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Estrogen receptors regulate the estrous behavior induced by progestins, peptides, and prostaglandin E_2

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ABSTRACT

The role of classical estrogen receptors (ERs) in priming female reproductive behavior has been studied previously; however, the participation of this receptor during activation of estrous behavior has not been extensively studied. The purpose of this work was to test the possibility that the facilitation of lordosis behavior in estrogen-primed rats by progesterone (P) and its 5α- and 5β-reduced metabolites, gonadotropin-releasing hormone (GnRH), leptin, prostaglandin E_2 (PGE_2) and vagino-cervical stimulation (VCS) involves interactions with classical ERs by using the selective ER modulator, tamoxifen. To further assess the role of ERs, we also explored the effects of the pure ER antagonist, ICI182780 (ICI), on estrous behavior induced by P and GnRH. Ovariectomized, estrogen-primed rats (5 μg estradiol benzoate 40 h earlier) were injected intraventricularly with the above-mentioned compounds, or they received VCS. All compounds and VCS effectively facilitated estrous behavior when tested at 60, 120 or 240 min after infusion or application of VCS. Intraventricular infusion of tamoxifen (5 μg), 30 min before, significantly attenuated estrous behaviors induced in estradiol-primed rats by P, most of its 5α- and 5β-reduced metabolites, GnRH, and PGE_2, but not by VCS. Although there was a trend for reduction, tamoxifen did not significantly decrease lordosis in females treated with 5β-pregnan-3,20-dione. ICI also inhibited lordosis behavior induced by P and GnRH at some testing intervals. These results suggest that activation of classical ERs participates in the triggering effects on estrous behavior induced by agents with different chemical structures that do not bind directly to ERs.

Introduction

Progestins and some non-steroidal agents, such as gonadotropin-releasing hormone (GnRH; Beyer et al., 1982, 1995; Foreman and Moss, 1977; González-Flores et al., 2006; González-Mariscal and Beyer, 1988; Ramírez-Orduña et al., 2007; Riskind and Moss, 1983; Sakuma and Pfaff, 1980; Wu et al., 2006) and prostaglandin E_2 (PGE_2; Beyer et al., 1997; Dudley and Moss, 1976; Hall and Luttge, 1977; Rodriguez-Sierra and Komisaruk, 1977) facilitate lordosis behavior in ovariectomized (ovx) rats primed with estradiol (E_2). The time course of lordosis facilitation is similar to that induced by progesterone (P; see Moralí and Beyer, 1979). None of these agents have affinity for either classical estrogen receptors (ERs) or progestin receptors (PRs).

Several researchers (Beyer et al., 2003; Blaustein, 2003; Mani et al., 1997) proposed that the PR acts as a common molecular mediator for the various agents that activate lordosis behavior. This idea is supported by the finding that administration of the PR antagonist, RU486, blocks estrous behavior normally induced by P, several ring A-reduced progestins (Beyer et al., 1995), GnRH, cyclic nucleotides, PGE_2 (Beyer et al., 1997), leptin (García-Juárez et al., 2011), dopamine (Mani et al., 1996), and by vagino-cervical stimulation (VCS; Auger et al., 1997; González-Flores et al., 2008) in ovx rats primed with E_2. This role of PR as a common molecular mediator in lordosis behavior is unlikely to be due to its classic transcriptional action at the genome, but rather by rapidly activating signaling systems such a protein kinases involved in the expression of female sexual behavior in rats (Beyer et al., 2003; Mani and Portillo, 2010; Mani et al., 1996, 1997).

Recent findings show that the activation of the Src–PR–mitogen-activated protein kinase (MAPK) complex is essential for the facilitation of lordosis behavior by P and other agents (González-Flores et al., 2010;

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Lima-Hernández et al., 2012). The mechanism by which P stimulates the Src–MAPK pathway has been well studied in some cells possessing both ER and PR (for review see, Boonyaratanakornkit et al., 2008; Edwards, 2005; Lange, 2004), and many laboratories now suggest that functional c-Src is required for intracellular signaling initiated by both growth factors and steroid hormones. For example, PR through its N-terminus binds directly to SH3 domains in c-Src and associates via the N-terminal with ER, whereas ERs interact with the SH2-domain of c-Src (Ballare et al., 2003; Boonyaratanakornkit et al., 2001; Migliaccio et al., 2007). These interactions are required for Src–MAPK activation by progestins and estrogens (Boonyaratanakornkit et al., 2001; Faivre et al., 2005; Migliaccio et al., 1998) in that the antiprogestin RU486 and antiestrogens, such as tamoxifen and ICI182780, inhibit steroid stimulation of the Src–MAPK complex.

In the present study we hypothesized that the classical ER participates in the facilitation of lordosis not only by P, as was reported by several workers in rodents (Etgen, 1979; Kow and Pfaff, 1975; Micevych and Sinchak, 2013), but also by ring A-reduced progestins, by non-steroidal agents such as GnRH, PGE_2 and leptin, and by VCS. For this purpose, an infusion of tamoxifen, an agent that has a mixed agonist/antagonist action at ERs, or the pure ER antagonist ICI182780 (ICI) was administered intracerebroventricularly (icv) shortly before application of VCS or infusion of agents that facilitate lordosis.

Material and methods

Animals and surgeries

We used a total of 194 Sprague–Dawley female rats (240–280 g body weight), bred in our colony in Tlaxcala, Mexico. They were maintained under controlled temperature (23 ± 2 °C) and light conditions (14:10 L:D) with Purina rat chow and water provided ad libitum. Rats were bilaterally ovx under anesthesia with xylazine (4 mg/kg) and ketamine (80 mg/kg) and housed in acrylic cages in groups of four. Two weeks after ovariectomy, the females were anesthetized with xylazine and ketamine, placed in a Kopf stereotaxic instrument (Tujunga, CA), and implanted with a 22 gauge stainless steel guide cannula (Plastics One, Roanoke, VA) into the right lateral ventricle following coordinates from the atlas of Paxinos and Watson (2006) (A/P +0.80 mm, M/L −1.5 mm, D/V −3.5 mm with respect to the bregma). A stainless steel screw was fixed to the skull, and both cannula and screw were attached to the bone with dental cement. A 30 gauge insert cannula provided with a cap was introduced into the guide cannula to prevent clogging and contamination. Females in which infusions missed the ventricle were excluded from the study. All subjects received an antibiotic (penicillin 22,000 UI/kg) and an anesthetic (lidocaine 5%) for three days after surgery. Animal care and all the experimental procedures adhered to NIH guidelines and the Mexican Law for the Protection of Animals.

Drugs

E_2 benzoate (E_2B), P, 5α-pregnan-3,20-dione (5α-DHP), 5α-pregnan-3α-ol-20-one (5α,3α-Pgl), 5β-pregnan-3,20-dione (5β-DHP), 5β-pregnan-3β-ol-20-one (5β,3β-Pgl), GnRH, PGE_2, leptin (rat recombinant, purity of >97%), ICI (7α-[9-(4,4,5,5,5-pentafluoropentylsulfinyl) nonyl]estra-1,3,5-(10)-triene-3,17β-diol) and tamoxifen were purchased from Sigma Chemical, Inc. (St. Louis, MO). It should be noted that tamoxifen did not produce generalized behavioral suppression, and this compound has been widely used in studies of female sexual behavior. Moreover, it has been reported that tamoxifen can function as a neuroprotectant against methamphetamine-induced neurotoxicity in females (Dluzen and Mickley, 2005). In that study, tamoxifen increased several locomotor behaviors, such as horizontal activity, number of movements and total distance traveled.

Experiment 1

Effect of tamoxifen on estrous behavior induced by P and 5α- and 5β-reduced progestins

This experiment was performed to assess the participation of ERs in estrous behavior induced by progestins with a variety of structures. One week after cannula placement, all females were injected s.c. with 5 μg of E_2B. At 39.5 h after E_2B either tamoxifen or oil vehicle was infused, and 40 h after E_2B progestins were infused. All compounds were infused icv in a volume of 1 μl delivered through a plastic catheter (Clay Adams; PE 10 No. 7401) fitted to a Hamilton syringe (10 μl) inserted into the guide cannula. Progestins and tamoxifen were dissolved in sesame oil and injected at the following doses: 130 ng for P (n = 8), 5β-DHP (n = 8) and 5β,3β-Pgl (n = 8) and 13 ng for 5α-DHP (n = 8) and 5α,3α-Pgl (n = 9). These doses were selected from dose–response curves previously established in our laboratory (González-Flores et al., 2006). Other groups of females were injected icv at 39.5 h after E_2B with 5 μg of tamoxifen and 30 min later received the following progestins: 130 ng of P (n = 9), 5β-DHP (n = 9) and 5β,3β-Pgl (n = 9) and 13 ng of 5α-DHP (n = 11) and 5α,3α-Pgl (n = 9). The dose of tamoxifen was selected from previous studies showing its effectiveness for interfering with ER activation (McKenna et al., 1992).

Experiment 2

Effect of tamoxifen on estrous behavior induced by GnRH and leptin

This experiment was performed to assess the participation of ER in estrous behavior induced by GnRH and leptin. One week after cannula placement, all females were injected s.c. with 5 μg of E_2B and 39.5 h later received an icv infusion of vehicle (oil) followed at 40 h by icv GnRH or leptin as described in Experiment 1. GnRH was dissolved in distilled water and injected at a concentration of 50 ng/1 μl (n = 9). Leptin (1 mg) was initially dissolved in 111 μl of Tris (10 mM, pH = 8). From this original concentration, dilutions were made to obtain the selected leptin dose of 3 μg/1 μl (n = 8). The doses of both drugs were selected from previous studies in our laboratory (García-Juárez et al., 2011). Other groups of females were injected icv at 39.5 h after E_2B with 5 μg of tamoxifen and 30 min later received 50 ng/1 μl of GnRH (n = 9) or 3 μg/1 μl of leptin (n = 10).

Experiment 3

Effect of tamoxifen on estrous behavior induced by PGE_2 and VCS

This experiment was performed to assess the participation of ERs in estrous behavior induced by PGE_2 and VCS. One week after cannula placement, all females in the PGE_2 groups were injected s.c. with 5 μg of E_2B, and 39.5 h later, some rats received vehicle (oil, n = 8) or tamoxifen (n = 9). At 40 h after E_2B, rats received icv infusions of PGE_2 as described in Experiment 1. PGE_2 was injected at a concentration of 1 μg/1 μl of saline. Forty hours after E_2B injection and 30 min after infusion of vehicle (oil), 9 females received VCS (a 150-g force applied against the cervix using a glass plunger from a 1 ml syringe inserted intra-vaginally). The VCS was applied either alone or concurrently with manual flank stimulation (palpations applied with the finger and thumb to both flanks and with the palm of the hand to the perineal area). Either form of stimulation was applied for 5 s. Other females were injected icv at 39.5 h after E_2B with 5 μg of tamoxifen and 30 min later received VCS (n = 9).