Analytical Pharmacology: How Numbers Can Guide Drug Discovery

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ABSTRACT: The unique ways in which pharmacological data compares to mathematical models are described. Examples show that insights into agonist action (prediction of agonism *in vivo*) and antagonist mechanism of action (orthosteric vs allosteric) can be gained that assist in the candidate selection process for new drugs in drug discovery and development efforts. In addition, the impact of component processes on complex physiological systems can be delineated, such as the effects of the hepatic system on whole body clearance in pharmacokinetics and prediction of drug–drug interactions. Finally, models are instrumental in the procurement of universal drug parameters that can be used in medicinal chemistry-based structure–activity relationships. The revitalization of these ideas under the banner of “Analytical Pharmacology” may serve to re-emphasize these concepts over qualitative description and lead to a better foundation for drug discovery.

KEYWORDS: mathematical models, drug discovery, drug structure activity relationships, drug receptor theory

INTRODUCTION

There are two general concepts that underpin the pharmacology of drug action. The first is that drugs interact with variable physiology to yield different behaviors; that is, the same drug can produce different effects in different organ systems through interaction with the same receptor. The second is a corollary to the first idea, namely that what is needed to accurately characterize drugs as modifiers of physiology are scales that are system-independent, that is, that are not unique to the measuring system where they are obtained. This is because drugs are rarely if ever discovered and characterized in the systems in which they will be used; therefore, a scale must be available to predict activity in all therapeutic systems from data obtained in test systems. Pharmacological principles have been applied to provide models and scales to characterize drug activity for therapeutic predictions, and this essentially converts descriptive data (what we see in an experiment) into predictive data (what will occur in all systems). One of the first formalizations of these ideas was the creation of a subdiscipline articulated by Nobel Laureate Sir James Black as “Analytical Pharmacology” at the Department of Pharmacology, University College London, and later at the James Black Institute at Kings College, London.¹ This paper will illustrate how the precepts and principles defined by Analytical Pharmacology can yield quantitative data that elucidate mechanisms of drug action in the drug discovery and development process in a system independent manner.

The process of determining the mechanism of action of a drug and determining parameters for its activity for all systems involves comparing experimental dose response data to pharmacological models. The best models have rules which then can be used to quantify activity and make predictions for

drug behavior in other systems; the models with rules are mathematical equations that predict drug activity over a range of concentrations. The verisimilitude of observed data to model prediction is a major tool in pharmacology to define drug action. A major function of pharmacological models is to provide a capability to control and modify tissue sensitivity; this feature carries over into many applications of models to predict drug effect.

Volume Control: How Tissues Control Sensitivity to Agonism. One of the most complex and mysterious properties of drugs is their ability to activate cells repeatedly without a change in the drug itself, referred to as the process of “agonism”. However, whether a drug will or will not produce observable agonism is controlled by two factors: (1) the intrinsic efficacy of the agonist, a drug effect and (2) the sensitivity of the system. The best description of agonism available is the Black/Leff operational model,² and it is useful to describe tissue sensitivity to agonism through this model. The Black/Leff operational model is arguably one of the most important models in pharmacology since it provides a physiologically plausible model of pharmacological agonism that can yield predictive scales of agonist activity. According to this model, responses to agonist ($[A]$) is given by²

$$\text{response} = \frac{[A]^n \tau_A^n E_m}{[A]^n \tau_A^n + ([A] + K_A)^n} \quad (1)$$

where agonist affinity is given by the equilibrium-dissociation constant of the agonist–receptor complex (K_A) and the agonist efficacy is denoted τ_A . The Hill coefficient for the

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concentration curve is n , and E_m is the maximal window of response for the assay. The operational model essentially expresses tissue response as the product of the cell described as a virtual enzyme the substrate being the amount of agonist-receptor complex. Tissue sensitivity is incorporated in the efficacy term τ which is defined as $[R_T]/K_E$ where $[R_T]$ is the receptor density in the tissue and K_E the 'Michaelis-Menten constant for the cell as an enzyme converting the agonist receptor stimulus to cellular response. Tissue sensitivity can be altered either through changes in receptor density or the relative stoichiometry of the cytosolic components of the stimulus-response system in the cell (i.e., concentration of G protein, β -arrestin, etc.) which translates to a change in K_E . The effect of changing tissue sensitivity on agonist response is shown in Figure 1. The Black/Leff model is used as a repeated

Figure 1. Effect of changing sensitivity through changing receptor density and/or transduction efficiency of receptor stimulus on agonist concentration response curves. Open circles show the EC_{50} values (concentrations of agonist producing 50% maximal response to the agonist).

motif in other pharmacological models (i.e., the functional allosteric model, competitive, and noncompetitive antagonist model) to predict drug effect in tissues of varying sensitivity. Thus, tissue “volume control” can be adjusted in these models through adjustment of the value τ_A (*vide infra*). This model then can be used to reveal agonist behavior beyond that shown in a single experiment in a test system. One area where this is especially useful is in the differentiation between efficacy-dominant vs affinity-dominant agonists.

Efficacy- vs Affinity-Dominant Agonist Potency. Since there are no *a priori* rules to dictate the dependence of efficacy and affinity on chemical structure, the comparison of different agonists can be capricious if varying effects on efficacy and affinity are involved in agonism. This model can be used to illustrate the fundamentally different effects that changes in efficacy and affinity can have on concentration–response curves. Drugs that produce such observable physiological response (agonists) are characterized by two different scales; potency (EC_{50} , the molar concentration of agonist producing 50% maximal response) as the location parameter along the concentration axis of concentration–response curves and the maximal response to the agonist (a scale describing full to partial agonism). Agonist potency ratios (ratios of EC_{50} values) have long been used to quantify the relative activity of agonists in a system-independent manner but this scale cannot be used to compare full and partial agonists. This is because the potencies of full and partial agonists change differently with changes in the system sensitivity; therefore, potency ratios are not linear with system sensitivity when comparing these types of molecules. This problem can be eliminated by fitting the Black/Leff operational model of agonism² to the data. In fact, the Black/Leff operational model is the first available model to allow the comparison of full to partial agonism.

Figure 2. Efficacy- vs affinity-dominant agonism. (Left Panel) Concentration response curves to the muscarinic agonists carbachol (filled circles) and oxotremorine (open circles) in guinea pig ileum (“sensitive tissue”). After treatment with phenoxybenzamine to alkylate muscarinic receptors, this tissue is >99% less sensitive to muscarinic agonist stimulation. Top right panel shows the curves for carbachol in untreated (filled circles) and POB treated (filled triangles) tissue. Bottom right panel shows the curves for oxotremorine in untreated (open circles) and POB treated (open triangles) tissue. It can be seen that the same chemical treatment selectively eliminates the curve for oxotremorine. Analysis with the Black/Leff operational model shows that oxotremorine is an affinity-dominant and carbachol is an efficacy-dominant agonist. Data redrawn from ref 5.

Models can be useful to delineate the important factor in a multifactorial system yielding an observed effect; one of these is agonist potency. Specifically, agonist is the ratio of agonist affinity and efficacy in the form:⁵

$$EC_{50} = K_A / (1 + \tau_A) \quad (2)$$

It can be seen that a high potency can be achieved through a high affinity (low K_A value) or a high efficacy (high τ_A value).⁴ While it would not be evident which of these factors is the important feature from a single estimate of potency (EC_{50}) for any full agonist, the effect of tissue sensitivity on agonism would identify the important dominant driver of agonist potency and this, in turn, would predict important features of the agonism that would be produced *in vivo*. Specifically, if agonist potency is dominated only by high affinity, then the agonist will be more subject to differences in tissue sensitivity than if efficacy is the dominating factor. For example, Figure 2 shows the effects of two agonists, oxotremorine and carbachol in a sensitive tissue; it can be seen that oxotremorine is 3-fold more potent than carbachol but both agonists produce the full maximal response.⁵ However, when the sensitivity of the tissue is reduced (in this case through chemical alkylation of the receptors), then in the less sensitive tissue, carbachol still yields an agonist response while the response to oxotremorine, the more potent agonist, disappears. This is because oxotremorine potency is dominated by high affinity while the potency of carbachol is dominated by high efficacy. The point of this discussion is that if only a relative effect in a high sensitivity assay was used to determine agonism, then both agonists appear to be identical. However, *in vivo*, where both agonists would encounter a wide range of tissue sensitivities, the effects will be quite different due to the fact that each agonist is dominated by a different pharmacological property. Specifically, the affinity-dominant agonist (oxotremorine) would show more tissue-dependent variation in agonism than the efficacy-dominant (carbachol) agonist. Fitting data to the operational model is a way of delineating the dominating factors in the agonism of oxotremorine and carbachol to predict the relative robustness of the agonism of each agonist to different nuances in tissue sensitivity. By modifying τ_A in the Black/Leff operational model ("volume control") agonism can be simulated under different conditions of tissue sensitivity to make these predictions.

In general, fitting the Black/Leff model to agonist concentration–response data can isolate differences in efficacy and/or affinity. The prediction of agonism *in vivo* can be a difficult process since the production of visible response in test systems is linked to both the efficacy of the agonist and the sensitivity of the test system. As with the example showing the dichotomous behavior of carbachol and oxotremorine, it can be difficult to predict whether a low efficacy agonist may produce response *in vivo* or function as an antagonist. For instance, the low efficacy β -adrenoceptor ligand prenalterol produces nearly an 80% maximal response in guinea pig right atria but functions as a silent β -blocker in the less sensitive *extensor digitorum longus* muscle of the guinea pig.⁶ The Black/Leff operational model can be very useful in this setting as it allows a quantification of agonist efficacy (as a ratio of a known standard, often the natural agonist) which can then be used to scale new test agonists. For example, if it is known that a defined weak agonist produces a low level response in humans *in vivo*, then that agonist can function as a scale for all new agonist candidates; that is, it can be assumed that any new

agonist with an operational model efficacy greater than the known weak agonist will produce response in humans as well.

The multiparameter nature of potency observations such as the EC_{50} also can obscure valuable structure–activity trends for medicinal chemists. Specifically, since the EC_{50} is a ratio of affinity and efficacy (see eq 2) then divergent trends in affinity and efficacy can mask effects of changing structure on agonism. Figure 3 shows a simulation for four compounds for which

Figure 3. Simulation of agonist potency for four agonists labeled CMPD 1 to 4. These agonists are assigned affinities and efficacies to yield pEC_{50} values according to eq 2. The values are chosen to show that in a series of agonists with decreasing efficacy and respectively increasing affinity, the resulting pEC_{50} values belie any difference between the agonists.

changes in chemical structure produce no change in agonist potency (pEC_{50}). However, a dissection of the respective affinities and efficacies for these agonists shows a rich structure activity relationship of changing affinity and efficacy with changing chemical structure. This illustrates the value of converting descriptive data to predictive data and obtaining pharmacological parameters through comparison to models.

Antagonist Selectivity and Mode of Action. Pharmacological models also can provide insights into antagonist behavior. For example, Schild analysis⁷ can provide a unique window into drug–receptor interactions that can address the question of antagonist specificity. Schild analysis yields a predicted behavior of a receptor system when a simple competitive antagonist and a receptor interact according to mass action kinetics. Thus, antagonist data can be analyzed by comparing the shifts to the right of the dose response curves to the Schild model for simple competitive antagonism in the form of the Schild equation:⁷

$$\log(DR - 1) = \log[B] + pK_B \quad (3)$$

where DR is the dose ratio for EC_{50} concentrations of agonist in the presence and absence of antagonist, [B] is the antagonist concentration, and pK_B is the negative log equilibrium dissociation constant of the receptor–antagonist complex. It can be seen that eq 3 is an equation for a straight line having a slope of unity. If this is not observed in a particular system then it indicates that another process interferes with the antagonism. One such process could be the interaction of the agonist with a heterogeneous receptor population; this may be important to determine for the prediction of therapeutic antagonism by the antagonist.⁸

Another application of Schild analysis is in the differentiation of orthosteric competitive binding from allosteric binding; that is, do two antagonists compete for the same binding site as the agonist? This can be done with a procedure termed "resultant analysis".⁹ This employs the construction of a Schild plot from Schild plots whereby the addition of one antagonist ("test" antagonist) to a system provides an analysis of the potency of another antagonist ("reference" antagonist) to produce a

Figure 4. Effects of a PAM agonist ($\alpha = 10$, $\beta = 10$, $\tau_B = 0.0033 \tau_A$, $K_B = 1 \mu\text{M}$) in functional systems of three different sensitivities. (A) Curves calculated with eq 5 with $\tau_A = 500$, $K_A = 1 \mu\text{M}$; responses in absence (solid line) and presence (dotted line) of 100 nM PAM agonist. Sensitization with no effect on maximal response is observed along with direct agonism from the PAM agonist. (B) Same conditions in system with 10% of the receptors as the system in panel A (90% reduction in receptors). Sensitization with no effect on basal or maximal responses observed. (C) Same conditions but in a less sensitive system (0.2% of the receptors as the system in panel A); less sensitization and an increased maximal response is observed.

predicted dextral displacement of the Schild plot of the reference antagonist. These displacements are then used to construct a resultant plot, the intercept of which should be the potency of the test antagonist. The value of this method is that it is very sensitive to aberrations of competitive binding and can differentiate competitive orthosteric binding from allosteric binding.¹⁰

Determining Parameters To Quantify Drug Action. A primary function of pharmacology is to concisely capture drug activity in regular numerical scales so that medicinal chemists may link changes in the structure of molecules to their biological activity. Pharmacological models can be extremely useful in this regard since they can summarize complex behaviors. One example of this is the functional allosteric model which can reduce complex allosteric effects of modulators to universal parameters. Allosteric modulators bind to receptors and enzymes to modify their tertiary conformation and essentially change their very nature. This is seen as changes in the binding affinity and effects of ligands as they bind to the protein. For example, changes in the responses to agonists by allosteric modulators can be described by an amalgam model of the Stockton/Ehlert allosteric binding model^{11,12} and the Black/Leff operational model² to yield:^{13–15}

$$\text{response} = \frac{([A]/K_A \tau_A (1 + \alpha\beta[B]/K_B) + \tau_B[B]/K_B) E_m}{[A]/K_A (1 + \alpha[B]/K_B + \tau_A (1 + \alpha\beta[B]/K_B) + [B]/K_B (1 + \tau_B) + 1} \quad (4)$$

Where the allosteric modulator [B] has a receptor–ligand equilibrium dissociation constant K_B and a possible direct efficacy τ_B . The modulator changes the affinity of the agonist by a cooperativity factor α and changes the efficacy of the agonist by a factor β . Fitting concentration–response curves to this model can concisely summarize the activity of modulators and greatly simplify structure–activity relationships. For example, Figure 4 shows the effects of a positive allosteric modulator agonist (PAM agonist) on the responses to an agonist in three different systems of varying sensitivity. It can be seen that different effects are seen in each system as a result of the same PAM agonist and it is difficult to reconcile these effects from one single mechanism across systems. In sensitive tissues (Figure 4A), the PAM agonist produces a 16% direct agonist response and a 10-fold sensitization to the agonist; in a less sensitive system with 90% fewer receptors (Figure 4B) the

PAM agonist produces no agonist response and a 10-fold sensitization to the agonist while in an even less sensitive tissue the PAM agonist produces a 100% increase in maximal response and little sensitization (Figure 4C). Therefore, depending on the sensitivity of the system, the scale for PAM agonist activity is variable. However, these diverse effects can be quantified by a single set of molecular parameters from the functional allosteric model which fits all of the data with the allosteric parameters $\alpha = 10$, $\beta = 10$, $\tau_B = 0.33\tau_A$, and $K_B = 1 \mu\text{M}$. Thus, irrespective of the system used, medicinal chemists have a unified scale to characterize the activity of the PAM agonist.

As discussed previously, the Black/Leff operational model can be used to quantify the affinity and efficacy of agonists but recent concepts relating to the biased signaling of receptors has added a new dimension to these types of analyses. Specifically, it has been shown that agonists stabilize different and unique active state conformations of receptors and when this occurs, different agonists emphasize different signaling mechanisms coupled to a given receptor. Under these circumstances, the affinity and efficacy of the agonist for each of these pleiotropic signaling pathways can be quantified with the Black/Leff operational model (with indices such as $\Delta\log(\tau/K_A)$) and compared to give estimates of the bias of any given agonist for a set of signaling pathways through values of $10^{\Delta\log(\tau/K_A)}$.¹⁶

Using Quantification To Determine Mechanism of Drug Action. Quantitative pharmacological models can assist in the determination (or confirmation) of drug mechanism of action. For instance, as seen in Figure 4, allosteric modulators can produce complex effects on agonist response. In the example shown, the agonist concentration–response curves can be fit to eq 4 to yield the parameters α , β , K_B , and τ_B . It can also be seen that eq 4 is a stringent standard relating the location and maxima of the concentration curves to the concentration of allosteric modulator. This stringency can be used as a test of drug mechanism or pharmacological assay. For example, a profile of direct agonism and sensitization of agonist response can be demonstrated for the muscarinic receptor PAM agonist BQCA^{17,18} both in muscarinic M1 receptor-mediated calcium transient or inositol phosphate hydrolysis (IP1) assays. However, whereas a consistent fit to the model to yield a single set of α , β , K_B , τ_B values can be obtained with IP1 data, no consistent fit can be seen with the calcium data thereby illustrating the hemiequilibrium nature of the calcium effect and how it cannot adequately capture the allosteric PAM

agonist properties of BQCA. Failure to adhere to the quantitative criteria of the model indicates that the calcium concentration response data were inaccurate for determining either mode of action or quantitative activity for BQCA.¹⁹

Models can be used to further characterize drug action. For instance, when an effect such as receptor noncompetitive antagonism is observed for an antagonist, it may be due to an orthosteric (pseudoirreversible persistent receptor occupancy by the antagonist) or allosteric mechanism. Extending the concentration–response data and fitting the data to models can reveal valuable insights to guide drug candidate selection. A special type of allosteric antagonism is produced by molecules called PAM antagonists (PAM effects increasing agonist affinity through α and NAM effects through effects on efficacy, β) and these have special properties that can make them exceptional blockers of agonism *in vivo*.²⁰ Thus, a fit of the concentration response curves to an agonist obtained in the absence and presence of a range of concentration of PAM antagonist can show a subtle diminution of the agonist EC₅₀ as antagonism takes place which is unique and not seen with any other mechanism of receptor antagonism (see example for the PAM antagonist ifenprodil in Figure 5). This translates to an

Figure 5. Effects of PAM antagonists. (A) Concentration response curves to NMDA in the absence (filled circles) and presence of the PAM antagonist ifenprodil (0.1 μM, open circles) and (1 μM, filled triangles). Note the diminishing maximal response and concomitantly diminishing EC₅₀ values for NMDA. (B) Dose ratios (DR, log scale) for agonist concentration response curves produced by various antagonists as a function of the concentrations of antagonist (log scale). All antagonists produce increases in the DR values as they produce antagonism except noncompetitive antagonists in low receptor reserve systems which produce DR values = 1. Only PAM antagonists actually produce fractional DR values (see diminution of EC₅₀ values for ifenprodil in panel A) as shown with the dotted line. Open circle values from ifenprodil effects shown in panel A. Data redrawn for ifenprodil from ref 21.

actual increase in potency of the antagonist with increased concentration of agonist through a positive cooperativity effect; that is, the agonist increases the affinity for the antagonist.²¹ Thus, ifenprodil “seeks and destroys” agonist-bound receptors to selectively reverse *in vivo* agonism. If a new antagonist candidate molecule is identified as having PAM antagonist properties, then a number of beneficial advantages for *in vivo* activity are predicted. For example, the 5-HT₃ receptor PAM antagonist palonosetron used for the treatment of nausea possesses an inordinately long *t*_{1/2} resulting in favorable target coverage. This is due to the increased affinity of palonosetron for the receptor in the presence of *in vivo* ambient levels of 5-HT in the brain.^{22,23}

Quantitative pharmacological models also can be used to unveil hidden favorable effects of other drugs such as positive

allosteric modulators (PAMs) aimed at revitalizing failing physiological systems. As discussed previously, functional allosteric models quantify separate effects of PAMs on agonist affinity (α -cooperativity) and efficacy (β -effects). Usually in systems testing PAM effects on natural neurotransmitters and hormones, these agonists are full agonists since they naturally are of high efficacy. In such systems, β -effects cannot be differentiated from α -effects since β -effects are revealed through increases in maximal agonist response and no change in the maximal response to high efficacy agonists can be seen (see Figure 5A,B). However, differentiation of these mechanisms may be critical to the assessment of the therapeutic potential of a PAM aimed to revitalize a failing system since an increased affinity will do little if there is little signal present (see Figure 6). An increase in the efficacy of the residual signal remaining is required therefore β -PAMs are preferred over α -PAMs. Manipulation of the therapeutic assay system can be done to reveal β -PAM activity through a reduction of assay sensitivity. For example, chemical alkylation of muscarinic receptors with the β -haloalkylamine phenox-ybenzamine (POB) reduces the maximal response to the normally full agonist acetylcholine to then allow the PAM amiodarone to demonstrate a positive β -effect (increased efficacy of acetylcholine)²⁴ (see Figure 6). This technique has also been shown to be extremely useful in delineating effects on affinity vs efficacy for PAMs and NAMs as shown in studies on the allosteric effects of the modulators ML380 and ML375 on muscarinic M5 receptors.^{25,26} It should be noted that the key variable in these types of experiments is the alteration of receptor density therefore any means that can achieve this, such as inducible receptor expression systems, also would be amenable. The functional allosteric model then can be used to quantify the relative α and β effects of PAMs to assess preferred effects on efficacy for therapeutic exploitation.

“Test Driving” Models To Reveal Drug Activity. There are two principles that help guide the effective use of quantitative models in pharmacology: (1) use as many probes as possible as the stimuli for the system and (2) use the model to express a wide range of different variables for the system, that is, concentration. These two ideas have been exemplified in studies of allosteric vs orthosteric drug function (see Figure 7A). This can be important in drug discovery efforts as allosteric antagonists can resemble orthosteric antagonists in many *in vitro* settings yet display different properties *in vivo*. A useful practice is to test the model with as many active probes as possible, and in the case of pharmacological models this amounts to testing as many agonists and/or radioligands as is practical. This has special relevance to the determination of mode of action of drugs. Specifically, receptor antagonists may block the effects of agonists either through competition with the agonist binding site (antagonism occurs when the antagonist binds to the agonist binding site and precludes agonist binding) which is referred to as an orthosteric mode of action, or it may bind to another site on the receptor to modify the protein conformation and thus inhibit either agonist binding or function to achieve blockade (referred to as allosteric function). A unique property of allosteric systems is the variability different allosteric molecules may have on the protein structure that can then translate into variability in the antagonism of different probes such as agonists and radioligands. This is in contrast to orthosteric systems which show uniformity in antagonism of all agonists; that is, it does not matter what agonist or radioligand is precluded from binding,

Figure 6. Effects of PAMs on agonist affinity and efficacy. (Top panel) Simulation of the effects of a PAM that only increases the affinity of the agonist (α PAM) and one that increases only the efficacy of the agonist (β PAM). (Bottom left) Effects of the muscarinic receptor PAM amiodarone on responses to acetylcholine. Curve shown for acetylcholine in the absence (filled squares) and presence (open squares) of amiodarone ($30\ \mu\text{M}$). (Bottom right) After alkylation of a portion of the muscarinic receptors with phenoxybenzamine (POB, $1\ \mu\text{M}$), the sensitivity of the system to acetylcholine is reduced and it is a partial agonist (filled circles). Amiodarone reveals its β -PAM character by increasing the efficacy of acetylcholine such that an increased maximal response is observed (open squares). Data redrawn from ref 24.

Figure 7. Definitive features of allosteric receptor systems. Panel A shows the mode of action of orthosteric antagonists (antagonist and agonist compete for the same binding site on the receptor) and allosteric antagonists (allosteric antagonist binds to its own site on the receptor and affects agonist binding through a change in conformation of the receptor). (Panel B) Probe dependence. While the allosteric antagonist aplaviroc blocks the binding of the chemokine [^{125}I]-MIP-1 α , it has no effect on the binding of the chemokine [^{125}I]-RANTES. Data redrawn from ref 27. (C) Saturation of effect. The allosteric antagonist C7/3'-phth produces dextral displacement of concentration response curves to acetylcholine in a manner identical to a competitive antagonist (to yield a linear Schild regression) until concentrations greater than $10\ \mu\text{M}$ which saturate the allosteric binding site for C7/3'-phth; this causes a cessation to the blockade and a curvilinear saturation of the Schild regression for C7/3'-phth. Data redrawn from ref 28.

the potency of the antagonist will be the same. This property of allosteric systems is called probe dependence and it can be seen in effect with the blockade of chemokine radioligands to the CCR5 receptor and the interaction of these with the allosteric HIV-1 entry inhibitor aplaviroc.²⁷ As seen in Figure 7B, while aplaviroc blocks the binding of the chemokine [^{125}I]-CCL3, it does not block the alternative probe chemokine

[^{125}I]-CCL5; this is behavior not consistent with orthosteric binding and thus identifies aplaviroc as an allosteric antagonist.

The saturation of effect is another property of allosteric systems not shared by orthosteric systems. Orthosteric competitive antagonism predicts a linear regression of the degree of antagonism (i.e., Schild regressions as predicted by eq 3- [7]). As shown in Figure 7C, the muscarinic receptor antagonist C7/3'pth provides such a regression when tested

Figure 8. PK–PD modeling of drug–drug interactions *in vivo*. The “Net Effect Model” creates a system where reversible, irreversible (time-dependent, TDI) enzyme inhibition can occur with concomitant induction of hepatic liver enzymes. Curves to a “perpetrator” drug interacting with a previously prescribed “victim” drug show the changes in the exposure (area under the curve, AUC) of the victim drug as a range of concentrations of the perpetrator drug are added to the system. Top panel shows the model and the component separate equations determining the various processes for reversible enzyme inhibition (A), time dependent enzyme inhibition (B), and enzyme induction (C). (Panel A) Parameters entered to simulate reversible enzyme inhibition (solid line curve) vs time-dependent enzyme inhibition (dotted line); it can be seen that the model predicts similar but more sensitive effects to the perpetrator with time-dependent inhibition. (Panel B) Simulation for reversible enzyme inhibition (broken line) and added enzyme induction (solid line) showing the offsetting effects of enzyme induction. (Panel C) Simulation for reversible enzyme inhibition at the hepatic level (broken line) and with added enzyme inhibition in the GI tract during drug absorption (i.e., Cyp 3A4 inhibition in human GI tract)- solid line. The potentiating effect of enzyme inhibition during absorption is illustrated.

at concentrations ranging from 0.3 μ M to 10 μ M and for this range of concentration testing C7/3'ptph appears to be an orthosteric competitive antagonist. However, extension of the concentrations tested from 30 μ M to 300 μ M clearly shows a deviation from this linear regression and, in fact, adheres to the model for allosteric surmountable antagonism. This limiting antagonism results from the saturation of the allosteric binding site, an effect that is not operative for competing orthosteric ligands. Further experiments confirmed the allosteric nature of C7/3'ptph illustrating the value of extending the concentration range for the test.²⁸

Putting Individual Processes into an *in Vivo* Physiological Context. Another valuable application of quantitative comparison of data to models is the evaluation of the impact of a single component process in a complex system. In pharmacokinetics, hepatic drug clearance is an important process whereby drugs are metabolized to polar products that are eventually excreted by the renal system *in vivo*. While the effects of the hepatic system can be estimated *in vitro* with the Michaelis–Menten enzyme equation, the impact on clearance *in vivo* is also determined by the hepatic blood flow; these considerations are incorporated in Rowland's hepatic clearance equation:²⁹

$$\text{clearance} = Q \times \left[\frac{f_u \text{CL}_i}{Q + f_u \text{CL}_i} \right] \quad (5)$$

where Q is liver blood flow, CL_i is intrinsic hepatic clearance (given by the Michaelis–Menten equation to simulate the liver

as an enzyme) and f_u the fraction of drug not plasma protein bound. The power of this equation is that it helps interpret the effect of hepatic metabolism of a drug under different pathological conditions. For example, a drug with low intrinsic hepatic clearance (4.2 L/h) will have a whole body hepatic clearance according to Rowland's equation of 4.0 L/h. In cardiovascular patients who might have a 50% reduction in liver blood flow, the clearance for this drug will be minimally affected ($\text{CL} = 3.83$ L/h; a 4% change). However, in patients with compromised hepatic function (i.e., cirrhosis) which may reduce CL_i by 50%, the effect on whole body hepatic clearance is large ($\text{CL} = 2.05$ L/h; a 50% decrease). A reverse effect is seen for a drug with high hepatic clearance (flow limited clearance). For a drug with $\text{CL} = 1500$ L/h the whole body clearance in healthy volunteers is 84.6 L/h; in cardiovascular patients (50% reduction in Q) this clearance is severely decreased ($\text{CL} = 43.7$ L/h; a 48% decrease) while in liver disease patients clearance is minimally affected ($\text{CL} = 80.1$ L/h; a 5% decrease). Thus, Rowland's equation puts the isolated estimate of the hepatic metabolism into a physiological context where it can be seen to have varying importance depending on the state of the physiology.

This concept is extended in pharmacokinetic-pharmacodynamic models that include other mechanisms *in vivo*. For example, a comprehensive *in vivo* model for drug–drug interactions (DDI) due to reversible, time-dependent hepatic enzyme inhibition and hepatic enzyme induction is the Net Effect Model (also known as the Mechanistic Static Model).³⁰ As shown in Figure 8, the changes in the exposure (referred to

as the AUC, expressed as the area under the curve of the plasma-time curve *in vivo*) for a “victim” drug in the presence of another “perpetrating” drug producing an effect on hepatic clearance is a complex function of increased drug levels due to enzyme inhibition and decreased drug levels due to enzyme induction. As shown in Figure 8A, a time-dependent (irreversible) hepatic enzyme inhibition increases the sensitivity of the DDI with concentration of the perpetrator drug larger than that seen with a reversible enzyme inhibitor. Figure 8B shows the ameliorating effect of concomitant enzyme induction and inhibition by the perpetrating drug. Figure 8C shows the dramatic increase in DDI when the enzyme inhibition occurs both in the liver and the GI tract (such as inhibition of the cytochrome P450 enzyme CYP3A4 which is present both in the human liver and the GI tract). The point of these simulations is that the holistic effect of individual processes measured with *in vitro* assays on the *in vivo* pharmacokinetics of a drug can be visualized with quantitative models to assist in evaluating the importance of the individual processes.

CONCLUSIONS

Since the early publications of Clarke,^{31,32} pharmacology has been rooted in quantitative models and these have been used to generate numbers to guide medicinal chemists and biologists. However, many of these techniques have been reduced to descriptions of qualitative behaviors of drugs. For example, the strict agreement between the need for agonists to have the same potency ratios for the same receptor (in the process of receptor identification) has commonly devolved into the need for the same “rank order of potency”. This devalues the procedure since agonists may have the same rank order of potency but still have different quantitative potency ratios; this would identify different, not similar, receptors for the agonists. The renewed rigorous adherence to the numbers generated from pharmacological models was reintroduced into pharmacology by James Black and others and spawned the moniker “Analytical Pharmacology” as a subdiscipline. The re-emphasis of numerical scales has coincided with a Renaissance into new information describing drug and receptor mechanisms; it is anticipated that this will form a happy confluence resulting in an increase in new therapeutic drugs.

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Notes

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REFERENCES

- (1) Kenakin, T., and Christopoulos, A. (2011) Analytical pharmacology: the impact of numbers on pharmacology. *Trends Pharmacol. Sci.* 32, 189–96.
- (2) Black, J. W., and Leff, P. (1983) Operational models of pharmacological agonism. *Proc. R. Soc. Lond. B Biol. Sci.* 220, 141–162.
- (3) Black, J. W., Leff, P., Shankley, N. P., and Wood, J. (1985) An operational model of pharmacological agonism: the effect of $E/[A]$ curve shape on agonist dissociation constant estimation. *Br. J. Pharmacol.* 84, 561–571.
- (4) Kenakin, T. P. (1984) The relative contribution of affinity and efficacy to agonist activity: organ selectivity of noradrenaline and oxymetazoline with reference to the classification of drug receptors. *Br. J. Pharmacol.* 81 (1), 131–41.
- (5) Kenakin, T. (1997) *Pharmacologic Analysis of Drug-Receptor Interaction*, 3rd ed., pp 1–491, Lippincott-Raven.
- (6) Kenakin, T. P. (1985) Prenalterol as a selective cardiostimulant: differences between organ and receptor selectivity. *J. Cardiovasc. Pharmacol.* 1, 208–210.
- (7) Arunlakshana, O., and Schild, H. O. (1959) Some quantitative uses of drug antagonists. *Br. J. Pharmacol. Chemother.* 14, 48–58.
- (8) Kenakin, T. P. (1992) Tissue response as a functional discriminator of receptor heterogeneity: effects of mixed receptor populations on Schild regressions. *Mol. Pharmacol.* 41, 699–707.
- (9) Black, J. W., Gerskowitch, V. P., Left, P., and Shankley, N. P. (1986) Analysis of competitive antagonism when this property occurs as part of a pharmacological resultant. *Br. J. Pharmacol.* 89, 547–555.
- (10) Kenakin, T., and Boselli, C. (1989) Pharmacologic discrimination between receptor heterogeneity and allosteric interaction: resultant analysis of gallamine and pirenzepine antagonism of muscarinic responses in rat trachea. *J. Pharmacol. Exp. Ther.* 250, 944–52.
- (11) Stockton, J. M., Birdsall, N. J., Burgen, A. S., and Hulme, E. C. (1983) Modification of the binding properties of muscarinic receptors by gallamine. *Pharmacol.* 23, 551–557.
- (12) Ehlert, F. J. (1988) Estimation of the affinities of allosteric ligands using radioligand binding and pharmacological null methods. *Mol. Pharmacol.* 33, 187–194.
- (13) Kenakin, T. (2005) New concepts in drug discovery: collateral efficacy and permissive antagonism. *Nat. Rev. Drug Discovery* 4, 919–927.
- (14) Ehlert, F. J. (2005) Analysis of allosterism in functional assays. *J. Pharmacol. Exp. Ther.* 315, 740–754.
- (15) Price, M. R., Baillie, G. L., Thomas, A., Stevenson, L. A., Easson, M., Goodwin, R., McLean, A., McIntosh, L., Goodwin, G., Walker, G., et al. (2005) Allosteric modulation of the cannabinoid CB1 receptor. *Mol. Pharmacol.* 68, 1484–1495.
- (16) Kenakin, T. P., Watson, C., Muniz-Medina, V., Christopoulos, A., and Novick, S. (2012) A simple method for quantifying functional selectivity and agonist bias. *ACS Chem. Neurosci.* 3, 193–203.
- (17) Ma, L., Seager, M. A., Wittmann, M., Jacobson, M., Bickel, D., Burno, M., et al. (2009) Selective activation of the M1 muscarinic acetylcholine receptor achieved by allosteric potentiation. *Proc. Natl. Acad. Sci. U. S. A.* 106, 15950–15955.
- (18) Mistry, S. N., Jorg, M., Lim, H., Vinh, N. B., Sexton, P. M., Capuano, B., Christopoulos, A., Lane, J. R., and Scammells, P. J. (2016) 4-Phenylpyridin-2-one derivatives: A novel class of positive allosteric modulator of the M1 muscarinic acetylcholine receptor. *J. Med. Chem.* 59, 388–409.
- (19) Bdioui, S., Verdi, J., Pierre, N., Trinquet, E., Roux, T., and Kenakin, T. (2018) Equilibrium Assays Are Required to Accurately Characterize the Activity Profiles of Drugs Modulating Gq-Protein-Coupled Receptors. *Mol. Pharmacol.* 94, 992–1006.
- (20) Kenakin, T., and Strachan, R. T. (2018) PAM-Antagonists: A Better Way to Block Pathological Receptor Signaling? *Trends Pharmacol. Sci.* 39, 748–765.
- (21) Kew, J. N. C., Trube, G., and Kemp, J. A. (1996) A novel mechanism of activity-dependent NMDA receptor antagonism describes the effect of ifenprodil in rat cultured cortical neurons. *J. Physiol.* 497, 761–772.
- (22) Lummis, S. C., Sepulveda, M. I., Kilpatrick, G. J., and Baker, J. (1993) Characterization of [3 H]meta-chlorophenylbiguanide binding to 5-HT $_3$ receptors in N1E-115 neuroblastoma cells. *Eur. J. Pharmacol.* 243 (1993), 7–11.
- (23) Saito, M., and Tsukuda, M. (2010) Review of palonosetron: emerging data distinguishing it as a novel 5-HT $_3$ receptor antagonist for chemotherapy-induced nausea and vomiting. *Expert Opin. Pharmacother.* 11, 1003–1014.
- (24) Stahl, E., Elmslie, G., and Ellis, J. (2011) Allosteric Modulation of the M3Muscarinic Receptor by Amiodarone and N-Ethylamiodarone: Application of the Four-Ligand Allosteric Two-State Model. *Mol. Pharmacol.* 80, 378–388.

- (25) Berizzi, A. E., Bender, A. M., Lindsley, C. W., Conn, P. J., Sexton, P. M., Langmead, C. J., and Christopoulos, A. (2018) Structure–Activity Relationships of Pan-Gαq/11 Coupled Muscarinic Acetylcholine Receptor Positive Allosteric Modulators ACS Chem. ACS Chem. Neurosci. 9, 1818–1828.
- (26) Berizzi, A. E., Gentry, P. R., Rueda, P., Den Hoedt, S., Sexton, P. M., Langmead, C. J., and Christopoulos, A. (2016) Molecular Mechanisms of Action of M5Muscarinic Acetylcholine Receptor Allosteric. Mol. Pharmacol. 90, 427–436.
- (27) Watson, C., Jenkinson, S., Kazmierski, W., and Kenakin, T. (2005) The CCR5 receptor-based mechanism of action of 873140, a potent allosteric noncompetitive HIV entry inhibitor. Mol. Pharmacol. 67, 1268–82.
- (28) Lanza, A., Christopoulos, A., and Mitchelson, F. (1996) Interactions of agonists with an allosteric antagonist at muscarinic acetylcholine M2 receptors. Eur. J. Pharmacol. 316, 27–32.
- (29) Rowland, M., and Tozier, T. N. (1995) *Clinical Pharmacokinetics: Concepts and Applications*, pp 1–727, Lippincott.
- (30) Huang, S. M., and Rowland, M. (2012) The Role of Physiologically Based Pharmacokinetic. Clin. Pharmacol. Ther. 91, 542–549.
- (31) Clark, A. J.. (1933), *The mode of action of drugs on cells*, Edward Arnold, London.
- (32) Clark, A. J.. (1937) *General pharmacology*, Springer, Berlin.