

2

CHAPTER

Simple Ant-agonism – Simple Intervention

2.1. Classical Ant-agonism

2.1.1. Towards Intervention

By introducing a secondary ligand as well as a secondary binding site, Chapter 2 examines inhibitors, also called ant-agonists. We shall deal with several aspects of a second ligand interfering with the binding of a primary ligand and/or with the function induced by a primary ligand. Inhibitory processes may be competitive or non-competitive. Competitive ant-agonism only needs a single binding site, whereas non-competitive ant-agonism requires at least two binding sites. Un-competitive ant-agonism is a special case of the non-competitive reaction scheme. These concepts are dealt with in Sub-chapter 2.4. Meanwhile, the interaction between two ligands and a receptive unit may also involve a cross-correlation, as exemplified in a so-called ‘intervention’ model described in the following section and detailed further in Sub-chapter 2.5.

2.1.2. The Intervention Scheme

Having introduced a secondary site and a secondary ligand, we must also operate with a possible *interaction* between the two binding sites as ligands bind. The *interaction* may be stimulatory or inhibitory for the subsequent processes of binding or function. Here, the *interaction* is expressed in a single system constant c (Fig. 2.1A). Constant c above unity is stimulatory, while below unity it is inhibitory. At present, in channel studies this type of interaction may be resolved into 18-system constants (Burzomato et al. 2004; Colquhoun 2006).

In order to cover both the stimulatory as well as the inhibitory aspect of *interaction* in a two-ligand/two-site reaction scheme (TLTS) as in Fig. 2.1A, and at the same time clearly differentiate it from the allosteric type of

reaction scheme, which involves a TLTS with additional two-states and spontaneous activity (see Chapters 7 and 15), I have decided to designate the one-state TLTS reaction scheme in Fig. 2.1A – with binding site *interaction* but without spontaneous activity – as the *intervention* model. In this scheme, ligands participating in changing the binding or function of primary ligands will be referred to as *interventors*, reserving the term ‘modulators’ for secondary ligands in allosteric reaction schemes with two-sites and two-states (Chapter 14). The *intervention* model is depicted in Fig. 2.1A together with a simple *two-state* model with a single binding site shown in Fig. 2.1B, an allosteric model.

Derivation of formulas for the *intervention* model in enzymology started early with Michaelis (1914) and continued in the 1950s (Table 2.1) (Laidler & Socquet 1950; Segal et al. 1952; Botts & Morales 1953; Friedenwald & Maengwyn-Davies 1954a,b, pp. 154–190; Ariëns et al. 1955; Laidler 1956, 1958, pp. 78–93; Webb 1963, pp. 54–60; Cleland 1963; Hijazi & Laidler 1973; Laidler & Bunting 1973; Cha 1975).¹

In enzymology, intervention reaction schemes with negative interaction are mostly listed under the term ‘mixed-inhibition’ or just ‘non-competitive’ (Webb 1963; Cha 1975; Segel 1975, 1993, Chapter 4; Cornish-Bowden 1995, 2004; Varon et al. 2002), while a stimulatory aspect in the intervention models is ill-fated as it is hardly observed, and therefore only sporadically dealt with as ‘non-competitive synergism’, ‘partial non-competitive’, or ‘mixed activation’ (Ariëns et al. 1964; Mahler & Cordes 1966, p. 252; Segel 1975, 1993, Chapter 5; Cornish-Bowden 1995, 2004). A more direct approach to the *intervention* model is the so-called

¹ Laidler (1956, 1958) operated with *simple non-competitive* reactions, which in this book are equal to non-competitive reactions, and *non-competitive* reactions equal the present *intervention* scheme.

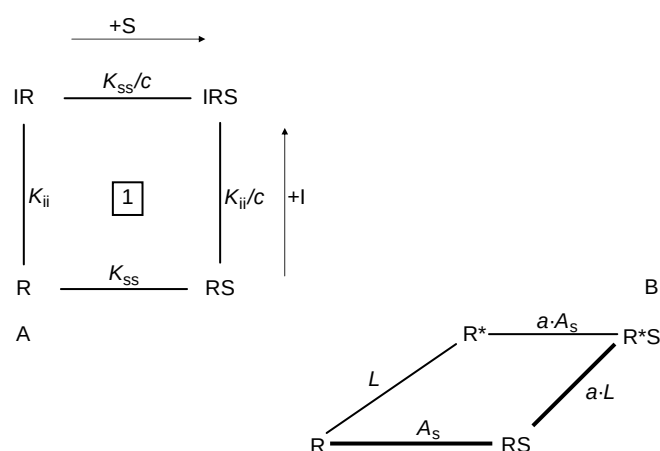


Figure 2.1. Two simple reaction schemes. (A) The **Intervention model** where an interaction constant c may be (a) zero equal to ‘competitive inhibition’, (b) unity equal to ‘non-competitive inhibition’, (c) above zero and below unity equal to ‘negative intervention’ and above unity equal to ‘positive intervention’. Although the intervention model has two binding sites, it is not an allosteric model, it is a pro-allosteric model. Allostery further requires un-liganded activity, the same as spontaneous activity (see Chapter 15). (B) The simple **two-state model** with a single binding site. This model is also referred to as the cyclic two-state model with spontaneous activity (Chapter 5). This two-state model is not an allosteric model. Allostery also requires at least two binding sites (see Chapter 15). S = primary ligand, I = interventor, K_{ss} = dissociation constant for S at primary s -site, and K_{ii} = dissociation constant for interventor ligand at intervention i -site.

“general modifier mechanism of Botts and Morales” and its formulation (Botts & Morales 1953; Garzia-Canoves et al. 1987; Topham & Brocklehurst 1992; Shou et al. 1999, Shou 2002; Varon et al. 2002).

In pharmacology, the intervention reaction schemes with site *interaction* were formulated by De Lean and co-workers (De Lean et al. 1980; Burgisser et al. 1982; Wreggett & De Lean 1984) as the ternary complex-model (TCM), specifically related to G protein coupled receptors. More recently, the TCM has appeared as an ‘allosteric’ TCM (Ehlert 1988; Christopoulos 2002; Christopoulos & Kenakin 2002; Avlani et al. 2004; Kenakin 2004a, Section 6.8.7, 2004b). Although terms including ‘allosteric’ have been sorted out (May & Christopoulos 2003; May et al. 2004, 2007a), a term such as ‘allosteric ternary complex-model’ for plain TCM is still confusing (Scheer et al. 1996; Daeflfer & Landry 2000).

In modeling, including mixed-inhibition, mixed activation, TCM and ‘allosteric’ TCM, I recommend a new term for these kinds of reaction schemes – namely the *intervention* model. See Sub-chapter 2.7 for more details on the development of the *intervention* scheme.

2.1.3. Auto-ant-agonism

In this chapter, I have opted to examine ant-agonism or inhibition by compounds that are different from the

Table 2.1. On the roots to formulate the heterotropic mutually inclusive one-state model, HE-MI-OSM, and the homotropic mutually inclusive one-state model, HO-MI-OSM

Reference	Names or models for intervention	Comment
Michaelis (1914)	Substrate inhibition	Function
Haldane (1930)	Substrate inhibition	Function
Wyman (1948, 1964)	Theory of linked function	Binding HO-MI-OSM
Laidler and Socquet (1950)	Non-competitive adsorption	Functional
Segal et al. (1952)	An intervention scheme	Functional
Botts and Morales (1953)	An intervention scheme	Functional
Friedenwald and Maengwyn-Davies (1954)	An intervention scheme	Functional
Ariëns et al. (1955, 1964)	Non-competitive synergism	Functional HO-MI-OSM
Laidler (1956, 1958)	An intervention scheme	Functional
Webb (1963)	Mixed inhibition	Functional
Mahler and Cordes (1966)	Partial non-competitive	Functional
Cheng and Prusoff (1973)	Non-competitive	Functional
Cornish-Bowden (1974, 1995, 2004, p 134)	General modifier mechanism	Functional
Segel (1975, 1993)	Mixed inhibition and activation	Functional
Cha (1975)	Non-competitive	Binding
Jacobs and Cuatrecasas (1976)	Mobile receptor	Functional
Tolkovsky and Levitzki (1978)	Floating receptor model	Functional
Birdsall et al. (1978)	Theory of linked function	Binding
Boeynaems and Dumont (1980, pp. 143–147)	Pseudo-competitive ant-agonism/mixed ant-agonism	Binding
De Lean et al. (1980)	Ternary complex model	Binding and function
Stockton et al. (1983)	Ternary complex	Binding
Wreggett and De Lean (1984)	Ternary complex	Binding
Ehlert (1988)	‘Allosteric’	Binding/functional
Mackay (1990)	Ternary complex, G proteins	Functional, steady-state
Topham (1990)	Mixed inhibition	Functional
Wells (1992)	Heterotropic co-operativity	Binding
Costa et al. (1992)	Negative ant-agonism	Functional
Samama et al. (1993)	Extended TCM	Functional
Trzeciakowski (1999)	Standard TCM, G proteins	Binding/function
Shou et al. (2001), Shou (2002)	Partial inhibition**	Functional HO-MI-OSM
Present text on one-state models	Intervention models	Binding/function

*See Chapters 5 and 14 for use of the term ‘allosteric’ in general and in particular.

** ‘Partial inhibition’ frequently used in enzymology have different meanings, see, e.g., Webb (1963) and Segel (1975, 1993). Segel’s Chapters 5 and 6 are on activation in HE-MI-OSM.

primary ligands, the agonists, while Chapter 3 will deal with self-inhibition. In self-inhibition, substrates or agonists at low concentration activate the function of relevant receptive units, enzymes, or transporters and so forth, while at increasing concentrations even in the absence of

other ligands the agonist or substrate starts to ant-agonize its own action – this is also designated auto-ant-agonism, auto-inhibition, or *negative auto-intervention*. This self-inhibition is understood to take place from a secondary binding site in the receptive unit.

Means of obtaining parameters such as dissociation constants from experimental data are discussed in Chapter 4 for *agonism*, *ant-agonism*, and *intervention* as well as for *auto-ant-agonism* and *auto-intervention* in one-state models.

Inverse agonists also inhibit function, but they ant-agonize inherent activity by binding at the primary site. Since inverse agonists inhibit constitutive activity, they are therefore best treated in two-state models with spontaneous activity, which appears in Chapter 5 under complex agonism.

2.1.4. Inhibitors and Reversibility

In general, when a drug lowers one or more of the activities of a system, a cell, or a protein molecule, it is called an **inhibitor**. Inhibitors may be either reversible or irreversible. Often, the reversibility of an inhibitor is decided by the speed with which the interaction between the inhibitor and its target decays after washout of the ant-agonistic drug. The time of decay is held against the time of experimentation. That is to say, if the inhibitory effect does not reverse significantly during the observation period after the inhibitory compound is removed from the system, the compound is said to be an ‘irreversible inhibitor’. For instance, regain of less than 10% of an initial response in the observation period following washout of a drug will probably be taken to mean that the drug has bound almost irreversibly or is distributed more permanently in an unstirred compartment, thereby preventing a reasonably short duration for washout and equilibration.

During washout, likely to be an exponential decay of binding or diffusion-limitation, the lapse of time to revert and regain 63% of a response equals one time constant, τ .² Thus, to name a compound ‘reversible’ typically requires an observation period in the range of a time constant or more for its washout process. The non-competitive-like behavior of exponential decay or diffusion-limitation was explained by Rang (1966) in his description of dissociation-limited and biophase models.

In this context, a designation of ‘irreversible inhibitor’ may be employed about a high affinity ant-agonist not displaced by an agonist during its exposure that is too short to allow the displacement, also known

as ‘insurmountable ant-agonism’ (see Sub-chapter 2.8) (Jenkinson 2003, p. 53).

2.1.5. Ant-agonists, Interventors, and Activators

Reversible inhibitors that affect agonist-induced activity are referred to as **ant-agonists**, and can be competitive, non-competitive, or un-competitive. Thus, for a compound to be an ant-agonist, it is required that it only works in the presence of an agonist, more or less preventing the agonist from activating or binding to its receptive unit. On the other hand, ‘inhibitor’ will, in general, refer to irreversible as well as reversible inhibitors of both agonist-induced and agonist-independent activity. The term ‘inhibitor’ is thus conveniently used for drugs that operate by irreversibly blocking spontaneous or agonist-induced activity, while ‘ant-agonist’ covers for reversible inhibitors dependent on agonist-induced activity (Table 2.2A), or eventually reversible inhibitors of a negative effect of inverse agonists.

Interventor molecules work at secondary sites in systems assumed not to possess spontaneous activity, i.e., in one-state models, similar to non-competitive ant-agonists. Meanwhile, interventors can be both inhibitory and stimulatory (Table 2.2A).

Drugs increasing the activity beyond constitutive or agonist-induced activities may be referred to as stimulators or **activators**. This is true in both binding and functional studies. Activators operate reversibly (Table 2.2A).

As demonstrated later, allosteric modulation at aberrant binding sites renders new possibilities in binding and function for the classification of drugs as activators, positive modulators, or inhibitors equal to negative modulators (Table 2.2A and Chapter 14).

Molecules that enhance binding or function in a constitutive manner will be referred to as **enhancers**. Auxiliary and accessory proteins, such as co-factors, beta-subunits, regulator-of-G protein-signaling (RGS), receptor-activity-modifying-proteins (RAMPs), scaffolding molecules, and chaperones (Sexton et al. 2001; Blumer & Lanier 2003; Chidiak & Roy 2003; Morfis et al. 2003; Rebois & Hébert 2003; Fukoto et al. 2004; Udawela et al. 2004, 2006; Abramow-Newerly et al. 2006; Hill et al. 2008) that also have an activating function will fall under the category of enhancers. Several of these proteins are in fact part of the receptor complex (Hay et al. 2006).

Table 2.2A is a preliminary and tentative summary of agonists, ant-agonist, inhibitors, interventors, inverse agonists, modulator molecules, activators, and enhancers. Ant-agonists and interventors are examined this chapter. Inverse agonists and neutral ant-agonists are first treated in Chapter 5, and modulator molecules in Chapters 7, 14 and 15.

² Here, one time constant, τ , is the time it takes to regain 63% of the inhibited function. Percentage regain for an exponential function is: regain (%) = $[1 - e^{-\tau}] \cdot 100$, where e is the base of natural logarithms = 2.718. For an observation period equal 1τ , the regain is 63%.

Table 2.2A. Activators, Inhibitors, Ant-agonists, Interventors, Modulators, Enhancers, and Repressors

Type of interaction	Site of binding	Model	Augmentation by	Attenuation by	Auto-effects pos or neg by
Reversible	Primary	All	Agonist	Ant-agonist	–
	Primary	All	Agonist/ neutral ant-agonist	Neutral ant-agonist/ inverse agonist	–
	Secondary	OSM	Pos interventor**	Neg interventor**	Pos/neg auto-interventor
General	Secondary	TSM	Pos modulator***	Neg modulator***	Pos/neg auto-modulator****
	Not specified	–	Activator*	Inhibitor*	Auto-activator, auto-inhibitor
	Not specified	–	Enhancer/ Derepressor	Desensitizer Repressor	–

*The terms ‘activator’ and ‘inhibitor’ may also be used as general designators for augmenters and attenuators in all cases, including regulators of G protein-signaling (RGSs) and receptor-activity-modifying-proteins (RAMPs).

**If neither positive nor negative, we have a ‘neutral interventor’ equal to a non-competitive ant-agonist. Binding is unchanged.

***When neither positive nor negative, it is a ‘neutral modulator’, not the same as a ‘neutral ant-agonist’.

****The process is designated positive or negative co-operativity.

2.1.6. Multi-functional Ligands

The complexity increases when ligands have more than one function. Activators may work synergistically with the possibility of positive co-operativity. Furthermore, ligands may modify the function or binding of other agonists while at the same time they are themselves substrates for enzyme reactions, transported species for mechanisms of pumping, transportees for co-, anti-, or uni-porters, if not ions funneled through a channel. At receptors, ligands may be either modulators or interventors – depending on the assumed reaction scheme and its modeling – while at the same time they are also agonists in their own right. Such drugs now rank as possible ‘ago-modulators’ (Schwartz & Holm 2006) or ‘allosteric agonists’ (May et al. 2007b). Table 2.2B is a tentative proposal for the naming of multi-functional drugs (see Sub-chapter 4.6 for more details).

2.2. Simple Inhibition

2.2.1. Ant-agonism

In general, ant-agonism is generated by compounds inhibiting the effects of agonists – ‘ant-agonists’. In classic literature on the subject, two possible forms of ant-agonism were often imagined, a competitive and a non-competitive ant-agonism.

With conceptions from the classic era, among others, Segel in his book ‘Enzyme Kinetics’ (1975, 1993, pp.

100–160) has presented ant-agonism as competitive, non-competitive, and un-competitive and explained them on a spatio-structural ground complete with derived formulations for each type (Fig. 2.2) (reproduced from Segel 1975, 1993, Figs. III-1.1–5, III-12, III-13, III-15, and III-21).

2.2.2. Simple Ant-agonism Meets Two-state Models

In the last few decades, ideas about how ligands can prevent agonist from activating receptors, enzymes from catalyzing substrates, or transporters from transporting transportees have been turned upside-down. Over the last fifteen years, theories have changed, especially in pharmacology, due to recognition of the importance of explicitly incorporating expressions for the spontaneous switching between active and non-active states of **un-liganded** receptive units, ‘two-state model’ (Leff 1995). Thus, competitive ant-agonism is now revitalized and viewed in light of inverse agonists stabilizing the non-active receptor state or new receptor states (Milligan et al. 1995; Vilardaga et al. 2005; Bond & Ijzerman 2006; Dowling et al. 2006). A basic description of such dose-responses at equilibrium, or in another word *synagics*, is best done with a so-called cyclic-two-state-model (cTSM), which I shall deal with in detail in Part II on complex agonism and ant-agonism (Chapter 5).

Here we will derive the equations for simple ant-agonism, i.e., classic competitive, non-competitive and un-competitive ant-agonism (Sub-chapter 2.4). Furthermore, we shall develop a so-called mixed-inhibition/mixed activation model into its complete potential in the form of the above-mentioned *intervention* model (Sub-chapter 2.5).

But first, a definition of what I call a ‘full response’ (see Section 2.2.3), and, in addition, a presentation of a complicated looking reaction scheme – a four-pane reaction scheme with receptive units in a single state, a so-called four-pane one-state model (FP-OSM) described in Sub-chapter 2.3.

Table 2.2B. Possible Terms for Multi-functional Drugs

Ligands	Different	Single
Modelers choice	Heterotropic system	Homotropic system
One-state model (-OSM)	Ago-interventor	Ago-auto-interventor = auto-interventor
Two-state model (-TSM)	Ago-allosteric modulators* allosteric agonists [§]	Ago-co-operative = co-operative drug

*See Schwartz and Holst (2006).

[§]May et al. (2007b).

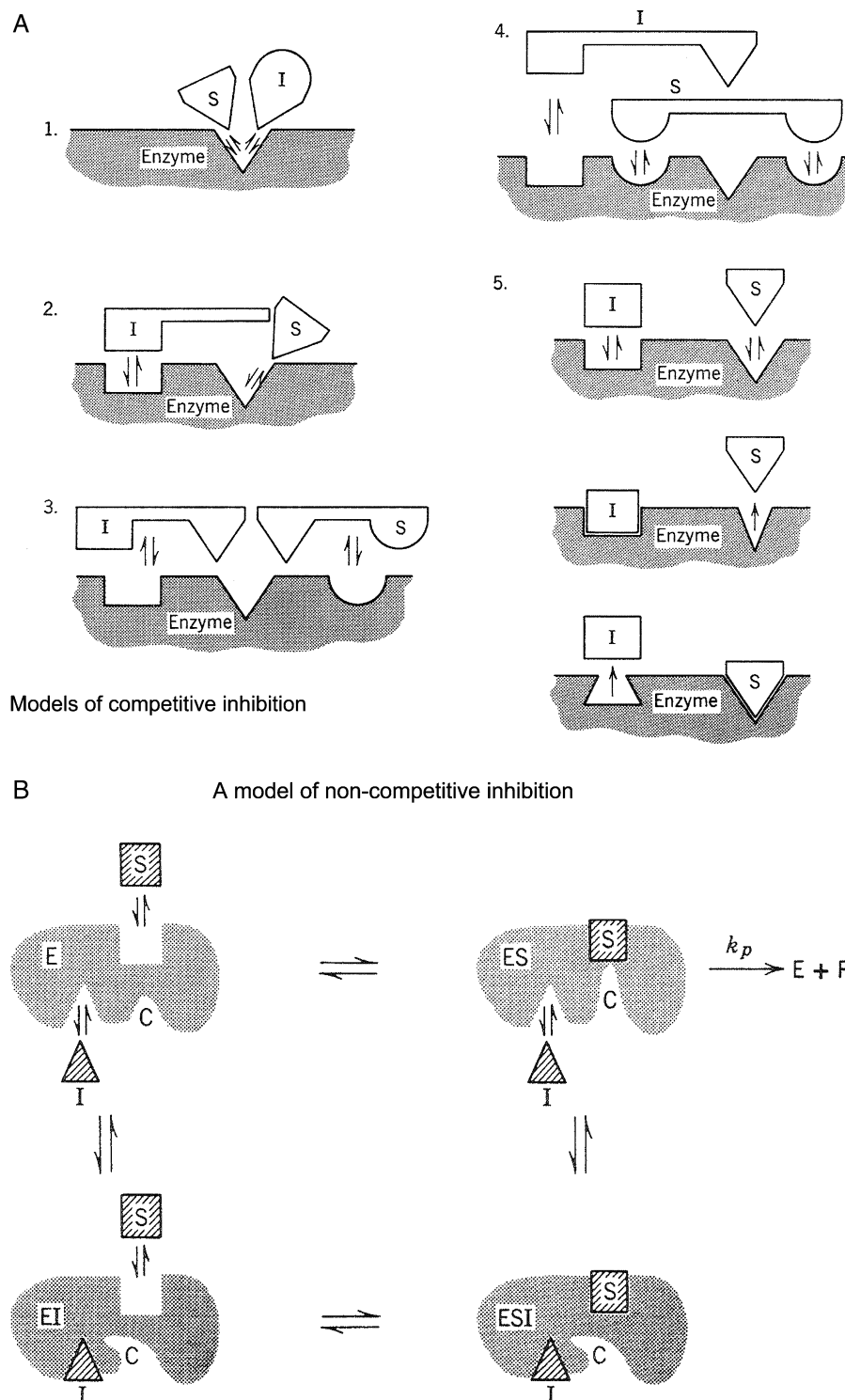
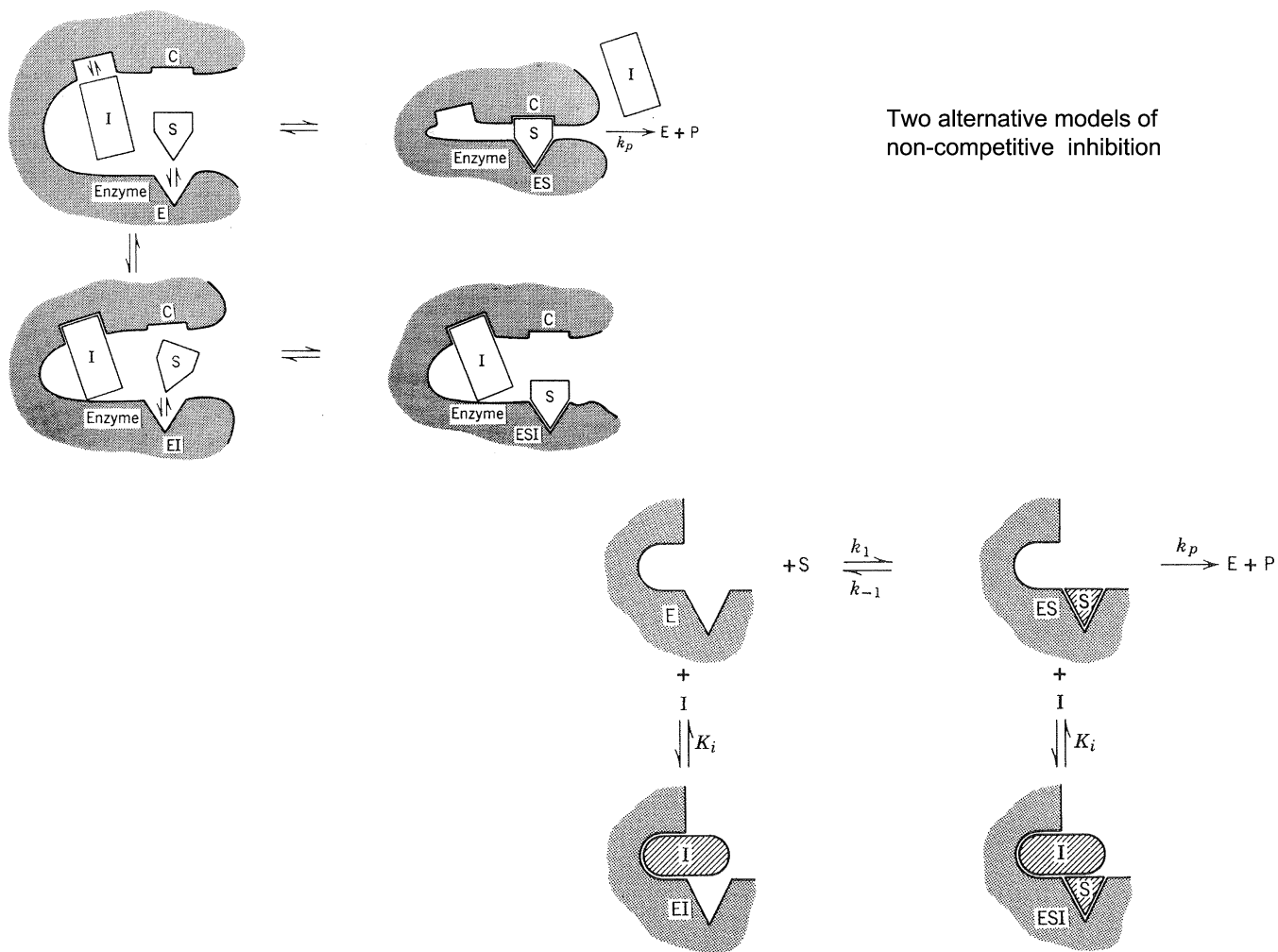


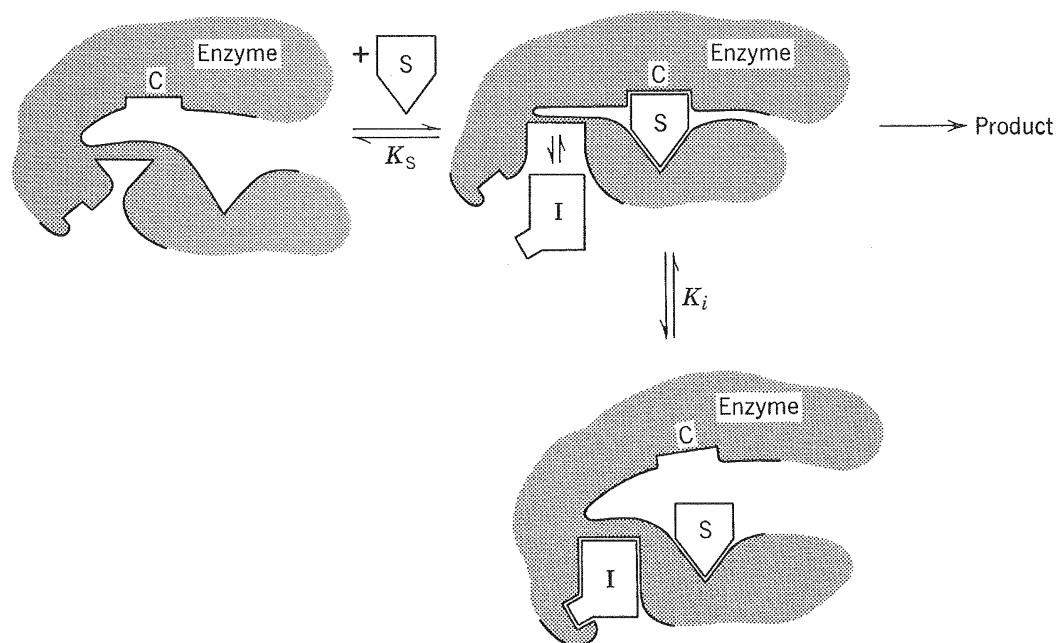
Figure 2.2. Physical models for competitive, non-competitive, and un-competitive inhibition. Panel A: models on competitive ant-agonism. Panels B and C: models for the non-competitive inhibition. Panel D: a model for the un-competitive inhibition (see text). Reproduced from Segel (1975, 1993) with permission.

C

Figure 2.2 (Continued)



D



2.2.3. The 'Full Response' Obtained at a Fixed Concentration of an Agonist

An agonist-induced response at a fixed agonist concentration, S' , is given by the **load** equation and represented in an [agonist]-vs-response plot, where the curve intersects with the 'y-axis for ant-agonists' before an ant-agonist I is introduced, i.e., for $[I] = 0$ (Fig. 2.3). This response by the fixed agonist concentration S' , we will call the *full response*. In a given situation, it is the full response that ant-agonists can work on. The full response is not necessarily the total response or maximal effect that can be obtained with higher concentrations of the agonist. That is, experiments of ant-agonism are carried out at a given agonist concentration, S' , which in the absence of ant-agonists yields a *full response*. The full response is lowered when an ant-agonist is introduced. The full response (FR) may be equated as:

$$\text{Full response} = FR = TR \cdot \frac{S'}{S' + K_{ss}}, \quad (2.1)$$

where TR is the total or maximal response, and K_{ss} is the equilibrium dissociation constant for ligand S . S' indi-

cates a fixed concentration of S . Subscripts are explained in Box 2.1 (at the end of Chapter 2).

2.3. The Four Pane Reaction Scheme and Simple Inhibition

2.3.1. The Four-pane and One-state Model (FP-OSM)

To understand the following arguments about simple ant-agonism, it seems advantageous to introduce a reaction scheme as depicted in Fig. 2.4. Initially, this reaction scheme may look daunting with its nine-receptor conformations and eight independent system constants. But careful scrutiny reveals its basic simplicity. For the moment, we will ignore the lower half of the reaction scheme in Fig. 2.4, thus forgetting about all conformation where ligand S appears on the left side (site) of R (the receptive unit). This means that agonists are not allowed to bind to a secondary ant-agonistic (intervention) site at the left side of R .

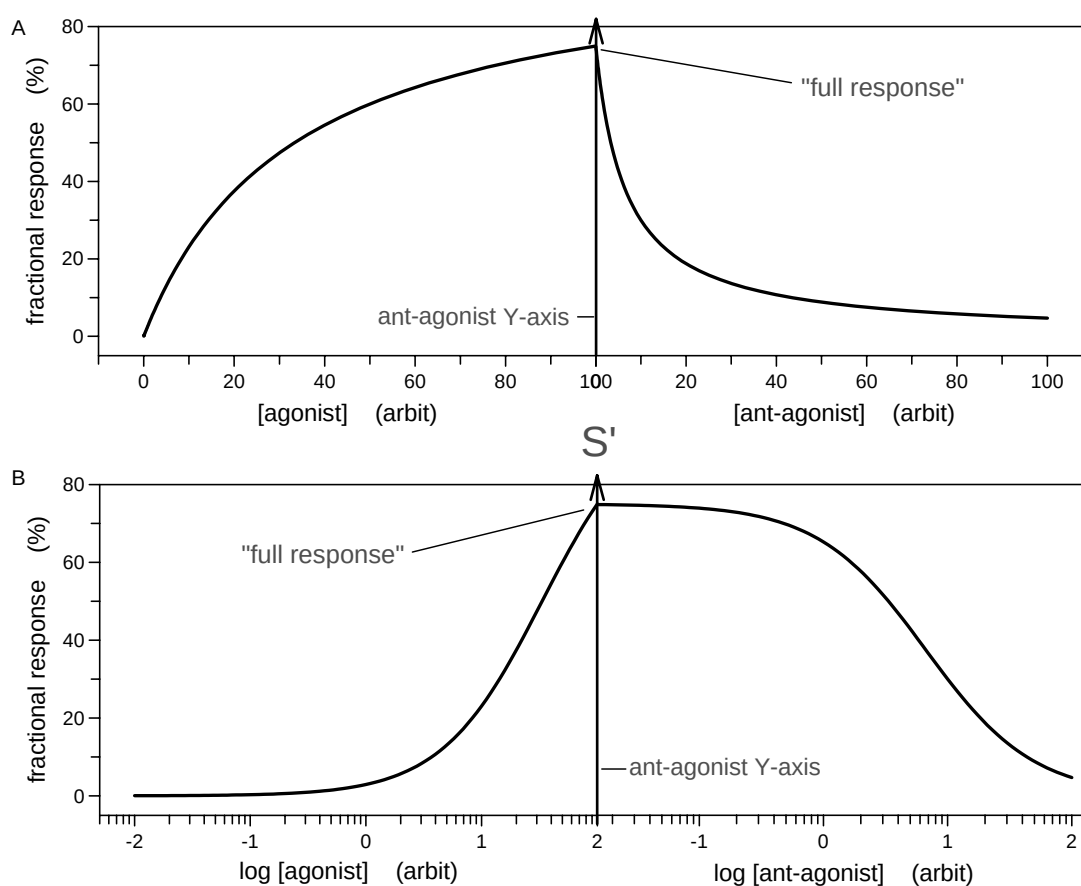


Figure 2.3. Illustration of the concept 'full response'. A fixed agonist concentration S' brings the system response to a given activity (or occupancy) level indicated at the 'Y-axis for ant-agonists' as **full-response**. At the right of the Y-axis for ant-agonists the concentration of ant-agonists starts to increase from zero and abolishes the full-response at high enough concentrations. Linear concentration scale in (A) and logarithmic concentration scale in (B).

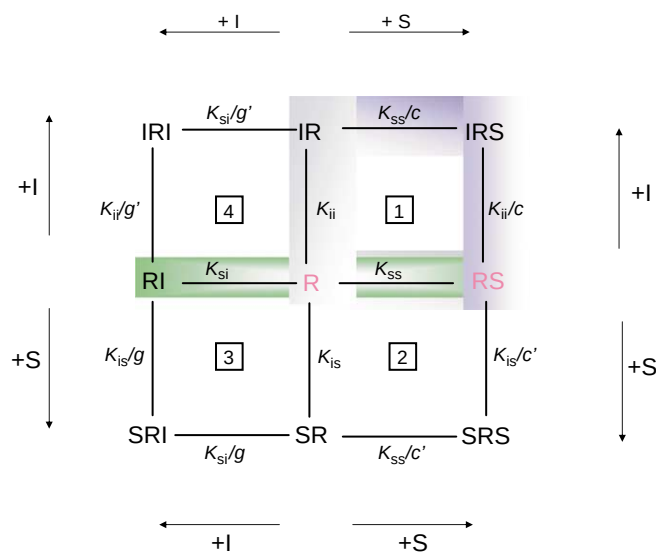
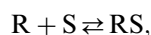


Figure 2.4. The **four-pane one-state model (FP-OSM)**. This model consists of nine independent receptor conformations (not states) and has eight different and independent system constants, either as association constants A_{xx} or dissociation constants K_{xx} (see Box 2.1) and interaction constants c , c' , g , and g' .

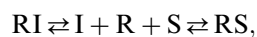
In Fig. 2.4 the reaction scheme for a simple agonism with receptor symbols colored in maroon are easily identifiable:



which was introduced in Chapter 1.

Next, identify the following three partial ‘ant-agonistic’ reaction schemes in the four-pane window scheme (Fig. 2.4):

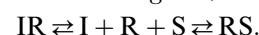
1. The position at the right of the receptor symbol R is an agonist binding site, which may bind either the agonist S or an ant-agonist I. Binding the agonist can activate the receptor or stabilize the receptor in an active state. When an ant-agonist I binds to this site, it prevents activation by preventing the agonist from binding. Possible receptor conformations are R, RS or RI. We can write its reaction scheme and get:



which is the reaction depicted in the middle, horizontal line of Fig. 2.4 (highlighted in green). Notice that all bound agents are to the right of receptor symbol R. S and I bind in a so-called mutually exclusive manner; they cannot bind simultaneously. Only the RS conformation is functionally active.

2. In Fig. 2.4 it may also be assumed that the receptor has two instead of only one binding site. At the

position to the left of the receptor symbol R there is an additional binding site for ant-agonists/interventors. So far, we assume that the ant-agonist/interventor site to the left of R cannot bind an agonist. Further, here we will assume that the ant-agonist/interventor I cannot bind at the agonist binding site. Binding at the agonist binding site is only for agonists and binding at the ant-agonist/interventor binding site is only for ant-agonists/interventors. This generates three possible receptor conformations R, IR, and RS, and for this we can also identify and extract a reaction scheme. The reaction scheme can be identified as the part highlighted in gray in the four-pane reaction scheme in Fig. 2.4, which is written as:



Again, the complex RS alone renders functional activity while the binding of agonists and ant-agonist/interventor are mutually exclusive.

3. Still assuming that agonists cannot bind to the ant-agonist/interventor site and ant-agonists/interventors cannot bind to the agonist site, there is still another possibility, namely that when an agonist is bound at the agonist site, an ant-agonist/interventor can *simultaneously* bind at the ant-agonist/interventor site. Binding of agonists and interventors is *not* mutually exclusive. This yields four possible receptor conformations, R, RS, IR, and IRS with a reaction scheme as shown in the upper right quadrant of Fig. 2.4 (quadrant labeled 1 and tinted in gray, green, and blue). This quadrant is equal to the reaction scheme in Fig. 2.1A; and again, RS is the only functionally active conformation.

With these three partial reaction schemes as possibilities, we are ready for the simple inhibition with competitive, non-competitive, and un-competitive ant-agonists.

2.4. Competitive and Non-competitive Ant-agonism

This Sub-chapter is relevant for functional studies to be analyzed by competitive and non-competitive theories. In an attempt to keep matters simple and separate, some of the parallel theories for binding studies are postponed to Sub-chapter 2.5, where the *intervention* model is also described.

As indicated in Sections 2.3.1.2 and 2.3.1.3, when a ligand binds to the secondary site it should more appropriately be termed an interventor instead of an ant-agonist. But, in the following I will stay with the term

‘ant-agonist’ and follow the classical terminology for development of the competitive, non-competitive, and un-competitive ant-agonism. The term ‘agonist’ may be replaced with ‘substrate’ or ‘transportee’.

2.4.1. Competitive Ant-agonism Type I and Its Dissociation Constant

In classic competitive ant-agonism, only the RS conformation is active, and both agonists and ant-agonists can bind to the agonist binding site of R, that is to the right of symbol R. There is no secondary binding site. This is equal to reaction scheme $RI \rightleftharpoons I + R + S \rightleftharpoons RS$ in Section 2.3.1.1, and is also depicted in Fig. 2.4 (see Segel 1975, 1993, Fig. III-1). Binding is mutually exclusive. There is no simultaneous binding of agonist and ant-agonist. The pertinent equilibrium dissociation constants are indicated with subscripts as follows: K_{ss} for the agonist S interaction and K_{si} for the ant-agonist I interaction at the agonist binding site. Note that with this choice of signification in the subscripts, the first position in the subscript signifies the binding site on the receptive unit, here s, while the second position in the subscript indicates the ligand bound to the site; s for the agonist and i for the ant-agonist. Box 2.1 specifies how these symbols are used and the conventions contained therein are used throughout the text.

2.4.2. Competitive Ant-agonism Type II and Its Dissociation Constant

As illustrated in Section 2.3.1.2, in case there are two separate binding sites on the receptive unit, one for agonists and another for ant-agonists, with the conditions that only agonists can bind at the agonist binding site and only ant-agonists can bind at the ant-agonistic binding site, then the reaction scheme $IR \rightleftharpoons I + R + S \rightleftharpoons RS$ is still without the possibility of simultaneous binding. Binding is mutually exclusive, and as indicated, only the RS conformation is functional. This reaction scheme is designated type II competitive ant-agonism. The scheme may be found in Figs. 2.1A and 2.4 (Segel 1975, 1993; Figs. III-1.2-5).

In the type II reaction scheme for competitive ant-agonism, binding of ant-agonists at the ant-agonistic binding site has its own dissociation constant K_{ii} , which is different from K_{si} of the type I competitive ant-agonism. However, the type II competitive ant-agonism will be difficult to discern from the competitive type I ant-agonism described in Section 2.4.1.

Mutually exclusive binding is the virtue of type I and II competitive ant-agonism, and since both take place in a one-state model, their acronym is ME-OSM.

2.4.3. Non-competitive Ant-agonism and Its Dissociation Constants

In classic non-competitive ant-agonism there is also a secondary site on the receptive unit R, indicated by positioning ligand symbols to the left of R, where only ant-agonists can bind. This renders an IR complex. At the agonist site only agonists can bind resulting in an RS complex. The difference between non-competitive ant-agonism and the just described competitive ant-agonism type II in Section 2.4.2 is the ability of the receptive unit in non-competitive ant-agonism to adapt binding of both agonists and ant-agonist at the same time. Binding is *not* mutually exclusive, binding is mutually *inclusive* (Levitski 1978). Meanwhile, activation of the receptive unit is prevented when an ant-agonist is bound, no matter what, thus only the RS conformation is active. Non-competitive ant-agonism also takes place in a one-state model and due to its mutual inclusiveness its acronym is MI-OSM.

This is the situation depicted and highlighted in Figs. 2.1A and 2.4-1 (Segel 1975, 1993; Figs. III-12, III-13, III-15), with four receptor conformations, R, RS, IR, and IRS. The pertinent dissociation constants are indicated with subscripts as follows: K_{ss} for the agonist interaction with the agonist binding site and K_{ii} for the ant-agonist interaction with the ant-agonist binding site, in accordance with the above-mentioned conventions (Box 2.1). Compared with dissociation constants when no other ligand is bound, the equilibrium dissociation constant for binding of a ligand may be different when other ligands are already bound to the receptive unit. For the reaction scheme in Fig. 2.4-1, this condition is indicated by a factor c . The c factor will be examined in more detail in Sections 2.5.1-3, but for now we will assume that c is unity. Let an *already* bound ligand be indicated with a *superscript*, where the first position of the superscript indicates to which site the already-bound ligand is bound, and a second position in the superscript indicates which type of ligand is ligated, for instance agonists = s and ant-agonists = i. Thus K_{ii}^{ss} is the dissociation constant for an ant-agonistic ligand to bind at the ant-agonist binding site when an agonist is already bound to its agonist binding site (Box 2.1). When $c = 1$, we have that $K_{ii} = K_{ii}^{ss}$ and $K_{ss} = K_{ss}^{ii}$. An explanation for $c \neq 1$ will be detailed in Sub-chapter 2.5 describing the intervention scheme.

2.4.4. From Reaction Scheme to Formulation

As soon as a specific reaction scheme has been defined, it is possible to derive its mathematical formulation. To formulate our reaction schemes, we need the system constants and the receptor conformations (see Figs. 2.1A, 2.4-1, and Box 2.1).

The parameters are:

$R_{\max}$ or $B_{\max}$	maximal effect or maximal binding
K_{ss}	equilibrium dissociation constant at the primary site for agonist S
K_{si}	equilibrium dissociation constant at the primary site for ant-agonist I
or	
K_{ii}	equilibrium dissociation constant at the secondary site for ant-agonist I
c	intervention constant—first discussed in detail in Sub-chapter 2.5,
and the conformations of the receptive unit are:	
R	
RS	$R \cdot S / K_{ss}$
RI	$R \cdot I / K_{si}$
or	
IR	$R \cdot I / K_{ii}$
and	
IRS	$R \cdot (S / K_{ss}) \cdot I / K_{ii}$

For the following formulations, in order to simply the equations, the letters S and I may also stand for the normalized ligand concentrations, S/K_{ss} and I/K_{si} , as for instance in Eq. 2.2a.

For reasons that will become obvious later, here, I first transcribe the reaction scheme for non-competitive ant-agonism into an equation before a transcription of the reaction schemes for competitive ant-agonism into its formulations.

2.4.5. Formulation of Functional Non-competitive Ant-agonism

A situation can be described as a non-competitive type of ant-agonism when a ligand solely binds to a secondary binding site and results in non-active receptive units even if an agonist is bound or not bound to its binding site on the receptive unit – the primary site (Section 2.4.3). For this, four receptor conformations are involved (see Figs. 2.1A or 2.4-1). To start, we will assume that the dissociation constants are identical for reactions $R+S \rightleftharpoons RS$ and $IR+S \rightleftharpoons IRS$, i.e., $c=1$, which as mentioned in Section 2.4.3 simplifies the analysis. Let us illustrate the issue by formulating a distribution equation involving all four receptor conformations of non-competitive ant-agonism and how they relate. With TR as the total response and ar as the actual response, we have:

$$\frac{ar}{TR} = \frac{RS}{R + RS + IR + IRS} = \frac{S}{1 + S + I + I \cdot S}, \quad (2.2a)$$

in which the S and I stand for the normalized ligand concentration. Eq. 2.2a can be rewritten to:

$$\frac{ar}{TR} = \frac{S}{(1 + S) \cdot (1 + I)} = \frac{S}{(1 + S)} \cdot \frac{1}{(1 + I)}, \quad (2.2b)$$

or in explicitly normalized versions of Eqs. 2.2a and 2.2b, they become:

$$\begin{aligned} \frac{ar}{TR} &= \frac{\frac{S}{K_{ss}}}{1 + \frac{S}{K_{ss}} + \frac{I}{K_{ii}} + \frac{S}{K_{ss}} \cdot \frac{I}{K_{ii}}} \\ &= \frac{\frac{S}{K_{ss}}}{\left(1 + \frac{S}{K_{ss}}\right) \cdot \left(1 + \frac{I}{K_{ii}}\right)}. \end{aligned} \quad (2.3)$$

We may rewrite Eq. 2.2b as:

$$ar = TR \cdot \frac{S}{1 + S} \cdot \frac{1}{1 + I}. \quad (2.4a)$$

When there is no antagonist $[I] = 0$, from Eq. 2.4a we have $ar = TR \cdot S / (1 + S)$ or:

$$ar_{I=0} = TR \cdot \frac{S}{1 + S}. \quad (2.4b)$$

This again is a **load** expression. It yields responses obtained at a varying concentration of agonist S.

When the agonist concentration is fixed at S' , the agonist-induced response is the intersection at the 'ant-agonist' y-axis in Fig. 2.3. In addition, when the agonist concentration is fixed at S' , Eq. 2.4b can be written as a new version equal to Eq. 2.1, i.e.,

$$ar'_{I=0} = TR \cdot \frac{S'}{1 + S'} \vee FR = TR \cdot \frac{S'}{1 + S'}. \quad (2.4c)$$

Thus, the actual response ar' for $I = 0$ is now equal to the *full response*, i.e., $ar'_{I=0} = FR$.

In a given situation, this full response is the response that ant-agonists can work on.³

So, the analysis is now carried out at a given agonist concentration S' , while varying the ant-agonist concentration. For any ant-agonist concentration, the obtained responses can be referred to as 'actual remaining activity' (ara). A formula for the ara , as the ant-agonist concentration varies, is obtained by combining Eqs. 2.4a and 2.4c:

³ 'Full response', given in Eq. 2.1, is not necessarily the maximal response that can be obtained with higher concentrations of the agonist (Section 2.2.3).

$$ara = ar' \cdot \left(\frac{1}{1 + I} \right). \quad (2.4d)$$

As explicitly demonstrated by Eq. 2.4d, not surprisingly, *ara* only varies with the term $1/(1 + I)$ when *S* is fixed, as depicted in Fig. 2.3. The level of fixed agonist concentration *S'* determines from what level on the *y*-axis the response is reduced by ant-agonists, but note, without affecting the determination of K_{ii} for the ant-agonist on the *x*-axis. This conclusion works for the theory of functional studies with simple non-competitive ant-agonism.

For the moment, observe without insight that in case the above-mentioned *c* coefficient is not equal to unity, as assumed, then the measured K_{ii} will be a function of *S*, as demonstrated later for the intervention scheme in Sub-chapter 2.5.

2.4.6. Formulation of Binding in Non-competitive Ant-agonism

In the theory of non-competitive ant-agonism for binding experiment, it may be observed that it has a different formulation from the functional ant-agonism, since both conformations RS and IRS will be involved (Figs. 2.1A and 2.4-1). Both type I and II competitive ant-agonism are not hampered by this discrepancy between binding and functional studies in their formulations due to mutual exclusiveness in binding between agonist and ant-agonist (see Sections 2.4.9 and 2.4.10 and Sub-chapter 2.5).

For *binding* studies assuming a non-competitive reaction scheme we may derive the following equation:

$$\frac{ar}{TR} = \frac{RS + IRS}{R + RS + IR + IRS} = \frac{S}{S + I}. \quad (2.4e)$$

This means, in contrast to functional studies, that binding is completely independent of possible non-competitive ant-agonists (cf. Table 2.3).

2.4.7. Actual Remaining Activity (ara) in Non-competitive Inhibition

In functional studies, non-competitive activity drops as the ant-agonist concentration increases. The *ara*, which decreases as the ant-agonist concentration increases, was obtained in Eq. 2.4d by combining Eqs. 2.4a and 2.4c. Inserting *FR* for *ar'* in Eq. 2.4d we may write:

$$ara = FR \cdot \left(\frac{1}{1 + I} \right), \quad (2.5)$$

and in an explicitly normalized form:

$$ara = FR \cdot \left(\frac{1}{1 + \frac{I}{K_{ii}}} \right). \quad (2.6)$$

In a normalized and linear-linear co-ordinate system, the dose-response relation of the *ara* is viewed as a horizontally-flipped law of adsorption-desorption (**load**). That is, it is reciprocal to a dose-response following the **load** (Fig. 2.5A). In a normalized semi-log co-ordinate system, a plot of simple dose-responses of actual remaining activity has the form as shown in Fig. 2.5B.

2.4.8. The Actual Inhibited Response (air) in Non-competitive Inhibition

Converse to the *ara*, the actual size of inhibited response (*air*) rises with increasing ant-agonist concentration. Given the scenario in Section 2.4.5 for functional studies, where the activity is induced by a fixed concentration of an agonist, *air* is the size of the eliminated response. In functional studies, *air* rises as a function of the actual ant-agonist concentration and follows a simple **load**, still with *FR* as a 'maximal' obtainable response, now in the presence of an ant-agonist. We may equate the *air* as:

$$\begin{aligned} air &= FR - ara = FR - FR \cdot \left(\frac{1}{1 + I} \right) \\ &= FR \cdot \left(\frac{I}{1 + I} \right), \end{aligned} \quad (2.7)$$

and in an explicitly normalized form:

$$air = FR \cdot \left(\frac{\frac{I}{K_{ii}}}{1 + \frac{I}{K_{ii}}} \right). \quad (2.8)$$

This equation is plotted in Fig. 2.5C and is a normal oriented Langmuirian dose-response relationship of the **load** type. The inhibitory response is rising with increasing concentration of the ant-agonist, and in a normalized semi-log co-ordinate system this simple ant-agonism has the form as shown in Fig. 2.5D.

We can summarize Sections 2.4.7 and 2.4.8 as follows:

$$air = FR \cdot (I/(I + K_{ii})), \quad (2.9a)$$

and

$$ara = FR - FR \cdot (I/(I + K_{ii})) = FR \cdot (K_{ii}/(I + K_{ii})), \quad (2.10a)$$

which are the equations for **actual ant-agonistic** or **inhibitory response** (*air*) and **actual remaining activity** (*ara*), both as a function of the increasing ant-agonist

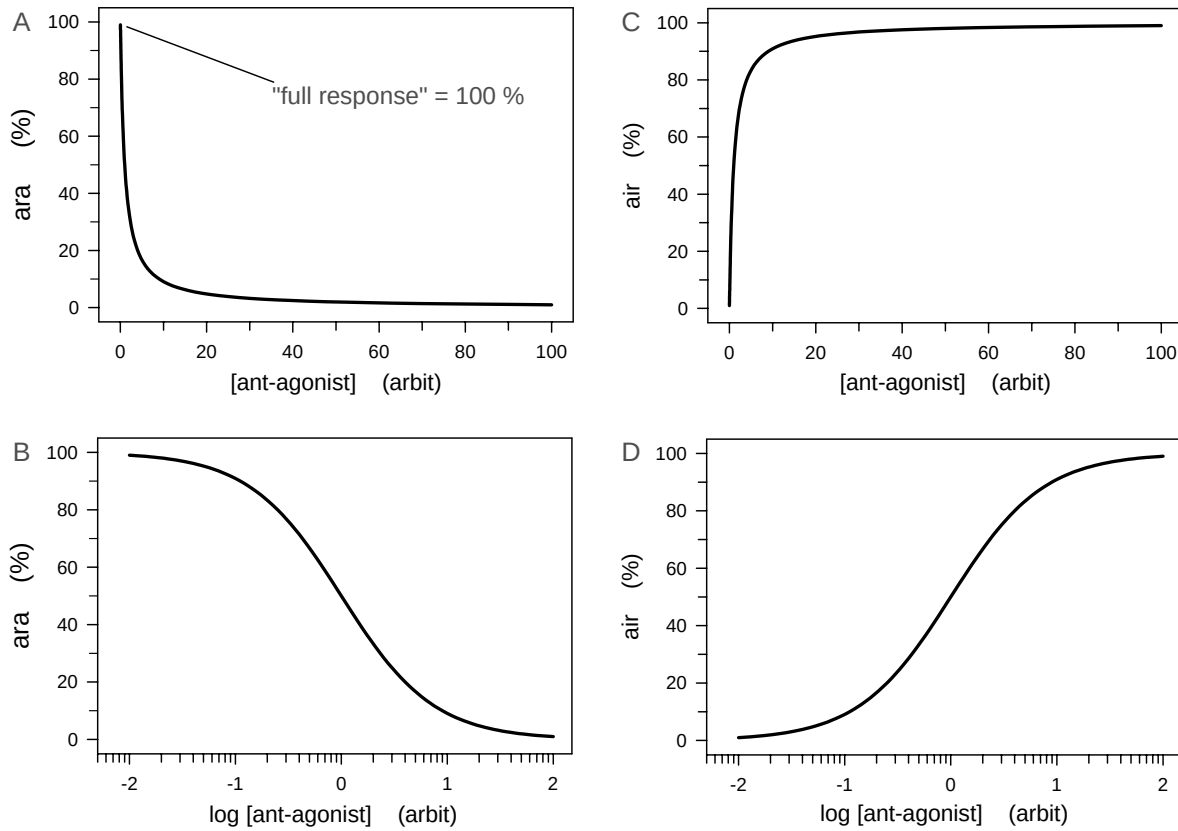


Figure 2.5. Non-competitive inhibition ($c = 1$) in a functional scheme. Panel A is a linear-linear and Panel B is a semi-log plot for the actual remaining activity (ara) in non-competitive ant-agonism as the concentration of the ant-agonist increases (Eq. 2.6). Panel C is a linear-linear and panel D is a semi-log plot of the actual inhibitory response (air) in non-competitive ant-agonism as the concentration of the ant-agonist increases (Eq. 2.8).

concentrations $[I]$ in the functionally non-competitive reaction scheme.

The comparable formulations of theory for non-competitive ant-agonism in *binding* studies, started in Section 2.4.6, are developed later in Section 2.5.1 and summarized in Table 2.3.

2.4.9. Formulation of Competitive Ant-agonism Type I

Now we turn to competitive ant-agonism. Although merely three receptor conformations are involved in the scheme for competitive ant-agonism (see reaction scheme in Section 2.3.1 and mid-horizontal line in Fig. 2.4), it is slightly more complicated than the non-competitive ant-agonism with four involved conformations. With a fixed agonist concentration S' , the ant-agonist competes with the agonist at the activating site in a mutually exclusive manner.

Below is the formulation for a fractional response of simple competitive ant-agonism as the ant-agonist concentration varies, while the agonist concentration is fixed at S' , and with the dissociation constants tacitly assumed:

$$\frac{ar'}{TR} = \frac{R \cdot S'}{R + R \cdot S' + R \cdot I} = \frac{S'}{1 + S' + I}, \quad (2.11)$$

or in an explicitly normalized fashion by including dissociation constants:

$$\frac{ar'}{TR} = \frac{\frac{S'}{K_{ss}}}{1 + \frac{S'}{K_{ss}} + \frac{I}{K_{si}}} = \frac{S'}{S' + K_{ss} \cdot \left(1 + \frac{I}{K_{si}}\right)}. \quad (2.12)$$

We can identify that when $I = 0$, we have the full response $FR = ar' = TR \cdot (S' / (S' + K_{ss}))$. With a fixed agonist concentration $[S']$, it is only the so-called apparent dissociation $\text{app}K_{ss}$, equal to $K_{ss} \cdot (1 + I/K_{si})$ in the denominator of Eq. 2.12 that varies as the $[I]$ concentration varies, not the FR .

However, when $[S]$ is not fixed, the right-hand term in Eq. 2.12 has the form of a **load** dose-response expression with an apparent dissociation constant for the ant-agonist, $\text{app}K_{si} = IC_{50}$, which varies with the concentration of both the agonist and the ant-agonist; thus $IC_{50} = K_{ss} \cdot (1 + I/K_{si})$ is a function of both I and S . The effect of S on the IC_{50} is most easily seen from a reformulation of Eq. 2.12:

Table 2.3. Summary of the Relative Maximal Responses ($R_{\max}$) and Maximal Occupancies ($B_{\max}$) and the Apparent Dissociation Constant for Agonist S, $\text{app}K_{\text{ss}}$, in the Simplest Mutually Exclusive and Inclusive Reaction Schemes

Summary of Sections 2.4.5–6; 2.4.9; 2.4.10; and 2.5.1	Competitive I+II ant-agonism $c=0$, mutually exclusive (Figs. 2.8A–2.9A)	Non-competitive ant-agonism $c=1$, mutually inclusive (Figs. 2.8C–2.9C)	Intervention model, intervention $c>0$, mutually inclusive (Figs. 2.8–2.9)
Study type	Parameters		
	Fractional response $B_{\max}/R_{\max}$ for high [S]		
Occupancy	1 surmountable	1, independent of [I]	1 surmountable
Effect	1 surmountable	<1 ; $1/(1+I/K_{\text{ii}})$	<1 ; $1/(1+(I/K_{\text{ii}}) \cdot c)$
	Apparent agonist dissociation constant $\text{app}K_{\text{ss}} = \text{EC}_{50}$		
Occupancy [§]	$K_{\text{ss}} \cdot (1+I/K_{\text{i}})^{\dagger}$	K_{ss}	$\frac{K_{\text{ss}} \cdot (1+I/K_{\text{ii}})^{\#}}{(1+(I/K_{\text{ii}}) \cdot c)}$
Effect	$K_{\text{ss}} \cdot (1+I/K_{\text{i}})^{\dagger}$	K_{ss}	$\frac{K_{\text{ss}} \cdot (1+I/K_{\text{ii}})^{\#}}{(1+(I/K_{\text{ii}}) \cdot c)}$

Mutually inclusive inhibition = non-competitive ant-agonism, and mutually exclusive inhibition = competitive ant-agonism.

[†] $K_{\text{i}} = K_{\text{si}}$ or K_{ii} .

[#]For $c>1$ $\text{app}K_{\text{ss}} < K_{\text{ss}}$; for $c<1$ $\text{app}K_{\text{ss}} > K_{\text{ss}}$. Compare these two equations with similar equations for 'allosteric' ligand binding and effect in Ehlert (1988). The co-lateral intervention constant c here is equal to the inverse of constant α in Ehlert's paper.

[§]Special: for competitive ant-agonism type I with $c \gg 1$ and $1/c \approx I/K_{\text{ii}}$ all concentration-occupancy relationships lie on top of each other.

$$\frac{ar}{TR} = \frac{1}{1 + \frac{K_{\text{ss}}}{S} \cdot \left(1 + \frac{I}{K_{\text{si}}}\right)}. \quad (2.13)$$

Here $(K_{\text{ss}}/S) \cdot (1+I/K_{\text{si}})$ in the denominator of Eq. 2.13 is a type of relative apparent dissociation constant dependent on the level of both [S] and [I].

Recap that we have assumed there is a steric hindrance for the agonist to bind at the agonist site and activate the receptive unit when an ant-agonist is bound to the agonist site of R. This was for function.

In *occupancy*, the same equations as Eqs. 2.11 and 2.12 may be derived for comparable theories for binding studies. Therefore, conclusions about system constants for binding assays in competitive inhibition are the same as above (cf. Table 2.3).

2.4.10. Formulation of Competitive Ant-agonism Type II

As specified in Section 2.4.2, there is another reaction scheme that leads to competitive ant-agonism (Fig. 2.4-1, grey marking). We assume that there is a steric hindrance for the agonist to bind at the agonist site and activate the receptive unit when an ant-agonist is bound to an ant-agonist site on R. Development of the formulation for this type of reaction scheme is straightforward. It should result in:

$$\frac{ar'}{TR} = \frac{R \cdot S'}{R + R \cdot S' + R \cdot I} = \frac{S'}{1 + S' + I}, \quad (2.14)$$

or in explicitly normalized form:

$$\frac{ar'}{TR} = \frac{S'}{S' + K_{\text{ss}} \cdot \left(1 + \frac{I}{K_{\text{ii}}}\right)}. \quad (2.15)$$

There is an overlap between the above type I competitive ant-agonism in Section 2.4.9, and the formulations for the present regime of type II competitive ant-agonism in functional and binding studies. Compare Eq. 2.11 with Eq. 2.14 and Eq. 2.12 with Eq. 2.15; only K_{ii} is replaced with K_{si} . This is contrary to the non-competitive ant-agonism where formulation from functional studies deviates from that of binding studies.

In *occupancy*, as mentioned, in either case where these two types of competitive ant-agonism operate, one with a single binding site and the other with two binding sites, the same equations as Eqs. 2.14 and 2.15 may be derived for comparable theories on competitive binding studies. Therefore, the conclusions above on system constants for functional studies are the same as for binding studies when competitive (cf. Table 2.3).

Due to complete thermodynamic reversibility in the competitive inhibition type II reaction scheme (Fig. 2.4-1, in grey), the binding of an inhibitor to the secondary binding site can be surmounted by binding enough agonists to the primary binding site. Accordingly, also for occupancy studies, the two types of competitive ant-agonism will be hard to discern experimentally (for 'surmount-ability', see Section 2.8.2).

Competitive ant-agonism may also be developed for a system equal to the 'cross' in Fig. 2.4. In this type of

reactions scheme, which is competitive with five receptor conformations, R, RS, IR, SR, and RI, there will be a discrepancy between function and binding, since only RS is functional, while both RS and SR count for binding. Producing formulations for this ‘cross’ reaction scheme and implementing its analysis are left to the reader.

2.4.11. Actual Remaining Activity and Actual Inhibitory Response for Competitive Ant-agonism

For the competitive reaction schemes type I and type II, the relative and actual remaining activity (*ara*) is determined at a fixed concentration of the agonist S' , and an increasing concentration of an ant-agonist I, according to the equation for either type of competitive ant-agonism. Thus, for *ara* as a fraction in type I competitive ant-agonism, we have:

$$ara = \frac{S'}{S' + K_{ss} \cdot (1 + I/K_{si})}. \quad (2.10b)$$

Eq. 2.10*b* is plotted in linear-linear and semi-log plots in Fig. 2.6A+B. Compare this equation with Eq. 2.10*a* for non-competitive ant-agonism.

If we equate the fractional level from which a competitive ant-agonist can operate, *viz.* $S'/(S' + K_{ss}) = FR$, then we can formulate the reverse function of *ara*, equal to the relative and actual inhibitory response (*air*) as a function of increasing ant-agonist concentration, i.e.,

$$air = FR - \frac{S'}{S' + K_{ss} \cdot (1 + I/K_{si})}. \quad (2.9b)$$

By incorporating the constant *FR* into the fractional term, we get:

$$air = \frac{FR \cdot [S' + K_{ss} \cdot (1 + I/K_{si})] - S'}{S' + K_{ss} \cdot (1 + I/K_{si})}, \quad (2.9c)$$

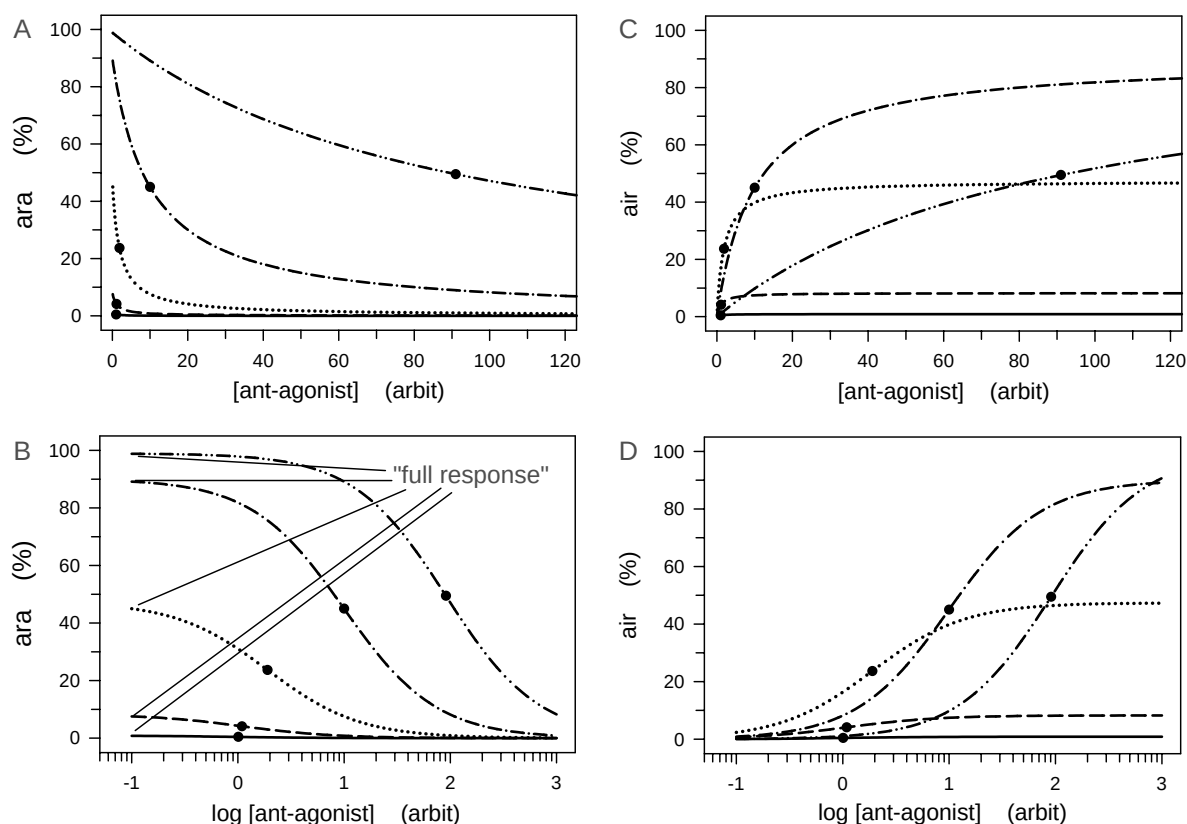


Figure 2.6. Competitive ant-agonism ($c=0$) for both function and occupancy. Panel A is linear-linear and panel B is semi-log plots for the actual remaining activity (*ara*) in competitive ant-agonism as the concentration of the ant-agonist increases (Eq. 2.10*b*). Panel C is linear-linear and panel D is semi-log plots of the actual inhibitory response (*air*) in competitive ant-agonism as the concentration of the ant-agonist increases (Eq. 2.9*d*). The full response is varied in five steps by varying S' from 10^{-2} (—) to 10^2 (---) in steps of 10 yielding five different curves. Plots are for both competitive ant-agonism type I and type II, and the filled circles indicate the K_{si} or K_{ij} on the x-axes.

and the nominator of Eq. 2.9c can be reformulated to $FR \cdot (I \cdot K_{ss}) / K_{si}$. Inserting the value for FR , the *air* is thus equal to:

$$air = \frac{S'}{S' + K_{ss}} \cdot \frac{I \cdot K_{ss} / K_{si}}{S' + K_{ss} \cdot (1 + I / K_{si})} \quad (2.9d)$$

Eq. 2.9d is plotted in linear-linear and semi-log plots in Fig. 2.6C + D.

Notice that Eqs. 2.10b and 2.9d are both valid for any value of $S' \geq 0$ and are mirror images of each other (compare Figs. 2.6A + B with 2.6C + D).

The equations here in Section 2.4.11 are for both function and occupancy.

For competitive ant-agonism type II, we simply replace K_{ss} in Eqs. 2.10b and 2.9b–d with K_{ii} .

2.4.12. Competitive Versus Non-competitive Studies. A Summary

It is traditional to conceive of the competitive and non-competitive reaction schemes as opposites. This is based on the fact that in concentration-binding and dose-response studies of simple competitive ant-agonism (Section 2.4.9 and 2.4.10), it is only the apparent agonist-binding constant, $\text{app}K_{ss} = \text{EC}_{50}$, which changes as the ant-agonist concentration increases, while the maximal agonist binding or maximal agonist-dependent response obtainable are unaffected at all ant-agonist concentrations. In simple competitive ant-agonism, the inhibitory effect of increasing an ant-agonist concentration on maximal binding or response of an agonist is always surmountable with high enough agonist concentrations as described in Sections 2.4.9 and 2.4.10.

Meanwhile, in the simple non-competitive ant-agonism of dose-response relations, only the maximal agonist response in the presence of a so-called non-competitive ant-agonist will be lower than the maximum of an agonist-dependent response in the absence of an ant-agonist, while the apparent agonist equilibrium dissociation constant, $\text{app}K_{ss}$, is unaffected by the presence of a non-competitive ant-agonist and equal to K_{ss} (cf. Section 2.4.5, Fig. 2.5, and Table 2.3).

On the contrary, for non-competitive binding, neither the maximal binding nor the agonist dissociation constant is affected (Table 2.3). This is not the conventional understanding of ‘non-competitive ant-agonism’.

2.5. On Simple Ant-agonism and Simple Intervention

In this sub-chapter, you will learn that when we develop a certain theory in the form of a reaction scheme to

Table 2.4. Effects in the Intervention Model on $\text{app}K_s$ and $B_{\max}$ or $R_{\max}$ for Increasing Concentration of Interventor [I] (Compare with Table 2.6)

	$\text{app}K_s$	$B_{\max}$ or $R_{\max}$ (%)	Comment
Binding $c=0$	$\uparrow\uparrow$	100	Competitive
$0 < c < 1$	$\uparrow$	100	
$c=1$	K_s	100	Non-competitive
$c > 1$	$\downarrow\downarrow$	100	
Function $c=0$	$\uparrow\uparrow$	100	Competitive
$0 < c < 1$	$\uparrow$	$\rightarrow 0$	
$c=1$	K_s	$\rightarrow 0$	Non-competitive
$c > 1$	$\downarrow\downarrow$	$\rightarrow 0$	

Know your theoretical tools and what they can accomplish.

illustrate our concepts of competitive and non-competitive ant-agonism, as described in Sub-chapter 2.4, such a reaction scheme does not always yield the expected solutions. Further, the solutions may deviate between functional experiments and binding studies. This will be clear when we summarize the conclusions about simple ant-agonism and simple intervention in Tables 2.3 and 2.4. The theory developed and examined in this sub-chapter is a so-called *intervention model*. In this model the constant c , which was kept = 1 in Sub-chapter 2.4, is now allowed to vary.

2.5.1. Intervention and Non-competitive Reaction Schemes

The non-competitive reaction scheme and variants thereof were presented in Sections 2.4.5, 2.4.6, 2.4.7, and 2.4.8. A condition for these schemes was the assumption that the constant c equaled unity. Here, we will analyze the impact of constant c varying freely above as well as below unity in the full cyclic scheme of Figs. 2.1A or 2.4.1.

When the co-lateral intervention constant is equal to unity, $c = 1$, it is the same as saying that binding of an interventor to a secondary site does not change the binding of an agonist to its orthosteric binding site and *vice versa* when an agonist binds to the orthosteric binding site it does not change the equilibrium binding constant for an interventor to its binding site, the secondary binding site. Observe in this connection, that a non-competitive ant-agonist/inhibitor is not the same as a neutral ant-agonist.⁴

The non-competitive ant-agonist/inhibitor should in a model-related sense be a ‘neutral interventor’ (see Table 2.2).

⁴ A neutral ant-agonist (see Milligan et al. 1995; Milligan & Bond 1997; Richard et al. 2001; Foucaud et al. 2006) is something other than a non-competitive ant-agonist, α : non-competitive ant-agonists/inhibitors are defined by $c = 1$, while neutral ant-agonists are defined by system constant $b = 1$. For constants b and c , see for instance the allosteric two-state model (ATSM) in Chapter 7.

However, that c is equal to unity is unlikely to be the general case, since one would expect an ant-agonist/inhibitor, on binding to the secondary binding site, to cause a conformational change in the receptive unit, and thereby a change in agonist-dependent binding and activity. A quantification of this expected change is taken care of by the co-lateral intervention constant c , as shown in Figs. 2.1A and 2.4-1, equal to a full intervention reaction scheme.

Function. Therefore, in functional studies, when c is different from unity, both the apparent agonist dissociation constant, $\text{app}K_{ss}$, and the maximal agonist-dependent response, R_{max} , are affected. As already stated, in classic theory this is referred to as *mixed*-inhibition (ant-agonism). Thus, for $c \neq 1$ (Fig. 2.4-1) which is the most likely condition for ant-agonism/inhibition of mutually inclusive ant-agonists/inhibitors, the relationship of dose-response by agonist in the presence of an ant-agonist/interventor will most likely demonstrate mixed behavior, i.e., both R_{max} and K_{ss} are altered concomitantly.

2.5.2. Switch from 'Ant-agonist' to 'Interventor' in Binding and Function

For intervention schemes the fractional *binding* of an agonist as the interventor concentration varies is given by:

$$\frac{\text{occupancy}}{\text{total}} = \frac{S}{S + K_{ss} \cdot \left(\frac{1 + I/K_{ii}}{1 + (I/K_{ii}) \cdot c} \right)}, \quad (2.16)$$

and, as the concentration of an interventor changes, the fractional *activity*, which is dependent on an agonist, is:

$$\frac{\text{response}}{\text{total}} = \frac{S/(1 + (I/K_{ii}) \cdot c)}{S + K_{ss} \cdot \left(\frac{1 + I/K_{ii}}{1 + (I/K_{ii}) \cdot c} \right)}. \quad (2.17)$$

Now I is not only symbol for an ant-agonist/inhibitor but also for an interventor ligand.

Examples of semi-log plots of Eq. 2.16 for binding-against-agonist concentration are shown in Fig. 2.7, and

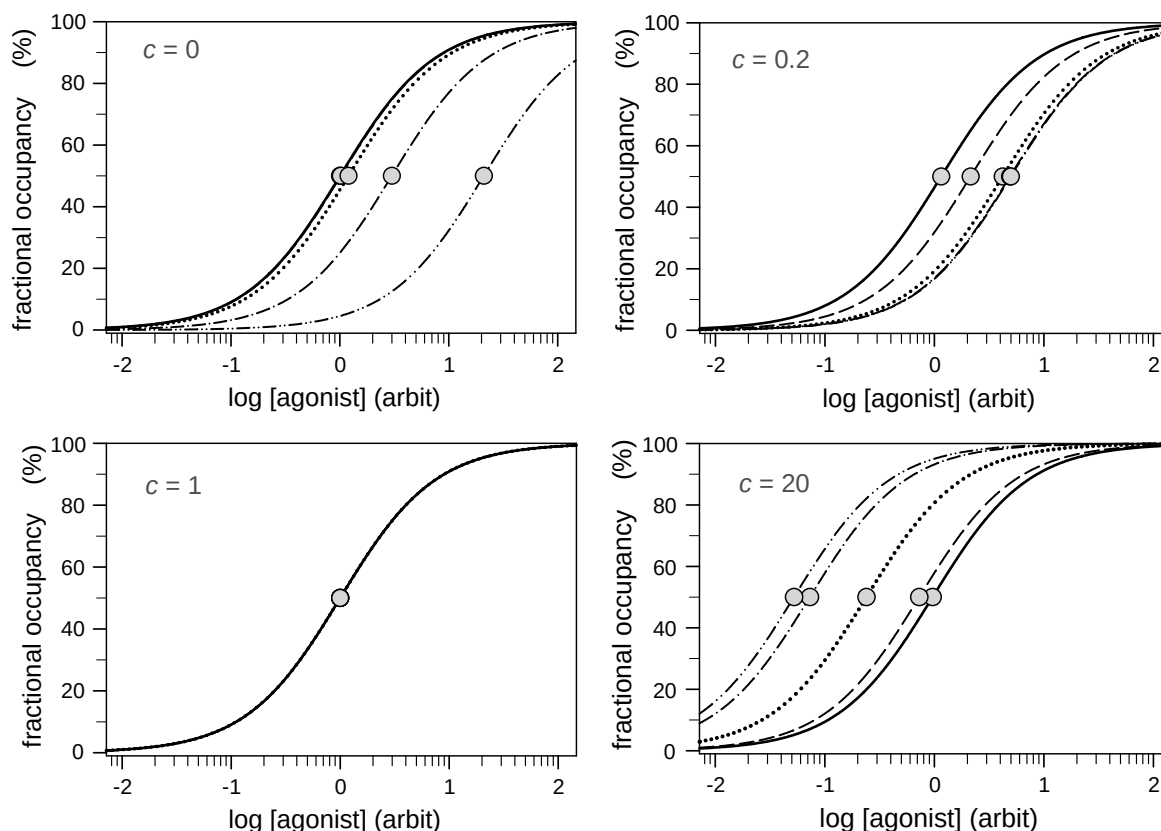


Figure 2.7. Plots of the intervention model for **binding**. In the first three panels the interventor concentration $[I]$ is varied by a factor 10 in five steps between $2 \cdot 10^{-1}$ (—) and $2 \cdot 10^3$ (-----). In the last panel ($c=20$), the interventor concentration $[I]$ is varied by a factor 10 in five steps between $2 \cdot 10^{-3}$ (—) and $2 \cdot 10$ (-----) yielding five plots in each graph. In $c=0$, the plots are for the competitive situation. In $c=0.2$, the plots are for a negative intervention situation. In $c=1$, the plots are for a non-competitive situation. In $c=20$, the plots are for a positive intervention situation. Tinted circles indicate the ED_{50} at 50% of the effective binding (EB_{50}). K_{ss} =dissociation constant for S at primary s-site and K_{ii} =dissociation constant for interventor ligand at intervention i-site are both =1.

response-against-agonist concentration (Eq. 2.17), is shown in Fig. 2.8 when the intervencor concentration $[I]$ is varied in five steps and with four different values for constant c as well.

From Eq. 2.16 for concentration-occupancy it is fairly straightforward to deduce that the apparent dissociation constant $\text{app}K_{ss}$ for intervention is equal to $K_{ss} \cdot (1 + I/K_{ii}) / (1 + (I/K_{ii}) \cdot c)$, and thus may be above or below K_{ss} and that the maximal occupancy is reached at 100% as the agonist concentration S increases (Fig. 2.7).

For intervention in the dose-effect relationship (Eq. 2.17, Fig. 2.8), we can show that the apparent dissociation constant $\text{app}K_{ss} = K_{ss} \cdot (1 + I/K_{ii}) / (1 + (I/K_{ii}) \cdot c)$ may be above or below K_{ss} and the fractional effect at high agonist concentration is always <1 in the presence of an intervencor for $c > 0$: $R_{\max} = 1 / (1 + (I/K_{ii}) \cdot c)$ (Fig. 2.8). Thus, the intervencor appears as a non-surmountable inhibitor.

For both the binding and functional schemes, it is worth noting that the non-competitive ant-agonism/

inhibition appears for $c = 1$ (Figs. 2.7C and 2.8C), and competitive ant-agonism/inhibition type II for $c = 0$ (Figs. 2.7A and 2.8A). Thus, non-competitive and competitive ant-agonism are merely special cases of the more general intervention theory.

In non-competitive ant-agonism/inhibition only the $R_{\max}$ in functional studies is affected (Fig. 2.8C), while neither the maximal binding $B_{\max}$ nor its agonist equilibrium dissociation constant K_{ss} are affected (Fig. 2.7C). The fractional effect at high $[S]$ in functional studies will be $R_{\max} = 1 / (1 + (I/K_{ii}))$, and in binding studies the occupancy reaches maximum and is always completely independent of $[I]$ (Tables 2.3 and 2.4). Obviously when $c = 1$ – the non-competitive condition – then $\text{app}K_{ss}$ is equal to K_{ss} for both occupancy and response studies (Table 2.4). System constants for all these derived conditions, based on theories, each from a portion of the four-pane reaction scheme with one state as in Fig. 2.4-1, are summed up in the Section 2.5.3.

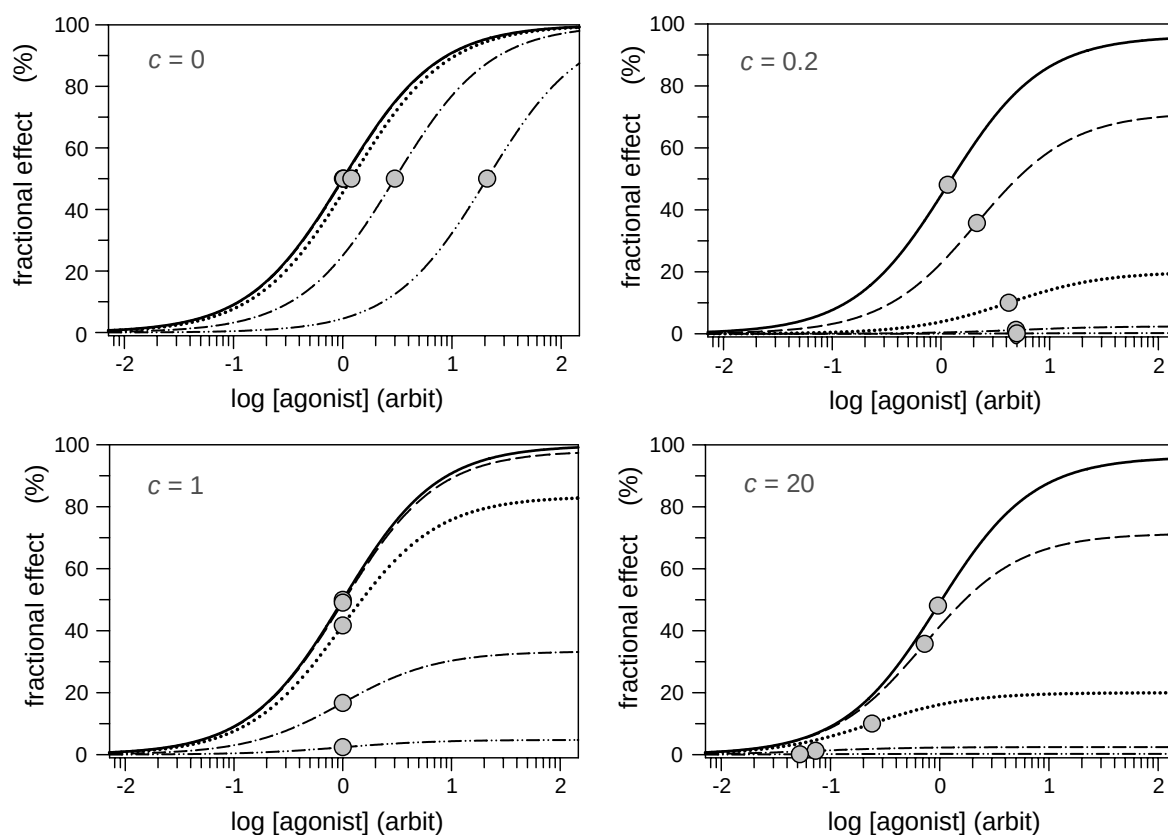


Figure 2.8. Plots of the intervention model for **function**. In the first three panels the intervencor concentration $[I]$ is varied by a factor 10 in five steps between $2 \cdot 10^{-1}$ (—) and $2 \cdot 10^3$ (— · — · —). In the last panel ($c = 20$), the intervencor concentration $[I]$ is varied by a factor 10 in five steps between $2 \cdot 10^{-3}$ (—) and $2 \cdot 10$ (— · — · —) yielding five plots in each graph. In $c = 0$, the plots are for a competitive situation. In $c = 0.2$, the plots are for a negative intervention situation. In $c = 1$, the plots are for a non-competitive situation. In $c = 20$, the plots are for a positive intervention situation. Tinted circles indicate the ED_{50} at 50% of the effective response (ER_{50}). K_{ss} = dissociation constant for S at primary s-site and K_{ii} = dissociation constant for intervencor ligand at intervention i-site are both = 1.

2.5.3. Summary of Parameter Characteristics from Simple Intervention

Based on both mutually exclusive and inclusive one-state models, ME-OSM and MI-OSM, with an intervenor different from the agonist, we reach a conclusion on the various effects an intervenor may have on agonist-induced maximal binding or maximal response and on agonist affinity, $1/K_{ss}$, with different types of intervention, as analyzed in Sections 2.4.5–6 and 2.4.9–10, and for intervention in Sections 2.5.1 and 2.5.2. Examples of plots of the intervention model are shown in Figs. 2.7 and 2.8. Conclusions from these sections are summarized in Tables 2.3, 2.4 and 2.5. These results may be compared with similar solutions and conclusions reached by other authors on competitive and non-competitive inhibition as well as mixed-inhibition and -activation (Ariëns et al. 1964, pp. 287–321; Cheng & Prusoff 1973; Laidler & Bunting 1973; Segel 1975, 1993 – mixed-inhibition, pp. 136–143; Levitzki 1978, pp. 11–27; Dixon & Webb 1979, pp. 332–353; Varon et al. 2002; Cornish-Bowden 2004, Chapter 5; Shou 2005). Examples in enzymology are of a more complex nature as they include product formation and additional pathways.

2.5.4. The Un-competitive Reaction Scheme

The suggested reaction scheme for un-competitive⁵ ant-agonism/inhibition (see Segel 1975, 1993, Fig. III-21; Cornish-Bowden 1995, 2004) is a special case, a shortened version of the MI-OSM described in Section 2.4.5 (Fig. 2.4-1). In the *shortened* version of the MI-OSM, the receptive unit conformation IR is excluded. Now, the un-competitive reaction scheme has the parameter $c = 1$. In the ‘un-competitive’ reaction of the *shortened* version of MI-OSM, the intervenor cannot bind to the unliganded receptive unit. This means that the receptive unit must first bind an agonist before a second intervenor ligand can bind. That is, an ordered reaction. Parameter c is allowed to vary above and below unity in the *shortened* version of MI-OSM, but in reality, in the classical un-competitive scheme $c = 1$. It is subsumed into the dissociation constant K_{ii} and we can put c as 1. The reaction scheme for the shortened version of the mutually inclusive one-state model is:



where only the R'S conformation is active or activatable. R' and R'' in this reaction scheme signify conformations different from R and from each other.

We can derive the fractional concentration-binding and dose-response equations for this shortened mutually-

Table 2.5. Summary of the Relative Maximal Responses (R_{max}) and Maximal Occupancies (B_{max}) and the Apparent Dissociation Constant for Agonist S, $appK_{ss}$, in Two Simple Mutually Inclusive Reaction Schemes, the *Shortened* Heterotropic Mutually Inclusive One-state Model (amp-HE-MI-OSM) and the Full Heterotropic Mutually Inclusive One-state Model (HE-MI-OSM)

Type of study with $c \neq 1$	amp-HE-MI-OSM	HE-MI-OSM
Parameters		
Fractional response B_{max}/R_{max} (in%) for high [S]		
Concentration-occupancy	100; surmountable	100; surmountable
Dose-response	$100/(1 + (I/K_{ii}) \cdot c)$	$100/(1 + (I/K_{ii}) \cdot c)$
Apparent dissociation constant $appK_{ss} = EC_{50}$		
Concentration-occupancy	$K_{ss}/(1 + (I/K_{ii}) \cdot c)$	$\frac{K_{ss} \cdot (1 + I/K_{ii})^{\#}}{(1 + (I/K_{ii}) \cdot c)}$
Dose-response	$K_{ss}/(1 + (I/K_{ii}) \cdot c)$	$\frac{K_{ss} \cdot (1 + I/K_{ii})^{\#}}{(1 + (I/K_{ii}) \cdot c)}$

For the ‘un-competitive’ situation, amp-HE-MI-OSM, parameter K_{ii} varies with c as there are only two independent system constants (see Figs. 2.9 and 2.10).
[#]For $c > 1$ $appK_{ss} < K_{ss}$; for $c < 1$ $appK_{ss} > K_{ss}$.

inclusive and one-state reaction scheme. For *binding* one gets:

$$\frac{\text{occupancy}}{\text{total}} = \frac{S}{S + K_{ss}/(1 + (I/K_{ii}) \cdot c)}, \quad (2.18)$$

and for the *functional* shortened MI-OSM it is:

$$\frac{\text{response}}{\text{total}} = \frac{S/(1 + (I/K_{ii}) \cdot c)}{S + K_{ss}/(1 + (I/K_{ii}) \cdot c)}. \quad (2.19)$$

The system parameters for the fractional binding and response of the shortened MI-OSM are summarized in Table 2.5, its behavior in Table 2.6, and examples of plots are presented in Fig. 2.9 for occupancy and Fig. 2.10 for function. All panels in Figs. 2.9 and 2.10 are for the ‘un-competitive’ situation because when c varies it is the same as K_{ii} varies.

Table 2.6. Effects of the Shortened Intervention Model on $appK_s$ and B_{max} or R_{max} for Increasing Concentration of Intervenor [I] (Compare with Table 2.4)

	$appK_s$	B_{max} or R_{max} (%)	Comment
Binding $c=0$	K_s	100	Competitive
$0 < c < 1$	$\downarrow$	100	
$c=1$	$\downarrow\downarrow$	100	Un-competitive
$c > 1$	$\downarrow\downarrow\downarrow$	100	
Function $c=0$	K_s	100	Competitive
$0 < c < 1$	$\downarrow$	$\rightarrow 0$	
$c=1$	$\downarrow\downarrow$	$\rightarrow 0$	Un-competitive
$c > 1$	$\downarrow\downarrow\downarrow$	$\rightarrow 0$	

⁵ The term ‘un-competitive’ was introduced by Ebersole et al. (1944).

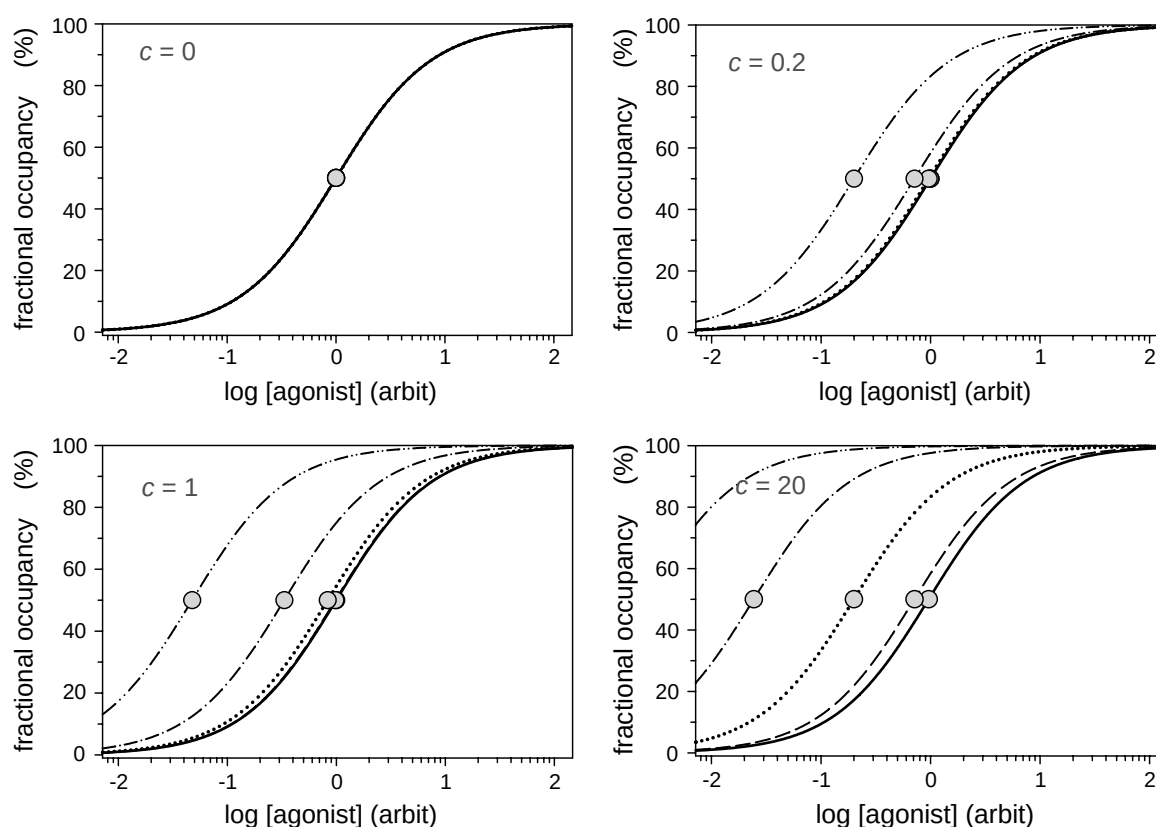


Figure 2.9. Plots of the *shortened* intervention model for **binding**. In all four panels the intervener concentration $[I]$ is varied by a factor 10 in five steps between $2 \cdot 10^{-1}$ (—) and $2 \cdot 10^3$ (-·-·-) yielding five plots in each graph. In $c=0$, the plots are for an ‘ordered’ competitive situation. In $c=0.2$, the plots are for the negative shortened intervention situation. In $c=1$, the plots are for an ‘ordered’ non-competitive, un-competitive, situation. In $c=20$, the plots are for the positive shortened intervention situation. Tinted circles indicate the ED_{50} at 50% of the effective **binding** (EB_{50}). K_{ss} = dissociation constant for S at primary s-site and K_{ii} = dissociation constant for intervener ligand at intervention i-site are both = 1.

2.5.5. Conclusions for Both Complete and Shortened MI-OSMs

For comparison, Table 2.6 lists the parameter characteristics based on the complete heterotropic MI-OSM together with the equivalent terms for the shortened heterotropic MI-OSM.

With a definition of ‘un-competitive’ as a concomitant lowering of both the maximal response and the affinity for agonists in the presence of an intervener, if we base our modeling of such a behavior on either the shortened or the complete MI-OSM, the conclusion is that only in the functional versions of these models is the ‘un-competitive’ definition fulfilled. In the binding versions of these models, we simply have ‘competitive’ ant-agonism (Tables 2.3, 2.4, 2.5 and 2.6).

A theory for non-competitive behavior in functional studies is described by the heterotropic MI-OSM when the co-lateral intervention constant is equal to unity, $c = 1$, while non-competitive behavior in binding studies does not have a correlate in MI-OSM (Table 2.3).

Competitive ant-agonism/inhibition both in binding and functional studies may be analyzed by the ME-OSM.

Strangely enough, competitive behavior in binding studies can also be analyzed by the shortened MI-OSM as well as the complete MI-OSM for all values of c (Tables 2.3 and 2.4).

2.6. Co-lateral Intervention Coefficient and Co-lateral Binding

2.6.1. ‘Co-lateral’⁶ in Two Terms

Caveat: The terms ‘co-lateral intervention coefficient’ and ‘co-lateral binding’ are two totally different concepts.

Co-lateral intervention coefficient c appears in the intervention reaction scheme (Fig. 2.4-1) with mutually inclusive binding, meaning that both an agonist and an intervener can bind simultaneously to their respective binding sites and affect binding or function of the other ligand. Meanwhile, they cannot bind co-laterally in this scheme.

⁶ Co-lateral or collateral? ‘Co-lateral’ is chosen in favor of ‘collateral’.

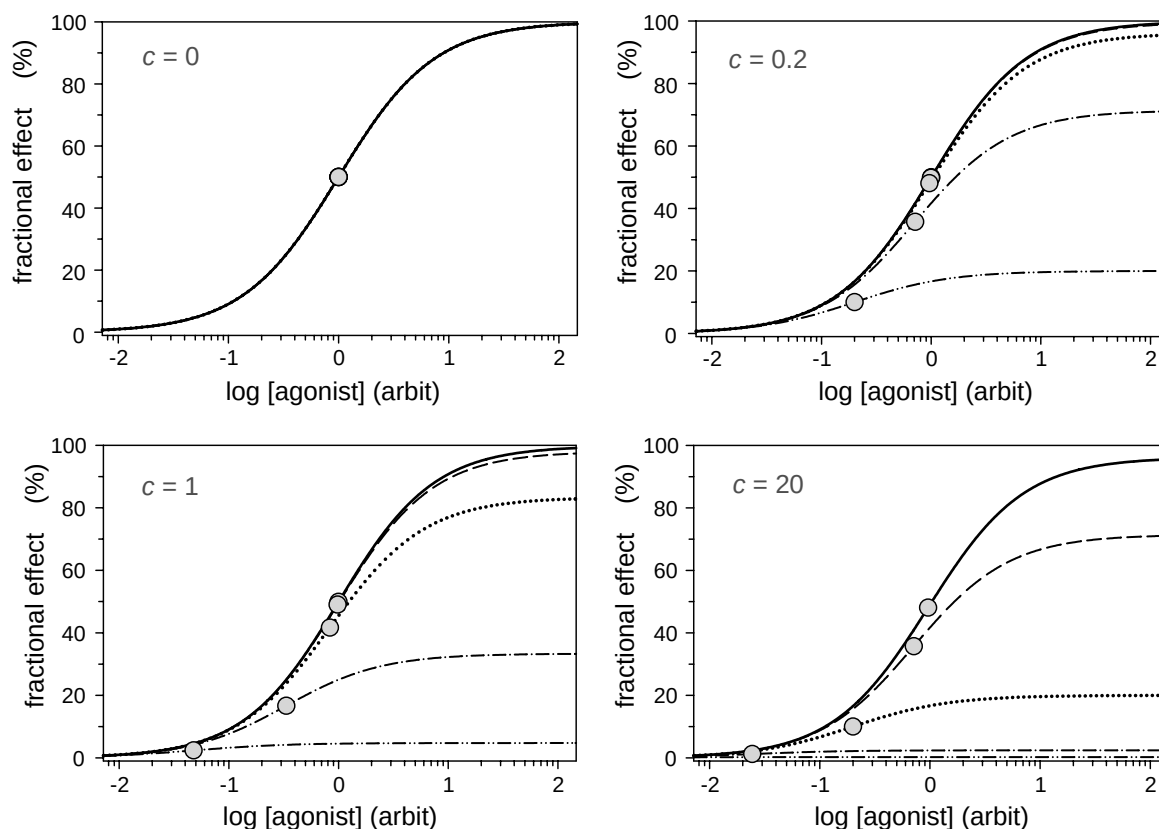


Figure 2.10. Plots of the *shortened* intervention model for **function**. In all four panels the intervener concentration $[I]$ is varied by a factor 10 in five steps between $2 \cdot 10^{-1}$ (—) and $2 \cdot 10^3$ (-·-·-) yielding five plots in each graph. All plots are for variations in agonist concentration. In $c=0$, the plots are for an 'ordered' competitive situation. In $c=0.2$, the plots are for the negative shortened intervention situation. In $c=1$, the plots are for an 'ordered' non-competitive, un-competitive, situation. In $c=20$, the plots are for the positive shortened intervention situation. Tinted circles indicate the ED_{50} at 50% of the effective **response** (ER_{50}). K_{ss} = dissociation constant for S at primary s-site and K_{ii} = dissociation constant for intervener ligand at intervention i-site are both = 1.

Co-lateral binding signifies that the substrate or agonist is allowed to bind to both the primary binding site as well as the secondary binding site (Figs. 2.4-2 and 2.4-3, and Section 2.6.3) and that the intervener can also bind to both binding sites, including the orthosteric binding site (Figs. 2.4-3 and 2.4-4). The two ligands can switch sites, and this ability of ligands to switch sites is defined as *co-lateral binding*. Thus, co-lateral binding goes beyond 'mutually inclusive' binding, which only covers simultaneity in binding.

2.6.2. Co-lateral Intervention Constants and Auto-intervention

In this chapter, ant-agonism and intervention by ligands have, thus far, solely been induced by molecules other than the agonists – i.e., hetero-intervention. We shall deal further with intervention that is elicited by the agonist itself. This type of intervention is also known as 'auto-intervention' (Fig. 2.4-2), and will be examined in Chapter 3. In this reaction scheme there is an obligatory co-lateral binding, RS or SR and SRS.

The co-lateral intervention constant for hetero-intervention is described by c , while the co-lateral intervention constant for auto-intervention is designated as c' , in order to differentiate between the two co-lateral intervention constants (Table 2.7). See Figs. 2.4-1 and 2.4-2 of the four-pane scheme for reasons to operate with both c and c' .

2.6.3. Co-lateral Binding and Auto-intervention

A reaction scheme with co-lateral binding as well as mutually inclusive binding – simultaneous binding for both agonists and intervenors – is illustrated in Fig. 2.4. It is the complete four-pane one-state-model (FP-OSM). The FP-OSM was used to derive the reaction schemes for competitive ant-agonism/inhibition types I and II (ME-OSMs) and for non-competitive and un-competitive ant-agonism/inhibition as well, and, furthermore, expanding into intervention models where both c and c' are above 0 (MI-OSMs). In Chapter 3, I describe an auto-intervention model where only one type of ligand (substrate, agonist) can bind to both an orthosteric and a secondary site

Table 2.7. Models With and Without Co-lateral Intervention and With and Without Co-lateral Binding

Type of model	Co-lateral	
	Intervention constant	Binding
ME-OSM (Chapter 2)	None ($c=0$)	No
HE-MI-OSM (Chapter 2)	c	No
HO-MI-OSM (Chapter 3)	c'	Yes
FP-OSM (Chapter 2)	Both c and c'	Yes
ATSM (Chapter 7)	c	No
HOTSM (Chapter 7)	c'	Yes

simultaneously. It is an auto-intervention one-state model (HO-MI-OSM), with co-lateral binding for the ligand (Fig. 2.4-2). Obviously, for interventors that can bind to orthosteric as well as secondary binding sites, there is also co-lateral binding, equal to the upper left quadrant of the FP-OSM (Fig. 2.4-4).

2.6.4. Co-lateral Binding, Co-lateral Intervention and the HOTSM/ATSM

After the FP-OSM has been transformed to its two-state variant, a four-pane two-state model (FP-TSM; see Figs. 5.9 and 7.2), it will be employed to derive the homotropic two-state model (HOTSM, Fig 7.1B) in Part II, Chapter 7 (Bindslev 2004), which is a part of the FP-TSM with co-lateral binding. However, note that an allosteric two-state model (ATSM) (Hall 2000), which is also part of the FP-TSM, and likewise described in Chapter 7, does not allow co-lateral binding for the interventor, modulator or agonist (Fig. 7.1A and Table 2.7). This, as opposed to the lower right quadrant in the FP-OSM for a primary ligand (Fig. 2.4-2), and the upper left quadrant of the FP-OSM for a secondary ligand, an interventor (Fig. 2.4-4). In these two quadrants co-lateral binding is allowed and comparable to co-lateral binding in the FP-TSM for an agonist and a modulator (Fig. 7.2).

2.6.5. Differences Between Intervention and Negative Allostery

Negative allostery is first defined in Chapter 15 and demonstrated in Fig. 15.10. Briefly, in functional studies of negative allostery, a modulator molecule can lower the maximal response similar to an interventor in the intervention scheme (Fig. 2.8 and Table 2.3).

The difference between negative intervention and negative allosteric modulation is that allosteric modulation conceptually is based on two-state models, while the intervention including un-competitive and non-competitive ant-agonism/inhibition is in the realm of one-state models. Two-state against one-state models is the difference. For the sake of organizing models in a meaningful and manageable lattice that is also crystal clear, the

crucial concept of the difference between two-state and one-state is further discussed in Chapters 5 and 15. If it is not *the* key issue of the book, it is one of them.

2.7. A History on the Development of Ternary-complex Models

2.7.1. Early Years

From 1914 models were suggested for the action (function) of enzymes altered through two different substrates or ligands that interfere with the action of each other (Michaelis 1914; Burk 1944 cited by Ebersole et al 1944; and by Friedenwald & Maengwyn-Davis 1954a, pp. 163). Burk termed the aberrant behavior “anti-competitive coupling activation”. Formalism for ternary complexes with two substrates was developed in the following years (Laidler & Socquet 1950; Friedenwald & Maengwyn-Davis 1954a,b, pp. 154–190; Webb 1963; Mahler & Cordes 1966; Cheng & Prusoff 1973; Segel 1975, 1993; Birdsall et al. 1978; Tolkovski & Levitzki 1978; Dixon & Webb 1979, pp. 79–82). In pharmacological literature, similar and not always complete derivations starting with Ariëns et al. (1955) appeared in the 1970s and 1980s (Jacobs & Cuatrecasas 1976; Boeynaemes & Dumont 1980; Burgisser et al. 1982; Stockton et al. 1983).

2.7.2. Terminology for the Intervention Scheme

The fully reversible reaction scheme for two ligands binding at a single receptive unit is presented in Figs. 2.1A and 2.4-1, and appears in different settings. I call it *the intervention model*. In principle, it is a heterotropic, one-state ternary-complex model that is also ‘inclusive’, which indicates that it allows binding of two ligands simultaneously. All these features are also part of the non-competitive reaction scheme presented in Sub-chapter 2.4. The difference between a non-competitive reaction scheme and the intervention scheme is that constant c may deviate from unity in the intervention scheme.

The intervention scheme in Fig. 2.4-1 is described by numerous authors, and is given different names depending on the text it appears in (Laidler 1956, 1958; Mahler & Cordes 1966; Segel 1975, 1993; Birdsall et al. 1978; Dixon & Webb 1979; De Lean et al. 1980; Stockton et al. 1983; Wreggett & De Lean 1984; Ehlert 1988; Costa et al. 1992; Cornish-Bowden 1995, 2004; Varon et al. 2002; Shou 2005; see additional references in Table 2.1). For instance, it is now standard in pharmacology to refer to the scheme as ‘The ternary-complex model’, TCM, when invoked for ligand and G protein interaction with a G protein-coupled receptor (De Lean et al. 1980), and as the ‘allosteric ternary-complex model’ when explicitly

dealing with parameter c (Ehlert 1988). Meanwhile in enzymology, when focusing on both its functional as well as ant-agonistic possibilities, it is referred to as the ‘general modifier mechanism’ in order to avoid the term ‘competitive’ (Topham 1990; Topham & Brocklehurst 1992), while when merely paying attention to its ant-agonistic aspects it is referred to as the ‘mixed-competitive reaction scheme’ (e.g., Webb 1963; Laidler & Bunting 1973; Segel 1975, 1993, Chapters 5 and 6; Dixon & Webb 1979; Cornish-Bowden 2004, Chapter 5) or the ‘general non-competitive inhibition mechanism’ (Cha 1975). In enzymology in general, there are added reaction paths of one-way product formation in relation to the intervention scheme in Fig. 2.1A, and often the term ternary-complex model in enzymology refers to other types of reaction schemes (Cornish-Bowden 1995, 2004, Chapter 7).

Initially, and in particular with Ehlert’s derivations in 1988 for ligand interaction in pharmacology, we got the full picture of formalism for the intervention model with two different ligands at receptors, a primary ligand and a secondary ligand, a G protein (Ehlert 1988; see also Costa et al. 1992).

Ehlert (1988) has treated the ternary-complex model in much detail and derived its formulation both for binding and functional experimentation. Ehlert’s derivations were performed with the specific purpose of extracting affinity constants of occupancy and activity for GPCRs on binding ligands, including G proteins. Meanwhile, with regard to the terminology in this book, Ehlert’s use of the word ‘allosteric’ is misleading. Observed from a modeler’s view, Ehlert’s ‘allostery’ is unfortunately purported by others (Kenakin 2004a,b, 2007; Christopoulos et al. 2004, Vauquelin & von Mentzer 2007, Fig. 213).

I want to use a novel expression to designate the reaction scheme in Fig. 2.4-1. This in order to avoid imprecise designation and confounding connotations, such as (1) ‘inhibition’ and ‘competition’ used in enzymology, and (2) ‘ternary-complex’ and ‘allosteric ternary-complex’ as used in receptology. Therefore, I suggest to call the reaction scheme in Fig. 2.4-1 ‘the intervention scheme’, hopefully leaving room for possible interactive measures as well as associations that include both positive and negative effects in the epithet ‘intervention’. A designation that fully covers the reaction scheme in Fig. 2.4-1 is a “heterotropic mutually inclusive one-state ternary-complex model with interaction parameter $c \geq 0$ – possibly different from unity – and with no ‘co-lateral binding’ of ligands”. This is too cumbersome, justifying a short designator as ‘intervention’ (see Subchapter 2.6 for more on co-lateral binding).

Note that the intervention scheme in Fig. 2.1A is an integral part of the FP-OSM (Fig. 2.4-1), a reaction scheme in which co-lateral binding is allowed in some quadrants.

Can you find the quadrants in Fig. 2.4 where the co-lateral binding is allowed? The answers appear in Section 2.6.1.

2.7.3. Auto-intervention One-state Model

For the homotropic mutually inclusive and intervention one-state model (HO-MI-OSM) in binding and action with identical ligands in a ternary complex, derivations started early on with the equation by Hill (1910), the formulation by Adair (1925), and Haldane’s ‘substrate inhibition’ (Haldane 1930), and was then followed by Pauling and Wyman, culminating with a derivation given by Laidler (Laidler & Hoare 1949; Laidler 1956, 1958, pp. 77–80) invoking an intervention constant equal to c as described in more detail in Chapter 3.

2.7.4. The Name of the Game

In this section, in an attempt to clarify which model is treated, I shall use the designation HE-MI-OSM for the simple TCM with heterologous ligands and HO-MI-OSM for the simple TCM with homologous ligands. Thus, the simplest TCM with full reciprocity where two different ligands interact simultaneously (mutually inclusive) (Figs. 2.1A and 2.4-1) but not co-laterally (see Section 2.6.2) is HE-MI-OSM. When two identical ligands interact, the model is the HO-MI-OSM (Fig. 2.4-2). The HE-MI-OSM is described in Sub-chapter 2.5, while the HO-MI-OSM is treated in Chapter 3. The behavior of parameters in the two models, HE-MI-OSM and HO-MI-OSM, for both binding and function are listed in Tables 2.3, 2.4, 2.5, and 3.1.

2.7.5. The HE-MI-OSM Looks Like the HO-MI-OSM

It is worth noting that plots of a HE-MI-OSM may become equal to plots for the HO-MI-OSM. In case the two different ligand, agonist and intervener, in the HE-MI-OSM are combined in a mixture and this mixture is used for changing the ligand concentration, then $[S]$ and $[I]$ are changed simultaneously and the plots become identical to plots of the HO-MI-OSM (Fig. 2.11) (see Chapter 3 for more details).

2.8. Surmountable and Insurmountable Synagics

2.8.1. The Development of ‘Surmountable’

The two terms ‘surmountable’ and ‘insurmountable’ were originally introduced for functional assays by Gaddum et al. (1955) to characterize the behaviour of

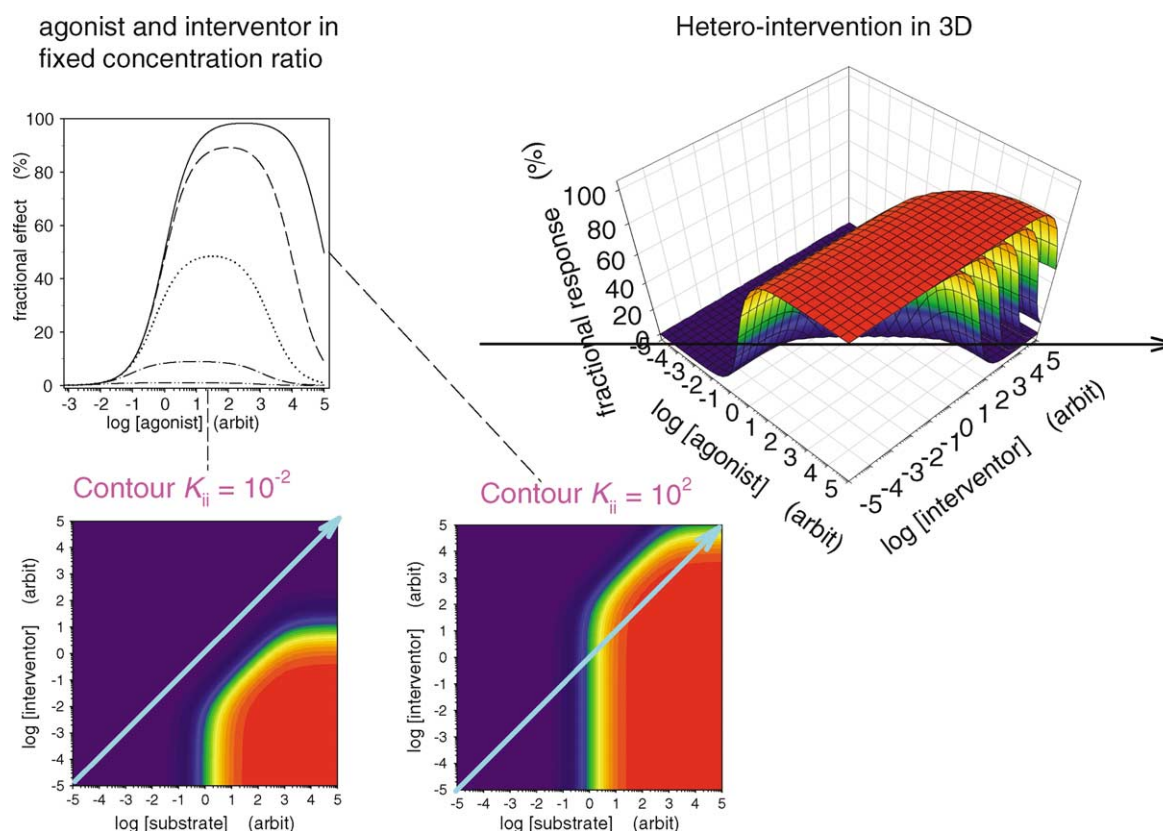


Figure 2.11. Plots of the intervention model in three-dimensions. Upper right panel shows dose-response plots for the intervention model in 3-D, where the substrate (agonist) and the interventor concentrations are the independent variables and also vary independently of each other. The interventor concentration is varied by a factor 10 in five steps from 10^{-2} to 10^2 yielding five 3-D surface plots. In this model (Fig. 2.4-1) there is no co-lateral binding possible. The two lower panels are contour plots of the model for $1/K_{ii} = 10^{-2}$ and $1/K_{ii} = 10^2$. With a fixed concentration ratio between substrate (agonist) and interventor a 2-D graph as in the upper left panel may appear with an interventor concentration varied by a factor 10 in five steps from 10^{-2} (—) to 10^2 (-----). A graph similar to the 2-D graph will also be the consequence in case a single ligand (agonist) is capable of co-lateral binding that may lead to 'auto-ant-agonism' (substrate inhibition) (see Figs. 3.2 and 3.3 and auto-intervention functions in Chapter 3). Maximal response = 100% and co-lateral intervention coefficient $c = 10^{-3}$. K_{ss} = dissociation constant for S at primary s-site and K_{ii} = dissociation constant for interventor ligand at intervention i-site are both = 1.

dose-responses equal to that expected for competitive (surmountable) and non-competitive (insurmountable) ant-agonism/inhibition. Surmountable synagics were characterized by a rightward shift in dose-responses always reaching the maximum level by a high enough agonist concentration (Fig. 2.7), while insurmountable synagics had decreasing maximum responses as the ant-agonist concentration increased, independent of increasing agonist concentrations (Fig. 2.8).

The physical realities behind observed surmountable and insurmountable functional dose-responses are much more diverse than merely being explained on the grounds of competitive and non-competitive synagics. Vauquelin et al. (2002) provide a good review on the subject, however, their use of the concept 'allosteric', as based on a so-called Kaumann model (Kaumann & Frenken 1988), is not in line with its use in this text. The Kaumann model does not include un-liganded

activity, which is a hallmark of allosteric models. Reasons for insurmountable dose-responses such as dissociation/limitation or diffusion/limitation (biophase) were detailed by Rang (1966) and, recently, a detailed description for assessing system constants in insurmountable analysis has reappeared (Kenakin et al. 2006).

The concepts of surmountability/insurmountability remain (Christopoulos 2001; Takezako et al. 2004; Mathiesen et al. 2006). Today, it is often assumed to relate to non-equilibrium or hemi-equilibrium explanations and therefore more often suggests an analysis of time-dependence in the models to analyse this kind of non-competitive-like behaviour (Paton & Rang 1965; Lew et al 2000; Avlani et al. 2004; Mathiesen et al. 2006, Kenakin et al. 2006; Vauquelin & Szczuka 2007), while others lean towards other explanations of a conformational change in the receptor *per se*, one being so-called 'network-leaning' (Takezako et al. 2004).

2.8.2. Summary of the ‘Surmountable’

The two types of simple ant-agonism/inhibition, competitive and non-competitive, may be summarized in a ‘narrow’ manner by statements of ‘surmount-ability’:

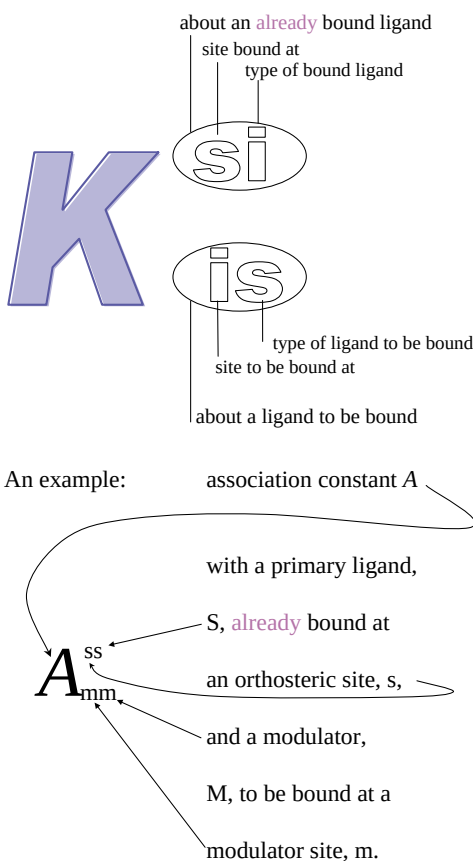
In *non-competitive ant-agonism/inhibition*, the relative effect of ant-agonists/inhibitors is always **independent** of varying concentrations of the agonist evoking a response. No matter what the value of a fixed concentration of an agonist, S' , the full response is always reduced by the fraction $1/(1+I)$ in the presence of non-competitive

ant-agonists/inhibitors. The ant-agonism/inhibition is said to be **insurmountable** (Figs. 2.7C and 2.8C).

In *competitive ant-agonism/inhibition*, the relative effect of ant-agonists/inhibitors is always **dependent** on the concentrations of the agonist evoking a response, with an apparent dissociation constant for the ant-agonist/inhibitor K_{xi} equal to $(K_{ss}/S) \cdot (1 + I/K_{xi})$, where K_{xi} is either K_{si} or K_{ii} . The ant-agonism/inhibition is said to be **surmountable**, since with an increasing concentration of S , the effect of competitive ant-agonism/inhibition may

Box 2.1. Some papers on HAP- and Mon-land models for allostery and co-operativity

Parameters. The letter K is most often used to indicate an equilibrium *dissociation* constant and the letter A an equilibrium *association* constant. The present text varies between association and dissociation constants as most convenient. At K and A , subscripts are nearly always included as signifiers, while superscripts are often omitted. For subscripts and superscripts, see below.



Ligands. A typical symbol for ligands such as substrates, agonists or transportees (transported molecules) is S , while inhibitors, ant-agonists or interventors are designated with I , and modulators equal to allosteric ligands with M .

Receptive units. The symbol for a receptive unit as an enzyme, a receptor, or a transporter is usually R . Receptive units often have more than one binding site. For a receptive unit with two binding sites, see, e.g., Fig. 2.1A. In reaction schemes, symbols to the right of the R term mean that they stand for ligands bound to a primary site on the receptive unit, for instance RS . When ligand symbols are to the left of the symbol R they indicate binding to a secondary site on the receptive unit, for instance IR . A primary binding site is also termed an ‘orthosteric’

Box 2.1 (Continued)

site, and a secondary binding site may be termed an ‘intervention’, ‘modulation’, or ‘allosteric’ site. For a receptive unit with two states in un-liganded conformations, see, e.g., Fig. 2.1B. These are represented by, for instance, the following two symbols: R and R*, where R is the reactive (not yet active) conformation and R* is an active conformation.

Subscripts and superscripts. Symbols and conventions are described for subscripts and superscripts at K and A . See Box 2.1 figure with K as an example.

Subscripts may contain one or two signifiers. In case only one position is filled it refers to the ligand *to be bound*, for instance K_s is a dissociation constant for ligand S to be bound. If both positions in the subscript are presented, the first signifier indicates the site to be occupied, and the second signifier indicates the ligand to be bound.

The primary binding site is signified by s, and the secondary binding site is signified by i or m. Signifier i for the secondary site is related to intervention models (see text), while signifier m is related to the secondary site in allosteric models.

Three examples: (1) A_{ss} designates an association constant with the primary binding site to be occupied by ligand S; (2) K_{is} designates a dissociation constant for binding to the secondary binding site by a ligand S; and (3) A_{ii} indicates an association constant for binding to the secondary site of ligand I.

Superscripts. When a superscript is included, the same rules as those for subscripts apply except it is now for ligands *already bound* to the receptive unit, see Box 2.1 figure. The K in Box-figure signifies a dissociation constant for ligand S ready to bind to a secondary site in a receptive unit to which another ligand I is already bound at the primary binding site.

For practice, first write (A) the equilibrium dissociation constant for an agonist S binding to a secondary binding site, assuming intervention models, and include the reaction scheme for such a process. Next write (B) the dissociation constant for a modulator, i.e., assuming allosteric models, binding at a primary site with an agonist already bound to the secondary site. The solutions are at the bottom of the Box.

Additional examples of parameter signifiers. The conformational constant for a switch between a reactive (not yet active) receptor state R and an active receptor state R* is usually designated with an L . Thus, $R^*/R = L$. In case an agonist is already bound to an orthosteric site m the receptive unit before a conformational change, this is indicated in superscript. Thus, for instance, L^s , L^{ss} , or L^i are for $RS \rightleftharpoons R^*S$ as in the dC&K model (Chapter 5), and L^{is} is for $SR \rightleftharpoons SR^*$ as in the homotropic two-state model (Chapter 7).

(A) $K_{is}^s: R + S \rightleftharpoons SR$, notice S at the left of R in the SR complex.
(B) $K_{ii}^{is}: R + S \rightleftharpoons SR$, notice S at the left of R in the SR complex.

always be overcome (Figs. 2.7A and 2.8A). Since its introduction (Gaddum et al. 1955), the concepts of being surmountable/insurmountable have developed and, currently, seem to be employed in a more loose sense than defined above (Vauquelin et al. 2002 and references therein; Vauquelin & Szczuka 2007).

REFERENCES

- Abramow-Newerly M, Roy AA, Nunn C & Chidiac P. RGS proteins have a signalling complex: interactions between RGS proteins and GPCRs, effectors, and auxiliary proteins. *Cell Signal* 18: 579–591, 2006.
- Ariëns EJ. *Molecular Pharmacology. The Mode of Action of Biologically Active Compounds*. New York: Academic Press, 1964.
- Ariëns EJ, Simonis AM & de Groot WM. Affinity and intrinsic-activity in the theory of competitive- and non-competitive inhibition and an analysis of some forms of dualism in action. *Arch Int Pharmacodyn* 100: 298–322, 1955.
- Avlani V, May LT, Sexton PM & Christopoulos A. Application of a kinetic model to the apparently complex behavior of negative and positive allosteric modulators of muscarinic acetylcholine receptors. *J Pharmacol Exp Ther* 308: 1062–1072, 2004.
- Bindsløv N. A homotropic two-state model and auto-antagonism. *BMC Pharmacol* 4: 11 (pp 1–22), 2004.
- Birdsall B, Burgen AS, Rodrigues de Miranda J & Roberts GC. Cooperativity in ligand binding to dihydrofolate reductase. *Biochemistry* 17: 2102–2110, 1978.
- Blumer JB & Lanier SM. Accessory proteins for G protein-signaling systems: activators of G protein signaling and other nonreceptor proteins influencing the activation state of G proteins. *Receptors Channels* 9: 195–204, 2003.
- Boeynaems JM & Dumont JE. *Outlines of Receptor Theory*. Amsterdam: Elsevier, 1980.
- Bond RA & Ijzerman AP. Recent developments in constitutive receptor activity and inverse agonism, and their potential for GPCR drug discovery. *Trends Pharmacol Sci* 27: 92–96, 2006.
- Botts J & Morales M. Analytical description of the effects of modifiers and of enzyme multivalency upon the steady state catalyzed reaction rate. *Trans Faraday Soc* 49: 696–707, 1953.

- Burgisser E, De Lean A & Lefkowitz RJ. Reciprocal modulation of agonist and antagonist binding to muscarinic cholinergic receptor by guanine nucleotide. *Proc Nat Acad Sci USA* 79: 1732–1736, 1982.
- Burzomato V, Beato M, Groot-Kormelink PJ, Colquhoun D & Sivilotti LG. Single-channel behavior of heteromeric $\alpha 1\beta$ glycine receptors: an attempt to detect a conformational change before the channel opens. *J Neurosci* 24: 10924–10940, 2004.
- Cha S. Tight-binding inhibitors-I. Kinetic behavior. *Biochem Pharmacol* 24: 2177–2185, 1975.
- Cheng Y-C & Prusoff WH. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 per cent inhibition (I_{50}) of enzymatic reaction. *Biochem Pharmacol* 22: 3099–3108, 1973.
- Chidiac P & Roy AA. Activity, regulation, and intracellular localization of RGS proteins. *Receptors Channels* 9: 135–147, 2003.
- Christopoulos A. From 'captive' agonism to insurmountable antagonism: demonstrating the power of analytical pharmacology. *Clin Exp Pharmacol Physiol* 28: 223–229, 2001.
- Christopoulos A. Allosteric binding sites on cell-surface receptors: novel targets for drug discovery. *Nat Rev Drug Discov* 1: 198–210, 2002.
- Christopoulos A & Kenakin T. G protein coupled receptor allosterism and complexing. *Pharmacol Rev* 54: 323–374, 2002.
- Christopoulos A, May LT, Avlani VA & Sexton PM. G-protein-coupled receptor allosterism: the promise and the problem(s). *Biochem Soc Trans* 32: 873–877, 2004.
- Cleland WW. The kinetics of enzyme-catalyzed reactions with two or more substrates or products. *Biochim Biophys Acta* 67: 104–137, 1963.
- Colquhoun D. Agonist-activated ion channels. *Br J Pharmacol* 147: S17–S26, 2006.
- Cornish-Bowden A. A simple graphical method for determining the inhibition constants of mixed, uncompetitive and non-competitive inhibitors. *Biochem J* 137: 143–144, 1974.
- Cornish-Bowden A. *Fundamentals of Enzyme Kinetics*. London: Portland Press, 1995.
- Cornish-Bowden A. *Fundamentals of Enzyme Kinetics*. Colchester: Portland Press Ltd., 2004.
- Costa T, Ogino Y, Munson PJ, Onaran HO & Rodbard D. Drug efficacy at guanine nucleotide-binding regulatory protein-linked receptors: thermodynamic interpretation of negative antagonism and of receptor activity in the absence of ligand. *Mol Pharmacol* 41: 549–560, 1992.
- Daeffler L & Landry Y. Inverse agonism at heptahelical receptors: concept, experimental approach and therapeutic potential. *Fundam Clin Pharmacol* 14: 73–87, 2000.
- De Lean A, Stadel JM & Lefkowitz RJ. A ternary complex model explains the agonist specific binding properties of the adenylate cyclase coupled beta-adrenergic receptor. *J Biol Chem* 255: 7108–7117, 1980.
- Dixon M & Webb EC. *Enzymes*. London: Longman Group Ltd., 1979.
- Dowling MR, Willets JM, Budd DC, Charlton SJ, Nahorski SR & Challiss RA. A single point mutation (N514Y) in the human M3 muscarinic acetylcholine receptor reveals differences in the properties of antagonists: evidence for differential inverse agonism. *J Pharmacol Exp Ther* 317: 1134–1142, 2006.
- Ebersole ER, Guttentag C & Wilson PW. Nature of carbon monoxide inhibition of biological nitrogen fixation. *Arch Biochem* 3: 399–418, 1944.
- Ehlert FJ. Estimation of the affinities of allosteric ligands using radioligand binding and pharmacological null methods. *Mol Pharmacol* 33: 187–194, 1988.
- Foucaud M, Tikhonova IG, Langer I, Escrieut C, Dufresne M, Seva C, Maigret B & Fourmy D. Partial agonism, neutral antagonism, and inverse agonism at the human wild-type and constitutively active cholecystokinin-2 receptors. *Mol Pharmacol* 69: 680–690, 2006.
- Friedenwald JS & Maengwyn-Davies GD. Elementary kinetic theory of enzymatic activity. First order theory. In: *The Mechanism of Enzyme Action*, edited by McElroy WD & Glass B. Baltimore: Johns Hopkins Press, 1954a, pp. 154–179.
- Friedenwald JS & Maengwyn-Davies GD. Elementary kinetic theory of enzymatic activity. Second order theory. In: *The Mechanism of Enzyme Action*, edited by McElroy WD & Glass B. Baltimore: Johns Hopkins Press, 1954b, pp. 180–190.
- Fukuto HS, Ferkey DM, Apicella AJ, Lans H, Sharmeen T, Chen W, Lefkowitz RJ, Jansen G, Schafer WR & Hart AC. G Protein-coupled receptor kinase function is essential for chemosensation in *C. elegans*. *Neuron* 42: 581–593, 2004.
- Gaddum JH, Hameed KA, Hathway DE & Stephens FF. Quantitative studies of antagonists for 5-hydroxytryptamine. *Q J Exp Physiol* 40: 49–74, 1955.
- Garcia Canovas F, Tudela J, Martinez Madrid C, Varon R, Garcia Carmona F & Lozano JA. Kinetic study on the suicide inactivation of tyrosinase induced by catechol. *Biochim Biophys Acta* 912: 417–423, 1987.
- Haldane JBS. *Enzymes*. London: Longmans, Green & Co., 1930.
- Hall DA. Modeling the functional effects of allosteric modulators at pharmacological receptors: an extension of the two-state model of receptor activation. *Mol Pharmacol* 58: 1412–1423, 2000.
- Hay DL, Christopoulos G, Christopoulos A & Sexton PM. Determinants of BIBN4096BS affinity for CGRP and amylin receptors; the role of RAMP1. *Mol Pharmacol* 70: 1984–1991, 2006.
- Hijazi NH & Laidler KJ. Transient-phase and steady-state kinetics for enzyme systems involving two substrates. *Can J Biochem* 51: 832–840, 1973.
- Hill C, Brownlie Z, Davey J, Milligan G & Ladds G. Isolation and characterization of novel human RGS mutant displaying gain-of-function activity. *Cell Signal* 20: 323–336, 2008.
- Jacobs S & Cuatrecasas P. The mobile receptor hypothesis and 'cooperativity' of hormone binding. Application to insulin. *Biochim Biophys Acta* 433: 482–495, 1976.
- Jenkinson DH. Classical approaches to the study of drug-receptor interactions. In: *Textbook of Receptor Pharmacology*, edited by Foreman JC & Johansen T. Boca Raton: CRC Press, 2003, pp. 3–62.
- Kaumann AJ & Frenken M. ICI 169,369 is both a competitive antagonist and an allosteric activator of the arterial 5-hydroxytryptamine₂ receptor system. *J Pharmacol Exp Ther* 245: 1010–1015, 1988.
- Kenakin T. *A Pharmacology Primer: Theory, Application, and Methods*. Amsterdam: Elsevier Academic Press, 2004a.
- Kenakin T. Allosteric modulators: the new generation of receptor antagonist. *Mol Interv* 4: 222–229, 2004b.
- Kenakin T. Allosteric agonist modulators. *J Recept Signal Transduct Res* 27: 247–259, 2007.
- Kenakin TP, Jenkinson S & Watson C. Determining the potency and molecular mechanism of action of insurmountable antagonists. *J Pharmacol Exp Ther* 319: 710–723, 2006.
- Laidler KJ. General steady-state equations in enzyme and other catalyzed reactions. *Trans Faraday Soc* 52: 1374–1382, 1956.
- Laidler KJ. *The Chemical Kinetics of Enzyme Action*. London: Oxford University Press, 1958.
- Laidler KJ & Socquet IM. Kinetic laws in catalysed systems. II. Reactions involving two substrates or a substrate and a coenzyme. *J Phys Colloid Chem* 54: 530–536, 1950.
- Laidler KJ & Bunting PS. *The chemical kinetics of enzyme action*. Oxford: Oxford University Press, 1973.

- Leff P. The two state model of receptor activation. *Trends Pharmacol Sci* 16: 89–97, 1995.
- Levitzki A. *Quantitative Aspects of Allosteric Mechanisms*. Berlin: Springer-Verlag, 1978.
- Lew MJ, Ziogas J & Christopoulos A. Dynamic mechanisms of non-classical antagonism by competitive AT(1) receptor antagonists. *Trends Pharmacol Sci* 21:376–381, 2000.
- MacKay D. Interpretation of relative potencies, relative efficacies and apparent affinity constants of agonist drugs estimated from concentration-response curves. *J Theor Biol* 142: 415–427, 1990.
- Mahler HR & Cordes EH. *Biological Chemistry*. London: Harper & Row, Ltd., 1966.
- Mathiesen JM, Christopoulos A, Ulven T, Royer J, Campillo M, Heinemann A, Pardo L & Kostenis E. On the mechanism of interaction of potent, surmountable and insurmountable antagonists with the prostaglandin D2 receptor CRTH2. *Mol Pharmacol* 69: 1441–1453, 2006.
- May LT & Christopoulos A. Allosteric modulators of G-protein-coupled receptors. *Curr Opin Pharmacol* 3: 551–556, 2003.
- May LT, Avlani VA, Sexton PM & Christopoulos A. Allosteric modulation of G protein-coupled receptors. *Curr Pharm Des* 10: 2003–2013, 2004.
- May LT, Leach K, Sexton PM & Christopoulos A. Allosteric modulation of G protein-coupled receptors. *Annu Rev Pharmacol Toxicol* 47: 1–51, 2007a.
- May LT, Avlani VA, Langmead CJ, Herdon HJ, Wood MJ, Sexton PM & Christopoulos A. Structure-function studies of allosteric agonism at M2 muscarinic acetylcholine receptors. *Mol Pharmacol* 72: 463–476, 2007b.
- Michaelis L. Zur theorie der elektrolytischen dissociation der fermenten. *Biochem Zeitschrift* 60: 91–96, 1914.
- Milligan G & Bond RA. Inverse agonism and the regulation of receptor number. *Trends Pharmacol Sci* 18: 468–474, 1997.
- Milligan G, Bond RA & Lee M. Inverse agonism: pharmacological curiosity or potential therapeutic strategy? *Trends Pharmacol Sci* 16: 10–13, 1995.
- Morfis M, Christopoulos A & Sexton PM. RAMPs: 5 years on, where to now? *Trends Pharmacol Sci* 24: 596–601, 2003.
- Paton WD & Rang HP. The uptake of atropine and related drugs by intestinal smooth muscle of the guinea-pig in relation to acetylcholine receptors. *Proc R Soc Lond B Biol Sci* 163: 1–44, 1965.
- Rang HP. The kinetics of action of acetylcholine antagonists in smooth muscle. *Proc R Soc Lond B Biol Sci* 164: 488–510, 1966.
- Rebois RV & Hebert TE. Protein complexes involved in heptahelical receptor-mediated signal transduction. *Receptors Channels* 9: 169–194, 2003.
- Richard F, Barroso S, Martinez J, Labbe-Jullie C & Kitabgi P. Agonism, inverse agonism, and neutral antagonism at the constitutively active human neurotensin receptor 2. *Mol Pharmacol* 60: 1392–1398, 2001.
- Samama P, Cotecchia S, Costa T & Lefkowitz RJ. A mutation induced activated state of the beta 2 adrenergic receptor. Extending the ternary complex model. *J Biol Chem* 268: 4625–4636, 1993.
- Scheer A, Fanelli F, Costa T, De Benedetti PG & Cotecchia S. Constitutively active mutants of the alpha 1B-adrenergic receptor: role of highly conserved polar amino acids in receptor activation. *EMBO J* 15: 3566–3578, 1996.
- Schwartz TW & Holst B. Allo-allosteric modulation and other types of allostery in dimeric 7TM receptors. *J Recept Signal Transduct Res* 26: 107–128, 2006.
- Segal HL, Kachmar JF & Boyer PD. Kinetic analysis of enzyme reactions. I. Further considerations of enzyme inhibition and analysis of enzyme activation. *Enzymologia* 15: 187–198, 1952.
- Segel IH. *Enzyme Kinetics. Behavior and Analysis of Rapid Equilibrium and Steady-state Enzyme Systems*. New York: Wiley & Sons (reissued 1993), 1975.
- Sexton PM, Albiston A, Morfis M & Tilakaratne N. Receptor activity modifying proteins. *Cell Signal* 13: 73–83, 2001.
- Shou M. Kinetic analysis for multiple substrate interaction at the active site of cytochrome P450. *Methods Enzymol* 357: 261–276, 2002.
- Shou M. Prediction of pharmacokinetics and drug-drug interactions from in vitro metabolism data. *Curr Opin Drug Discov Dev* 8: 66–77, 2005.
- Shou M, Mei Q, Ettore MW Jr, Dai R, Baillie TA & Rushmore TH. Sigmoidal kinetic model for two co-operative substrate-binding sites in a cytochrome P450 3A4 active site: an example of the metabolism of diazepam and its derivatives. *Biochem J* 340: 845–853, 1999.
- Shou M, Lin Y, Lu P, Tang C, Mei Q, Cui D, Tang W, Ngui JS, Lin CC, Singh R, Wong BK, Yergey JA, Lin JH, Pearson PG, Baillie TA, Rodrigues AD & Rushmore TH. Enzyme kinetics of cytochrome P450-mediated reactions. *Curr Drug Metab* 2: 17–36, 2001.
- Stockton JM, Birdsall NJ, Burgen AS & Hulme EC. Modification of the binding properties of muscarinic receptors by gallamine. *Mol Pharmacol* 23: 551–557, 1983.
- Takezako T, Gogonea C, Saad Y, Noda K & Karnik SS. ‘Network leaning’ as a mechanism of insurmountable antagonism of the angiotensin II type 1 receptor by non-peptide antagonists. *J Biol Chem* 279: 15248–15257, 2004.
- Tolkovsky AM & Levitzki A. Mode of coupling between the betaadrennergic receptor and adenylate cyclase in turkey erythrocytes. *Biochemistry* 17: 3795–3810, 1978.
- Topham CM. A generalized theoretical treatment of the kinetics of an enzyme-catalysed reaction in the presence of an unstable irreversible modifier. *J Theor Biol* 145: 547–572, 1990.
- Topham CM & Brocklehurst K. In defence of the general validity of the Cha method of deriving rate equations. The importance of explicit recognition of the thermodynamic box in enzyme kinetics. *Biochem J* 282: 261–265, 1992.
- Trzeciakowski JP. Stimulus amplification, efficacy, and the operational model. Part II-ternary complex occupancy mechanisms. *J Theor Biol* 198: 347–374, 1999.
- Udawela M, Hay DL & Sexton PM. The receptor activity modifying protein family of G protein coupled receptor accessory proteins. *Semin Cell Dev Biol* 15: 299–308, 2004.
- Udawela M, Christopoulos G, Tilakaratne N, Christopoulos A, Albiston A & Sexton PM. Distinct receptor activity modifying protein (RAMP) domains differentially modulate interaction with calcitonin receptors. *Mol Pharmacol* 70: 1750–1760, 2006.
- Varon R, Garcia-Canovas F, Garcia-Moreno M, Valero E, Molina-Alarcon M, Garcia-Meseguer MJ, Vidal de Labra JA & Garrido-del Sol C. Kinetic analysis of the general modifier mechanism of Botts and Morales involving a suicide substrate. *J Theor Biol* 218: 355–374, 2002.
- Vauquelin G & Szczuka A. Kinetic versus allosteric mechanisms to explain insurmountable antagonism and delayed ligand dissociation. *Neurochem Int* 51: 254–260, 2007.
- Vauquelin G, Van Liefde I & Vanderheyden P. Models and methods for studying insurmountable antagonism. *Trends Pharmacol Sci* 23: 514–518, 2002.
- Vauquelin G & von Mentzer B. G protein-coupled receptors. Molecular pharmacology from academic concept to pharmaceutical research. Chichester, Wiley & Sons, 2007.
- Vilardaga JP, Steinmeyer R, Harms GS & Lohse MJ. Molecular basis of inverse agonism in a G protein-coupled receptor. *Nat Chem Biol* 1: 25–28, 2005.

- Walz D & Caplan SR. Energy coupling and thermokinetic balancing in enzyme kinetics: microscopic reversibility and detailed balance revisited. *Cell Biophys* 12: 13–28, 1988.
- Webb JL. *Enzyme and Metabolic Inhibitors. Vol 1. General Principles of Inhibition*. New York: Academic Press, 1963.
- Weber G. Ligand binding and internal equilibria in proteins. *Biochemistry* 11: 878, 1972.
- Weber G. Energetics of ligand binding to proteins. *Adv Protein Chem* 29: 1–83, 1975.
- Wells JW. Analysis and interpretation of binding at equilibrium. In: *Receptor–ligand Interactions. A Practical Approach*, edited by Hulme EC. Oxford: IRL Press at Oxford University Press, 1992, pp. 289–395.
- Wreggett KA & De Lean A. The ternary complex model. Its properties and application to ligand interactions with the D2-dopamine receptor of the anterior pituitary gland. *Mol Pharmacol* 26: 214–227, 1984.
- Wyman J. Heme proteins. *Adv Protein Chem* 4: 407–531, 1948.
- Wyman J. Linked functions and reciprocal effects in hemoglobin: A second look. *Adv Protein Chem* 19: 223–286, 1964.
- Wyman J. Allosteric linkage. *J Am Chem Soc* 89: 2202–2218, 1967.