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Progesterone-facilitated lordosis of estradiol-primed mice is attenuated by knocking down expression of membrane progestin receptors in the midbrain

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ABSTRACT

Evidence is emerging of the role of membrane progestin receptors (referred to as mPRs herein: members of Progestin and AdipoQ Receptor (Paqr) family) as a novel brain target in mammals, such as rats. In the present study, the role of mPRs in mice was assessed to further elucidate the conservation of this mechanism across species. The brain target investigated was the midbrain ventral tegmental area (VTA) given its described role for rapid actions of progestins for reproduction. Studies tested the hypothesis that if mPRs are required for progestin-facilitated lordosis through actions in the VTA, then knockdown of mPRs in the VTA will attenuate lordosis. Ovariectomized (OVX) mice were subcutaneously injected with estradiol (E_2) and progesterone (P_4), and infused with antisense oligodeoxynucleotides (AS-ODNs) to mPRα (Paqr7) and/or mPRβ (Paqr8) or vehicle to the lateral ventricle or VTA. Mice were assessed for reproductive behavior (lordosis and aggression/rejection quotients) in a standard mating task. Results supported our hypothesis. $E_2 + P_4$-facilitated lordosis was significantly reduced, and aggression/rejection increased, with infusions of mPRα, mPRβ, or mPRαβ AS-ODNs to the lateral ventricle, compared to vehicle. $E_2 + P_4$-facilitated lordosis was significantly decreased, and aggression/rejection increased, with mPRβ or mPRαβ AS-ODNs to the VTA of C57/BL6 mice. Both mPRα and mPRβ AS-ODNs reduced lordosis, and increased aggression/rejection, of wildtype (C57/BL6x129) mice, but not nuclear PR knockout mice. Thus, mPRs may be a novel target of progestins for reproductive behavior of mice.

1. Introduction

One approach to further understand the mechanisms and brain targets of ovarian hormones for their functional effects is to use a model that includes a behavioral output that is dependent upon these hormones, and manipulate actions of hormones in brain regions of interest. One such well-characterized model utilized to investigate these questions for ovarian steroids is reproductive behavior of female mice. Reproductive behaviors of female rodents depend on ovarian hormones, such as 17β-estradiol (E_2) and progesterone (P_4) and environmental stimuli. Cyclic increases in E_2, followed by P_4, are associated with sexual receptivity of mice. Ovariectomy attenuates cyclic increases in these hormones and sexual receptivity of mice [1]. Administration of hormone regimen that produce circulating concentrations, akin to that observed during behavioral estrus, reliably reinstate sexual receptivity commensurate to that which can be observed over the estrous cycle [2–5]. Thus, this model of reproductive behavior in mice is utilized to address questions about the mechanisms and brain targets of ovarian hormones; a focus, in the present study, is the actions and brain targets of progestins, such as P_4.

One mechanism of progestins to consider "genomic" signaling involving classical progestin receptors (PRs), which were traditionally considered to be located in the nucleus (and will thus be referred to as nPRs herein), and have actions as transcriptional factors, regulating gene transcription and translation. There is also "non-genomic" signaling of progestins, which can include classical, or nPRs, that are tethered to the membrane and have actions in this location of the cell, and other transmembrane steroid and

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neurotransmitter targets; reproductive behavior has been one model utilized to understand these different, and potentially complementary, mechanisms of progestins. Among E_2-primed rodents, P_4 has both genomic and non-genomic effects in the ventral medial hypothalamus (VMH) and midbrain ventral tegmental area (VTA) to mediate mating. As an example of the genomic actions of progestins, in the VMH, P_4's classical actions involving nPRs and induction of gene transcription are important for modulation of reproductive responses [6]. P_4's actions in the VTA, an area of the brain with few non-E_2 induced nPRs, influences the intensity and duration of sexual receptivity of rodents exclusively through non-genomic, rapid actions at neuronal membranes, such as via neurotransmitter targets, including GABA and dopamine [7–9]. Another potential target of interest is the membrane progestin receptor, which is a member of the Progestin and AdipoQ Receptor (Paqr) family, identified by Zhu and colleagues [10,11]. These receptors (referred to as mPRs herein) alter progestin binding and rapid non-genomic signaling in various *in vitro* expression systems, such as *Escherichia coli*, yeast, and mammalian cell lines. Actions involving mPRs for functional effects, such as those effects for reproduction, have received much less attention to date compared to these *in vitro* models.

The hypothesis tested in the present series of experiments was that mPRα (Paqr7) and mPRβ (Paqr8), two of the most common variants of mPRs, are targets of progestins for reproductive behavior of mice. A preliminary probe assessed 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 naturally sexually-receptive mice. Experiments were conducted to assess reproductive responses of OVX, hormone-primed mice following manipulations of mPRα and mPRβ with infusions of AS-ODNs to the lateral ventricle or to the VTA. These comparisons were done to begin to address site specificity of these effects. Moreover, if the AS-ODN treatment was producing other side effects, the notion was that these would be particularly apparent with lateral ventricle infusions. We also examined these effects across different strains of mice. Mice were replete in nPRs (C57/BL6 in Experiments 1 and 2, or PRKO wildtypes on a C57/BL6x129/SvEv background in Experiment 3) or lacking functional nPRs (PRKO mice in Experiment 4) to begin to ascertain if there may be interactions with classical nuclear PRs. We predicted that if mPRs are involved in P_4's non-genomic actions in the VTA for reproduction, knocking down mPRs in the midbrain VTA will selectively reduce lordosis responses of OVX, E_2- and P_4-primed mice.

2. Experimental

2.1. Experimental overview

A pilot experiment assessed mPR expression in peripheral and central tissues of proestrous C57/BL6 mice ($n = 2$). For Experiment 1, OVX C57/BL6 mice were administered E_2 and P_4 and infused with control ($n = 15$), mPRα ($n = 13$), mPRβ ($n = 13$) or mPRαβ ($n = 15$) AS-ODNs to the lateral ventricle. For Experiment 2, OVX C57/BL6 mice were administered E_2 and P_4 and infused with control ($n = 9$), mPRα ($n = 9$), mPRβ ($n = 10$) or mPRαβ ($n = 14$) AS-ODNs to the VTA. For Experiment 3, OVX PRKO wildtypes on a C57/BL6x129 background were administered E_2 and P_4 and infused with control ($n = 11$), mPRα ($n = 15$), mPRβ ($n = 13$) or mPRαβ ($n = 16$) AS-ODNs to the VTA. For experiment 4, PRKO mice were administered E_2 and P_4 and infused with control ($n = 13$), mPRα ($n = 12$), mPRβ ($n = 11$) or mPRαβ ($n = 13$) AS-ODNs to the VTA. For Experiments 1–4, mice were behaviorally tested and tissues were collected from a subset of animals to verify effects of AS-ODN infusions. A control experiment assessed specificity of effects by determining extent of behavioral responses following mPR manipulations in OVX, C57/BL6 mice administered E_2 only and infused with control ($n = 12$), mPRα anti-sense deoxynucleotides (AS-ODN; $n = 4$), mPRβ AS-ODN ($n = 5$) or mPRαβ AS-ODN ($n = 5$) to the lateral ventricle. These methods utilizing live animals (surgery, drug manipulations, behavioral testing, euthanasia) 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).

2.2. Housing of animal subjects

Subjects were 8–10 week old, female mice that were C57/BL6, PRKO, or their wildtype counterparts on a C57/BL6x129 background ($n = 272$). Mice were group-housed (4/5 per cage) in polycarbonate cages ($26 \times 16 \times 12$ cm) in a temperature-controlled room (21 ± 1 °C) in the Laboratory Animal Care Facility. Mice were maintained on a 12/12-h reversed light cycle (lights off at 8:00 am) with continuous access to Purina Mice Chow and tap water in their home cages.

2.3. Mouse strain and genotyping

C57/BL6 mice, bred in our colony, were used for Experiments 1 and 2. For Experiments 3 and 4, wildtype (+/+) or homozygous (−/−) PRKO knockout mice were derived from heterozygous (+/−) breeder pairs from a colony that maintained our animal facility. Genotyping was determined by genomic DNA isolated from tails and analyzed by polymerase chain reaction (PCR) modified from Jackson Laboratory protocol and per previous methods to determine the genotype of mice [4,12–15]. PCR was performed by denaturing the DNA at 95 °C for 5 min, followed by 30 cycles of amplification: 94 °C for 1 min, 60 °C for 1 min, 72 °C for 1 min and a final primer extension step at 72 °C for 10 min. The following PR specific primers were used: P1 (5′-TAGACAGTGTCTTAGA CTCGTTGTTG-3′), P2 (5′-GATGGGCACATGGATGAAATC-3′), and a neo gene- specific primer, N2 (5′-GCATGCTCCAGACTGCCTTGG GAAA-3′). Bands of approximately 565 and 500 base pairs were amplified for wild-type and PRKO, respectively.

2.4. Estrous cycle determination

For the expression study, mice were cycled daily. To determine what stage of the estrous cycle each mouse was in, vaginal epithelium of experimental mice was obtained by lavage and examined under a light microscope daily between 0700 and 0900. After two weeks of regular, 4–5 day cycles, tissues were collected of mice when in proestrus or behavioral estrus. Mice were considered in proestrus when their vaginal epithelium had characteristic nucleated cells, 4–5 days following the previous lavage of this type.

2.5. mPR expression

Mice were left intact and cycled and had tissues collected when they were sexually-receptive (in proestrus), associated with high E_2 and P_4 levels. Whole brains and peripheral tissues were collected at University of Albany. Expression of mPRα and mPRβ were determined at East Carolina State University with reverse transcriptase PCR (RT-PCR) (from tissues from University of Albany) which were frozen immediately following collection and dissection, and shipped on dry ice overnight. Expression of mPRα and mPRβ was determined by RT-PCR for brain, spleen, heart, lungs, kidney, liver, and intestines. In brain, expression was examined in prefrontal cortex, hippocampus, amygdala, hypothalamus, and