Published in final edited form as:

Neuropharmacology. 2010 January ; 58(1): 44–49. doi:10.1016/j.neuropharm.2009.07.006.

Oxytocin and/or steroid hormone binding globulin infused into the ventral tegmental area modulates progesterone-mediated lordosis

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Summary

Estradiol (E₂) and progesterone (P₄) have classical, steroid receptor-mediated actions in the ventral medial hypothalamus to initiate lordosis of female rodents. P₄ and the P₄ metabolite and neurosteroid, 5 α -pregnan-3 α -ol-20-one (3 α ,5 α -THP), have non-classical actions in the midbrain ventral tegmental area (VTA) to modulate lordosis. We investigated the role of steroid hormone binding globulin (SHBG) and oxytocin in the VTA as mechanisms for these effects. Rats were ovariectomized and surgically-implanted with bilateral guide cannulae aimed at the VTA. Rats were E₂-primed (10 μ g/0.2 ml) at hour 0, and administered 100 (Experiments 1 and 2), 500 (Experiment 3), or 0 (Experiment 1 and 4) μ g/0.2 ml P₄ at hour 44. At hour 47.5, rats received bilateral infusions to the VTA, and tested for lordosis 30 minutes post-infusion. Experiment 1: rats were infused with sterile saline vehicle or SHBG (4.5 pg/ μ l) to the VTA. SHBG, compared to vehicle, to the midbrain VTA significantly increased lordosis in E₂- and P₄-primed, but not E₂-primed, rats. Experiment 2: rats were infused with bilateral infusions of sterile saline or oxytocin (1.0 pg/ μ l). Compared to vehicle, oxytocin to the VTA increased lordosis. Experiment 3: rats were administered bilateral intra-VTA infusions of saline or an oxytocin receptor antagonist, d(CH₂)₅[Tyr(Me)²,Thr⁴,Tyr-NH^{9,2}] (1.2 pg/ μ l). Compared to vehicle, the oxytocin receptor antagonist to the VTA attenuated lordosis of E₂- and P₄-primed rats. Experiment 4: rats were E₂-primed and infused with vehicle, oxytocin, or oxytocin antagonist. There were no effects of these manipulations in E₂-primed rats. Thus, SHBG and/or oxytocin may have actions in the VTA for progesterone-facilitated lordosis.

Keywords

neurosteroids; midbrain; 5 α -pregnan-3 α -ol-20-one; allopregnanolone

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1. Introduction

Reproductive behavior of female rodents, characterized by the lordosis posture, is mediated by actions of the ovarian steroids, estradiol (E_2) and progesterone (P_4). These steroids have classical (i.e. cognate steroid receptor-mediated) actions or non-classical actions in various brain regions to modulate lordosis of female rodents. Of interest is the role of neuropeptides for these effects.

Progestogens have classical actions via progesterin receptors (PRs) in the ventral medial hypothalamus (VMH) to initiate lordosis (Pleim et al., 1993; Rubin and Barfield, 1983). Upregulation of PRs in the VMH by E_2 and/or P_4 coincide with expression of lordosis (Ahdieh et al., 1986; Pleim et al., 1989). PR ligands enhance lordosis (Glaser et al., 1985; Vathy et al., 1987), and PR blockers attenuate lordosis (Etgen and Barfield, 1986; Vathy et al., 1989). P_4 to knockout mice does not facilitate lordosis (Frye and Vongher, 1999). Effects of P_4 only occur when P_4 is able to enter the cell, and, bind PRs in the VMH (Frye and Gardiner, 1996; Frye et al., 2000). The VMH is a target of neuropeptides, such as oxytocin, for lordosis. There is high expression of oxytocin receptors in the VMH (Ostrowski, 1998), which can be increased by E_2 (De Kloet et al., 1985). There is high co-expression of estrogen receptors and oxytocin receptors (Devidze et al., 2005). Infusions of oxytocin enhance, and oxytocin receptor antagonists reduce, lordosis, when administered intracerebroventricularly (ICV) or to the VMH (Arletti and Bertolini, 1985; Benelli et al., 1994; Caldwell et al., 1994; Gorzalka and Lester, 1987; McCarthy et al., 1994; Pedersen and Boccia, 2002; Schumacher et al., 1989). These data support role of progestins acting at PRs, and some involvement of oxytocin, in the VMH for lordosis.

The medial preoptic area (MPOA) is another central target of steroids for lordosis. There are E_2 -induced PRs in the MPOA (Parsons et al., 1982). P_4 , or its metabolites, enhance lordosis when administered to the MPOA (Beyer et al., 1988). Unlike what is observed in the VMH, there is some indication that progestins in the MPOA may have effects at PRs and/or at the membrane. Although free P_4 is most efficacious, there are some effects of P_4 conjugated to membrane-impermeable bovine serum albumin (BSA) to enhance lordosis, compared to BSA alone, when applied to the MPOA (Frye et al., 1996). Oxytocin in the MPOA may modulate lordosis, and other reproduction-related behaviors. First, oxytocin levels are increased in the MPOA of ovariectomized rats administered E_2 and P_4 (Caldwell et al., 1988). Second, binding of oxytocin receptors in the MPOA is increased with hormone-priming, mating, gestation, and maternal behavior (Bealer et al., 2006; Caldwell, 1992; Caldwell et al., 1992; Pedersen et al., 1994). Third, infusions of oxytocin ICV or to the MPOA enhance lordosis (Arletti and Bertolini, 1985; Benelli et al., 1994; Caldwell et al., 1989; 1994). Fourth, an oxytocin receptor antagonist to the MPOA-lateral hypothalamus attenuates lordosis (Caldwell, 1992; Caldwell et al., 1990). Furthermore, steroid hormone binding globulin (SHBG), or SHBG+oxytocin, infusions to the MPOA-anterior hypothalamus of ovariectomized E_2 -primed rats enhances lordosis, compared to that observed in rats infused with vehicle or oxytocin alone (Caldwell et al., 2000; 2002). Thus, there is a role of progestins, oxytocin, and SHBG in the MPOA for lordosis.

Another brain region that has received attention as a target of progestogens for lordosis is the ventral tegmental area (VTA). The VTA has unique properties to support its role as a site of non-classical steroid action for lordosis, particularly the intensity and duration of the lordosis response. Unlike the VMH and MPOA, there are few E_2 -induced, intracellular PRs in the VTA of adult rodents (Frye, 2001ab). Ligands of PRs do not facilitate, and blockers do not attenuate, lordosis when applied to the VTA (Frye et al., 2000; Pleim and Barfield, 1988). P_4 to the VTA of PR knockout mice facilitates lordosis (Frye and Vongher, 1998). Free P_4 , or P_4 conjugated to BSA or horseradish peroxidase, enhance lordosis, despite not freely entering the cell (Frye,

2001ab). P₄ or P₄:BSA increase cell firing in <60 secs and facilitate lordosis within 2–5 mins, a short timeframe that may preclude classical, genomic effects (Frye, 2001ab). Thus, these data support a role of non-classical actions of progestogens in the VTA for lordosis.

A question is whether the effects of progestogens in the VTA for lordosis involve oxytocin and/or SHBG. The VTA has high expression of oxytocin receptors (Vaccari et al., 1998). There are modest effects of oxytocin manipulations on sexual behavior of ovx, E₂-primed rats when administered to the VTA before P₄-priming (Caldwell et al., 1994). In the present study, we investigated the effects of manipulating SHBG and oxytocin in the VTA of ovariectomized E₂ and/or P₄-primed rats. We hypothesized that SHBG and oxytocin may be involved in progestogens-facilitated lordosis in the midbrain VTA.

2. Materials and Methods

2.1. Experimental Procedures

All procedures utilized in the present study involving experimental rats were done in accordance with The National Institute of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985), and with approval from The University at Albany Institutional Animal Care and Use Committee. All efforts were made to use the fewest number of rats in these experiments and to minimize suffering of rats utilized, and *in vivo* techniques as alternatives were considered, but found lacking for this experiment.

2.2. Animal Subjects and Housing

Rats were bred and raised in The University at Albany- Laboratory Animal Care Facility in the Social Sciences Building or the Life Sciences Research Building. Original breeders were obtained from Taconic Farms, Germantown, NY. Experimental subjects were adult (approximately 55 days of age, weighing 200–250 grams) female Long-Evans rats (N=75). Rats were group-housed (4–5 per cage), under reversed-lighting conditions (lights off between 0800 and 20:00 hrs). Rats had unlimited access to rodent chow and tap water in their home cages.

2.3. Surgery

For surgical procedures, rats were anesthetized with xylazine (12 mg/kg IP Bayer Corp., Shawnee Mission, KS, USA) and ketamine (80 mg/kg, IP; Fort Dodge Animal Health, Fort Dodge, IA, USA). Rats were ovariectomized using standard methods to remove the primary peripheral source of E₂ and P₄, the ovaries. Immediately following ovariectomy, rats were surgically implanted with bilateral guide cannulae. Cannulae were made from modified thinwalled, 23-gauge stainless steel needles (with 30-gauge removable inserts) aimed at the VTA (as adapted from Paxinos and Watson (1996); from bregma, AP = −5.3, ML = ± 0.4, DV = −7.0). Rats recovered from these surgical procedures for one week, a time in which they were assessed for their complete recovery from anesthesia, surgery, and cannulation. This is important because rats receive bilateral cannulae and the potential for damaging effects of these implants for brain function could be a concern. As such, rats were assessed for their ability to cage climb, right themselves, and respond normatively to external stimuli, groom themselves, and gain weight following surgery, as indicators of full recovery from surgery (Marshall and Teitelbaum, 1974). Rats were also assessed for normative motor behavior in an Activity Monitor. In the present study, all rats met criteria for healthy and normal recovery from surgery and bilateral cannulae implantation.

2.4. Hormone-priming

In each Experiment, ovariectomized rats were hormone-primed with 17β -E₂ (10 µg/ 0.2 ml in corn oil vehicle; Steraloids, Rhode Island) at hour 0. At hour 44, rats were administered subcutaneous injections of 100 (Experiment 1 and 2), 500 (Experiment 3), or 0 (Experiments 1 and 4) µg P₄. E₂ and P₄ were administered via subcutaneous injections to the nape of the neck.

2.5. Drugs and Infusions

In each Experiment, at hour 47.5, rats received bilateral infusions to the VTA, and were tested for lordosis and motor behavior 30 minutes post-infusion. Drugs were infused to rats under minimal hand-held restraint by an experienced researcher. Bilateral infusions were done by inserting a 30-gauge needle, which was connected to a 5 µl Hamilton Syringe (Reno, NV, USA) with PE-20 tubing, into the guide cannulae. Drugs were then administered at a rate of 1 µl/min, and the infusion needle was left in place for 60 secs to minimize displacement of infusate. In Experiment 1, rats were infused with bilateral 1 µl infusions of sterile water vehicle or purified SHBG (4.5 pg/µl; Fitzgerald International, Inc., Concord, MA) to the VTA. In Experiment 2 and 4, rats were infused with bilateral 1 µl infusions of sterile saline or oxytocin (1.0 pg/µl). In Experiment 3 and 4, rats were administered bilateral intra-VTA infusions of sterile saline or an oxytocin receptor antagonist, d(CH₂)₅[TYR(ME)²,Thr⁴,Tyr-NH^{9,2}] (1.2 pg/µl; Bachem Bioscience Inc., King of Prussia, PA).

2.6. Assessment of Lordosis

Lordosis of experimental rats was determined following intra-VTA infusions, using established methods (Frye et al., 2000; Frye and Walf, 2009) in a 50 × 25 × 30 cm mating chamber, with approximately 20 cm of woodchip shavings on the bottom of the chamber. The percentage of times female rats assumed the lordosis posture (lordosis quotients), in response to mounting by a sexually-experienced male, were used to quantify sexual receptivity of female rats. Rats were assessed during a 10-minute test period, or after ten mounts were made, whichever came first.

2.7. Site Analyses

In Experiments 1, 2, and 4, to verify placement of cannulae, experimental rats were deeply anesthetized with sodium pentobarbital (150 mg/kg or to effect; intraperitoneal route). Rats were then perfused with 0.9% saline, followed by 10% formalin, and brains were removed from the skull and then stored in 10 % formalin. Before freezing and slicing on a microtome, brains were stored in a 30% sucrose-saline solution for 2–3 days. Brains were then frozen and sliced on a cryostat (40 µm) at the level of the midbrain VTA. Brain slices were affixed to glass slides and stained with cresyl violet. Researchers, blind to the experimental condition and behavioral data of the animals, examined infusion location by light microscopy. In Experiment 3, rats' brains were collected without fixation by formalin, and were visually analyzed to verify cannulae location (as per Frye and Rhodes, 2008). Infusion sites located dorsal to the mammillary peduncle, ventral to the red nucleus, medial to the medial lemniscus or substantia nigra and lateral to the interfascicular nucleus were considered to be within the VTA. See Figure 1 for depiction of cannulae tracks and intended infusion site. Only rats that received infusions to the VTA, and not the two rats with infusions to the substantia nigra, which demonstrated a different pattern of behavior, had data included in the data analyses.

2.8. Statistical analyses

Lordosis quotients, 30 mins after infusions, were analyzed with analyses of variance (ANOVA). For Experiment 1, a two-way ANOVA (with P₄ and drug condition as the between subjects variables) was utilized. For Experiments 2, 3, and 4, a one-way ANOVA was utilized.

Main effects were considered significant when $p < 0.05$, and Fisher PLSD comparisons were used *as post hoc* tests to determine group differences. A tendency was considered when $P < 0.10$.

3. Results

3.1. Experiment 1

There were main effects of SHBG ($F(1,27) = 15.64$, $p < 0.01$) and P_4 ($F(1,27) = 7.70$, $p < 0.01$) to increase lordosis quotients of ovariectomized, E_2 - and P_4 -primed rats, compared to vehicle (Figure 2). There were no significant interactions between these variables.

3.2. Experiment 2

Oxytocin infusions tended to increase lordosis quotients ($F(1,14) = 3.15$, $p < 0.09$; Figure 3) of ovariectomized, E_2 - and P_4 -primed rats, compared to vehicle.

3.3. Experiment 3

Infusions of an oxytocin antagonist, compared to vehicle, significantly decreased lordosis quotients ($F(1,16) = 4.82$, $p < 0.04$; Figure 4) of ovariectomized, E_2 - and P_4 -primed rats.

3.4. Experiment 4

Neither infusions of oxytocin, nor an oxytocin antagonist, significantly altered lordosis quotients (Figure 5) of ovariectomized, E_2 -primed rats.

4. Discussion

The present data supported our hypothesis that SHBG and oxytocin in the VTA would be involved in some of P_4 's effects for lordosis of ovariectomized rats. Administration of SHBG, compared to vehicle, to the midbrain VTA significantly increased lordosis in ovariectomized, E_2 - and P_4 -, but not E_2 -, primed rats. Compared to vehicle infusions to the VTA, oxytocin infusions tended to increase, and oxytocin receptor antagonist significantly decreased, lordosis of ovariectomized E_2 and P_4 , but not E_2 -primed, rats. Together, these findings suggest that oxytocin and SHBG have actions in the midbrain VTA for progestogens-facilitated lordosis of female rats.

These results confirm previous studies on the role of SHBG and oxytocin for reproduction-related behaviors of female rodents. There is high expression of oxytocin receptors in the VTA (Vaccari et al., 1998). Oxytocin receptor binding is increased in the VTA with maternal behavior of rats (Pedersen et al., 1994). An oxytocin receptor blocker reduces the expression of maternal behaviors of rats (Pedersen et al., 1994). There are modest effects of oxytocin manipulations on sexual behavior of ovx, E_2 -primed rats when administered to the VTA before P_4 -priming (Caldwell et al., 1994). Similar to what was observed in the present study, oxytocin to the VTA did not enhance lordosis of E_2 -primed rats (Caldwell et al., 1989). Sexual receptivity of female rats is also enhanced by SHBG to the medial preoptic area or medial basal hypothalamus (Caldwell et al., 2000), or the VTA in the present study. We found similar effects of SHBG to only increase P_4 -facilitated lordosis when infused to the VTA. In the brain, production of SHBG occurs mainly in the paraventricular nucleus and supraoptic nucleus, which also contain oxytocinergic neurons (Buijs, 1978; Sofroniew, 1985; Wang et al., 1990), suggesting that these factors may work in concert. It may be that in the VTA P_4 's actions for lordosis are mediated, in part, by SHBG. The potential for interactions of SHBG with oxytocin receptors is of interest.

These data extend previous findings on the brain targets of progestogens and oxytocin for lordosis. Previous studies demonstrated a role of oxytocin in the VMH for lordosis of rodents

(Arletti and Bertolini, 1985; Benelli et al., 1994; Caldwell et al., 1994; Gorzalka et al., 1987; Schumacher et al., 1989; McCarthy et al., 1994; Pedersen and Boccia, 2002). There is some indication that oxytocin in the MPOA may modulate lordosis, and other reproduction-related behaviors (Arletti and Bertolini, 1985; Bealer et al., 2006; Benelli et al., 1994; Caldwell et al., 1984; 1989; Caldwell, 1992; Caldwell et al., 1990; 1992; Pedersen et al., 1994). Indeed, these effects of oxytocin in these central sites are not limited to female reproductive behavior. Among male rats, infusions of oxytocin to the VTA, or hypothalamus, facilitates erectile function by activating mesolimbic dopaminergic neurons (Melis et al., 2007; Succu et al., 2007; 2008). It may be that oxytocin and dopamine signaling in these regions mediates both anticipatory and consummatory phases of sexual behavior. The present study adds to this literature to demonstrate that the VTA may be another target of oxytocin, and SHBG, for lordosis of rodents mediated by P₄. However, other experiments, which were beyond the scope of the present study, are necessary to more directly investigate the possibility of cooperation between oxytocin, SHBG, and dopamine signaling in the VTA for progesterone-facilitated lordosis. For example, effects of co-administration of oxytocin and SHBG would provide some insight into whether there are interactions with these systems. In the present study, this could not be directly assessed as the lordosis responses were near maximum. As well, the capacity for oxytocin replacement to reverse the effects of the oxytocin antagonist would provide integral information about the mechanisms for these effects. Future studies will address these points.

Another question of future investigations will be the role of the P₄ metabolite, and neurosteroid, 5 α -pregnan-3 α -ol-20-one (3 α ,5 α -THP), for these effects. P₄'s effects in the VTA are dependent upon formation and activity of 3 α ,5 α -THP in the VTA (reviewed in Frye and Walf, 2009). 3 α ,5 α -THP is devoid of affinity for PRs, and can exert rapid, non-classical actions involving neurotransmitter receptors and their downstream signaling cascades to facilitate lordosis (Frye and Walf, 2009). In the VTA, oxytocin receptors are another membrane-bound protein that may be a target for progesterone's actions for lordosis, involving G-protein-initiated intracellular signaling cascades. In support, oxytocin receptors are G-protein coupled receptors (Shojo and Kaneko, 2000), and have been localized to the midbrain of rats (Ostrowski, 1998). *In vitro* studies demonstrate that P₄ alters oxytocin-stimulated calcium signaling in a G-protein-independent manner (Burger et al., 1999). It may be that P₄-mediated changes in calcium signaling are due to direct interactions between P₄ and oxytocin receptors. In support, physiological levels of P₄ reduce the ligand binding capacity of the oxytocin receptors transfected into cells by more than 50% (Zingg et al., 1998). In this study, P₄ also reduced oxytocin-induced inositol phosphate production and intracellular calcium mobilization by 85 and 90%, respectively. Given the essential role of 3 α ,5 α -THP in the midbrain VTA in mediating lordosis, whether the mechanisms for these effects involve SHBG and oxytocin is of great interest.

In summary, the present data demonstrate that SHBG and oxytocin in the VTA are likely targets of progesterone in the VTA for lordosis. The relevance of this effect to other social behaviors is of interest. Oxytocin has clear effects to enhance affiliative behaviors (Carter, 2007). Indeed, it may be that quality social relationships improve health and/or well-being, but these effects can often be confounded with other trophic actions of steroids in the body. An example of this is parity, in which women experience robust hormonal fluctuations (progesterone, oxytocin, etc), and their trophic actions, during pregnancy and postparturition, and then intense social bonding with their offspring. Parity is also associated with changes in breast cancer risk. Thus, further investigation of progesterone's actions involving SHBG and oxytocin for socially-relevant behaviors has clinical significance.

Acknowledgments

This research was supported in part by grants from the National Science Foundation (03-16083) and National Institute of Mental Health (MH06769801). The technical assistance provided by Danielle Osborne, Jason Paris, and Sandy Petralia is greatly appreciated.

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Fig. 1. Diagram of cannulae tracks to the VTA of a cresyl violet stained rat brain sliced at the level of the VTA (left) and drawing from brain atlas of this region (hatch marks; right).

Fig. 2.

The effects of SHBG manipulations for lordosis quotients of ovariectomized rats administered E₂ (10 µg/0.2 ml) and/or P₄ (100 µg/0.2 ml)-primed rats, mean (+ sem). Rats administered E₂ SC and vehicle to the VTA (n=7), E₂ SC and SHBG to the VTA (n= 8), E₂+P₄ SC and vehicle to the VTA (n=8), and E₂+P₄ SC and SHBG to the VTA (n=8) were compared. A line above the bars indicates significant effect of P₄ to increase lordosis compared to vehicle (p<0.05). * indicates significant effect of SHBG to increase lordosis compared to vehicle (p<0.05).

Fig. 3.

The effects of oxytocin infusions for lordosis quotients of ovariectomized, E₂ (10 µg/0.2 ml) and P₄ (100 µg/0.2 ml)-primed rats, mean (+ sem). Rats administered E₂+P₄ SC and vehicle to the VTA (n=7), and E₂+P₄ SC and oxytocin to the VTA (n=9) were compared. * indicates significant effect of oxytocin to increase lordosis compared to vehicle (p<0.05).

Fig. 4.

The effects of oxytocin receptor antagonist infusions for lordosis quotients of ovariectomized, E₂ (10 µg/0.2 ml) and/or P₄ (500 µg/0.2 ml)-primed rats, mean (+ sem). Rats administered E₂+P₄ SC and vehicle to the VTA (n=9), and E₂+P₄ SC and oxytocin antagonist to the VTA (n=9) were compared. * indicates significant effect of oxytocin receptor antagonist to reduce lordosis compared to vehicle (p<0.05).

Fig. 5.

The effects of oxytocin manipulations for lordosis quotients of E₂ (10 µg/0.2 ml)-primed rats, mean (+ sem). Rats administered E₂ SC and vehicle to the VTA (n=8), E₂ SC and oxytocin to the VTA (n=8), and E₂ SC and oxytocin antagonist to the VTA (n=9) were compared. There were no significant main effects.