HHS Public Access

Author manuscript

Eur J Pharmacol. Author manuscript; available in PMC 2018 July 29.

Published in final edited form as:

Eur J Pharmacol. 2018 March 15; 823: 79–86. doi:10.1016/j.ejphar.2018.01.039.

Involvement of the N/OFQ-NOP system in rat morphine antinociceptive tolerance: are astrocytes the crossroad?

Laura Micheli[a], **Elena Lucarini**[a], **Francesca Corti**[a], **Roberto Ciccocioppo**[b], **Girolamo Calò**[c], **Anna Rizzi**[c], **Carla Ghelardini**[a], and **Lorenzo Di Cesare Mannelli**[a]

[a]Department of Neuroscience, Psychology, Drug Research and Child Health - Neurofarba - Pharmacology and Toxicology Section, University of Florence, Florence, Italy

[b]School of Pharmacy, Pharmacology Unit, University of Camerino, Camerino, Italy

[c]Department of Medical Sciences, Section of Pharmacology and National Institute of Neuroscience, University of Ferrara, Italy

Abstract

The development of tolerance to the antinociceptive effect is a main problem associated with the repeated administration of opioids. The progressively higher doses required to relieve pain reduce safety and exacerbate the side effects of classical opioid receptor agonists like morphine. Nociceptin/orphanin FQ (N/OFQ) and its NOP receptor constitute the fourth endogenous opioid system that is involved in the control of broad spectrum of biological functions, including pain transmission. Aim of this work was to evaluate the relevance of the N/OFQ-NOP system in morphine antinociceptive action and in the development of morphine tolerance in the rat. Continuous spinal intrathecal infusion of morphine (1 – 3 nmol/h) evoked analgesic effects for 5 days in wild type animals. The same doses infused in NOP(−/−) rats showed a lower analgesic efficacy, while the onset of tolerance was delayed to day 9. N/OFQ (1 – 3 nmol/h), continuously infused in NOP(+/+) animals, showed an analgesic profile similar to morphine. Immunohistochemical analysis of the dorsal horn of the spinal cord of morphine tolerant NOP(+/+) rats showed an increased number of Iba1- and GFAP-positive cells (microglia and astrocytes, respectively). Interestingly, microglia but not astrocyte activation was observed in NOP(−/−) morphine tolerant rat. A selective activation of astrocytes was observed in the dorsal horn of wild type N/OFQ tolerant rats.

The antinociceptive effect of morphine partially depends by the N/OFQ-NOP system that participates in the development of morphine tolerance. In particular, NOP receptors are involved in morphine-induced astrocyte activation, and N/OFQ per se increases astrocyte density.

Corresponding author: Lorenzo Di Cesare Mannelli, Dept. of Neuroscience, Psychology, Drug Research and Child Health - NEUROFARBA - Pharmacology and Toxicology Section, University of Florence, Viale Pieraccini 6, 50139, Florence, Italy. Phone: +39-055-2758395, lorenzo.mannelli@unifi.it.

Conflict of interest statement

All authors declare no conflict of interest

Keywords

morphine tolerance; N/OFQ; NOP receptor; NOP(−/−) rats; glial cells

1. Introduction

Morphine is one of the most powerful drug used in pain relief and remains a preferred and commonly prescribed analgesic for pain management (Denlinger et al., 2014). Although it shows remarkable efficacy for treating chronic pain conditions, there are several limitations to its use (Yuill et al., 2016), including rapid development of tolerance, which is characterized by a decrease in antinociceptive efficacy (Self and Nestler, 1995). To overcome tolerance and achieve equivalent pain relief, the opioid dose is progressively escalated which can exacerbate the unwanted effects (Thorn et al., 2015). Mechanisms of tolerance are complex and involves changes in receptors, ion channels cell and neuronal networks functions (Ji, 2010; Johansson et al., 2010; Zhang et al., 2015). According to a classical view these re-arrangements occur at the neuronal level, however growing evidence indicates a potential role of glia cells that are activated by repeated administrations of morphine (Di Cesare Mannelli et al., 2015; Horvath et al., 2009; Mika, 2008; Mika et al., 2009; Raghavendra et al., 2003; Song and Zhao, 2001; Watkins et al., 2005). Morphine treatment induces astrocyte and microglia changes resulting in increased production of many soluble factors such as proinflammatory cytokines and chemokines, neurotrophic factors, prostaglandins, nitric oxide, complement proteins, free radicals, neurotoxins and excitatory amino acids that counteract opioid analgesia (Eidson and Murphy, 2013; Hameed et al., 2010). The pharmacological inhibition of microglia and astrocyte with minocycline and fluorocitrate, respectively, attenuates the onset of tolerance in rats (Cui et al., 2008; Song and Zhao, 2001).

Nociceptin/orphanin FQ (N/OFQ), a heptadecapeptide from the opioid family, and its cognate NOP receptor (previously called the opioid receptor-like, ORL1), are present in nociceptive pathways in spinal cord and brain with a complex role (Schroder et al., 2014, Zeilhofer and Calo, 2003). N/OFQ differentially modulates nociception depending upon site of administration, dose, assay, and animal species. N/OFQ injected intracerebroventricularly (i.c.v) decreases the pain threshold in the hotplate and tail-flick assays (Meunier et al., 1995; Reinscheid et al., 1995). Moreover, after the i.c.v. administration, N/OFQ attenuates morphine analgesia (Mogil et al., 1996). On the contrary, intrathecal (i.t.) administration of the peptide shows acute antinociceptive activity in the tail-flick and in the paw pressure tests (Micheli et al., 2015a; Xu et al., 1996). Similarly, in models of neuropathic and inflammatory pain, i.t. injections of N/OFQ have also been shown to produce anti-hyperalgesic (Micheli et al., 2015b) and anti-allodynic effects and to potentiate morphine antihyperalgesia (Courteix et al., 2004; Hao et al., 1998; Yamamoto et al., 1997). Despite these studies prove the properties of N/OFQ to evoke antinociceptive effect after spinal administration, no definitive data are reported regarding the involvement of the NOP system in the onset of opioid analgesic morphine tolerance.

Aiming to evaluate the involvement of the NOP system in the onset of morphine tolerance, morphine was administered in NOP(+/+) and NOP(−/−) animals through an intrathecal catheter that allowed a continuous infusion of the compounds at the lumbar level of the spinal cord. The glial activation profile was studied in the dorsal horn in comparison to the same treatment with N/OFQ.

2. Materials and Methods

2.1. Animals

Male Sprague-Dawley and Wistar rats were supplied by Envigo (Varese, Italy). Male NOP(−/−) rats, supplied by GenOway (Lyon, France), were generated in a Brown Norway background and subsequently backcrossed on a Wistar background for four generations as previously described in detail (Homberg et al., 2009). For knockout studies Wistar rats were used as controls and indicated as NOP(+/+). Animals, weighing approximately 280–300g at the beginning of the experimental procedure, were housed in Ce.S.A.L (Centro Stabulazione Animali da Laboratorio, University of Florence) and used at least one week after their arrival. Four rats were housed per cage (size 26 × 41 cm) kept at 23 ± 1°C with a 12 h light/dark cycle, light at 7 a.m; were fed a standard laboratory diet and tap water *ad libitum.* All animal manipulations were carried out according to the Directive 2010/63/EU of the European parliament and of the European Union council (22 September 2010) on the protection of animals used for scientific purposes. The ethical policy of the University of Florence complies with the Guide for the Care and Use of Laboratory Animals of the US National Institutes of Health (NIH Publication No. 85–23, revised 1996; University of Florence assurance number: A5278–01). Formal approval to conduct the experiments described was obtained from the Animal Subjects Review Board of the University of Florence. Experiments involving animals have been reported according to ARRIVE guidelines (McGrath and Lilley, 2015). All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Intrathecal catheterization

Rats were anesthetized with 2% isoflurane and intrathecal catheter was surgically implanted according to the Yaksh & Rudy (1976) method. Rats were shaved on the back of the neck and placed in the stereotaxic frame with the head securely held between ear bars. The skin over the nap of the neck was cleaned with ethyl alcohol and incised for 1 cm. The muscle on either side of the external occipital crest was detached and retracted to expose about 3–4 mm^2 of the atlanto-occipital membrane. The membrane was incised by a needle, which led to the escape of cerebrospinal fluid. The caudal edge of the cut was lifted and about 7.0 cm of 28G polyurethane catheter (0.36 mm outer diameter; 0.18 mm inner diameter; Alzet, USA) was gently inserted into the intrathecal space in the midline, dorsal to the spinal cord until the lumbar enlargement. The exit end of the catheter was connected to the osmotic pumps (Alzet, USA) for continuous infusion. Then pumps were subcutaneously fixed on the back of the head. The incision site in the skin was sutured with polyamide wire and animals were allowed to recover for 24 h before the study began.

All animals used during behavioral tests did not shown motor impairment induced by surgical operation for the catheter implantation. The evaluation of potential motor dysfunctions were investigated using Rota rod test (Ugo Basile, Varese, Italy). The animals that represented any kind of motor disability (approximately 15%) were excluded from the behavioral measurements.

2.3. Drug intrathecal treatments

Morphine hydrochloride was from S.A.L.A.R.S., Como, Italy. N/OFQ was synthesized and purified as previously described in details (Arduin et al., 2007; Guerrini et al., 1997). All compounds were dissolved in sterile saline solution.

For continuous infusion, compound concentrations were adjusted to release 1 and 3 nmol/h morphine or N/OFQ from a mini-osmotic pump ALZET 1002 (USA) with a flow rate 0.25 μL/h. Final solutions were filtered by a 0.22 μm pore size Hydrophilic PVDF membrane (Millipore, Italy). Mini-pumps were attached to the described polyurethane catheters and filled with compounds or vehicle solutions, respectively. All mini-osmotic pumps were incubated in sterile saline solution overnight at 37 °C.

2.4. Paw Pressure test

The nociceptive threshold in the rat was determined with an analgesimeter (Ugo Basile, Varese, Italy), according to the method described by Leighton et al. (1988). Briefly, a constantly increasing pressure was applied to a small area of the dorsal surface of the paw using a blunt conical probe by a mechanical device. Mechanical pressure was increased until vocalization or a withdrawal reflex occurred while rats were lightly restrained. Vocalization or withdrawal reflex thresholds were expressed in grams. Rats scoring below 35 g or over 75 g during the test before the surgical procedure for the catheter implantations were rejected. For analgesia measures, mechanical pressure application was stopped at 180 g. All experiments were performed by a researcher blind to drug treatments.

2.5. Rota-rod test

The Rota-rod apparatus (Ugo Basile, Varese, Italy) consisted of a base platform and a rotating rod with a diameter of 6 cm and a non-slippery surface. The rod was placed at a height of 25 cm from the base. The rod, 36 cm in length, was divided into 4 equal sections by 5 disks. Thus, up to 4 rats were tested simultaneously on the apparatus, with a rod-rotating speed of 10 r.p.m. The integrity of motor coordination was assessed on the basis of the time the animals kept their balance on the rotating rod up to a maximum of 120 s. The number of falls from the rod was also measured. After a maximum of 6 falls, the test was suspended and the time was recorded.

2.6. Immunofluorescence staining

After the disappearance of the antinociceptive effects (day 6 for morphine-treated NOP(+/+); day 9 for morphine-treated NOP(−/−); day 8 for morphine-treated NOP(+/+)) animals from each treatment group were killed using a guillotine. The L4/L5 segments of the spinal cord were exposed from the lumbar vertebral column via laminectomy and identified by tracing the dorsal roots from their respective dorsal root ganglia (DRG). As previously described by

(Di Cesare Mannelli et al., 2013; Di Cesare Mannelli et al., 2014; Tomassoni et al., 2013), formalin-fixed cryostat sections (7 μm) were washed 3 × phosphate-buffered saline (PBS), 0.3% Triton X-100 for 5 min and then were incubated, at room temperature, for 1 h in blocking solution (PBS, 0.3% Triton X-100, 5% albumin bovine serum; PBST). Slices were incubated overnight at 4°C in PBST containing rabbit primary antisera. The primary antibody used was directed against ionized calcium binding adapter molecule 1 (Iba1; rabbit, 1:1000; Wako, Richmond, VA, USA) for microglial staining or against glial fibrillary acidic protein (GFAP; rabbit, 1:1000; DAKO, Carpinteria, CA, USA) for astrocyte staining. The following day, slides were washed 3 × PBS, 0.3% Triton X-100 for 5 min and then sections were incubated in goat anti-rabbit IgG secondary antibody labeled with Alexa Fluor 568 (1:500; Invitrogen, Carlsbad, USA) and DAPI (4', 6-diamidin-2-fenilindolo; 1:2000; Life Technologies-Thermo scientific, Rockford, IL, USA), a nuclei marker, in PBST at room temperature for 2 h, in the dark. After 3 × PBS 0.3% Triton X-100 wash for 10 min, slices were mounted using ProLong Gold (Life Technologies-Thermo scientific, Rockford, IL, USA) as a mounting medium.

Negative control sections (no exposure to the primary antisera) were processed concurrently with the other sections for all immunohistochemical studies, in order to exclude the presence of nonspecific immunofluorescent staining or cross-immunostaining. Images were acquired by using an Olympus BX63 microscope equipped with an Olympus XM10 camera and coupled to CellSense Dimension Software (Olympus, Milan, Italy).

Quantitative analysis of GFAP and Iba1-positive cells was performed by collecting three independent fields through a 20X 0.40NA objective in the dorsal horn of each rat spinal cord. GFAP and Iba1-positive cells were counted using the "cell counter" plugin of ImageJ (NIH, Bethesda, Maryland, USA).

2.7. Statistical analysis

Behavioral measurements were performed on 8 rats for each treatment carried out in 2 different experimental sets. Results were expressed as means ± S.E.M. and the analysis of variance was performed by two way ANOVA. A Bonferroni's significant difference procedure was used as post-hoc comparison. *P* values of less than 0.05 or 0.01 were considered significant. Data were analyzed using the "Origin 9" software (OriginLab, Northampton, USA).

3. Results

We compared the analgesic effects of morphine (1 nmol/h and 3 nmol/h) induced by a continuous intrathecal (i.t.) infusion in NOP(+/+) and NOP(−/−) rats. Control groups were treated with sterile saline solution. To allow a constant delivery of the substance over days, mini-osmotic pumps connected to catheters were used. Pain threshold was evaluated every day by Paw pressure test, beginning 24 h after pump implant (Fig. 1A and 1B). Animals treated with vehicle displayed a stable pain threshold over the time course of the experiment (10 days). No difference in the basal pain threshold between NOP(+/+) and NOP(−/−) rats was highlighted.

On day 1, morphine elicited a higher antinociceptive effect in NOP(+/+) animals in comparison to the same dose infused in NOP(−/−) rats. A superior effect in NOP(+/+) rats was maintained for 2 and 4 days depending on the treatment with 1 or 3 nmol/h, respectively. In NOP(+/+) rats morphine effectiveness rapidly decreased (dose-dependently) losing significance on day 5 (Fig. 1A and 1B). In NOP(−/−) animals, the antinociceptive effect of morphine, even lower, was more stable and longer lasting for both dosages used (1 and 3 nmol/h). Efficacy was lost on day 9 (Fig. 1A and 1B). Results obtained by the continuous intrathecal infusion of the endogenous peptide N/OFQ (1 and 3 nmol/h) in NOP(+/+) rats are shown in Fig. 2. N/OFQ efficacy was slightly lower than morphine on day 1 but the decrease in the following days was lesser drastic. Moreover, the antinociceptive effect was maintained till day 7. As previously reported, N/OFQ does not induce analgesia in NOP(−/−) animals (Micheli et al., 2015a).

After the disappearance of the antinociceptive effects (day 6 for morphine-treated NOP(+/+) at the dose of 3 nmol/h; day 9 for morphine-treated NOP(−/−) at both dosages (1 and 3 nmol/h); day 8 for nociceptin/OFQ-treated NOP(+/+)) animals from each treatment group were killed for spinal cord collection. The dorsal horn were collected and glial cells analysed by immunohistochemistry at the lumbar level. In wild type animals, morphine, infused at the dose of 3 noml/h, increased the number of both Iba1- and GFAP-positive cells in comparison to control (Fig. 3A and 4A). Differently, the microglial component only was increased by morphine (1 and 3 nmol/h) in NOP(−/−) rats whereas astrocytes were unmodified by treatment (Fig. 3B and 4B).

In Fig. 5, the effect of N/OFQ on glial cells is shown. The intrathecal infusion of N/OFQ (1 nmol/h and 3 nmol/h) did not alter the number of Iba-1 positive cells (Fig. 5B). On the contrary, the density of GFAP-positive cells was significantly increased (Fig. 5B).

4. Discussion

Results showed that genetic deletion of NOP receptor is associated with reduction in the analgesic effect and tolerance development of morphine. Knocking out the NOP receptor, morphine is less efficient in increasing the pain threshold but the effect is longer lasting in comparison with wild type animals. Moreover, immunohistochemistry revealed that lack of NOP receptor is also associated with reduced activation of GFAP-positive cells occurring in morphine tolerant rats indicating a potential role of NOP/OFQ system in regulation of tolerance through modulation of astrocytes-related mechanisms. This hypothesis if further supported by results in wild type rats in which N/OFQ infusion was associated with activation of astrocytes.

The lower analgesic efficacy of morphine in NOP(−/−) rats agree with our previous evidence after an acute intrathecal injections in rats (Micheli et al., 2015a). Conversely, other authors reported similar analgesic effects of morphine in NOP(+/+) and NOP(−/−) in mice (Mamiya et al., 2001; Ueda et al.,1997) suggesting a different role of N/OFQergic signaling in rats and mice. On the other hand, a partial loss of tolerance liability to morphine analgesia was observed also in mice lacking the NOP receptor gene (Ueda et al., 1997).

The development of morphine tolerance and dependence is also associated with changes of the NOP receptor system. For instance, in wild type mice, chronic administration of morphine induced an increase of NOP gene expression in the spinal cord while the administration of J- 113397, a non-peptide N/OFQ antagonist (Bigoni et al., 2000; Ozaki et al., 2000), prevented the development of morphine tolerance (Caputi et al., 2014; Chung et al., 2006; Ueda et al., 2000). Accordingly, the concentration of endogenous N/OFQ increased in the rat ventricular cerebrospinal fluid, in periaqueductal gray and amygdala in a time-dependent manner during the development of morphine tolerance (Yuan et al., 1999).

During the development of morphine tolerance similar increases were observed also for cholecystokinin (Becker et al., 2000; Lucas et al., 1999) and neuropeptide FF (Malin et al., 1990), both of which have been shown to contrast the analgesics effects of opioid in the central nervous system. Hence, engagement of antiopioid peptide systems may represent a key mechanism responsible for the development of tolerance to the analgesic effects of morphine (Harrison et al.,1998).

The demonstration that N/OFQ blocks opioid receptor-mediated stress-induced antinociception (Mogil et al., 1996) and also decreases the antinociceptive effect of morphine and other opioid receptor agonists (Lutfy et al., 2001; Mogil et al., 1996; Morgan et al., 1997; Tian et al., 1997) has led to the suggestion that N/OFQ may act as an anti-opioid peptide in the brain (Mogil et al., 1996). Among several mechanisms proposed to explain morphine tolerance, the activation of glial cells assumes a main role (Cui et al., 2008; Eidson and Murphy, 2013; Ferrini et al., 2013; Song and Zhao, 2001). To the best of our knowledge, there are no findings regarding the relationship between glial cells and N/OFQ in the onset of morphine tolerance.

In the present study we reported the glial response in the dorsal horn of the spinal cord in NOP(+/+) and NOP(−/−) rats tolerant to morphine. We performed the same analysis in NOP(+/+) rats tolerant to N/OFQ. Wild type animals, tolerant to morphine after the intrathecal continuous infusion of the alkaloid at the dose of 3 nmol/h, showed glial activation characterized by an increase in cell density without morphological alterations of both microglia and astrocytes. This results are in accord with previous evidence (Di Cesare Mannelli et al., 2015). On the contrary, in wild type rats tolerant to N/OFQ the number of astrocyte cells is statistically increased whereas microglia density is not altered. Neverthless, when the NOP receptor is deleted morphine treatment selectively activates microglia but not astrocytes. A NOP-mediated morphine-induced astrocyte activation may be hypothesize. On the other hand, fluorocitrate, a nonselective metabolic inhibitor of astrocytes, partially reversed the development of morphine tolerance in rats (Song and Zhao, 2001). Moreover, astrocytes express NOP receptor and cytokine production by astrocytes can be inhibited by N/OFQ, an effect which represents one mechanism by which N/OFQ may reduce pain sensitivity (Fu et al., 2007). Furthermore, N/OFQ mRNA is increased in astrocytes treated with morphine (Takayama and Ueda, 2005), further, various proinflammatory cytokines as those increased during morphine treatment (eg., tumor necrosis factor and interleukin-1) can enhance astrocyte concentrations of immunoreactive N/OFQ and N/OFQ mRNA (Buzas et al., 2002; Mallimo and Kusnecov, 2013). In this view, our data suggest that lacking NOP receptor the ancillary role of N/OFQ in morphine antinociception is lost but losing the NOP-

dependent astrocyte signal tolerance development may be delayed. The topic deserves further studies to clarify the role of N/OFQ signaling in astrocyte and to attempt a NOP modulation for enhancing morphine antinociceptive activity. In particular, an in vivo study with the continuous i.t. infusion of morphine and a NOP receptor antagonist will be performed to highlight the link between the onset of morphine tolerance and the NOP system.

5. Conclusion

In conclusion the antinociceptive effect of morphine seems to be partially dependent from the NOP system since rats lacking NOP receptor are less sensitive to the analgesic effect of this opioid agonist. Interestingly results revealed also that receptor deletion contributes to delay the development of morphine tolerance possibly through mechanisms associated with astrocytes activation.

Acknowledgements

This research was founded by the Italian Ministry of Instruction, University and Research (FIRB grant to CT), the University of Florence (FAR grant to CG), and the University of Ferrara (FAR grant to GC) and grant NIH/NIAAA AA014351 (to RC).

References

Arduin M, Spagnolo B, Calo G, Guerrini R, Carra G, Fischetti C, Trapella C, Marzola E, McDonald J, Lambert DG, Regoli D, Salvadori S, 2007 Synthesis and biological activity of nociceptin/orphanin FQ analogues substituted in position 7 or 11 with Calpha, alpha-dialkylated amino acids. Bioorg. Med. Chem 15(13), 4434–43.17490886

Becker C, Pohl M, Thiébot MH, Collin E, Hamon M, Cesselin F, Benoliel JJ, 2000 Delta-opioid receptor-mediated increase in cortical extracellular levels of cholecystokinin-like material by subchronic morphine in rats. Neuropharmacology. 39(2), 161–71.10670411

Bigoni R, Calo G, Rizzi A, Guerrini R, De Risi C, Hashimoto Y, Hashiba E, Lambert DG, Regoli D, 2000 In vitro characterization of J-113397, a non-peptide nociceptin/orphanin FQ receptor antagonist. Naunyn. Schmiedebergs. Arch. Pharmacol 361(5), 565–8.10832612

Buzas B, Rosenberger J, Kim KW, Cox BM, 2002 Inflammatory mediators increase the expression of nociceptin/orphanin FQ in rat astrocytes in culture. Glia. 39, 237–246.12203390

Caputi FF, Di Benedetto M, Carretta D, Bastias del Carmen Candia S, D'Addario C, Cavina C, Candeletti S, Romualdi P, 2014 Dynorphin/KOP and nociceptin/NOP gene expression and epigenetic changes by cocaine in rat striatum and nucleus accumbens. Prog. Neuropsychopharmacol. Biol. Psychiatry. 49, 36–46.24184686

Chung S, Pohl S, Zeng J, Civelli O, Reinscheid RK, 2006 Endogenous orphanin FQ/nociceptin is involved in the development of morphine tolerance. J. Pharmacol. Exp. Ther 318, 262–267.16595734

Courteix C, Coudoré-Civiale MA, Privat AM, Pélissier T, Eschalier A, Fialip J., 2004 Evidence for an exclusive antinociceptive effect of nociceptin/orphanin FQ, an endogenous ligand for the ORL1 receptor, in two animal models of neuropathic pain. Pain. 110(1–2):236–45.15275773

Cui Y, Liao XX, Liu W, Guo RX, Wu ZZ, Zhao CM, Chen PX, Feng JQ, 2008 A novel role of minocycline: attenuating morphine antinociceptive tolerance by inhibition of p38 MAPK in the activated spinal microglia. Brain. Behav. Immun 22(1), 114–23.17919885

Denlinger CS, Ligibel JA, Are M, Baker KS, Demark-Wahnefried W, Friedman DL, Goldman M, Jones L, King A, Ku GH, Kvale E, Langbaum TS, Leonardi-Warren K, McCabe MS, Melisko M, Montoya JG, Mooney K, Morgan MA, Moslehi JJ, O'Connor T, Overholser L, Paskett ED, Raza M, Syrjala KL, Urba SG, Wakabayashi MT, Zee P, McMillian N, Freedman-Cass D, 2014 National

Comprehensive Cancer Network. Survivorship: pain version 1.2014. J. Natl. Compr. Canc. Netw 12(4), 488–500.24717568

Di Cesare Mannelli L, Pacini A, Bonaccini L, Zanardelli M, Mello T, Ghelardini C, 2013 Morphologic features and glial activation in rat oxaliplatin-dependent neuropathic pain. The Journal of Pain. 14(12),1585–1600.24135431

Di Cesare Mannelli L, Pacini A, Matera C, Zanardelli M, Mello T, De Amici M, Dallanoce C, Ghelardini C, 2014 Involvement of α7 nAChR subtype in rat oxaliplatin-induced neuropathy: effects of selective activation. Neuropharmacology. 79, 37–48.24225197

Di Cesare Mannelli L, Corti F, Micheli L, Zanardelli M, Ghelardini C, 2015. Delay of morphine tolerance by palmitoylethanolamide. Biomed. Res. Int 2015;2015:894732.25874232

Eidson LN, Murphy AZ, 2013 Persistent peripheral inflammation attenuates morphine-induced periaqueductal gray glial cell activation and analgesic tolerance in the male rat. J. Pain 14, 393–404.23395474

Ferrini F, Trang T, Mattioli TA, Laffray S, Del'Guidice T, Lorenzo LE, Castonguay A, Doyon N, Zhang W, Godin AG, Mohr D, Beggs S, Vandal K, Beaulieu JM, Cahill CM, Salter MW, De Koninck Y, 2013 Morphine hyperalgesia gated through microglia-mediated disruption of neuronal Cl^- homeostasis. Nat. Neurosci 16, 183–92.23292683

Fu X, Zhu ZH, Wang YQ, Wu GC, 2007 Regulation of proinflammatory cytokines gene expression by nociceptin/orphanin FQ in the spinal cord and the cultured astrocytes. Neuroscience. 144, 275–285.17069983

Guerrini R, Calo G, Rizzi A, Bianchi C, Lazarus L, Salvadori S, Temussi P, Regoli D, 1997 Address and message sequences for the nociceptin receptor: a structure-activity study of nociceptin-(l-13)-peptide amide. J. Med. Chem 40, 1789–93.9191955

Hameed H, Hameed M, Christo PJ, 2010 The effect of morphine on glial cells as a potential therapeutic target for pharmacological development of analgesic drugs. Curr. Pain. Headache. Rep 14(2), 96–104.20425198

Hao JX, Xu IS, Wiesenfeld-Hallin Z, Xu XJ, 1998 Anti-hyperalgesic and anti-allodynic effects of intrathecal nociceptin/orphanin FQ in rats after spinal cord injury, peripheral nerve injury and inflammation. Pain. 76(3), 385–93.9718257

Harrison LM, Kastin AJ, Zadina JE, 1998 Opiate tolerance and dependence: receptors, G-proteins, and antiopiates. Peptides. 19(9), 1603–30.9864069

Homberg JR, Mul JD, De Wit E, Cuppen E, 2009 Complete knockout of the nociceptin/orphanin FQ receptor in the rat does not induce compensatory changes in mu, delta and kappa opioid receptors. Neuroscience 163, 308–315.19527777

Horvath RJ, DeLeo JA, 2009 Morphine enhances microglial migration through modulation of P2X4 receptor signaling. J Neurosci. 29, 998–1005.19176808

Ji RR, 2010 Targeting microglial purinergic signaling to improve morphine analgesia. Pain. 150, 377–8.20584568

Johansson T, Elfverson M, Zhou Q, Nyberg F, 2010 Allosteric modulation of the NMDA receptor by neurosteroids in rat brain and the impact of long term morphine administration. Biochem. Biophys. Res. Commun 401, 504–8.20869946

Leighton GE, Rodriguez RE, Hill RG, Hughes J, 1988 k-opioid agonist produce antinociception after i.v. and i.c.v. but not intrathecal administration in the rat. Br. J. Pharmacol 93, 553–560.2836010

Lucas GA, Hoffmann O, Alster P, Wiesenfeld-Hallin Z, 1999 Extracellular cholecystokinin levels in the rat spinal cord following chronic morphine exposure: an in vivo microdialysis study. Brain Res. 821(1), 79–86.10064790

Lutfy K, Hossain SM, Khaliq I, Maidment NT, 2001 Orphanin FQ/nociceptin attenuates the development of morphine tolerance in rats. Br. J. Pharmacol 134, 529–534.11588106

Malin DH, Lake JR, Hammond MV, Fowler DE, Rogillio RB, Brown SL, Sims JL, Leecraft BM, Yang HY, 1990 FMRF-NH2-like mammalian octapeptide: possible role in opiate dependence and abstinence. Peptides. 11(5), 969–72.2284204

Mallimo EM, Kusnecov AW, 2013 The role of orphanin FQ/nociceptin in neuroplasticity: relationship to stress, anxiety and neuroinflammation. Front. Cell. Neurosci 7, 173.24155687

Mamiya T, Noda Y, Ren X, Nagai T, Takeshima H, Ukai M, Nabeshima T, 2001 Morphine tolerance and dependence in the nociceptin receptor knockout mice. J Neural Transm. 108, 1349–61.11810400

McGrath JC, Lilley E, 2015 Implementing guidelines on reporting research using animals (ARRIVE etc.): new requirements for publication in BJP. Br. J. Pharmacol 172(13), 3189–3193.25964986

Meunier JC, Mollereau C, Toll L, Suaudeau C, Moisand C, Alvinerie P, Butour JL, Guillemot JC, Ferrara P, Monsarrat B, 1995 Isolation and structure of the endogenous agonist of opioid receptor-like ORL1 receptor. Nature. 377, 532–535.7566152

Micheli L, Di Cesare Mannelli L, Guerrini R, Trapella C, Zanardelli M, Ciccocioppo R, Rizzi A, Ghelardini C, Calo G, 2015a Acute and subchronic antinociceptive effects of nociceptin/orphanin FQ receptor agonists infused by intrathecal route in rats. Eur. J. Pharmacol 754, 73–81.25704616

Micheli L, Di Cesare Mannelli L, Rizzi A, Guerrini R, Trapella C, Calo G, Ghelardini C, 2015b Intrathecal administration of nociceptin/orphanin FQ receptor agonists in rats: A strategy to relieve chemotherapy-induced neuropathic hypersensitivity. Eur. J. Pharmacol 766, 155–62.26450087

Mika J, Wawrzczak-Bargiela A, Osikowicz M, Makuch W, Przewlocka B, 2009 Attenuation of morphine tolerance by minocycline and pentoxifylline in naive and neuropathic mice. Brain. Behav. Immun 23, 75–84.18684397

Mika J, 2008 Modulation of microglia can attenuate neuropathic pain symptoms and enhance morphine effectiveness. Pharmacol. Rep 60, 297–307.18622054

Mogil JS, Grisel JE, Reinscheid RK, Civelli O, Belknap JK, Grandy DK, 1996 Orphanin FQ is a functional anti-opioid peptide. Neuroscience. 75(2), 333–7.8930999

Morgan MM, Grisel JE, Robbins CS, Grandy DK, 1997 Antinociception mediated by the periaqueductal gray is attenuated by orphanin FQ. Neuroreport. 8(16), 3431–4.9427301

Ozaki S, Kawamoto H, Itoh Y, Miyaji M, Iwasawa Y, Ohta H, 2000 A potent and highly selective nonpeptidyl nociceptin/orphanin FQ receptor (ORL1) antagonist: J-113397. Eur. J. Pharmacol 387(3), R17–8.10650183

Raghavendra V, Rutkowski MD, DeLeo JA, 2002 The role of spinal neuroimmune activation in morphine tolerance/hyperalgesia in neuropathic and sham-operated rats. J. Neurosci 22, 9980–9989.12427855

Reinscheid RK, Nothacker HP, Bourson A, Ardati A, Henningsen RA, Bunzow JR, Grandy DK, Langen H, Monsma FJ, Civelli O, 1995 Orphanin FQ: a neuropeptide that activates an opioidlike G protein-coupled receptor. Science. 270, 792–794.7481766

Schröder W, Lambert DG, Ko MC, Koch T, 2014 Functional plasticity of the N/OFQ-NOP receptor system determines analgesic properties of NOP receptor agonists. Br. J. Pharmacol 171(16), 3777–800.24762001

Self DW, Nestler EJ, 1995 Molecular mechanisms of drug reinforcement and addiction. Annu. Rev. Neurosci 18, 463–495.7605071

Song P, Zhao ZQ, 2001 The involvement of glial cells in the development of morphine tolerance. Neurosci. Res 39, 281–286.11248367

Takayama N, Ueda H, 2005 Morphine-induced overexpression of prepro-nociceptin/orphanin FQ in cultured astrocytes. Peptides. 26, 2513–2517.15990199

Thorn DA, Zhang Y, Li JX, 2016 Effects of the imidazoline I2 receptor agonist 2-BFI on the development of tolerance and behavioral/physical dependence to morphine in rats. Br. J. Pharmacol 173(8), 163–72.

Tian JH, Xu W, Fang Y, Mogil JS, Grisel JE, Grandy DK, Han JS, 1997 Bidirectional modulatory effect of orphanin FQ on morphine-induced analgesia: antagonism in brain and potentiation in spinal cord of the rat. Br. J. Pharmacol 120(4), 676–80.9051307

Tomassoni D, Amenta F, Amantini C, Farfariello V, Di Cesare Mannelli L, Nwanko IE, Marini C, Tayebati SK, 2013 Brain activity of Thioctic acid enantiomers: In vitro and in vivo studies in an animal model of cerebrovascular injury. International Journal of Molecular Sciences. 14(3), 4580–4595.23443159

Ueda H, Inoue M, Takeshima H, Iwasawa Y, 2000 Enhanced spinal nociceptin receptor expression develops morphine tolerance and dependence. J. Neurosci 20(20), 7640–7.11027224

Ueda H, Yamaguchi T, Tokuyama S, Inoue M, Nishi M, Takeshima H, 1997 Partial loss of tolerance liability to morphine analgesia in mice lacking the nociceptin receptor gene. Neurosci. Lett 237, 136–8.9453234

Watkins LR, Hutchinson MR, Johnston IN, Maier SF, 2005 Glia: Novel counter-regulators of opioid analgesia. Trends. Neurosci 28, 661–669.16246435

Xu XJ, Hao JX, Wiesenfeld-Hallin Z, 1996 Nociceptin or antinociceptin: potent spinal antinociceptive effect of orphanin FQ/nociceptin in the rat. Neuroreport. 7(13), 2092–4.8930965

Yaksh TL, Rudy TA, 1976 Chronic catheterization of the spinal subarachnoid space. Physiol Behav. 17, 1031–6.14677603

Yamamoto T, Nozaki-Taguchi N, Kimura S, 1997 Effects of intrathecally administered nociceptin, an opioid receptor-like1 (ORL1) receptor agonist, on the thermal hyperalgesia induced by carageenan injection into the rat paw. Brain. Res 754(1–2), 329–32.9134994

Yuan L, Han Z, Chang JK, Han JS, 1999 Accelerated release and production of orphanin FQ in brain of chronic morphine tolerant rats. Brain. Res 826(2), 330–4.10224316

Yuill MB, Zee ML, Marcus D, Morgan DJ, 2016 Tolerance to the antinociceptive and hypothermic effects of morphine is mediated by multiple isoforms of c-Jun N-terminal kinase. Neuroreport. 27(6), 392–6.26914092

Zeilhofer HU, Calô G, 2003 Nociceptin/orphanin FQ and its receptor—potential targets for pain therapy? J. Pharmacol. Exp. Ther 306, 423–429.12721334

Zhang X, Bao L, Li S, 2015 Opioid receptor trafficking and interaction in nociceptors. Br. J. Pharmacol 172, 364–74.24611685

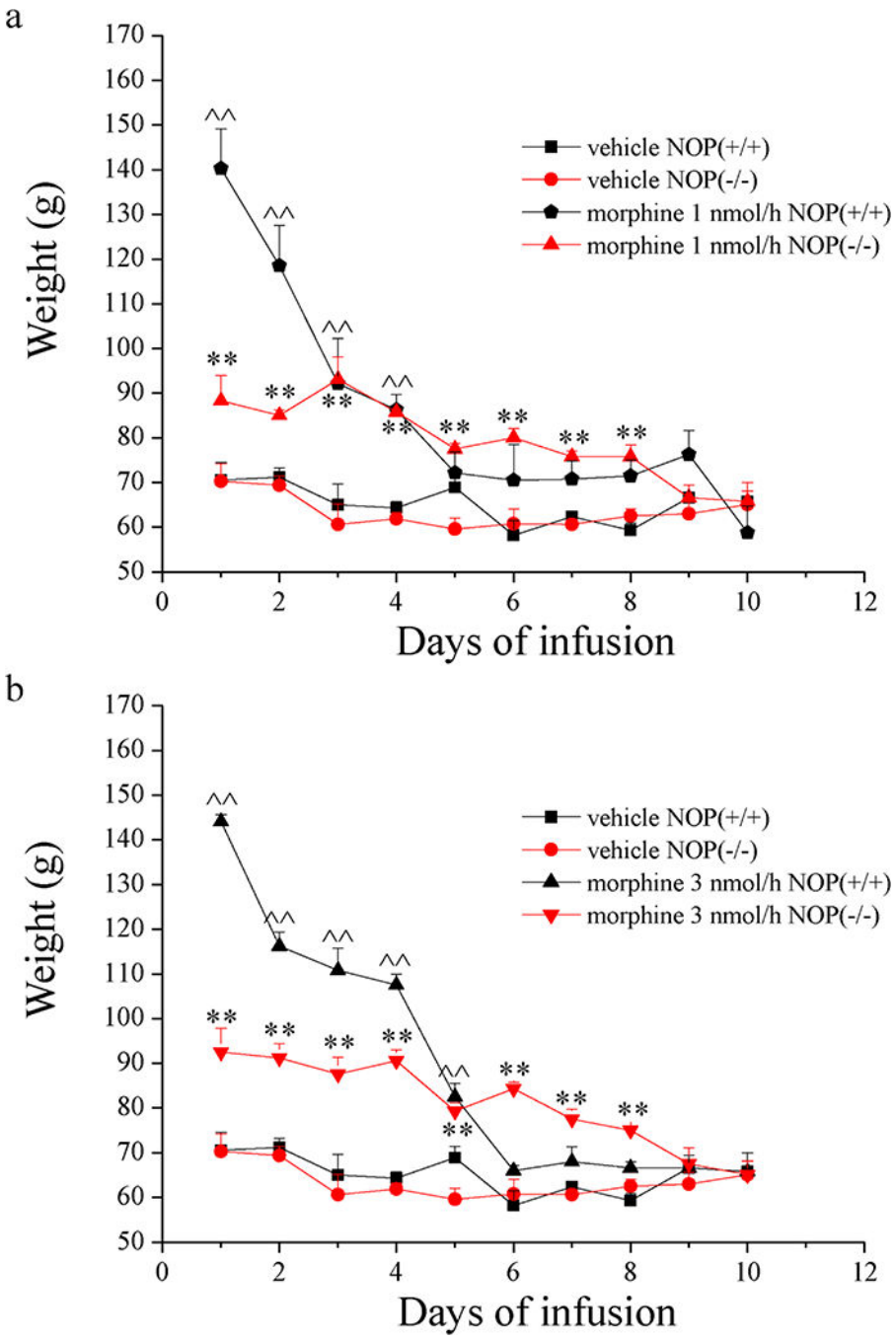

Fig. 1.

Analgesic effect induced by continuous intrathecal infusion of morphine in NOP(+/+) and in NOP(−/−) rats. a) The opioid compound was dissolved in sterile saline solution and infused in two different groups of animals both at the dose of 1 nmol/h with osmotic pumps until day 10. Vehicle NOP(+/+) and vehicle NOP(−/−) groups were infused with sterile saline solution. The nociceptive threshold was evaluated every day by Paw pressure test, starting on the 1st day of treatment. b) The opioid compound was dissolved in sterile saline solution and infused in two different groups of animals, both at the dose of 3 nmol/h with osmotic pumps

until day 10. Vehicle NOP(+/+) and vehicle NOP(−/−) groups were infused with sterile saline solution. The nociceptive threshold was evaluated every day by Paw pressure test, starting on the 1st day of treatment. Each value represents the mean of 8 rats performed in 2 different experimental sets. Data are shown as mean ± S.E.M; ^^P<0.01 vs vehicle NOP(+/+) infused rats; **P<0.01 vs vehicle NOP (−/−) infused rats.

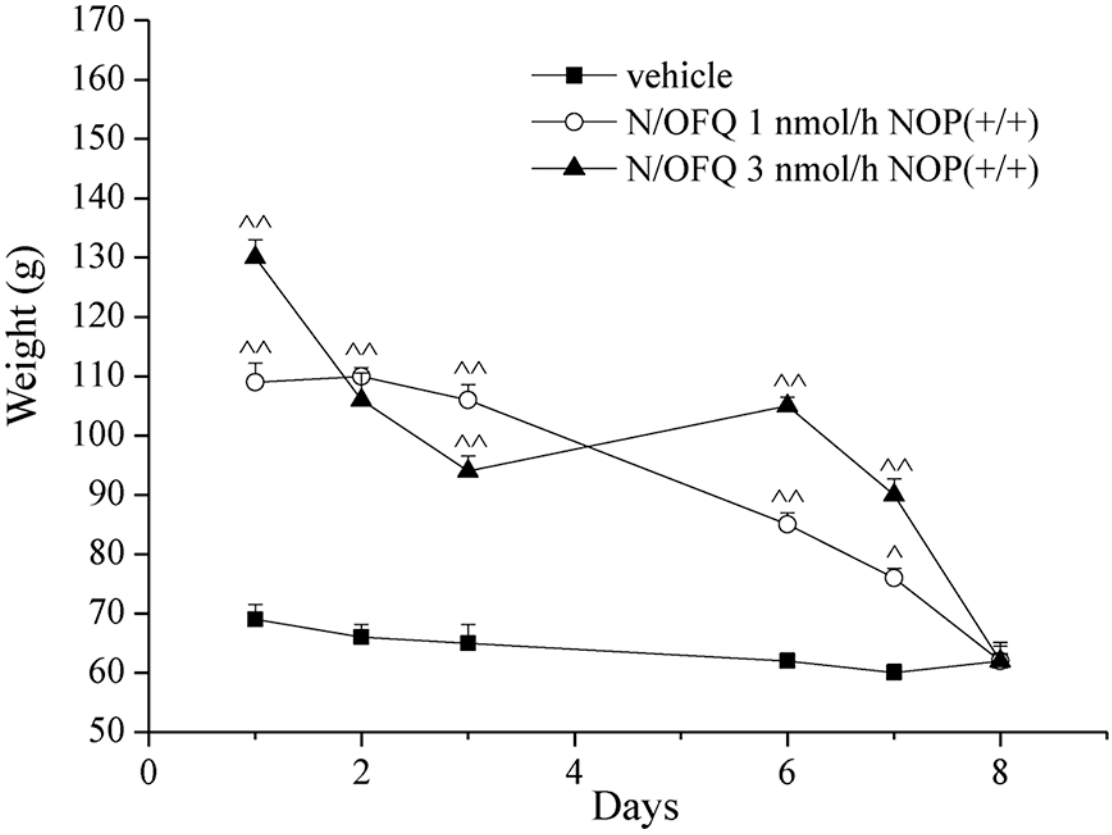

Fig. 2.

Effect induced by continuous intrathecal infusion of N/OFQ in NOP (+/+) rats. The compound was dissolved in sterile saline solution and administered in two different groups of animals at the dosages of 1 and 3 nmol/h with osmotic pumps until day 8. Vehicle NOP (+/+) group was infused with sterile saline solution. The nociceptive threshold was evaluated by Paw pressure test, starting the 1st day of treatment. Each value represents the mean of 8 rats performed in 2 different experimental sets. Data are shown as mean ± S.E.M; ^P<0.05 and ^^P<0.01 vs vehicle NOP (+/+) infused rats.

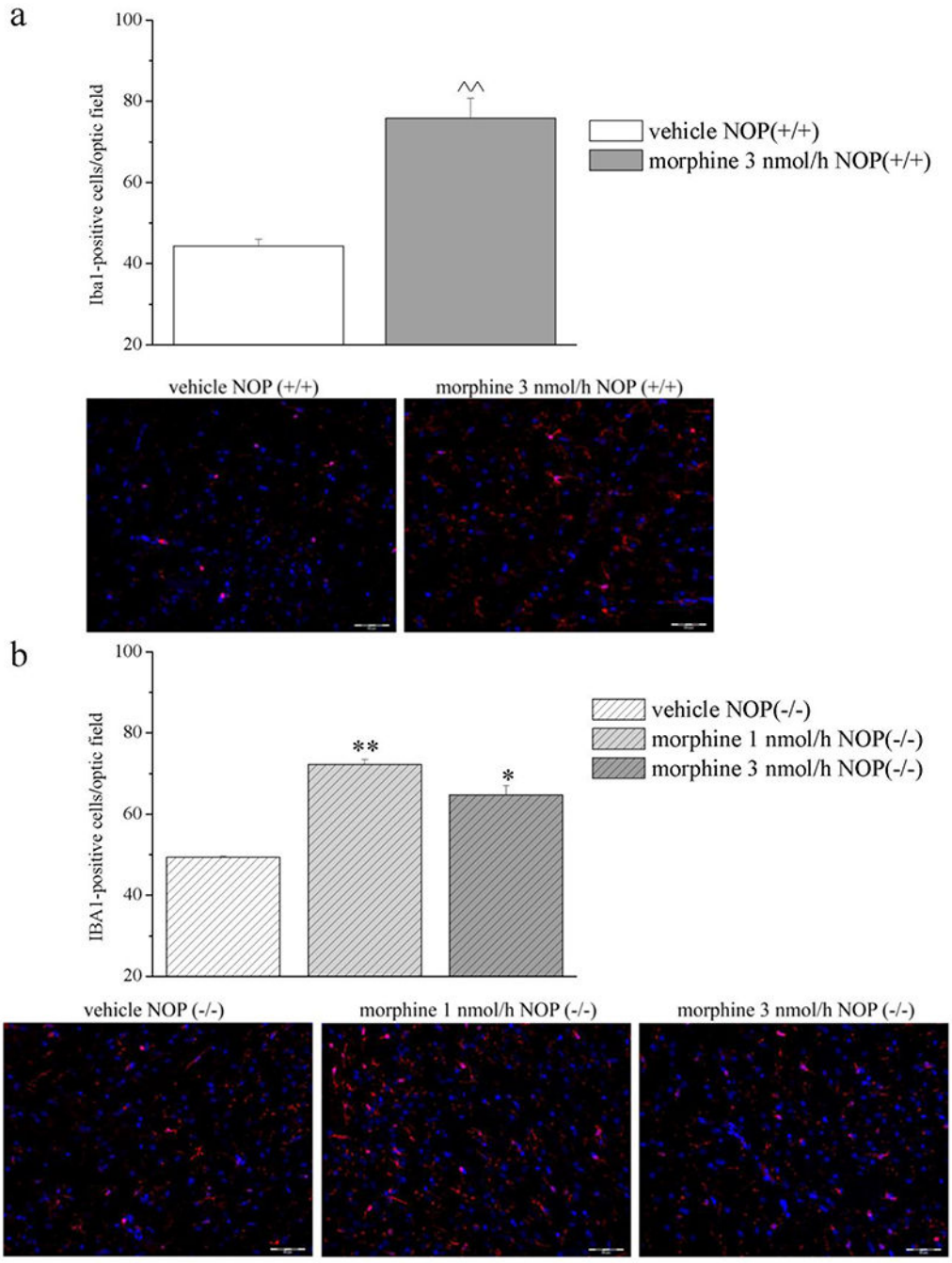

Fig. 3.

Iba1-positive cell density in the dorsal horn of the spinal cord of morphine NOP(+/+) and NOP(−/−) tolerant rats. a) Morphine 3 nmol/h was continuously intrathecal infused in NOP(+/+) rats until the onset of tolerance and immunohistochemical analysis was performed on day 6. Representative images of merged Iba1-labeled microglia cells (red), plus DAPI-labeled cell nuclei (blue), scale bar: 50 μm. b) Morphine 1 nmol/h and 3 nmol/h was continuously intrathecal infused in NOP(−/−) rats until the onset of tolerance and immunohistochemical analysis was performed on day 9. Representative images of merged

Iba1-labeled microglia cells (red), plus DAPI-labeled cell nuclei (blue), scale bar: 50 μm. Each value represents the mean of 4 rats performed in 2 different experimental sets. Data are shown as mean ± S.E.M; ^^P<0.01 vs vehicle NOP(+/+) infused rats. *P<0.05 and **P<0.01 vs vehicle NOP(−/−) infused rats.

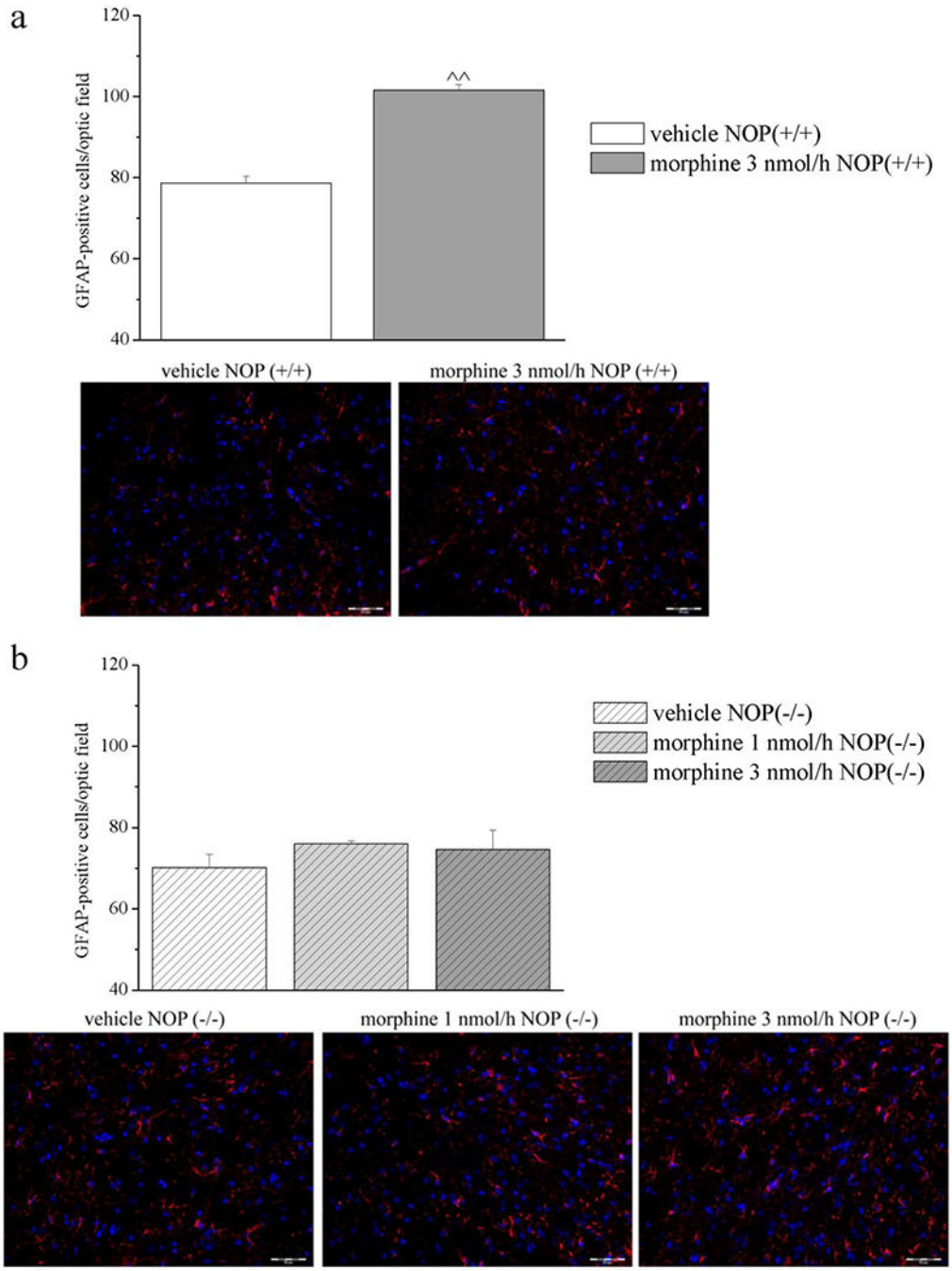

Fig. 4.

GFAP-positive cell density in the dorsal horn of the spinal cord of morphine NOP(+/+) and NOP(−/−) tolerant rats. a) Morphine 3 nmol/h was continuously intrathecal infused in NOP(+/+) rats until the onset of tolerance and immunohistochemical analysis was performed on day 6. Representative images of merged GFAP-labeled astrocytes cells (red), plus DAPI-labeled cell nuclei (blue), scale bar: 50 μm. b) Morphine 1 nmol/h and 3 nmol/h was continuously intrathecal infused in NOP(−/−) rats until the onset of tolerance and immunohistochemical analysis was performed on day 9. Representative images of merged

GFAP-labeled astrocytes cells (red), plus DAPI-labeled cell nuclei (blue), scale bar: 50 μm. Each value represents the mean of 4 rats performed in 2 different experimental sets. Data are shown as mean ± S.E.M; ^^P<0.01 vs vehicle NOP(+/+) infused rats.

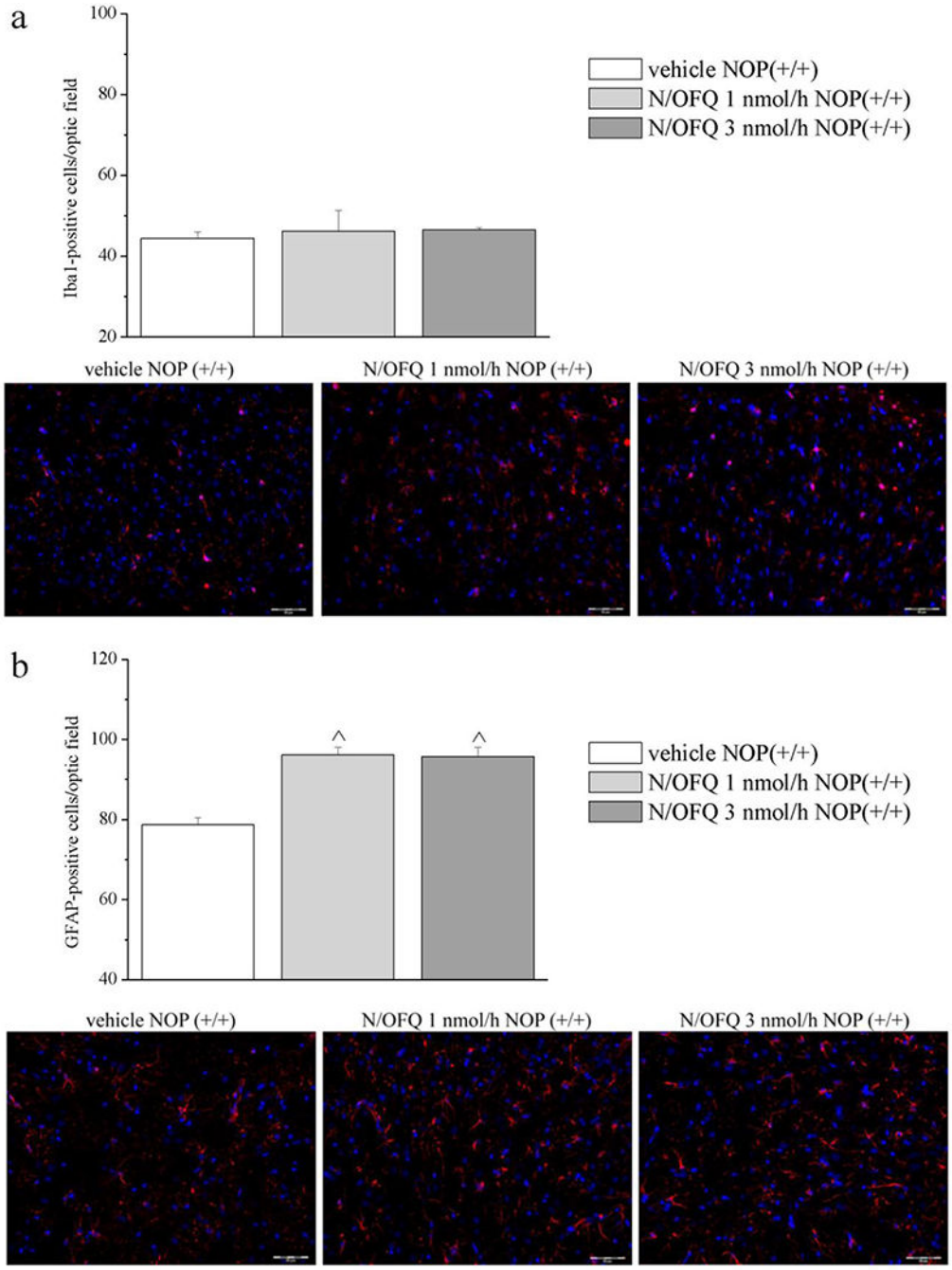

Fig. 5.

Iba1-positive cell density and GFAP-positive cell density in the dorsal horn of the spinal cord of N/OFQ NOP(+/+) tolerant rats. a) N/OFQ 1 nmol/h and 3 nmol/h was continuously intrathecal infused in NOP(+/+) rats until the onset of tolerance and immunoistochemical analysis were performed on day 8. Representative images of merged Iba1-labeled microglia cells (red), plus DAPI-labeled cell nuclei (blue), scale bar: 50 μm. b) N/OFQ 1 nmol/h and 3 nmol/h was continuously intrathecal infused in NOP(+/+) rats until the onset of tolerance and immunoistochemical analysis were performed on day 4. Representative images of

merged GFAP-labeled astrocytes cells (red), plus DAPI-labeled cell nuclei (blue), scale bar: 50 μm. Each value represents the mean of 8 rats performed in 2 different experimental sets. Data are shown as mean ± S.E.M; ^P<0.05 vs vehicle NOP(+/+) infused rats.