Functional Relevance of GPCR Dimers

Receptors Work as Single-receptor Monomers or as Aggregates?

Practically all reports about G protein-coupled receptors (GPCRs) start with two statements, one describing that they are the largest family of proteins in chordates and the other that they are the targets for >40% of commercial drugs. Their importance is well valued since they regulate the senses of smell, touch, taste and vision, as well as mediate the actions of numerous neurotransmitters and hormones (e.g. gonadotropins and adrenaline). In addition, there is a number of "orphan" receptors for which no ligands and/or specific functions have yet been identified.

An unsolved question in the field of GPCR is whether the receptors work as single-receptor monomers or as aggregates (dimers or oligomers). This debate increases, incidentally through GPCR-dependent mechanisms, the heartbeat and pulse of pharmacologists.

GPCR dimers have been observed for >30 years on immunoblots of tissue or cell extracts and often interpreted as biochemical artifacts. This confusing phenomenon remained without functional relevance until rather recently, when it was discovered that some members of GPCRs class C, i.e. GABA and taste receptors (T1R), form "forced" heterodimers. One of the receptors (GABAB\(^1\) or T1R\(^1\) or T1R\(^2\)) requires association with the other receptor type (GABAB\(^2\), or T1R\(^3\)) for proper trafficking through the endoplasmic reticulum (ER) and to reach the plasma membrane. Moreover, because the 2\(^{nd}\) receptor type cannot bind ligand, its role is solely as a transport chaperon to the cell surface [1] (fig. 1). Still no one at that point could have imagined the plethora of combinations possible between GPCRs of the same (homodimerization, fig. 2) or different type (heterodimerization), and even with non-GPCRs, membrane proteins and smaller molecules (unfaithful-dimerization).

By now many GPCRs have been found as dimers or higher order oligomers prior to, during, or after ligand binding and activation, but also during their desensitization and internalization (reviewed in [2]). Moreover, one of the most compelling pieces
of evidence for GPCR complexes with other molecules comes from reports showing that the GPCR calcitonin-receptor-like receptor (CRLR) can associate with at least two of receptor activity modifying proteins (RAMP).

When associated with RAMP1 it functions as the calcitonin gene-related peptide (CGRP), but when associated with RAMP2 it functions as the adrenomedullin receptor [3] (fig. 3).

Despite the wealth of in vitro data, very little has been done to determine if dimers or oligomers have functional relevance in the physiological context, in particular in the case of homodimers. To determine the functionality of homodimerization, clever studies have been performed in cell cultures using mutant receptors, where one is unable to bind the ligand while the other is signaling-deficient. Both alone are nonfunctional, but when coexpressed they are able to form complementary dimers and respond to ligand-activation. If dimerization has physiological relevance then the signaling responses upon of the functional complementation of non-functional receptors should be (at least partially) comparable to that of the WT receptor. This means that most of the intricate cellular responses (generation of cAMP, activation of kinases, and eventually specific-gene transcription) should function after complementation.

Earlier studies have shown that using complementary luteinizing hormone (LH) receptor mutants, one binding- and the other signaling-deficient, it is possible to generate LH-dependent cAMP response in vitro. We applied the same principle to living animals in vivo, by creating transgenic (TG) mice with bacterial artificial chromosomes (BACs) containing the entire genomic LHR gene, and expressing either a binding- or a signaling-deficient mutant of LHR. These TG mice were crossed to the LHR knockout (LuRKO) background [4], and we finally had mice that expressed either one or both of the LHR mutants in the LuRKO background.
Both single-TG/LuRKO mice were hypogonadal and infertile, similar to LuRKO mice. However, when both receptor mutants were coexpressed in the absence of functional endogenous LHR, all measured LHR functions were normalized in the studied male mice and their phenotype was indistinguishable from wild-type controls, including gonadal development, spermatogenesis, and fertility [5]. This provided the first evidence that GPCR homodimerization is not only a physical phenomenon, but it also has physiological relevance.

The regulation of GPCRs seems to be more complex than previously thought, in particular because each cell type may present with a variety of potential cooperation partners. On the other hand, this makes perfect sense in light of pleomorphic receptor functions in different environments, cell-types and the mix of simultaneous extracellular signals.

GPCR dimerization should not be a dim scenario, but rather an opportunity for pharmacologists, allowing the design of drugs that can:

- act at different stages of the life cycle of a GPCR (e.g. rescuing a mutant GPCR trapped in the ER, or trapping a harmful GPCR such as constitutively active mutants)
- selectively block or activate an individual signaling pathway (biased agonism)
- un-dimerize (dimer-breaker) or dimerize GPCRs to inhibit or enhance their responses
- desensitize GPCRs by directing them to the internalization pathway.

There is evidence that such goals can be achieved. A recent example is that blocking the δ-opioid receptor, involved in the tolerance and dependence of the µ-opioid receptor agonist (e.g. morphine), also blocks the side-effects of morphine without reducing analgesia [6]. Of note, these two opioid receptors form heterodimers.

The more we understand about the structure, function and cooperativity of GPCRs the more likely will it be that we can design selective drugs, which would be more specific, have fewer side effects, and act only on the specific pathway(s). The potential for such medicines is immense now when personalized medical treatment is becoming a reality.

References

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