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An operational model for GPCR homodimers and its application in the analysis of biased signaling

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Q2 **G-protein-coupled receptors are one of the most important protein superfamilies as drug targets in drug discovery programs. Their interactions with ligands are influenced by their homomerization. In this study, we propose an operational model for receptor homodimers, which includes constitutive receptor activity. Distinct functional response curves can be obtained from this model, which can satisfactorily depict typical complex experimental data as biphasic and bell-shaped curves. Operational parameters in the model can provide mechanistic explanations for observed functional complexity associated with the cooperativity and intrinsic efficacy of ligands. Because the model presented here is derived from the conceptual framework of operational models, it takes advantage of the body of knowledge coming from the widespread use of this type of modeling. The operational homodimer model can also explain the biased signaling dependent on ligand concentration. In conclusion, this operational homodimer model has a wide range of applications in pharmacological research.**

Introduction

G-protein-coupled receptors (GPCRs) are integral cell membrane proteins responsible for many biological processes. Extracellular agonists bind to these receptors and influence their conformations, thus altering the propensity of receptors to bind to G proteins, β-arrestins or other transducer proteins and promoting signal transduction within the cell. In this way, GPCRs respond to extracellular stimuli and help to direct the information flow from outside to inside the cell [1]. The analysis of drug–GPCR interactions is fundamental in academia and the pharmaceutical industry. Because of the involvement of GPCRs in many physiological processes, their malfunctioning is the cause of many diseases. As a consequence, research on GPCRs is central to drug discovery programs. Of note, as of November 2017, GPCRs are the primary targets of ~35% of approved drugs in the USA and the European Union [2].

It is widely accepted that GPCRs often form oligomers that can be physiologically relevant [3]. The formation of oligomers affects the binding of extracellular ligands to GPCRs and enables the ligands to produce a wider and more complex range of functional responses [4]. Homodimerization is the simplest case of receptor oligomerization. The allosteric interactions between the bound ligands in the respective protomers can lead to cooperativity effects that can affect the binding and the function of the receptor [5,6]. Ligand binding cooperativity in a receptor dimer context has been the subject of modeling approaches [7,8]. Durroux considered the possibility of two different receptor dimer states, one in which receptor protomers can crosstalk and the other in which they do not [7]. Casadó *et al*. comprehensively compared procedures to fit binding data from saturation isotherms and competition assays within a receptor dimer model with the traditional way of fitting data [8]. The issue of fitting the binding data was reviewed by Giraldo [9], who analyzed various empirical and mechanistic models. Moreover, the functional response induced by ligands can also be assessed by mathematical models. There have Q3

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been some mathematical models for signaling mediated by GPCR homodimers [10–12]. However, no operational model has been proposed for this purpose. The operational model of agonism was presented in 1983, but it considered the receptor as a monomer [13]. This model describes the receptor signaling as a two-step process, one for ligand binding and the other for transducing the ligand–receptor complex into the functional response. This model has been widely used to analyze the functional effects of ligands and, remarkably, the issue of biased agonism [14,15]. Nevertheless, it cannot explain the behavior of inverse agonists. This is because the constitutive activity of the receptor is not integrated into the model. Subsequently, there have been extensions of the operational model by incorporating constitutive receptor activity, but they are still intended for the monomer [16,17]. Therefore, here, we aim to propose an operational model for GPCR homodimers. This new model considers constitutive receptor activity and thus is intended to describe the function of inverse agonists.

One GPCR can perform its functions through a variety of pathways. The same ligand is likely to generate different effects on different pathways. When distinct ligands bind to the receptor, it is possible that different sets of downstream pathways will be affected. Biased signaling occurs for many ligands binding to GPCRs in the human body and has great significance in biological functions [1]. Therefore, we have applied the developed operational homodimer model to the dissection of biased signaling by considering different pathways that are associated with one receptor homodimer. Remarkably, the model assists in the understanding of concentration-dependent effects of a ligand on different pathways.

An operational model for GPCR homodimers

Q4 Figure 1 illustrates an operational model for a GPCR homodimer consisting of two protomers: R. The combination of the two protomers in the homodimer mediates the functional response. A is a ligand for each of the protomers. ARR and RRA are equivalent. The parameters in this model have the following definitions. K is the equilibrium dissociation constant for the binding of ligand A to the free homodimer. α represents the binding cooperativity between the two ligands. For α, values less than, equal to and greater than 1 mean negative, neutral and positive binding cooperativities, respectively. E denotes the absolute functional response and E_m is the maximum possible functional response of the system. f denotes the fractional functional response ($f = E/E_m$). S is the stimulus for the functional response. A rectangular hyperbolic function converts stimulus S into fractional response f. K_E is the value of S for half of E_m and thus represents the efficiency of transducing stimulus into fractional response. ε_{AA} and ε_A are the intrinsic efficacies of A–A combination and A, respectively. δ is introduced to measure the functional interaction: $\varepsilon_{AA} = \varepsilon_A * \varepsilon_A * \delta$; meaning that δ is the activation cooperativity between A and A in the ARRA complex. χ is a parameter used to account for the basal fractional response. $\chi = [RR]_T/K_E$, with $[RR]_T = [RR] + [ARR] + [RRA] + [ARRA]$. Given that the constitutive activity of the receptor is considered here, this model can be used to analyze the functional effects of inverse agonists. This feature distinguishes our model from many other models.

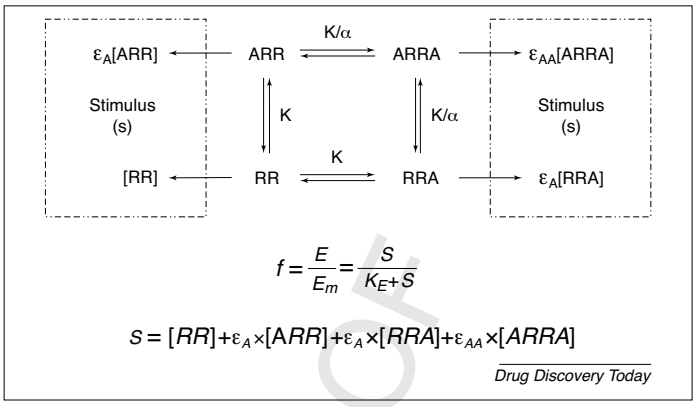

FIGURE 1

An operational homodimer model. K = [A] * [RR]/[ARR]; K = [A] * [RR]/[RRA]; K/α = [A] * [ARR]/[ARRA]; K/α = [A] * [RRA]/[ARRA]. K is the equilibrium dissociation constant for the singly-bound receptor dimer. α is the constant for binding cooperativity. f denotes the fractional functional response ($f = E/E_m$), with E and E_m being the absolute functional response and the maximum possible functional response of the system, respectively. S is the stimulus for the functional response. K_E is the value of S for half of E_m and thus represents the efficiency of transducing stimulus into fractional response. ε_{AA} and ε_A are the intrinsic efficacies of A–A combination and A, respectively.

Based on above relationships, Eq. (1) for the fractional response f of the homodimer model depicted in Fig. 1 can be obtained (for more detail, see Supplementary material online).

$$f = \frac{\chi\left(K^2 + 2K\varepsilon_A[A] + \alpha\delta\varepsilon_A{}^2[A]^2\right)}{K^2(\chi+1) + 2K(\chi\varepsilon_A + 1)[A] + \alpha(\chi\delta\varepsilon_A{}^2 + 1)[A]^2} \quad (1)$$

The asymptotic response when $[A] \to \infty$ (f_∞) is calculated as:

$$f_\infty = \lim_{[A]\to\infty} f = \frac{\chi\delta\varepsilon_A{}^2}{\chi\delta\varepsilon_A{}^2 + 1} \quad (2)$$

The basal fractional response of the system (f_{basal} = f for [A] = 0) is:

$$f_{basal} = \frac{\chi}{\chi+1} \quad (3)$$

As displayed by Eq. (3), f_{basal} is determined by χ and rises as χ increases. Therefore, χ is associated with the intrinsic ability of a signaling system without ligands to generate the functional response. Eq. (2) shows that f_∞ is positively correlated with χ, δ and ε_A. It is interesting to note that f_{basal} and f_∞ are influenced by χ. This means that f_{basal} and f_∞ are interrelated and highlights the importance of the measurement of basal response in the analysis of ligand intrinsic efficacy.

Different parameter values lead to functional response curves with different shapes

According to the model for the receptor homodimer (Fig. 1, Eq. (1)), a variety of curves with differing shapes can be obtained when different values are assigned to the parameters. To better understand how the parameter values affect the functional response curves, we conducted some simulations using the following values and special attention was paid to the comparison between ε_{AA}, ε_A and 1.

The nine curves in Fig. 2 share the values for χ, K and α: $\chi = 0.5$, $K = 10^{-8}$ and $\alpha = 0.001$, so there is a negative binding cooperativity between the two ligands binding to the homodimer. The following nine curves have distinct Q5 values of ε_{AA} and ε_A: (1) $\varepsilon_{AA} = 100$, $\varepsilon_A = 10$; (2) $\varepsilon_{AA} = 10$, $\varepsilon_A = 10$; (3) $\varepsilon_{AA} = 1$, $\varepsilon_A = 10$; (4) $\varepsilon_{AA} = 10$, $\varepsilon_A = 1$; (5) $\varepsilon_{AA} = 1$, $\varepsilon_A = 1$; (6) $\varepsilon_{AA} = 0.4$, $\varepsilon_A = 1$; (7) $\varepsilon_{AA} = 1$, $\varepsilon_A = 0.4$; (8) $\varepsilon_{AA} = 0.4$, $\varepsilon_A = 0.4$; (9) $\varepsilon_{AA} = 0.1$, $\varepsilon_A = 0.4$. The basal fractional response depends exclusively on the χ parameter, which reflects the constitutive receptor activity. Because χ is constant in all the

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