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Small molecules targeting heterotrimeric G proteins

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ABSTRACT

G protein-coupled receptors (GPCRs) represent the largest family of cell surface receptors regulating many human and animal physiological functions. Their implication in human pathophysiology is obvious with almost 30–40% medical drugs commercialized today directly targeting GPCRs as molecular entities. However, upon ligand binding GPCRs signal inside the cell through many key signaling, adaptor and regulatory proteins, including various classes of heterotrimeric G proteins. Therefore, G proteins are considered interesting targets for the development of pharmacological tools that are able to modulate their interaction with the receptors, as well as their activation/deactivation processes. In this review, old attempts and recent advances in the development of small molecules that directly target G proteins will be described with an emphasis on their utilization as pharmacological tools to dissect the mechanisms of activation of GPCR-G protein complexes. These molecules constitute a further asset for research in the "hot" areas of GPCR biology, areas such as multiple G protein coupling/signaling, GPCR-G protein preassembly, and GPCR functional selectivity or bias. Moreover, this review gives a particular focus on studies *in vitro* and *in vivo* supporting the potential applications of such small molecules in various GPCR/G protein-related diseases.

1. Introduction

GPCRs represent the largest cell surface and membrane receptor family mediating cellular responses of a large variety of activating extracellular signals (Bockaert and Pin, 1998; Katritch et al., 2013; Lefkowitz, 2013; Pierce et al., 2002). This illustrates the central role of GPCRs in many physiological functions, making them one of the largest families of drug targets (Eglen and Reisine, 2011; Hauser et al., 2017; Lappano and Maggiolini, 2011; Lundstrom, 2009; Spiegel and Weinstein, 2004). Indeed, the attractiveness of GPCRs as drug targets for the pharmaceutical industry led to the development of many selective small molecules that are proposed to directly modulate the function of GPCRs. Moreover, considerable advances in our understanding of the molecular pharmacology of GPCRs and their signaling and regulation have significantly contributed to the explosion of drug discovery programs (Kobilka, 2011; Limbird, 2004). The structural insights obtained from the recently reported crystallographic GPCR structures, as well as the advances in the field of chemical and computational biology, will certainly contribute to further advances in GPCR drug discovery (Hauser et al., 2017; Kobilka, 2011; Rasmussen et al., 2011a; Rosenbaum et al., 2009). Moreover, recently antibodies and nanobodies targeting GPCRs constitute a promising way towards the development of new generations of more selective drugs to fine tune GPCR function (Ayoub et al., 2017; Hutchings et al., 2010; Rasmussen et al., 2011a, 2011b). All these advances have contributed to the development of new classes of drugs with better pharmacological properties in terms of selectivity, efficacy and safety.

Since heterotrimeric G proteins play the pivotal role in GPCR activation and signaling, they have been obvious targets for researchers wanting to modulate GPCR function (Bockaert, 1991; Bonacci et al., 2006; Cabrera-Vera et al., 2003; Johnston et al., 2008; Smrcka, 2013). Indeed, GPCRs signal mostly through their intimate physical and functional coupling with various classes of guanosine di/tri-phosphate (GDP/GTP) nucleotide-binding proteins (G proteins) including the major ones, Gs, Gi/o, Gq/11, and G12/13 proteins (Bockaert, 1991; Bockaert et al., 1987; Cabrera-Vera et al., 2003; Gilman, 1987; Oldham and Hamm, 2008). At the molecular level, GPCR-G protein coupling occurs in a well ordered cycle where ligand binding promotes receptor activation followed by receptor-G protein interaction resulting in GDP to GTP exchange on the Gα subunit (Fig. 1). This is followed by subunit dissociation and/or conformational changes within the Gα/Gβγ trimer and each subunit triggers the activation of multiple intracellular pathways (such as cyclic adenosine 3′,5′-monophosphate (cAMP), inositol trisphosphate (IP3), Ca^{2+}, diacylglycerol (DAG), mitogen-activated protein kinase (MAP kinase), Rho/Rac), which control important physiological responses (Fig. 1) (Bockaert, 1991; Cabrera-Vera et al., 2003; Ghanouni et al., 2001; Oldham and Hamm, 2008). For regulation of G protein activation and its termination, this is mediated by the GTPase activity of G proteins, as well as regulators of G protein signaling (RGSs) that stimulate GTP hydrolysis on the Gα subunit (Fig. 1) (De Vries et al.,

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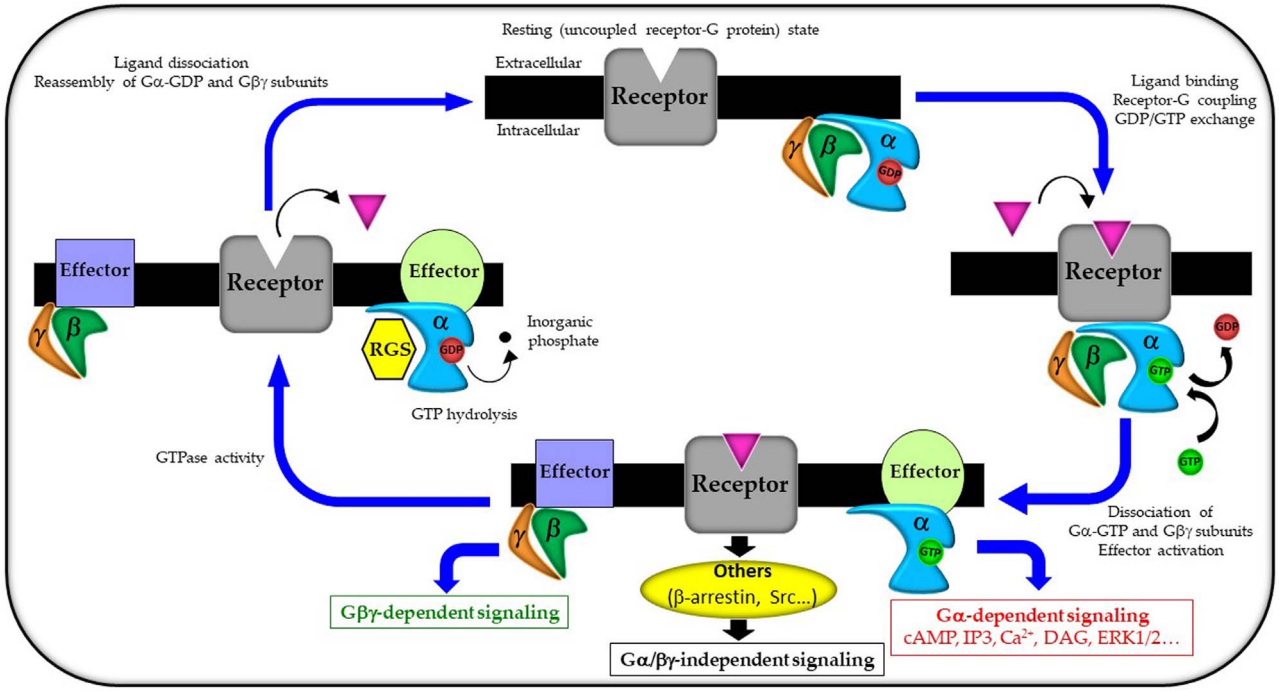

Fig. 1. The classical GPCR-G protein activation/deactivation cycle. In the absence of activation, the Gα-GDP and Gβγ subunits of the heterotrimeric G proteins are associated with each other without necessarily physically interacting with the receptor. Upon a ligand binding to its GPCR, conformational changes occur within the receptor leading to an intimate interaction and coupling with the heterotrimeric G protein. This results in G protein activation characterized by GDP/GTP exchange followed by the dissociation between Gα-GTP complex from the Gβγ subunits. Each activated subunit is then able to interact with and modulate the function of its specific intracellular effectors leading to canonical Gα- and Gβγ-dependent, but also Gα/Gβγ-independent signaling pathways (*e.g.* β-arrestin, Src). As a regulation mechanism, the GTPase activity of Gα hydrolyzes GTP to GDP and along with the involvement of selective RGSs, this inactivates the Gα subunit. Finally, the Gα-GDP and Gβγ subunits re-associate to be ready for another GPCR-G protein activation cycle.

2000). A further mechanism of GPCR regulation involves the phosphorylation of GPCRs by specific G protein coupled-receptor kinases (GRKs) followed by their interaction with β-arrestins causing GPCR desensitization and internalization (Luttrell and Lefkowitz, 2002; Moore et al., 2007; Reiter and Lefkowitz, 2006). Of course, this classical view of GPCR-G protein activation and desensitization appears very simplistic since it is now evident that GPCRs are able to engage various G protein-independent signaling pathways (Fig. 1) (Lefkowitz and Shenoy, 2005; Luttrell and Lefkowitz, 2002; Reiter and Lefkowitz, 2006). Indeed, upon their internalization GPCRs promote intracellular signaling pathways including β-arrestin- and Src-dependent responses and even intracellular G protein-dependent responses (Lefkowitz and Shenoy, 2005; Luttrell and Lefkowitz, 2002; Reiter and Lefkowitz, 2006). Overall, this highlights the complexity of GPCR pharmacology and signaling systems, where G proteins still play the central role in GPCR function.

2. G proteins as key targets

As a key element in GPCR function, G proteins constitute interesting molecular targets. This has led to the development of many pharmacological tools used for better understanding of GPCR function and more importantly for their potential use as therapeutic targets to treat GPCR-linked disorders. Indeed, several approaches for pharmacological targeting of G proteins have been used, and many selective and non-selective peptides and small molecules that target either Gα or Gβγ subunits have been developed. These tools have been used in mechanistic-based studies to understand GPCR-G protein signaling, as well as for their potential application in treating GPCR-linked diseases (Table 1). In the current topical context of GPCR-biased signaling, G protein-selective molecules present a major asset to better dissect GPCR signaling and its implication in pathophysiology (Fig. 2). In this review, a particular emphasis will be placed on small molecules targeting G proteins and their different modes of action (Fig. 2). In addition, their pharmacological effects on G protein activation in various *in vitro* and *in vivo* models will be described (Table 1).

3. Small molecules targeting Gα subunits

3.1. From toxins to small molecules

From our knowledge of the key role of G proteins in GPCR signaling and function, it is obvious that molecules that interfere or directly interact with G proteins and modulate their activation would be of great interest. This was amazingly illustrated by the famous harmful bacterial toxins, *cholerae* toxin (CTX), *Bordetella pertussis* toxin (PTX), heat-labile enterotoxin of *Escherichia coli*, and *Pasteurella multocida* toxin (PMT), which differentially act on the Gα subunit of heterotrimeric G proteins and thereby cause severe infections (Aktories, 2011; Mangmool and Kurose, 2011; Orth and Aktories, 2010). Their common mode of action is based on the chemical modification of specific and critical residues on the Gα subunits involved either in the GTP hydrolysis reaction (CTX and PMT) or in the physical interaction of the G proteins with GPCRs (PTX) (Table 2). In addition to their infectious potential, these toxins have been extensively used to study GPCR/G protein-mediated signaling, especially to dissect the involvement of each of the different classes of G proteins in GPCR biology and physiology.

Furthermore, these toxins strongly inspired scientists to develop other molecules that can bind and interfere with Gα protein activation and its physical and functional interaction with either GPCRs or Gβ/Gγ subunits. In fact, over the years G proteins have constituted interesting targets for the development of molecules that can bind the different G protein subunits and modulate their activation/deactivation cycle. This is mainly based on our knowledge of the molecular and structural events involved in both GPCR-Gα protein and Gα-Gβγ physical interactions and their activation upon ligand binding and receptor activation. Consequently, many peptides and small molecules capable of binding to Gα subunits in a more or less selective manner have been