BIOCHEMICAL PHARMACOLOGY 71 (2006) 1663–1670

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Commentary

Some implications of receptor theory for in vivo assessment of agonists, antagonists and inverse agonists

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ARTICLE INFO

Keywords:
Inverse agonist
Agonist
Antagonist
Receptor theory
In vivo
Constitutive activity

ABSTRACT

Drug effects can be classified into three major phenotypes: agonist, antagonist and inverse agonist. Agonist and inverse agonist effects are associated with receptor activation and inactivation, respectively, whereas antagonism implies that a drug produces no effect when administered alone but blocks the effects of agonists and inverse agonists. Attention has only recently begun to focus on the theoretical and clinical implications of inverse agonists, and studies of inverse agonism have also stimulated revisions in receptor theory. This commentary addresses two specific issues related to the application of receptor theory to studies of inverse agonists in vivo. First, principles of receptor theory suggest that increasing drug doses produce a graded pharmacological stimulus that is transduced by receptor-containing tissue into a biological response. However, assays vary in their ability to detect those responses, and any given assay provides only a narrow window on the full range of underlying drug effects. Consequently, in vivo assessment of inverse agonists will benefit from development of assays sensitive to graded inverse agonist effects. Second, detection of inverse agonist effects requires some preexisting level of receptor activity (or tone). This tone can result from at least two sources: (a) endogenous ligands for the receptor, or (b) constitutive receptor activity. Strategies for discriminating these two sources of tone will also contribute to the in vivo assessment of inverse agonist effects. Studies with intermediate efficacy ligands may be especially helpful in this regard, because their effects are differentially influenced by endogenous agonist tone versus constitutive receptor tone.

The terms ''agonist'', ''antagonist'', and most recently, ''inverse agonist'' have evolved to describe different profiles of drug effects. By convention, an agonist is considered to be a drug that activates receptors to produce a measurable effect in some assay. A full agonist produces a maximal effect under a given set of conditions, whereas a partial agonist produces a detectable but submaximal effect. Conversely, an inverse agonist produces an effect opposite to that of an agonist, and inverse agonists can also be ''full'' or ''partial''. An antagonist produces no effect on its own but blocks the effects of both agonists and inverse agonists. In the first study to demonstrate inverse agonist activity at a G-protein-coupled receptor (the delta opioid receptor), the delta opioid [D-Ala-^{2}-D-Leu-^{5}]enkephalin increased GTPase activity in membranes from a cell line that expresses delta receptors (i.e. an agonist effect), whereas ICI174864 reduced GTPase activity below basal levels (i.e. an inverse agonist effect), and the effects of both drugs were blocked by MR2266 (an antagonist effect) [1]. Inverse agonists have now been identified for numerous receptors, including many G-protein-coupled-receptors, and inverse agonists are also being actively explored for their therapeutic potential in certain disease states [2–6]. As interest in inverse

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0006-2952/$ – see front matter
doi:10.1016/j.bcp.2005.12.038

agonists grows, it will become increasingly important to develop strategies for their in vivo assessment in studies of whole-animal physiology and behavior. The purpose of this commentary is to consider issues related to the in vivo assessment of agonist, antagonist and inverse agonist effects from the perspective of receptor theory.

Drugs produce their effects by binding to receptor proteins and influencing the behavior of the tissue in which the receptor resides. These changes in behavior can be measured proximal to the receptor (e.g. changes in activation of G-proteins directly associated with the receptor) or at levels more remote from the receptor (e.g. changes in the behavior of a whole organism). Receptor theory comprises a collection of evolving models designed to describe and quantify this process. One early articulation of receptor theory, known as occupation theory (for review, see [7,8]), has been especially influential in the design, conduct and interpretation of pharmacological studies in vivo [9], and as a result, this commentary begins with a consideration of the content and predictions of occupation theory.

Occupation theory relates drug concentration to tissue response according to the equation [7,8]:

$$\text{response} = f\left\{\frac{[A]}{[A]+K_A}\varepsilon[Rt]\right\} \quad (1)$$

where [A] is the concentration (or dose) of the drug, K_A the dissociation constant of the drug for the target receptor, [A]/([A] + K_A) the fraction of receptors occupied by [A], ε the intrinsic efficacy of the drug—a measure of the ability of a drug to activate a receptor and its associated transduction mechanisms, [Rt] the receptor density (number of receptors per unit of tissue) and f is a function that relates the initial receptor response to downstream tissue responses.

In this equation, the independent variable is [A], the dependent variable is the response being measured, and the relationship between [A] and response is governed by two drug-specific variables (K_A and ε) and two tissue-specific variables (Rt and f). The response and the variables [A], K_A and [Rt] can be objectively defined and quantified. In contrast, intrinsic efficacy ε (hereafter referred to simply as efficacy) is conceptualized as a *relative* term that cannot be measured directly. Rather, efficacy is defined as the *relative* ability of a given drug, in comparison to other ligands, to modulate receptor-associated transduction processes. For the purposes of the equation, efficacy can be considered to vary along a continuum from 0 to 1, with "0" indicating a drug that binds to a receptor but does not activate associated transduction mechanisms, and "1" indicating a drug that maximally activates those mechanisms. [*Note*: The variable f denotes an amplification function that varies depending on the endpoint under investigation [8]. For the purposes of this commentary, an important feature of this function is that it may be saturable, reflecting an upper limit in the ability of the tissue to produce a response—see below.]

This equation from occupation theory can be used to generate theoretical curves that illustrate the impact of efficacy on dose–response curves [7,8]. For example, Fig. 1 shows theoretical dose–response functions for a series of drugs with affinity for a given target receptor but with different efficacies at that receptor. Under these conditions, any drug with $\varepsilon > 0$ will produce an agonist effect, and the maximal effect varies as a function of ε. However, these idealized drug effects are modulated by at least two factors in the production and measurement of in vivo drug effects.

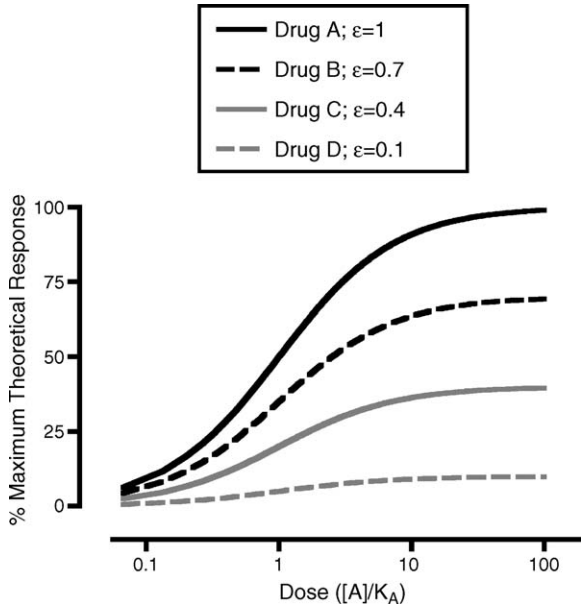


Fig. 1 – Theoretical effects of drugs with varying efficacies (ε_A = 0.1–1.0). Abscissae: drug dose or concentration expressed as [A]/K_A. (Note than when [A] = K_A and [A]/K_A = 1, then 1/2 of available receptors are occupied). Ordinate: percent maximum theoretical response assuming no constraints on response production or response detection. All lines were drawn from Eq. (1), with tissue parameters of Rt = 100 and $f(x) = x$.

1. Constraints on response detection as a determinant of drug effects

First, this model supposes that the tissue is able to generate graded effects across all drug doses from the lowest active dose sufficient to bind a single receptor to a maximal dose that binds all receptors. Moreover, this model supposes the existence of an experimental procedure that can detect and differentiate effects across this entire range. In practice, neither premise holds [7]. Some threshold level of tissue response is required before a signal can be detected by the experimental procedure used to measure the response, and this threshold can be considered the floor of the assay system. Differentiable effects may occur beneath this floor, but the assay is not sensitive to them. Similarly, there is typically a ceiling to the maximal response that a tissue can generate (related to the function "f" above) and that an experimental procedure can measure. This is especially true in in vivo studies, where the emergence of toxic or competing effects may limit the range of doses that can be tested and the range of effects that can be safely and reliably measured. Again, differentiable effects may be possible above the ceiling, but the assay system is not sensitive to these differences. Ultimately, the actual response that is measured in a given experiment

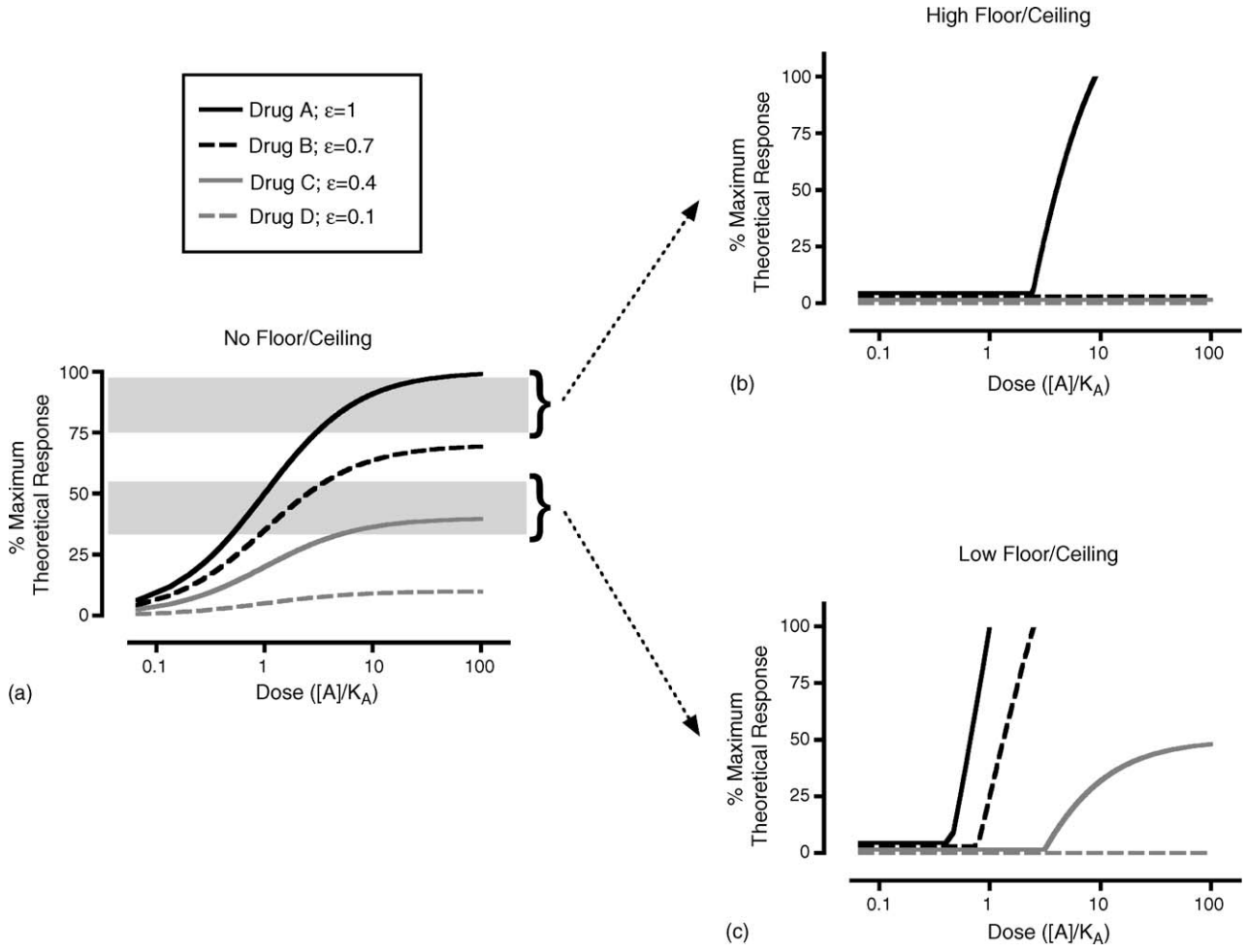


Fig. 2 – Effects of drugs with varying efficacies (ε_A = 0.1–1.0) when constraints on response production or detection are imposed by specific assay conditions. Abscissae: drug dose or concentration expressed as [A]/K_A. Ordinate (a): percent maximum theoretical response. Ordinates (b and c): percent maximum actual response in specific assays with constraints on sensitivity and maximum effect that define a floor and ceiling for possible responses. The areas shown in the gray boxes on the left panel are magnified in the right panels. All other details as in Fig. 1.

will be constrained by the floor and ceiling. Moreover, different assays may have different floors and ceilings and provide different windows onto the underlying drug actions. The potential impact of these factors on empirically determined dose–response curves is illustrated in Fig. 2. An assay with a high floor and ceiling will retain sensitivity to relatively high efficacy drugs, but such an assay may be less sensitive or insensitive to lower efficacy drugs. In the example in Fig. 2 (part b), the high floor/ceiling assay would retain sensitivity to drug A, but be insensitive to drugs B–D, and these drugs would function as antagonists of drug A. In an assay with a lower floor and ceiling, drug A will still produce a maximal effect, but its dose–effect curve is shifted to the left. In addition, lower efficacy drugs may also produce maximal or submaximal effects. In the example of the low floor/ceiling assay in Fig. 2 (part c), drug B now also functions as a full agonist, whereas drug C is a partial agonist, and drug D remains ineffective and would continue to function as an antagonist. Thus, the phenotype of a drug as agonist, partial agonist or antagonist may vary as a function of the constraints of the assay system in which drug effects are examined.

The impact of these factors on behavioral drug effects is nicely illustrated in procedures used to assess the potential analgesic effects of ligands acting at mu opioid receptors [10,11]. In a typical procedure, animals are exposed to a noxious and putatively painful stimulus (e.g. a heat stimulus), and the latency to a withdrawal response is measured as the behavioral endpoint. Such withdrawal responses are considered as evidence of ''nociception'' (i.e. the ability to detect the presence of a noxious stimulus), and nociception in animals is thought to be related to pain in humans. Drugs can then evaluated for their ability to attenuate the withdrawal response. This type of effect is called ''antinociception'' (because it opposes the nociceptive response), and antinociception is thought to be related to analgesia in humans. An important determinant of mu opioid effects in these procedures is the intensity of the noxious stimulus used to elicit the withdrawal response. When low intensity noxious stimuli are used, the assay functions as a low floor/ceiling assay, and low efficacy mu opioids may produce partial or full agonist effects. Conversely, when high intensity noxious stimuli are used, the assay functions as a high floor/ceiling assay, and low efficacy

mu opioids may fail to produce detectable effects. Moreover, under these conditions, low efficacy mu opioids function as competitive antagonists. Systematic use of a range of stimulus intensities permits accurate classification of mu opioids across a wide range of efficacies.

2. Preexisting receptor activity (tone) as a determinant of drug effects

A second simplification of the original model of occupation theory lies in its assumption that target receptors in a tissue are quiescent prior to delivery of the drug. Again, though, this premise does not appear to hold under many circumstances. Rather, drug effects on receptor activity are often integrated with some preexisting level of receptor activity, which can be referred to as the ''tone'' of the receptor system. There are at least two sources of tone, and of interest for the present discussion, the effects of drugs may vary as a function of that source.

2.1. *Tone due to endogenous ligands*

One source of tone is receptor activation by other receptor ligands. In particular, the principle function of receptors is to mediate responses to endogenous hormones and neurotransmitters, and drugs produce their effects in vivo in part by competing with these endogenous ligands. An example in the study of opioid antinociception would be that some stressors are thought to promote the release of endogenous, high efficacy opioids [12,13], and effects of exogenously administered drugs in stressed animals will be superimposed on a baseline of endogenous opioid effects. A competitive interaction between a drug and an endogenous ligand can be modeled using occupation theory [7,8], according to the equation:

$$\text{response} = f\left\{\left(\frac{[A]\varepsilon_A}{[A]+K_A(1+E/K_E)}+\frac{[E]\varepsilon_E}{[E]+K_E(1+A/K_A)}\right)[Rt]\right\} \quad (2)$$

In this equation, [E] is the concentration of an endogenous ligand, K_E the dissociation constant of that ligand for the receptor, and ε_E is the efficacy of the ligand at the receptor. Tone in this case is defined by the contribution of the endogenous ligand to total response when no drug is present (i.e. [A] = 0: tone = $[E]\varepsilon_E/([E]+K_E)$). The impact of tone resulting from a high efficacy endongenous ligand ($\varepsilon_E = 1$) on effects for drugs of varying efficacies is shown in Fig. 3 (assuming a constant concentration of endogenous ligand [E] and variable concentrations of the drug [A]). In each case, the thin dotted line shows the basal response in the system due to endogenous agonist tone. When endogenous ligand is absent and [E] = 0 (low tone, Fig. 3a), Eq. (2) reduces to Eq. (1), and the effects of drugs are identical to those described above in Figs. 1 and 2. Under conditions of medium tone (e.g. $[E]/K_E = 0.5$, Fig. 3b), the basal response is elevated. Relatively high efficacy drugs (e.g. drugs A and B) may produce further receptor

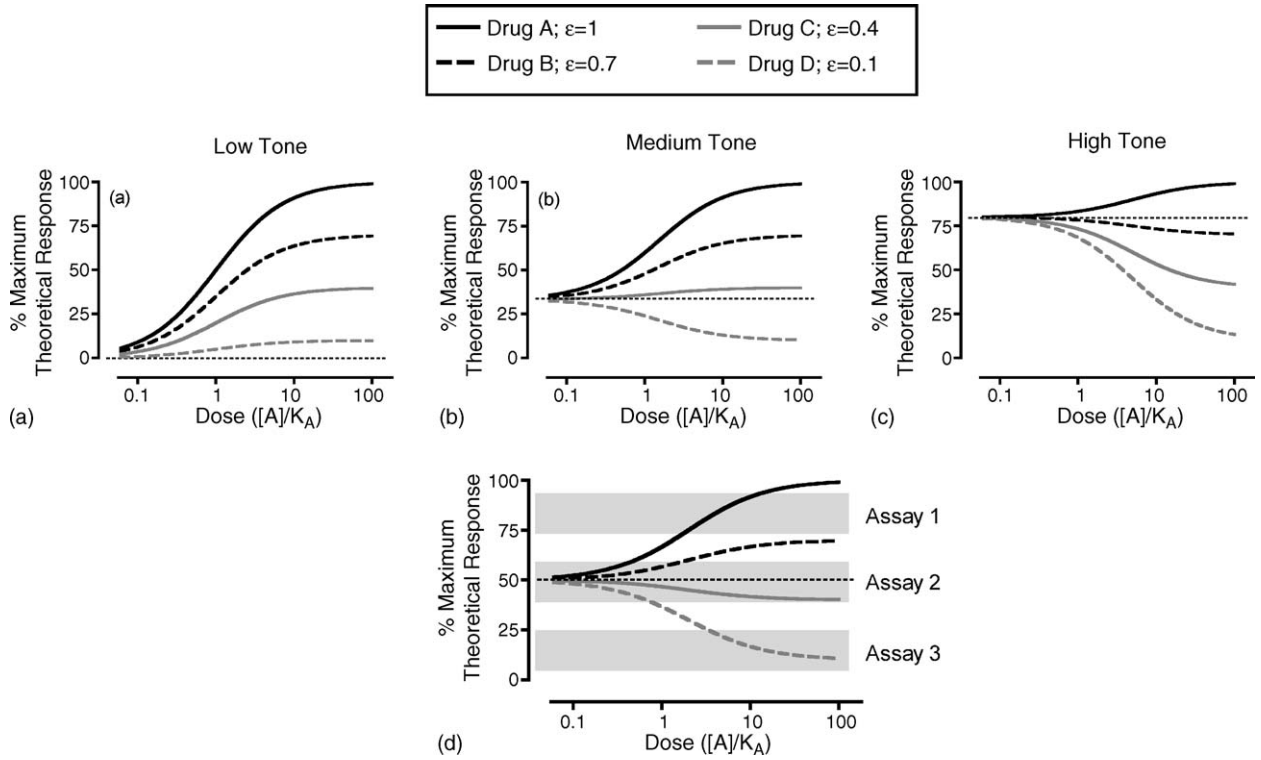


Fig. 3 – Theoretical effects of drugs with varying efficacies (ε_A = 0.1–1.0) in tissues with (a) low, (b) medium, or (c) high endogenous agonist tone. Abscissae: drug dose or concentration expressed as $[A]/K_A$. Ordinates: percent maximum theoretical response. All lines were drawn from Eq. (2), with endogenous agonist parameters set at $[E]/K_E$ = 0, 0.5 or 4 and ε_E = 1.0 and tissue parameters set at Rt = 100 and $f(x) = x$. (d) As above with $[E]/K_E$ = 1 and gray boxes to show possible constraints on underlying drug effects imposed by three different assays.

activation, which could be manifested as measurable agonist effects. However, low efficacy drugs (e.g. drug D) may decrease receptor activation below the basal response and produce effects opposite to those of an agonist (i.e. inverse agonist effects). Finally, drugs with an intermediate efficacy such that $\varepsilon_A \cong$ tone (e.g. drug C) may produce a response indistinguishable from the basal response. This latter type of drug would function as an antagonist of drugs producing both agonist and inverse agonist effects. Under conditions of high tone (e.g. $[E]/K_E = 4$, Fig. 3c), the basal response is further elevated. Now, only high efficacy drugs (e.g. drug A) may retain the ability to produce an agonist effect, whereas drugs of intermediate or low efficacy may all decrease receptor activation below the basal response and produce inverse agonist effects, with the greatest inverse agonist effects being produced by the lowest efficacy drugs.

As a final caveat, it should be noted that the phenotype of drugs under these conditions of varying endogenous agonist tone will depend on the floor and ceiling constraints of the assay used to measure the response. For example, Fig. 3d shows how three different assays might provide three different windows onto underlying drug effects in a tissue with intermediate tone ($[E]/K_E = 1$). In assay 1, drug A would function as a full agonist (because it produces a maximal theoretical response that exceeds the ceiling of the assay), but all the other drugs would be inactive when given alone, and would function as antagonists of drug A (because they fail to produce a sufficient response to exceed the threshold, or floor, of the assay). In assay 2, drugs A and B would function as full agonists, whereas drugs C and D would function as full inverse agonists. Finally, in assay 3, drug D would function as a full inverse agonist, and the other drugs would be ineffective and would function as antagonists of drug D.

There are several implications of this modeling exercise. The first is that the net effect of a drug on receptor activation is dependent on the endogenous agonist tone, and as a result, the phenotype of a drug as agonist, antagonist or inverse agonist is dependent on endogenous agonist tone. In particular, an agonist effect requires $\varepsilon_A >$ tone, and an inverse agonist effect requires $\varepsilon_A <$ tone. A drug for which $\varepsilon_A \cong$ tone would always function as an antagonist, but antagonist effects might also be observed for other drugs incapable of producing the threshold effect for a given assay (e.g. drugs B–D in assay 1 of Fig. 3d; drugs A–C in assay 3 of Fig. 3d). A second and related point is that the phenotype of any given drug is also dependent on the constraints of the assay used to measure the response. For example, drug C in the figures above ($\varepsilon_A = 0.4$) could function as a full agonist in a tissue with low tone and monitored using a sensitive assay with a low floor and ceiling; as an antagonist under a whole host of conditions; and as a full inverse agonist in a tissue with high tone and an assay sensitive to inverse agonist effects. However, despite this dependence of drug effects on tone and assay parameters, the rank order of maximal agonist effects is preserved across conditions such that $A \geq B \geq C \geq D$ (with an opposite rank order for inverse agonist effects $D \geq C \geq B \geq A$). This principle can be exploited to characterize the relative efficacies of unknown drugs across a set of known tissue/assay systems, or to characterize the properties of unknown tissue/assay systems using a set of known drugs. A violation of this rank order (e.g. an assay in which the rank order of maximal effects was $C > D > A \geq B$) would suggest that the effect is being mediated by a novel receptor–effector system.

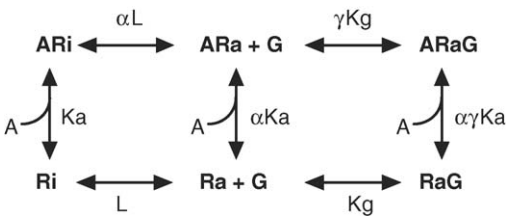
2.2. Tone due to constitutive receptor activity

A second potential source of tone is constitutive receptor activity, which is defined as receptor activity in the absence of ligand at the drug binding site. In practice, constitutive receptor activity would include activity resulting from any process other than the binding of ligands to the drug binding site, such as activity resulting from mutations in amino acid sequence, receptor phosphorylation, or allosteric changes in receptor conformation consequent to interactions with other proteins. Concepts of constitutive receptor activity arose from empirical findings that could not be accommodated by simple occupation theory, and this has stimulated the development of newer models [14]. Before considering the impact of tone due to constitutive activity on drug effects, the basic tenets of one of these models will be briefly reviewed.

In occupation theory, a drug A binds to a receptor R to produce an effect. In the case of G-protein-coupled receptors, the initial effect involves the binding of the drug–receptor complex to a G-protein. This set of chemical reactions can be written:

$$A + R \leftrightarrow AR + G \leftrightarrow ARG$$

where A, R and G denote the ligand, inactive receptor and G-protein, respectively, AR denotes the ligand-bound and activated receptor, and ARG denotes the bound and activated G-protein. This has been referred to as a ''ternary complex'' (because three components A, R and G are involved), and at a molecular level, it assumes that binding of A to R induces a conformational change in the structure of R that in turn permits association to and activation of G. Notably, this conceptualization of drug/receptor interactions does not permit the receptor to bind to the G-protein in the absence of a drug or endogenous ligand. However, it is theoretically reasonable to suppose that receptor structures are sufficiently flexible and dynamic to permit assumption of active states in the absence of agonist, and a growing body of research conducted primarily using neurochemical measures in transfected cell lines has provided compelling evidence to suggest that this can occur. These studies have led to the ''extended ternary complex'' model [2–4,14], which can be diagrammed as follows:



where R_i and R_a denote inactive and active forms of the receptor, respectively. Interconversion between each of these states is governed by equilibrium constants. Thus, L is the allosteric constant for interconversion between R_i and R_a, and K_g is the dissociation constant for the equilibrium between

$R_a + G$ and R_aG. It is these constants that govern the constitutive ability of a receptor to isomerize to an active state and bind to and activate a G-protein (and produce a response) in the absence of ligand. The effects of a ligand are superimposed on this preexisting level of constitutive activity. Thus, ligand A binds to R_i, R_a and R_aG with dissociation constants K_a, αK_a and $\alpha\gamma K_a$, respectively. αL governs the equilibrium between AR_i and AR_a, and γK_g specifies the relative affinity of the G-protein for AR_a versus R_a. In this model, the efficacy of a ligand is represented by the constants α and γ, which govern (a) the relative selectivity of the ligand for the active state of the receptor (higher α implies greater affinity for R_a versus R_i), and (b) the relative affinity of the G-protein for AR_a (higher γ implies greater affinity for AR_a versus R_a). If α and γ are both greater than 1, then the drug will increase receptor activation relative to baseline levels of constitutive activity and may function as a full or partial agonist depending on the constraints of the tissue and assay system. If α and γ are both less than 1, then the drug will decrease receptor activation relative to baseline constitutive activity, and may function as an inverse agonist. Lastly, if α and γ are both equal to 1, then the presence of the drug has no effect on the relative distribution of R_i and R_a or on the promotion of G-protein binding and activation, and as a result, such a drug would have no effect alone. [An additional possibility is that either α or γ is greater than 1 and the other constant is less than 1. Such dissociation constants may yield a ''protean agonist'' phenotype [2], but this type of compound will not be considered further here.] Overall, the ability of a drug to drive these equilibria to formation of AR_aG and R_aG and to produce a response is given by the following equation [4]:

$$\text{response} = \frac{L[G]/K_g(1+\alpha\gamma[A]/K_a)}{[A]/K_a(1+\alpha L(1+\gamma[G]/K_g))+L(1+[G]/K_g)+1} \quad (3)$$

where response is defined as the proportion of total receptors in the AR_aG or R_aG states. This model (and more sophisticated derivatives) have been described in detail elsewhere [2,3], but for the purposes of this commentary, two features of the extended ternary complex model are especially relevant. First, as noted above, it allows for active receptor states that can bind to and activate G-proteins in the absence of drug or endogenous ligand. As a result, this model permits variable levels of tone independent of the levels of endogenous ligands. Second, the valence of a drug effect on receptor activation is independent of the level of tone (for drugs with α and $\gamma > 1$, α and $\gamma = 1$ or α and $\gamma < 1$). This critical concept is illustrated in Fig. 4. This figure shows the effects of four drugs with variable levels of efficacy in tissues with low, medium or high levels of constitutive receptor activity (low, medium and high tone). Drugs W and X have high efficacy ($\alpha = 10$, $\gamma = 10$) and intermediate efficacy ($\alpha = 3$, $\gamma = 3$), respectively. These drugs would increase receptor activation relative to baseline across all conditions, and in appropriately sensitive assays, these drugs may produce agonist effects. Drug Y has no efficacy ($\alpha = 1$, $\gamma = 1$), and it produces no change in response relative to baseline, regardless of the level of constitutive activity. However, drug Y will antagonize the effects of both higher or lower efficacy drugs at all levels of constitutive activity. Drugs that produce this phenotype have been called ''neutral antagonists'', to indicate that antagonist effects are present at all levels of (and hence ''neutral'' to) constitutive activity. Neutral antagonism can also be differentiated from ''conditional antagonism'' (i.e. antagonism present only under particular conditions of assay sensitivity or endogenous agonist tone, see above). Finally, drug Z has low efficacy relative to baseline ($\alpha = 0.1$, $\gamma = 0.1$), and it will decrease receptor activation at all levels of constitutive activity. Under appropriate assay conditions, drug D may produce inverse agonist effects.

Two additional points are also notable in Fig. 4. First, although the valence of drug effects does not change across levels of constitutive activity, the magnitude (and hence the detectability) of drug effects does vary. For example, at low levels of constitutive activity, a high efficacy ligand may produce a large and easily detected change in effect from baseline. However, if constitutive activity is very high, then further increases in receptor activation may be small relative to baseline and may be harder to detect. The reciprocal holds for drugs with low efficacy that decrease receptor activation. Such reductions in receptor activation are likely to be small and difficult to detect under low levels of constitutive activity

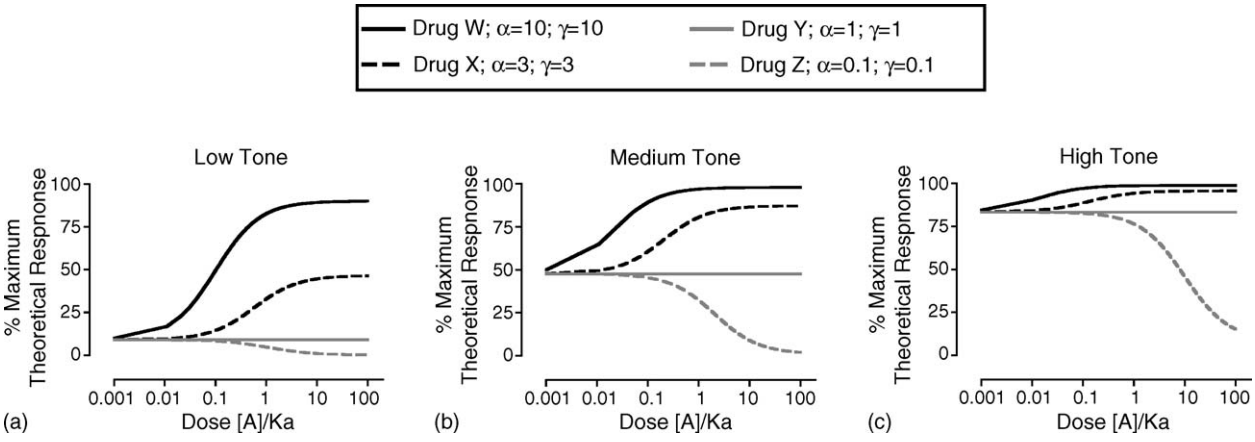


Fig. 4 – Theoretical effects of drugs with varying efficacies (α = 0.1–10, γ = 0.1–10) under conditions of low (left panel), medium (center panel) or high (right panel) constitutive receptor activity. Abscissae: drug dose or concentration expressed as $[A]/K_A$. Ordinates: percent maximum theoretical response. All lines were drawn from Eq. (3) with parameters set at $G = 10$, $K_g = 1$ and $L = 0.01$ (a), $L = 0.1$ (b) or $L = 1$ (c).

and much easier to detect at higher levels. Indeed, the discovery of inverse agonist activity at G-protein coupled receptors relied on the development of sensitive assays in systems with high levels of constitutive activity due to high levels of receptor expression [1]. A second key point relates to effects produced by intermediate efficacy drugs such as drug X in Fig. 4. When tone increases due to an increase in constitutive receptor activity, an intermediate efficacy ligand becomes more potent and produces maximal effects more closely approaching those produced by a high efficacy ligand. Moreover, the valence of drug X never changes. Exactly the opposite occurs when tone results from receptor activation by a high efficacy endogenous ligand (see Fig. 3). Specifically, as endogenous agonist tone increases, intermediate efficacy ligands become less potent and less effective relative to high efficacy ligands, and the phenotype of an intermediate efficacy ligand may change from agonist to inverse agonist. This dependence of intermediate efficacy ligand effects on the source of tone suggests that these drugs may be extremely useful in vivo for discriminating the source of tone in a receptor system.

3. Implications

The ability of drugs to produce agonist, antagonist or inverse agonist effects in vivo depends not only on features inherent to the drug, but also on characteristics of the assay system and on the magnitude and source of preexisting levels of receptor activity, or tone. The dependence of drug phenotypes on tissue and assay conditions indicates that, at the very least, care should be taken to cultivate an awareness of the constraints that any particular assay imposes on the detection of underlying drug effects. The resourceful pharmacologist will go further and exploit this situation to develop a range of assays that provide a systematic series of windows on underlying drug effects. One example of this approach has been the use of multiple stimulus intensities in studies of antinociception to create assays with an orderly variation in efficacy requirements for assessment of opioid effects [9–11]. As interest in inverse agonists grows, it will become increasingly important to develop assays that permit graded detection of inverse agonist effects. For example, exposure to high efficacy ligands appears to be one manipulation that promotes constitutive activity of mu opioid receptors [15], and recent studies have manipulated the degree of opioid exposure in an effort to produce graded levels of constitutive activity sensitive to varying degrees of inverse agonism [16,17].

The dependence of drug effects on levels of preexisting receptor activity (i.e. tone) also poses challenges and opportunities to the in vivo pharmacologist. This commentary focused particular attention on the differential impact of tone due to constitutive receptor activity and tone due to endogenous ligands. In the last decade, there has been a sharp growth in research on the physiological relevance and clinical implications of constitutive receptor activity. This research has stimulated development of increasingly refined models of drug action (e.g. the extended ternary complex model), which provide a theoretical basis for novel drug phenotypes, such as neutral antagonists and inverse agonists. However, it is critical to realize that constitutive activity is only one source of tone in vivo, and preexisting receptor activity in the whole animal may also result from varying levels of endogenous ligands. This dual source of tone is likely to introduce some complexity into the task of drug classification. In vitro, for example, it is possible to examine receptor activity in the absence of other ligands, and the term ''inverse agonist'' has been reserved for drugs that decrease constitutive receptor activity. In this context, blockade of effects produced by another high efficacy ligand (e.g. a high efficacy endogenous agonist) would be considered antagonism rather than inverse agonism. In vivo, however, it is rarely possible to study receptors in isolation from their endogenous ligands, and a phenotype of inverse agonism (i.e. induction of effects opposite to those of an agonist) may reflect the presence of endogenous agonist rather than constitutive receptor activity. Distinguishing these different sources of tone and evaluating their relative contributions to organismal states and drug effects are likely to be important topics for pharmacological research in the coming years [16,18]. The theoretical models presented in this commentary suggest that intermediate efficacy ligands may be especially useful for investigating tone due to constitutive activity versus endogenous agonists. Specifically, an increase in constitutive activity is implicated if a manipulation *increases* the potency of an intermediate efficacy ligand and *increases* the ability of such a drug to produce maximal effects similar to those of a high efficacy ligand. Conversely, an increase in endogenous agonist tone would be indicated if a manipulation *decreases* the potency of an intermediate efficacy ligand, *decreases* the maximal effects of such a drug relative to a high efficacy ligand, and *reverses* the valence of effects from agonist to inverse agonist.

As a final cautionary note, it should be acknowledged that at least four other factors are likely to complicate interpretation of in vivo drug effects. First, the tone in a receptor system in vivo is likely to reflect an integration of both endogenous ligand effects and constitutive receptor activity rather than one or the other alone. Moreover, tone from either source in a primary receptor system may also converge with tone from other secondary receptor systems to influence baseline values in a given assay, and as a result, tone in these secondary receptor systems may also influence drug effects. Second, for behavioral studies conducted in whole animals, the magnitude of tone is unlikely to be constant as in the models above, but rather will vary both spatially (across neuroanatomical regions) and temporally. The variation in tone across neuroanatomic subregions may be especially important, because it suggests that tone (and as a result drug profiles) may vary for different effects mediated by anatomically distinct receptor populations [16]. Third, manipulations that influence constitutive receptor activity (the parameters L and K_g in Eq. (3)) may also influence drug-related parameters of the extended ternary complex model (i.e. α and γ). For example, exposure to high efficacy ligands is thought to increase constitutive activity of mu opioid receptors [15], but this same manipulation may also reduce the parameter γ, as indicated by reduced coupling between agonist-bound receptors and G-proteins [19]. Finally, models of receptor theory have evolved primarily to describe drug effects on receptors coupled to a single effector system (e.g. G-proteins). However, receptors

may be coupled to multiple effector pathways contributing to multiple effects, and parameters governing drug–receptor–effector interactions are likely to vary across effector pathways [4,20,21]. Thus, relative effects of a series of drugs mediated by a given receptor on one effector system may differ from relative effects mediated by the same receptor on a different effector system. Further advances in receptor theory will permit more sophisticated modeling of these factors and generation of testable hypotheses that will contribute to drug development.

Acknowledgement

This work was supported in part by R01-DA11460 from the National Institute on Drug Abuse, NIH.

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