PAIN 141 (2009) 283–291

PAIN

www.elsevier.com/locate/pain

Local peripheral opioid effects and expression of opioid genes in the spinal cord and dorsal root ganglia in neuropathic and inflammatory pain

Ilona Obara [a,1], Jan Rodriguez Parkitna [b,1], Michal Korostynski [b], Wioletta Makuch [a], Dorota Kaminska [b], Barbara Przewlocka [a], Ryszard Przewlocki [b,*]

[a] Department of Pain Pharmacology, Institute of Pharmacology PAS, Smetna 12, 31-343 Krakow, Poland
[b] Department of Molecular Neuropharmacology, Institute of Pharmacology PAS, Smetna 12, 31-343 Krakow, Poland

ARTICLE INFO

Article history:
Received 9 April 2008
Received in revised form 31 October 2008
Accepted 1 December 2008

Keywords:
Opioids
Pain
Neuropathy
Inflammation
Spinal cord
Dorsal root ganglia

ABSTRACT

We investigated the efficacy of local intraplantar (i.pl.) injection of peptide and non-peptide μ-, δ- and κ-opioid receptor agonists in rat models of inflammatory and neuropathic pain. Locally applied agonists dose-dependently reduced formalin-induced flinching of the inflamed paw and induced antiallodynic and antihyperalgesic effects in sciatic nerve ligation-induced neuropathic pain. These effects were mediated by peripheral opioid receptors localized at the side of tissue/nerve injury, as was demonstrated by selective and non-selective opioid receptors antagonists. The ED_{50} dose range of μ- and κ-agonists required to induce analgesia in neuropathy was much higher than the ED_{50} for inflammation; moreover, only δ-agonists were effective in the same dose range in both pain models. Additionally, effective antinociception was achieved at a lower dose of peptide, compared to non-peptide, opioids. Such findings support the use of the peripheral administration of opioid peptides, especially δ-agonists, in treating chronic pain. Furthermore, in order to assess whether adaptations in the expression of opioid genes could underlie the clinical observation of reduced opioid effectiveness in neuropathic pain, we analyzed the abundance of opioid transcripts in the spinal cord and dorsal root ganglia (DRG) during the neuropathy and inflammation. Nerve injury down-regulated mRNA for all types of opioid receptors in the DRG, which is predicted to decrease in the synthesis of opioid receptors to possibly account for the reduced effectiveness of locally administered opioids in neuropathy. The obtained results differentiate inflammatory and neuropathic pain and provide a novel insight into the peripheral effectiveness of opioids in both types of pain.

1. Introduction

The main challenge in the therapy of chronic pain is providing relief without causing adverse central effects. While opioids efficiently alleviate acute and inflammatory pain, neuropathic pain appears to be resistant to opioid actions, except at high opioid doses that might heighten risk for side effects. Besides their central mechanisms of action, opioids also exert analgesia through peripheral mechanisms. This alternative mechanism allows for antinociception after the application of small, systemically inactive doses of opioids directly into injured peripheral tissue [14,23,24,35,39,43] and/or the injection of opioids with a limited access to the central nervous system, both minimize adverse central actions [9,11,21]. The effects of locally applied opioids are dose-dependent and reversible by opioid receptor antagonists [23,24,35,39,43]. The mechanism involves peripheral opioid receptors, which are synthesized in the cell bodies of primary afferent sensory neurons located in the dorsal root ganglia (DRG), and respective proteins, which are present on peripheral afferent axons of sensory neurons [6,28,42]. The strong antinociceptive effectiveness of locally applied opioids in inflammatory pain [13,35,43] is associated with enhanced axonal transport of opioid receptors toward the periphery, increased mRNA transcription and a higher opioid receptor density in DRG, as well as increased μ-opioid receptor binding and G protein coupling during inflammation [13,20,30,47]. On the other hand, only a few reports demonstrated antinociception after local opioid application in neuropathic pain [23,24,39]. Also, reports regarding the expression of opioid receptors in the DRG of neuropathic animals are contradictory since they demonstrate both an increase in the expression of the μ-opioid receptors [39] and a decrease in the number of cells expressing functional μ-opioid receptors [17,31]. This decrease might be partially explained by the migration of receptors to the peripheral nerve endings, which is observed in inflammatory pain [20]. Interestingly, an increase in DRG mRNA levels for the κ-opioid receptor was postulated to be associated with the development of

* Corresponding author. Tel.: +48 12 6623218; fax: +48 12 6374500.
E-mail address: nfprzewl@cyf-kr.edu.pl (R. Przewlocki).
[1] These authors contributed equally to this work.

0304-3959/$34.00
doi:10.1016/j.pain.2008.12.006

mechanical allodynia in mice [37]. Conversely, since most of the studies to date focused on the changes in spinal cord mRNA expression, little is known about the expression of the mRNAs encoding precursors of opioid peptides in the DRG and their potential involvement in neuropathic pain. Thus, our aim was to comprehensively evaluate the peripheral antinociceptive effects of μ-, δ- and κ-opioid receptor agonists after their local (i.pl.) administration in two distinct rodent models of pain, inflammation neuropathy (formalin injection) and neuropathy (chronic constriction injury – CCI to sciatic nerve) 12–16 days after injury (at time when inflammatory symptoms have lessened). We employed peptide and non-peptide selective agonists and correlated their activity to their chemical structures, as well as to their receptor selectivity, after local administration. Additionally, in order to define if and how molecular differences associated with inflammatory and neuropathic pain may affect opioid-induced peripheral analgesia, we measured the abundance of transcripts encoding opioid receptors and their corresponding peptides in the spinal cord and DRG during early and late time points during the development of inflammatory (complete Freund's adjuvant – CFA) and neuropathic (CCI) pain.

2. Materials and methods

2.1. Animals

Experiments were performed on male Wistar rats (300–350 g), individually housed in cages lined with sawdust bedding, under a standard 12-h/12-h light/dark cycle (08:00–20:00 h) with food and water available *ad libitum*. All experiments were conducted during the light cycle, between 8:00 and 13:00. All experiments were performed according to the NIH Guide for Care and Use of Laboratory Animals and recommendation of IASP [47], and were approved by the Local Bioethics Committee.

2.2. Drugs and substances

Chemicals and their sources were as follows: morphine hydrochloride – Polfa (Kutno, Poland); DAMGO, naloxone methiodide – SIGMA Chemical Co. (St. Louis, MO, USA); DSLET, SNC-80, Dyn A, ICI-199.441, naltrindole hydrochloride – TOCRIS (Northpoint, UK); cyprodime – Helmut Schmidhammer, Innsbruck, Austria; formaldehyde – Odczynniki SA (Lublin, Poland); complete Freund's adjuvant (CFA) – Calbiochem (Darmstadt, Germany).

All drugs were dissolved in sterile water for injection (*Aqua pro injectione,* Polfa, Poland) and injected intraplantarly (i.pl.), directly into the injured hindpaw in a volume of 20 μl. Each dose was administered to 8–10 rats per group. Opioid receptor antagonists were co-injected i.pl. with the highest effective dose of each agonist used. Control animals were injected i.pl. with the same volume of sterile water and were tested according to the same schedule as described below. After completion of the experiment, the animals were killed by CO_2 asphyxiation.

2.3. Inflammatory pain

2.3.1. Formalin test

The formalin test was used as a model of tonic inflammatory pain (without a neuropathic component). Pain-related behavior induced by local administration of formalin is characterized by the occurrence of two characteristic phases of increased pain sensitivity in rats. The first phase relates to a direct stimulation of nociceptors while the second phase leads to the development of a localized inflammatory response. Fifteen minutes after i.pl. administration of opioids, the rats were lightly anesthetized by inhalation of halothane (2–3% v/v oxygen mixture, 5 L/min) for 2–3 min in a Plexiglas chamber. Then, rats were injected s.c. with 100 μl of a 12% formalin solution or sterile water (in case of the control group) into the dorsal part of the right hindpaw as was described previously [11,21,44]. The intensity of pain-induced behavior (number of paw shakes) in Wistar rats after 12% formalin injection is of similar magnitude as the pain response induced by 5% formalin treatment in Sprague–Dawley rats (e.g. late phase −41.2 ± 5.3 vs. 43 ± 4.9, respectively) as was reported in our earlier studies [44].

2.3.1.1. Behavioral testing – pain behavior. After formalin injection, each rat was placed in a single wire cage for observation of the formalin-injected paw. Pain-related behavior was quantified by counting incidence of spontaneous flinches, shakes and jerks of the inflamed paw, and each incidence was recorded as one episode of pain behavior. In some cases, especially in phase I of the response, the intensity of the formalin-induced shakes is very high and manifested as bursts of paw-shaking lasting 1–3 s. When a 1–3 s burst of paw-shaking was observed, this was recorded also as one episode of pain behavior. However, in the majority of our observations, and especially in the phase II, we were able to count each individual paw shake as a single episode. Pain reactions were continuously counted for each individual animal for 90 min and then totaled over characteristic periods: 5–15 min (first phase), 35–50 and 75–90 min (second phase) after formalin administration. In a separate experiment, the non-selective opioid receptor antagonist – naloxone methiodide (QNX, 43 nmol i.pl.) or the selective μ-,cyprodime (CYP, 274 nmol i.pl.), δ-,naltrindole (NT, 94 nmol i.pl.) and κ-,5′-guanidinonaltrindole (GNTI, 0.2 nmol i.pl.) opioid receptor antagonists were co-injected with the highest dose of each opioid agonist (15 min before formalin injection) and the observation scheme was the same as described above.

2.3.2. Edema measurement

The paw volume was measured using a plethysmometer (Ugo Basile, Varese, Italy). The measurements were made 30 min prior to formalin injection, as well as 50 and 90 min after the induction of inflammation.

2.3.3. Complete Freund's adjuvant-induced inflammation

In the analyses of opioid gene expression during the development of inflammatory pain, the inflammation was induced by the injection of complete Freund's adjuvant (CFA). For this, 0.15 ml of undiluted original CFA solution (Calbiochem, Darmstadt, Germany) was administered s.c. into the plantar surface of the right hind limb of rats under brief halothane anesthesia (2–3% v/v in oxygen mixture, 5 L/min for 2–3 min in a Plexiglas chamber) according to the method described by Stein et al. [34]. The inflammation remained confined to the inoculated paw throughout the observation period. The opioid gene expression was measured on the 3rd and 14th day after inoculation.

2.4. Neuropathic pain

2.4.1. Surgery

Peripheral neuropathy was induced by chronic constriction injury (CCI) as described by Bennett and Xie [3] with slight modification [21–23]. The sciatic nerve injury was performed under sodium pentobarbital anesthesia (60 mg/kg, i.p.). The biceps femoris and the gluteus superficialis were separated, and the right sciatic nerve was exposed. Proximal to the sciatic trifurcation, about 7 mm of nerve was freed of adhering tissue and the injury was produced by tying four loose ligatures (4/0 silk, 1 mm spacing) around the sciatic nerve, until they elicited a brief twitch in the respective hind limbs. This twitch prevented us from applying too strong a ligation. The total length of nerve affected was 4–5 mm. Testing

procedures were conducted on days 12–16 after CCI. This time point was selected as injury-induced inflammatory processes are reported to have waned by this time, thus the pain reflects mainly neuropathy.

2.4.2. Behavioral testing

For the assessment of tactile allodynia, rats were tested for their foot withdrawal threshold in response to mechanical stimuli using von Frey filaments (Stoelting, Wood Dale, IL, USA), which are used to apply an innocuous stimulus – slight pressure to the skin. Animals were placed in a plastic cage with a wire net floor and were allowed to habituate 5 min before the experiment. The filaments were applied to the midplantar surface of the ipsilateral hindpaw as described previously [7,21,23]. The strength of the von Frey stimuli ranged from 0.2 to 26 g. The measurements were carried out 15, 30 and 60 min after i.pl. drug administration, at 12–14 days after the sciatic nerve ligation.

For the assessment of thermal hyperalgesia, the Hargreaves test was used as described in our previous study [21]. Rats were tested for paw withdrawal latency (PWD) to a noxious thermal stimulus using an Analgesia Meter (mod 33, IITC INC., Landing, NJ). The animals were placed in Plexiglas cubicles with a glass plate as a floor. After 5 min of habituation, a noxious thermal stimulus (temperature) was focused onto the plantar aspect of a hindpaw until the animal lifted a paw away from the heat source. A cut-off latency of 20 s was used to avoid tissue damage. The measurements were carried out 15, 30 and 60 min after i.pl. drug administration, at 12–14 days after the sciatic nerve ligation.

In a separate experiment, the effects of selective μ-,cyprodime (CYP, 274 nmol i.pl.), δ-,naltrindole (NT, 94 nmol i.pl.) and κ-,5′-guanidinonaltrindole (GNTI, 0.2 nmol i.pl.) opioid receptor antagonists were assessed. In addition, the effects of the non-selective opioid receptor antagonist naloxone methiodide (QNX, 43 nmol i.pl.), which does not cross the blood–brain barrier and therefore blocks only peripheral opioid receptors were tested. The antagonists were co-injected with the highest dose of each opioid agonist and the observation scheme was the same as described above.

2.5. Gene expression studies

2.5.1. qPCR analysis of gene expression

Animals were sacrificed either on the 3rd or 14th day after nerve ligation or complete Freund adjuvant injection. A group of naive animals was used as a reference. Ipsilateral DRG L4–L6 and the corresponding sections of the dorsal, ipsilateral part of the lumbar spinal cord were removed immediately after sacrificing the animal. DRGs were frozen on dry ice and pooled (each pool consisting of 7–10 ganglia), while spinal cord sections were immediately homogenized in 1 ml of Trizol reagent (Invitrogen, Carlsbad, CA, USA). Total RNA was isolated following the method described by Chomczynski and Sacchi [8]. Reverse transcription Real-Time PCRs (qPCR) were performed using Applied Biosystems TaqMan assays, with TaqMan Reverse Transcription Reagents and FG TaqMan PCR Master Mix (Applied Biosystems, Foster, CA, USA). Reactions were run on a Real-Time PCR iCycler device (BioRad, Hercules, CA, USA) with the 3.0a software version. The following TaqMan assays were used: Rn00571351_m1 (prodynorphin), Rn00561699_m1 (δ_1-opioid receptor), Rn00565144_m1 (μ_1-opioid receptor), Rn00567737_m1 (κ_1-opioid receptor), Rn00567566_m1 (preproenkephalin), Rn00595020_m1 (proopiomelanocortin) and Rn01527838_g1 (hypoxanthine guanine phosphoribosyl transferase, Hprt). Threshold cycle values were calculated automatically with default parameters. The abundance of opioid receptor and peptide precursor mRNAs was calculated as described previously [26] using Hprt as a reference.

2.6. Statistical analysis

The results of behavioral experiments were evaluated by the analysis of variance (ANOVA), followed by Bonferroni tests, and presented as a percentage of the maximal possible antinociceptive effect (%MPE ± SEM) using the equation: %MPE = [(TL − BL)/(CUT-OFF − BL)] × 100, BL, baseline latency; TL, respective test value or as mean ± SEM. Each group included 8–10 animals. RT-qPCR data were analyzed using one-way ANOVAs followed by Tukey post-test. A value of $p < 0.05$ vs. respective control group was considered to be statistically significant. The Litchefield and Wilcoxon method was used to determine the antinociceptive dose necessary to produce a 50% response (ED_{50}) with 95% confidence limits on quantal data [38].

3. Results

3.1. Behavioral studies

3.1.1. Inflammatory pain

Formalin injection induced a biphasic incidence of spontaneous flinches, shakes and jerks of formalin-injected paw (Fig. 1 – control). Local, intraplantar (i.pl.) administration of morphine (53, 264, 396 nmol), DAMGO (1.9, 3.8 nmol), DSLET (14, 42, 70 nmol), SNC-80 (11, 44, 111 nmol) dynorphin A (0.5, 2.3, 4.6 nmol) and ICI-199.441 (0.7, 2.3, 7 nmol) reduced formalin-induced pain behavior (Fig. 1A). The strongest antinociceptive effect observed in both phases of the formalin test was obtained after the highest dose of the μ-opioid receptor agonist – DAMGO (first phase: 82.1 ± 10.9% of inhibition of pain behavior, second phase: 75.3 ± 8.8% of inhibition of pain behavior). Also, the δ-opioid receptor agonist DSLET was very potent, relative to the other compounds, at reducing pain behavior in the first phase of the formalin test (75.8 ± 6.8% of inhibition of pain behavior). Both the κ-opioid receptor agonists dynorphin A and ICI-199.441 were effective not only as antinociceptive substances, especially in the second phase of formalin test (60.1 ± 6.8% and 69.3 ± 9.2% of inhibition of pain behavior, respectively), but also significantly decreased the formalin-induced edema as measured 90 min after formalin administration ($F_{13,110} = 7.5$, $P < 0.0001$; Table 1). In fact, the effects of these κ-opioid receptor agonists were similar to that produced by the highest doses of morphine (Table 1).

The antinociceptive effect produced by all agonists was reversed by i.pl. administration of the non-selective peripheral opioid receptor antagonist, naloxone methiodide, which was co-injected with the highest dose of each of the agonists (Fig. 1B). Only the analgesic effect induced by ICI-199.441 in the second phase of formalin test was not inhibited by naloxone methiodide. Additionally, selective opioid receptor antagonists (cyprodime for μ-, naltrindole for δ-, GNTI for κ-opioid receptor) blocked the analgesic effects induced by all agonists used (Fig. 1B).

3.1.2. Neuropathic pain

Chronic constriction injury (CCI) to the sciatic nerve resulted in mechanical allodynia demonstrated by significantly lower thresholds to von Frey filaments, and thermal hyperalgesia as evidenced by a significantly shorter latency to withdraw the paw from the heat stimulus. Both symptoms characteristic for neuropathic pain appeared right after nerve injury and lasted up to 3 weeks, as was observed in our earlier studies [33]. These changes were observed only in paws ipsilateral but not contralateral to CCI.

Tactile allodynia and thermal hyperalgesia were dose-dependently decreased by morphine (528, 1056 nmol), DAMGO (2, 4, 9.5 nmol), DSLET (22, 56, 111 nmol), SNC-80 (22, 66, 111 nmol) dynorphin A (2.3, 4.6, 14 nmol) and ICI-199.441 (7, 23, 47 nmol)

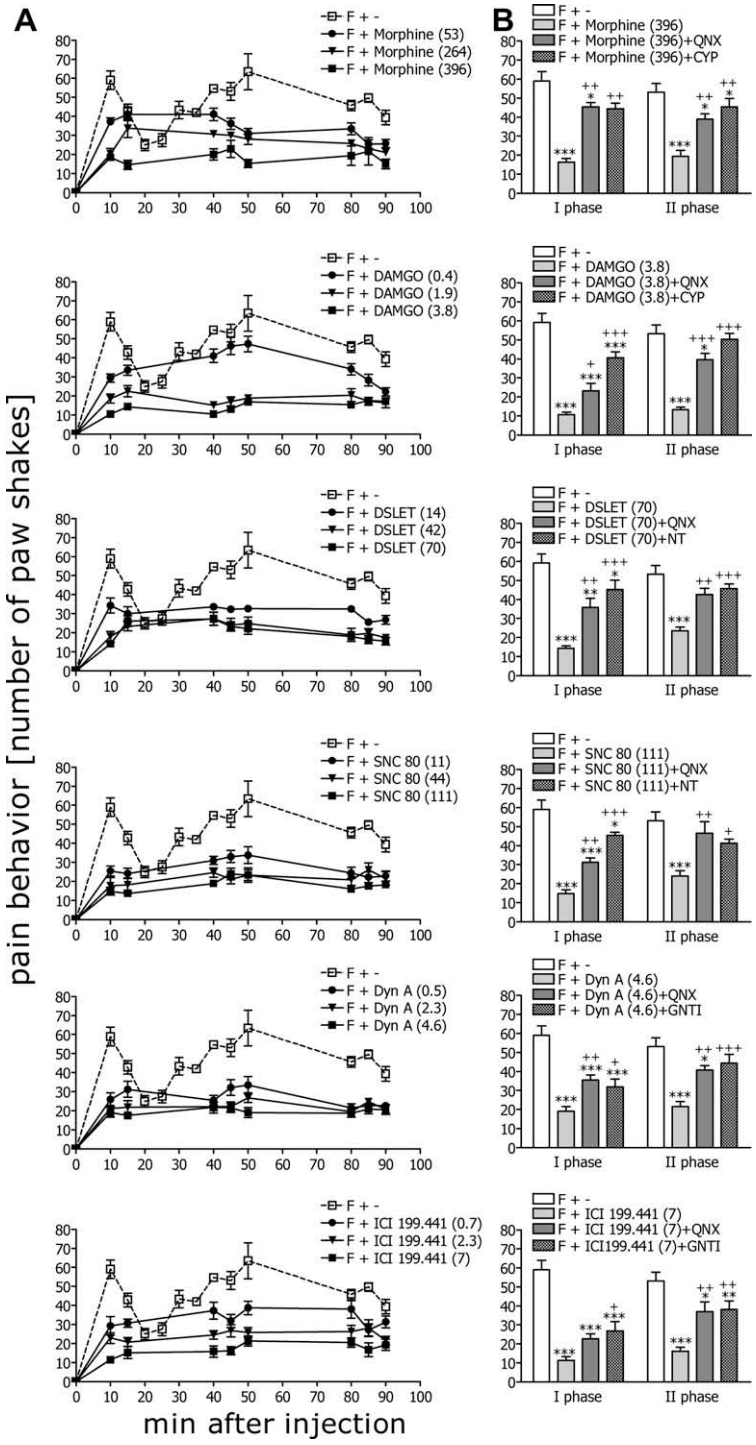

Fig. 1. Effect of intraplantar (i.pl.) administration of μ-, δ- and κ-opioid receptor agonists in the formalin test in rat. Opioid agonists were administered i.pl. 15 min before the formalin injection, and the number of paw flinches, expressed as pain behavior, was counted during a 5 min observation period over the first (5–15 min) and the second phase (35–90 min) of formalin test. (A) Effect of morphine (53, 264, 396 nmol), DAMGO (0.4, 1.9, 3.8 nmol), DSLET (14, 42, 70 nmol), SNC-80 (11, 44, 111 nmol) dynorphin A (0.5, 2.3, 4.6 nmol), ICI-199.441 (0.7, 2.3, 7 nmol) in the formalin test in the rats. (B) Effect of i.pl. administered antagonists: naloxone methiodide (QNX; 43 nmol) on the antinociceptive effects of all agonists used; cyprodime (CYP; 274 nmol) on effect of morphine and DAMGO; naltrindole (NT; 94 nmol) on effect of DSLET and SNC-80; GNTI (0.2 nmol) on effect of dynorphin A and ICI-199.441 in the first and second phase of the formalin test in rats. Data are presented as means ± SEM, $n = 8–10$. The asterisk (*) denotes significance vs. saline-treated group $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ (ANOVA with Bonferroni post-test). The ($^{+}$) denotes significance between groups treated with the agonist alone vs. groups treated with the agonist and antagonist; $^{+}P < 0.05$; $^{++}P < 0.01$; $^{+++}P < 0.001$ (ANOVA with Bonferroni post-test).

i.pl. injected into the injured paw (Fig. 2A and Table 2). The strongest (DSLET) and also long-lasting (SNC-80) effect was observed after δ-opioid receptor agonists in both tests; however, the κ-opioid receptor agonist ICI-199.441 was also very potent in reversing thermal hyperalgesia (Table 2).

The i.pl. administration of μ-, δ- and κ-opioid receptor agonists into injured paws did not significantly change the nociceptive thresholds of the contralateral uninjured paws in response to thermal stimulation (Table 2). Moreover, when the agonists at doses comparable to the highest doses used locally (i.pl.) were injected

Table 1
The measurements of the edema by plethysmometry after intraplantar (i.pl.) administration of the highest dose of μ-, δ- and κ-opioid receptor agonists. The volume (in ml) of the inflamed hindpaw was measured 50 and 90 min after formalin administration. Data are presented as means ± SEM, $n = 8–10$. The asterisk (∗) denotes significance vs. control volume of paw $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ (ANOVA with Bonferroni post-test).

Ligands (dose in nmol)	Volume (% of control ± SEM)	
	50 min	90 min
Control	154.2 ± 12.6	201.9 ± 9.2
Morphine (396)	156.3 ± 11.8	158.0 ± 10.3**
DAMGO (3.8)	190.2 ± 7.1	185.3 ± 3.6
DSLET (70)	192.7 ± 2.3	195.7 ± 4.5
SNC-80 (111)	189.7 ± 5.1	180.8 ± 5.5
Dynorphin A (4.6)	144.8 ± 4.2	141.3 ± 4.9**
ICI-199.441 (7)	153.2 ± 8.3	163.8 ± 7.4*

s.c. into the wrinkle of the neck (i.e. at a site distal to the side of injury), this treatment did not induce any significant alterations in pain thresholds (data not shown).

In both tests, the antiallodynic and antihyperalgesic effects produced by all substances tested were reversed by i.pl. administration of the non-selective peripheral opioid receptor antagonist naloxone methiodide, as well as by selective opioid receptor antagonists (cyprodime for μ-, naltrindole for δ-, GNTI for κ-opioid receptor) (Fig. 2B and Table 2).

3.1.3. Comparison of ED_{50} values for agonists of all types of opioid receptors in inflammatory and neuropathic pain models

When the ED_{50} values were compared, the inhibition of pain-related symptoms by different peptide and non-peptide opioid receptor agonists seemed to be higher in the second, than in the first, phase of the formalin test. Only the ED_{50} value calculated for morphine was higher in the first, compared to the second, phase of the formalin test (Table 3). Comparing the ED_{50} values for inflammatory and neuropathic pain revealed that μ- and κ-opioid receptor agonists are effective at 5.1–11.8 times higher doses in neuropathic pain than in the second phase of formalin test (follow the data in Table 3). Interestingly, the same ED_{50} doses for both types of pain were obtained for δ-opioid receptor agonists only (Table 3). Comparing the chemical structure of the used agonists, the strongest effect in neuropathic pain was observed after peptide agonists of μ-, δ- and κ-opioid receptors than after non-peptide agonists (Table 3).

3.2. Gene expression studies

3.2.1. Expression of opioid receptors and corresponding prohormones in the CFA model of inflammatory pain

Trace amounts of all types of opioid receptor transcripts were detected in the dorsal spinal cord (Table 4). Their abundances were less than a thousandth of Hprt mRNA levels, and the injection of CFA had no effect on them. Much higher levels of opioid receptor transcripts were detected in the DRG and, with the exception of increased μ-opioid receptor mRNA in the L6 DRG two weeks after the induction of inflammation ($F_{4,19} = 11.71$, $P < 0.05$, post-test CFA14 vs. naive $P < 0.01$), CFA had no significant effects on DRG mRNA levels (Fig. 3). In accordance with a previous report [10], inflammation led to an increase in prodynorphin mRNA in the dorsal spinal cord, compared to naive rats on days 3 and 14 after injection, however, the change did not reach significance ($F_{4,15} = 3.01$, $P < 0.05$, post-test n.s.; Table 4). The abundance of proenkephalin mRNA was reduced at both time points tested ($F_{4,13} = 14.95$, $P < 0.0001$, all post-tests vs. naive $P < 0.01$), while proopiomelanocortin transcript levels were unchanged (Table 4). We consistently detected low levels of all opioid peptide precursor mRNAs in DRG (Fig. 3).

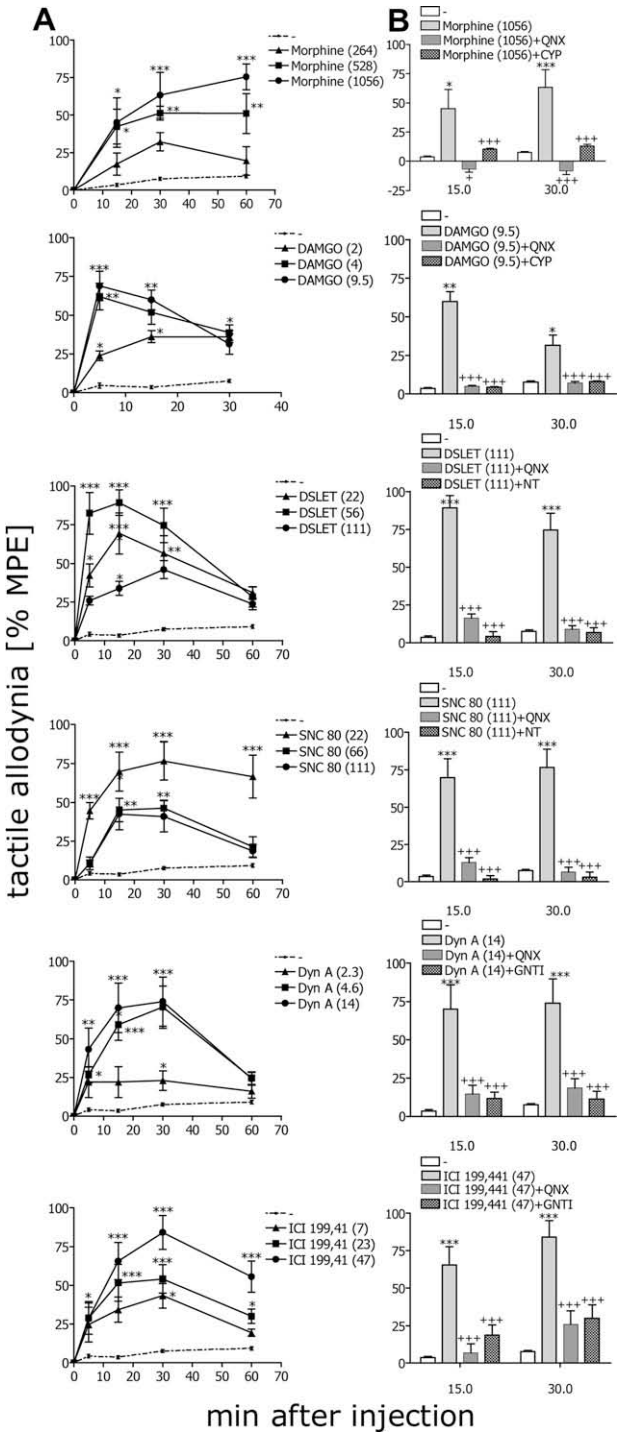

Fig. 2. Effect of intraplantar (i.pl.) administration of μ-, δ- and κ-opioid receptor agonists in neuropathic rats 12–16 days after sciatic nerve ligation. (A) Effect of morphine (264, 528, 1056 nmol), DAMGO (2, 4, 9.5 nmol), DSLET (22, 56, 111 nmol), SNC-80 (22, 66, 111 nmol) dynorphin A (2.3, 4.6, 14 nmol), ICI-199.441 (7, 23, 47 nmol) in mechanical allodynia estimated by von Frey filaments and measured 5, 15, 30 and 60 min after drug administration. (B) Effect of i.pl. administered antagonists: naloxone methiodide (QNX; 43 nmol) on the antinociceptive effects of all agonists used; cyprodime (CYP; 274 nmol) on effect of morphine and DAMGO; naltrindole (NT; 94 nmol) on effect of DSLET and SNC-80; GNTI (0.2 nmol) on effect of dynorphin A and ICI-199.441 in mechanical allodynia estimated with von Frey filaments. Data are presented as means ± SEM, $n = 8–10$. The asterisk (∗) denotes significance vs. saline-treated group $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ (ANOVA with Bonferroni post-test). The ($^{+}$) denotes significance between groups treated with the agonist alone vs. groups treated with the agonist and antagonist; $^{+}P < 0.05$; $^{++}P < 0.01$; $^{+++}P < 0.001$ (ANOVA with Bonferroni post-test).

Table 2
The effect of the highest dose of μ-, δ- and κ-opioid receptor agonists administered intraplantarly (i.p.l.) on thermal hyperalgesia in neuropathic rats as measured in ipsi- and contralateral hindpaw in paw withdrawal test (Hargreaves test) as well as the effect of naloxone methiodide (QNX; 43 nmol) co-administered with all agonists used. Data are presented as means ± SEM in %MPE, $n = 8–10$. The asterisk (*) denotes significance vs. saline-treated group $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ (ANOVA with Bonferroni test). The (+) denotes significance between groups treated with the agonist alone vs. groups treated with the agonist and antagonist; $^{+}P < 0.05$; $^{++}P < 0.01$; $^{+++}P < 0.001$ (ANOVA with Bonferroni post-test).

Ligands (dose in nmol)	15 min			30 min		
	Ipsi	Contra	Ipsi + QNX	Ipsi	Contra	ipsi + QNX
Control	6.4 ± 1.8	10.5 ± 4.3	7.5 ± 2.8	9.1 ± 2.9	9.8 ± 3.7	7.3 ± 2.6
Morphine (1056)	28.1 ± 5.1	8.0 ± 2.8	11.3 ± 2.1	45.7 ± 4.8*	11.6 ± 4.5	12.6 ± 3.1^{++}
DAMGO (9.5)	40.0 ± 4.7*	4.3 ± 0.6	2.1 ± 0.8^{+++}	48.9 ± 8.4**	13.3 ± 2.8	3.4 ± 1.3^{+++}
DSLET (111)	53.4 ± 9.3***	17.6 ± 7.8	1.7 ± 0.5^{+++}	54.3 ± 9.9**	12.6 ± 9.6	20.3 ± 8.1^{+}
SNC-80 (111)	57.9 ± 9.8***	7.3 ± 2.3	18.5 ± 6.8^{+}	68.5 ± 9.4***	11.9 ± 4.2	31.1 ± 9.9^{++}
Dynorphin A (14)	56.9 ± 9.9***	7.8 ± 5.6	15.2 ± 7.6^{+++}	35.7 ± 9.7*	7.4 ± 3.6	2.6 ± 0.9^{+}
ICI-199.441 (47)	64.0 ± 9.9***	3.9 ± 1.8	5.3 ± 2.9^{+++}	68.9 ± 9.9***	9.9 ± 7.2	10.5 ± 4.9^{+++}

CFA treatment caused decrease in abundance of proenkephalin mRNA in L6 and moderately L5 DRG on day 3 after injection (L6: $F_{4,19} = 9.57$, $P < 0.001$, post-test CFA3 vs. naive $P < 0.05$; L5: $F_{4,19} = 14.80$, $P < 0.0001$, post-test CFA vs. naive n.s.). No significant changes in the abundance of prodynorphin and proopiomelanocortin mRNA were observed in DRG after CFA inoculation.

3.2.2. Expression of opioid receptors and the corresponding prohormones in CCI model of neuropathic pain

While the abundance of opioid receptors in spinal cord was unaffected by CCI (Table 4), we found a 2- to 3-fold decrease in their mRNA levels in the DRG (Fig. 3). This decrease was observed at both time points tested and was most pronounced in L5 DRG for the μ- ($F_{4,18} = 24.63$, $P < 0.0001$, CCI14 vs. naive $P < 0.001$) and κ-opioid receptors ($F_{4,19} = 11.05$, $P < 0.0001$, CCI14 vs. naive $P < 0.001$). Analysis of transcript levels of opioid prohormones revealed that proopiomelanocortin mRNA in the dorsal lumbar spinal cord was decreased on day 3 after sciatic nerve ligation ($F_{4,14} = 10.78$, $P < 0.001$, post-test CCI3 vs. naive $P < 0.001$) but returned to the levels observed in naive animals on the 14th day (Table 4). Also, the abundance of proenkephalin mRNA in the lumbar spinal cord was significantly down-regulated at both time points after neuropathic pain induction ($F_{4,13} = 14.95$, $P < 0.001$ both post-tests $P < 0.001$), similar to what was observed after CFA injection. In line with previous reports [18,27], prodynorphin mRNA levels followed an opposite course, with a non-significant tendency to increase on day 3 after CCI ($F_{4,15} = 3.07$, $P < 0.05$, post-test n.s.). Interestingly, we found a robust increase in the abundance of prodynorphin mRNA in the DRG after CCI (Fig. 3). A significant, greater than 10-fold increase, was found in each assayed lumbar DRG (i.e. L5: $F_{4,18} = 63.04$, $P < 0.001$, CCI3 vs. naive $P < 0.001$). In contrast, the abundance of proenkephalin mRNA was decreased both on day 3 and on day 14 in L5 and L6 DRGs ($F_{4,19} = 14.80$; $P < 0.001$ and $F_{4,19} = 9.57$; $P < 0.001$, all post-tests $P < 0.01$). We observed no appreciable changes in the abundance of proopiomelanocortin mRNA levels in the DRG (Fig. 3).

Table 3
Comparison of antinociceptive potency of the μ-, δ- and κ-opioid receptor agonists after their intraplantar (i.p.l.) administration in inflammatory (first phase: 5–10 min, second phase: 40–45 min) and neuropathic pain (15 min after injection; von Frey test 14–16 days after nerve injury). The results are shown as ED_{50} value with 95% confidence limits (CL) determined on the quantal data.

Ligands	ED_{50} (95% CL) (nmol, i.p.l.)		
	Inflammatory pain		Neuropathic pain
	I phase	II phase	
DAMGO	0.4 (0.3–0.6)	1.1 (0.9–1.36)	5.6 (2.8–10.9)
Morphine	149 (1.8–1187)	108 (69–168)	554 (419–733)
DSLET	19.1 (15.1–24.4)	27.3 (18.3–40.8)	22.8 (18.1–28.7)
SNC-80	4.2 (1.9–8.9)	26.3 (15.6–44.1)	53.8 (17.5–165.2)
Dynorphin A	0.2 (0.1–0.5)	1.0 (0.7–1.7)	5.3 (0.5–60.9)
ICI-199.441	0.8 (0.5–1.2)	1.5 (0.9–2.4)	17.7 (12.7–24.7)

4. Discussion

In the first part of this study, local intraplantar (i.pl.) injection of low doses of μ-, δ- and κ-opioid receptor agonists, which are ineffective when administered s.c., produced antinociception in a model of inflammation induced by formalin injection and in a model of neuropathic pain developed after sciatic nerve ligation. The agonist-induced antinociceptive activity in both types of pain models was dose-dependent and was inhibited by the non-selective brain-inpenetrant antagonist naloxone methiodide, indicating that the effects were mediated by peripheral opioid receptors present within the injured tissue. Moreover, the effects of opioid agonists were blocked by antagonists selective for each type of opioid receptors, further confirming the contribution of μ-, δ- and κ-opioid peripheral receptors. This is consistent with our earlier studies and those of others [13,23,24,35,39] suggesting the involvement of specific opioid receptor types in opioid-induced peripheral antinociception. Besides their peripherally mediated antinociceptive effects, locally applied κ-agonists and morphine (but not δ-agonists) indicated also anti-inflammatory potential as they decreased formalin-induced edema of the inflamed paw. The effect induced by μ- and κ-opioids might relate to the inhibition of substance P and CGRP release from C fibers [5,35,41].

The employment of both peptide and non-peptide agonists, selective for each type of opioid receptors, allowed for a comprehensive assessment of the antinociceptive potency of locally applied opioids. The ED_{50} analysis demonstrated that much higher doses of μ- and κ-agonists are required to produce antinociception in neuropathic than in inflammatory pain. Thus, our result confirms earlier observations that opioid-induced analgesia in neuropathic pain could be achieved after higher agonist doses than in acute and/or inflammatory pain [1,4,31]. Degeneration of the C fibers is followed by a decrease in the synthesis of all types of opioid receptors at both the spinal cord and DRG levels, and this is often suggested as a main reason for lower opioid effectiveness in neuropathic pain [17,31,45,46]. In contrast, inflammation-induced enhanced axonal transport of opioid receptors toward periphery is preceded by an increase in mRNA transcription, thus causing a higher receptor density and enhanced opioid antinociception at the injured site [13,20,30,47]. Moreover, lower pH of inflamed tissue may increase the effects of opioids by affecting of opioid receptor interactions with G proteins and with intracellular signaling proteins [32,48] and therefore, ligand–receptor interaction can be more efficient in inflamed tissue. Interestingly, the ED_{50} of δ-agonists were comparable in both neuropathic and inflammatory pain. Thus, the potent antinociceptive effects of δ-agonists point to the

Table 4
The changes in the expression of opioid receptors and corresponding prohormones in the spinal cord from animals 3 or 14 days after the injection of complete Freund's adjuvant (CFA) or chronic constriction injury (CCI). The relative abundances of transcripts encoding opioid receptors and corresponding precursors. Data are presented as means ± SEM from 4 to 5 samples each pooled from 7 to 10 spinal cords. The C denotes significance vs. naive animals; $^{C}P < 0.001$ (ANOVA with Tukey post-test). The a denotes significance between CCI 3 vs. CFA 3, or CCI 14 vs. CFA 14; $^{a}P<0.05$ (ANOVA with Tukey post-test).

	Naive	CFA 3	CFA 14	CCI 3	CCI 14
μ-Opioid receptor	$0.18 \pm 0.05 \times 10^{-3}$	$0.27 \pm 0.08 \times 10^{-3}$	$0.19 \pm 0.04 \times 10^{-3}$	$0.12 \pm 0.06 \times 10^{-3a}$	$0.12 \pm 0.02 \times 10^{-3}$
δ-Opioid receptor	$0.09 \pm 0.03 \times 10^{-3}$	$0.12 \pm 0.03 \times 10^{-3}$	$0.07 \pm 0.003 \times 10^{-3}$	$0.09 \pm 0.02 \times 10^{-3}$	$0.06 \pm 0.008 \times 10^{-3}$
κ-Opioid receptor	$0.22 \pm 0.06 \times 10^{-3}$	$0.27 \pm 0.07 \times 10^{-3}$	$0.30 \pm 0.01 \times 10^{-3}$	$0.17 \pm 0.03 \times 10^{-3}$	$0.17 \pm 0.01 \times 10^{-3a}$
Proopiomelanocortin	0.03 ± 0.003	0.02 ± 0.003	0.03 ± 0.004	0.007 ± 0.001^{Ca}	0.030 ± 0.004
Proenkephalin	8.61 ± 0.49	5.97 ± 0.25^{C}	5.10 ± 0.17^{C}	5.42 ± 0.29^{C}	5.41 ± 0.39^{C}
Prodynorphin	0.88 ± 0.11	1.45 ± 0.31	0.99 ± 0.11	1.73 ± 0.23	1.29 ± 0.12

peripheral δ-receptor as an interesting target in searching for new peripherally active analgesics for chronic pain therapy. This pathway is of particular interest since neuropathy observed in patients is often coupled with inflammatory symptoms [2,19] and opioids can act through the opioid receptors present on immune cells (e.g. macrophages) which migrate to the inflamed/injured tissue [13,24,35]. Further analysis of ED_{50} doses demonstrated also a higher efficiency of peptides, in comparison with non-peptide agonists, in both types of pain. Higher antinociceptive effectiveness of opioid agonists with peptide structures might be here explained only by their slower distribution from the site of injection and lower diffusion to the adjacent tissues, which results in higher local concentration in the peripheral target tissue. However, further studies are required to explain the specific mechanisms underlying the observed peptide effect. Nevertheless, the current observations clearly indicate that local treatment with peptide agonists is beneficial as they are antinociceptive in neuropathic pain and have limited CNS penetration.

In the second part of this study, distinct changes in the endogenous opioid system were demonstrated in the development of chronic inflammatory (CFA injection) and neuropathic pain (CCI to sciatic nerve) – two distinct models of long-lasting pain, that are known to differently regulate the endogenous opioid system. This approach thereby provides a better comparison of long-term adaptations in gene expression. Our comprehensive analysis revealed that the expression of all types of opioid receptor mRNA in the DRG was decreased in neuropathic pain and unaltered in CFA-induced inflammatory pain. Similar to the research conducted by others [25,42], our results indicate that CFA-induced inflammation did not change the mRNA levels of opioid receptors in DRG nor in spinal cord. In neuropathic pain, the decrease might simply correspond to a reduction in opioid receptor proteins in the neurons in which they are expressed. In line with our study, a decrease in the abundance of opioid receptor mRNA has been previously observed in the DRG of rats after axotomy [46]. Our results, as well as previous reports, indicate that lower efficacy of opioid receptor agonists in neuropathic pain may relate to the decreased abundance of opioid receptors mRNA at the level of spinal cord and peripheral neurons [17,25,31,46]. Interestingly, a level of δ-opioid receptor mRNA (which may indicate a lower synthesis of δ-receptors) does not

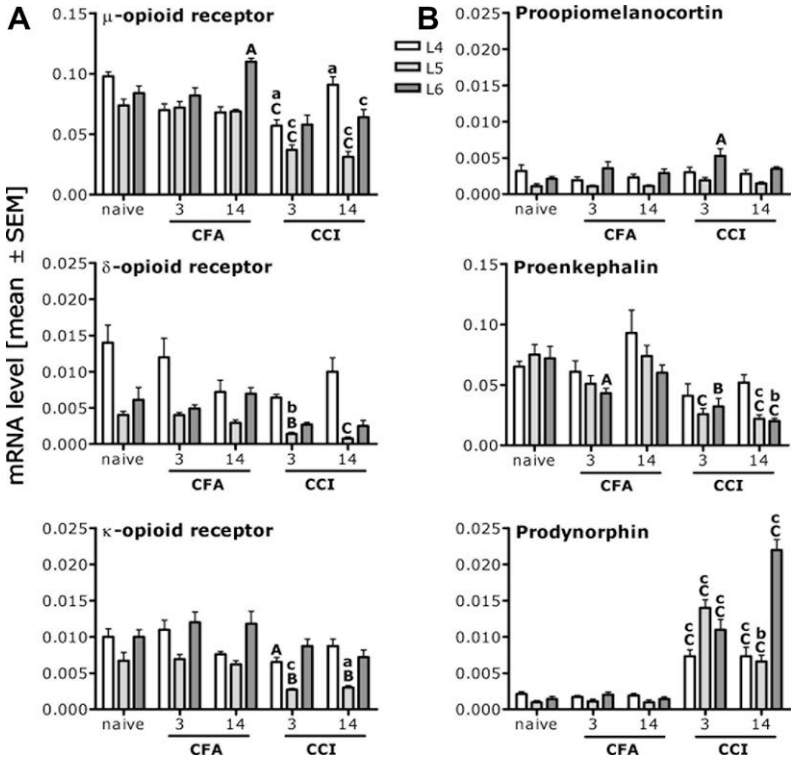

Fig. 3. The changes in expression of opioid receptors and the corresponding prohormones in DRG. The bar graphs show abundances of opioid receptors and opioid peptide precursor transcripts normalized to Hprt. Each bar represents mean value from 4 to 5 samples each pooled from 7 to 10 ganglions. Empty bars represent the L4 DRG, light gray bars correspond to L5 DRG and dark gray to L6 DRG. Results shown correspond to samples derived from animals 3 or 14 days after the injection of complete Freund's adjuvant (CFA) or chronic constriction injury (CCI). Data are presented as means ± SEM. The A,B,C denote significance vs. naive animals; $^{A}P < 0.05$; $^{B}P < 0.01$; $^{C}P < 0.001$ (ANOVA with Tukey post-test). The a,b,c denote significance between CCI 3 vs. CFA 3, or CCI 14 vs. CFA 14; $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$ (ANOVA with Tukey post-test).

affect the peripheral effectiveness of δ-receptor agonists in neuropathic pain. Simultaneously with a decrease of δ-opioid receptors mRNA, we observed also a decrease of proenkephalin transcript abundance. This result might suggest that the availability of endogenous agonists is, in part, responsible for a putative increase in δ-receptor sensitivity in neuropathic pain. However, further studies need to be performed to clarify this phenomenon.

Alterations in opioid peptide gene expression after chronic pain may influence nociceptive transmission, as well as opioid-induced peripheral antinociceptive effects. It was shown that hyperalgesia and allodynia can be associated with the alterations of dynorphin at the spinal level [18,22,40]. Our qPCR analysis found a trend toward increases in the abundance of prodynorphin mRNA in lumbar spinal cord, which is in accordance with a previously reported significant up-regulation after both inflammation of the hindpaw [10] and sciatic nerve injury [18,27]. The functional role of dynorphin was demonstrated in transgenic mice where the deletion of the *prodynorphin* gene blocked the development of neuropathic pain symptoms [12,42]. It appears that dynorphins, depending on the dose, may induce antinociception through κ-receptors [29,36, current results] or act pronociceptively by a non-opioid mechanism involving the NMDA receptors [4,22,40].

Besides the elevated level of spinal prodynorphin mRNA, this paper demonstrates for the first time a profound increase of prodynorphin mRNA abundance in the DRG after sciatic nerve ligation. While it should be emphasized that the abundance of dynorphin in the DRG is minute, it is interesting to speculate whether it may contribute to the development of neuropathic pain. A recent study demonstrated that dynorphin induces calcium influx via voltage-sensitive calcium channels in sensory neurons by activating bradykinin receptors in the spinal cord [16]. A similar mechanism may operate at the peripheral nerve ending and, through the increase of dynorphin expression in the DRG, might contribute to the maintenance of neuropathic pain. Our studies indicate also that nerve injury decreases pro-opiomelanocortin mRNA in the spinal cord. Interestingly, Kurrikoff et al. [15] suggested that this decrease is involved in the antagonistic interaction between non-opioid (cholecystokinin) and opioid systems, which regulate pain sensitivity and the development of neuropathy.

In summary, the results presented provide broad evidence for an involvement of peripheral mechanisms in opioid-induced analgesia in inflammatory and neuropathic pain. Employment of agonists selective to different types of opioid receptors provides new targets for the treatment of chronic pain. The important targets could be either the δ-receptor because its agonists were similarly effective in both neuropathy and inflammation or the κ-receptor because its agonists displayed both antinociceptive efficacy and anti-inflammatory properties. Moreover, opioid ligands with a peptide structure might have therapeutic potential as peripherally active analgesics. Our findings demonstrate also long-lasting differences in the activity of endogenous opioid systems under chronic inflammatory and neuropathic pain, which might correlate with different effectiveness of locally applied opioid agonists under neuropathy. The changes in the prodynorphin system, observed only in neuropathic pain, might also have an important role in the development and maintenance of neuropathic pain symptoms. Thus, the obtained results differentiate the pharmacology of neuropathy and inflammation and provide evidence regarding the effectiveness of peripheral opioids in chronic, especially neuropathic, pain.

Acknowledgements

This work was supported by Polish MNSW Scientific Network funds and a statutory funds from the Ministry of Sciences Research and Information Technology (Warszawa, Poland). The contribution of Foundation for Polish Science (FNP) to I. Obara is greatly acknowledged. The authors like to thank Dr. Helmut Schmidhammer from University of Innsbruck, Austria for providing us with cyprodime as well as Dr. Karen Szumlinski from University of California, Santa Barbara, USA for grammatical assistance during manuscript preparation. The authors disclose no conflict of interest in respect of this work.

References

[1] Arner S, Meyerson BA. Lack of analgesic effect of opioids on neuropathic and idiopathic forms of pain. Pain 1988;33:11–23.
[2] Baron R. Mechanisms of disease: neuropathic pain – a clinical perspective. Nat Clin Pract Neurol 2006;2:95–106.
[3] 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.
[4] Bian D, Ossipov MH, Ibrahim M, Raffa RB, Tallarida RJ, Malan Jr TP, et al. Loss of antiallodynic and antinociceptive spinal/supraspinal morphine synergy in nerve-injured rats: restoration by MK-801 or dynorphin antiserum. Brain Res 1999;831:55–63.
[5] Binder W, Machelska H, Mousa S, Schmitt T, Riviere PJ, Junien JL, et al. Analgesic and antiinflammatory effects of two novel kappa-opioid peptides. Anesthesiology 2001;94:1034–44.
[6] Coggeshall RE, Carlton SM. Receptor localization in the mammalian dorsal horn and primary afferent neurons. Brain Res Brain Res Rev 1997;24:28–66.
[7] Colburn RW, DeLeo JA, Rickman AJ, Yeager MP, Kwon P, Hickey WF. Dissociation of microglial activation and neuropathic pain behaviors following peripheral nerve injury in the rat. J Neuroimmunol 1997;79:163–75.
[8] Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction. Anal Biochem 1987;162:156–9.
[9] DeHaven-Hudkins DL, Dolle RE. Peripherally restricted opioid agonists as novel analgesic agents. Curr Pharm Des 2004;10:743–57.
[10] Dubner R, Ruda MA. Activity-dependent neuronal plasticity following tissue injury and inflammation. Trends Neurosci 1992;15:96–103.
[11] Furst S, Riba P, Friedmann T, Timar J, Al-Khrasani M, Obara I, et al. Peripheral versus central antinociceptive actions of 6-amino acid-substituted derivatives of 14-O-methyloxymorphone in acute and inflammatory pain in the rat. J Pharmacol Exp Ther 2005;312:609–18.
[12] Gardell LR, Ibrahim M, Wang R, Wang Z, Ossipov MH, Malan Jr TP, et al. Mouse strains that lack spinal dynorphin upregulation after peripheral nerve injury do not develop neuropathic pain. Neuroscience 2004;123:43–52.
[13] Janson W, Stein C. Peripheral opioid analgesia. Curr Pharm Biotechnol 2003;4:270–4.
[14] Kabli N, Cahill CM. Anti-allodynic effects of peripheral delta opioid receptors in neuropathic pain. Pain 2007;127:84–93.
[15] Kurrikoff K, Koks S, Matsui T, Bourin M, Arend A, Aunapuu M, et al. Deletion of the CCK2 receptor gene reduces mechanical sensitivity and abolishes the development of hyperalgesia in mononeuropathic mice. Eur J Neurosci 2004;20:1577–86.
[16] Lai J, Luo MC, Chen Q, Ma S, Gardell LR, Ossipov MH, et al. Dynorphin A activates bradykinin receptors to maintain neuropathic pain. Nat Neurosci 2006;9:1534–40.
[17] Li JL, Kaneko T, Mizuno N. Effects of peripheral nerve ligation on expression of mu-opioid receptor in sensory ganglion neurons: an immunohistochemical study in dorsal root and nodose ganglion neurons of the rat. Neurosci Lett 1996;214:91–4.
[18] Malan TP, Ossipov MH, Gardell LR, Ibrahim M, Bian D, Lai J, et al. Extraterritorial neuropathic pain correlates with multisegmental elevation of spinal dynorphin in nerve-injured rats. Pain 2000;86:185–94.
[19] Moriwaki K, Yuge O, Tanaka H, Sasaki H, Izumi H, Kaneko K. Neuropathic pain and prolonged regional inflammation as two distinct symptomatological components in complex regional pain syndrome with patchy osteoporosis – a pilot study. Pain 1997;72:277–82.
[20] Mousa SA, Zhang Q, Sitte N, Ji R, Stein C. Beta-endorphin-containing memory-cells and mu-opioid receptors undergo transport to peripheral inflamed tissue. J Neuroimmunol 2001;115:71–8.
[21] Obara I, Makuch W, Spetea M, Schütz J, Schmidhammer H, Przewlocki R, et al. Local peripheral antinociceptive effects of 14-O-methyloxymorphone derivatives in inflammatory and neuropathic pain in the rat. Eur J Pharmacol 2007;558:60–7.
[22] Obara I, Mika J, Schafer MK, Przewlocka B. Antagonists of the kappa-opioid receptor enhance allodynia in rats and mice after sciatic nerve ligation. Br J Pharmacol 2003;140:538–46.
[23] Obara I, Przewlocki R, Przewlocka B. Local peripheral effects of mu-opioid receptor agonists in neuropathic pain in rats. Neurosci Lett 2004;360:85–9.
[24] Pertovaara A, Wei H. Peripheral effects of morphine in neuropathic rats: role of sympathetic postganglionic nerve fibers. Eur J Pharmacol 2001;429:139–45.
[25] Porreca F, Tang QB, Bian D, Riedl M, Elde R, Lai J. Spinal opioid mu receptor expression in lumbar spinal cord of rats following nerve injury. Brain Res 1998;795:197–203.

[26] Proudnikov D, Yuferov V, LaForge KS, Ho A, Jeanne Kreek M. Quantification of multiple mRNA levels in rat brain regions using real time optical PCR. Brain Res Mol Brain Res 2003;112:182–5.
[27] Przewlocki R, Haarmann I, Nikolarakis K, Herz A, Hollt V. Prodynorphin gene expression in spinal cord is enhanced after traumatic injury in the rat. Brain Res 1988;464:37–41.
[28] Przewlocki R, Hassan AH, Lason W, Epplen C, Herz A, Stein C. Gene expression and localization of opioid peptides in immune cells of inflamed tissue: functional role in antinociception. Neuroscience 1992;48:491–500.
[29] Przewlocki R, Shearman GT, Herz A. Mixed opioid/nonopioid effects of dynorphin and dynorphin related peptides after their intrathecal injection in rats. Neuropeptides 1983;3:233–40.
[30] Puehler W, Zollner C, Brack A, Shaqura MA, Krause H, Schafer M, et al. Rapid upregulation of mu opioid receptor mRNA in dorsal root ganglia in response to peripheral inflammation depends on neuronal conduction. Neuroscience 2004;129:473–9.
[31] Rashid MH, Inoue M, Toda K, Ueda H. Loss of peripheral morphine analgesia contributes to the reduced effectiveness of systemic morphine in neuropathic pain. J Pharmacol Exp Ther 2004;309:380–7.
[32] Selley DE, Breivogel CS, Childers SR. Modification of G protein-coupled functions by low-pH pretreatment of membranes from NG108-15 cells: increase in opioid agonist efficacy by decreased inactivation of G proteins. Mol Pharmacol 1993;44:731–41.
[33] Starowicz K, Bilecki W, Sieja A, Przewlocka B, Przewlocki R. Melanocortin 4 receptor is expressed in the dorsal root ganglions and down-regulated in neuropathic rats. Neurosci Lett 2004;358:79–82.
[34] Stein C, Millan MJ, Herz A. Unilateral inflammation of the hindpaw in rats as a model of prolonged noxious stimulation: alterations in behavior and nociceptive thresholds. Pharmacol Biochem Behav 1988;31:451–5.
[35] Stein C, Machelska H, Schafer M. Peripheral analgesic and antiinflammatory effects of opioids. Z Rheumatol 2001;60:416–24.
[36] Stevens CW, Yaksh TL. Dynorphin A and related peptides administered intrathecally in the rat: a search for putative kappa opiate receptor activity. J Pharmacol Exp Ther 1986;238:833–8.
[37] Sung B, Loh HH, Wei L. Association of kappa opioid receptor mRNA upregulation in dorsal root ganglia with mechanical allodynia in mice following nerve injury. Neurosci Lett 2000;291:163–6.
[38] Tallarida RJ, Murray RB. Manual of pharmacological calculations with computer programs. NY: Springer; 1987.
[39] Truong W, Cheng C, Xu QG, Li XQ, Zochodne DW. Mu opioid receptors and analgesia at the site of a peripheral nerve injury. Ann Neurol 2003;53:366–75.
[40] 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.
[41] Walker JS. Anti-inflammatory effects of opioids. Adv Exp Med Biol 2003;521:148–60.
[42] Wang H, Wessendorf MW. Equal proportions of small and large DRG neurons express opioid receptor mRNAs. J Comp Neurol 2001;429:590–600.
[43] Whiteside GT, Boulet JM, Walker K. The role of central and peripheral mu opioid receptors in inflammatory pain and edema: a study using morphine and DiPOA ([8-(3,3-diphenyl-propyl)-4-oxo-1-phenyl-1,3,8-triaza-spiro[4.5]dec-3-yl]-acetic acid). J Pharmacol Exp Ther 2005;314:1234–40.
[44] Wordliczek J, Banach M, Labuz D, Przewlocka B. Intrathecal administration of doxepin attenuated development of formalin-induced pain in rats. J Neural Transm 2005;112:1321–9.
[45] Xiao HS, Huang QH, Zhang FX, Bao L, Lu YJ, Guo C, et al. Identification of gene expression profile of dorsal root ganglion in the rat peripheral axotomy model of neuropathic pain. Proc Natl Acad Sci USA 2002;99:8360–5.
[46] Zhang X, Bao L, Shi TJ, Ju G, Elde R, Hökfelt T. Down-regulation of mu-opioid receptors in rat and monkey dorsal root ganglion neurons and spinal cord after peripheral axotomy. Neuroscience 1998;82:223–40.
[47] Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 1983;16:109–10.
[48] Zollner C, Shaqura MA, Bopaiah CP, Mousa S, Stein C, Schafer M. Painful inflammation-induced increase in mu-opioid receptor binding and G-protein coupling in primary afferent neurons. Mol Pharmacol 2003;64:202–10.