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Possible Role of Inhibitory Glycinergic Neurons in the Regulation of Lordosis Behavior in the Rat

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SANDOVAL, Y., B. KOMISARUK AND C. BEYER. *Possible role of inhibitory glycinergic neurons in the regulation of lordosis behavior in the rat.* PHARMACOL BIOCHEM BEHAV **29**(2) 303–307, 1988.—Strychnine sulfate (3, 9 or 27 μg in 0.5 μl saline) was bilaterally infused into the ventromedial hypothalamic nucleus (VMH) of ovariectomized sexually inexperienced rats primed 40 hr earlier with 4 μg of estradiol benzoate (EB). This dose of EB induced only weak lordosis behavior in 25% of the subjects (Ss). Strychnine at the 3 and 9 μg dosages, but not at 27 μg, induced intense lordosis behavior, but no proceptivity, in most estrogen-primed Ss (69% in 3 μg, 94% in 9 μg). Ovariectomized adrenalectomized EB-primed Ss also displayed significant lordosis behavior (59%) following infusion of 9 μg of strychnine into the VMH. Strychnine (9 μg) failed to stimulate lordosis in ovariectomized Ss that were not estrogen-primed. Administration of 5 μg EB followed 40 hr later by 2 mg of progesterone (P) elicited intense lordosis behavior in most Ss. Bilateral injections into the VMH of glycine (100 μg), β-alanine (100 μg) or taurine (50 μg) to rats that were already displaying estrous behavior (>80 LQ) in response to the sequential administration of EB and P failed to depress lordosis when tested between 5 min and 60 min postinjection. Similarly, glycine (20 or 100 μg) injected into the VMH of estrogen-primed, ovariectomized rats within 15 minutes of a 2 mg SC injection of P failed to interfere with the subsequent response to this steroid when tested 2 and 4 hr after P. The results suggest that strychnine injected into the VMH facilitates lordosis behavior in estrogen-primed rats by removing a tonic inhibitory effect exerted by glycinergic neurons on VMH neurons. Failure of glycine or glycine agonists, when injected into the VMH, to inhibit lordosis induced by EB and P may be due to either their rapid removal from the synaptic region or to the inability of the local restricted injections of amino acids to inhibit all the VMH neurons activated by the systemic administration of the steroids.

Ventromedial hypothalamic nucleus Glycine Strychnine Taurine β-Alanine Rat Lordosis

THE ventromedial hypothalamic nucleus (VMH) participates in the regulation of female sexual behavior in rodents [2, 17, 23]. Lesions involving the VMH reduce the expression of lordosis in hormonally-treated rats (see [23]). Moreover, implants into the VMH of estrogen in ovariectomized rats [2,28] or of progesterone (P) in ovariectomized, estrogen-primed rats [2, 26, 29] induce lordosis behavior. The expression of lordosis is related to an increased firing of some VMH neurons. Thus, electrical stimulation of VMH facilitates lordosis in estrogen primed rats [22] while blocking VMH action potentials by tetrodotoxin inhibits lordosis in the estrogen treated rat [8]. Therefore, it appears likely that neurotransmitters that decrease VMH neuronal firing will also inhibit the display of lordosis in response to male mounting. Glycine is an inhibitory neurotransmitter present in the hypothalamus [7,32] that exerts strong inhibitory effects on VMH neurons when microiontophoretically applied to this nucleus [4, 6, 25]. However, no information exists on the possible influence of glycinergic neurons in the hypothalamic control of lordosis. In this study we investigated the ability of strychnine, a potent specific glycine antagonist [3, 10, 13] to facilitate lordosis behavior in estrogen-primed rats when injected into the VMH. The ability of injections into the VMH of glycine and two putative glycine agonists, β-alanine, and taurine [10], to inhibit the effect of P in ovariectomized, estrogen-primed rats was also tested.

METHOD

Young, sexually inexperienced, female Wistar rats (220–240 g) were used in this study. Subjects (Ss) were kept in

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TABLE 1

EFFECT OF STRYCHNINE INFUSION INTO THE VMH ON THE LORDOSIS BEHAVIOR[A] OF OVARIECTOMIZED, AND OVARIECTOMIZED-ADRENALECTOMIZED, ESTROGEN-PRIMED RATS[B]

Group No.	Treatment	No. Rats Ovariectomized	Percent Rats Showing Lordosis and Mean Lordosis Quotient ± Standard Deviation Time After Infusion 15 min	2 hr	4 hr	8 hr	24 hr
			(25%)	(25%)	(25%)	(25%)	(8%)
1	EB + Saline	12	6±11	10±18	8±17	10±18	3±11
			(31%)	(46%)	(69%)	(38%)	(8%)
2	EB + 3 μg St.	13	15±28	30±37	43±35*	17±33	3±10
			(25%)	(94%)	(94%)	(38%)	(8%)
3	EB + 9 μg St.	16	11±26	71±28†	65±31*	16±23	2±7
			(27%)	(36%)	(18%)	(9%)	(0%)
4	EB + 27 μg St.	11	13±29	10±15	4±9	4±11	0
	Ovariectomized and Adrenalectomized						
			(20%)	(20%)	(20%)	(13%)	(13%)
5	EB + saline	15	11±26	14±25	14±23	10±16	12
			(18%)	(41%)	(59%)	(23%)	(4%)
6	EB + 9 μg St.	27	10±22	24±35	44±44*	9±12	6

[A]Lordosis quotient LQ=Lordosis/10 mounts × 100.

[B]All rats received a subcutaneous injection of 4 μg estradiol benzoate at time 0. Infusions of saline or strychnine in 0.5 μl of saline were performed 40 hr after estradiol benzoate.

*$p<0.05$ Mann-Whitney U-test.

†$p<0.01$ Mann-Whitney U-test.

isolation cages, fed Purina and water ad lib and maintained in a room at 23°C with a reversed light cycle (14 hr light-10 hr dark). Ss were ovariectomized under ether anesthesia at least three weeks before treatment. Two weeks after ovariectomy some rats were anesthetized with pentobarbital and mounted in a Kopf stereotaxic apparatus. The skull was exposed and two stainless steel guide cannulae (23 ga) were lowered into the brain through two small holes drilled in the skull with a dental burr. Coordinates for bilaterally-symmetrical cannula placement were selected from the König and Klippel stereotaxic atlas of the rat [12]. Guide cannulae were fixed to the skull with acrylic dental cement. At least one week of recovery was allowed before experimentation. In some rats (groups 5 and 6) bilateral adrenalectomy was performed the same day of the cannula implantation. Adrenalectomized rats were provided with saline ad lib for drinking.

Intrahypothalamic (IH) injections were administered through 30 ga insert cannulae that were introduced into the guide cannulae. The length of the insert cannulae was adjusted to end flush with the guide cannulae. The insert cannula was connected to a 5 μl Hamilton syringe that was driven by a micromanipulator. Injection volumes were 0.5 μl. Rats were lightly anesthetized with ether to perform the intrahypothalamic injections, the duration of which was approximately 30 sec on each side. Ss recovered from the injection procedure within 5 min. Estradiol benzoate (EB), progesterone (P), strychnine sulfate, glycine, β-alanine and taurine were purchased from Sigma. Steroids were dissolved in sesame oil; strychnine, glycine, β-alanine and taurine in distilled water.

Experiment 1

The ability of IH strychnine to facilitate lordosis behavior in ovariectomized or ovariectomized-adrenalectomized estrogen-primed rats was studied in this experiment. Ovariectomized (but not adrenalectomized) rats received a SC injection of 4 μg EB (no P) in 0.2 ml of sesame oil (time 0) followed 40 hr later by one of the following IH injections: *group 1*, saline (12 Ss); *group 2*, 3 μg strychnine (13 Ss); *group 3*, 9 μg strychnine (16 Ss); *group 4*, 27 μg strychnine (11 Ss). Ss were tested for lordosis behavior with vigorous male rats 15 min, 2, 4 and 8 hours after either saline or strychnine administration. Ovariectomized and adrenalectomized rats received (40 hr after EB) IH injections of either saline (*group 5*, 15 Ss) or 9 μg strychnine (*group 6*, 27 Ss). This dose of strychnine (9 μg) was found to be the most effective in facilitating lordosis in ovariectomized estrogen-primed Ss. The effect of 9 μg strychnine on lordosis in ovariectomized, but not estrogen-primed rats was also tested (*group 7*, 8 Ss).

Experiment 2

The ability of IH glycine, β-alanine or taurine to inhibit ongoing lordosis behavior was tested in ovariectomized rats treated with EB and P. Lordosis behavior was induced in ovariectomized rats by the sequential administration of 4 μg

TABLE 2

EFFECT OF INFUSION OF SALINE OR GLYCINE INTO THE VMH* ON THE BEHAVIORAL RESPONSE OF OVARIECTOMIZED ESTROGEN-PRIMED RATS TO THE SC ADMINISTRATION OF 2 mg OF P

Group No.	Treatment	No. Rats	Percent Rats Showing Lordosis and Mean Lordosis Quotient ± Standard Deviation 2 hr	4 hr	8 hr
			(72%)	(100%)	(100%)
12	EB + P + saline	11	54±42	93±16	96±12
			(85%)	(100%)	(80%)
13	EB + P + Glycine 20 μg	20	66±37	85±26	74±44
			(60%)	(60%)	(80%)
14	EB + P + Glycine 100 μg	16	66±28	67±42	78±26

*Glycine was infused within 15 min of P administration.

EB and 2 mg P (30 hr after EB). Four hours after P, when the Ss were expressing intense lordosis behavior (LQ >80), they received injections, bilaterally into VMH, of 0.5 μl of saline (*group 8*, 8 Ss) 100 μg glycine (*group 9*, 8 Ss), 100 μg of β-alanine (*group 10*, 8 Ss) or 50 μg taurine (*group 11*, 8 Ss). Taurine was injected in a smaller dosage due to its relative insolubility. Testing was resumed 5 min after the amino acid injections, and LQs were determined at 5, 15, 30 and 60 min postinjection.

Experiment 3

The ability of IH glycine to antagonize the facilitatory effect of P on ovariectomized EB-primed rats was tested in this experiment. Ovariectomized rats received, 40 hr after 4 μg EB, a SC injection of 2 mg P. Within 15 minutes after P, one of the following IH injection treatments was given: *group 12*, saline (18 Ss); *group 13*, 20 μg glycine (20 Ss); *group 14*, 100 μg glycine (8 rats). Rats were tested for lordosis behavior 2, 4 and 8 hr after P.

Behavioral Tests

Ss were tested for lordosis behavior in a circular Plexiglas cage (53 cm diameter) with selected vigorous males. Each female received 10 vigorous mounts per test. Receptivity was quantified by the lordosis quotient (LQ=number of lordoses per 10 mounts × 100). LQs in the various groups were compared using the Mann-Whitney U-test. Proportions of responsive individuals were compared by the Chi-square test.

At the completion of the experiments, implanted rats were sacrificed by overexposure to ether and the brain was perfused via the heart with 10% formalin. The brains were subsequently removed for histological analysis. They were embedded in paraffin, cut every 200 μg, and stained with hematoxylin-eosin. Only Ss showing cannula placement in the VMH or within 0.5 mm of this nucleus were included in the experimental analysis; the number of Ss/group specified above represents only these rats.

RESULTS

Experiment 1

Facilitation of lordosis behavior by strychnine infused into the VMH. Table 1 shows the effect of the IH administration of saline and of three dosages of strychnine on the lordosis behavior of ovariectomized rats primed with 4 μg EB. Only 25% of the saline-infused rats displayed lordosis behavior, and the LQs were uniformly low across all tests. Strychnine at 3 and 9 μg dose levels, but not at the highest dose (27 μg), induced a significant elevation in LQ that was evident 2 hr after its administration in the 9 μg dose group. At this time, LQ values of the 9 μg group were significantly higher than those of the saline group. At 4 hr the 3 μg group first became significantly greater than the saline group, and did not differ significantly from the 9 μg group, both of which remained significantly greater than the saline group. LQ in rats receiving the highest strychnine dose (27 μg) did not differ from that of saline injected rats. No overt signs of sickness were noted in any strychnine treated Ss. Ovariectomized-adrenalectomized EB-treated rats also responded to the IH infusion of 9 μg strychnine with significantly elevated levels of LQ (Table 1). At 2 hr but not 4 hr these rats showed a significantly lower LQ than ovariectomized rats that had received 9 μg strychnine. Strychnine (9 μg) infused into the VMH of ovariectomized non-estrogen-treated rats failed to induce lordosis (group 7) (maximum LQ=8±14 SD at 2 hr).

Experiment 2

Effect of the administration of amino acids during ongoing lordosis behavior. In ovariectomized rats that received sequential administration of EB and P, the IH infusion of saline (group 8), 100 μg glycine (group 9), β-alanine (group 10), or of 50 μg taurine (group 11) 4 hr after P failed to depress the intense lordotic behavior (selected at LQ >80) intentionally induced by the steroids.

Experiment 3

Effect of the administration of glycine 15 min after P (before lordosis appears) on subsequent lordosis behavior. As shown in Table 2, starting 15 min after systemic P injection in estrogen-primed rats, injections of 20 or 100 μg glycine (groups 13 and 14) into the VMH failed to reduce lordosis behavior significantly, compared to saline controls (group 12) when tested at 2, 4, or 8 hr.

DISCUSSION

Various brain areas, e.g., the cerebral cortex, septum or medial preoptic area, exert tonic inhibitory influences on the expression of lordosis. Lesions in some of these structures can facilitate lordosis behavior in estrogen-primed rats [5, 11, 20, 24] while electrical stimulation of some of them, e.g., preoptic area, tends to depress it [23]. Since these brain areas have rich direct and indirect connections with the VMH [21,30] it is possible that their inhibitory action on lordosis is mediated through modulation of the firing of VMH neurons. Electrophysiological studies have shown that the inhibitory effect exerted on the activity of VMH neurons by telencephalic structures, e.g., the amygdala, are mediated by inhibitory interneurons [18, 19, 25]. It is tempting to speculate that their inhibition is mediated through interneurons using GABA or glycine neurotransmitters. GABA and glycine, the two major inhibitory neurotransmitters, are

present in substantial amounts in the hypothalamus of the rat [7,32], and their microiontophoretic administration into the hypothalamus drastically inhibits neuronal firing [6,16]. The present findings show that the intrahypothalamic administration of strychnine facilitates lordosis behavior in ovariectomized or ovariectomized-adrenalectomized, estrogen-primed rats. This effect was most likely due to a local action on hypothalamic neurons since spread to the ventricles must have been minimal with the small volume used in this study (0.5 μl). Moreover motor effects that are obtained with infusions of very low amounts of strychnine (1 μg) in either the ventricles or the perispinal space [3] were absent from our animals, even those receiving the highest dose of strychnine (27 μg). Strychnine increases spontaneous firing rates [16] and selectively antagonizes the inhibitory responses elicited by glycine in hypothalamic neurons [6,16]. Therefore, it is likely that IH strychnine infusion increases the rate of firing of VMH neurons. The effect of strychnine on lordosis was not immediate but gradual, as when neurons are excited by direct electrical stimulation [22]. The failure of the large dose of strychnine (27 μg) to stimulate lordosis cannot be explained by the blockade of glycine postsynaptic receptors. This process would be expected to lead to an increase in VMH neuronal firing and lordosis facilitation. The high concentration of strychnine may have interfered with the action of other neurotransmitters important for the expression of lordosis, e.g., acetylcholine, but further studies are required to clarify this observation.

Since strychnine stimulated lordosis, it could have been anticipated that IH glycine or glycine agonists would have attenuated the expression of lordosis induced in ovariectomized rats by the sequential administration of EB and P. However, IH glycine failed to either depress (experiment 2) or prevent (experiment 3) the display of lordosis behavior induced by E and P. Similarly, β-alanine and taurine, that also inhibit hypothalamic neuronal firing [4], did not interfere with the expression of lordosis when administered to highly estrous rats. Some explanations can be proposed to account for these observations. It is possible that the IH injection of glycine and the other amino acids failed to produce a sufficiently prolonged depression of VMH neuronal activity to inhibit lordosis. Extremely efficient uptake systems have been reported for glycine and β-alanine [9], and these mechanisms rapidly terminate the action of inhibitory neurotransmitter at the postsynaptic site. However, an increase in prolactin secretion has been reported following intrahypothalamic injections of glycine in rats [1], thus indicating that this procedure can lead to significant effects even when rapid removal of the inhibitory neurotransmitter occurs in the synapses [9]. In our opinion, a more likely explanation is based on the fact that P may act at other brain regions besides the VMH to facilitate lordosis in estrogen primed rats (mesencephalic tegmentum [14,17], habenula [31]). Therefore, effects on these structures by systemic injections of optimal doses of EB and P probably induced lordosis despite the probable depression of the activity of the VMH by the infusion of the inhibitory neurotransmitters. This possibility is strongly supported by the finding that bilateral lesions of the VMH in the rat do not suppress lordosis in response to the combined treatment of estrogen and P [15]. In summary, the present results support the idea of the existence of tonic inhibitory neural systems controlling sexual behavior in the female rat, and suggest that glycine may be one of the inhibitory factors involved in the control of lordosis at the hypothalamic level.

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