Prazosin blocks the glutamatergic effects of N-methyl-D-aspartic acid on lordosis behavior and luteinizing hormone secretion in the estrogen-primed female rat

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Abstract

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We have observed that intracerebroventricular (*icv*) injection of selective N-methyl-D-aspartic acid (NMDA)-type glutamatergic receptor antagonists inhibits lordosis in ovariectomized (OVX), estrogen-primed rats receiving progesterone or luteinizing hormone-releasing hormone (LHRH). When NMDA was injected into OVX estrogen-primed rats, it induced a significant increase in lordosis. The interaction between LHRH and glutamate was previously explored by us and another groups. The noradrenergic systems have a functional role in the regulation of LHRH release. The purpose of the present study was to explore the interaction between glutamatergic and noradrenergic transmission. The action of prazosin, an $\alpha 1$- and $\alpha 2b$-noradrenergic antagonist, was studied here by injecting it *icv* (1.75 and 3.5 µg/6 µL) prior to NMDA administration (1 µg/2 µL) in OVX estrogen-primed Sprague-Dawley rats (240-270 g). Rats manually restrained were injected over a period of 2 min, and tested 1.5 h later. The enhancing effect induced by NMDA on the lordosis/mount ratio at high doses (67.06 ± 3.28, N = 28) when compared to saline controls (6 and 2 µL, 16.59 ± 3.20, N = 27) was abolished by prazosin administration (17.04 ± 5.52, N = 17, and 9.33 ± 3.21, N = 20, P < 0.001 for both doses). Plasma LH levels decreased significantly only with the higher dose of prazosin (1.99 ± 0.24 ng/mL, N = 18, compared to saline-NMDA effect, 5.96 ± 2.01 ng/mL, N = 13, P < 0.05). Behavioral effects seem to be more sensitive to the α-blockade than hormonal effects. These findings strongly suggest that the facilitatory effects of NMDA on both lordosis and LH secretion in this model are mediated by α-noradrenergic transmission.

Introduction

Glutamic acid is the main excitatory amino acid neurotransmitter in the mammalian brain (1). In previous studies, we observed that the activation of lordosis induced by progesterone and luteinizing hormone-releasing hormone (LHRH) in ovariectomized (OVX) estrogen-primed rats can be blocked by the intracerebroventricular (*icv*) administration of 7-amino-phosphonoheptanoic acid (AP-7), a selective N-methyl-D-aspartic-acid (NMDA)-glutamatergic antagonist (2). The *icv* administration of NMDA to OVX estrogen-primed rats induces an increase in lordosis and plasma LH (3). This effect on lordosis appears not to be mediated by LHRH, since a selective LHRH antagonist did not affect the lordosis/mount ratio (L/M), but the effect was decreased by AP-7 (3). The endocrine effect appears to be mediated by LHRH, since *icv* administration of a selective LHRH antagonist blunted the secretion of LH induced by NMDA (3). We previously raised the possibility that the NMDA effects might be mediated by other neurotransmitters (3).

On the basis of these considerations and of the relevance of noradrenergic transmission in lordosis and LH release (4), we investigated the possible involvement of noradrenergic transmission in NMDA-induced effects on these two variables. The aim of the experiments described here was to identify possible interactions between glutamatergic and noradrenergic transmission in lordosis. These interactions were considered because noradrenaline is released by glutamate (5-7). The stimulatory actions of NMDA appear to be hormone dependent (3). Estradiol appears to regulate the number of α1- but not ß- or α2-noradrenergic receptors in the hypothalamus of female rats (8). For this reason, we studied the sexual behavior and LH release of female rats previously treated with different doses of a noradrenergic receptor antagonist in order to establish or not a relationship between behavioral and hormonal effects.

Material and Methods

Animals

Female Sprague-Dawley rats (240-270 g) were ovariectomized under ether anesthesia and kept on a reverse light cycle (lights on from 19:00-7:00 h) in a temperature-controlled (22 ± 2ºC) room. Animals were allowed access to food and water *ad libitum*. Two weeks prior to the experiments, the receptivity of the rats was determined. Animals were injected subcutaneously (*sc*) with 20 µg estradiol benzoate (EB) 48 h before testing, and 1 mg progesterone *sc* 4 h before testing in order to select responsive rats. In the experiments, only EB *sc* 48 h before testing was used for priming, and progesterone was not administered. Mating tests were conducted in a rectangular arena illuminated by a red light during the dark period, in the morning (8:00-10:00 h). Two stud males (300-400 g) were introduced into the arena at least 10 min before the female. The behavioral tests ended when the test female had received 30 male copulatory acts or after 15 min.

The L/M ratio was established for each rat. Only responsive rats with an L/M of at least 80% (criterion of inclusion) in previous tests were selected for stereotaxic implantation of a 23-gauge stainless steel guide cannula into the 3rd brain ventricle under ether anesthesia. Coordinates for cannula implantation were: A-C: bregma; V: 8.5 mm; lateral: midline (9). Localization of the cannula was confirmed when cerebrospinal fluid flowed from the cannula after removal of an inner stylet used to prevent such leakage. The cannula was cemented to the skull. After surgery, the animals were housed individually and left undisturbed during recovery. Drugs were injected *icv* through a 30-gauge stainless steel tube fitted into the guide cannula and connected to a 10-µL Hamilton

syringe with a polyethylene catheter. In all cases, a volume between 2 and 6 µL of solution was delivered over a period of 2 min. During *icv* injections, animals were manually restrained. Each animal was tested only once.

Experiments

In the experiments, the effect of prazosin on lordosis induced by NMDA was studied. Rats implanted with an *icv* guide cannula received an *sc* injection of 20 µg EB in 0.2 mL corn oil. They were injected 48 h later with saline (SAL, two injections of 6 and 2 µL, respectively), SAL (6 µL) and NMDA (1 µg/2 µL), or prazosin (1.75 and 3.50 µg/6 µL) and NMDA (1 µg/2 µL), and tested for lordosis 1.5 h later. In all cases, the injections were separated by a 10-min interval. In each mating session, rats injected with drugs and controls were tested simultaneously.

Lordosis was evaluated 1.5 h after administration of the drugs. To determine LH release during copulatory behavior, rats were sacrificed by decapitation immediately after completion of the lordosis tests, and blood was collected from the trunk. Rats used for LH determination in blood samples were randomly selected. Plasma was separated and stored frozen (-70ºC) until assay for LH by radioimmunoassay. LH was measured with a double-antibody radioimmunoassay performed with materials and according to the instructions of the kits provided by the National Hormone & Pituitary Program (Harbor-UCLA Medical Center, Torrance, CA, USA). The intra- and inter-assay coefficients of variation were 9 and 11%, respectively. Data are reported as ng/mL serum after comparison to NIDDK-rLH-RP1 reference samples.

Drugs

The following drugs were used: NMDA (Research Biochemicals International, Na-

tick, MA, USA), and an α1- and α2b-adrenoceptor antagonist (10), prazosin (1-(4-amino-6,7-dimethoxy-2-quinazolinyl)-4-(2-furanylcarbonyl) piperazine hydrochloride (Research Biochemicals International). They were dissolved in saline and the pH was adjusted to near physiological pH. EB and progesterone (Sigma-Aldrich, St. Louis, MO, USA) were dissolved in corn oil.

Data analysis

The L/M data were analyzed by the Kruskal-Wallis test followed by the Dunn test. Hormone values were analyzed by ANOVA 1 followed by the Student Newman-Keuls test. In all cases, the level of significance was set at $P < 0.05$. Data are reported as means ± SEM.

Results

L/M ratios were modified by injection of the drugs tested (Kruskal-Wallis = 53.89, d.f. = 3). The injection of NMDA (SAL-NMDA, N = 28) increased the L/M ratio to a very significant extent compared to control (SAL-SAL, N = 27, $P < 0.001$, Figure 1). The injection of both doses of prazosin (N = 17 for the lower dose, and N = 20 for the higher dose) decreased the scores to control levels ($P < 0.001$, Figure 1).

Plasma LH concentrations were modified by the treatments [(F = 3,461, d.f. treatment = 3, d.f. residual = 45)]. They were significantly increased by NMDA (SAL-
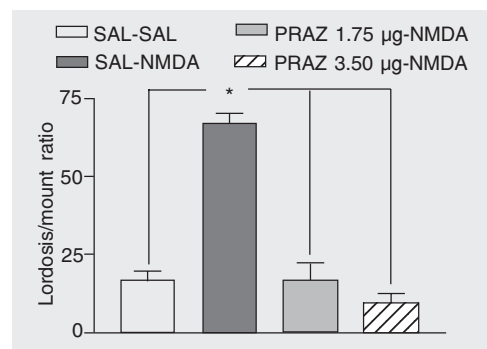

Figure 1. Prazosin inhibition of the NMDA-induced increase of lordosis in ovariectomized estradiol-primed female rats. SAL = saline; NMDA = N-methyl-D-aspartic acid; PRAZ = prazosin. Groups: SAL (6 µL)-SAL (2 µL), N = 27; SAL (6 µL)-NMDA (1 µg/2 µL), N = 28; PRAZ (1.75 µg/6 µL)-NMDA (1 µg/2 µL), N = 17; PRAZ (3.50 µg/6 µL)-NMDA (1 µg/2 µL), N = 20. *P < 0.001 compared to other groups (Kruskal-Wallis test followed by Dunn test).

NMDA, N = 13, P < 0.05) compared to control (SAL-SAL, N = 12, Figure 2). The administration of prazosin at the lower dose (1.75 μg prazosin-NMDA, N = 6) did not significantly reduce the increase of LH values induced by NMDA. No differences were observed between the lower dose of prazosin and the SAL-NMDA group, or the SAL-SAL group. However, a significant decrease in LH values was observed when the higher dose of prazosin was administered (3.5 μg prazosin-NMDA, N = 18, P < 0.05) compared to the NMDA group (SAL-NMDA), leading to plasma concentrations of LH that were equivalent to those of controls.
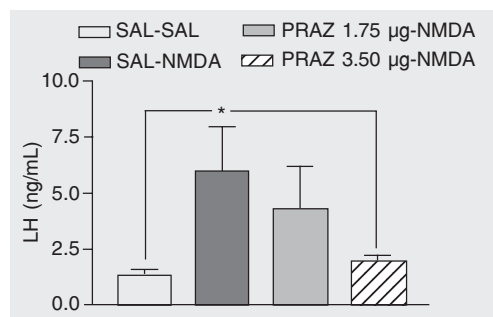
Discussion

The results presented here support the view that the enhancing effects of NMDA glutamatergic neurotransmission on mating behavior and LH release are mediated by α-noradrenergic transmission, thus extending our previous observations (3). The increase in L/M ratio induced by NMDA was clearly decreased by prazosin administration (P < 0.001 for both doses). Plasma LH levels were decreased only by the higher dose of prazosin (P < 0.05). Another interesting finding is that behavioral effects seem to be more sensitive to adrenergic blockade than the hormonal effects. In other studies, the role of α1-receptors has been linked to lordosis and LH release (4). Prazosin has been used in female Rhesus macaques to antagonize NMDA-induced LH release (11). In the cited study (11), however, copulatory behavior

Figure 2. Prazosin inhibition of the NMDA-induced increase in plasma luteinizing hormone (LH) values in ovariectomized estradiol-primed female rats. SAL = saline; NMDA = N-methyl-D-aspartic acid; PRAZ = prazosin. Groups: SAL (6 μL)-SAL (2 μL), N = 12; SAL (6 μL)-NMDA (1 μg/2 μL), N = 13; PRAZ (1.75 μg/6 μL)-NMDA (1 μg/2 μL), N = 6; PRAZ (3.50 μg/6 μL)-NMDA (1 μg/2 μL), N = 18. *P < 0.05 compared to SAL-SAL and PRAZ 3.50 μg-NMDA groups (ANOVA 1 test followed by Student Newman-Keuls test).

was not investigated and drugs were administered during the luteal phase of the menstrual cycle, before administration of NMDA. The present study extends these results, establishing a comparison between behavioral and hormonal effects.

We have observed that the enhancement of lordosis induced by NMDA is not mediated by LHRH, which is involved in NMDA-mediated endocrine effects (3). The present findings support the idea that a noradrenergic mechanism could mediate these effects. The hormonal status of the animals appears to play an important role since, under our previous experimental conditions, in OVX female rats not primed with EB, NMDA had no effects on lordosis and decreased plasma LH levels (3). Estradiol priming increases α1-receptors, which have facilitatory effects on the parameters considered here (4,8,12). The effects of NMDA could be mediated by noradrenaline release since α1-blockade interferes with the facilitatory effects on mating behavior and plasma LH values (4). Excitatory pathways appear to mediate these effects by α1-adrenoceptors, and these receptors use cyclic adenosine monophosphate as second messenger (12). The blockade of LH secretion by α1-adrenergic receptor antagonists has been described (13), and brain-stem catecholaminergic neurons are activated by mating in the female rat (14). The different influences of hormonal status on noradrenergic receptors may explain why both stimulatory (3) and inhibitory (15,16) effects of excitatory amino acids on lordosis have been reported.

Axo-axonic contacts and stimulation of distal neurones could be mediating these effects. Excitatory amino acids are present in the presynaptic boutons of several important hypothalamic nuclei (1), and noradrenaline is released in response to excitatory amino acids in the rat mediobasal hypothalamus (5-7) and at other brain sites. Glutamate-stimulated noradrenaline release has been described in minislices of the bed

nucleus of the stria terminalis (17) and superfused rat hippocampal synaptosomes, suggesting the presence of NMDA receptors in hippocampal noradrenergic axon terminals (18). Similar results have also been observed in slices of the prefrontal cortex (19). These findings support the idea that functions elicited by NMDA receptors are mediated by noradrenergic release in several brain areas.

It is possible that glutamatergic neurons act on distal noradrenergic neurons (through axo-somatic contacts), since applying NMDA to the locus ceruleus (LC) increases noradrenaline release in other brain areas (20). Conversely, LC lesions decrease noradrenaline input into the medial preoptic area and medial basal hypothalamus, blocking the LH, follicle-stimulating hormone and prolactin preovulatory surge (21). The activation by genital-somatosensory signals (22) of midbrain and brainstem noradrenergic neurons, which project to the mediobasal hypothalamus, promotes the release of LHRH from nerve terminals in the median eminence. Similarly, it has recently been pointed out that LHRH release depends, at least in part, on LC noradrenergic inputs to the medial preoptic area and median eminence by activation of LHRH neurons (23). Furthermore, the LC appears to have an intrinsic cyclic activity which is amplified by ovarian steroids (23). Interaction of NMDA and noradrenaline, and noradrenaline and LHRH, appears to occur in different brain areas. In the present study we injected the drugs *icv*, but in future studies it would be enlightening to define the zones involved more precisely.

The present results strongly suggest the noradrenergic mediation of facilitatory NMDA-induced effects on lordosis and plasma LH values. Our findings support the idea that the stimulatory effect of NMDA in this model could be mediated by α-noradrenergic receptors.

Acknowledgments

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