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COMMENTARY

Opioid and cannabinoid receptors: friends with benefits or just close friends?

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μ-Opioid (MOP) and cannabinoid CB1 receptors mediate overlapping pharmacological responses in clinically important areas such as drug abuse and pain management, and functional interactions between agonists at these receptors have long been recognized. In the present issue of this Journal, Rios and co-workers have provided the first strong evidence that the two receptors interact directly when coexpressed in the same cells. The authors report a close physical association between MOP and CB1 receptors and novel pharmacological interactions of MOP and CB1 agonists. They argue that MOP/CB1 heterodimer formation explains these interactions. If correct, the direct interaction of MOP and CB1 pharmacophores in a quaternary complex would provide real benefits by opening the potential for development of novel MOP/CB1 small molecules or new strategies for use of current ligands. However, a lot more evidence will be required before the heterodimer interpretation can be accepted. If it turns out that MOP and CB1 receptors do not readily form hetero-oligomers, the study by Rios and co-workers shows that they are still friends but there may be few benefits.
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The demonstration by Rios *et al*. (2006) of a close relationship between μ-opioid (MOP) and cannabinoid CB1 receptors adds to the list of GPCRs that may directly influence each other *via* protein–protein interactions. This is significant because MOP and CB1 signaling systems mediate overlapping pharmacological responses in clinically important areas such as drug abuse and pain management. However, what remains to be established is just how close the two receptors are. Rios *et al*. (2006) argue that their data suggest CB1 and MOP receptors form heterodimers. If this interpretation is correct, the direct interaction of MOP and CB1 pharmacophores in a quaternary complex opens the possibility of the development of novel small molecules or new strategies for use of currently available ligands that interact with MOP–CB1 receptor oligomers. If, on the other hand, MOP and CB1 receptors are just close friends but do not actually form hetero-oligomers, the results are less exciting but may still have significant implications for interacting signaling cascades.

Dimer formation of GPCRs is beyond doubt and appears obligatory for membrane expression and function (see Milligan & Bouvier, 2005). Heterodimer formation is needed for the function of some GPCRs (e.g. $GABA_B$ receptors) and there is strong evidence that it can occur between less closely related GPCR monomers (see Milligan *et al*., 2004). Devi's group has previously shown a range of more or less direct interactions among the opioid receptors themselves (Rios *et al*., 2001). MOP–DOP and KOP–DOP interactions have been reported in single cells but similar MOP–KOP interactions have not been found. Devi's group has also shown similar interactions between opioid receptors and β_2- (Jordan *et al*., 2001) or α_2-receptors (Jordan *et al*., 2003). Significantly, the pharmacological behavior of opioid receptor containing hetero-oligomers was found to be unusual and to exhibit altered signaling by coapplied agonists (usually inhibition of maximal responses) and antagonists (potentiation of agonists actions by antagonists of the partner) as well as modified receptor trafficking. In some cases, the hetero-oligomer interpretation is compelling, such as the 'dominant negative' blockade of all agonist-induced internalization when β_2-receptors are coexpressed with non-internalizing KOP receptors (Jordan *et al*., 2001). With other receptor pairs, altered agonist efficacies could be due either to formation of hetero-oligomers or downstream effects on G-protein signaling or recruitment of other regulatory proteins. These latter possibilities are very difficult to rule out.

Optical methods, such as measurement of bioluminescence resonance energy transfer (BRET) between a luminescent photon and proximal acceptor fluorophore, can help to resolve these questions (Milligan & Bouvier, 2005; Pfleger & Eidne 2006). Rios *et al*. (2006) used BRET as the first line of evidence for opioid–CB1 receptor association. If their interpretation is correct, the results suggest that CB1-receptors hetero-oligomerize with all of the three major opioid receptors. The positive BRET signal reported here is consistent with a close proximity of receptors (<100 Å) and hetero-oligomer formation, but it does not prove such complexes exist. Beyond establishing a separation of less than 100 Å, the BRET data do not indicate the actual proximity of opioid–CB1 receptors, the affinity of hetero-associations relative to homodimers or the number of receptors involved in the putative heterocomplexes. The cellular compartment giving rise to the BRET signal is not identifiable, making it difficult to rule out BRET interactions among intracellular degradation products or misfolded receptors. These issues are very difficult to resolve, but more detailed resonance energy transfer experiments can provide

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much stronger support for hetero-oligomer formation (Milligan & Bouvier, 2005; Pfleger & Eidne, 2006). In particular, saturation BRET techniques quantify the affinity of hetero-*versus* homo-oligomer interactions and can rule out nonspecific artifacts, so establishing the likely biological significance of the interaction. This is important; if hetero-oligomers form at much lower affinity than homo-oligomers, then it is unlikely that they exist in significant numbers relative to homomers. Saturation BRET has shown heterointeractions between KOP and DOP receptors to be of at least as high affinity as KOP homomeric interactions (see Milligan & Bouvier, 2005), suggesting significant hetero-oligomer formation.

Other evidence would also greatly strengthen the case for importance of opioid–CB1 hetero-oligomers. Reciprocal co-immunoprecipitation of partner receptors provides necessary but not sufficient evidence for heteromer formation. Further, transactivation of closely associated hetero-oligomers can be inferred from functional complementation studies, where coexpression is performed with each of the partners possessing nonequivalent, nonfunctional mutations and restoration of function is examined when one partner unable to bind ligand is coexpressed with another that is unable to signal (Milligan & Bouvier, 2005).

It is intriguing that agonist coactivation of CB1 and MOP receptors, when expressed either heterologously or natively in the same cells, consistently produces a response smaller than activation of either receptor alone, regardless of endpoint (Rios *et al*., 2006). However, other interpretations of these results include disruption of the availability of G-protein subunits, associated regulatory proteins or downstream signaling cascades by the two receptors in relatively close proximity. Regardless of which interpretation is correct, it will be worthwhile to further investigate CB1–MOP interactions using agonist, antagonist and inverse agonist combinations in neurons and other cell types where both receptors are abundantly expressed. This should be relatively straightforward because CB1 and MOP receptors are Gi/o coupled, and are preferentially expressed in the perisynaptic zones of overlapping neural populations.

If correct, the hetero-oligomer interpretation for CB1–MOP partners may lead to novel pharmacological tools to specifically target systems that natively coexpress opioid and CB1 receptors. Indeed, the potential significance of direct opioid receptor interactions is already yielding novel experimental agents. Strong evidence for the existence for DOP–MOP and DOP–KOP heteromers prompted Portoghese's group to develop bivalent ligands linked by aliphatic bridges of various lengths for DOP–KOP (Xie *et al*., 2005) and DOP–MOP partners (Daniels *et al*., 2005). Only bivalent ligands with specific bridge lengths roughly consistent with distances between the pharmacophores predicted by some dimer models revealed novel pharmacological interactions that either mimicked previously unexplained pharmacological subtypes of DOP and KOP receptors reported *in vivo* or, for DOP–MOP ligands, displayed opioid actions that were unanticipated by conventional concepts of opioid pharmacology. Those findings are consistent with the existence of hetero-oligomers but do not prove that heterodimers are responsible for the novel actions, which could also be to stabilize low-affinity receptor–receptor interactions by bivalent ligands. However, they do suggest that physically close interactions can provide potential pharmacological benefits if the relationships are close enough.

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