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MINIREVIEW

Efficacy as a Vector: the Relative Prevalence and Paucity of Inverse Agonism

Terry Kenakin

Systems Research, Department of Assay Development and Compound Profiling, GlaxoSmithKline Research and Development, Research Triangle Park, North Carolina

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ABSTRACT

This article describes the expected phenotypic behavior of all types of ligands in constitutively active receptor systems and, in particular, the molecular mechanisms of inverse agonism. The possible physiological relevance of inverse agonism also is discussed. Competitive antagonists with the molecular property of negative efficacy demonstrate inverse agonism in constitutively active receptor systems. This is a phenotypic behavior that can only be observed in the appropriate assay; a lack of observed inverse agonism is evidence that the ligand does not possess negative efficacy only if it can be shown that constitutive receptor activity is present. In the absence of constitutive activity, inverse agonists behave as simple competitive antagonists. A survey of 105 articles on the activity of 380 antagonists on 73 biological G-protein-coupled receptor targets indicates that, in this sample dataset, 322 are inverse agonists and 58 (15%) are neutral antagonists. The predominance of inverse agonism agrees with theoretical predictions which indicate that neutral antagonists are the minority species in pharmacological space.

Inverse agonism was first observed by Costa and Herz (1989) for the opioid antagonist ICI 174864 in a recombinant receptor system. Ligand-mediated depression of basal benzodiazepine receptor [(a non-G-protein-coupled receptor (GPCR)] activity had been reported 5 years before (Braestrup et al., 1982; Wood et al., 1984). Similarly, studies on muscarinic receptors (Burgisser et al., 1982) and dopamine receptors (De Lean et al., 1982) demonstrated guanine nucleotide effects opposite of those seen with agonists (indicative of inverse agonism). However, the study by Costa and Herz (1989) was the first instance in which receptor-mediated inverse agonism as a phenotypic behavior was observed for GPCRs. Specifically, it was seen that an elevated basal level of response in NG108-15 cells was reduced by ICI 174864 in a concentration-dependent manner and that this effect was competitively blocked by another opioid receptor antagonist (MR2266). Importantly, the potency of MR2266 in blocking this inhibition of basal activity by ICI 174864 was equal to its potency in blocking the positive effects of the opioid agonist [D-Ala^2,D-Leu^5]-enkephalin; i.e., the inverse agonism was blocked selectively by opioid antagonists. This pattern clearly showed that the phenomenon was not caused by trace amounts of opioid agonist in the system but rather that it was an effect specific to the receptor and ICI 174864. The reversal of constitutive activity by antagonists is an active event, and thus this phenomenon was referred to as *agonism*; the fact that it is the opposite of a normal agonist response gives it a vector quality, giving the qualifier of *inverse* agonism. Accordingly, because agonism is associated with efficacy, the molecular property responsible is termed *negative efficacy*. When first reported, this extraordinary finding was considered with skepticism only because it required unique experimental conditions, namely constitutively active receptor systems, to be observed. At the time, these were not widely accessible to the greater pharmacological community. With increased and widespread availability of these systems has come a greater appreciation of the ubiquity of inverse agonism.

It is important to note that inverse agonism is a phenotypic behavior, not a molecular property in itself. Although a molecular mechanism is responsible for the behavior (see *Ligand Phenotypic Behavior*), the experimental conditions must be appropriate for the effect to be seen; i.e., nonobservance of inverse agonism does not necessarily imply absence

ABBREVIATIONS: ICI 174864, ([*N,N′*-diallyl-Tyr^1,Aib^{2,3}]Leu^5-enkephalin; GPCR, G-protein-coupled receptor; ETC, extended ternary complex; VIP, vasoactive intestinal peptide; MR2266, (−)-5,9-diethyl-2-(3-furylmethyl)-2′-hydroxy-6,7-benzomorphan.

of negative efficacy. As mentioned above, this probably is the reason for differences of opinion regarding the paucity and prevalence of inverse agonism. The true frequency of inverse agonism could only be evaluated when pharmacologists were on a level playing field (i.e., when there was universal and common access to constitutively active receptor systems). It is important to separate the discussion of inverse agonism as a phenotypical behavior of antagonists and as a molecular property. As a starting point, it is useful to discuss the molecular mechanisms believed to give rise to inverse agonism.

Negative Efficacy in GPCR Models. The most simple model of GPCR function is the extended ternary complex (ETC) model (Samama et al., 1993) shown in Fig. 1. This model evolved from the ternary complex model for GPCRs first derived by De Lean and colleagues (1980) and was modified to accommodate the observation of constitutive GPCR activity. There are two molecular mechanisms that can lead to inverse agonism in terms of this model; these involve the magnitudes and vector properties of the efficacy parameters α and γ. It should be noted that these constants reflect modifications of microaffinity constants that cannot be measured independently. However, it is useful to describe the stepwise effects of receptor isomerization and binding to ligands and G-proteins in conceptual terms with the ETC model as a tool for discussing negative efficacy.

Effect of α. It can be seen from Fig. 1 that the receptor is able to spontaneously form an active state ($[R_a]$) which then can go on to activate G-proteins; the propensity to do this is governed by the allosteric constant L ($[R_a]/[R_i]$). It can also be seen that ligands (denoted [A]) can affect the equilibrium by having selective affinity for one of the receptor conformations (value of α such that the affinity of [A] for $[R_i]$ is K_a, whereas the affinity for $[R_a]$ is αK_a). It can be shown that the relative amounts of $[R_i]$ and $[R_a]$ are affected by the presence or absence of [A] (presuming [A] has affinity for the receptor) if the equilibrium between the two protein conformations is not constrained by external factors. Thus, the effect of a saturating concentration of [A] on the relative amounts of $[R_i]$ and $[R_a]$ is given as (Chen et al., 2000):

$$\frac{\rho_\infty}{\rho_0} = \frac{\alpha(1 + L)}{(1 + \alpha L)} \quad (1)$$

where $\rho\infty$ and ρ_0 refer the fraction of receptors in the $[R_a]$ state in the presence and absence of a saturating concentration of ligand [A]. It can be seen from eq. 1 that if $\alpha > 1$, the ligand will enrich state $[R_a]$ (increase the concentration of the active state at the expense of the inactive one and thus produce positive agonism). Conversely, if $\alpha < 1$, then the ligand will reduce the amount of any spontaneously produced $[R_a]$ and thus reduce any constitutive agonism produced by that species (i.e., it will be an inverse agonist). Thus, one simple molecular mechanism for inverse agonism is a selective affinity for the inactive state of the receptor $[R_i]$ ($\alpha < 1$).

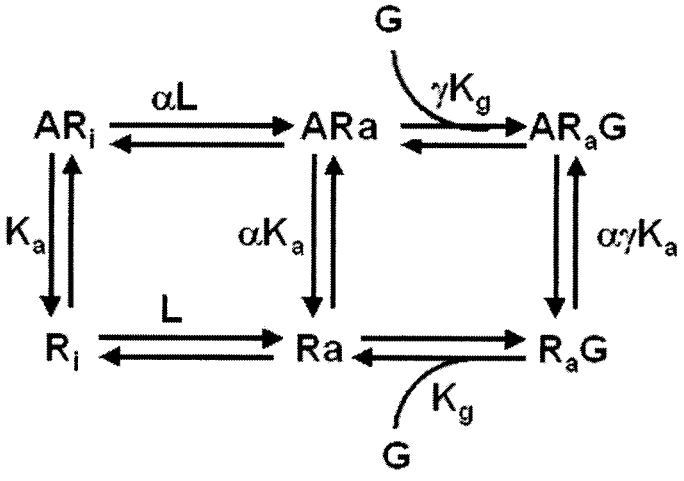


Fig. 1. ETC model of G-protein-coupled receptor function. Receptors are assumed to exist in active ([Ra]) and inactive ([Ri]) conformations in equilibrium driven by an allosteric constant L. The active state can spontaneously activate G-protein ([G]) to produce a constitutively active response producing species $[R_aG]$. Ligands have an affinity association constant (K_a) for $[R_i]$ and αK_a for $[R_a]$. G-proteins have an affinity association constant (K_g) for $[R_a]$ and γK_g for ligand-bound species $[AR_a]$.

Effect of γ. The influence of ligands on GPCR activation extends beyond the effects on the relative proportion of the receptor active state. It can be seen from Fig. 1 that through the parameter γ, the affinity of the ligand-bound receptor for G-proteins differs from that of the spontaneously formed receptor active state (i.e., the presence of the ligand makes a difference in the molecular properties of the receptor with respect to G-protein activation). The ligand dependence of γ leads to the formation of ligand-specific receptor active states and the ability of the ETC model to predict an infinite number of receptor states without modification to three or greater state models (Kenakin, 2003). As with the parameter α, if $\gamma > 1$, then the affinity of the ligand-bound receptor will be greater for G-proteins and a positive effect on G-protein activation will result. However, if $\gamma < 1$, then the ligand-bound receptor will have a lower affinity for the G-protein; this too can be a mechanism for inverse agonism under certain experimental circumstances. An interesting effect of protean agonism (positive agonism in quiescent nonconstitutively active systems and inverse agonism in constitutively active systems) can be produced by ligands with $\alpha > 1$ and $\gamma < 1$ (see next section).

Ligand Phenotypic Behavior. The ETC model predicts different behaviors for ligands depending on the values for α and γ and the interplay of these with the constitutive activity of the system (magnitude of L). The experimentally accessible parameter available to control system sensitivity to inverse agonism is receptor density. Thus, high levels of receptor expression allow tangible values of L to produce significant levels of spontaneously activated receptor to the point at which elevated basal responses can be observed. For example, a value of L = 0.001 in a system expressing 10,000 receptors will produce $[L/(L + 1)] \times 10{,}000 = 10$ receptors in the active state; this may be below the threshold for observation. However, if there are 1,000,000 receptors expressed, then 1000 would be in the active state and this may exceed the threshold for observation of elevated basal responses; i.e., constitutive activity will be observed.

It should be stressed that the molecular properties of the ligand (magnitudes of α and γ) are characteristic parameters defining the efficacy of the ligand for the receptor type. Thus, all ligands in pharmacological space can be thought of as having finite values for both affinity and efficacy (even if efficacy is 0, as would be the case if $\alpha = \gamma = 1$), and the relative magnitudes of these determines the properties of the

ligand in pharmacological systems (Fig. 2). How these molecular parameters interact with the system is what leads to observed behavior of the ligands in pharmacological assays.

The resulting behaviors of the systems under the influence of ligands varies in a manner dependent on the setpoint of the system (value of L); this defines the phenotypic behavior of the ligand in the system. Figure 3 shows the effects of six types of ligands of defined molecular types (set values of α and γ) on receptor systems of varying constitutive activity. Figure 3A shows a high positive efficacy agonist ($\alpha \gg 1$, $\gamma \gg 1$). It can be seen that positive agonism most likely will be observed in most if not all systems, and the full system maximum is produced in these cases. Figure 3B shows the effects of a ligand of lower-magnitude α and γ values. In this case, partial positive agonism probably will be observed in most systems (although full agonism can be seen in very efficiently coupled systems and silent competitive antagonism in very poorly coupled systems).

If a ligand does not differentiate receptor conformations with respect to affinity, the $[R_a]$, $[R_i]$, or $[R_aG]$ state of the receptor ($\alpha = \gamma = 1$), then it will produce no active redistribution of basal receptor states in the systems (Fig. 3C). This will be true irrespective of the setpoint of the system (i.e., both constitutively and nonconstitutively active systems), and these types of ligands will present a profile of neutral competitive antagonism in all receptor systems.

Negative efficacy is the opposite vector of positive efficacy (scales of α and $\gamma < 1$) with the same range of phenotypic behavior. Thus, there can be partial (Fig. 3D) and full (Fig. 3E) inverse agonists depending on the setpoint of the system. It is important to note that inverse agonists function as simple competitive antagonists in nonconstitutively active receptor systems. The question of full and partial inverse agonism is a great deal more complicated than the same for positive agonists. This is because of the variability in the scale of systems in which inverse agonism can be seen (see next section).

An interesting, and to this point not often observed, phenotype is protean agonism (Fig. 3F). This is where the ligand produces a receptor active state that is of lower efficacy than the natural spontaneously produced state [Ra] (i.e., when $\alpha > 1$ and $\gamma < 1$). These ligands produce positive agonism in quiescent nonconstitutively active systems and inverse agonism when the system is constitutively active. This phenotype was defined theoretically (Kenakin, 1995, 1997, 2001) and has been observed in a limited number of ligands at this time (Chidiac et al., 1996; Jansson et al., 1998; Fathy et al., 1999; Pauwels et al., 2002).

Conditional Activity of Inverse Agonism. In general, the maximal effects of ligands are defined operationally in systems in which the limits of the system can be determined. Thus, full agonism is observed when the stimulus to the system produced by the agonist saturates one or more of the biochemical cascades that convert stimulus to response. The natural agonist usually furnishes this scale, thereby giving a readily accessible standard with which experimental agonists can be compared. In addition, there often are physiological ways in which such system maxima can be defined (i.e., potassium contraction of vascular smooth muscle, forskolin stimulation of cyclic AMP, etc.). Unlike the situation with positive agonists, the availability of clearly classified full inverse agonists still is somewhat limited.

Constitutive activity is the difference between the basal response of a system containing active-state receptors and the same system without the receptors. However, to make this type of measurement requires the comparison of the system in different pharmacological states (i.e., transfected versus not transfected with receptors, or with low versus high levels of receptors). This can pose practical difficulties balancing system variance and true receptor-mediated differences. More often, the presence of constitutive activity is defined operationally as the difference between the basal response of the system in the absence and presence of an inverse agonist. Because constitutive activity defines inverse agonism, there is a clear risk of circular reasoning. This practical problem leads to the imperative notion that a lack of

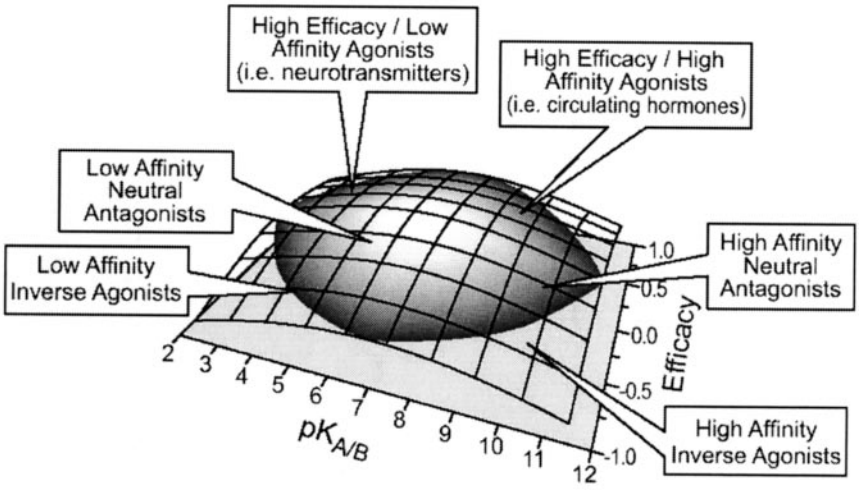


Fig. 2. Surface depicting pharmacological ligand space. All ligands have affinity (denoted as $pK_{A/B}$ where pK refers to $-$log value and K_A and K_B refer to equilibrium dissociation constants of agonists and antagonists, respectively), and efficacy (expressed as intrinsic activity). This latter scale ranges from -1 (full maximum inverse agonism) and 0 (no pharmacological effect observed) to 1.0 (full maximum positive agonism). The magnitude of a ligands affinity and efficacy (molecular properties of the ligand) determines the observed pharmacological effect of the ligand in any given system. The interaction of these molecular properties with the setpoints of the system determine the phenotypical response to the ligand in the system in terms of classifications such as full agonism, partial agonism, neutral antagonism, partial inverse agonism, and full inverse agonism. Vertical axis has no mechanistic significance.

observation of inverse agonism does not necessarily imply the absence of negative efficacy. In fact, it places the burden of proof on the definition of neutral antagonism; i.e., the lack of effect on basal response must be shown in a constitutively active system (for which inverse agonism can be shown for other ligands) before true neutral antagonism can be associated with a ligand.

The operational definition of inverse agonism poses another problem for ligand taxonomy. Specifically, the degree of inverse agonism (maximal effect) observed is totally dependent on the level of constitutive activity in the system (i.e., the difference between the constitutive and nonconstitutive basal response). This can be an important experimental variable leading to confusion in the definition of inverse agonists. For example, very low levels of constitutive activity will yield low values of negative intrinsic activity for powerful full inverse agonists; i.e., a 10% inhibition of a 10% elevated baseline response can be a full response for a full inverse agonist. This same ligand may produce a 100% reversal of a fully constitutive system as well; this apparently capricious behavior can lead to confusion in ligand taxonomy and to difficulty in unambiguously defining full and partial inverse agonism.

The Importance of Sensitivity of Measurement. Another potential problem in the study of inverse agonism and constitutive activity is the sensitivity of the measuring system. Specifically, there must be sufficient amounts of constitutive species (the [RaG] species consisting of an activated receptor spontaneously coupled to and activating a G-protein) (Fig. 1) to be detectable (visualization of constitutive activity) and also to be seen to be altered (inverse agonism). Although this can occur theoretically in binding studies, functional systems typically are much more sensitive indicators of these effects. Sensitivity in this case refers to the ordinate changes in drug effect (i.e., either response or amount of bound receptor) and not the concentration at which these effects take place. For example, in efficiently coupled functional systems, it may only require 1% of the existing receptors to be activated by high-efficacy agonists to produce a full physiological response. Thus, an effective expansion of scale is seen whereby a 1% change in levels of [RaG] produces the complete range of functional response of the system.

In tightly coupled functional assays, the effects of even a low concentration of $[R_aG]$ will result in measurable levels of constitutive activity. This amplification can be demonstrated with simple models. The most simple system for spontaneous constitutive activity for GPCRs is shown in Scheme 1.

Thus, a receptor forms an active-state R_a from an inactive-state R_i to couple to G-protein to produce the constitutively active species $[R_aG]$. It can be shown that the dependence of constitutive activity on receptor number is

$$\frac{[R_aG]}{[G_{tot}]} = \frac{[R_i]}{[R_i] + (K_G/L)} \quad (2)$$

This defines a hyperbolic relationship between receptor density and constitutive activity. The sensitivity of such a system to constitutive activity is given as the ratio of the equilibrium dissociation constant of the activated receptor/

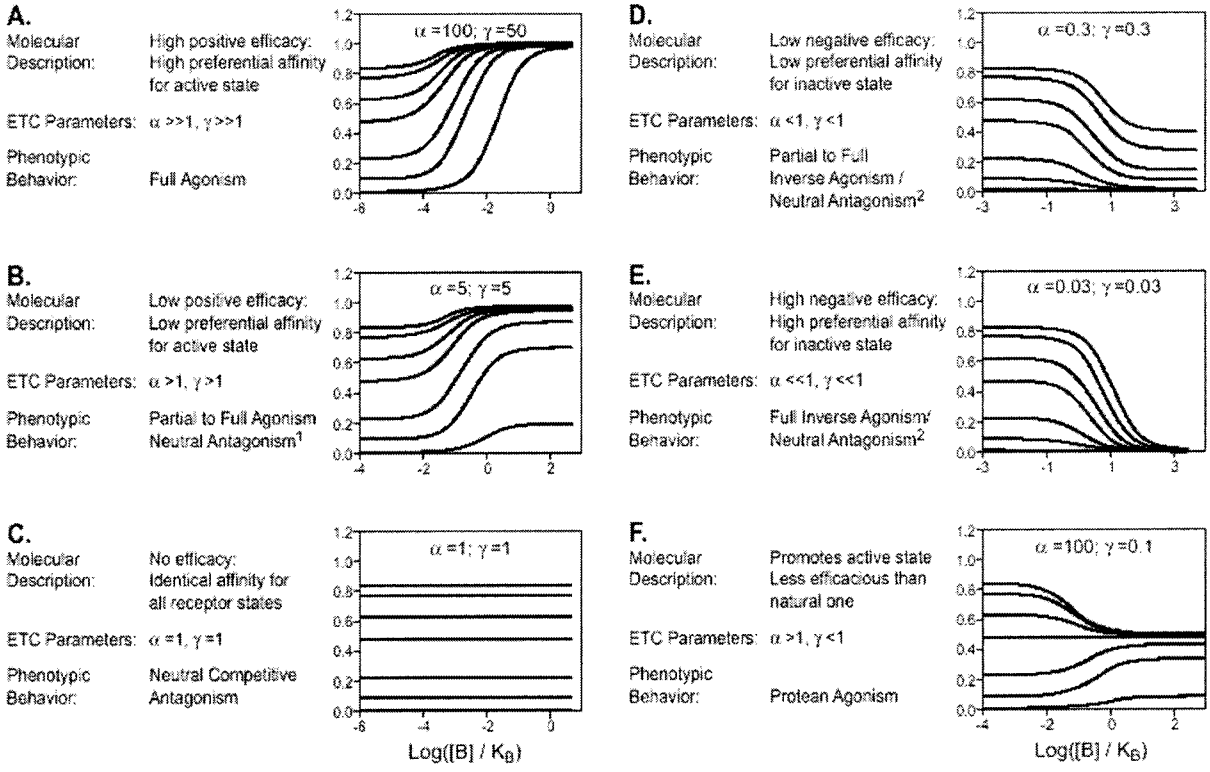


Fig. 3. Levels of response producing species ([RaG] and/or [ARaG]) calculated with the ETC model for ligands of defined molecular values of α and γ. Curves represent systems of varying magnitudes of L (varying levels of constitutive activity: values of L = 0.001, 0.01, 0.02, 0.1, 0.2, 0.5, and 1.0). Neutral antagonism is observed for low positive-efficacy ligands (B) in poorly coupled systems; the curve is coincident with abscissal axis and cannot be seen in the figure. See text for discussion of individual profiles. In poorly coupled receptor systems, ligands with low positive values for α and γ (partial agonists with low intrinsic efficacy) may function as neutral competitive antagonists.

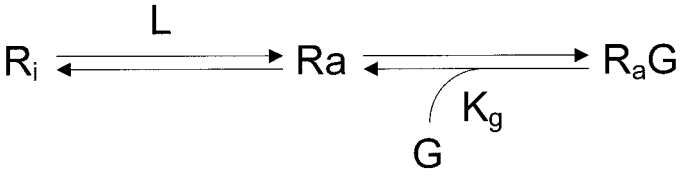
G-protein complex and the allosteric constant of the receptor (K_G/L). If this active receptor species is processed through another hyperbolic forcing function relating receptor stimulus to tissue response (to yield the 99% receptor reserve for function), the curve defining constitutive activity as a function of receptor density is considerably shifted to the left. Under these circumstances, the curve defining constitutive activity (C.A., as a fraction of the maximal response of the system denoted Max.) is given by

$$\frac{\text{C.A.}}{\text{Max.}} = \frac{[R_i]}{[R_i](1+\beta) + \beta(K_G/L)} \quad (3)$$

where β refers to the functional coupling constant for receptors. The sensitivity of the constitutive activity to receptor density then is given by $(\beta/(1+\beta)) \times K_G$/L. It can be seen that for nonzero values of β, the sensitivity of the constitutive activity of a functional system will *always* be greater than for a binding system. Also, if the functional coupling is efficient ($\beta \ll 1$), inverse agonism will be much more sensitive in the functional system than in a binding system ($(\beta/(1+\beta)) \rightarrow \beta$); i.e., the system is much more responsive to spontaneous activation of receptors. Figure 4A shows a synoptic receptor system of K_G/L = 0.1, in which the initial stimulus is processed through a stimulus-response forcing function to produce a 100-fold amplification of signal to functional response. It can be seen that the relationship between constitutive activity and receptor expression (abscissae) is 100-fold more sensitive when observed through functional tissue response than through detection of [RaG] through binding. Figure 4B shows the effects of a full inverse agonist on this system at a defined level of receptor expression ([R] = 0.005); it can be seen that the inverse agonism is nearly undetectable through binding but is quite evident with functional response.

Inverse Agonists: Rare Species or the Common Currency of Antagonism? When the inverse agonist activity of ICI 174864 was reported by Costa and Herz (1989), it was the first discovery of negative efficacy in a GPCR ligand. Whereas the excellent agreement of the blockade of the effect with MR2266 confirmed that it was a direct opioid-receptor interaction, there was no way, at the time, to gauge the wider prevalence of inverse agonism. The relative paucity of reports of inverse agonism was in keeping with the paucity of studies on the receptor system required to detect the effect.

In theory, it would be expected that inverse agonism or positive partial agonism (but not neutral antagonism) would predominate in the world of competitive antagonists. This is because the thermodynamic forces that control ligand affinity and efficacy are the same; therefore, these properties of biologically active ligands should be related (Onaran and Costa, 1997; Onaran et al., 2000). In fact, a probabilistic model of receptor activation has shown that, on a thermodynamic level, affinity and efficacy (the ability of the molecule to change the behavior of the receptor toward the host) are related (Kenakin and Onaran, 2002). In general, it would be predicted that all ligands with affinity for receptors should produce either positive agonism or inverse agonism; i.e., to not do so would require that the ligand recognize at least two ([Ri] and [Ra]) and probably a third ([RaG]) conformational species of the receptor as being identical. In addition, there is both theoretical and experimental evidence that when ligands bind to receptors, they bias and change the conformations of the receptor. The theoretical rationale for this idea comes from the notion that receptors fold and unfold tertiary conformation in different regions at different times, making it likely that, instead of a single [Ra] state for G-protein activation, there is an ensemble of microconformations that are all capable of producing the same pharmacological effect (in this case, G-protein activation) but having different overall tertiary conformations. In fact, it could be speculated that ensembles for the complete array of receptor behaviors (dimerization, oligomerization, internalization, and interaction with other membrane or cytosolic proteins) exist and that ligands take on pharmacological properties by creating conformations coincident with these pharmacological ensembles (Kenakin 2002a,b). In any case, the lack of observation of changes of receptor conformation cannot be taken as evidence that changes do not occur with ligand binding, only that the detection system is inadequate to notice.



Scheme 1.

Proteins are subject to constant local unfolding reactions occurring independently of each other; such interactions can be observed with NMR-detected hydrogen/deuterium exchange (Jeng et al., 1991; Bai et al., 1995; Milne et al., 1999). Recently, experimental evidence for these effects also has been provided by experiments with fluorescence lifetime spectroscopy. Specifically, a fluorescent probe covalently bound to cysteine 265 of the β_2-adrenoceptor indicates a range of molecular microenvironments for the fluorescent probe bound to the protein (Ghanouni et al., 2001). These changes in the protein environment of the probe indicate that the receptor adopts a Gaussian distribution of states reflecting the distribution of protein microconformations (Fig. 5). Interestingly, the binding of the neutral β-adrenoceptor antagonist alprenolol changes the distribution of these conformations (Fig. 5). This indicates that although no pharmacological effects can be seen with this ligand as it binds to the receptor, it does change receptor conformational states by binding.

Given these data, it would be probable that all antagonists would fall into the category of either partial agonist or inverse agonist. A survey of 380 antagonist-receptor pairings from 105 articles on 73 biological GPCR targets indicates that 322 are inverse agonists and 58 (15%) are neutral antagonists (Supplemental Table 1: http://molpharm.aspetjournals.org/cgi/content/full/65/1/DC4). Included data for this survey met the following criteria: 1) the experimental system had to be shown to be able to demonstrate inverse agonism for some ligand; 2) biological targets were included for diversity and not necessarily for physiological relevance; i.e., constitutively active mutants were included to address the question can a given GPCR recognition unit differentiate inverse agonism and neutral antagonism; and 3) partial agonists were excluded, only inverse agonists and neutral antagonists were compared. This survey includes antagonists prescribed clinically, and of those, all are inverse agonists. The data shown in Table 1 support the theoretical prediction that antagonists generally can discern receptor con-

formations; i.e., neutral antagonists are the minority category of ligand. This sample of data are mainly composed of studies with family A receptors (90%) and indicates no specific preference for G-proteins (35%/40%/25% $G_s/G_i/G_q$ proteins); it is not clear whether this spectrum reflects intrinsic properties of these proteins or a biased sample of receptors and G-protein systems amenable to experimental study.

Physiological Relevance of Inverse Agonism. Presently, the physiological importance of constitutive activity (and/or inverse agonism) is unclear. However, in highly efficiently coupled tissues with large receptor reserves for the endogenous agonist, this may be an important factor in the pharmacological control of tissue function. This also will depend on the relative proclivity with which different receptors spontaneously form active states (magnitude of L). There is evidence to suggest that the ability of different receptors to produce constitutive activity varies with receptor type (Chen et al., 2000). The physiological relevance of inverse agonism can be discussed on two levels. The first concerns the acute physiological effects of inverse agonists on organ function. Clearly, for this to be a factor, the tissue must be under a tonic constitutive receptor activity. There are suggestions that chronic elevated tone may be an important feature of some diseases. For example, in severe Jansen-type metaphyseal chondrodysplasia, ligand-independent constitutive activity of parathyroid receptors may be the cause of the observed severe hypercalcemia and hypophosphatemia (Schipani et al., 1995). Similarly, constitutive receptor activity may be relevant in other conditions such as retinitis pigmentosa, hyperthyroidism (Spiegel, 1996), or autoimmune diseases (de Ligt et al., 2000). Pathology can also introduce a constitutive species such as viral infection with Kaposi's sarcoma-associated herpes virus; this can lead to expression of a constitutively active chemokine receptor. The result is cell proliferation and continued viral replication (Arvanitakis et al., 1997; Rosenkilde and Schwartz, 2000). An intriguing link between receptor overexpression, cell transformation, and cancer growth may involve GPCR constitutive activity. Thus, long-term elevation of second messengers has been linked to cell transformation (Lyons et al., 1990; Weinstein et al., 1990; Spiegel et al., 1993), and it has been shown that receptors can be the source [i.e., 5-hydroxy-

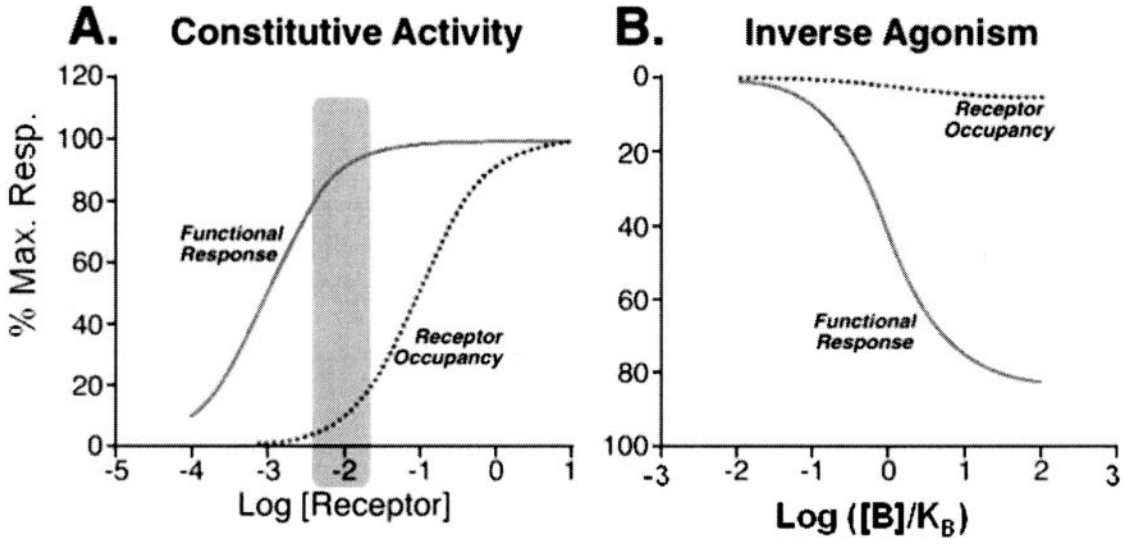


Fig. 4. Constitutive activity and inverse agonism. A, constitutive activity expressed as the fraction of receptors in the active state that are spontaneously coupled to G-protein ([RaG]) according to eq. 2 as the curve in the dotted line and that same concentration of active-state species processed through a stimulus-response function imposing a 99% receptor reserve for functional activity ($\beta = 0.01$). Constitutive activity expressed as a fraction of the maximal response in the system according to eq. 3. B, an example of the activity of a full inverse agonist in a system of [R] = 0.005 in A. It can be seen that the inverse agonism is much more obvious when functional (as opposed to receptor) levels are monitored.

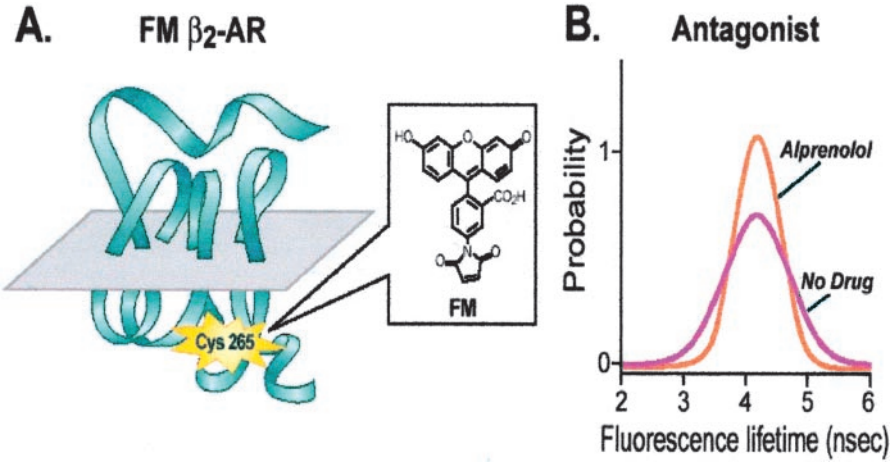


Fig. 5. Direct visualization of multiple receptor states with fluorescence lifetime spectroscopy. A, location of covalently linked fluorescent probe (Cys265) on β_2-adrenoceptors. B, Gaussian distributions of fluorescent lifetimes of a probe covalently linked to the β_2-adrenoceptor via Cys265. The variation in the lifetimes is indicative of changing protein environment (formation of microconformations). The distribution is changed by the addition of the receptor antagonist alprenolol, showing that the binding of the neutral antagonist produces changes in protein microconformations. Data are redrawn from Ghanouni et al. (2001) with permission.

tryptamine-2C receptors (Julius et al., 1988) and α_{1B}- and α_1-adrenoceptors (Allen et al., 1991)]. Some tumors greatly overexpress receptors such as those for VIP (Couvineau and Laburthe, 1985; el Battari et al., 1988; Svoboda et al., 1988; Lee et al., 1990; Sreedham et al., 1991; Virgolini et al., 1994) and bombesin (Cuttita et al., 1985; Maruno et al., 1989; Mahmoud et al., 1991; O'Dorisio et al., 1992; Moody et al., 1993; Kroog et al., 1995). In fact, imaging with ^{125}I-labeled octreotide radiolabeled ligand for VIP is a method of locating tumors (Krenning et al., 1989; Lamberts et al., 1990). The extreme levels of receptors expressed in some tumors (i.e., 100,000-fold the natural level in pancreatic epitheliod carcinoma) (Virgolini et al., 1994) theoretically should produce constitutive cellular response that could lead to tumor growth. Antagonists for VIP have been found to reduce tumor growth (Gozes et al., 1991; Moody et al., 1993; Virgolini et al., 1994), although it is not clear whether the effect is through inverse agonism or blockade of locally released VIP from the tumor. However, the very high levels of receptor expression in tumors would suggest that constitutive activity is operative in this pathology and that inverse agonists may be uniquely valuable in the retardation of tumor growth (Kenakin, 2001) .

A second setting for unique features of inverse agonists may not involve short-term effects on elevated basal cellular response but rather the molecular properties of inverse agonists. For example, the selective affinity of the inverse agonists cimetidine and metiamide for histamine H2 receptors has been linked to tolerance to H2 receptor blockade (Nwolko et al., 1990; Wilder-Smith et al., 1990; Deakin and Williams, 1992). This tolerance is believed to occur through the elevation of histamine receptor levels (Nwolko et al., 1991). In contrast, no tolerance and/or change in receptor levels were seen with long-term treatment with the neutral histamine H2 receptor antagonist burimamide (Smit et al., 1996). The inhibition of the spontaneous formation of receptor active states and the subsequent phosphorylation and internalization of the receptor by inverse agonists could be a mechanism by which inverse agonists interfere with the natural cycle of receptor synthesis, membrane expression, and internalization. This, coupled with normal receptor synthesis and expression, could lead to an increased surface density of receptor in the presence of chronic inverse agonism (Milligan and Bond, 1997). Receptor up-regulation has been associated with inverse agonism of β_2-adrenoceptors (MacEwan and Milligan, 1996) and α_2-adrenoceptors (Lee et al., 1997). In addition, up-regulation of G-proteins also has been noted with inverse agonism (Berg et al., 1999; Nagaraja et al., 1999). From these data, negative efficacy could be considered an undesirable property (leading to tolerance). However, the overall rate of receptor synthesis and degradation in the cell would be an important factor in determining whether inverse agonists would cause tolerance to antagonism through increases in receptor levels; it would be expected that this would be dependent on the receptor type, cell, and degree of negative efficacy.

In general, current evidence suggests that negative efficacy is a molecular property that will interact with various receptor systems to cause a difference in some cases and no difference in others. Similarly, in those instances in which negative efficacy is expressed, there may be conditions in which this is a useful property [i.e., reduce pathologically induced constitutive activity (Seifert and Wenzel-Seifert, 2002)] or an undesirable property (tolerance to antagonism). The particular impact of negative efficacy on therapeutics probably will be assessed in retrospect with epidemiological tools. For example, it has been postulated that the classification of "typical" and "atypical" antipsychotics may be related to the fact that these classes of ligand coincide with inverse agonism (atypical) and neural antagonism (typical) activity for 5-hydroxytryptamine-2C receptors (Herrick-Davis et al., 2000).

Conclusion

The widespread availability of constitutively active receptor systems has revealed the full spectrum of drug efficacy. It can be seen that ligands with affinity for receptors, through selective affinity for receptor microconformations, most likely produce bias in total receptor tertiary structure either to states that activate G-proteins (to produce full or partial agonism) or states that do not activate G-proteins (to produce inverse agonism). From a limited survey, it seems that a minority of ligands (one in seven) induces no bias (to produce neutral antagonism). It should be noted that these classifications relate only to G-protein activation and not to other GPCR behaviors; therefore, a neutral antagonist for physiological response may still produce positive or negative effects on other GPCR-mediated processes.

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Address correspondence to: Dr. Terry Kenakin, Department of Assay Development and Compound Profiling, GlaxoSmithKline Research and Development, 5 Moore Drive, Research Triangle Park, NC 27709. E-mail: terry.p.kenakin@gsk.com