Pharmacologic Analysis of Drug-Receptor Interaction

Second Edition

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To my wife Debbie
for what went before
and for what is to come

Preface to First Edition

The receptor can be considered a crossroads where a vast array of hormones, autacoids, neurotransmitters, drugs, and other foreign substances meet the cell and transmit their messages to another vast array of stimulus-response mechanisms. In this sense, the study of drug-receptor interactions touches all aspects of pharmacology, and a great many aspects of biochemistry and physiology. Thus, by necessity, the discipline of drug-receptor kinetics enters the lives of scientists concerned with a variety of biological mechanisms, forcing them to go into the realm of kinetic processes in diffusion-restricted systems observed in an indirect manner.

This volume considers the pharmacologic analysis of the interactions of drugs and drug receptors as a problem of classification, the ultimate aim of which is to define the properties of drugs on biological systems. In the process, this same exercise is capable of classifying physiological processes as well. Unlike many treatises on drug-receptor pharmacology, little emphasis is placed on the structure of the receptor, the definition of its nature being subordinate to its function as a looking glass into the properties of drugs and physiological mechanisms.

For the pharmacologist, the techniques for study of drug-receptor interaction are major tools in the quest for new drugs of therapeutic benefit. Definition of the properties of drugs is an essential part of this process, and from this standpoint, every newly discovered selective drug *begins* the pharmacologist's primary task of classification. Thus, the discovery of the drug becomes both the endpoint (delivery of a therapeutic entity for the benefit of humans) and the starting point (defining what makes it a benefit to humans) for pharmacologic research. It will be axiomatic that this latter process is essential to the former.

TERRY KENAKIN

Preface

The first edition of this book concentrated primarily on the classification of drugs and drug receptors in functional systems. Over the intervening years the face of pharmacology has changed immensely, and now the systems available to study drugs and drug responses can be customized and, in some cases, even created. The state of the art of pharmacology as a discipline owes much to this new technology. Perhaps because we sometimes fail to fathom the complexities of chemical perturbation of cellular function, drug selectivity can be a mystery, and one interim way to classify selective drugs is to pass the mysterious property on to the cellular recognition site, i.e., the receptor. This edition discusses the procedures available to determine whether selectivity is a function of the receptor or a result of the collective properties in the drug, receptor, and tissue host.

At present, cells can be made to express drug-receptor proteins at will, with the apparent promise of systems in which drug activity can be quantified without complication. The true import of the findings in such systems remains to be seen and may, in fact, depend on the verisimilitude of the surrogate systems to those operative in humans. The verdict on how well the new technology will ease the process of drug design for therapeutic benefit in humans lies in the accuracy of the quantification of drug effect. This edition also concerns the criteria by which drug selectivity can be ascribed to receptor selectivity and the robustness of the resulting classifications in the therapeutic arena. It has relevance to pharmacologists, physiologists, and cell biologists interested in the strengths and limitations of drugs as chemical scalpels used to dissect biological systems.

TERRY KENAKIN

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Drug-Receptor Theory

That combining group of the protoplasmic molecule to which the introduced group is anchored will hereafter be termed receptor.

—PAUL EHRLICH, 1909

DEFINITION OF THE "KEY"

When a medicinal chemist synthesizes a compound that does something extraordinary to a physiological system, this compound enters an elite class of chemicals and becomes classified as a drug. Let us suppose that this drug has one and only one property in its interactions with all physiological systems and thus possesses the property of specificity. Under these circumstances it can be used to perturb a variety of physiological and biological systems, and by observing the way these systems accommodate the perturbations, we can gain physiological knowledge. In short, the drug becomes a "key." It will be one premise of this book that the obvious value of such keys can lead to tacit assumptions of specificity and that the challenge of these assumptions is a critical function of pharmacologists.

Experience demonstrates that drugs more often are selective rather than specific, in that a specific activity is incontrovertibly linked to a concentration range (therapeutic window). Therefore, a drug is useful therapeutically if the dosage is kept within the range in which only the desired property is expressed. If this range is exceeded, other properties of the drug may complicate the therapy; as stated by Walter Straub (1874–1944), "there is only a quantitative difference between a drug and a poison." A drug discovered to possess one property, when subjected to scrutiny, often is found to have another. For instance, cocaine is known mainly as a central nervous system stimulant and local anesthetic, but it also is a potent inhibitor of the uptake of catecholamines by nerves. Hydrocortisone is a well-known anti-inflammatory drug, but it also blocks the ability of muscle to take up and degrade catecholamines. *It is the major thesis of this book that pharmacologists should be concerned primarily with the discovery of and quantification of*

the properties of drugs, not the physiological systems with which they interact—in essence, the definition of the key. This is not a new idea. It was proposed more than a century ago by the German pharmacologist Rudolf Buchheim (1820–1879) in his statement that "we pharmacologists must acquire a knowledge of the tools which we use" (4). Not only pharmacologists should be concerned with the classification of drugs, but also every biological scientist who uses drugs to delineate physiological mechanisms.

There are at least three reasons that pharmacology should be concerned with the definition of the key. First, if specificity is erroneously assumed for a given drug, then all subsequent classifications utilizing this drug are in danger of being irrelevant. Considering the numbers of drugs, receptors, tissues, and species, this could lead to an enormous amount of contradictory data. For example, suppose a new drug is classified as being a stimulant of a particular cellular mechanism on the basis of limited data and then is shown to produce a stimulant response in an organ previously thought, on the basis of other data, not to possess that particular mechanism. An interesting dilemma presents itself: Does one reclassify the drug or reclassify the organ? If an error is made at this point, a second dissimilitude occurs, because all subsequent classifications using either the misclassified drug or organ will be incorrect as well.

The third reason for questioning specificity is the possible therapeutic benefit of the process. Let us assume that a drug has been discovered that produces a specific action *in vivo* and that current classifications of the known receptors cannot explain the specificity attained. One point of view assumes that the drug is a key that has unlocked another secret of physiology; it has led to the discovery of a new receptor. Considering the limited numbers of hormones, autacoids, and neurotransmitters that cells have to deal with, a parsimonious view of membrane receptors would discourage such speculation unless it is inescapable. In the process of defining possible receptor heterogeneity, exploration of other possible properties of the drug that could be responsible for the selectivity would be warranted. Thus, an alternative point of view would seek to explain the selective effect of the drug in terms of a more commonly encountered phenomenon, namely, multiple drug properties. In this setting, the selectivity would result from the drug acting on two or more biological mechanisms that interact in the host (whether this be cell, tissue, or whole body) in a complex manner. The definition of such selectivity theoretically could suggest new and better ways to attain selectivity, because delineation of the various processes would suggest to the synthetic organic chemist new structures for future molecular design. If a drug is selective because it interacts with a newly discovered cellular process, the chemist has a limited data base on which to design analogues of the drug, namely, those structures synthesized en route to the drug. If, on the other hand, the selectivity of the drug is found to result from interaction of the drug with two already known cellular processes, then all other chemical

structures known to interact with these cellular processes become relevant to the drug design process.

DRUG NOMENCLATURE

The label attached to a drug often determines how it is used, and some guidelines to drug nomenclature should be noted before discussion of the properties of drugs. Some ambiguities involved in the utilization of drugs as tools arise from their sometimes protean nature, a property that often can be controlled by limiting the concentration, because it becomes evident when selectivity windows are exceeded. In general, a drug is known for and used for its most prominent property, although it may have other properties. This necessitates a procrustean approach to the attainment of pharmacologic specificity. Like the giant Procrustes in Greek mythology, who made his hapless victims lie on a bed and either stretched or truncated them until they fit the bed,[1] pharmacologists often find themselves truncating the concentration ranges of their drugs in order to achieve some measure of specificity. For example, yohimbine can be thought of as a competitive antagonist of α_2-adrenoceptors, α_1-adrenoceptors, serotonin receptors, and acetylcholinesterase, as well as a local anesthetic (Fig. 1.1A). An even more extreme case of multiple personality is exhibited by amitriptyline, which boasts no fewer than six prominent properties (Fig. 1.1B). However, by using yohimbine within a concentration range of 6 to 60 nM and amitriptyline between 8 and 60 nM, selective α_2-adrenoceptor blockade and histamine H-1 receptor blockade, respectively, can be achieved. Thus, a window of selectivity is obtained by judicious restriction of concentration. An example of how the nature of a drug can change when these windows are exceeded is provided by clonidine, which at concentrations below 30 nM selectively depresses electrically stimulated twitch contraction of the rat anococcygeus muscle (Fig. 1.2). When 30 nM clonidine is added to the organ bath, the selectivity window for this property is exceeded, and postsynaptic stimulation, resulting in a powerful tissue contraction, is observed. In general, the concentration of a drug at which a particular activity is observed is critical to characterization of the event, and selective labels must always be qualified by quantitative limits.

Perhaps nowhere is the protean nature of drugs better revealed than in the variations seen in drug stimulant activities. By definition, a drug that produces stimulation is labeled an agonist, whereas that which blocks the effects of an agonist is an antagonist. The tissue producing the response determines the observed maximal response, and the powerful drugs that produce the same maximal responses in any given tissue are termed full agonists, i.e., they produce the maximal tissue response. Those drugs that produce a re-

[1] Good for Procrustes, until Theseus did the same to him.

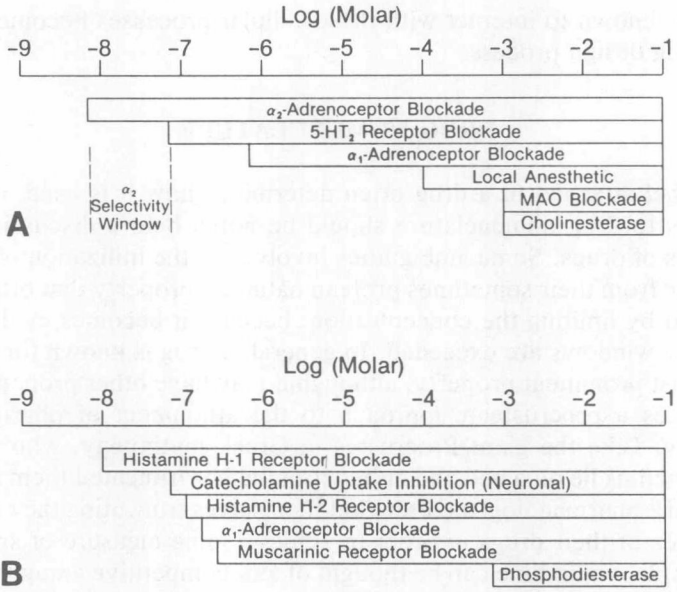

FIG. 1.1. The necessity for a procrustean approach to specificity. **A:** Yohimbine concentration ranges (on a logarithmic molar scale) necessary for activity for a series of autonomic receptors and functions. **B:** Similar data for amitriptyline.

sponse that is less than the tissue maximum are termed partial agonists. Thus, a hazardous nomenclature results that is based on biological tissues with all their variances. Clearly, a sensitive tissue may show a drug to be a full agonist, whereas in a less sensitive tissue the same drug may be a partial agonist. Conceivably, in a rather insensitive tissue, the drug can produce no

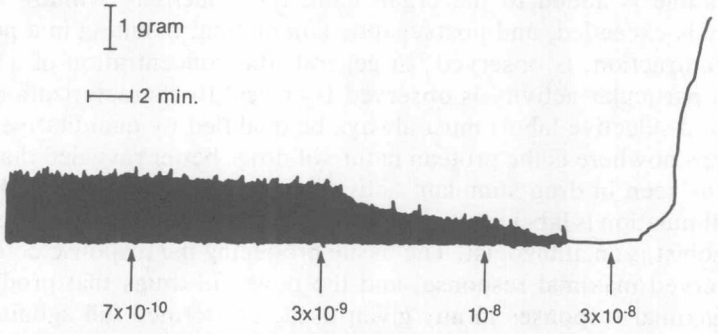

FIG. 1.2. Effects of clonidine on electrically stimulated isometric twitch in rat anococcygeus muscle. Concentrations between 0.7 and 10 nM depress twitch by α_2-adrenoceptor-mediated inhibition of neurotransmission. At a concentration of 30 nM, clonidine produces a sustained contraction by α_1-adrenoceptor activation. (From ref. 19.)

response at all and be an antagonist. For example, prenalterol can be shown to be nearly a full agonist in thyroxine-treated guinea pig right atria, a weak partial agonist in cat papillary muscle, and a pure competitive antagonist in canine coronary artery (Fig. 1.3). Tissues are amplifiers of the effects of drugs and can vary dramatically in their threshold and maximal limits of amplification. To discuss this point further requires definition of the two fundamental properties of drugs: affinity and intrinsic efficacy. The affinity of a drug is the tenacity with which it binds to its biological receptor on the cell membrane. Clark (5), in his classic treatment of drug-receptor theory, discussed affinity as the ability of drugs to be "fixed" to cells. In statistical terms, the affinity is the probability of a drug molecule binding to a free drug receptor at any given instant. The intrinsic efficacy of a drug is that inherent property that imparts the biological signal to the drug receptor (and thus to the cell) to result in a biological response. Intrinsic efficacy is a property of the drug, not the tissue, and is a very important parameter in the classification of drugs and drug receptors (*vide infra*). Thus, the affinity gets the drug to the receptor, and the intrinsic efficacy determines what it does when it gets there. The pharmacologist observes what the tissue does with the signal; it can greatly amplify it to yield a response, or it may deem the signal too weak to bother about and not give a response.

The production of a biological response by a drug can be thought of as the addition of a weight to one side of a lever balance. The weight is the intrinsic efficacy of the drug, an inviolate property. As the weight is added to the lever, the opposite end is displaced in proportion to the weight (Fig. 1.4). Where, along the lever, we view this process determines what displacement we observe. If we equate the magnitude of displacement with maximal response, the different vantage points along the lever represent different tissues. Thus, referring to Fig. 1.4, tissue I would demonstrate very little response for the drug, tissue II would show the drug to be a partial agonist, and tissue III a full agonist. Considering the multitude of tissues and variations in intrinsic efficacies of drugs, it can be seen that the designations full agonist,

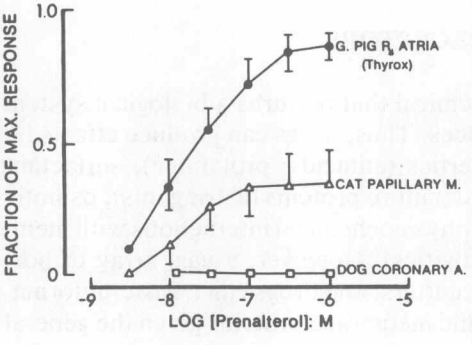

FIG. 1.3. Concentration-response curves in isolated tissues for prenalterol. Ordinate: Responses as fractions of maximal responses to the full agonist isoproterenol. Abscissa: Logarithms of molar concentrations of prenalterol. Responses of right atria from thyroxine-pretreated guinea pigs [(•)N = 4], cat left atria (N = 6), and canine coronary [(•)N = 4]. Bars represent SEM. (Adapted from refs. 16 and 17.)

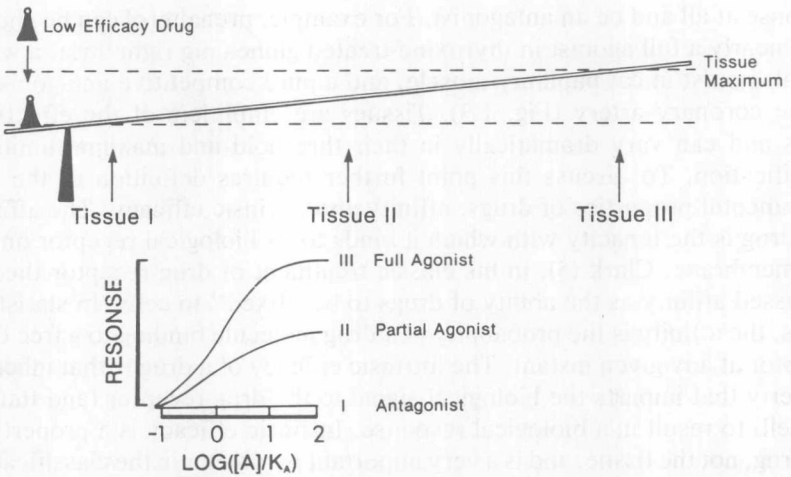

FIG. 1.4. An operational view of intrinsic efficacy. **Top:** The drug is considered a mass with a given weight corresponding to its intrinsic efficacy. The tissue response is considered to be displacement of the lever as a result of placement of the weight to the left of the fulcrum. The downward displacement can be thought of as the stimulus, with the upward displacement the amplified maximal response. Where along the lever this process is viewed determines the amplification factor; these vantage points correspond to different tissues. **Bottom:** The predicted dose-response curves to the drug in tissues I, II, and III, these corresponding to the labeled vantage points.

partial agonist, and antagonist may sometimes be ambiguous. In practice, most drugs have intrinsic efficacies that either greatly exceed tissue limits (thus uniformly are full agonists) or are well below tissue thresholds (antagonists). Therefore, ambiguities arise only with drugs of intermediate intrinsic efficacies (partial agonists).

In summary, it would seem to be prudent to interpret drug labels in terms of the nature of the drug activity and extent of selectivity (or claimed specificity) with some latitude, keeping in mind the dependence of these factors on the nature of the test system and the concentration of the drug.

DRUG RECEPTORS

The definition of a drug as any chemical that perturbs a biological system suggests a broad category of substances. Thus, drugs can produce effects by virtue of their acidic or basic properties (antacids, protamine), surfactant properties (amphotericin), ability to denature proteins (astringents), osmotic properties (laxatives, diuretics), and physicochemical interactions with membrane lipids (general and local anesthetics). However, a vast array of hormones, autacoids, toxins, neurotransmitters, and drugs can transfer information to cells by interaction with specific membrane proteins given the general

name receptors. The concept of specific sites residing on cell membranes with cognitive and transitive properties for drugs emerged at the turn of the century as a result of studies by Ehrlich (1854–1915), through his experiments with tissue stains, snake venoms, and bacterial toxins, and Langley (1852–1926), who studied the effects of pilocarpine and atropine on salivary secretion.

There are certain extraordinary properties of drug actions that invite, if not compel, the postulate of a specific receptor on a cell membrane capable of binding drugs and serving also as a transducer for biological stimuli. First, many drug responses are obtained at very low concentrations. Calculations from studies of atropine binding to guinea pig ileum suggest that only 0.02% of the cell surface is composed of specific receptors for acetylcholine. Thus, if a muscle cell were compared to a sphere the size of the earth, an area the size of Iceland would contain the complete acetylcholine-receptor population. Clark (1885–1941) calculated that the area on a frog heart cell covered by a concentration of acetylcholine sufficient to reduce heart rate by 50% would be 0.001%, or an area the size of the Caribbean island of Jamaica if the heart cell were the size of the heart. Such extraordinary potencies suggest specific receptors linked to amplification processes. Second, responses to drugs can be very selectively blocked by other drugs of specific chemical structures. For example, the antihistaminic drug mepyramine blocks isolated tissue responses to histamine at concentrations of 0.4 nM, whereas concentrations 30,000 times higher (12 μM) are required to block the effects of acetylcholine. Third, the selectivities of drugs as stimulants and antagonists are extremely dependent on chemical structures, and very small changes in the structures of drugs can lead to profound changes in pharmacologic activities. Thus, extension of the methylene side chain of alkyltrimethylammonium salts by one methylene bridge (*n*-propyl to *n*-butyl) changes the potency for stimulation of guinea pig ileum by a factor of 145 (Fig. 1.5A). Addition of a single chlorine atom to pheniramine produces a 10-fold enhancement of potency for antihistaminic activity (Fig. 1.5B). The dependence of activity on structure impressed Clark, who wrote that "the most interesting feature of drug action is the extraordinary specificity of action of drugs and the manner in which slight changes in chemical constitution alter their action." These data, and much recent biochemical data, have led to important operational concepts concerning the receptor, a protein usually embedded in the plasma membrane that serves to recognize drugs and transmit their information to the cell.

In general the membrane-bound receptors currently known can be divided by structural, biochemical, and functional data into families (see Fig. 1.6). One such family consists of G protein–linked receptors, which contain seven membrane-spanning regions (as deduced from hydropathicity data) joining extracellular and intracellular loops of amino acids. These receptors transmit information to the cell by binding to membrane-bound coupling proteins that,

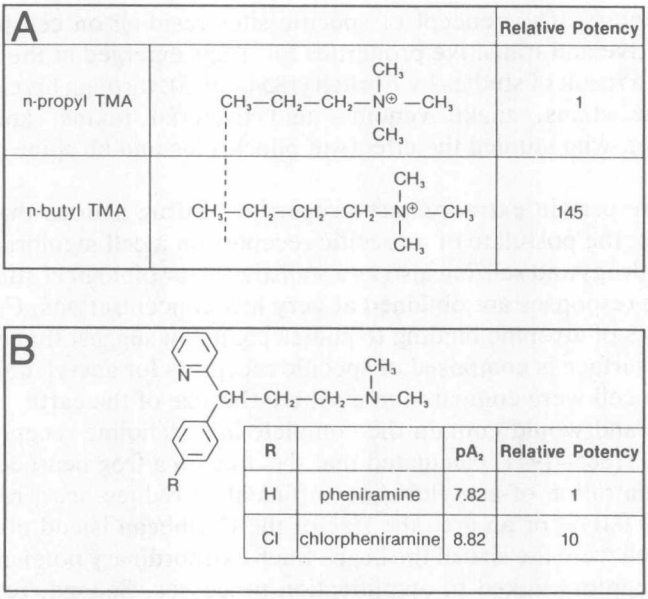

FIG. 1.5. Dependence of drug potency on chemical structure. **A:** Addition of one methylene bridge to *n*-propyltrimethylammonium (to form *n*-butyltrimethylammonium) increases the potency by a factor of 145. Shown are the concentrations producing 50% maximal response in guinea pig ileum. (Data from ref. 25.) **B:** Addition of a single chlorine atom on the pararing position of pheniramine increases antihistaminic potency by a factor of 10. Shown are the pA_2 values minus logarithms of the molar concentrations of antagonist producing a twofold shift to the right in a concentration-response curve to histamine in guinea pig ileum. (Data from ref. 23.)

when activated by agonist-receptor complexes, initiate biochemical cellular processes. These coupling proteins utilize guanine nucleotides and thus are called G proteins. Many receptors for neurotransmitters (i.e., α-adrenoceptors and β-adrenoceptors for catecholamines) and hormones are G protein–linked receptors. Another class of receptor are multisubunit proteins that contain ion channels and thus transmit ions from the extracellular space into the cytosol. For example, nicotinic receptors for acetylcholine allow passage of more than 10^6 sodium ions per second into the cell when activated by cholinergic agonists. Another family of receptor contains enzymatic activity. Insulin, endothelial growth factor, and platelet-derived growth factor are among the substances that utilize receptors linked to tyrosine kinase activity. Yet another group of receptors have membrane-bound guanylate-cyclase enzymatic activity (i.e., atrial natriuretic factor).

The common properties of all of these receptors are the two that are essential for receptors, namely, that of *recognition* of extracellular substances and *transduction* of information of those substances to the intracellular machinery.