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A new paradigm in the functioning of G-protein-coupled receptors

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Aggregation and oligomerization have often been a complicating factor in the study of membrane proteins. Recent results from a number of laboratories, however, point out to a novel mechanism of function of probably the most abundant and important class of membrane proteins – the G-protein-coupled receptors – which involves heterodimer formation of two receptor molecules in the membrane. G-protein-coupled receptors (GPCRs) constitute a superfamily of proteins whose function is to transmit information across a cell membrane from the extracellular environment to the interior of the cell, thus providing a mechanism of communication between the exterior and the interior of the cell[1-5]. Such a process requires that the signal transduction be specific to the initiating stimulus and have well-defined intracellular sequence of events. GPCRs represent the single largest family of cell surface receptors involved in signal transduction. This receptor superfamily includes over 2000 receptors which respond to a variety of molecules such as neurotransmitters, hormones, taste and odorant molecules, and even photons, thus mediating a multitude of functions[1]. These transmembrane receptors act as key players in diverse physiological processes such as neurotransmission, cellular metabolism, secretion, cellular differentiation and growth, and inflammatory and immune responses. GPCRs therefore represent major targets for the development of novel drug candidates in all clinical areas[4]. It is estimated that 30% of clinically prescribed drugs function as either agonists or antagonists at GPCRs, which points out to their immense therapeutic potential[1].

In response to diverse stimuli which include light, hormones and neurotransmitters, GPCRs activate heterotrimeric guanine-nucleotide-binding proteins (G-proteins) by facilitating the exchange of GTP for GDP bound to the G_α subunit. Typically, an agonist-stimulated receptor activates as many as several hundred G-proteins which in turn activate a variety of downstream effectors such as enzymes, ion channels, and intracellular signalling pathways such as the MAP kinase cascade. All GPCRs share some structural features[1]. In general, they have an extracellular N-terminal domain, seven transmembrane domains which form the transmembrane core with three exoloops and three cytoloops and an intracellular C-terminal domain. The ligand is thought to bind within the transmembrane segments or to the extracellular N terminus[1]. In response to ligand binding, the cytoplasmic portion of the receptor undergoes a conformational change, allowing interaction with the G-proteins (which are localized in the cytoplasmic side of the membrane through fatty acyl membrane anchors) thereby transmitting the signal across the membrane. The G-proteins carry the signal forward to various intracellular messengers.

The molecular mechanism involved in GPCR function, i.e. the coupling of extracellular signal and intracellular response, represents a very active area of research in signal transduction and has become the research focus of an ever-increasing number of laboratories. Till very recently, it was generally believed that receptor dimerization could play a crucial role in the molecular events leading to GPCR activation[7]. Such dimerization was thought to involve receptors of the same kind, i.e. homodimerization of GPCRs was implicated in the activation of GPCRs. However, a few recent reports[8-10] have pointed out a rather novel concept that such dimerization could actually involve receptors of different types and classes. This adds a new twist to the mechanism of GPCR activation and raises interesting possibilities especially in drug research. In one of the first reports of this kind, Kuner *et al*[8]. reported that the $GABA_B$ receptor is a heterodimer of two different GABA receptors – the $GABA_BR1$ and $GABA_BR2$. GABA (γ–aminobutyric acid) is a neurotransmitter whose regulation is implicated in a number of mental disorders such as epilepsy and anxiety. Interestingly, neither of the monomers, $GABA_BR1$ and $GABA_BR2$, is functional on its own but can bring about the physiological effects of GABA when co-expressed together. It thus appeared for the first time that interaction of the two receptor molecules and the formation of the heterodimer was a key step in receptor activation and signal transduction. A similar study was reported soon involving two different serotonin or 5-hydroxytryptamine (5-HT) receptors -the $5\text{-}HT_{1B}$ and $5\text{-}HT_{1D}$ receptors[9]. Serotonin is yet another important neurotransmitter and plays a crucial role in many cognitive and behavioral processes. It was shown in this study that the $5\text{-}HT_{1B}$ and $5\text{-}HT_{1D}$ receptors form homodimers when expressed alone and heterodimers when co-expressed. Interestingly, no heterodimers were observed when membranes expressing one receptor subtype were mixed with membranes expressing only the other type, indicating that the heterodimerization in the cellular environment may be due to a specific mechanism and is not a result of non-specific aggregation[9]. One of the recent reports in this area goes one step further and shows that even very distantly related receptors such as the dopamine D2 receptor and the somatostatin SST5 receptor can form heterodimers[10]. The most interesting aspect of this latest report is that, unlike the GABA or the serotonin (5-HT) receptors, these two receptors are pharmacologically quite different and yet they are co-expressed in striatum and pyramidal neurons of the cortex and form heterodimers, as shown by fluorescence resonance energy transfer (FRET). A novel feature of this heterodimerization is that unlike $GABA_B$ heterodimerization where the agonist has no effect in the formation of the heterodimer, dopamine D2 and somatostatin SST5 receptors would heterodimerize only when neurotransmitter agonist for either receptor were present. In other words, it is a ligand-induced heterodimerization of GPCRs.

What are the implications of such heterodimerization in GPCRs especially keeping in mind their role as major drug targets? The prospect of ligand-induced heterodimerization can have great implications if it turns out to be a general phenomenon in GPCR functioning. There would be a plethora of possible GPCR heterodimers opening new avenues for therapeutics. This would also offer an attractive mechanism for increasing the diversity of cellular responses to extracellular signals or stimuli. Unravelling the functional significance of these protein-protein interactions poses a challenging task.

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