Review

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Functional studies cast light on receptor states

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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, or 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.

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 (Figure 1A) [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 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 1B). As the size of the receptor population increases to 200, activation is nearly constant in time after reaching equilibrium (Figure 1C). 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 1D). 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 1D). 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, 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 1A,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.

By 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

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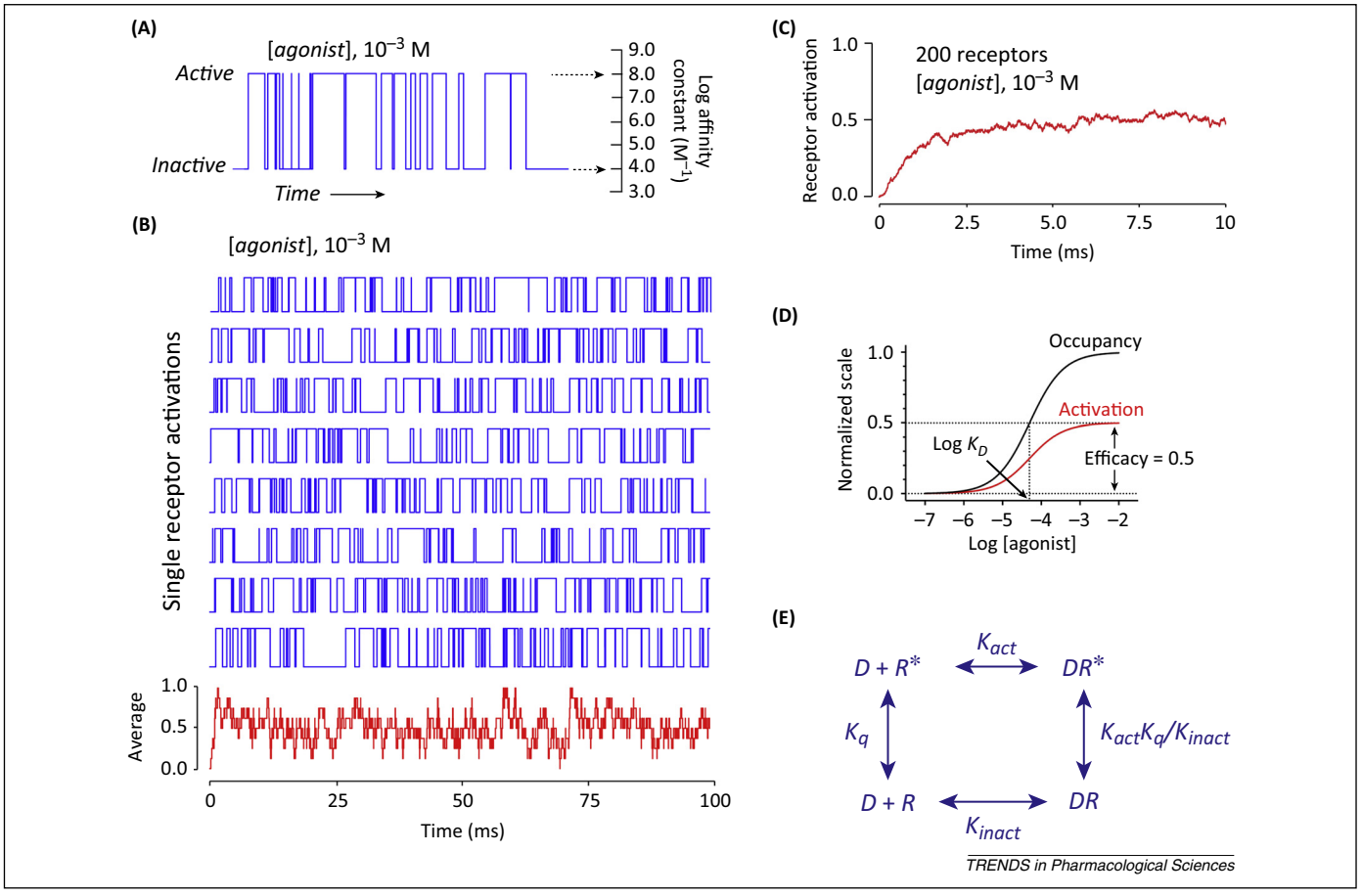

Figure 1. Relationship between receptor state and population parameters. **(A)** Simulation of single-receptor activity in time and in the presence of agonist (10^{-3} M). The affinity constants of the agonist for the active and inactive states are indicated on the ordinate scale on the right. A continuous 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 (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 (C) (about 0.5 at 7.5–10 ms). 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 ($K_q = R^*/R$).

ion channels of the Cys-loop and glutamate families [11]. However, 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 various 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 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. By 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