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Nitric oxide and ERK/MAPK mediation of estrous behavior induced by GnRH, PGE₂ and db-cAMP in rats

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Abstract

We tested the hypothesis that GnRH, PGE₂ and db-cAMP act via the nitric oxide (NO)-cGMP and MAPK pathways to facilitate estrous behavior (lordosis and proceptivity) in estradiol-primed female rats. Estradiol-primed rats received intracerebroventricular (icv) infusions of pharmacological antagonists of NO synthase (L-NAME), NO-dependent soluble guanylyl cyclase (ODQ), protein kinase G (KT5823), or the ERK1/2 inhibitor PD98059 15 min before icv administration of 50 ng of GnRH, 1 µg of PGE₂ or 1 µg of db-cAMP. Icv infusions of GnRH, PGE₂ and db-cAMP enhanced estrous behavior at 1 and 2 hr after drug administration. Both L-NAME and ODQ blocked the estrous behavior induced by GnRH, PGE₂ and db-cAMP at some of the times tested. The protein kinase G inhibitor KT5823 reduced PGE₂ and db-cAMP facilitation of estrous behavior but did not affect the behavioral response to GnRH. In contrast, PD98059 blocked the estrous behavior induced by all three compounds. These data support the hypothesis that the NO-cGMP and ERK/MAPK pathways are involved in the lordosis and proceptive behaviors induced by GnRH, PGE₂ and db-cAMP. However, cGMP mediation of GnRH-facilitated estrous behavior is independent of protein kinase G.

Keywords

NO; MAPK; Estrous behavior; GnRH; PGE₂; cAMP

1. INTRODUCTION

A number of endogenous signaling molecules, including gonadotropin hormone releasing hormone (GnRH), prostaglandin E₂ (PGE₂) and cyclic AMP (cAMP), can substitute for progesterone (P) to facilitate lordosis behavior in ovariectomized (OVX) rats primed with estradiol (E₂) [1–9]. The cellular mechanism through which these compounds induce female sexual behavior in rats is unclear. The temporal characteristics of the responses induced by these agents, e.g., latency to display lordosis, are similar to those of P, suggesting a common

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mechanism of action [see 7,8,10,11,12]. There is also evidence that these agents facilitate estrous behavior (lordosis and proceptive behaviors) by activating the progesterin receptor (PR), because administration of the PR antagonist RU486 blocks the lordosis and proceptivity induced by all of these agents [11].

A number of second messengers, including nitric oxide (NO), also play an important role in the activation of sexual behavior in female rats treated with E₂ and P [13–16]. We recently found that the NO pathway participates in the induction of female sexual behavior by P, its ring A-reduced metabolites, and by vaginocervical stimulation [17,18]. NO synthase (NOS) is expressed in diverse hypothalamic areas [19–21] and in the pituitary lobes [22–26] where it can be regulated by E₂, raising the possibility that NO may act in both the brain and pituitary as a neuroendocrine regulator of reproductive function. For example, NO acts as a signal transducer in norepinephrine-induced PGE₂ release from hypothalamic explants [27]. Both norepinephrine and PGE₂ regulate the pulsatile release of GnRH and participate in the preovulatory GnRH/LH surge [27–29]. Thus the NO system may participate in the regulation of GnRH release as well as in female sexual behavior.

The second messenger cAMP has also been implicated in neuroendocrine control of reproductive function. GnRH acting on GnRH type 1 receptors and PGE₂ acting on its receptors associate with Gas, which activates the adenylyl cyclase-cAMP-protein kinase A (PKA) cascade [30–32]. Administration of cAMP systemically or directly into the brain facilitates lordosis behavior in E₂-primed rats [10,11,33–38]. Mani et al. [36] also found that a PKA blocker decreased the facilitatory effect of P on lordosis behavior of OVX, E₂-treated rats. Similarly, we reported recently that intracerebroventricular (icv) injections of Rp-cAMPS, a PKA inhibitor, significantly depressed both lordosis and proceptive responses induced by GnRH, PGE₂ and db-cAMP [38]. Thus, these agents can also use the cAMP-PKA signaling pathway to elicit their stimulating effect on estrous behavior in rats.

Other intracellular signaling pathways, such as protein kinase G (PKG) [13,15,17,34,39,40], mitogen-activated protein kinase (MAPK) [40–42] and protein kinase C [43–45], have also been implicated in the expression of estrous behavior in OVX, E₂-primed rats. For example, administration of KT5823, an inhibitor of PKG, or PD98059, an inhibitor of the extracellular signal regulated kinase (ERK) family of MAPKs, blocked estrous behavior induced by P and ring A-reduced progestins [17,40] or by vaginocervical stimulation [46]. Interestingly, GnRH and PGE₂ can activate several of these pathways in different cell lines [47,48]. GnRH activates phospholipases D and A2 [49,50], produces cGMP [51,52] and under some circumstances, activates tyrosine kinases and the MAPK cascade [53,54]. PGE₂ also binds to distinct receptors to induce either activation of the MAPK pathway via G_{aq} or G_{ai} or to increase cAMP synthesis and subsequently activation of PKA [32,55].

The goal of the present studies was to determine whether the NO, PKG and ERK/MAPK intracellular signaling pathways, which have been implicated in progesterin facilitation of estrous behavior, also play a role in the facilitation of female reproductive behaviors by GnRH, PGE₂ and cAMP. In the Experiment 1, we used inhibitors of NOS and soluble guanylyl cyclase to explore if the NO pathway is indispensable for GnRH, PGE₂ and db-cAMP stimulation of estrous behavior. In Experiment 2, we assessed the possible role of PKG and ERK1/2 in the estrous-facilitating action of GnRH, PGE₂ and cAMP by administering KT5823, a potent blocker of PKG, and PD98059, a potent blocker of ERK1/2.

2. MATERIALS AND METHODS

2.1. Animals

A total of 171 animals were used in this study. Animals were sexually inexperienced female Sprague Dawley rats (240–280 g) bred in our colony. They were kept at $23 \pm 2^\circ\text{C}$ with a reversed light–dark cycle (14 h light, 10 h dark, lights on at 2300 h). They were fed with Purina rat chow and water ad libitum. Animal care and all the experimental procedures adhered to the Mexican Law for the Protection of Animals.

2.2. Surgical procedures

Females were bilaterally OVX under ether anesthesia, injected with penicillin (22,000 I.U./kg) and housed 4 females 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). Females were implanted with a stainless steel cannula (22 gauge, 17 mm long) in the right lateral ventricle using the following coordinates: A/P +0.80 mm, M/L –1.5 mm, D/V –3.5 mm with respect to bregma [56]. A stainless steel screw was fixed to the skull, and both the cannula and screw were attached to the bone with dental cement. An insert cannula (30 gauge) provided with a cap was introduced into the guide cannula to prevent clogging and contamination.

2.3. Hormone and inhibitor treatment

One week after cannula implantation, all females received a sc injection of 5 μg of E_2 benzoate (E_2B ; Sigma-Aldrich, St. Louis, MO). About 40 hr later, they received icv infusion of vehicle or one of the following inhibitors: the NOS inhibitor, NG-nitro-L-arginine methyl ester (L-NAME), the specific inhibitor of soluble guanylyl cyclase, 1H-[1,2,4] oxadiazolo[4, 3-a] quinoxalin-1-one (ODQ), the PKG inhibitor KT5823, or the ERK1/2 inhibitor, PD98059. Fifteen min after infusion of vehicle or inhibitors, vehicle, GnRH, PGE_2 or db-cAMP were administered by icv infusion. GnRH, PGE_2 , db-cAMP and L-NAME were prepared in sterile saline; ODQ, KT5823 and PD98059 were prepared in 10% DMSO. All agents were infused in a volume of 1 μl into the right lateral ventricle through the guide cannula over 1 min, and another 1 min was allowed for drug diffusion before the removal of the infusion needle. L-NAME was purchased from RBI (Natick, MA). ODQ was obtained from Tocris Cookson (St. Louis, MO), and KT5823 and PD98059 were purchased from CalBiochem (La Jolla, CA). PGE_2 and db-cAMP were purchased from Sigma, and GnRH was purchased from Peninsula Laboratories (Belmont, CA).

2.4. Experiment 1. Effect of icv infusion of GnRH, PGE_2 and db-cAMP on estrous behavior in E_2B -primed-female rats

E_2B -primed animals were randomly assigned to receive an infusion of 50 ng of GnRH, 1 μg of PGE_2 or 1 μg of db-cAMP. These doses were selected from dose response curves previously established by our laboratory [38]. Because different inhibitors were dissolved in different vehicles, separate control animals were primed with E_2B and infused icv with one of these vehicles alone or vehicle followed 15 min later by drug. The numbers of animals in each of these groups were: saline (n= 8); 10% DMSO (n= 8); saline+GnRH (n= 9); 10% DMSO+GnRH (n= 9); saline+ PGE_2 (n= 9); 10% DMSO+ PGE_2 (n= 9); saline+db-cAMP (n= 8); 10% DMSO +db-cAMP (n= 9).

2.5. Experiment 2. Effect of NO pathway inhibitors on estrous behavior induced by GnRH, PGE_2 or db-cAMP in E_2B primed-female rats

E_2B -primed female rats were infused icv with 500 μg of L-NAME or 22 μg of ODQ 15 min before infusion of GnRH, PGE_2 or db-cAMP. The doses of these two inhibitors were selected on the basis of our previous work [13,15,17]. The numbers of animals in these groups were:

L-NAME+GnRH (n= 8); L-NAME+PGE₂ (n= 9); L-NAME+db-cAMP (n= 9); ODQ+GnRH (n= 9); ODQ+PGE₂ (n= 8); ODQ+db-cAMP (n= 9). The vehicles that were tested in Experiment 1 are the vehicles used to dissolve the inhibitors in this experiment; thus, the controls included 9 females infused with 1 µl of 10% DMSO (the solvent used with ODQ) and 9 females infused with 1 µl saline (the solvent used with L-NAME). These two control treatments were combined for statistical analysis, because they did not differ significantly from each other with respect to either lordosis or proceptivity scores.

2.6. Experiment 3. Effect of PKG and ERK1/2 inhibitors on estrous behavior induced by GnRH, PGE₂ or db-cAMP in E₂-B-primed female rats

E₂B-primed female rats were infused icv with 0.12 µg of KT5823 or 3.3 µg of PD98059 15 min before infusion of GnRH, PGE₂ or db-cAMP. The doses of these inhibitors were selected on the basis of our previous work [40–42]. The numbers of animals in these groups were: KT5823+GnRH (n= 8); KT5823+PGE₂ (n= 8); KT5823+db-cAMP (n= 8); PD98059+GnRH (n= 9); PD98059+PGE₂ (n= 8); PD98059+db-cAMP (n= 9). The control group comprised 8 females (different from those used in Expt. 2) infused with 1 µl of 10% DMSO.

2.7. Behavioral testing

Behavioral testing was initiated 1 hr after icv infusion of GnRH, PGE₂ or db-cAMP. Females were placed in a circular plexiglas arena (53 cm in diameter) with a vigorous male. Receptivity for each female was determined as a lordosis quotient [$LQ = (\text{number of lordosis} / 10 \text{ mounts}) \times 100$]. The intensity of lordosis was quantified according to the lordosis score (LS) proposed by Hardy and De Bold [32]. This scale ranged from 0 to 3 (0= no lordosis; 3= maximal lordosis posture) for each individual response and, consequently, from 0 to 30 for each female that received ten mounts from the male [43].

Proceptivity was evaluated by determining the incidence of hopping, darting, and ear-wiggling across the whole receptivity test [57]. We considered an animal proceptive when showing two of these behaviors during the testing period. This criterion was used because in our Sprague–Dawley rats, only a small proportion of animals will display all three proceptive behaviors together. This may be due to the fact that our Sprague–Dawley rats rarely (<10%) show darting in our testing conditions. Females were tested at 1, 2 and 4 hr after GnRH, PGE₂ and db-cAMP infusions.

2.8. Histology

After the last behavioral test, the animals were anesthetized with halothane, 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 right lateral ventricle. Seven animals whose cannula was not in the ventricle were not included in the data analysis.

2.9. Data analysis

Individual rats were administered only one experimental drug, although they were tested repeatedly after icv infusions. Because our major interest was in the duration of behavioral inhibition in drug-treated compared to vehicle-treated animals at each time point rather than in the change over time, separate statistical comparisons were performed at each time point across groups. For example, comparisons in Experiment 1 were carried out separately at each post-infusion time between animals receiving drug and animals receiving the appropriate vehicle in the absence of the drug [e.g., DMSO+GnRH was compared to DMSO alone]. Animals infused only with different vehicles (saline and 10% DMSO) were also compared at each time, and no significant differences were found (see Table 1). Therefore, in later experiments, animals treated with the two vehicles were combined for statistical purposes.

Because the distribution of LQ values in some groups was not normally distributed, a Wilcoxon-Mann Whitney U test rather than ANOVA was used to compare the control versus the different experimental groups at each time point. Fisher's exact probability test was used to compare the proportion of proceptive females at each time interval [58,59]. Differences were considered statistically significant if $P < 0.05$.

3. RESULTS

3.1. Experiment 1. Effects of LHRH, PGE₂ and db-cAMP on estrous behavior of E₂B-primed rats

Table 1 shows the LQ values and percentage of proceptive rats in OVX, E₂B-primed females observed at 1, 2 and 4 hr following the infusion of vehicles alone or of vehicles plus GnRH, PGE₂ or db cAMP. Neither saline nor DMSO alone induced any reproductive behaviors. All three test agents induced a significant facilitation of lordosis, which in nearly all cases was already present at the 1 hr test and reached its maximal value at 2 hr. Proceptive behavior was also displayed at the 1 hr test by same animals (none in vehicle groups) and was maximal at 2 hr.

3.2. Experiment 2. Inhibitors of NOS and soluble guanylyl cyclase reduce sexual behavior induced by GnRH, PGE₂, and dbcAMP in E₂B-treated rats

Because the effect of the agents employed did not differ between the vehicles employed (saline or 10% DMSO), control data obtained with the two solvents was combined and compared with the estrous behavior observed in separate rats receiving GnRH, PGE₂ or db-cAMP. As shown in Fig. 1, administration of the NOS inhibitor, L-NAME, and the specific inhibitor of soluble guanylyl cyclase, ODQ significantly attenuated GnRH-dependent increases in LQ at 1 and 2 hr; ODQ continued to inhibit GnRH-induced lordosis at 4 hr (Fig. 1A). LQ induced by PGE₂ was inhibited significantly by L-NAME only at 2 hr (Fig. 1B). On the other hand, ODQ clearly reduced the LQ induced by PGE₂ at all three times tested. The lordosis behavior induced by db-cAMP was blocked by L-NAME at 2 and 4 hr (Fig. 1C). As was the case for GnRH and PGE₂, ODQ inhibited lordosis induced by db-cAMP at all three times tested.

In addition, proceptive behavior induced by GnRH, PGE₂, and db-cAMP was significantly suppressed by both inhibitors at 2 hr post-administration (Fig. 2). Both inhibitors continued blocking the proceptivity induced by GnRH and db-cAMP at 4 hr. We did not include control groups treated with L-NAME or ODQ alone, because previous studies showed that these compounds did not increase lordosis and proceptive behaviors [17].

3.3. Experiment 3. Effects of KT5823 and PD98059 on estrous behavior induced by LHRH, PGE₂, and dbcAMP in E₂B-treated rats

The PKG inhibitor KT5823 did not interfere with the stimulatory effect of GnRH on lordosis behavior at any time point, but it blocked the stimulatory effect of PGE₂ at 1 hr and of db-cAMP at 2 hr (see Fig. 3). Similarly, KT5823 reduced the proceptivity induced by PGE₂ at 1 hr and by db-cAMP at 2 hr.

Administration of the ERK1/2 inhibitor PD98059 (Fig. 3) significantly decreased lordosis induced by GnRH and PGE₂ at both 1 and 2 hr post injection, and this inhibition was still significant at 4 hr for GnRH-facilitated lordosis. PD98059 blocked db-cAMP-induced lordosis only at 2 hr. The time course of the inhibitory effect of PD98059 on proceptivity also varied with the chemical tested. PD98059 significantly suppressed proceptive behaviors induced by GnRH and PGE₂ at 2 hr and by PGE₂ at 1 hr. A decrease in the proportion of proceptive animals was also observed in females treated with db-cAMP, but this decrease did not reach statistical significance.

4. DISCUSSION

The present study shows that icv infusion of GnRH or PGE₂ elicits lordosis and proceptive behaviors in E₂B-primed rats with temporal characteristics similar to those obtained with icv infusion of db-cAMP. These results agree with previous experiments administering these chemicals both through intracerebral and sc routes [4,7–9,11,33,38,60–64]. The data also show that the icv infusion of a NOS inhibitor, L-NAME, and an inhibitor of NO-stimulated guanylyl cyclase, ODQ, significantly attenuates the lordosis behavior induced by GnRH, PGE₂ and db-cAMP, especially at the 1 and 2 hr tests. These findings support the hypothesis that the NO pathway is involved in the lordosis induced by these agents. Previous studies have shown that the NO system is important, if not essential, for the enhancement of estrous behavior in E₂B-primed rats by progestins, adrenergic agonists and vaginocervical stimulation [13–17,40,65]. The NO pathway also modulates male sexual behavior [66] as well as the secretion of various hormones such as GnRH [25,27,67], corticotropin-releasing hormone [68], luteinizing hormone [24], and prolactin [69]. Brain NOergic activity, in turn, is regulated by a variety of stimuli affecting hormone secretion including gonadectomy [70], lactation [71], and stress [72]. These observations raise the possibility that NO-producing neurons are activated in female rats during mating and may help integrate the genitosensory stimulation that leads to neuroendocrine responses [65].

Our laboratories and others have shown that downstream kinases such as MAPK (specifically ERK) and PKG participate in the facilitation of estrous behavior in E₂-primed rats by progestins, including 5 α -reduced metabolites of P, and by vaginocervical stimulation [40–42, 73]. The present results strongly suggest that activation of the ERK family of MAPKs is involved in GnRH, PGE₂ and db-cAMP-induced estrous behavior in E₂-primed rats, because PD98059 interfered with the behavioral actions of all three agents. Both GnRH and PGE₂ have been reported to activate the ERK/MAPK cascade in several tissues [55,74–76]. For example, activation of distinct PGE₂ receptor subtypes (EP1–EP4) can stimulate the MAPK pathway via G α_q or G α_i , or elevate cAMP via G α_s , leading to activation of PKA [55]. There is evidence that cAMP can stimulate MAPK signaling through mechanisms involving src, B-Raf, Rap1, Ras and various phosphatases (for review see [77,78]). Finally, cAMP might also activate Ras and Rap1 through PKA-independent pathways involving guanine nucleotide exchange factors for Rap1 and Ras [77,79].

It is interesting that administration of KT5823, a PKG inhibitor, failed to inhibit the effect of GnRH on lordosis behavior despite the fact that two NO pathway inhibitors, L-NAME and ODQ, significantly reduced GnRH-stimulated lordosis. This is unlikely to be a problem with the dose of KT5823 as this drug did inhibit lordosis induced by PGE₂ and db-cAMP. Thus, the cGMP produced by GnRH may be acting independently of PKG, perhaps by cross-activating PKA or regulating phosphodiesterase (PDE) activity [80]. PDEs are the enzymes responsible for hydrolysis of cAMP and cGMP. Both a cGMP-activated PDE (type III) and cGMP-inhibited PDE (type II) are present in various cells, where they can decrease or increase cAMP levels, respectively, in response to cGMP [27,33]. This mechanism is consistent with observations that administration of theophylline, a PDE inhibitor, enhances the behavioral effects of GnRH in E₂-primed, OVX rats [33,81,82]. Icv injection of Antide, a GnRH-1 receptor antagonist, inhibits lordosis behavior in E₂-primed rats infused with icv GnRH [9,83]. The GnRH-1 receptor can be linked to G α_s , which activates the adenylyl cyclase-cAMP-PKA cascade [30,31]. Indeed, we recently found that Rp-cAMPS, a potent inhibitor of PKA, blocked estrous behavior induced by GnRH, PGE₂ and db-cAMP in E₂-primed female rats [38]. Finally, cGMP can regulate cell function by interacting with cGMP receptor proteins other than PKG, such as cyclic nucleotide gated channels.

It is important to acknowledge that icv administration of drugs can influence both circumventricular organs and more distant regions throughout the brain. Therefore, each agent that we injected could be acting at different brain areas to elicit their effects. Similarly, NO is a highly diffusible signaling agent that can act not only in the cells in which it is generated but on neighboring cells as well (see [22]). Although the three agents used to induce lordosis (GnRH, PGE₂ and dbcAMP) have been reported to act in or around the ventromedial hypothalamus [2,7,10], this fact does not rule out the possibility that the inhibitors used in this study acted on other brain structures that make up the efferent pathways mediating these behavioral responses or in brain regions modulating these responses. Infusion studies directed to specific brain areas are needed to identify their specific sites of action. It is possible that hypothalamic NO neurons implicated in lordosis could be a target for glutamate action in the hypothalamus [84–87]. Glutamate-induced GnRH release from hypothalamic fragments in vitro is blocked by a competitive inhibitor of NOS as well as by the NO scavenger hemoglobin [86]. Similar results were found in immortalized GnRH neurons [see 87]. However, infusion of glutamate and certain glutamate receptor agonists into the ventromedial hypothalamus inhibits lordosis [84], suggesting that the predominant site of NO action may be outside of this hypothalamic nucleus.

It is tempting to speculate that the PR is one of the molecules phosphorylated and activated by PKG and MAPK that leads to enhancement of estrous behavior, because the antiprogesterin RU486 interferes with the facilitatory effect of GnRH and PGE₂ on lordosis behavior [11]. However, the phosphorylation sites so far reported in PRs are exclusive targets of MAPK and not of PKG [83,88]. However, PKG can activate MAPK [89–91], and we have shown that cGMP facilitation of lordosis is blocked by both RU486 and ERK/MAPK inhibitor PD98059 [13,39]. Nonetheless, the participation of PKG and MAPK in the facilitation of estrous behavior likely involves molecules in addition to PRs.

In summary, the NO-cGMP pathway, via PKG-dependent and independent mechanisms, as well as the ERK/MAPK pathway, represent important mechanisms by which progestins and several neurotransmitters/neuromodulators, including GnRH and PGE₂, can facilitate estrous behavior in estrogen-primed rodents. It is likely that the enhancement of female sexual behavior by different agents depends on the coordinate action of several signals acting at different brain sites and different cellular levels, for example, membrane receptors, generation of second messengers and activation of protein kinases. These multiple signals may in turn operate through a variety of molecules, including but not limited to PRs.

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Figure 1.

The facilitation of lordosis behavior in E₂B-primed rats produced by (A) GnRH (50 ng); (B) PGE₂ (1 µg); and (C) db-cAMP (1 µg) is antagonized by icv infusion of L-NAME (500 µg) and ODQ (22 µg). Drugs and vehicles were infused into the right lateral ventricle 15 min before application of GnRH, PGE₂ or db-cAMP. Vehicle data from rats receiving saline or 10% DMSO were combined, because no significant differences were obtained with the two solvents (see Table 1). ***P* < 0.001; **P* < 0.01; +*P* < 0.05 vs. corresponding group receiving vehicles alone (closed circle).

Figure 2.

The facilitation of proceptive behaviors in E₂B-primed rats produced by (A) GnRH (50 ng); (B) PGE₂ (1 µg); and (C) db-cAMP (1 µg) is antagonized by icv infusion of L-NAME (500 µg) and ODQ (22 µg). Drugs and vehicles were infused into the right lateral ventricle 15 min before application of GnRH, PGE₂ or db-cAMP. Vehicle data were combined (saline, 10% DMSO). **P* < 0.01; +*P* < 0.05 vs. corresponding group receiving vehicles alone (closed circle).

Figure 3.

The facilitation of lordosis behavior in E₂B-primed rats produced by (A) GnRH (50 ng); (B) PGE₂ (1 µg); and (C) db-cAMP (1 µg) is antagonized by icv infusion of the PKG inhibitor KT5823 (0.12 µg) or the MAPK inhibitor PD98059 (3.3 µg). Drugs and 10% DMSO were infused into the right lateral ventricle 15 min before application of GnRH, PGE₂ or db-cAMP. ***P* < 0.001; **P* < 0.01; +*P* < 0.05 vs. 10% DMSO alone.

Figure 4.

The facilitation of proceptive behaviors in E₂B-primed rats produced by (A) GnRH (50 ng); (B) PGE₂ (1 µg); and (C) db-cAMP (1 µg) is antagonized by icv infusion of the PKG inhibitor KT5823 (0.12 µg) or the ERK1/2 inhibitor PD98059 (3.3 µg). Drugs and 10% DMSO were infused into the right lateral ventricle 15 min before application of GnRH, PGE₂ or db-cAMP. **P* < 0.01; +*P* < 0.05 vs. 10% DMSO alone.

Table 1

Effects of icv infusion of different vehicles alone and vehicles with GnRH, PGE₂ and db-cAMP on sexual behavior (lordosis and proceptivity) in OVX rats primed with 5 µg of E₂B.

Test time	N	LQ Mean ± SE	1 hr % Proceptive Females	LQ Mean ± SE	2 hr % Proceptive Females	LQ Mean ± SE	4 hr % Proceptive Females
Saline	8	5 ± 2	0	4 ± 2	0	2 ± 1	0
DMSO	8	7 ± 5	0	7 ± 6	0	14 ± 6	0
Saline+GnRH	9	59 ± 8*	33	82 ± 5**	55 ⁺	92 ± 3**	77*
DMSO + GnRH	9	62 ± 6*	22	71 ± 7**	44	56 ± 11*	11
Saline+PGE ₂	9	58 ± 6*	22	81 ± 8**	55 ⁺	73 ± 6**	22
DMSO+PGE ₂	9	61 ± 12*	44	73 ± 7*	66*	42 ± 12	11
Saline+Db-cAMP	8	61 ± 11**	37	82 ± 4**	50 ⁺	63 ± 9**	37
DMSO+Db-cAMP	9	42 ± 12	22	68 ± 9*	44	50 ± 10*	33

⁺ P< 0.05;

* P< 0.01;

** P<0.001 vs animals receiving only saline or DMSO.