CROSSTALK

The Journal of Physiology

CrossTalk opposing view: Weighing the evidence for class A GPCR dimers, the jury is still out

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Evidence that class A G protein-coupled receptors (GPCRs) can assemble as dimers or higher-order oligomers has steadily accumulated for two decades. However, the structural properties of these oligomers and the relevance of oligomerization to receptor function are still unclear. Until these issues are clarified there will be legitimate cause to question the significance of interactions between class A protomers.

Structure

Unlike many proteins that dimerize or oligomerize, class A GPCRs are typically monomers in detergent solutions, suggesting that interactions between protomers are weaker than those that hold other quaternary structures together. More importantly, this means that inferences drawn from preparations that start with solubilized receptors rely on the assumption that protomers will reassemble in a manner that mimics their behaviour in a membrane. This assumption undermines confidence in interactions studied with biochemical methods such as immunoprecipitation, as well as the relevance of dimeric structures observed with x-ray crystallography. The presence of dimers in precipitates and crystals does not mean that similar dimers necessarily exist in cells any more than their absence implies that they do not.

Because studying transmembrane proteins outside of membranes produces ambiguous results, the field has relied heavily on biophysical methods to demonstrate the existence of GPCR oligomers in cells. Resonance energy transfer (RET) methods based on bioluminescence (BRET), fluorescence (FRET) and time-resolved fluorescence (TR-FRET) have been especially popular. The principal weakness of RET for this application is that it reports proximity instead of association *per se*, and random proximity can generate easily detectable RET signals between proteins that are restricted to membranes (Lan *et al.* 2012). Identifying signals that represent specific association is not trivial, and conflicting results have been obtained. For example, stable dimers should produce RET that is equally efficient at all receptor densities. This has been observed in some RET studies (Mercier *et al.* 2002; Salahpour & Masri, 2007) but not others (James *et al.* 2006; Kawano *et al.* 2013), even when the same receptors are being studied. In addition, several reports have shown TR-FRET efficiency that increases with receptor density (Maurel *et al.* 2008; Albizu *et al.* 2010; Alvarez-Curto *et al.* 2010), although this has been largely ignored. Most efforts to identify specific RET signals rely on theoretical models that require several assumptions regarding homogeneous expression of receptors within populations of cells. We have found that similar RET signals can be obtained with a wide variety of membrane proteins, provided they are expressed at similar levels in the same subcellular compartments (Lan *et al.* 2012). Against this background, specific signals between GPCRs do not clearly stand out.

The data obtained thus far have not produced a consistent picture of the interface between protomers for any class A dimer or oligomer. The ternary structure of these receptors is highly conserved, and it seems likely that a functionally important and stable quaternary structure would also be conserved. Identification of a consensus dimer interface would lend support to the idea that dimers are biologically relevant, and would suggest experiments that could be done to test functional significance. However, structural models of GPCR dimers vary widely, and crosslinking and mutagenesis studies have pointed to a number of potential interfaces (Guo *et al.* 2005, 2008; Hu *et al.* 2012; Knepp *et al.* 2012). This may reflect the fact that dimers are actually transient, and protomers therefore sample several different interfaces.

How might the quaternary structure of class A GPCRs be more clearly elucidated? Improved proximity-based methods will probably be useful, as will live-cell methods based on affinity rather than proximity. The latter methods detect protein interactions by measuring the effects of immobilized

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DOI: 10.1113/jphysiol.2014.272997

membrane proteins on the mobility of other proteins (Dorsch *et al.* 2009; Fonseca & Lambert, 2009; Gavalas *et al.* 2013). Another promising development has been the application of single-molecule imaging methods to this question (Hern *et al.* 2010; Kasai *et al.* 2011; Calebiro *et al.* 2013). One early outcome of these studies has been confirmation of the idea that class A dimers are much less stable than previously imagined, with individual dimers lasting for tens of milliseconds to a few seconds at most. As the methods used to study interactions between GPCRs are refined, it will also be important to place these interactions into the proper context. For example, it will be important to know if GPCRs interact on a similar time scale with other membrane proteins, and how often these proteins actually encounter each other in their native environments, where they may be more or less concentrated than in model cells. This will require a much more detailed picture of native membrane micro-environments than is currently available.

Function

The search for the functional significance of GPCR oligomers has been wide-ranging, but has not converged on a common functional correlate. The core functions of these receptors, namely ligand binding, G protein activation and arrestin recruitment, can be efficiently carried out by isolated monomers (Hanson *et al.* 2007; Whorton *et al.* 2007, 2008; Bayburt *et al.* 2011). Instances of cooperative ligand binding have been attributed to dimerization, but effects on binding do not always conform to thermodynamic expectations of dimers (Birdsall, 2010), and alternative explanations can often explain the data equally well. For example, competition for a common pool of G proteins can produce apparent negative cooperativity between GPCRs (Chabre *et al.* 2009). Both positive and negative functional interactions between receptors have been described for individual heteromers in transfected cells (Milligan, 2013), but as yet no unifying principle of communication between different protomers has emerged.

Most studies aimed at the functional consequences of GPCR dimerization assess signalling at the level of a second messenger or events even further removed from receptor activation. Unfortunately, such studies are often confounded by the complexity of signalling pathways, which present many opportunities for receptors to interact functionally (crosstalk) without interacting physically. We have observed crosstalk using methods we developed to measure signalling exclusively from defined dimers (Han *et al.* 2009; Urizar *et al.* 2011), but how these findings will apply to receptors interacting in their native environment remains unclear. A reductionist approach may ultimately produce the most satisfying demonstration of direct functional interactions between protomers. It is now possible to directly observe the conformational states sampled by purified class A GPCRs in solution (Kahsai *et al.* 2011; Mary *et al.* 2012; Nygaard *et al.* 2013). If similar studies can be carried out in preparations that permit lateral interactions within a lipid bilayer, then it should be possible to determine how one protomer affects the conformations sampled by another. Once interactions between protomers are understood at this reduced level, corresponding changes in pharmacology or signalling could be examined in more intact preparations.

Of course it is possible that lateral interactions between protomers would not significantly affect the conformational dynamics of either protomer, although only a limited number of potential interfaces could mediate such ‘conformationally silent’ interactions. In this case the impact of oligomerization might be limited to trafficking or chaperoning functions. Here too it will be important to clearly distinguish direct effects from effects on trafficking and biogenesis mediated by intermediate processes.

Summary

Biochemical and biophysical studies have not settled the most fundamental questions regarding the structure and function of class A GPCR dimers. The methods currently used to address these questions have significant known limitations and are not themselves completely understood. Until well-defined and conserved structural and functional features are identified there will be room for reasonable doubt about the existence of class A dimers and oligomers as discrete entities capable of transmitting unique signals.

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Additional information

Competing interests

None declared.

Funding

The authors' research is supported by grants from the National Institute of General Medical Sciences (GM078319), National Institute of Drug Abuse (DA022413), and National Institute of Mental Health (MH54137).

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