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REVIEW

Functional plasticity of the N/OFQ-NOP receptor system determines analgesic properties of NOP receptor agonists

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Despite high sequence similarity between NOP (nociceptin/orphanin FQ opioid peptide) and opioid receptors, marked differences in endogenous ligand selectivity, signal transduction, phosphorylation, desensitization, internalization and trafficking have been identified; underscoring the evolutionary difference between NOP and opioid receptors. Activation of NOP receptors affects nociceptive transmission in a site-specific manner, with antinociceptive effects prevailing after peripheral and spinal activation, and pronociceptive effects after supraspinal activation in rodents. The net effect of systemically administered NOP receptor agonists on nociception is proposed to depend on the relative contribution of peripheral, spinal and supraspinal activation, and this may depend on experimental conditions. Functional expression and regulation of NOP receptors at peripheral and central sites of the nociceptive pathway exhibits a high degree of plasticity under conditions of neuropathic and inflammatory pain. In rodents, systemically administered NOP receptor agonists exerted antihypersensitive effects in models of neuropathic and inflammatory pain. However, they were largely ineffective in acute pain while concomitantly evoking severe motor side effects. In contrast, systemic administration of NOP receptor agonists to non-human primates (NHPs) exerted potent and efficacious antinociception in the absence of motor and sedative side effects. The reason for this species difference with respect to antinociceptive efficacy and tolerability is not clear. Moreover, co-activation of NOP and μ-opioid peptide (MOP) receptors synergistically produced antinociception in NHPs. Hence, both selective NOP receptor as well as NOP/MOP receptor agonists may hold potential for clinical use as analgesics effective in conditions of acute and chronic pain.

Abbreviations

CCI, chronic constriction injury; CFA, complete Freund's adjuvant; CPP, conditioned place preference; DOP, δ-opioid peptide; DRG, dorsal root ganglion; i.c.v., intracerebroventricular; i.pl., intraplantar; i.t., intrathecal; KOP, κ-opioid peptide; MOP, μ-opioid peptide; N/OFQ, nociceptin/orphaninFQ; NHP, non-human primate; NOP, nociceptin/orphaninFQ opioid peptide; NST, nocistatin; PAG, periaqueductal grey; RVM, rostral ventromedial medulla; SNL, spinal nerve ligation; WDR, wide dynamic range

Introduction

In 1994, soon after the cloning of μ-, δ- and κ-opioid receptors (MOP, DOP and KOP, respectively), several groups identified a GPCR with high homology to opioid receptors (Bunzow *et al.*, 1994; Fukuda *et al.*, 1994; Mollereau *et al.*, 1994; Nishi *et al.*, 1994; Wang *et al.*, 1994; for receptor nomenclature see Alexander *et al.*, 2013a), but very low affinity for opioid

ligands. Thus, this receptor was named opioid receptor like 1 (ORL1). In 1995, two groups independently identified the endogenous ORL1-ligand, a heptadecapeptide that was named nociceptin (Meunier *et al.*, 1995) for its ability to elicit hyperalgesia after supraspinal administration in mice and orphanin FQ (Reinscheid *et al.*, 1995) for its ability to recognize a previous orphan receptor and for its first and last amino acid residues [F (Phe) and Q (Gln)]. Following identification of nociceptin/orphanin FQ (N/OFQ) as the endogenous agonist of ORL1, the receptor was renamed nociceptin opioid peptide receptor and abbreviated as NOP receptor, considered a subcategory of the opioid peptide receptor family by IUPHAR (Cox *et al.*, 2014). However, N/OFQ acts at the molecular and cellular level in very much the same way as opioids to produce pharmacological effects that sometimes differ from, and even oppose, those of opioids. In fact, activation of NOP receptors translates into a very complex pharmacology of pain modulation leading to either pronociceptive or antinociceptive activity, depending on the route of administration, pain model and species employed. Furthermore, functional expression of the NOP receptor system has been shown to display a high degree of plasticity and is up-regulated under conditions of chronic pain (Briscini *et al.*, 2002; Chen and Sommer, 2006). Importantly, systemic administration of selective NOP receptor agonists exerted potent and efficacious analgesia in non-human primate (NHP) models of acute and inflammatory pain in the absence of side effects (Ko *et al.*, 2009; Podlesnik *et al.*, 2011; Cremeans *et al.*, 2012; Sukhtankar *et al.*, 2014). Activation of NOP receptors has been demonstrated to be devoid of reinforcing effects, but to inhibit opioid-mediated reward in rodents and NHPs (Ciccocioppo *et al.*, 2000; Rutten *et al.*, 2010; Podlesnik *et al.*, 2011). Strong opioids acting at MOP receptors continue to be widely used to treat moderate to severe acute and chronic pain. However, their therapeutic window is limited by severe side effects such as nausea and vomiting, constipation, dizziness, somnolence, respiratory depression, physical dependence and abuse (Zollner and Stein, 2007). Reduced effectiveness of MOP receptor agonists under conditions of chronic and neuropathic pain further narrows their therapeutic window (Rosenblum *et al.*, 2008; Labianca *et al.*, 2012). Hence, strong analgesics for the treatment of moderate to severe chronic nociceptive and neuropathic pain are urgently needed (Kissin, 2010). Both NOP receptor selective and bifunctional NOP/MOP receptor agonists have been proposed to have clinical value as analgesics with reduced abuse liability as compared with opioids (Lin and Ko, 2013; Toll, 2013). This review will focus on the functional expression and plasticity of the N/OFQ-NOP receptor system and its interaction with opioid receptors in relation to analgesia.

NOP receptor structure

Despite high sequence similarity of the NOP receptor to opioid receptor subtypes (63–65%), opioid peptides have very low affinity for the NOP receptor. However, the endogenous NOP receptor ligand N/OFQ shares sequence homology with other opioid peptides such as the endogenous κ-ligand dynorphin A, but does not interact with opioid receptors. Previous biochemical studies attributed this distinct selectivity profile to three residue positions in the binding pocket of the NOP receptor that differs from all other opioid receptors: Ala^{216} (Lys in others), Gln^{280} (His in others) and Thr^{305} (Ile in others). Recently, the three-dimensional crystal structures of the NOP receptor and of the opioid receptors MOP, DOP and KOP have been identified (Granier *et al.*, 2012; Manglik *et al.*, 2012; Thompson *et al.*, 2012; Wu *et al.*, 2012), revealing atomic details of ligand–receptor recognition and selectivity. In fact, the crystal structure of the human NOP receptor, solved in complex with the peptide mimetic antagonist compound-24 (C-24) revealed substantial conformational differences in the binding pocket regions between the NOP receptor and opioid receptors (Thompson *et al.*, 2012). The crystal structure of the NOP receptor provides evidence that the three residues Ala^{216}, Gln^{280} and Thr^{305} point towards the interior of the binding pocket and that Gln^{280} and Thr^{305} are involved in C-24 interaction. Remarkably, the NOP receptor binding pocket with Gln^{280} does not have any hydrogen-bonding water molecules, whereas in opioid receptors, this glutamine is replaced by histidine forming a hydrogen bond network with two water molecules. This has consequences for the terminal structural moieties of binding peptide ligands, as the hydrophobic and hydrophilic parts of the binding pockets of opioid receptors and NOP receptor are different. In the case of opioid peptides with a Tyr–Gly–Gly–Phe sequence, the hydroxy group of the phenols are involved in the hydrogen bond network, whereas in nociceptin with Phe–Gly–Gly–Phe sequence that has no hydroxy group, hydrophobic interactions are preferred as in C-24. Furthermore, it has been shown that the three NOP receptor-specific residue changes are involved in a large-scale reshaping of the binding pocket. In opioid receptors, Lys^{227} is located at the entrance of the ligand binding pocket and involved in salt bridges with the side chains of Asp^{223} and Glu^{297}. In the NOP receptor, Lys is replaced by alanine, preventing these stabilizing ionic interactions and leading to an outward shift of the extracellular half of helix V in the NOP crystal structure, and an inward shift of helix VI, reshaping the entrance to the pocket. Thus, despite high sequence homology between the NOP receptor and opioid receptors, marked differences in endogenous ligand selectivity between these receptors go in hand with substantial changes in the structure of their binding pockets, underscoring the evolutionary differences between NOP and opioid receptors.

N/OFQ precursor

N/OFQ is produced from a larger precursor pre-pro-N/OFQ (ppN/OFQ) composed of 176 amino acids that is located on chromosome 8p21 in humans (Mollereau *et al.*, 1996). ppN/OFQ encodes two additional peptides, N/OFQ-II (17 amino acids) and nocistatin (NST; 30 amino acids in human) (Calo' *et al.*, 2000). PCR-based evidence for N/OFQ mRNA turnover is in fact measuring the precursor and hence these additional cleavage products. The target site(s) for N/OFQ-II and NST are the subject of intense debate. N/OFQ-II has antinociceptive actions (Rossi *et al.*, 1998; 2002) and increases locomotor activity in rodents (Florin *et al.*, 1997). The precise cellular target site is unknown. There is more interest and data for

NST (Okuda-Ashitaka *et al.*, 1998), which is described, in general, as an anti-N/OFQ peptide where reversal of N/OFQ effects on glutamate release (Nicol *et al.*, 1998), the antimorphine effect in the brain (Zhao *et al.*, 1999) and impairment of learning and memory (Hiramatsu *et al.*, 2008) have been reported. There are a range of direct actions that are beyond the scope of this paper. What is clear is that NST does not interact with the NOP receptor (Neal *et al.*, 2003), but the cellular target again remains unknown (Johnson and Connor, 2007). One study from Okuda-Ashitaka *et al.* (1998) attempts to address this using NST-conjugated affinity latex beads to uncover NST binding partners. One target of interest was NIPSNAP1 (4-nitrophenylphosphate domain and non-neuronal SNAP25-like protein homologue 1), a protein involved in vesicle trafficking, and it was shown that NST inhibition of N/OFQ-induced tactile allodynia is absent in NIPSNAP1 knockout mice (Okuda-Ashitaka *et al.*, 2012). What is clear from this discussion is that tissues capable of producing and releasing N/OFQ are also producing a potential antagonist of these actions; the relative amounts and actions at different sites are at present unclear.

Signal transduction of the NOP receptor

Similar to opioid receptors, the NOP receptor has been shown to inhibit adenylate cyclase, to activate inwardly rectifying potassium channels, and to close calcium channels via coupling to *Pertussis* toxin (PTX)-sensitive Gi/o proteins (Ma *et al.*, 1997; Margas *et al.*, 2008). However, in contrast to opioid receptors, the NOP receptor has also been shown to couple to PTX-insensitive G-proteins, such as Gz, G16 or Gs (Chan *et al.*, 1998; Klukovits *et al.*, 2010).

According to cell type or tissue, numerous studies revealed that the activated NOP receptor triggers a variety of intracellular signalling events, including modulation of adenylate cyclase activity (Ma *et al.*, 1997; Klukovits *et al.*, 2010) and activation of PKC (Lou *et al.*, 1997), PLA_2 (Fukuda *et al.*, 1998) and PLC (Lou *et al.*, 1997), ERK1/2 (Fukuda *et al.*, 1997; Lou *et al.*, 1997), p38 MAPK (Zhang *et al.*, 1999), JNK (Chan and Wong, 2000), and NF-κB (Donica *et al.*, 2011). Moreover, it has been reported that STAT3 may be involved in the transduction of NOP receptor signalling (Wu *et al.*, 2003).

NOP receptor activation reduces neuronal excitability and neurotransmitter release by inhibition of presynaptic, voltage-gated calcium channels (Connor *et al.*, 1996b; Knoflach *et al.*, 1996) and activation of inwardly rectifying potassium channels (Connor *et al.*, 1996a; Vaughan *et al.*, 1997). NOP receptor activation has been shown to inhibit the release of a wide range of neurotransmitters including noradrenaline, dopamine, 5-HT, ACh and glutamate (Nicol *et al.*, 1996; 1998; 2002; Schlicker and Morari, 2000). This NOP receptor-mediated reduction in neurotransmitter release is the basis for its modulation of many biological functions that rely on synaptic transmission, including nociception, anxiety and reward.

Indeed, activation of Gi-coupled NOP receptors has been shown to inhibit $Ca_v2.2$ N-type calcium channels (for nomenclature see Alexander *et al.*, 2013b) to attenuate nociception. However, it has been demonstrated that the sensitivity of N-type channels to G–protein-mediated inhibition is regulated by alternative splicing of the exons 37a and 37b in $Ca_v2.2$ pre-mRNAs (Raingo *et al.*, 2007). The most common form of G–protein-mediated inhibition of N-type currents is voltage-dependent and requires Gβγ, which binds directly to $Ca_v2.2$ (both channel variants 37a and 37b) and is independent of Src tyrosine kinase. Voltage-independent inhibition is unique to the $Ca_v2.2$-e37a isoform, requires Src tyrosine kinase-mediated phosphorylation of the C-terminal Y1747 and is independent of Gβγ-binding. Activation of Gi-coupled NOP receptor produces free Gβγ-subunit mediating a signalling pathway involving PI-3K and Src-kinase (Hawes *et al.*, 1998). Activated Src-kinase then plays a role in the phosphorylation and inhibition of the nociceptor-specific exon 37a splice isoform of $Ca_v2.2$. The 37b splice isoform of $Ca_v2.2$ lacks the Src-specific tyrosine phosphorylation site and therefore can only be inhibited via direct Gβγ-binding. The e37a splice variant of $Ca_v2.2$ is highly enriched in nociceptors of dorsal root ganglia (Bell *et al.*, 2004) leading to an increased cellular sensitivity to inhibition by activated MOP receptors and behavioural sensitivity to spinal morphine-induced analgesia (Andrade *et al.*, 2010).

Interaction of NOP receptors with N-type calcium channels

Recent data support the concept that NOP receptors and N-type calcium channels can form signalling complexes, which are internalized into vesicular compartments after prolonged NOP receptor activation, effectively inhibiting calcium influx into the cell (Altier *et al.*, 2006; Evans *et al.*, 2010). However, a recent study examining the effect of NOP receptor activation on N-type calcium channels in a highly N/OFQ-sensitive subpopulation of rat dorsal root ganglion (DRG) and spinal cord neurons found that, although N/OFQ treatment inhibited primary afferent excitatory postsynaptic currents on dorsal horn neurons, it did not induce internalization of N-type calcium channels in the cell body or nerve terminals of DRG neurons (Murali *et al.*, 2012). Other studies revealed that the NOP receptor associates with and inhibits N-type calcium channels, even in the absence of N/OFQ (Beedle *et al.*, 2004). Thus, although there is agreement over the ability of the N/OFQ-NOP receptor system to inhibit N-type calcium channels, the precise mechanism by which the NOP receptor regulates calcium channel activity, and whether this involves NOP receptor-mediated internalization of these channels is still under debate.

Cellular NOP receptor expression and function in tissues

The NOP receptor and its ligand N/OFQ are widely expressed in the CNS and in the peripheral nervous system as well as in many peripheral organs and the immune system in rodents, NHPs and humans (reviewed in Mollereau and Mouledous, 2000; Civelli, 2008). In particular, the NOP receptor is

expressed in DRG, in the dorsal and ventral horns of the spinal cord, in the forebrain, including cortical areas, olfactory regions, the thalamus, and a variety of limbic structures, such as the hippocampus, septum, the bed nucleus of the stria terminalis, the diagonal band of Broca, the habenula, the amygdaloid complex, and in several nuclei of the hypothalamus, that are involved in the processing of emotional stimuli. The NOP receptor is also localized in 5-hydroxytryptaminergic, noradrenergic, and dopaminergic nuclei, such as the raphe complex, the locus coeruleus, the nucleus of the solitary tract, the ventral tegmental area, and the substantia nigra (Neal *et al.*, 1999a; Mollereau and Mouledous, 2000). A similar pattern of N/OFQ and NOP receptor expression in human and rodent CNS has been observed (Peluso *et al.*, 1998; Berthele *et al.*, 2003; Witta *et al.*, 2004).

NOP receptor expression has also been found in other peripheral tissues, namely in rodent and human intestines (Menzies *et al.*, 1999; Agostini *et al.*, 2009; Li *et al.*, 2013), in human blood lymphocytes (Wick *et al.*, 1995), in several peripheral sensory and sympathetic ganglia from guinea-pigs (Kummer and Fischer, 1997) and rats (Xie *et al.*, 1999), in rabbit retina (Neal *et al.*, 1997) and in rat heart (Giuliani *et al.*, 1997; Dumont and Lemaire, 1998). In line with this wide distribution of the N/OFQ-NOP receptor system, N/OFQ modulates many physiological responses/systems, including anxiety (Jenck *et al.*, 1997), food intake (Pomonis *et al.*, 1996), learning and memory (Sandin *et al.*, 1997), locomotor activity (Reinscheid *et al.*, 1995; Florin *et al.*, 1996), respiratory (Corboz *et al.*, 2000; 2001; Takita *et al.*, 2003; Takita and Morimoto, 2008; Singh *et al.*, 2013), immune (Peluso *et al.*, 2001; Serhan *et al.*, 2001), urinary bladder (Lecci *et al.*, 2000; Lazzeri *et al.*, 2001; 2006), cardiovascular and renal functions (Kapusta *et al.*, 1997). NOP receptors identified on pre- and/or postganglionic sympathetic and parasympathetic nerve fibres and innervating blood vessels and heart are involved in the cardiovascular effects of N/OFQ, and might play a role in the pathophysiology of inflammation, arterial hypertension and cardiac or brain circulatory ischaemia (Malinowska *et al.*, 2002; Serrano-Gomez *et al.*, 2011; Brookes *et al.*, 2013; Thompson *et al.*, 2013).

Splice variants of the NOP receptor

There are several publications describing NOP receptor splice variants. For example, RT-PCR and RNAse protection studies of NOP receptor transcripts in the human CNS as well as human immune cells identified a splice variant of the human NOP receptor lacking 15 nucleotides at the junction between exons 1 and 2 (Halford *et al.*, 1995; Peluso *et al.*, 1998). This splice variant (Δ15hNOP receptor) encodes a receptor that lacks a -YVILR- motif in the N-terminal portion of the first intracellular loop. Analysis of the distribution of this shorter receptor isoform demonstrated no significant difference from that of the full-length human NOP receptor (Peluso *et al.*, 1998). However, the short (truncated) form displays a marked reduction in binding affinity for a range of NOP receptor ligands and this is coupled with loss of function (Pan *et al.*, 1998; Xie *et al.*, 2000; Curro *et al.*, 2001).

Remarkably, four additional NOP receptor splice variants have been identified, including a rat variant that contains a 81 bp insertion between the second and third coding exons (Wang *et al.*, 1994), and three splice variants isolated from mouse brain with insertions of 34 bp (KOR-3a), 98 bp (KOR-3b), and 139 bp (KOR-3c) between exons 1 and 2 (Pan *et al.*, 1998). The expression of the three variants in the mouse brain varies markedly among brain regions with a distribution quite distinct from the NOP receptor itself, indicating region-specific NOP receptor splicing. However, whether these splice variants have a functional relevance beyond ligand decoy awaits further investigation.

NOP receptor internalization and trafficking

For many GPCRs, it is widely known that sustained agonist activation can lead to receptor phosphorylation and internalization (Pierce and Lefkowitz, 2001). Activation of NOP receptors with N/OFQ produces rapid and robust receptor internalization over time (Spampinato *et al.*, 2001; 2002; 2007; Corbani *et al.*, 2004; Baiula *et al.*, 2013). Recently, it was demonstrated that NOP receptor phosphorylation at carboxyl terminal serine 363 by GPCR kinase 3 plays an important role in N/OFQ-induced NOP receptor desensitization, β-arrestin2-recruitment, internalization and arrestin-dependent JNK MAPK signalling (Zhang *et al.*, 2012). This finding is consistent with previous data suggesting that arrestin binding to GPCRs may enable MAPK activation, and acts to regulate receptor function (Bohn *et al.*, 2004; Bruchas *et al.*, 2006; Shenoy and Lefkowitz, 2011). Similar to opioid receptors, the NOP receptor has been shown to efficiently recycle to the plasma membrane after agonist-induced internalization (Spampinato *et al.*, 2007). Together, these findings indicate, that NOP receptors are regulated via similar, but unique mechanisms as compared with opioid receptors.

Heterodimerization of NOP receptors with opioid receptors

NOP receptors have also been shown to form heterodimers with MOP, DOP or KOP receptors (Pan *et al.*, 2002; Wang *et al.*, 2005; Evans *et al.*, 2010). The NOP/opioid receptor heterodimers were co-internalized after N/OFQ or opioid agonist treatment indicating that the heterodimers can be trafficked together as a complex (Evans *et al.*, 2010). The formation of MOP/NOP receptor heterodimers was found to impair MOP receptor-activated signalling pathways (Mandyam *et al.*, 2003; Wang *et al.*, 2005). Therefore, it seems reasonable to suggest, that MOP/NOP receptor heterodimerization may lead to the impairment of MOP receptor-mediated biological effects in the brain contributing to NOP receptor-mediated antiopioid effects. However, a recent study revealed that the internalization of NOP receptor/$Ca_v2.2$ complexes following prolonged exposure to N/OFQ may be dependent on the formation of NOP/MOP receptor heterodimers (Evans *et al.*, 2010). Moreover, the NOP receptor was shown to function as

a molecular link that allows MOP receptors to trigger N-type channel internalization. These findings indicate that formation of NOP/MOP receptor heterodimers affects receptor function with consequences for NOP receptor- and MOP receptor-mediated N-type calcium channel regulation. Indeed, the search for drug molecules that target MOP/NOP receptor heterodimers has already met with some success. Recently, iodobenzoylnaltrexamide (IBNtxA) has been shown to target MOR1G/NOP receptor heterodimers, displaying a full analgesic response without the side effects of opioids (Majumdar *et al.*, 2011). MOR1G is a truncated, six-transmembrane variant of the MOP receptor, which lacks exon 1, and is generated from a second, upstream promoter associated with exon 11. The exon 11-associated MOR-1G splice variant alone is insufficient to generate the IBNtxA binding site and requires heterodimerization with NOP receptors. Collectively, these findings strongly indicate that pharmacological and signalling properties of NOP/MOP receptor heterodimers are different from those of the individual receptors.

Functional expression of the N/OFQ-NOP receptor system in acute pain

In rodents, N/OFQ and NOP receptor expression has been extensively studied at both transcript and protein levels using a combination of *in situ* hybridization, radioligand binding and immunohistochemical approaches. Their constitutive expression pattern has been described and reviewed elsewhere (Anton *et al.*, 1996; Neal *et al.*, 1999a,b; 2001; Mollereau and Mouledous, 2000). Because of the widespread distribution of NOP receptors and the pleiotropic effects of N/OFQ, the NOP receptor holds promise for numerous therapeutic applications (Lambert, 2008; Calo and Guerrini, 2013). In this review, we specifically focus on the functional expression of the N/OFQ-NOP receptor system in relation to nociception and its regulation under conditions of chronic neuropathic and inflammatory pain. Functional NOP receptors are expressed at peripheral, spinal and supraspinal sites of the ascending and descending pain pathways (Table 1, Figure 1). N/OFQ reduced capsaicin-induced nociception after peripheral administration in mice (Sakurada *et al.*, 2005) and exerted spinal antinociceptive effects in the tail flick test in rats (Xu *et al.*, 1996; Tian *et al.*, 1997a) and mice (King *et al.*, 1997). Moreover, spinal N/OFQ potentiated systemic morphine antinociception (Tian *et al.*, 1997a). However, when administered intracerebroventricularly (i.c.v.), N/OFQ was pronociceptive in the hot plate test (Meunier *et al.*, 1995) and tail flick test (Reinscheid *et al.*, 1995) in mice. Other authors found that i.c.v. N/OFQ was not effective alone, but reversed antinociception induced by systemic and i.c.v. morphine in the tail flick test in rats (Tian *et al.*, 1997a) and mice (King *et al.*, 1998) respectively. The potent truncated N/OFQ analogue [Phe1psi(CH2-NH)Gly2]N/OFQ(1-13)-NH2 was antinociceptive after intrathecal (i.t.) administration whereas it exerted pronociceptive effects when administered i.c.v. in the rat tail flick test (YQ Wang *et al.*, 1999b). In the mouse tail withdrawal assay, i.c.v. N/OFQ and [Phe1psi(CH2-NH)Gly2]N/OFQ(1-13)-NH2 were pronociceptive *per se* and inhibited i.c.v. morphine antinociception (Calo *et al.*, 1998). In addition, evidence was provided showing that low doses of N/OFQ induce nociceptive behaviour after peripheral and i.t. administration in naïve mice (Inoue *et al.*, 1999; Sakurada *et al.*, 1999). In summary, when comparing different routes of local administration, NOP receptor agonists elicited either antinociceptive or pronociceptive effects as well as potentiated or counteracted opioid-mediated antinociception depending on the site of action in rodent models of acute pain.

The effects of N/OFQ on nociceptive processing in rodents after peripheral, spinal and supraspinal administration as well as the underlying cellular and molecular mechanisms have been reviewed in detail elsewhere (Grisel and Mogil, 2000; Moran *et al.*, 2000; Mogil and Pasternak, 2001; Zeilhofer and Calo, 2003). In the peripheral nervous system, N/OFQ was shown to inhibit neurotransmitter release (Giuliani *et al.*, 2000), which might explain its inhibitory effect on substance P-mediated nociceptive flexor reflex in mice (Inoue *et al.*, 1999).

In the spinal cord, N/OFQ analgesia is mediated by inhibiting excitatory glutamatergic nociceptive transmission via activation of pre- and postsynaptic NOP receptors. The majority of NOP receptors in rat spinal cord seems to be expressed on intrinsic neurons (Le Cudennec *et al.*, 2002). Presynaptic NOP receptors are located in primary afferents arising from small- and medium-sized DRG neurons, corresponding to C- and Aδ-fibres respectively (Chen and Sommer, 2006). Activation of presynaptic NOP receptors inhibited N-type Ca^{2+} channels leading to reduced transmitter release from central terminals of primary afferent fibres (Helyes *et al.*, 1997), thereby attenuating excitatory nociceptive input to the rat spinal cord dorsal horn (Lai *et al.*, 1997; Liebel *et al.*, 1997; Luo *et al.*, 2002; Murali *et al.*, 2012). Dorsal horn spinothalamic tract neurons convey nociceptive signals to supraspinal sites. N/OFQ hyperpolarized and hence inhibited electrical activity of these neurons by increasing K^+ currents (Lai *et al.*, 1997; Luo *et al.*, 2001) secondary to postsynaptic NOP receptor activation. Consequently, N/OFQ inhibited A– and C–fibre-mediated compound action potentials in an *ex vivo* rat hemisected spinal cord preparation (Faber *et al.*, 1996) as well as C-fibre-evoked wind-up of rat spinal dorsal horn neurons *in vivo* (Stanfa *et al.*, 1996). Interestingly, N/OFQ inhibited C–fibre-evoked responses more potently than A–fibre-evoked responses (Faber *et al.*, 1996; Luo *et al.*, 2002). Furthermore, N/OFQ inhibited excitatory amino acid-evoked responses of rat trigeminal dorsal horn neurons by a postsynaptic mechanism (Wang *et al.*, 1996).

We have already demonstrated that spinal NOP receptor activation elicits an antinociceptive response via mechanisms similar to those described for opioids; pre- and post-synaptic inhibition at the first synapse in the pain pathway. Moreover, that supraspinal NOP receptor activation produces an anti-opioid action resulting in a hyperalgesic response. Anti-opioid actions cover opiates produced by stress in the original description of N/OFQ by Meunier *et al.* (1995), several peptide and non-peptide opioids selective for opioid receptors (Mogil *et al.*, 1996b; Chen *et al.*, 2007) and electroacupuncture (Tian *et al.*, 1997b). As an anti-opioid N/OFQ is not unique as there is evidence of similar actions for other

Table 1

Functional expression of the NOP receptor in rodent nociceptive system and effects of N/OFQ and NOP receptor agonists in rodent models of acute pain

		NOP receptor expression		Function of N/OFQ and NOP receptor agonists		
Region		**mRNA**	**Protein**	***In vitro***	***In vivo***	
Supraspinal	Thalamus	Many thalamic nuclei [ISH] (Neal *et al.*, 1999a)	Many thalamic nuclei [RLB] (Neal *et al.*, 1999a)		↓ firing rate of thalamic neurons [*in vivo* electrophysiology rat] (Albrecht *et al.*, 2001)	i.c.v.: no effect alone, but reduced i.c.v. DAMGO-induced antinociception [tail withdrawal mouse] (Mogil *et al.*, 1996b) i.c.v.: no effect alone, but reversed systemic and i.c.v. morphine-induced antinociception in rats [tail flick] (Tian *et al.*, 1997a) and mice [tail flick] (King *et al.*, 1998), [tail withdrawal] (Mogil *et al.*, 1996a) i.c.v.: pronociceptive alone, inhibited i.c.v. morphine-induced antinociception [tail withdrawal mouse] (Calo *et al.*, 1998) i.c.v. N/OFQ(1-13): pronociceptive [tail flick rat] (YQ Wang *et al.*, 1999b) i.c.v.: pronociceptive [hot plate mouse] (Liu *et al.*, 2006) i.c.v.: pronociceptive [tail flick mouse] (Wang *et al.*, 2006)
	Amygdala	Many amygdaloid nuclei [ISH] (Neal *et al.*, 1999a)	Many amygdaloid nuclei [RLB] (Neal *et al.*, 1999a), [IHC] (Anton *et al.*, 1996)	↑ GIRK current in CeA neurons [patch clamp] (Meis and Pape, 1998) ↓ IPSC in CeA neurons [patch clamp] (Roberto and Siggins, 2006) ↑ K^+ current, hyperpolarized CeA-PAG projection neurons [patch clamp] (Chen *et al.*, 2009) (Chieng and Christie, 2010)	m.i.: antinociceptive [tail flick rat] (Shane *et al.*, 2001)	
	PAG	High expression through entire extent [ISH] (Neal *et al.*, 1999a)	Dense N/OFQ binding [RLB] (Neal *et al.*, 1999a)	↑ K^+ current, ↓ IPSCs and EPSCs [patch clamp] (Vaughan *et al.*, 1997) ↓ N-, P/Q-type Ca^{2+} current [patch clamp] (Connor and Christie, 1998)	m.i.: reduced intra-PAG morphine-induced antinociception [tail flick rat] (Morgan *et al.*, 1997) m.i.: antinociceptive [tail flick rat] (Shane *et al.*, 2003) m.i.: pronociceptive [tail flick rat] (Lu *et al.*, 2010)	
	RVM	High expression in C1, A1 cell group [ISH] (Neal *et al.*, 1999a)	Moderate N/OFQ binding in C1, A1 cell group [RLB] (Neal *et al.*, 1999a)	↑ K^+ current, ↓ Ca^{2+} current in primary and secondary RVM neurons [patch clamp] (Vaughan *et al.*, 2001)	m.i.: no effect alone, but reduced intra-RVM DAMGO-induced antinociception [tail flick rat] (Heinricher *et al.*, 1997) m.i.: no effect alone, but reduced intra-PAG DAMGO-induced antinociception [tail flick rat] (Pan *et al.*, 2000)	

Spinal	Intrinsic neurons	Lamina I, II, X, ventral horn [ISH] (Pettersson *et al.*, 2002) Dorsal, ventral horn [ISH] (Mika *et al.*, 2003)	Dorsal horn [RLB] (Pettersson *et al.*, 2002) Majority of N/OFQ binding sites spared by rhizotomy (Le Cudennec *et al.*, 2002)	↓ A- and C-fibre responses [hemisected spinal cord] (Faber *et al.*, 1996) Hyperpolarized lamina II neurons by ↑ K^+ conductance [patch clamp] (Lai *et al.*, 1997) ↓ glutamate-, kainate-, quisqualate-induced currents in DH neurons [patch clamp] (Shu *et al.*, 1998) ↑ GIRK current in lamina II neurons [patch clamp] (Luo *et al.*, 2001)	i.t.: ↓ C-fibre evoked wind-up of DH neurons [*in vivo* electrophysiology rat] (Stanfa *et al.*, 1996) i.t.: antinociceptive, potentiated s.c. morphine-induced antinociception [tail flick rat] (Tian *et al.*, 1997a) i.t. N/OFQ(1-13): antinociceptive [tail flick rat] (YQ Wang *et al.*, 1999b)
	Proximal PAF (presynaptic)		Rhizotomy reduced N/OFQ binding sites by 18% [RLB] (Le Cudennec *et al.*, 2002)	↓ Aδ-, C-fibre EPSCs of lamina I and lamina II neurons [patch clamp] (Lai *et al.*, 1997; Liebel *et al.*, 1997; Luo *et al.*, 2002; Murali *et al.*, 2012)	
Peripheral	DRG neuron (cell body)	Large neurons [ISH] (Pettersson *et al.*, 2002) Co-localization in majority of SP- and CGRP-positive neurons [ISH, RT-PCR] (Mika *et al.*, 2003)	Medium and large neurons [RLB] (Pettersson *et al.*, 2002) Small and medium neurons [IHC] (Chen and Sommer, 2006)	↓ T-type Ca^{2+} currents in medium-sized neurons [patch clamp] (Abdulla and Smith, 1997) ↓ N-type Ca^{2+} currents (Abdulla and Smith, 1998) in small IB4 negative neurons [patch clamp] (Murali *et al.*, 2012)	
	Distal PAF (dendritic)	Not in skin [ISH] (Pettersson *et al.*, 2002)			s.c. in the tail: antinociceptive [tail flick mouse] (Kolesnikov and Pasternak, 1999) i.pl.: ↓ i.pl. capsaicin-induced nociception in mice (Sakurada *et al.*, 2005) i.pl. Ro64-6198: no effect on mechanical and thermal nociception [PPT, PWL rat] (Obara *et al.*, 2005)

No data are published for fields that are left blank. ↑, activation/increase; ↓, inhibition/decrease; CeA, central nucleus of amygdala; CGRP, calcitonin gene-related peptide; DAMGO, (D-Ala^2, N-Me-Phe^4, Gly-ol)-enkephalin; DH, dorsal horn; EPSC, excitatory postsynaptic current; GIRK, G–protein-activated inward rectifier K^+; IB4, isolectin B4; IHC, immunohistochemistry; IPSC, inhibitory postsynaptic current; ISH, *in situ* hybridization; m.i., microinjected; PAF, primary afferent fibre; PPT, paw pressure test; PWL, paw withdrawal latency; RLB, radioligand binding; Ro64-6198, selective NOP receptor agonist; SP, substance P.

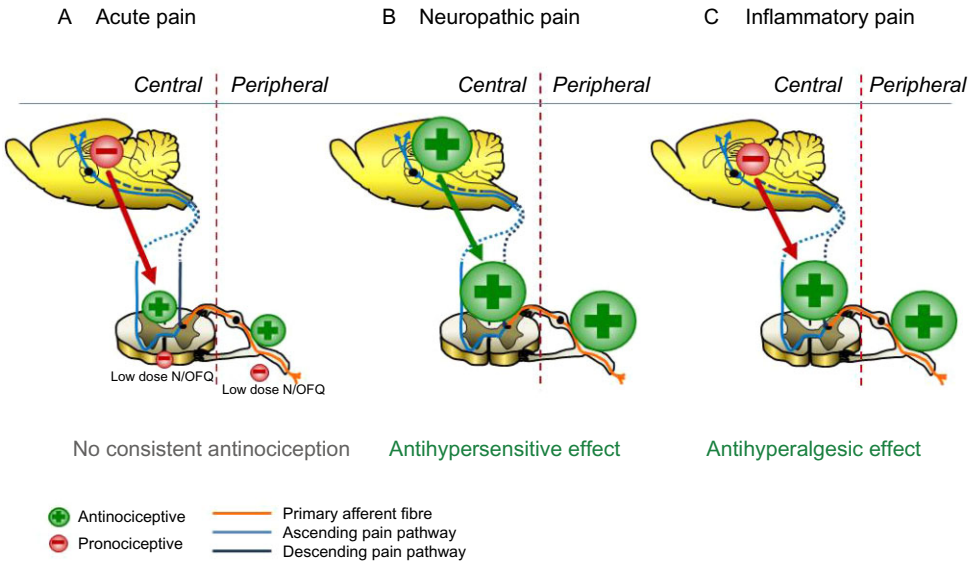

Figure 1

Schematic presentation summarizing the effects of NOP receptor activation on nociceptive processing at peripheral, spinal and supraspinal sites, and resulting analgesic effects of systemically administered NOP receptor agonists under conditions of acute, neuropathic and inflammatory pain in rodents. (A) NOP receptor agonists were largely ineffective in acute pain after systemic administration as activation of supraspinal NOP receptors counteracted spinally and peripherally mediated antinociception. Pronociceptive effects were also elicited by low concentrations of N/OFQ at peripheral and spinal sites. (B) In contrast, systemic administration of NOP receptor agonists elicited antihypersensitive effects in neuropathic pain as, here, activation of supraspinal NOP receptors did not counteract, but contributed to analgesic efficacy. In addition, peripheral, spinal and supraspinal NOP receptors were up-regulated and functionally sensitized. (C) Inhibition of nociceptive processing elicited by activation of functionally sensitized peripheral and spinal NOP receptors is hypothesized to overcome pronociceptive effects of supraspinal NOP receptor activation, thus leading to antihyperalgesic efficacy after systemic administration of NOP receptor agonists in inflammatory pain. Larger symbols indicate up-regulation/functional sensitization of NOP receptors.

peptides such as cholecystokinin (Heinricher *et al.*, 2001). This apparent hyperalgesia results from an interaction with descending inhibitory control machinery from the rostral ventromedial medulla (RVM) back to the spinal dorsal horn. In its simplest form, this can be explained based on the activity of two types of cells located in the RVM; OFF (or primary) cells and ON (or secondary) cells. OFF cells project from the RVM back to the spinal cord and increased activity reduces afferent inflow – resulting in 'descending inhibition'. In the RVM, OFF cells are tonically inhibited by ON cells via GABAergic mechanisms (Fields, 2004). MOP receptors are located on the ON cells where activation with drugs like morphine (or endogenous opioids) inhibits their activity (Heinricher *et al.*, 1994). This effectively takes the brakes from the system (disinhibits) and allows the OFF cells to fire leading to a supraspinal MOP receptor-mediated antinociceptive response (Heinricher *et al.*, 1994). NOP receptors are located in both the ON and OFF cells, but in terms of explaining their anti-opioid actions their location on, and inhibition of OFF cells takes primacy (Heinricher *et al.*, 1997). Direct inhibition of OFF cell firing would effectively reverse any disinhibition of this subset of cells produced by either endogenous or exogenous MOP receptor agonists (Heinricher *et al.*, 1997). The resulting anti-opioid action would produce hyperalgesia. This is described schematically in several publications (Pan *et al.*, 2000; Zeilhofer and Calo, 2003; Lambert, 2008). The effect of chronic pain conditions on the functionality and interaction between the ON and OFF cells has not been investigated in rodents. In addition, it is not clear whether this circuitry also exists in the RVM of NHPs and humans.

The net effect of systemically administered NOP receptor agonists on nociception is proposed to depend on the relative contribution of peripheral, spinal and supraspinal sites of action, which in turn may depend on experimental conditions (Figure 1). While systemic administration of the potent and selective non-peptide NOP receptor agonist Ro64-6198 exerted antinociceptive effects in the mouse hot plate test, it increased pain sensitivity in the mouse tail flick test, an effect attributed to a supraspinally mediated inhibition of stress-induced analgesia (Reiss *et al.*, 2008). Ro64-6198 was not antinociceptive in the rat tail flick test (Jenck *et al.*, 2000; Dautzenberg *et al.*, 2001), or in the tail immersion test in mice after systemic administration (Kotlinska *et al.*, 2003). In agreement with these latter studies, Ro64-6198 did not change mechanical and thermal nociceptive thresholds of naïve rats after i.t. and intraplantar (i.pl.) administration in the paw pressure and paw withdrawal latency test respectively (Obara *et al.*, 2005). While systemic Ro64-6198 was not convincingly antinociceptive across a range of rodent models of acute pain, it consistently produced severe motor side effects over the same dose range (Higgins *et al.*, 2001; Varty *et al.*, 2005; Reiss *et al.*, 2008).

As in rodents, peripherally and spinally delivered peptide NOP receptor agonists inhibited thermal nociception in NHPs (Ko *et al.*, 2002; 2006; Ko and Naughton, 2009; Hu *et al.*, 2010). Interestingly, pronociceptive effects after periph-

eral and spinal administration of low doses of N/OFQ were not evident in NHPs (Ko *et al.*, 2002; Ko and Naughton, 2009). The effect of supraspinally delivered NOP receptor agonists on nociception has not yet been investigated in NHPs. However, in sharp contrast to results obtained in rodents, systemic administration of Ro64-6198 to NHPs exerted potent and fully efficacious thermal antinociception in the absence of motor and sedative side effects (Ko *et al.*, 2009; Podlesnik *et al.*, 2011; Cremeans *et al.*, 2012). Hence, a profound species difference exists between rodents and NHPs with respect to antinociceptive efficacy and tolerability of systemically administered selective NOP receptor agonists (Table 2). The reason for this functional difference is not yet clear. Although radioligand binding autoradiography also demonstrated widespread expression of NOP receptors in the brain and spinal cord of NHPs, subtle differences in areas relevant for nociception were observed as compared with the expression pattern in rodents. Specifically, lower NOP receptor expression was observed in raphe nuclei and in the spinal cord dorsal horn in NHPs compared with rodents (Bridge *et al.*, 2003). Therefore, it is tempting to speculate that the functional species difference described earlier might result from differences in NOP receptor expression and from a different functional effect of NOP receptors on nociceptive processing particularly in supraspinal pain circuits. Elucidating the consequences of NOP receptor activation on functionally characterized cells of such neuronal networks in the NHP and human RVM should be one focus of future research. Moreover, investigating the effect of supraspinally delivered NOP receptor agonists on nociception in NHPs will further contribute to our understanding of species differences in antinociceptive efficacy of systemically administered NOP receptor agonists and is, therefore, highly warranted.

Also in humans, both N/OFQ and NOP receptor mRNA and protein has been detected in nociceptive structures of the CNS and peripheral nervous system (Peluso *et al.*, 1998; Mollereau and Mouledous, 2000; Berthele *et al.*, 2003; Witta *et al.*, 2004). The pattern of NOP receptor expression in humans was in general agreement with that seen in NHPs (Peluso *et al.*, 1998; Bridge *et al.*, 2003). It has been suggested that NHP models of nociception can provide a translational bridge for research and development of NOP receptor and opioid receptor-related ligands (Lin and Ko, 2013). However, clinical proof-of-concept trials testing for analgesic properties of NOP receptor agonists after systemic administration to assess their therapeutic potential for diverse pain indications are still urgently awaited.

Functional expression and regulation of the N/OFQ-NOP receptor system in neuropathic pain

N/OFQ elicited potent and efficacious antihypersensitive effects in rodent models of neuropathic pain (Table 3). For example, spinally delivered N/OFQ inhibited thermal hyperalgesia as well as mechanical allodynia and hyperalgesia in the rat chronic constriction injury (CCI) model (Yamamoto *et al.*, 1997c; Corradini *et al.*, 2001; Courteix *et al.*, 2004) and reduced mechanical allodynia in the rat spinal nerve ligation (SNL) model (Ju *et al.*, 2013). N/OFQ selectively inhibited mechanical hyperalgesia in CCI rats while it had no effect on mechanical pain thresholds in naïve rats (Courteix *et al.*, 2004). Of more interest, pre-emptive i.t. administration of N/OFQ delayed the development of thermal hyperalgesia and decreased expression of c-Fos in the rat CCI model (Yamamoto *et al.*, 2000). Similar to N/OFQ, Ro64-6198 inhibited mechanical and cold allodynia after peripheral and spinal administration in rats subjected to CCI, whereas Ro64-6198 had no effect on mechanical and thermal pain thresholds in naïve animals (Obara *et al.*, 2005). This is mirrored by the fact that microiontophoretically applied N/OFQ inhibited spontaneous and noxious mechanically evoked activity of spinal wide dynamic range (WDR) neurons in CCI, but not in sham and intact rats (Sotgiu *et al.*, 2004). This result is in line with the observation of increased inhibition of N-type Ca^{2+} channel currents by N/OFQ in DRG neurons after sciatic nerve section (Abdulla and Smith, 1998). Furthermore, the antinociceptive potency of spinal N/OFQ in the tail flick test was greater in mice with diabetic polyneuropathy than in non-diabetic mice (Kamei *et al.*, 1999). This neuropathy-related functional sensitization of the NOP receptor system might be explained, at least in part, by an up-regulation of the NOP receptor. Indeed, NOP receptor mRNA was up-regulated in ipsilateral lumbar (L)5-L6 DRG and lumbar spinal cord of rats displaying mechanical allodynia 7 days after induction of CCI with transcript levels returning to baseline as allodynia resolved at day 15 (Briscini *et al.*, 2002). Moreover, the number of NOP receptor mRNA positive cells increased also in the rat periaqueductal grey (PAG) and RVM 7–14 days after CCI (Ma *et al.*, 2005). N/OFQ immunoreactivity was found to be increased in rat cingulate cortex, but not in the PAG and RVM, 14 days after CCI (Rosen *et al.*, 2000) and in rat amygdala and PAG 36 days after SNL (Sun *et al.*, 2001). Both NOP receptor protein and N/OFQ immunoreactivity seemed to be up-regulated in small- and medium-sized L4 DRG neurons in rats 7 and 14 days after partial sciatic nerve transection (Chen and Sommer, 2006). Therefore, the peripheral, spinal and supraspinal N/OFQ-NOP receptor system seems to respond to nerve injury with a reversible and temporally coordinated up-regulation at discrete sites of the nociceptive pathways in rodents.

The moderately selective non-peptide NOP receptor agonists SR14150 and SR16835 displayed NOP, but not MOP receptor-dependent antiallodynic efficacy in SNL mice at rather high systemic doses (Khroyan *et al.*, 2011). In addition, the selective non-peptide NOP receptor agonists GRT-TA2210 and Ro65-6570 also exerted potent antiallodynic effects in the mouse CCI model after spinal, supraspinal and systemic administration (Linz *et al.*, 2013). It is especially noteworthy that, after supraspinal administration, NOP receptor agonists showed antiallodynic efficacy in a model of neuropathic pain. This is in sharp contrast to their lacking or even pronociceptive efficacy under conditions of acute pain (see earlier) and inflammatory pain (see later). It might be hypothesized that this qualitative change in supraspinal NOP receptor functionality in rodent models of neuropathic pain translates into antihypersensitive efficacy after systemic administration as activation of both spinal and supraspinal NOP receptors sustain efficacy (Figure 1B). In line with this notion, systemic administration of Ro65-6570 exerted

Table 2

Comparison of analgesic properties of N/OFQ and NOP receptor agonists between rodents and NHPs

Administration route	Pain modality	Pharmacological action	Rodents	NHPs
Peripheral	Acute pain	Antinociceptive effects	s.c. N/OFQ in the mouse tail Local antinociception inhibited by naloxone (Kolesnikov and Pasternak, 1999)	
		No antinociceptive effects	i.pl. Ro64-6198 in naïve rats (Obara *et al.*, 2005)	s.c. N/OFQ in the NHP tail (Ko *et al.*, 2002)
		Biphasic (low dose: nociceptive vs. high dose: antinociceptive)	i.pl. N/OFQ (0.01 fmol–1 nmol) in the nociceptive flexor test in mice (Inoue *et al.*, 1999)	
		No biphasic effects		s.c. N/OFQ (1 pg–30 μg) in the NHP tail withdrawal assay (Ko *et al.*, 2002)
	Capsaicin allodynia	Antiallodynic effects	i.pl. N/OFQ in naïve mice (Sakurada *et al.*, 2005)	s.c. N/OFQ in the NHP tail Local antinociception inhibited by J-113397 (Ko *et al.*, 2002)
	Neuropathic pain	Antihypersensitive effects	i.pl. N/OFQ and Ro64-6198 in CCI rats (Obara *et al.*, 2005) Local antihypersensitive effect inhibited by Nphe (Obara *et al.*, 2005)	
Spinal	Acute pain	Antinociceptive effects	i.t. N/OFQ in the rat and mouse tail flick assay (Xu *et al.*, 1996; King *et al.*, 1997; Tian *et al.*, 1997a) Spinal antinociception inhibited by naltrexone (King *et al.*, 1997) and UFP-101 (Nazzaro *et al.*, 2007) in mice, by naloxone in rats (Jhamandas *et al.*, 1998), by MOP and DOP receptor antagonists in rats (Yu *et al.*, 2002)	i.t. N/OFQ in the NHP tail withdrawal assay (Ko *et al.*, 2006; Ko and Naughton, 2009) Spinal antinociception inhibited by J-113397, not naltrexone (Ko *et al.*, 2006)
		No antinociceptive effects	i.t. Ro64-6198 in naïve rats (Obara *et al.*, 2005)	
		Biphasic (low dose: nociceptive vs. high dose: antinociceptive)	i.t. N/OFQ (fmol) in mice (Inoue *et al.*, 1999; Sakurada *et al.*, 1999)	
		No biphasic effects		i.t. N/OFQ (1 fmol–1 μmol) in the NHP tail withdrawal assay (Ko and Naughton, 2009)
	Capsaicin allodynia	Antiallodynic effects	i.t. N/OFQ in rats with mechanical allodynia (Nozaki-Taguchi and Yamamoto, 2005)	i.t. UFP-112 in NHPs with thermal allodynia (Hu *et al.*, 2010)
	Neuropathic pain	Antihypersensitive effects	See details in Table 3	

Table 2

Continued

Administration route	Pain modality	Pharmacological action	Rodents	NHPs
Systemic	Acute pain	Antinociceptive effects	i.p. Ro64-6198 in the mouse hot plate test (Reiss *et al.*, 2008) Systemic antinociception was absent in NOP receptor knockout mice (Reiss *et al.*, 2008)	s.c. Ro64-6198 in the NHP tail withdrawal assay (Ko *et al.*, 2009) i.v. Ro64-6198 in the NHP tail withdrawal assay (Ko, 2004; Podlesnik *et al.*, 2011) i.m. Ro64-6198 and SCH221510 in the NHP tail withdrawal assay (Cremeans *et al.*, 2012) Systemic antinociception inhibited by J-113397 (Ko *et al.*, 2009)
		No antinociceptive effects	i.p. Ro64-6198 in the rat tail flick test (Jenck *et al.*, 2000; Dautzenberg *et al.*, 2001) i.p. Ro64-6198 in the mouse tail immersion test (Kotlinska *et al.*, 2003)	
		Pronociceptive effects	i.p. Ro64-6198 in the mouse tail flick assay (Reiss *et al.*, 2008)	
		Potentiation of MOP receptor agonist-induced antinociception	Ro64-6198 potentiated morphine antinociception in an additive manner (Reiss *et al.*, 2008)	Ro64-6198 potentiated buprenorphine antinociception in a synergistic manner (Cremeans *et al.*, 2012)
	Capsaicin allodynia	Antiallodynic effects		s.c. Ro64-6198 in the NHP thermal allodynia assay (Ko *et al.*, 2009)
	Neuropathic pain	Antihypersensitive effects	i.v. GRT-TA2210 and Ro65-6570 in CCI mice (Linz *et al.*, 2013) i.v. and i.p. Ro65-6570 in neuropathic mice and rats (Schiene *et al.*, 2013)	
	Inflammatory pain	Antihyperalgesic effects	i.v. GRT-TA2210 in the mouse formalin test (Linz *et al.*, 2013) i.v. Ro65-6570 in the rat formalin and CFA assays (Schiene *et al.*, 2013)	s.c. Ro64-6198 in the NHP carrageenan assay (Sukhtankar *et al.*, 2014)

No data are published for fields that are left blank. GRT-TA2210, selective NOP receptor agonist; J-113397, selective NOP receptor antagonist; Nphe, [Nphe1]N/OFQ(1-13)NH2, a selective NOP receptor antagonist; Ro64-6198, Ro65-6570, selective NOP receptor agonists; SCH-221510, selective NOP receptor agonist; UFP-101, selective NOP receptor antagonist; UFP-112, selective NOP receptor agonist.

potent and efficacious antihyperalgesic and antiallodynic effects in mouse and rat models of mono- and poly-neuropathic pain without confounding locomotor side effects (Schiene *et al.*, 2013). Exploring the influence of chronic neuropathic pain conditions on the expression and functional interaction of NOP receptors with the ON–OFF cell circuitry in the rodent RVM is needed to further substantiate this concept.

For ethical reasons, models of neuropathic pain are only sparsely available in NHPs. While analgesics like pregabalin ($\alpha 2\delta$ Ca^{2+} channel subunit modulator) and duloxetine (serotonin-noradrenaline re-uptake inhibitor) have been shown to exert antiallodynic efficacy in a cynomolgus monkey L7 SNL model (Hygate *et al.*, 2012a,b), no published data are available on effects of NOP receptor agonists in NHP models of neuropathic pain or on the regulation of N/OFQ

Table 3

Functional expression of the NOP receptor in rodent nociceptive system and effects of N/OFQ and non-peptide NOP receptor agonists under conditions of neuropathic pain

		NOP receptor expression		Function of N/OFQ and NOP receptor agonists	
Region		**mRNA**	**Protein**	***In vitro***	***In vivo***
Supraspinal	Thalamus				
	Amygdala				
	PAG	Up-regulated 7–14 days after CCI [ISH] (Ma *et al.*, 2005)			Endogenous N/OFQ tone maintained, since m.i. UFP-101, inhibited mechanical allodynia [CCI rat] (Scoto *et al.*, 2009) i.c.v. GRT-TA2210, Ro65-6570: ↓ cold allodynia [CCI mouse] (Linz *et al.*, 2013)
	RVM	Up-regulated 7–14 days after CCI [ISH] (Ma *et al.*, 2005)			
Spinal	Intrinsic neurons	Up-regulated in lumbar enlargement 7 days after CCI [RT-PCR] (Briscini *et al.*, 2002)			i.t.: ↓ thermal hyperalgesia [partial sciatic nerve injury rat, CCI rat] (Yamamoto and Nozaki-Taguchi, 1997; Yamamoto *et al.*, 1997c) i.t.: ↓ mechanical and cold allodynia [sciatic nerve injury rat] (Hao *et al.*, 1998) i.t.: ↓ flexor reflex [sciatic nerve transection rat] (Xu *et al.*, 1999) i.t.: antinociceptive in diabetic poly-neuropathy [tail flick mouse] (Kamei *et al.*, 1999) i.t.: ↓ mechanical allodynia and hyperalgesia [CCI rat] (Corradini *et al.*, 2001; Briscini *et al.*, 2002; Courteix *et al.*, 2004) i.t.: synergistic interaction with i.t. morphine to suppress mechanical hyperalgesia [CCI rat] (Courteix *et al.*, 2004)
	Proximal PAF (presynaptic)				i.t.: ↓ spontaneous and mechanically evoked activity of lamina V WDR neurons [CCI rat] (Sotgiu *et al.*, 2004) i.t. Ro64-6198, N/OFQ: ↓ mechanical and cold allodynia [CCI rat] (Obara *et al.*, 2005) i.t.: ↓ mechanical allodynia [SNL rat] (Ju *et al.*, 2013) i.t. GRT-TA2210, Ro65-6570: ↓ cold allodynia [CCI mouse] (Linz *et al.*, 2013)
Peripheral	DRG neuron (cell body)	Up-regulated in L5-L6 7 days after CCI [RT-PCR] (Briscini *et al.*, 2002)	Up-regulated 7 days after sciatic nerve injury [IHC] (Chen and Sommer, 2006)	↑ inhibition of N-type Ca^{2+} current after sciatic nerve section [patch clamp] (Abdulla and Smith, 1998)	
	Distal PAF (dendritic)				i.pl. Ro64-6198, N/OFQ: ↓ mechanical and cold allodynia [CCI rat] (Obara *et al.*, 2005)

No data are published for fields that are left blank. ↑, activation/increase; ↓, inhibition/decrease; GRT-TA2210, selective NOP receptor agonist; IHC, immunohistochemistry; ISH, *in situ* hybridization; m.i., microinjected; PAF, primary afferent fibre; Ro64-6198, Ro65-6570, selective NOP receptor agonists; UFP-101, selective NOP receptor antagonist.

or NOP receptor expression in NHPs under neuropathic conditions.

Functional expression and regulation of the N/OFQ-NOP receptor system in inflammatory pain

N/OFQ also elicited potent and efficacious antihypersensitive effects in rodent models of inflammatory pain (Table 4). N/OFQ exerted antihyperalgesic effects in a rat model of trinitrobenzene sulfonic acid (TNBS)-induced colonic hyperalgesia after peripheral administration. Interestingly, peripheral injection of the NOP receptor-selective peptide antagonist UFP-101 not only inhibited the effect of N/OFQ, but exacerbated visceral hyperalgesia when administered alone (Agostini *et al.*, 2009).

In the rat formalin test, N/OFQ was antinociceptive after i.t. administration, whereas it exerted pronociceptive effects and antagonized opioid analgesia when administered i.c.v. (Erb *et al.*, 1997; Yamamoto *et al.*, 1997a; Zhu *et al.*, 1997; Hao and Ogawa, 1998; JL Wang *et al.*, 1999a). The ability of NOP receptors to bidirectionally modulate nociception in a site-specific manner was also corroborated in the mouse formalin test where UFP-101 exerted antinociceptive and pronociceptive effects after i.c.v. and i.t. administration respectively (Rizzi *et al.*, 2006). This supports the notion of endogenous N/OFQ tone mediating spinal antinociception and supraspinal pronociception. Likewise, in the rat complete Freund's adjuvant (CFA)-induced arthritis model, [Phe1psi(CH2-NH)Gly2]N/OFQ(1-13)-NH2 induced hyperalgesia, and similar to N/OFQ, inhibited systemic morphine antinociception after i.c.v. administration (Bertorelli *et al.*, 1999). In the rat model of carrageenan-induced inflammation, i.t. N/OFQ inhibited thermal hyperalgesia (Yamamoto *et al.*, 1997b; Hao *et al.*, 1998) and the nociceptive flexor reflex (Xu *et al.*, 1999). Hence, in rodent models of inflammatory pain, NOP receptor agonists also elicited antinociceptive or pronociceptive effects depending on spinal or supraspinal sites of action, respectively (Figure 1C).

N/OFQ-mediated inhibition of C-fibre evoked responses of rat spinal dorsal horn neurons was increased 4 h after carrageenan treatment, which is indicative of a functional sensitization of spinal NOP receptors under inflammatory conditions (Carpenter *et al.*, 2000). Indeed, several studies

Table 4

Functional expression of the NOP receptor in rodent nociceptive system and effects of N/OFQ under conditions of inflammatory pain

		NOP receptor expression		N/OFQ function	
Region		**mRNA**	**Protein**	***In vitro***	***In vivo***
Supraspinal	Thalamus Amygdala PAG RVM				i.c.v.: ↑ pain response, antagonized opioid analgesia [formalin test rat] (Zhu *et al.*, 1997; JL Wang *et al.*, 1999a) i.c.v.: antagonized morphine analgesia [CFA inflammation rat] (Bertorelli *et al.*, 1999) i.c.v.: no inhibition of colonic hyperalgesia [colorectal distension in TNBS-treated rats] (Agostini *et al.*, 2009)
Spinal	Intrinsic neurons		Up-regulation in L4 dorsal horn (laminae I, II) 4 days after CFA [RLB] (Jia *et al.*, 1998)		i.t.: analgesic [formalin test rat] (Erb *et al.*, 1997; Yamamoto *et al.*, 1997a; Hao and Ogawa, 1998; YQ Wang *et al.*, 1999a) i.t.: ↓ thermal hyperalgesia, ↓ flexor reflex [carrageenan inflammation rat] (Yamamoto *et al.*, 1997b; Hao *et al.*, 1998; Xu *et al.*, 1999)
	Proximal PAF (presynaptic)				↑ inhibition of C-fibre evoked responses of spinal neurons 4h after carrageenan [*in vivo* electrophysiology rat] (Carpenter *et al.*, 2000)
Peripheral	DRG neuron (cell body)		Up-regulation at day 1 and 7 after CFA [IHC] (Chen and Sommer, 2006; 2007)		
	Distal PAF (dendritic)				i.v.: inhibition of articular mechanosensitivity [carrageenan-induced knee joint inflammation, rat] (McDougall *et al.*, 2000) i.p.: inhibition of colonic hyperalgesia [colorectal distension in TNBS-treated rats] (Agostini *et al.*, 2009)

No data are published for fields that are left blank. ↑, activation/increase; ↓, inhibition/decrease; IHC, immunohistochemistry; PAF, primary afferent fibre; RLB, radioligand binding.

reported increased expression of N/OFQ and NOP receptors after inflammatory challenges in rodents. As rapidly as 0.5–3 h after carrageenan-induced inflammation, a transient increase in ppN/OFQ mRNA expression was observed in transient receptor potential cation channel, subfamily V 1-positive rat DRG neurons (Andoh *et al.*, 1997; Itoh *et al.*, 2001). N/OFQ immunoreactivity was increased in rat dorsal spinal cord, cingulate cortex and hypothalamus 14 days after carrageenan inflammation (Rosen *et al.*, 2000). NOP receptor protein was found to be up-regulated in rat superficial dorsal spinal cord 4 days after CFA-induced inflammation using radioligand binding (Jia *et al.*, 1998). Both NOP receptor protein and N/OFQ immunoreactivity seemed to be up-regulated in rat DRG neurons 7 days after CFA inflammation (Chen and Sommer, 2006).

Systemic administration of GRT-TA2210 displayed full antihyperalgesic efficacy in the mouse formalin test (Linz *et al.*, 2013), whereas Ro65-6570 exerted moderate antihyperalgesic efficacy in the rat models of formalin- and CFA-induced inflammatory pain without confounding locomotor side effects (Schiene *et al.*, 2013). The selective non-peptide NOP receptor agonist SCH-221510 showed anti-inflammatory and analgesic activity in a mouse model of TNBS-induced inflammatory bowel disease after systemic administration (Sobczak *et al.*, 2013; 2014). Systemically administered selective NOP receptor agonists displayed antihyperalgesic efficacy in inflammatory pain, but lacked antinociceptive efficacy in acute pain in rodents. This pain state-dependent functionality of the N/OFQ-NOP receptor system is mirrored in mice carrying a global knockout of either ppN/OFQ or the NOP receptor, or both. These mice displayed normal sensitivity to acute pain, but showed increased inflammatory hyperalgesia compared with their wild-type littermates (Depner *et al.*, 2003).

In rhesus monkeys, systemic Ro64-6198 attenuated carrageenan-induced thermal hyperalgesia an order of magnitude more potently than it blocked acute thermal nociception (Sukhtankar *et al.*, 2014) reminiscent of the increased potency of spinal N/OFQ in carrageenan-treated rats (Carpenter *et al.*, 2000). It would be interesting to investigate whether functional sensitization of the N/OFQ-NOP receptor system observed in NHPs under inflammatory conditions is accompanied by altered expression as described for rodents.

Interaction of NOP and MOP receptors in relation to analgesia

Early immunohistochemical studies in rodents demonstrated an overlapping distribution, but no co-localization of N/OFQ and the NOP receptor with opioid peptides and the MOP receptor, respectively, in areas involved in nociceptive processing (Schulz *et al.*, 1996; Monteillet-Agius *et al.*, 1998). However, doubts were raised with respect to the specificity of the NOP receptor antibody used (Neal *et al.*, 1999a). Indeed, a patch clamp study analysing N/OFQ- and morphine-induced suppression of N-type Ca^{2+} channels in rat DRG neurons demonstrated that NOP receptors may be functionally co-expressed with MOP receptors on the very same neuron (Abdulla and Smith, 1998). Cellular co-expression and heterodimerization of NOP and MOP receptors was also found in human neuroblastoma cells (Mandyam *et al.*, 2003). In the rat RVM, NOP and MOP receptors are functionally co-expressed in ON cells, whereas only NOP, but no MOP receptors are expressed in OFF cells (Pan *et al.*, 2000; Vaughan *et al.*, 2001). The functional consequences of this site-specific pattern of NOP and MOP receptors being either co-expressed or showing segregated cellular expression are believed to underlie spinal antinociceptive and supraspinally mediated pronociceptive actions of N/OFQ. Hence, NOP and MOP receptors interact directly at the level of individual cells as well as indirectly at the neuronal circuitry level. Effects of N/OFQ were not inhibited by the opioid receptor antagonist naloxone in *in vitro* (Faber *et al.*, 1996; Abdulla and Smith, 1997; 1998; Lai *et al.*, 1997; Liebel *et al.*, 1997; Shu *et al.*, 1998) and *in vivo* (Xu *et al.*, 1996; Sotgiu *et al.*, 2004) electrophysiological studies. However, behavioural studies reported discrepant results. Although naltrexone, a MOP receptor antagonist, inhibited spinal N/OFQ analgesia in the tail flick and formalin test (King *et al.*, 1997; Hao and Ogawa, 1998), the MOP receptor antagonists naloxone (Erb *et al.*, 1997; Yamamoto *et al.*, 1997a) and β-funaltrexamine (Kamei *et al.*, 1999) did not. I.t. N/OFQ increased withdrawal latencies to thermal and mechanical stimuli in naïve rats, antinociceptive effects that were attenuated by i.t. NOP, MOP and DOP, but not KOP receptor antagonists (Jhamandas *et al.*, 1998; Yu *et al.*, 2002). In the rat SNL model, spinal MOP as well as DOP and KOP receptors contributed to the antiallodynic effects of i.t. N/OFQ (Ju *et al.*, 2013). Another line of evidence elucidating complex interactions between NOP and opioid receptors comes from studies with knockout mice in acute heat nociception and diabetic heat hyperalgesia (Christoph *et al.*, 2013). From the above, it seems clear that MOP as well as other opioid receptors interact with NOP receptors in a complex manner and may contribute to NOP receptor-mediated analgesia depending on experimental conditions.

Indeed, initial studies demonstrated that spinal N/OFQ increased systemic and spinal morphine analgesia in rodent models of acute and neuropathic pain (Tian *et al.*, 1997a; Courteix *et al.*, 2004). Isobolographic analysis indicated that spinal NOP and MOP receptors interact synergistically to inhibit mechanical hyperalgesia in the rat CCI model (Courteix *et al.*, 2004), whereas combining systemic subthreshold doses of Ro64-6198 and morphine reduced pain sensitivity in an additive manner in the mouse hot plate test (Reiss *et al.*, 2008). Subsequent studies in NHPs reported that spinal N/OFQ potentiated spinal morphine-induced antinociception (Ko and Naughton, 2009), and that a combination of inactive spinal doses of the selective peptide NOP receptor agonist University of Ferrara Peptides [(pF)Phe^{4}Aib^{7}Arg^{14}Lys^{15}] N/OFQ-NH2 (UFP)-112 and morphine produced antihyperalgesia (Hu *et al.*, 2010). Interestingly, this antihyperalgesic effect was completely antagonized only by a combination of the NOP receptor antagonist J-113397 with the MOP receptor antagonist naltrexone whereas either antagonist alone tended to partially inhibit antihyperalgesic efficacy without reaching statistical significance (Hu *et al.*, 2010). Most importantly, co-activation of NOP and MOP receptors after systemic administration of respective agonists synergistically produced antinociception in NHPs as revealed by isobolographic analysis (Cremeans *et al.*, 2012). These findings

suggest that NOP/MOP receptor agonists may hold potential for clinical use as analgesics with efficacy in acute and chronic pain. Furthermore, as both modes of action contribute to analgesia, the relative dose of each component may be reduced, thus potentially leading to an improved side effect profile of NOP/MOP receptor agonists over selective MOP receptor agonists (Lin and Ko, 2013; Toll, 2013). Recently, the bifunctional NOP/MOP receptor agonists BU08028 and SR16435 were shown to exert antihyperalgesic and antiallodynic effects after spinal administration in mouse models of inflammatory and neuropathic pain with potencies higher than those of selective NOP and MOP receptor agonists. Antiallodynic efficacy of both BU08028 and SR16435 was partially inhibited by either spinal J-113397 or naltrexone, but only the combined administration of both antagonists completely inhibited this response (Sukhtankar *et al.*, 2013). The increased potencies of bifunctional NOP/MOP receptor agonists as compared with selective NOP and MOP receptor agonists in inflammatory and neuropathic pain is hypothesized to result from a (supra)additive interaction between both mechanisms of action at the spinal level. Under inflammatory conditions both spinal NOP (see earlier) as well as MOP receptors (Maekawa *et al.*, 1996) are up-regulated, whereas under conditions of neuropathic pain, functional sensitization of the NOP component (see earlier) might compensate for reduced contribution of the MOP component to spinal analgesia (Ossipov *et al.*, 1995; Porreca *et al.*, 1998; Rashid *et al.*, 2004; Kohno *et al.*, 2005), thus retaining good efficacy and potency for bifunctional NOP/MOP receptor agonists also in neuropathic pain. In addition, spinal SR16435 showed delayed development of analgesic tolerance to antiallodynic efficacy as compared with a MOP receptor agonist, an advantageous effect also attributed to co-activation of NOP and MOP receptors (Sukhtankar *et al.*, 2013). Whereas SR16435 was equally rewarding as morphine (Khroyan *et al.*, 2007), another bifunctional NOP/MOP receptor agonist, SR14150, did not display rewarding properties in the rat conditioned place preference (CPP) paradigm (Toll *et al.*, 2009). This latter observation is in line with previous reports demonstrating that i.c.v. N/OFQ (Ciccocioppo *et al.*, 2000) and systemic Ro65-6570 (Rutten *et al.*, 2010) reduced, whereas pharmacological blockade or genetic ablation of the NOP receptor (Rutten *et al.*, 2011) increased the rewarding properties of systemic morphine in the rat CPP test. Clearly, the rewarding properties of bifunctional NOP/MOP agonists are determined by the relative affinities and activities at NOP and MOP receptors (Toll, 2013). In summary, these findings indicate that combined NOP/MOP receptor agonists may prove to be effective analgesics in acute and chronic pain with reduced tolerance and abuse liability as compared with selective MOP receptor agonists.

Translational approaches to the N/OFQ-NOP receptor system

In addition to a demonstration of sufficient compound exposure to the site of action allowing target engagement, another important aspect of translational research is the demonstration of functional target modulation across species (Morgan *et al.*, 2012).

Functional NOP receptor modulation might be monitored by suitable blood-borne biomarkers; for example, i.v. administration of N/OFQ to naïve rats increased the expression of CD11b on circulating neutrophils (Brookes *et al.*, 2007). Moreover, i.c.v. N/OFQ has been reported to transiently increase plasma prolactin levels in rats (Bryant *et al.*, 1998), an effect related to the NOP receptor-mediated inhibition of tuberoinfundibular dopaminergic neurons of the hypothalamic–pituitary circuitry (Chesterfield *et al.*, 2006). Interestingly, systemic administration of the KOP receptor agonist spiradoline has been shown to elevate plasma prolactin levels both in rats and humans (Chang *et al.*, 2011). Whether this also holds true after systemic administration of NOP receptor agonists should be addressed in future research.

Central NOP receptor modulation has also been reported as an inhibition of spontaneous bursting in the rat electroencephalogram (EEG) after i.v. administration of the selective NOP receptor agonist Ro65-6570 (Byford *et al.*, 2007). Assessment of EEG activity changes induced by dosing of investigational compounds (pharmaco-EEG) is an established assessment in a number of species, including humans. A prerequisite for recording pharmaco-EEG in NHPs and humans is that the target needs to be expressed cortically to allow signal detection by external electrodes. Cortical expression of NOP receptors in NHPs and humans has recently been demonstrated using a PET approach (Kimura *et al.*, 2011; Lohith *et al.*, 2012; Hostetler *et al.*, 2013). In a double-blind, placebo-controlled study in visceral pain patients suffering from chronic pancreatitis, the analgesic pregabalin induced EEG changes, with individual changes correlating with brief pain inventory composite scores. In contrast, placebo treatment was devoid of EEG effects (Graversen *et al.*, 2012). Thus, for pregabalin, pharmaco-EEG has been successfully used as a biomarker for functional target modulation that was also related to analgesic efficacy. Whether pharmaco-EEG may be suited to detect functional activation of NOP receptors in humans requires detailed investigation.

Finally, identification of biomarkers for efficacy of NOP receptor activation with utility across species and allowing prediction of treatment outcomes in humans is the ultimate aim of translational research in this area. In a recent elegant study, it was demonstrated that responses of spinal cord WDR neurons of naïve rats evoked by suprathreshold thermal stimulation translate to intensities of perceived heat pain in healthy volunteers (Sikandar *et al.*, 2013). Spinal N/OFQ inhibited evoked responses of WDR neurons both in naïve rats (Stanfa *et al.*, 1996) and in rats with carrageenan-induced inflammation and CCI-induced mononeuropathic pain (Carpenter *et al.*, 2000; Sotgiu *et al.*, 2004). Interestingly, these studies showed that the N/OFQ-mediated inhibition of spinal nociceptive processing was enhanced after induction of inflammatory and neuropathic pain states. Therefore, it is tempting to speculate that NOP receptor agonists might be effective analgesics also in human conditions of acute and chronic pain. Indeed, several analgesics like morphine and fentanyl (MOP receptor agonists), and pregabalin and tapentadol (MOP receptor agonist/noradrenaline re-uptake inhibitor) attenuated evoked WDR neuron responses in naïve animals as well as in rat models of chronic pain after spinal, and more importantly, from a clinical perspective, also after systemic administration (Homma *et al.*, 1983; Suzukawa

et al., 1983; Urch *et al.*, 2005; Bee and Dickenson, 2008; Bee *et al.*, 2011). As described earlier, systemically administered NOP receptor agonists exert analgesic effects in models of acute and inflammatory pain in NHPs, whereas in rodents, they are largely ineffective in models of acute pain, but effective in inflammatory and neuropathic pain. Therefore, elucidating the ability of systemically administered NOP receptor agonists to inhibit evoked activity of rat WDR neurons, depending on pain condition and stimulus modality, will be a next step to define whether this rodent approach holds translational predictability for the analgesic activity of NOP receptor agonists in NHPs and, eventually, in humans.

The property of NOP receptor agonists to modulate sensory processing of nociceptive input at various neuraxial sites in rodent and NHP models of evoked pain responses is well documented. Demonstrating the ability of an analgesic to affect spontaneous pain as well as the affective component of pain perception has been suggested to be more predictive for analgesic efficacy and improvement of quality of life in pain patients (Rice *et al.*, 2008; Mao, 2012). Indeed, spinal N/OFQ was reported to inhibit spontaneous activity of WDR neurons (Sotgiu *et al.*, 2004) as well as spontaneous pain behaviour in rats (Sun *et al.*, 2004). With respect to the affective dimension of pain, the central nucleus of the amygdala is believed to represent one neuroanatomical substrate where an emotional connotation is added to the pain experience (Neugebauer *et al.*, 2004). Effective analgesic treatments that also reduce comorbidities of chronic pain like anxiety are urgently needed. Most interestingly, negative emotion may even lead to or exacerbate pain, a relationship, which is also encoded in the amygdala (Wiech and Tracey, 2009). N/OFQ and its receptor were detected in the amygdala of rodents (Neal *et al.*, 1999a,b), NHPs (Bridge *et al.*, 2003) and humans (Peluso *et al.*, 1998; Witta *et al.*, 2004). In rats, N/OFQ has been reported to increase inwardly rectifying K^+ currents in central amygdala neurons (Meis and Pape, 1998), and to exert anxiolytic-like effects when administered i.c.v. or microinjected in the central amygdala (Jenck *et al.*, 1997; Vitale *et al.*, 2006; Uchiyama *et al.*, 2008). Furthermore, selective non-peptide NOP receptor agonists were also reported to induce anxiolytic-like effects in rodents after systemic administration (Jenck *et al.*, 2000; Varty *et al.*, 2005; 2008; Hayashi *et al.*, 2009; Lu *et al.*, 2011; Goeldner *et al.*, 2012). Likewise, an anxiogenic-like phenotype was demonstrated in NOP receptor-knockout mice (Gavioli *et al.*, 2007) and rats (Rizzi *et al.*, 2011), further supporting a role for the N/OFQ-NOP receptor system in modulating anxiety-related behaviour. Hence, it is possible that NOP receptor agonists may also exert anxiolytic effects in chronic pain patients.

Conclusions

Although the N/OFQ-NOP receptor system shares similarities with opioid receptor systems, pronounced differences exist at the molecular, cellular and behavioural level. The N/OFQ-NOP receptor system modulates nociceptive processing in a site-dependent manner with antinociceptive effects dominating at peripheral and spinal sites and pronociceptive effects at supraspinal sites in rodents. In addition, the system is subject to functional regulation under conditions of chronic pain and interacts with opioid receptor systems to produce powerful analgesia. Whereas systemic administration of NOP receptor agonists exerts potent and efficacious antinociception in NHPs, they largely lack efficacy in rodent models of acute pain. The intriguing plasticity of the N/OFQ-NOP receptor system in pain states and the interaction with MOP receptors offers new avenues of investigation in the field of opioid research. Although there are still many specific gaps in our understanding of rodent N/OFQ-NOP receptor pain pharmacology, focusing future research on investigations of antinociceptive efficacy and tolerability in NHPs is probably more compelling. Specifically, elucidating effects of supraspinally administered NOP receptor agonists on NHP pain processing and exploring mechanisms of functional NOP receptor sensitization in inflammatory pain states clearly deserves further attention. Finally, establishing a robust translational trajectory, especially for measures of target modulation and analgesic efficacy from rodents to NHPs and eventually to humans will be the key to successfully moving NOP receptor agonists into appropriate clinical proof-of-concept trials to investigate their potential as innovative analgesics in diverse pain indications.

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Conflict of interest

D. G. L. held consultancies with Grünenthal GmbH. M. C. K. holds consultancies with Grünenthal GmbH. W. S. and T. K. are employees of Grünenthal GmbH.

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