Could adding constitutive receptor activity redefine biased signaling quantification?

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Abstract

Biased signaling, differential activation of distinct signaling pathways, is currently at the center of pharmacology. Reliable characterization of biased ligands requires robust scales applicable to all ligand classes: agonists, neutral antagonists and inverse agonists. To this end, constitutive receptor activity should be included in the models.

Quantification of GPCR biased signaling

G protein-coupled receptors (GPCRs) are key players of cell signaling that are found inserted in the cell membrane and transmit the signals embodied in the structure of ligands from outside to inside the cell [1]. GPCRs signal not only through G proteins but also through other proteins, e.g., β-arrestin [2]. Differential engagement of these multiple signaling pathways (biased agonism) may have therapeutic implications. For example, typical opioid analgesics (such as morphine) directed to the μ-opioid receptor yield beneficial effects through the G protein but unwanted effects through β-arrestin [3]. Similarly, it was suggested that antipsychotic drugs selectively activating β-arrestin via the dopamine D2 receptor might present benefits versus simple inverse agonists [4]. Likewise, β-adrenergic receptor blockers like carvedilol functioning as antagonists of G protein–mediated signaling and agonists of β-arrestin–mediated signaling could have clinical benefits [5]. Moreover, it has been reported that β-arrestin–biased agonists of the angiotensin type I receptor stimulate cardiac contractility while also antagonizing some harmful effects of the receptor mediated through G proteins [5].

Robust scales to quantify biased agonism are a fundamental need in pharmacology. These scales should have certain properties, as pointed out by Kenakin et al., [6]: “It is essential for such a scale

not to be affected by differences in receptor density, as these are system factors that often vary between cell types". To define a scale of biased agonism a mathematical model is needed. The Black and Leff operational model of agonism (Box 1) considers two steps for the generation of a pharmacological effect [7]: the binding of agonist to the receptor and transduction of binding into effect. A single parameter in the model, τ or operational efficacy, quantifies the ability to generate a pharmacological effect. τ determines the maximum response to a particular agonist thus differentiating full and partial agonists (Box 1 Eq. 5) and also affects the potency of the ligand (Box 1 Eq. 6). Because τ is proportional to receptor density (Box 1 Eq. 3), $\Delta\log(\tau)$ between two agonists in a particular pathway is independent of receptor density and provides a scale with which to quantify differences between agonists. If we take one of these agonists as the reference ligand, $\Delta\Delta\log(\tau)$ may serve as a scale for biased agonism between pathways [8].

However, the concentration-effect curve is also influenced by the equilibrium dissociation constant K_A because, as a component of agonist potency (Box 1 Eq. 6), it too determines the location of the curve along the concentration axis. Consequently, the ratio of τ to K_A, termed the transduction coefficient $\log(\tau/K_A)$, has been proposed to quantify biased agonism [6]. For the reasons outlined above, since $\Delta\log(\tau/K_A)$ is independent of receptor density in a given pathway it is an appropriate scale to quantify the differences between the agonist of interest and the reference ligand. Finally, $\Delta\Delta\log(\tau/K_A)$ measures biased agonism between two pathways for the particular agonist.

The classical operational model of agonism [7] (Box 1) does not account for constitutive receptor activity, that is, signaling by receptors in the absence of ligands, and thus inverse agonists, ligands which yield a response lower than constitutive receptor activity, are excluded from the analysis of biased agonism. Since inverse agonists are part of the ligand space, their potential therapeutic

effects should not be excluded from routine screening analyses of biased agonism. In a recent paper [9] we used a model, the Slack and Hall operational model [10], which includes constitutive receptor activity (Box 2), and allows for the quantification of ligand bias independently of 'system bias'. Both the free receptor and the ligand-bound receptor produce a stimulus (Box 2 Eq. 2). ε is the intrinsic efficacy of the ligand: the value of ε determines whether ligands are agonists ($\varepsilon > 1$), neutral antagonists ($\varepsilon = 1$), or inverse agonists ($\varepsilon < 1$). It was proposed [9] that $\log(\varepsilon)$ and $\log(\varepsilon/K_A)$ can work as scales for biased agonism. Importantly, because both ε and K_A are ligand-receptor molecular properties, these scales do not depend on receptor density and thus a reference compound is not required to eliminate system dependence.

Receptor bias

We refer to the inherent bias of the receptor in the absence of ligands as 'receptor bias'. As shown in Box 2 Eq. 5, the basal response depends on E_m, n and χ. E_m is the maximum response of the system, n is a slope parameter and χ is the ratio of $[R]_T$, the total receptor concentration, to K_E, the value of the stimulus that produces half-maximal effect. χ determines the value of the basal response. Increasing χ from 0 makes the effect increase from 0 towards E_m. Thus, χ is an appropriate parameter to quantify receptor bias. As mentioned above, because χ is proportional to receptor density then standardization is needed. One method would be to measure the constitutive receptor activity along each pathway simultaneously or at an overlapping range of receptor densities to allow comparison of activity at a specific receptor concentration. In a similar way to previous studies which used τ [8] and τ/K_A [6] for quantification of biased signaling, we propose χ (at constant receptor density) as a property to identify receptor bias between two pathways.

Ligand bias

A given receptor can be unbiased ($\Delta\log(\chi) = 0$) or biased ($\Delta\log(\chi) \neq 0$) between two pathways. Addition of a ligand may alter the bias of the receptor by changing its inherent tendency to favor a given pathway. To quantify ligand bias we need to remove the receptor bias component. When using the log (τ) [8] or the log (τ/K_A) [6] scales a reference ligand is needed to remove the effects of receptor density and/or tissue properties related to transduction efficiency and coupling [6]. This is because τ contains both the receptor density and the tissue component of K_E in its definition (Box 1). An alternative way to quantify ligand bias, which does not need a reference ligand, is to use intrinsic efficacy. Scales based on $\log(\varepsilon)$ or $\log(\varepsilon/K_A)$ [9] can serve this purpose (Box 2). Because standardization within pathways is unnecessary, a single Δ measures ligand bias between two pathways. However, the $\Delta\log(\varepsilon/K_A)$ scale can produce counter-intuitive results. As stated in [9], when the affinities differ $\log(\varepsilon/K_A)$ cannot differentiate between the two properties of a ligand which is an inverse agonist for one pathway ($\varepsilon_1 = 0.01$ and $K_{A1} = 10^{-6}$) and a neutral antagonist for another ($\varepsilon_2 = 1$ and $K_{A2} = 10^{-4}$). In this case, $\log(\varepsilon/K_A)$, the bias of this ligand, would be zero, yet it can be argued that this is not the case since the ligand clearly has differential effects. This problem needs further development which is beyond the scope of this article.

Concluding remarks

Reliable quantification of biased signaling is fundamental for drug discovery programs. Consideration of constitutive receptor activity widens their scope by including inverse agonists. Inclusion of biased agonism within the framework of a mathematical model, such as the Slack and

Hall operational model of agonism [10], ensures that the pharmacological properties derived from constitutive receptor activity would be properly analyzed within a robust theoretical framework. Yet, the value of a pharmacological model resides in its applicability. We propose that the approach presented here should be incorporated into screening analyses of biased agonism. Thus, testing the practicality of quantitative approaches based on constitutive receptor activity in real pharmacological assays will let us know their feasibility.

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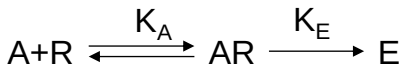
Competing interests

The authors declare no competing interests.

Box 1

The Black and Leff operational model of agonism

The Black and Leff operational model of agonism [7] is depicted in Scheme 1.



Scheme 1. An agonist, A, binds to receptor, R, to form agonist-receptor complex, AR, which transmits a signal leading to the observed pharmacological effect, E. K_A is the agonist-receptor equilibrium dissociation constant and K_E is a constant determining the efficiency of signal transduction by AR.

The concentration of occupied receptors, [AR], is given by Eq. 1.

$$[AR] = \frac{[R]_T[A]}{K_A + [A]} \qquad (1)$$

With $[R]_T = [R] + [AR]$

A logistic equation is proposed for the transduction of receptor occupancy into effect:

$$E = \frac{E_m[AR]^n}{K_E{}^n + [AR]^n} \qquad (2)$$

Where E_m is the maximum effect of the system and n is a factor related to the slope of the concentration-effect curves. K_E is the value of [AR] which elicits E=(1/2)Em. Low values of K_E result in higher values of E for a given [AR].

The concentration-effect equation (3) results from the substitution of Equation 1 into 2.

$$E = \frac{E_m \tau^n [A]^n}{(K_A + [A])^n + \tau^n [A]^n} \qquad (3)$$

With $\tau = \frac{[R]_T}{K_E}$.

In the Black & Leff model (Scheme 1), the receptor exerts the pharmacological effect only after the binding of the agonist. Thus, signaling by the receptor by itself, constitutive receptor activity, is not included in the model and the basal response (E when [A] equals 0) is 0.

$$E_{[A]=0} = 0 \qquad (4)$$

The response at saturating concentrations of ligand is:

$$E_{[A]\to\infty} = \frac{E_m \tau^n}{1+\tau^n} \qquad (5)$$

The τ parameter determines the ligand's efficacy. High τ values give $E_{[A]\to\infty}$ values close to E_m (full agonists) whereas low τ values give $E_{[A]\to\infty}$ values significantly lower than E_m (partial agonists).

τ and K_A determine ligand potency as reflected by EC_{50}, the [A] value causing half $E_{[A]\to\infty}$.

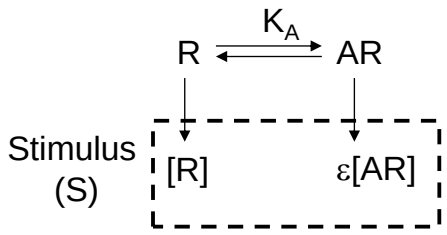
$$EC_{50} = \frac{K_A}{\left(2+\tau^n\right)^{1/n} - 1} \qquad (6)$$

High potency corresponds to low values of EC_{50}. Low EC_{50} values result from low K_A and high τ parameters.

Box 2

The Slack and Hall operational model of agonism

The Slack and Hall operational model of agonism [10] is depicted in Scheme 1.



Scheme 1. An agonist, A, binds to receptor, R, to form the agonist-receptor complex, AR. K_A is the agonist-receptor equilibrium dissociation constant. Both R and AR produce a stimulus: [R] and ε[AR], respectively. ε measures the 'intrinsic efficacy' of A.

The concentration of occupied receptors, [AR], is given by Eq. 1.

$$[AR] = \frac{[R]_T[A]}{K_A + [A]} \qquad (1)$$

With $[R]_T = [R] + [AR]$.

The stimulus S is the sum of the stimuli produced by R and AR.

$$S = [R] + \varepsilon[AR] \qquad (2)$$

A logistic equation is proposed for the transduction of stimulus into effect:

$$E = \frac{E_m S^n}{K_E{}^n + S^n} \qquad (3)$$

Where E_m is the maximum effect of the system and n is a factor related with the slope of the concentration-effect curves. K_E is the value of S which elicits E=(1/2)Em. Low values of K_E result in larger values of E for a given S.

The concentration-effect equation (4) results from the combination of Equations 1 to 3.

$$E = \frac{E_m \chi^n (K_A + \varepsilon[A])^n}{(K_A + [A])^n + \chi^n (K_A + \varepsilon[A])^n} \qquad (4)$$

With $\chi = \frac{[R_T]}{K_E}$.

In contrast with the Black & Leff model (Box 1), the free receptor is able to exert an effect. Thus, signaling by the receptor alone, constitutive receptor activity, is included in the model and the basal response (E when [A] equals 0) is not 0.

$$E_{[A]=0} = \frac{E_m \chi^n}{1+\chi^n} \qquad (5)$$

The response at saturating concentrations of the ligand is:

$$E_{[A]\to\infty} = \frac{E_m \varepsilon^n \chi^n}{1+\varepsilon^n \chi^n} \qquad (6)$$

The combined parameter $\varepsilon\chi$ determines the efficacy of the system. High $\varepsilon\chi$ values give $E_{[A]\to\infty}$ values close to E_m (full agonists) whereas low $\varepsilon\chi$ values give $E_{[A]\to\infty}$ values significantly lower than E_m (partial agonists).

The potency of the ligand is:

$$EC_{50} = \frac{K_A\left(\left(2\varepsilon^n\chi^n + \varepsilon^n + 1\right)^{1/n} - \left(\chi^n + \varepsilon^n\chi^n + 2\right)^{1/n}\right)}{\varepsilon\left(\chi^n + \varepsilon^n\chi^n + 2\right)^{1/n} - \left(2\varepsilon^n\chi^n + \varepsilon^n + 1\right)^{1/n}} \qquad (7)$$