

I

PART

ONE-STATE MODELS: SIMPLE AGONISM AND ANT-AGONISM

I.1. A Prelude

What happens when two drugs are given simultaneously? This book is about the analysis of data from ligand–receptor interactions obtained from dose–response experiments at equilibrium and about how theoretical schemes of mechanisms can be adopted to these data and with a main aim on how to simulate physically what happens when two drugs are given simultaneously. This for a comparison with reality and for a better implementation of combinational drug therapy. In essence, the entire book is an elaboration of the Michaelis–Menten equation.

The various concepts of ligand–receptor interaction were first developed during a so-called classic era ending *circa* 1945, and then during a post-classic era, starting *circa* 1965, with what could be called a transition period between 1945 and 1965. Concepts from the classic era were mostly concerned with occupancy or function as a *two-step mechanism* while the post-classic era, besides binding and function, was and still is preoccupied with states of conformational changes for the un-liganded receptive unit, a *two-state mechanism*.

I.2. One, Two, and Many Steps or States

Concepts from the classic and post-classic eras are entwined. In Part I, concepts from the classic era are described in detail, delineating the *step* of binding *per se*. Concepts such as efficacy and partial agonism are also introduced. These latter concepts were developed in the transition period. Efficacy is related to conformational change in protein molecules that function as receptive units and effectors. Part II deals with the *state* of

conformational change in un-liganded receptive units. A switch from one to two or more conformational states for an un-liganded receptive unit was introduced and expanded in the post-classic period.

Other terms for an initial classification of concepts from the classic era could be processes with *single-state* receptors or simple agonism and simple ant-agonism (cf. title of Part I), while for the post-classic era terms such as complex agonism and complex ant-agonism or *two-state* processes within a receptive unit are dealt with in Part II.

Terms are tentatively tabulated in a somewhat dogmatic manner in Table I.1 in an attempt to illuminate the distinction between the two eras, their concepts, and our terminology so far.

I.3. Synagic and Synagics

In this book, a dose-response relation will also be referred to as a ‘synagic’ relation. The term synagic is derived from the Greek substantive ‘synagoge’ meaning concentration, meeting, or gathering. We know this word from ‘synagogue’. The verb to concentrate is ‘synagein’. Instead of the somewhat cumbersome ‘synagagic’ as an adjective, we abbreviate it to synagic. The noun synagic signifies that concentration of a ligand is a driving force and the independent variable, rather than time as the independent variable. Note that synagic only refers to dose-response relations at equilibrium and steady-state (see Preface).

Time is a tacit independent variable in the adjectives ‘kinetic’ and ‘dynamic’. Therefore, throughout most of the text, at equilibrium, synagic replaces kinetic and dynamic. Incidentally, remember that ‘thermodynamics’

Table I.1. Periods and concepts developed for interaction between ligands and receptive units

Part in the book	Era	Agonism and ant-agonism	Process in focus	States for un-liganded receptive unit
Part I	Classic	Simple	Occupancy	Single-
Part II	Post-classic	Complex	Conformation	Two- or multi-

When the subject is complex agonism, all the tabulated adjectives of Part II may come into play. Note, that simple ant-agonism may involve two **sites** on the receptive unit, as discussed in Part I Chapter 2, while mechanisms due to two **states** solely belong to the complex agonism and complex ant-agonism dealt with in Part II. Here, 'states' refer to the number of conformations for an **un-liganded** receptive unit.

most often refer to equilibrium situations, but not always (Katchalsky & Curran 1965), and not necessarily to dose-responses.

So, synaptic and synagics are specific terms that stand for concentration-occupancy and dose-responses at equilibrium.

I.4. Equilibrium and its Formulation

This tome is about the use of theories in the analysis of experimental dose-response data obtained at equilibrium. For the analysis, equilibrium theories are expressed as equations, synaptic equations.

The formulations of concentration-dependent equilibria are based on a conservation principle, which means that the receptive units of interest, e.g., all activated receptors or actual response (ar), are expressed as a fraction of the total of all receptive units or total response (TR). Thus, expressions such as ' $ar/TR = \dots$ ' will commonly appear. Formulations with $ar/TR = \dots$ are also known as 'distribution equations'.

On equilibria, JBS Haldane wrote "The key to a knowledge of enzymes is the study of reaction velocities, not of equilibria" (Haldane 1930, p. 3), and, of course, one cannot assess the order of events in kinetic schemes by measurements at equilibrium (Fersht 1999, p. 125). Nevertheless, the present book tries to disprove Haldane's statement, arguing that theoretical analysis of concentration-dependent equilibria as well as time-dependent velocities are key tools to our understanding of transducing molecules and their function. In addition, since the emphasis of this book is on equilibria, nearly all parts herein deal with dose-responses at equilibrium, that is, synagics.

I.5. Binding versus Function

In dose-response studies it is paramount to realize what type of experimental set-up is to be analyzed. When embarking on an analysis of agonism or ant-agonism at equilibrium, the first question to be answered is whether the experiments are performed as a binding response of a ligand against a change in concentration of the ligand, or as a functional response by a ligand against a change

in concentration of the ligand. Recognition of the need to discern between binding and functional experiments is crucial (see, e.g., Sections 2.4.5, 2.4.6, and 2.4.12, and Table 5.1 in Chapter 5). Obviously, just because there is a conformational change when a ligand binds, this conformational change is not necessarily the one which activates the receptive unit for function. Meanwhile, since theories on binding and function have many overlapping and identical expressions (Sections 2.4.9 and 2.4.10), their analyses are easily confounded. For this reason, they must be sharply separated at all times. The very first question is, therefore, are your experiments based on *binding* or on *functional* observations?

I.6. Reciprocity

Lately, a clear realization that conformational changes also influence the binding-occupancy process has been established. Thus, if binding of a ligand induces a conformational change necessary for the functional activity, then that conformational change will affect the binding process. This mutual interaction is known



Figure I.1. Associative semiotics ... reciprocity? Geckoman © 1996. Benita Epstein, with permission.

as reciprocity. Mechanistic reciprocity is a fundamental principle in all physico-chemical processes, including irreversible processes (Onsager 1931a,b). At equilibrium, and also in non-equilibrium situations, so-called cross-coefficients L_{mn} and L_{nm} are equal for $m \neq n$:

$$L_{mn} = L_{nm} \quad (\text{for } m \neq n) \quad (\text{I.1})$$

when local entropy production is the sum of all effects.¹

The consequences of reciprocity for the interaction between receptive molecules and ligand molecules

have been discussed eloquently in a seminal paper (Colquhoun 1998). Colquhoun stated aptly that “If binding affects activation, then activation must affect binding”. The reciprocity here is physical (Colquhoun 1987) and different from the associative reciprocity of Fig. I.1. Examples of a tight survey of reciprocity and its cross-coefficients for irreversible processes are given in Katchalsky and Curran (1965) and DeLaage (1975), and on “linked function” in hemoglobin by Wyman (1964) and Wyman and Gill (1990).

¹ Effect here is the product of flow and force. For more details on cross-coefficients, see for instance Katchalsky and Curran (1965).

4 Part I: One-state Models: Simple Agonism and Ant-agonism