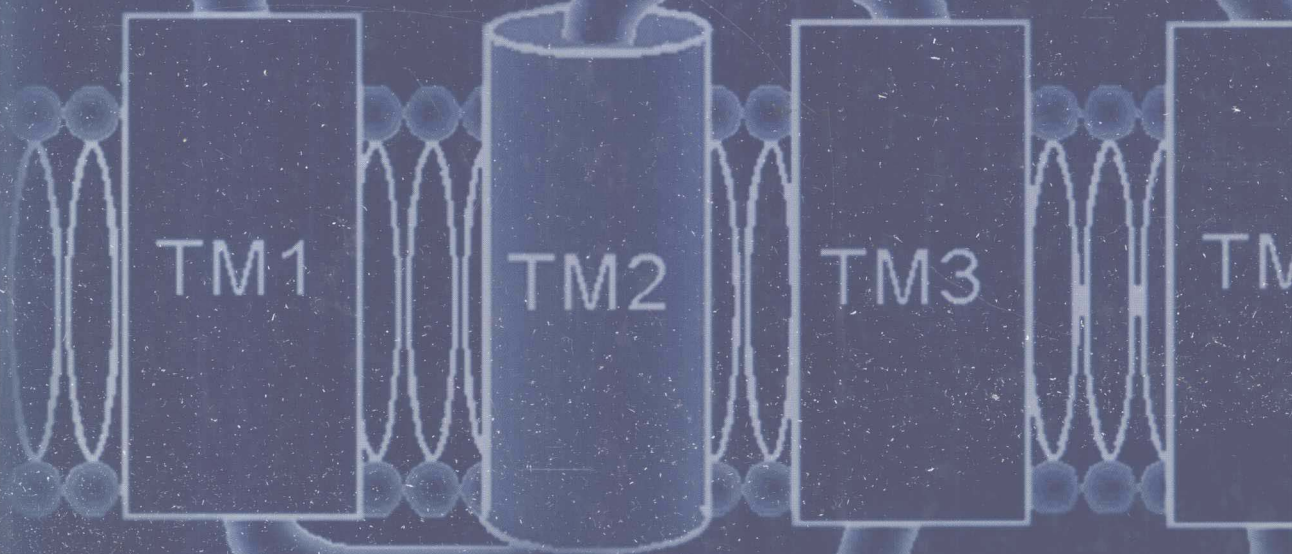
TEXTBOOK *of* RECEPTOR PHARMACOLOGY

Second Edition



Edited by

John C. Foreman & Torben Johansen

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Preface

For about four decades now, a course in receptor pharmacology has been given at University College London for undergraduate students in their final year of study for the Bachelor of Science degree in pharmacology. More recently, the course has also been taken by students reading for the Bachelor of Science degree in medicinal chemistry. The students following the course have relied for their reading upon a variety of sources, including original papers, reviews, and various textbooks, but no single text brought together the material included in the course. Also, almost continuously since 1993, we have organized courses for graduate students and research workers from the pharmaceutical industry from the Nordic and European countries. In many cases, generous financial support from the Danish Research Academy and the Nordic Research Academy has made this possible. These courses, too, were based on those for students at University College London, and we are grateful for the constructive criticisms of the many students on all of the courses that have shaped this book.

The first edition of the book provided a single text for the students, and the enthusiasm with which it was received encouraged us to work on a second edition. There have been very significant steps forward since the first edition of this book, particularly in the molecular biology of receptors. These advances are reflected in the rewritten chapters for the section of this book that deals with molecular biology. At the same time, we realized that in the first edition we included too much material that was distant from the receptors themselves. To include all the cellular biology that is consequent upon a receptor activation is really beyond the scope of any book. Hence, we have omitted from the second edition the material on intracellular second messengers such as calcium, the cyclic nucleotides, and phospholipids. The second edition now concentrates on cell membrane receptors themselves, together with their immediate signal transducers: ion channels, heterotrimeric G-proteins, and tyrosine kinases.

The writers of the chapters in this book have been actively involved in teaching the various courses, and our joint aim has been to provide a logical introduction to the study of drug receptors. Characterization of drug receptors involves a number of different approaches: quantitative description of the functional studies with agonists and antagonists, quantitative description of the binding of ligands to receptors, the molecular structure of drug receptors, and the elements that transduce the signal from the activated receptor to the intracellular compartment.

The book is intended as an introductory text on receptor pharmacology but further reading has been provided for those who want to follow up on topics. Some problems are also provided for readers to test their grasp of material in some of the chapters.

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The Editors

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Section I

Drug–Receptor Interactions

1 Classical Approaches to the Study of Drug–Receptor Interactions

Donald H. Jenkinson

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1.1 INTRODUCTION

The term *receptor* is used in pharmacology to denote a class of cellular macromolecules that are concerned specifically and directly with chemical signaling between and within cells. Combination of a hormone, neurotransmitter, or intracellular messenger with its receptor(s) results in a change in cellular activity. Hence, a receptor must not only recognize the particular molecules that activate it, but also, when recognition occurs, alter cell function by causing, for example, a change in membrane permeability or an alteration in gene transcription.

The concept has a long history. Mankind has always been intrigued by the remarkable ability of animals to distinguish different substances by taste and smell. Writing in about 50 B.C., Lucretius (in *De Rerum Natura, Liber* IV) speculated that odors might be conveyed by tiny, invisible "seeds" with distinctive shapes which would have to fit into minute "spaces and passages" in the palate and nostrils. In his words:

> Some of these must be smaller, some greater, they must be three-cornered for some creatures, square for others, many round again, and some of many angles in many ways.

The same principle of complementarity between substances and their recognition sites is implicit in John Locke's prediction in his *Essay Concerning Human Understanding* (1690):

> Did we but know the mechanical affections of the particles of rhubarb, hemlock, opium and a man, as a watchmaker does those of a watch, ... we should be able to tell beforehand that rhubarb will purge, hemlock kill and opium make a man sleep.

(Here, *mechanical affections* could be replaced in today's usage by *chemical affinities*.)

Prescient as they were, these early ideas could only be taken further when, in the early 19th century, it became possible to separate and purify the individual components of materials of plant and animal origin. The simple but powerful technique of fractional crystallization allowed plant alkaloids such as nicotine, atropine, pilocarpine, strychnine, and morphine to be obtained in a pure form for the first time. The impact on biology was immediate and far reaching, for these substances proved to be invaluable tools for the unraveling of physiological function. To take a single example, J. N. Langley made great use of the ability of nicotine to first activate and then block nerves originating in the autonomic ganglia. This allowed him to map out the distribution and divisions of the autonomic nervous system.

Langley also studied the actions of atropine and pilocarpine, and in 1878 he published (in the first volume of the *Journal of Physiology*, which he founded) an account of the interactions between pilocarpine (which causes salivation) and atropine (which blocks this action of pilocarpine). Confirming and extending the pioneering work of Heidenhain and Luchsinger, Langley showed that the inhibitory action of atropine could be overcome by increasing the dose of pilocarpine. Moreover, the restored response to pilocarpine could in turn be abolished by further atropine. Commenting on these results, Langley wrote:

> We may, I think, without too much rashness, assume that there is some substance or substances in the nerve endings or [salivary] gland cells with which both atropine and pilocarpine are capable of forming compounds. On this assumption, then, the atropine or pilocarpine compounds are formed according to some law of which their relative mass and chemical affinity for the substance are factors.

If we replace *mass* by *concentration*, the second sentence can serve as well today as when it was written, though the nature of the law which Langley had inferred must exist was not to be formulated (in a pharmacological context) until almost 60 years later. It is considered in Section 1.5.2 below.

J. N. Langley maintained an interest in the action of plant alkaloids throughout his life. Through his work with nicotine (which can contract skeletal muscle) and curare (which abolishes this action of nicotine and also blocks the response of the muscle to nerve stimulation, as first shown by Claude Bernard), he was able to infer in 1905 that the muscle must possess a "receptive substance":

> Since in the normal state both nicotine and curari abolish the effect of nerve stimulation, but do not prevent contraction from being obtained by direct stimulation of the muscle or by a further adequate injection of nicotine, it may be inferred that neither the poison nor the nervous impulse acts directly on the contractile substance of the muscle but on some accessory substance.
>
> Since this accessory substance is the recipient of stimuli which it transfers to the contractile material, we may speak of it as the receptive substance of the muscle.

At the same time, Paul Ehrlich, working in Frankfurt, was reaching similar conclusions, though from evidence of quite a different kind. He was the first to make a thorough and systematic study of the relationship between the chemical structure of organic molecules and their biological actions. This was put to good use in collaboration with the organic chemist A. Bertheim. Together, they prepared and tested more than 600 organometallic compounds incorporating mercury and arsenic. Among the outcomes was the introduction into medicine of drugs such as salvarsan that were toxic to pathogenic microorganisms responsible for syphilis, for example, at doses that had relatively minor side effects in humans. Ehrlich also investigated the selective staining of cells by dyes, as

well as the remarkably powerful and specific actions of bacterial toxins. All these studies convinced him that biologically active molecules had to become bound in order to be effective, and after the fashion of the time he expressed this neatly in Latin:

*Corpora non agunt nisi fixata.**

In Ehrlich's words (*Collected Papers*, Vol. III, *Chemotherapy*):

> When the poisons and the organs sensitive to it do not come into contact, or when sensitiveness of the organs does not exist, there can be no action.
>
> If we assume that those peculiarities of the toxin which cause their distribution are localized in a special group of the toxin molecules and the power of the organs and tissues to react with the toxin are localized in a special group of the protoplasm, we arrive at the basis of my side chain theory. The distributive groups of the toxin I call the "haptophore group" and the corresponding chemical organs of the protoplasm the 'receptor.' ... Toxic actions can only occur when receptors fitted to anchor the toxins are present.

Today, it is accepted that Langley and Ehrlich deserve comparable recognition for the introduction of the receptor concept. In the same years, biochemists studying the relationship between substrate concentration and enzyme velocity had also come to think that enzyme molecules must possess an "active site" that discriminates among various substrates and inhibitors. As often happens, different strands of evidence had converged to point to a single conclusion.

Finally, a note on the two senses in which present-day pharmacologists and biochemists use the term *receptor*. The first sense, as in the opening sentences of this section, is in reference to the whole receptor macromolecule that carries the binding site for the agonist. This usage has become common as the techniques of molecular biology have revealed the amino-acid sequences of more and more signaling macromolecules. But, pharmacologists still sometimes employ the term *receptor* when they have in mind only the particular regions of the macromolecule that are concerned in the binding of agonist and antagonist molecules. Hence, *receptor occupancy* is often used as convenient shorthand for the fraction of the binding sites occupied by a ligand.**

1.2 MODELING THE RELATIONSHIP BETWEEN AGONIST CONCENTRATION AND TISSUE RESPONSE

With the concept of the receptor established, pharmacologists turned their attention to understanding the quantitative relationship between drug concentration and the response of a tissue. This entailed, first, finding out how the fraction of binding sites occupied and activated by agonist molecules varies with agonist concentration, and, second, understanding the dependence of the magnitude of the observed response on the extent of receptor activation.

Today, the first question can sometimes be studied directly using techniques that are described in later chapters, but this was not an option for the early pharmacologists. Also, the only responses that could then be measured (e.g., the contraction of an intact piece of smooth muscle or a change in the rate of the heart beat) were indirect, in the sense that many cellular events lay between the initial step (activation of the receptors) and the observed response. For these reasons, the early workers had no choice but to devise ingenious indirect approaches, several of which are still important. These are based on "modeling" (i.e., making particular assumptions about) the two

* Literally: entities do not act unless attached.

** *Ligand* means here a small molecule that binds to a specific site (or sites) on a receptor macromolecule. The term *drug* is often used in this context, especially in the older literature.

relationships identified above and then comparing the predictions of the models with the actual behavior of isolated tissues. This will now be illustrated.

1.2.1 The Relationship between Ligand Concentration and Receptor Occupancy

We begin with the simplest possible representation of the combination of a ligand, A, with its binding site on a receptor, R:

$$A + R \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} AR \tag{1.1}$$

Here, binding is regarded as a bimolecular reaction and k_{+1} and k_{-1} are, respectively, the *association rate constant* (M^{-1} s^{-1}) and the *dissociation rate constant* (s^{-1}).

The law of mass action states that the rate of a reaction is proportional to the product of the concentrations of the reactants. We will apply it to this simple scheme, making the assumption that equilibrium has been reached so that the rate at which AR is formed from A and R is equal to the rate at which AR dissociates. This gives:

$$k_{+1}[A][R] = k_{-1}[AR]$$

where [R] and [AR] denote the concentrations of receptors in which the binding sites for A are free and occupied, respectively.

It may seem odd to refer to receptor concentrations in this context when receptors can often move only in the plane of the membrane (and even then perhaps to no more than a limited extent, as many kinds of receptors are anchored). However, the model can be formulated equally well in terms of the proportions of a population of binding sites that are either free or occupied by a ligand. If we define p_R as the proportion free,* equal to $[R]/[R]_T$, where $[R]_T$ represents the total concentration of receptors, and p_{AR} as $[AR]/[R]_T$, we have:

$$k_{+1}[A]p_R = k_{-1}p_{AR}$$

Because for now we are concerned only with equilibrium conditions and not with the rate at which equilibrium is reached, we can combine k_{+1} and k_{-1} to form a new constant, $K_A = k_{-1}/k_{+1}$, which has the unit of concentration. K_A is a *dissociation equilibrium constant* (see Appendix 1.2A [Section 1.2.4.1]), though this is often abbreviated to either *equilibrium constant* or *dissociation constant*. Replacing k_{+1} and k_{-1} gives:

$$[A]p_R = K_A p_{AR}$$

Because the binding site is either free or occupied, we can write:

$$p_R + p_{AR} = 1$$

Substituting for p_R:

* p_R can be also be defined as N_R/N, where N_R is the number of receptors in which the binding sites are free of A and N is their total number. Similarly, p_{AR} is given by N_{AR}/N, where N_{AR} is the number of receptors in which the binding site is occupied by A. These definitions are used when discussing the action of irreversible antagonists (see Section 1.6.4).

$$\frac{K_A}{[A]} p_{AR} + p_{AR} = 1$$

Hence,*

$$p_{AR} = \frac{[A]}{K_A + [A]} \tag{1.2}$$

This is the important *Hill–Langmuir equation*. A. V. Hill was the first (in 1909) to apply the law of mass action to the relationship between ligand concentration and receptor occupancy at equilibrium and to the rate at which this equilibrium is approached.** The physical chemist I. Langmuir showed a few years later that a similar equation (the *Langmuir adsorption isotherm*) applies to the adsorption of gases at a surface (e.g., of a metal or of charcoal).

In deriving Eq. (1.2), we have assumed that the concentration of A does not change as ligand receptor complexes are formed. In effect, the ligand is considered to be present in such excess that it is scarcely depleted by the combination of a little of it with the receptors, thus [A] can be regarded as constant.

The relationship between p_{AR} and [A] predicted by Eq. (1.2) is illustrated in Figure 1.1. The concentration of A has been plotted using a linear (left) and a logarithmic scale (right). The value of K_A has been taken to be 1 μM. Note from Eq. (1.2) that when $[A] = K_A$, $p_{AR} = 0.5$; that is, half of the receptors are occupied.

With the logarithmic scale, the slope of the line initially increases. The curve has the form of an elongated S and is said to be *sigmoidal*. In contrast, with a linear (arithmetic) scale for [A], sigmoidicity is not observed; the slope declines as [A] increases, and the curve forms part of a rectangular hyperbola.

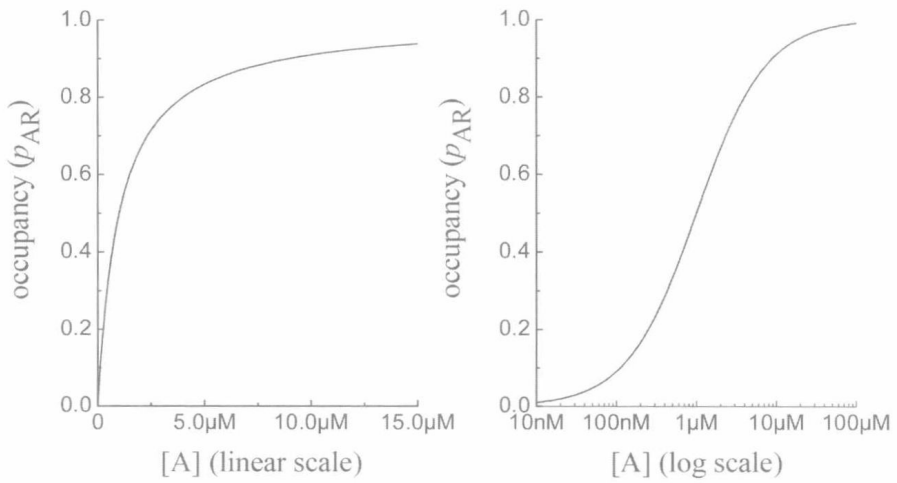

FIGURE 1.1 The relationship between binding-site occupancy and ligand concentration ([A]; linear scale, left; log scale, right), as predicted by the Hill–Langmuir equation. K_A has been taken to be 1 μM for both curves.

* If you find this difficult, see Appendix 1.2B at the end of this section.

** Hill had been an undergraduate student in the Department of Physiology at Cambridge where J. N. Langley suggested to him that this would be useful to examine in relation to finding whether the rate at which an agonist acts on an isolated tissue is determined by diffusion of the agonist or by its combination with the receptor.

Equation (1.2) can be rearranged to:

$$\frac{p_{AR}}{1-p_{AR}} = \frac{[A]}{K_A}$$

Taking logs, we have:

$$\log\left(\frac{p_{AR}}{1-p_{AR}}\right) = \log[A] - \log K_A$$

Hence, a plot of log (p_{AR} /(1 − p_{AR})) against log [A] should give a straight line with a slope of one. Such a graph is described as a *Hill plot*, again after A. V. Hill, who was the first to employ it, and it is often used when p_{AR} is measured directly with a radiolabeled ligand (see Chapter 5). In practice, the slope of the line is not always unity, or even constant, as will be discussed. It is referred to as the *Hill coefficient* (n_H); the term *Hill slope* is also used.

1.2.2 The Relationship between Receptor Occupancy and Tissue Response

This is the second of the two questions identified at the start of Section 1.2, where it was noted that the earliest pharmacologists had no choice but to use indirect methods in their attempts to account for the relationship between the concentration of a drug and the tissue response that it elicits. In the absence at that time of any means of obtaining direct evidence on the point, A. V. Hill and A. J. Clark explored the consequences of assuming: (1) that the law of mass action applies, so that Eq. (1.2), derived above, holds; and (2) that the response of the tissue is linearly related to receptor occupancy. Clark went further and made the tentative assumption that the relationship might be one of direct proportionality (though he was well aware that this was almost certainly an oversimplification, as we now know it usually is).

Should there be direct proportionality, and using y to denote the response of a tissue (expressed as a percentage of the maximum response attainable with a large concentration of the agonist), the relationship between occupancy* and response becomes:

$$\frac{y}{100} = p_{AR} \tag{1.3}$$

Combining this with Eq. (1.2) gives an expression that predicts the relationship between the concentration of the agonist and the response that it elicits:

$$\frac{y}{100} = \frac{[A]}{K_A + [A]} \tag{1.4}$$

This is often rearranged to:

$$\frac{y}{100-y} = \frac{[A]}{K_A} \tag{1.5}$$

* Note that no distinction is made here between *occupied* and *activated* receptors; it is tacitly assumed that all the receptors occupied by agonist molecules are in an active state, hence contributing to the initiation of the tissue response that is observed. As we shall see in the following sections, this is a crucial oversimplification.