Neuropharmacology 63 (2012) 427–433

Contents lists available at SciVerse ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Sex differences in the Nociceptin/Orphanin FQ system in rat spinal cord following chronic morphine treatment

Yong Zhang [a], Courtney L. Donica [b], Kelly M. Standifer [a,b,c,*]

[a] Department of Pharmaceutical Sciences, College of Pharmacy, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73126, USA
[b] Oklahoma Center for Neuroscience, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73126, USA
[c] Department of Cell Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73126, USA

ARTICLE INFO

Article history:
Received 12 September 2011
Received in revised form
18 April 2012
Accepted 23 April 2012

Keywords:
N/OFQ
Morphine
Sex difference
Tolerance
Spinal cord
NOP receptor

ABSTRACT

Nociceptin/Orphanin FQ (N/OFQ) appears to contribute to the development of morphine tolerance, as blockade of its actions will block or reverse the process. To better understand the contribution of N/OFQ to the development of morphine tolerance, this study examined the effect of chronic morphine treatment on levels of N/OFQ and levels and activity of the N/OFQ peptide (NOP) receptor in spinal cord (SC) from male and female rats. Both male and female Wistar rats showed less responsiveness to morphine after subcutaneous injection of escalating doses of morphine (10, 20, 40, 60 and 80 mg/kg, respectively) twice daily for five consecutive days. Male rats were more tolerant to the antinociceptive actions of morphine than females. The N/OFQ content of SC extracts was higher in females than in males, regardless of treatment; following chronic morphine treatment the difference in N/OFQ levels between males and females was more pronounced. N/OFQ content in cerebrospinal fluid (CSF) was reduced 40% in male and 16% in female rats with chronic morphine exposure, but increased in periaqueductal grey of both sexes. Chronic morphine treatment increased NOP receptor levels 173% in males and 137% in females, while decreasing affinity in both. Chronic morphine increased the efficacy of N/OFQ-stimulated [^{35}S]GTPγS binding to SC membranes from male rats, consistent with increased receptor levels. Taken together, these findings demonstrate sex differences in N/OFQ–NOP receptor expression and NOP receptor activity following chronic morphine treatment. They also suggest interplay between endogenous N/OFQ and chronic morphine treatment that results in nociceptive modulation.

1. Introduction

Numerous studies support the existence of sex differences in the response to opioid drugs in both humans and laboratory animals. In general, females have been observed to be more sensitive to experimental pain and less sensitive to morphine analgesia (Kepler et al., 1991; Craft et al., 1999; Sarton et al., 2000; Cook and Nickerson, 2005; Ji et al., 2006; Wang et al., 2006; Dahan et al., 2008). Female and male gonadal steroids are key factors influencing sexual dimorphism in pain and analgesia (for reviews, Aloisi and Bonifazi, 2006; Dahan et al., 2008). Sex differences in the development of tolerance to morphine in rodents have been reported, though with some controversy. Some studies revealed greater morphine tolerance in male rats (Badillo-Martinez et al., 1984; Craft et al., 1999; South et al., 2001), which was challenged by other studies (Barrett et al., 2001; Holtman et al., 2004). The discrepancy between studies may be due to several factors such as means of drug administration, type of nociceptive assay utilized, hormonal status, species, and experimental design.

The NOP receptor was first cloned and identified as LC132 (Bunzow et al., 1994), opioid receptor-like 1 receptor (ORL-1; Mollereau et al., 1994), XOR1 (Chen et al., 1994), ROR-C (Fukuda et al., 1994) and KOR-3 (Pan et al., 1995). Its endogenous ligand, N/OFQ was identified within the next year by two groups. One group named it Nociceptin (Meunier et al., 1995) and the other called it Orphanin FQ (Reinscheid et al., 1995). N/OFQ exhibits a variety of complex actions; in particular, it is an important endogenous modulator of nociceptive processing (Lambert, 2008). Contrary to its anti-opioid activity at the supraspinal level (Mogil et al., 1996; Tian et al., 1997; Murphy et al., 1999; Ciccocioppo et al., 2000), N/OFQ possesses analgesic properties when administered spinally (Tian et al., 1997; King et al., 1997; Yamamoto et al., 1997). Several lines of evidence strongly suggest a role for N/OFQ

* Corresponding author. Department of Pharmaceutical Sciences, College of Pharmacy, University of Oklahoma Health Sciences Center, P.O. Box 26901, Oklahoma City, OK 73126-0901, USA. Tel.: +1 405 271 6593x47333; fax: +1 405 271 7505.
E-mail addresses: Yong-Zhang@ouhsc.edu (Y. Zhang), Courtney-Donica@ouhsc.edu (C.L. Donica), Kelly-Standifer@ouhsc.edu (K.M. Standifer).

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doi:10.1016/j.neuropharm.2012.04.028

in the development and maintenance of morphine tolerance at the supraspinal level. For example, intracerebroventricular (i.c.v.) injections of anti-sera directed against N/OFQ reduced analgesic tolerance associated with chronic morphine treatment (Tian and Han, 2000). Morphine tolerance was blocked in N/OFQ knock-out mice or by systemic administration of the NOP receptor antagonist J-113397 (Ueda et al., 2000; Chung et al., 2006), and intravenous administration of NOP receptor antagonist SB-612111 reversed morphine tolerance (Zaratin et al., 2004). There is a dynamic regulation of the gene itself during exposure to morphine. Pro-N/OFQ mRNA was increased in rat brain regions following both acute and chronic morphine administration (Romualdi et al., 2002). Chronic morphine treatment increased levels of N/OFQ in rat brain perfusate and in the periaqueductal gray (PAG), a region of rat brain associated with activation of the descending analgesic pathway (Yuan et al., 1999). However, it is still not clear how the production and release of endogenous N/OFQ in brain is involved in morphine tolerance, or how the development of morphine tolerance alters the pharmacological characteristics of the NOP receptor and N/OFQ.

Recent studies noted that intrathecal N/OFQ produces anti-nociception to acute heat, NMDA administration or mustard oil stimuli in male rats, diestrous or OVX female rats but not in pro-estrous or estradiol-treated OVX female rats (Claiborne et al., 2006, 2009). This supports the hypothesis that the endogenous N/OFQ–NOP system is involved in the sexual dimorphism of pain. However, it is unclear whether N/OFQ is involved in the more clinically significant problems of sex-related differences in response to chronic morphine treatment. The present study approached this question from the perspective of the spinal cord to investigate the effect of chronic morphine treatment on the N/OFQ–NOP receptor system. We provide new evidence of plastic changes of N/OFQ and NOP receptor in morphine tolerance, and confirmed that the N/OFQ system as a valuable candidate for development of new strategies for better use of existing pain medications.

2. Methods

2.1. Materials

The following drugs and materials were purchased from or provided by the sources indicated: [^{3}H]N/OFQ and morphine HCl (Chemistry and Physiological Systems Research Branch of the National Institute on Drug Abuse, Bethesda, MD); N/OFQ RIA kit (Phoenix Pharmaceuticals, Belmont, CA).

2.2. Animals

Adult male and female Wistar rats weighing 200–250 g were provided by Charles River Labs (Wilmington, MA). Animals were housed in the animal facility under a 12-h light: 12-h dark cycle (lights on at 0600 h) with free access to food and water. After arrival, rats were acclimated to the animal facility for at least 1 week before experiments were initiated. Experimental protocols were approved by the Institutional Animal Care and Use Committee of the University of Oklahoma Health Sciences Center. All experiments conformed to the guidelines of the International Association for the Study of Pain. Every effort was made to minimize animal discomfort and reduce the number of animals used.

2.3. Behavioral testing

Experiments were performed in a climate-controlled room. A tail flick unit including a tail temperature sensor (IITC Life Science Inc., Woodland Hills, CA) was used to assess the nociceptive sensitivity by radiant heat tail-flick latency (TFL) assay, with the lamp set at 25% active intensity. Rats were kept in a plastic restrainer with hind limbs and tail extended. The lamp in the tail flick unit was turned off as soon as the rat flicked its tail and the time lapse between the onset of irradiation and the flick of the tail was noted. Values from 3 measurements with 5 min intervals were averaged as the basal TFL. Tail temperature was monitored by Tail Temperature probe for every test, and a cut-off limit of 12 s was set to prevent any tissue damage.

2.4. Chronic morphine treatment

Female ($n = 15$) and male ($n = 14$) Wistar rats (200–250 g) received subcutaneous (s.c.) injections of escalating doses of morphine (10, 20, 40, 60 and 80 mg/kg, respectively) twice a day (08:30 h and 17:30 h) for 5 days similar to the procedure described by Yuan et al. (1999). Saline-treated control animals (female: $n = 15$; male: $n = 14$) received an equivalent volume of saline. The development of morphine tolerance was tested between 09:00 h and 12:00 h on the 6th day. Basal TFL in all rats was measured after s.c. injection of saline. Animals then received s.c. injection of 10 mg/kg morphine and TFL was measured again after 30 min. At the end of the experiment, animals were euthanized with an overdose of inhaled isoflurane. CSF and spinal cord tissues were immediately taken and kept in −80 °C for analysis.

2.5. Radioimmunoassay of N/OFQ-immunoreactivity (N/OFQ-IR)

The procedure for peptide extraction has been described previously (Walker et al., 2002) and modified. After TFL was determined in the 6th day, rats were anesthetized as described above. CSF from each rat was withdrawn by inserting a 26-gauge needle into the cisterna magna and immediately stored at −80 °C. Acetic acid (0.5 M, 200 μl) was preheated to ~95 °C, added to each sample of spinal cord tissue and boiled for 10 min before cooling on ice for 2 min. Samples were homogenized and reheated at 95 °C for 5 min, cooled on ice, then centrifuged at 15,000 g for 15 min at 4 °C. Supernatant was dried in a vacuum centrifuge, and stored at −80 °C until assay. N/OFQ content in CSF and tissue was determined by RIA kit, according to the protocol suggested by manufacturer, and is presented as N/OFQ-IR. All samples and standards were assayed in duplicate. The sensitivity of the assay was <10 pg/ml; non-specific binding was 2.9%. There was no cross-reactivity with Dynorphin A (1–17), enkephalin or β-endorphin. Concentration of soluble protein present in the spinal cord extract was determined by the Bradford method (Bradford, 1976) using a Pierce protein assay kit. Total amount of N/OFQ was calculated and expressed as pg/μg protein. RIA curves and data have been analyzed using GraphPad Prism 5.0 software.

2.6. [^{3}H]N/OFQ receptor binding assay

Spinal cord dorsal horn tissue was thawed and homogenized in 50 mM Tris–HCl (pH 7.4). The homogenates were centrifuged at 35,000× g for 25 min at 4 °C and the membrane pellets were suspended in assay buffer (50 mM Tris–HCl pH 7.4, 0.5% bovine serum albumin, 0.1% bacitracin). Membrane proteins (40–50 μg) were incubated with various concentrations of [^{3}H]N/OFQ from 0.9 nM to 6 nM in 250 μl assay buffer at 30 °C for 1 h. The reaction was terminated by filtration and washing through GF/C filters presoaked with 0.1% polyethyleneimine. [^{3}H]N/OFQ binding was determined by scintillation spectroscopy. Non-specific binding (<20% of the total binding) was determined in the presence of 50 μM cold N/OFQ and was subtracted from the total binding.

2.7. [^{35}S]GTPγS binding assay

NOP receptor activity was determined by [^{35}S]GTPγS binding assay. Spinal cord dorsal horn tissue was dissected on ice from 6 rats in each group. Membrane protein was prepared (Odagaki and Toyoshima, 2006) and [^{35}S]GTPγS binding assay was conducted as described (Baker et al., 2000). Aliquots (25 μl) of the diluted rat spinal cord membranes equivalent to 3 μg protein were incubated at 30 °C for 60 min in 100 μl of 50 mM Tris–HCl buffer containing 0.2 nM [^{35}S]GTPγS, 0.5% BSA, 10 μM GDP, 0.1 mM EDTA, 0.2 mM DTT, 5 mM $MgCl_2$, and 100 mM NaCl in the presence of 10^{-9}–10^{-5} M N/OFQ. The reaction was terminated by rapid filtration through glass fiber filters using a Brandel cell harvester with three washing with 5 ml of ice-cold washing buffer (5 mM KPO_4, pH 7.4). Radioactivity was determined by liquid scintillation spectroscopy. Non-specific binding was measured in the presence of 100 μM unlabeled GTPγS, which was subtracted from the total binding to define the specific [^{35}S]GTPγS binding.

2.8. Data analysis

Data are expressed as mean ± S.E.M. Statistical comparisons of behavioral and neurochemical data were performed with two-way analysis of variance (ANOVA) followed by Bonferroni's posttest. Where necessary, Bonferroni correction was employed to adjust for multiple comparisons. All analyses were conducted with GraphPad Prism 5.0 software. A $p < 0.05$ was considered statistically significant.

3. Results

3.1. Sex difference in the development of chronic morphine tolerance in rats

After five consecutive days of saline or morphine (10, 20, 40, 60, 80 mg/kg, s.c., twice daily) treatment, rats of both sexes and in both