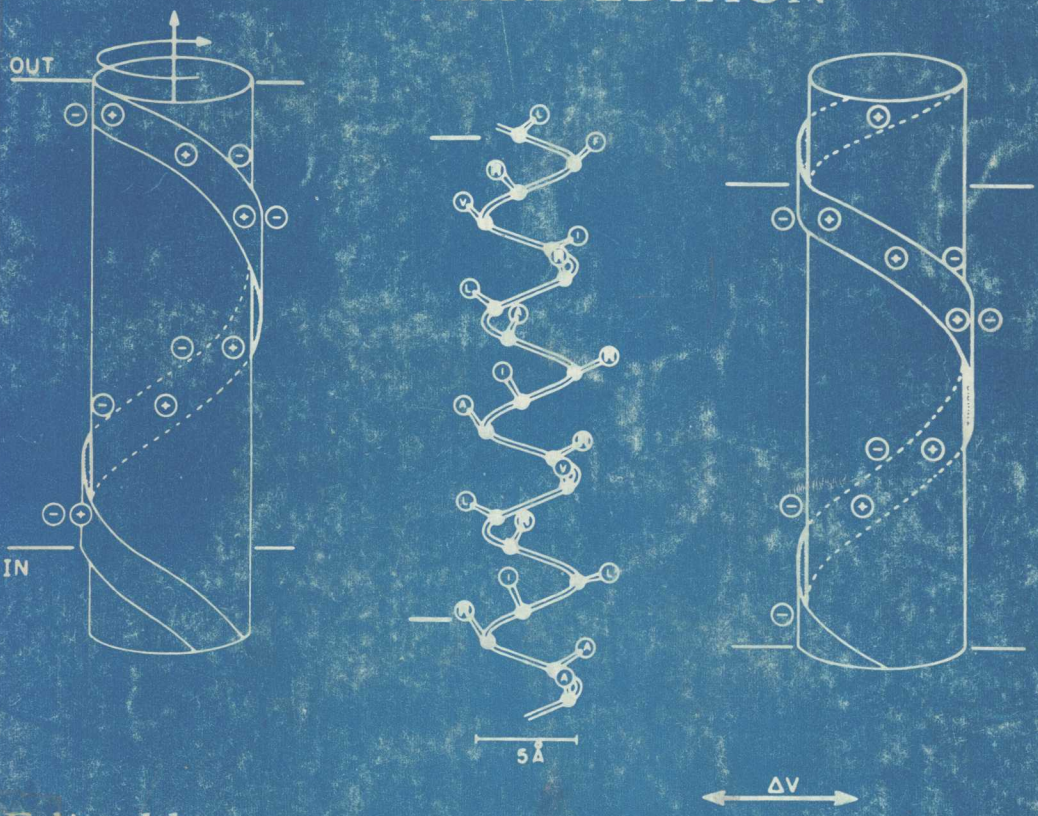
PRINCIPLES OF DRUG ACTION

The Basis of Pharmacology

THIRD EDITION



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To Avram Goldstein,
who developed many of the principles
of drug action presented herein

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Preface

In the 16 years since the publication of the second edition of *Principles of Drug Action*, there have been tremendous advances in understanding drug action. A textbook devoted to basic principles and major research directions within pharmacology is even more essential for the student of the pharmacologic sciences today. Often pharmacologic texts are organized around organ systems, the practice of clinical medicine, or classes of drugs; this approach makes it difficult to identify the chemical and biologic concepts that underlie pharmacology and are essential to understanding this science. We hope this book will serve to define pharmacology as a continually evolving discipline and emphasize its important place within the biomedical sciences.

In undertaking this revision, our aim was to present current concepts of pharmacology in an easily used textbook form. To achieve the goal of comprehensively presenting the basic principles without substantially increasing the length of the text, we deleted the chapters on drug toxicity, drug development, and drug evaluation. In their place, we added new chapters that further elucidate fundamental concepts in pharmacology. This edition begins with two new chapters dealing with chemical and physical bases of pharmacologic specificity and a review of the molecular basis of drug action for the various classes of receptors and other sites of drug action. This section is followed by a chapter on drug absorption, distribution, and elimination and one on the time course of drug action. These two chapters update the only substantial portion of the text that was retained from the previous edition. A chapter on the principles and pathways of drug metabolism is followed by another entirely new chapter reviewing recent advances in defining the structures of the drug-metabolizing enzymes and their regulation. These advances have been spurred on by the new techniques of molecular biology. Other chapters on pharmacogenetics, drug resistance, carcinogenesis, and drug tolerance and physical dependence also reflect the application of new technology to these areas of pharmacology. In all, we estimate that about 70 percent of the material in this edition is new; the remaining 30 percent covers basic concepts that are unlikely to change substantially as our knowledge of drug action expands.

The breadth of modern pharmacology does not allow one pharmacologist to be an expert in all areas of the discipline. To make this edition as authoritative and accurate as previous editions, contributing authors were selected to provide expertise in each subject. To achieve an integrated perspective of the previous editions, we made a concerted effort to edit the chapters to achieve a consistency in scientific content and style. Throughout the text, we have followed the first two editions in illustrating principles with specific examples that do not require any specialized knowledge of pharmacology on the part of

the reader. The framework of pharmacology is presented in such a way that scientists in any branch of biology, chemistry, or medicine will find it readable and will be able to understand those factors that determine drug action.

We thank the original authors, Avram Goldstein, Lewis Aronow, and Sumner Kalman, for their support in publishing this third edition. We hope that their high standards of accuracy, scientific sophistication, and writing style have been maintained.

William B. Pratt, M.D.
Palmer Taylor, Ph.D.

Contents

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Molecular Basis of Pharmacologic Selectivity

Palmer Taylor
Paul A. Insel

THE LIGAND-MACROMOLECULE COMPLEX

The common event in the initiation of pharmacologic responses is the formation of a complex between the ligand, or drug, molecule and its site of action. Since most pharmacologic responses are mediated through *receptors*, recognition of the more mobile drug molecules by the cellular receptor is the critical element determining the specificity of the response. Moreover, these same considerations extend to chemical neurotransmission and to responses to hormones and other mediators within the body. Thus, neurotransmitters, hormones, other extracellular signals, and many intracellular mediators initiate cellular responses by forming complexes with receptors. In an even broader perspective, complex formation is common to many fields of the biologic sciences, and it is only when we consider the subsequent fate of the complex that the individual fields diverge. For example, an enzymologist views complex formation (e.g., formation of the Michaelis complex between enzyme and substrate) as an event leading to a reaction product via a change in the covalent structure of the substrate, while an immunologist looks at the formation of an antigen-antibody complex as an event that initiates sequelae such as antigen ingestion by macrophages or disruption of a cell surface. In classical pharmacology, drug-macromolecule complex formation is linked typically to a contractile, hemodynamic, or secretory event; however, in recent years the capacity to identify intracellular mediators, to monitor complex formation on cell surfaces, and to detect voltage changes in individual cells has enabled investigators to examine events more proximal to complex formation than the physiologic response. An essential goal of pharmacologic research is to identify intermediate steps in the response and to obtain a quantitative understanding of the linkage between drug-receptor complex formation and the ultimate functional response of the cell or organ. Details of such coupling mechanisms will be developed in Chapter 2.

Intrinsic to complex formation is the ability of the macromolecule to recognize ligands of a particular structure, which imparts the necessary specificity for physiologic function as well as for pharmacologic intervention. From the time that drugs could be characterized structurally, modification of structure of the drug and structural correlations with pharmacologic activity (i.e., the study of structure-activity relationships[1–5]) have formed the basis for major research endeavors in synthetic organic chemistry conducted by the pharmaceutical industry and many academic institutions.

The specificity of pharmacologic responses does not reside in ligand recognition alone, since many molecules show a differential capacity for initiating a response upon binding or complex formation. This is the basis for distinguishing among *agonists* (agents that can elicit a maximal response), *partial agonists* (agents that elicit a response that at maximum is less than the maximal response to another agonist on the same receptor) and *antagonists* (agents that occupy the receptor but fail to elicit a response). This differential capacity to transduce responses is attributed either to conformational changes in the receptor or to different states of association of the receptor with active complexes of coupling proteins.

With the above considerations of ligand recognition and signal transduction, we have defined the features that distinguish receptors from other macromolecules in biologic systems. Thus, a formal definition of *receptors* should encompass both their unique recognition properties and their primary function to transduce the binding of ligands into a cellular response.

Definition and Classification of Receptors

A strict definition of receptors connotes that the macromolecule has been designed by nature to confer a response or transduce a signal to a naturally occurring ligand. Thus, a receptor for neurotransmitter or hormone should be distinguished from serum albumin although both types of molecules can influence drug action. As will become evident in subsequent chapters, serum albumin can transport drugs in the circulation to various organs, and it can also sequester drugs, preventing them from gaining access to their site of action. Albumin might then be considered an *acceptor* site for the drug rather than a true receptor.

Pharmacologic Classification of Receptors

Receptors are commonly classified by the mediator to which they respond and hence by their chemical specificity. The mediator may be a hormone, neurotransmitter, drug, growth factor, paracrine, or an intracellular messenger. The name given to a receptor is usually derived from this classification (e.g., cholinergic receptor, insulin receptor). In some cases the endogenous compound to which the receptor responds is not known, and it is therefore classified according to an exogenous agent to which it responds. This has occurred in the case of opiate (morphine) receptors, which are now known to respond to a range of naturally occurring opioid peptides (endorphins, enkephalins, and dynorphins). Thus, this basis for classification requires modifications as additional knowledge is acquired.

The classification based solely on the endogenous agent eliciting response was found long ago to be inadequate to explain the effects of various agonists and antagonists on animals and tissues. In 1914 Sir Henry Dale proposed that acetylcholine exerts two distinct actions, termed *nicotinic* and *muscarinic*, because of the resemblance of acetylcholine's responses to those of the plant alkaloids nicotine and muscarine. This necessity for *sub-*

classification of receptors upon which neurotransmitters or hormones act has been found to be the rule rather than the exception. Indeed, both the nicotinic and muscarinic cholinergic receptors have been further subclassified according to their responses to agonists and antagonists, and this has proved useful in designing cholinergic drugs of improved selectivity. Another early example of receptor subclassification is that of division of adrenergic receptors into α and β subtypes, as proposed by Ahlquist in 1948 on the basis of quantitative measurements of potency for a series of natural and synthetic agonists. In general it is most useful to have both selective agonists and antagonists to study the receptor subclasses. Pharmacologic classifications of receptors are critical to structure-activity considerations.

Biochemical or Biophysical Classification of Receptors

Another useful classification of receptors is based on the cellular *responses* elicited. This depends on the tremendous advances in molecular pharmacology since the late 1950s, which have permitted an understanding of receptors and the responses they produce at a biochemical and biophysical level. Many different types of responses can be studied for a single receptor, ranging from biochemical responses, such as changes in cyclic AMP concentrations or glucose production, to electrical or mechanical responses, such as membrane depolarization and muscle contraction. The understanding of receptor mechanisms at a molecular level requires a knowledge of the initial stimulus that the receptor produces in the cell or tissue.

Molecular or Structural Classification of Receptors

Such a classification is inherent to the primary amino acid sequence of the receptor, which may now be deduced from the sequence of the gene encoding the protein. Ultimately, one would hope for a convergence in the classification of receptors such that a receptor of known sequence could be expressed in a cell previously deficient in that or related receptors but possessing the necessary cellular machinery to confer a response. The ensuing cellular, biochemical, or biophysical responses and the chemical specificity of the receptor to a sufficient number of discriminating ligands could then be correlated with structure. Hence, each receptor could be defined on the basis of these three parameters for classification.

Other Classifications of Receptors

Receptors may also be classified by their anatomic location. Often nature's design has been convenient for this taxonomy since different tissues will express a predominant receptor subtype. Smooth (involuntary) muscle typically contracts in response to acetylcholine, and this is mediated by muscarinic receptors, while skeletal muscle in the voluntary motor system contracts in response to acetylcholine by activation of nicotinic receptors. Neurotransmission in the efferent or outward direction in the autonomic nervous system proceeds through a ganglion containing a synapse. Chemical neurotransmission occurs between preganglionic and postganglionic fibers. The primary transmitter is acetylcholine, and the initial electrical event (depolarization) is elicited through nicotinic receptors. These nicotinic receptors differ in chemical specificity and primary structure from the nicotinic receptors in skeletal muscle. Because of this they have been termed N_1 and N_2 or N_N and N_M (for neuronal and muscle, respectively). Efferent neurotransmission in the voluntary motor nervous system, in contrast to the autonomic nervous system, involves no ganglion.

Receptors also might be classified as *extracellular* or *intracellular*. Since polar molecules such as biogenic amines, amino acids, peptides, and proteins cannot rapidly transverse membranes, receptors for such molecules reside on cell surfaces. A major task for cells is the transduction of signals across cell membranes. Typically, extracellular receptors contain only a portion of their structure on the extracellular surface and contain domain(s) integral to the membrane or the cytoplasmic surface. This facilitates signaling across the cell membrane either through opening of an ion channel or through a change in conformation. Intracellular receptors are typically found for substances that are sufficiently nonpolar to cross the membrane (e.g., steroids) or that are generated within the cell itself.

The General Problem of Structure-Activity Relationships

Our considerations of the structure and conformation of drug molecules will attempt to bridge the disciplines of medicinal chemistry and pharmacology. Many of our considerations will require a background knowledge of bonding forces and protein structure, which will not be covered in this chapter. The reader might be referred to one of several texts on protein structure for this information.

Even though manipulation of the structure of the ligand (drug, hormone, or neurotransmitter) has usually proved inadequate for describing the precise mechanism of specificity of drug action, the ligand is usually the only component of the complex that is readily accessible to structural modification. This is beginning to change with the application of recombinant DNA technology to pharmacologic systems, whereby site-specific mutagenesis and the development of chimeric receptor constructs permit defined modifications of receptor structure (cf. Ch. 2). Nevertheless, a comprehensive understanding of pharmacologic selectivity and responses cannot be dissociated from a knowledge of the fundamentals of molecular structure.

Structure-activity relationships might be viewed from four distinct perspectives of study, which depend largely on how well we know the structure of the target site for drug action.

1. *An unidentified target molecule or site*: Studies in this arena must be largely correlative, and the best example is the action of general anesthetics. As developed in this chapter and in Chapter 2, correlations of the chemical structure of a ligand or a physical parameter inherent to it with biologic activity enable one to exclude potential sites of drug action but do not usually allow one to define a unique site. This arises because distinct subcellular loci (e.g., a phospholipid bilayer, a hydrophobic pocket on a protein, and an interface between a membrane lipid and a membrane-associated protein) may have similar physical properties.
2. *A target site that has been identified but whose primary structure (amino acid sequence) is unknown*: Sites of drug action falling in this category include the majority of drug-receptor systems. Studies of structure-activity relationships can be used to infer certain characteristics of the recognition site on the macromolecule. Information about the number of binding sites, the nature of binding forces, steric and size limitations of the recognition site, and requirements for activation can be deduced for these systems. Newer computational and graphic methods aided by computers enable one to estimate binding energies for congeneric series of compounds and to predict conformations of the bound state of the ligand-macromolecule complexes. The rapid advances in recombinant DNA technology should limit the number

of receptors studied at this level since primary structures of various receptors are being rapidly elucidated.

3. *A target site whose primary but not tertiary structure is known*: If the primary structure is known, some information on tertiary structure and location of binding sites can be inferred from sequence homology with proteins of known three-dimensional structure, from site-specific labeling of the recognition site, or from characteristic structural features of particular domains of the molecule. Such considerations further delimit the variables and increase the knowledge base from which structure-activity studies can be pursued. For example, homologous sequences between the guanine nucleotide-binding, or G proteins, involved in signal transduction and the oncogene *ras* and between the G protein-coupled receptors and bacteriorhodopsin provide critical reference points for structural predictions, since crystal structures have been solved for *ras* and bacteriorhodopsin (cf. Ch. 2).
4. *A target site of known tertiary structure*: Only in this case is structural information available at the atomic level. Few examples that relate to the specificity of pharmacologic agents exist to date, but such information greatly increases the power of computational and graphic methods for detailing structure-activity relationships and predicting activities of untested structures. To date, most of our knowledge of structures of macromolecules comes from x-ray crystallography[6]. Although nuclear magnetic resonance (NMR) has the potential for achieving comparable structural resolution[7], at present this technology is restricted to proteins of smaller mass ($\leq 10^4$ daltons). In Chapter 2 we examine the structure of complexes of selective chemotherapeutic agents with various dihydrofolate reductases. Such three-dimensional structures provide a detailed frame of reference for examining defined protein mutants with different inhibitor selectivity and also for designing inhibitors with enhanced selectivity of action.

Distinguishing Receptor Types and Subtypes Using Structure-Activity Relationships

The delineation of nicotinic and muscarinic subtypes of acetylcholine receptors actually preceded the structural characterization of the two alkaloids (nicotine and muscarine). However, a knowledge of the structure of these alkaloids and many congeners allows one to infer distinctive features of the respective receptors.

Nicotinic agonists have common structural features, shown at the top of Figure 1-1. In addition to a cationic center, there is a group, whose van der Waals radius is 5.9 Å distant, that can act as an acceptor for a hydrogen bond. In acetylcholine and some of its congeners this is the carbonyl oxygen, but in nicotine it is the pyridine nitrogen. In the muscarinic series (Fig. 1-1 bottom) the hydrogen bond acceptor is an oxygen atom located 4.4 Å from the center of positive charge. This is the ester oxygen in acetylcholine and the ring oxygen in muscarine. In addition, a methyl group corresponding to the acetyl methyl in acetylcholine or the ring methyl in muscarine strongly reinforces the interaction. These intersite distances suggest that acetylcholine binds to the nicotinic and muscarinic receptors in rather different conformations, and studies with conformationally rigid analogs of acetylcholine that are agonists further support this contention.

The disparate structures of the prototypical cholinergic antagonists *d*-tubocurarine and atropine for the nicotinic and muscarinic subtypes of receptors, respectively, are indicative of even greater diversity in the recognition sites of the respective receptors. It is now

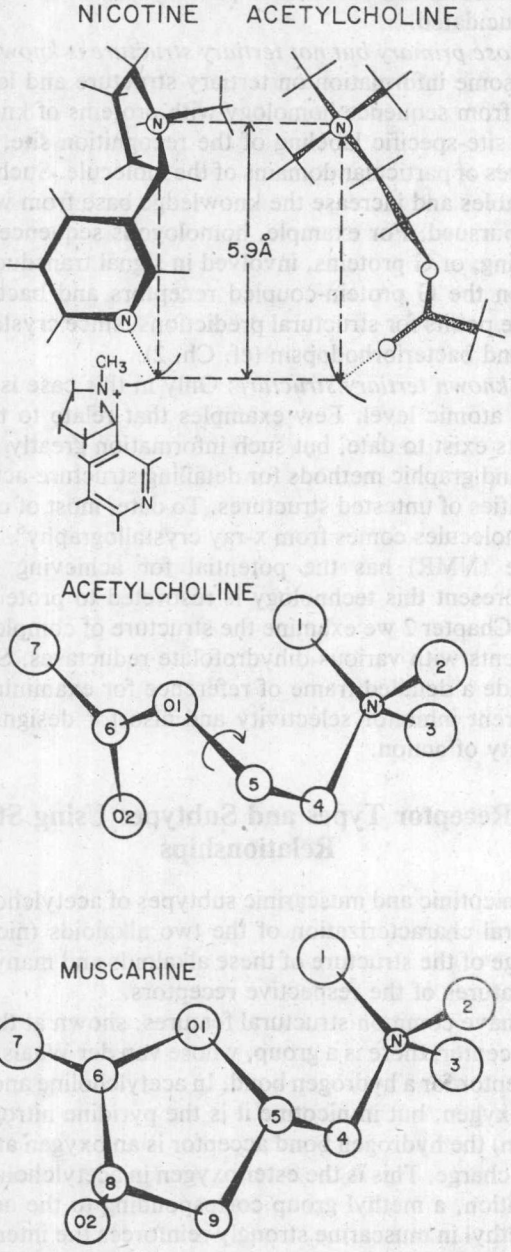

Fig. 1-1. Relationship of the molecular structures of nicotine and muscarine to that of acetylcholine. (Top) 1-Nicotine and corresponding conformation of acetylcholine, with 5.9 Å distance between cationic N and hydrogen bond acceptor group. (Bottom) L(+)-Muscarine and the corresponding conformation of acetylcholine, with 4.4 Å distance between cationic N and H-bond acceptor group 01. Group 7 is CH_3. (From Beers and Reich[8] and Chothia and Pauling[9], with permission.)