0022-3565/03/3062-423–429$7.00
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
JPET 306:423–429, 2003

Vol. 306, No. 2
46979/1077450
Printed in U.S.A.

Perspectives in Pharmacology

Nociceptin/Orphanin FQ and Its Receptor—Potential Targets for Pain Therapy?

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Received February 24, 2003; accepted April 16, 2003

ABSTRACT

The neuropeptide nociceptin, also called orphanin FQ (N/OFQ), is the endogenous agonist of the N/OFQ peptide receptor (NOP receptor). Both N/OFQ and the NOP receptor share a high degree of homology with classical opioid peptides and opioid receptors, respectively, and use similar signal transduction pathways as classical opioids. The NOP receptor has thus been regarded as the fourth member of the opioid receptor family. Despite this close relationship, 7 years of research have demonstrated that the N/OFQ system has a distinct pharmacological profile and serves different physiological functions. In particular, its role in the control of pain and analgesia at different levels of integration appears quite different from that of classical opioids. The recent development of specific antagonists at the NOP receptor and of NOP receptor or N/OFQ precursor knock-out mice have generated new insights into the role of N/OFQ in pain processing and help to evaluate the N/OFQ-NOP system as a potential target for new analgesic drugs.

While acute—particularly post-traumatic and postsurgical pain can be treated satisfactorily with available analgesics in most cases—chronic inflammatory and neuropathic pain often responds only poorly. The identification of novel targets for analgesic therapy is therefore a major focus in current pharmacological research. Chronic pain states are frequently accompanied by an increased pain sensitivity, which can appear as hyperalgesia, an increased sensitivity to noxious stimuli, or allodynia, a painful sensation of usually innocuous stimuli. N/OFQ has repeatedly been implicated in the development and/or modulation of both phenomena and has thus attracted significant attention in current pain research.

The discovery of N/OFQ is a prominent example of so called reverse pharmacology in which a receptor was known before the corresponding ligand could be identified (Civelli et al., 1998). Soon after the discovery of the δ opioid (DOP) receptor by expression cloning molecular biologists have not only identified the expected μ and κ (MOP and KOP) opioid receptors, but also a fourth unperceived receptor, which does not bind classical opioids. The human homolog of this receptor has therefore been termed opioid receptor-like 1 receptor (Mollereau et al., 1994). Only 1 year after its discovery, a 17 amino acid neuropeptide was identified as the endogenous agonist by two independent groups and called nociceptin (Meunier et al., 1995) or orphanin FQ (Reinscheid et al., 1995). Both the peptide and its receptor, now called N/OFQ peptide (NOP) receptor, share a high degree of homology with classical opioid peptides (namely dynorphin A) and classical opioid receptors (namely the κ opioid peptide receptor). A first series of behavioral tests has suggested that this peptide was pro- rather than antinociceptive, when injected intracerebroventricularly (i.c.v.). This effect led Meunier et al. (1995) to coin the term nociceptin. Reinscheid et al. (1995) called the peptide orphanin FQ, indicating that the peptide activated a receptor previously classified as an orphan recep-

Article, publication date, and citation information can be found at http://jpet.aspetjournals.org.
DOI: 10.1124/jpet.102.046979.

ABBREVIATIONS: N/OFQ, nociceptin/orphanin FQ; MOP, μ opioid peptide receptor; NOP receptor, N/OFQ peptide receptor; CNS, central nervous system; RVM, rostral ventromedial medulla; NST, nocistatin; KOP, κ opioid peptide receptor; DOP; δ opioid peptide receptor; Ro 64-6198, (1*S*,3a*S*)-8-(2,3,3*a*,4,5,6-hexahydro-1*H*-phenalon-1-yl)-1-phenyl-1,3,8-triza-spiro[4.5]decan-4-one; J-113397, (1-[3*R*,4*R*)-1-cyclooctymethyl-3-hydroxymethyl-4-piperidyl]-3-ethyl-1,3-dihydro-2*H*-benzimidazol-2-one; JTC-801, *N*-(4-amino-2-methylquinolin-6-yl)-2-(4-ethylphenoxymethyl)-benzamide monohydrochloride.

tor, whereas the letters F and Q designate the first and last amino acid of this peptide, phenylalanine and glutamine.

The NOP receptor belongs to the large family of G-protein coupled (7 transmembrane) receptors. After agonist binding the NOP receptor activates G-proteins of the G_i/G_o type, causing an inhibition of cAMP production, activation of potassium currents and inhibition of Ca^{2+} currents, in particular of the N-type (Moran et al., 2000). At the cellular level, all these actions favor inhibition of neuronal activity and a reduction in transmitter release. The cellular signal transduction pathway initiated by NOP receptor activation thus appears nearly identical to that of classical opioids. Despite this close relationship, however, the role of N/OFQ in pain processing appears considerably more complex than that of classical opioids.

The available data suggest that N/OFQ can interfere with nociceptive processing at least at three different levels of integration, where it may modulate nociception in opposite directions: the spinal cord, supraspinal sites, and peripheral endings of primary afferent nerve fibers (nociceptors). Consistent with a role in pain processing, expression of both the NOP receptor and the N/OFQ peptide is particularly intense in several areas involved in nociception including the spinal cord dorsal horn, the nucleus raphe magnus, and the periaqueductal gray (Neal et al., 1999a,b). Cellular effects of N/OFQ have been thoroughly studied in these CNS areas and much of our picture how N/OFQ exerts its different effects on pain processing is based on these results.

Modulation of Nociceptive Processing by Exogenous N/OFQ

Supraspinal Effects of N/OFQ. As outlined above, pain modulating effects of N/OFQ were first studied at supraspinal sites in which it was pronociceptive, hyperalgesic, or proallodynic after i.c.v. injection (Meunier et al., 1995; Reinscheid et al., 1995). These results came rather unexpected since they were in obvious contrast to the well documented analgesic effects of classical opioids, in particular of μ-agonists. Subsequent studies yielded a wide diversity of results including hyperalgesia, antianalgesia, or even analgesia. Meanwhile considerable agreement has been reached that N/OFQ's most prominent role in supraspinal pain modulation is a "functional opioid antagonism" directed against many different opioid receptor agonists (Mogil and Pasternak, 2001). Small amounts of N/OFQ injected directly into the rostral ventromedial medulla (RVM) reverse [D-Ala^2,MePhe^4,Gly-ol^5]-enkephalin-induced antinociception (Heinricher et al., 1997; Pan et al., 2000). It has subsequently been speculated that N/OFQ might also reverse stress-induced opioid-mediated antinociception (Rizzi et al., 2001). Since behavioral testing in pain models, in particular i.c.v. and i.t. injections, expose animals to acute stress, the apparent pronociceptive action seen in the initial studies may thus be interpreted as the reversal of stress-induced antinociceptive effects rather than as a genuine pronociceptive or hyperalgesic effect (Mogil et al., 1996).

A cellular model explaining the antiopioidergic action of supraspinal N/OFQ is based on seminal studies by Heinricher et al. (1997) and Pan et al. (2000). The brain stem, in particular the nucleus raphe magnus of the RVM, appears as a major site of supraspinal N/OFQ effects on pain processing. In this brain region, different types of neurons, so called ON and OFF cells, can be distinguished. ON cells fire immediately before a nociceptive reaction, while OFF cells are inhibited by the GABAergic ON cells and therefore silent at the same time. Activation of OFF cells induces spinal antinociception via descending antinociceptive tracts. μ opioids inhibit ON cells and thereby cause a subsequent disinhibition of the antinociceptive OFF cells. By contrast, N/OFQ inhibits nearly all cell types in the RVM (Vaughan et al., 2001). Via a direct inhibition of OFF cells, N/OFQ counteracts the disinhibitory effects of μ agonists on these cells and thereby reverses opioid-induced supraspinal analgesia. The same mechanism may also account for the apparent hyperalgesic effect of N/OFQ, providing a cellular basis for the reversal of stress-induced analgesia by N/OFQ. These studies demonstrate that the net effects of N/OFQ on nociception at supraspinal sites strongly depend on the activation state (resting versus sensitized) of pain controlling neuronal circuits.

Spinal Effects of N/OFQ. Neither the analgesic effect of classical opioids nor the diverse actions of N/OFQ on pain processing can solely be explained by its supraspinal actions. Many lines of evidence indicate that the spinal cord is an equally important CNS area for nociceptive processing and its modulation by N/OFQ and classical opioids. In particular, the superficial layers of the spinal cord dorsal horn, where thin- and unmyelinated primary afferent nerve fibers terminate, represent an important structure for nociceptive processing. This area constitutes the first site of synaptic integration in the pain pathway. Although the first functional studies on N/OFQ concentrated on supraspinal sites, subsequent studies have shown that mRNA and protein of both the N/OFQ precursor ppN/OFQ and of the NOP receptor are highly expressed in this structure (e.g., Neal et al., 1999a,b). While hyperalgesic or antianalgesic effects of N/OFQ dominate after supraspinal injection, most studies report an antinociceptive action of N/OFQ after spinal application in a wide variety of animal models of pain—at least in nanomolar doses. The models tested include, among others, acute thermal and mechanical pain and tonic inflammatory as well as neuropathic pain (for reviews, see Mogil and Pasternak, 2001; Calò et al., 2000b).

Fast excitatory and inhibitory neurotransmission in the spinal cord is mediated by the amino acids L-glutamate and glycine (together with GABA), respectively. Several reports have demonstrated that N/OFQ inhibits excitatory glutamatergic neurotransmission without affecting GABA or glycine receptor-mediated synaptic responses (Zeilhofer et al., 2000; Luo et al., 2002). This inhibition is naloxone-insensitive and absent in $NOP^{-/-}$ mice (Ahmadi et al., 2001), indicating that it is specifically mediated by the NOP receptor. N/OFQ does not affect the responsiveness of postsynaptic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid or *N*-methyl-D-aspartate receptors but decreases the synaptic release of L-glutamate, i.e., acts via a presynaptic site. Inhibition of glutamatergic transmission by N/OFQ is thus remarkably reminiscent of the spinal analgesic mechanism of classical opioids and most likely underlies the well accepted antinociceptive action of spinally applied N/OFQ. Nevertheless, it should be noted that synaptic connections targeted by N/OFQ and classical opioids such as methionin-enkephalin are not identical (Monteillet-Agius et al., 1998).

In the light of these findings, it is interesting that very low (atto- to femtomole) doses of N/OFQ can also elicit a prono-

ciceptive in particular proallodynic action in the spinal cord (for a review, see Ito et al., 2001). Nevertheless, a convincing cellular mechanism for this spinal pronociceptive action has not been identified yet. A reduction in synaptic glycine release has been suggested (Ito et al., 2001), but a large set of data meanwhile unambiguously indicates that N/OFQ does not interfere with GABAergic or glycinergic neurotransmission at the level of the spinal cord (Zeilhofer et al., 2000; Ahmadi et al., 2001; Luo et al., 2002). An alternative explanation might be that N/OFQ specifically inhibits synaptic glutamate release from low threshold mechanical (Aβ) fibers. These fibers control, via glycinergic interneurons, the activity of so called wide dynamic range neurons. Although this hypothesis could easily explain how a reduction in glutamate release (from Aβ-fibers) induces allodynia, Luo et al. (2002) have demonstrated that N/OFQ interferes primarily with the release of glutamate from nociceptive C- and Aδ-fibers.

Alternatively, N/OFQ might induce the release of pronociceptive mediators in the spinal cord similar to what has been described in the periphery (see below). Two studies by Inoue et al. (1998, 1999) have reported that N/OFQ induces a Ca^{2+}-dependent release of substance P not only from the peripheral but also from the central terminals of primary afferent nociceptive nerve fibers. The underlying signal transduction is only incompletely understood, but the available data provide indirect evidence that N/OFQ may trigger phospholipase C- and IP_3-dependent Ca^{2+} release—at least in heterologous expression systems (for a review, see Moran et al., 2000). Although unproven, another intriguing hypothesis is that a reduction in synaptic glutamate release not only inhibits fast excitatory neurotransmission via α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid and *N*-methyl-D-aspartate receptors but also activation of—perhaps inhibitory—metabotropic glutamate receptors. Taken together, the cellular mechanisms of the pronociceptive effects of N/OFQ in the spinal cord are still rather obscure, whereas inhibition of excitatory synaptic transmission presents as a clearly defined cellular mechanism underlying the spinal analgesia.

The combination of opioid-like analgesia by N/OFQ at the level of the spinal cord with functional opioid antagonism at supraspinal sites, where most of the unwanted effects of classical opioids arise, has promoted the idea that NOP receptor agonists might be better tolerated centrally acting analgesics. It should however be noted that the only well studied nonpeptide NOP receptor agonist Ro 64-6198 was anxiolytic, but not antinociceptive in acute pain models (Jenck et al., 2000). Nevertheless, further studies with other NOP receptor agonists and in chronic pain models are desirable.

Peripheral Effects of N/OFQ. Compared with studies investigating CNS effects of N/OFQ, only a very limited number of reports have addressed peripheral actions of N/OFQ on nociceptors. Both the N/OFQ and the NOP receptor are expressed at rather low levels in dorsal root ganglion cells, but NOP receptor expression increases after sciatic nerve injury (Briscini et al., 2002). A functional role of peripheral NOP receptors has been demonstrated by Inoue et al. (1998), who reported that N/OFQ injected intraplantar at very low (femtomole) doses induced Ca^{2+}-dependent substance P release thereby evoking nociceptive flexor reflexes. At higher (nanomole) doses, N/OFQ prevented substance P induced flexor reflexes in mice (Inoue et al., 1999) and capsaicin-induced thermal nociception in monkeys (Ko et al., 2002a) suggesting that an additional antinociceptive effect of N/OFQ also exists in the periphery.

Other ppN/OFQ Products

Like other neuropeptides, N/OFQ is released after proteolytic cleavage from a larger precursor polypeptide called prepro-N/OFQ (ppN/OFQ; Mollereau et al., 1996; Nothacker et al., 1996). In addition to the cleavage sites necessary for the release of N/OFQ, ppN/OFQ contains further sites, which may give rise to other neuropeptides with potential biological activity. Nocistatin (NST; Okuda-Ashitaka et al., 1998), which is probably the most extensively studied neuropeptide among these, has repeatedly been shown to antagonize many of the effects of N/OFQ in vivo and also to possess pain modulating activity per se.

NST reduces the release of glycine and GABA in the spinal cord dorsal horn but has no effect on the release of L-glutamate (Zeilhofer et al., 2000). This inhibition is completely retained in mice lacking the NOP receptor (Ahmadi et al., 2001), indicating that NST's action does not require NOP receptors. Pretreatment of spinal cord slices with pertussis toxin prevented the inhibition, indicating that NST acted via a so far unidentified seven transmembrane receptor. At nanomolar doses, NST induces robust pronociceptive effects after i.t. injection in rats implanted with chronic intrathecal catheters (Zeilhofer et al., 2000), while an antinociceptive effect is observed after low (femtomole) doses of NST (Okuda-Ashitaka et al., 1998). In the spinal cord dorsal horn, inhibition by N/OFQ and NST of excitatory and inhibitory synaptic transmission, respectively, probably accounts for the functional antagonism of N/OFQ and NST seen in vivo. Figure 1 shows how N/OFQ and NST interfere with synaptic transmission and nociceptive processing in the spinal cord and at supraspinal sites. At a first glance, it appears surprising that two neuropeptides with apparently opposing effects on nociception originate from the same precursor peptide. Presently, however, it is not known whether both peptides are indeed released from the same neuron and, if so, whether they are always coreleased. ppN/OFQ, like other neuropeptides, may be processed in a cell type-specific manner depending on the expression of specific proprotein convertases (Hallberg and Nyberg, 2003). In addition, different peptides may be transported to and released from distinct nerve terminals even within the same neuron (Fumagalli and Zanini, 1985).

Synthetic NOP Receptor Antagonists

The studies discussed above have used exogenously applied N/OFQ and do therefore not provide information about the physiological function of endogenous N/OFQ. To address this question, both NOP receptor antagonists (summarized in Table 1) and mice lacking either the NOP receptor or the N/OFQ precursor protein ppN/OFQ (Table 2) have been employed by different groups.

Local i.c.v. injection of NOP receptor peptide antagonists ([Nphe^1]N/OFQ(1-13)NH_2, and [Nphe^1,Arg^{14},Lys^{15}]N/OFQNH_2, also known as UFP-101) was antinociceptive (or antihyperalgesic?) in the tail withdrawal test (Calò et al., 2000a, 2002). These results are in excellent agreement with the previous

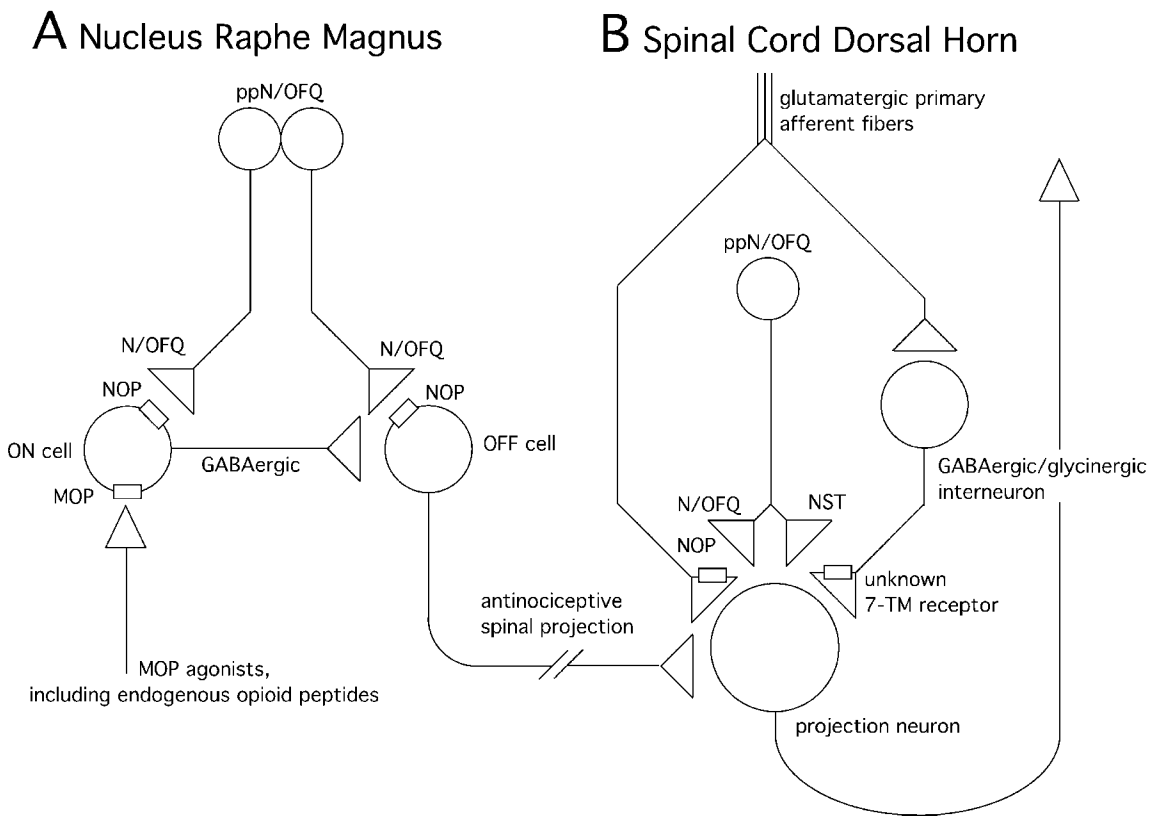

Fig. 1. Schematic drawing showing how N/OFQ modulates synaptic transmission in the nucleus raphe magnus of the brain stem (A) and in the spinal cord dorsal horn (B). A, in the brain stem, MOP agonists including endogenous opioid peptides inhibit so-called secondary or ON cells. These cells are GABAergic and, in turn, inhibit descending antinociceptive OFF or primary cells. By inhibiting the ON cells, μ-opioids cause a disinhibition of OFF cells and elicit thereby antinociception. By contrast, N/OFQ inhibits both cell types at the same time. Under resting conditions, N/OFQ probably exerts no net effect on nociception. However, when MOP receptors on ON cells are activated N/OFQ can reverse this analgesia by inhibiting in addition OFF cells. B, in the spinal cord, N/OFQ selectively inhibits the release of glutamate and leaves the release of the inhibitory neurotransmitters glycine and GABA unaffected. Inhibition of glutamate release and the subsequent inhibition of nociceptive transmission through the spinal cord probably underlie the analgesic effect of nanomolar doses of spinally applied N/OFQ. NST by contrast reduces selectively the release of GABA and glycine via so far unknown 7-transmembrane receptor, but spares the release of glutamate. Panel A was modified from Pan et al. (2000) with permission.

TABLE 1
In vivo pro- and antinociceptive effects of NOP receptor antagonists

Compound B (J-113397)			
i.t., i.c.v.	Rat formalin test	Pronociceptive in phase II	Yamamoto et al. (2001)
Systemic, i.p.		No effect	
Local peripheral	Capsaicin-induced thermal nociception in monkeys	No effect on its own	Ko et al. (2002a)
Systemic, s.c.t.	Mouse tail-flick test	No effect	Ozaki et al. (2000)
Systemic, s.c.t.	Mouse tail-flick test and tail pinch	Attenuation of tolerance to morphine	Ueda et al. (2000)
JTC-801			
i.t.	Mouse formalin test	Antinociceptive	Muratani et al. (2002)
Systemic, i.v./p.o.	Rat formalin test	Antinociceptive (naloxone insensitive)	Yamada et al. (2002)
Systemic, p.o.	Mouse hot plate and rat formalin test	Antinociceptive (naloxone insensitive)	Shinkai et al. (2000)
[Nphe^1]N/OFQ(1-13)NH_2			
i.c.v.	Mouse tail withdrawal test	Antinociceptive (naloxone insensitive)	Caló et al. (2000)
i.t.	Rat chronic constriction injury	No effect	Corradini et al. (2001)
i.t.	Flexor reflex in spinalized rats	Facilatory (pronociceptive)	Xu et al. (2002)
i.c.v.	Mouse tail withdrawal test	Potentiation of morphine antinociception and attenuation of tolerance to morphine	Rizzi et al. (2000)
i.c.v.	Mouse tail-flick test and hot plate	Antinociceptive (ineffective in NOP$^{-/-}$ mice), no tolerance development	Di Giannuario et al. (2001)
UFP-101			
i.c.v.	Tail withdrawal test	Antinociceptive	Calò et al. (2002)

studies using supraspinal N/OFQ injections. They support a contribution of endogenous N/OFQ to the induction or maintenance of supraspinal hyperalgesia/antianalgesia. By contrast, spinal application of J-113397 (also known as compound B; Ozaki et al., 2000) increased pain related behavior in the second phase of the formalin test (Yamamoto et al., 2001) and [Nphe^1]N/OFQ(1-13)NH_2 facilitated the C-fiber evoked flexor reflex in spinalized rats (Xu et al., 2002), which

TABLE 2
Nociceptive phenotypes of mice lacking the NOP receptor or the ppN/OFQ polypeptide

ppN/OFQ$^{-/-}$	Antinociceptive (higher stress-induced analgesia in group house males) in the tail-flick test; no nociceptive phenotype in individually housed males	Köster et al. (1999)
	Tail-flick test, antinociceptive in young mice (6–8 weeks), but hyperalgesic in adult mice (>12 months)	Mogil and Pasternak (2001)
	Unchanges tolerance to morphine	Kest et al. (2001)
	Normal responses in phase I, but increased responses in phase II of the formalin test, increased thermal sensitivity after zymosan A injection	Depner et al. (2003)
NOP$^{-/-}$	No nociceptive phenotype in the tail-flick and acetic acid writhing test	Nishi et al. (1997)
	No nociceptive phenotype tail-flick and tail pintch test; no nociceptive phenotype hot plate, tail-flick, electric foot shock, acetic acid writhing test	Ueda et al. (1997) Mamiya et al. (1998)
	Increased nociceptive flexor responses upon intraplantar injection of bradykinin or substance P	Inoue et al. (2003)
	Normal responses in phase I, but increased responses in phase II of the formalin test, increased thermal sensitivity after zymosan A injection	Depner et al. (2003)

is consistent with the antinociceptive effect of spinally applied N/OFQ. Nevertheless, it should be noted that another NOP receptor antagonist (JTC-801) has repeatedly been shown by different groups to be antinociceptive after spinal application in the mouse and rat formalin test and in the mouse hot plate assay (Shinkai et al., 2000; Muratani et al., 2002; Yamada et al., 2002). At present, it is not entirely clear whether this apparent antinociceptive effect is indeed mediated via an antagonism at NOP receptors. Unlike the peptide NOP receptor antagonists, JTC-801 exhibits a rather poor (4-fold) selectivity for the NOP receptor over the MOP receptors in the rat (Yamada et al., 2002). It would be very instructive if these experiments were repeated in NOP receptor deficient mice. Although the available data are still quite limited, the majority of reports are at least consistent with the view that not only exogenously injected but also endogenous N/OFQ contributes to spinal analgesia, but is pronociceptive at supraspinal sites.

Systemic administration of nonpeptide NOP receptor antagonists could in principle clarify, whether spinal analgesia or supraspinal antianalgesia of endogenous N/OFQ dominates in vivo. Unfortunately, such studies have not yet provided a consistent view. J-113397 was inactive in one study (Ozaki et al., 2000), while JTC-801 produced analgesia in two other studies (Shinaki et al., 2000; Yamada et al., 2002) . Part of these discrepancies may be due to the limited selectivity of JTC-801 in the rat and to rather unfavorable pharmacokinetic properties of J-113397 including poor distribution in vivo.

Genetically Modified Mice

Genetically modified mice present another tool to address the physiological role of N/OFQ in pain processing. Mice lacking the NOP receptor exhibit normal nociceptive thresholds and normal behavior in the acute pain tests including the tail-flick, hot-plate, and writhing test (Nishi et al., 1997; Ueda et al., 1997; Mamiya et al., 1998). The nociceptive phenotype of ppN/OFQ$^{-/-}$ mice has been characterized less extensively. Köster et al. (1999) have reported a normal nociceptive responsiveness in these mice under baseline conditions (i.e., in the absence of inflammation or on-going painful stimulation). An analgesic phenotype was observed, however, when the mice were exposed to a stressful environment (Köster et al., 1999). This apparent analgesia has thus been attributed to a higher stress susceptibility of the knock-out mice compared with their wild type littermates. The situation becomes even more complicated as an increased sensitivity to painful stimulation has been reported in an independently generated strain of ppN/OFQ$^{-/-}$ mice, which has been attributed to the different ages at which the mice were tested. Analgesia was seen in younger (6–8 weeks) and hyperalgesic responses in older (>12 months) mice (Mogil and Pasternak, 2001). Interpretation of the data obtained with the ppN/OFQ$^{-/-}$ is complicated by the potential lack of ppN/OFQ products other than N/OFQ, including NST (Okuda-Ashitaka et al., 1998). A systematic comparison of the nociceptive phenotypes of NOP receptor and ppN/OFQ knock-out mice has been undertaken recently. Depner et al. (2003) showed that both types of knock-out mice as well as double knock-outs show normal sensitivity in acute pain tests but an increased nociceptive sensitivity to tonic pain in the formalin test and the Hargreaves model.

Despite these conflicting results, most researchers in the field agree that NOP$^{-/-}$ and ppN/OFQ$^{-/-}$ mice do not exhibit altered nociceptive behavior under resting conditions. In this respect, NOP receptor and ppN/OFQ knock-out mice resemble those lacking μ opioid receptors, which also show only subtle changes in their sensitivity to "physiological" pain (Kieffer and Gaveriaux-Ruff, 2002). Thus, the apparent lack of a nociceptive phenotype does not preclude an involvement of N/OFQ in endogenous pain control. Interestingly, when NOP$^{-/-}$ mice are exposed to tonic painful stimulation as in the formalin test or the Hargreaves model of thermal hyperalgesia, they show increased nociceptive responses (Depner et al., 2003; Inoue et al., 2003). Genetic ablation of NOP receptors is therefore in good agreement with the pharmacological study by Yamamoto et al. (2001), who found that spinally injected J-113397 increased nociceptive behavior in the second, but not in the first, phase of the formalin test. Baseline N/OFQ levels appear to be too low to affect pain sensitivity and acute painful stimulation, e.g., in the tail-flick test, is too short to elicit sufficient release of N/OFQ. One might therefore argue that tonic nociceptive input is required for N/OFQ release in the spinal cord. Unfortunately, only very little is known about the upstream innervation of ppN/OFQ expressing neurons in the spinal cord and elsewhere in the CNS. It is therefore at present unknown, which neuronal circuits drive the release of N/OFQ and of other potential ppN/PFQ products. A few studies have so far analyzed endogenous N/OFQ levels in the serum or cerebrospinal fluid of patients. One study (Ko et al., 2002b) indeed found increased N/OFQ blood levels in patients suffering from chronic pain compared with acute pain or healthy subjects. Furthermore, prolonged nociceptive stimulation may not only evoke release

of N/OFQ but probably also induces expression of N/OFQ in the spinal cord dorsal horn (Rosén et al., 2000) and in primary afferent nociceptive neurons (Itoh et al., 2001).

If one considers the involvement of N/OFQ in stress adaptation (Köster et al., 1999; Jenck et al., 2000), it is probably not too far fetched to assume that acute stress might release N/OFQ from supraspinal sites (Devine et al., 2003). As most pain testing in animals is accompanied by some stress exposure, it is not unexpected that supraspinal (i.c.v.) injection of NOP receptor antagonists produced analgesia in most studies, i.e., reversed the antianalgesic action of endogenous N/OFQ released in response to stress exposure.

Addictive Properties and Development of Tolerance Addictive or rewarding properties classify classical opioids as drugs with significant abuse potential. Unlike classical opioid analgesics, neither N/OFQ nor the NOP receptor agonist Ro-64-6198 show rewarding or aversive properties in the rat conditioned place preference test (Devine et al., 1996; Le Pen et al., 2002) suggesting that they lack addictive properties. N/OFQ might even posses antirewarding properties directed against several different agents including ethanol and morphine (Ciccocioppo et al., 2000). Despite this apparent lack of rewarding properties, tolerance develops rapidly against the spinal analgesic effect of N/OFQ (Hao et al., 1997). Interestingly, the anxiolytic properties of Ro 64-6198 (Dautzenberg et al., 2001) as well as the antihyperalgesic effects of i.c.v. injected [Nphe^1]N/OFQ(1-13)NH$_2$ (Di Giannuario et al., 2001) do not undergo tolerance development.

Apart from its genuine pain modulatory effects, the N/OFQ system has attracted considerable interest through its possible involvement in opioid dependence and tolerance. Several articles have suggested that at the systems level the release of antiopioid substances might play a major role (Rothman, 1992). The functional opioid antagonistic effect of N/OFQ has led researchers to speculate about a role of N/OFQ in the development of this phenomenon. Meanwhile, there is considerable experimental evidence that N/OFQ may indeed be crucially involved—at least in mice. Two articles by Ueda et al. (1997, 2000) have shown that NOP receptor deficient mice develop significantly (50%) less reduction in morphine analgesia during a 5 days treatment. A strong reversal of morphine tolerance was also seen in mice systemically treated with the NOP receptor antagonist J-113397 (Ueda et al., 2000) or treated i.c.v. with [Nphe^1]N/OFQ(1-13)NH$_2$ (Rizzi et al., 2000). Both genetic ablation of NOP receptors and NOP receptor antagonism with J-113397 also attenuated withdrawal symptoms evoked by naloxone injection in morphine dependent mice (Ueda et al., 2000; Kest et al., 2001). Given the many different mediators that have been implicated in morphine tolerance/dependence, further studies will have to verify that N/OFQ's role is really as critical as the present studies suggest.

Summary More than 7 years after its discovery, our understanding of the role of the N/OFQ-NOP receptor system in pain processing is far from being complete. Nevertheless, it has already become clear that NOP receptor ligands will exhibit a pharmacological profile quite different from the currently available analgesics. They may for example combine analgesic efficacy with anxiolytic and antidepressant activity and may potentially lack rewarding properties and tolerance development. Potential indications in pain therapy might include the use of NOP receptor agonists as solely spinally acting analgesics, perhaps only in well defined subgroups of patients, and the use of NOP receptor antagonists alone or in combination with morphine, which might reduce the development of tolerance.

Acknowledgments

We apologize to all whose work could not be cited due to space limitations.

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