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DRUG EFFICACY AT G PROTEIN–COUPLED RECEPTORS

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■ **Abstract** Efficacy has been defined in receptor pharmacology as a proportionality factor denoting the amount of physiological response a given ligand imparts to a biological system for a given amount of receptor occupancy. While first defined in terms of response, the concept can be expanded to a wide variety of G protein–coupled receptor (GPCR) behaviors, which includes pleiotropic interaction with multiple G proteins, internalization, oligomerization, desensitization, and interaction with membrane auxilliary proteins. Thus, there can be numerous types of efficacy, and different ligands can have a range of efficacies for different receptor behaviors. This review discusses the use of the efficacy concept in GPCR models based on the thermodynamic linkage theory and also in terms of the protein ensemble theory, in which macroaffinity of ligands for an ensemble of receptor microstates produces a new ligand-bound ensemble. The pharmacological characteristics of the ligand emerge from the intersection of the ligand-bound ensemble with the various ensembles defining pharmacological receptor behaviors. Receptor behaviors discussed are activation of G proteins; ability to be phosphorylated, desensitized, and internalized; formation of dimers and oligomers; and the interaction with auxiliary membrane and cytosolic proteins. The concepts of ligand-specific receptor conformation and conditional efficacy are also discussed in the context of ligand control of physiological response.

WHAT IS EFFICACY?

From the time it was perceived that some chemicals produced pharmacological responses and others did not came efforts of receptor pharmacologists to classify and quantify these two apparently different properties of drugs. Thus, proportionality factors such as intrinsic activity (1) were introduced to differentiate agonists from antagonists. The term efficacy was first used by R. P. Stephenson to denote the property of a drug that caused it to activate a receptor and produce pharmacological response (2). Stephenson's vantage point was tissue response. However, emerging G protein–coupled receptor (GPCR) technology shows us that receptors have a rich texture of behavior, whereas tissue activation is only part of a repertoire of

responses. GPCRs are now known to associate with various different G proteins, and this G protein pleiotropy appears to be specific, even with respect to the combination of G protein subunits available to the receptor (3). For example, somatostatin receptors in GH_3 cells inhibit calcium channels through activation of $G\alpha_{02}\beta_{1\gamma 3}$. The same pathway is activated by M_4 muscarinic receptors, but apparently only through the activation of a different complex of Gi subunits, namely, $G\alpha_{o1}\beta_{\gamma 4}$ (4). In addition, GPCRs are known to demonstrate various behaviors, all of which may be relevant to their function. Thus, GPCRs also can desensitize, internalize, homodimerize, heterodimerize, form clusters, and associate with other membrane proteins, as well as couple to G proteins to induce physiological response. In light of this plethora of activities, what should be the measure of efficacy?

In some cases, there are relationships between these various receptor behaviors. For example, studies from several receptor types indicate that receptor internalization is positively correlated with receptor activation, i.e., the more efficacious an agonist is for producing response, the more likely it will produce receptor internalization (5–7). However, there is divergence in what is commonly thought of as efficacy and this receptor behavior. For example, the cholecystokinin receptor antagonist D-Tyr-Gly-[(Nle^{28,31},D-Trp^{30})cholecystokinin-26-32]-phenethyl ester does not produce receptor stimulation but does produce profound receptor internalization (8). This shows a lack of efficacy for cholecystokinin-mediated tissue response that differs from a clearly positive efficacy for receptor internalization. Similarly, HIV-1 mediated infection of healthy cells, leading to AIDS, is known to occur through the interactions of the viral coat protein gp120, cell membrane proteins CD4, and the chemokine receptor CCR5 (9–12). Two possible therapeutically relevant approaches to blocking this process are an allosteric modification of CCR5, so that ternary complex formation between gp120, CD4, and CCR5 cannot occur, and the removal of CCR5 from the cell surface through induction of CCR5 receptor internalization (13). The chemokine peptide, regulated on activation, normal T cell–expressed and -secreted (RANTES), produces chemotaxis (14) and also blocks HIV-1 infection through interaction with CCR5 (9). RANTES produces CCR5 internalization as well as receptor activation (9). The RANTES analogue, aminooxypentane-RANTES (AOP-RANTES), does *not* produce CCR5-mediated chemotaxis (14) but does promote rapid internalization of CCR5 receptors with inhibition of receptor recycling back to the cell surface (15–17). This latter property makes AOP-RANTES a potent inhibitor of HIV-1 infection (14, 16). In this context, the lack of efficacy of AOP-RANTES for producing primary chemotaxis does not correlate with a clear efficacy for CCR5 internalization and protection against HIV-1 infection, a very relevant therapeutic activity.

In light of these ideas, a general definition to be used throughout this review is the following: Efficacy is the property of a molecule that causes the receptor to change its behavior toward the host cell. In this review, efficacy is described operationally in terms of qualitative and quantitative properties of ligands and their relationship to therapeutic application. The relationship between receptor structure and efficacy is beyond the scope of this review (for further reference see 18–21).

MATHEMATICAL AND OPERATIONAL TREATMENT OF EFFICACY

Historically, efficacy was considered to be a proportionality factor grading response and receptor occupancy (1, 2, 22). There is no biochemical correlate to this proportionality factor, but rather it is a dimensionless term designed to quantify ratios of the power of different agonists to induce physiological response. In terms of the theory of GPCR activation, little mechanistic information can be gained from this model. In spite of this fact, there are practical advantages to treating efficacy as an operational ratio, and these are discussed later in this review.

Models of GPCR function have been described in terms of thermodynamic linkage theory, whereby various receptor species are linked together in a system, and the energy of transitions between the species made to be independent of the pathway between them (23–25). Two such related models incorporate the ability of receptors to spontaneously form active states (capable of activating G proteins), namely, the extended ternary complex (ETC) model (26) (see Figure 1*A*) and the cubic ternary complex (CTC) model (27–29) (see Figure 1*B*). The CTC model is a variation of the ETC model that allows the inactive-state receptor to interact with G proteins. This model is more complete thermodynamically, but it is also more complex. It is not clear to what extent the added complexity of the CTC model better accommodates experimental findings with GPCRs, but one of these models may be better suited than the other to describe various receptors, and the suitability may relate to the receptor type and stoichiometrical makeup of the system (30). In any case, such equilibrium models, rooted in thermodynamic linkage theory, relate efficacy to factors modifying the affinity of the receptor for the G protein (31–33). Thus, in the ETC model, the terms α and γ control the modification of affinity of the active-state receptor R_a for the G protein (see Figure 1*A*). In terms of the CTC model, a third term, namely δ, is added to α and γ to describe modification of the active-state receptor affinity for G protein produced by binding of a ligand (see Figure 1*B*). The terms α, γ, and (for the CTC model) δ are ligand related and describe the change in the receptor affinity, for the G protein, imparted by the ligand. In the context of equilibrium models rooted in linkage theory, this is one definition of efficacy. Therefore, a ligand with a high value for γ would promote formation of the active-state receptor R_a (*vide infra* for the thermodynamics of how this occurs). A ligand with a high value for γ would produce a selectively higher affinity of the receptor for G protein when the receptor is ligand bound. In terms of the CTC model, a high value for δ would indicate that the combination of the receptor and the G protein in concert with ligand binding would promote a high-affinity ternary complex capable of stimulus production. In terms of these equilibrium models, α, γ, and δ could function as mathematical correlates for efficacy.

It is relevant to point out that the ETC and CTC models are often referred to as two-state models. This is correct from the standpoint of the unliganded forms of the receptor but clearly incorrect when a ligand is introduced into the system. With binding of the ligand, the receptor can take on a new affinity for the G protein

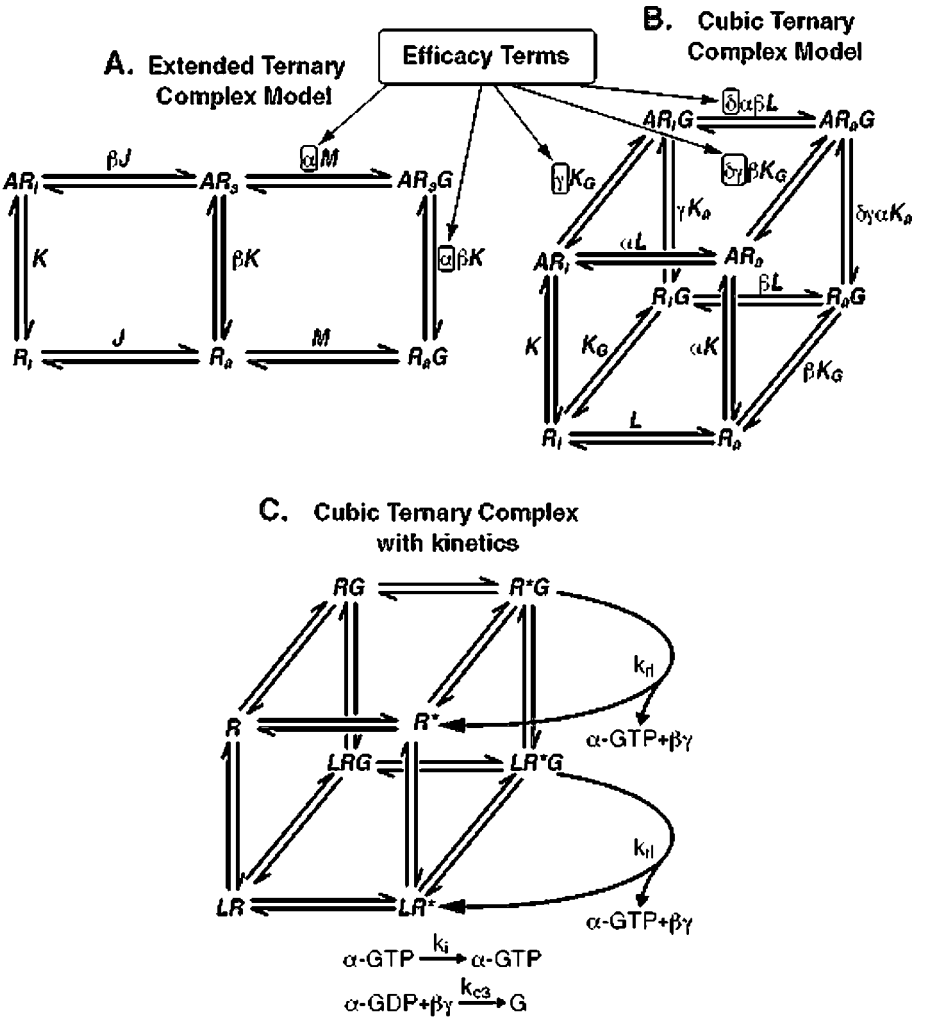

Figure 1 Models of GPCRs as described with linkage theory. (*A*) Extended Ternary Complex theory as presented by Samama et al. 1993 (26). The receptor exists in an inactive (Ri) and active (Ra) state; G proteins (mediating physiological response) bind only to the active-state Ra. The addition of a ligand [A] forms three corresponding ligand-bound species with differing proclivities to form the active state and affinities for G proteins. (*B*) A thermodynamically more complete but more complex model (termed the cubic ternary complex model, CTC) whereby the inactive state of the receptor also can form a complex with the G protein that does not signal (27–29). (*C*) The CTC model with the kinetic addition of the catalytic exchange of GDP and GTP and subsequent activation of G protein. The rate constant k_{rl} adds a description of the quality of the ligand-receptor complex, in terms of what characteristics it imparts to the ability of the G protein to exchange GDP and GTP (35).

(through unique values of α, γ, and δ) and thus, it could become a totally different protein species. Under these circumstances, both the ETC and CTC models are potentially infinite state models when they form an intersection set with the set of infinite ligands.

EFFICACY AS A DIRECTIONAL VECTOR

Before the discovery of constitutive receptor activity, efficacy was considered to be an active property in an on-off mode, i.e., a molecule could have or not have the property that induced a measurable physiological response. The discovery, by Costa & Herz (35), that receptors could spontaneously produce a physiological response and that ligands could reverse this process changed this concept and showed that efficacy had direction, i.e., it could be negative as well as positive. Therefore, a ligand that stabilized the inactive form of the receptor would reduce levels of RaG (Figure 1) in an active process of reversal. These ligands are referred to as inverse agonists. It is now known that a great many apparently silent competitive antagonists are in fact inverse agonists and that this property can only be detected in systems that are constitutively active, i.e., the direction of the efficacy vector cannot be determined without the appropriate scale (vide infra). This concept of vectorial direction of efficacy should be kept in mind for all interactive properties of receptors (i.e., formation of dimers, binding to auxiliary proteins) in the sense that ligands may stabilize conformations that cancel natural behaviors of receptors as well as promote them.

THE QUALITY OF EFFICACY

While the foregoing discussion somewhat describes the concept of efficacy, in terms of the power of a ligand to induce response in biochemical systems, it still does not consider the core meaning of the term. Specifically, these models simply describe changes in affinity of the receptor for G protein through ligand binding. They do not in any way consider the quality of the receptor change when ligand is bound in terms of propensity to produce pharmacological response. It is known that point mutation (for example, mutations of Phe303 in the α_{1B}-adrenoceptor) in receptors can preserve high affinity for G proteins but eliminate the ability of receptor agonists to produce G protein activation (34). This suggests that there are conformations that can separate G protein binding from activation.

A step toward describing the ability of a receptor complex to induce activation can be made by considering kinetics in the model as well as simple equilibrium binding. Thus, the addition of the kinetics of GDP-GTP exchange after receptor activation of G proteins gives a measure of discerning different qualities of ligand-bound active-state receptors in activating G proteins through the magnitude of the constant k_{rl} (see Figure 1*C*) (36–39). Thus, not only can the affinity of the receptor for G protein be described by thermodynamic constants (α, γ, δ), but the ability

of the resulting complex to induce GDP-GTP exchange can be linked to a ligand through an additional constant k_{rl}. The constant describes the rate of GDP-GTP exchange imparted by the activated receptor upon ligand binding (see Figure 1*C*). However, even in the context of equilibrium, steady-state, and kinetic models, the concept of efficacy is still rooted in a relationship between the physical states of the receptor protein and the thermodynamics of interaction between proteins and the host system. A different view of protein dynamics and behavior can give insight into what is considered to be pharmacological efficacy.

ENSEMBLE THEORY IN GPCR DYNAMICS

A model that treats native-state proteins as an ensemble of conformational states is termed ensemble theory (40, 41). Nuclear magnetic resonance–detected hydrogen/deuterium exchange (42, 43) indicates that proteins are subject to constant local unfolding reactions occurring independently of each other in different regions of the protein (44–46). Some microstates may simply be comprised of the protein with different selected regions that are locally unfolded. These interactions are constantly formed and broken by changes in thermal energy (47–49). A surface can be created by the coordinates of microconformations and free energy of the protein to form an "energy landscape." Thus, receptors traverse an energy landscape (50–51) for the distribution of the various possible energy states (conformations) upon the number of conformations possible. The existence of numerous receptor conformations at any given instant in time can be calculated with this model. At any point in time, the enumeration of the various numbers of conformations in any given state, summed over all possible receptor states, yields a Gaussian distribution (see Figure 2*A*). The introduction of a ligand, which selectively binds to different receptor conformations, creates a new Gaussian distribution of receptor states, which is dependent on this differential affinity (Figure 2*A*). In essence, the ligand enters what could be termed a conformational cafeteria of numerous microstates. It will bind most to those for which it has the highest affinity, and at equilibrium, liganded forms of the ensemble will be created according to mass action. Like a cafeteria, the system will replenish those conformations taken by the ligand, and this will be at the expense of those for which the ligand has less affinity. Thus, the average activity of the receptor protein will be turned into an ensemble biased by the affinity of the ligand for those conformations (52). This produces redistribution of the entire ensemble, yielding a new one unique to the collection of microaffinities of the ligand for the native ensemble (Figure 2*A*).

This also can be thought of in terms of changes in Gibbs free energy. For a ligand [A], the free energy of an arbitrary state in the ensemble will be changed by an amount proportional to its binding affinity for that ligand (53):

$$\Delta G_i = \Delta G_i^0 - RT \ln \frac{(1 + K_{a,i}[A])}{(1 + K_{a,0}[A])} \qquad 1.$$

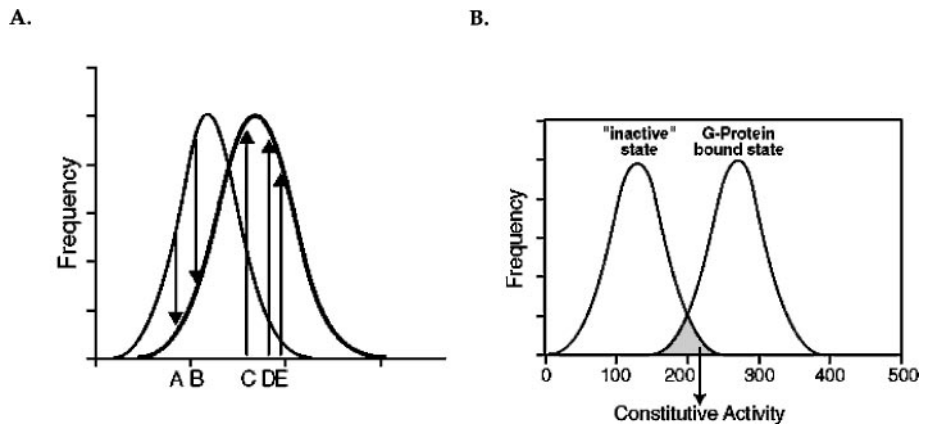

Figure 2 (*A*) Two normal distributions of receptor microstates. The distribution furthest left represents the native ensemble of receptor states. The binding of a ligand with macroaffinity depletes some of these states (states A and B; downward arrows) and enriches others (C, D, and E; upward arrows) according to the microaffinity of the ligand for the various states in the native ensemble. The result is a new ligand-bound receptor ensemble (shown to the right of the native ensemble). (*B*) Two receptor ensembles of receptor states, namely, the native inactive state and the G protein–bound signaling state. The shaded intersection between the two ensembles represents common microstates that spontaneously exist and can spontaneously activate G protein. These make up GPCR constitutive activity.

where ΔG_i^0 is the free energy of an arbitrary state i in the absence of ligand; $K_{a,0}$ is the binding constant to the reference state ("inactive" state in the ensemble); and $K_{a,i}$ is the binding constant to state i (the favored state). In the equation above, a larger-association binding constant for the favored state increases the negative term, producing a net decrease in free energy, and therefore, the formation of a new ensemble.

The relative abundance of microstates in different ensembles is unknown, but there is experimental evidence to allow some speculation on the relative number of microstates in the inactive- and active-state ensembles. Specifically, the inactive-state ensemble may contain fewer microstates. Mutation experiments have shown that select regions of the intracellular loops of GPCRs interact with G proteins to produce activation (54–56). Small peptide fragments, synthesized to mimic these regions, can produce G protein activation independently (57, 58). Thus, the conformations of the inactive states prevent accessibility of these regions to G proteins to preclude spontaneous activation. It can be surmised that the number of conformations that keep these regions inaccessible is less than that of the conformations that expose the regions to the cytoplasmic surface; i.e., the inactive state is more constrained (59). Point mutation often produces a constitutively active receptor, which suggests that the inactive states are constrained by the type of tertiary structure

present in the native protein. The difference is defined by the energy barrier denoted by the allosteric constant L, i.e., the Gibbs free energy required to change a relatively stable inactive conformation into an active one. With point mutation, the innate tertiary structure of the native receptor protein could be altered such that this free-energy barrier is reduced and active states then could be formed more freely. There are numerous point mutations in several categories of GPCRs that readily produce constitutive receptor activity (20, 21, 26, 60–62). It is as if disruption of the core tertiary structure of the receptor leads to reduction of the favorable free energy for maintenance of receptor structure and progression to other structures as a function of thermal energy. In keeping with this idea, studies on the rate of denaturation of constitutively mutated β_2-adrenoceptors indicate that this active state is more structurally unstable than its native counterpart (62).

Just as native-state protein is thought to exist in a collection of microstates, so too will other states, either ligand-induced or spontaneously occurring, exist in a collection of microstates. Thus, there could be ensembles defined for pharmacological function such as activation of a given G protein, internalization, dimerization, cluster formation, phosphorylation, and other behaviors. While there can be interdependence between some of these ensembles (i.e., activation and internalization), there need not be. It is more probable that the various ensembles defining pharmacological behavior of receptors will not be identical because different regions of the receptor protein are involved.

The pharmacological behavior of a receptor system toward a ligand depends on the degree of intersection of the ligand-formed ensemble with the various behavior ensembles. For example, Figure 2*B* shows two system ensembles, the native inactive state and a G protein–bound ensemble that produces a pharmacological response. The shaded intersection between the two ensembles represents the microconformations in common between the two. Thus, as thermal energy causes the formation of an ensemble that is common to the signaling one, a spontaneous G protein signal will be produced. This is defined as constitutive receptor activity (see Figure 2*B*). The magnitude of the pharmacological activity is determined by the degree of intersection. Although ligands may form different ensembles, if the degree of intersection of those with a G protein–signaling ensemble is the same, then the ligands will have identical signaling efficacy (63).

LIGAND-SELECTIVE RECEPTOR STATES

If a strict two-state model of receptor activation is adopted, then the simplest model of efficacy is enrichment of the spontaneously formed (constitutive) active receptor state through ligand binding (conformational selection). The creation of another state (a third or more) is extraneous to such a model and would require extraordinary evidence to be supportable. However, if an ensemble view is taken, an opposite conclusion is reached. If there is an ensemble of conformations that do not interact with G proteins [inactive state(s)] and another that produces activation (the activation ensemble), then to adhere to the idea that a model whereby

a ligand simply enriches the constitutive active state requires the model to form the exact same ensemble as the native activation one. Thus, such a ligand would have to have identical affinities for every microconformation in the native active-state base ensemble. The probability of such an occurrence is extremely low. This predicts that there will be differences in ensembles made by different ligands.

How these ligand-based ensembles intersect with the various ensembles for G protein activation, internalization, dimerization, etc. (all behaviors of GPCRs) determines what pharmacological properties the ligand will have. Again, it is predicted that different ligands would have differing propensities (efficacies) to induce various receptor behaviors. The range of intersections of the ligand-bound ensemble then dictates both the quantity and quality of the efficacy of the ligand and subsequently the pharmacological properties of the ligand. For example, Figure 3*A* shows four hypothetical native ensembles, one for the inactive resting state, one for signaling (a G protein–bound RaG state), one representing conformations that internalize the receptor, and one representing conformations that form receptor dimers. Shown also in this figure are three theoretical ligands each forming their

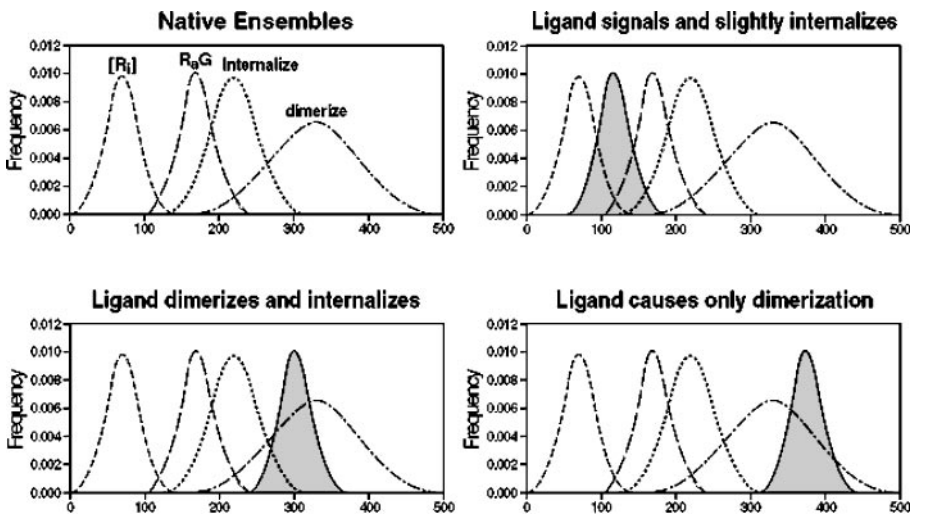

Figure 3 Pharmacological characteristics of three hypothetical ligands each forming their own ligand-bound receptor ensemble. (*Upper left panel*) Four receptor-state ensembles are shown. The native inactive states, states that bind to and activate G proteins (producing response), an ensemble favoring internalization of the receptor, and an ensemble conducive to receptor dimerization. (*Upper right panel*) This ligand produces an ensemble intersecting with the signaling and internalization ensemble, thus having a pharmacological profile of producing response and internalization. (*Lower left panel*) This ligand does not signal but does internalize and dimerize receptors. (*Lower right panel*) This ligand produces only dimerization.

own ligand-bound ensemble. Thus, one ligand could signal and slightly internalize receptors (Figure 3*B*), another could dimerize and internalize (Figure 3*C*), and yet another only dimerize receptors (Figure 3*D*). This mechanism links the thermodynamics of ligand binding to the biological activity of ligands and shows how different ligands can possess different kinds of efficacy. It also suggests that efficacy can very much be a ligand-dependent property. It is relevant, at this point, to review the experimental evidence for ligand-selective receptor conformations.

LIGAND-SELECTIVE RECEPTOR CONFORMATIONS: RECEPTOR SIGNALING

There are data to suggest that agonist-induced active-state conformations differ from the unliganded active states. For example, in TG4 murine myocytes genetically engineered to overexpress β_2-adrenoceptors (64, 65), cyclic AMP and cardiac contractility are elevated, and this elevation is sensitive to the β_2-adrenoceptor inverse agonist ICI 118551. Cyclic AMP also mediates calcium entry through L-type calcium channels with comparable sensitivity in both wild-type and TG4 myocytes. However, in TG4 myocytes, cyclic AMP elevation through constitutively activated β_2-adrenoceptors does not activate calcium channels. On the other hand, through activation of receptors by the β_2-adrenoceptor agonist zinterol, cyclic AMP elevation does activate the channels. This suggests that the receptor conformation produced by zinterol is different from that produced constitutively (66). Similarly, certain mutations of dopamine D_2 receptors abolish dopamine activation of the receptor but not activation by other dopamine agonists, which suggests that these agonists produce different conformations interacting with G proteins (67).

One approach to the detection of agonist-selective receptor active states is to monitor multiple receptor/G protein coupling. It is known that different regions of the cytosolic loops of receptors interact with different G proteins (68–72). It also is known that point mutation produces constitutive receptors that can selectively couple to different G proteins. For example, the C116F mutation of the β_2-adrenoceptor induces selective coupling to the Na^+/H^+ exchanger over cyclic AMP (73). Similarly, mutation of Asp^{79} to asparagine in the α_{2A}-adrenoceptor selectively uncouples effects on K^+ currents but not on cyclic AMP and voltage sensitive Ca^{2+} channels (74). Conversion of Cys^{128} to Phe in the α_{1B}-adrenoceptor induces selective constitutive activity for activation of phospholipase C but not phospholipase A2 (75).

The directing of receptor-induced signals to various cellular pathways through different receptor conformations is termed stimulus trafficking (76–78). True stimulus trafficking should not be confused with differential activation of different pathways through differences in signal strength. A powerful agonist may produce a stimulus sufficient to activate two pathways, whereas a weaker agonist may only stimulate the most sensitive one. For example, the opioid agonist DAla^2-DLeu^5]enkephalin (DADLE) produces stimulation of GTPase and also inhibition

of basal adenylate cyclase in NG 108–115 cells. However, upon alkylation of the receptor population (to diminish signal strength), the least sensitive response (GTPase response) is eliminated while the most sensitive response still remains (79). In view of these findings, the best evidence for true stimulus trafficking is obtained when both agonists produce both responses and when their relative activities on the two pathways differ, either through differences in relative potency, or better, through true reversals of rank order of potency.

Evidence of possible ligand-selective active states for signaling can be found in discontinuities in the relative potency of agonists on the same receptor for differing signals. Striking reversals in the rank order of agonist potency have been observed for pituitary adenylate cyclase-activating polypeptide receptors (80), dopamine D2 receptors (81), and drosophila tyramine receptors (82). A study of substance P analogues on neurokinin-1 receptors shows substance P to be 2.1 times more potent than the analogue [P3^{E}met(O2)^{11}]SP for producing cyclic AMP through neurokinin-1 receptor activation; however, it is 0.11 times less potent than the analogue for producing phosphoinositol hydrolysis through activation of the same receptor (83). A recent and novel approach has been the study of β_2-adrenoceptor/Gα-fusion proteins. Thus, while the relative potency of isoproterenol (ISO) and dichloroisoproterenol (DCI) for the β_2-adrenoceptor fusion protein $G_{s\alpha}$ (short form) is ISO > DCI, the potency is reversed to DCI > ISO for the fusion protein $G_{\alpha i3}$ (84). Similarly, the relative potency of dobutamine (DOB) and ephedrine (EPH) changes from DOB > EPH for $G_{\alpha s}$ to EPH > DOB for $G_{\alpha i3}$ (84).

Another unique approach utilizes [^{35}S]-GTPγS binding /immunoprecipitation in CHO cells expressing mucarinic receptors (85). With this technique it was found that the mucarinic receptors activate both $G_{i/0}$– and $G_{q/11}$–G proteins, and it was found that the agonist pilocarpine produces a cellular response dominated by $G_{i/0}$, whereas methacholine response activates both types of G protein nearly equally ($G_{q/11}$ slightly > $G_{i/0}$.).

Some of the most compelling evidence for differential receptor active states is agonist-dependent reversal of maximal responses for various signaling pathways. Since the maximal response is dependent only on efficacy (affinity is not an issue because the receptor population is saturated), these data indicate molecular differences in the ability of the receptor active state to maximally stimulate a given G protein. One example of this type of data has been obtained with human 5-HT_{2C} receptors. When transfected into CHO cells, these receptors mediate phospholipase C-mediated inositol phosphate accumulation (IP accumulation) and phospholipase A2-mediated arachadonic acid release. The agonist (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) produces a higher maximal stimulation than the 5-HT agonist quipazine for arachadonic acid release (86), thus indicating that DOI has a greater efficacy than quipazine for arachadonic acid release. However, the effects on the maximal responses for these agonists are reversed for IP accumulation (quipazine > DOI). This suggests that the active state formed by DOI (arachadonic release–preferring) is different from that produced by quipazine (IP accumulation–preferring). Another study showing differences in the rank order of

maximal stimulation of two G proteins (G_s and G_i) has been reported for cannabinoid receptors in CHO cells (87). Thus, while the agonists anandamide (AN), CP-55,940 (CP), HU-210 (HU), tetrahydrocannabinol (THC), and WIN-55212-2 (WIN) have rank-order maximal response for stimulation of G_i (WIN > CP > HU > AN > THC), this order changes for stimulation of G_s (WIN > HU > CP > THC > AN).

The detection of stimulus trafficking in cellular systems is an indirect indicator of ligand-specific receptor active state conformations. A direct study of ligand-induced receptor conformational change has been presented by Gether et al. (60). Thus, agonists and antagonists were tested on β_2-adrenoceptors, covalently labeled with an environment-sensitive fluorophore (4-[(iodoacetoxy)ethylmethylamino]-7-nitro-2,1,3-bezoxadiazol). Conformational changes in the protein altered observed fluorescence. An analysis of these data indicate that different agonists, such as DCI and DOB, have similar intrinsic activities but different effects on fluorescence (63). As shown in Figure 3, this suggests different ensembles of active states but similar degrees of intersection with the signaling ensemble.

PROTEAN AGONISM

In theoretical terms a ligand that produces an active receptor conformation of lower activity than the naturally formed constitutive one(s) can be detected by observation of positive agonism in a quiescent (nonconstitutively active) system and negative agonism in a constitutively active receptor system. Such ligands have been described in theoretical terms as protean, after the Greek God Proteus, who could change shape at will (88–90). Specifically, protean ligands would, by virtue of producing a low-efficacy active-state receptor, produce positive agonism in a quiescent system and inverse agonism in a constitutively active system. This latter activity would result from the conversion of the highly efficacious naturally constitutively active receptor state to the ligand-bound less active state.

Linkage theory can define the pharmacological properties of a protean ligand. For example, in the CTC model, a ligand with $\alpha > 1$ but $\gamma < 1$ could function as a protean ligand under specific circumstances. Such a ligand would favor the formation of an active state of the receptor (as defined as having a higher affinity for G protein than the inactive state) but produce a ligand-bound state of lower affinity for the G protein than the naturally formed spontaneous active state. In terms of the CTC model, protean agonism can be simulated by a variety of conditions, including changes in receptor/G protein stoichiometry and changes in affinity between receptors and G protein (i.e., desensitization), (88, 89, 91, 92).

Woolf & Linderman have described a specific relationship between parameters in the CTC model that could yield protean agonism (93). Thus, the following equality can be defined:

$$\alpha\delta\gamma + \gamma[\mathrm{G}]/\mathrm{K_G}(\alpha\delta - 1) + \alpha\mathrm{L}(\delta\gamma - 1) = \vartheta \qquad 2.$$

When $\vartheta > 1$, the ligand produces positive agonism; $\vartheta = 1$, neutral antagonism; and $\vartheta < 1$, inverse agonism. It can be seen that changes in [G] can change the value of ϑ from >1 to <1. Therefore, in any one system, with an appropriate level of [G], the ligand could function as a positive agonist while in another (lower level of [G]), it could function as an inverse agonist. This is, by definition, protean agonism.

In general, the experimental observation of protean agonism can furnish circumstantial evidence of the formation of ligand-selective receptor active states. Such activity has been noted for β_2-adrenoceptor ligands labetolol, DCI, and pindolol (94). Specifically, while these ligands produce positive agonism in sf9 cells transfected with β_2-adrenoceptors, the ligands produce inverse agonism in constitutively active membranes made from the same cells. Additionally, the inducement of β_2-adrenoceptor desensitization in transfected sf9 cells can convert the normally positive stimulation, produced by dichloroisoproterenol, to a negative agonism (95).

STIMULUS-BIASED ASSAYS

An extension to using G proteins to detect different receptor active states is to engineer specialized systems to do so. Referred to as stimulus-biased assay systems, these are hosts with identical cellular backgrounds except for stably transfected enriched levels of Gα-subunit proteins (96). These hosts were used in a study of the human calcitonin receptor type 2 (hCTR2), which is pleiotropic with respect to the G proteins with which it can interact (G_i, G_s, and G_q-96). In these particular experiments, transfection of hCTR2 into wild-type HEK 293 cells and HEK 293 cells stably enriched with $G_{\alpha i}$- and $G_{\alpha s}$-subunits allowed comparison of the relative potency of calcitonin agonists. A basic principle in the use of relative agonist potency in receptor classification is that this parameter is a unique identifier of agonist-receptor pairs. Deviation from a characteristic relative agonist potency would constitute evidence for differences in receptors. The basic tenet of this approach is that all of the agonists induce response in the same way, i.e., they form the same active-state conformation of the receptor. Therefore, if it is known that agonists have different relative potencies for a single given receptor in two different systems, then this basic tenet is not supported. In fact, this constitutes evidence to show that the various agonists involved produce different active-state conformations.

In these studies, the relative potency of eight calcitonin agonists did not change when studied in wild-type and $G_{\alpha i}$-enriched HEK cells. However, dramatic differences, including changes in the rank order of agonist potency, were observed when wild-type and $G_{\alpha s}$-enriched cells were used (96). Thus, the potency ratio for rat amylin and porcine calcitonin changed from 4.6 in wild-type cells to 84 in $G_{\alpha s}$-enriched cells. In general, the relative rank order of potency changed from [Eel Calcitonin > Salmon Cal > Porcine Cal > Rat Cal = Chicken CGRP = Human Cal > Rat Amylin > Rat CGRP] to [Porcine Cal > Human Cal > Rat Cal > Eel

Cal > Salmon Cal > Chicken CGRP > Rat CGRP > Rat Amylin]. Such findings cannot be explained with the assumption that all eight agonists produce the same receptor state (96).

KINETICS OF RECEPTOR ACTIVATION

Another experimental approach used to detect ligand-selective receptor active states is the study of the kinetics of adenylate cyclase activation. Thus, the rate of activation of adenylate cyclase, in the presence of limiting GTP concentrations, showed a differential rate of heterotrimer dissociation for different β_2-adrenoceptor agonists (98). In another study, the efficacy of β_2-adrenoceptor agonists correlated well with the promotion of GTP hydrolysis but did not correlate for inosine triphosphate (ITP) hydrolysis. The differences in the ability of these agonists to hydrolyze GTP vs. ITP are consistent with the production of different receptor active states (99).

LIGAND-SELECTIVE CONFORMATIONS AND THERAPEUTIC UTILITY

The formation of ligand-selective receptor active states that traffic receptor stimulus to different cellular pathways adds another dimension to how agonists of differing efficacies can be utilized therapeutically. For receptors that are pleiotropic with respect to the number of G proteins with which they interact, different active-state conformations may preferentially stimulate different cellular response pathways. Thus, ligand structure may begin to manipulate not only the quantity of efficacy but also the quality of efficacy of agonists. Presently, it is not clear how these effects can be systematically exploited for therapeutic advantage. However, it could be that agonists which produce different receptor active states, and thus, preferentially activate different G proteins, may induce a subset of physiological responses normally controlled by a given receptor subtype. Under these circumstances, active-state selective agonists may offer another level of receptor selectivity (100).

RECEPTOR PHOSPHORYLATION AND DESENSITIZATION

As discussed previously, there are receptor systems where the processes of receptor activation and receptor desensitization parallel each other, i.e., there is a correlation between the strength of stimulus imparted by an agonist and the ability of the agonist to cause phosphorylation and desensitization of receptors (5–7, 101–105). However, this correlation is not uniform across all receptor types, and these exceptions also may furnish evidence for ligand-selective receptor conformations.

Separate active-state receptor conformations have been postulated for natural vs. synthetic ligands. For example, while angiotensin II produces activation, phosphorylation and internalization of angiotensin II receptors in transfected CHO-K1 cells, the analogue [Sar^1,Ile^4,Ile^8]AngII does not activate or internalize the receptor but does promote robust receptor phosphorylation (106). This suggests that this analogue produces a conformation not conducive to activation or internalization but one that is readily phosphorylated. Similarly, while morphine opioid receptor complexes can be phosphorylated in vitro by protein kinase A PKA catalytic subunit, DAMGO-receptor complexes cannot (107). Another exception to the rule that desensitization parallels activation can be found in the μ opioid agonists methadone and l-α-acetyl methadone. These agonists produce disproportionate desensitization and receptor phosphorylation when compared to response. This thereby suggests ligand-specific receptor conformations (108). Similarly, in studies with μ opioid receptors, methadone and buprenorphine have been shown to have desensitizing properties different from those of morphine (108). Differences in the desensitizing capabilities also can be inferred from observing recovery from desensitization. For example, the recovery from prolonged activation of 5-HT_3 receptors is mono-exponential with partial agonists but sigmoidal (indicating three and possibly four steps) for full agonists (109). These different abilities to desensitize and signal may have utility in the treatment of tolerance (102, 108).

INTERNALIZATION

Another prominent behavior of GPCRs is internalization from the cell surface to the interior of the cell. Internalization can be followed either by observation of re-emergence of receptors to the surface or degradation. While being a natural activity of receptors linked to signaling, internalization also may be a separate and therapeutically useful activity in itself. Ligands that selectively induce receptor internalization may have utility in the prevention of HIV-1 infection. This is because internalization may remove critical coreceptors for membrane fusion and subsequent HIV-1 infection, namely, CXCR4 (15, 110) and/or CCR5 (14, 16). In fact, this approach may be superior to blocking the receptor since it would circumvent possible rapid emergence of resistant HIV-variants through therapeutic pressure and mutation (111–113).

As discussed previously, the data with the cholecystokinin antagonist analogue D-Tyr-Gly-[($Nle^{28,31}$,D-Trp^{30})cholecystokinin-26-32]-phenethyl ester indicates that the ability to signal and the ability to induce receptor internalization clearly can be separated (8), i.e., there can be separate efficacies to induce these two behaviors. It also is known that while enkephalins and morphine both stimulate μ and δ opioid receptors, enkephalins, not morphine, induce receptor internalization (114). Thus, a possible new therapeutic efficacy for ligands to be explored is the induction of GPCR internalization as an activity independent of the production of physiological response.

GPCR OLIGOMERIZATION

Another potential behavior of GPCRs is the formation of homodimers, heterodimers and higher order oligomers. Evidence for homodimer formation has been reported for cannabinoid receptors (115), adenosine A1 receptors (116, 117), δ-opioid receptors (118, 119), β_2-adrenoceptors (120, 121), somatostatin receptors (122), calcium-sensing receptors (123–125), muscarinic receptors (126), dopamine receptors (116, 127–131), serotonin receptors (130, 132–134), and chemokine receptors (17, 135). Heterodimer formation between $GABA_BR1$ and $GABA_BR2$ has been described (136, 137). Similarly, studies have demonstrated heterodimerization between dopamine and somatostatin receptors (138), dopamine and serotonin receptors (130), adenosine and dopamine receptors (117), 5-HT_{1B} and 5-HT_{1B} receptors (130, 133), and κ and δ opioid receptors (139).

There are some data to link receptor oligomerization to physiological or pathophysiological events. For example, CCR5 chemokine receptor clustering and dimerization may be required for HIV-1 infection (140, 141). In general, it still is unclear to what extent behaviors such as homo- and heterodimerization and the formation of higher order oligomers play a role in the physiological function of GPCRs. In some cases, agonists have been shown to increase dimerization (117, 120–122, 130). However, this may not always be the case. For opioid δ and κ receptors, δ-selective agonists produce monomerization of dimers (118). For the CCR5 chemokine receptor, ligand-induced homodimerization is required for calcium mobilization, chemotaxis, and receptor internalization (17). In cells coexpressing δ and κ opioid receptors, dimerization produces a leftward shift in the dose-response curves for inhibition of adenylate cyclase when compared to expression of the single receptor types alone (139). From the standpoint of ligand efficacy, what could be important is the relative proclivity of molecules to induce oligomerization behaviors. For example, it is interesting to note that while the chemokine derivative AOP-RANTES does not produce chemotaxis, it does promote CCR5 dimerization (17).

GPCR dimerization can produce different profiles of cellular sensitivity to agonists and antagonists. For example, for a decade it was known that the profile of opioid agonists and antagonists for δ and κ opioid receptors was not standard in some natural systems. This led to the postulate that yet undiscovered subtypes of these receptors existed (142, 143). However, large-scale searches for the subtype cDNA failed to detect the novel subtypes. An alternative hypothesis, for which there now is considerable evidence, is that the δ and κ opioid receptor form heterodimers in some systems and that these heterodimers possess different pharmacological profiles for ligands (119, 139). In agreement with this idea is the fact that δ-knockout mice lose the complex apparent subtype profiles as well (143). There also is evidence to suggest that heterodimers, when compared to single GPCRs, have differential sensitivity toward ligands. For example, δ-opioid receptors are known to rapidly internalize in the presence of the agonist etorphine (145–147). However, in cells expressing δ and κ heterodimers, etorphine is unable to induce opioid receptor internalization (139).

In general, there are many data to suggest that GPCRs form oligomers and that these may have different pharmacological properties from the single receptors of which they are made. It also is possible that ligands influence the formation and dissolution of these oligomers, thereby constituting another form of efficacy. Heterodimers involved in such ligand activity constitutes conditional efficacy (see next section) in that the system must provide part of the required species for the ligand efficacy to be fully operational.

RECEPTOR GENOTYPE VS. PHENOTYPE: "CONDITIONAL" EFFICACY

Human recombinant GPCR systems have, in some ways, obviated the gap between therapeutic screening and testing in animal receptor systems and the human therapeutic endpoint. Also, with the creation of recombinant systems in surrogate host cells has come an understanding of the subtle difference between receptor genotype and phenotype. Thus, a given cDNA for a GPCR can be introduced into different cellular backgrounds and produce completely different profiles with respect to sensitivity to agonists and antagonists.

There are instances where the relationship between a defined receptor genotype and a tissue receptor phenotype has been observed but not yet elucidated. For example, the putative β_4-adrenoceptor subtype has a distinct pharmacological profile that differs from other β-adrenoceptor subtypes, namely, activation by the agonist CGP 12177, resistance to beta-blockade, and activation by the β_3-adrenoceptor phenethanolamine agonists (148). However, no gene for this receptor has been found. A survey of existing evidence suggests strongly that the putative β_4-adrenoceptor is a phenotypic state of the β_1-adrenoceptor (149). In keeping with this idea, the β_4-adrenoceptor phenotype is absent in β_1-adrenoceptor knockout mice (150).

Cellular background can affect ligand efficacy through direct physical interaction. GPCRs interact with auxiliary proteins and these interactions can completely change the receptor phenotype. For example, a heat-sensitive macromolecular entity, extracted with detergent from PC-12 cells, facilitates the coupling of $\alpha_{2A/D}$-adrenoceptors to G proteins (151). Similarly, a protein-coupling factor has been reported for adenosine receptors (152). Receptor activity modifying proteins (RAMPs) can change ligand activity of calcitonin, calcitonin gene related peptide (CGRP) and adrenomedullin receptors (153–157) when coexpressed in the same cell. Thus, RAMP3 changes the relative order of potency of human calcitonin and rat amylin on human calcitonin receptor type 2 (hCTR2) and, interestingly, the affinity of the antagonist peptide AC66 as measured with Schild analysis (157). When ligands form ensembles that can interact with these auxiliary proteins, they can be said to have conditional efficacy, i.e., an effect that is made manifest only in the systems containing the auxiliary proteins. In this sense, the formation of receptor heterodimers to form new pharmacological species (discussed in the previous

section) also is conditional since it will not take place if the heterogeneous receptor partner is not present. Conditional efficacy differs from conventional efficacy in that the intersection of the ligand-bound ensemble and the ensemble that favors interaction of the receptor with the auxiliary protein produces a new ensemble through physical association. Thus, the interaction of the ligand-bound (or unbound) receptor and the auxiliary protein forms a new protein species with its own set of pharmacological behaviors.

RELATIVE PREVALENCE OF "EFFICACY" IN CHEMICAL SPACE

As discussed earlier, every ligand with macroaffinity for a receptor should form a new receptor ensemble. This does not necessarily mean that every pharmacological detection system will make the new ensemble observable. Early indications of the hidden nature of this fact came with the discovery of inverse agonists. The first inverse agonists for GPCRs were described in a classic study by Costa & Herz (35) with δ opioid receptors transfected into NG108-15 cells. Thus, the peptide ligand ICI 174864 ([N,N′-diallyl-Tyr^{1},Aib^{2,3}]Leu^{5}-enkephalin) depressed constitutive receptor-related elevated basal cyclic AMP responses in a concentration-dependent manner. This effect was blocked competitively with a neutral antagonist, which indicates that it was not simply the result of residual agonist in the receptor compartment. This defines ICI 174864 as an inverse agonist possessing negative efficacy, i.e., a selective affinity for the inactive-state ensemble. However, the detection of the inverse agonist effects of ICI 174864 would not have been detected had the system not been constitutively active. Many subsequent studies have shown that competitive antagonists, previously thought to possess no efficacy because they were tested in nonconstitutively active quiescent receptor systems, do in fact reveal negative efficacy when tested in constitutively active systems. This underscores the principle that efficacy can be detected only in an appropriate system, and the absence of observed efficacy does not necessarily imply that it is not a property of a molecule. For example, the peptide ligand AC512 produces simple competitive antagonism of human calcitonin responses in HEK 293 cells stably transfected with human calcitonin receptor type 2 (158). In this study, the system was not constitutively active and response was measured as changes in cellular metabolism with microphysiometry. However, when AC512 was tested in constitutive active *Xenopus laevi* melanophores transiently transfected with hCTR2, a concentration-dependent active depression of constitutive activity was observed in real time. Thus, in the appropriate system, the negative efficacy of AC512 was revealed (159).

The absence of a direct effect by a ligand on a receptor may only mean that the method of detection of effect is inadequate. For example, while β-adrenoceptor antagonists appear to do nothing to receptors in many receptor preparations, covalent fluorescent probe experiments with the β_2-adrenoceptor indicate that these antagonists actually do produce active conformational changes in the receptor

protein (60). This returns to the importance of vantage point in experimental science and how it defines what one observes.

THE OPERATIONAL MEASUREMENT OF EFFICACY

In terms of the thermodynamics of receptor function and the behavior of receptor protein, efficacy is a complex and multifaceted molecular property. Moreover, the intimate relationship between affinity and efficacy suggests that it may not be possible to independently measure ligand efficacy in a way that predicts ligand behavior across all receptor systems. Given these constraints, it could be argued that the concept of efficacy is not useful in a practical sense. A converse view is that measuring some operational output dependent on efficacy can still be useful in practical terms and that it can be practiced without a full understanding of the protein dynamics involved.

In general, if the quantitity of response is compared to the receptor occupancy, then the relative power of one agonist to induce response over another can be estimated. For example, if it could be determined that agonist A produces 50% maximal system response through occupation of 5% of the available receptors, whereas agonist B requires 50% of the receptors, then it could be estimated that agonist A has ten times the ability to impart response that B has, i.e., the relative efficacy of A/B is 10. This is the basis for the method of Furchgott, a widely utilized method, for determination of relative efficacy (22, 160). Specifically, the responses to agonists are expressed as a function of their respective receptor occupancies (occupancy-response curves are constructed) and the ratio of the location parameters of the curves used as a measure of relative efficacy. However, a prerequisite to the correct use of this method is an accurate value for the affinity of the agonist for the receptor (to determine correct receptor occupancy). Since affinity and efficacy are related mechanistically, it is unclear to what extent unambiguous measurement of the affinity of efficacious ligands can be made. For example, consider the binding of an agonist A to a receptor R to produce an activated receptor complex AR^*:

$$A + R \overset{K_A}{=\!=\!=} AR \overset{\varsigma}{=\!=\!=} AR^* \qquad 3.$$

where K_A is the equilibrium dissociation constant of the ligand-receptor complex (1/true affinity), and ς is a term quantifying the ability of the ligand to induce receptor isomerization to the active state (conversion from R to R^*), in essence the magnitude of ligand efficacy. With such a scheme, the observed affinity of A for the receptor would be augmented by the ability of the ligand to isomerize the receptor to a different species (161). Thus, the affinity observed for ligand A is given as:

$$K_{obs} = \frac{K_A}{(1 + \varsigma)} \qquad 4.$$

It can be seen from Equation 4 that the presence of positive efficacy will increase the apparent affinity of the ligand for the receptor (the observed equilibrium dissociation constant of the agonist-receptor complex will be $<K_A$). Given this limitation, the comparison or response as a function of receptor occupancy does not yield an accurate measure of relative efficacy.

Complications of efficacy-dependent affinity can be obviated by the comparison of relative agonist maximal responses. These are obtained at saturating levels of receptor occupancy, and affinity ceases to be an issue. In terms of linkage theory (according to the ETC and CTC models of GPCRs—see Figure 1), the relative maximal responses to two agonists A and B are (162):

Extended Ternary Complex Model:

$$\text{Rel. Max.} = \frac{\gamma_A\alpha_A[1 + \alpha_B L(1 + \gamma_B\beta[G]/K_G)]}{\gamma_B\alpha_B[1 + \alpha_A L(1 + \gamma_A\beta[G]/K_G)]} \quad 5.$$

Cubic Ternary Complex Model:

$$\text{Rel. Max.} = \frac{\delta_A\gamma_A\alpha_A[1 + \alpha_B L + \gamma_B[G]/K_G(1 + \delta_B\alpha_B\beta L)]}{\delta_B\gamma_B\alpha_B[1 + \alpha_A L + \gamma_A[G]/K_G(1 + \delta_A\alpha_A\beta L)]} \quad 6.$$

It can be seen from Equations 6 and 7 that the relative maxima are controlled by efficacy terms α, γ, and δ and that affinity (K_A) plays no role in the control of maximal response. A caveat to the universal application of relative maximal response measurements as estimates of relative efficacy are instances where the system can modify these estimates through extreme values of L and/or [G] (see 162 for further details). However, in general, the relative maximal response of agonists can be used as an indicator of the relative maximal efficacy and can be a very useful operational tool.

A major practical problem in attempts to utilize the maximal response of agonists is the limited response window of receptor systems. Thus, if the stimulus exceeds a certain value in a given system, it saturates the stimulus-response capabilities of the system. When this occurs the system returns the system maximal response, not the stimulus maximal response. Under these circumstances, a group of high-efficacy agonists would demonstrate full agonism (the system maximal response), and no distinction between the agonists can be made. A corollary to this is that the efficacy of a group of full agonists should not be assumed to be equal; rather this simply shows that the stimulus-production capability of the agonists exceeds the particular response-producing capabilities of the stimulus-response machinery. The saturation of stimulus-response mechanisms (which leads to uniform imposition of the system maximal response on all high-efficacy agonists) becomes more evident the further down the stimulus-response chain the response is observed. For GPCRs, the earliest opportunity to measure stimulus is the rate of the GDP-GTP exchange reaction on the G protein. This results in separation of the G protein heterodimer and subsequent activation of effectors such as adenylate cyclase and ion channels. A cascade ensues involving production of second

messengers and activation of other response pathways. Each of these steps amplifies the previous one and may be saturable, thereby decreasing the possibility of detection of differences in the initial activation step (and thus differences in efficacy). For example, Figure 4 shows a stimulus-response cascade beginning with the activation of G protein (Figure 4*A*) continuing through a succession of stimulus-response reactions (Figure 4*B,C*). It can be seen from this simulation that agonists 1, 2, and 3 produce differing amounts of initial stimulus to the system as measured by GDP-GTP exchange (Panel 4A). However, as the result of this initial activation is processed through stimulus-response reactions [simulated for step 4B by a saturable hyperbolic function equaling stimulus/(stimulus +3)], it can be seen that the differentiation between the maximal stimulus-producing capability of agonists 2 and 3 is lost. Further down the stimulus-response chain, all three agonists appear to be of equal efficacy, when in fact they are not (Figure 4*C*).

The practical consequences of operationally estimating the power (efficacy) of an agonist to induce response is the prediction of response production across physiological systems in vivo. The potency of agonists depends on both efficacy and affinity. Agonists can be potent because of high affinity or high efficacy. Although the activity of these agonists may be equal in highly coupled receptor systems, the agonists will differ as receptor density and/or receptor-coupling efficiency decreases. In general, the response to high-efficacy agonists will be less sensitive to changes in receptor density and/or receptor-coupling efficiency than lower-efficacy agonists. For example, if agonist A requires 3% of the receptors in a given system to produce maximal system response, whereas agonist B requires 30%, then diminution of the receptor density to 20% of control will not decrease the maximal response to agonist A but will decrease it to agonist B. While the dose-response curve to agonist A will shift to the right, an increase in the dosage of this agonist would overcome the effects of receptor diminution and regain the maximal response. This will not be true for agonist B, to which no increase in the dosage will retain the maximal response.

In agonist therapy, the sensitivity, or lack of sensitivity, to changes in receptor density and/or coupling efficiency can be therapeutically relevant. For example, an efficacy-dominant agonist (high efficacy) might be a better choice for β-adrenoceptor agonist treatment of the symptoms of asthma since receptor desensitization could be overcome with an increase in dose for acute bronchodilation. On the other hand, a low-efficacy agonist might be a better choice for selective stimulation of a highly coupled pathway, i.e., a greater organ selectivity may be observed with a low-efficacy agonist. Knowledge of the relative dependence of a given ligand's observed potency on efficacy vs. affinity at secondary receptors also may be useful to predict side effects. The side-effect profile of a therapeutic agonist usually is determined in surrogate test systems. From this, a judgement of the side-effect potential for the agonist is made. The differential sensitivity of agonism on efficacy vs. affinity would suggest that a low efficacy at secondary receptors (those mediating side-effects) is preferred (163).

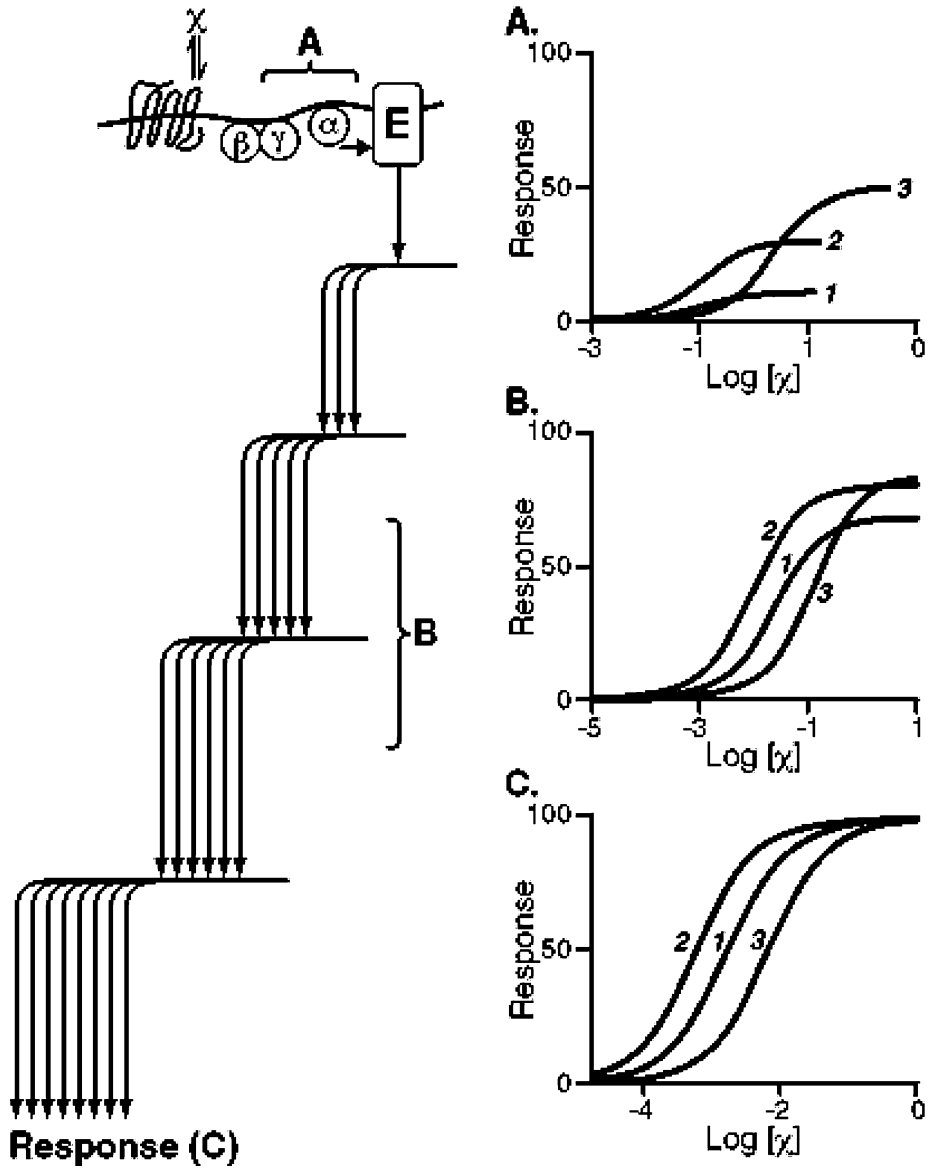

Figure 4 The saturation of steps in the stimulus-response mechanisms of receptor systems obscures the ability to observe differences in efficacy. (*Panel A*) The stimulus to three hypothetical agonists of relative efficacy Agonist 3 = 1, Agonist 2 = 0.6, and Agonist 1 = 0.2 (as measured by maximal stimulation of GDP-GTP exchange) is shown. (*Panel B*) The stimulus produced in *Panel A* is processed through a hyperbolic saturable stimulus-response function of output = input/(input + 3). (*Panel C*) The output of *Panel B* is further processed through another saturable stimulus-response function of output = input/(input + 0.03).

CONCLUSIONS

This paper has described the ability of ligands to change the behavior of GPCRs in terms of an abstract property, or more correctly, collection of properties known as efficacy. The given pharmacological profile of a ligand depends on its ability to stabilize a defined set of receptor conformations. Specifically, the number of those ligand-stabilized conformations that corresponds to pharmacologically active conformations determines the overall pharmacological profile of the ligand. The recognition of the numbers of receptor behaviors that possibly can be influenced by ligand binding should lead to an increase in the utility of ligands to induce therapeutically useful activity.

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