Available online at www.sciencedirect.com
ScienceDirect
Hormones and Behavior 51 (2007) 406–412
Hormones and Behavior
www.elsevier.com/locate/yhbeh

Release of orphanin FQ/nociceptin in the medial preoptic nucleus and ventromedial nucleus of the hypothalamus facilitates lordosis

Kevin Sinchak *, Phoebe Dewing, Misty Cook, 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, CA 90095-1763, USA

Received 30 May 2006; revised 22 November 2006; accepted 13 December 2006
Available online 22 December 2006

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. NOPs are also expressed in the medial preoptic nucleus (MPN), however, their 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 (DOP)-, and κ-opioid (KOP)) are expressed in the MPN, while only DOP and KOP are expressed in the VMH (but see Vathy et al., 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 et al., 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 et al., 1995; Mollereau et al., 1994; Neubig et al., 2003; Wang et al., 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

* Corresponding author. Department of Biological Sciences, California State University, Long Beach, Long Beach, CA 90840, USA. Fax: +1 562 985 8878.
E-mail address: ksinchak@csulb.edu (K. Sinchak).

0018-506X/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.yhbeh.2006.12.008

opioid ligands (endorphins, enkephalins, and dynorphins) and is not affected by naloxone antagonism, a hallmark of classic opioid receptors (Fukuda et al., 1994; Lachowicz et al., 1995; Lee et al., 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 et al., 1995; Reinscheid et al., 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 et al., 1997; Butour et al., 1997; Dooley and Houghten, 1996; Guerrini et al., 1997; Reinscheid et al., 1996; Shimohigashi et al., 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 et al., 1999a,b; Sinchak et al., 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 et al., 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-h light/dark cycle (lights on at 0600 h). 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 4 days for the duration of the experiment with 2 μg of EB (Micevych et al., 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 h 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 h after EB treatment, or sequential injections of 2 μg EB and 26 h later 500 μg progesterone and tested 30 h 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 min 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 × 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/min 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 1 min 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 4 days with 2 μg EB and then 30 h 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 min 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