Research is essentially a dialogue with Nature. The important thing is not to wonder about Nature’s answer—for she is always honest—but to closely examine your question to her.

A. Szent-Györgi, a paraphrase
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PREFACE

In this, the third edition of *Cell Surface Receptors: A Short Course on Theory and Methods*, I have tried to link theoretical insights into drug-receptor interactions described in mathematical models with the experimental strategies to characterize the biological receptor of interest. I continue to need to express my indebtedness to my earlier tutelage in these areas by Pierre DeMeyts and Andre DeLean, which occurred during my postdoctoral years as a member of Robert J. Lefkowitz’s laboratory at Duke University. Other concepts, particularly classical approaches to defining and characterizing receptors, I learned from Joel G. Hardman while teaching a course together at Vanderbilt School of Medicine on receptor theory and signal transduction mechanisms. My national colleagues also have been terrific teachers, including Terry Kenakin (Glaxo Smith Kline), Harvey Motulsky (GraphPad Software, Inc.) and Rick Neubig (University of Michigan). In the end, of course, the motivation of preparing such a text is for the students, whose contagious enthusiasm encourages efforts to meet their needs. I hope this text is of value to investigators—at whatever stage of their career they find themselves—who want to identify, characterize and understand the biology of a receptor of interest.

I prepared this revision just prior to taking a sabbatical from Vanderbilt University. Vanderbilt has been a wonderfully supportive and intellectually stimulating place to work and to continue to learn. I am grateful to Eric Woodiwiss, for his technical support in preparing the manuscript and the figures, and to Harold Olivey, Ph.D., a former student in my courses at Vanderbilt, who read and thoughtfully critiqued the text. Without their help, I suspect this edition would not have materialized from draft to completion.

The study of receptors has changed considerably over the period of the publication of the three editions of this book. The cloning of several genomes makes it unlikely that preparations of receptors now or in the future will arise from their purification as trace proteins from native tissues, but rather from a myriad of molecular approaches. Nonetheless, understanding the molecular mechanisms and ultimately the *in vivo* biology of these receptors means that investigators will engage in molecular, cellular and ultimate in vivo strategies. To work across this continuum means that we must be forever grateful to the remarkable insights of those early describers of receptor theory and the criteria expected for biologically relevant receptors. We are the beneficiaries of their genius, simply fleshing out a skeleton, a conceptual framework, that preceded us by decades.
A good question is never answered. It is not a bolt to be tightened into place but a seed to be planted and bear more seed toward the hope of greening the landscape of idea. *John Ciardi*
1. INTRODUCTION TO RECEPTOR THEORY

Much of the conceptual framework regarding how to study receptor function evolved from pharmacological investigation of drug action. Consequently, the historical account of the development of receptor theory in this chapter will emphasize early investigations of drug action rather than (for example) physiological studies of hormone action. However, the reader must keep in mind that the term drug can be defined as any chemical agent that affects living processes. Drugs bind to receptors presumably designed for interaction with endogenous hormones and neurotransmitters or other regulatory agents. **Agonist** drugs are analogous to endogenous hormones and neurotransmitters in the sense that they elicit a biological effect, although the effect elicited may be stimulatory or inhibitory. Different agonists activate receptors along a continuum of effectiveness; those which induce or stabilize less productive conformations are termed **partial agonists**, a property which will be discussed in considerable detail later in this chapter. In contrast, **antagonist** drugs are defined as agents that block receptor-mediated effects elicited by hormones, neurotransmitters, or agonist drugs by competing for receptor occupancy. Antagonists, as initially defined, were competitive inhibitors of receptor occupancy by agonists, having no intrinsic activity in their own right. However, more recently, antagonist agents have been observed to have negative intrinsic activity, or behave as **inverse agonists**, and decrease
“basal” (agonist-independent, or constitutive) receptor activity. Still other antagonists of function mediate their effects by interacting with another, allosteric, site rather than in the binding pocket of the native agonist (defined as the orthosteric site) (Christopoulos and Kenakin [2002]; Kenakin [2004]; Neubig et al. [2003]). The properties of agents that interact via the orthosteric binding sites of the receptors are shown schematically in figure 1-1.

**Figure 1-1.** Schematic representation of the functional consequences of the binding of drugs at the site of binding of endogenous ligands (the orthosteric site). Agents which activate the receptor are agonists, and can elicit fully efficacious or partially efficacious (partial agonist) properties. *Partial agonists can either elicit a full response, but with lower efficiency or efficacy than full agonists, or, as shown in this schematic, elicit a submaximal response compared to a full agonist, even when fully occupying the receptor population. The properties of partial agonists and the theories that describe their behavior are considered in detail in later sections of the chapter. Classic, or null, antagonists occupy the agonist binding pocket and block receptor-mediated function by blocking agonist occupancy and subsequent agonist-elicited responses. Inverse agonists, or negative antagonists, stabilize inactive receptor conformations and decrease “basal” receptor activation in a dose-dependent manner.

**ORIGIN OF THE RECEPTOR CONCEPT**

Contemporary scientists take it as a “given” that biological substances such as hormones and drugs elicit their effects via interaction with specific receptors in a manner analogous to the interaction of substrates with enzymes. This
dogma was not always self-evident, but evolved from the remarkable insights of early scientists exploring a number of fundamental living processes.

Although Claude Bernard (1813-1878) never used the term receptor, he pioneered a pattern of scientific investigation that permitted clarification of the specificity and selectivity of drug action, particularly in regard to the locus of a drug effect. Bernard had a very unpretentious question: he simply wanted to know how the arrow poison curare worked. It was effective when "administered" by an arrow but, interestingly (at least to Bernard), was ineffective when taken by mouth. His early studies explained the importance of the route of administration of this drug for its lethal effects by demonstrating that although curare was unaltered functionally by saliva, gastric juice, bile, or pancreatic juice, it was not absorbed by the gastrointestinal tract, thus accounting for its harmlessness when swallowed. Bernard then wanted to understand just how curare effected its lethal paralysis. It was his impression from general observations that curare did not affect the sensory nerves, but instead altered motor nerve function. By an ingenious group of experiments, he determined that curare blocked the ability of motor nerves to control muscular contraction. Bernard noticed that, after injecting curare under the skin on the back of the frog, the frog showed progressively fewer reflex movements. If he skinned the hind legs of the frog that had been exposed to curare and isolated the lumbar nerve, he could produce no contraction of the leg muscles by stimulating the nerve electrically, whereas he could produce violent contractions if the same electrical stimulus were applied directly to the muscle. Bernard concluded from these experiments that muscle contractility is distinct from the nervous system that produces it and that curare removes the neural control of muscular function (cf. Bernard [1856]).

Bernard did not talk about receptors per se, but he did demonstrate that the ability of a drug to elicit its effects depends on its access to a particular location. As a result of his findings, Bernard encouraged investigators not to focus studies of drugs on organs but on organ systems, for example, the nervous system or the muscular system. Similarly, he believed that the mechanism of drug toxicity would be better elucidated by focusing on the drug-mediated death of these organ systems, rather than on the death of the organ itself. His own experiments revealed the existence of a neuromuscular "junction" prior to the demonstration of the muscular endplate as a discrete anatomic structure.

It may have been a physicist, rather than a physician or biological scientist, who first provided evidence for molecular interactions between two substances that had physiological consequences; Stokes (1864) observed that spectral changes occurred when oxygen was removed from, or subsequently reintroduced to, blood, implicating a complex between oxygen and hemoglobin. However, the biological concept of receptors is generally
attributed to Paul Erhlich (1854-1915), although the word receptor (receptive substance) was coined by one of Erhlich’s contemporaries, J. N. Langley. Erhlich was a remarkable individual whose scientific career spanned (and even spawned) several biomedical disciplines. One overriding principle was common to all of Erhlich’s investigative endeavors, and that was selectivity.

Erhlich’s earliest work involved the distribution of lead in the body, particularly its preferential accumulation in the central nervous system. He had been inspired by a publication of Heubel on lead poisoning, which demonstrated that there were significant differences in the amount of lead found in various organs of animals that had succumbed to lead poisoning. When Heubel exposed the isolated organs of normal animals to dilute solutions of lead, the organs demonstrated the same differential uptake of lead as had been noted in vivo. In Erhlich’s continuation of these studies, he realized that it was impossible to use a microscope to determine the basis for this differential selectivity of lead uptake in different tissues. Consequently, he changed his experiments to investigate the differential staining of tissues by dyes, as this could be easily detected. He continued to pursue the question of the basis for selectivity, from a more general standpoint. Erhlich’s studies on dye distribution originated the concept of “vital staining,” and his morphological distinction of leukocytes as acidophilic, basophilic, neutrophilic, or non-granular (based on the relative uptake of dyes of varying chemical constitution) is still in practice today. It was Erhlich’s impression that although staining of dead tissue gave information regarding its anatomical structure, the staining of live tissue (i.e., “vital staining”) provided insight into the properties and functions of living cells.

Erhlich’s most acclaimed studies were his subsequent experiments in immunochemistry, cited as the basis for the Nobel Prize in Medicine awarded to him in 1908. By neutralizing the activity of toxins following incubation of toxins with anti-toxins in a test tube, Erhlich demonstrated that antigen-antibody interactions are direct chemical encounters and not generalized phenomena requiring the biological processes ongoing in a whole animal. From these observations Erhlich developed his “side chain theory” to explain the chemical basis for the immune response. He described the antigen as possessing two active areas: the haptophore (which functioned as the anchorer) and the toxophile (which functioned as the poiser). He postulated that mammalian cells possess “side chains” that are complementary to certain chemical groups on the haptophore domain of the antigen, and thus serve as the basis for “anchoring” the antigen to the cell. This side chain-haptophore interaction thus gives the “toxophile” portion of the antigen access to cells that possess the appropriate side chains. Pictures reproduced from Erhlich’s original notebooks show the side chains drawn with -NH₂ and -SH moieties, thus underscoring his assertion that the basis for these selective interactions between antigen and antibody was a chemical one. Quite clearly, his side
chain theory also could explain earlier observations concerning the preferential uptake of lead into the central nervous system and the principle governing vital staining of living cells. Erhlich conjectured that the normal function of cellular side chains was the binding of cell nutrients, and that the affinity of toxic substances for these groups was the fortuitous analogy between the structure of the exogenous toxic substance and the endogenous nutrient. Inherent in Erhlich's side chain theory was the burgeoning concept of specific cell surface receptors as the basis for targeting bioactive agents to the appropriate cell for response.

Erhlich turned his attention from large molecules, such as toxins, to low molecular weight molecules in a series of investigations that earned him recognition as the “father of chemotherapy” (see Albert [1979]). He believed that since the pharmaceutical industry could produce a number of small molecules (e.g., analgesics, antipyretics, and anesthetics) which appeared, at least functionally, to differentiate among various tissues in human beings, it also should be possible to design small molecules that differentiated between human beings and parasites (Erhlich [1913]). His initial studies pursuing this postulate shifted from the protozoan (Trypanosoma) to the bacterium (Treponema) when Hata showed that the latter organism could produce syphilis in rabbits. Thus, with a model system allowing more detailed studies of chemotherapeutic principles, Erhlich invited Hata to leave Tokyo and join him as a colleague in Frankfurt. Erhlich realized that a particular organism (i.e., Trypanosoma versus Treponema) was not critical for furthering his studies, because the basis of his experiments on differentiating host from parasite relied only on a general principle: that the parasite, as an incessantly motile organism, had a higher rate of metabolism than its host and presumably would be differentially sensitive to the toxic effects of arsenicals. Erhlich’s work with a family of arsenical compounds revealed that agents were never entirely specific for the parasite (i.e., he never found his “magic bullet”) and, at increasing concentrations, all agents studied had deleterious effects on the host. As a result of this finding, he introduced the term chemotherapeutic index, which he defined as the ratio of the minimal curative dose to the maximal tolerated dose. Second, Erhlich maintained that the haptophobic and toxophilic principles that guided immunochemistry also pertained in chemotherapy. Thus, he believed that small molecules also possessed distinct domains for binding to the target cell versus taking part in cellular nutrition or respiration. His own studies established that the arsenoxide group of arsenicals was essential for the lethal effect of these agents and that the chemical substituents on the arsenoxide group were responsible for uptake of the agent. The need first to “bind” the arsenical explained the basis for resistance to arsenicals by particular strains of trypanosomes, i.e., these strains were unable to recognize certain substituents on the phenyl ring attached to the arsenic.
All of Erhlich’s studies on the basis of selectivity often are distilled into his often-quoted dictum, *corpora non agunt nisi fixata* (agents cannot act unless they are bound). Consequently, Erhlich’s own advice regarding the pursuit of chemotherapeutic agents was to focus on the haptophore group, as it was the *conditio sine qua non* for therapeutic action.

J. N. Langley (1852-1926), of Cambridge University, was a contemporary of Erhlich who studied the chemical basis for autonomic transmission and neuromuscular communication. Langley extended Bernard’s studies, which identified curare as a blocker of neuromuscular transmission, by demonstrating that curare also blocked chemical stimulation of the frog gastrocnemius muscle by nicotine, even after severance and degeneration of its motor nerves. However, even under curare “blockade” direct electrical stimulation of denervated muscle could elicit contraction. The mutually antagonistic effects of curare and nicotine, as well as the ability of direct electrical stimulation of the muscle to bypass the effects of curare, led Langley to conclude that nicotine and curare act on the same substance, which is neither nerve nor muscle. Langley called this postulated substance the “receptive substance” (Langley [1909]). The concept of mutual antagonism implying a common site of action was noted by Langley as well as by other contemporaries (e.g., Luchsinger in 1877 and after) for the effect of pilocarpine (agonist) and atropine (antagonist) on contraction of the heart (1909) and on secretion of saliva from the submaxillary gland of the dog (1878). Luchsinger was the first to apply the term “mutual antagonism” to the observed counter-regulatory effects. (See Langley [1878] for a translation from the German of Luchsinger’s results and interpretations.) However, Langley emphasized that mutual antagonism depended on the relative concentrations of drugs added and that it had limits. For example, he observed that if he applied extremely large doses of pilocarpine to the artery of the submaxillary gland, secretion was blocked, i.e., pilocarpine could be made to mimic the physiological effect of atropine. Langley also realized that limits to mutual antagonism might be dictated not only by the properties of the receptive substance but also by other secondary effects of the drugs, such as drug-elicited changes in blood flow.

In summarizing his experimental findings, Langley concluded that the effects of the drugs he had observed could reasonably be assumed to result from the existence of some substance(s) in the nerve endings or glands with which both atropine and pilocarpine are capable of forming “compounds.” He further postulated that these compounds (complexes) are formed according to some law by which the relative concentration of the drugs and their affinity for the receptive substance are critical factors. Thus, Langley first stated the concept of drug-receptor interaction and predated the algebraic description of these interactions as a consequence of mass action law. Langley observed that the height of the contraction elicited as a result of nicotine interacting with a
receptive substance depends on the rate of combination of nicotine with this substance as well as the duration of the resulting contraction, and that "saturable" effects on contractility could be observed. Langley actually postulated that if the combination of nicotine with the receptive substance were slow enough and the duration of contraction brief enough, a complete saturation of the receptive substance might occur without eliciting a visible contraction (Langley [1909]).

Despite persuasive evidence that receptors that are specific for particular drugs or endogenous substances do exist and thus determine the selectivity of biological responses to these agents, not all contemporaries or successors of Erhlich and Langley concurred. H. H. Dale (1875-1968) believed that the differential effectiveness of adrenaline analogs in mimicking sympathetic functions in varying tissues could be due to a chemical process, and did not necessarily imply the existence of specific chemical receptors on target tissues. He stated in 1910 that it was equally probable that the limiting factor determining the selective response to various substances might be the ease with which those substances reached their site of action. Thus, he appeared to favor the distributive rather than the interactive properties of a drug in determining its target cell selectivity, although Dale himself acknowledged that his own results could provide no decisive evidence one way or the other (cf. Dale [1914]).

**OCCUPANCY THEORY**

A. J. Clark (1885-1941) introduced a more quantitative approach to the description of receptor selectivity and saturability (Clark [1926a,b]). Based on his studies of antagonism between acetylcholine and atropine in a variety of muscle preparations, Clark postulated that drugs combine with their receptors at a rate dependent on the concentration of drug and receptor, and that the resulting drug-receptor complex breaks down at a rate proportional to the number of complexes formed (Clark [1927]). This statement implied that drug-receptor interactions obey the principles of mass action and thus could be described by the same isotherms used by Langmuir to describe adsorption of gases onto metal surfaces. Based on Clark's principles, a mathematical expression can be provided to describe drug-receptor interactions:

\[
\text{rate of combination} = k_1 A (1 - Y) \quad (1.1)
\]
\[
\text{rate of dissociation} = k_2 Y \quad (1.2)
\]

where \( k_1 = \text{rate constant for combination} \)
\( k_2 = \text{rate constant for dissociation} \)
As will be described in further detail in chapter 2, J. H. Gaddum later extended this mathematical relationship to describe and analyze the competitive antagonism between adrenaline and ergotamine in the rabbit uterus (Gaddum [1926, 1937, 1957]).

At equilibrium, the rate of combination equals the rate of dissociation:

\[ k_1A(1 - Y) = k_2Y \]

and

\[ \frac{k_1}{k_2} = \frac{Y}{A(1 - Y)} \]

defining \( K \), the equilibrium association constant, as \( k_1/k_2 \), and rearranging the above relationship yields

\[ Y = \frac{KA}{1 + KA} \quad (1.3) \]

Equation 1.3 relates the concentration of drug applied, \( A \), to the proportion of receptors occupied by the drug at equilibrium, \( Y \). This algebraic relationship describing fractional receptor occupancy as a function of drug concentration is analogous to the quantitative relationships between enzyme and substrate introduced by Michaelis and Menten.

**RELATIONSHIP BETWEEN OCCUPANCY AND RESPONSE**

A. J. Clark extended his hypothesis about the relationship between occupancy and response by postulating that the fraction of receptors occupied, \( Y \), was directly proportional to the response of the tissue. To substantiate this postulate, Clark provided evidence from his studies on acetylcholine-induced contraction of isolated frog *rectus abdominis* muscle and acetylcholine-inhibited contraction of electrically stimulated frog ventricular muscle. If receptor occupancy correlated linearly with receptor-mediated response, then the above equations made certain predictions of what would be expected for the slope of log concentration-response relationships.
Since \( K = \frac{Y}{A(1-Y)} \), then rearrangement yields

\[
A = \frac{Y}{(1-Y) \cdot K}
\]

and predictions could be made about the ratio of drug concentrations eliciting \( x\% \) versus \( y\% \) of response. For example, Clark often compared the ratio of [drug] eliciting 16\% versus 84\% of a maximal response. If the fraction of receptors occupied correlates directly with the maximal response elicited, then the ratio of drug concentration eliciting 16\% versus 84\% of maximal response should be around 28 fold, as shown algebraically below:

\[
\frac{A_{84}}{A_{16}} = \frac{.84}{.16K} = \frac{.16}{.84K} = 28
\]

Although some early data of Clark and others describing concentration-response relationships in various contractile systems were consistent with the postulate that the fraction of receptors occupied (implied to be equivalent to the dose of drug added) correlated directly with the fractional response elicited, certain data conflicted with this straightforward relationship between occupancy and effect. First, the slope of the concentration-response relationships reported often was steeper (although sometimes shallower) than predicted from equation 1.3. Second, a number of examples existed in which application of supramaximal concentrations of stimulatory agents did not elicit a maximal contractile response. The latter findings suggest that even saturating occupancy of a receptor population by certain agonist agents might not necessarily elicit a maximal physiological effect (Clark [1937]).

Comparing dose-response relationships for a homologous series of drug analogs often revealed that some agents in the series failed to elicit the same maximal effect, even at supramaximal concentrations. Raventos and Clark (1937) and later Ariëns (1954), Stephenson (1956) and others observed that a dualism of behavior was noted for compounds in a homologous series of quaternary ammonium salts in a variety of muscle preparations. These salts had the basic structure:

\[
(CH_3)_3 \hat{N} - R
\]
When the substituent, $R$, was butyl or corresponded to lower members of the series, a maximal muscle contraction was elicited. In contrast, only a weak contraction could be elicited by hexyl and heptyl analogs. Furthermore, the hexyl and heptyl analogs behaved as antagonists when applied to the muscle simultaneously with the butyltrimethylammonium compound.

Ariëns found a similar dualistic behavior of phenylethylamines (chemically related to epinephrine) in elevating blood pressure in decapitated cats. Ariëns drew attention to this enigma: How can a substance which is postulated to interact with a single receptor nonetheless elicit both agonistic and antagonistic effects? He introduced the term intrinsic activity to describe the ability of an agent to elicit its pharmacological effect. He expressed the relationship between the agonist effect ($E_A$) elicited by drug $D$ and the concentration of drug-receptor complexes ($DR$) as:

$$E_A = \alpha[DR]$$

(1.4)

and defined $\alpha$ as the “proportionality constant” or “intrinsic activity” of the particular drug, where intrinsic activity was meant to be a constant determining the effect elicited per unit of $DR$ complex formed. Ariëns still did not alter the fundamental principles of A. J. Clark in his initial definition of intrinsic activity. The maximal effect of a given drug still required occupancy of the entire receptor population. The only nuance was that some drugs, even at maximal occupancy, might elicit a biological effect less than that considered to be “maximal” for the system under study. Consequently, this early definition of intrinsic activity proposed by Ariëns addressed the anomalous observation that apparently maximal receptor occupancy by some agonists did not elicit a maximal response. However, this conceptualization still could not explain dose-response relationships that were steeper than predicted by mass action law.

R. P. Stephenson (1956) introduced a major conceptual advance in understanding the quantitative relationship between receptor occupancy and receptor-elicited effects. Stephenson argued that even A. J. Clark’s own experimental findings were not in accord with a linear relationship between occupancy and effect. Stephenson concurred that equation 1.3 ($y = \frac{KA}{1 + KA}$) is the probable relationship between the concentration of drug introduced and the concentration of drug-receptor complexes formed. However, Stephenson argued that there was no experimental justification for extending this relationship by supposing that equation 1.3 describes a general relationship between the concentration of drug added and the response of the tissue. R. F. Furchgott (1955, 1964) also emphasized that a non-proportionality between occupancy and response was commonly observed. When Stephenson
tabulated the slopes of concentration-response curves already reported in the literature, he observed that these slopes typically were steeper than would be predicted if the percentage of maximal response elicited were to correspond directly to the percentage of receptors occupied.

Stephenson (1956) postulated three principles governing receptor-mediated functions that could explain the previous anomalous observation that agonist-response curves often were steeper than the dose-response relationships predicted by simple mass action law. In addition, Stephenson offered an explanation for the observed progressive variation in the agonistic properties of a homologous series of drugs.

1. A maximum effect can be produced by an agonist when occupying only a small proportion of the receptors.
2. The response is not linearly proportional to the number of receptors occupied.
3. Different drugs may have varying capacities to initiate a response and consequently occupy different proportions of the receptors when producing equal responses. This property is referred to as the efficacy of the drug. In this setting, a pure competitive antagonist would have zero efficacy.

Stephenson described the relationship between occupancy and response as follows:

\[ S = \text{stimulus given to the tissue} \]
\[ S = e \cdot y \]

where \( e \) = efficacy
\( y \) = fractional receptor occupancy
\( R \) = response of a tissue and \( R = f(S) \)

indicating that the response is some function (albeit quantitatively unknown) of stimulus \( S \).

If \( S = e \cdot y \), then, by mass action law (cf. equation 1.3),

\[ S = \frac{eKA}{1 + KA} \]  

(1.5)

Stephenson stated that for an "active agonist," i.e., one with high efficacy and having to occupy only a small portion of the receptors to elicit a maximal
response, $KA$ would be small relative to 1. In this situation, equation 1.5 reduces to:

\[ S = eKA \]  

(1.6)

This definition of efficacy is distinct from that originally proposed by Ariëns. However, Ariëns later changed his definition of intrinsic activity to one formally equivalent to this efficacy term of Stephenson (Van Rossum and Ariëns [1962]).

To test the validity of his postulates regarding various efficacies for different agonists, Stephenson carried out two separate lines of investigation. First, he evaluated the concentration-response for the "full agonists" (which he called "active agonists") acetylcholine and histamine in eliciting contraction of the guinea pig ileum. Stephenson quantitated these data based on the ratios of drug concentrations needed to elicit certain graded responses. Based on Clark's hypothesis, for example, the ratios of the concentration of agonist eliciting 80% versus 20% contraction should be 16 (see earlier algebraic determination of these concentration ratios), and those for 20% versus 50% and 50% versus 80% contraction should be 4. Stephenson noted that the values he obtained were considerably less than the predicted values of 4, and noted this same discrepancy when he calculated agonist ratios from contractile data already published in the literature. (An exception was the concentration-response relationship of adrenaline for contracting rabbit aorta strips published by Furchgott and Bhadrakom in 1953.) Stephenson thus concluded that many agonists elicit a far greater contractile response than would be predicted based on the extent of receptor occupancy.

In a second series of experiments, Stephenson studied the series of alkyltrimethylammonium salts, introduced by Raventos and Clark, on contraction of the guinea pig ileum. He noted that the lower homologs (e.g., butyltrimethylammonium) behaved like acetylcholine, an agonist, whereas higher homologs acted like atropine, an antagonist. He interpreted this antagonism as a property expected for a drug with low efficacy. Thus, the drug produces a response much lower than maximal even when occupying all or nearly all of the receptors. However, because a drug with low efficacy can nonetheless occupy the receptors, it decreases the response elicited by a drug with high efficacy when added simultaneously. Stephenson termed these low-efficacy drugs "partial agonists" because they possessed properties intermediate between agonists and antagonists. (These partial agonists are what Ariëns [1954] referred to as drugs with a dualism of action or mixed agonists/antagonists.) The ability of partial agonists to antagonize agonist effects formed a basis for determining the affinity of partial agonists for the receptor. This methodology will be described in further detail in chapter 2.
CONCEPT OF SPARE RECEPTORS

The finding that some agonists could elicit maximal physiological effects by occupying only a small fraction of the total receptor population suggested that there were “spare receptors.” Avraim Goldstein (1974) offered a tenable teleological explanation for such a phenomenon. In circumstances where the desired response must be rapid in onset and in termination (as in neurotransmission), a spare receptor capacity provides a mechanism for obtaining a response at a very low concentration of an agonist that nonetheless has a relatively low affinity for the receptor. Sensitivity to low drug concentrations is achieved by the spare receptor capacity. The low affinity (i.e., low $K_A$) of the drug assures its more rapid rate of dissociation, since $K_A = k_1/k_2$. Alternatively, if sensitivity to low concentrations of agonist were achieved by a high affinity of the drug for the receptor, then the rate of reversal of the effect would necessarily be slow.

Documentation of the existence of spare receptors, however, came not from studies of agonist concentration-response profiles but instead from studies of receptor antagonism. Several examples of so-called anomalous antagonism had been described that simply could not be explained by A. J. Clark’s hypotheses or by the equations describing simple competitive antagonism introduced by Gaddum. To evaluate the nature of a particular drug’s antagonistic effects, agonist concentration-response curves were obtained in the presence of increasing concentrations of the antagonist. A rightward parallel shift of these curves was consistent with reversible competitive antagonism, and estimates of receptor affinity for the antagonist could be obtained by the method of Schild (see chapter 2) or by Lineweaver-Burk plots, as had been popularized in enzyme kinetic studies. However, as pointed out by M. Nickerson (and other contemporaries who obtained similar findings in other systems), one occasionally could obtain evidence consistent with reversible competitive antagonism when other data nonetheless suggested that reversible competitive interactions were not a likely explanation for the nature of the antagonism (see Furchgott [1955]). For example, β-haloalkylamines, such as dibenamine, were known to block histamine and catecholamine receptors irreversibly, since blockade of contraction by β-haloalkylamines never could be reversed despite extensive washing of the isolated tissue preparation. Except at higher concentrations of these antagonists, however, data for the blockade of histamine-induced contractions resembled that expected for reversible, competitive antagonism: a shift to the right of the agonist concentration-response curve with no change in the slope of the curve or the maximal effect elicited. Only at high concentrations of β-haloalkylamines was a decrease in both the slope and
maximal effect of the agonist finally detected for histamine-induced effects. Nickerson is credited with explaining these anomalous antagonisms by demonstrating that receptor occupancy is not necessarily the limiting factor in tissue activation, i.e., that spare receptors exist. As an example, Nickerson demonstrated in 1956 that occupancy of only 1% of the histamine receptor population in guinea pig ileum was required to elicit maximal contractile effects, suggesting the existence of a large receptor reserve for histamine receptors in this tissue. Receptor reserves were not always so dramatic, however. Furchgott (1955) noted that for epinephrine there was only a shift of half a log unit, if anything, before a decrease in maximal response was observed following β-haloalkylamine exposure.

The impact of receptor reserve, or “spare receptors,” on the sensitivity of a system to agonist is most readily (and dramatically) revealed in heterologous receptor systems where receptor density can be controlled in a straightforward fashion. Here, increases in receptor expression often are noted to be paralleled by a decrease in the concentration of agonist eliciting 50% of maximal response, defined as EC$_{50}$ (e.g. Whaley et al. [1994]). As predicted by receptor theory, the efficacy of partial agonists also is increased as receptor density is increased (Tan et al. [2003]).

OPERATIONAL MODELS OF PHARMACOLOGICAL AGONISM

Black and Leff (1983) developed a mathematical model, dubbed the operational model for agonism, in an effort to provide quantitative descriptors for the frequently observed nonlinear relationship between occupancy and response, or effect (cf. figure 1-2). This model assumes that agonist $A$ binds to receptors $R$ in a bimolecular reaction obeying mass action law, such that:

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

(1.7)

where $R_o$=total receptor concentration

$K_A$=equilibrium association constant M$^{-1}$, the reciprocal of which defines affinity.

The relationship given in equation 1.7 takes the form of a rectangular hyperbola: $y = mx/(a + b)$. Thus, a plot of $[A]$ on the $x$ axis versus $[AR]$ on the $y$ axis will resemble a rectangular hyperbola.