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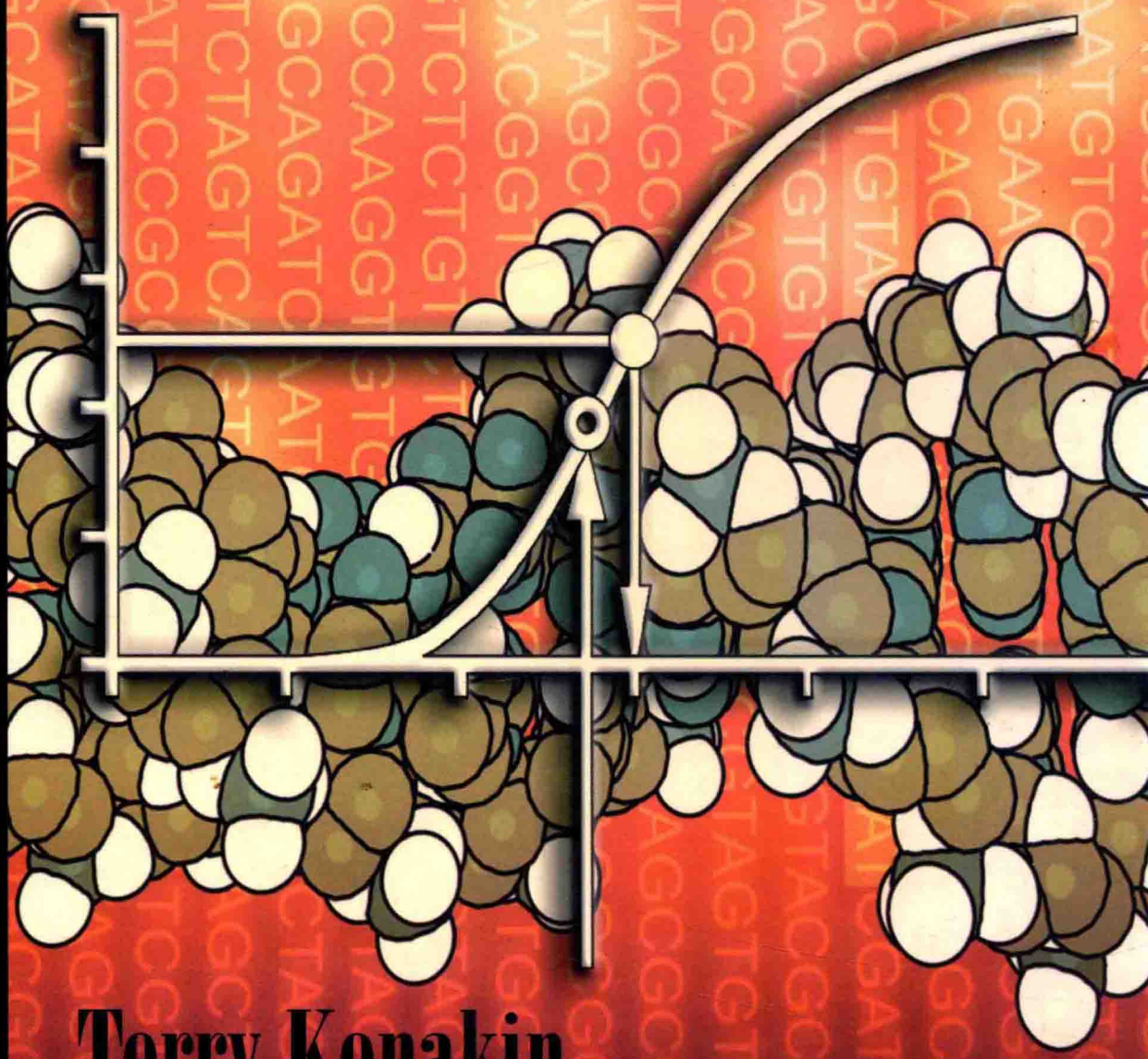
A Pharmacology Primer

Theory, Application, and Methods



Terry Kenakin

A PHARMACOLOGY PRIMER: THEORY, APPLICATION, AND METHODS

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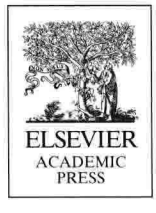

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A PHARMACOLOGY PRIMER: THEORY, APPLICATION, AND METHODS

As always...for Debbie

"…more ceterum censeo is perhaps necessary in order to rouse pharmacology from its sleep. The sleep is not a natural one since pharmacology, as judged by its past accomplishments, has no reason for being tired."

Rudolph Bucheim (1820–1879)

I am indebted to GlaxoSmithKline Research and Development for support during the preparation of this book and for the means and scientific environment to make the science possible.

T.P.K.
Research Triangle Park, NC 2003

Foreword

If scientific disciplines can be said to go in and out of vogue, pharmacology is exemplary in this regard. The flourishing of receptor theory in the 1950s, the growth of biochemical binding technology in the 1970s, and the present resurgence of interest in defining cellular phenotypic sensitivity to drugs have been interspersed with troughs such as that brought on by the promise of the human genome and a belief that this genetic road map may make classical pharmacology redundant. The fallacy in this belief has been found in experimental data showing the importance of phenotype over genotype, which underscores a common finding with road maps; they are not as good as a guide who knows the way. Pharmacology is now more relevant to the drug discovery process than ever as the genome furnishes a wealth of new targets to unravel. Biological science often advances at a rate defined by the technology of its tools (i.e., scientists cannot see new things in old systems without new eyes). A veritable explosion in technology coupled with the great gift of molecular biology have definitely given pharmacologists new eyes to see.

This book initially began as a series of lectures at GlaxoSmithKline Research and Development on receptor pharmacology aimed at increasing the communication between pharmacologists and chemists. As these lectures developed it became evident that the concepts were useful to biologists, not specifically trained in pharmacology. In return, the exchange between chemists and biologists furnished new starting points from which to view the pharmacological concepts. It is hoped that this book will somewhat fill what could be a gap in present biological sciences, namely the study of dose–response relationships and how cells react to molecules.

Terry P. Kenakin
Research Triangle Park, 2003

Contents

1

What Is Pharmacology

I would in particular draw the attention to physiologists to this type of physiological analysis of organic systems which can be done with the aid of toxic agents.

—CLAUDE BERNARD (1813–1878)

1.1 About This Book

Essentially this is a book about the methods and tools used in pharmacology to quantify drug activity. Receptor pharmacology is based on the comparison of experimental data to simple mathematical models with a resulting inference of drug behavior to the molecular properties of drugs. From this standpoint, a certain understanding of the mathematics involved in the models is useful, but it is not imperative. This book is structured such that each chapter begins with the basic concepts, then moves on to the techniques used to estimate drug parameters, and finally, for those so inclined, the mathematical derivations of the models used. Understanding the derivation is not a prerequisite to understanding the application of the methods or the resulting conclusion; these are included for completeness and are for readers who wish to pursue exploration of the models. In general, facility with mathematical equations is definitely not required for pharmacology; the derivations can be ignored to no detriment to the use of this book.

Second, the symbols used in the models and derivations, on occasion, duplicate each other (e.g., α is an extremely popular symbol). However, the use of these multiple symbols has been retained because this preserves the context of where these models were first described and used. Also, changing these to make them unique would cause confusion if these methods are to be used beyond the framework of this book. Therefore, care should be taken to consider the actual nomenclature of each chapter.

Third, an effort has been made to minimize the need to cross-reference different parts of the book; when a particular model is described, the basics are reiterated somewhat to minimize the need to read the relevant but different part of the book where the model is initially described. Although this leads to a small amount of repeated description, it is believed that this will allow for a more uninterrupted flow of reading and use of the book.

1.2 What Is Pharmacology?

Pharmacology as a separate science is approximately 120–140 years old. The relationship between chemical structure and biological activity began to be studied systematically in the 1860s [1]. It began when physiologists, using chemicals to probe physiological systems, became more interested in the chemical probes than the systems they were probing. *Pharmacology* can be an all encompassing term for the study of drugs on living systems in relation to their therapeutic value. Within this discipline is the study of absorption, metabolism, distribution, and excretion of drugs (*pharmacokinetics*) and the study of the interaction of drugs with living systems (*pharmacodynamics*). A differentiation of physiology and pharmacology was given by the pharmacologist Sir William Paton in 1986 [2]:

> If physiology is concerned with the function, anatomy with the structure, and biochemistry with the chemistry of the living body, then pharmacology is concerned with the changes in function, structure, and chemical properties of the body brought about by chemical substances.

Many works about pharmacology essentially deal in therapeutics associated with different organ systems in the body. Thus, in many pharmacology texts, chapters are entitled drugs in the cardiovascular system, the effect of drugs on the gastrointestinal system, central nervous system, and so on. However, the underlying principles for all of these is the same, namely the pharmacodynamic interaction between the drug and the biological recognition system for that drug. Therefore, a prerequisite to all of pharmacology is an understanding of the basic concepts of dose–response and how living cells process pharmacological information. This generally is given the term *receptor pharmacology*, where receptor is a term referring to any biological recognition unit for drugs (e.g., membrane receptors, enzymes, DNA).

With such knowledge in hand, readers will be able to apply these principles to any branch of therapeutics effectively. This book treats dose–response data generically and demonstrate methods by which drug activity can be quantified across all biological systems irrespective of the nature of the biological target.

The human *genome* is now widely available for drug discovery research. Far from being a simple blueprint of how drugs should be targeted, it has shown biologists that receptor *genotypes* (i.e., properties of proteins resulting from genetic transcription to their amino acid sequence) are secondary to receptor *phenotypes* (how the protein interacts with the myriad of cellular components and how cells tailor the makeup and functions of these proteins to their individual needs). Since the arrival of the human genome, receptor pharmacology as a science is more relevant than ever in drug discovery. Current drug therapy is based on less than 500 molecular targets, yet estimates utilizing the number of genes involved in multifactorial diseases suggest that the number of potential drug targets range from 5000 to 10,000 [3]. Thus, current therapy is using only 5–10% of the potential trove of targets available in the human genome.

A meaningful dialogue between chemists and pharmacologists is the single most important element of the drug discovery process. The necessary link between medicinal chemistry and pharmacology has been elucidated by Paton [2]:

> For pharmacology there results a particularly close relationship with chemistry, and the work may lead quite naturally, with no special stress on practicality, to therapeutic application, or (in the case of adverse reactions) to toxicology.

Chemists and biologists reside in different worlds from the standpoint of the type of data they deal with. Chemistry is an exact science with physical scales that are not subject to system variance. Thus, the scales of measurement are transferrable. Biology deals with the vagaries of complex systems that are not completely understood. Within this scenario, scales of measurement are much less constant and much more subject to system conditions. Given this, a gap can exist between chemists and biologists in terms of understanding and also in terms of the best method to progress forward. In the worst circumstance, it is a gap of credibility emanating from a failure of the biologist to make the chemist understand the limits of the data. Usually, however, credibility is not the issue and the gap exists due to a lack of common experience. This book was written in an attempt to limit or, hopefully, eliminate this gap.

1.3 The Receptor Concept

One of the most important concepts emerging from early pharmacological studies is the concept of the *receptor*. Pharmacologists knew that minute amounts of certain chemicals had profound effects on physiological systems. They also knew that very small changes in the chemical composition of these substances could lead to huge differences in activity. This led to the notion that something on or in the cell must specifically read the chemical information contained in these substances and translate it into a physiological effect. This something was conceptually referred to as the "receptor" for that substance. Pioneers such as Paul Ehrlich (1854–1915; Fig. 1.1A) proposed the existence of "chemoreceptors" (actually he proposed a collection of "amboceptors," "triceptors," "polyceptors") on cells for dyes. He also postulated that the chemoreceptors on parasites, cancer cells, and microorganisms were different from a healthy host and thus could be exploited therapeutically. The physiologist turned pharmacologist John Newport Langley (1852–1926; Fig. 1.1B), during his studies with the drug jaborandi (which contains the alkaloid pilocarpine) and atropine, introduced the concept that receptors were switches that received and generated signals and that these switches could be activated or blocked by specific molecules. The originator of quantitative receptor theory, the Edinburgh pharmacologist Alfred Joseph Clark (1885–1941; Fig. 1.1C) was the first to suggest that the data, compiled from his studies of the interactions of acetylcholine and atropine, resulted from the unimolecular interaction of the drug and a substance on the cell surface. He articulated these ideas in the classic work "The mode of action of drugs" on cells [4] later revised as the "Handbook of Experimental Pharmacology" [5]. As put by Clark in 1937:

> It appears to the writer that the most important fact shown by a study of drug antagonisms is that it is impossible to explain the remarkable effects observed except by assuming that drugs unite with receptors of a highly specific pattern . . . No other explanation will, however, explain a tithe of the facts observed.

Clark's next step formed the basis of receptor theory by applying chemical laws to systems of "infinitely greater complexity" [4]. It is interesting to note the scientific atmosphere in which Clark published these ideas. The dominant ideas between 1895 and 1930 were based on theories such as the law of phasic variation essentially stating that "certain phenomena occur frequently." Homeopathic theories such as the Arndt–Schulz law and Weber–Fechner law were based on loose ideas around surface tension of the cell membrane but there was little physicochemical basis to these ideas [6]. In this vein, prominent pharmacologists of the day such as Walter Straub (1874–1944) suggested that a general theory of chemical binding between drugs and cells utilizing receptors was "going too far . . . and . . . not admissible" [6]. The impact of Clark's thinking against these concepts cannot be overemphasized to modern pharmacology.

Drug receptors can exist in many forms from cell surface proteins to enzymes, ion channels, membrane transporters, DNA, and cytosolic proteins (see Fig. 1.2). There are examples of important drugs for all of these. This book deals with general concepts that can be applied to a range of receptor types, but most of the principles are illustrated with the most tractable receptor class known in the human genome, namely *seven transmembrane* **(7 TM)** *receptors*. These receptors are named for their characteristic structure, which consists of a single protein chain that traverses

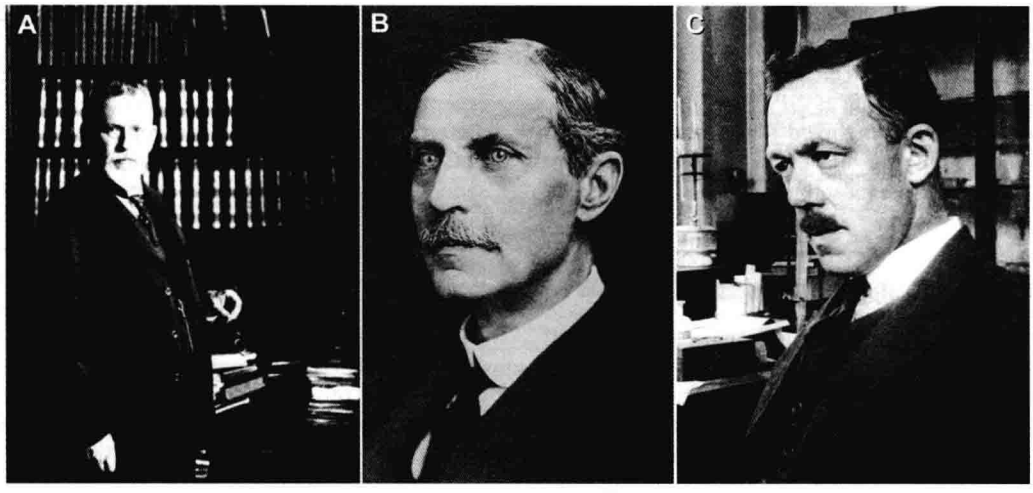

FIGURE 1.1 Pioneers of pharmacology. (A) Paul Ehrlich (1854–1915). Born in Silesia, Ehrlich graduated from Leipzig University to go on to a distinguished career as head of institutes in Berlin and Frankfurt. His studies with dyes and bacteria formed the basis of early ideas regarding the recognition of biological substances by chemicals. (B) John Newport Langley (1852–1926). Although he began reading mathematics and history in Cambridge in 1871, Langley soon took to physiology. He succeeded the great physiologist M. Foster to the chair of physiology in Cambridge in 1903 and branched out into pharmacological studies of the autonomic nervous system. These pursuits led to germinal theories of receptors. (C) Alfred J. Clark (1885–1941). Beginning as a demonstrator in pharmacology in King's College (London), Clark went on to become professor of pharmacology at University College, London. From there he took the chair of pharmacology in Edinburgh. Known as the originator of the modern receptor theory, Clark applied chemical laws to biological phenomena. His books on receptor theory formed the basis of modern pharmacology.

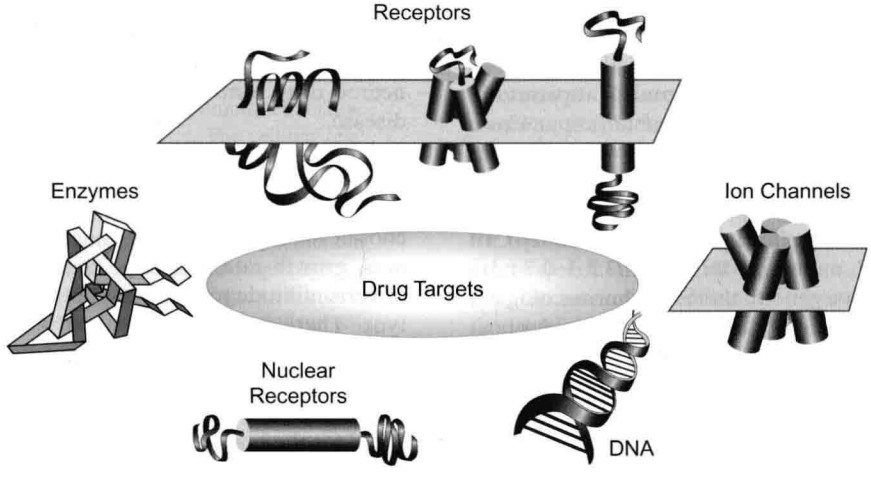

FIGURE 1.2 Schematic diagram of potential drug targets. Molecules can affect the function of numerous cellular components both in the cytosol and on the membrane surface. There are many families of receptors that traverse the cellular membrane and allow chemicals to communicate with the interior of the cell.

the cell membrane seven times to produce extracellular and intracellular loops. Because these receptors activate G-proteins to elicit response, they are also commonly referred to as *G-protein–coupled receptors* **(GPCRs)**. There are between 800 and 1000 [7] of these in the genome (the genome sequence predicts 650 GPCR genes of which approximately 190 [on the order of 1% of the genome of superior organisms] are categorized as known GPCRs [8] activated by some 70 ligands). In the United States in the year 2000, nearly half of all prescription drugs were targeted toward 7TM receptors [3]. These receptors, accounting for 1–5% of the total cell protein, control a myriad of

physiological activities. Because they are on the cell surface, they are tractable for drug discovery; therefore, drugs do not need to penetrate the cell to produce effect. In the study of biological targets such as GPCRs and other receptors, a "system" must be employed that accepts chemical input and returns biological output. It is worth discussing such receptor systems in general terms before their specific uses are considered.

1.4 Pharmacological Test Systems

Molecular biology has transformed pharmacology and the drug discovery process. As recently as 10 years ago, screening for new drug entities was carried out in surrogate animal tissues. This necessitated a rather large extrapolation spanning differences in genotype and phenotype. The belief that the gap could be bridged came from the notion that the chemicals recognized by these receptors in both humans and animals were the same (see later). Receptors are unique proteins with characteristic amino acid sequences. While *polymorphisms* (spontaneous alterations in amino acid sequence; see later) of receptors exist in the same species, in general, the amino acid sequence of a natural ligand binding domain for a given receptor type largely may be conserved. There are obvious pitfalls of using surrogate species receptors for the prediction of human drug activity, and it never can be known for certain whether an agreement for estimates of activity for a given set of drugs ensures accurate prediction for all drugs; the agreement is very much drug and receptor dependent. For example, the human and mouse α_2-adrenoceptor are 89% homologous and thus are considered very similar from the standpoint of an amino acid sequence. Furthermore, the affinities of the α_2-adrenoceptor antagonists atipamezole and yohimbine are nearly indistinguishable (atipamezole human α_2-C10 $K_i = 2.9 \pm 0.4$ nM, mouse α_2-4H $K_i = 1.6 \pm 0.2$ nM; yohimbine human α_2-C10 $K_i = 3.4 \pm 0.1$ nM, mouse α_2-4H $K_i = 3.8 \pm 0.8$ nM). However, there is a 20.9-fold difference for the antagonist prazosin (human α_2-C10 $K_i = 2034 \pm 350$ nM, mouse α_2-4H $K_i = 97.3 \pm 0.7$ nM) [9]. Such data highlight a general theme in pharmacological research, namely that a hypothesis, such as one proposing two receptors to be identical with respect to their sensitivity to drugs are the same, cannot be proved, only disproved. Although a considerable number of drugs could be tested on the two receptors (thus supporting the hypothesis that their sensitivity to all drugs is the same), this hypothesis is immediately disproved by the first drug that shows differential potency on the two receptors. The fact that a series of drugs tested shows identical potencies may only mean that the wrong sample of drugs has been chosen to unveil the difference. Thus, no general statements can be made that any one surrogate system is completely predictive of activity on the target human receptor; this will always be a drug-specific phenomenon.

The link between animal and human receptors is the fact that both proteins recognize the endogenous transmitter (e.g., acetylcholine, norepinephrine), and, therefore, the hope is that this link will carry over into other drugs that recognize the animal receptor. This imperfect system formed the basis of drug discovery until human *cDNA* for human receptors could be used to make cells express human receptors. These engineered (recombinant) systems now are used as surrogate human receptor systems and the leap of faith from animal receptor sequences to human receptor sequences is not required (i.e., the problem of differences in genotype has been overcome). However, cellular signaling is an extremely complex process, and cells tailor their receipt of chemical signals in numerous ways. Therefore, the way a given receptor gene behaves in a particular cell can differ in response to the surroundings in which that receptor finds itself. These differences in phenotype (i.e., properties of a receptor produced by interaction with its environment) can result in differences in both the quantity and the quality of a signal produced by a concentration of a given drug in different cells. Therefore, there is still a certain, although somewhat lesser, leap of faith taken in predicting therapeutic effects in human tissues under pathological control from surrogate recombinant or even surrogate natural human receptor systems. For this reason, it is a primary requisite of pharmacology to derive system-independent estimates of drug activity that can be used to predict therapeutic effects in other systems.

A schematic diagram of the various systems used in drug discovery, in order of how appropriate they are to therapeutic drug treatment, is shown in Fig. 1.3. As discussed earlier, early functional experiments in animal tissue have now largely given way to testing in recombinant cell systems engineered with human receptor material. This huge technological step greatly improved the predictability of drug activity in humans, but it should be noted that there are still many factors that intervene between the genetically engineered drug testing system and the pathology of human disease.

A frequently used strategy in drug discovery is to express human receptors (through *transfection* with human cDNA) in convenient surrogate host cells. These host cells are chosen mainly for their technical properties (i.e., robustness, growth rate, stability) and not with any knowledge of verisimilitude to the therapeutically targeted human cell type. There are various factors relevant to the choice of surrogate host cell, such as a very low background activity (i.e., a cell cannot be used that already contains a related animal receptor for fear of cross-reactivity to molecules targeted for the human receptor). Human receptors often are expressed in animal surrogate cells; the main idea here is that the cell is a receptacle for the receptor, allowing it to produce physiological responses, and that activity can be monitored in pharmacological experiments. In this sense, human receptors expressed in animal cells are still a theoretical step distanced from the human receptor in a human cell type. However, even if a human surrogate is used (and there are such cells available), there is no definitive evidence that a surrogate human cell is any more predictive of a natural receptor activity than an animal cell when compared with the complex receptor behavior in its natural host cell type expressed under pathological conditions. The receptor phenotype dominates in the end organ, and the exact

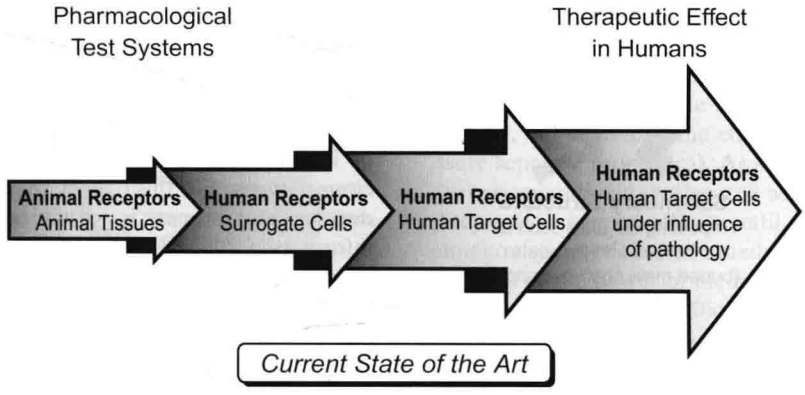

FIGURE 1.3 A history of the drug discovery process. Originally, the only biological material available for drug research was animal tissue. With the advent of molecular biological techniques to clone and express human receptors in cells, recombinant systems supplanted animal isolated tissue work. It should be noted that these recombinant systems still fall short of yielding drug response in the target human tissue under the influence of pathological processes.

differences between the genotypic behavior of the receptor (resulting from the genetic makeup of the receptor) and the phenotypic behavior of the receptor (due to the interaction of the genetic product with the rest of the cell) may be cell specific. Therefore, there is still a possible gap between the surrogate systems used in the drug discovery process and the therapeutic application. Moreover, most drug discovery systems utilize receptors as switching mechanisms and quantify whether drugs turn on or turn off the switch. The pathological processes that we strive to modify may be subtler. As put by pharmacologist Sir James Black [10]:

> angiogenesis, apoptosis, inflammation, commitment of marrow stem cells, and immune responses. The cellular reactions subsumed in these processes are switch like in their behavior ... biochemically we are learning that in all these processes many chemical regulators seem to be involved. From the literature on synergistic interactions, a control model can be built in which no single agent is effective. If a number of chemical messengers each bring information from a different source and each deliver only a subthreshold stimulus but together mutually potentiate each other, then the desired information-rich switching can be achieved with minimum risk of miscuing.

Such complex end points are difficult to predict from any one of the component processes, leading to yet another leap of faith in the drug discovery process.

Even when an active drug molecule is found and activity is verified in the therapeutic arena, there are factors that can lead to gaps in its therapeutic profile. When drugs are exposed to huge populations, genetic variations in this population can lead to the discovery of *alleles* that code for mutations of the target (isogenes), which can lead to a variation in the drug response. Such polymorphisms can lead to resistant populations (e.g., resistance of some asthmatics to β-adrenoceptor bronchodilators) [11]. In the absence of genetic knowledge, these therapeutic failures for a drug could not be averted easily, because they in essence resulted from the presence of new biological targets not considered originally in the drug discovery process. However, with new epidemiological information becoming available, these polymorphisms can now be incorporated into the drug discovery process.

Two theoretical and practical scales can be used to make system-independent measures of drug activity on biological systems. The first is a measure of the attraction of a drug for a biological target, namely its *affinity* for receptors. Drugs must interact with receptors to produce an effect, and *affinity* is a chemical term used to quantify the strength of that interaction. The second is much less straightforward and is used to quantify the degree of effect imparted to the biological system after the drug binds to the receptor; this is termed *efficacy*. This property was named by R. P. Stephenson [12] within classical receptor theory as a proportionality factor for tissue response produced by a drug. There is no absolute scale for efficacy; rather it is dealt with in relative terms (i.e., the ratio of the efficacy of two different drugs on a particular biological system can be estimated and, under ideal circumstances, will transcend the system and be applicable to other systems as well). It is the foremost task of pharmacology to use the translations of a drug effect obtained from cells to provide system-independent estimates of affinity and efficacy. Before a specific discussion of affinity and efficacy, it is worth considering the molecular nature of biological targets.

1.5 The Nature of Drug Receptors

Although some biological targets such as DNA are not protein in nature, most receptors are. It is useful to consider the properties of receptor proteins to provide a context for the interaction of small molecule drugs with them. An important property of receptors is that they have a three-dimensional structure. Proteins usually are composed of one or more peptide chains; the composition of these

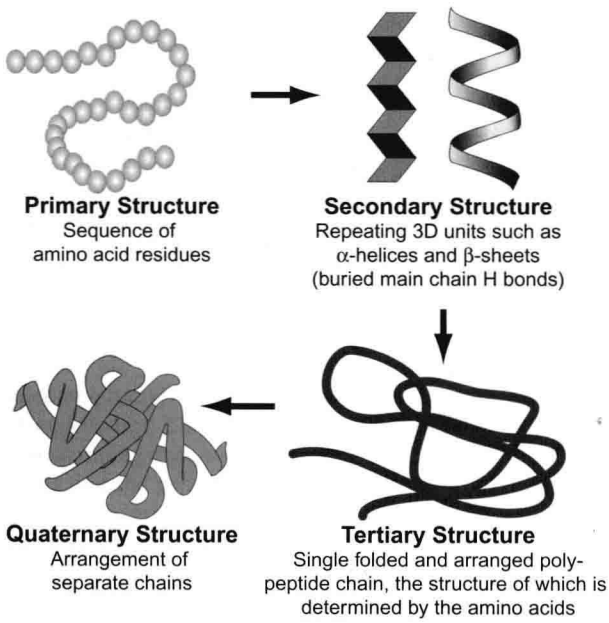

FIGURE 1.4 Increasing levels of protein structure. A protein has a given amino acid sequence to make peptide chains. These adopt a three-dimensional structure according to the free energy of the system. Receptor function can change with changes in tertiary or quaternary structure.

chains makes up the primary and secondary structure of the protein. Proteins are also described in terms of a tertiary structure, which defines their shape in three-dimensional space, and a quaternary structure, which defines the molecular interactions between the various components of the protein chains (Fig. 1.4). It is this three-dimensional structure that allows the protein to function as a recognition site and an effector for drugs and other components of the cell; in essence, the ability of the protein to function as a messenger shuttling information from the outside world to the cytosol of the cell. For GPCRs, the three-dimensional nature of the receptor forms binding domains for other proteins, such as *G-proteins* (these are activated by the receptor and then go on to activate enzymes and ion channels within the cell; see Chapter 2), and endogenous chemicals, such as neurotransmitters, hormones, and autacoids that carry physiological messages. For other receptors, such as ion channels and single transmembrane enzyme receptors, the conformational change *per se* leads to a response either through an opening of a channel to allow the flow of ionic current or the initiation of enzymatic activity. Therapeutic advantage can be taken by designing small molecules to utilize these binding domains or other three-dimensional binding domains on the receptor protein in order to modify physiological and pathological processes.

1.6 System-Independent Drug Parameters: Affinity and Efficacy

The process of drug discovery relies on the testing of molecules in systems to yield estimates of biological activity in an iterative process of changing the structure of the molecule until optimal activity is achieved. This book shows that there are numerous systems available to do this and that each system may interpret the activity of molecules in different ways. Some of these interpretations can appear to be in conflict with each other, leading to apparent capricious patterns. For this reason, the way forward in the drug development process is to use only system-independent information. Ideally, scales of biological activity should be used that transcend the actual biological system in which the drug is tested. This is essential to avoid confusion and also because it is quite rare to have access to the exact human system under the control of the appropriate pathology available for *in vitro* testing. Therefore, the drug discovery process necessarily relies on the testing of molecules in surrogate systems and the extrapolation of the observed activity to all systems. The only means to do this is to obtain system-independent measures of drug activity, namely affinity and efficacy.

If a molecule in solution associates closely with a receptor protein it has affinity for that protein. The area where it is bound is the binding *domain* or *locus*. If the same molecule interferes with the binding of a physiologically active molecule, such as a hormone or a neurotransmitter, (i.e., if the binding of the molecule precludes activity of the physiologically active hormone or neurotransmitter), then the molecule is referred to as an *antagonist*. Therefore, a pharmacologically active molecule that blocks the physiological effect is an antagonist. Similarly, if a molecule binds to a receptor and produces its own effect, it is termed an *agonist*; it is also assumed to have the property of efficacy. Efficacy is detected by the observation of a pharmacological response. Therefore, agonists have both affinity and efficacy.

Classically, an agonist response is described in two stages, the first being the initial signal imparted to the immediate biological target, namely the receptor. This first stage is composed of formation, either through interaction with an agonist or spontaneously, of an active state receptor conformation; this initial signal is termed the *stimulus* (Fig. 1.5). This stimulus is perceived by the cell and processed in various ways through successions of biochemical reactions to the end point, namely the *response*. The sum total of the subsequent reactions is referred to as the *stimulus–response mechanism* or cascade (see Fig. 1.5).

Efficacy is a molecule-related property; that is, different molecules have different capabilities to induce a physiological response. The actual term for the molecular aspect of the response-inducing capacity of a molecule is *intrinsic efficacy* (see Chapter 3 for how this term evolved). Thus, every molecule has a unique value for its intrinsic efficacy (in cases of antagonists this could be zero). The different abilities of molecules to induce a response are illustrated in Fig. 1.6. Figure 1.6 shows dose–response curves for four 5-HT (serotonin) agonists in rat jugular vein. It can be seen that if response is plotted as a function of the percentage receptor occupancy, different receptor occupancies for the different agonists lead to different levels of response. For example, while 0.6 g force can be generated

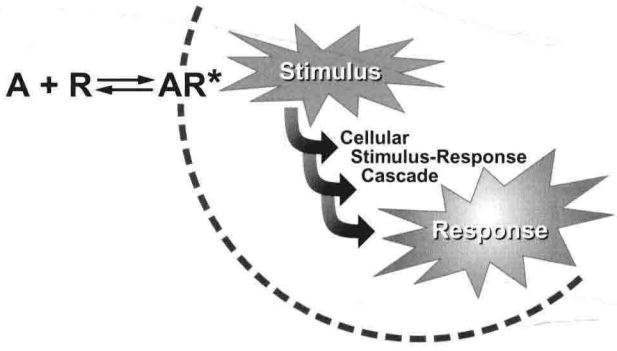

FIGURE 1.5 Schematic diagram of response production by an agonist. An initial stimulus is produced at the receptor as a result of agonist–receptor interaction. This stimulus is processed by the stimulus–response apparatus of the cell into an observable cellular response.

by 5-HT by occupying 30% of the receptors, the agonist 5-cyanotryptamine requires twice the receptor occupancy to generate the same response, that is, the capability of 5-cyanotryptamine to induce response is half that of 5-HT [13]. These agonists are then said to possess different magnitudes of intrinsic efficacy.

It is important to consider affinity and efficacy as separately manipulatable properties. Thus, there are chemical features of agonists that pertain especially to affinity and other features that pertain to efficacy. Figure 1.7 shows a series of key chemical compounds made *en route* to the histamine H_2 receptor antagonist cimetidine (used for healing gastric ulcers). The starting point for this discovery program was the knowledge that histamine, a naturally occurring autacoid, activates histamine H_2 receptors in the stomach to cause acid secretion. This constant acid secretion is what prevents the healing of lesions and ulcers. The task was then to design a molecule that would antagonize the histamine receptors mediating acid secretion and prevent histamine H_2 receptor activation to allow the ulcers to heal. This task was approached with the knowledge that molecules, theoretically, could be made that retained or even enhanced affinity, but decreased the efficacy of histamine (i.e., these were separate properties). As shown in Fig. 1.7, molecules were consecutively synthesized with reduced values of efficacy and enhanced affinity until the target histamine H_2 antagonist cimetidine was made. This was a clear demonstration of the power of medicinal chemistry to separately manipulate affinity and efficacy for which, in part, the Nobel prize in medicine was awarded in 1988.

1.7 What Is Affinity?

The affinity of a drug for a receptor defines the strength of an interaction between the two species. Forces controlling the affinity of a drug for the receptor are thermodynamic (enthalpy as changes in heat and entropy as changes in the state of disorder). The chemical forces between the components of the drug and the receptor vary in importance in relation to the distance the drug is away from the receptor binding surface. Thus, the strength of electrostatic forces (attraction due to positive and negative charges and/or complex interactions between polar groups) varies as a function of the reciprocal of the distance between the drug and the receptor. Hydrogen bonding (the sharing of a hydrogen atom between an acidic and basic group) varies in strength as a function of the fourth power of the reciprocal of the distance. Also involved are van der Waals forces (weak attraction between polar and nonpolar molecules) and hydrophobic bonds (interaction of nonpolar surfaces to avoid interaction with water). The combination of all of these forces causes the drug to reside in a certain position within the protein binding pocket; this is a position of minimal free energy. It is important to note that drugs do not statically reside in one uniform position. As thermal energy

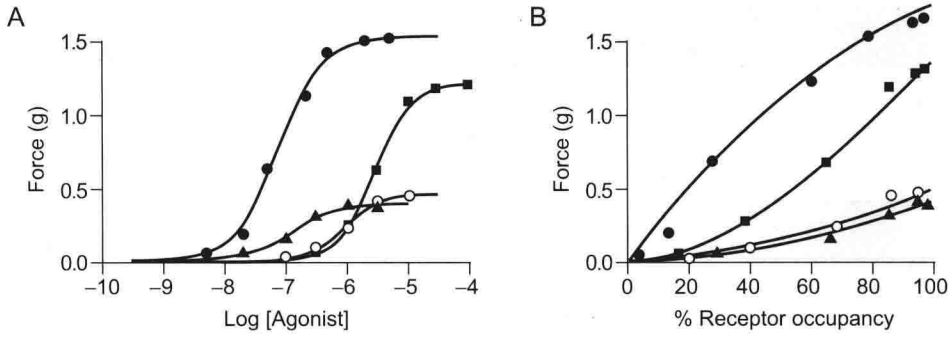

FIGURE 1.6 Differences between agonists producing contraction of a rat jugular vein through the activation of 5-HT receptors. (A) Dose–response curves to 5-HT receptor agonists 5-HT (●), 5-cyanotryptamine (■), *N*,*N*-dimethyltryptamine (○), and *N*-benzyl-5-methoxytryptamine (▲). Abscissae: logarithms of molar concentrations of agonist. (B) Occupancy response curves for curves shown in A. Abscissae: percentage receptor occupancy by the agonist as calculated by mass action and the equilibrium dissociation constant of the agonist–receptor complex. Ordinates: force of contraction in g. Data drawn from Leff *et al.* [13].

"...we knew the receptor bound histamine, so it was a matter of keeping affinity and losing efficacy..."
Sir James Black, 1996

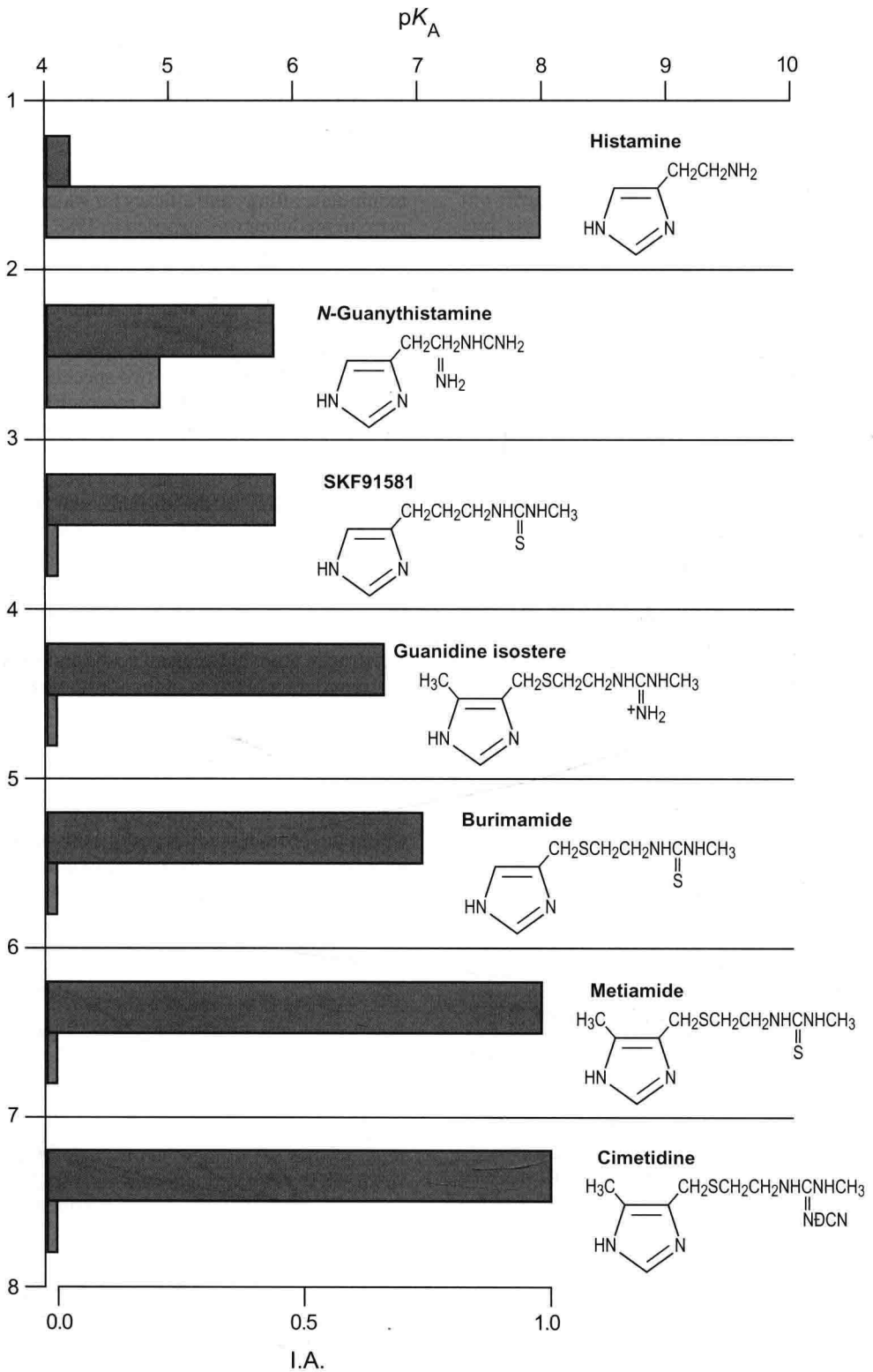

FIGURE 1.7 Key compounds synthesized to eliminate the efficacy (green) and to enhance the affinity (burgundy red) of histamine for histamine H_2 receptors to make cimetidine, one of the first histamine H_2 antagonists of use in the treatment of peptic ulcers. Quotation from James Black [10].