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Functional Studies Cast Light on Receptor States

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Abstract

Contemporary analysis of the functional responses of G protein-coupled receptors (GPCRs) usually addresses drug-receptor interactions from the perspective of the average behavior of the receptor population. This behavior is characterized in terms of observed affinity and efficacy. Efficacy is a measure of how well a drug activates the receptor population and observed affinity a measure of how potently a drug occupies the receptor population. The latter is quantified in terms of the dissociation constant of the ligand-receptor complex. At a deeper level of analysis, drug-receptor interactions are described in terms of ligand affinity constants for active and inactive receptor states. Unlike observed affinity and efficacy, estimates of receptor state affinity constants are unperturbed by G proteins, guanine nucleotides and other signaling proteins that interact with the receptor. Recent advances in the analysis of the functional responses of GPCRs have enabled the estimation of receptor state affinity constants. These constants provide a more fundamental measure of drug-receptor interactions and are useful in analyzing structure-activity relationships and in quantifying allosterism, biased signaling and receptor-subtype selectivity.

Keywords

Receptor state affinity constants; Functional studies; Agonist bias; Allosterism; Single receptor behavior

A single-receptor view of drug action

Drug-receptor interactions are often illuminated when viewed from the perspective of single receptors. Single receptors isomerize between active and inactive states depending on the nature of the ligand bound to them (Figures 1*a*)[1–4]. When unbound, most receptors remain inactive except for occasional fleeting activations (constitutive activity). These activations have greater frequency and longer duration when the receptor is bound with an agonist. Agonists bind to both receptor states, but they extend the mean duration of the active state because of their higher affinity for it. For the purpose of measuring drug action, receptor states are defined by their activity and affinity for specific ligands [5,6]. Certainly there are numerous vibrating conformations of each state as well as additional evanescent transition states.

In contemporary analysis of G protein-coupled receptors (GPCRs), the frame of reference is usually the receptor population [7–10]. For a population of eight receptors, activation in the presence of agonist approaches a mean level with considerable relative variation (Figure 1*b*). As the size of the receptor population increases to 200, activation is nearly constant in time after reaching equilibrium (Figure 1*c*). Unlike the bound or unbound condition of a single receptor, occupancy of a population of receptors is represented by a graded variable ranging from zero to one (Figure 1*d*). The observed dissociation constant (K_D) designates the position of the ligand-occupancy function on the log ligand-concentration scale. For a specific population of receptors, both half-maximal occupancy and receptor activation occur at an agonist concentration equivalent to the value of K_D (Figure 1*d*). The ability of a ligand to activate the receptor population is represented by the parameter, efficacy, which is defined as the fraction of the occupied receptor population in the active state. For example, if 30% of the receptor population is occupied and one-third of these ligand-receptor complexes are in the active state, then the value of efficacy is 0.33.

Although the observed affinity constant (K_{obs}; $1/K_D$) determines receptor occupancy, no stable receptor structure having an agonist affinity constant of K_{obs} exists. Rather, there are at least two structures (active and inactive states) characterized by affinity constants of K_{act} and K_{inact}, respectively (Figure 1*a* and e). The value of K_{obs} represents a weighted average of the values for K_{act} and K_{inact} (Table 1). Hence, K_{obs} might better be termed, occupancy constant.

In contrast, the relationship between the efficacy and the activation state of single receptors is simple. If the time that a single ligand-receptor complex spends in the active state is divided by the total time that the receptor is occupied, the result is a unitless fraction between zero and one that represents the probability that the ligand-receptor complex is in the active state. This probability is equivalent to the population concept of efficacy defined above.

Recently, methods for estimating receptor state parameters from functional assays on GPCRs have been described. In this review, I explain some intuitive relationships between receptor state and population parameters and briefly review the experimental paradigms from which state parameters can be estimated.

A model for GPCR activation

The simulation depicted in Figure 1 adequately portrays activation of the soluble ligand-binding domain of the dimeric metabotropic glutamate receptor 4 [4]. An analogous model with two cooperatively linked orthosteric sites would resemble the behavior of many ligand-gated ion channels of the cys-loop and glutamate families [11]. But how does the simulation relate to a receptor coupled to G proteins?

The interactions among orthosteric ligand (*D*), receptor states (*R* and *R**), G protein and guanine nucleotide have been described using the quaternary complex model [12,13]. Its most recent description includes GTPase activity, the guanine nucleotides GTP and GDP, and three states of G protein [14]. The latter correspond to the crystal structures of GDP-bound holoprotein (inactive, *G*) [15], GTP-bound G_α subunit (active, $G\alpha**$) [16], and agonist-occupied

receptor-G protein complex (exchange, $G*$) [3]. The exchange state exhibits high affinity for the active state of the receptor ($R*$) and low affinity for GTP and GDP. For a variety of conditions, simulations with this model identify the form of the agonist-receptor complex that initiates signaling. This component is the active state of the agonist-receptor complex bound with the exchange state of the GDP-occupied G protein (quaternary complex, $DR*G*GDP$) (Figure 2a). In the presence of GTP, the quaternary complex rapidly exchanges GTP for GDP, causing the resulting GTP-bound G_α and loosely associated $G_{\beta\gamma}$ subunits to dissociate from the receptor. Thus, the quaternary complex is the immediate precursor of activated G proteins (GTP-G_α and $G_{\beta\gamma}$), and it represents the biophysical correlate of receptor activation (i.e., stimulus function of Stephenson [9] and Furchgott [17]). It follows that the concentration of agonist generating half-maximal formation of $DR*G*GDP$ is equivalent to the agonist's K_D value ($1/K_{obs}$) and that the fraction of the agonist-occupied receptor population in the $DR*G*GDP$ complex is proportional to efficacy (ε) (Figure 2b). The value of these population parameters can change depending on the G protein, its relative abundance and the concentrations of guanine nucleotides. In contrast, estimates of ligand-affinity constants for a receptor state involved in signaling through a specific G protein are unaffected by variation in the concentrations of G protein and guanine nucleotide [13,14,18].

Relationship between population parameters and receptor state affinity constants

When a ligand induces a protein to assume a different conformation, some of the intrinsic binding energy associated with the induced state is used to cause the conformational change [19]. Hence, the observed affinity constant of a ligand for the receptor population can be much less than its affinity for the state that it induces. The amount of agonist-induced activation of a GPCR can be expressed as a ratio (activation ratio, R_{act}) equivalent to the fractional amount of ligand-receptor complexes in the active state (efficacy, ε) divided by the fractional amount of unoccupied receptors in the active state (constitutive activity, ε_0) ($R_{act} = \varepsilon/\varepsilon_0$). If the observed affinity constant of the agonist-receptor complex is multiplied by the activation ratio, the result is the value of the active state affinity constant (Figure 3) [20]:

$$K_{act} = K_{obs} R_{act} \quad\quad 1$$

For example, consider a highly efficacious agonist with an observed affinity constant (K_{obs}, $1/K_D$) of 10^5 M^{-1} (log K_{obs}, 5.0). If the fraction of the population of agonist-occupied receptors in the active state (ε) is 0.5 and that of the unoccupied receptor population (ε_0) is only 10^{-4}, then the activation ratio (R_{act}) is 5×10^3. Multiplying K_{obs} (10^5 M^{-1}) by R_{act} (5×10^3) yields the value of the affinity constant for the active state (K_{act}, 5×10^8 M^{-1}, log K_{act}, 8.70). An analogous calculation can be used for estimation of K_{inact} [18].

This concept can be restated as a corollary for ligand-induced conformational changes [21]. That is, the affinity constant of a ligand for a particular receptor state (K_j) is equivalent to the product of the observed affinity (K_{obs}) and the fraction of the population of ligand-

receptor complexes in the state (ε_j) divided by the fraction of the unoccupied receptor population in the same state (ε_{0-j}):

$$K_j = K_{obs} \frac{\varepsilon_j}{\varepsilon_{0-j}} \qquad 2$$

Estimates of ε and ε_0 are unneeded for these calculations. When functional data are analyzed with the operational model, τ values can be estimated (τ and τ_0,) that are proportional to ε and ε_0, respectively [20]. Hence, one can estimate the activation ratio (R_{act}) as τ/τ_0, and therefore:

$$K_{act} = K_{obs} \frac{\tau}{\tau_0} \qquad 3$$

A more robust approach is to analyze the appropriate functional data using nonlinear regression analysis with a version of the operational model in which K_{act} is substituted for $\tau K_{obs}/\tau_0$ or the total stimulus function is expressed in terms of receptor state parameters instead of population parameters [13,20]. Additional relationships between receptor state and population parameters are given in Ehlert and Griffin [13] and Table 1.

When applied to the phosphoinositide response of the human M_3 muscarinic receptor, this analysis yielded estimates of 4×10^7 and 10^4 M^{-1} for the K_{act} and K_{inact} values of the efficacious agonist, oxotremorine-M [20]. The analogous estimates for carbachol were 1.6×10^7 M^{-1} and 5.5×10^3 M^{-1}. Because acetylcholine has tenfold-greater potency than carbachol for eliciting M_3 responses [22], the results suggest a K_{act} value of approximately 10^8 M^{-1} for acetylcholine. Nearly the same K_{act} value was estimated for acetylcholine at the muscle-type nicotinic receptor (5×10^7 M^{-1}) [1] using single channel analysis, suggesting that similar binding pockets have evolved for acetylcholine on muscarinic and nicotinic receptors [23]. An affinity constant of 10^8 M^{-1} represents a binding energy of about 11 kcal mol^{-1} or 1.1 kcal mol^{-1} per non-hydrogen atom of acetylcholine, which is similar to that of the biotin-steptavidin interaction (1.2 kcal mol^{-1} per non-hydrogen atom of biotin).

The K_{obs} value of epinephrine for the β_2 adrenergic receptor (binding assay estimate) increases 1000-fold in the presence of G_s or an antibody stabilizing the active receptor state [24], indicating the more than 1000-fold selectivity of isoproterenol for the active state (i.e., $K_{act} > K_{obs} > K_{inact}$).

A relative estimate of the active state affinity constant

An easy state parameter to estimate in functional studies is a relative value of the active state affinity constant. For the case of two full agonists, A and B, relative affinity for the active state (K_{act-B}/K_{act-A}) is equivalent to the corresponding ratio of potencies (EC_{50-A}/EC_{50-B}) [26,25]. For full and partial agonists, the ratio of equiactive agonist concentrations approaches a constant limiting value at low concentrations of the agonists ($EAMR$) [26]. $EAMR$ was later termed RA_i and defined as the product of affinity and efficacy of a given agonist (εK_{obs}) expressed relative to that of a standard agonist ($\varepsilon' K_{obs}'$) [27,28]:

$$RA_i = \frac{\varepsilon K_{obs}}{\varepsilon' K_{obs}'} = \frac{\tau K_{obs}}{\tau' K_{obs}'} = \frac{K_{act}}{K_{act}'} \quad 4$$

As shown above, the efficacy terms can be replaced with the appropriate τ values from the operational model. The RA_i value, raised to the exponent m (transducer slope factor), was also shown to be equivalent to the ratio of initial slopes of two concentration-response curves [27]. Subsequently, the RA_i value was shown to be equivalent to the active state affinity constant of an agonist (K_{act}), expressed relative to that of a standard agonist (K_{act}') [25], which is also indicated above and can be easily derived from equation 3. Kenakin and coworkers [29–31] have used the term, transducer ratio, for τ/K_D, and the variable, $\quad \tau/K_D$, for RA_i.

The RA_i value can be estimated from two or more agonist concentration-response curves even if there is insufficient information to estimate the observed affinity (K_{obs}), relative efficacy (ε/ε'), τ value or even the product, τK_{obs}, for each agonist. It is, nonetheless, always possible to estimate RA_i. Figure 4 shows an example of a simulation that illustrates this point for two partial agonists. Panel *a* shows the concentration-response curves of two agonists. Because both drugs are partial agonists, it is impossible to estimate any of the individual parameters of the operational model with any degree of accuracy including the maximal response of the system, observed affinity (K_{obs}), the τ value nor the product, τK_{obs}, of either agonist. Nonetheless, the log RA_i value $\pm$ SEM of agonist 2 relative to agonist 1 can be estimated (-0.96 ± 0.062) using regression methods described previously [27,32]. This value is nearly the same as that used in the simulation (log RA_i, -1.0).

Panels *b* – *e* show the results of the analysis after the responses measured at the higher concentrations of agonist are progressively removed, one at a time, from each successive panel in alphabetical order. Remarkably, it is still possible to estimate the RA_i value with reasonable accuracy after the four largest response values are removed from each curve (panel *e*) even though it is impossible to estimate the EC_{50} and E_{max} values of the curves as well as any of the primary parameters of the operational model except the composite parameter $\tau K_{obs}/\tau' K_{obs}'$ (RA_i). This result illustrates the fundamental nature of the active state affinity constant.

Analysis of allosterism yields all of the receptor state parameters

Allosterism is defined by a subcommittee of the International Union of Basic and Clinical Pharmacology as a modification in the properties of a ligand caused by the binding of a second ligand at a distinct site [33]. This mechanism can account for reciprocal modulation in ligand binding. It can also account for effects on ligand efficacy that are unrelated to a change in the conformation of the receptor. For example, an allosteric inhibitor could bind to the open state of a ligand-gated ion channel and plug the channel, causing an increase in orthosteric agonist affinity and an inhibition of channel function (open channel block). Similarly, an allosteric inhibitor could bind to the active state of a GPCR and competitively displace the G protein resulting in increased orthosteric binding affinity and decreased efficacy.

A more restrictive way of defining allosterism involves determining if the allosteric effect is indistinguishable from a change in the isomerization constant of the unoccupied receptor [5]. The isomerization constant (K_q) defines the spontaneous equilibrium between the unoccupied active ($R*$) and inactive (R) states of the receptor ($K_q = R*/R$). An allosteric ligand that acts in this manner has the effect of altering the isomerization constant by the factor, K_f/K_e, in which K_f and K_e denote the affinity constants of the allosteric ligand for active and inactive receptor states (Figure 5a) [5,34]. Here, the effects of such a ligand are termed, purely allosteric. Some candidate purely allosteric agonists include the M_1 and M_2 selective ligands described by Christopoulos and coworkers [35,36].

For example, consider a receptor having an isomerization constant of 10^{-4} (Figure 5b). In the absence of ligands, the fraction of the receptor population in the active state (constitutive activity, ε_0) would be approximately 10^{-4} ($\varepsilon_0 = K_q/(1 + K_q)$) (Table 1). In the presence of a purely allosteric modulator, with threefold selectivity for the active receptor state ($K_f/K_e = 3$), the receptor would behave as if its isomerization constant has increased threefold. In most instances, it would be difficult to measure the associated increase in constitutive activity (threefold), but not the corresponding increase in the affinity (1.5-fold) and efficacy (twofold) of an efficacious agonist with 3000-fold selectivity for the active state (Figure 5c). The combined effects would be obvious in a sensitive output assay for GPCRs (Figure 5d).

If a purely allosteric agonist is available for a GPCR, or alternatively, if the GPCR exhibits constitutive activity and the action of the modulator is purely allosteric, then it is possible to analyze the allosteric interaction and estimate the receptor state affinity constants of the orthosteric (K_{act}, K_{inact}) and allosteric (K_f, K_e) ligands, the observed isomerization constant of the unoccupied receptor ($K_{q\text{-}obs}$), the observed sensitivity constant of the transducer function of the operational model ($K_{E\text{-}obs}$), and all of the population parameters for the allosteric interaction [13]. Almost any output assay for GPCRs can be used in the analysis. The essential requirements of the protocol include measuring the independent effects of the allosteric and orthosteric ligands under control conditions and their interaction under conditions of partial receptor inactivation or reduced receptor expression. The various state parameters that can be obtained from this analysis are described in Ehlert and Griffin [13].

Having estimated the observed isomerization ($K_{q\text{-}obs}$) and sensitivity ($K_{E\text{-}obs}$) constants (see Table 1 for definitions) of a particular output response, an investigator could estimate the receptor state affinity constants of various additional orthosteric and allosteric agonists (test ligands) through analysis of their individual concentration-response curves. In this analysis, global nonlinear regression analysis is done on two sets of data: 1) the allosteric interaction described in the prior paragraph for a single full agonist and 2) a series of concentration-response curves for test agonists [13]. For any full agonist in this latter group, an additional concentration-response curve measured under the condition of reduced receptor expression or partial receptor inactivation is needed for estimation of K_{inact}.

One of the easiest parameters to extract from functional studies on allosterism is the affinity constant of an allosteric ligand for the active state of a GPCR. It is always possible to estimate the product of maximal changes in the allosteric modulatory effects on the affinity ($K_{obs\text{-}max}$, α) and efficacy (ε_{max}, β_1) of an agonist in functional assays [37,38]. This

cooperative effect was initially designated as "1/B", but has been renamed using the variable γ_1 (γ_2 denotes the maximal scalar effect of the orthosteric ligand on the affinity and efficacy of the allosteric ligand) [13].

The simulation in Figure 6 illustrates how these parameter are related to allosteric modulation of receptor activation (panel a) and the resulting output response (panel b). Figure 6c shows the allosteric effect, expressed as the product of the observed changes in the affinity and efficacy of the orthosteric ligand (K_{obs} ε), plotted against the concentration of modulator. The maximal value of K_{obs} ε is equivalent to γ_1. Figure 6d illustrates that receptor occupancy by the allosteric ligand is equivalent to the normalized K_{obs} ε value (K_{obs} $\varepsilon - 1$ divided by the maximal value of K_{obs} $\varepsilon - 1$) [13]. Lazareno and Birdsall [39] have described the analogous relationship for occupancy and allosteric modulation in ligand binding affinity.

Unlike the reciprocal allosteric effects that orthosteric and allosteric ligands have on their respective binding affinities (α), γ_1 is determined only by the allosteric ligand. It is equivalent to the ratio of the efficacy of the allosteric ligand (ε_A) divided by the efficacy of the unoccupied receptor (ε_0) [13]. It can also be defined using the corresponding τ values from the operational model (i.e., $\gamma_1 = \tau_A/\tau_0$). Thus, γ_1 is analogous to the activation ratio (R_{act}) mentioned above in connection with orthosteric ligands. It can be shown that the product of the observed affinity of the allosteric ligand ($K_{obs\text{-}A}$) and γ_1 is equivalent to the affinity constant of the allosteric ligand for the active state [13]:

$$K_f = \gamma_1 K_{obs-A} = \frac{\varepsilon_A K_{obs}}{\varepsilon_0} = \frac{\tau_A K_{obs}}{\tau_0} \quad 5$$

Both γ_1 and K_{obs} can be estimated from data like those shown in Figure 6b using global nonlinear regression analysis with the appropriate regression equation [13,40]. Alternatively, the regression equation can be written in terms of state parameters and estimates of K_f can be obtained directly without using equation 5 [13].

Implications for drug discovery

With estimates of an agonist's receptor state affinity constants in hand, an investigator has a means of comparing the activity of an agonist at different receptor subtypes and determining its ability to persuade a given receptor to signal through different pathways. Different receptor coupling proteins provide a window for estimating agonist affinity for effector-selective states of the receptor [20]. These estimates depend only on the active and inactive states of the receptor involved in triggering the response. This brief review has focused mainly on G protein signaling, but the same considerations apply to arrestin signaling [41,42]. Additional complications arise with arrestin signaling, however, regarding the ligand-dependent rate of receptor phosphorylation by GRK and potential receptor dephosphorylation at the plasma membrane before recruitment of arrestin [43–45].

The RA_i estimate has been used as a means of detecting agonist bias through different signaling pathways [25,28,32,46]. The rationale is based on the assumption that the active state

is the first cause of downstream responses, and that different relative estimates of K_{act} (RA_i) imply different active states that mediate the different responses. Although the difference between the RA_i values (log RA_i) of an agonist for eliciting two different responses is useful for detecting bias, neither the RA_i value itself nor the component of the RA_i value reflecting the εK_{obs} value (e.g., τK_{obs}) of a given agonist is a measure of the ligand's ability to transduce a signal. Rather, εK_{obs} is equivalent to the product of the active state affinity constant and constitutive activity ($\varepsilon K_{obs} = K_{act}\varepsilon_0$) [20]. Similarly, τK_{obs} is equivalent to $K_{act}\tau_0$ (see equation 3).

The population definition of efficacy gives rise to somewhat unexpected behavior at the very low end of the scale. For example, the efficacy of a neutral antagonist is equivalent to [21] constitutive activity (ε_0) and that of an inverse agonist is between ε_0 and zero [20]. The efficacies of neutral antagonists and inverse agonists, or their corresponding τ values, can be estimated in functional assays that report constitutive activity [20] or through the allosteric approach reviewed in the prior section. It follows that RA_i values can be estimated for both inverse agonists and neutral antagonists and that the RA_i value of an inverse agonist can be larger than that of an agonist. Nonetheless, the   log RA_i value of an inverse agonist accurately reflects the log difference in its affinity constants for the active receptor state, relative to that of the standard agonist, when estimated for different output assays.

The activation ratio (R_{act} or γ_1) is a useful parameter for estimating receptor signaling through a particular pathway, particularly if the response is a natural one of clinical importance. Incidentally, this parameter can also be calculated as K_{act}/K_{obs} (see equation 1). Changes in the concentration of GTP or G protein can influence constitutive receptor activity more so than agonist efficacy [20]. Thus, the R_{act} value (τ/τ_0) of an agonist for a response can vary depending on the expression level of signaling components.

If the goal is drug screening using native and nonnative cellular assays, then the selectivity of a drug for the active receptor state (i.e., K_{act}/K_{inact}) is a better estimate of pathway activation. This parameter is invariant for a particular signaling pathway and has previously been suggested as a measure of efficacy at the receptor state level of analysis [6]. There is no problem with having two different definitions of ligand efficacy, based on single-receptor or population analysis, provided that the level of analysis is clearly specified.[21] Here I refer to the ratio, K_{act}/K_{inact}, as the induction ratio (R_{induct}). An agonist could have similar K_{act}/K_{inact} values for two different pathways but exhibit a bias for one pathway because of its higher affinity for both the active and inactive states of its preferred pathway. Thus, knowledge of the individual estimates of K_{act} and K_{inact} is useful in understanding biased signaling, and an absolute estimate of K_{act} is better than a relative one (RA_i).

If the agonist first binds to the same inactive state of the receptor when initiating signaling through different pathways, then the corresponding differences in log R_{induct} (log R_{induct}) would be equivalent to   log RA_i. Although it might seem unlikely that K_{inact} would vary for the same ligand-receptor complex when signaling through different pathways, it is possible that it does and that changes in log K_{inact} underlie a component of agonist efficacy in some instances. Differences in a ligand's K_{inact} value could give rise to biased antagonism. The binding pocket and cytosolic ends of helix 5 and 6 of the β_2-adrenergic receptor are thought

to undergo dynamic changes in the inactive state [47], which could provide the basis for differences in K_{inact}.

While estimates of K_{inact} require more data than RA_i, future studies employing the methods described above in the section on allosterism could yield a database of observed receptor isomerization (K_{q-obs}) and sensitivity (K_{E-obs}) constants for various signaling pathways in defined cells and tissues used in drug screening. These values would enable investigators to estimate K_{act} and K_{inact} from agonist concentration-response data as described above.

Conclusions

The past few years have witnessed a surge in our understanding of receptor structure, which will surely continue as more active and inactive receptor structures are solved. The population analysis that has driven pharmacology over the past few decades is insufficient for advancing analysis of receptor function in the present era. A scientist interested in designing a more potent analog of a drug, for example, might dock the parent drug onto the active and inactive receptor structures, *in silico*, and determine how an added substituent interacts with a specific amino acid side chain in both structures. Estimating receptor state affinity constants in functional assays provides a means of verifying conclusions drawn from such *in silico* investigations. By analogy, receptor state analysis improves structure-activity relationship studies, which currently relate drug structure to potency (EC_{50}) or observed binding affinity (K_i value). Knowing how modification of a ligand structure alters its affinity for active and inactive receptor structures provides more useful information in these analyses, particularly regarding pathway induction (K_{act}/K_{inact}) and bias. Hence, functional analysis of receptor states represents an adjunct to structural analysis.

Receptor states are the first cause of pharmacological effects, and hence their ligand-affinity constants are the ultimate measures of drug action because they provide an estimate of how well a drug turns on a receptor.

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Highlights

Ligand affinity and efficacy are determined by G protein-coupled receptor state constants.

Relative active state affinity constants can be estimated from concentration-response curves.

Analysis of allosteric interactions can provide estimates of all receptor state parameters.

Receptor state parameters are useful for measuring biased signaling and receptor selectivity.

Estimates of receptor state parameters are unperturbed by receptor coupling proteins.
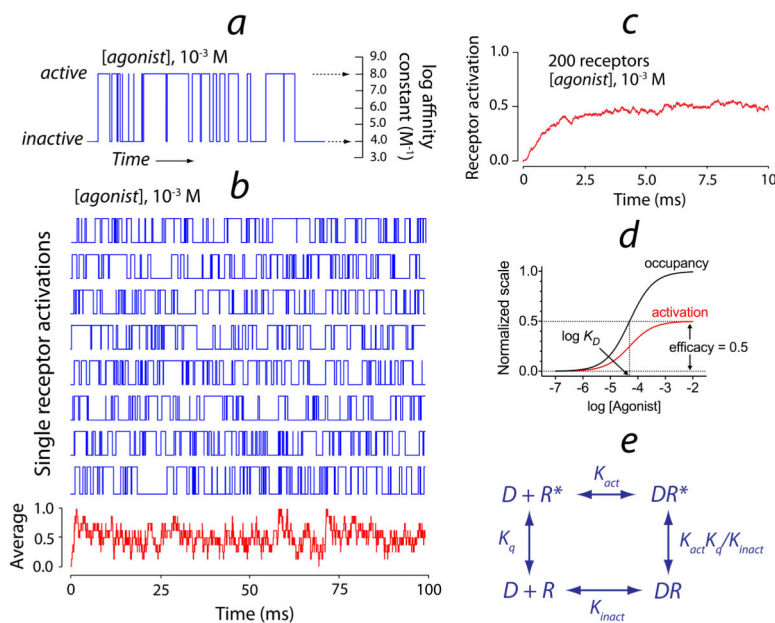
Figure 1. Relationship between receptor state and population parameters

a, Simulation of single receptor activity in time and in the presence of agonist (10^{-3} M). The affinity constants of the agonist for the active and inactive states are indicated on the ordinate scale on the right. A continuous Markov model was used to simulate receptor isomerization, using an isomerization constant of 10^{-4} for the unoccupied receptor (K_q, see Table 1) as described previously [21]. *b*, Simulation of an ensemble of eight receptors using the approach described in *a*, assuming that agonist was added at time zero. The lowest trace represents the average activity of the eight receptors. *c*, The average activation of an ensemble of 200 receptors. The simulation was derived as shown in the lowest trace in panel *b*, except that the receptor population was increased to 200. *d*, Receptor occupancy and activation plotted against the agonist concentration for a large population of receptors. Receptor activation is defined as the average activity of all of the receptors. For example, at an agonist concentration of 10^{-3} M, the activation level is equivalent to the equilibrium value shown in panel *c*, (about 0.5 at 7.5 to 10 msec). The parameters K_D (dissociation constant) and ε (efficacy) are defined in the text. *e*, Two-state model used to generate the simulations shown in *a*–*c*. The scheme shows the equilibrium of ligand (*D*) with active (*R**) and inactive (*R*) states of the receptor. K_{act} denotes the affinity constant of *D* for the active state, K_{inact}, the corresponding value for the inactive state, and K_q, the isomerization constant of the unoccupied receptor ($K_q = R^*/R$).
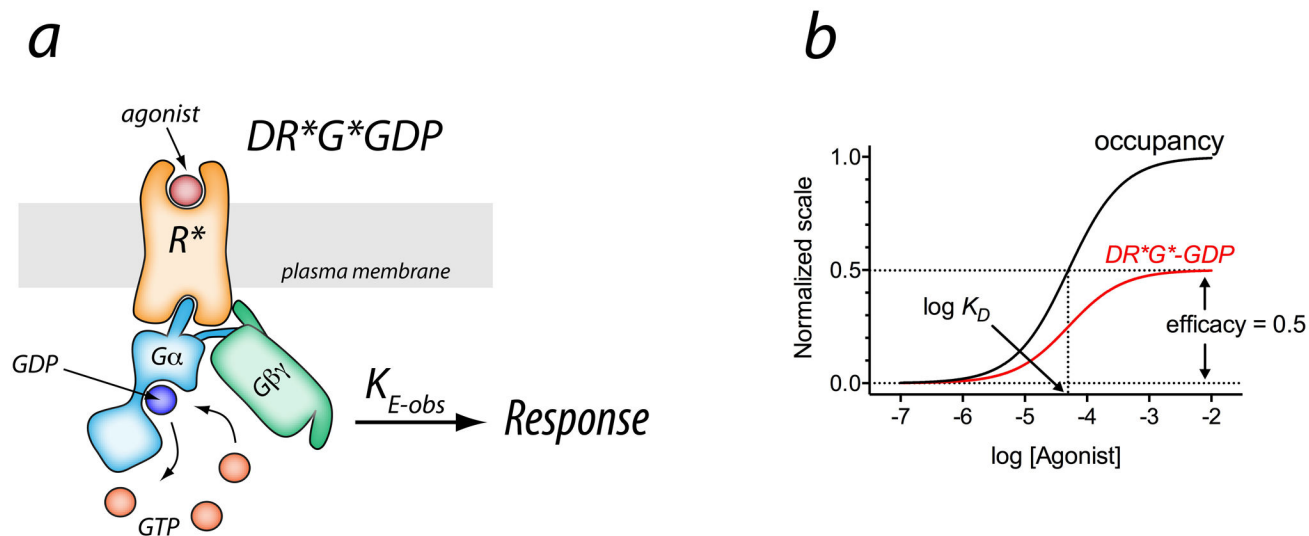
Figure 2. Generation of a stimulus by the active state of a GPCR

a, The quaternary complex consists of the active state of the receptor (R^*) bound with agonist (D) and the exchange state of the G protein (G^*) bound with GDP (DR^*G^*GDP). The parameter $K_{E\text{-}obs}$ determines the observed sensitivity of the transducer function of the operational model (see Table 1). *b*, The graph shows a plot of receptor occupancy and the fraction of the occupied receptor population in the form of the active state of the quaternary complex. Efficacy is defined as the fraction of the population of occupied receptor complexes in the active state of the quaternary complex. The dissociation constant (K_D) denotes the concentration of agonist that yields both half-maximal receptor occupancy and half-maximal formation of DR^*G^*GDP.
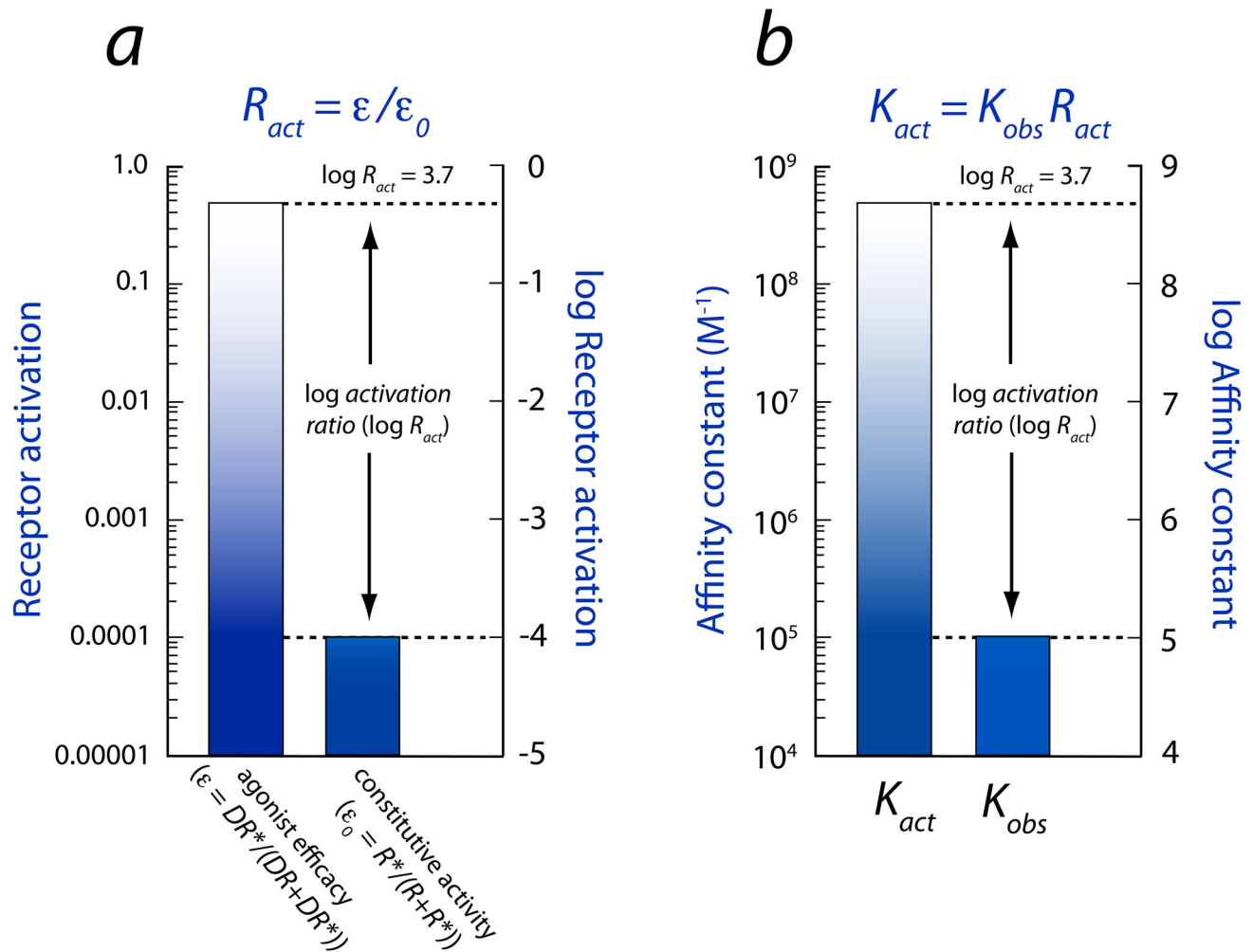
Figure 3. Relationship between the activation ratio (R_{act}) and the observed (K_{obs}) and active state (K_{act}) affinity constants

a, The activation ratio (R_{act}) is defined as the ratio of agonist efficacy (ε) divided by constitutive activity (ε_0). The histogram shows the fractional values of agonist efficacy and constitutive activity. The ordinate scales have been adjusted to reflect the fractional values (left ordinate) and the corresponding logarithms (right ordinate). The log difference between ε and ε_0 is equivalent to log R_{act}. *b*, The relationship between the active state affinity constant (K_{act}) and the product of the activation ratio (R_{act}) and the observed affinity constant (K_{obs}). The histogram shows the values of K_{act} and K_{obs}. The ordinates have been adjusted to reflect the values of the affinity constants (left ordinate) and their corresponding logarithms (right ordinate). The log difference between the K_{act} and K_{obs} is equivalent to log R_{act}.
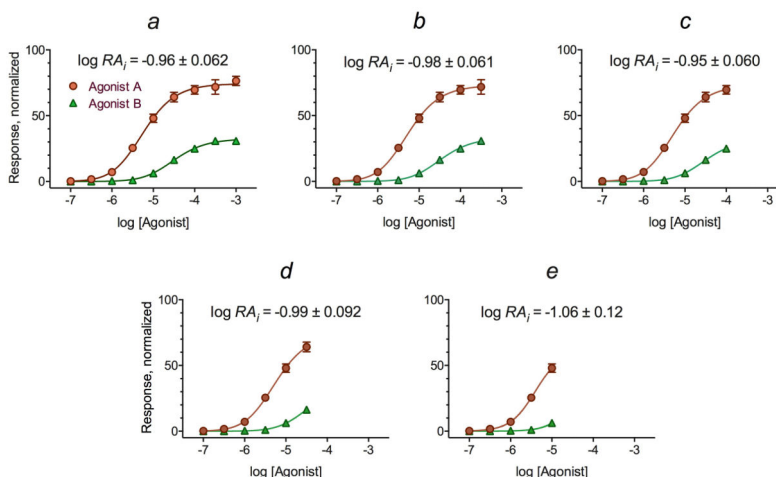
Figure 4. Estimation of the log RA$_i$ value of partial agonist B, expressed relative to that of partial agonist A

The concentration-response curves of two partial agonists (A and B), having different log observed affinity constants (K_A, 5.0 and K_B, 4.5) and log τ values (τ_A, 0.3 and τ_B, 0.03) were simulated using the operational model with values of 1.0 for the transducer slope factor (m) and maximum response of the system (M_{sys}) and a 10% random error. The mean values ± SEM of four simulated replicates are shown. The simulated data were analyzed by global nonlinear regression analysis using a form of the operational model described in Ehlert [32], having parameters of M_{sys}, m, log K_A, log R (log $\tau_A K_A$), log K_B and log RA_i ($\tau_B K_B / \tau_A K_A$). The theoretical curves represent the least-squares fit to the data. In each case, an accurate value of log RA_i was estimated as shown in the plot. In contrast, it was impossible to obtain accurate estimates of the other parameters. The plots differ with regard to the number of data points used in the regression analysis (9 – 5 for panels *a – e*, respectively).
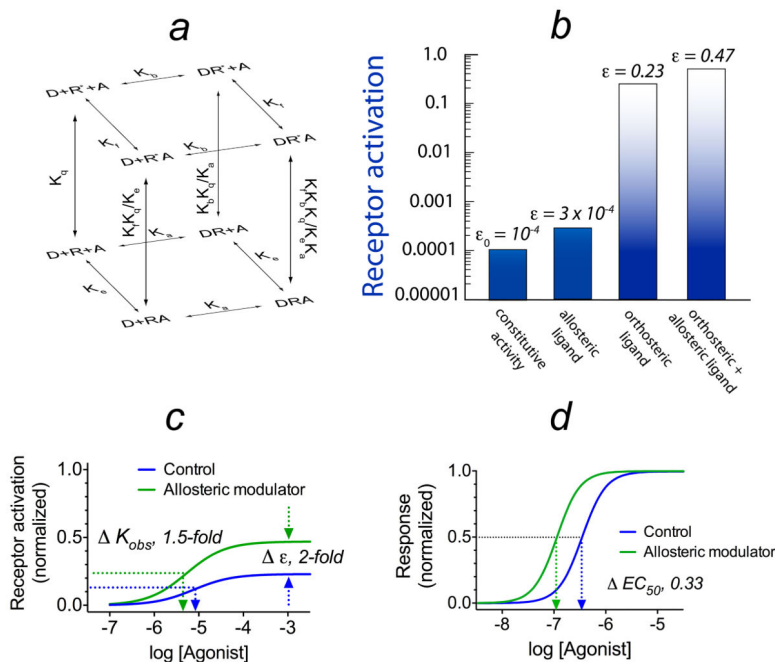
Figure 5. Purely allosteric effects are indistinguishable from a change in the isomerization constant of the unoccupied receptor

a, A simplified form of the Monod Wyman and Changeux model [5] for allosterism. The K_{act} and K_{inact} of the orthosteric ligand are denoted by K_b and K_a, respectively, and those of the allosteric ligand, by K_f and K_e. The back face of the cube represents the interaction of the orthosteric agonist with the receptor and is the same as that shown in Figure 1*e*. The front side of the cube illustrates agonist binding when the receptor is occupied by the allosteric ligand. The data in *b–d* were simulated with this model using the following parameter values: K_b, 3×10^8 M^{-1}; K_a, 10^5 M^{-1}; K_f, 3×10^5 M^{-1}; K_e, 10^5 M^{-1} and K_q 10^{-4}. *b*, The histogram shows the fractional values of constitutive activity (ε_0), the efficacies (ε) of the allosteric ligand and orthosteric agonist, and their combined effect on receptor activation. *c*, Receptor activation plotted against the agonist concentration in the absence and presence of an allosteric modulator. The parameter, K_{obs}, denotes the observed affinity constant of the agonist measured in the presence of allosteric modulator (K_{obs}') divided by that measured in its absence (K_{obs}) ($K_{obs} = K_{obs}'/K_{obs}$). The parameter, ε, is calculated in an analogous manner ($\varepsilon = \varepsilon'/\varepsilon$). The log K_{obs} values of the agonist in the absence and presence of modulator were 5.11 and 5.28, respectively. The corresponding values for efficacy were 0.231 and 0.474. *d*, The influence of allosteric modulation on the output response of a GPCR. The concentration-response curves were generated using the operational model with a sensitivity constant (K_E) of 0.01 and a transducer slope factor (*m*) of 2.0. The stimulus inputs to the model were the simulated activation curves in panel *c*.
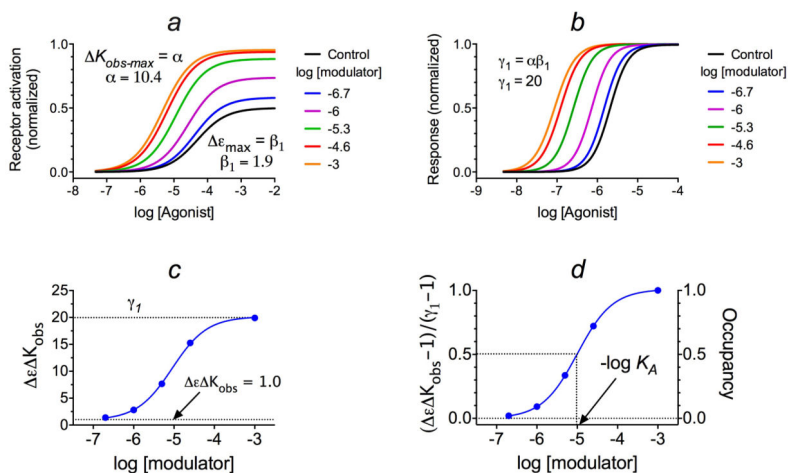
Figure 6. The influence of allosteric modulation on observed affinity, efficacy and the output response of the agonist

a, Effect of allosteric modulation on receptor activation by an orthosteric agonist. The simulated data were derived with the model shown in Figure 5*a* using the following parameter estimates: K_b, 10^8 M^{-1}; K_a, 10^4 M^{-1}; K_f, 2×10^6 M^{-1}; K_e, 10^5 M^{-1} and K_q 10^{-4}. The parameters, $K_{obs-max}$ and ε_{max}, represent the maximal values of K_{obs} and ε (defined in the legend to Figure 5*b*) measured at receptor saturating concentrations of allosteric modulator. These maximal values are also denoted with the variables α and β_1, respectively. *b*, The receptor activation functions generated in panel *a* were used as input to an operational model to simulate the concentration-response curves. The values of the transducer slope factor (*m*) and sensitivity constant (K_E) were 2.0 and 0.01, respectively. *c*, The effect of allosteric modulation on the product of the changes in observed affinity and efficacy of the orthosteric ligand (K_{obs} ε). The maximal change in K_{obs} ε is denoted by γ_1 and the value in the absence of modulator is equivalent to 1.0. *d*, A normalized K_{obs} ε value can be derived by subtracting one from each value and dividing these by the maximum K_{obs} ε value minus one. These normalized values are plotted (left ordinate) against the allosteric modulator concentration to yield receptor occupancy by the modulator (right ordinate). The parameter K_A denotes the observed affinity constant of the allosteric modulator in the absence of orthosteric ligand.

Table 1

Receptor state and population parameters and their reciprocal relationships[1]

Parameter	Definition	Equation
Receptor states		
K_{act}	Active state affinity constant (units, M^{-1})	$\dfrac{\varepsilon K_{obs}}{\varepsilon_0},\ \dfrac{\tau K_{obs}}{\tau_0}$
K_{inact}	Inactive state affinity constant (units, M^{-1})	$K_{obs}\left(\dfrac{1-\varepsilon}{1-\varepsilon_0}\right),\ K_{obs}\left(\dfrac{1-\tau K_{E-obs}}{1-\tau_0 K_{E-obs}}\right)$
$K_{q\text{-}obs}$	Observed isomerization constant. Its value is perturbed from that of the isolated receptor (K_q) by G protein and guanine nucleotides.	$\dfrac{\varepsilon_0}{1+\varepsilon_0},\ \dfrac{\tau_0 K_{E-obs}}{1+\tau_0 K_{E-obs}}$
Receptor population		
K_{obs}	Observed affinity constant (units, M^{-1})	$\dfrac{K_{inact}+K_{act}K_{q-obs}}{1+K_{q-obs}}$
ε	Efficacy of ligand, fraction of the ligand-occupied receptor population in the active state	$\dfrac{1}{1+\dfrac{K_{inact}}{K_{act}K_{q-obs}}}$
ε_0	Constitutive activity, fraction of the unoccupied receptor population in the active state	$\dfrac{K_{q-obs}}{1+K_{q-obs}}$
Transducer function		
M_{sys}	The maximum of the output response for an agonist with an infinite K_{act}/K_{inact} ratio	
K_E	Sensitivity constant of the transducer function (units, receptor concentration, R_T)	
m	Transducer slope factor	
Composite		
$K_{E\text{-}obs}$	$\dfrac{K_E}{R_T T_{max}}$, T_{max} denotes maximal efficacy of an agonist with an infinite K_{act}/K_{inact} ratio	
τ	$\varepsilon/K_{E\text{-}obs}$	
τ_0	$\varepsilon_0/K_{E\text{-}obs}$	
RA_i	Estimate of K_{act}, expressed relative to that of a standard agonist (K_{act}')	$\dfrac{K_{act}}{K_{act}'},\ \dfrac{\varepsilon K_{obs}}{\varepsilon' K_{obs}'},\ \dfrac{\tau K_{obs}}{\tau' K_{obs}'}$

[1] Equations are from Ehlert and coworkers [13,18,20,26].