Published in final edited form as:
Drug Alcohol Depend. 2012 March 1; 121(3): 167–172. doi:10.1016/j.drugalcdep.2011.10.025.

Functional Relevance of μ-δ Opioid Receptor Heteromerization: A Role in Novel Signaling and Implications for the Treatment of Addiction Disorders

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Abstract

Morphine and other opiates are among the most widely prescribed and clinically useful medications for the treatment of chronic pain. However, the applicability of these compounds has been severely hampered by the rapid development of tolerance and physical dependence that typically accompanies their repeated use. A growing body of evidence has implicated the regulated functioning of μ-δ opioid receptor heteromers in both the modulation of morphine-mediated antinociception, and in the limitation of undesirable side effects resulting from chronic opiate exposure. Moreover, μ-δ heteromers exhibit unique ligand binding characteristics and signaling properties, indicating that pharmacological targeting of the μ-δ heteromer may represent a novel therapeutic approach for the management of chronic pain and addiction disorders. Therefore, the present review will attempt to summarize the latest relevant findings regarding the regulation and functional characteristics of the μ-δ heteromer both *in vitro* and *in vivo*.

Keywords

Opiate receptor; endorphin; G protein coupled receptor; heterodimer; oligomerization; opiate addiction; opiate tolerance; opiate dependence

1. Introduction

G-protein coupled receptors (GPCRs), also referred to as seven-transmembrane receptors, are a large family of cell surface proteins that have been implicated in almost all physiological processes. This is accomplished by their ability to bind a diverse array of molecules, and then convert these surface stimuli into intracellular signaling cascades via

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This paper was presented in a symposium at the Behavior, Biology, and Chemistry: Translational Research in Addiction meeting on March 5, 2011 in San Antonio, TX entitled “New concepts in mu-opioid pharmacology -implications for addiction and its management.”

Contributors SDS conducted literature researches and wrote the manuscript; LAD provided mentorship and guidance in its preparation and help write the manuscript. All authors contributed to and have approved the final manuscript prior to submission.

Conflict of Interest All authors declare that they have no conflicts of interest.

their binding of heterotrimeric guanosine proteins (G proteins; Rozenfeld and Devi, 2011). Previously, it was believed that these receptors functioned only in their monomeric form, with each GPCR being associated with a distinct G-protein-mediated signaling cascade. Recent evidence from converging methodologies suggests that GPCRs can heterodimerize under both normal and pathophysiological conditions. While the existence of such heterodimers is still being actively debated (Viladarga et al., 2009; Kenakin et al., 2010), accumulating evidence demonstrates that many GPCRs have the ability to form functional dimers, oligomers, and even heteromers. Many have suggested that such heteromerization may represent a common mechanism by which GPCR activity and signaling may be modulated (Devi, 2001; Angers et al, 2002; George et al., 2002; Prinster et al, 2005; Miligan, 2007). While numerous examples of functional GPCR heteromers have been documented in recent years (Prinster et al., 2005; Miligan, 2007), heteromers composed of opioid receptors have garnered particular interest as novel therapeutic targets for the modulation of antinociception, as well as their role in tolerance and addiction to opiate compounds.

The three presently known opioid receptors, μ, δ, and κ, are all members of the family A GPCRs, and as such exhibit many of the same functional characteristics as other family A receptors. All three opioid receptors are coupled to $G_{i/o}$ proteins, inhibit adenylyl cyclase and cAMP activity *in vivo*, and are functionally sensitive to pertussis toxin (Cox, 2010). As with other members of this class of receptors, the opioid receptors have been shown to undergo both homo- and heterotypic interactions in heterologous cell lines (Jordan and Devi, 1999; George et al., 2000; Gomes et al., 2000). The results of such studies have revealed the existence of opioid receptor heteromers possessing unique ligand binding characteristics and signaling properties that are distinct from those identified for the individual constituents of the heteromer in question (Rios et al., 2001; Gomes et al., 2003; Gomes et al., 2004). Of particular clinical relevance, however, are heteromers composed of the μ and δ opioid receptors. Work from our laboratory and others have provided substantial evidence for the existence of opioid receptor heteromers using a variety of direct and indirect methods. As will be addressed in the present review, μ-δ heteromers are now known to modulate μ-agonized antinociception, in addition to significantly modulating the development of tolerance to the antinociceptive effects of opiate compounds. Importantly, modulating the functional characteristics of these receptor heteromers may represent a novel therapeutic target for the management of chronic pain as well as opiate addiction disorders (Rozenfeld and Devi, 2003). Consequently, this review will attempt to summarize what is presently known about the μ-δ heteromer, in addition to the latest emerging ideas implicating the μ-δ heteromer as a novel target for the treatment of chronic pain and addiction disorders.

2. Indirect Evidence of μ-δ Receptor Heteromers

2.1 Pharmacology and the μ-δ Receptor

Pharmacological tools provided some of the earliest evidence supporting the existence of heteromers of the μ and δ receptors, when it was observed that endogenous δ-selective ligands, such as Leu-enkephalin, and exogenous synthetic analogues of Leu-enkephalin (FK33824) had the ability to synergistically enhance low levels of morphine-mediated analgesia in a dose dependent manner *in vivo* (Lee et al., 1980). Subsequent to these initial findings, additional research observed that pretreatment with the selective δ-antagonist naltrindole prior to acute- or multiple administrations of morphine sulfate resulted in a robust attenuation of tolerance to morphine (Abdelhamid et al., 1991). Compounding evidence for the pharmacological interaction between the μ and δ opioid receptors was the finding that mice lacking the δ opioid receptor do not exhibit tolerance to the antinociceptive effects of morphine. This study in particular demonstrated, for the first time, that an interaction of the μ and δ opioid receptors is required for the development of tolerance to

morphine *in vivo* (Zhu et al., 1999). Further supporting the necessity of this heteromeric interaction in the development of tolerance, mice possessing a constitutive deletion of the δ opioid receptor were recently found to exhibit enhanced context-dependent sensitivity to the locomotor-activating effects of morphine. Additionally, when exposed chronically to morphine, these mice demonstrated an impaired ability to develop tolerance to these locomotor-activating effects relative to wildtype controls (Chefer and Shippenberg, 2009). These findings further suggest a critical role of the δ opioid receptor, and its interactions with the μ opioid receptor, in the mechanisms by which tolerance is developed. Similar recent research documenting the involvement of δ receptors in pain processing and opioid analgesia also further supports the notion of *in vivo* interactions between μ and δ opioid receptors (Beaudry et al., 2009; Gavériaux-Ruff et al., 2011.)

Recent studies have further linked the μ and δ opioid receptors through pharmacological activity *in vivo*. Administration of nanogram doses of the μ-selective antagonists CTOP and CTAP, or the δ-selective antagonist naltrindole all demonstrated the ability to synergistically enhance analgesia elicited by either a sub-maximal (5 μg/kg) or maximal (15 μg/kg) dose of morphine. Co-administration of these antagonists with morphine not only enhanced analgesia, but also prevented the development of tolerance and the accompanying loss of morphine efficacy (Abul-Husn et al., 2007). Recent findings have also supported the hypothesis that heteromeric interactions between the μ and δ opioid receptors can significantly modulate the conditioned rewarding effects of opioid administration. Consequently, a number of recent investigations have examined the impact of δ opioid receptors on morphine-induced conditioned place preference, demonstrating a significant relationship between antagonism of the δ opioid receptor (Shippenberg et al., 2009; Billa et al., 2010) or deletion of the δ opioid receptor gene (Le Merrer et al., 2010) and impaired conditioned place preference in response to morphine administration. Intriguingly, utilizing subcellular fractionation of the hippocampus, Billa and colleagues also observed a significant upregulation of both δ-opioid receptor dimers in the postsynaptic fraction, and the μ-opioid receptor in the total homogenate of animals that exhibited disrupted morphine-induced conditioned place preference in response to blockade of δ receptors with the δ antagonist naltriben (Billa et al., 2010). These results dovetail nicely with an emerging concept that has implicated trafficking and surface expression of the δ opioid receptor as playing a pivotal role in the generation of these phenomena.

Cahill and colleagues demonstrated that chronic morphine exposure, both *in vitro* and *in vivo*, resulted in increased surface expression of the δ opioid receptor in both cultured cortical neurons and the dorsal horn of the spinal cord respectively, and that increased surface expression of the δ opioid receptor *in vivo* was associated with an increase in deltorphin-II mediated antinociception (Cahill et al., 2001; Morinville et al., 2003). Gendron and colleagues demonstrated similar increases in the surface expression of the δ opioid receptor following chronic morphine treatment in cultured neurons of the dorsal root ganglion (Gendron et al., 2006). Though these lines of research have focused on trafficking of the δ receptor to the cell surface, more recent evidence suggests that the application of δ selective antagonists, such as naltriben, can prevent endocytosis of the μ-δ heteromer complex, leaving μ and δ homomers unaffected, all while simultaneously preserving the ability of the heteromeric complex to signal (Milan-Lobo and Whistler, 2011). That such effects were truly dependent upon specific and selective activation of the μ opioid receptor was further demonstrated in studies conducted with μ opioid receptor knockout mice. In these later studies, chronic escalating morphine administration failed to produce detectable increases in plasma membrane expression of the δ opioid receptor. Similarly, the concomitant potentiation of deltorphin-II mediated analgesia was also undetected (Morinville et al., 2003). Taken together, these studies suggest that the activities of the μ and δ receptors are inextricably linked together pharmacologically, that the pharmacological

synergism observed between these two opioid receptors is dependent upon increased surface expression of δ opioid receptors as a direct consequence of activation of μ opioid receptors (Cahill et al, 2001; Morinville et al., 2003), and that tolerance to and physical dependence on morphine requires the functioning of both the μ and δ receptors.

Building upon studies demonstrating that the analgesic effects of morphine administration may be preserved *in vivo* by the administration of antagonists of the δ-opioid receptor, a recent and intriguing study sought to study the *in vivo* pharmacology of μ-δ heteromers through the use of bivalent ligands; artificial compounds containing derivatives of a μ-receptor agonist and a δ-antagonist (MDAN) coupled by molecular spacers of differing lengths. Reasoning that the μ and δ binding sites on the heteromer should have a specific separation distance, then MDAN (μ-δ agonist-antagonist) compounds with varying spacer lengths ought to exhibit variable pharmacological and antinociceptive properties (Daniels et al., 2005). Indeed, the authors confirmed that both the development of tolerance and dependence to chronic morphine exposure, as well as analgesic potency, varied as a function of spacer length, with longer MDAN compounds that had spacers of roughly 22Å (MDAN19-MDAN 21) exhibiting enhanced analgesia and no discernable tolerance when compared to chronic morphine administration (Daniels et al., 2005). The authors go on to suggest that this dependence on the spacer length distance is further evidence that μ-δ heteromers are required for, and play a critical role in, the development of tolerance and dependence to opiate compounds.

Still further pharmacological evidence for the existence of the μ-δ heteromer comes from studies that have demonstrated a clear synergism between agonist and antagonist effects in heterologous expression systems *in vitro*. These studies have also further clarified the debate regarding the nature of the μ-δ heteromer forming interaction; there are a number of means by which such an interaction could occur however, but intriguing evidence lends support to the notion that the aforementioned effects are driven by a physical interaction between the two GPCRs, in fact, a true heteromeric receptor interaction. To date, most of this data has been generated by the co-expression of the μ and δ receptor in heterologous cell lines (Gomes et al., 2003).

2.2 Co-expression in Heterologous Cell Lines

By elucidating evidence that μ and δ receptors are co-localized together, in conjunction with the previously addressed pharmacological syngergy between the two receptors, further evidence of the *in vivo* feasibility of heteromeric complexes of μ and δ receptors has emerged. To date, a number of studies have demonstrated the heteromeric interaction between the μ and δ receptors in heterologous cell lines (Jordan and Devi, 1999; Gomes et al., 2000; George et al., 2000). Among the earliest of these studies, work from our laboratory demonstrated that interacting complexes could be isolated from heterologous cells expressing recombinant receptors or from endogenous tissue expressing native opioid receptors (Jordan and Devi, 1999; Gomes et al., 2000; George et al., 2000). Mirroring studies investigating the *in vivo* pharmacological syngergy of μ and δ receptor ligands, such studies also demonstrated *in vitro* that, upon co-expression of the receptors, extremely low doses of either μ or δ receptor selective ligands had the ability to significantly increase ligand binding and signaling through the of δ or μ receptor respectively. Interestingly, these results mirror the *in* vivo pharmacology of the μ-δ heteromer outlined above (Abdelhamid et al., 1991; Zhu et al., 1999; Cahill et al., 2001; Morinville et al., 2003; Abul-Husn et al., 2007). Additional follow-up studies in which HEK-293 cells were transfected with μ-luciferase and δ-yellow fluorescent protein not only confirmed these pharmacological properties of the μ-δ heteromer, but further revealed their close physical proximity through the use of a bioluminescence resonance energy transfer assay (Gomes et al., 2003). In support of these findings with BRET, these studies further demonstrated the physical

interaction between the μ and δ receptors through co-immunoprecipitation experiments. Cells were transfected with either *myc* or *Flag* tagged μ and δ receptors alone, or with a combination of *myc*-δ and *Flag*-μ. Lysates from these cells were then immunoprecipitated with antibodies directed against myc, and the resultant precipiates probed with anti-*Flag* antibodies via Western blotting. Only when the receptors were co-expressed (*myc*-δ-*Flag*-μ) was a distinct band corresponding to the heteromer visible at ~ 150 kDa (Gomes et al., 2003). While these experiments have gone a long way towards demonstrating the existence of the physical interaction between these two receptors, yet another study has furthered our understanding of how these interactions might physically occur.

Similar to other heteromers of GPCRs (Borroto-Escuela et al., 2010), the physical interaction between μ and δ receptors may depend critically upon the distal carboxy tails of both receptors (Fan et al., 2005). In this study, the authors demonstrated that truncation of the distal carboxy tail of the δ receptor, either by deletion of the last 15 amino acid residues, or deletion of all residues after Ser^{344}, resulted in graded changes in the ability of the μ-δ heteromer to bind μ-selective ligands and activate μ–specific signaling cascades. In the first mutation, binding of μ-selective ligands still elicited heteromer specific signaling cascades. When all residues of the δ receptor following Ser^{344} were deleted, however, neither receptor had the ability to physically interact, nor could specific μ-δ heteromer signaling cascades be detected (Fan et al., 2005). More recent findings however, have suggested an additional mechanism by which μ and δ receptors may physically interact to form functional μ-δ heteromer. Though the findings of this study will be described in greater detail below, He and colleagues have presented convincing evidence that the first transmembrane spanning region of the μ opioid receptor may be critical in both promoting the physical interaction between μ and δ opioid receptors, in addition to stabilizing the μ-δ heteromer complex (He et al., 2011). Taken together with the previously discussed studies however, the present body of research robustly demonstrates that physical interaction and formation of the μ-δ heteromer is at least possible. That such a physical interaction occurs between the two receptors, however, is insufficient to confirm that these putative μ-δ heteromers function and signal as unique entities. It might be possible, for instance, that the μ and δ receptors physically interact while still utilizing the same signal transduction pathways as their protomeric counterparts. As a result, additional studies have been undertaken in order to elucidate the signal transduction cascades that are unique to the μ-δ heteromer.

2.3 Unique Signaling Properties and Trafficking of the μ-δ heteromer

In addition to furthering our understanding of the physical requirements for interactions to occur between the μ or δ opioid receptors, Fan and colleagues demonstrated that the association between these two receptors via their distal carboxy tails may also contribute to generation of the unique pharmacology of these receptor heteromers by altering the G-protein signaling cascade initiated upon their activation (Fan et al., 2005). As discussed previously, μ and δ opioid receptors are coupled to $G\alpha_i$ proteins, and, upon activation, inhibit adenylyl cyclase in a pertussis toxin sensitive manner (Cox, 2010). Interestingly, these authors not only demonstrated that deltorphin-II may represent a potentially selective agonist of the μ-δ heteromer, but through [^{35}S]GTPγS assays, that μ-δ heteromer signaling is coupled to the pertussis toxin insensitive $G\alpha_z$ (Kabli et al., 2010). It is likely, however, that the heteromer would couple and signal through distinct signal transduction pathways under different conditions. Support for this hypothesis has been advanced by recent data from our laboratory suggesting that heterodimerization of the μ and δ opioid receptors leads to a shift from $G\alpha_i$- to β-arrestin2-mediated signaling rather than the suspected switch to $G\alpha_z$ (Rozenfeld and Devi, 2007).

In the aforementioned studies, within cells that co-expressed both μ- and δ-opioid receptors, β-arrestin2 was observed to co-localize with μ-δ heteromers at the plasma membrane in a

significant majority of the cells. More importantly, this surface co-localization did not occur in cells lacking either of the opioid receptors. Of equal importance was the observation that β-arrestin2 could only be found to co-immunoprecipitate in cells co-expressing the μ and δ opioid receptors, and that these effects occurred across a range of cell lines (Rozenfeld and Devi, 2007). Expanding upon these findings, we then explored the kinetics of ERK phosphorylation, comparing the temporal dynamics of ERK phosphorylation in cells expressing either the μ-δ heteromer or μ opioid receptors alone. While cells that expressed μ receptors alone exhibited a rapid but transient increase in the generation of phosphorylated ERK (pERK) following activation by the μ receptor agonist DAMGO, in cells co-expressing both opioid receptors, this initial peak of pERK was followed by a slower, more persistent second stage of ERK phosphorylation. Moreover, the duration of this second phase of ERK phosphorylation was directly dependent upon the expression levels of the μ-δ heteromer; the greater the surface expression of the heterodimer relative to homodimers, the more prolonged the time course of ERK phosphorylation (Rozenfeld and Devi, 2007). Using siRNA knockdown of β-arrestin2, we then demonstrated that knockdown of β-arrestin2 results in expression of pERK in a manner similar to that associated with activation of μ opioid receptors alone (Rozenfeld and Devi, 2007). Together, these data suggest that the degree of heterodimerization present in a cell has the ability to regulate both levels of heteromer expression in addition to the spatiotemporal regulation of ERK phosphorylation, and that such modulation of pERK occurs in a β-arrestin2 dependent manner (Rozenfeld and Devi, 2007). The involvement of β-arrestin2 in morphine tolerance and the generation of μ-δ heteromers have been further established by investigations conducted with β-arrestin2 knockout mice. In these studies it was observed that β-arrestin2 knockout mice fail to develop tolerance to the antinociceptive effects of chronic morphine exposure. Additionally, it has been reported that the β-arrestin2 knockout mice also exhibited significantly diminished withdrawal responses to submaximal doses of morphine (Raehal and Bohn, 2011). While the combined results of the aforementioned studies clearly establish the unique signaling characteristics of the μ-δ heteromer, the next critical question to be addressed was the precise manner in which surface expression of the μ-δ heteromer might be regulated.

Given their established roles in tolerance and dependence to opiates, understanding the molecular mechanisms by which these novel μ-δ heteromers are trafficked to the cell surface is a question of critical importance. The first piece of this puzzle was uncovered when it was observed that co-expression and association of the δ receptors with μ opioid receptors led specifically to the intracellular retention of the μ receptor, and concomitantly, a reduction in the surface expression of both receptors in a range of cell lines, suggesting that μ-δ heteromers are formed intracellularly before being trafficked to the cell surface (Décaillot et al., 2008). Importantly, these putative heteromeric receptors were being retained in the Golgi apparatus, but when both opioid receptors were expressed together along with a specific receptor transport protein (RTP4), an increase in the surface expression of the μ-δ heteromer was observed, in addition to diminished ubiquination and proteasomal degradation of the receptor heteromer (Décaillot et al., 2008). Though convincing, divergent findings from Law and colleagues has suggested that μ and δ opioid receptors may come together only at the cell surface, where they then form functional heteromers (Law et al., 2005). These conflicting results could be due in part, however, to the use of different expression systems utilized between the two studies. Further research will be needed in order to explore the degree to which RTP4 and other chaperone proteins may be upregulated in response to morphine exposure, and also the precise nature of their involvement in the heterodimerization process. Taken together cell surface targeting of the μ-δ heteromers via association with chaperone proteins or perhaps transient protein-protein interactions at the cell surface are two promising mechanisms by which modulation of both expression and function of μ-δ heteromers could occur in the *in vivo* setting.

While much has been learned about the function, pharmacology, and subcellular localization of the μ-δ heteromer *in vitro*, knowledge of how these findings relate to *in vivo* heteromer dynamics remains relatively sparse. For the most part, this paucity of information has been caused by a lack of the appropriate tools required to fully explore such questions in an *in vivo* model system. Though a number of studies have convincingly demonstrated precise co-localization of both the μ and δ opioid receptors (Cheng et al., 1997; Wang et al., 2010), further supporting the concept of their heteromerization, direct physical detection of μ-δ heteromers has remained elusive. The issue of direct detection of μ-δ heteromers is of significant importance, especially in light of a recent study that failed to support the findings from previous reports that documented significant co-localization between the μ and δ opioid receptors. Combining immunohistochemistry, using antibodies directed against GFP and μ opioid receptors, with a knock-in mouse model possessing δ opioid receptors tagged with enhanced green fluorescent protein (δeGFP), Scherrer and colleagues observed that significant co-localization of δeGFP with endogenous μ receptors occurred in less than 5% of dorsal root ganglion neurons (Scherrer et al., 2009). There are, however, a number of factors that may account for the apparent disparity between these results and previously documented findings of significant co-localization between the two receptors. For instance, the GFP antibody that was utilized in this study is also known to exhibit a higher avidity for GFP than the μ antibody shows towards μ receptors. More importantly, it is known that these knock-in mice overexpress δ opioid receptors (Scherrer et al., 2006), and additionally exhibit increased surface expression of δ receptors as a consequence of the GFP tag at their C-terminus (Wang et al., 2008). Taken together with the observation that increased surface expression of δ opioid receptors alters and attenuates maturation of μ receptors (Décaillot et al., 2008), it is likely that the reported lack of co-localization between μ and δ opioid receptors (Scherrer et al., 2009) was a consequence of an overestimated abundance of δeGFP receptors relative to that of endogenous μ receptors (Gupta et al., 2010). Though the discussion regarding such emergent findings persists, progress towards a resolution of this debate has begun with the recent generation of monoclonal antibodies selectively targeting the μ-δ heteromer, thereby allowing direct detection of the heteromer in fixed tissue for the first time (Gupta et al., 2010).

3. Direct Evidence of μ-δ Receptor Heteromers

With their established role in the development of analgesic tolerance, and interest peaking in the μ-δ heteromer as a potential therapeutic target for the management of chronic and/or neuropathic pain, it is of great interest to chart the localization of these heteromers across the neuroaxis. To facilitate such studies, using a subtractive immunization strategy, we generated monoclonal antibodies that selectively recognize the μ-δ heteromer in order to directly detect its regulation and expression following treatment of mice with a chronic escalating morphine treatment paradigm. This escalating morphine paradigm has reliably been shown to produce tolerance to the antinociceptive effects of morphine (Décaillot et al., 2006). We found that, relative to saline injected controls, mice that had received chronic escalating morphine treatment exhibited a significant increase in immunofluorescent intensity and density of μ-δ heteromers in both the medial nucleus of the trapezoid body (MNTB, an auditory relay nucleus) and in the rostral ventral medulla (RVM, a major relay nucleus involved in the transmission of the perception of pain). Using ELISA, additional increases in heteromer abundance were also observed in membranes derived from the cortex, nucleus accumbens, hypothalamus, and the ventral tegmental area. Moreover, such increases were seen only in animals subjected to chronic escalating morphine treatments, and not in those that received acute doses of morphine (Gupta et al., 2010).

An additional application of this new monoclonal antibody can be found in the ability to further probe μ-δ heteromer specific pharmacology *in vitro*, using the antibody to selectively

block binding and signaling of clinically relevant agonists of the μ or δ receptors that may have the potential to act on the receptor heteromer. Using this approach, we demonstrated that the monoclonal antibody could indeed prevent low doses of δ antagonists from potentiating the binding and signaling of μ receptor agonists and vice versa (Gupta et al., 2010). As such, these novel antibodies are among the most useful tools to date in order to better probe and understand the *in vivo* functioning of the μ-δ heteromer.

In a recent study, another useful tool to probe the functioning of the μ-δ heteromer was described (He et al., 2011). Here, the authors demonstrated that the potential heteromeric interaction between μ and δ receptors could be disrupted by expressing an interfering fusion protein, consisting of the first transmembrane region of the μ opioid receptor (MOR^{TM1}) and the TAT peptide, in cultured DRG neurons. Importantly, it should be noted that these findings build upon earlier computational studies that utilized three-dimensional homology modeling of full length μ and δ opioid receptor dimers to ultimately predict that the most likely interface point supporting the formation of the μ-δ heteromer would be through MOR^{TM1} (Liu et al., 2009). In conjunction with predictions from additional computational modeling studies of the μ-δ dimer (Provasi et al., 2010; Johnston et al., 2011), these findings suggest that it is MOR^{TM1} that may be responsible for promoting and maintaining the heteromeric interaction between the μ and δ opiate receptors (He et al., 2011). This novel tool was then used to demonstrate that co-trafficking of μ and δ receptors into postendocytotic degradation pathways in response to activation of δ opiate receptors complexed as part of a μ-δ heteromer could also be disrupted in cultured DRG neurons, in turn leading to desensitization of μ opioid receptors (He et al., 2011). More importantly, however, their results revealed that disruption of the μ-δ complex using the interfering peptide could also disrupt the μ-δ heteromer in spinal cord and small DRG neurons *in vivo*, resulting in enhanced antinociception in response to morphine, as well as an overall reduction in tolerance to the drug (He et al., 2011). Taken together, the use of these novel probes only hint at what future studies may reveal regarding the functioning of the μ-δ heteromer, and potential therapeutic applications for the management of chronic and/or neuropathic pain.

4. Conclusions

To date, heteromers of the μ and δ receptors have been found *in vivo* through the use of monoclonal antibodies, where their expression has been shown to be upregulated by chronic morphine exposure (Cahill et al., 2001; Morinville et al., 2003; Abul-Husn et al, 2007; Gupta et al., 2010). Morever, these heteromers are not only upregulated by morphine, but modulation of their functioning appears to play a critical role in the development of antinociceptive tolerance and in opiate dependence (Abdelhamid et al., 1991; Zhu et al., 1999; Cahill et al., 2001; Morinville et al., 2003; He et al., 2011). With emergent evidence suggesting that downregulation of the μ opioid receptors has the potential to reduce consumption of ethanol (Lasek et al., 2007), it is possible that the μ-δ heteromer may play a significant role in the regulated use of and addiction to compounds beyond opiates alone. In light of these findings, μ-δ heteromers have become promising and desirable targets for the pharmacological management of chronic and/or neuropathic pain (Rozenfeld and Devi; 2010), and studies such as those involving the development of novel bivalent ligands designed to selectively target these heteromers (Daniels et al., 2005) are leading the way in this field. In the quest for enhanced management of chronic pain, as well as the treatment of addiction disorders, future studies will need to expand our knowledge of the signaling cascades utilized by the μ-δ heteromer *in vivo*. Lastly, further research focused on the development of compounds that selectively target the μ-δ heteromer will be of tremendous benefit.

Acknowledgments

Role of Funding Source This study was partly supported by funds from NIH grants DA 008863 and DA 019521 (to LAD)

We thank Drs. R. Rozenfeld and I. Gomes for critical reading of this manuscript and for helpful suggestions in the preparation of the final manuscript.

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