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The delta opioid receptor: an evolving target for the treatment of brain disorders

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Abstract

Compared to the better-known mu opioid receptor, delta opioid receptors have been relatively understudied. However, the development of highly selective delta opioid agonists, and the availability of genetic mouse models have extended our knowledge of delta opioid receptors *in vivo*. Here we review recent developments in the characterization of delta opioid receptor biology, and aspects of delta opioid receptor function that have potential for therapeutic targeting. Preclinical data have confirmed that delta opioid receptor activation reduces persistent pain and improves negative emotional states; clinical trials have been initiated to assess the effectiveness of delta opioid agonists in chronic pain and depression. Further, a possible role for these receptors in neuroprotection is being investigated. The usefulness of targeting delta opioid receptors in drug abuse remains open, and there is an emerging role of these receptors in impulse control disorders. Finally, the recent demonstration of biased agonism at the delta opioid receptor *in vivo* opens novel perspectives towards targeting specific therapeutic effects through drug design.

The opioid system

Discovery of the opioid system stems from the use and abuse of opium in ancient history. Opium has strong pain-relieving properties, and also produces euphoria. Morphine, considered the prototypic opiate, is the most active constituent of opium, and remains the most widely used pain killer in contemporary medicine despite strong adverse effects [1, 2]. Heroin, a diacetylated morphine derivative, is a major drug of abuse with strong societal impact. These powerful substances act on nervous system receptors, whose existence was established in 1973 and molecular characterization achieved in the late 1990s. These receptors are activated by a family of endogenous opioid peptides whose first members were isolated in 1975, and genes identified in the late 1970's. Altogether, the opioid receptor family consists of three receptors, mu, delta, and kappa, encoded by the *Oprm1*, *Oprd1*, and *Oprk1* genes respectively; and neuropeptides processed from large precursor proteins, encoded by *Penk*, *Pdyn* and *Pomc* genes. Due to high sequence homology, the *Oprl1* gene,

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encoding the Nociceptin/OrphaninFQ receptor, is classified as a member of the opioid receptor gene family (http://www.iuphar-db.org/DATABASE/FamilyIntroductionForward?familyId=50). However this receptor, qualified as “opioid-like receptor”, does not respond to opioids pharmacologically, and will not be further discussed in this review. Opioid receptors and peptides are broadly expressed throughout the peripheral and central nervous systems and have been the subject of intense investigation for several decades (for recent review see [1]).

The opioid system plays a central role in pain control, and is a key player in hedonic homeostasis, mood and well-being. This system also regulates responses to stress, and a number of peripheral physiological functions including respiratory, gastrointestinal, endocrine and immune systems. In the past two decades, refinement of pharmacological tools and availability of genetic approaches have clarified the specific role of each opioid receptor in many aspects of opioid-related behaviors, physiology and disorders. At present, the notion that mu opioid receptors mediate both analgesic and addictive properties of clinically useful and abused opiates is well accepted. Mu opioid receptor activation strongly inhibits severe acute pain, and is a major target for post-operative and cancer pain management [2]. Mu opioid receptors are also central for reward processing [3], representing a main factor in the initiation of addictive behaviors. The activation of kappa opioid receptors also produces antinociception in preclinical models [4]. These receptors, however, oppose mu receptors in the regulation of hedonic homeostasis. Kappa agonists are strongly aversive and potentially hallucinogenic, which limits the therapeutic potential of centrally-acting kappa opioid agonists in pain treatment. Recently, mounting preclinical evidence supports the notion that kappa receptor blockade may beneficially alleviate stress responses, reduce drug craving and remediate depressive states [5-7].

Delta opioid receptors (also known as delta opioid receptors, δ receptors, DORs, or DOP receptors in the IUPHAR nomenclature) appear increasingly attractive, both from the perspective of receptor function *in vivo* and therapeutic potential. Along with the development of highly selective delta opioid agonists and rapid progress in mouse mutagenesis approaches targeting the *Oprd1* gene, the previously reported roles of delta opioid receptors have been clarified and novel functions have emerged. Here we focus on recent advances in delta opioid receptor biology, and the potential of delta opioid agonists in the treatment of neurological and psychiatric disorders. Rather than being exhaustive, this review highlights selected aspects of delta opioid receptor function at cellular and behavioral levels, which have evolved recently and hold promise for biomedical research.

Molecular and cellular aspects of the delta opioid receptor

Opioid receptors are seven-transmembrane proteins which belong to the G protein-coupled receptor (GPCR) superfamily. Upon binding of exogenous opiates or endogenous opioid peptides, opioid receptors are activated and convey extracellular stimulation to multiple intracellular effectors, including ion channels and second messengers that ultimately decrease neuronal activity [8]. The three opioid receptors show highly homologous protein sequences, and *in vitro* mutagenesis studies have identified a common opioid receptor binding pocket within the helical transmembrane core for mu, delta and kappa receptors. The analysis of mutant receptors *in vitro* has further demonstrated the importance of extracellular domains for delta opioid receptor selectivity [9], and identified helical domain-mediated mechanisms for delta opioid receptor activation [10]. More recently, delta opioid receptors have been proposed to act as heterodimers with opioid receptors or other GPCRs in cellular models, and the possibility that delta opioid agonists may act at delta receptor-GPCR heteromers with distinct pharmacological properties *in vivo* is being explored [11-13].

As for most GPCRs, stimulation of the delta opioid receptor triggers intracellular signaling via G protein-dependent or independent pathways. Receptor signaling is typically accompanied by desensitization, a complex feedback regulatory process whereby receptor responsiveness decreases upon continued agonist stimulation. GPCR internalization, trafficking and redistribution is considered one of the key mechanisms underlying this regulatory response [14] and major progress has been achieved recently regarding the dynamics and implications of delta opioid receptor internalization *in vivo*. Delta opioid receptor internalization has been observed *in vitro* for several ligands, including endogenous opioids [15,16]. Studies in cellular models indicate that internalized GPCRs may either recycle to the cell surface, or be further processed along several distinct endocytic pathways (see [17] and references therein). Unlike the mu opioid receptor, which rapidly recycles to the cell surface, delta opioid receptors are targeted for lysosomal degradation ([15] and refs therein) via the Endosomal Sorting Complex Required for Transport (ESCRT) machinery using ubiquitination-dependent or independent mechanisms [18]. Other factors upstream of the ESCRT pathway also contribute to delta opioid receptor proteolysis, including G protein coupled receptor Associated Sorting Proteins (GASPs) that were shown to interact directly with delta opioid receptor, as well as other GPCRs [19,20].

Until recently it was difficult to study the consequences of agonist-induced delta opioid receptor internalization and degradation *in vivo*. The advent of a novel knock-in mouse model in which endogenous delta opioid receptors are replaced by fluorescently-tagged delta opioid receptors (DOR-eGFP, [21]) has allowed correlating ligand-induced receptor internalization in live neurons with receptor function *in vivo*. DOR-eGFP mice express fully functional delta opioid receptors, which are directly visible *in vivo*. In these mice the prototypic delta opioid agonist, SNC80 [22], produced robust receptor internalization at behaviorally active concentrations [15,21]. In a model of inflammatory pain, acute SNC80 induced strong analgesia, together with receptor internalization and G-protein uncoupling, resulting in transient acute behavioral desensitization [15]. Chronic SNC80 ultimately produced widespread receptor degradation, as was anticipated from cellular studies, leading to generalized behavioral tolerance to all agonist effects ([23], Figure 1).

In contrast to SNC80, treatment with the non-internalizing delta opioid agonist ARM390 produced remarkably different effects *in vivo.* Acute treatment with ARM390 did not induce behavioral desensitization, and delta opioid receptors remained G protein-coupled on the cell surface [15]. Chronic treatment nevertheless produced analgesic tolerance, even though no obvious receptor change was detectable at the cellular level, and other behavioral effects of delta opioid agonists were maintained [23]. In this case, analgesic tolerance resulted from receptor internalization-independent mechanisms that occurred specifically in pain pathways ([23] and Figure 1). In other tolerance studies, SNC80 induced differential tolerance for convulsant, locomotor, and antidepressant responses [24], and the novel delta opioid agonists SB-235863 and JNJ-20788560 did not induce analgesic tolerance in animal models of chronic pain [25,26]. In the future, the exact relationship between agonist-induced internalization, behavioral effects of agonists and the distinct forms of tolerance should be determined for a number of different delta opioid agonists (see Table 1), in order to establish whether *in vivo* internalization has any predictive value for drug efficacy in clinically relevant experimental settings.

The differential tolerance induced by high- and low-internalizing delta opioid agonists provides an interesting illustration of biased agonism *in vivo*. This concept, also referred to as functional selectivity, stems from the observation that distinct agonists acting at the same receptor can engage different active receptor conformations, leading to agonist-dependent signaling or regulatory responses [27,28]. Biased agonism has profound implications in understanding the complexity of GPCR pharmacology and facilitating drug development

[29-32]. Evidence for this phenomenon is primarily based on *in vitro* experiments using recombinant cell systems, and a current challenge in GPCR research is to demonstrate the physiological relevance of agonist-biased responses *in vivo*. Only a few studies have demonstrated behavioral consequences of biased agonism, and a prime example is the finding that hallucinogenic properties of some, but not all 5HT2A agonists result from ligand-biased activation of the $G_{i/o}$-Src pathway in cortical neurons [33]. In opioid receptor research, kappa [34] and mu [35] agonist-biased activation of the *c-jun* cascade was shown to engage specific desensitization mechanisms that had differential impacts on opioid analgesia and tolerance. Ligand-directed trafficking at the mu receptor was also proposed to predict opioid tolerance and dependence [36]. Altogether *in vivo* biased agonism has now been demonstrated for all three opioid receptors, opening the way to innovative therapeutic strategies based on differential signaling/trafficking properties of opioid drugs. In the case of delta opioid receptors, agonist-biased research may lead to the discovery of drugs with targeted efficacy in either pain, mood enhancement or potentially other disorders discussed further in this review. Additionally, biased agonism may offer a strategy to avoid adverse properties of delta opioid agonists, such as potential convulsions at high doses (see below). The characterization of biased activities of delta opioid agonists is just beginning.

The potential for delta opioid agonists for the treatment of chronic pain

Mu opioid receptor agonists are the most commonly used drugs for the treatment of pain. However, there are several severe drawbacks with the use of mu agonists, such as respiratory depression, sedation, constipation and abuse liability. In addition, mu agonists show variable efficacy in the treatment of chronic pain, partly due to the development of tolerance [37], and there is a debate on whether opioid drugs are useful for chronic non-cancer pain [38]. In contrast to agonists of mu receptors, delta opioid agonists weakly modulate acute nociception [4]. Remarkably however, delta opioid agonists are effective under experimental conditions involving persistent pain [39-42], stress [43] or chronic morphine treatment [44-47]. Mechanisms underlying increased delta opioid agonist efficacy under those specific conditions include higher receptor number and externalization to the cell surface, or better receptor coupling to signaling effectors [39].

Genetic approaches have corroborated the importance of delta opioid receptors in chronic pain rather than acute nociception. Delta opioid receptor knockout mice showed no, or only subtle, modification of pain thresholds in models of acute pain [48-51], but displayed increased neuropathic [51] and inflammatory pain [48], demonstrating the existence of an endogenous delta opioid receptor activity that reduces chronic pain. Notably, knockout mice were also insensitive to nortriptyline, a tricyclic antidepressant drug that fully reverses mechanical allodynia in an animal model of neuropathic pain [52]. The latter data suggest that delta opioid receptors reduce chronic pain downstream of aminergic systems.

Altogether therefore, pharmacological and genetic data highlight delta opioid agonists as a promising alternative to mu analgesics in the treatment of chronic pain (for reviews see [39,41,53]. More recently, the identification of several novel systemically active delta opioid agonists has confirmed the potential of delta opioid receptors as a useful target for chronic pain (see [4,22,25,26,54-59] and Table 1) and some of these drugs are under clinical trials (http://clinicaltrials.gov/ct2/results?term="delta+opioid").

Opioid receptors exert their antinociceptive activities at several levels of pain pathways, including the periphery, spinal cord, brain stem and higher-order brain centers ([60], and refs therein). Receptor binding and *in situ* hybridization studies, as well as the analysis of DOR-eGFP knock-in mice (see above), show delta opioid receptor expression in dorsal root ganglia (DRGs) and spinal cord, as well as limbic forebrain structures ([3,21,61] and Figure 2), suggesting a role for these receptors in primary pain processing as well as emotional and

cognitive aspects of pain. Contrasting with mu receptors, delta opioid receptor density is extremely low in nociceptive circuits of the mid-brain (eg. periaqueductal grey) and brainstem (eg. raphe magnus). In the mouse peripheral nervous system, delta opioid receptors are expressed in all DRG cell types, including Aδand C nociceptive neurons, where limited co-expression with mu receptors was described [62], and possible functional interaction with these receptors is currently being debated [12,63]. Notably there are species differences and, in contrast to rodents, delta opioid receptor expression is limited to small diameter neurons in human DRGs [64].

Understanding the role of delta opioid receptors in peripheral neurons has gained importance [65,66], and the respective contribution of peripheral versus central receptors has been addressed recently. Delta opioid receptors were eliminated genetically in Nav1.8^{+} sensory neurons that include unmyelinated C and thinly myelinated Aδ nociceptive neurons. Conditional mutant mice showed increased mechanical sensitivity and strongly reduced analgesic responses to delta opioid agonist, in both models of chronic inflammatory and neuropathic pain [60]. These findings confirm the reported role of peripheral delta opioid receptors in the tonic inhibition of mechanical allodynia [62], and demonstrate that peripheral delta opioid receptors are essential to mediate analgesic effects of delta opioid agonists. The development of peripherally-acting delta drugs, therefore, is a feasible strategy for treating nociceptive hypersensitivity associated with chronic pain while avoiding drug-induced central effects.

In conclusion, chronic pain was identified as the first therapeutic area of interest for delta opioid agonists and, although only briefly summarized here, has been heavily documented. Most efforts to develop novel delta compounds stem from pain research. These novel drugs together with recent genetic tools (Table 1), have allowed the expansion of delta opioid receptor research to other disease areas that are discussed below.

The potential of delta opioid agonists for the treatment of emotional disorders

Genetic deletion of the delta opioid receptor [50] or preproenkephalin [67,68] was shown to increase levels of anxiety and depressive-like behavior. These emotional alterations were specifically associated to delta opioid receptors, because kappa and mu receptor knockout mice tested in parallel did not show this phenotype [50]. In accordance, pharmacological blockade of delta opioid receptors by naltrindole, the prototypic delta antagonist, also increased anxiety [69], together with blood corticosterone levels; and this effect was reversed by the agonist SNC80 [70]. Data from receptor ablation or blockade, therefore, concur to indicate that endogenous delta opioid receptor activity improves emotional states.

Accordingly, activation of the delta opioid receptor, either endogenously or by agonist treatment, improved emotional responses. The enkephalinase inhibitor RB101, which increases endogenous levels of opioid peptides, reduced anxiety levels and depressive-like responses even in the absence of mu receptors, and in a naltrindole-reversible manner [71,72]. Focusing on anxiety, several authors showed that systemic SNC80 [73] and UFP-512 [74] decreased levels of anxiety comparable to the prototypic anxiolytic drug diazepam. Microinjections of DPDPE into the amygdala produced a comparable response, which was blocked by naltrindole [75]. The latter observation suggests that delta opioid receptors modulate anxiety at the level of amygdala circuits, a main area for emotional processing and a major site of delta opioid receptor expression ([21] and Figure 2). Other studies have specifically shown the potential of delta opioid agonists as antidepressant drugs. SNC80 [24,69,73,76], NIH11082 [77], UFP512 [74] and ADL5859 [57] inhibited depressive-like behavior, and these effects were comparable to that of prototypic

antidepressant drugs, including selective serotonin reuptake inhibitors and tricyclic antidepressants [73,77].Interestingly, acute delta opioid agonist treatment increased BDNF expression in the frontal cortex, similar to classic antidepressants [78], suggesting a common mechanism for the two classes of drugs.

The potential for delta opioid agonists for the treatment of anxiety and depression has raised substantial interest (**see** Table 1), and clinical trials in this area are ongoing. At the forefront of this effort is AstraZeneca, with one compound (AZD2327) which has successfully completed phase I studies and is currently being assessed in a phase II trial on patients with anxious major depressive disorder (ClinicalTrials.gov ID: NCT00759395).

In the future, the ability of delta opioid receptors to enhance mood in emotional disorders may be considered in the context of pathological pain conditions, known to induce depressive states. The dual activity of delta opioid receptors in both reducing pain and improving mood is particularly attractive in terms of setting novel therapeutic strategies. This research field is still in its infancy, and the finding that delta opioid receptors are down-regulated in the frontal cingulate cortex and amygdala of animals with peripheral neuropathy [79,80] provides a first indication that targeting delta opioid receptors may efficiently contribute to the management of pain-induced emotional disorders.

The potential for delta opioid agonists in neurological disorders

The notion that delta opioid receptors have neuroprotective activity is currently being examined [1]. Deprivation of oxygen and blood supply induces neuronal death, and delta opioid receptor activation appears beneficial in situations of ischemia or hypoxia (review [81]). Hypoxia decreased delta opioid receptor expression, and delta opioid receptor activation counteracted ischemia-induced disruption of ionic homeostasis (review [82]). One strategy to protect neurons from harmful oxidative stress is preconditioning, which consists of exposing cells to sub-lethal amounts of stress leading to enhanced cellular resistance to subsequent stress or injury. Delta opioid receptor blockade inhibited hypoxic preconditioning-induced protection (see reviews [81,82]). Furthermore, delta activation also prevented neurodegeneration in the retina induced by elevation of intraocular pressure [83]. Delta opioid receptors may finally be involved in neuroprotective mechanisms in the context of Alzheimer's [84] and Parkinson's diseases [85].

The role of delta opioid receptors in reward and addiction

Delta opioid receptors are heavily expressed in corticolimbic areas of the brain (Figure 2**),** and the role of delta opioid receptors in reward processes and in the development of addictive behaviors is complex and debated (for recent reviews see [86-88]). Recent data have clarified a number of issues, and the conclusions are summarized here.

Drug addiction originates from the recreational use of drugs, which possess rewarding (or euphoria-producing) properties. The disorder develops upon repeated brain exposure to the drug and excessive stimulation of reward systems. In rodent models, drug reward is classically evaluated using drug-induced conditioned place preference (CPP) or drug self-administration (SA). The switch from drug use to drug abuse is reflected in these models, where adaptive behavioral modifications become detectable following chronic drug exposure. For example, sensitization to drug-induced CPP or extinction of drug SA reflects increased or decreased drug-seeking, respectively. Delta opioid agonists, antagonists, and knockout mice have been tested in these behavioral models and interactions between delta opioid receptors and several drugs of abuse have been investigated.

Whether direct delta opioid receptor activation produces reward is equivocal from pharmacological evidence. Several delta opioid agonists or enkephalinase inhibitors elicit reward, whereas others are ineffective ([89-91] and for review see [86]). Hence, the abuse liability of delta opioid agonists remains controversial. However, several pharmacological studies suggest that delta opioid receptors regulate rewarding or addictive properties of drugs acting at mu opioid receptors or other non-opioid receptor targets. First, delta opioid agonists enhance, while delta antagonists block morphine reward, as well as sensitization to morphine-CPP or heroin-SA ([90,92] and ref therein; [93,94]). Further, pharmacological blockade of delta opioid receptor reduces the rewarding properties of cocaine, methamphetamine, and MDMA (see in [93,95,96] and ref therein). Brain site-specific infusion of delta opioid agonists, antagonists or enkephalinase inhibitor also modify cocaine-SA or cocaine-seeking behavior [97,98]. Therefore, although a number of reports failed to observe any effect of delta antagonists on rewarding properties of cocaine or nicotine [86,99], there is evidence to suggest that delta opioid receptors influence rewarding and addictive effects of psychostimulants. Delta opioid receptors also modulate behavioral effects of alcohol. Opposite results on ethanol intake were described using delta opioid agonists either systemically (decrease) or locally administered in the brain (increase) ([89,100] and ref therein). Site-specific delta antagonist injection disrupted reward properties of ethanol [101] and the two delta antagonists naltriben and BNTX oppositely modified ethanol intake, suggesting differential activity of the two ligands at the receptor [89]. Delta opioid receptor blockade finally reduced cue- or context-induced alcohol seeking [86,102].

Delta opioid receptor gene knockout in mice represent another approach to probe receptor function in drug reward and addiction-related behaviors. Morphine CPP was decreased in mutant animals [92,95], suggesting a role for delta opioid receptors in either morphine reward or conditioned contextual learning. Interestingly, the same [95] and another [103] study showed preserved morphine-SA in mutant mice indicating that rewarding properties of morphine, and motivation to obtain the drug are intact in these mice. These data, together with the observation that both morphine CPP (appetitive stimulus) and lithium conditioned place aversion (aversive stimulus) are impaired in delta opioid receptor knockout mice [95], suggested that delta opioid receptors do not contribute to morphine reward, but are involved and may facilitate contextual learning. The CPP deficit was not evident in other place conditioning studies, showing unchanged CPP to 3,4-methylenedioxymetamphetamine (ecstasy) or D9-tetrahydrocannabinol ([104], and ref in [3]).

Delta opioid receptor knockout mice also showed enhanced voluntary ethanol consumption ([89], and refs in [3]), and innate anxiety levels of mutant mice were reduced after alcohol self-administration [3] providing support for delta opioid receptors as a vulnerability factor in alcohol abuse. Within this line, delta opioid agonists may be effective in preventing relapse by reducing emotional alterations that emerge during withdrawal periods. Accordingly, delta opioid agonists diminished cocaine withdrawal-induced anxiety [105,106]. Also, the delta opioid agonist TAN-67, although not effective in decreasing anxiety-like behavior in naive mice, showed anxiolytic effects in ethanol-withdrawn mice [107].

In conclusion, data from both pharmacological and genetic approaches indicate that delta opioid receptor activity modulates rewarding activities of a number of drugs of abuse, and influences several aspects of addictive behaviors, including drug-seeking, emotional responses and learning processes. Intriguing is the recent finding that delta opioid receptors play a key role in motor impulsivity [108,109], suggesting a possible implication of these receptors in yet another facet of addiction, (i. e. the loss of control over drug intake involving alteration of inhibitory controls) (See Figure 2 and [110,111]). Delta drugs may

therefore target several distinct components of this complex relapsing disorder, and the question of whether delta opioid agonists or antagonists may be therapeutically useful in the treatment of drug abuse remains open.

Proconvulsant effects of delta opioid agonists

Altogether, delta opioid receptor activation appears beneficial in several disease conditions that include chronic pain, emotional disorders and perhaps some aspects of addiction. Worth mentioning, however, is the fact that delta opioid receptor activation may lead to convulsions. Delta opioid agonist-induced seizures are mild, closer to a model of absence seizures rather than grand mal, and are prevented by drugs that attenuate absence seizure in humans [112]. Convulsant effects are dependent on intact delta opioid receptor function [113], and were originally considered a caveat to the development for delta drugs. Interestingly however, convulsive effects of SNC80 can be separated from other effects [76]**.** In addition, tolerance has been shown to develop rapidly to the convulsant effects of SNC80 [24], and many of the recently developed delta opioid agonists presented in Table 1 showed no convulsant effects at analgesic doses. Therefore, the proconvulsant activity of delta opioid agonists appears to be avoidable or surmountable.

Concluding remarks

Delta opioid receptor pharmacology has recently progressed and many novel highly selective delta opioid agonists with potent *in vivo* activities are now available (Table 1). Beneficial effects of delta opioid agonists have been clearly established in areas of chronic pain and emotional disorders, while the usefulness of delta drugs (agonists or antagonists) in neuroprotection, as well as addictive or impulse disorders, remains to be clarified. Moreover other areas such as learning and memory, in which delta opioid receptors may play a role, are currently being investigated. Importantly, the demonstration that biased agonism at the delta opioid receptor allows the discrimination of delta opioid agonist efficacies in stimulant, analgesic and anxiolytic responses offers novel perspectives for targeted therapies. Altogether, new discoveries in the field of delta opioid receptor biology at the cellular and behavioral level, combined with advanced medicinal chemistry will doubtless open new avenues in several disease areas.

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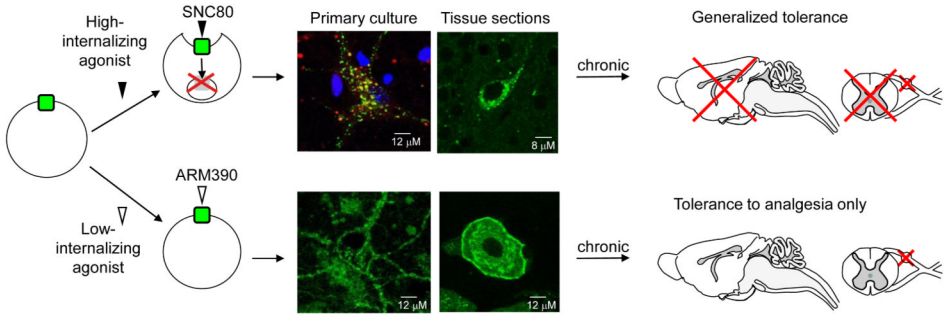


Figure 1. ***In vivo*** **consequences of biased agonist-induced trafficking at the delta opioid receptor: two mechanisms towards tolerance**

SNC80 binding to the delta opioid receptor results in massive receptor internalization in primary neurons from DOR-eGFP mice (representative neuron from a hippocampal primary culture, top left panel), as well as throughout central and peripheral nervous systems (representative image from a hippocampal section, top right panel). Following internalization, delta opioid receptors are targeted towards degradation (top left panel), as shown by co-localization of DOR-eGFP fluorescence (green) with the lysosomal marker, lysotracker (red). In contrast, ARM390 does not produce significant internalization of DOR-eGFP, either in primary neurons from DOR-eGFP mice (hippocampal primary culture, bottom left panel) or in central and peripheral nervous systems (dorsal root ganglia section bottom right panel). *In vivo*, a single injection of the high-internalizing, but not the low-internalizing agonist, produces acute behavioral desensitization [15]. However, chronic treatment with both agonists produces analgesic tolerance, regardless of internalization potency. The high-internalizing agonist down-regulates delta opioid receptors throughout the nervous system leading to generalized tolerance. In contrast, the low-internalizing agonist only affects delta responses at the level of the dorsal root ganglia, resulting in a pain-specific tolerance. In this case, non-analgesic delta opioid agonist effects (anxiolytic effects, for example) remain intact after chronic treatment [23]. Drug design for low-internalizing delta opioid agonists may therefore be helpful in therapeutic strategies targeting chronic psychiatric disorders.

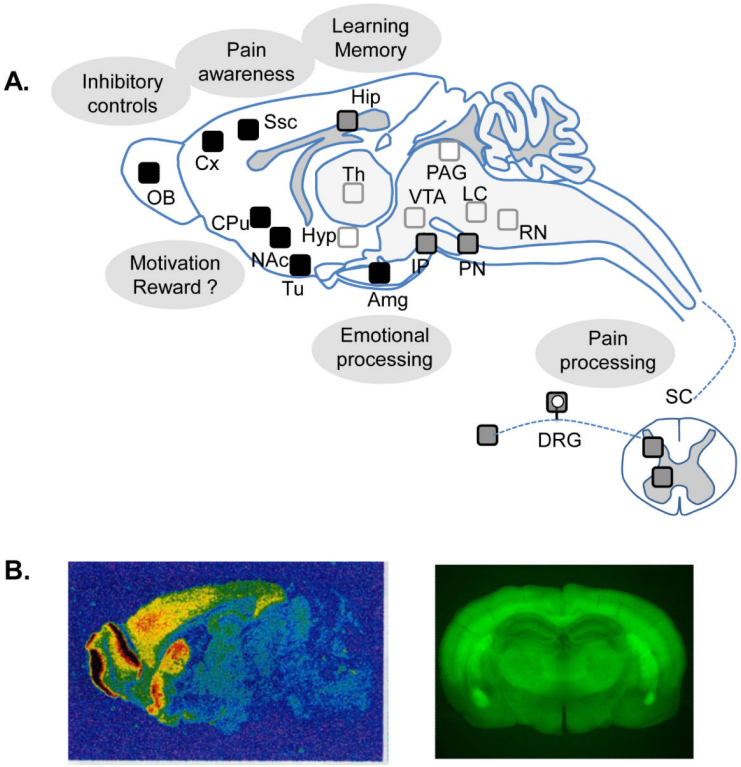
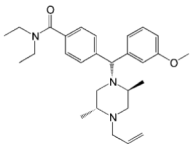
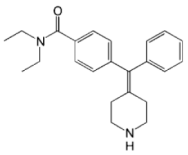
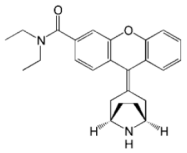
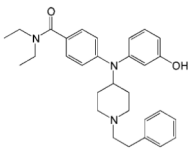
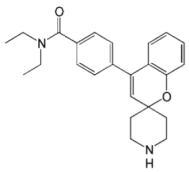
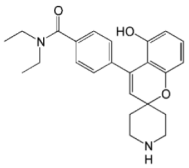
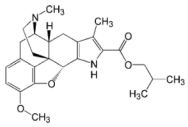
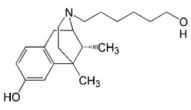


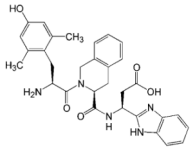
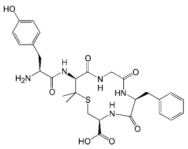
Figure 2. Delta opioid receptors in the central and peripheral nervous systems as potential targets for neurologic and psychiatric disorders

A. Schematic representation of delta opioid receptor binding sites. Delta opioid receptors are particularly abundant (black squares) in the olfactory bulb, cortex, amygdala and striatum (caudate putamen and nucleus accumbens). Delta opioid receptors are also expressed at moderate level in the interpeduncular and pontine nuclei, hippocampus as well as spinal cord and dorsal root ganglia (grey squares), and at a much lower level in hypothalamus, thalamus, mesencephalon, and brain stem (open squares) (Adapted from [3]). Brain areas of high delta opioid receptor expression are involved in several neural processes whose dysfunction may lead to neurological or psychiatric conditions, including pain (pain processing and awareness), anxiety and depression (emotional processing), addictive and impulse disorders (motivation and reward, learning and memory, inhibitory controls). There is a high expression of delta opioid receptors in dopaminergic terminals (striatum), and the role of delta opioid receptors in reward and motivation is complex. **B.** Delta opioid receptor binding sites visualized by ligand autoradiography ([^{3}H]deltorphin I, sagittal section left, courtesy of Ian Kitchen) or in knockin fluorescent delta opioid receptor reporter mice (coronal section, [21]). Abbreviations: Amg: amygdala; Cpu: Caudate putamen; Cx, cortex; DRG: dorsal root ganglia; FC: frontal cortex; Hip: hippocampus; Hyp: hypothalamus; IP, interpeduncular nucleus; LC: locus coeruleus; NAc: nucleus accumbens; OB: olfactory bulb; PN, pontine nucleus; PAG: periaqueductal gray; RN: raphe nucleus; SC: spinal cord; Ssc: somatosensorial cortex; Th: thalamus; Tu: olfactory tubercle; VTA: ventral tegmental area.

Table 1

Tools to Study the Delta Opioid Receptor

Small Molecule Agonists (a)	Chemical Structure	Summary	Reference For in vivo data
SNC80		- Classic non-peptide delta opioid receptor agonist - Analgesic, antidepressant and anxiolytic effects - Induces receptor internalization *in vivo*	[15, 22, 48, 69, 73]
AR-M100390		- Analgesic effect - No receptor internalization *in vivo*	[15]
JNJ-20788560		- Analgesic effect	[25]
Compound 8e		- Analgesic effect	[55]
ADL5747		- Analgesic effect - Clinical trial phase II ongoing	[56]
ADL5859		- Analgesic and antidepressant effects - Clinical trial phase II completed	[57]
SB-235863		- Analgesic effect	[26]
NIH 11082		- Analgesic and antidepressant effects	[54, 77]

Small Molecule Agonists (a)	Chemical Structure	Summary	Reference For in vivo data
UFP-512		- Antidepressant and anxiolytic effects	[74]
$DV_L^2DA_L^5LanEnk$		- Analgesic effect	[58,59]

Genetic Tools	Summary	Reference
Delta receptor knockout mice	- Conventional delta opioid receptor knockout mouse - Anxiety and depressive-like behavior - Enhanced hyperalgesia under chronic pain state - SNC80 analgesia abolished	[48-51]
Delta receptor conditional knockout mice	- Deletion of delta opioid receptors in $Na_v1.8$+ sensory neurons - Enhanced hyperalgesia under chronic pain state - SNC80 analgesia reduced	[60]
DOR-eGFP knock-In mice	-Functional delta opioid receptor in fusion with Green Fluorescent Protein in place of native receptor - Receptor visible *in vivo* at subcellular level	[15, 21]

(a) Prototypic (SNC80) and novel delta opioid receptor agonists. These ligands have been selected based on (1) high *in vivo* delta selectivity determined by blockade with the prototypic delta receptor antagonist naltrindole, or using delta receptor knockout mice, (2) small molecular weight (<600), (3) systemic activity *in vivo*.