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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], and **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

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.

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

Keywords

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

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 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′-TTGTTTGCAGAGACCCTGTG-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′-CGCTCTTCTGGAAGCCGTACATCTATG-3′. The sequence for the mPRβ AS-ODN was: 5-GACTGGAAAGTAAGTAGGTGGCTGGCTGGTCCTC-3′.

Behavioral tasks—Rats were tested sequentially in the following tasks (open field, elevated plus maze, social interaction, and paced mating), in the order listed. For behavioral testing, assessments of each subject were made by an experienced experimenter with the

Any-Maze video-tracking system (Stoelting, Wood Dale, IL). Rats were tested in smaller cohorts, with different experienced experimenters collecting the data; the concordance rate between experimenters and the video-tracking system was high (95%), with no evidence to suggest that there were large differences between experimenters' assessments. Experimenters were not fully knowledgeable about the conditions of rats, or hypothesized outcome of these studies.

For the open field testing, rats were placed in the open field, observed for 5 min, while the number spent in central and peripheral squares (summed for total) was recorded (Frye et al., 2000; McCarthy et al., 1995). The open field (76 × 57 × 35 cm) has a 48-square grid floor (6 × 8 squares, 9.5 cm/side), and the central squares (all but the 24 perimeter squares) are illuminated from overhead.

For the elevated plus maze, rats were placed at the junction of the open and closed arms, and the number of entries, and amount of time spent on the open and closed arms, were recorded for 5 min (Frye et al., 2000, 2008; Walf and Frye, 2007). The elevated plus maze consists of 2 arms (49 cm long, 10 cm wide), enclosed by walls 30 cm high, and 2 exposed arms, elevated 50 cm off the ground.

For the social interaction task, experimental and conspecific rats are placed in opposite corners of the open field (described above). An ovariectomized rat was utilized as the conspecific in this task. Time spent by the experimental rat engaging in social interaction (crawling over and under partner, sniffing, following with contact, anogenital investigation, tumbling, boxing and grooming) with the conspecific was recorded for 5 min (Frye et al., 2000, 2008).

In the paced mating task, the female's pacing of male's contacts for an ejaculatory series was evaluated in a chamber with a partition, which divides the chamber equally (Frye and Erskine, 1990; Frye and Orecki, 2002a,b). Males are relegated to one side and the frequency of lordosis, proceptivity, and aggression as well as a qualitative measure of lordosis (lordosis ratings (LRs) on a 3 point scale according to Hardy and DeBold, 1971) are calculated (Frye and Erskine, 1990; Frye et al., 1998). Lordosis quotients (LQs) were determined by calculating the total number of lordosis responses / total number of mounts by the male. Proceptivity quotients (PQs) were determined by calculating the incidence of proceptive behaviors (hopping, darting, and ear wiggling) / total number of mounts by the male. Aggression quotients (AQs) were determined by calculating the incidence of aggression / rejection behaviors / total number of mounts by the male.

Tissue collection and measurement of plasma E_2 and P_4 levels—Immediately after testing, rats were rapidly decapitated. Trunk blood and whole brain were collected to measure hormone levels and examine the expression of mPRα and mPRβ, respectively. Plasma levels of E_2 and P_4 were determined with radioimmunoassay using previously described methods (Frye et al., 2009) to validate that physiological levels were achieved with the systemic dosing of E_2 and P_4 that were utilized. We found that there were proestrous-like levels of E_2 in plasma of ovx rats administered E_2 (52.5 ± 4.9 pg/ml) or E_2 and P_4 (51.0 ± 4.5 pg/ml). As expected, levels of P_4 were higher in plasma of rats

administered systemic P_4 (24.0 ± 2.8 ng/ml) and reached proestrous-like levels, compared to rats that were administered E_2 alone (11.9 ± 2.0 ng/ml).

Infusion placements and site-specificity—Frozen brains were sliced at the level of the midbrain and cut in 100 micron slices. These slices were inspected visually using a dissecting microscope as previously described (Frye et al., 2009). All rats with ICV infusions were found to have infusions to the ventricle. Eleven of the rats with intended infusions to the VTA had infusions to other sites. Data from rats that had infusions to sites other than the VTA did not show the same pattern of behavior as did rats with infusions to the VTA (see Table 1): their data were excluded from the overall analyses. To determine efficacy of AS-ODNs, mPRα and mPRβ expression was examined in micropunches from the VTA which were taken from the slices and in some cases included additional tissues from the surrounding midbrain. Tissues were placed in RNAlater (Qiagen) until homogenizations and extractions to prevent degradation and were used for determining mPRα and mPRβ expression with quantitative PCR (qPCR). Expression resulting from ICV and VTA AS-ODN infusions was determined in 6–9 and 4–6 rats per condition, respectively.

qPCR—Standard qPCR methods were utilized (Zhu et al., 2003a). For extractions, total RNA was isolated from tissue using the Qiagen RNeasy Micro Kit (Valencia, CA) according to the manufacturer's protocol. Reverse transcription was carried out using Oligo$(dT)_{20}$ and the Superscript III First-Strand Synthesis System for RT-PCR from Invitrogen (Carlsbad, CA). qPCR was performed using Bio-Rad SYBR Green Supermix (Hercules, CA) and the following gene-specific primers (Integrated DNA Technologies, Coralville, IA): β-actin forward (5′-GCTCGTCGTCGACAACGGCT-3′), β-actin reverse (5′-CAAACATGATCTGGGTCATCTTCTC-3′), mPRα forward (5′-TGTGGCCGTGTACCAGTTT-3′) and reverse (5′-TGATACAGAAGGGCGGGAT-3′) and mPRβ forward (5′-GAGTGGACTGCACCTCTGTC-3′) and reverse (5′-CTCCTCGGGGTTCAGTAGGA-3′). Reactions were run on an Applied Biosystems 7900HT and analyzed using the comparative cycle time (DeltaDeltaCT) method (Applied Biosystems, Foster City, CA). The fold change in comparison to vehicle controls of the delta CT values of mPR versus actin is depicted for the differences in the number of cycles for expression as observed from subjects in each condition (Livak and Schmittgen, 2001; Schmittgen and Livak, 2008).

Statistical analyses—One-way analyses of variances (ANOVAs) were used to examine effects of infusion condition (control, mPRα, β, αβ AS-ODN groups) on behavior and mPR RNA expression. When the α level for statistical significance was reached $p \leq 0.05$, or a trend was observed $p < 0.10$, post hoc tests were used to examine group differences.

Results

Experiment 1—brain and periphery expression patterns of mPRα and mPRβ

Analyses of samples from proestrous rats demonstrated that mPRβ was expressed in the brain, but was undetectable in the peripheral tissues (spleen, heart, lung, kidney, liver,

intestines) of rats (Fig. 1). The expression of mPRα was observed in all tissues of proestrous rats at 25 PCR cycles, and the expression of mPRβ was observed at 30 cycles. Further characterization of expression of mPRs in the central nervous system (prefrontal cortex, hippocampus, amygdala, hypothalamus, midbrain) was completed using RT-PCR (Fig. 1). The expression patterns of both receptors were strong in the midbrain region.

Experiment 2: ICV infusions of mPRβ, mPRα or mPRαβ AS-ODNs attenuate sexual receptivity

Although AS-ODN infusions did not significantly alter behavior in the open field, social interaction or elevated plus maze (see Table 2, top), there were significant effects of ICV AS-ODN infusions on reproductive behaviors. As Fig. 2 (top) illustrates, infusions of mPRα, mPRβ, and mPRαβ AS-ODNs significantly reduced incidence of lordosis ($F(3, 32) = 11.85$, $p < 0.01$). Other parameters of reproductive behaviors (see Table 2, middle), such as intensity of lordosis ($F(3, 32) = 3.09$, $p < 0.01$) and solicitation behaviors ($F(3, 32) = 6.81$ $p < 0.01$) were reduced by AS-ODN infusions, and incidence of aggression was increased by AS-ODN infusion ($F(3, 32) = 5.69$, $p < 0.01$), compared to control infusions. Rats infused ICV with mPRα, mPRβ, or both AS-ODNs had lower mPRα expression in the midbrain compared to those in the control condition ($F(3, 26) = 4.56$, $p < 0.01$) (see Table 2, bottom). ICV infusions of mPRβ AS-ODNs also reduced mPRβ expression. In contrast, ICV infusions of mPRα ODN increased the expression of mPRβ in the midbrain (Table 2, bottom).

Experiment 3: VTA infusions of mPRβ or mPRαβ, but not mPRα, AS-ODNs attenuate sexual receptivity

Infusion to the VTA of AS-ODN did not significantly alter behavior in the open field, social interaction or elevated plus maze (see Table 3, top). There was a significant effect of VTA infusions on LQs ($F(3, 54) = 20.05$, $p < 0.01$). Rats infused with mPRβ or mPRαβ had significantly lower LQs compared to the control infusion condition (see Fig. 2, bottom). Infusions of mPRβ or mPRαβ to the VTA significantly reduced LRs ($F(3, 54) = 14.25$, $p < 0.01$), PQs ($F(3, 54) = 14.62$, $p < 0.01$), and increased AQs ($F(3, 54) = 4.49$, $p < 0.01$) compared to control (see Table 3, middle). Rats infused with mPRα, mPRβ, and mPRαβ AS-ODNs had significantly reduced mPRα expression in the midbrain ($F(3, 24) = 13.22$, $p < 0.01$) compared to vehicle. Rats infused with mPRβ or mPRαβ AS-ODNs compared to control had significantly lower mPRβ ($F(3, 15) = 4.59$, $p < 0.01$) expression in the midbrain, while mPRα AS-ODNs increased mPRβ expression (see Table 3, bottom). Moreover, the effects on sexual behavior were not observed among rats that were primed only with E_2 when mPR AS-ODNs significantly downregulated the expression of mPRs (Table 4).

Discussion

The hypothesis that mPRs are involved in P_4's actions in the VTA, relevant for reproductive behavior of E_2-primed rats, was supported. First, mPRα and mPRβ were expressed in the midbrain and knockdown was observed with AS-ODN infusions to the lateral ventricle and VTA. Second, reductions in incidence and intensity of lordosis behavior were observed with lower expression of mPRβ, either alone or in conjunction with mPRα in the midbrain. Third,

these effects were site-, behavior-, and hormone-specific. Site-specificity is demonstrated by more circumspect effects observed with VTA, compared to ICV, infusions and the different patterns of effects observed with infusions to sites outside the VTA. There were no salient effects of AS-ODN infusions on open field, social interaction or elevated plus maze behavior, albeit the expression of mPRs in hippocampus, a brain region that may be central for mediating behavior in these tasks, was not examined. As well, no effects of AS-ODN infusions were observed without P_4 priming. Together, these findings suggest that mPRs in VTA may be specific targets in the VTA for membrane actions of P_4 to facilitate lordosis.

The present data confirm and extend the literature on the role of non-genomic effects of progestins in the VTA for lordosis. The previously published studies of mPRs were almost exclusively focused on the functions and signaling of mPRα. The unique functions of other mPR isoforms, particular mPRβ, have not been clearly demonstrated. We believe that our study is the first to report that mPRβ may play a major role in progestin-facilitated lordosis in a rodent model. We have previously reported that traditional signaling via nPRs in this region is not required for P_4's actions. First, there are very few nPRs in the VTA of adult rodents and those that are localized to the VTA are not E_2-induced (Blaustein, 2003; Munn et al., 1983). Second, actions of P_4 at the few nPRs in the VTA are not required for facilitation of lordosis. P_4 enhances lordosis when applied to the VTA of mutant mice lacking nPRs, or of rats that have nPRs knocked down with AS-ODNs (Frye and Vongher, 2001; Frye et al., 2000). Third, the rapidity of effects of progestins applied directly to the VTA suggests that these actions do not require genomic signaling of nPRs. Other groups have also shown how important this brain region is for natural rewards, like mating, as well as the effects of drugs of abuse for sexual behavior (Pitchers et al., 2010). Some of these effects in our model, and others, involve signal transduction pathways associated with G-protein coupled receptors in the VTA (reviewed in Frye and Walf, 2008; Pitchers et al., 2010). As such, mPRs, which are G-protein coupled receptors, as putative membrane targets in the VTA, were investigated here.

There is some evidence for the role of ovarian steroids for mPR expression. In support, among ovx and E_2-primed rats, expression of mPRβ, as measured by in situ hybridization and histochemistry, was greater than mPRα in the hippocampus, lateral and medial septum, thalamus, and regions of the midbrain (Intlekofer and Petersen, 2011). Similar results were shown in a recent study comparing expression of mPRα and mPRβ in the hypothalamus, midbrain and forebrain. E_2-treatment decreased mPRβ expression in the medial septum (Zuloaga et al., 2012). Both mPRα and mPRβ were increased during proestrus, compared to diestrus, of rats in the mediobasal hypothalamus (Liu and Arbogast, 2009). In the present study, we saw that reduction of mPRβ in the midbrain was particularly effective in attenuating lordosis of rats. This occurred with ICV infusions, which knocked down mPRα and mPRβ in the VTA, or intra-VTA infusions of AS-ODNs, which only knocked down mPRβ. Another interesting pattern was observed with mPRα AS-ODNs ICV or to the VTA increasing expression of mPRβ in the midbrain. We cannot rule out the importance of mPRs in other brain regions in mediating reproductive and other behaviors. For example, mPRs may also be expressed in regions nearby the VTA that were not investigated in this study, such as the midbrain central gray (a brain structure, which has also previously been implicated in the hormonal regulation of lordosis behavior in female rats; McCarthy et al.,

1995). The present findings provide strong support for a role of mPRβ in the VTA in mediating lordosis and may even suggest that mPRα may influence mPRβ signaling to mediate the expression of progestin-facilitated lordosis in this region.

In addition to the midbrain VTA, the hypothalamus is an important brain region in mediating progestin-facilitated lordosis. The hypothalamus has high expression of classic targets of progestins, nPRs, which are involved in reproductive behaviors of female rodents. Additionally, there is some evidence that P_4 may also have actions independent of nPRs, involving non-classical targets, such as neurotransmitters (e.g. GABA, glutamate), and signal transduction pathways, in the VMH for lordosis (Balasubramanian et al., 2008a,b; Frye, 2001c; Georgescu and Pfaus, 2006a,b; González-Flores et al., 2010; Hoffman et al., 2002). A question not addressed in the present study was the role of mPRs in the VMH as a membrane target for progestins' actions for lordosis. Perhaps hormone-primed rats had attenuated lordosis responding following lateral ventricle infusions of mPRα and/or mPRβ AS-ODNs coincident with reduction in mPRs in the hypothalamus. Indeed, both VTA and ICV infusions of mPRα AS-ODNs resulted in an increased expression of mPRβ in the midbrain, but only ICV infusions of mPRα AS-ODNs reduced lordosis. This suggests to us that mPRα in the hypothalamus and mPRβ in the VTA may be important for progestin-facilitated lordosis. A limitation of this study was that we were unable to systematically examine expression of hypothalamic mPRs. As such, a question for further research is the complementary roles of mPRα in the hypothalamus and mPRβ in the VTA underlying rapid actions of progestins for lordosis.

In the present study, there were no differences in behaviors other than reproductive behavior. Given these null effects, and that we do not have data on mPR knock down in other regions, we cannot rule out the possibility that mPRs may play a role in hippocampally-mediated behaviors. Indeed, there is considerable clinical evidence that supports the need for further understanding progestin-sensitive behaviors (Girdler et al., 2012). The selective expression of mPRβ in the brain, but not in the body, implies that it may be possible in the future to target these receptors without the liability of trophic effects in peripheral reproductive tissues. As such, future studies manipulating mPRs in regions, such as the hippocampus, are of considerable interest. In these experiments, we tried to verify our knock down of mPR following lateral ventricle and midbrain VTA infusions of AS-ODNs in experimental rats using qPCR. The approach that we utilized for taking tissue punches of these regions in a subset of subjects and extracting RNA for qPCR precluded examination of mPR proteins. It is notable that we saw modest changes in RNA expression following AS-ODN infusions; yet, robust behavioral changes. We hope to address the shortcoming of these approaches in our future work.

Currently, strong evidence exists for two main groups of PRs mediating non-genomic progestin actions. One candidate is the nPRs acting through non-genomic signaling pathways (Bayaa et al., 2000; Tian et al., 2000). The nPR, despite having well-defined transcriptional activity (Schwartz et al., 1977), is also thought to have a separate non-genomic signaling function. The nPRs have non-genomic function in breast cancer signaling and *Xenopus* oocyte maturation (Bayaa et al., 2000; Boonyaratanakornkit et al., 2001, 2007; Faivre et al., 2005), but these non-genomic effects of nPRs in the brain for behavior are still

being elucidated. Another candidate is the mPRs, which were characterized a decade ago (Zhu et al., 2003a,b) and investigated here. mPRs have been grouped into a unique receptor class called the PAQR family, which contains 5 unique mPR members (α ~ ε, class II receptors), 4 adiponectin receptor like (class I receptors), and 2 hemolysin-like receptors (Smith et al., 2008; Tang et al., 2005; Thomas et al., 2007). The mPRα and mPRβ are the most well-studied of the mPRs (Dosiou et al., 2008; Dressing and Thomas, 2007; Hanna et al., 2006; Karteris et al., 2006; Thomas et al., 2005), and expression/localization and functional effects of mPRγ, mPRδ, and mPRε are still relatively unknown (Nutu et al., 2007; Zhu et al., 2003a). In support, the mPRα was first characterized in spotted seatrout and studied most extensively of all mPRs. Biochemical analyses have shown that the transcripts and proteins of mPRα are expressed abundantly in the brain, spinal cord, testis, kidney, uterus, and ovary in vertebrates including fish, mice and human (Hanna et al., 2006; Labombarda et al., 2010; Lemale et al., 2008; Sleiter et al., 2009; Zhu et al., 2003b). The potential functions of mPRα have been suggested to mediate rapid progestin signaling in final oocyte maturation in fish oocytes (Thomas et al., 2005; Tokumoto et al., 2006; Zhu et al., 2003b), sperm motility in fish (Tubbs and Thomas, 2009), P_4 signaling in human myometrial cells, breast cancer cells and lymphocytes (Dosiou et al., 2008; Dressing and Thomas, 2007; Karteris et al., 2006; Thomas et al., 2007), reproductive activity in rat and sheep reproductive tissues (Ashley et al., 2006; Cai and Stocco, 2005), and rapid gonadotropin releasing hormone secretion among mice (Sleiter et al., 2009). As well, mPRβ has been proposed to be involved in the controlled beating of cilia of the fallopian tubes of mice and humans (Nutu et al., 2009). A question that had yet to be addressed was the functional role of mPRs in a whole rodent model. The present data suggest that mPRβ may be the membrane target for these rapid effects of P_4 in the VTA. These findings extend what has previously been reported in the literature regarding mPR expression, and suggest a functional role of mPRβ for a hard-wired behavior in a brain region that is conserved across species, the midbrain. Of interest, are the relative roles of nPRs and mPRs for their involvement in rapid functional effects of progestins. Moreover, the extent to which there may be conserved functional effects in other species, such as mice, is the subject of ongoing investigation.

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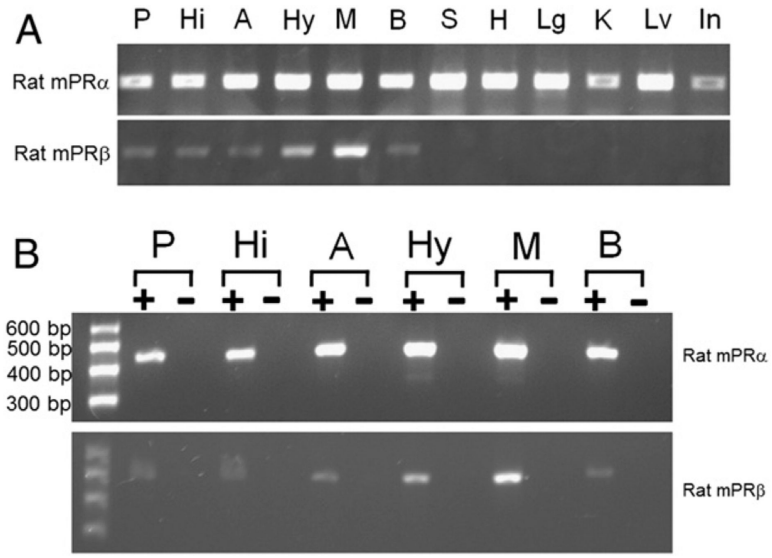

Fig. 1.

Expression of membrane progestin receptor α (mPRα, Paqr7) and mPRβ (Paqr8) transcripts in proestrous rat peripheral tissues and brain regions, as analyzed by RT-PCR. P: prefrontal cortex; Hi; hippocampus; A: amygdale; Hy: hypothalamus; M: midbrain; B: brain; S: spleen; H: heart; Lg: lungs; K: kidney; Lv: liver; and In: intestines. Samples were prepared for all tissues samples using same procedure with (+, indicated in Panel B) or without Superscript III (−, indicated in Panel B). Specific PCR products amplified from the transcripts with expected size were observed. There was no evidence of genomic DNA contamination.

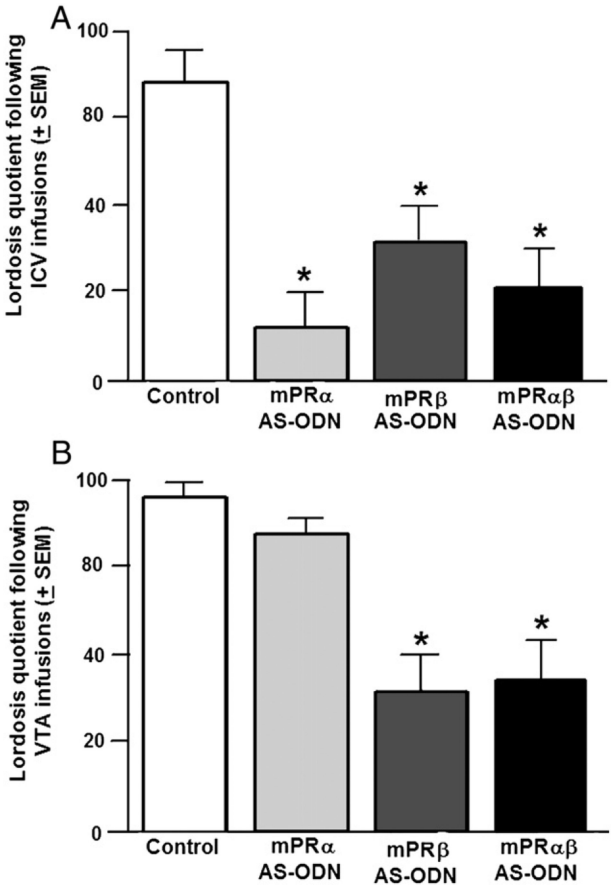

Fig. 2.

Mean (±SEM) lordosis quotients (LQs) of E_2- and P_4-primed rats infused with vehicle (control), mPRα antisense oligonucleotides (AS-ODNs), mPRβ AS-ODNs, or both mPRα/β AS-ODNs intracerebroventricularly (top panel A) or to the VTA (bottom panel B).

*Indicates significant difference from control group ($p < 0.05$).

Table 1

Missed sites/infusions outside the VTA: Behavioral measures in the open field, elevated plus maze, and social interaction task, and effects on reproductive behaviors (lordosis ratings, proceptivity and aggression quotients) of rats that were infused with antisense oligonucleotides (AS-ODNs) outside of the ventral tegmental area (missed sites).

Brain infusion	**Infusions to sites other than the VTA**			
Condition	**Veh**	**mPRα AS-ODN**	**mPRβ AS-ODN**	**mPRαβ, AS-ODN**
n	**0**	**2**	**5**	**4**
Total entries (#)	-	284 ± 68	234 ± 20	287 ± 19
Central entries (#)	-	72 ± 21	55 ± 20	80 ± 13
Open arm time (s)	-	23 ± 6	5±3	22 ± 5
Social interaction (s)	-	51 ± 8	73 ± 18	57 ± 9
Lordosis quotient	-	0±0	85 ± 10	86 ± 11
Lordosis rating	-	0±0	1.8 ± 0.2	2.0 ± 0.3
Proceptivity quotient	-	0±0	79 ± 8	78±14
Aggression quotient	-	35 ± 35	28 ± 19	33 ± 4

Table 2

Infusions to the lateral ventricle: Behavioral measures in the open field, elevated plus maze, and social interaction task, and effects on reproductive behaviors (lordosis ratings, proceptivity and aggression quotients) and fold changes in midbrain mPR expression of ovx, E_2- and P_4-primed rats infused with mPRα, mPRβ and mPRαβ antisense oligonucleotides (AS-ODN) or vehicle to the lateral ventricle. Average fold changes of mPR transcripts were obtained using $2^{-\Delta\Delta C_T}$ (Schmittgen and Livak, 2008). Minus (–) before the number indicates average fold decrease compared to the control.

Brain infusion	**Brain infusion-ICV infusion condition**			
Condition	**Veh**	**mPRα AS-ODN**	**mPRβ AS-ODN**	**mPRαβ AS-ODN**
n	**8**	**9**	**9**	**10**
Total entries (#)	148 ± 29	200 ± 20	183 ± 18	167 ± 20
Central entries (#)	33 ± 7	47 ± 6	41 ± 8	41 ± 8
Open arm time (s)	34 ± 11	48 ± 14	31 ± 12	17 ± 6
Social interaction (s)	75 ± 8	41 ± 10	64 ± 11	77 ± 15
Lordosis rating	1.8 ± 0.3	0.5 ± 0.3*	0.7 ± 0.3*	0.6 ± 0.2*
Proceptivity quotient	68.5 ± 15.6	7.5 ± 5.3*	18.4 ± 10.6*	15.8 ± 8.0*
Aggression quotient	15.3 ± 10.9	32.0 ± 14.2	79.8 ± 10.9*	66.6 ± 12.3*
MPRα fold change	-	–1.5 ± 0.3*	–1.5 ± 0.2*	–1.4 ± 0.1*
MPRβ fold change	-	2.1 ± 1.9	–1.3 ± 1.0	1.1 ± 1.6

*Indicates a statistically significant effect compared to control group ($p < 0.05$).

Table 3

Infusions to the VTA, E_2- and P_4-priming: Behavioral measures in the open field, elevated plus maze, and social interaction task, and effects on reproductive behaviors (lordosis ratings, proceptivity and aggression quotients) and fold changes in mibrain mPR expression of ovx, E_2- and P_4-primed rats infused with mPRα, mPRβ and mPRαβ anti-sense oligonucleotide (ODN) or vehicle to the VTA. Average fold changes of mPR transcripts were obtained using $2^{-\Delta\Delta C_T}$ (Schmittgen and Livak, 2008). Minus (–) before the number indicates average fold decrease compared to the control.

Brain infusion	**Effects of AS-ODN infusions to the VTA**			
Condition	**Veh**	**mPRα AS-ODN**	**mPRβ AS-ODN**	**mPRαβ AS-ODN**
n	**16**	**14**	**13**	**13**
Total entries (#)	245 ± 17	264 ± 17	229 ± 20	254 ± 24
Central entries (#)	60 ± 8	64 ± 9	47 ± 7	56 ± 8
Open arm time (s)	12 ± 3	10 ± 3	10 ± 3	23 ± 9
Social interaction (s)	71 ± 6	70 ± 7	84 ± 7	80 ± 6
Lordosis rating	2.3 ± 0.1	1.7 ± 0.2	0.7 ± 1.0*	0.7 ± 0.2*
Proceptivity quotient	96 ± 3	68 ± 10*	25 ± 11*	30 ± 11*
Aggression quotient	3±2	9 ± 4*	30 ± 11*	35 ± 11*
MPRα fold change	-	–1.0 ± 0.2*	–1.0 ± 0.1*	–1.4 ± 0.1*
MPRβ fold change	-	2.6 ± 1.8	–2.9 ± 1.6*	–1.3 ± 0.2*

*Indicates a statistically significant effect compared to control group ($p < 0.05$).

Table 4

Infusions to the VTA, E_2-only priming. Behavioral measures in the open field, elevated plus maze, and social interaction task, and effects on reproductive behaviors (lordosis ratings, proceptivity and aggression quotients) and fold changes in mibrain mPR expression of ovx, E_2-primed rats infused with AS-ODN to the VTA. Average fold changes of mPR transcripts were obtained using $2^{-\Delta\Delta C_T}$ (Schmittgen and Livak, 2008). Minus (–) before the number indicates average fold decrease compared to the control.

Brain infusion	**Effects of AS-ODN infusions to the VTA**			
Condition	**Veh**	**mPRα AS-ODN**	**mPRβ AS-ODN**	**mPRαβ AS-ODN**
n	**16**	**13**	**13**	**10**
Total entries (#)	255 ± 20	262 ± 16	280 ± 19	265 ± 30
Central entries (#)	58 ± 6	65 ± 8	70 ± 11	48 ± 10
Open arm time (s)	8±3	19 ± 5	17 ± 5	10±5
Social interaction (s)	88 ± 9	89 ± 12	90 ± 11	98 ± 9
Lordosis quotient	17 ± 5	10 ± 7	15 ± 5	15±7
Lordosis rating	0.3 ± 0.1	0.2 ± 0.2	0.5 ± 0.2	0.3 ± 0.1
Proceptivity quotient	3±2	7±7	1±1	1±1
Aggression quotient	38 ± 11	33 ± 12	58 ± 11	41 ± 11
MPRα fold change	-	–1.4 ± 0.2*	–1.0 ± 0.2*	–1.8 ± 0.3*
MPRβ fold change	-	–2.2 ± 0.5	–5.9 ± 2.0	–2.1 ± 0.4

* Indicates a statistically significant effect compared to control group ($p < 0.05$).