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The mass action equation in pharmacology

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The mass action equation is the building block from which all models of drug–receptor interaction are built. In the simplest case, the equation predicts a sigmoidal relationship between the amount of drug–receptor complex and the logarithm of the concentration of drug. The form of this function is also the same as most dose–response relationships in pharmacology (such as enzyme inhibition and the protein binding of drugs) but the potency term in dose–response relationships very often differs in meaning from the similar term in the simple mass action relationship. This is because (i) most pharmacological systems are collections of mass action reactions in series and/or in parallel and (ii) the important assumptions in the mass action reaction are violated in complex pharmacological systems. In some systems, the affinity of the receptor R for some ligand A is modified by interaction of the receptor with the allosteric ligand B and concomitantly the affinity of the receptor for ligand B is modified to the same degree. When this occurs, the observed affinity of the ligand A for the receptor will depend on both the concentration of the co-binding allosteric ligand and its nature. The relationships between drug potency in pharmacological models and the equilibrium dissociation constants defined in single mass action reactions are discussed. More detailed knowledge of efficacy has led to new models of drug action that depend on the relative probabilities of different states, and these have taken knowledge of drug–receptor interactions beyond Guldberg and Waage.

Introduction

The mass action equation is the building block from which all models of drug–receptor interaction are built. The present review considers the assumptions underlying the application of the equation to complex pharmacological systems, the consequences of violations of the underlying assumptions and ways of overcoming the problems that arise.

The mass action equation

The mass action equation was given in the work of Guldberg and Waage [1], as follows:

$$A + B \rightleftharpoons A' + B' \quad (1)$$

Guldberg and Waage introduced the idea that the rates of reactions, both forward and backward, depended on the 'active masses' (i.e. the concentrations) of reactants in the mixture ('…the amount of substance in the sphere of action' or, put another way, the concentration in the medium) [2]. Thus, at equilibrium, the product of the active masses on one side of the equation divided by the product of the active masses on the other side of the equation is a constant, independent of the amount of substances at the start of the reaction. This model has been applied to pharmacology, and it underpins *all* models of drug activity. Specifically, it defines the relationship between the quantity of the drug ([A]) and the amount of drug–target complex ([AR]) formed; this latter species is the initiator of pharmacological activity in all physiological processes.

It is useful to demonstrate how the law of mass action is used to determine [AR] from the amount of drug ([A]) and receptor ([R]), where the receptor is the drug target (receptor, ion channel, enzyme). If the drug A and the receptor R react with a rate constant k_1 (units = $s^{-1}M^{-1}$), the rate of association of the drug with the receptor is given by k_1 [A] [R]. Similarly, the rate constant for dissociation of the drug from the receptor is denoted as k_2 (units s^{-1}) and defines a rate of dissociation of the drug from the receptor of

k_2 [AR]. The receptor conservation equation when the stoichiometry of binding is 1:1 (accounting for all species of receptor) is given as $[R_T] = [R] + [AR]$, where $[R_T]$ is the total number of receptors and [R] the concentration of free receptors (not bound by ligand). At equilibrium, the rate of association of drug to the receptor is equal to the rate of dissociation:

$$k_1 [A] [R] = k_2 [AR] \quad (2)$$

Defining a ratio K_A as k_2/k_1:

$$K_A = \frac{[A]([R_T] - [AR])}{[AR]} \quad (3)$$

This reduces to the mass action equation as applied to pharmacology:

$$[AR] = \frac{[A] [R_T]}{[A] + [K_A]} \quad (4)$$

Equation 4 is the equation of a rectangular hyperbola, which defines a sigmoidal curve on a semi-logarithmic scale (see Figure 1). Specifically, this equation describes a relationship whereby the product of the reaction (in the case of equation 4, the drug–target complex) is a ratio of the product of one of the reactants (drug concentration [A]) multiplied by the maximal output capability of the system (given by $[R_T]$) and the sum of the reactant ([A]) and a potency factor (apparent dissociation constant K_A) which, in the simplest case, is a measure of the amount of reactant needed to carry the process to half-maximal completion (see Figure 1). In the case of equation 4, the potency factor is the equilibrium dissociation constant of the drug–target complex. In terms of target binding, when $[A] = K_A$, half of the target sites are bound with drug. Sigmoidal semi-logarithmic binding relationships form a main working tool of pharmacology, the potency term in the denominator being a major parameter of drug characterization. Specifically, the potency term K_A locates the binding curve along the concentration axis. While the mass action reaction yields a relationship of the same general form, it is fortuitous that the potency observed in a binding curve sensitivity term is also the formal term defined by the law of mass action (namely, K_A); there are instances where this is not the case. Complexity in pharmacological binding may be due to two factors. The first is that pharmacological systems may be complex mixtures of mass action reactions (*vide infra*) and the second is that real pharmacological systems violate some of the basic assumptions essential to the mass action reaction. The application of the law of mass action to pharmacology must be considered in light of the assumptions made in the derivation of this law for chemical reactions. In terms of the pharmacological application, the following assumptions are made:

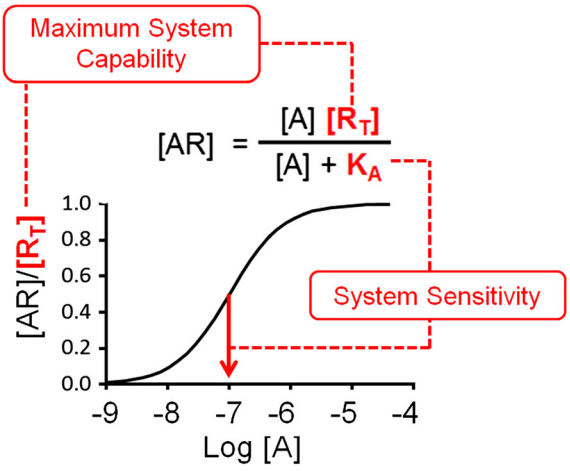


Figure 1

The mass action equation as applied to the binding of a drug A to receptor R, to form complex AR. The amount of AR complex plotted as a logarithmic function of the concentration yields a characteristic sigmoidal curve. The sensitivity of the system to A is given by the K_A term (apparent dissociation constant) and gives the location parameter of the curve along the x axis. The maximal ordinate value is given by $[R_T]$, the maximal amount of receptor in the system. This curve closely resembles the dose–response relationships for many drugs

1. All receptors are equally accessible to ligands – violation of this assumption leads to incomplete assessment of ligand binding.
2. The binding is reversible – violation of this assumption precludes calculation of valid K_A values.
3. Receptors are either free or bound to ligand, and there is no more than one affinity state, or states of partial binding (the ligand and receptor must exist in only two states, bound or unbound) – violation of this assumption leads to ambiguity in the assignment of potency values.
4. Binding does not alter the ligand or receptor – violation of this assumption also leads to ambiguity in the assignment of potency values (system-dependent potency). When a ligand binds to a receptor and changes its conformation, this is an expression of pharmacological efficacy.

It will be seen that some of these assumptions are violated in the application of the mass action equation to complex pharmacological systems, and it is worth identifying these violations in order to understand the meaning of the parameters obtained from applying mass action to pharmacological models.

Mass action processes in series and parallel

The essential feature of the law of mass action as applied to drug binding to a target (equation 4) is that the balance between the amounts of product ([AR]) and the amounts

of the reactants ([A] + [R]) is determined by a simple ratio of the rates of two reactions (see Figure 2A). If another process interferes with any of the amounts of these species, then the balance is changed and becomes dependent on more than just the simple rates k_1 and k_2 (violation of assumption 4). Therefore, if another reaction removes the product of the first reaction ([AR] is depleted by another process) or if the level of the reactants is altered by another reaction, then the amount of [AR] will cease to be solely determined by k_1 and k_2; this can occur if the complete system involves series or parallel mass action processes.

Some pharmacological systems ostensibly follow simple mass action kinetics but are in effect simple mass action reactions connected in series; series mass action reactions lead to more complex interpretation of potency values beyond simple K_A values as described by equation 4. In a series mass action system, a second mass action reaction removes the product of the first reaction, and therefore the rate of the overall reaction depends upon the two processes (see Figure 2B). For example, if ligand binding promotes the binding of the ligand-occupied receptor to another species such as a G protein in the cell membrane (i.e. if the protein 'isomerizes' [3] to becomes another thermodynamic species,

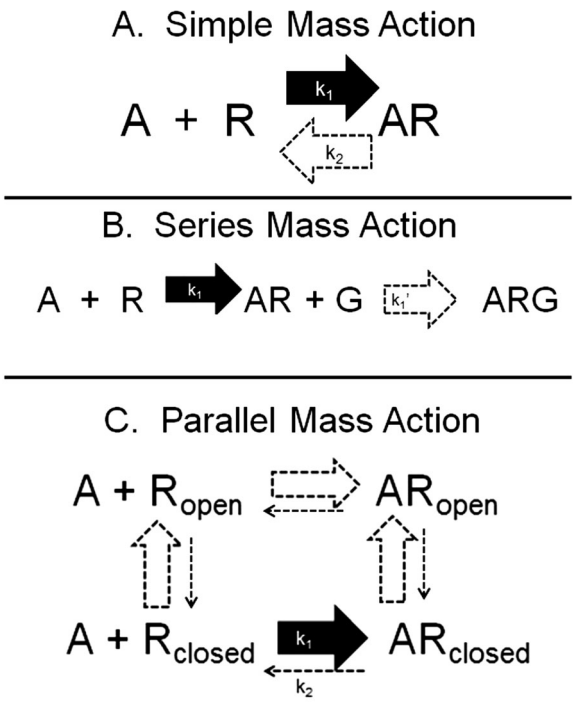


Figure 2

Pharmacological systems as combinations of the simple mass action reaction scheme (A). (B) A series mass action system where the product of the first reaction becomes the reactant for the second. (C) A system where two series mass action reactions are aligned in parallel with each other, with a thermodynamic link between them. In this case, a protein (ion channel) can exist in an open and closed conformation and drug A can interact with both of them

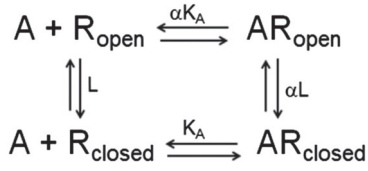
which then has a different reactivity to G proteins), then the overall observed affinity of that ligand can be dependent upon these interactive processes – i.e. the observed potency of the ligand will not be equal to the equilibrium dissociation constant as defined in equation 4.

Defining K_A as k_{a2}/k_{a1} and K_G as k_{g2}/k_{g1}, the sensitivity factor for the complete reaction (R to ARG) is not given by K_A but rather by a term:

$$K_{obs} = \frac{K_A}{1 + [G]/K_G} \quad (5)$$

It can be seen from equation 5 that for all nonzero values of [G], $K_{obs} < K_A$; that is, the effective affinity of the ligand for the receptor system will be greater than its affinity for the receptor when it cannot interact with the secondary membrane component (in this case, the G protein). This has been shown experimentally in many systems. For example, the clinically used β-adrenoceptor agonist for asthma, salbutamol (albuterol), shows a five-fold loss of affinity when complexation of the receptor with a G protein is cancelled by addition of GTPγS [4]. Other pharmacological systems comprise parallel mass action reactions; under these circumstances, the interpretation of ligand potency becomes a complex result of the makeup of the receptor system, not just the binding of the ligand to the receptor protein. In parallel mass action systems, the reactants as well as the products may be depleted by a second mass action reaction (see Figure 2C). In these more complex systems (i.e. ion channels or two-state receptor systems), the rate of the overall reaction is an amalgam of the micro-rate constants of the individual mass action processes.

The alteration of equilibria between protein states by ligand binding can be illustrated by two states in equilibrium with each other as R_{closed} and R_{open}, controlled by an allosteric constant L, which is defined as the ratio R_{open}/R_{closed}:



(6)

Under these circumstances, the sensitivity factor for the complete system does not depend solely on K_A (where $K_A = k_2/k_1$) but rather on the differential activity of the ligand for both target species, defined as the term α. This is the ratio of the affinity of A for R_{open} *vs.* its affinity for R_{closed}:

$$K_{obs} = \frac{K_A(1 + L)}{1 + \alpha L} \quad (7)$$

Violation of receptor immutability (assumption 4)

A key violation of the simple mass action scheme is that the receptor does not change upon ligand binding. If

the drug protein target consists of a system of interconvertible protein conformations (see below) then the binding of drugs in that system will necessarily change the mix of receptors available for drug binding. In series and parallel mass action reactions where there is a thermodynamic bridge between species (making them interconvertible, such as through the constant L in scheme 6), the differential affinity of the ligand A for the two receptor states (defined as the term α, where $\alpha \neq 1$) will necessarily lead to a change in the system from that present in the absence of the ligand, through the ratio ρ_∞/ρ_0 (where ρ_0 is the fraction of receptors in the R_{open} state in the absence of ligand, and ρ_∞ is the fraction in the presence of a saturating concentration of ligand) in the two-state equation, a process referred to as 'conformational selection' [5]:

$$\frac{\rho_\infty}{\rho_0} = \frac{\alpha(1+L)}{(1+\alpha L)} \tag{8}$$

A mixture of receptor conformations will re-equilibrate according to Le Chatelier's principle (if a chemical system is displaced from equilibrium, changes will act to minimize the deviation from equilibrium) towards enrichment of the protein species for which the ligand has the greater affinity. Therefore, if $\alpha > 1$, the active state (R_{open}) will be enriched and if $\alpha < 1$, then the inactive state (R_{closed}) will be enriched. This idea is the basic mechanism of drug efficacy and currently is thought to be how a ligand causes a change in a receptor system to initiate an increase in an active receptor species that then elicits a cellular response.

The seven transmembrane receptors (7TMRs) are major drug targets in therapeutic pharmacology. Such receptors reside on the membrane surface of cells and mediate chemical signalling from the environment to the cytoplasm of the cell. They are characterized by having seven transmembrane helices joined by extracellular and cytoplasmic protein loops; their main function is to bind hormones, neurotransmitters and autacoids to form a complex, which then has consequences on the functions of cells. Teleologically speaking, the main function of 7TMRs is to change their shape (conformation) in response to interactions with extracellular ligands and intracellular signalling proteins. In pharmacological systems, drug targets such as 7TMRs do not stay in static conformations but rather exist in ensembles of different conformations, and these conformations are interchangeable according to the available free energy of the system [6–11].

With a greater number of possible conformations in the ensemble comes a much greater increase in the likelihood that drug binding will change the nature of the system. For example, the effect of a saturating concentration of ligand on the fraction of receptors not in any chosen state (e.g. state *i*) in an ensemble of one to *n* states is given by:

$$\frac{\rho_\infty}{\rho_0} = \frac{\sum_{i=1}^{n} \alpha_{i+1} L_{i+1} \left(1 + \sum_{i=1}^{n} L_{i+1}\right)}{\left(1 + \sum_{i=1}^{n} \alpha_{i+1} L_{i+1}\right) \sum_{i=1}^{n} L_{i+1}} \tag{9}$$

From equation 9 it can be seen that only in the case where $\alpha_1 = \alpha_2 = \alpha_3 = ... = \alpha_i = 1$ (the ligand has the same affinity for all states) will the ratio of states not change upon ligand binding. This suggests that binding is not a passive process and that the binding of a ligand will change the energy landscape of the receptor ensemble. A corollary of this idea is the notion that the ability of a ligand to change the receptor ensemble is a far more prevalent drug property than thought previously, when there were limited pharmacological assays available to measure drug response.

Under these circumstances, drugs bind not to a single protein species but rather to a collection of tertiary conformationally different proteins (i.e. an ensemble). Probability partition functions show that ligand binding is not a passive process but rather that it can actively modify these ensembles [10–12]. Although there may be numerous parallel mass action processes, and although drug binding itself may change the nature of the binding species (series mass action processes), the overall effect of ligand binding in such a system can still resemble a simple single mass action process and yield a curve such as that shown in Figure 1 – i.e. the dose–response relationship can be modelled by a rectangular hyperbola that yields an apparently sigmoid curve after log transformation. However, as with the examples shown in Figure 2B and 2C, the meaning of the sensitivity term differs from the simple K_A parameter shown in equation 4.

Specific pharmacological models

The mass action relationship forms the basis of all major drug activity models in pharmacology. For example, it describes the initial binding of substrates to enzymes (Figure 3A) [13]. Various combinations of mass action equations form other models of important pharmacological systems. Series mass action processes describe the binding of 7TMRs to agonists and subsequently to G proteins; the model for agonism in general – namely, the Black–Leff operational model [14] – is described by series mass action equations for the binding of agonist to receptor and the receptor to signal coupling proteins in cells (Figure 3B). Parallel mass action equations describe the control of ion channel opening and also for the allosteric function of 7TMRs (Figure 3C). While all of these models describe a sigmoidal function on a semi-logarithmic scale for drug concentration much in the same way as the mass action

equation (Figure 1), the meaning of the sensitivity term varies according to the specific model. The ascription of the sensitivity term from the curves generated by these models to a simple mass action reaction constant is obviously inappropriate (i.e. see Table 1).

The sensitivity term in the sigmoidal relationships described by these models (i.e. the K_A term in equation 4) is usually an important parameter in pharmacology as it equates to drug *potency* – i.e. how much drug must be present in the target compartment to achieve a given drug response. There are two major areas where drug potencies are important therapeutic parameters: (1) the EC_{50} of an agonist, defined as the concentration producing a half-maximal response and (2) the K_B of antagonists, defined as the concentration of antagonist producing 50% occupancy of receptors. It is worth comparing the relationship of these parameters to mass action reactions.

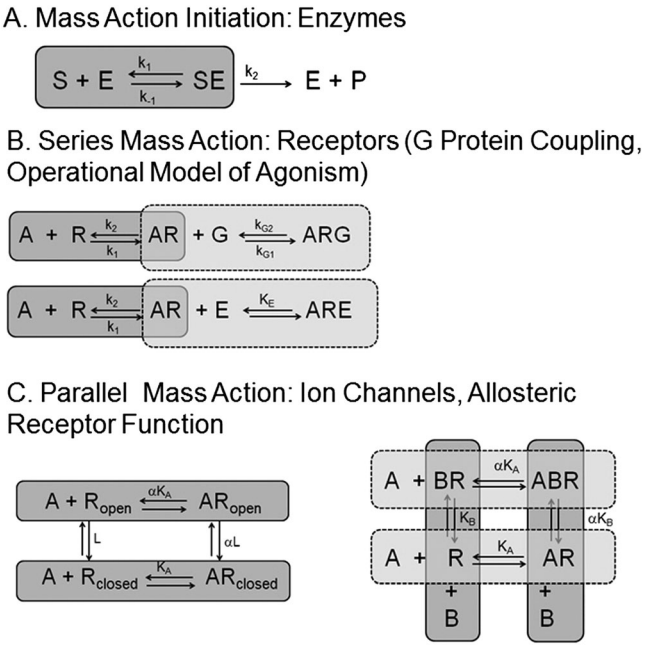


Figure 3

Series and parallel mass action reactions specified as distinct pharmacological models. (A) Mass action binding of a substrate to an enzyme begins the process of enzyme catalysis in the Michaelis–Menten model [13] for enzyme function. Once the substrate is bound, the enzyme catalyses the reaction to production of product through k_2 (also referred to as k_{cat}). (B) Two common settings for series mass action reactions are the agonist-mediated production of ternary complexes for G protein-coupled receptors and agonism as described by the Black–Leff operational model [14]. (C) Two common parallel mass action reactions involve drug activation of ion channels and the allosteric model for seven transmembrane receptors [27, 28]

EC_{50} values for agonist potency

Pharmacological agonism is the process whereby agonists bind to receptors to cause them to change their behaviour toward the host cell, the outcome being a change in cellular function. Conceptually, this can be thought of as the binding of a drug A to a receptor R to form an AR complex, which then changes its nature to AR* to alter the cellular response:

$$A + R \underset{K}{\rightleftharpoons} AR \underset{\varphi}{\overset{\gamma}{\rightleftharpoons}} AR^* \qquad (10)$$

The processes controlled by γ and ϕ (the conformational state of the 7TMR is not confined to AR) will modify the observed affinity of the system for A and will be quite different from the K_A parameter defined by equation 4 [3]. If receptor R converts to R* upon binding of ligand A, the observed affinity of the ligand for the system is not defined by K_A (equation 4) but rather an affinity that

Table 1

Meaning of the sensitivity term for some common pharmacological models

Target	Term	Definition	Pharmacological significance
A. Enzymes	K_m	$K_m \frac{k_{-1}+k_2}{k_1}$	Sensitivity of enzyme to substrate concentration – i.e. concentration of substrate causing enzyme rate to attain 50% of the maximum reaction velocity (Vmax)
B. Ion channels	EC_{50}	$EC_{50} = \frac{K_A(1+L)}{(1+\alpha L)}$	Concentration of ligand producing the opening of half of the channels
C. 7TMRs (GPCRS)	K_{obs}	$K_{obs} = \frac{K_A}{(1+[G]/K_G)}$	Concentration of binding ligand that causes half-maximal production of ternary complex between ligand, receptor and G protein
D. 7TMRs	EC_{50}	$EC_{50} = \frac{K_A}{(1+[R_T]/K_E)}$	Concentration of agonist producing 50% maximal response
E. 7TMRs	EC_{50}	$EC_{50} = \frac{K_A(1+[B]/K_B)}{(1+\alpha[B]/K_B)+\tau_A(1+\alpha\beta[B]/K_B)}$	Concentration of agonist producing 50% maximal response in the presence of an allosteric modulator

A. Michaelis–Menten enzyme kinetics: k_1 = rate of substrate-enzyme association, k_{-1} rate of substrate-enzyme dissociation, k_2 = rate of product formation once substrate bound. B. Ion channel opening: α is the differential affinity of the ligand for the open ion channel, L is the ratio of active state receptors and inactive state receptors, K_A the equilibrium dissociation constant of the ligand-reeptor complex. C. Seven transmembrane receptor (7TMR) G protein binding; K_G is the equilibrium dissociation constant of the receptor–G protein complex. D. 7TMR function: $[R_T]$ is the receptor density, K_E is the equilibrium dissociation constant of the ligand–receptor complex and the response elements of the cell. GPCRS, G protein-coupled receptors. E. 7TMR allosteric function: τ_A is the efficacy of the agonist, α the effect of the allosteric ligand [B] on agonist affinity, β is the effect of the allosteric ligand on agonist efficacy, K_B is the equilibrium dissociation constant of the allosteric modulator–receptor complex.

depends on the rate of the transformation to the R* state. Under these circumstances, the complete reaction is not characterized by the simple K_A term but rather by an amalgam term describing the series mass action processes [3]:

$$K_{obs} = \frac{K}{1 + \frac{\gamma}{\phi}} \quad (11)$$

By definition, the change of the receptor to an active state is a reflection of the ligand's efficacy and it is useful to discuss the concept of the efficacy and EC_{50} of a full agonist in terms of the standard model for agonism in pharmacology – namely, the Black–Leff operational model [14, 15]:

$$\text{Response} = \frac{[A]\ \tau\ Em}{[A]\ (1 + \tau) + K_A} \quad (12)$$

where the efficacy, τ, is $[R_T]/K_E$, which is the total number of receptors divided by the equilibrium dissociation constant ($K_E = \gamma/\varphi$ from equation 4) of the agonist-bound receptor–response element E complex. The Black–Leff model can be thought of as a series mass action system, whereby the agonist-bound and activated receptor unites with elements in the cell to produce a cellular response:

$$A + R \underset{}{\overset{K_A}{\rightleftharpoons}} AR + E \overset{K_E}{\rightleftharpoons} ARE \quad (13)$$

In terms of this model, the primary measure of agonist potency – namely, the EC_{50} – is given by equation 14:

$$K_{obs} = \frac{K_A}{1 + \frac{[R_T]}{K_E}} \quad (14)$$

This produces the well-known efficacy-mediated displacement of functional agonist dose–response curves to the left of the binding curve (see Figure 4).

In addition to agonism being a series mass action system, receptors are known to be pleiotropic with respect to the response elements with which they can interact within cells. Thus, drugs can have many different efficacies, a phenomenon referred to as 'pluridimensional efficacy' [16]. For example, calcitonin receptors can interact with at least three G proteins in the membrane (G_s, G_q and G_i), thereby creating a more diverse series of mass action reactions (see Figure 5). This pleiotropy with respect to mass action reactions may be an important aspect of the therapeutic value of drugs in the clinic. For instance, β-blockers theoretically should be valuable in the treatment of heart failure but, of the many β-blockers run in clinical trials, only a handful have shown statistically significant improvement. It has been proposed that the multiple actions of carvedilol (i.e. causing blockade of β_2- and α_1-adrenoceptors and endothelin responses, and having antiproliferative and antioxidant effects) determine its clinical efficacy [17].

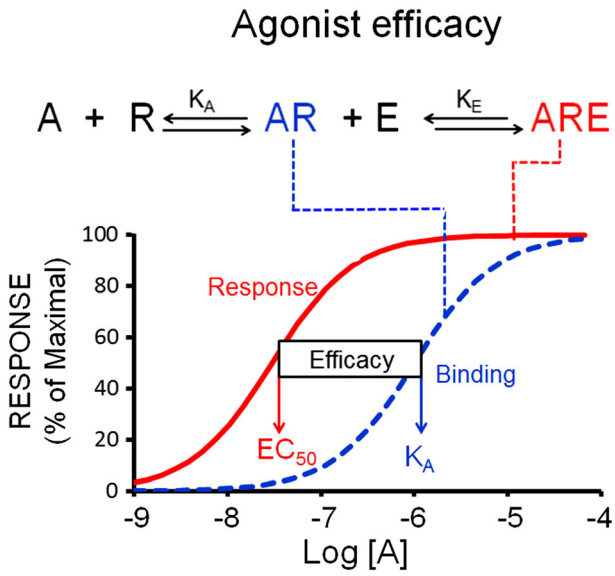


Figure 4

The sinistral displacement of functional activity curves for agonists from the initial binding curve. The property of efficacy causes the concentration of agonist producing 50% maximal response (EC_{50}) to be of a lower magnitude than the binding constant K_A. In the Black–Leff operational model, it is assumed that the initial agonist–receptor complex AR interacts with response elements in the cell (denoted as E) to produce a further complex that causes cellular response. The sensitivity factor for this second reaction is denoted K_E and represents numerous saturable processes within the cell cytosol. Efficacy in this model is given as τ, where $\tau = [R_T]/K_E$, ([RT] is the total number of receptors

In addition, the alteration of receptor conformation with agonist binding (according to scheme 10) can produce even greater diversity of agonist signalling through the imposition of signalling bias [18–20]. Specifically, there is abundant evidence that some ligands stabilize unique states within the ensemble of normal receptor systems, to cause differential activation of the pleiotropic response elements in cells and thereby produce unique signalling patterns. For example, while the natural substance angiotensin II causes activation of Gq protein and β-arrestin through activation of angiotensin receptors, the analogue TRV120027 stabilizes a conformation that only activates β-arrestin (see Figure 6). This property may make TRV120027 better for the treatment of heart failure [21, 22].

K_B values for antagonist target coverage

The main characteristic parameter for antagonists of receptors, enzymes, or ion channels is the K_B – namely, the apparent equilibrium dissociation constant of the antagonist–receptor complex. It can be seen from the mass action equation (equation 4) that when the

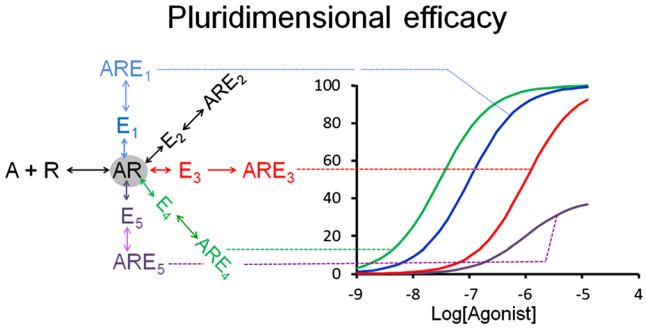


Figure 5

Receptors are often pleiotropically linked to numerous signalling systems in the cell, and the activation of each of these can generate a separate concentration–response curve for drug effect. The key to observing these is the availability of separate assays for each of the responses. Panel A. Schematic showing the interacting species of ligand (A), receptor® and various response coupling elements labeled E1 to E5. Panel B. Simulated concentration-response curves for interaction of the ligand-bound receptor with the various response element

antagonist concentration is equal to K_B, the receptor occupancy for the antagonist is 50% of the total receptor population. This is a characteristic condition as it represents a twofold diminution of agonist stimulation to the system and generally characterizes the threshold for receptor antagonism. A common experimental approach to the measurement of antagonist potency values K_B is through biochemical radioligand binding, most notably for the target class 7TMRs. Owing to the apparent simplicity of binding experiments, equation 4 is often directly applied to experimental data, with the expectation that the sensitivity term obtained is an accurate measure of K_A: in some cases this may be correct but in many others it is not. It is worth examining why, even in binding experiments, the K_A term in equation 4 is often not what is obtained in pharmacological binding experiments.

The relationship defining the binding of a drug to a biological target was first presented by A.V. Hill [23] and adopted by pharmacologists 6 years later in another hyperbolic relationship made popular by the chemist Irving Langmuir, referred to as the adsorption isotherm [24]. Langmuir had applied this equation to the adsorption of gas molecules onto surfaces, describing the interactions as an attraction of molecules to the surface (a process he denoted as 'condensation') and diffusion away from the surface (a process he described as 'evaporation'). As discussed by the originators, this equation is often inappropriately applied to complex processes. For example, Hill described the equation only to determine '…whether an equation *of this type* can satisfy all the observations, [rather] than to base any direct physical meaning on … K_A' [italics in original] [25]. Langmuir was primarily interested in the adsorption of gases to metal surfaces, a non-interactive surface that is quite

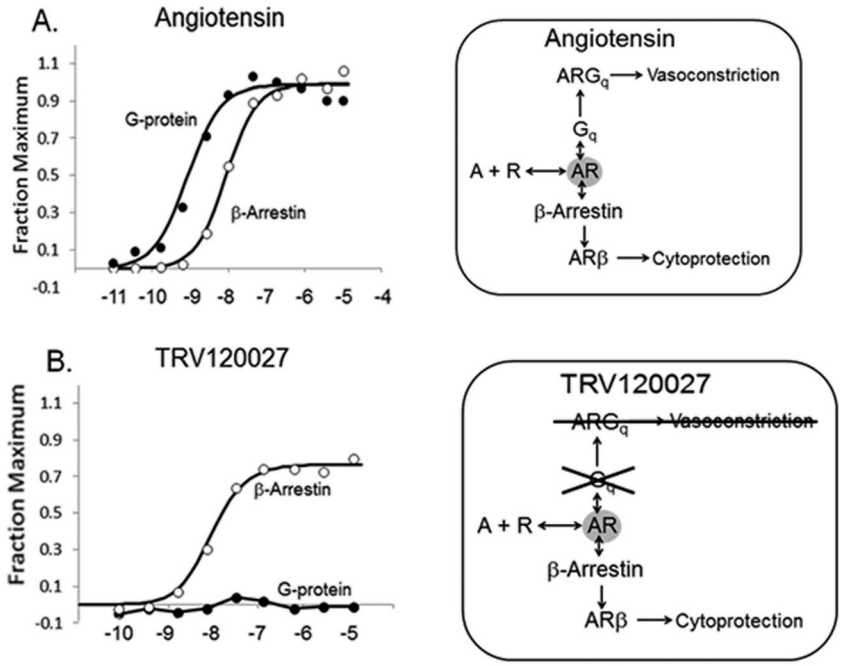


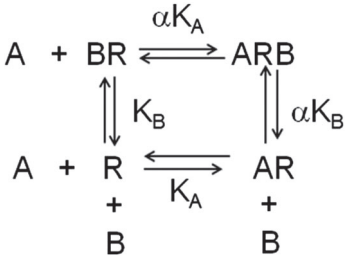
Figure 6

The stabilization of unique receptor states can lead to the selective activation of some of the pleiotropic signalling mechanisms linked to the receptor. The activation of angiotensin receptors by angiotensin II normally leads to the activation of the G_q protein and interaction of the receptor with β-arrestin. The structural analogue TRV120027 stabilizes a conformation of the receptor that causes interaction of the receptor with β-arrestin, not G_q. This phenomenon is generally referred to as biased receptor signalling. Data for TRV120027 are redrawn from [36]. Panel A: Concentration response curves for angiotensin activation of two signaling pathways (G-protein and β-arrestin- see left panel). Panel B: Biased signaling for TRV120027 showing activation of β-arrestin but no activation of G protein

different from receptor protein. In his paper, in describing the use of the equation, Langmuir stipulated that any heterogeneity in the surface (such as might be found for activated charcoal) could alter the interpretation of the parameters obtained: '…but it is evident that [the] Equation, which appl[ies] to adsorption by plane surfaces, could not apply to adsorption by charcoal.' [24]. As noted previously, the fact that receptors do not have a uniform conformation, but rather exist in ensembles of various conformations, makes the 'binding surface' for ligands nonhomogeneous. Therefore, while a process of mass action binding takes place for drug action, the drug may encounter a mixture of receptor conformations, all with varying affinities for the drug. The different conformations in the ensemble are interchangeable, and ligands may selectively stabilize some conformations over others. If this occurs, binding may change the relative proportions of these conformations, previously discussed as conformational selection [5]. In view of the heterogeneity of receptor systems in conformational ensembles, quite unlike Langmuir's inert surface, the mass action equation will not describe a single binding process. Probability partition functions with virtual data have shown that affinity and efficacy (as defined by the changing of receptor conformation) are necessarily correlated [12].

7TMRs are allosteric proteins – i.e. they co-bind a ligand in the extracellular space domain and a signalling protein in the cytosolic domain of the cell. These interactions depend on each other, in that the affinity of both the ligand and the signalling protein depend on the binding of the other species. Thus, agonism is the process whereby extracellular ligands such as hormones or neurotransmitters stabilize conformations of the receptor that facilitate binding and activation of cellular signalling proteins. The transfer of energy from the ligand domain to the signalling domain of the receptor constitutes an 'allosteric vector' [26], which is bidirectional, in that the binding of a signalling protein to a receptor will concomitantly alter the affinity of the receptor for a ligand in other regions of the protein.

The allosteric protein binding model can be described as a system of parallel mass action binding reactions depicting the allosteric binding of two ligands, A and B, to a single receptor protein, R (see Figure 2C) [27, 28]:



(15)

In these systems, the affinity of receptor R for ligand A is modified by interaction of the receptor with the allosteric ligand B by the factor α, and concomitantly the affinity of the receptor for ligand B is modified to the same degree (namely, α) to ensure conservation of energy by the binding of ligand A. When this occurs, the observed affinity of ligand A for the receptor will depend on both the concentration of the co-binding allosteric ligand and its nature by the expression:

$$K_{obs} = \frac{K_A(1 + [B]/K_B)}{(1 + \alpha[B]/K_B)} \quad (16)$$

Several allosteric ligands produce effects on ligand receptor affinity through interactions at extracellular and intracellular sites. The striking effects of co-binding species are demonstrated by differences in the X-ray crystallographic structure of the β_2_adrenoceptor bound and not bound to a nanobody simulating a G protein [29, 30]. Experiments with ghrelin receptors in lipid discs have shown changes in the conformations of the receptors on addition of G_q to nanodiscs [31]. Thus, addition of β-arrestin causes formation of different receptor conformations, as seen with exponential fluorescent lifetime decay analysis [31]. Similarly, changes in the conformations of κ-opioid receptors with the binding of $G_{\alpha 16}$ and/or $G_{\alpha i2}$ G protein subunits have been shown, using the substituted cysteine accessibility method, to produce an 18-fold change in affinity for the ligand salvinorin [32]. The effect of receptors forming different membrane complexes with signalling proteins has been labelled 'receptor distribution' [33] and underscores the heterogeneity of the drug target species in functional pharmacological experiments. Given these effects, apparently simple radioligand binding experiments with 7TMRs produce apparent K_A values that may have no relationship to the true micro-affinities of the ligands for the receptor in the physiological environment.

In general, the nature of 7TMRs is very different from the binding surface defined by equation 4 (i.e. the available binding sites on 7TMRs most often do not satisfy either of the prerequisites of uniformity and non-interaction) and it is these differences that require re-evaluation of the simple mass action equation as it applies to the description of drug action.

Beyond Guldberg and Waage

All of the models discussed thus far are so-called 'linkage' models, in that the various species of receptor protein present are defined, and linkages between them made to equate and conserve energy transfer. While this seemed to suffice when there were simple readouts of ligand efficacy, they showed their inadequacy as technology progressed to the point where multiple assays report the complex behaviour of 7TMRs (efficacy is pluridimensional [16]). Linkage models cannot

accommodate the clear evidence that these efficacies are linearly related. For example, previously it had been assumed that receptor internalization followed receptor activation because the only scale for internalization was the elimination of response. When separate assays were available to measure receptor internalization directly, it was seen that many antagonists which did not produce receptor activation caused an active receptor internalization [34, 35]. This overall independence in efficacy requires more flexible models of receptor function than the existing linkage models. Molecular dynamic models fill the gap through the description of protein 'ensembles', where a collection of receptors with differing tertiary conformations comprise the systems that interact with cells and ligands [6–9]. If such systems were to be frozen in time for any one instant, it would be seen that a collection of slightly different receptor conformations, of similar free energy, co-exist; ligands selectively stabilize those for which they have the highest affinity and these are selected at the expense of others – i.e. ligands produce a new receptor ensemble for interaction with the cytosolic signalling machinery [10–12]. Figure 7 shows an ensemble view of receptor function. As receptors do not change conformation at a uniform rate in every region of the protein, there will be a collection of different global conformations, corresponding to specific cytosolic states capable of activating signalling proteins. Under these circumstances, a Boltzmann distribution of states will be the so-called 'active' state of the receptor. Ligands will have a range of affinities for these various states and will selectively stabilize the states for which they have the highest affinity (at the expense of others). Thus, upon ligand binding, the ensembles will take on a ligand-specific nature (see Figure 7). Note that there is no forced linearity between pharmacological efficacies – i.e. G protein activation does not need to precede receptor internalization; this fits new experimental evidence showing that efficacies can be independent [37].

In contrast to linkage models, probability models do not specify the protein species present but rather describe the possibility of a given protein state changing into another state. For example, a model proposed by Onaran and Costa [10] and Oneran *et al*. [11] begins with one receptor state (referred to as [Ro]) and defines the affinity of a ligand [A] and a G protein [G] for that state as:

$$^{A}ko = [ARo]/[Ro][A] \quad (17)$$

and

$$^{G}ko = [GRo]/[Ro][G] \quad (18)$$

The probability of the receptor being in that state is denoted p_o, while the probability of the receptor forming another conformation [R1] is defined as p_1. The probability ratio for forming state R1 (denoted p_1) *vs*. Ro (probability p_0) is given as j_1, where $j_1 = p_1/p_o$; the value j controls the energy of transition between the states. The relative probability of forming state [R_1] with ligand binding is denoted $^{A}j_1 = {}^{A}p_1/{}^{A}p_o$ and with G protein binding as $^{G}j_1 = {}^{G}p_1/{}^{G}p_o$. This defines vectors describing fractional stabilization of states binding either ligand (defined $^{A}b_1 = {}^{A}j_1/j_i$) or G protein ($^{G}b_1 = {}^{G}j1/ji$), where the magnitude of b is unique for each receptor state. Therefore, values of b constitute ligand affinity and efficacy. With these probabilities and vectors, the following operators are:

$$\Omega = 1 + \Sigma j_i \quad (19)$$

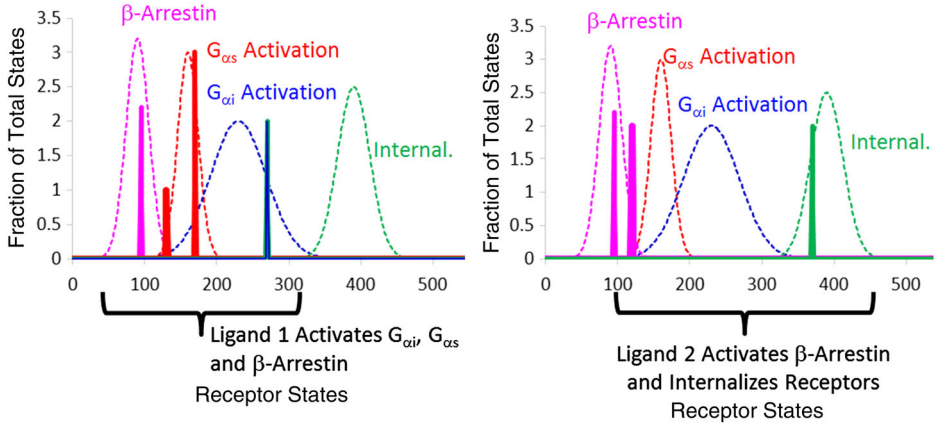


Figure 7

Receptor conformational ensembles as Boltzmann distributions of tertiary states. Shown are natural ensembles that lead to various physiological responses such as activation of $G_{\alpha s}$, $G_{\alpha i}$ protein, β-arrestin interaction and receptor internalization. As different ligands bind to the ensemble, certain conformations are stabilized through selective high affinity at the expense of other conformations. If the stabilized conformation coincides with a physiologically active conformation within the ensemble, this confers that efficacy upon the ligand. Shown are two different ligands. Ligand 1 activates $G_{\alpha s}$, $G_{\alpha i}$ and β-arrestin (but does not internalize receptors) while ligand 2 activates β-arrestin and causes receptor internalization

$$\Omega_A = 1 + \Omega\,\Sigma^A b_i p_i \quad (20)$$

$$\Omega_G = 1 + \Omega\,\Sigma^G b_i p_i \quad (21)$$

$$\Omega_{AG} = 1 + \Omega\,\Sigma^A b_i{}^G b_i p_i \quad (22)$$

where i refers to the specific conformational state and the superscripts A and G refer to the ligand and G protein-associated forms, respectively. Under these circumstances, macroaffinity is given by:

$$\text{Macroaffinity}\quad (K) \quad = {}^A k_0 \Omega_A (\Omega)^{-1} \quad (23)$$

where the interaction free energy between ligand and a reference microstate of the receptor is related to k_0 and efficacy is given by:

$$\text{Efficacy}\quad (\alpha) \quad = \quad (\Omega\Omega_{AG})(\Omega_A\Omega_G)^{-1} \quad (24)$$

This model has been used to simulate the effects of ligand binding on receptor ensembles and shows that ligand affinity is positively correlated with ligand efficacy [12]. These functions also can be used to describe biased signalling owing to the stabilization of different receptor ensembles that go on to activate different signalling cascades [37, 38].

Conclusions

It can be seen that there are most often differences between the potencies of agonists or antagonists measured in pharmacological experiments and the simple equilibrium dissociation constant of a drug–receptor complex defined by mass action. This occurs even with such apparently simple experiments such as radioligand binding of ligands to 7TMRs, where the apparent K_A value is really an amalgam of values describing the complex ensemble of receptors and their allosteric co-binding partners. However, the mass action reaction, defined many years ago, still forms the basis of all models describing drug action in pharmacology.

Competing Interests

The author has completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declares: no support from any organization for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years; no other relationships or activities that could appear to have influenced the submitted work.

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