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Lordosis facilitated by GPER-1 receptor activation involves GnRH-1, progestin and estrogen receptors in estrogen-primed rats

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ABSTRACT

The present study assessed the participation of membrane G-protein coupled estrogen receptor 1 (GPER-1) and gonadotropin releasing hormone 1 (GnRH-1) receptor in the display of lordosis induced by intracerebroventricular (icv) administration of G1, a GPER-1 agonist, and by unesterified 17β-estradiol (free E_2). In addition, we assessed the participation of both estrogen and progestin receptors in the lordosis behavior induced by G1 in ovariectomized (OVX), E_2-benzoate (EB)-primed rats. In Experiment 1, icv injection of G1 induced lordosis behavior at 120 and 240 min. In Experiment 2, icv injection of the GPER-1 antagonist G15 significantly reduced lordosis behavior induced by either G1 or free E_2. In addition, Antide, a GnRH-1 receptor antagonist, significantly depressed G1 facilitation of lordosis behavior in OVX, EB-primed rats. Similarly, icv injection of Antide blocked the stimulatory effect of E_2 on lordosis behavior. In Experiment 3, systemic injection of either tamoxifen or RU486 significantly reduced lordosis behavior induced by icv administration of G1 in OVX, EB-primed rats. The results suggest that GnRH release activates both estrogen and progestin receptors and that this activation is important in the chain of events leading to the display of lordosis behavior in response to activation of GPER-1 in estrogen-primed rats.

1. Introduction

Although lordosis in ovariectomized (OVX) rats typically requires sequential treatment with 17β-estradiol (E_2) and progesterone (P), it can also be induced by high or repeated doses of E_2 alone (Beyer et al., 1971; Boling and Blandau, 1939; Davidson et al., 1968; Zemlan and Adler, 1977). We and others recently also showed that unesterified 17β-estradiol (free E_2) administered subcutaneously (sc; Domínguez-Ordóñez et al., 2015) or intracerebroventricularly (icv; Domínguez-Ordóñez et al., 2015; Long et al., 2014) facilitates lordosis behavior in OVX-E_2 benzoate (EB)-primed rats in the absence of P.

The cellular mechanism by which E_2 enhances this behavior is unclear. However, lordosis behavior induced 39.5 h after EB priming by acute sc and intracerebral E_2 administration is reduced by tamoxifen (TMX), a selective estrogen receptor modulator that has both antagonist and agonist actions on estrogen receptor α and β (ERα/β) and agonist effects on G-protein coupled estrogen receptor 1 (GPER-1; Filardo et al., 2000; Filardo et al., 2002; MacGregor and Jordan, 1998; Revankar et al., 2005; Vivacqua et al., 2006) and by 5 mg of RU486, a classical progestin receptor (PR) antagonist, when administered sc 1 h before E_2 (Domínguez-Ordóñez et al., 2015). This implicates the participation of both steroid receptors in the short latency behavioral response to E_2. It is important to note that TMX has brain region-specific effects on lordosis (Howard et al., 1984). For example, TMX reduced lordosis behavior induced by P when injected into the ventromedial hypothalamus, but not when administered into the preoptic area or interpeduncular region (Howard et al., 1984). Moreover, Long et al. (2017) showed that TMX and ICI 182,780 (ICI) infused into the arcuate nucleus facilitate lordosis within 30 min in EB-primed animals. These effects are blocked by pretreatment with the GPER-1 antagonist G15, indicating that they are mediated by GPER-1. Furthermore, RU486 administered sc at 48 h after EB-priming facilitated female sexual behavior in Long-Evans rats (Pleim et al., 1990). However, when administered 1 h before P, the PR antagonist reduced P facilitation of lordosis, suggesting that RU486 has a dual effect; it may act as an antagonist in the presence of P and as an agonist in its absence.

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Most of the biological effects of estrogens are mediated by the classical ERs, ERα and ERβ, which up- or down- regulate the expression of their target genes by binding to site-specific DNA sequences (estrogen response elements) and/or specific co-regulatory proteins, including co-activators and co-repressors. The role of each ER subtype in the expression of lordosis behavior has been explored using ER knockout female mice, antisense oligonucleotides, and ER subtype-specific agonists and antagonists (Dewing et al., 2007; Kudwa and Rissman, 2003; Mazzucco et al., 2008; Ogawa et al., 1999; Ogawa et al., 1998; Rissman et al., 1999). We recently reported that ERα and ERβ agonists each induce lordosis behavior in estrogen-primed rats, and both ER subtype-specific antagonists reduce lordosis behavior induced by free E_2 (Domínguez-Ordóñez et al., 2016).

More recently, E_2 has been found to act on non-classical receptors localized within cell membranes or the endoplasmic reticulum (Domínguez and Micevych, 2010; Filardo and Thomas, 2012; Gaudet et al., 2015; Revankar et al., 2005). One example of this is GPER-1, also known as GPR30, which plays an important role in mediating the rapid effects of E_2 (Filardo et al., 2000, 2002; Filardo and Thomas, 2012; Gaudet et al., 2015). For example, this receptor regulates the release of gonadotropin releasing hormone (GnRH) from hypothalamic neurons (Noel et al., 2009; Qiu et al., 2008; Rudolf and Kadokawa, 2013). Stimulation of GnRH neurons with G1, a selective agonist (Bologa et al., 2006), stimulates GnRH release, and treatment with G15 (GPER-1 antagonist), blocks E_2-induced GnRH release (Kenealy and Terasawa, 2012). Furthermore, icv administration of either E_2 or G1 significantly increases lordosis 30 min after administration to EB-primed rats, while pretreatment with G15 blocks E_2 and G1 facilitation of sexual receptivity (Long et al., 2014). Thus, GPER-1 participates in the acute E_2 facilitation of lordosis behavior estrogen-primed rats. GPER-1 is highly expressed in areas involved in the expression of lordosis, such as the arcuate nucleus, medial preoptic nucleus and ventromedial hypothalamus (Brailoiu et al., 2007; Hazell et al., 2009; Long et al., 2014; Qiu et al., 2008; Rudolf and Kadokawa, 2013).

GnRH also facilitates lordosis behavior in E_2-primed rodents that have been either OVX or OVX and adrenalectomized (Moss and McCann, 1973; Pfaff, 1973). This effect of GnRH on female sexual behavior is mediated by the GnRH-1 receptor, because Antide, a GnRH-1 receptor antagonist, blocks lordosis behavior in OVX, E_2-primed rats induced by several agents including GnRH, ring A-reduced progestins, and vaginocervical stimulation (Gómora-Arrati et al., 2008). PRs also participate in lordosis behavior induced by GnRH, because RU486 inhibits GnRH-induced lordosis and proceptive behaviors (Beyer et al., 1997).

The present study assessed the participation of GPER-1 and GnRH-1 receptors in the display of lordosis induced by icv administration of G1, a GPER-1 agonist, and by free E_2 in OVX, estrogen-primed rats. Experiment 1 was designed to confirm prior reports that GPER-1 facilitates lordosis in OVX, estrogen-primed rats through the administration of G1 and by the icv infusion of the antagonist, G15. Because the cellular mechanism by which GPER-1 induces lordosis behavior has not been well clarified, and because GPER-1 promotes GnRH release (Terasawa and Kenealy, 2012), Experiment 2 tested the ability of the GnRH antagonist, Antide, to interfere with G1 and E_2 facilitation of lordosis. Because G1 may induce lordosis behavior through GnRH release and subsequent activation of PRs and perhaps ERs, in Experiment 3, we tested the ability of the selective ER modulator, TMX, and the PR antagonist, RU486, to block lordosis facilitated by G1 in OVX, estrogen-primed rats.

2. Methods

2.1. Animals

A total of 126 female rats were used in this study. Animals were sexually inexperienced, female Sprague Dawley rats (240–280 g) bred in our colony at Centro de Investigación en Reproducción Animal-Panotla. Animals were housed in a reversed light–dark cycle (14 h light, 10 h dark, lights on at 2300 h) and a controlled temperature (23 ± 2 °C) environment. They were fed Purina Rodent Laboratory Chow 5001 and water ad libitum. Several males were used during sexual behavior testing (see below).

2.2. Surgical procedures

Female rats were bilaterally OVX under anesthesia with xylazine (4 mg/kg) and ketamine (80 mg/kg) and group housed (4/cage). One week 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 length) into the right lateral ventricle following the Paxinos and Watson (2006) atlas coordinates: anteroposterior + 0.80 mm, mediolateral − 1.5 mm, dorsoventral − 3.5 mm with respect to bregma. A stainless steel screw was fixed to the skull, and both the 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 each surgical procedure, rats were injected with penicillin (165,000 IU/kg of procaine benzyl penicillin and 55,000 IU/kg of crystalline benzyl penicillin), and this continued for 3 days after surgery. After surgery, rats were housed individually in plastic cages with food and water available ad libitum for recovery until the test day. During this time, animals did not show any apparent discomfort due to isolation.

All of the experiments were performed under the guidelines of the Mexican Law of Animal Protection (NOM-062-ZOO-1999) under the approval and supervision of the Institutional Committee for the Use and Care of Laboratory Animals of Centro de Investigación y de Estudios Avanzados.

2.3. Behavioral testing

Tests for sexual behavior were conducted by placing females in a circular Plexiglas arena (53 cm diameter) with a male after drug administration as discussed below. The lordosis quotient [LQ = (number of lordosis / 10 mounts) × 100] and lordosis score (LS) were used to assess receptive behavior in response to the first 10 mounts. LS refers to the intensity of lordosis, which is quantified according to Hardy and DeBold (1971). This scale ranges from 0 to 3 for each individual response and consequently, from 0 to 30 for each female that received 10 mounts. In all experiments, the rats were tested at 30, 120, and 240 min after E_2 or G1 infusion by an experimenter blind to treatment groups.

2.4. Chemicals

E_2 benzoate (EB), unesterified 17β-E_2 (E_2) and the GnRH-1 antagonist, Antide (acetyl-D-Ala(2-naphthyl)-D-Phe(4-Cl)-D-Ala(3-pyridyl)-Ser-Lys(Nε-nicotinoyl)-D-Lys(Nε-nicotinoyl)-Leu-Lys(Nε-isopr-opyl)-Pro-D-Ala-NH2) were purchased from Sigma Chemicals (St. Louis, MO). The GPER-1 agonist G1 (±)-1-[(3aR*,4S*,9bS*)-4-(6-Bromo-1,3-benzodioxol-5-yl)-3a,4,5,9b-tetrahydro-3H-cyclopenta[*c*]quinolin-8-yl]-ethanone and the GPER-1 antagonist G15 (3aS*,4R*,9bR*)-4-(6-Bromo-1,3-benzodioxol-5-yl)-3a,4,5,9b-3H-cyclopenta[*c*]quinoline were purchased from Tocris Cookson (St. Louis, MO). PR antagonist, RU486, and the ER antagonist, TMX, were also purchased from Sigma Chemicals (St. Louis, MO).

EB, E_2 and TMX were dissolved in sesame oil vehicle. G1, G15 and Antide were dissolved in 10% DMSO, and RU486 was dissolved in sesame oil:benzyl benzoate:benzylic alcohol (80:15:5). All drugs and vehicles infused icv were administered in a volume of 1 μl. To avoid unnecessary duplication and thus minimize the numbers of animals killed, important animal welfare concerns, the same groups of vehicle (DMSO) control animals were used throughout Experiments 1 and 2.