Published in final edited form as:
Horm Behav. 2007 March ; 51(3): 406–412.

RELEASE OF ORPHANIN FQ/NOCICEPTIN IN THE MEDIAL PREOPTIC NUCLEUS AND VENTROMEDIAL NUCLEUS OF THE HYPOTHALAMUS FACILITATES LORDOSIS

Kevin Sinchak[1], **Phoebe Dewing**, **Misty Cook**, and **Paul Micevych**
Department of Neurobiology, Mental Retardation Research Center and Laboratory of Neuroendocrinology, Brain Research Institute, David Geffen School of Medicine at UCLA, Los Angeles, California 90095-1763

Abstract

Opioid regulation of reproduction has been widely studied. However, the role of opioid receptor-like 1 receptor (NOP; also referred to as ORL-1 and OP4) and its endogenous ligand orphanin FQ/nociceptin (OFQ/N) have received less attention despite their extensive distribution throughout nuclei of the limbic-hypothalamic system, a circuit that regulates reproductive behavior in the female rat. Significantly, the expression of both receptor and ligand is regulated in a number of these nuclei by estradiol and progesterone. Activation of NOP in the ventromedial nucleus of the hypothalamus (VMH) of estradiol-primed nonreceptive female rats facilitates lordosis. NOP's are also expressed in the medial preoptic nucleus (MPN), however, its roles in reproductive behavior have not been studied. The present experiments examined the role of NOP in the regulation of lordosis in the MPN and tested whether endogenous OFQ/N in the MPN and VMH mediates reproductive behavior. Activation of NOP by microinfusion of OFQ/N in the MPN facilitated lordosis in estradiol-primed sexually nonreceptive female rats. Passive immunoneutralization of OFQ/N in either the MPN or the VMH reduced lordosis in estradiol-primed females, but had no effect on lordosis in estradiol + progesterone primed sexually receptive rats. These studies suggest that OFQ/N has a central role in estradiol-only induced sexual receptivity, and that progesterone appears to involve additional circuits that mediate estradiol + progesterone sexual receptivity.

Keywords

orphanin FQ; nociceptin; OFQ; opioids; ORL-1; opioid receptor-like receptor; NOP; lordosis; estrogen; progesterone

INTRODUCTION

Steroid regulation of sexual reproduction involves the activation and inhibition of numerous transmitter/receptor systems in the medial preoptic nucleus (MPN) and the ventromedial nucleus of the hypothalamus (VMH). In particular, endogenous opioid peptides in the MPN and VMH have been shown to play an important role in this regulation of sexual receptivity in the female rat (Sinchak and Micevych, 2003). All classic opioid receptors (μ-(MOP), δ-

[1]Corresponding Author: Kevin Sinchak, Ph.D. Department of Biological Sciences, California State University, Long Beach, Long Beach, CA 90840, Phone: (562) 985-8649, Fax: (562) 985-8878, ksinchak@csulb.edu.

(DOP), and κ-opioid (KOP)) are expressed in the MPN, while only DOP and KOP are expressed in the VMH (but see Vathy, van der Plas, Vincent, and Etgen, 1991). Naloxone, an antagonist of classic opioid receptors, infused either intracerebroventricular or site specifically into the MPN facilitates sexual receptivity in estradiol primed females, indicating that the overriding tone of opioid activity is inhibitory to sexual receptivity (Acosta-Martinez and Etgen, 2002; Sinchak and Micevych, 2003). Consistent with this observation, activation of both the MOP and DOP in the MPN has been shown to inhibit lordosis (Sinchak and Micevych, 2001; Sinchak, Mills, Eckersell, and Micevych, 2004b). However, activation of DOP in the VMH facilitates lordosis (Acosta-Martinez and Etgen, 2002; Sinchak and Micevych, 2003).

Until recently, it was assumed that all classes of opioid receptors and their endogenous opioid ligands were known. However, in the mid-1990s a new opioid system has been described. Orphanin FQ-nociceptin (OFQ/N) is the endogenous ligand of the opioid receptor-like receptor-1 (NOP; also known as ORL-1 or OP4; Brit J Pharm, 2003; Lachowicz, Shen, Monsma, and Sibley, 1995; Mollereau, Parmentier, Mailleux, Butour, Moisand, Chalon, Caput, Vassart, and Meunier, 1994; Neubig, Spedding, Kenakin, and Christopoulos, 2003; Wang, Johnson, Imai, Persico, Ozenberger, Eppler, and Uhl, 1994). NOP, a G_i/G_o-coupled opioid receptor, was discovered to have high sequence and structural homology to opioid receptors, especially the KOP, and affect lordosis behavior. However, NOP exhibits little affinity for binding endogenous classical opioid ligands (endorphins, enkephalins, and dynorphins) and is not affected by naloxone antagonism, a hallmark of classic opioid receptors (Fukuda, Kato, Mori, Nishi, Takeshima, Iwabe, Miyata, Houtani, and Sugimoto, 1994; Lachowicz et al., 1995; Lee, Nicholson, and McKnight, 1997; Mollereau et al., 1994; Pfaus and Pfaff, 1992; Wang et al., 1994). The only known endogenous ligand for NOP is OFQ/N, which is similar in structure to dynorphin A, a KOP endogenous ligand (Meunier, 1997; Meunier, Mollereau, Toll, Suaudeau, Moisand, Alvinerie, Butour, Guillemot, Ferrara, Monsarrat, and et al., 1995; Reinscheid, Higelin, Henningsen, Monsma, and Civelli, 1998), but lacks the NH_2 terminal amino acid tyrosine necessary for activation of the MOP, DOP, or KOP receptors. Instead, the NH_2 terminal amino acid of OFQ/N is replaced with a phenylalanine (Meunier, 1997; Meunier et al., 1995; Reinscheid et al., 1998), allowing for specific binding to the NOP receptor (Ardati, Henningsen, Higelin, Reinscheid, Civelli, and Monsma, 1997; Butour, Moisand, Mazarguil, Mollereau, and Meunier, 1997; Dooley and Houghten, 1996; Guerrini, Calo, Rizzi, Bianchi, Lazarus, Salvadori, Temussi, and Regoli, 1997; Reinscheid, Ardati, Monsma, and Civelli, 1996; Shimohigashi, Hatano, Fujita, Nakashima, Nose, Sujaku, Saigo, Shinjo, and Nagahisa, 1996).

Both OFQ/N and NOP are expressed throughout the reproductive circuits of the limbic system and hypothalamus of both male and female rats (Neal, Mansour, Reinscheid, Nothacker, Civelli, Akil, and Watson, 1999; Neal, Mansour, Reinscheid, Nothacker, Civelli, and Watson, 1999; Sinchak, Romeo, and Micevych, 2006). NOP is expressed throughout the limbic-hypothalamic lordosis regulating circuit, particularly in the VMH and the MPN. Furthermore, NOP mRNA expression is increased in the MPN and VMH when treated with estradiol + progesterone or estradiol only, respectively. OFQ/N mRNA is expressed in a number of nuclei that project to the MPN or VMH, and likewise its expression is regulated by ovarian hormones (Sinchak et al., 2006). In the VMH, activation of NOP facilitates sexual receptivity, lordosis (Sinchak, Hendricks, Baroudi, and Micevych, 1997; Sinchak and Micevych, 2003), but whether NOP in the MPN also regulates sexual receptivity is not known. These studies investigated the role OFQ/N in regulating sexual receptivity in the MPN and VMH. First, the role of NOP and OFQ/N in the MPN on steroid facilitated lordosis behavior was investigated. OFQ/N was infused into the MPN of estradiol primed females to test the hypothesis that in the MPN activation of NOP facilitates lordosis. Second, to determine whether endogenous OFQ/N regulates lordosis, OFQ/N in either the MPN or VMH was immunoneutralized. Passive immunoneutralization of OFQ/N in either the MPN or VMH demonstrated that part of the

mechanism of steroid facilitation of sexual receptivity is mediated through the release of OFQ/N in these regions.

METHODS and MATERIALS

Animals

Adult male and ovariectomized (OVX) female (200–225 g) Long-Evans rats were purchased from Charles River (Portage, MI). Females were bilaterally OVX by the supplier. Upon arrival male and female rats were segregated by sex and housed 2 per cage in a 12/12 hr light/dark cycle (lights on at 0600 hr). Food and water were provided *ad libitum*. All experimental procedures were approved by the Chancellor's Animal Research Committee at the University of California, Los Angeles.

Guide Cannulae Implantation Surgery

Female rats were anesthetized with isoflurane (2–3% in equal parts oxygen and nitrous oxide) and bilateral guide cannulae (24 gauge; Plastics One Inc., Roanoke, VA), directed either at the MPN (coordinates from bregma: anterior −0.1 mm, lateral 0.9 mm, and ventral −4.5 mm from dura; tooth bar: −3.3 mm) or the VMH (coordinates from bregma: anterior −1.7 mm, lateral 0.8 mm, ventral −7.7 mm from dura; tooth bar: −3.3 mm), were implanted using standard stereotaxic procedures. The cannulae were secured to the skull with dental acrylic and stainless steel bone screws. Stylets were placed in the guide cannulae which protruded less than 0.5 mm beyond the opening of the guide cannulae. Animals were single housed after surgery, received antibiotics orally via the drinking water (Baytril, 0.52 mg/ml; Bayer) and allowed to recover 7 days prior to behavioral testing.

Steroid Priming and Behavioral Test Design

17β-estradiol benzoate (EB) and progesterone were dissolved in safflower oil and injected subcutaneously in a total volume of 0.1 ml each. To test whether drug treatments facilitate lordosis, females were cycled once every four days for the duration of the experiment with 2 μg of EB (Micevych, Eckersell, Holland, and Smith, 1996), which produces physiological circulating proestrous levels of estradiol in the rat (Asarian and Geary, 2002) but does not induce sexual receptivity (Sinchak et al., 1997; Sinchak and Micevych, 2001). Female rats were tested for sexual receptivity during the second steroid treatment cycle after surgery to confirm responsiveness to steroids by giving a subsequent injection of 500 μg of progesterone 26 hours after EB. In the third steroid treatment cycle after cannulae implantation, females were treated with 2 μg EB only. Experiments in which treatments were expected to inhibit lordosis, sexual receptivity in females was induced with either 5 μg EB injections weekly (Babcock et al 1988) and tested for lordosis 48 hours after EB treatment, or sequential injections of 2 μg EB and 26 hours later 500 μg progesterone and tested 30 hours after EB treatment.

Sexual receptivity was measured by placing each female in a Plexiglas™ testing arena with a stimulus male, who was acclimated to the testing arenas for at least 30 minutes prior to testing. The male was allowed to vigorously mount the female 10 times. The number of times that the female displayed a lordosis posture: lifting of the head, arching of the back, movement of the tail to one side, when mounted by a male was recorded. For each female, a lordosis quotient (LQ) was calculated (number of lordosis displays/number of mounts X 100) as a measure of sexual receptivity.

Site Specific Infusions

Site specific infusions of OFQ/N and antibodies were performed with an infusion pump (Harvard Instruments) at a rate of 0.5 μl/minute in a total volume of 0.5 μl for the OFQ/N study

or 1.0 μl volume for immunoneutralization studies. The microinjection needle protruded 2 mm past the opening of the guide cannulae and remained in the cannulae approximately one minute after injection to allow for diffusion of drugs/antibodies from the injectors. Stylets were reinserted into the guide cannulae following microinfusion and animals returned to their home cage until time of behavioral testing.

Experiment 1: OFQ/N facilitation of lordosis in the MPN

To determine whether activation of NOP by OFQ/N in the MPN facilitates sexual receptivity, females were treated once every four days with 2 μg EB and then 30 hours after the last injection on the third cycle all females received bilateral microinjections into the MPN of either OFQ/N (2, 10, or 25 nmol/side) or aCSF vehicle only. Animals were tested for sexual receptivity 10 and 60 minutes after microinjection. Animals received different drug treatments in successive tests, however each animal did not receive all the drug treatments. Data were analyzed by two-way ANOVA followed by Student-Newman-Keuls (SNK) post hoc analyses for main effects and interactions that were significant, where $p < 0.05$ was considered significant.

Experiment 2: Inhibition of lordosis by passive immunoneutralization of OFQ/N in MPN and VMH

To test whether OFQ/N is necessary for steroid facilitation of lordosis in either the MPN or VMH, sexual receptivity was induced in OVX rats by treating with either 2 μg EB and 500 μg progesterone cycled every 4 days or 5 μg EB on a weekly basis. Antibodies against OFQ/N were site specifically infused bilaterally into either the MPN or VMH (1:10,000 or 1:10, respectively; Phoenix, Belmont, CA) 30 hours (EB + progesterone) or 48 hours (EB only) after the steroid treatment. Preimmune normal rabbit serum (NRS) diluted to the same concentrations as the OFQ/N anti-serum was infused into control animals. Females were tested for sexual behavior with stimulus males, as described in Experiment 1, and LQ scores recorded both 10 and 90 minutes after microinjections. Data were analyzed by two-way ANOVA followed by Student-Newman-Keuls (SNK) post hoc analyses for main effects and interactions that were significant, where $p < 0.05$ was considered significant.

Confirmation of Guide Cannulae Placement

At the end of the behavioral series, animals were anesthetized with sodium pentobarbital (100 mg/kg) and transcardially perfused with chilled 0.9% saline, and then 4% paraformaldehyde dissolved in 0.2 M Sorenson's phosphate buffer (pH 7.4). Brains were removed and placed into fixative overnight at 4°C. The fixative was replaced with 15% sucrose in phosphate buffer to cryoprotect the tissue. Brains were blocked, sectioned at 20 μm on a cryostat (Zeiss, Microm, Thornwood, NY) and collected into a chamber filled with phosphate buffer saline. Sections were mounted onto Superfrost Plus slides (Fisher, Pittsburgh, PA), stained with thionin, dehydrated, and cover slipped with Permount (Fisher). Injection sites were mapped and verified with bright-field illumination (Figure 1).

RESULTS

Experiment 1: OFQ/N facilitates lordosis in the MPN

Microinfusion of OFQ/N into the MPN facilitated the display of lordosis in 2 μg EB primed nonreceptive female rats. Within 10 minutes of infusion, OFQ/N (25 nmol) significantly increased the LQ compared to aCSF controls (ANOVA, $F = 29.8$, $df = 1,76$, $p < 0.001$; SNK, $p = 0.007$; Figure 2). Furthermore, LQ was increased 60 minutes after either 2, 10, or 25 nmol of OFQ/N infusion into the MPN of 2 μg EB primed nonreceptive female rats compared to aCSF controls ($p = 0.034$, $p = 0.009$, and $p = 0.014$, respectively; Figure 2), which is similar to our previous findings in the VMH (Sinchak et al., 1997). However unlike the female rats

infused with OFQ/N in the VMH, these MPN infused rats did not exhibit a loss in muscle tone or locomotor activity after OFQ/N microinjections (Sinchak et al., 1997).

Experiment 2: Passive immunoneutralization of OFQ/N in either MPN or VMH inhibits lordosis

To determine whether endogenous OFQ/N acts in the MPN and/or VMH to facilitate steroid induced sexual receptivity of rats, NRS (control) or antibodies against endogenous OFQ/N were infused into either the MPN or VMH of maximally receptive OVX rats, primed with either EB (5 μg) or EB (2 μg) + progesterone (500 μg). Immunoneutralization of OFQ/N in the MPN significantly reduced sexual receptivity (LQ) in animals that were primed with 5 μg EB compared to animals that were infused with NRS 10 minutes after infusion (ANOVA, $F = 12.1$, $df = 1,27$, $p = 0.002$; SNK, $p < 0.001$; Figure 3). The inhibitory effects of the OFQ/N anti serum were still significant after 90 minutes (SNK, $p = 0.038$; Figure 3). However, the OFQ/N antibodies had no effect on lordosis in estradiol + progesterone primed sexually receptive rats (10 min LQ (sem): aCSF = 92.2 (8.1), OFQ/N antibody 88.0 (7.7); 90 min LQ: aCSF = 95.6 (8.1), OFQ/N antibody = 88.8 (7.7); $p > 0.5$).

Immunoneutralization of OFQ/N using the same antibody in the VMH significantly reduced LQ in animals that were primed with 5 μg EB compared to animals that were infused with NRS when tested 10 minutes after infusions (ANOVA, $F= 5.72$, $df = 1,49$, $p = 0.021$; SNK, $p = 0.036$; Figure 3). The inhibitory effects of the OFQ/N anti serum were gone after 90 minutes (SNK, $p = 0.23$; Figure 3). Similar to the MPN, immunoneutralization of OFQ/N in the VMH also had no effect on LQ in estradiol + progesterone primed sexually receptive rats (10 min LQ (sem): aCSF = 92.5 (5.2), OFQ/N antibody 97.8 (2.7); 90 min LQ: aCSF = 100.0 (0.0), OFQ/N antibody = 97.8 (3.0); $p > 0.5$).

DISCUSSION

The major finding of these experiments is that OFQ/N acts in the MPN to facilitate sexual receptivity in nonreceptive, estradiol-primed female rats. This facilitation was similar to the effect observed in the VMH (Sinchak et al., 1997). Further, immunoneutralization of OFQ/N in the MPN and VMH of estradiol-primed sexually receptive rats indicated that endogenous OFQ/N is involved in modulation of sexual receptivity via the NOP. Thus, the present study demonstrates that activation of NOP in the MPN facilitates lordosis, and that the release of the endogenous ligand, OFQ/N, in both the MPN and VMH is important for facilitation of lordosis.

In the present studies, infusion of OFQ/N in the MPN facilitated lordosis in estradiol-primed nonreceptive rats and immunoneutralization of OFQ/N in either the MPN or VMH inhibited sexual receptivity in estrogen primed sexually receptive rats. These results support the idea that estradiol treatment facilitates lordosis via the release of OFQ/N that activates its cognate receptor, NOP, in the MPN and VMH. These data are congruent with our previous studies that demonstrated estradiol and progesterone up regulate and activate the OFQ/N-NOP system within brain regions important for lordosis (Sinchak et al., 1997; Sinchak and Micevych, 2003; Sinchak et al., 2006). In the present study, we used a higher estradiol dose (5 μg) and longer exposure (48 h). Previously, we demonstrated that 30 h after treatment with estradiol (2 μg) alone NOP expression increased in the VMH, and subsequent progesterone significantly increased NOP expression in the MPN that was initiated by estradiol (Sinchak et al., 1997; Sinchak and Micevych, 2003; Sinchak et al., 2006). Estradiol and progesterone treatment also increases NOP binding and G protein functional coupling in the VMH and MPN (Micevych and Quesada unpublished observations). In our previous studies the 2 μg dose of estradiol and exposure time neither facilitated lordosis nor induced release of OFQ/N measured by internalization/activation of NOP in the VMH (Sinchak and Micevych, 2003). A 5 μg dose of estradiol has been effective at facilitating lordosis and the observation that immunoneutralization of OFQ/N to inhibit lordosis in these rats indicates that the higher dose

of estradiol and longer exposure facilitates lordosis through the release of OFQ/N in the MPN and VMH to activate NOP. This is similar to the actions of estradiol combined with progesterone that facilitates lordosis and induces the internalization/activation of NOP in the VMH as a part of the ligand activated desensitization process (Sinchak et al., 1997; Sinchak and Micevych, 2003; Sinchak et al., 2006). Thus, these studies support the hypothesis that steroid facilitation of sexual receptivity involves the release of OFQ/N in the VMH and MPN.

In light of estradiol + progesterone induced NOP internalization/activation via the release of OFQ/N (Sinchak et al., 1997; Sinchak and Micevych, 2003; Sinchak et al., 2006), the inability of the anti-OFQ/N serum to attenuate lordosis in the estradiol + progesterone primed rats was unexpected. Since estradiol-only lordosis is independent of progesterone receptors or their activation, progesterone may activate additional lordosis circuits (Mani, Allen, Lydon, Mulac-Jericevic, Blaustein, DeMayo, Conneely, and O'Malley, 1996; Mani, Fienberg, O'Callaghan, Snyder, Allen, Dash, Moore, Mitchell, Bibb, Greengard, and O'Malley, 2000). It is possible then, that estradiol treatment activates an OFQ/N circuit, and subsequent progesterone activates another lordosis facilitating circuit that is not dependent on OFQ/N. So while OFQ/N is released in the MPN and VMH by either estradiol (5 μg) or estradiol + progesterone treated animals, only the estradiol treated animals require OFQ/N release for lordosis. Another possibility is that estradiol + progesterone facilitation of lordosis may induce the release of an endogenous ligand that activates NOP that is not OFQ/N or recognized by the OFQ/N anti-serum. This seems unlikely given the present state of knowledge that OFQ/N is the only endogenous ligand for NOP. Alternatively, compared to estradiol-only facilitation of lordosis, estradiol + progesterone treatment may produce very high release of OFQ/N in both the VMH and MPN that could not be counteracted by the infusion of OFQ/N antiserum. This may be especially true within the VMH, where we used a higher concentration of OFQ/N anti-serum since initial trials with the lower concentrations had no affect on behavior (data not shown). Compared to the MPN, the VMH has higher levels of NOP mRNA expression throughout the nucleus (Sinchak et al., 2006), and the number of OFQ/N afferents are likely to be greater in quantity as well. In addition, this OFQ/N would act on estrogen-induced NOP that are more efficient G protein functional coupling because of the subsequent the actions of progesterone stimulation (Micevych and Quesada unpublished observations). Thus, the inability of OFQ/N immunoneutralization to block estradiol + progesterone facilitation of lordosis, may not be because NOP activation is not necessary or other circuits have been activated, but because the OFQ/N release and NOP responsiveness were increased beyond the capabilities of the OFQ/N anti-serum to neutralize enough ligand to block behavior.

In terms of opioid circuits regulating lordosis, progesterone blocks the estradiol-induced MOP-mediated inhibition of lordosis (Sinchak and Micevych, 2003; Sinchak and Micevych, 2001). Passive immunoneutralization of enkephalin attenuated estradiol + progesterone induced lordosis (Sinchak, Dewing, and Micevych, 2005) suggesting that endogenous enkephalins, the ligands for DOP, are also involved in regulating lordosis behavior. In the present studies, we demonstrate that NOP activity in the MPN opposes the actions of MOP and DOP in the MPN, where activation of either MOP or DOP inhibits lordosis (Sinchak and Micevych, 2001; Sinchak et al., 2004b); reviewed in (Sinchak and Micevych, 2003). Estradiol rapidly activates MOP in the MPN and maintains the activation of this lordosis-inhibitory circuit until animals are treated with progesterone which facilitates sexual receptivity. Progesterone activation of the OFQ/N-NOP circuits may facilitate lordosis by attenuating MOP activation in the MPN and activating lordosis promoting circuits downstream of MOP. Evidence for this is that OFQ/N acts in the MPN to facilitate lordosis when the MOP system is activated, indicating that NOP activation is either downstream of the MOP inhibition, or within the MPN to reduce MOP inhibitory tone. The MPN OFQ/N neurons and receives OFQ/N afferents from steroid responsive regions which include the posterodorsal medial amygdala, arcuate nucleus, and bed nucleus of the stria terminalis (Sinchak, Hipschman, Cook, and Micevych, 2004a). Moreover,

OFQ/N acts within the mediobasal hypothalamus, which contains the VMH and arcuate nucleus of the hypothalamus, to inhibit β-endorphinergic neurons that project to the MPN (Nguyen, Sinchak, Dewing, and Micevych, 2006). OFQ/N release, stimulated by progesterone, may inhibit an estradiol-stimulated multisynaptic lordosis-inhibitory circuit that involves activation of MOP in the MPN. Estradiol stimulates neuropeptide Y release (NPY) in the arcuate nucleus that activates NPY-Y1 receptors on β-endorphin neurons leading to the release of β-endorphin in the MPN, which inhibits sexual receptivity (Mills, Sohn, and Micevych, 2004). This is supported by OFQ/N inhibition of β-endorphin neurons that project to the MPN (Wagner, Ronnekleiv, Grandy, and Kelly, 1998). Moreover, infusions of OFQ/N into the region of the arcuate nucleus reverse the estradiol-induced internalization (activation) of MOP in the MPN (Nguyen et al., 2006). Together, these data indicate that OFQ/N-NOP systems act at several loci to modulate the steroid induced onset and regulation of sexual receptivity in the female rat.

The VMH neurons do not synthesize OFQ/N, and any activation of these receptors requires inputs from steroid sensitive, reproduction regulatory regions, such as posterior dorsal medial amygdala and MPN; (Sinchak et al., 1997; Sinchak and Micevych, 2003; Sinchak et al., 2006). Since the NOP activates an inward rectifying K^+ channel (Wagner et al., 1998), OFQ/N facilitation of lordosis within the VMH is likely through relieving inhibitory tone to lordosis facilitating circuits that project to the MPN and/or periaquaductal gray which may include substance P and GABA systems (Dornan, 1990; Dornan, Malsbury, and Penney, 1987; Frye and DeBold, 1992; Frye, Mermelstein, and DeBold, 1993; McCarthy, 1991). Further study is needed to clarify the relationship of this novel, lordosis regulating opioid system with other neurotransmitter/neuropeptide systems.

Acknowledgements

The authors thank Drs. C. J. Evans and N.T. Maidment of the Hatos Research Center, Department of Psychiatry & Biobehavioral Sciences, UCLA, for their support. The research was supported by DA13185.(Abdulla and Smith, 1998)

References

Abdulla FA, Smith PA. Axotomy reduces the effect of analgesic opioids yet increases the effect of nociceptin on dorsal root ganglion neurons. J Neurosci 1998;18(23):9685–94. [PubMed: 9822729]

Acosta-Martinez M, Etgen AM. The role of delta-opioid receptors in estrogen facilitation of lordosis behavior. Behav Brain Res 2002;136(1):93–102. [PubMed: 12385794]

Ardati A, Henningsen RA, Higelin J, Reinscheid RK, Civelli O, Monsma FJ. Interaction of [H-3]orphanin FQ and I-125-Tyr14-orphanin FQ with the orphanin FQ receptor: Kinetics and modulation by cations and guanine nucleotides. Molecular Pharmacology 1997;51(5):816–824. [PubMed: 9145920]

Asarian L, Geary N. Cyclic estradiol treatment normalizes body weight and restores physiological patterns of spontaneous feeding and sexual receptivity. Horm Behav 2002;42:461–471. [PubMed: 12488112]

Brit J Pharm. Nomenclature guidelines for authors. British Journal of Pharmacology 2003;138:725–729.

Butour JL, Moisand C, Mazarguil H, Mollereau C, Meunier JC. Recognition and activation of the opioid receptor-like ORL 1 receptor by nociceptin, nociceptin analogs and opioids. European Journal of Pharmacology 1997;321(1):97–103. [PubMed: 9083791]

Dooley CT, Houghten RA. Orphanin FQ: receptor binding and analog structure activity relationships in rat brain. Life Sci 1996;59(1):L23–9.

Dornan WA, Akesson TR, Micevych PE. A substance P projection from the VMH to the dorsal midbrain central gray: Implication for lordosis. Brain Research Bulletin 1990;25:791–796. [PubMed: 1705159]

Dornan WA, Malsbury CW, Penney RB. Facilitation of lordosis by injection of substance P into the midbrain central gray. Neuroendocrinology 1987;45(6):498–506. [PubMed: 2441308]

Frye CA, DeBold JF. Muscimol facilitates sexual receptivity in hamsters when infused into the ventral tegmentum. Pharmacol Biochem Behav 1992;42(4):879–87. [PubMed: 1513871]

Frye CA, Mermelstein PG, DeBold JF. Bicuculline infused into the hamster ventral tegmentum inhibits, while sodium valproate facilitates, sexual receptivity. Pharmacol Biochem Behav 1993;46(1):1–8. [PubMed: 8255899]

Fukuda K, Kato S, Mori K, Nishi M, Takeshima H, Iwabe N, Miyata T, Houtani T, Sugimoto T. cDNA Cloning and Regional Distribution of a Novel Member of the Opioid Receptor Family. Febs Letters 1994;343(1):42–46. [PubMed: 8163014]

Guerrini R, Calo G, Rizzi A, Bianchi C, Lazarus LH, Salvadori S, Temussi PA, Regoli D. Address and message sequences for the nociceptin receptor: A structure-activity study of nociceptin-(1–13)-peptide amide. Journal of Medicinal Chemistry 1997;40(12):1789–1793. [PubMed: 9191955]

Lachowicz JE, Shen Y, Monsma FJ Jr, Sibley DR. Molecular cloning of a novel G protein-coupled receptor related to the opiate receptor family. J Neurochem 1995;64(1):34–40. [PubMed: 7798930]

Lee K, Nicholson JR, McKnight AT. Nociceptin hyperpolarises neurones in the rat ventromedial hypothalamus. Neuroscience Letters 1997;239(1):37–40. [PubMed: 9547166]

Mani SK, Allen JM, Lydon JP, Mulac-Jericevic B, Blaustein JD, DeMayo FJ, Conneely O, O'Malley BW. Dopamine requires the unoccupied progesterone receptor to induce sexual behavior in mice [published erratum appears. *Mol Endocrinol*. 1997, Apr 11;5(4):423]. Mol Endocrinol 1996;10(12): 1728–1737. [PubMed: 8961281]

Mani SK, Fienberg AA, O'Callaghan JP, Snyder GL, Allen PB, Dash PK, Moore AN, Mitchell AJ, Bibb J, Greengard P, O'Malley BW. Requirement for DARPP-32 in progesterone-facilitated sexual receptivity in female rats and mice. Science 2000;287(5455):1053–6. [PubMed: 10669419]

McCarthy MM, Masters DB, Fiber JM, López-Colomé A, Beyer C, Komisaruk BR, Feeder HH. GABAergic Control of Receptivity in the Female Rat. Neuroendocrinology 1991;53:473–479. [PubMed: 1870664]

Meunier JC. Nociceptin/orphanin FQ and the opioid receptor-like ORL1 receptor. European Journal of Pharmacology 1997;340(1):1–15. [PubMed: 9527501]

Meunier JC, Mollereau C, Toll L, Suaudeau C, Moisand C, Alvinerie P, Butour JL, Guillemot JC, Ferrara P, Monsarrat B, et al. Isolation and structure of the endogenous agonist of opioid receptor-like ORL1 receptor [see comments]. Nature 1995;377(6549):532–5. [PubMed: 7566152]

Micevych PE, Eckersell CB, Holland KL, Smith A. Induction of CCK mRNA levels in the limbic-hypothalamic circuit: Time course and site-specific effects of estrogen. J Neurobiol 1996;30:465–479. [PubMed: 8844510]

Mills RH, Sohn RK, Micevych PE. Estrogen-induced mu-opioid receptor internalization in the medial preoptic nucleus is mediated via neuropeptide Y-Y1 receptor activation in the arcuate nucleus of female rats. J Neurosci 2004;24(4):947–55. [PubMed: 14749439]

Mollereau C, Parmentier M, Mailleux P, Butour JL, Moisand C, Chalon P, Caput D, Vassart G, Meunier JC. ORL1, a novel member of the opioid receptor family. Cloning, functional expression and localization. FEBS Lett 1994;341(1):33–8. [PubMed: 8137918]

Neal CR, Mansour A, Reinscheid R, Nothacker HP, Civelli O, Akil H, Watson SJ. Opioid receptor-like (ORL1) receptor distribution in the rat central nervous system: Comparison of ORL1 receptor mRNA expression with I-125-[(14)Tyr]-orphanin FQ binding. Journal of Comparative Neurology 1999;412 (4):563–605. [PubMed: 10464356]

Neal CR, Mansour A, Reinscheid R, Nothacker HP, Civelli O, Watson SJ. Localization of orphanin FQ (nociceptin) peptide and messenger RNA in the central nervous system of the rat. Journal of Comparative Neurology 1999;406(4):503–547. [PubMed: 10205026]

Neubig RR, Spedding M, Kenakin T, Christopoulos A. International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification. XXXVIII. Update on terms and symbols in quantitative pharmacology. Pharmacol Rev 2003;55(4):597–606. [PubMed: 14657418]

Nguyen Y, Sinchak K, Dewing P, Micevych P. Orphanin FQ/nociceptin regulation of steroid activation of μ-opioid receptors and sexual behavior in rat models. Journal of Investigative Medicine 2006;54 (Supplement 1):#171.

Pfaus JG, Pfaff DW. μ-, δ-, and κ-opioid receptor agonists selectively modulate sexual behaviors in the female rat: Differential dependence on progesterone. Horm Behav 1992;26(4):457–73. [PubMed: 1335955]

Reinscheid RK, Ardati A, Monsma FJ, Civelli O. Structure-Activity Relationship Studies on the Novel Neuropeptide Orphanin FQ. Journal of Biological Chemistry 1996;271(24):14163–14168. [PubMed: 8662940]

Reinscheid RK, Higelin J, Henningsen RA, Monsma FJ, Civelli O. Structures that delineate orphanin FQ and dynorphin A pharmacological selectivities. Journal of Biological Chemistry 1998;273(3):1490–1495. [PubMed: 9430687]

Shimohigashi Y, Hatano R, Fujita T, Nakashima R, Nose T, Sujaku T, Saigo A, Shinjo K, Nagahisa A. Sensitivity of opioid receptor-like receptor ORL1 for chemical modification on nociceptin, a naturally occurring nociceptive peptide. J Biol Chem 1996;271(39):23642–5. [PubMed: 8798582]

Sinchak K, Dewing P, Micevych P. Immunoneutralization of beta-endorphin and met-enkephalin in the medial preoptic area of the hypothalamus facilitates lordosis in female rats. Society for Neuroscience Meetings. 2005

Sinchak K, Hendricks DG, Baroudi R, Micevych PE. Orphanin FQ/nociceptin in the ventromedial nucleus facilitates lordosis in female rats. Neuroreport 1997;8(18):3857–60. [PubMed: 9462455]

Sinchak K, Hipschman S, Cook M, Micevych P. Nociceptin/orphanin FQ afferents of the medial preoptic nucleus. Society for Neuroscience. 2004aProgram No. 423.17

Sinchak K, Micevych P. Visualizing activation of opioid circuits by internalization of G protein-coupled receptors. Molecular Neurobiology 2003;27(2):197–222. [PubMed: 12777688]

Sinchak K, Micevych PE. Progesterone blockade of estrogen activation of μ-opioid receptors regulates reproductive behavior. J Neurosci 2001;21(15):5723–5729. [PubMed: 11466444]

Sinchak K, Mills RH, Eckersell CB, Micevych PE. Medial preoptic area delta-opioid receptors inhibit lordosis. Behav Brain Res 2004b;155(2):301–6. [PubMed: 15364490]

Sinchak K, Romeo HE, Micevych PE. Site-specific estrogen and progestin regulation of orphanin FQ/nociceptin and nociceptin opioid receptor mRNA expression in the female rat limbic hypothalamic system. J Comp Neurol 2006;496(2):252–68. [PubMed: 16538678]

Swanson, LW. Brain Maps: Structure of the Rat Brain. 3. Elsevier; San Diego: 2004.

Vathy I, van der Plas J, Vincent PA, Etgen AM. Intracranial dialysis and microinfusion studies suggest that morphine acts in the ventromedial hypothalamus to inhibit female rat sexual behavior. Horm Behav 1991;25(3):354–66. [PubMed: 1657761]

Wagner EJ, Ronnekleiv OK, Grandy DK, Kelly MJ. The peptide orphanin FQ inhibits beta-endorphin neurons and neurosecretory cells in the hypothalamic arcuate nucleus by activating an inwardly-rectifying K+ conductance. Neuroendocrinology 1998;67(2):73–82. [PubMed: 9508037]

Wang JB, Johnson PS, Imai Y, Persico AM, Ozenberger BA, Eppler CM, Uhl GR. cDNA cloning of an orphan opiate receptor gene family member and its splice variant. FEBS Lett 1994;348(1):75–9. [PubMed: 8026588]

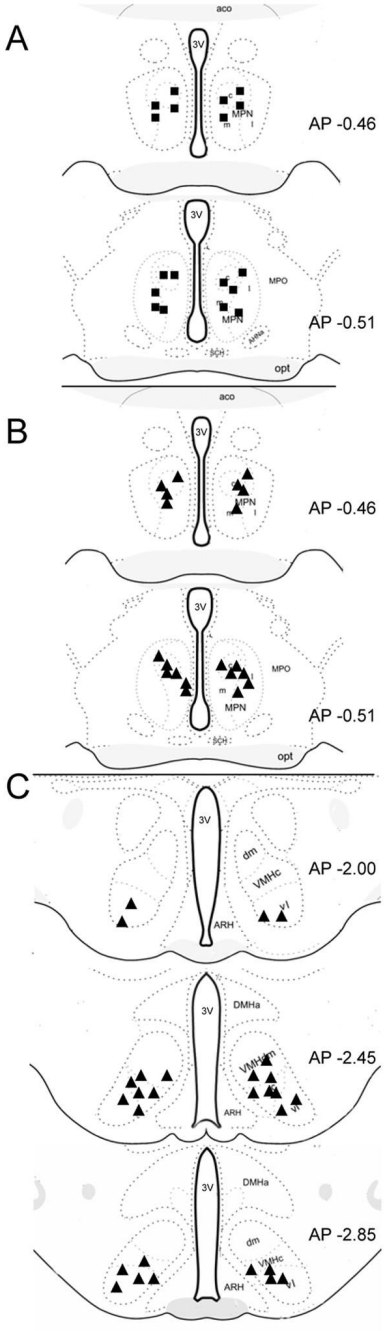

Figure 1.

Maps of cannulae placements for drug or antibody infusion sites in the medial preoptic nucleus (MPN) or ventromedial nucleus of the hypothalamus (VMH) for experiments 1 (■) and 2 (▲). In experiment 1, bilateral cannulae were aimed at the MPN for the infusion of either orphanin FQ (OFQ) or artificial cerebrospinal fluid (aCSF) (A). In experiment 2, antibodies against OFQ or normal rabbit serum were infused into groups of animals that with bilateral cannulae aimed at either MPN (B) or VMH (C). 3V, third ventricle; aco, anterior commissure; AHNa, anterior hypothalamus nucleus, anterior; ARH, arcuate nucleus of the hypothalamus; DMHa, anterior dorsomedial nucleus of the hypothalamus; MPNc, central; MPNl, lateral; MPNm, medial; MPO, medial preoptic area; opt, optic tract; SCH, suprachiasmatic nucleus; VMHdm,

dorsomedial; VMHc, central; VMHvl, ventrolateral. AP – Anterior/posterior coordinates from bregma (mm). Modified from Swanson, 2004 (Swanson, 2004).

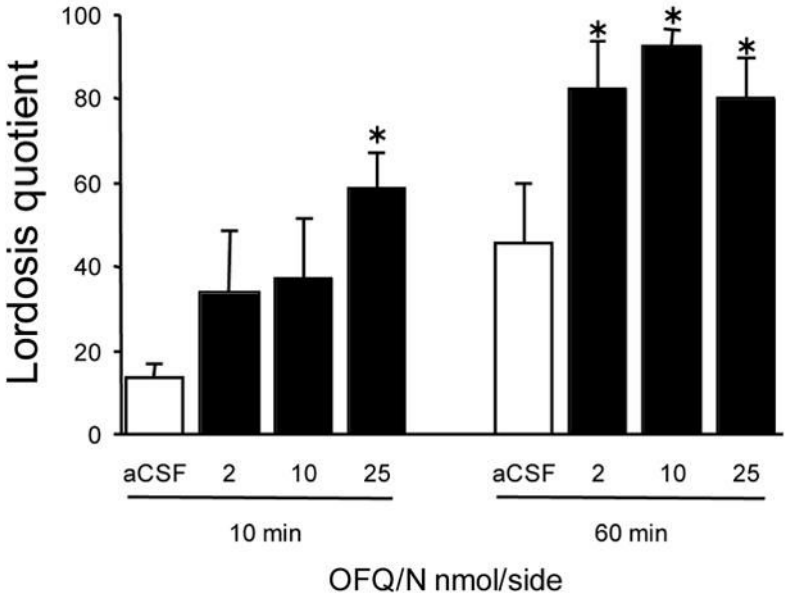

Figure 2.

Infusion of nociceptin into the medial preoptic area (MPO) of estrogen primed OVX females facilitates lordosis. OVX females were implanted with bilateral cannulae aimed at the medial preoptic nucleus (MPN) and treated with 2 μg EB and 30 hr later infused bilaterally with either artificial cerebrospinal fluid (aCSF) or nociceptin (2, 10, or 25 nmol dissolved in 0.5 μl aCSF/per side). Sexual receptivity (lordosis) was measured 10 and 60 min after infusion. Infusion of nociceptin (25 nmol) facilitated lordosis within 10 min compared to aCSF treated rats. All three doses of nociceptin facilitated lordosis 60 min after infusion compared to aCSF treated rats. * = significantly greater than aCSF group within time (SNK $p < 0.05$).

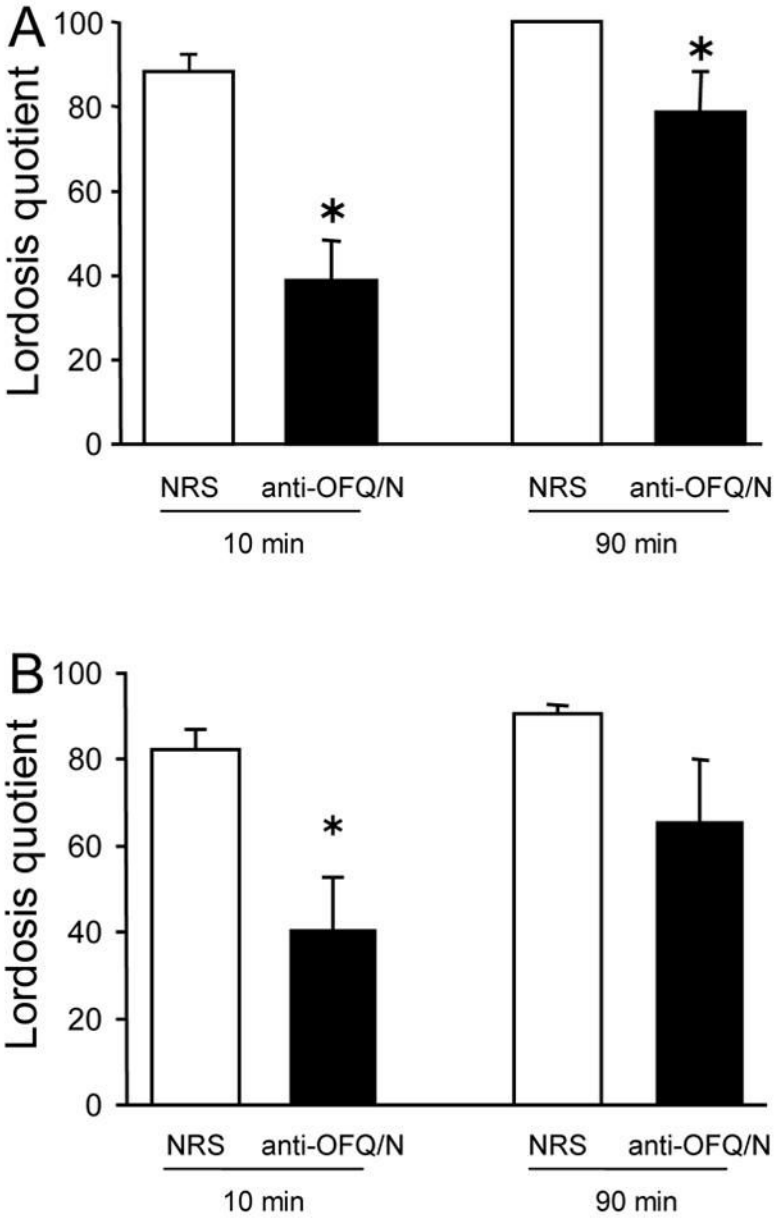

Figure 3.

Immunoneutralization of OFQ/N in A) the medial preoptic area (MPO) or B) the ventromedial nucleus of the hypothalamus (VMH) inhibits lordosis in estrogen primed OVX rats. Ovariectomized females with bilateral cannulae aimed at the medial preoptic nucleus (MPN) were treated with 5 μg EB and 48 hr later infused into either the MPO or VMH with either normal rabbit serum (NRS) or OFQ/N antibody (anti-OFQ/N; Phoenix 1:10,000 or 1:10 respectively). Total volume of infusion was 1 μl per side. Animals displayed a significant decrease in lordosis compared to control NRS infused animals 10 and 90 min after infusion. (* = significantly less than NRS group within in time, $p < 0.05$).