Available online at www.sciencedirect.com
SCIENCE DIRECT®
Pharmacology, Biochemistry and Behavior 78 (2004) 171–178
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Morphine and endomorphin-1 differently influence pronociceptin/orphanin FQ system in neuropathic rats

Joanna Mika[a,b], Martin K.H. Schäfer[b], Ilona Obara[a], Eberhard Weihe[b], Barbara Przewlocka[a,*]

[a]*Department of Molecular Neuropharmacology, Institute of Pharmacology, Polish Academy of Sciences, 12 Smetna Street, 31-343 Cracow, Poland*
[b]*Department of Molecular Neuroscience, Institute of Anatomy and Cell Biology, Philipps University, 35033 Marburg, Germany*

Received 11 November 2003; received in revised form 26 February 2004; accepted 5 March 2004

Available online 21 April 2004

Abstract

In the present study, we investigated the influence of intrathecal (i.t.) administration of morphine and endomorphin-1 on the level of pronociceptin/orphanin FQ and opioid receptor-like 1 (ORL1) receptor mRNAs in the lumbar part of the spinal cord in the rat model of neuropathic pain. The ligation of the sciatic nerve did not change the levels of pronociceptin/orphanin FQ and ORL1 receptor mRNAs in laminae I–VI of the dorsal horn when measured by in situ hybridisation 2 and 7 days after the nerve injury, but ORL1 receptor mRNA level in the ventral horn was significantly increased. Two μ-opioid receptor agonists, morphine and endomorphin-1, whose effectiveness in neuropathic pain is different, also disparately influenced nociceptin/orphanin FQ system in this pain model, inasmuch as an increase in pronociceptin/orphanin FQ and ORL1 receptor mRNAs was observed in laminae I–VI after morphine administration (5 μg i.t.) but not after endomorphin-1 treatment (5 μg i.t.). Moreover, the injection of ORL1 receptor antagonists (PheΨ; 30 μg i.t.) before morphine potentiated the effect of morphine in neuropathic pain model. Therefore, the activation of the endogenous nociceptin/orphanin FQ system, which is known to exhibit antiopioidergic activity, apart from its analgesic action, could be the reason for lower responsiveness to morphine in neuropathic pain.

Keywords: Nociceptin/orphanin FQ; ORL1 receptor; Endomorphin-1; Morphine; Neuropathic pain; Rat

1. Introduction

In recent years, nociceptin/orphanin FQ has been discovered and described, after its receptor, opioid receptor-like 1 (ORL1) receptor had been characterised in the course of investigations aimed to clone κ-opioid receptor subtypes (Meunier, 1997; Reinscheid et al., 1995). Nociceptin/orphanin FQ precursor is encoded by pronociceptin/orphanin FQ gene discovered recently in mice, rats, and humans (Houtani et al., 1996). Nociceptin/orphanin FQ, which shares some structural similarities with prodynorphin-derived peptides, is a nonopioid peptide with no action on opioid receptors (Hao et al., 1998). Northern blot analysis demonstrated the presence of nociceptin/orphanin FQ in the central nervous system, as well in the brain as in the spinal cord of mice and humans. In situ hybridisation and immunohistochemical studies demonstrated expression of nociceptin/orphanin FQ and ORL1 receptor in various brain regions, dorsal and ventral horns of the spinal cord, and in dorsal root ganglia (Mollereau et al., 1996; Meunier, 1997; Narita et al., 2002). Detailed analysis of the distribution of nociceptin/orphanin FQ and ORL1 receptor mRNA revealed their presence in the nociceptive pathways (thalamus, central grey, spinal dorsal horn). Concurrent physiological and behavioural studies in ORL1 receptor knockout mice suggested an important role of ORL1 receptor in nociception (Houtani et al., 1996). Electrophysiological and behavioural data have indicated that intrathecal (i.t.) nociceptin/orphanin FQ has an analgesic action (Hao et al., 1998; Xu et al., 1996; Erb et al., 1997; Yamamoto and Nozaki-Taguchi, 1997; Yamamoto et al., 1997a,b,c; Meunier, 1997) in contrast to its intracerebroventricular administration, which leads to hyperalgesia (Zhu et al., 1997).

Interestingly, in addition to their antinociceptive activity, the nociceptin/orphanin FQ, similarly to dynorphin (Laughlin et al., 1997), shows an ability to induce hyperalgesia and allodynia after intrathecal administration at the doses different from those exhibiting analgesic potential (Minami et al., 2000). This effect was described for low doses of nociceptin/orphanin FQ (Yamamoto et al., 1997c). Some authors

* Corresponding author. Tel.: +48-12-6623298; fax: +48-12-6374500.
E-mail address: przebar@if-pan.krakow.pl (B. Przewlocka).

0091-3057/$ – see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2004.03.005

have suggested antiopioid action of nociceptin/orphanin FQ. For example, Okuda-Ashitaka et al. (1998) reported that nociceptin/orphanin FQ administration in rats could also inhibit analgesic effects of opioids, but the mechanism of this action remains unclear. In spite of evidence that nociceptin/orphanin FQ contributes to nociceptive transmission (Briscini et al., 2002; Yamamoto et al., 1997c; Vanderah et al., 1996), its role in neuropathic pain is still relatively poorly understood.

Interestingly, neuropathic pain, peripheral axotomy, or spinal cord injury leads to neuroplastic alterations at the level of the spinal cord, which result in the reduction of morphine effectiveness (Ossipov et al., 1995a,b, 1996). In contrast, endomorphin-1, which binds with high affinity to the μ-opioid receptor (Zadina et al., 1997) and displays no activity in μ-opioid receptor knockout mice (Narita et al., 1999), produced analgesia in neuropathic pain models in rats and mice and was effective at the same doses as in acute pain (Przewlocka et al., 1999). The reason for the differences in the effects of these two agonists of μ-opioid receptor in neuropathic pain remains unclear. The question that arises is whether nociceptin/orphanin FQ system can influence the above phenomena.

The aim of our study was to investigate the changes in endogenous pronociceptin/orphanin FQ system in neuropathic pain model in rats. In particular, we attempted to find out whether the pronociceptin/orphanin FQ system (which plays an important role in nociceptive transmission and, in some situations, exhibits antiopioid activity) is responsible for different effectiveness of μ-opioid receptor agonists in neuropathic pain. Therefore, we examined the influence of morphine and endomorphin-1, which have different analgesic effectiveness in neuropathic pain, on the expression of pronociceptin/orphanin FQ and ORL1 receptor mRNAs by in situ hybridisation and investigated the effect of ORL1 receptor antagonist on the reduced morphine effectiveness in neuropathic pain.

2. Materials and methods

2.1. Animals

Male Wistar rats (200–350 g) were housed in single cages lined with sawdust, under a standard 12:12-h light–dark cycle (lights on from 0800 h) with food and water ad libitum. The experiments had the approval of the Institute's Animal Research Committee and were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

2.2. Surgical preparation

The rats were chronically implanted with intrathecal catheters under pentobarbital (60 mg/kg i.p.) anaesthesia. They were placed on the stereotaxic table, and an incision was made in the atlanto–occipital membrane. A catheter (PE 10, Clay Adams, Sparks, MD) was carefully introduced to the subarachnoid space at the rostral level of the spinal cord lumbar enlargement (L4–L6) according to Yaksh and Rudy (1976). Intrathecal injections were performed 7–8 and 14–15 days after the intrathecal implantation.

2.3. Sciatic nerve injury

Sciatic nerve injury was performed under pentobarbital anaesthesia 5–6 days after the intrathecal implantation of catheters. The right sciatic nerve was ligated (four ligatures) at a position 27 mm distal to the sciatic notch. In the sham-operated group (used as the control for biochemical experiment only), identical dissection was performed, except that the sciatic nerve was not ligated. The procedure has been described in detail by Bennett and Xie (1988). All animals with ligation to the sciatic nerve developed long-lasting allodynia. The behavioural and biochemical experiments where carried out 2 days after sciatic nerve injury, so this means that intrathecal administration started 7–8 days after intrathecal implantation (additionally, the biochemical experiments were carried out 7 days after the injury, i.e., 14–15 days after intrathecal implantation).

2.4. Drugs

Endomorphin-1 (Tyr–Pro–Trp–Phe–NH_2) was synthesised by Geza Toth (Biological Research Center of Hungarian Academy of Sciences, Szeged, Hungary), morphine hydrochloride was obtained from Polfa-Poland, while [Phe^1-Ψ(CH_2-CH)-Gly^2]NOCICEPTIN/ORPHANIN FQ(1–13)-NH_2, the ORL1 receptor antagonist (PheΨ), was from Sigma (St. Louis, MO, USA). The drugs were dissolved in distilled water and injected in a volume of 5 μl, followed by an injection of 10 μl of solvent to flush the catheter. Control animals were injected intrathecally with solvent and were tested according to the same time schedule as described for the experimental groups. Endomorphin-1 and morphine were administered intrathecally at doses of 5 μg/5 μl, whereas PheΨ was injected intrathecally at a dose 30 μg/5 μl, followed 1 min later by morphine at a dose of 25 or 50 μg/5 μl. Our previous experiment showed that 30 μg of PheΨ antagonised analgesic effect of Ro646198, an ORL1 receptor agonist. When Ro646198 was given at 10 μg 30 min after intrathecal administration, the result in the von Frey test was 42.2 ± 4.9%MPE (percent of maximum possible effect), while treatment with PheΨ + Ro646198 yielded 13.5 ± 1.2%MPE.

2.5. Probes

Reverse transcription–polymerase chain reaction (RT–PCR) cloning from the rat brain RNA was used to obtain specific probes for pronociceptin/orphanin FQ mRNA (nt. 199-809; Acc. Nr. S79730) and ORL1 receptor mRNA (nt.

406-1186; Acc. Nr. U01913). The amplified cDNA fragments of the rat pronociceptin/orphanin FQ and ORL1 receptor were subcloned into the pGEMT vectors (Promega). The sequence identity was confirmed by double-stranded DNA sequencing (Seqlab, Göttingen). Probes with antisense and sense orientation were generated from linearized vector constructs by in vitro transcription as described earlier (Melton et al., 1984) using the appropriate RNA polymerases. Radioactive riboprobes specific for pronociceptin/orphanin FQ mRNA and ORL1 receptor mRNA were double-labelled by [^{35}S]-UTP and [^{35}S]-CTP. After transcription, the probes were subjected to mild alkaline hydrolysis as described by Angerer et al. (1987).

2.6. Tissue preparation for in situ hybridisation

The spinal cords (L4–L5) of rats with sciatic nerve ligation and sham-operated animals were dissected and frozen on dry ice 2 and 7 days after nerve injury. Based on in situ hybridisation results, we chose Day 2 for the following biochemical study to see the effects of intrathecal injection of morphine and endomorphin-1. We used the same doses of morphine and endomorphin-1 (5 μg i.t.) to compare their influences on the endogenous pronociceptive system. One hour after the administration, the spinal cord was removed and frozen on dry ice. The collected tissue was cut into 12-μm-thick slices on a cryostatic microtome (Leica Microsystems, Nussloch, Germany) and processed for in situ hybridisation.

2.7. In situ hybridisation

In situ hybridisation was performed as described previously (Schafer et al., 1993). Frozen serial sections were thaw-mounted on adhesive slides and stored at − 70 °C. All following steps were performed at room temperature. Tissues were fixed on the slide by immersion in ice-cold 4% phosphate-buffered formaldehyde solution for 1 h and then washed in 10 mM phosphate-buffered saline (PBS), pH 7.4, three times for 10 min each and once in PBS containing 0.4% Triton X-100. After a short rinse in distilled water, the sections were washed in 0.1 M triethanolamine pH 8.0 (Sigma, Deisenhofen, Germany) followed by the second wash in 0.1 M triethanolamine pH 8.0 containing acetic anhydride (0.25% vol/vol) for 10 min at room temperature. After the incubation in 2 × saline/sodium citrate (SSC), the sections were dehydrated in graded alcohols (50%, 70%) and were air-dried.

Radioactive probes were diluted with hybridisation buffer (3 × SSC, 50 mM $NaPO_4$, 10 mM dithiothreitol, 1 × Denhardt's solution, 0.25 g/l yeast tRNA, 10% dextran sulphate, and 50% formamide) to obtain a final concentration of 5×10^4 dpm/μl, and 30 to 50 μl of hybridisation solution was applied to each section; then, the slides were cover-slipped and were incubated for 14 h at 60 °C. The slides were washed in 2 × SSC and 1 × SSC for 20 min each, followed by incubation in RNase buffer (10 mM Tris, pH 8.0; 0.5 M NaCl; 1 mM EDTA) containing 1 unit/ml of RNase T1 and 20 μg/ml of RNase A (Boehringer, Mannheim, Germany) for 30 min at 37 °C. The slides were washed at room temperature in 1 ×, 0.5 ×, and 0.2 × SSC for 20 min each, at 60 °C in 0.2 × SSC for 60 min, and at room temperature in 0.2 × SSC and distilled water for 10 min each. The tissue was dehydrated in 50% and 70% 2-propanol.

2.8. Behavioural tests

2.8.1. Mechanical allodynia (von Frey test)

Foot withdrawal threshold in response to a mechanical stimulus was measured by the use of von Frey filaments (Stoelting, IL, USA), which are used to apply slight pressure to the skin (1–26 g). The animals were placed in plastic cages with wire net floors and were allowed to habituate for 5 min before the experiment. A von Frey filament was applied to the midplantar surface of the paw, as described by Chaplan et al. (1994), starting with the smallest filament. Each probe was applied to the foot until it bent, and the smallest filament eliciting a foot withdrawal response was considered the threshold stimulus. The measurements were performed 30 min after intrathecal injection of drugs.

2.8.2. Thermal cold allodynia

For assessment of thermal allodynia in rats, the latency of hind limb withdrawal evoked by nonnoxious thermal stimulation (0 °C cold water allodynia test) was determined according to the method that was previously described in detail by Hedley et al. (1995) and Hunter et al. (1997). Each animal was placed onto a metal stage submerged to a depth of 1.5 cm in ice-cold water (0 °C). Animals with injured sciatic nerve in every case took out from the cold water only the ipsilateral hind paw after 3.09 ± 0.55 s. The control animals were moving in a cage trying to escape but never took even one paw from the water. In the repeated cold allodynia test with a 30-s cutoff time, the reactivity remained unchanged in control groups, which convinced us that the tissue injury was not present in this procedure. We also parallelly conducted other behavioural tests, and the reactivity of the paw was not changed. The measurements were taken 30 min after intrathecal injection.

2.9. Data analysis

Analysis of in situ hybridisation autoradiograms was conducted using the MCID M4 image analysis system (Imaging Research, Ontario, Canada). The measurements were carried out in the dorsal (laminae I–VI) and ventral horns (laminae VII–IX). The biochemical data are presented as means ± S.E.M. (the results from 30–60 sections), and behavioural data are presented as %MPE, using the equation $\%MPE=[(BL-TL)/(BL-Cutoff)] \times 100\%$, where BL is the baseline latency and TL is the respective

test latency. A group included 8–10 animals for the behavioural study and 3–4 animals for biochemical experiments. The results were statistically assessed by an analysis of variance (ANOVA). * $P < .05$ indicates a significant difference versus the respective control group. Intergroup differences were analysed by Bonferroni test.

3. Results

3.1. The effects of sciatic nerve injury on the activity of nociceptin/orphanin FQ system

The in situ hybridisation study showed no changes in the pronociceptin/orphanin FQ as well as ORL1 receptor mRNA levels in the laminae I–VI of the lumbar part (L4–L6) of the spinal cord when measured on Days 2 and 7 after the injury (Table 1). The level of ORL1 receptor mRNA in the ventral horn (laminae VII–IX) of the spinal cord was significantly increased in comparison with sham-operated rats (Figs. 1 and 2). No significant differences between intact and sham-operated animals in the ventral horn were observed (data not shown).

3.2. The effects of μ-opioid receptor agonists in sciatic-nerve-injured rats on the activity of nociceptin/orphanin FQ system

Our earlier study (Przewlocka et al., 1999) showed that in behavioural model of neuropathic pain, endomorphin-1 had statistically significant antinociceptive effects at the same dose (5 μg i.t.) as in acute pain. In contrast, morphine at the dose of 5 μg i.t. was found to be ineffective, but the effect was obtained with much higher doses of 25–50 μg i.t. For in situ hybridisation, we used the same doses of morphine and endomorphin-1 (5 μg i.t.) to visualise the biochemical differences in the endogenous pronociceptive system, which are the reason for the lack of morphine

Table 1
The influence of ligation of the sciatic nerve on the level of pronociceptin/orphanin FQ and ORL1 receptor mRNAs in the laminae I–VI of the lumbar spinal cord on Day 2 and 7 after the nerve injury

	Ipsi	Contra
Level of pronociceptin/orphanin FQ mRNA (ROD)		
Control	0.149 ± 0.006	0.147 ± 0.004
Sham	0.145 ± 0.008	0.141 ± 0.009
Ligation (2 days)	0.144 ± 0.007	0.138 ± 0.008
ligation (7 days)	0.148 ± 0.010	0.149 ± 0.010
Level of ORL1 mRNA (ROD)		
Control	0.180 ± 0.006	0.181 ± 0.005
Sham	0.176 ± 0.006	0.179 ± 0.004
Ligation (2 days)	0.178 ± 0.007	0.176 ± 0.006
Ligation (7 days)	0.178 ± 0.006	0.177 ± 0.005

The level of mRNA is presented as a mean of relative optical density (ROD) ± S.E.M. from 30–60 slices (3–4 animals per group). No significant differences were observed (ANOVA, Bonferroni test).

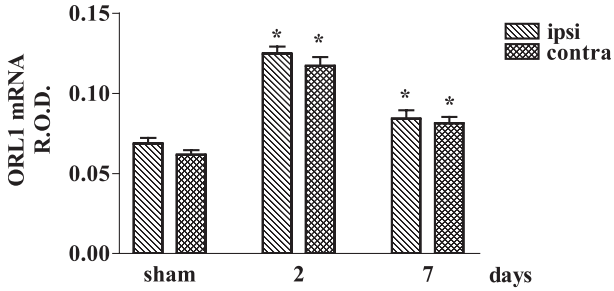

Fig. 1. The influence of ligation of the sciatic nerve on the level of ORL1 receptor mRNA in the ventral horn (laminae VII–IX) of the lumbar spinal cord on Days 2 and 7 after sciatic nerve injury. The level of mRNA is presented as a mean of relative optical density (ROD) ± S.E.M. from 30–60 slices (3–4 animals per group). * $P < .05$ as compared with the sham-operated group of animals (ANOVA, Bonferroni test).

effectiveness in neuropathic pain, and to compare their influences. In situ hybridisation results showed higher expression of pronociceptin/orphanin FQ mRNA in the laminae I–VI of the lumbar part (L4–L6) of the spinal cord 1 h after intrathecal administration of morphine (5 μg). The effect was more pronounced on the ipsilateral side, but a significant increase was also observed on the contralateral side. In contrast, no such changes were observed after

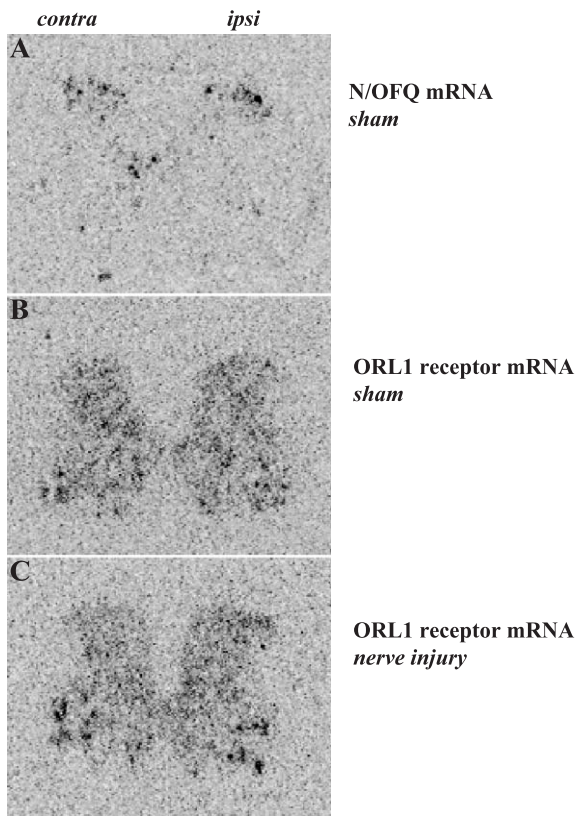

Fig. 2. Photomicrographs illustrating the pattern of (A) pronociceptin/orphanin FQ (N/OFQ) and (B,C) ORL1 receptor expression in the lumbar part of the spinal cord in (B) sham-operated animals and (C) 2 days after nerve injury, when an increase in the level of ORL1 receptor mRNA in the ventral horns was observed.

endomorphin-1 treatment (5 μg). Similarly, morphine (5 μg), but not endomorphin-1 (5 μg), influenced the level of ORL1 receptor mRNA in the laminae I–VI of the lumbar spinal cord on both ipsilateral and contralateral side, as measured 1 h after intrathecal drug administration, 2 days following ligation of the sciatic nerve (Fig. 3).

3.3. The influences of ORL1 receptor antagonist PheΨ on the effects of morphine in rats with sciatic nerve injury

Inasmuch as morphine efficacy in neuropathic pain is attenuated and our biochemical experiments showed different influence of morphine, but not endomorphins on the expression of N/ORQ and ORL1 mRNAs, we examined if PheΨ ORL1 receptor antagonist (described by Lin et al., 2000 as efficacious and selective antagonist of ORL1 receptor in vivo) can modulate the effects of morphine. In contrast to morphine, endomorphins did not influence nociceptin system. Therefore, the PheΨ action was not checked in animals treated with endomorphins. In our experiments, PheΨ itself, at a dose of 30 μg i.t., did not influence allodynia but antagonised the effect of Ro646198, a selective agonist of ORL1 receptor (see Methods section and Fig. 4). The effect of ORL1 receptor antagonists PheΨ

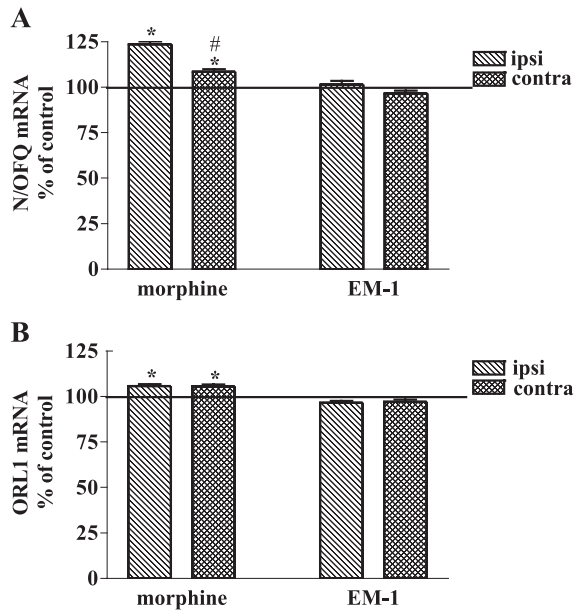

Fig. 3. The influence of morphine (5 μg) and endomorphin-1 (EM-1; 5 μg) on the level of (A) pronociceptin/orphanin FQ (N/OFQ) and (B) ORL1 receptor mRNA in the laminae I–VI of the lumbar spinal cord, measured 1 h after intrathecal drug administration, on Day 2 after ligation of the sciatic nerve. The results are presented as percent of control ± S.E.M. from 30–60 slices (3–4 animals per group). The level of mRNA in the control group (rats subjected to sciatic nerve injury injected with solvent) presented as relative optical density (ROD) was for N/OFQ: ipsi 0.144 ± 0.001, contra 0.138 ± 0.002; and for ORL1 receptor: ipsi 0.178 ± 0.001, contra 0.176 ± 0.001. These values were taken as 100%. $^{*}P<.05$ as compared with the group of animals subjected to sciatic nerve ligation and injected with solvent (ANOVA, Bonferroni test); $^{\#}P<.05$ as compared with the ipsilateral site (ANOVA, Bonferroni test).

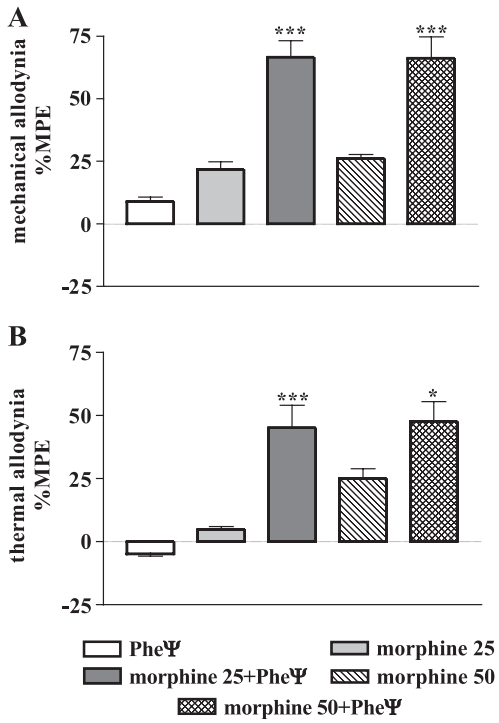

Fig. 4. The effect of PheΨ (30 μg) administered before morphine (25 and 50 μg) on its effects in rats after ligation of the sciatic nerve as measured in (A) mechanical (von Frey) and (B) thermal allodynia tests. The reaction in the control group (rats subjected to sciatic nerve injury injected with morphine at 25 μg: 10.4 ± 1.4 g in mechanical allodynia, 5.6 ± 1.2 s in thermal allodynia; morphine at 50 μg: 13.5 ± 2.4 g in mechanical allodynia, 12.4 ± 1.7 s in thermal allodynia) were considered 100%. The results are shown as %MPE ± S.E.M. of 8–10 animals per group. $^{*}P<.05$; $^{***}P<.001$ as compared with the group of animals subjected to sciatic nerve ligation and injected with morphine (ANOVA, Bonferroni test).

(30 μg i.t.) on analgesic efficacy of morphine (25 and 50 μg i.t.) was measured in mechanical (von Frey) and thermal allodynia tests (cold water allodynia) after ligation of the sciatic nerve. We observed that the effect of morphine administered intrathecally at doses of 25 and 50 μg amounted only to 24%MPE and 26%MPE, respectively, in mechanical allodynia test. Injection of PheΨ (30 μg i.t.) before morphine potentiated about three times (67 and 66%MPE) the effect of morphine (Fig. 4A). In thermal allodynia test, morphine alone, at doses of 25 and 50 μg, exhibited dose-dependent effects corresponding to 5%MPE and 25%MPE, respectively. The injection of PheΨ (30 μg i.t.) before morphine administration at doses of 25 and 50 μg i.t. significantly increased antiallodynic effects of morphine to 46%MPE and 48%MPE, respectively (Fig. 4B).

4. Discussion

The nociceptin/orphanin FQ system seems to be involved in the modulation of acute nociceptive stimulation, as well as in chronic pain processes, e.g., in inflammation (Andoh et al., 1997; Jia et al., 1998) and neuropathy (Briscini et al.,

2002). Our in situ hybridisation study showed significant increase in ORL1 receptor mRNA levels in the ventral horns 2 and 7 days after the sciatic nerve injury. This effect can possibly be explained by involvement of the nerve-injury-activated trophic factors. It was reported that nociceptin/orphanin FQ gene expression in the brain structures and in primary astrocyte tissue cultures was regulated by ciliary neurotrophic factor (CNTF), which is an injury-induced factor in the brain (Buzas et al., 1999). Thus, it can be speculated that the sciatic nerve injury could induce an increase in the expression of ORL1 receptor on motoneurons in the spinal cord via CNTF-related mechanisms. Surprisingly, no changes in the level of pronociceptin/orphanin FQ mRNA were observed in the laminae I–VI of the dorsal horn in this model of neuropathic pain. The lack of changes was also observed by Briscini et al. (2002). Using the semiquantitative RT–PCR assay, these authors showed that ORL1 receptor was up-regulated in the ipsilateral lumbar enlargement in the allodynic animals. Our in situ hybridisation results correspond with these data and additionally show that the changes in ORL1 receptor occur not in the dorsal but in the ventral horn of the ipsilateral lumbar enlargement.

An increase in the level of ORL1 receptor mRNA measured by RT–PCR (which does not describe the location of the change) in the mouse spinal cord tissue was reported to occur during morphine tolerance and dependence (Ueda et al., 2000). In our experiments, the changes in nociceptin/orphanin FQ system associated with neuropathic pain were limited to motoneurons in the ventral horns, but the changes after intrathecal administration of morphine were observed in the superficial laminae involved in the regulation nociceptive transmission. It is well known that the antinociceptive efficacy of intrathecally administered morphine is decreased in rats with nerve injury (Ossipov et al., 1995a,b; Nichols et al., 1997; Yaksh et al., 1995; Bian et al., 1995). In addition, clinical studies have generally indicated that neuropathic pain is partly resistant to morphine (Twycross, 1982; Arner and Myerson, 1988). It has been suggested that ineffectiveness of morphine in models of neuropathic pain is due to a reduced number of presynaptic opioid receptors, resulting from degeneration of primary afferent neurones, subsequent to nerve damage (Ossipov et al., 1995a,b). Such reduction in the number of μ-opioid receptors may in fact be an important factor in diminishing the efficacy of morphine in neuropathic pain. However, in our experiments, the levels of μ-opioid receptor mRNA in laminae I–VI were not changed (data not shown). Recently, also Briscini et al. (2002) observed no changes in the level of μ-opioid receptor mRNA in the lumbar spinal cord enlargement and in L5–L6 dorsal root ganglia after nerve injury, which was evidenced with the RT–PCR method. Therefore, we suggest that lower responsiveness to morphine in neuropathic pain may result from other causes than reduced number of presynaptic opioid receptors, e.g., from the increased activity of antiopioidergic systems like dynorphin (Laughlin et al., 1997) or nociceptin/orphanin FQ systems. Indeed, our results showing that morphine administration in neuropathic pain increased the activity of nociceptin/orphanin FQ system support this suggestion. It is possible that morphine administration induces nociceptin release and that activation of the nociceptin system is responsible for the observed behavioural effect. This initial activation is followed by a lower level of peptide. This may be the stimulus for the increase in biosynthetic activity and increase in the nociceptin mRNA level. We hypothesised that the activity of this system could antagonise the morphine action in neuropathic pain. Moreover, the activity of the nociceptin/orphanin FQ system remains at the control level after endomorphin-1 administration, which can explain why the effectiveness of endomorphin-1 is not changed in neuropathic pain. In fact, low antiallodynic effect of morphine in neuropathic pain could be potentiated by ORL1 receptor antagonist, as was demonstrated in our experiment. The influence of the nociceptin/orphanin FQ system on the opioid effects during neuropathic pain seems to be very interesting, resembling, to some extent, antiopioidergic actions of dynorphin. Dynorphin injected intraventricularly in mice did not elicit antinociceptive effects itself but decreased analgesic action of morphine and β-endorphin (Friedman et al., 1981). Nociceptin/orphanin FQ also possesses antiopioidergic activity. Intraventricular administration of nociceptin/orphanin FQ in mice led to hyperalgesia in the hot plate test (Meunier, 1997), reversed stress induced analgesia, and antinociceptive actions of morphine and several other μ- and κ-opioid receptor agonists (Meunier, 1997). Zhu et al. (1997) also observed that nociceptin/orphanin FQ potentiated formalin-induced pain behaviour and showed that nociceptin/orphanin FQ antagonised morphine analgesia in rats. In behavioural experiments, we observed that pretreatment with the ORL1 receptor antagonist (PheΨ) significantly restored morphine efficacy in the rats with sciatic nerve injury. Moreover, a significant influence of morphine on the level of pronociceptin/orphanin FQ and ORL1 receptor mRNA was observed. All these results strongly suggest the influence of the pronociceptin/orphanin FQ system on the effects of morphine in neuropathic pain, which can be one of the reasons of lower morphine effectiveness in this type of pain. These results seem puzzling inasmuch as nociceptin/orphanin FQ itself exhibits dose-dependent, opioid-like analgesic activity in many tests, but it also antagonises morphine action in other models.

Another possible explanation is that opioids maintain their efficacy, but the concurrent expression of hyperalgesia counteracts antinociception, which in consequence produces an impression of tolerance (Vanderah et al., 2001). This has been evidenced for NMDA receptor activation, which possesses pain-facilitative components, masking opioid analgesia in rats (Chen et al., 1995; Celerier et al., 1999). Following axotomy, nociceptin/orphanin FQ inhibits Ca^{+2} transmission and accelerates K^{+} transmission, causing an increase in neuronal excitability (Abdulla and Smith, 1997).

Nociceptin/orphanin FQ diminishes glutamatergic transmission and indirectly influences the activation of the NMDA receptors, which resembles nonopioidergic action of dynorphin to some extent (Liebel et al., 1997; Yamamoto and Nozaki-Taguchi, 1997). It seems that hyperalgesia in neuropathic pain could be induced, on one hand, by the increased activity of endogenous antiopioidergic systems as dynorphin and nociceptin/orphanin FQ systems and, on the other, by the NMDA-receptor-related effects. This conclusion is supported in the present study by the experiment with the ORL1 receptor antagonist (PheΨ), which showed that pretreatment with this antagonist potentiated the antiallodynic effect of morphine. The paper published recently (Kotlinska et al., 2003) revealed that the non-peptidergic ORL1 receptor full agonist, Ro646198, inhibited morphine antinociception, which fits very well with our results.

In summary, this study demonstrated for the first time that the level of ORL1 receptor mRNA significantly increased in the ventral horn in the rat model of neuropathic pain. The presence of these receptors in the ventral horn was evidenced, but their role in motoneuron function has not been clarified. It may be suggested that the activation of higher number of ORL1 receptors on motoneurons could facilitate pain-induced motor reactions. No changes in pronociceptin/orphanin FQ mRNA were observed in the dorsal horn. Interestingly, intrathecal administration of morphine, but not endomorphin-1, enhanced the expression of nociceptin/orphanin FQ and ORL1 receptor mRNAs in the superficial laminae involved in the ascending nociceptive transmission. Therefore, the morphine-induced activation of pronociceptin/orphanin FQ system could be the reason for its lower responsiveness in rats with the sciatic nerve ligation. This study has shown that morphine, but not endomorphin-1, activates the nociceptin/orphanin FQ system, which can be one of the reasons for different antinociceptive properties of μ-opioid receptor agonists at the spinal cord level during neuropathic pain.

Acknowledgements

This research was supported by statutory funds from the State Committee for Scientific Research (KBN; Warsaw) and partly by German Research Foundation (DFG; SFB 297). The authors would like to thank Prof. Geza Toth from Biological Research Center of Hungarian Academy of Sciences, Szeged, Hungary, for generously providing them with endomorphin-1.

References

Abdulla FA, Smith PA. Nociceptin inhibits T-type Ca2+ channel current in rat sensory neurons by a G-protein-independent mechanism. J Neurosci 1997;17(22):8721–8.

Andoh T, Itoh M, Kuraishi Y. Nociceptin/orphanin FQ gene expression in rat dorsal root ganglia induced by peripheral inflammation. NeuroReport 1997;8:2793–6.

Angerer LM, Cox KH, Angerer RC. Demonstration of tissue-specific gene expression by in situ hybridization. Methods Enzymol 1987; 152:649–61.

Arner S, Myerson BA. Lack of analgesic effect on neuropathic and idiopathic forms of pain. Pain 1988;33:11–23.

Bennett GJ, Xie YK. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. Pain 1988;33: 87–107.

Bian D, Nichols ML, Ossipov MH, Lai J, Porreca F. Characterization of the antiallodynic efficacy of morphine in a model of neuropathic pain in rats. NeuroReport 1995;6:1981–4.

Briscini L, Corradini L, Ongini E, Bertorelli R. Up-regulation of ORL-1 receptors in spinal tissue of allodynic rats after sciatic nerve injury. Eur J Pharmacol 2002;447:59–65.

Buzas B, Symes AJ, Cox BM. Regulation of nociceptin/orphanin FQ gene expression by neuropoietic cytokines and neurotrophic factors in neurons and astrocytes. J Neurochem 1999;72:1882–9.

Celerier E, Laulin J, Larcher A, Le Moal M, Simonnet G. Evidence for opiate-activated NMDA processes masking opiate analgesia in rats. Brain Res 1999;847(1):18–25.

Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL. Quantitative assessment of tactile allodynia in the rat paw. J Neurosci Methods 1994;53(1):55–63.

Chen L, Gu Y, Huang LY. The mechanism of action for the block of NMDA receptor channels by the opioid peptide dynorphin. J Neurosci 1995; 15:4602–11.

Erb K, Liebel JT, Tegeder I, Zeilhofer HU, Brune K, Geisslinger G. Spinally delivered nociceptin/orphanin FQ reduces flinching behaviour in the rat formalin test. NeuroReport 1997;8:1967–70.

Friedman HJ, Jenm MF, Changm JK, Lee NM, Loh HH. Dynorphin, a possible modulatory peptide on morphine or beta-endorphin analgesia in mouse. Eur J Pharmacol 1981;69:357–60.

Hao JX, Xu IS, Wiesenfeld-Hallin Z, Xu XJ. Anti-hyperalgesic and anti-allodynic effects of intrathecal nociceptin/orphanin FQ in rats after spinal cord injury, peripheral nerve injury and inflammation. Pain 1998;76:385–93.

Hedley LR, Martin B, Waterbury LD, Clarke DE, Hunter JC. A comparison of the action of mexiletine and morphine in rodent models of acute and chronic pain. Proc West Pharmacol Soc 1995;8:103–4.

Houtani T, Nishi M, Takeshima H, Nukada T, Sugimoto T. Structure and regional distribution of nociceptin/orphanin FQ precursor. Biochem Biophys Res Commun 1996;219:714–9.

Hunter JC, Gogas KR, Hedley LR, Jacobson LO, Kassotakis L, Thompson J, et al. The effect of novel anti-epileptic drugs in rat experimental models of acute and chronic pain. Eur J Pharmacol 1997;324:153–60.

Jia Y, Linden DR, Serie JR, Seybold VS. Nociceptin/orphanin FQ binding increases in superficial lamina of the rat spinal cord during persistent peripheral inflammation. Neurosci Lett 1998;250:21–4.

Kotlinska J, Wichmann J, Rafalski P, Talarek S, Dylag T, Silberring J. Non-peptidergic OP4 receptor agonist inhibits morphine antinociception but does not influence morphine dependence. NeuroReport 2003;14(4): 601–4.

Laughlin TM, Vanderah TW, Lashbrook J, Nichols ML, Ossipov MH, Porreca F, et al. Spinally administered dynorphin A produces long-lasting allodynia: involvement of NMDA but not opioid receptors. Pain 1997;72:253–60.

Liebel JT, Swandulla D, Zeilhofer HU. Modulation of excitatory synaptic transmission by nociceptin/orphanin FQ in superficial dorsal horn neurones of the neonatal rat spinal cord. Br J Pharmacol 1997;121:425–32.

Lin B, Waterman R, Lippton H. Nociceptin receptor activation produces nitric oxide-mediated systemic hypotension. Life Sci 2000;66(6): PL99–PL104.

Melton DA, Krieg PA, Rebagliati MR, Maniatis T, Zinn K, Green MR. Efficient in vitro synthesis of biologically active RNA and RNA hy-

bridization probes from plasmids containing a bacteriophage SP6 promoter. Nucleic Acids Res 1984;25:7035–56.
Meunier JC. Nociceptin/orphanin FQ and the opioid receptor-like ORL1 receptor. Eur J Pharmacol 1997;340:1–15.
Minami T, Okuda-Ashitaka E, Mori H, Sakimura K, Watanabe M, Mishina M, et al. Characterization of nociceptin/orphanin FQ-induced pain responses in conscious mice: neonatal capsaicin treatment and *N*-methyl-D-aspartate receptor GluRepsilon subunit knockout mice. Neuroscience 2000;97(1):133–42.
Mollereau C, Simons MJ, Soularue P, Liners F, Vassart G, Meunier JC, et al. Structure, tissue distribution, and chromosomal localization of the prepronociceptin/orphanin FQ gene. Proc Natl Acad Sci U S A 1996;93:8666–70.
Narita M, Mizoguchi H, Narita M, Sora I, Uhl GR, Tseng LF. Absence of G-protein activation by mu-opioid receptor agonists in the spinal cord of mu-opioid receptor knockout mice. Br J Pharmacol 1999;126:451–6.
Narita M, Mizoguchi H, Khotib J, Suzuki M, Ozaki S, Yajima Y, et al. Influence of a deletion of protein kinase C gamma isoform in the G-protein activation mediated through opioid receptor-like-1 and mu-opioid receptors in the mouse pons/medulla. Neurosci Lett 2002;331:5–8.
Nichols ML, Lopez Y, Ossipov MH, Bian D, Porreca F. Enhancement of the antiallodynic and antinociceptive efficacy of spinal morphine by antisera to dynorphin A (1–13) or MK-801 in a nerve-ligation model of peripheral neuropathy. Pain 1997;69:317–22.
Okuda-Ashitaka E, Minami T, Tachibana S, Yoshihara Y, Nishiuchi Y, Kimura T, et al. Nocistatin, a peptide that blocks nociceptin/orphanin FQ action in pain transmission. Nature 1998;392:286–9.
Ossipov MH, Lopez Y, Nichols ML, Bian D, Porreca F. Inhibition by spinal morphine of the tail-flick response is attenuated in rats with nerve ligation injury. Neurosci Lett 1995a;199:1–4.
Ossipov MH, Lopez Y, Nichols ML, Bian D, Porreca F. The loss of antinociceptive efficacy of spinal morphine in rats with nerve ligation injury is prevented by reducing spinal afferent drive. Neurosci Lett 1995b;199:87–90.
Ossipov MH, Kovelowski CJ, Wheeler-Aceto H, Cowan A, Hunter JC, Lai J, et al. Opioid antagonists and antisera to endogenous opioids increase the nociceptive response to formalin: demonstration of an opioid kappa and delta inhibitory tone. J Pharmacol Exp Ther 1996;277:784–8.
Przewlocka B, Mika J, Labuz D, Toth G, Przewlocki R. Spinal analgesic action of endomorphins in acute, inflammatory and neuropathic pain in rats. Eur J Pharmacol 1999;367:189–96.
Reinscheid RK, Nothacker HP, Bourson A, Ardati A, Henningsen RA, Bunzow JR, et al. Orphanin FQ: a neuropeptide that activates an opioid-like G protein-coupled receptor. Science 1995;270:792–4.
Schafer MK, Day R, Cullinan WE, Chretien M, Seidah NG, Watson SJ. Gene expression of prohormone and proprotein convertases in the rat CNS: a comparative in situ hybridization analysis. J Neurosci 1993;13:1258–79.
Twycross RG. Morphine and diamorphine in the terminally ill patient. Acta Anaesthesiol Scand 1982;74:128–34.
Ueda H, Inoue M, Takeshima H, Iwasawa Y. Enhanced spinal ORL1 receptor expression develops morphine tolerance and dependence. J Neurosci 2000;20:7640–7.
Vanderah TW, Laughlin T, Lashbrook JM, Nichols ML, Wilcox GL, Ossipov MH, et al. Single intrathecal injections of dynorphin A or des-Tyr-dynorphins produce long-lasting allodynia in rats: blockade by MK-801 but not naloxone. Pain 1996;68:275–81.
Vanderah TW, Ossipov MH, Lai J, Malan Jr TP, Porreca F. Mechanisms of opioid-induced pain and antinociceptive tolerance: descending facilitation and spinal dynorphin. Pain 2001;92:5–9.
Xu XJ, Hao JX, Wiesenfeld-Hallin Z. Nociceptin/orphanin FQ or antinociceptin/orphanin FQ: potent spinal antinociceptive effect of orphanin FQ/nociceptin/orphanin FQ in the rat. NeuroReport 1996;7:2092–4.
Yaksh TL, Rudy TA. Chronic catheterization of the spinal subarachnoid space. Physiol Behav 1976;17:1031–6.
Yaksh TL, Pogrel JW, Lee YW, Chaplan SR. Reversal of nerve ligation-induced allodynia by spinal alpha-2 adrenoceptor agonists. J Pharmacol Exp Ther 1995;272:207–14.
Yamamoto T, Nozaki-Taguchi N. Effects of intrathecally administered nociceptin/orphanin FQ, an opioid receptor-like1 receptor agonist, and *N*-methyl-D-aspartate receptor antagonists on the thermal hyperalgesia induced by partial sciatic nerve injury in the rat. Anesthesiology 1997;87:1145–52.
Yamamoto T, Nozaki-Taguchi N, Kimura S. Analgesic effect of intrathecally administered nociceptin/orphanin FQ, an opioid receptor-like1 receptor agonist, in the rat formalin test. Neuroscience 1997a;81:249–54.
Yamamoto T, Nozaki-Taguchi N, Kimura S. Effects of intrathecally administered nociceptin/orphanin FQ, an opioid receptor-like1 (ORL1) receptor agonist, on the thermal hyperalgesia induced by carageenan injection into the rat paw. Brain Res 1997b;754:329–32.
Yamamoto T, Nozaki-Taguchi N, Kimura S. Effects of intrathecally administered nociceptin/orphanin FQ, an opioid receptor-like1 (ORL1) receptor agonist, on the thermal hyperalgesia induced by unilateral constriction injury to the sciatic nerve in the rat. Neurosci Lett 1997c;224:107–10.
Zadina JE, Hackler L, Lin-Jun G, Kastin AJ. A potent and selective endogenous agonist for the μ-opiate receptor. Nature 1997;386:499–502.
Zhu CB, Cao XD, Xu SF, Wu GC. Orphanin FQ potentiates formalin-induced pain behavior and antagonizes morphine analgesia in rats. Neurosci Lett 1997;235:37–40.