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Opioid Receptors: Toward Separation of Analgesic from Undesirable Effects

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Abstract

The use of opioid analgesics for pain has always been hampered by their many side effects; in particular, the addictive liability associated with chronic use. Recently, attempts to develop analgesic agents with reduced side effects have targeted either the putative opioid receptor splice variants or the receptor heterooligomers. This review discusses the potential for receptor splice variant- and the hetero-oligomer-based discovery of new opioid analgesics. We also examine an alternative approach of using receptor mutants for pain management. Finally, we discuss the role of the biased agonism observed and the recently reported opioid receptor crystal structures in guiding the future development of opioid analgesics

Keywords

receptor splice variants; heteroligomers; receptor mutants as therapeutic targets

Receptors as targets for developing opioid analgesics without side effects

Opioids are the most potent and prevalently used analgesic agents for the treatment of severe acute, surgical and cancer pain, as indicated by the continued increase in global consumption of morphine from 7.2 tons in 1990 to 41 tons in 2010[1]. Still, the therapeutic use of morphine has been limited by its frequent side effects, such as nausea (30% of patients), constipation (23%), dizziness (20%), somnolence (18%), and vomiting (13%) observed in patients within pain studies (1). The side effects that have the highest impact on a physician's or patient's decision to use morphine for chronic pain treatment are the high addictive liability and drug-induced respiratory depression, which could lead to death. In 2006, opioids were involved in almost 40% of all poisoning fatalities (2). Therefore there is an urgency to develop compounds or treatment paradigms that will minimize the side effects of opioid analgesic agents.

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[1]From "Comments on the reported statistics on narcotics drugs, 2010" by International Narcotics Control Board, United Nations

From the initial identification of the high affinity stereo-selective binding sites, it was already apparent that the actions of the opioids are mediated by non-homogeneous receptor populations. The multiple opioid receptors were named by Martin and colleagues for the prototypic drugs that produced the physiological responses: mu (OPRM1), for morphine, and kappa (OPRK1), for ketocyclazocine (3). Subsequent analyses of the opioid activities in mouse vas deferens led to the discovery of the third or delta receptor (OPRD1, δ for deferens) (4). In the years leading to the molecular cloning of the μ-, κ- and δ-opioid receptors, pharmacological studies based on antagonists' selectivities and agonists' affinities led to the hypothesis that there are multiple opioid receptor subtypes, namely: $\mu_{1\text{-}3}$, $\kappa_{1\text{-}3}$ and $\delta_{1,2}$ (5). Among these multiple opioid receptor subtypes, the ones that gather the most attention are the μ_1 and μ_2 subtypes, as the majority of the *in vivo* morphine responses, including antinociception (pain relief), are absent in *oprm1*-null mice (6-8). Adding to their interest, the activation of μ_1 resulted in antinociceptive responses while μ_2 activation led to side effects such as respiratory depression (9, 10). An attractive hypothesis to eliminate side effects of the drugs involves identifying which responses are due to various receptor subtypes and developing ligands that are selective for particular subtypes. However, the molecular cloning of the receptor genes did not yield corresponding genes for various receptor subtypes. Importantly, the ablation of a single receptor gene such as *oprm1* eliminates all μ-opioid responses, as the deletion of *oprd1* or *oprk1* eliminates all δ- or κ-opioid responses.

In the past several years, two hypotheses have gained momentum in the field of opioid drug development: that receptor splice variants, or receptor heterodimers, create the receptor subtypes that exhibit distinct pharmacological responses. This review evaluates the supporting evidence for these hypotheses. We also examine alternative approaches in reducing the side effects of opioid drugs. Also, we discuss the probable implication of the recently resolved crystal structures of the opioid receptors and opioid-biased agonism on future attempts to separate the analgesic activities from the side effects of these drugs. Additional discussion on opioid receptor heterodimers as drug targets has been made in recent reviews (11-15).

Alternative splicing of the receptor genes

In contrast to some G protein-coupled receptors (GPCRs) such as ß$_2$-adrenergic receptor, all three opioid receptor genes have multiple exons, and splice variants of the receptors can be generated accordingly. For OPRM1, the mRNA is composed of exons 1, 2, 3, and 4. The first splice variant of OPRM1 was identified by Zimprich et al. (16), in which the final receptor protein exhibited a truncated carboxyl terminus and diminished ability to desensitize. Since then, numerous alternative splice variants of OPRM1 have been reported due to the identification of additional exons within the putative receptor gene (17). These splice variants are too numerous to be detailed individually, but they are generated through two main mechanisms: they are either expressed from an alternative promoter at exon 11, resulting in variants with different N terminus sequences or truncated receptors with 6 or 1 transmembrane (TM) region(s); or, they are created from the alternative splicing of exon 3 with exons other than exon 4 (Figure 1). Some of these splice variants are expressed at much lower levels than OPRM1, and have a brain region expression distribution that is distinct from that of OPRM1. For instance, OPRM1C and 1D are presynaptically located in the dorsal horn of the spinal cord whereas OPRM1 is located both pre- and post-synaptically (18-20). Furthermore, when some splice variants with different carboxyl terminus sequences were heterologously expressed in cell models they exhibited distinct pharmacological responses (21, 22), suggesting that the carboxyl tail sequences, or the cellular proteins associated with the carboxyl tail, influence the eventual receptor conformation. However, none of the splice variants can account for the μ_1 or μ_2 subtypes.

Nevertheless, the *in vivo* significance of some of these *oprm1* splice variants was illustrated using gene deletion studies. In mice lacking the exon 1 of *oprm1*, morphine was inactive while the antinociceptive activity of the morphine metabolite: morphine-6ß-glucuronide (M6G) and heroin were unexpectedly observed (23). The activities of M6G and heroin were demonstrated subsequently to involve the exon 11 splice variants; that is, those receptors having different N-termini or a truncated N-terminus relative to OPRM1 (Figure 1). Mice with exon 11 knocked out exhibited diminished heroin and M6G antinociceptive responses, while morphine and methadone activity was normal (24). Similarly, the inhibition of gastrointestinal transit by heroin and M6G was greatly diminished in exon 11 knockout mice while inhibition of gastrointestinal transit by morphine and methadone was not affected.

Such drug selectivity for the various splice variants represents an opportunity to identify or design compounds that could selectively activate a splice variant or its complex. This seems to the case with a recently reported compound: iodobenzoylnaltrexamide (IBNtxA). IBNtxA is a very potent analgesic; 10-fold more potent than morphine. However, in contrast to morphine, IBNtxA does not produce respiratory depression, and it inhibits gastrointestinal transit only modestly (25), two of the most concerning side-effects in the clinical use of opioid analgesics. Most interestingly, chronic administration of IBNtxA did not result in dependence or addictive responses, as demonstrated by the absence of withdrawal signs and the drug's motivational effects. Although the exact target of IBNtxA remains to be identified, the absence of IBNtxA responses in exon 11 knockout mice and the presence of IBNtxA activity in the triple knockout mice (i.e., the absence of OPRM1, OPRD1 and OPRK1 in these mice) suggest that IBNtxA must activate one of the exon 11 splice variants, as illustrated by the ability of IBNtxA to bind to the membrane of cells expressing the MOR1G variant (see Fig 1) and the orphanin F/Q receptor (ORL1), and not to cell membranes that express either of these receptors alone (25). These studies suggest that the separation of the side effects from the analgesic response can be achieved by targeting a subset of opioid receptor variants.

Receptor heterodimerization results in distinct pharmacological profiles

The ability of opioid receptors to homo- or heterodimerize has been the subject of intense investigation. Most of the data supporting hetero-dimerization are based on the apparent pharmacological profiles in cells expressing multiple opioid receptors. The heterodimerization of opioid receptors has been reported to alter ligand selectivity (26), switching signals from being Gi/Go- to Gz- (27) or ß-arrestin-dependent (28). Opioid receptor heterodimerization has resulted in the appearance of ligand activity, as demonstrated by the ability of 6-guanidionaltrindole (6-GNTI)a designed receptor antagonist to induce increase in the intracellular [Ca^{2+}] level in cells that express both OPRD1 and OPRK1, but not either alone (29).

In OPRM1/OPRD1 dimers agonist binding was shown to cause allosteric enhancement through inducing a cross-conformational switch among individual receptors (30). The conformation changes, as reflected in the intra- and intermolecular fluorescence resonance energy transfer (FRET), led to a decrease in activity within the putative OPRM1 and the α_2-adrenergic receptor heterodimers (31). These and other *in vitro* cell model studies, such as those showing that the ligand of one receptor can induce internalization of another receptor (see review by Rijn et al (32)), have implicated the probable distinct heterodimer pharmacological profiles. However, the reported opioid receptor subtypes' profiles have not been fully replicated in cells expressing various receptor heterodimers.

OPRM1/OPRD1 heterodimer's role in tolerance development

Nevertheless, there is some encouraging *in vivo* evidence in support of distinct activities of opioid receptor heterodimers. The heterodimer that attracts the most attention is OPRM1/OPRD1. Interaction between OPRM1 and OPRD1 is best exemplified by the classic studies which showed that administration of a δ-antagonist could block the development of tolerance to chronic morphine treatment (33), and also showed that mice with *oprd1* ablated did not develop morphine tolerance (34) and had altered addictive responses (35), (36). Such observations have prompted the designs of ligands or treatment paradigms that could alter the putative OPRM1/OPRD1 heterodimer activities. One such example is a class of bivalent ligands with 2 distinct pharmacophores: an OPRM1-selective agonist, oxymorphone, with the OPRD1-selective antagonist, naltrindole, joined together with flexible spacers between 16 and 21 atoms in length. Such bivalent ligands were shown to exhibit *in vivo* agonist activities ranging from 1.6- to 45-fold higher than morphine. Furthermore, mice treated chronically with bivalent ligands that had spacers of 19 atoms or higher did not exhibit tolerance or physical dependence, suggesting a role for these heterodimers in these chronic side-effects (37, 38). Accordingly, He et al. (39) interfered with the formation of OPRM1/OPRD1 heterodimers using a cell-permeable OPRM1 TM1-TAT peptide, and demonstrated that morphine antincociception was enhanced while tolerance development was reduced. It is their hypothesis that the activation of OPRD1 by its agonists such as deltorphin II or SNC80 will lead to internalization of heterodimers and OPRM1 degradation (39). Thus, the chronic side effects of morphine can be manipulated by regulating the heterodimers' formation.

Whether OPRM1/OPRD1 heterodimers are indeed the source of adverse chronic effects is not without controversy. The concurrent internalization of both OPRM1 and OPRD1 upon activation by OPRD1 (39) was not uniformly observed by others with different agonists (40). In order for the heterodimers to exhibit distinct pharmacological profiles, co-localization of both OPRM1 and OPRD1 in the same neuron and at the same sub-cellular location is a prerequisite. The 71-79% of small diameter dorsal root ganglion (DRG) neurons co-expressing OPRM1 and OPRD1 as reported (39) (41) (42) was not observed in the OPRD1-GFP knockin mice, in which only 36% of the non-myelinated DRG neurons contained both OPRD1 and OPRM1 (43). Whether the observed difference was the result of differential distribution of OPRD1 due to fusion with GFP remains to be resolved. Electron microscopy ultrastructural analyses of receptor distribution in the striatum and superficial layers of the cervical spinal cord did not reveal a high percentage of intracellular co-localization of OPRM1 and OPRD1 (44, 45). However, by developing a monoclonal antibody that recognizes only the OPRM1/OPRD1 heterodimers, Gupta et al. (42) reported the presence of these heterodimers in membranes from the cortex, nucleus accumbens, hypothalamus and ventral tegmental area. Furthermore, heterodimer levels in the medial nucleus of the trapezoid body (MNTB, an auditory relay nucleus) and the rostral ventral medulla (RVM) within the descending pain pathway increased after morphine treatment. These data clearly support the *in vivo* existence of heterodimers, but they appear to contradict the reported ability of morphine to internalize the heterodimers within the *in vitro* cell model studies (32). Hence, the significance of the observed changes in heterodimer level and the role of heterodimers in the chronic drug effects remain to be resolved in future studies.

Drugs targeting the receptor heterodimers

Despite the unresolved *in vivo* existence of heterodimers, the development of drug molecules that target heterodimers has already found some success. For example, in addition to the above mentioned 6-GNTI, which appears to be selective for OPRD1/OPRK1

heterodimers and is a very potent spinal analgesic (29), the molecule IBNtxA is devoid of side effects and might target the MOR1G/ORL1 heterodimers, as suggested by Majumdar et al. (25). Another example of targeting a specific heterodimer, and thereby eliminating side effects, is the recent observation that MOR1D and gastrin-releasing peptide receptor (GRPR) can form heterodimers and mediate the itch sensation. Pruritus (itching) is one of the side effects observed with morphine administration, and is especially noticeable with intraspinal administration. In their studies, Li et al. (46) reported that MOR1D and GRPR formed heterodimers, and morphine induced the internalization of both MOR1D and GRPR. Itch sensation was blocked by GRPR inhibitors and was absent in GRPR knockout mice. However, morphine-mediated analgesia was retained in GRPR-null mice. The injection of a Tet peptide containing the MOR1D C-terminal sequence RNEEPSS into the spinal cord reduced heterodimer formation and the itch sensation. Although other GPCRs such as the family of orphan receptors known as Mrg/SNSR or Mas-related G-protein coupled receptors (Mrgprs) that are expressed exclusively in the peripheral sensory neurons could also be involved in the itch sensation (47), disrupting heterodimer formation might be a viable approach for the reduction of this effect of morphine. However, the heterodimer interface must be well defined before peptides or molecules that interfere with the formation of heterodimers can be designed. As is apparent from the crystal structures of the opioid receptors, a definitive heterodimer interface might not be obvious.

Factors affecting dimer formation and stability

Insights from receptors' crystal structures

Whether ligands are activating a subset of opioid receptor splice variants or heterodimers, it is clear that receptor structures must be considered in the future design of ligands. Recently, the X-ray crystal structures of antagonist-bound inactive forms of the OPRM1, OPRD1 and OPRK1 receptors were reported (48-50). Unlike Class A GPCRs such as S1P1 (51) or Rhodopsin (52), which have their binding pockets largely shielded from the extracellular milieu, the binding pocket in all three opioid receptor structures is more open. This is largely due to the unique conformation of the EC-2 loop, which forms a β-hairpin that is pulled back from the bundle in each case despite its formation of a disulfide bridge with C3.25 (Figure 2). The openness of these binding pockets may be a contributing factor to the success of bivalent opioid ligands (53). There are also similarities between the binding pockets of OPRM1, OPRD1 and OPRK1 structures, a probable reason for the difficulties encountered in designing receptor-subtype-specific agonists. The protonated nitrogen of each antagonist in the X-ray structures interacts with D3.32 (Figure 2), a residue that is also the primary interaction site in cationic neurotransmitter GPCRs (54). On the intracellular side of all three receptors, transmembrane helix 3 (TMH3) and TMH6 are held in close proximity by an interaction between R3.50 and T6.34 (55) (Figure 2). This interaction mimics the R3.50-D/E6.30 "ionic lock" that promotes the inactive state in most Class A GPCRs. The most striking difference between the OPRM1, OPRD1 and OPRK1 structures is in the position of TMH1. In the OPRK1, TMH1 is pulled away from the bundle on the extracellular side in a manner similar to that seen in the β2-adrenergic receptor (56) (Figure 2, red helix). This conformation is promoted by the presence of the bulky residue M2.57, which prevents the top of TMH1 from moving closer to the TMH bundle (Figure 2).

Such differences in structure could be critical in putative heterodimer formation among these opioid receptors, especially since TMH1 has been found to be part of the interface in certain dimers and oligomers (see below). A cysteine crosslinking study of the inverse agonist-bound dopamine D2 receptor identified a symmetric TMH4-TMH5 homodimer interface (57) consistent with the atomic force microscopy (AFM)-based model of the dimer interface of dark state rhodopsin (58). An additional TMH1/TMH1 interface was later found for D2 in its oligomeric state (59) and a different set of TMH4 residues was found to form

the dimeric interface in activated D2 (60). The symmetric TMH4-TMH5 interface has been predicted computationally for numerous GPCRs (for a review, see (61)), including OPRD1 (62).

At first inspection, the OPRM1 receptor crystallized as intimately associated pairs, with two different interfaces: TMH5-TMH6 and TMH1–TMH2–Hx8, distinct from previous observations. The geometry of this dimer allows a close interaction between the T4-lysozyme inserted in the IC-3 loop of each monomer to promote crystal formation. The interface defined by TMH5 and TMH6 in the OPRM1 structure is much more extensive than the one defined by TMH1–TMH2–Hx8 (48). The OPRK1 receptor crystallized as parallel dimers with a single interface: TMH1–TMH2–Hx8 (49). However, the OPRD1 crystallized with only an anti-parallel arrangement of receptor molecules (50). Importantly, it is unclear if the oligomeric interfaces observed in GPCR crystal structures have physiological relevance. For instance, for crystallization, receptors are purified and detergent solubilized as monomers, which may limit their physiological relevance. Furthermore, the formation of parallel or antiparallel dimers occurs during crystallogenesis and probably reflects differences in the most energetically favorable interactions under crystallography conditions, rather than physiological conditions (50). In addition, modifications made to the GPCR structure (such as IC-3 loop insertion of T4-lysozyme to promote crystallization) may also favor certain interfaces. This may be the case for the OPRM1 receptor structure, which shows a close association of the T4-lysozyme inserts (48). However, the interfaces that are emerging from crystallographic data of unrelated GPCRs suggest the propensity of certain similar interfaces to form dimers, indicating that the observed opioid receptor interfaces may indeed be physiologically relevant. For example, the TMH5-TMH6 interface found in the OPRM1 (48) has also been found in five crystal structures of the CXCR4 receptor complexed with small molecule and cyclic peptide antagonists (63). Furthermore, the putative dimer interfaces observed with these opioid receptor crystal structures could also be interfaces for the formation of heterodimers with other GPCRs, and therefore influence the ability of ligands designed for such heterodimers to stabilize and activate the receptor.

Post-translational modifications

In addition to the influence of helix sequences in determining the most likely dimer interface and thus the stability of the receptor oligomers, post-translational modifications of the receptor also contribute to dimer stability. Palmitoylation is a post-translational modification that typically occurs in the C terminus of a GPCR. Cholesterol--palmitoyl interactions at the C-terminal (Hx8) residue, C7.68 (Figure 2), are present in the crystallographic TMH1-Hx8 dimeric interface of the β_2-adrenergic receptor crystal structure (β_2-AR) (56, 64). In this interaction, cholesterol is sandwiched between the receptor and the palmitoyl chain. Rat OPRM1 has two cysteines [C7.63(346) and C7.58(351)] in its C-terminus and another cysteine at the intracellular end of TMH3, C3.55(170) (Figure 2). Recent results have shown that C3.55(170) (rather than the C-terminal cysteines) is the major palmitoylation site in OPRM1 (65). Mutation of this cysteine attenuated receptor signaling and decreased the amount of cholesterol associated with the receptor signaling complex. In addition, palmitoylation was found to stabilize the receptor signaling complex and enhance morphine-induced signaling (65). In this case, the homodimer interface was proposed to be a symmetric TMH4-TMH5 interface (65) as seen in the D2 receptor (57) and rhodopsin (66). It bears mentioning that no palmitoylation sites are seen in any of the opioid receptor crystal structures. This is likely due to detergent solubilization of receptor in preparation for crystallization. Regardless of where the palmitoylation sites are located within the opioid receptors, however, differences in receptor palmitoylation could influence the ultimate stability of homo- or heterodimers, and therefore influence the signaling outcomes of ligands designed for such receptor oligomers.

Alternative approaches to reduce side effects via receptor activation

In addition to ligands that target a specific receptor, other approaches such as local administration of opioids have been used to reduce the subjective effects of these drugs (67, 68). An approach to reduce the development of tolerance by taking advantage of ligand-induced receptor endocytosis has been proposed by Whistler and co-workers. They hypothesize that ligands that can induce receptor endocytosis and recycling will induce less desensitization than those ligands that cannot, thus manipulating the Relative Activity versus Endocytosis (RAVE) ratio of the ligands (69, 70). By generating a mouse line that replaced the OPRM1 carboxyl tail sequence with that of OPRD1, Whistler and co-workers demonstrated that tolerance and addiction to morphine were reduced in these mice because morphine can cause internalization of the mutant receptor (71). Importantly, a cocktail that consisted of morphine and a small dose of methadone retained the antinociceptive response to morphine but did not promote morphine dependence (72, 73), because it promoted OPRM1 internalization. Thus, it is conceivable that such cocktails can be designed for clinical treatment of chronic pain.

Recently, another approach utilizing a mutant OPRM1 to eliminate the drug's side effects has been reported. In this mutant receptor, in which the conserved Ser residue in TM4 (S4.54) was substituted with either Leu or Ala, classical opiate antagonists such as naloxone or naltrexone activated the receptor without altering the agonist activity (74). Naloxone and naltrexone produced antinociceptive responses in a S4.54A substitution knockin mouse without eliciting any of the chronic effects such as tolerance and dependence (75). The chronic effects were avoided because the opioid antagonists could activate the mutant receptor while inhibiting OPRD1 (76, 77). When a double-stranded adeno-associated virus type 2 (dsAAV2) was used to deliver the S4.54A OPRM1 mutant into the spinal cord of wild type or *oprm1* null mice (78, 79), or other parts of pain pathway such as the ventral lateral periaqueductal gray (PAG) area (80), expression of the mutant receptor and subsequent antagonist-induced antinociceptive responses persisted for months, without development of measurable tolerance or dependence. So far, this approach involves the delivery and expression of a transgene in all cells surrounding the injection sites. The use of neural-specific promoters or even the OPRM1 promoters will limit the expression of the dsAAV2-delivered transgene to nociceptive neurons that normally express the opioid receptor. Such a gene therapy approach could be a viable method for the elimination of the side effects of opioid drugs, perhaps most applicable in terminal cancer pain.

Concluding remarks

In addition to the opioid receptor crystal structures, other factors that could contribute to the receptor activities, and therefore the eventual pharmacological profiles, must also be considered during the design of drug molecules that eliminate side effects. The association of GPCRs with cellular proteins that affect their functions is well documented (81-83). Opioid receptors are no exception. Interaction of an opioid receptor with cellular proteins such as ß-arrestin, riboporin1 and GRIN1 will determine its cellular location (84). Translocation of the receptor among microdomains can influence the coupling between the receptor and signaling proteins, such as Gα or ß-arrestin (65, 85). The cellular cholesterol level could not only affect the formation of microdomains such as lipid rafts but could also facilitate the formation of the homodimers and thereby affecting the signaling and the *in vivo* analgesic responses (86-89). All these and other factors could contribute to the biased agonism that has been reported for all three opioid receptors (90, 91). The observed biased agonism is mainly due to the differential coupling of the receptor with cellular proteins such as G protein or ß-arrestin. But the consequences of such differential coupling can be significant; for instance, OPRM1 biased agonism can control microRNAs that regulate

neuron differentiation and targeting (89), and OPRK1 biased agonism can specifically activate p38 MAP kinase in the dorsal raphe nucleus (92), which is implicated in the addiction process (86, 90, 91, 93). The differential *in vivo* response can be the consequence of the eventual cellular location of the activated protein kinases (94). Therefore, as summarized in Figure 3, for the future design of drug molecules or paradigms for reducing the side effects of opioid drugs to succeed, not only do the putative targets of these molecules need to be identified, (whether they are splice variants, heterodimers or receptor subtypes), but also the cellular proteins that interact with the activated receptor and the cellular locations of the activated proteins need to be considered. The ultimate goal will be the design of a drug molecule that will target a specific receptor subtype, whether it is a splice variant or a heterodimer between two opioid receptor types or between an opioid receptor and another GPCR. Thereby, the activation of a specific cellular pathway will result in the desired analgesic effect of the drug without any side effects. This has been and always will be the holy grail of opioid research.

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Highlights

- Novel development strategies separate analgesic efficacy from opioid side effects.
- Targeting receptor splice variants or receptor heterodimers could exhibit high analgesic efficacy with minimal side effects.
- “Drug cocktails” to alter receptor trafficking and antagonist-activated receptor mutants are other viable approaches to minimize side effects.
- Future drug designs should be guided by the resolved receptors’ crystal structures and the *in vivo* consequences of biased agonism.

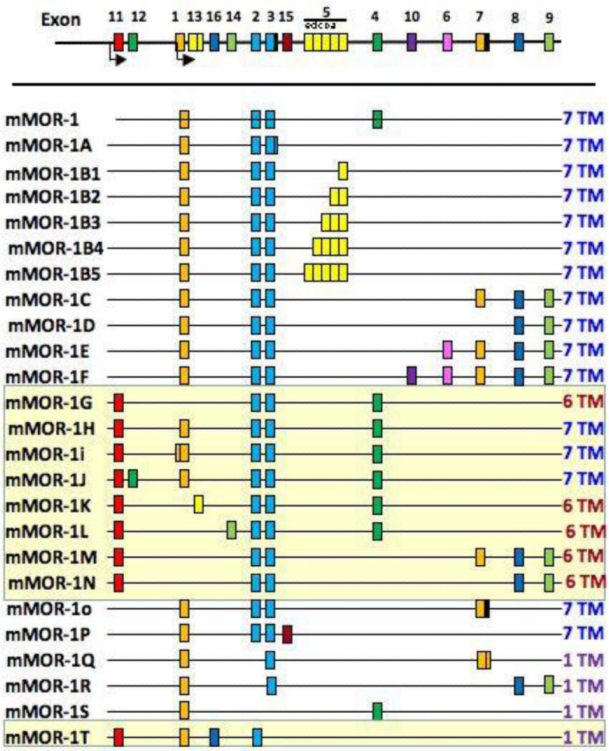

Figure 1.

Schematic representation of various mouse OPRM1 splice variants. The mouse gene is presented with various exons (color boxes) in schematic form and not to scale, with splice variants shown underneath. The splice variants generated by the exon11 promoter (designated by the yellow background) are distinct from those generated by the promoter associated by exon1, which are traditional 7-TM GPCRs. The exon11-associated variants include several full length 7-TM receptors, and both 6-TM and 1-TM variants as indicated. Reproduced with permission of Figure 1 in an article by Majumdar et al, Proc. Natl. Acad. Sci. USA 108: 19778-19783 (2011).

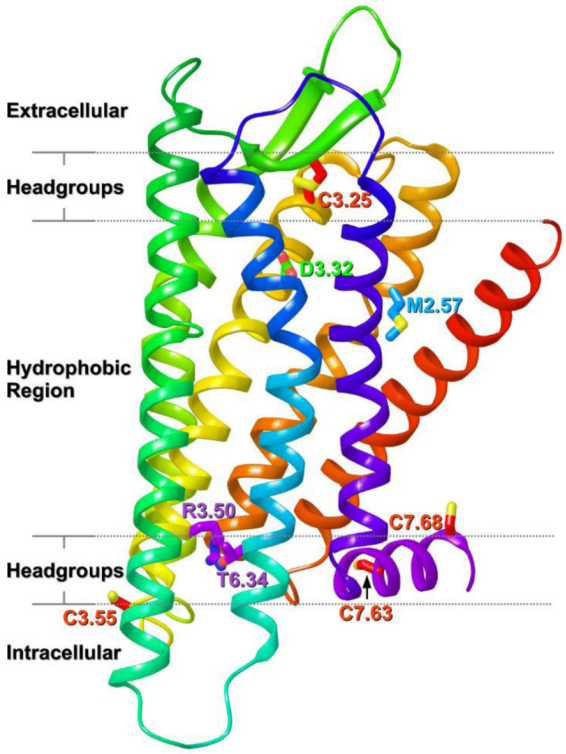

Figure 2.

The OPRK1 receptor crystal structure is shown with the approximate location of lipid bilayer components delineated by dashed lines. The seven transmebrane helix (TMH) bundle is colored from red to blue (TMH1, red; TMH2, orange, TMH3, yellow; TMH4, light green; TMH5, green;TMH6, blue;TMH7, dark blue). The helical segment that begins the C terminus and is oriented parallel to the membrane is Helix 8 (purple). Extracellular loop 2 (EC-2 loop; colored green) forms a β-hairpin structure that is pulled back from the bundle despite its formation of a disulfide bridge with C3.25.

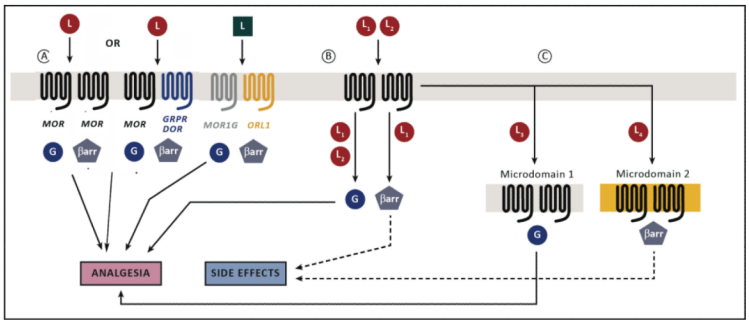

Figure 3.

Schematic representations of future designs of opioid analgesic drugs without side effects. In (A), a ligand could be designed to interact with specific heterodimers of OPRM1 (MOR) and other GPCRs, or with a heterodimer formed between a specific MOR splice variant and other GPCR, such as ORL1. In (B) ligands can be designed to select for signaling pathways that will result in analgesia and no side effect, as in the case ligand L_2 and not ligand L_1. In (C), ligands can also be designed to alter the microdomain distribution of the receptor resulting in analgesia and minimal side effects, as in the case ligand L_3.