administration of the GABA transaminase inhibitor, sodium-n-dipropylacetate (DPA), suppressed lordosis in normal 4-day cycling rats, and caused elevated GABA concentrations in the hypothalamus and the olfactory bulbs Since olfactory bulb lesions, like septal lesions, facilitate the display of lordosis in steroid hormone treated rats, it was speculated that the olfactory bulb and the septum form part of a system exerting an inhibitory influence on the lordosis behavior through a GABAergic mechanism [1]

In order to study the role of GABAergic transmission for the expression of lordosis, we undertook two series of experiments In the first series, lordosis was induced in OVX rats by sequential treatment with estrogen and progesterone Through cannulae implanted in the ventromedial hypothalamic nucleus (VMH) or in the lateral septum (LS), either GABA or the potent GABA agonist, muscimol, were injected It was hypothesized that an increase in GABAergic activity would inhibit the lordosis behavior The second series of experiments was aimed at investigating whether a decrease in central GABAergic activity would facilitate the lordosis behavior To that purpose, OVX rats were treated with a subthreshold dose of estrogen Picrotoxin, a potent GABAergic antagonist, was thereafter administered either intrahypothalamically or systemically To control for behavioral effects due to adrenal secretions, some of the animals were adrenalectomized (ADX) in addition to OVX

METHOD

General

Sexually inexperienced female Wistar rats (200–250 g body wt) were used The animals were maintained in a room with an inverted light-dark cycle (lights on at 2200 hr and off at 1000 hr) at 22°C The animals were fed with commercial rat chow and water ad lib They were OVX under methohexital (Brietal® 50 mg/kg, Lilly) anesthesia two weeks before the treatment ADX was performed in some of the animals under Brietal® anesthesia at least 6 days before the treatment Saline for drinking was available ad lib for ADX rats

Animals were anesthetized with pentobarbital (Nebumal®

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TABLE 1

MEDIAN LORDOSIS QUOTIENT (LQ) FOLLOWING TREATMENT WITH MUSCIMOL INJECTED EITHER INTO THE VENTROMEDIAL HYPOTHALAMIC NUCLEUS (VMH) OR THE LATERAL SEPTAL AREA (LS) OR INJECTED IP, AND AFTER TREATMENT WITH GABA INJECTED INTO THE VENTROMEDIAL HYPOTHALAMIC NUCLEUS

			Lordosis Quotient				
Groups	Treatment	N	Pre-drug	Post-drug 1	10	30	60 minutes
1	50 ng/cannula muscimol VMH	7	100	—	100	100	—
2	50 ng/cannula muscimol LS	6	95	—	95	100	—
3	50 mg/kg muscimol IP	8	100	—	—	100	100
4	1 0 μg/cannula GABA VMH	7	100	85	95	—	—

Females were ovariectomized and treated with estradiol benzoate (1 25 μg/rat) followed 44 hr later by progesterone (1 0 mg/rat) 4 hr before drug administration Comparisons were made between pre-drug and post-drug LQ values, Wilcoxon T-test [13]

TABLE 2

EFFECTS OF PICROTOXIN ON LORDOSIS BEHAVIOR IN ESTROGEN-PRIMED RATS

				Lordosis Quotient				
Groups	Treatment		N	10	30	60	120	240 minutes
5	0 5 μl/cannula saline VMH	ovx	9	00	30	—	10	30
6	50 ng/cannula picrotoxin VMH	ovx	8	55*	65*	—	95†	80
7	2 0 ml/kg saline IP	ovx	12	—	00	00	00	40
8	1 0 mg/kg picrotoxin IP	ovx	6	—	20	25	75†	50
9	0 5 μl/cannula saline VMH	adx+ovx	6	05	15	—	15	30
10	50 ng/cannula picrotoxin VMH	adx+ovx	6	00	00	—	05	10
11	2 0 ml/kg saline IP	adx+ovx	8	—	00	00	00	05
12	1 0 mg/kg picrotoxin IP	adx+ovx	8	—	00	00	00	05

Table shows median lordosis quotient Rats were ovariectomized (ovx) or ovariectomized and adrenalectomized (ovx+adx) Estradiol benzoate 1 25 μg/rat was injected 44 hr before testing Picrotoxin or saline were injected into the ventromedial hypothalamus (VMH) or IP Comparisons were performed between saline and picrotoxin treated groups Mann-Whitney U-test, *$p<0$ 05, †$p<0$ 02 [13]

40 mg/kg IP, Aco Lakemedel) The skull was exposed, and two guiding cannulae were implanted aimed either at the VMH or the LS Coordinates were as follows VMH, 0 5 mm from the middle line and 2 6 mm posterior to bregma, LS, 0 5 mm from the middle on the level of bregma [8] Cannulae were fixed to the skull with dental acrylic cement At least four days were allowed for recovery Intrabrain injections were made using an injection cannula (27 g) adapted to a microliter Hamilton syringe Dorsoventral coordinates were 9 0 mm and 4 0 mm from the brain surface to the VMH and the LS respectively

Compounds were purchased from Sigma Chemical Co Estradiol benzoate (EB) and progesterone (P) were dissolved in sesame oil and injected subcutaneously in 0 1 ml GABA, muscimol and picrotoxin were dissolved in saline, and injected in 0 5 μl doses when administered intracerebrally and in 2 0 ml doses when administered IP

Rats were tested for lordosis behavior in a circular Plexiglas arena Sires were sexually experienced males Ten mounts were allowed of each female and the lordosis quotient (LQ = number of lordosis/10 mounts × 100) was determined Group comparisons were performed with the Mann Whitney U test or the Wilcoxon T test [13]

After the completion of the behavioral tests, the animals were decapitated and their brains were removed and stored in a freezer The frozen brains were cut in 60 μm slices on a microtome and inspected for localization of the cannulae tracks Only those animals in which the cannula tracks were followed to intended brain nuclei were accepted for further analysis

Experiment 1 Effects of Muscimol and GABA on EB + P-Induced Lordosis Behavior

In this experiment, rats were brought into sexual receptivity by sequential administration of EB (1 25 μg/rat, −44 hr) and P (1 0 mg/rat) Four hours after P administration, rats were tested for lordosis behavior and a pretreatment evaluation of LQ was established After this test, rats received one of the treatments indicated in Table 1 In this experiment,

animals were tested for lordosis behavior 10 and 30 minutes after intrahypothalamic, or 30 and 60 minutes after systemic muscimol administration GABA treated rats were tested for lordosis behavior 1 and 10 minutes after the intrahypothalamic injections Testing schedule was decided on the basis of reports showing maximal drug effects at times indicated [5,6]

Experiment 2 Effects of Picrotoxin on Lordosis Behavior in EB Primed Rats

In this experiment, the animals were primed with 1 25 μg/rat EB, a dose which is not effective in inducing lordosis behavior *per se* Forty-four hours later, rats received one of the treatments indicated in Table 2 No pretest LQ's were established because of the presence of saline treated controls Animals in this experiment were tested for lordosis behavior at 10, 30, 120 and 240 minutes after intrahypothalamic picrotoxin or saline injection and at 30, 60, 120 and 240 minutes after systemic administration of either saline or picrotoxin

RESULTS

Experiment 1 Effects of Muscimol and GABA on EB + P-Induced Lordosis Behavior

Table 1 shows the results of this experiment Intracerebral (Groups 1 and 2) or systemic (Group 3) administration of muscimol did not suppress the lordosis behavior induced by EB + P As shown in Table 1, injection of GABA into the VMH (Group 4) did not affect the lordosis behavior

Experiment 2 Effects of Picrotoxin on Lordosis Behavior in EB Primed Rats

Table 2 shows the results of this experiment Treatment with saline did not induce lordosis behavior under any of the conditions studied (Groups 5, 7, 9 and 11) Intrahypothalamic or systemic administration of picrotoxin facilitated the elicitation of lordosis behavior in OVX (Groups 6 and 8) but not in OVX-ADX rats (Groups 10 and 12)

DISCUSSION

According to present findings, intrahypothalamic injection of picrotoxin facilitated the expression of lordosis in OVX but not in OVX-ADX rats, suggesting the dependence upon adrenal secretions for this response This result is in line with the observation that treatment with picrotoxin or other GABA antagonists, stimulates ACTH release [9] which, in turn, facilitates the display of lordosis [4] by stimulating adrenal steroid release

None of the two models used in this study to investigate a role of GABAergic transmission for the expression of lordosis behavior provided any evidence in favour of a GABAergic influence on this behavior Thus, an increase in central GABAergic activity by administration of GABA and muscimol failed to inhibit lordosis behavior induced by combined estrogen and progesterone treatment Furthermore, a decrease in central GABAergic activity caused by treatment with picrotoxin, did not induce lordosis in OVX-ADX estrogen primed rats

McGinnis *et al* [11] reported that injection of picrotoxin in the substantia nigra suppressed the facilitatory effect on the lordosis behavior which normally accompanies septal lesions in estrogen-primed rats They speculated that GABAergic neurones control dopamine turnover rate thereby determining the expression of lordosis behavior This model, however, does not consider the basic fact that lordosis behavior is induced by gonadal hormones Al Satli *et al* [1] observed that an increase of GABA levels by DPA administration in late afternoon of proestrous, in normal 4-day cycling rats, blocked the sexual receptivity which normally occurs in this phase of the estrous cycle This effect was attributed to a direct inhibitory effect of the GABAergic system on neural mechanism mediating the lordosis behavior However, pharmacological manipulation of the GABAergic system has been demonstrated to affect anterior pituitary hormone secretions [3], and hence the hypothesis cannot be excluded that the effect of DPA on the lordosis behavior was due to alterations in ovarian progesterone secretions related to variations in the gonadotrophin release

Present findings do not exclude the possibility that manipulation of the GABAergic transmission by injections of compounds affecting this system in other brain regions that the VMH and the LS, influence the hormonal induction of lordosis behavior Further work should be undertaken to investigate this idea

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