REVIEW

CHALLENGES FOR OPIOID RECEPTOR NOMENCLATURE

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Summary:

Recent developments in the study of the structure and function of opioid receptors raise significant challenges for the definition of individual receptor types and the development of a nomenclature that precisely describes isoforms that may subserve different functions *in vivo*. Presentations at the 2013 meeting of the INRC in Cairns, Australia, considered some of the new discoveries that are now unraveling the complexities of opioid receptor signaling. Variable processing of opioid receptor messenger RNAs may lead to the presence of several isoforms of the μ receptor. Each opioid receptor type can function either as a monomer or as part of a homo- or heterodimer or higher multimer. Additionally, recent evidence points to the existence of agonist bias in the signal transduction pathways activated through μ receptors, and to the presence of regulatory allosteric sites on the receptors. This brief review summarizes the recent discoveries that raise challenges for receptor definition and the characterization of signal transduction pathways activated by specific receptor forms.

Almost two decades ago, the International Union of Pharmacology (IUPHAR) established a nomenclature committee to standardize the definitions and characterize the properties of receptors activated by neurotransmitters, hormones, cytokines and many drugs - this committee is known by the acronym NC-IUPHAR. In turn NC-IUPHAR established subcommittees to make recommendations on specific receptors and to develop a database defining the receptor systems and drug targets coded by the human genome with references to the most appropriate experimental models and the best selective radioligands, agonists and antagonists. This database can be found at http://www.iuphar-db.org; it is a mine of useful information for almost all receptors and ion channels.

The well-established Greek symbol terminology, μ,δ, and κ, for the first three types of opioid receptors to be identified, was proposed by Bill Martin, Hans Kosterlitz and their co-workers (Martin et al, 1976; Gilbert et al, 1976; Lord et al, 1977) in the mid-1970s. In 1996, an alternative terminology was proposed by an NC-IUPHAR subcommittee (Dhawan et al, 1996) but this terminology was not accepted by the field and is no longer used. A reconstituted Opioid Receptor Nomenclature Subcommittee (ORNS) proposed a return to the original nomenclature for opioid receptors, and added additional recommendations relating to the opioid receptor family and the receptor that is selectively activated by the endogenous ligand, nociceptin/orphanin FQ (abbreviated here as N/OFQ). The recommended revised terminology and abbreviations were accepted by NC-IUPHAR, and can be found on the NC-IUPHAR website: http://www.iuphar-db.org/DATABASE/FamilyIntroductionForward?familyId=50. The recommended nomenclature is briefly summarized in Table 1.

The close structural homologies between the three classic types of opioid receptors, μ, δ, and κ, and the more recently discovered receptor for N/OFQ have been confirmed by the recent reports of the crystal structures of each of these receptors when complexed with antagonists (Manglik et al, 2012; Granier et al, 2012; Wu et al, 2012; Thompson et al, 2012). They are clearly members of one family of proteins, with the differences between the receptor types arising by gene duplication events during evolution. It is thus appropriate to group these receptors as a single receptor family. NC-IUPHAR policy is to name receptors after their endogenous ligands, and to require that the abbreviation selected to represent a receptor family is two letters when there would be potential for confusion with other receptors if a single letter were to be used. Given the existence of receptors for oxytocin (OT) and orexins (OX), the family name selected by NC-IUPHAR for opioid receptors is OP (i.e., Opioid Peptide receptors). The Greek symbol terminology for the three receptors of the opioid receptor family that were first discovered, μ, δ, and κ, is retained, so these become the μ receptor, the δ receptor and the κ receptor (or μOP receptor, δOP receptor, and κOP receptor). Since it is sometimes inconvenient or impractical to use the Greek symbols, alternative abbreviations recognized by NC-IUPHAR are MOP receptor, DOP receptor, and KOP receptor. By analogy, the fourth member of the family becomes the NOP receptor (for nociceptin opioid peptide receptor). Note that in the NC-IUPHAR system the letter R for receptor is

never used as part of the receptor name since this adds no information to the terminology; the context usually makes clear that the terminology refers to a receptor. The widely used abbreviations MOR, DOR and KOR are therefore inconsistent with the NC-IUPHAR standards for receptor nomenclature; the ORNS recommends that these abbreviations should not be used to describe opioid receptor types. A summary of the recommended nomenclature and abbreviations for opioid receptors types is presented in Table 1.

Some investigators have questioned whether the NOP receptor should be classified as a member of the opioid receptor family, perhaps influenced by Hans Kosterlitz' dictum, frequently repeated by him at INRC meetings during the 1970s and 1980s, that if a receptor mediated action is not antagonized by naloxone, then the action should not be called an opioid receptor mediated effect. This insistence on a rigid procedural definition of an "opioid" was valuable at the time. For example, it became apparent that the actions of drugs at the sigma receptor, originally identified by Bill Martin as an opioid receptor (Martin et al, 1976; Gilbert et al, 1976), were not antagonized by naloxone (in contrast to Martin's original claim) and should not be called opioid. Subsequent studies have established that the sigma receptor exists, but as a protein that is very different in structure and function from the μ,δ, and κ receptors (Seth et al, 1998). Furthermore, many of the ligands that activate this receptor have very different structures from the endogenous ligands for the opioid receptors (Hayashi & Su, 2005). Kosterlitz' dictum need not be applied to the entire OP receptor family. The NOP receptor, unlike sigma receptors, is very similar in structure and in most functions to the other OP receptors. Actions of N/OFQ through the NOP are not antagonized by naloxone, but the amino acid sequence of N/OFQ indicates that this peptide is closely related structurally to the endogenous opioid peptides, probably derived during evolution by gene duplications among the opioid peptide gene family in much the same way as the various OP receptor forms diverged by gene duplication during evolution (Nothacker et al, 1996). As noted above, the NOP receptor crystal structure closely resembles the crystal structures of the μ, δ, and κ receptors and is more similar to these than to other GPCRs. Thus, in contrast to sigma receptors, NOP receptors display primary, secondary and tertiary structural similarity to other members of the OP receptor family, and are activated by an endogenous ligand that has a primary structure that is closely related to that of the endogenous ligands for the μ, δ, and κ receptors. Additionally, the NOP receptor employs a repertoire of signal transduction pathways that is very similar to the set of pathways activated by the three classic opioid receptors. These structural and functional considerations trump the absence of sensitivity to naloxone antagonism and clearly necessitate the assignment of the NOP receptors to the OP receptor family. The NOP receptor should be considered a subcategory of the OP receptor family with atypical low affinity for the classic opioid peptides (the enkephalins, β-endorphin and dynorphin) and insensitivity to antagonism by naloxone.

Several issues that have implications for opioid receptor classification and nomenclature were discussed during the 2013 INRC meeting. An area with potential significance for OP receptor classification is the growing evidence that the signal transduction pathways that are activated by agonists acting at the same receptor type are not always identical. Evidence that individual agonist ligands may preferentially direct the functional response

elicited by their common receptor to different transduction pathways was the subject of a plenary lecture by Arthur Christopoulos on biased agonism at GPCRs. The main emphasis of Christopoulos' talk was on other GPCRs, not specifically on opioid receptors, but other speakers addressed biased agonism at OP receptors. Eamonn Kelly from Bristol showed unambiguously that certain agonists at MOP receptors bias the response towards either G protein- or β-arrestin-mediated transduction pathways. The signaling pathway repertoire that can be activated by these transducers is also expanding. For example, Wendy Walwyn presented evidence that δ and NOP receptors can activate cofilin, an actin-modulating protein, via β-arrestin, ROCK and LIMK. Until recently it had been assumed that any ligand that could activate a receptor would induce essentially the same cellular response, with the major differences in response relating to the relative efficacies of different agonists. Now that biased agonism at OP receptors is an established fact, apparent differences in the responses induced by agonists that act at the same receptor type do not require the postulation of separate receptor sub-types for each agonist; the same receptor may be differentially biased by each agonist to mediate different transduction pathways.

Functional studies of OP receptors in the 1980 and 1990s suggested the existence of subtypes of the major OP receptor forms; specifically differences in the relative potencies of selected agonists at δ receptors and their differential sensitivities to certain antagonists led to claims of the existence of subtypes of δ receptor (see review by Zaki et al, 1996). At μ receptors, the actions of some agonists are reported to be more readily antagonized by the irreversible antagonist, naloxonazine, than others (Pasternak & Wood, 1988; Paul et al, 1989). These observations led to the proposal that there are subclasses of δ and μ receptor, named δ_1, δ_2, μ_1 and μ_2, but no evidence for the existence of more than one gene for the δ-or μ receptors exists despite careful homology searches of the genome. Knock-out of the δ receptor gene is reported to abolish the activity of ligands preferentially acting at both δ_1 and δ_2 sites (Filliol et al, 2000). There are also proposals for the existence of subtypes of κ receptor, based on relative agonist potencies for selected actions that appear to be mediated by κ receptors (Rothman et al, 1989). However, a triple knockout of μ, δ, and κ receptors completely abolishes binding and function of all opioid ligands (Clark et al., 2002; Martin et al, 2003), indicating that these ligands require at least one of the three receptor members of the OP receptor family for activity.

It is possible that some or all of the data leading to the proposal that there are subtypes of μ, δ, and κ receptors might be explained by biased agonism. Agonist potency ratios are now only interpretable if the experimental system from which the data is obtained is fully defined, including not only the receptor type mediating the actions, but also the cell type(s), experimental conditions and the signal transduction systems mediating the measured effects. Examining differences in the relative potencies of a series of agonists in different cell or tissue preparations or *in vivo* was historically an important approach to the identification of heterogeneity of many receptor types. It was this type of evidence that was used in part to support the proposed δ receptor subtypes (Zaki et al., 1996). However, because of the possibility of biased agonism, differential agonist potency or efficacy can no longer be regarded as strong enough evidence to postulate the existence

of non-identical receptors as the mediators of these actions, although antagonist dissociation constants continue to provide more robust evidence of receptor heterogeneity. These conclusions have significance for the receptor databases. Agonist potency ratios are of value in the context of highly defined experimental systems but must be interpreted with caution. The possible existence of opioid receptor subtypes should be reexamined in the light of recent studies demonstrating biased agonism at opioid receptors

Apparent receptor heterogeneity might also be induced by interactions of receptors with interacting proteins or modulating ligands. In 1997, Cvejic & Devi and her colleagues reported that OP receptors could form homodimers, and in 1999 they showed the formation of functional heterodimers with ligand binding properties that differed from those displayed by either of the individual receptor types (Jordan & Devi, 1999). In the case of the δ–κ receptor heterodimer, the ligand binding properties were found to match the properties of the putative κ_2 subtype (Zukin et al., 1988). Subsequently many other groups have confirmed the existence of opioid receptor dimers and higher-order forms (oligomers), and the observation has been extended to many other GPCRs (Milligan, 2009). Indeed, several non-opioid GPCRs, including chemokine and serotonin receptors have now been reported to form functional heterodimers with opioid receptors (Hebert 2008; Cussac et al, 2012; Rozenfeld and Devi, 2010). But to date it has not been unambiguously demonstrated that the reported μ, δ, and κ receptor heterogeneity can be accounted for by receptor heterodimerization. Towards this end, reagents that allow detection and evaluation of the endogenous OP heterodimers are being generated and these have begun to show promising results; δ–μheterodimer-selective antibodies have been useful in revealing morphine-induced upregulation of this heterodimer in the brain and in demonstrating heterodimer-directed signal trafficking (Rozenfeld & Devi, 2007). Ligands selectively targeting the heterodimer have helped demonstrate allosteric modulation of ligand binding and signaling by heterodimerization (Gomes et al, 2011; 2013) as well as the exploration of the pharmacological properties of heterodimers *in vivo* (Daniels et al, 2005; Milan-Lobo, et al, 2013). Finally, cell-permeable peptides that selectively disrupt the δ–μ heterodimer have helped address the contribution of this heterodimer to opioid pharmacology (He et al, 2011). Reagents such as these will be valuable in addressing the extent to which receptor heterogeneity could be attributed to opioid receptors heterodimers in biological systems.

Agonist actions at many GPCRs are additionally subject to either positive or negative regulation by ligands acting through allosteric regulatory sites on the GPCR. The existence of allosteric modulators of the μ receptor (Burford et al., 2013) was discussed at the meeting by Andrew Alt and John Traynor. Positive and negative allosteric compounds binding to a GPCR change receptor conformation to either enhance or inhibit orthosteric agonist binding and receptor activation; positive allosteric modulators may also show agonist effects, such compounds are allosteric agonists. A key finding is that the allosteric modulator-occupied receptor can have differential affinity for some, but not all orthosteric ligands, resulting in probe dependence***;*** moreover, the allosteric modulator may induce, or change the direction of, signaling bias. Thus differential sensitivity of the activation of receptors by diverse agonists to allosteric regulation offers another potential

explanation for the apparent differences in the actions of different agonists at the same receptor. These observations point to the need for additional research describing more completely allosteric modulatory sites on each OP receptor. Because there are changes in relative agonist affinity and/or efficacy, and perhaps induction of a signaling bias, the allosteric modulator-bound receptor may be considered a novel entity. On the other hand allosteric modulators only subtly alter receptor conformation and so an OP receptor bound to an allosteric modulator remains an OP receptor, based on the structural and functional arguments discussed earlier. Nonetheless, the fact that allosteric modulators can differentially change the ability of agonists to bind to and activate the receptor and may have agonist actions on their own presents new challenges for OP receptor nomenclature, and in particular for opioid ligand nomenclature.

The role of alternative transcription from a single gene as a potential basis for OP receptor subtypes is also controversial. Gavril Pasternak and others have shown that there is more than one transcription start site on the μ receptor gene and multiple alternative mRNA splicing pathways, resulting in multiple transcripts coding for proteins with different primary structures (Abbadie et al, 2000; Koch et al, 2001; Kvam et al, 2004). It is suggested that these variant receptor forms can account for the apparent functional heterogeneity of μ receptors (Pasternak & Pan, 2013) There are no reports of multiple start sites and alternative transcripts for the δ and κ receptor genes. Thus, the alternative transcript hypothesis is only plausible as a potential explanation for the apparent heterogeneity of μ receptors but, with few exceptions (e.g. Liu et al., 2011), there remains much uncertainty about the levels of expression of the variant mRNA forms for this receptor, their stability in the cell, and the properties of any proteins expressed from these mRNA variants. The presence of functional receptor proteins derived from variant transcripts (arising from different transcription start sites or alternative mRNA splicing) from a single receptor gene requires further study. If confirmed then a consistent nomenclature differentiating the variant forms of a single receptor will need to be developed.

Sequence variations within opioid receptor genes might cause the expressed receptors to display properties that distinguish these receptors from those of the most frequently expressed receptor form. There are numerous single nucleotide polymorphisms (SNPs) in human opioid receptor genes, but most are rare and none are known to alter the conformations of the expressed receptor (Mague & Blendy, 2010). Only one polymorphism in the coding region of human opioid receptor genes is known to occur with relatively high frequency (rs 1799971; varying from 40 to 50% is some Asian populations to 5% or less in African Americans: Gelernter et al, 1999) and its known functional consequences are limited. A change of adenosine to guanosine in position 118 (A118G) of the coding region (exon 1) of the human μ opioid receptor gene results in the expression of a receptor with aspartic acid (Asp) in position 40 instead of asparagine (Asn); this change removes a potential glycosylation site. A transgenic mouse line in which guanosine is replaced by adenosine in the equivalent position of the mouse μ receptor gene (A112G, expressing N38D) resulted in expression of receptors with similar ligand binding properties but reduced levels of expression of the receptor mRNA and reduced receptor protein levels relative to the wild-type receptor (Mague & Blendy,

2009). This is consistent with other reports that downstream signaling is impaired in the variant form relative to the wild-type human μ receptor (Mague & Blendy ,2010; Oertel et l 2012). The mechanism for the reduced level of expression may relate to increases in methylation of the 118G μ receptor gene. Oertel et al (2012) report that the degree of gene methylation at positions +117 and adjacent downstream methylation sites was higher in heroin-using subjects expressing the 118G variant than in 118A expressing subjects. In 118G subjects, chronic heroin use was not associated with elevated levels of μ receptor expression, but in 118A subjects chronic heroin use induced an upregulation of μ receptor expression in the thalamus and a cortical region. Increased receptor methylation in the 118G receptor gene may impede receptor up-regulation in response the drug exposure, suggesting an epigenetic regulation of the level of expression of μ receptors (Oertel et al, 2012). This work requires confirmation in a larger set of subjects. To date however, there are no reports indicating that functional opioid receptors with altered ligand binding or signal transduction properties are produced as a result of polymorphisms in opioid receptor genes.

Some opioid drugs have been reported to bind to non-opioid receptor proteins such as filamin A which interacts directly with μ receptors (Wang HY et al. 2008), or to the toll-like receptor-4 (TLR-4) (Hutchinson et al., 2011) that does not interact directly with opioid receptors. Naturally, the structural requirements for interaction of opioids with these proteins are very different from their binding to classical opioid receptors. Nonetheless the interactions might be important, e.g., direct actions of opioids on the TLR-4 complex have been proposed to activate microglia to mediate many of the adverse effects of morphine (Hutchinson et al., 2011). However, this potential mechanism has been ruled out in other studies of morphine-induced microglial activation (Ferrini et al., 2013; Fukugawa et al., 2013) and the affinity of interaction of opioids with TLR-4 mechanisms is at least several orders of magnitude weaker than their interaction with MOPr (Wang et al., 2012), questioning their pharmacological relevance. Thus the functional relevance of binding of some opioid drugs to proteins other than the opioid receptors is not clearly established. Since these interactions do not involve direct binding to opioid receptors, it is not feasible to define them within the framework of an opioid receptor nomenclature, but investigators need to be aware that ligands for opioid receptors, like many other receptor ligands, can interact with other proteins with possible functional consequences, whether with very high affinity as for filamin A (Wang HY et al, 2008) or with low affinity as for TLR4 (Wang X et al, 2012).

Concluding comments:

There has been a growing consensus on use of the recommended opioid receptor nomenclature shown in Table 1 since publication of the most recent NC-IUPHAR recommendation. If and until the accepted nomenclature for opioid receptors is revised to encompass the proposed variant forms of μ, δ, and κ receptors based on more stringent criteria that take into account the additional variables in receptor properties outlined above, we suggest that the simple classification in Table 1 be used by all authors.

Beyond μ, δ, κ and NOP receptors, a description of opioid receptor subtypes such as μ_1 or μ_2 is not recommended unless they are described as putative. We do not consider that the evidence for opioid receptors subtypes that has been offered to date provides unambiguous evidence of independent functional receptors that are not variant forms of the major opioid receptors. Until a new nomenclature for opioid receptor variants encompassing possible alternative transcription start sites or splice sites, receptor homo- and hetero-multimers, positive and negative allosteric regulation, and biased agonism is established and accepted by the research community, any such proposed variants should be fully described. Evidence of activation of signaling pathway(s) not previously associated with opioid receptors, the identification of novel allosteric regulatory site(s), and the establishment of novel polymeric structures should not be considered sufficient justification for modification of the current nomenclature.

Table 1. NC-IUPHAR Approved Nomenclature for Opioid Peptide Receptors

Receptors activated by opiate drugs respond physiologically to endogenous opioid peptides; they are therefore Opioid Peptide receptors, the receptor family being designated by the two-letter abbreviation OP.

Current NC-IUPHAR Approved Nomenclature[1]	Other (non-approved) Nomenclature	Presumed Endogenous Ligand(s)
μ, mu, or MOP	MOR, OP_3	β-endorphin (not selective[3]) enkephalins (not selective[3]) endomorphin-1[2] endomorphin-2[2]
δ delta, or DOP	DOR, OP_1	enkephalins (not selective[3]) β-endorphin (not selective[3])
κ, kappa, or KOP	KOR, OP_2	dynorphin A dynorphin B α-neoendorphin
NOP	ORL1, OP_4	nociceptin/orphanin FQ (N/OFQ)

Footnotes:

1. The well-established Greek terminology for opioid receptor types using the descriptors μ, δ, and κ is recommended, but where Greek symbols are not permitted or impractical, the use of mu, delta or kappa, or MOP, DOP, or KOP is permissible.

2. No mechanism for the endogenous synthesis of endormorphins has been identified; their status as *endogenous* ligands for the μ receptor is tentative.

3. "Not selective" indicates that these ligands are not strongly selective for the specific receptor types indicated; they may have sufficient affinity and efficacy at other opioid receptors to exert pharmacological effects through the non-preferred site. For example, the enkephalins are listed as non-selective ligands for both μ and δ receptors. However, all ligands in this table have very low affinity and efficacy at non-opioid GPCRs.

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Table of Abbreviations:

DOP, δ receptor, a member of the OP receptor family
GPCR, G-protein coupled receptor
INRC, International Narcotics Research Conference
IUPHAR, International Union of Basic and Clinical Pharmacology
KOP, κ receptor, a member of the OP receptor family
LIMK, Lim domain kinase
MOP, μ receptor, a member of the OP receptor family
NC-IUPHAR, International Union of Basic and Clinical Pharmacology Receptor Nomenclature Committee
N/OFQ, nociception/orphanin FQ
NOP, N/OFQ receptor, a member of the OP receptor family
ORNS, Opioid Receptor Nomenclature Subcommittee
OP, NC-IUPHAR abbreviation for opioid receptor family
ROCK, Rho-associated protein kinase

Conflict of Interest Statement:

The authors all assert that they have no financial conflicts of interest.