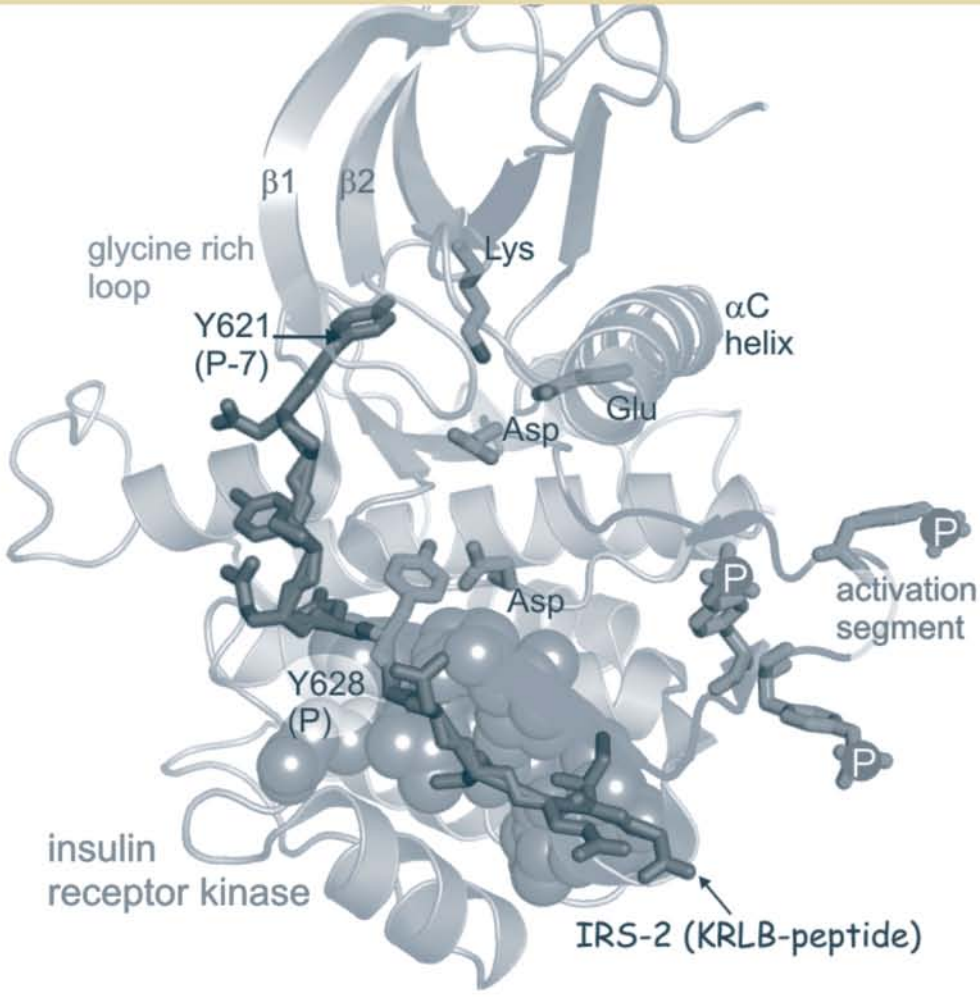
TEXTBOOK OF RECEPTOR PHARMACOLOGY



THIRD EDITION

CRC Press
Taylor & Francis Group

EDITED BY
JOHN C. FOREMAN
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Preface

For about five decades, a course in receptor pharmacology has been offered at University College London for undergraduate students in their final year of study for their 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. Beginning in 1993, we 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 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 further editions. There have been 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 the book that deals with molecular biology. The book concentrates on cell membrane receptors themselves, together with their immediate signal transducers: ion channels, heterotrimeric G-proteins, and tyrosine kinases.

The chapter authors 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, including: 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.

John C. Foreman

Torben Johansen

Alasdair J. Gibb

Editors

John C. Foreman, Ph.D., D.Sc., M.B., B.S., F.R.C.P., is emeritus professor of pharmacology at University College London (UCL). After qualifying in medicine, he spent two years as visiting instructor of medicine at Johns Hopkins University School of Medicine in Baltimore, Maryland, before joining the permanent staff at University College London in the Pharmacology Department. He has been a visiting professor at the University of Tasmania and the University of Southern Denmark.

Dr. Foreman's research interests have included the role of bradykinin receptors in the human nasal airway, the control of microvascular circulation in human skin, and the mechanism of activation of dendritic cells. He served two terms as an editor of the *British Journal of Pharmacology* and was an associate editor of *Immunopharmacology.* He has published 170 research papers as well as reviews and contributions to books.

Torben Johansen, M.D., Dr. Med. Sci., is docent of pharmacology, Department of Physiology and Pharmacology, Institute of Medical Biology, Faculty of Health Sciences, University of Southern Denmark. Dr. Johansen obtained his M.D. degree in 1970 from the University of Copenhagen and became a research fellow in the Department of Pharmacology of Odense University in 1970, lecturer in 1972, and senior lecturer in 1974. Since 1990, he has been docent of pharmacology. In 1979, he was a visiting research fellow for three months at the University Department of Clinical Pharmacology, Oxford University, and in 1998 and 2001 he was a visiting research fellow at the Department of Pharmacology, University College London. In 1980, he did his internship in medicine and surgery at Odense University Hospital. He obtained his Dr. Med. Sci. in 1988 from Odense University.

Dr. Johansen is a member of the British Pharmacological Society, the Physiological Society, the Scandinavian Society for Physiology, the Danish Medical Association, the Danish Pharmacological Society, the Danish Society for Clinical Pharmacology, and the Danish Society for Hypertension. He has published 70 research papers in refereed journals. His current major research interests are N-methyl-D-aspartate (NMDA) receptors in the stubstantia nigra in relation to cell death in Parkinson's disease and also ion transport and signaling in mast cells in relation to intracellular pH and volume regulation.

Alasdair J. Gibb, B.Sc., Ph.D., is reader in pharmacology at University College London. He graduated in biochemistry and pharmacology and completed his Ph.D. at the University of Strathclyde, Glasgow, United Kingdom, studying the mechanisms of action of neuromuscular-blocking drugs. After two years of postdoctoral research at the Australian National University in Canberra, he came to the Pharmacology Department at UCL in 1986 to take up a postdoctoral fellowship. Dr. Gibb was appointed lecturer in pharmacology in 1990. He is currently vice dean for teaching in biosciences at UCL. He is one of the coordinators of the Wellcome Trust four-year Ph.D. program in neuroscience at UCL and a lecturer and joint organizer of the International Brain Research Organization (IBRO) Visiting Lecture Team Programme, a UNESCO and Grass Foundation–funded program that delivers neuroscience teaching to postgraduate students in developing countries around the world. He currently leads the General and Advanced Receptor Theory Workshop of the British Pharmacological Society Diploma in Pharmacology and is a course leader on the British Pharmacological Society short course on Translational Pharmacology.

Dr. Gibb is a member of the British Pharmacological Society and the Physiological Society. He is a past member of the Medical Research Council New Investigator Awards Panel, editor and distributing editor of the *Journal of Physiology*, and coordinator of the Ion Channels Special Interest Group of the Physiological Society, and is currently an editor of the *British Journal of Pharmacology*. He has published more than 50 research papers, reviews, and contributions to books. Dr. Gibb's current main research interest is in the pharmacology and function of NMDA receptors.

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* During production of the third edition of this book Sir James Black died on March 22, 2010. James Black was a pioneer in the application of careful quantitative pharmacology to the development of new therapeutic agents. He developed the first of the β-blocking drugs, propranolol, to relieve angina pectoris which subsequently became for many years a mainstay in the treatment of high blood pressure. His approach was to take the structure of the natural hormone and then in collaboration with medicinal chemists, make chemical analogues that would be effective antagonists with selective affinity for the receptor of interest, while lacking the agonist efficacy of the natural hormone. He used a similar approach to develop histamine H_2 antagonists for the treatment of stomach and duodenal ulcers. He shared the 1988 Nobel Prize for Physiology or Medicine with George H. Hitchings and Gertrude Elion. β-blockers and H_2-receptor antagonists have benefited millions of patients and often saved lives. By his example, Black made a massive contribution to establishing the importance of receptor pharmacology in drug discovery.

Professor Black graduated in medicine from St. Andrews University in 1946 and, after academic posts at the University of Glasgow, joined ICI as a pharmacologist (1958–1964) where he worked on the development of β-blockers. After working with Smith, Kline and French, where he developed the H_2 antagonists, he became Professor of Pharmacology at University College London (1973–1977), before joining Wellcome as the Director of Therapeutic Research (1978–1984). Since 1984, he was a professor of Analytical Pharmacology at King's College London.

Section I

Drug-Receptor Interactions

1 Classical Approaches to the Study of Drug-Receptor Interactions

Donald H. Jenkinson

CONTENTS

1.1 INTRODUCTION

1.1.1 Some History

The term *receptor* is used in pharmacology to denote a class of cellular macromolecules that are concerned directly and specifically in chemical signaling between and within cells. The combination of a hormone, neurotransmitter, or intracellular messenger with its receptor(s) results in a change in cellular activity. Hence a receptor has not only to recognize the particular molecules that activate it but also, when recognition takes place, to alter cell function by causing, for example, a change in membrane permeability, enzyme activity, or 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 ~50 B.C., Lucretius (in *De Rerum Natura*, *Liber IV*) speculated that odors might be conveyed by tiny, invisible "seeds" with distinctive shapes that 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 be taken further only 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 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 brilliant 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* with *concentration*, the second sentence can serve as well today as when it was written, though the nature of the law that 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.

J. N. Langley maintained an interest in the action of plant alkaloids throughout his life. From 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 act 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 a quite 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 Alfred 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, for example, syphilis, at doses that had relatively minor side effects in man. 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.

* Literally: Entities do not act unless attached.

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 between different substrates and inhibitors. As often happens, different strands of evidence had converged to point to a single conclusion.

Finally, a note on the two ways in which present-day pharmacologists and biochemists use the term *receptor.* The first, as in the opening sentences of this section, is to refer to the entire macromolecule, often with several subunits, that carries the binding site(s) for the agonist. This usage has become common as advances in molecular biology have revealed the amino acid sequences and structures 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.

Though the first question can now often be studied directly using techniques described in later chapters, 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 heartbeat) 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 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 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

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

* For a fuller account, see Prüll, Maehle, and Halliwell (2009).

† *Ligand* here means 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.

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

It may well seem odd to refer to receptor *concentrations* in this context when receptors can often move only in the plane of the membrane (and then perhaps to no more than a limited extent, since many kinds of receptors are anchored). However, the model can be formulated just as well, or better, 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 an *equilibrium dissociation constant* (see Appendix 1.2.1), 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

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

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 Equation (1.2), we have assumed that the concentration of the ligand 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 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 Equation (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

* 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 terms are used when we come to discuss the action of irreversible antagonists (Section 1.6.4).

† If you find this difficult, see Appendix 1.2.2 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. See Colquhoun (2006) for a fuller account.

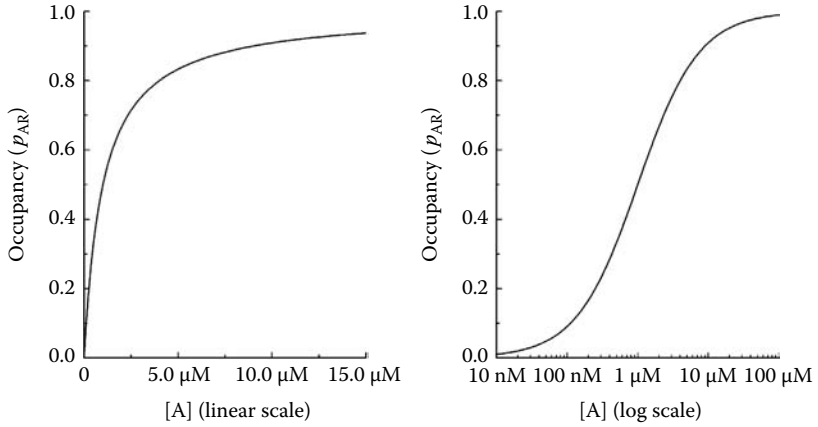

FIGURE 1.1 The relationship between binding-site occupancy and ligand concentration ([A]; linear scale, left; log scale, right), as predicted by the Hill–Langmuir equation. K_A has been set to 1 μM for both curves.

K_A has been taken to be 1 μM. Note from Equation (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 hence is said to be *sigmoidal*. In contrast, with a linear (arithmetic) scale for [A], there is no sigmoidicity: The slope declines as [A] increases, and the curve forms part of a rectangular hyperbola.

Equation (1.2) can be rearranged to

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

Takings 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 unity. Such a graph is described as a *Hill plot*, again after A. V. Hill, who was the first to employ it, and 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 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 Equation (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 Equation (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

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

Taking logs,

$$\log\left(\frac{y}{100 - y}\right) = \log\ [A] - \log\ K_A$$

One approach to testing the applicability of this expression (and so of Equation 1.4) is to measure a series of responses (y) to different concentrations of A and then plot log (y/(100 – y)) against log [A]. If Equation (1.4) holds, a straight line with a slope of unity should be obtained. Also, were all the underlying assumptions to be correct, the value of the intercept of the line on the abscissa (i.e., when the response is half-maximal) would give an estimate of K_A. A. J. Clark was the first to explore this using the responses of isolated tissues, and Figure 1.2 illustrates some of his findings. Figure 1.2a shows that Equation (1.4) provides a reasonably good fit to the experimental values. Also, Clark's values for the slopes of the Hill plots in Figure 1.2b are quite close to unity (0.9 for the frog ventricle, 0.8 for the rectus abdominis†).

While these findings are in keeping with the simple model that has been outlined, *they do not amount to proof that it is correct.* Also, later studies with a variety of tissues have shown that many concentration-response relationships cannot be fitted by Equation (1.4). For example, the Hill coefficient is almost always greater than unity for responses mediated by ligand-gated ion channels (see Appendix 1.2.3 and also Chapter 6). What is more, it is now known that in many tissues the maximal response (e.g., contraction of intestinal smooth muscle) can occur when an agonist such as acetylcholine occupies less than a tenth of the available receptors, rather than all of them as postulated in Equation (1.3). By the same token, when an agonist is applied at the concentration (usually termed the $[A]_{50}$ or EC_{50}) needed to give a half-maximal response, receptor occupancy may be as little as 1% in some tissues,‡ rather than the 50% to be expected were the response to be directly proportional to occupancy. An additional complication is that many tissues contain enzymes (e.g., cholinesterase) or uptake processes (e.g., for noradrenaline) for which agonists are substrates. Because of this, the agonist concentration in the inner regions of an isolated tissue may be much less than that applied in the external solution.

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* 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 observed tissue response. As we shall see in the following sections, this is a crucial oversimplification.

† These experiments have been reanalyzed by Colquhoun (2006).

‡ For evidence on this, see Section 1.6 on irreversible antagonists.

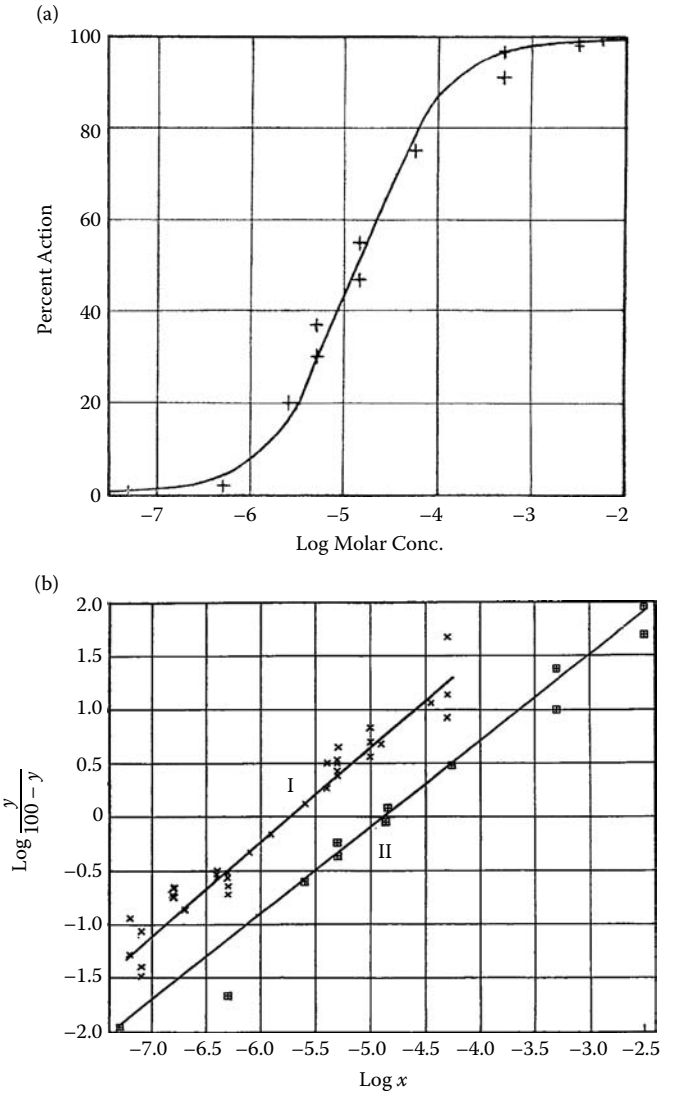

FIGURE 1.2 Upper: Concentration-response relationship for the action of acetylcholine in causing contraction of the frog rectus abdominis muscle. The curve has been drawn using Equation (1.4). Lower: Hill plots for the action of acetylcholine on frog ventricle (curve I) and rectus abdominis (curve II). (Adapted from Clark, A. J., *J. Physiol*., 61, 530–547, 1926.)

For all these reasons, pharmacologists have had to abandon (sometimes rather reluctantly and belatedly) not only their attempts to explain the shapes of the dose-response curves of complex tissues in terms of the simple models first explored by Clark and by Hill, but also the hope that the value of the concentration of an agonist that gives a half-maximal response might provide even an approximate estimate of K_A. Nevertheless, as Clark's work showed, the relationship between the concentration of an agonist and the response of a tissue commonly has the same general form shown in Figure 1.1. In keeping with this, concentration-response curves can often be described *empirically*, and at least to a first approximation, by the simple expression

$$y = y_{max} \frac{[A]^{n_H}}{[A]_{50}^{n_H} + [A]^{n_H}} \tag{1.6}$$

This is usually described as the *Hill equation* (see also Appendix 1.2.3). Here n_H is again the *Hill coefficient* and y and y_{max} are respectively the observed response and the maximum response to a large concentration of the agonist, A. $[A]_{50}$ is the concentration of A at which y is half maximal. Because it is a constant for a given concentration-response relationship, it is sometimes denoted by K. While this is algebraically neater (and was the symbol used by Hill), it should be remembered that K in this context does not necessarily correspond to an equilibrium constant. Employing $[A]_{50}$ rather than K in Equation (1.6) helps to remind us that the relationship between [A] and response is here being *described* rather than *explained* in terms of a model of receptor action. The difference is important.

1.2.3 The Distinction between Agonist Binding and Receptor Activation

To end, we return to models of receptor action and to a further limitation of the early attempts to account for the shapes of concentration-response curves. As already noted, the simple concepts expressed in Equations (1.3) and (1.4) do not distinguish between the *occupation* and the *activation* of a receptor by an agonist. This distinction, it is now appreciated, is crucial to the understanding of the action of agonists and partial agonists. Indeed all contemporary accounts of receptor activation take as their starting point a mechanism of the following kind*:

$$\underset{\text{inactive}}{\overset{\text{vacant}}{A + R}} \overset{K_A}{\rightleftharpoons} \underset{\text{inactive}}{\overset{\text{occupied}}{AR}} \overset{E}{\rightleftharpoons} \underset{\text{active}}{\overset{\text{occupied}}{AR^*}} \tag{1.7}$$

Here the occupied receptors can exist in two forms, one of which is inactive (AR) and the other active (AR*) in the sense that its formation leads to a tissue response. AR and AR* can interconvert (often described as isomerization), and at equilibrium the receptors will be distributed between the R, AR, and AR* conditions.† The position of the equilibrium between AR and AR*, and hence the magnitude of the maximum response of the tissue, will depend on the value of the equilibrium constant E‡. Suppose that a very large concentration of the agonist A is applied, so that all the binding sites are occupied, that is, the receptors are in either the AR or the AR* state. If the position of the equilibrium strongly favors AR, with few active (AR*) receptors, the response will be relatively small. The reverse would apply for a very effective agonist. This will be explained in greater detail in Sections 1.4.3 through 1.4.7, where we will also look into the quantitative relationship between agonist concentration and the fraction of receptors in the active state.

APPENDICES TO SECTION 1.2

Appendix 1.2.1 Equilibrium, Dissociation, and Affinity Constants

Confusingly, the terms *equilibrium*, *dissociation*, and *affinity constant* are all in current use to express the position of the equilibrium between a ligand and its receptors. The choice arises because the ratio of the rate constants k_{-1} and k_{+1} can be expressed either way. In this chapter we take K_A to be k_{-1}/k_{+1}, and it is then strictly an *equilibrium dissociation constant*, often abbreviated to either *dissociation constant* or *equilibrium constant*. The inverse ratio, k_{+1}/k_{-1}, gives the *association equilibrium constant*, which is usually referred to as the *affinity constant*.

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* This will be described as the del Castillo–Katz scheme since it was first applied to receptor action by J. del Castillo and B. Katz (University College London) in 1957 (see also Section 1.4.3).

† The scheme is readily extended to include the possibility that some of the receptors may be active even in the absence of an agonist (see Section 1.4.7).

‡ This constant is sometimes denoted by L or by K_2. E has been chosen for this introductory account because of the relation to efficacy and also because it is the term used in a seminal review by Colquhoun (1998) on binding, efficacy, and the effects thereon of receptor mutations.

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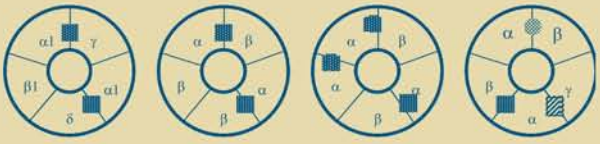

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