OPIOID-INDUCED HYPERALGESIA: CELLULAR AND MOLECULAR MECHANISMS

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Abbreviations: 5-HT, serotonin ; Ab, antibody ; β 2-AR, β 2-adrenergic receptor ; β -FNA, β -funaltrexamine ; BDNF, brain derived neurotrophic factor ; CaMKII, calmodulin kinase 2 ; CCK, cholecystokinin ; CD11b, cluster of differentiation molecule 11b ; CNS ,central nervous system ; COX2, cyclooxygenase 2 ; CTOP, D-Phe-Cys-Tyr-D-Orn-Thr-Pen-Thr-NH₂ ; Ctrls, controls ; CxCR, chemokine receptor ; CxCL, chemokine ; DAMGO, Tyr-D-Ala-Gly-NMe-Phe-Gly-ol ; DOR, delta-opioid receptor ; DRG, dorsal root ganglia ; EAAT3, excitatory amino acid transporter-3 ; EPSC, excitatory post synaptic potential ; ERK, extracellular signal-regulated kinase ; GABA, gamma aminobutyric acid ; GFAP, glial fibrillary acidic protein ; GLAST, glutamate aspartate transporter ; GLT-1, glutamate transporter 1; GluN2B, NMDA receptor subunit 2B ; GPCR, G protein coupled receptor ; HDAC, histone deacetylase ; icv., intracerebroventricular; IL-1 β , interleukin-1 β ; IL, interleukin ; it., intrathecal; JNK, c-Jun N-terminal kinase ; KCC2, K⁺/Cl⁻ cotransporter ; LPS, lipopolysaccharide ; LTP, long-term potentiation ; M3G, morphine-3-glucuronide ; M6G, morphine-6-glucuronide ; MCP-1, monocyte chemoattractant protein 1; MOR, mu-opioid receptor ; mTOR, mammalian target of rapamycin ; NF- κ B, nuclear factor κ B ; NK-1, neurokinin 1 ; NMDA, N-methyl-D-aspartic acid ; NPFF, neuropeptide FF ; NOP, nociceptin ; OIH , opioid-induced hyperalgesia ; OFQ, orphanin FQ ; P2X4R, purinergic receptor P2X; P65, protein 65 ; PAG, periaqueductal gray matter ; PI3K, phosphoinositide 3 kinase ; PKC, protein kinase C ; PKA, protein kinase A ; PLC, phospholipase C ; PNS, peripheral nervous system ; PTX, pertussis toxin ; RVM, rostral ventral medulla ; S1P, sphingosine-1-phosphate ; SAHA, suberoylanilide hydroxamic acid ; sc., subcutaneous; SDF-1, stromal derived factor 1 ; SNL, sciatic nerve ligation; TLR4, toll like receptor 4 ; TM, transmembrane domain ; TNF- α , tumor necrosis factor- α ; TrkB, tyrosine kinase B ; TRPM8, transient receptor potential member 8 ; TRPV1, transient receptor potential vanilloid 1 ;

Abstract

Opioids produce strong analgesia but their use is limited by a paradoxical hypersensitivity named opioid-induced hyperalgesia (OIH) that may be associated to analgesic tolerance. In the last decades, a significant number of preclinical studies have investigated the factors that modulate OIH development as well as the cellular and molecular mechanisms underlying OIH. Several factors have been shown to influence OIH including the genetic background and sex differences of experimental animals as well as the opioid regimen. Mu opioid receptor (MOR) variants and interactions of MOR with different proteins were shown important. Furthermore, at the cellular level, both neurons and glia play a major role in OIH development. Several neuronal processes contribute to OIH, like activation of neuroexcitatory mechanisms, long-term potentiation (LTP) and descending pain facilitation. Increased nociception is also mediated by neuroinflammation induced by the activation of microglia and astrocytes. Neurons and glial cells exert synergistic effects, which contribute to OIH. The molecular actors identified include the Toll-like receptor 4 and the anti-opioid systems as well as some other excitatory molecules, receptors, channels, chemokines, pro-inflammatory cytokines or lipids. This review summarizes the intracellular and intercellular pathways involved in OIH and highlights some mechanisms that may be challenged to limit OIH in the future.

Keywords: Opioid-induced hyperalgesia; morphine; opiates; mu-opioid receptor; neuroinflammation; pain

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INTRODUCTION

Chronic pain is a major health issue in our society that clearly impacts quality of life. 30-40% of the population in United States, and equivalent in Europe, suffer from chronic pain and its total cost has been estimated at 560-635 billion dollars annually (see Renfrey et al., 2003, Breivik et al., 2006, Johannes et al., 2010, Breivik et al., 2013). Even if research progresses and new targets appear for treating acute and chronic pain, opioids still represent the gold standard analgesics. However, opioid treatments induce several adverse side effects among which analgesic tolerance and opioid-induced hyperalgesia are of major importance. Analgesic tolerance corresponds to a progressive decrease of analgesia produced by a given dose of opiate upon chronic administration, resulting in the need to increase the opiate dosage in order to maintain the initial analgesic effect. Opioid-induced hyperalgesia (OIH) refers usually to the development of hypersensitivity to painful stimuli observed upon chronic opiates administration. Therefore, the treatment supposed to relief the patient's pain will, in an opposite way, generate a more exacerbated pain sensation. In clinic, these two phenomena are conflicting since the development of analgesic tolerance will lead to increase opiate dosage, which in turn will induce an enhancement of OIH. In this review we will focus our discussion on OIH, observed in both human and animal models (see Angst and Clark, 2006, see Silverman, 2009, see Crofford, 2010, Chen et al., 2014, Johnson et al., 2014, Hayhurst and Durieux, 2016). OIH is well established in humans in different types of pain such as post-surgical pain (Fletcher and Martinez, 2014), low back pain (Chu et al., 2006), cancer pain (Carullo et al., 2015), musculoskeletal pain (Crofford, 2010), but also in healthy volunteers (see Fishbain et al., 2009, see Ruscheweyh et al., 2011, Mauermann et al., 2016) and opioid addicts (Compton et al., 2001). Hence, the clinician faces a dilemma to treat or not chronic pain with opioids, known to have a beneficial effect at first, with a risk of noxious consequences.

In clinic, diverse strategies are used in order to reduce OIH. First, the practitioner can change the type of opiate used, i.e. opiate switching, in order to limit the side effects due to a specific opiate (see Mercadante and Arcuri, 2005, see Mercadante and Bruera, 2016). This alternative is used in 21 to 44% of patients with cancer pain and considered successful when the pain felt is decreased of at least 33% (Mercadante et al., 2009). Another possibility is to block the glutamatergic system, which is known to be implicated in OIH (see Lee et al., 2011). Ketamine, a classical antagonist of a glutamate receptor, have been shown to be effective in preventing the development of OIH (see Ramasubbu and Gupta, 2011), however its chronic administration also produce adverse side effects (Cvrcek, 2008). Finally, usage of non-opioid medication including pregabalin, propofol or COX2 inhibitors can be efficient to prevent OIH (see Lee et al., 2011), but clinical data are limited.

Although well characterized in humans, OIH has been less studied than analgesic tolerance in preclinical models. Most opiate molecules commonly used in clinic have been shown to induce OIH, such as morphine, fentanyl and remifentanyl (see Angst and Clark, 2006). Moreover, heroin also leads to OIH, as shown in heroin-dependent users before and under methadone or buprenorphine maintenance therapy (Compton et al., 2012). However, the kinetic of action, metabolism and potency of the different opiate molecules can greatly influence the strength and rapidity of OIH

development. Morphine, fentanyl and methadone are strong agonists of the mu-opioid receptor, when codeine and oxycodone for example are considered as moderate agonists. In addition, morphine has a low intrinsic efficacy whereas fentanyl has a high intrinsic efficacy (Duttaroy and Yoburn, 1995). Therefore, switch from moderate to strong agonists and from low to high intrinsic activity agonists may diminish the adverse side effects and increase the potency of the analgesia.

As in humans, diverse opiates can produce OIH in preclinical models, including fentanyl (Celerier et al., 2000), remifentanyl (Aguado et al., 2015), morphine (Mao et al., 1994 and references cited in table 2), and codeine (Johnson et al., 2014). In addition, OIH can also be induced by DAMGO, a synthetic opioid peptide selectively activating mu-opioid receptor (Drdla et al., 2009). Different animal models are available to study OIH. For example, a single fentanyl injection will induce OIH during two to three days (Elhabazi et al., 2012, Laboureyras et al., 2014), repeated morphine administrations will induce long lasting OIH with a return to basal nociceptive threshold around 10 days after the last administration (Richebe et al., 2005, Elhabazi et al., 2014), and after 6 days of daily injections of 1 μ g of DAMGO in the paw, mice develop hypersensitivity to mechanical stimuli (Rowan et al., 2014a). These models enable to identify the molecular mechanisms underlying OIH as well as to find novel molecules that block its development.

Although OIH is not yet completely understood, different mechanisms have been identified for this adaptive process including sensitization of primary afferent neurons and enhanced release of glutamate by these primary afferents, hyperexcitability of second order neurons to excitatory neurotransmitters, and up-regulation of nociceptive neuromodulators by descending pain controls (see Chu et al., 2008, see Lee et al., 2011). More recently, not only neurons but also glial cells have been shown to participate, being activated concomitantly with OIH and involving diverse targets and receptors on both microglia and astrocytes (see Hutchinson et al., 2011). These mechanisms are summarized in figure 1.

In this review we will focus non-exhaustively on the different factors that can influence OIH as well as on the newest mechanisms discovered over the last decade, in term of cellular and molecular implication.

FACTORS INFLUENCING OIH

The role of mu-opioid receptor

Mu receptor variants

The mu-opioid receptor (MOR) is the main target of opiates used in clinic (Matthes et al., 1996). MOR activation leads to analgesia, but also to adverse effects such as hyperalgesia (see Lee et al., 2011). It has been proposed that these two opposite effects are due to specific isoforms of the receptor. MOR is a seven transmembrane domains (7TM) G Protein Coupled Receptor (GPCR) encoded by the OPRM1 gene in human, named *Oprm1* in mouse. Since its initial molecular characterization as a 7TM receptor encoded by 4 exons, several variants have been discovered (see Law et al., 2013, see Pasternak and Pan, 2013, Convertino et al., 2015). In humans, alternative

splicing mechanisms result in 7TM, 6TM and 1TM receptor variants. The role of these variants, their relative expression levels as well as distribution in the human nervous system, have not been extensively explored yet. However, 6TM MOR is studied because of its peculiar properties on nociception. The 6TM isoform lacks residues in the N-terminal region corresponding to the extracellular tail and first transmembrane domain. Activation of the 6TM receptor induces excitatory cellular effects by activating Gs (Gris et al., 2010), while activation of the 7TM receptor inhibits neurons because of Gi activation. Moreover specific silencing of MOR-1K variant in mice led to a decrease of morphine-induced hyperalgesia compared to wild-type animals suggesting that the Gs coupling of this 6TM MOR isoform could be important for the development of OIH (Convertino et al., 2015, Oladosu et al., 2015). From these results, it was hypothesized that specific MOR isoforms could be involved in OIH while the classical 7TM receptor would be responsible for analgesia. Interestingly, a β 2-adrenergic (β 2-AR) antagonist reversed hyperalgesia induced either by chronic morphine or by a 6TM-MOR selective agonist in mice suggesting that the β 2-AR antagonist disrupts the 6TM-MOR- β 2-AR heterodimer signalling producing OIH (Samoshkin et al., 2015). These new findings may be put in relation to previous genetic analyses that identified a haplotype block containing the β 2-AR that was associated to OIH (Liang et al., 2006).

Interestingly, patients display differential reactions to morphine in relation to their genetic variability (Lotsch and Geisslinger, 2010). For example, patients homozygous for OPRM1 (μ receptor) gene A118G show less analgesia to morphine (see De Gregori et al., 2012). These findings suggest that a case-by-case analysis and understanding of OIH in patients may help solving this problem.

Finally, MOR is known to be present in the central and peripheral nervous systems (CNS, PNS). The analgesic effects of MOR activation on either CNS or PNS have been explored, and the analysis of mice with a conditional MOR deletion in peripheral Nav1.8+ sensory neurons has revealed the involvement of these receptors in opioid-induced analgesia (Weibel et al., 2013, see Stein, 2016). In the future, the investigation of particular opioid receptor populations or splice variants in OIH may be determined by using similar genetic approaches and/or injection of opioids in specific areas.

Functional interactions of MOR with other GPCRs

In case of tolerance to analgesia, desensitization of MOR has been observed (see Dang and Christie, 2012, see Williams et al., 2013). Desensitization corresponds to molecular changes in receptor signalling following repeated exposure to agonists, resulting in a loss of effectiveness or a biased answer to agonist binding (Dang and Christie, 2012, Williams et al., 2013). Two types of desensitization can be distinguished, homologous desensitization, i.e. the activation of a receptor type provokes its own desensitization (i.e MOR activation desensitizes MOR), and heterologous desensitization when the activation of another receptor desensitizes MOR. Heterologous desensitization has been described between MOR and chemokine receptors above all (see Melik Parsadaniantz et al., 2015). For instance, Heinisch and collaborators studied MOR-CxCr4 and

MOR-Cx3Cr1 interactions in the periaqueductal gray matter (PAG), and recorded heterologous desensitization of these receptors by whole cell patch clamp electrophysiology (Heinisch et al., 2011). Neurons treated with the respective CxCr4 or Cx3Cr1 agonists CXCL12 or CX3CL1 displayed decreased morphine-induced electrophysiological activity *in vitro*. In addition, intraspinal CXCL12 administration diminished morphine analgesia, while a CXCR4 antagonist potentiated morphine analgesia, revealing a role for a MOR–CxCr4 crosstalk in the spinal cord *in vivo* (Rivat et al., 2014). From a molecular point of view, chemokine receptors activate protein kinase C (PKC), which phosphorylates the intracytoplasmic tail of MOR (see Williams et al., 2013). Different mechanisms occur for homologous and heterologous MOR phosphorylation on different serine or threonine residues, which have been reviewed recently (see Mann et al., 2015). MOR phosphorylation uncouples MOR from Gi. Then, G protein receptor kinase (GRK) and arrestin are recruited to MOR, leading to MOR internalization and therefore to analgesic tolerance (see Williams et al., 2013). Chemokine receptor activation can also lead to activation of the ERK pathway that decreases nociceptive thresholds and hence induces hyperalgesia (see Melik Parsadaniantz et al., 2015).

MOR heterodimers and OIH

As a member of the family of GPCRs, MOR can interact with other GPCRs and form heterodimers (Gomes et al., 2013). High hopes are awarded to the comprehension of opioid receptors heterodimers function. Delta-opioid receptor (DOR) may be involved in OIH, and contrasting results have been obtained on the role of DOR in analgesic tolerance. Indeed, pretreatment with DOR antagonists reversed analgesic tolerance (Beaudry et al., 2015), while tolerance was lost or maintained in DOR knockout mice (Zhu et al., 1999, Scherrer et al., 2009). Heterodimers of MOR and DOR receptors have been characterized, and novel ligands have been developed with mu-agonist and delta-antagonist properties that produce longer analgesia (Harland et al., 2015) or analgesia without development of tolerance (Mosberg et al., 2014). The interaction between MOR and DOR remains a field of investigation and the novel pharmacology of MOR-DOR heterodimers has been reviewed recently (see Ong and Cahill, 2014, see Fujita et al., 2015). Also, a synergy between MOR and $\alpha 2$ -adrenoreceptors was evidenced for analgesia and analgesic tolerance (Milne et al., 2008, Chabot-Dore et al., 2015). MOR heterodimers with other GPCRs have been mainly described in cellular assays. However, some of them have been evidenced *ex vivo* by either physical association or co-localization in neurons, as recently reviewed (see Massotte, 2015). These findings are of great interest because they open a new field of development for analgesic drugs with potency comparable to MOR agonists, but devoid of tolerance and OIH induced by classical opiates. However, the influence of MOR heterodimers on OIH and the capacity of new ligands targeting these heterodimers to limit the development of OIH remain mostly unknown.

G protein activation

Another mechanism underlying OIH consists in a potential shift of MOR coupling from Gi to Gs. Analgesic properties of MOR are initiated by Gi activation in neurons, resulting in neuron hyperpolarization and inhibition of nociception. This classical MOR Gi-mediated pathway seems to be counteracted in OIH by other signalling pathways. Under spinal nerve ligation (SNL)-induced neuropathic condition, MOR coupling to Gs increases in the spinal cord, repeated oxycodone augments SNL-induced MOR-Gs coupling and ultra-low dose naltrexone attenuates this Gs coupling, as well as oxycodone-evoked OIH (Largent-Milnes et al., 2008). The role of Gs has also been evidenced by the use of the Gi protein signalling-disrupter Pertussis toxin (PTX). PTX treatment induces ADP-ribosylation of Gi proteins and therefore prevents their interaction with GPCRs, leading to increased coupling of MOR to Gs protein. Hyperalgesia induced by acute morphine is correlated with an increase of spinal MOR coupling to Gs, and is reversed by ultra-low dose of naloxone (Tsai et al., 2009). In contrast, Gi was found involved rather than Gs in the periaqueductal gray of mice by using an OIH paradigm induced by ultra-low dose of morphine (Bianchi et al., 2009). Further, $G\alpha_{i1-3}$ and $G\alpha_o1$ are necessary in this OIH paradigm, as shown by an antisense oligodeoxynucleotide approach (Bianchi et al., 2011). Altogether these data indicate that Gi and Gs involvement in OIH may depend upon the dose and chronicity of morphine, as well as on the nervous system site under study.

Pharmacological blockade of MOR

Because opiates are MOR agonists, and as analgesic tolerance and OIH occur following prolonged treatments with opiates, the prevention of OIH by opioid antagonists has been investigated. Two opioid receptor antagonists were mainly used: naloxone and naltrexone. These compounds displayed no effect on the nociceptive threshold when they were administered alone under non-pathological conditions but efficiently blocked opioid-induced analgesia (Celerier et al., 1999, Juni et al., 2006, Corder et al., 2013, Sanna et al., 2014, 2015a). A pre-treatment with naloxone or with the selective MOR antagonist CTOP prevented low dose opiate OIH (Sanna et al., 2014, 2015a, Sanna et al., 2015b), thereby demonstrating MOR involvement in the development of this acute hyperalgesia. In the other way, naltrexone did not prevent and even aggravate OIH when using 1.6 or 10 mg/kg morphine pellets (Juni et al., 2006). Also, naltrexone induced hyperalgesia when it was administered before a morphine analgesic dose (Swartjes et al., 2012). These studies used different protocols for inducing hyperalgesia and for measuring nociception as well as naltrexone vs naloxone (Table 1) that may explain the divergent results.

MOR has been shown mandatory for morphine analgesia (see Gaveriaux-Ruff, 2013, see Pasternak and Pan, 2013) but its role in OIH is still unclear as shown in table 1 with the contrasting effects of opioid antagonists on OIH. In addition, some studies show no involvement of opioid receptors in OIH. For example, naloxone had no effect on hyperalgesia induced by remifentanyl infusion in healthy human volunteers (Chu et al., 2011). Furthermore, toll like receptor-4 (TLR4) has been proposed as a mediator of OIH as shown by naltrexone effects on TLR4 (Hutchinson et al., 2010, Hutchinson et al., 2011, see Grace et al., 2015). The neuroinflammatory aspects of OIH

including studies on TLR4 are summarized in the section “neuroinflammatory mechanisms in OIH” of this review.

Other factors influencing OIH

OIH is a phenomenon observed in humans and modelled in experimental animals. In both clinical and preclinical studies, pain perception depends on the gender (Hampton et al., 2015), and on genetic profiles (see Denk et al., 2014). As summarized in the following paragraphs, OIH is also under the influence of sex, species and genetic background, and depends of the dose of opioid used.

Sex

Most of the teams working on pain or OIH have used males rather than females (see Mogil, 2012). However, a few teams have reported different profiles of OIH between males and females, although a sex-dependent factor for OIH is not clearly established yet. In their paper, Holtman and colleagues, (Holtman and Wala, 2005, 2007) have found increased morphine induced-hyperalgesia in females as compared to males, in agreement with higher analgesic tolerance in females (Hopkins et al., 2004). Of note, the studies by Holtman and colleagues have been performed on rats while most other studies were on mice, suggesting that the species also constitutes a factor influencing OIH. In comparison, no sex difference was observed for OIH in C57BL/6J mice under morphine infusion (40mg/kg/d or 1.6mg/kg/d; (Juni et al., 2010)). Interestingly, in a previous paper, the same authors noticed a stronger hyperalgesia in CD-1 females as compared to males, for morphine infusion at 1.6mg/kg/d but not at 40mg/kg/d (Juni et al., 2008). These results suggest a potential interaction for the influences of sex, genetic background and opioid dose (see Bodnar and Kest, 2010). Recently, the supraspinal MC1R melanocortin receptor antagonist and the NMDA receptor antagonist MK-801 were shown to selectively reverse morphine-induced hyperalgesia in males and females mice, respectively (Arout et al., 2015b). These results suggest that MC1R and NMDAR may underlie the qualitative sex difference observed in the development of morphine-induced hyperalgesia in mice.

Genetic background

The influence of the genetic background on OIH has been clearly demonstrated in rodent models. Liang and colleagues have compared 23 strains of inbred mice in a chronic morphine protocol (40mg/kg/d for 4 days), and have found strain differences for OIH (Liang et al., 2006, Liang et al., 2014b). As an example, C57BL/6J mice displayed a strong hyperalgesia whereas 129/S mice presented no or a weak OIH under a same morphine protocol (Liang et al., 2006, Liang et al., 2014b, Oladosu et al., 2015). Furthermore, single nucleotide polymorphisms at the multi-PDZ-domain protein 1 (MPDZ/MUPP1) gene may contribute to this mouse strain variation in OIH, as the SNPs correlated with the OIH phenotypes, and heterozygous mutant mice for MPDZ displayed less

morphine-induced hyperalgesia (Donaldson et al., 2016). An influence of the strain in the development of OIH has also been observed in rats (Laboureyras et al., 2014).

Opiate regimen

It has been shown that the dose of opiate administered impacts both analgesia and hyperalgesia. In this context, a morphine dose below 1mg/kg can be considered as a low dose in rodents. Low as well as ultra-low morphine doses induced acute hyperalgesia (Bianchi et al., 2011, Milne et al., 2013, Alizadeh et al., 2014, Sanna et al., 2015a, Sanna et al., 2015b) while a treatment over 1mg/kg was rather analgesic (Sanna et al., 2014). The same applies to buprenorphine for which doses over 20 μ g/kg elicited analgesia in rats, whereas a dose of 0.1 μ g/kg induced hyperalgesia (Wala and Holtman, 2011). Similarly to OIH elicited by chronic high opioid doses, hyperalgesia induced by ultra-low dose of opiates could also be affected by the sex (Holtman and Wala, 2005, 2007). Interestingly, repeated treatment with low doses of morphine produced tolerance to hyperalgesia (Holtman and Wala, 2005, Wala et al., 2011, Milne et al., 2013) while a chronic treatment with analgesic doses of morphine is classically known to cause hyperalgesia (see Lee et al., 2011). It is noteworthy that low doses of opiates also led to the development of OIH and higher post-operative morphine consumption in chronic pain patients with osteoarthritis scheduled for orthopedic surgery (Hina et al., 2015).

Classically, in rodent models, opiate systemic administration is performed through intraperitoneal, subcutaneous and intramuscular injections (see Jin et al., 2015). Intravenous administrations are mainly used to obtain fast answers (Kranke et al., 2015). Additionally, the role of specific brain regions can also be explored by injections into the areas themselves. Opiates produce analgesia, analgesic tolerance and OIH via the different routes, intraperitoneal (Tumati et al., 2012), subcutaneous (Bianchi et al., 2011), intrathecal (Milne et al., 2013 and many other papers) and intradermally (Araldi et al., 2015).

Opiate metabolites

The main metabolites obtained following *in vivo* morphine administration are morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G). In human, morphine is converted into 44-55% of M3G and 9-10% of M6G (see Andersen et al., 2003, Frolich et al., 2011), and numerous metabolites have been identified in blood of patients treated with morphine (see Laux-Biehlmann et al., 2013). M6G is estimated to be 2-5 times more analgesic than morphine (see Collier et al., 2009, see Lotsch, 2009), whereas M3G is considered as hyperalgesic and devoid of any analgesic property (see Komatsu et al., 2009, Due et al., 2012, Swartjes et al., 2012). Codeine can also produce hyperalgesia, and it is known that about 10% of this opiate is metabolized into morphine (Johnson et al., 2014).

MECHANISMS AND TARGETS DESCRIBED FOR OIH AT THE NEURONAL LEVEL

At the neuronal level, several possible mechanisms for opioid-induced hyperalgesia have been studied including (i) sensitization of primary afferent neurons (ii) sensitization of second order neurons to excitatory neurotransmitters and (iii) adaptation of pain descending control leading to up-regulation of nociceptive neuromodulators and enhanced release of glutamate by primary afferents (see Chu et al., 2008). In the last few years, several novel neuronal targets have been involved in these phenomena. This section will particularly focus on the most recent studies describing neuronal mechanisms and targets associated with development of OIH. They are summarized in figure 2.

Mechanisms described for OIH

Long-term potentiation

Long-term potentiation (LTP) is a sensitization of homosynapses leading to an enhanced strength of the synapse and its signal transduction. This will lead to a hypersensitivity of the system and so may contribute to hyperalgesia in models of persistent pain. LTP has been shown to occur at synapses between C fibers and neurons from the superficial layers of the spinal cord dorsal horn (Liu and Sandkuhler, 1997), being a prime site of the nociceptive processing. This phenomenon can also occur at the level of heterosynapses inducing a spread sensitization to the neighbouring synapses leading to secondary hyperalgesia (Scanziani et al., 1996). The link between LTP and hyperalgesia induced by persistent pain has been revealed by drugs able to block LTP that can also prevent hyperalgesia, such as ketamine (Klein et al., 2007, Zhou et al., 2010) or minocycline (Zhong et al., 2010). More recently, LTP has also been shown to be involved in OIH. Indeed, the withdrawal following remifentanyl infusion has been shown to lead to a potentiation of the synapse between nociceptive C fibers and neurons in superficial dorsal horn of the spinal cord (Drdla et al., 2009). In another study, the withdrawal from the three opiates morphine, fentanyl and remifentanyl induced an increase in C fibers field potential (Drdla-Schutting et al., 2012). Other arguments are in favour of a role of LTP in OIH including the fact that the three opioid receptors are present on primary afferents C fibers and in neurons of the superficial spinal dorsal horn (Besse et al., 1990) and that LTP and OIH share common signalling pathways. For example, the neurotrophin BDNF that is released by glial cells and plays a role in the development of OIH (Ferrini et al., 2013) has been shown to be involved in the maintenance of LTP (Zhou et al., 2010). Moreover, secondary messengers such as CaMKII, PKC, PKA and PLC are part of both OIH and LTP hypersensitivity signalling, and their inhibition can prevent LTP (Chen and Huang, 1991, Yang et al., 2004, Drdla et al., 2009). Also of major importance, LTP and OIH require NMDA receptors (see Mayer et al., 1999, see Sandkuhler, 2009) and can be prevented by NMDA receptor antagonists including ketamine (Benrath et al., 2005, Haugan et al., 2008), and MK801 (Frankiewicz et al., 1996, Li et al., 2001). Finally, glial cells and pro inflammatory cytokines are mediators of LTP and OIH. Regarding LTP itself, IL-1 β and TNF- α can enhance the frequency and/or amplitude of spontaneous EPSCs leading to neuroexcitation in the spinal cord (Zhang et al., 2011). These

cytokines will act mostly through the activation of glial cells (although some neurons also express their receptors) to induce the release of other mediators that will trigger LTP and hyperalgesia (Gruber-Schoffnegger et al., 2013).

Taken together, these data argue in favour of an association between LTP and OIH.

Hyperalgesic priming

Hyperalgesic priming is a mechanism that was first identified in an inflammatory pain model in which the short lasting hyperalgesia induced by carrageenan in rats was shown to induce a phenomenon of latent pain sensitization in primary nociceptors that resulted in a long lasting increased response to inflammatory mediators including prostaglandin E2, 5-hydroxytryptamine or adenosine A2 receptor agonist (Aley et al., 2000). Latent pain sensitization was further prevented by the administration of a PKC epsilon inhibitor. In a recent work, Araldi and collaborators showed that intradermal injection of DAMGO induces mechanical hyperalgesia as well as a marked prolongation of the hyperalgesia induced by further administration of prostaglandin E2 (Araldi et al., 2015). They further showed that these two phenomena are attenuated by a PKA inhibitor and by antisense oligonucleotides against PLC- β 3. However, this effect was reversible, indicating that both PKA and PLC- β 3 are involved in the expression, but not the induction or maintenance of DAMGO-induced hyperalgesia and hyperalgesic priming. Moreover, G α i protein does not seem to be involved, as pertussis toxin did not affect the hyperalgesia produced by DAMGO while the G-protein β/γ both DAMGO-induced and hyperalgesic priming. Altogether, these data clearly highlight a critical role of primary nociceptors in the development of OIH and that repeated opioid exposure can also induce hyperalgesic priming like other nociceptive stimuli although with some differences in the mechanisms (Araldi et al., 2015).

Descending pain facilitation

The descending pain modulation from the periaqueductal gray matter (PAG) and rostral ventromedial medulla has clearly been involved in the facilitation of nociceptive signalling in different models of persistent pain (Donovan-Rodriguez et al., 2006, Svensson et al., 2006, Dogrul et al., 2009) as well as in the development of hyperalgesia and analgesic tolerance induced by opiates (Vanderah et al., 2001). The implication of descending pathways originating from the rostral ventromedial medulla (RVM) was investigated further in naïve and paw incision-sensitized rats. Local administration of lidocaine into RVM abolished fentanyl-induced hyperalgesia in both rat models, supporting a main role for descending facilitatory pathways in OIH (Rivat et al., 2009). Interestingly, glial cell-based mechanisms in PAG have been shown to participate to OIH (Eidson and Murphy, 2013b). In PAG, administration of an inhibitor of protein G $\beta\gamma$ signalling reversed acute hyperalgesia induced by a low dose of morphine. This low dose induced the specific coupling of G $\beta\gamma$ proteins to the phospholipase C (PLC) subunit β 3 whose activation appears associated to this form of OIH (Bianchi et al., 2009). In the PAG also, OIH induced by an ultra-low dose of morphine was associated with the phosphorylation of MAPK such as ERK1, whereas in the frontal cortex the same treatment induced phosphorylation of ERK1, ERK2 and JNK (Sanna et al., 2014).

Interestingly, the same kinases were not activated in the thalamus. Furthermore, ERK and JNK inhibitors administered icv. suppressed ultra-low dose morphine-induced hyperalgesia. Altogether, these results suggest that the activation of ERK and JNK in the descending facilitation pathway participates to OIH. In line with this, c-Fos expression was increased in the PAG by acute morphine or its metabolite M3G (Arout et al., 2014), indicating an activation of the pain descending circuits. Finally, a recent study showed that remifentanyl infusion in mice induced hyperalgesia and MOR protein downregulation in the PAG that was reversed by intracerebral administration of antisense oligonucleotides to the neuron-restrictive silencer factor (NRSF; Lu et al., 2015). NRSF oligonucleotides restored MOR expression, indicating that reduced MOR expression in the PAG under fentanyl infusion contributes to fentanyl OIH.

Molecular targets described for OIH

NMDA – glutamatergic system

OIH shares common mechanisms with chronic pain, and one of them is the involvement of the glutamatergic system and particularly of the NMDA glutamate receptors. NMDA receptors are located presynaptically on the central terminals of primary afferent neurons as well as postsynaptically on spinal dorsal horn neurons (Antal et al., 2008). Several studies showed that the sc., it. or icv. administration of a NMDA receptor antagonist diminishes or prevents OIH in both rats and mice (Larcher et al., 1998, Celerier et al., 2001, Li et al., 2001, Ohnesorge et al., 2013, Arout et al., 2015a). In addition, under OIH conditions, chronic morphine augmented NMDAR1 subunit expression in brain that was reversed by NMDA blockade (Ohnesorge et al., 2013). Furthermore, NMDA receptor antagonists given chronically together with morphine under OIH condition lowered β -arrestin 2 transcripts (Arrb2) in mouse whole brain (Ohnesorge et al., 2013). Arrb2 has previously been described to be upregulated in case of analgesic tolerance in the PAG, the cortex and the striatum (Bohn et al., 2000, Hurle, 2001, Jiang et al., 2006). This arrestin isoform is responsible of MOR desensitization, a mechanism described as a cause of analgesic tolerance (see Williams et al., 2013). These results demonstrated that NMDA-regulated Arrb2 activity is involved in both OIH and tolerance therefore strengthening the link between these two phenomena. With electrophysiology experiments, it has been demonstrated that a chronic opioid treatment increases presynaptic NMDA receptor activity in spinal dorsal horn as well as decreases the postsynaptic NMDA receptor activity (Zhao et al., 2012b). The hypothesis of Zhao and colleagues is that chronic opioid administration can induce a translocation of PKC to the plasma membrane leading to the activation of presynaptic NMDA receptor by the removal of the Mg^{2+} blocker from the receptor. This results in promoting NMDA receptor trafficking to the plasma membrane to increase its activity, ending up with a higher release of glutamate (Zhao et al., 2012b). Correlated with the observation of a decrease of the glutamate transporters GLAST and GLT1 after a sustained morphine administration (Mao et al., 2002), this will induce an enhancement of glutamate level in the synaptic cleft, and hence an overstimulation of the synapse that could lead to hyperalgesia.

A new study from Gong and collaborators underlines the importance of the peripheral nervous system in OIH and more precisely of DRG neurons through the upregulation of the glutamate transporter EAAT3 and NMDA receptor subunit GluN2B (Gong et al., 2016). Mu-opioid receptors are expressed mainly by small diameter neurons and by some larger diameter neurons of the DRG (Scherrer et al., 2009, Wang et al., 2010, Erbs et al., 2016). The recent work from Gong and coworkers shows that sustained morphine administration increased the excitability of small diameter DRG neurons but not of large neurons, which, when they are activated, can lead to an increase in intracellular calcium signalling and release of excitatory amino acids, resulting in an overexcitability of the fibers (Gong et al., 2016).

KCC2 and Cl⁻ homeostasis

Disruption of Cl⁻ homeostasis at the level of the dorsal horn spinal cord has been shown to be critical for the development of hyperalgesia in different persistent pain syndromes including inflammatory and neuropathic pain (see Zeilhofer et al., 2012). Trans-synaptic reduction in the expression of the potassium chloride exporter KCC2 is considered a key mechanism that leads to a shift in neuronal anion gradient associated with the development of hyperalgesia in neuropathic pain (Coull et al., 2003, Coull et al., 2005). Moreover, modulation of KCC2 expression results from the release of brain-derived neurotrophic factor (BDNF) by activated microglia and further TrkB receptor activation in lamina 1 neurons (Coull et al., 2005). This mechanism has recently been shown to underlie hyperalgesia induced by opiates but not analgesic tolerance (Ferrini et al., 2013; see below for details).

TRPV1

The capsaicin receptor TRPV1 is a non-selective cation channel activated by heat and chemical ligands that plays a role in transduction of noxious chemical and thermal stimuli (Caterina et al., 2000). This channel is present at both central and peripheral terminals of primary sensory neurons (see Julius, 2013). Several pieces of evidence indicate that TRPV1 is involved in the development of OIH. Chronic use of opiates can repeatedly stimulate TRPV1-expressing primary afferents and increase the nociceptive input leading to the sensitization of spinal dorsal horn neurons and hyperalgesia (Zhou et al., 2010). Different TRPV1 antagonists (e.g. AMG0347, SB366791) were able to suppress tolerance and OIH (Chen et al., 2008, Vardanyan et al., 2009, Zhou et al., 2010), TRPV1 KO mice did not develop thermal and tactile hypersensitivity after chronic morphine administration (Vardanyan et al., 2009) and TRPV1 mRNA is increased in DRG and spinal cord of rats after chronic morphine treatment. Moreover, the TRPV1 channel can be found in association with β -arrestin2, which permits its regulation through its desensitization and internalization (Por et al., 2012). Recently, MOR agonists have been shown to sequester β -arrestin2 to MOR therefore attenuating the TRPV1/ β -arrestin2 interaction to amplify TRPV1 activity in peripheral sensory neurons and contribute to symptoms of OIH (Rowan et al., 2014b). The same mechanism was

observed with DOR and the DOR-selective agonist SNC80 resulting in sensitization of TRPV1 and behavioural signs of OIH (Rowan et al., 2014a).

TRPM8

The menthol receptor TRPM8 is another member of the transient receptor potential channel (TRP) superfamily that has been shown to be responsible for mild cold sensation in mammals (Bautista et al., 2007). Like TRPV1, TRPM8 is expressed in primary nociceptors but in a distinct neuronal population in naïve animals, indicating that different cells may operate the detection of hot and cold noxious stimuli (see Basbaum et al., 2009). In a recent study, sustained stimulation of MOR has been shown to suppress TRPM8 activity in DRG neurons by promoting its internalization (Shapovalov et al., 2013). This phenomenon has been proposed to form the basis of cold analgesia induced by opiates. Moreover, both morphine cold analgesia and hyperalgesia were suppressed in TRPM8 deficient mice suggesting that functional interaction between mu-opioid receptor and TRPM8 are critical for both phenomena.

5HT-3 receptor

Among 5-HT receptors, the 5HT-3 subtype is the only ligand-gated cation channel with excitatory function. It is expressed both in spinal dorsal horn and primary afferent neurons and has been identified as an important player in the modulation of pain hypersensitivity by descending serotonergic neurons (see Lopez-Garcia, 2006). Recently, pharmacological blockade of 5-HT3 receptor with ondansetron by systemic or intrathecal administrations was shown to significantly prevent and reverse OIH and tolerance indicating that adaptations to chronic opiate treatments are also controlled by descending serotonergic processing from the rostral ventromedial medulla (Vera-Portocarrero et al., 2007, Bannister et al., 2011, Liang et al., 2011). In addition, Guo and colleagues have shown that selective activation of neuronal 5-HT3 receptor leads to hypereactivity of microglia and astrocytes, which result in spinal sensitization and pain hypersensitivity (Guo et al., 2014). These results suggest the existence of a link between descending facilitation and glial mechanisms in OIH.

EphrinB receptors

Ephrin receptors form the largest known subfamily of receptor tyrosine kinases. They are activated by membrane bound ligands called ephrins. EphrinB receptors have largely been shown to regulate the development of glutamatergic synapses and their plasticity in adult nervous system by interaction with NMDA receptors (Dalva et al., 2000, Henderson et al., 2001, Takasu et al., 2002). More recently, EphrinB receptor blockers have been shown to inhibit the induction and maintenance of hyperalgesia and allodynia induced by nerve injury as well as hyperexcitability of nociceptive small DRG neurons, sensitization of dorsal horn neurons and LTP of synapses between primary nociceptors (C fibers) and spinal neurons. A recent study further extends these observations

by showing that an antagonist of ephrinB receptor inhibits remifentanyl-induced hyperalgesia in rats (Xia et al., 2014). Moreover, similarly to what was observed in a model of nerve-injury, ephrinB1 and its receptor were found up-regulated in spinal cord dorsal horn of rats treated with remifentanyl.

mTOR

mTOR is a Serine/Threonine kinase forming two different complexes: mTORC1 with the protein raptor, is sensitive to the antibiotic rapamycin and is involved in protein translation through the regulation of phosphorylation/activation of downstream effectors such as p70S6K and 4E-BP1 (see Hay and Sonenberg, 2004, see Lutz et al., 2015). mTORC2, with the protein rictor, is rapamycin insensitive and can regulate the phosphorylation of PKB/Akt and PKC proteins (see Hay and Sonenberg, 2004). mTOR, p70S6K and 4E-BP1 mRNAs and proteins are present in DRG and spinal dorsal horn neurons but not in astrocytes or microglia (Xu et al., 2010). A recent study from Xu and colleagues focused on morphine-induced hyperalgesia and demonstrated that MOR activation induces the phosphorylation and thus activation of mTOR and its downstream effectors (Xu et al., 2014). Moreover, MOR, mTOR, PI3K/Akt and NMDA receptor subunit NR1 were shown to colocalize in neurons of the spinal dorsal horn and pharmacological inhibition of mTOR action decreased morphine tolerance and hyperalgesia (Xu et al., 2014). Altogether, these data are in favour of a role of mTOR activation in the spinal cord in morphine-induced hyperalgesia mechanism through the increase of protein translation in the spinal dorsal horn (Xu et al., 2014, Lutz et al., 2015, Xu et al., 2015).

NEUROINFLAMMATORY MECHANISMS IN OIH

Besides neuronal regulations, neuroinflammatory cells have been identified as key actors in these opioid-induced phenomena, and their role in OIH has been reviewed recently (see Grace et al., 2015). These cells include oligodendrocytes, astrocytes, microglia, perivascular macrophages, endothelial cells and infiltrating immune cells (see Grace et al., 2015, see Thomas et al., 2015), although most studies were carried on astrocytes and microglia. The neuroimmune mechanisms in OIH appear to constitute one facet of the glial cell contribution to chronic pain. Since the initial discussion on the tetrapartite synapse for central pain sensitization (see De Leo et al., 2006), several reviews on the role of neuroimmune activation in chronic pain have been published (see Stein and Machelska, 2011, Calvo et al., 2012, von Hehn et al., 2012, Ji et al., 2013, Mika et al., 2013, Grace et al., 2014, Ji et al., 2014, Austin et al., 2015, Old et al., 2015, Ren and Dubner, 2015, Yaksh et al., 2015).

Neuroimmune mechanisms for OIH have been first demonstrated by using inhibitors of glial cell activity. These inhibitors include the glial cell blockers pentoxifylline, propentofylline, fluorocitrate and minocycline, as well as proinflammatory cytokines antagonists (see Hutchinson et al., 2011). It is noteworthy that while many reports have described the importance of neuroinflammation in analgesic tolerance, only a dozen have focused on immune mechanisms for

OIH. The different studies, which report that blockade of immune activation diminishes OIH are summarized in table 2. Most articles show that inhibition of inflammation attenuates hyperalgesia induced by repeated morphine doses. Interestingly, the only one paper in which animals were chronically treated with remifentanyl showed that minocycline did not prevent the development of OIH (Aguado et al., 2015). Differences in drugs (remifentanyl versus other opiates) and administration protocols (intravenous versus other route of administration) may contribute to this divergent result. In any case, this raises the notion that both opioid type and administration schedule may have a significant impact on analgesic tolerance and OIH.

It is important to note that among papers describing a link between neuroinflammation and OIH, most of them used male rats or mice, and only two have involved female rats (Wilson et al., 2011, Due et al., 2012). However, sex has recently been reported to have a major influence on the immune mechanisms mediating pain (Sorge et al., 2015, Mapplebeck et al., 2016). In male mice, peripheral nerve injury induced an increase in P2X4 receptor expression and BDNF release by spinal microglia that activated TrkB receptor on neurons, leading to neuronal disinhibition and hyperalgesia. In contrast, female mice microglia did not show these responses, and the suppression of microglia activation reversed hypersensitivity in males but not in females. It has been suggested that, in females, peripheral nerve injury rather elicits T lymphocytes activation resulting in hyperalgesia (Sorge et al., 2015, Mapplebeck et al., 2016). Similarly, in male rats the microglial inhibitor minocycline increased morphine analgesia and lowered morphine-induced glial activation whereas it had no effect on morphine analgesia in female rats and tended to increase some glia activation markers (Posillico et al., 2015). As microglia appears to be more involved in pain and OIH in males as compared to females, upcoming studies focusing on neuroimmune mechanisms involved in the development of OIH should therefore consider analysing both males and females. Moreover, species, strain and opioid category will also have to be regarded in studies on neuroimmune mechanisms for OIH (see Table 2). Finally, as mentioned above for females, peripheral immune cells may also regulate OIH mechanisms. Indeed, in damaged tissues they release pronociceptive mediators and trigger analgesia by releasing endogenous opioids, therefore playing a dual role in pain control (see Machelska, 2011, see Sacerdote et al., 2012, see Ninkovic and Roy, 2013, see Basso et al., 2014)

Astrocytes and microglia contribute to OIH

At first, several papers showed that morphine exposure triggers astrocyte activation. Acute morphine exposure led to GFAP, IL-1 β and matrix metalloprotease-9 upregulation in DRG satellite glial cells, as shown by co-staining with GFAP (Berta et al., 2012). Furthermore, 5-7 days of chronic morphine was required for spinal astrocyte activation and IL-1 β upregulation in astrocytes (Berta et al., 2012, Berta et al., 2013). On the other hand, LPS-induced Ca²⁺ response in astrocytes was attenuated by the mu-agonist endomorphin in vitro (Block et al., 2013). A specific action on neurons and astrocytes vs microglia was shown following intrathecal chronic morphine in rats, as reflected by augmented levels of NF-kB-phospho-p-65 in both spinal neurons and astrocytes but not microglia (Bai et al., 2014). In this study, the TLR4 antagonist LPS-RS prevented p-65

phosphorylation as well as tolerance and OIH. A selective activation of astrocytes was also found in response to an ultra-low morphine dose inducing OIH (Sanna et al., 2015a). Morphine at 1 $\mu\text{g/kg}$ produced hyperalgesia and the activation of spinal astrocytes but not microglia. Astrocytic levels of p-JNK were increased by morphine while JNK and NMDA antagonists prevented the development of OIH (Sanna et al., 2015a). Similarly, buprenorphine at ultra-low dose (0.1 $\mu\text{g/kg}$) produced hyperalgesia and activated spinal astrocytes while microglia did not show enhanced reactivity (Gerhold et al., 2015). Moreover, spinal administration of 5-HT₂ receptors antagonists prevented descending facilitating pathways, diminished astrocytes activation and reversed buprenorphine-induced hyperalgesia (Gerhold et al., 2015). This indicates that ultra-low-dose of buprenorphine induced OIH through a 5-HT mediated mechanism in astrocytes (Gerhold et al., 2015).

Secondly, microglia have been shown activated upon chronic opioid administration. Chronic morphine and codeine treatments lead to augmented CD11b but not GFAP expression in trigeminal ganglia and spinal cord (Johnson et al., 2014). As well, microglia were shown *in vitro* to release proinflammatory cytokines and nitric oxide upon morphine or DAMGO stimulation (Merighi et al., 2013). Ferrini and colleagues have also identified a specific role of microglia in OIH (Ferrini et al., 2013). They showed that both microglia depletion by spinal anti-Mac-1-saporin treatment and the conditional inactivation of BDNF gene in microglia prevent chronic morphine OIH while sparing tolerance. They demonstrated that the microglia to neuron signalling cascade involves P2X₄R upregulation in microglia that release BDNF (see below for detailed mechanisms). Interestingly, these findings have identified a pathway that contributes to OIH without affecting analgesic tolerance, dissociating the two phenomena (Ferrini et al., 2013). The opposite has been observed in patients with low back pain that had analgesic tolerance without OIH (Chu et al., 2012, see Richebe et al., 2012). To conclude, these different studies indicate that a number of microglial actors and mechanisms participate in the development of OIH.

Finally, concurrent activation of astrocytes and microglia has also been observed under chronic morphine conditions. In particular, chronic morphine injections in rats activate both spinal microglia and astrocytes, together with TNF- α release (Tumati et al., 2012). In neuropathic animals also, both spinal astrocytes and microglia are activated by chronic morphine. Furthermore, glial activation and morphine analgesia are reduced following a chronic co-treatment with botulinum toxin, suggesting a potential clinical application (Vacca et al., 2013). Coincident activation of microglia and astrocytes also occurred in the ventrolateral periaqueductal gray matter (vlPAG) following repeated morphine, indicating that glial cells besides neurons are involved in OIH associated events in the PAG (Eidson and Murphy, 2013a). Also, persistent peripheral inflammation attenuated chronic morphine-induced activation of astrocytes and microglia in the vlPAG together with lowered analgesic tolerance, revealing a long-distance effect of peripheral injury on PAG mediated OIH mechanisms (Eidson and Murphy, 2013b). Accordingly, similar results were found on the role of peripheral inflammation on analgesic tolerance (Zollner et al., 2008). Lin and co-workers published a recent method for quantifying the different parameters of microglia and astrocyte activation found in OIH that will help in future studies focused on glia activation in OIH (Lin et al., 2015b). Interestingly, whereas morphine triggers OIH and astrocytes-microglia

activation, some novel endomorphin derivatives induce less tolerance together with a lack of OIH or glia activation (Zadina et al., 2015). This indicates that mu opioid agonists that possess good analgesic properties without glia-mediated adverse effects can be developed.

Molecular actors of OIH in neuroinflammatory cells

In the recent years, several novel glial targets have been associated with development of OIH. This section will focus on the studies describing these newly identified actors that are summarized in figure 3 for microglial cells and figure 4 for astrocytes.

Toll like receptor 4 (TLR4)

Among neuroimmune mechanisms, TLR4 activation has been shown to contribute to opioid-induced tolerance and hyperalgesia. Blockade of TLR4 activation potentiated and prolonged acute opioid analgesia (Hutchinson et al., 2010), suggesting a role in analgesic tolerance. Also, the TLR4 antagonist LPS-RS prevented OIH and tolerance as well as p-65 phosphorylation in the spinal cord (Bai et al., 2014). Furthermore, morphine and other opiates were reported to bind directly to - and activate TLR4 by molecular modelling and *in vitro* cellular assays (Hutchinson et al., 2010). In contrast, morphine, fentanyl, naltrexone and β -FNA lowered TLR4 activation in a similar functional cell assay (Stevens et al., 2013). The notion that morphine elicits analgesic tolerance and hyperalgesia through TLR4 activation raised much interest and debate (Skolnick et al., 2014, Watkins et al., 2014, see Grace et al., 2015). To our knowledge until now, four studies have investigated TLR4 implication in OIH by genetic approaches, through *in vivo* experiments with TLR4 deficient mice (Due et al., 2012, Ferrini et al., 2013, Johnson et al., 2014, Mattioli et al., 2014). Main features from these papers are summarized in Table 3. Two of these studies found no OIH in TLR4 mutants while the two others reported that OIH was preserved. In addition, two papers found intact analgesic tolerance in TLR4 deficient mice (Fukagawa et al., 2013, Mattioli et al., 2014) while tolerance was not investigated in the other OIH studies. These conflicting findings on the implication or lack of implication of TLR4 in OIH may be explained by disparities in experimental conditions including mouse strains, sanitary status that may activate TLR4 to different extents, morphine regimen as well as OIH evaluation. Besides, TLR9 was found involved in *in vitro* microglia responses to morphine, in a MOR-dependent manner (He et al., 2011). Also, TLR2, TLR5, TLR8 and microRNA-124 expression was regulated by morphine in microglia culture experiments (Qiu et al., 2015). Future analyses will be required to understand more deeply the implication of TLRs in opioid-induced analgesic tolerance and OIH.

Cytokines, chemokines and chemokine receptors

In addition to the TLRs, several cytokines, chemokines and chemokine receptors were identified as important actors in OIH or tolerance (see Melik Parsadaniantz et al., 2015). Neutralizing antibodies to MCP-1 or CX3CR1 administrated intrathecally reduced analgesic tolerance or OIH (Johnston et al., 2004, Zhao et al., 2012a). Activation of CXCR4 receptor by the chemokine CXCL12 counteracted acute morphine analgesia in naïve rats (Rivat et al., 2014). Along the same lines,

chronic morphine upregulated SDF1/CXCL12 and CXCR4 whereas CXCR4 blockade reversed OIH (Wilson et al., 2011). More broadly, the SDF1/CXCL12 - CXCR4 system is considered as a general modulator of chronic pain (Luo et al., 2016). Interestingly, a study on cancer patients reported increased CXCL1 levels in the cerebrospinal fluid of the opioid-tolerant group (Lin et al., 2015a). In the same paper, morphine analgesia in rats was decreased by CXCL1 infusion and improved by CXCL1- neutralizing antibody or CXCR2 antagonist administration (Lin et al., 2015a). These studies pointed to MCP-1/CCR2, CX3CL1/CX3CR1, SDF1/CXCR4 and CXCL1/CXCR2 as major chemokine systems involved in OIH. In addition, chemokines such as CCL3, CCL2, CCL5 and CXCL8 can alter the function of the mu-opioid receptor by desensitizing it through the activation of their respective GPCR receptors (Zhang et al., 2004). On the cytokine side, the IL-1 receptor antagonist IL-1ra (Johnston et al., 2004, Johnson et al., 2014), a combination of IL-1, TNF- α and IL-6 blockers (Lewis et al., 2010), as well as the anti-inflammatory cytokine IL-10 (Johnston et al., 2004) prevented OIH (Table 2). In connection with these results, TNF- α and IL-1 β were shown to contribute to high frequency stimulation-induced hyperalgesia and synaptic LTP potentiation by activating their receptors on glial cells in the spinal cord (Gruber-Schoffnegger et al., 2013).

ATP, P2X4 receptor and BDNF

Following the initial demonstrations that morphine promotes microglia migration via MOR and P2X4 receptor dependent signalling (Takayama and Ueda, 2005, Horvath and DeLeo, 2009), inhibition of P2X4 receptors activity on microglia was shown to prevent morphine analgesic tolerance (Horvath et al., 2010). Recently, genetic tools in mice have been used to confirm those findings and showed that OIH relies on two different mechanisms, a MOR-dependent upregulation of P2X4 receptors on microglia, and a MOR-independent gating of BDNF release (Ferrini et al., 2013). Spinal BDNF activates TrkB on neurons and downregulates the potassium-chloride co-transporter KCC2, leading to the disinhibition of GABAergic neurons and hyperalgesia (see Grace et al., 2015, see Trang et al., 2015). Interestingly, BDNF signalling from microglia has been previously shown important in learning and memory (Parkhurst et al., 2013), indicating a common mechanism for memory and persistent pain.

Tachykinin NK1 receptor

In the spinal cord, the tachykinin NK1 receptor has been shown to be involved in glia activation during OIH. Morphine withdrawal-induced glial cell activation and OIH are attenuated by the tachykinin NK1 receptor antagonist L-732,138, indicating a role for the tachykinin-NK1 receptor system in OIH. In addition, an opioid agonist-NK1 antagonist bivalent compound did not activate spinal glial cells and prevented OIH. This indicates that substance P-triggered glia activation and OIH with sustained morphine can be prevented by targeting opioid and NK1 receptors concomitantly (Tumati et al., 2012).

Sphingolipid ceramides

In glial cells, the sphingolipid ceramide system has been described as an actor in OIH. The sphingosine-1-phosphate (S1P) and its GPCR are expressed in the spinal cord and DRG, structures well known to be involved in nociceptive processing. Otherwise, there is an overlapping distribution of S1P receptor and mu-opioid receptor in different CNS regions like the striatum, amygdala or spinal cord (see Salvemini et al., 2013). OIH was shown correlated to sphingolipid ceramide upregulation in rat spinal astrocytes and microglia under chronic morphine infusion (Muscoli et al., 2010). Moreover, intrathecal administration of ceramide inhibitors prevented OIH as well as upregulation of S1P and inflammatory cytokines (TNF- α , IL1- β and IL-6; Muscoli et al., 2010). These results are in agreement with a previous study showing that the neuraminidase inhibitor oseltamivir blocked ganglioside-regulated morphine analgesic tolerance and low dose-morphine hyperalgesia in mice (Crain and Shen, 2004). Another argument for the implication of the ceramide pathway in OIH is that the activation of TLR4 (see above) leads to an enhanced production of ceramides by glial cells, suggesting that this receptor could be involved in OIH development by triggering the ceramide pathway. In addition to OIH, the sphingolipid pathway is currently considered as a therapeutic target for chronic pain management (see Salvemini et al., 2013).

Superoxides and peroxynitrites

Spinal peroxynitrite has been involved in OIH. Indeed, the superoxide-sparing peroxynitrite decomposition catalyst SRI-110 prevented morphine-induced hyperalgesia and analgesic tolerance (Little et al., 2013). SRI-110 lowered morphine-induced upregulation of TNF- α , IL-1 β and IL-6 while it increased IL-4 and IL-10 anti-inflammatory cytokines (Little et al., 2013). Therefore, restoring spinal mitochondrial function by removal of superoxides and peroxynitrites may increase the levels of anti-inflammatory mediators and ensuing pain relief.

OTHER SYSTEMS INVOLVED IN OIH

Several molecular targets have been described to play a role in the development of OIH in the past decades. However, for most of these targets the mechanism of action remains poorly understood. This section aims to summarize the current knowledge on these targets.

Anti-opioid peptides

Several neuropeptides have been shown to display pro-nociceptive activity that opposes anti-nociceptive action of opiates. They have been proposed to be part of a homeostatic equilibrium in which exogenous administration of opiates triggers the release of these peptides that in turn counteract the analgesic action of opiates thus leading to the development of hyperalgesia and analgesic tolerance. The most studied peptides include cholecystokinin, neuropeptide FF (NPFF) and OFQ/Nociceptin as well as the opioid peptide dynorphin. The anti-opioid action of these

peptides and their receptors has already been described in several reviews (see Rothman, 1992, McNally, 1999, Ossipov et al., 2003, Mouledous et al., 2010, Toll et al., 2016).

CCK has long been proposed to act as an endogenous anti-opioid (Faris et al., 1983). Several studies have shown that morphine triggers the release of CCK in the spinal cord (de Araujo Lucas et al., 1998, Gustafsson et al., 2001) and CCK receptor antagonists have been shown to prevent the development of antinociceptive tolerance to morphine (Dourish et al., 1990, Hoffmann and Wiesenfeld-Hallin, 1994). Moreover, pharmacological blockade of CCK2 receptors in the rostral ventral medulla (RVM) have been shown to block the development of hyperalgesia induced by continuous systemic morphine indicating that endogenous CCK activity in RVM may decrease spinal analgesic effect of opiates by activating descending pain facilitatory mechanism to exacerbate spinal nociceptive sensitivity (Xie et al., 2005).

Both neuropeptide FF and OFQ/nociceptin induce hyperalgesia and reverse morphine analgesia when administered intracerebroventrically, but display antinociceptive effect when administered intrathecally suggesting that these peptides could display both pro and antinociceptive properties (see Simonin, 2006, see Toll et al., 2016). However, systemic administration of NPFF receptor antagonist RF9 have been shown to completely prevent the development of hyperalgesia and analgesic tolerance induced by chronic opiates administration indicating that NPFF and its receptors represent a *bona fide* anti-opioid system (Simonin et al., 2006, Elhabazi et al., 2012). Although the consequence of systemic pharmacological blockade of OFQ/nociceptin receptor on the development OIH has not been investigated so far, systemic administration of a selective antagonist (J-113397) as well as the genetic blockade of NOP receptor or OFQ/nociceptin precursor genes produced a significant increase of mouse nociceptive behaviour in inflammatory pain models. These data indicate that for this system the spinal antinociceptive action prevails over supraspinal pronociceptive effects (Depner et al., 2003, Rizzi et al., 2006).

Kappa-opioid receptor and its endogenous ligand dynorphin have also been shown to counteract mu-opioid receptor action in different brain regions (see Pan, 1998). However, systemic administration of kappa-opioid receptor selective agonists produce analgesic effect indicating that, similarly to OFQ/nociceptin system, the antinociceptive action of kappa-opioid receptor and dynorphin prevails over anti-analgesic actions of this system (Simonin et al., 1998). Recently, the kappa receptor system has been shown implicated in pain aversion (see Cahill et al., 2014). Therefore, this system may also contribute to aversive mechanisms underlying hyperalgesia induced by chronic opiates.

GPCRs and intracellular pathways

In addition to the so-called anti-opioid peptides and their receptors several other GPCRs as well as intracellular targets have been involved in the development of OIH.

A genetic analysis in mice pointed to $\beta 2$ adrenergic receptor as an important player in the development of OIH, which was further confirmed by pharmacological and genetic blockade of this receptor (Liang et al., 2006, Samoshkin et al., 2015). These studies concluded that genetic variants

of $\beta 2$ adrenergic receptor gene could explain the differences observed between different strains of mice in the development of OIH.

Selective antagonist of melanocortin 4 receptor (HS104) has also been shown to prevent the development of analgesic tolerance induced by chronic morphine infusion in rats as well as hyperalgesia that was observed after drug withdrawal (Kalange et al., 2007). Moreover, pharmacological blockade of this receptor during the induction of morphine tolerance was further shown to reduce the activation of astrocytes as well as the expression of proinflammatory cytokines (IL-1 β , IL-6 and TNF- α) and to upregulate the anti-inflammatory cytokine IL-10 in the rat spinal cord (Niu et al., 2012).

At the intracellular level, protein kinase C γ was shown to play an important role in the development of OIH. Indeed, PKC γ deficient mice displayed an enhancement of fentanyl acute analgesia as well as a complete absence of secondary hyperalgesia (Celerier et al., 2004). This effect could be due to the fact that Ca²⁺-sensitive protein kinases C, particularly the γ isoform catalyzes the NMDA receptor phosphorylation upon stimulation of opioid receptors leading to sensitization to painful stimuli (Celerier et al., 2004). Moreover, thermal hypernociception induced by morphine in mice was further shown to be inhibited by the PLC inhibitor U73122 and the PKC blocker, calphostin C as well as by antisense phosphodiester oligonucleotides directed against PLC $\beta 3$ and PKC γ suggesting that PLC $\beta 3$ /PKC γ /NMDA pathway is stimulated upon morphine administration and play an opposing role in morphine analgesia (Galeotti et al., 2006). More recently, hyperalgesia induced by ultra-low dose of morphine was shown to be dependent on ERK1/2-c-JUN signalling pathway at different levels of the central nervous system including the spinal cord (Sanna et al., 2014, 2015a, Sanna et al., 2015b). In this latter region JNK was shown activated specifically in astrocytes suggesting that activation of neuronal MOR could lead to the activation of ERK1/2-c-JUN pathway in astrocytes, thus contributing to hyperalgesia induced by ultra-low doses of morphine.

EPIGENETICS AS A NOVEL MECHANISM FOR OIH

Epigenetic adaptations reflect the impact of the environment on DNA leading to a modulation of gene transcription. Two main mechanisms are involved: the cytosine methylation of the DNA leading to a repression of gene transcription and the histone acetylation, increasing the access for gene transcription. Histone deacetylases (HDAC) inhibitors are able to diminish pain sensitivity, indicating that epigenetic mechanisms are involved in chronic pain (see Denk et al., 2014, see Descalzi et al., 2015).

The first evidence for a link between OIH and epigenetic is that opiates users have an increase of methylation on the MOR promoter gene that will influence its transcription (see Doebring et al., 2011), suggesting that opiates may inhibit MOR gene demethylation (Doebring et al., 2013). Another study showed that modifications of the degree of histone acetylation induced by morphine in the spinal cord participate in the regulation of morphine tolerance, dependence and OIH (Liang et

al., 2013). Indeed, the concomitant treatment with chronic morphine and the HDAC inhibitor suberoylanilide hydroxamic acid (SAHA) enhances OIH in naïve mice (Liang et al., 2014a) and induce changes in BDNF and prodynorphin gene histones acetylation that will modify their expression (Liang et al., 2013, Liang et al., 2014a). On the other hand, the HDAC inhibitors trichostatin A and valproic acid have been shown to reverse neuropathy-induced decrease in morphine analgesia in mice (Uchida et al., 2015). Neuropathic damage decreased MOR expression in DRG, and the HDAC inhibitors restore morphine analgesia by upregulating MOR mRNA expression to non-injury levels. Both studies were performed on young adult C57BL/6 male mice, but Liang and collaborators used an escalating morphine treatment of pain-naïve mice whereas the study by Uchida and colleagues was performed with nerve-injured mice acutely treated with morphine. BDNF was also found upregulated following a repeated morphine treatment inducing OIH, with a downregulation of BDNF exon IV methylation level (Chao et al., 2016). In addition, the DNA methylation inhibitor 5-aza-2'-deoxycytidine upregulated BDNF expression and augmented OIH, suggesting the existence of an epigenetic control of BDNF gene expression in OIH (Chao et al., 2016). Very recently, epigenetic changes in BDNF and Prodynorphin genes were found in the spinal cord of mice with post-operative pain and OIH, which was shown attenuated by histone acetyltransferase inhibitor anacardic acid (Sahbaie et al., 2016). Altogether, these studies show a contribution of epigenetic in OIH that will be influenced by the animal pain state and opioid regimen.

Finally, epigenetic could represent a new track to create personalized treatments in order to limit the development of opiates side effects including OIH. Profiles of DNA methylation or acetylation are specific from an individual and can be obtained with a genetic diagnosis. Hence, knowing these personal characteristics could help to treat patients individually e.g. with HDAC inhibitors in addition to other drugs. However, epigenetic modifications are not specific from genes involved in pain pathways and OIH, therefore side effects could be also induced (see Doebling et al., 2011).

CONCLUSION

In this review, we present the numerous mechanisms and actors that have been recently identified in OIH. Interestingly, some of them are similar to those involved in chronic pain. In neuropathic or inflammatory pain models, a tetrasynapse composed of the central terminal of primary afferent neurons, projection neurons, astrocytes and microglia has been demonstrated in the spinal cord dorsal horn (see Grace et al., 2014). In non-pathological conditions, one of the main nociceptive mechanisms is the glutamatergic signalling transmitting the nociceptive message to supraspinal areas. This phenomenon is characterized by a short duration because of compensations and regulations by environmental cells. GABAergic inhibitory neurons and endogenous opioid systems are thus activated and decrease neuroexcitatory responses and thereby alleviate pain. Additionally, glutamate transporters present on neurons or astrocytes (EAAC or GLT-1 respectively), recapture

glutamate in the synapse cleft and so contribute to the decrease of neuroexcitation induced by the nociceptive stimulus, and participate to pain relief.

Both chronic pain and OIH show a disruption of these regulatory processes. In case of OIH, the recapture of glutamate by neurons or astrocytes is reduced, increasing synaptic concentrations of glutamate in the synaptic cleft and thus neuroexcitation by elevating intracellular Ca^{2+} concentrations through specific receptors activation. On the other hand, KCC2 channels activation will block the inhibitory GABA-R on neurons also leading to neuroexcitation. In addition to this context, LTP, induced by high Ca^{2+} intracellular concentrations, and reactive oxygen species participates to the maintenance of neurons hypersensitivity. Consequently, these activated neurons produce chemokines, which will in turn stimulate microglial cells that will release molecules responsible of neuroinflammation. Astrocytes are also activated in this context and feed this neuroinflammatory environment. The chronological order of tetrasynapse cells activation is currently under debate, but the fact that glial cells and neurons play a role in the development of OIH appears well established. Specific MOR variants, with different number of TM domains, have been shown to induce hyperalgesia under acute exposure to opiates. This suggests a change of the signalling pathways engaged in case of hyperalgesia compared to those involved in analgesia. In addition, other receptors could be involved in OIH including other GPCRs or TLR4.

The environment also appears to have a great impact on OIH development. According to the gender, age, genetic background, immune conditions, pain and opiate treatment history as well as the doses of opiates administered, OIH establishment, development and maintenance may differ. All these OIH influencing factors identified in preclinical studies make these observations close to the clinical conditions of OIH. Indeed, each patient is a unique case. The opiate treatment is initiated by different painful situations and each patient has his own health history with, or without opiate pre-exposure, his own genetic profile and life environment. This patient is a man or a woman, young or aged, and all these factors will influence the establishment of OIH, its intensity and persistence. Preclinical studies show that OIH implicates complex mechanisms, reflecting somehow the clinical reality, and providing hints for the development of strategies to avoid OIH.

Furthermore, tolerance to analgesia may be described as a consequence of OIH. Because OIH lowers the basal nociceptive level, analgesia will be masked by OIH, leading to analgesic tolerance. From a mechanistic point of view, this phenomenon may contribute to tolerance beside or in addition to the decrease of functional MOR induced by chronic exposure to opiates, contributing to the loss of analgesic effects of opiates (see Williams et al., 2013). In the future, the respective contribution of OIH and other mechanisms in the development of analgesic tolerance will need further investigation.

Finally, mechanisms that underlie OIH may also contribute to other adaptations associated with opiates. Particularly, the understanding of OIH mechanisms may help to link the nociceptive effects of opiates and their psychological adverse effects such as mood disorders and addiction. Indeed, it is tempting to speculate that development of hyperkatifeia, which corresponds to an enhanced sensitivity to negative emotions observed during withdrawal from opiates or drug abuse (see Shurman et al., 2010) could rely on similar mechanisms than OIH. In the future, it will be interesting to study more precisely the adaptations in brain areas involved in mood disorders in OIH conditions.

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TABLES

Table 1: Effect of opioid receptor antagonists on morphine-induced hyperalgesia in preclinical models

Kind of treatment	Dose	Administration	Effect	Opioid antagonist	Nociception test	Result on OIH	Reference
morphine ultra low dose	1 μ g/kg	Subcutaneous injection	Acute	CTOP, 0.001 μ g intrathecally immediately before morphine	Latency before paw licking on hot plate 52.5°C	Prevents OIH	(Sanna et al., 2014, 2015a, Sanna et al., 2015b)
				Naloxone, 1mg/kg intraperitoneally 15 min. before morphine	Latency before paw licking on hot plate 52.5°C	Prevents OIH	
Morphine low dose	1.6mg/kg	Osmotic pump	Chronic	Naltrexone, 30mg pellet 24h before morphine	Latency before tail withdrawal in tail immersion 47.3°C	Provokes OIH	(Juni et al., 2006, Juni et al., 2008, Juni et al., 2010)
Morphine high dose	40mg/kg						

Table 2 : Neuroimmune inhibitors decrease OIH

Drug name and dose	Chronic opioid name and dose	Species Sex	OIH	Tolerance	Reference
IL-1ra 100 μ g it.	Morphine 10 μ g it.	Rats Males	Reduced	Reduced	(Johnston et al., 2004)

Anti-Cx3cr1 Antibody 10 μ g it.	Morphine 10 μ g it.	Rats Males	Reduced	Reduced	(Johnston et al., 2004)
IL-10 by adenovirus 5 μ g it.	Morphine 10 μ g it.	Rats Males	Reduced	Reduced	(Johnston et al., 2004)
AMD3100 Cxcr4 antagonist 10 mg/kg	Morphine 10 mg/kg	Rats Females	Reduced	Not tested	(Wilson et al., 2011)
Minocycline 30-100 mg/kg i.p.	Remifentanyl 240 μ g /kg/h i.v.	Rats Males	No effect	No effect	(Aguado et al., 2015)
Mac-1 Ab-saporin 20-36 μ g it.	Morphine 10 mg/kg sc.	Rats Males	Reduced	No effect	(Ferrini et al., 2013)
Bdnf-cKO in microglia	Morphine 10-40 mg/kg sc.	Mice Males	Reduced	No effect	(Ferrini et al., 2013)
IL-1ra/sTNFR/ anti-IL6 Ab 100 μ g /30 μ g / 0.08 μ g it.	Morphine 10 mg/kg sc.	Rats Males*	Reduced	Reduced	(Raghavendra et al., 2002)
Propentofylline 1-10 μ g it.	Morphine 10 mg/kg sc.	Rats Males	Reduced	Reduced	(Raghavendra et al., 2004)
Pentoxifylline 50 mg/kg i.p.	Morphine 10-40 mg/kg sc.	Mice Males ^r	Reduced	Not tested	(Liang et al., 2008)
IL1ra 100 mg/kg i.p. Ibuprofen 15 mg/kg i.p.	Morphine 20 mg/kg Codeine 21 mg/kg	Mice Males	Reduced	Not tested	(Johnson et al., 2014)
LPS-RS 20 μ g it.	Morphine	Rats	Reduced	Reduced	(Bai et al., 2014)

	10 μ g it.	Males			
IL1ra 100 μ g it.	M3G 0.75 μ g it.	Rats Males	Reduced	No analgesia	(Lewis et al., 2010)
Compound 15 Tlr4 inhibitor	M3G 10 mg/kg i.p.	Rats Females	Reduced	No analgesia	(Due et al., 2012)

All studies were performed on naïve animals except in * Raghavendra et al. 2002 where rats underwent spinal nerve transection, and in †Liang et al. 2008 where OIH was followed by hind paw incision.

Table 3 : Opioid Induced Hyperalgesia studies on TLR4 deficient mice

Mouse lines	Opiate treatments	Sensitivity assays	Result	Reference
C57BL/6 Ctrl B10ScNJ mutants	Morphine-3 glucuronide 25 mg/kg	Von Frey filaments Hot plate	OIH in Ctrl No OIH in mutants	(Due et al., 2012)
Balb/c Ctrl TLR4-KO mutants	Morphine 20mg/kg Codeine 21mg/kg 2x/day/4 days	Von Frey filaments	OIH in Ctrl No OIH in mutants	(Johnson et al., 2014)
C3H/HeOuJ Ctrl C3H/HeJ mutants B10ScSNJ Ctrl B10ScNJ mutants	Morphine 10- 40mg/kg 2x/day/7 days	Von Frey filaments	OIH in Ctrl OIH in mutants	(Mattioli et al., 2014)
C3H/HeOuJ Ctrl C3H/HeJ mutants	Morphine 10- 40mg/kg 2x/day/7 days	Von Frey filaments	OIH in Ctrl OIH in mutants	(Ferrini et al., 2013)

LEGENDS TO FIGURES

Figure 1. Tetrasynapse activation contributes to OIH.

Morphine activates neurons, astrocytes and microglia by inhibiting glutamate recapture and inducing production of pro-inflammatory molecules. In response to its activation, each cell will

produce more excitatory substances such as pro-inflammatory chemokines, cytokines, ATP or NO contributing to the establishment and the maintenance of OIH.

ERK, Extracellular signal-regulated kinase; mTOR, mammalian target of rapamycin; NFkB, nuclear factor kappa B; NO, nitric oxide; LTP, long-term potentiation.

Figure 2. Neuronal pathways involved in OIH

Activation of neuronal MOR by its agonists (i.e. morphine) induces regulation of intracellular mechanisms involved in OIH. Morphine activates mTOR signalling taking part in neuroexcitation and OIH. Morphine can also block the glutamate transporter EAAC1 involved in glutamate recapture. Synaptic concentrations of glutamate are thus increased. Glutamate will play an important role, through its receptor NMDA and the LTP to provoke OIH. Other factors are involved such as TrkB, the BDNF receptor, which will diminish KCC2 action, modulating the Cl⁻ homeostasis, leading to a switch in the role of GABA from inhibitory to stimulatory and thus producing OIH. Activated glial cells and neurons by morphine will contribute to OIH by producing cytokines such as IL1 β or TNF- α involved in LTP. Altogether, these mechanisms participate to the establishment and persistence of OIH.

BDNF, brain derived neurotrophic factor; Cx3Cl1, chemokine Cx3Cl1; EAAC1, glutamate transporter EAAC1; GABA-R, Gamma Aminobutyric Acid receptor; GRK, G protein coupled receptor kinase; IL1 β , Interleukin beta; IP3, inositol triphosphate; KCC2, K⁺/Cl⁻ cotransporter 2; LTP, long-term potentiation; MOR, mu-opioid receptor; mTOR, mammalian target of rapamycin; NO, nitric oxide; NOS, nitric oxide synthase; NK1, neurokinin 1 receptor; NMDA-R, N-methyl-D-aspartic acid receptor; PKC, protein kinase C; SP, substance P; TNF α , Tumor Necrosis Factor alpha; TrkB, Tyrosine Kinase B; TRPV1, Transient Receptor Potential vanilloid 1.

Figure 3. Microglia activation contributes to OIH.

Morphine binds to its specific receptor MOR or to TLR4 and will activate molecular intracellular cascades. This will trigger the activation of microglial cells. In addition, diverse pro-inflammatory molecules such as chemokines, cytokines and ATP also participate in reinforcing this activation and OIH.

BDNF, brain derived neurotrophic factor; Cx3Cl1, chemokine Cx3Cl1; CX3Cr1, CX3C chemokine receptor 1; CCL, chemokines with CC motif; CXCL, chemokines with CXC motif; ERK, Extracellular signal-regulated kinase; IL, interleukin; MOR, mu-opioid receptor; NFkB, nuclear factor kappa B; NO, nitric oxide; P2X4-R, purinergic receptor P2X4; S1P, sphingosin-1-phosphate; TLR4, toll like receptor 4; TNF α , tumor necrosis factor alpha; R, receptor.

Figure 4. Astrocytes are actors of OIH.

Concomitant actions of opiates and cytokines induce intracellular cascades leading to production and release of cytokines that confer an activated state to astrocytes. In addition, morphine is able to

block the glutamate transporter (GLT-1) resulting in an increase of glutamate in the synaptic cleft, which will then reverberate on neuronal excitation.

GLT1, glutamate transporter 1; IL-1 β , Interleukin-1 β ; IL1R, interleukin 1 receptor; IL-6, Interleukin 6; JNK, junk kinase; MOR, mu-opioid receptor; NFkB, nuclear factor kappa B; NO, Nitric Oxide; P2Y-R, purinergic receptor P2Y; P65, protein 65; PKC, Protein Kinase C; TNF α , tumor necrosis factor alpha; TNF α R, tumor necrosis factor alpha receptor.