Hormones and Behavior 64 (2013) 539–545

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

Membrane progestin receptors in the midbrain ventral tegmental area are required for progesterone-facilitated lordosis of rats

Cheryl A. Frye [a,b,c,d,e,*], Alicia A. Walf [a,e,f], Amy S. Kohtz [a], Yong Zhu [g]

[a] Dept. of Psychology, The University at Albany-SUNY, Albany, NY 12222, USA
[b] Dept. of Biological Sciences, The University at Albany-SUNY, Albany, NY 12222, USA
[c] The Centers for Neuroscience, The University at Albany-SUNY, Albany, NY 12222, USA
[d] Life Science Research, The University at Albany-SUNY, Albany, NY 12222, USA
[e] Department of Chemistry, The University of Alaska-Fairbanks, IDeA Network of Biomedical Excellence (INBRE), 202 West Ridge Research Bldg., Fairbanks, AK 99775, USA
[f] Cognitive Science Department, Rensselaer Polytechnic Institute, Troy, NY 12180-3590, USA
[g] Department of Biology, East Carolina University, Greenville, NC 27858-4353, USA

ARTICLE INFO

Article history:
Received 12 February 2013
Revised 22 May 2013
Accepted 29 May 2013
Available online 12 June 2013

Keywords:
Lordosis
Progesterone
Neurosteroids
Progestin and AdipoQ Receptor (PAQR)
Ventromedial hypothalamus
Ventral tegmental area

ABSTRACT

Progesterone (P_4) and its metabolites, rapidly facilitate lordosis of rats partly through actions in the ventral tegmental area (VTA). The study of membrane progestin receptors (mPRs), of the Progestin and AdipoQ Receptor (PAQR) superfamily, has been limited to expression and regulation, instead of function. We hypothesized that if mPRs are required for progestin-facilitated lordosis in the VTA, then mPRs will be expressed in this region and knockdown will attenuate lordosis. First, expression of mPR was examined by reverse-transcriptase polymerase chain reaction (RT-PCR) in brain and peripheral tissues of proestrous Long–Evans rats. Expression of mPRα (paqr7) was observed in peripheral tissues and brain areas, including hypothalamus and midbrain. Expression of mPRβ (paqr8) was observed in brain tissues and was abundant in the midbrain and hypothalamus. Second, ovariectomized rats were estrogen (E_2; 0.09 mg/kg, SC), and P_4 (4 mg/kg, SC) or vehicle-primed, and infused with antisense oligodeoxynucleotides (AS-ODNs) targeted against mPRα and/or mPRβ intracerebroventricularly or to the VTA. Rats were assessed for motor (open field), anxiety (elevated plus maze), social (social interaction), and sexual (lordosis) behavior. P_4-facilitated lordosis was significantly reduced with administration of AS-ODNs for mPRα, mPRβ, or co-administration of mPRα and mPRβ to the lateral ventricle, compared to vehicle. P_4-facilitated lordosis was reduced, compared to vehicle, by administration of mPRβ AS-ODNs, or co-administration of mPRα and mPRβ AS-ODNs, but not mPRα AS-ODNs alone, to the VTA. No differences were observed for motor, anxiety, or social behaviors. Thus, mPRs in the VTA are targets of progestin-facilitated lordosis of rats.

© 2013 Elsevier Inc. All rights reserved.

Introduction

Steroid signaling is typically considered to be mediated through nuclear steroid receptors, which regulate gene transcription and translation. This classical "genomic" mechanism of steroid action involves binding of steroid receptors directly to DNA, altering transcription and synthesis of proteins. Genomic signaling mechanisms for steroid-mediated behaviors, such as mating, have been well-characterized, and can even be considered dogma in biological fields, resulting from decades of studies. Genomic steroid action is a relatively slow process that can take hours to days to elicit a biological response. However, unlike this classical steroid mechanism, many actions of steroids can occur much more rapidly and in the presence of inhibitors of transcription and/or translation. These rapid, "non-genomic," or non-classical, steroid actions have been demonstrated for all major classes of steroids, but the identity of these targets has been hotly contested (Lösel et al., 2003; Norman et al., 2004; Pietras and Szego, 1975; Thomas, 2008; Zhu et al., 2008). The onset and duration of reproductive behavior induced by estradiol (E_2) and progesterone (P_4) at least in part are mediated by this rapid non-genomic progestin signaling in female rodents (Caldwell, 2002; Delville, 1991; Frye, 2009). A research interest has been on the receptor targets for these effects.

In E_2-primed rodents, P_4 has classical and non-classical actions in the ventromedial hypothalamus (VMH) and midbrain ventral tegmental area (VTA) to mediate mating. Briefly, in the VMH, P_4's actions via nuclear progestin receptors (nPRs) and induction of gene transcription reflect the classical actions of ovarian steroids' modulation of reproductive responses (reviewed in Blaustein, 2003). P_4's actions in the VTA, an area of the brain with few E_2 induced nPRs, influence the intensity and duration of sexual receptivity of rodents

* Corresponding author at: Dept. of Psychology, The University at Albany-SUNY, Albany, NY 12222, USA.
E-mail address: cafrye@alaska.edu (C.A. Frye).

0018-506X/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.yhbeh.2013.05.012

exclusively through non-classical, rapid actions at neuronal membranes (reviewed in Frye, 2001a,b, 2009). In support, free P_4 and P_4 bound to large macromolecules, such as P_4:BSA, P_4:HRP conjugates, that are impermeable have similar effects to rapidly enhance lordosis when applied to the VTA (Frye and DeBold, 1993; Frye and Gardiner, 1996; Frye et al., 1992), suggesting that P_4 does not have to diffuse through the cell membrane to have its actions in the VTA. When administered directly to the VTA, P_4 increases cell firing in the VTA within 60 s, and facilitates lordosis within 5 min (Frye and Bayon, 1999). These effects are considered to occur in a shorter timeframe than is necessary for binding to nPRs and altering gene transcription (Pfaff and McEwen, 1983). Prior investigations have supported the notion that P_4's actions in the VTA for lordosis were through membrane receptors, such as $GABA_A$, dopamine type 1-like receptors or glutamatergic receptors, and their downstream signal transduction processes, rather than nPRs (see Frye and Walf, 2008 for a review). Of interest, and investigated in the current project, was whether some of the non-genomic actions of P_4 in the VTA may occur through the membrane progestin receptors (mPRs), of the Progestin and AdipoQ Receptor (PAQR) super-family.

In the present study, the hypothesis that mPRα (PAQR7) and mPRβ (PAQR8), two of the most common variants of mPRs, are targets of P_4 for mating and reproduction-related behavior was tested. First, whether there was expression of mPRα and mPRβ in peripheral tissues (spleen, heart, lungs, kidney, liver, intestines) and different brain regions (prefrontal cortex, hippocampus, amygdala, hypothalamus, and midbrain) of sexually-receptive rats was examined. Second, whether sexual receptivity (as defined by lordosis), and other behaviors (exploration, anxiety, social interaction), will be affected when expression of mPRs is altered following intracerebroventricular (ICV) or VTA infusions of mPRα and/or mPRβ antisense deoxynucleotides (AS-ODN) was examined. We predicted that if mPRs in the VTA are required for progestin-facilitated lordosis, then mPRs will be expressed in this region and knockdown will attenuate lordosis. Furthermore, if these effects are progestin-dependent, we expect to see a different pattern of results among rats that were E_2-primed alone, compared to those that were E_2- and P_4-primed.

Materials and methods

These methods were approved by the Institutional Animal Care and Use Committee at The University at Albany-SUNY and were conducted in accordance with ethical guidelines defined by the National Institutes of Health (NIH Publication No. 85-23).

Animal housing

Adult (55–60 days old), Long–Evans female rats (N = 167) were bred in the Life Sciences Laboratory Animal Care Facility at The University at Albany-SUNY (original stock obtained from Taconic, Germantown, NY, USA). Rats were housed in polycarbonate cages with woodchip bedding (45 × 24 × 21 cm) in a temperature-controlled room (21 ± 1 °C) and were maintained on a 12:12 h reversed light cycle (lights off at 08:00 h). Rats had continuous access to Purina Rat Chow and tap water in their home cages.

Experiment 1—mPR expression

Determination of estrous cycle stage

Adult, female rats had estrous cycle stage determined by assessment of vaginal cytology. The presence of nucleated and cornified cells was used to identify rats in proestrus, and the presence of heterogeneous cell types in smears taken was used to identify rats in diestrus. Rats had tissues collected when they were sexually-receptive in proestrus, which is an estrus stage associated with high E_2 and P_4 levels.

Tissue collection and RT-PCR

Whole brains and peripheral tissues were collected and expression of mPRα and mPRβ was determined with reverse transcriptase polymerase chain reaction (RT-PCR). The expression of mPRα and mPRβ was determined by RT-PCR for whole brain, spleen, heart, lungs, kidney, liver, and intestines, as well as in grossly-dissected brain regions (prefrontal cortex, hippocampus, amygdala, hypothalamus, and midbrain). These tissues were frozen immediately following their collection and dissection. Total RNA was extracted from snap-frozen tissue samples with TRIzol reagent (Invitrogen), homogenized using a sonicator (Sonic Dismembrator Model 100; Fisher Scientific), and purified following the manufacturer's instructions. Then, total RNA (1 μg) of each sample was reverse transcribed into cDNA in a 10-μl reaction using Superscript III (Invitrogen). As a negative control, samples were prepared using the same procedure except without Superscript III (RT minus). The PCR was conducted on the cDNA template for 25 or 30 cycles with an annealing temperature of 55 °C using mPRα- or mPRβ-specific primer pairs (mPRα forward: 5′-CTGCCCCTCTTCATCATTGT-3′; mPRα reverse: 5′-GAAAACCACCTGGCACTTGT-3′; mPRβ forward: 5′-TTGTTTGCAGAG ACCCTGTG-3′, mPRβ reverse: 5′-GAGGCTGCAGGTGAGGTAAG-3′). The PCR products were run on a 2% agarose gel and imaged using a Fluor Chem 8900 imaging station (Alpha Innotech, Santa Clara, CA).

Experiments 2 and 3—mPR knockdown and behavioral testing

Surgical protocol

Rats were administered xylazine (12 mg/kg) and ketamine (80 mg/kg) anesthesia for placement of bilateral guide cannulae aimed at the lateral ventricle (from bregma: AP −1.0, ML ± 1.0, DV −2.0, Exp 2) or VTA (from bregma: AP −5.3, ML ± 0.4, DV −7.0, Exp 3). Guide cannulae consisted of 23-gauge stainless steel needles with 30-gauge removable inserts. Immediately after stereotaxic surgery, rats were ovariectomized (ovx). Following surgery, rats were neurologically evaluated daily for their ability to right themselves, cage-climb, have proper muscle tone and reflexive responses to hind limb extension. Rats were also evaluated for weight gain after surgery. Only rats that passed neurological evaluations and gained weight following surgery were continued in the experiment. Rats were administered post-operative analgesic for 5 days following surgery.

Hormone-priming

In Experiment 2, using ICV administration of AS-ODNs, rats were E_2-primed with subcutaneous (SC) injections (0.09 mg/kg; in vegetable oil vehicle) 44–48 h prior to behavioral testing and then SC administered P_4 (4 mg/kg; in vegetable oil vehicle) 4–6 h before behavioral testing. Hormones were purchased from Steraloids (Newport, RI). In Experiment 3 using intra-VTA administration of AS-ODNs, rats were SC primed with E_2 and P_4, as described above, or SC primed with E_2 only.

Infusion condition

Rats were infused 44 h, 24 h, and immediately before behavioral testing. Infusions were saline control, mPRα AS-ODN, mPRβ AS-ODN, or mPRαβ AS-ODN to the lateral ventricle (Exp 2) or the VTA (Exp 3). Rats were tested once after receiving the three infusions over 44 h. The timing of these infusions was based upon the hormone-priming protocol that was utilized to mimic proestrous-increases in E_2 and P_4 and to counter the known instability of AS-ODNs so that these targets were knocked down during behavioral testing and hormone-priming. Full phosphorothioate AS-ODNs were synthesized, such that S-oligonucleotides were capped and remaining links were unmodified, purified by HPLC, and desalted by Invitrogen Life Technologies (Carlsbad, CA). The sequence for the mPRα AS-ODN was: 5′-CGCTCTTC TGGAAGCCGTACATCTATG-3′. The sequence for the mPRβ AS-ODN was: 5-GACTGGAAAGTAAGTAGGTGGCTGGCTGGTCCTC-3′.

Download English Version:

https://daneshyari.com/en/article/10301103

Download Persian Version:

https://daneshyari.com/article/10301103

Daneshyari.com