Commentary

Functional selectivity of G-protein-coupled receptors: From recombinant systems to native human cells

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ABSTRACT

In the mid 1990s, it was assumed that a two-state model, postulating an inactive (R) state and an active (R*) state provides the molecular basis for GPCR activation. However, it became clear that this model could not accommodate many experimental observations. Accordingly, the two-state model was superseded by a multi-state model according to which any given ligand stabilizes a unique receptor conformation with distinct capabilities of activating down-stream G-proteins and β-arrestin. Much of this research was conducted with the β$_2$-adrenoceptor in recombinant systems. At the molecular level, there is now no doubt anymore that ligand-specific receptor conformations, also referred to as functional selectivity, exist. This concept holds great potential for drug discovery in terms of developing drugs with higher selectivity for specific cells and/or cell functions and fewer side effects. A major challenge is the analysis for functional selectivity in native cells. Here, I discuss our current knowledge on functional selectivity of three representative GPCRs, the β$_2$-adrenoceptor and the histamine H$_2$- and H$_4$-receptors, in recombinant systems and native human cells. Studies with human neutrophils and eosinophils support the concept of functional selectivity. A major strategy for the analysis of functional selectivity in native cells is to generate complete concentration/response curves with a large set of structurally diverse ligands for multiple parameters. Next, correlations of potencies and efficacies are analyzed, and deviations of the correlations from linearity are indicative for functional selectivity. Additionally, pharmacological inhibitors are used to dissect cell functions from each other.

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1. Development of the concept of functional selectivity

G-protein-coupled receptors (GPCRs) constitute the most important class of drug targets; agonists activate GPCRs while antagonists block agonist effects [1,2]. In the mid 1990s, the two-state model of GPCR activation was developed [3–5]. According to this model, GPCRs isomerize between an inactive (R) state and in active (R*) state. Agonists stabilize the R* state, whereas inverse agonists stabilize the R and, thereby, reduce the agonist-independent GPCR activity. The classic (neutral) antagonists have no effect on the equilibrium between the two receptor states and block both the effects of agonists and inverse agonists [1]. Many of the seminal studies on the two-state model were conducted with the recombinantly expressed β$_2$-adrenoceptor (β$_2$AR). The β$_2$AR couples canonically to G$_s$-proteins and mediates activation of adenylyl cyclase, resulting in an increase in the intracellular concentration of the second messenger cyclic AMP and activation of down-stream effector proteins such as cAMP-dependent protein kinase (PKA) [6,7].

However, even in some of the first studies on the two-state model with the β$_2$AR, certain experimental observations could not be easily reconciled with the model. Specifically, the partial agonists ephedrine, dobutamine and salbutamol were similar effective at increasing adenylyl cyclase activity with at a constitutively active β$_2$AR mutant, but with respect to ternary complex formation, ligand efficacy varied considerably [3]. In addition, dichloroisoproterenol acted in opposite ways as partial agonist and partial inverse agonist at the β$_2$AR in intact Sf9 insect cells and cell membranes [4]. Moreover, ephedrine and dobutamine increased fluorescence signals in the purified β$_2$AR, whereas other partial agonists decreased fluorescence [5]. Based on these studies and data obtained with other GPCRs, an intense and productive discussion in the GPCR community began whether the two-state model is sufficient to explain the molecular mechanisms of GPCR activation [8,9]. This discussion resulted in experimental studies by numerous independent groups on multiple GPCRs to

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test the hypothesis whether a two-state model or a more complex multiple-state model can account for the mechanisms of GPCR activation. According to the multiple-state model, any given ligand stabilizes a unique receptor conformation that is then capable of directing coupling of the receptor towards specific G-proteins or β-arrestin [6,7,10–15]. The ability of a ligand to direct signalling towards a specific effector pathway is also referred to as functional selectivity (the term used in this Commentary) or biased signalling (Fig. 1). Functional selectivity has been the subject of several excellent reviews [6,7,10–15] and the reader is referred to these papers for a comprehensive overview on a field to which many groups have made important contributions. While initially, there were still doubts whether GPCRs adopt multiple ligand-specific conformations, there is now consensus in the GPCR research community that functional selectivity is applicable to virtually any GPCR studied so far.

A recent culmination of the research in the field has been the elucidation of high-resolution crystal structures of closely related serotonin receptor subtypes bound to various ligands exhibiting functional selectivity and showing clear structural differences [16]. Moreover, the first structures of GPCR–G-protein complexes [17] and β-arrestin-GPCR fragment structures have also been resolved recently [18]. Therefore, it is reasonable to expect that within the next years, numerous new crystal structures of different combinations of ligands with GPCRs and G-proteins or β-arrestin will be revealed and that these studies support the concept of functional selectivity. It can also be anticipated that an in-depth understanding of functional selectivity will offer multiple new opportunities for drug development, not only via orthosteric and allosteric GPCR ligand binding sites [1,712] but also via interaction surfaces between specific ligand/GPCR complexes and their coupling partners [18].
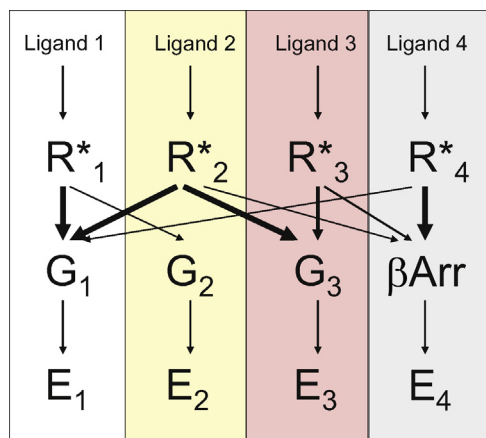

Fig. 1. The concept of functional selectivity. The concept of functional selectivity implies that any given ligand (for example, ligand 1- ligand 4, with the number of ligands being limited by the chemical space afforded by a given receptor) at a specific GPCR stabilizes a unique and ligand-specific conformation (R^*_1–R^*_4, again the number of receptor conformations being limited by the number of ligands binding to a receptor). These conformations differ from each other in their capacity to interact with down-stream G-proteins G_1–G_3 (currently 16 G-protein α-subunits are known [77]) or β-arrestin (βArr), also existing as multiple species [6]. As a result, effector systems modulated by G-proteins and β-arrestins are regulated in a ligand-specific manner. The concept of functional selectivity has been demonstrated at the level of purified receptors, recombinant systems (membranes and in intact cells) and, more recently, also in native human cells (see Table 1). The concept of functional selectivity holds great potential for future drug development and for reducing drug toxicity. The arrows are just shown for illustrative purposes and are not specifically focused on the addressed receptor species. In order to avoid the Figure from becoming too complicated, each G-protein is shown as single entity only and not as heterotrimer. The Figure also does not show that a G-protein via Gα and Gβγ subunits can regulate, in a stimulatory or inhibitory manner, the activity of multiple effectors [77,78].

From a therapeutic perspective, the concept of functional selectivity is of eminent relevance. It may become possible to develop drugs that stimulate only certain aspects of GPCR functions and, thereby, increase therapeutic efficacy while reducing unwanted effects [6,7,10–15]. This potential is particularly relevant for GPCRs that are widely expressed in the human body.

While the strategies and methodologies for future studies on functional selectivity at the molecular level are laid out very well, we face an important problem: How can we transfer the concept of functional selectivity from recombinant systems, purified and crystallized receptors to the human body at the cellular, organ and organismal level?

2. Scope of this commentary

The aim of this perspective is to discuss functional selectivity of three prototypical GPCRs, the β_2AR, and the histamine receptors H_2R and H_4R in recombinant systems and native human cells. The β_2AR and H_2R were chosen because these receptors are expressed in human neutrophils that can be obtained in large numbers for pharmacological studies [19,20]. The H_4R was selected because this receptor constitutes a "hot" drug target for anti-inflammatory drugs [21–23] and is a current focus of research on functional selectivity [24–26].

These three receptors chosen represent well-studied cases for a principle that is probably applicable to all other GPCRs. However, so far, very few studies on functional selectivity of GPCRs in native cells have been performed, a major challenge being the lower receptor expression level than in recombinant systems and, therefore, a lower signal-to-noise ratio. Evidently, a lower signal-to-noise ratio renders the analysis of partial agonists in terms of precise EC_{50} values and efficacies more difficult, implying the necessity to conduct larger experimental series than with cells expressing recombinant receptors. Hence, an important aspect of this article will also be to discuss strategies and challenges associated with the analysis of functional GPCR selectivity in native human cells.

3. The β_2AR as paradigm for a GPCR for analysis of functional selectivity: studies with recombinant systems, purified receptors and cardiomyocytes

Among all GPCRs, the β_2AR is the best-studied member with respect to functional selectivity. Table 1 summarizes representative studies on various recombinant systems convincingly demonstrating functional selectivity of the β_2AR. Following the initial observations on ligand-specific signalling of the β_2AR already discussed above [3–5], several studies corroborated the concept: Using β_2AR-Gα fusion proteins that ensure close proximity and effective interaction of the coupling partners [27], differences in potencies and efficacies of β_2AR ligands including inverse agonist/agonist switches were found depending on which nucleoside 5′-triphosphate was present for G-protein activation [28]. The multiple receptor states were visualized by plotting ligand efficacies with respect to nucleotide turnover against ligand efficacies with respect to adenylyl cyclase activation [28]. In case of the nucleotide guanosine 5′-triphosphate (GTP) the data correlated very well, whereas in case of inosine 5′-triphosphate (ITP), the correlation was poor [28]. These two-dimensional correlation plots or more complex multidimensional plots have been subsequently used by many groups to visualize ligand-specific receptor conformations in an intuitive manner [see, e.g. 6,10,11]. The Lefkowitz group has also developed various sophisticated mathematical models to quantify functional selectivity of ligands [29].

Using the GPCR–Gα fusion protein technique [27], substantial differences in the pharmacological profile of the β_2AR were