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FACILITATION OF ESTROUS BEHAVIOR BY VAGINAL CERVICAL STIMULATION IN FEMALE RATS INVOLVES α_1 -ADRENERGIC RECEPTOR ACTIVATION OF THE NITRIC OXIDE PATHWAY

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Abstract

In estrogen-primed female rats, vaginal cervical stimulation (VCS) provided by male intromissions or by an experimenter enhances estrous behaviors exhibited by females during subsequent mating with a male. We tested the hypothesis that α_1 -adrenergic receptors, acting via the nitric oxide-cGMP/protein kinase G pathway, mediate VCS-induced facilitation of female reproductive behaviors. Ovariectomized, estradiol-primed rats received intracerebroventricular (icv) infusions of vehicle or pharmacological antagonists 15 or 60 min before VCS. Estrous behaviors (lordosis and proceptivity) in the presence of a male were recorded immediately (0 min), and 120 min following VCS. First we verified that VCS, but not manual flank stimulation alone, enhanced estrous behaviors when females received icv infusion of the vehicles used to administer drugs. Increased estrous behavior was apparent immediately following VCS and persisted for 120 min. We then infused prazosin, phenoxybenzamine (α_1 -adrenergic receptor antagonists), yohimbine, idaxozan (α_2 -adrenergic receptor antagonists), or propranolol (β -adrenergic receptor antagonist) 15 min prior to the application of VCS in females primed with 5 μ g estradiol benzoate. Only α_1 -adrenergic antagonists inhibited VCS facilitation of estrous behavior, apparent 120 min after VCS. Finally, we administered specific inhibitors of soluble guanylyl cyclase, nitric oxide synthase or protein kinase G icv 15 or 60 min before VCS. All three agents significantly attenuated VCS facilitation of estrous behavior. These data support the hypothesis that endogenously released norepinephrine, acting via α_1 -adrenergic receptors, mediates the facilitation of lordosis by VCS, and are consistent with a mechanism involving α_1 -adrenergic activation of the nitric oxide/cGMP/protein kinase G pathway.

Keywords

Estrous behavior; vaginocervical stimulation; adrenergic receptors; nitric oxide; cGMP

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Introduction

Estradiol (E_2) primes the neural substrates that mediate estrous behaviors (lordosis and proceptive behaviors), and subsequently progesterone (P) acts on the E_2 -primed brain to trigger the onset and increase the intensity of these behaviors [2,14,15,22,45]. During a mating sequence in rats, females receive several mounts without vaginal intromission, mounts with intromission, and several ejaculations from a male, resulting in flank-perineal and vaginocervical stimulation (VCS). Normal VCS induces neuroendocrine and behavioral changes in female mammals that are critical to establish pregnancy [17,20,33] and accelerates the termination of sexual behavior in hamsters and rats [6,35]. Artificial VCS and extravaginal stimuli, such as flank-perineal stimulation, facilitate lordosis in ovariectomized (ovx) female rats primed with E_2 [38,54].

During the estrous cycle and in ovx, hormone-treated rats, VCS induces the release of several neurotransmitters [43] and signaling molecules, including norepinephrine (NE) and nitric oxide (NO), in CNS areas that regulate various reproductive processes [24,31,37]. Sensory information from the uterus and cervix is transmitted in the anterolateral columns of the spinal cord via afferent fibers in the hypogastric, pelvic, and vagus nerves [5,11,13,18,39]. Many of these fibers terminate in the brainstem regions that give rise to the ventral NE bundle (e.g., nucleus of the solitary tract, a major site of convergence of visceral sensory information). NE neurons in this region then project to the hypothalamus. Bilateral transection of the pelvic nerve [5] or lesions of the ventral NE bundle [34] decrease the lordosis response and completely prevent the occurrence of VCS-induced pseudopregnancy [19,21,41].

Although the cellular mechanisms underlying the stimulation of sexual behavior by VCS are not clear, it is known that both NE and NO participate in the expression of hormone-dependent female sexual behaviors [7–9,24,27,30,40,42,46]. Thus, microdialysis studies demonstrate that NE release increases dramatically in the ventromedial hypothalamus when copulation begins in E_2 - and progesterone-treated female rats. Moreover, VCS rather than chemosensory or flank-perineal stimuli evoke NE release in sexually receptive rats [24,58]. Studies in which NE, NE agonists, or NE antagonists were administered systemically or intracerebrally point to a critical role for hypothalamic α_1 -adrenergic receptors in the regulation of lordosis behavior in E_2 -primed rodents. For example, both NE- and P-facilitated lordosis is blocked by systemic or intrahypothalamic administration of the α_1 -adrenergic antagonist prazosin [8,12,23,25,27,40,46,58]. Erskine and colleagues (47) recently implicated α_1 -adrenoceptors in other responses to VCS by showing that infusion of an α_1 -adrenergic agonist as well as an α_2 -autoreceptor antagonist into the ventrolateral ventromedial hypothalamus facilitated pseudopregnancy induced by VCS while an opposite effect was found with administration of an α_1 antagonist.

The NO pathway also plays a crucial role in the facilitation of lordosis behavior by P and its ring A-reduced metabolites [7–9,30,42]. A major mediator of cellular responses to NO is cGMP, which is synthesized when soluble guanylyl cyclase is stimulated by NO [3,52,53]. Intracerebroventricular (icv) infusions of a selective inhibitor of NO-stimulated soluble guanylyl cyclase decrease lordosis behavior in ovx rats treated with E_2 plus P. Icv infusion of 8-bromo-cGMP also enhances reproductive behaviors in rats primed only with E_2 and reverses the inhibitory effects of the α_1 -adrenoreceptor antagonist prazosin on lordosis behavior [8]. NE and the α_1 -adrenoreceptor agonist phenylephrine stimulate cGMP accumulation in hypothalamic and preoptic area slices, but only if tissue is derived from ovx females pretreated with both E_2 and P. Thus, the NO/cGMP pathway most likely mediates the facilitatory effects of α_1 -adrenoreceptors on lordosis behavior in female rats, and prior exposure of the hypothalamus and preoptic area to both E_2 and P is required to link α_1 -adrenoceptors to this pathway. The present study tested the hypothesis that the α_1 -adrenoceptor-NO-cGMP pathway,

via activation of protein kinase G (PKG), is involved in the stimulation of female sexual behavior by VCS. This idea was tested by infusing selective inhibitors of adrenoceptor subtypes and of the NO-cGMP-PKG pathway into the lateral ventricle of ovx, E₂-primed female rats before application of VCS. VCS was administered using a vaginal probe that allowed us to deliver a quantifiable and reproducible stimulus to the vagina and cervix.

Material and methods

Animals and Surgery

Eighty seven adult Sprague–Dawley rats (200–260 g) bred in our colony in Tlaxcala were used and maintained on a 14-h light/10-h dark cycle with lights off at 1000 h. The females were bilaterally ovx under ether anesthesia and housed in groups of 4 per cage. Two weeks later, they were anesthetized with xylazine (4 mg/kg) and ketamine (80 mg/kg) and placed in a Kopf stereotaxic instrument (Tujunga, CA) for implantation of a stainless steel cannula (22 gauge, 17 mm long) into the right lateral ventricle following coordinates from the atlas of Paxinos and Watson [48] (A/P +0.80 mm, M/L -1.5 mm, D/V -3.5 mm with respect to bregma). A stainless steel screw was fixed to the skull and both cannula and screw were attached to the bone with dental cement. A dummy cannula (30 gauge) provided with a cap was introduced into the guide cannula to prevent clogging and contamination. Immediately after the cannula implantation the females were injected with penicillin (22,000 IU/kg). One week after surgery, all females received a s.c. injection of 5 µg of E₂ benzoate (E₂B; Sigma-Aldrich, St. Louis, MO) dissolved in 0.1 ml corn oil 40 h before drug infusions and manual flank stimulation (MFS)/VCS or MFS only. All drugs were purchased from Sigma-Aldrich and were administered icv in a volume of 2 µl through the guide cannula in the right lateral ventricle over 1 min, and another 1 min was allowed for drug diffusion before the removal of the infusion needle. All procedures used in these experiments followed the Mexican Law for the Protection of Animals and were approved by the Institutional Animal Care and Use Committee at CINVESTAV-Universidad Autónoma de Tlaxcala.

Behavioral Testing

Between 8 and 12 rats were assigned to each treatment group. Most females were tested twice with a two week interval between tests. These animals were incorporated into groups at random, but no rat received the same treatment twice. Control animals that received only MFS or that received MFS/VCS plus one of the vehicles used for drug infusion were tested only once. Forty hr after E₂B injection, females received either 150 g of pressure into the vagina-cervix through a calibrated vaginal probe [38] together with MFS or MFS only without VCS for approximately 5 sec. MFS consisted of palpations applied with the finger thumb to both flanks and with the palm of the hand to the perineal area of the rat. Immediately (0 min) and 120 min following these procedures (MFS with or without VCS), females were placed in a circular plexiglas arena (53 cm diameter) until they received 10 mounts with pelvic thrusting from an experienced stimulus male. The lordosis quotient [LQ = (number of lordosis/10 mounts) X 100] was used to assess lordosis behavior. The intensity of lordosis was quantified according to the lordosis score (scale of 0–3) proposed by Hardy and DeBold [35]. Proceptivity was analyzed by determining the incidence of hopping, darting and ear-wiggling across the whole receptivity test. Females were considered proceptive when showing any of these behaviors.

Experiment 1. Effect of icv infusions of adrenergic antagonists on MFS/VCS-induced sexual behavior

This experiment tested the effects of α and β adrenergic antagonists on the response to MFS/VCS. E₂B-primed animals were randomly assigned to receive an infusion of one adrenergic antagonist (α_1 , α_2 or β) 15 min before receiving MFS/VCS. Two weeks later females were tested again with a different α or β adrenergic antagonist. The α_1 -adrenergic antagonists were

prazosin and phenoxybenzamine; α_2 -adrenergic antagonists were yohimbine and idaxozan; and the β -adrenergic antagonist was propranolol. Because different drugs were dissolved in different vehicles (see below), separate control animals were primed with E₂B and infused icv with one of these vehicles just before application of MFS/VCS. Additional controls received VCS alone. The vehicles infused and the number of animals in these control groups were: MFS without VCS (n=12); MFS/VCS + saline (n=12); MFS/VCS plus propylene glycol (n=10); MFS/VCS plus sterile distilled water (n=10); and MFS/VCS plus 10% dimethylsulfoxide (DMSO; vehicle for drugs in Experiment 2) (n=9). The doses, vehicles and number of animals infused with adrenergic antagonists were: 160 μ g of prazosin (n= 9) prepared in saline containing 25% propylene glycol [8]; 5 μ g of phenoxybenzamine (n=9) dissolved in propylene glycol [28]; 5 μ g of idaxozan (n=8) dissolved in sterile distilled water; 5 μ g of yohimbine (n=9) dissolved in sterile distilled water [4,59]; and 5 μ g of propranolol (n=8) dissolved in saline.

Experiment 2. Effect of icv infusion of NO pathway inhibitors prior to MFS/VCS

This experiment tested the effects of different inhibitors of the NO-cGMP-PKG pathway on estrous behavior induced by MFS/VCS in E₂B-primed animals. E₂B-primed animals were infused with an NO pathway inhibitor at various times before receiving MFS/VCS; two weeks later females were tested again with a different NO pathway inhibitor. Fifteen min before MFS/VCS, E₂B-primed female rats were infused icv with the NO synthase inhibitor NG-nitro-L-arginine methyl ester (L-NAME), a specific inhibitor of soluble guanylyl cyclase, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ), or the specific PKG inhibitor KT5823. Drug-infused groups were compared with their corresponding vehicle-treated control groups (see description of Experiment 1). The doses, vehicles and number of animals utilized were: 500 μ g of L-NAME (n=8) dissolved in saline, 22 μ g of ODQ (n=8) and 0.12 μ g of KT5823 (n=8), both dissolved in 10% DMSO. The doses of L-NAME, ODQ and KT5823 were selected on the basis of our previous work [7–9,30]. L-NAME was purchased from Sigma-Aldrich. ODQ was obtained from Tocris Cookson (St. Louis, MO), and KT5823 was purchased from CalBiochem (La Jolla, CA). Additional animals received icv infusion of the same doses of L-NAME or ODQ (n=8/drug) 1 hr before application of MFS/VCS.

Verification of cannula placements

One day after the final test for lordosis, the animals were anesthetized with ether, and 1% methylene blue was administered through the cannula. The brain was removed and sectioned in the transverse plane to check the cannula position in the lateral ventricle. The animals whose cannulae were not in the ventricle were discarded from the experiment (6 animals).

Statistical analysis

To assess the effect of MFS vs MFS/VCS + vehicle in the first experiment, we used first the Kruskal Wallis test (significance level $P < 0.05$) for each of two the times tested. Subsequently, the results obtained at 0 and 120 min were compared using the Mann Whitney test (significance level $p < 0.05$). Proportion of females that displayed proceptivity was compared with the Fisher test. For the experiment involving adrenergic antagonists and vehicles, a Kruskal-Wallis test was performed to analyze lordosis behavior; when relevant the Mann Whitney test was used to compare lordosis behavior in specific groups receiving the experimental drug with control animals receiving the corresponding vehicle for each of the times tested (0 and 120 min). A similar procedure was used for analysis in experiments involving the NO-cGMP pathway inhibitors.

Results

Effect of MFS/VCS on the expression of female sexual behaviors

Table 1 shows that lordosis and proceptive behavior in the presence of a male were stimulated in ovx, E₂B-primed female rats that received MFS/VCS plus vehicle, but not in females that received MFS alone. The Mann Whitney test showed a significant increase in LQ immediately after MFS/VCS (0 min), but not after MFS alone, in each one of the vehicle-treated groups ($P < 0.05$ to $P < 0.001$; see Table 1). This effect persisted for at least 120 min after MFS/VCS. Between 30 and 40% of females that were infused with vehicle and received MFS/VCS also began to exhibit proceptive behavior immediately (0 min) after MFS/VCS (Table 1). A significantly greater proportion of females displayed proceptive behavior at 120 min post-MFS/VCS plus vehicle ($P < 0.01$) than at 0 min. Females that received MFS without VCS did not display proceptive behaviors at any time. Because females receiving only MFS did not exhibit reproductive behavior, we attribute the effects of combined MFS/VCS to the VCS component and refer to VCS alone in discussing the results.

Effect of icv infusions of α and β adrenergic antagonists on MFS/VCS-induced sexual behavior

Administration of prazosin (α_1 antagonist) into the lateral ventricle significantly attenuated the MFS/VCS-induced increase in LQ at 120 min ($P < 0.01$) post-MFS/VCS when compared to vehicle controls, but this difference was not significant at 0 min (Fig 1A). This inhibition of lordosis by prazosin persisted at 240 min post-MFS/VCS ($P < 0.05$; data not shown). A different α_1 antagonist, phenoxybenzamine, also attenuated MFS/VCS-induced increases in LQ at 120 min post-MFS/VCS ($P < 0.01$; Fig. 1A). In addition, MFS/VCS-induced proceptive behavior was significantly suppressed by both antagonists at 120 min post-MFS/VCS ($P < 0.05$; Fig 1B). We did not include control groups treated with prazosin or phenoxybenzamine without MFS/VCS, because previous studies showed that these compounds did not increase lordosis behavior [27,46].

We also tested the effect of α_2 -adrenergic antagonists (idaxozan and yohimbine) and a β -adrenergic antagonist (propranolol) on MFS/VCS-induced increases in LQ and proceptivity (Table 2). None of these agents significantly affected either lordosis or proceptive behavior induced by MFS/VCS. The non-significant tendency for yohimbine to decrease estrous behaviors may reflect its partial α_1 antagonist activity.

Effect of icv infusion of NO pathway inhibitors prior to MFS/VCS

In ovx, E₂B-primed females that received icv injections of L-NAME or ODQ 15 min prior to MFS/VCS (Fig. 2A), LQ scores were significantly reduced at 120 min ($P < 0.01$ and 0.001 respectively vs corresponding vehicle). However, neither L-NAME nor ODQ prevented the immediate (0 min) increase in lordosis behavior shown in control rats following MFS/VCS ($P > 0.05$; Fig. 2A). LQ scores in rats receiving MFS/VCS plus L-NAME or ODQ did not differ significantly from the group that received MFS without VCS (Table 1) at any time point. Administration of L-NAME and ODQ significantly reduced proceptivity at 120 min post-MFS/VCS (Table 3). Thus, 12% and 0% of females treated with L-NAME or ODQ, respectively, showed proceptive behavior, compared to 67% of the MFS/VCS plus vehicle controls. We did not include a control group treated with L-NAME or ODQ without MFS/VCS, because previous studies showed that these compounds did not increase lordosis behavior [30].

We also infused L-NAME or ODQ 60 min prior to MFS/VCS in some animals to determine whether a longer pre-exposure to these drugs would block the immediate increase in estrous behavior observed after MFS/VCS. Under these conditions, both L-NAME and ODQ attenuated the immediate increase (0 min) in LQ in addition to attenuating lordosis observed

at 120 min post-MFS/VCS (Fig 2B). At 240 min post-MFS/VCS, the LQ scores of the MFS/VCS plus L-NAME group were still significantly decreased ($P < 0.0$ vs. vehicle; data not shown). Both inhibitors completely blocked the MFS/VCS-induced increase in proceptive behavior observed at 120 min post-MFS/VCS ($P < 0.05$; Table 3).

Because PKG is the major target of NO-dependent cGMP synthesis, we next tested the effect of a PKG inhibitor, KT5823, on MFS/VCS-induced lordosis and proceptive behaviors (Fig. 2A). When KT5823 was infused icv 15 min prior to MFS/VCS, the MFS/VCS-induced increase in lordosis was significantly attenuated at 0 and 120 min post-MFS/VCS (0 min, $P < 0.05$; 120 min, $P < 0.001$ vs. vehicle). As shown in Table 3, KT5823 also prevented the MFS/VCS-induced increase in proceptive behavior observed at 120 min ($P < 0.01$).

Discussion

The present results support the hypothesis that VCS facilitates female sexual behaviors (lordosis and proceptivity) through release of NE, which then activates α_1 -adrenergic receptors. The data are also consistent with the hypothesis that α_1 -adrenoceptors mediate VCS facilitation of estrous behaviors via the NO-cGMP-PKG pathway. Administration of two different α_1 -adrenergic receptor antagonists (prazosin, phenoxybenzamine) as well as inhibitors of NO synthase (L-NAME), NO-stimulated guanylyl cyclase (ODQ), and PKG (KT5823) all attenuated the VCS-induced increase in lordosis and proceptive behaviors observed in E_2 -primed female rats during mating with a male. Sexually receptive female rats exhibit elevated release of NE in the ventromedial hypothalamus during mating tests with males [24]. When a vaginal mask was used to permit receptive females to receive flank-perineal stimulation but to prevent them from receiving VCS, elevated NE was not detected in the hypothalamus [24]. Therefore, VCS resulting from male intromissions appears to be necessary for the mating-induced release of NE in the ventromedial hypothalamus. Present findings are consistent with our previous conclusion that the released NE facilitates reproductive behaviors by a pathway involving α_1 -adrenergic receptor activation of the NO-cGMP-PKG pathway [8].

Our results are in agreement with previous studies showing that α_1 , but not α_2 - or β -adrenergic receptors, mediate NE stimulation of female sexual behavior. In the present study, the facilitatory effect of VCS on lordosis was blocked by icv infusions of the α_1 antagonists, prazosin and phenoxybenzamine, but not by β or α_2 antagonists. Consistent with a role for α_1 -adrenoceptors in the stimulation of lordosis, Nock and Feder [46] found that phenoxybenzamine (α_1 antagonist) reduced the facilitation of lordosis by the NE agonist clonidine in E_2 -primed females. Similarly, systemic or intrahypothalamic administration of the α_1 antagonist prazosin blocked the stimulation of lordosis by hormone treatment or by NE infusion into the ventromedial hypothalamus of female rats [23,27]. Likewise, administration of the α_1 agonists phenylephrine or methoxamine, but not the α_2 agonist clonidine or the β agonist isoproterenol, facilitated lordosis in E_2 -primed rats [4,40]. Intrahypothalamic application of antagonists specific for α_2 - and β -adrenergic receptors directly into the ventromedial hypothalamus also fails to attenuate E_2 and P-induced lordosis behavior [23]. Combined administration of the α_2 agonist clonidine and β agonist isoproterenol is reported to enhance lordosis behavior 15 min after intrahypothalamic infusion in rats [40]. Nonetheless, most evidence suggests that the predominant effect of β -adrenergic receptor activation in the hypothalamus is to inhibit lordosis in rats [28]. Interestingly, E_2 modifies activity of both β - and α_1 -adrenergic receptors in the hypothalamus and preoptic area, attenuating β -adrenergic while augmenting α_1 -adrenergic responses [25,49,57]. It is tempting to speculate that the attenuation of NE action at hypothalamic β receptors along with the potentiation of NE action at α_1 receptors are functionally related to E_2 priming of lordosis behavior.

There is little direct evidence that VCS induces the production specific second messengers. However, Meredith et al. [44] tested the hypothesis that VCS induces the phosphorylation of DARPP-32, a phosphoprotein activated by the cAMP-protein kinase A and cGMP-PKG systems [36,56]. They found that VCS increased the number of cells expressing phosphorylated DARPP-32 immunoreactivity in several brain areas related to lordosis expression (preoptic area, ventromedial hypothalamus, etc.). In agreement with these findings, we have preliminary evidence that several kinase systems participate in VCS-induced stimulation of estrous behavior (unpublished observations). The present results support the participation of cGMP-PKG pathway, which can be activated through α_1 -adrenoreceptors. Specifically, we demonstrated that ODQ and KT5823, selective inhibitors of soluble guanylyl cyclase and PKG, respectively, attenuated VCS-facilitated lordosis and proceptive behaviors. Taken together, these results suggest that some of the neuronal effects of VCS, and perhaps other social and environmental stimuli, are mediated by neurotransmitter-induced phosphorylation of different proteins, perhaps DARPP-32, progesterin receptors, or different coactivator proteins.

There is evidence that mating activates NOergic neurons in several brain areas important for regulation of sexual behavior [32,60]. Given the importance of NO in the regulation of female reproductive function, activated NOergic neurons in these brain areas may directly or indirectly be involved in integrating mating signals to trigger neuroendocrine changes. In the present study, we show that a NOS inhibitor (L-NAME), a specific inhibitor of NO-stimulated guanylyl cyclase (ODQ) and a PKG inhibitor (KT5823) all significantly attenuated lordosis behavior induced by VCS. cGMP is a key mediator of cellular responses to NO. Fernández-Guasti et al. [26] were the first to demonstrate the stimulatory effects of guanine nucleotides on lordosis in E_2 -primed rats, indicating a potential role of cGMP in the regulation of this behavior. The Etgen laboratory subsequently showed that PKG mediates the facilitation of lordosis by cGMP, and that the ability of cGMP to facilitate lordosis in E_2 -primed rats is blocked by the progesterin receptor antagonist RU 486 [9], suggesting that cGMP activates brain progesterin receptors. We have also shown that inhibitors of NO synthesis, of NO-stimulated guanylyl cyclase, and of PKG significantly attenuate lordosis behavior induced by P and two of its ring A-reduced metabolites [30]. These results, together with those obtained in the present study, support the hypothesis that the NO-cGMP-PKG pathway is involved in lordosis induced by membrane mechanisms.

It seems likely that the NO-cGMP-PKG signaling pathway is activated by NE (via α_1 adrenoreceptors) in response to VCS, and that this signaling pathway mediates the facilitatory effect of VCS on estrous behavior. Biochemically, α_1 -adrenoreceptors are well positioned to activate NO synthesis, a calcium-calmodulin activated enzyme, because they elevate intracellular calcium both by opening calcium channels and by mobilizing intracellular calcium stores [16]. Behavioral experiments showed that icv infusion of a cell-permeable cGMP analog reverses the inhibitory effect of the α_1 -adrenoreceptor antagonist prazosin on hormone-dependent lordosis behavior in female rats [8]. Moreover, E_2 and P together greatly enhanced NO-stimulated cGMP synthesis in female rat hypothalamic slices and in GT1-1 cells [10]. Interestingly, NE and phenylephrine (α_1 agonist) do not activate cGMP production in hypothalamic slices unless the tissue is prepared from animals exposed to both E_2 and progesterone [8]. These results suggest that NO-stimulated cGMP production acts downstream of α_1 -adrenoreceptor activation to enhance lordosis responsiveness and that several steps in this pathway are hormone-regulated.

We cannot rule out the possibility that estrous behavior induced by VCS in ovx, E_2 -primed female rats requires the release of P from the adrenal cortex. The adrenal cortex secretes P in response to mating stimuli [29,51,55], as well as to a variety of other stimuli [50]. Removal of the adrenals increases progesterin receptor levels in the brain [22]. VCS decreases progesterin receptor immunoreactivity within 1 h in ovx, E_2 -primed female rats; however, VCS does not

decrease progesterin receptor immunoreactivity in ovx, adrenalectomized E₂-primed female rats [1]. Because we find that stimulation of α_1 -adrenergic receptors in the hypothalamus only promotes cGMP synthesis if female rats are treated with both E₂ and P [8], adrenal P may be necessary for NE to facilitate VCS-induced estrous behaviors.

In conclusion, the present data support the hypothesis that NE released in the hypothalamus in response to VCS activates α_1 -adrenoreceptors. These receptors in turn couple to the NO-cGMP-PKG pathway to mediate the VCS-dependent facilitation of lordosis and proceptive behaviors observed in E₂-primed rats.

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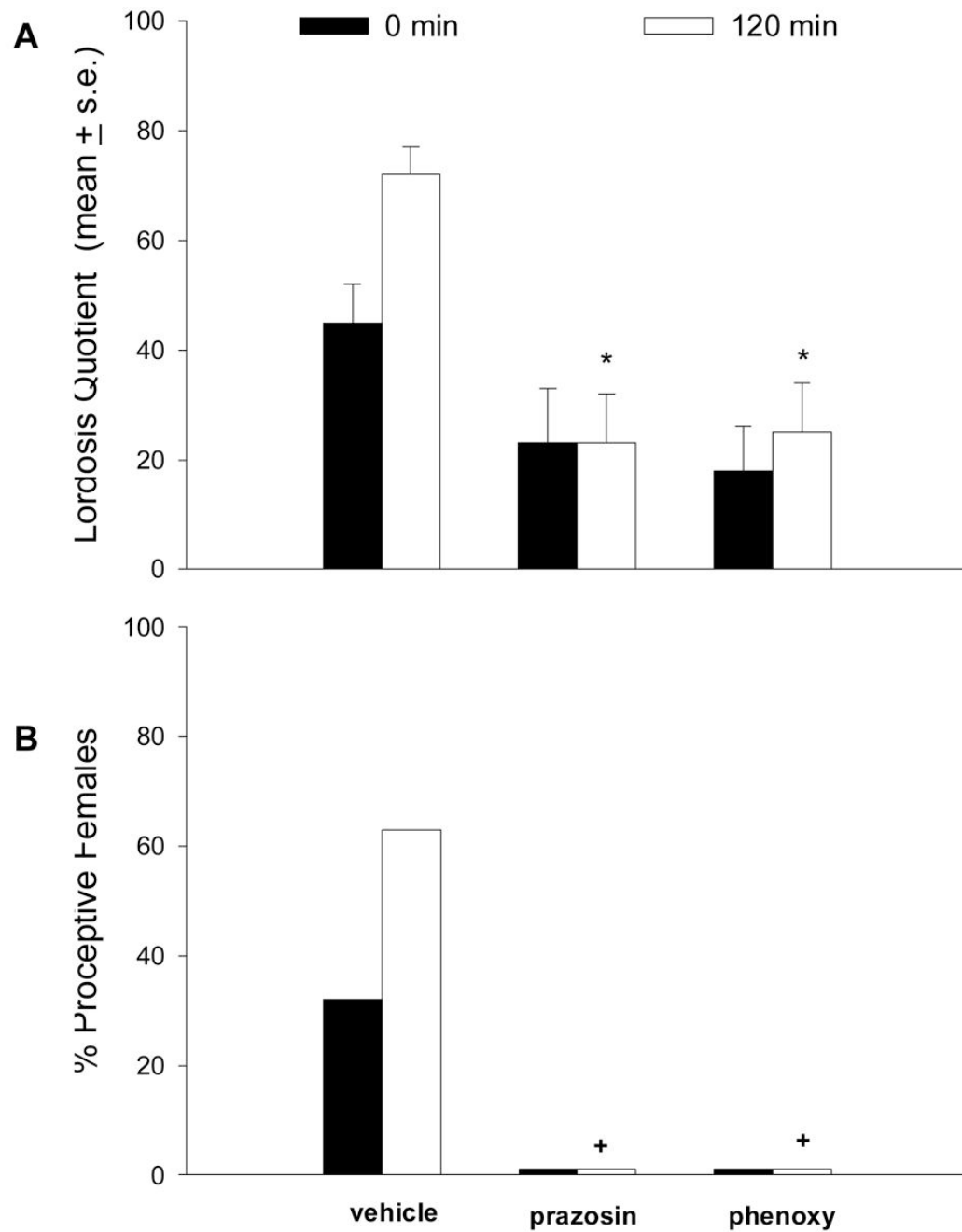


Fig. 1. The facilitation of lordosis (A) and proceptive behavior (B) in ovx, E₂B-primed rats produced by MFS/VCS is antagonized by icv infusion of the α_1 -adrenergic receptor antagonist prazosin (160 μ g) or phenoxybenzamine (phenoxy, 5 μ g). Drugs and vehicles were infused into the right lateral ventricle 15 min before application of MFS/VCS. Vehicle data are combined from the rows labeled saline and propylene glycol in Table 1. N = 8–12 rats per group. * $P < 0.01$; + $P < 0.05$ vs. corresponding group receiving MFS/VCS + vehicle.

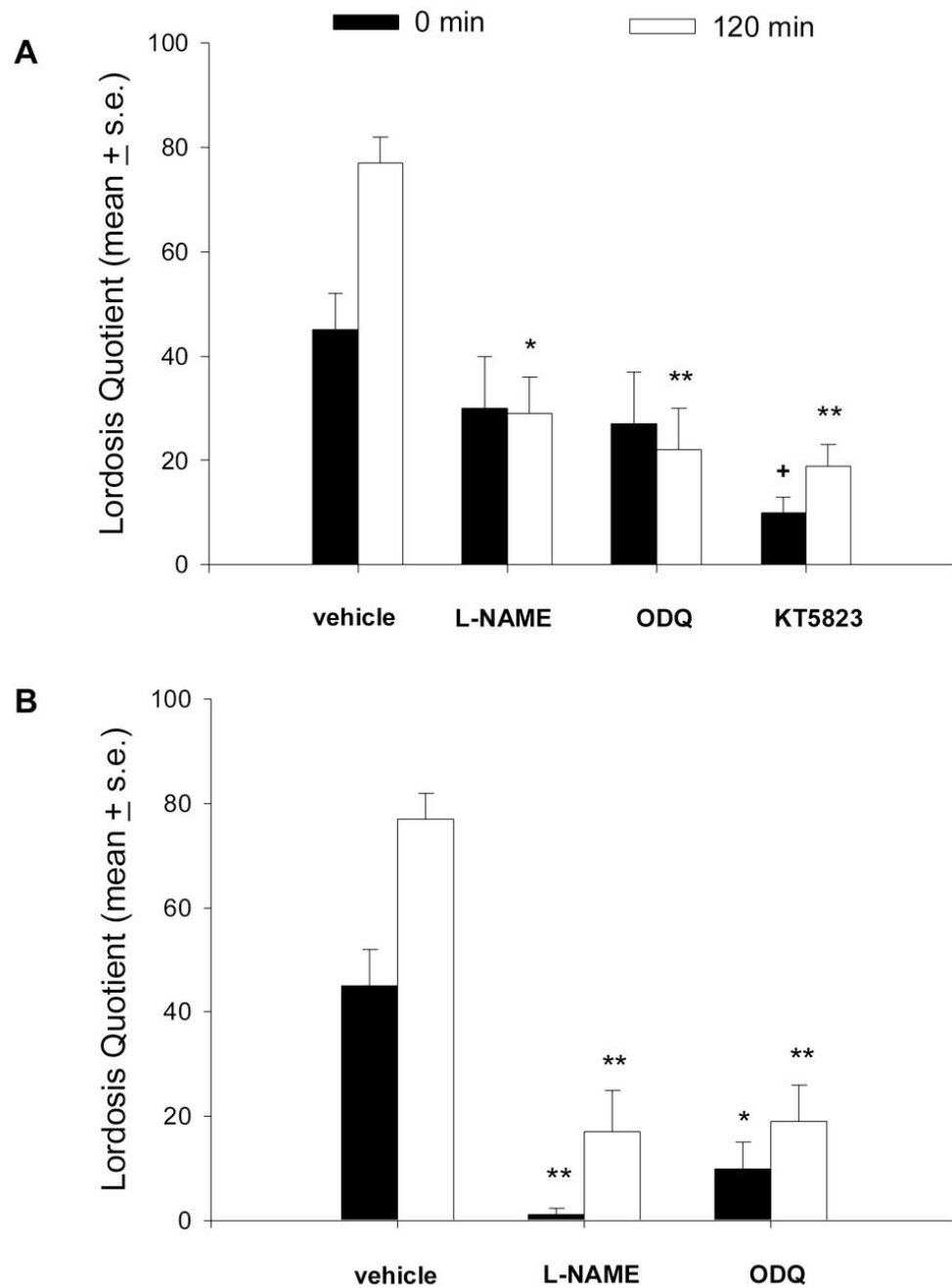


Fig. 2. The facilitation of lordosis in ovx, E2B-primed rats produced by MFS/VCS is antagonized by icv infusion of the NO synthase inhibitor LNAME (500 μ g), the soluble guanylyl cyclase inhibitor ODQ (22 μ g) or the PKG inhibitor KT5823 (0.12 μ g). Drugs and vehicle were infused into the right lateral ventricle 15 (A) or 60 min (B) before application of MFS/VCS. 1. N = 8–12 rats per group. ** $P < 0.001$; * $P < 0.01$; + $P < 0.05$ vs. corresponding group receiving MFS/VCS + vehicle.

Table 1

Infusion of different vehicles does not influence sexual behavior (lordosis and proceptivity) induced by VCS in ovx rats primed with E₂B

	N	LQ Mean ± SE	0 min % Proceptve Females	LQ Mean ± SE	120 min % Proceptive Females
Saline	12	49±10 [*]	33	74 ± 8 [*]	66 [*]
Water	10	52±9 ^{**}	40	74±10 [*]	70 [*]
Propylene Glycol	10	41±9 ⁺	30	70±7 [*]	60 [*]
DMSO	9	40±3 ⁺	33	80±5 ^{**}	66 [*]
MFS only	8	2.5±1.6	0	12±4	0

Estrous behavior in ovx female rats primed with 5 µg of E₂B was tested 0 and 120 min after application of VCS. Some animals received manual flank stimulation (MFS) without VCS. Different vehicles were administered into the right lateral ventricle 15 min before VCS.

⁺ P < 0.05;

^{*} P < 0.01;

^{**} P < 0.001 VS MFS only.

Table 2

Icv administration of α_2 (yohimbine and idaxozan) and β (propranolol) adrenergic antagonists did not affect VCS-facilitated lordosis and proceptive behavior of ovx rats primed with E₂B

	N	LQ Mean \pm SE	0 min % Proceptive Females	LQ Mean \pm SE	120 min % Proceptive Females
Vehicle	22	50 \pm 6	36	74 \pm 6	63
Yohimbine	9	40 \pm 8	33	48 \pm 14	33
Idaxozan	8	50 \pm 11	62	68 \pm 12	37
Propranolol	8	75 \pm 6	57	84 \pm 5	57

Estrous behavior in ovx female rats primed with 5 μ g of E₂B was tested 0 and 120 min after application of MFS/VCS. Vehicle data are combined from the rows labeled "saline" and "water" in Table 1. Adrenergic receptor antagonists (5 μ g) were administered into the right lateral ventricle 15 min before VCS.

Table 3

Icv administration of L-NAME, ODQ and KT5823 reduced proceptivity induced by VCS in ovx rats primed with 5 µg of E₂B

	N	0 min % Proceptve Females		120 min % Proceptve Females	
		15 minutes	60 minutes	15 minutes	60 minutes
Vehicle	21	33		67	
L-NAME 500 µg	8	12	0	12 ⁺	0 ⁺
ODQ 22 µg	8	12	0	0 ^{**}	0 ⁺
KT5823 0.12 µg	8	0		0 ^{**}	

Proceptive behavior in ovx female rats primed with 5 µg of E₂B was tested 0 and 120 min after application of VCS. Drugs or vehicle were infused into the right lateral ventricle 15 min before VCS as in Fig. 2A. Vehicle data are combined from the rows labeled “saline” and “DMSO” in Table 1. Other females were injected with L-NAME or ODQ 60 min before application of VCS (see Fig. 2B for lordosis data).

⁺ P<0.05;

^{**} P<0.001 vs. corresponding vehicle.