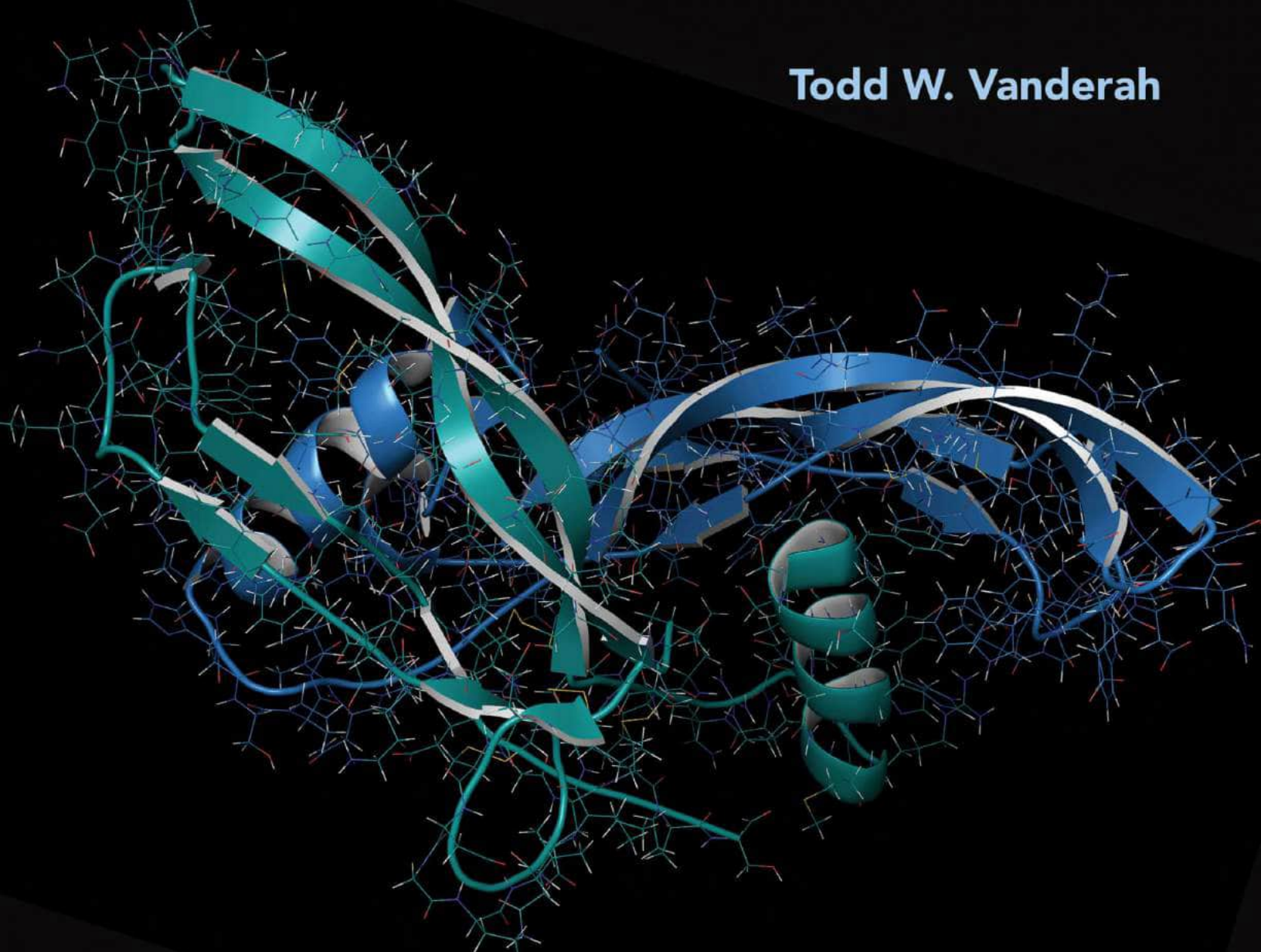


Todd W. Vanderah



KATZUNG's
BASIC & CLINICAL
PHARMACOLOGY

16th Edition

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Katzung's Basic & Clinical Pharmacology

Sixteenth Edition

Edited by

Todd W. Vanderah, PhD

Regents Professor and Chair

Department of Pharmacology

University of Arizona College of Medicine, Tucson



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Preface

The 16th edition of *Basic & Clinical Pharmacology* will recognize Dr. Bertram Katzung's legacy of contribution to this book by retitling the book as *Katzung's Basic & Clinical Pharmacology* and his mentorship of Dr. Todd W. Vanderah as editor for this edition. This edition continues the extensive use of full-color illustrations and expanded coverage of transporters, pharmacogenomics, and new drugs of all types emphasized in prior editions. The 16th edition also reflects the major expansion of large-molecule drugs in the pharmacopeia, with numerous new monoclonal antibodies and other biologic agents. Case studies accompany most chapters, and answers to questions posed in the case studies appear at the end of each chapter. The book is designed to provide a comprehensive, authoritative, and readable pharmacology textbook for students in the health sciences.

This edition continues the sequence used in many pharmacology courses and in integrated curricula: basic principles of drug discovery, pharmacodynamics, pharmacokinetics, and pharmacogenomics; autonomic drugs; cardiovascular-renal drugs; drugs with important actions on smooth muscle; central nervous system drugs; drugs used to treat inflammation, gout, and diseases of the blood; endocrine drugs; chemotherapeutic drugs; toxicology; and special topics. This sequence builds new information on a foundation of information already assimilated. For example, early presentation of autonomic nervous system pharmacology allows students to integrate the physiology and neuroscience they have learned elsewhere with the pharmacology they are learning and prepares them to understand the autonomic effects of other drugs. This is especially important for the cardiovascular and central nervous system drug groups. However, chapters can be used equally well in courses and curricula that present these topics in a different sequence. Within each chapter, emphasis is placed on discussion of drug groups and prototypes rather than offering repetitive detail about individual drugs. Selection of the subject matter and the order of its presentation are based on the accumulated experience of teaching this material to thousands of medical, pharmacy, dental, podiatry, nursing, and other health science students.

Health science curricula now place more emphasis on the clinical applications of therapeutic measures. Major features that make this book particularly useful in such curricula include sections that specifically address the clinical choice and use of drugs in patients and the monitoring of their effects—in other words, *clinical pharmacology* is an integral part of this text. Lists of the trade and generic names of commercial preparations available are provided at the end of each chapter for easy reference by the house officer or practitioner evaluating a patient's drug list or writing a prescription.

The 16th edition includes the novel medications that were quickly designed to prevent and overcome COVID-19 with emphasis on the importance of vaccines and antiviral drugs in pharmacology and clinical medicine.

Significant revisions in this edition include:

- Major revisions of the chapters on immunopharmacology; antiseizure, antipsychotic, antidepressant, antidiabetic, anti-inflammatory, and antiviral drugs; prostaglandins; and central nervous system neurotransmitters. Major additions on the treatment of COVID have been added in Chapter 49.
- Continued expansion of the coverage of general concepts relating to newly discovered receptors, receptor mechanisms, and drug transporters.
- Descriptions of important new drugs, especially biologicals, released through January 2022.
- Revised illustrations in full color that provide significantly more information about drug mechanisms and effects and help to clarify important concepts.

An important related educational resource is *Katzung's Pharmacology: Examination & Board Review*, 14th edition (Marieke Kruidering-Hall, Rupa L. Tuan, Todd W. Vanderah, Bertram G. Katzung, eds, McGraw Hill, 2024). This book provides a succinct review of pharmacology with almost 1000 sample examination questions and answers. It is especially helpful to students preparing for board-type examinations. A more highly condensed source of information suitable for review purposes is *USMLE Road Map: Pharmacology*, 2nd edition (Katzung BG, Trevor AJ: McGraw Hill, 2006). An extremely useful manual of toxicity due to drugs and other products is *Poisoning & Drug Overdose*, by Olson KR, ed; 8th edition, McGraw Hill, 2022.

The 16th edition marks the 42nd year of publication of *Basic & Clinical Pharmacology*. The widespread adoption of the first fifteen editions indicates that this book fills an important need. We believe that the 16th edition will satisfy this need even more successfully. Chinese, Croatian, Czech, French, Georgian, Indonesian, Italian, Japanese, Korean, Lithuanian, Portuguese, Spanish, Turkish, and Ukrainian translations of various editions are available. The publisher may be contacted for further information.

I wish to acknowledge the prior and continuing efforts of my contributing authors and the major contributions of the staff at Lange Medical Publications, Appleton & Lange, and McGraw Hill, and of our copy editor for this edition, Greg Feldman. I also wish to thank Tasneem Kauser for her contributions.

Suggestions and comments about *Katzung's Basic & Clinical Pharmacology* are always welcome. They may be sent to me in care of the publisher.

Todd W. Vanderah, PhD
Tucson, Arizona
July 2023

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SECTION I BASIC PRINCIPLES

C H A P T E R

1

Introduction: The Nature of Drugs & Drug Development & Regulation

Todd W. Vanderah, PhD, & Bertram G. Katzung, MD, PhD

CASE STUDY

A 78-year-old woman is brought to the hospital because of suspected aspirin overdose. She has taken aspirin for joint pain for many years without incident, but during the past year, she has exhibited signs of cognitive decline. Her caregiver finds her confused, hyperventilating, and vomiting. The caregiver finds an empty bottle of aspirin tablets and calls 9-1-1. In the

emergency department, samples of venous and arterial blood are obtained while the airway, breathing, and circulation are evaluated. An intravenous (IV) drip is started, and gastrointestinal decontamination is started. After blood gas results are reported, sodium bicarbonate is administered via the IV. What is the purpose of the sodium bicarbonate?

Pharmacology can be defined as the study of substances that interact with living systems through chemical processes. These interactions usually occur by binding of the substance to regulatory molecules and activating or inhibiting normal body processes. These substances may be chemicals administered to achieve a beneficial therapeutic effect on some process within the patient or for their toxic effects on regulatory processes in parasites infecting the patient. Such deliberate therapeutic applications may be

considered the proper role of **medical pharmacology**, which is often defined as the science of substances used to prevent, diagnose, and treat disease. **Toxicology** is the branch of pharmacology that deals with the undesirable effects of chemicals on living systems, from individual cells to humans to complex ecosystems (Figure 1–1). The nature of drugs—their physical properties and their interactions with biological systems—is discussed in part I of this chapter.

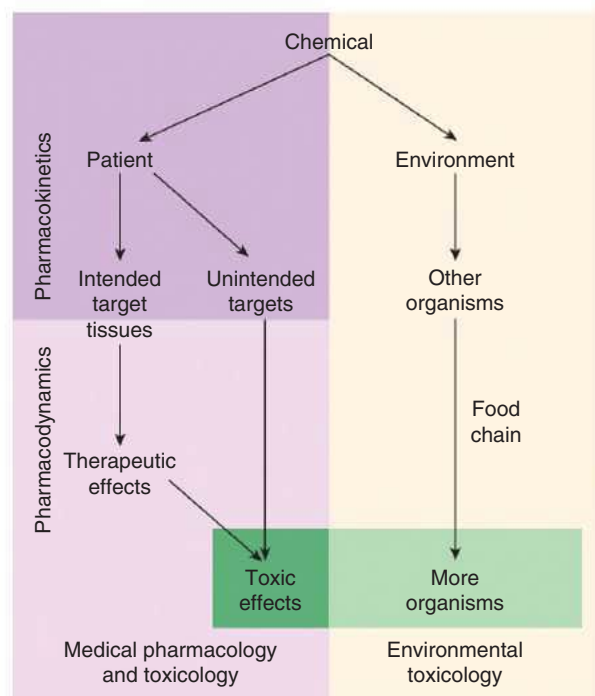


FIGURE 1–1 Major areas of study in pharmacology. The actions of chemicals can be divided into two large domains. The first (*left side*) is that of medical pharmacology and toxicology, which is aimed at understanding the actions of drugs as chemicals on individual organisms, especially humans and domestic animals. Both beneficial and toxic effects are included. Pharmacokinetics deals with the absorption, distribution, and elimination of drugs. Pharmacodynamics concerns the actions of the chemical on the organism. The second domain (*right side*) is that of environmental toxicology, which is concerned with the effects of chemicals on all organisms and their survival in groups and as species.

New drugs are added every year; they are needed for several reasons including: (1) increasing resistance by bacteria and other parasites; (2) discovery of new target processes in diseases that have not been adequately treated; and (3) recognition of new diseases. Furthermore, a dramatic increase has occurred in the number of *large molecule* drugs (especially antibodies) approved during the last two decades. The development of new drugs and their regulation by government agencies are discussed in part II.

THE HISTORY OF PHARMACOLOGY

Prehistoric people undoubtedly recognized the beneficial or toxic effects of many plant and animal materials. Early written records list remedies of many types, including a few that are still recognized as useful drugs today. Most, however, were worthless or actually harmful. Beginning about 1500 years ago, sporadic attempts were made to introduce rational methods into medicine, but none was successful owing to the dominance of systems of thought (“schools”) that purported to explain all of biology and disease without the need for experimentation and observation. These schools promulgated bizarre notions such as the idea that disease was caused by excesses

of bile or blood in the body, that wounds could be healed by applying a salve to the weapon that caused the wound, and so on.

Around the end of the 17th century, concepts based on observation and experimentation began to replace theorizing in physiology and clinical medicine. As the value of these methods in the study of disease became clear, physicians in Great Britain and on the Continent began to apply them systematically to the effects of traditional drugs used in their own practices. Thus, **materia medica**—the science of drug preparation and the medical uses of drugs—began to develop as the precursor to pharmacology. However, any real understanding of the mechanisms of action of drugs was prevented by the absence of methods for purifying active agents from the crude materials that were available and—even more—by the lack of methods for testing hypotheses about the nature of drug actions.

In the late 18th and early 19th centuries, François Magendie and his student Claude Bernard began to develop the methods of **experimental physiology** and **pharmacology**. Advances in chemistry and the further development of physiology in the 18th, 19th, and early 20th centuries laid the foundation needed for understanding how drugs work at the organ and cellular levels. Paradoxically, real advances in basic pharmacology during this time were accompanied by an outburst of unscientific claims by manufacturers and marketers of worthless “patent medicines.” Not until the concepts of rational therapeutics, especially that of the **controlled clinical trial**, were reintroduced into medicine—only about 60 years ago—did it become possible to adequately evaluate therapeutic claims.

Around the 1940s and 1950s, a major expansion of research efforts in all areas of biology began. As new concepts and new techniques were introduced, information accumulated about drug action and the biologic substrate of that action, the **drug receptor**. During the last 60 years, many fundamentally new drug groups and new members of old groups have been introduced. The last three decades have seen an even more rapid growth of information and understanding of the molecular basis for drug action. The molecular mechanisms of action of many drugs have now been identified, and numerous receptors have been isolated, structurally characterized, and cloned. In fact, the use of receptor identification methods (described in Chapter 2) has led to the discovery of many *orphan* receptors—receptors for which no ligand has been discovered and whose function can only be guessed. Studies of the local molecular environment of receptors have shown that receptors and effectors do not function in isolation; they are strongly influenced by other receptors and by companion regulatory proteins. Most recently, it has become clear that gravitational change, radiation, and other aspects of space *outside* the Earth’s environment require the development of *space medicine*. This has opened another important area for the future of pharmacology. One result of these discoveries is the confirmation that pharmacology represents an area where anatomy, biochemistry, genetics, physiology, pathology, clinical medicine, and the environment meet. Many problems that the health practitioner confronts can now be corrected or mitigated using pharmacologic tools.

Pharmacogenomics—the relation of the individual’s genetic makeup to his or her response to specific drugs—is becoming an important part of therapeutics (see Chapter 5). Decoding of the genomes of many species—from bacteria to humans—has led to

the recognition of unsuspected relationships between receptor families and the ways that receptor proteins have evolved. Furthermore, discovery of regulatory functions exerted by the neighbors of chromosomes and the *noncoding* regions of DNA on the expression of exons has opened a new area of possible manipulation of the genes—epigenetics—that control pharmacologic responses.

The discovery that small segments of RNA can interfere with protein synthesis with extreme selectivity has led to investigation of **small interfering RNAs (siRNAs)** and **micro-RNAs (miRNAs)** as therapeutic agents. Similarly, short nucleotide chains called **antisense oligonucleotides (ANOs)**, synthesized to be complementary to natural RNA or DNA, can interfere with the readout of genes and the transcription of RNA. Recently, the development of mRNA vaccines has led to new medications by activating one's own immune system to recognize and fight off viruses. New, efficient methods of DNA editing have made possible the modification of genes that encode proteins that are critical to biological function, representing a transformative means of generating medicines. Finally, development in the production of selective monoclonal antibodies to target selective proteins has led to a dramatic increase in *large molecule* therapeutics in the last 20 years (Table 1–1).

Unfortunately, the medication-consuming public is still exposed to vast amounts of inaccurate or unscientific information regarding the pharmacologic effects of chemicals. This results in the irrational use of innumerable expensive, ineffective, and sometimes harmful remedies and the growth of a huge “alternative health care” industry. Furthermore, manipulation of the legislative process in the United States has allowed many substances promoted for health—but not promoted specifically as “drugs”—to avoid meeting the Food and Drug Administration (FDA) standards described in the second part of this chapter. Conversely, lack of understanding of basic scientific principles in biology and statistics and the absence of critical thinking about public health issues have led to rejection of medical science, including vaccines, by a segment of the public and a tendency to assume that all adverse drug effects are the result of malpractice.

General principles that the student should remember are (1) that *all* substances can under certain circumstances be toxic; (2) that the chemicals in botanicals (herbs and plant extracts,

“nutraceuticals”) are no different from chemicals in manufactured drugs except for the much greater proportion of impurities in botanicals; and (3) that all dietary supplements and all therapies promoted as health-enhancing should meet the same standards of efficacy and safety as conventional drugs and medical therapies. That is, there should be no artificial separation between scientific medicine and “alternative” or “complementary” medicine. Ideally, all nutritional and botanical substances should be tested by the same types of randomized controlled trials (RCTs) as synthetic compounds.

■ GENERAL PRINCIPLES OF PHARMACOLOGY

THE NATURE OF DRUGS

In the most general sense, a drug may be defined as any substance that brings about a change in biologic function through its chemical actions. In most cases, the drug molecule interacts as an **agonist** (activator) or **antagonist** (inhibitor) with a specific target molecule that plays a regulatory role in the biologic system. This target molecule is called a **receptor**. Some of the new large molecule drugs (**biologicals**) act like receptors themselves and bind endogenous molecules. The nature of receptors is discussed more fully in Chapter 2. In a very small number of cases, drugs known as **chemical antagonists** may interact directly with other drugs, whereas a few drugs (**osmotic agents**) interact almost exclusively with water molecules. Drugs may be synthesized within the body (eg, **hormones**) or may be chemicals *not* synthesized in the patient's body (ie, **xenobiotics**). **Poisons** are drugs that have almost exclusively harmful effects. However, Paracelsus (1493–1541) famously stated that “the dose makes the poison,” meaning that any substance can be harmful if taken in the wrong dosage. **Toxins** are usually defined as poisons of biologic origin, ie, synthesized by plants or animals, in contrast to inorganic poisons such as lead and arsenic.

The Physical Nature of Drugs

To interact chemically with its receptor, a drug molecule must have the appropriate size, electrical charge, shape, and atomic composition. Furthermore, a drug is often administered at a location distant from its intended site of action, eg, a pill taken orally to relieve a headache. Therefore, a useful drug must have the necessary properties to be transported from its site of administration to its site of action. Finally, a practical drug should be inactivated or excreted from the body at a reasonable rate so that its actions will be of appropriate duration.

Drugs may be solid at room temperature (eg, aspirin, atropine), liquid (eg, nicotine, ethanol), or gaseous (eg, nitrous oxide, isoflurane, xenon). These factors often determine the best route of administration. The most common routes of administration are described in Chapter 3, Table 3–3. The various classes of organic compounds—carbohydrates, proteins, lipids, and smaller molecules—are all represented in pharmacology. As noted above,

TABLE 1–1 Development of large molecule therapeutic agents as a percentage of all new drug approvals, 2000–2020.

Year	Total New Drug Approvals	New Protein Drugs (~% of new drug approvals)	New MABs (~% of new drug approvals)
2000	63	3 (5%)	1 (2%)
2008	34	5 (15%)	1 (3%)
2010	49	8 (16%)	4 (8%)
2015	69	13 (19%)	9 (13%)
2017	63	10 (16%)	9 (14%)
2020	61	? (16%)	10 (16%)

MABs, monoclonal antibodies.

many proteins and some new mRNA vaccines have been approved while some oligonucleotides have also received approval for use depending on the country's regulation agencies.

A number of inorganic elements, eg, fluoride, lithium, iron, and heavy metals, are both useful and dangerous drugs. Many organic drugs are weak acids or bases. This fact has important implications for the way they are handled by the body, because pH differences in the various compartments of the body may alter the degree of ionization of weak acids and bases (see text that follows).

Drug Size

The molecular size of drugs varies from very small (lithium ion, molecular weight [MW] 7) to very large (eg, alteplase [t-PA], a protein of MW 59,050). Many antibodies are even larger; eg, eculumab, an antibody used in the management of migraine, has a MW of more than 145,000. However, most drugs have molecular weights between 100 and 1000. The lower limit of this narrow range is probably set by the requirements for specificity of action. To have a good "fit" to only one type of receptor, a drug molecule must be sufficiently unique in shape, charge, and other properties to prevent its binding to other receptors. To achieve such selective binding, it appears that a molecule should in most cases be at least 100 MW units in size. The upper limit in molecular weight is determined primarily by the requirement that most drugs must be able to move within the body (eg, from the site of administration to the site of action and then to the site of elimination). Drugs much larger than MW 1000 do not diffuse readily between compartments of the body (discussed under Permeation, in following text). Therefore, very large drugs (usually proteins) must often be administered directly into the compartment where they have their effect. In the case of alteplase, a clot-dissolving enzyme, it is administered directly into the vascular compartment by intravenous or intra-arterial infusion.

Drug Reactivity & Drug-Receptor Bonds

Drugs interact with receptors by means of chemical forces or bonds. These are of three major types: **covalent**, **electrostatic**, and **hydrophobic**. Covalent bonds are very strong and in many cases not reversible under biologic conditions. Thus, the covalent bond formed between the acetyl group of acetylsalicylic acid (aspirin) and cyclooxygenase, its enzyme target in platelets, is not readily broken. The platelet aggregation-blocking effect of aspirin lasts long (days) after free acetylsalicylic acid has disappeared from the bloodstream (about 15 minutes) and is reversed only by the synthesis of new enzyme in new platelets, a process that takes several days. Other examples of highly reactive, covalent bond-forming drugs include the DNA-alkylating agents used in cancer chemotherapy to disrupt cell division in the tumor.

Electrostatic bonding is much more common than covalent bonding in drug-receptor interactions. Electrostatic bonds vary from relatively strong linkages between permanently charged ionic molecules to weaker hydrogen bonds and very weak induced dipole interactions such as van der Waals forces and similar phenomena. Electrostatic bonds are weaker than covalent bonds.

Hydrophobic bonds are usually quite weak and are probably important in the interactions of highly lipid-soluble drugs with the lipids of cell membranes and perhaps in the interaction of drugs with the internal walls of receptor "pockets."

The specific nature of a particular drug-receptor bond is of less practical importance than the fact that drugs that bind through weak bonds to their receptors are generally more selective than drugs that bind by means of very strong bonds. This is because weak bonds require a very precise fit of the drug to its receptor if an interaction is to occur. Only a few receptor types are likely to provide such a precise fit for a particular drug structure. Thus, if we wished to design a highly selective drug for a particular receptor, we would avoid highly reactive molecules that form covalent bonds and instead choose a molecule that forms weaker bonds.

A few substances that are almost completely inert in the chemical sense nevertheless have significant pharmacologic effects. For example, xenon, an "inert" gas, has anesthetic effects at elevated pressures.

Drug Shape

The shape of a drug molecule must be such as to permit binding to its target site via the bonds just described. Optimally, the drug's shape is complementary to that of the target site (usually a receptor) in the same way that a key is complementary to a lock. Furthermore, the phenomenon of **chirality (stereoisomerism)** is so common in biology that more than half of all useful drugs are chiral molecules; that is, they can exist as enantiomeric pairs. Drugs with two asymmetric centers have four diastereomers, eg, ephedrine, a sympathomimetic drug. In most cases, one of these enantiomers is much more potent than its mirror image enantiomer, reflecting a better fit to the receptor molecule. If one imagines the receptor site to be like a glove into which the drug molecule must fit to bring about its effect, it is clear why a "left-oriented" drug is more effective in binding to a left-hand receptor than its "right-oriented" enantiomer.

The more active enantiomer at one type of receptor site may not be more active at another receptor type, eg, a type that may be responsible for some other effect. For example, carvedilol, a drug that interacts with adrenoceptors, has a single chiral center and thus two enantiomers (Table 1–2). One of these enantiomers, the (*S*)(–) isomer, is a potent β -receptor blocker. The (*R*)(+) isomer is 100-fold weaker at the β receptor. However, the isomers are approximately equipotent as α -receptor blockers. Ketamine, a chiral molecule with two isomers, was introduced as an anesthetic. When administered as

TABLE 1–2 Dissociation constants (K_d) of the enantiomers and racemate of carvedilol.

Form of Carvedilol	α Receptors (K_d , nmol/L ¹)	β Receptors (K_d , nmol/L)
(<i>R</i>)(+) enantiomer	14	45
(<i>S</i>)(–) enantiomer	16	0.4
(<i>R,S</i>)(±) enantiomers	11	0.9

¹The K_d is the concentration for 50% saturation of the receptors and is inversely proportionate to the affinity of the drug for the receptors.

Data from Ruffolo RR Jr, Gellai M, Hieble JP, et al: The Pharmacology of carvedilol. Eur J Clin Pharmacol 1990;38S2:S82-88.

an anesthetic, the racemic mixture is administered intravenously. In contrast, the (S) enantiomer has recently been approved for use in nasal spray form as a rapid-acting antidepressant.

Finally, because enzymes are usually stereoselective, one drug enantiomer is often more susceptible than the other to drug-metabolizing enzymes. As a result, the duration of action of one enantiomer may be quite different from that of the other. Similarly, drug transporters may be stereoselective.

Unfortunately, most studies of clinical efficacy and drug elimination in humans have been carried out with racemic mixtures of drugs rather than with the separate enantiomers. At present, less than half of the chiral drugs used clinically are marketed as the active isomer—the rest are available only as racemic mixtures. As a result, a majority of patients receive drug doses of which 50% is less active or inactive. A few drugs are currently available in both the racemic and the pure, active isomer forms. However, proof that administration of the pure, active enantiomer decreases adverse effects relative to those produced by racemic formulations has not been established.

Rational Drug Design

Rational design of drugs implies the ability to predict the appropriate molecular structure of a drug on the basis of information about its biologic receptor. Until recently, no receptor was known in sufficient detail to permit such drug design. Instead, drugs were developed through random testing of chemicals or modification of drugs already known to have some effect. However, the characterization of many receptors during the past three decades has changed this picture. A few drugs now in use were developed through molecular design based on knowledge of the three-dimensional structure of the receptor site. Computer programs are now available that can iteratively optimize drug structures to fit known receptors. As more becomes known about receptor structure, rational drug design will become more common.

Receptor Nomenclature

The spectacular success of newer, more efficient ways to identify and characterize receptors (see Chapter 2) has resulted in a variety of differing, and sometimes confusing, systems for naming them. This in turn has led to a number of suggestions regarding more rational methods of naming receptors. The interested reader is referred for details to the efforts of the International Union of Pharmacology (IUPHAR) Committee on Receptor Nomenclature and Drug Classification (reported in various issues of *Pharmacological Reviews* and elsewhere) and to Alexander SP et al: *The Concise guide to pharmacology* 2017/18: Overview. *Br J Pharmacol* 2017;174:S1. The chapters in this book mainly use these sources for naming receptors.

DRUG-BODY INTERACTIONS

The interactions between a drug and the body are conveniently divided into two classes. The actions of the drug on the body are termed **pharmacodynamic** processes (see Figure 1–1); the principles of pharmacodynamics are presented in greater detail in Chapter 2.

These properties determine the group in which the drug is classified, and they play the major role in deciding whether that group is appropriate therapy for a particular symptom or disease. The actions of the body on the drug are called **pharmacokinetic** processes and are described in Chapters 3 and 4. Pharmacokinetic processes govern the absorption, distribution, and elimination of drugs and are of great practical importance in the choice and administration of a particular drug for a particular patient, eg, a patient with heart failure who also has impaired renal function. The following paragraphs provide a brief introduction to pharmacodynamics and pharmacokinetics.

Pharmacodynamic Principles

Most drugs must bind to a receptor to bring about an effect. However, at the cellular level, drug binding is only the first in a sequence of possible steps:

- Drug (D) + receptor-effector (R) → drug-receptor-effector complex → effect
- D + R → drug-receptor complex → effector molecule → effect
- D + R → D-R complex → activation of coupling molecule → effector molecule → effect
- Inhibition of metabolism of endogenous activator → increased activator action on an effector molecule → increased effect

Note that the final change in function is accomplished by an **effector** mechanism. The effector may be part of the receptor molecule or may be a separate molecule. A very large number of receptors communicate with their effectors through coupling molecules, as described in Chapter 2.

A. Types of Drug-Receptor Interactions

Agonist drugs bind to and *activate* the receptor in some fashion, which directly or indirectly brings about the effect (Figure 1–2A). Receptor activation involves a change in conformation in the cases that have been studied at the molecular structure level. Some receptors incorporate effector machinery in the same molecule, so that drug binding brings about the effect directly, eg, opening of an ion channel or activation of enzyme activity. Other receptors are linked through one or more intervening coupling molecules to a separate effector molecule. The major types of drug-receptor-effector coupling systems are discussed in Chapter 2. **Pharmacologic antagonist** drugs, by binding to a receptor, compete with and prevent binding by other molecules. For example, acetylcholine receptor blockers such as atropine are antagonists because they prevent access of acetylcholine and similar agonist drugs to the acetylcholine receptor site and they stabilize the receptor in its inactive state (or some state other than the acetylcholine-activated state). These agents reduce the effects of acetylcholine and similar molecules in the body (Figure 1–2B), but their action can be overcome by increasing the dosage of agonist. Some antagonists bind very tightly to the receptor site in an irreversible or pseudoirreversible fashion and cannot be displaced by increasing the agonist concentration. Drugs that bind to the same receptor molecule but do not prevent binding of the agonist are said to act **allosterically** and may enhance (Figure 1–2C) or inhibit (Figure 1–2D) the action of

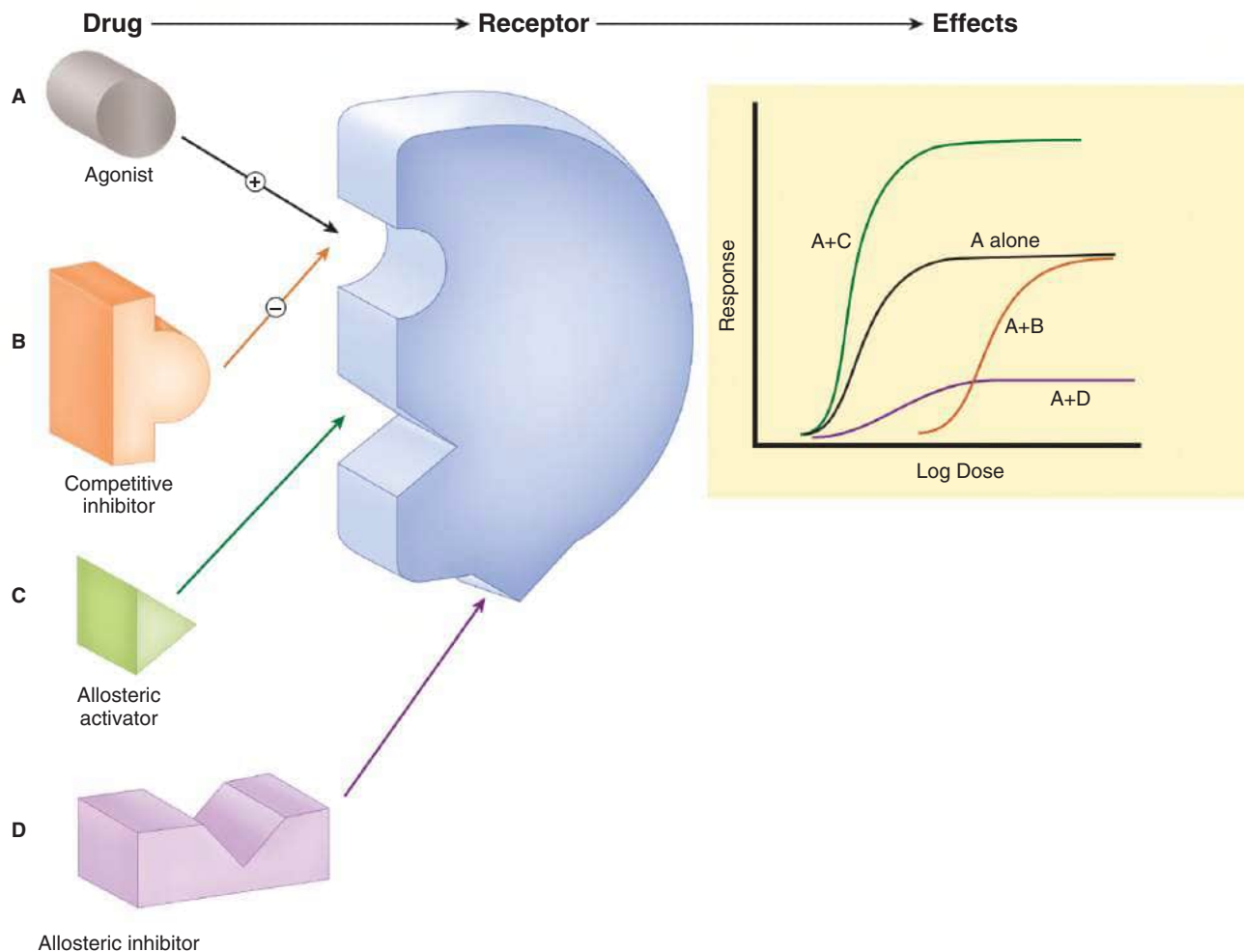


FIGURE 1-2 Drugs may interact with receptors in several ways. The effects resulting from these interactions are diagrammed in the dose-response curves at the right. Drugs that alter the agonist (**A**) response may activate the agonist binding site, compete with the agonist (competitive inhibitors, **B**), or act at separate (allosteric) sites, increasing (**C**) or decreasing (**D**) the response to the agonist. Allosteric activators (**C**) may increase the efficacy of the agonist or its binding affinity. The curve shown reflects an increase in efficacy; an increase in affinity would result in a leftward shift of the curve.

the agonist molecule. Allosteric inhibition is not usually overcome by increasing the dose of agonist.

B. Agonists That Inhibit Their Binding Molecules

Some drugs mimic agonist drugs by inhibiting the molecules responsible for terminating the action of an endogenous agonist. For example, acetylcholinesterase *inhibitors*, by slowing the destruction of endogenous acetylcholine, cause cholinomimetic effects that closely resemble the actions of cholinergic *agonist* molecules even though cholinesterase inhibitors do not bind or only incidentally bind to cholinergic receptors (see Chapter 7). Because they amplify the effects of physiologically released endogenous agonist ligands, their effects are sometimes more selective and less toxic than those of exogenous agonists.

C. Agonists, Partial Agonists, and Inverse Agonists

Figure 1-3 describes a useful model of drug-receptor interactions. As indicated, the receptor is postulated to exist partially in the inactive, nonfunctional form (R_i) and partially in the activated

form (R_a). Thermodynamic considerations indicate that even in the absence of any agonist, some of the receptor pool must exist in the R_a form some of the time and may produce the same physiologic effect as agonist-induced activity. This effect, occurring in the absence of agonist, is termed **constitutive** or **basal activity**. Agonists have a much higher affinity for the R_a configuration and stabilize it, so that a large percentage of the total pool resides in the R_a -D fraction and a large effect is produced. The recognition of constitutive activity may depend on the receptor density, the concentration of coupling molecules (if a coupled system), and the number of effectors in the system.

Many agonist drugs, when administered at concentrations sufficient to saturate the receptor pool, can activate their receptor-effector systems to the maximum extent of which the system is capable; that is, they cause a shift of almost all of the receptor pool to the R_a -D pool. Such drugs are termed **full agonists**. Other drugs, called **partial agonists**, bind to the same receptors and activate them in the same way but do not evoke as great a response, no matter how high the concentration. In the model in

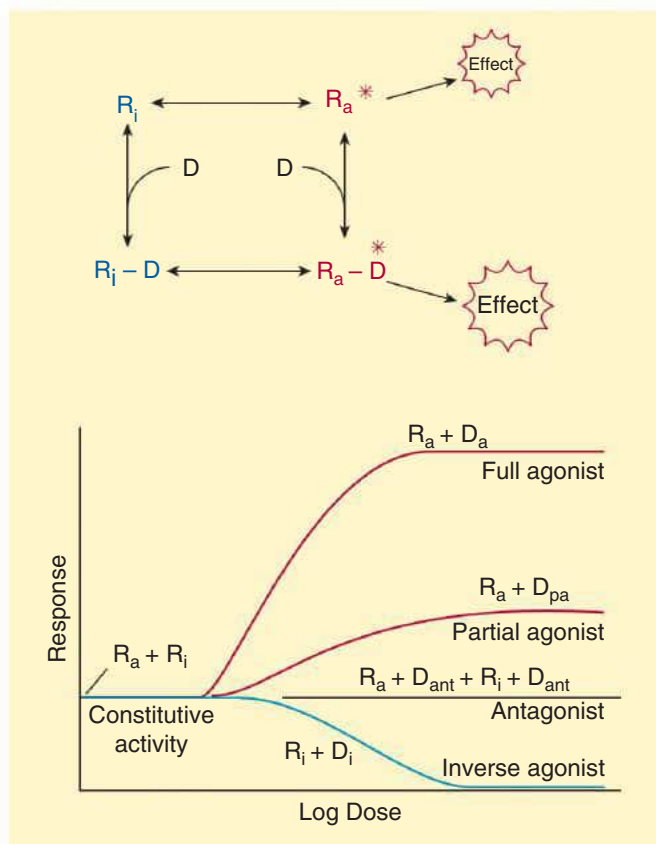


FIGURE 1-3 A model of drug-receptor interaction. The hypothetical receptor is able to assume two conformations. In the R_i conformation, it is inactive and produces no effect, even when combined with a drug molecule. In the R_a conformation, the receptor can activate downstream mechanisms that produce a small observable effect, even in the absence of drug (constitutive activity). In the absence of drugs, the two isoforms are in equilibrium, and the R_i form is favored. Conventional full agonist drugs have a much higher affinity for the R_a conformation, and mass action thus favors the formation of the R_a -D complex with a much larger observed effect. Partial agonists have an intermediate affinity for both R_i and R_a forms. Conventional antagonists, according to this hypothesis, have equal affinity for both receptor forms and maintain the same level of constitutive activity. Inverse agonists, on the other hand, have a much higher affinity for the R_i form, reduce constitutive activity, and may produce a contrasting physiologic result.

Figure 1-3, partial agonists do not stabilize the R_a configuration as fully as full agonists, so that a significant fraction of receptors exists in the R_i -D pool. Such drugs are said to have low **intrinsic efficacy**. Because they occupy the same receptor site, partial agonists can also prevent access by full agonists. Thus, pindolol, a β -adrenoceptor partial agonist, may act either as an agonist (if no full agonist is present) or as an antagonist (if a full agonist such as epinephrine is present). (See Chapter 2.) Intrinsic efficacy is independent of affinity (as usually measured) for the receptor.

In the same model, conventional antagonist action can be explained as fixing the fractions of drug-bound R_i and R_a in the same relative amounts as in the absence of any drug. In this situation, no change in activity will be observed, so the drug will appear

to be without effect. However, the presence of the antagonist at the receptor site will block access of agonists to the receptor and prevent the usual agonist effect. Such blocking action can be termed **neutral antagonism**.

What will happen if a drug has a much stronger affinity for the R_i than for the R_a state and stabilizes a large fraction in the R_i -D pool? In this scenario the drug will reduce any constitutive activity, thus resulting in effects that are the opposite of the effects produced by conventional agonists at that receptor. Such drugs are termed **inverse agonists** (see Figure 1-3). One of the best documented examples of such a system is the γ -aminobutyric acid ($GABA_A$) receptor-effector (a chloride channel) in the nervous system. This receptor is activated by the endogenous transmitter GABA and causes inhibition of postsynaptic cells. Conventional exogenous agonists such as benzodiazepines also facilitate the receptor-effector system and enhance GABA-like chloride entry into cells with sedation as the therapeutic result. This sedation can be reversed by conventional neutral antagonists such as flumazenil. Inverse agonists of this receptor system cause anxiety and agitation, the inverse of sedation (see Chapter 22). Similar inverse agonists have been found for β adrenoceptors, histamine H_1 and H_2 receptors, and several other receptor systems.

D. Duration of Drug Action

Termination of drug action can result from several processes. In some cases, the effect lasts only as long as the drug occupies the receptor, and dissociation of drug from the receptor automatically terminates the effect. In many cases, however, the action may persist after the drug has dissociated because, for example, some coupling molecule is still present in activated form. In the case of drugs that bind covalently to the receptor site, the effect may persist until the drug-receptor complex is destroyed and new receptors or enzymes are synthesized, as described previously for aspirin. In addition, many receptor-effector systems incorporate desensitization mechanisms for preventing excessive activation when agonist molecules continue to be present for long periods. (See Chapter 2 for additional details.)

E. Receptors and Inert Binding Sites

To function as a receptor, an endogenous molecule must first be **selective** in choosing ligands (drug molecules) to bind; and second, it must **change its function** upon binding in such a way that the function of the biologic system (cell, tissue, etc) is altered. The selectivity characteristic is required to avoid constant activation of the receptor by promiscuous binding of many different ligands. The ability to change function is clearly necessary if the ligand is to cause a pharmacologic effect. In some cases the function of the ligand may be to inhibit the endogenous actions of the receptor (antagonist and inverse agonist). The body contains a vast array of molecules that are capable of binding drugs, however, and not all of these endogenous molecules are regulatory molecules. Binding of a drug to a nonregulatory molecule such as plasma albumin will result in no detectable change in the function of the biologic system, so this endogenous molecule can be called an **inert binding site**. Such binding is not completely without significance, however, because it affects the

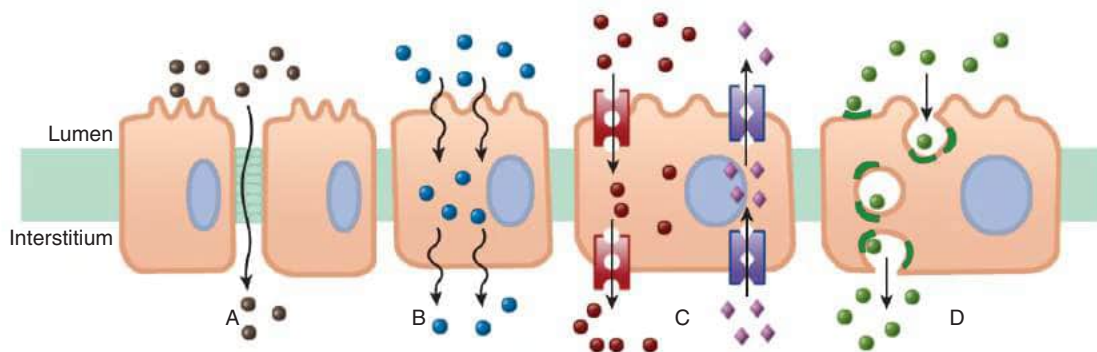


FIGURE 1-4 Mechanisms of drug permeation. Drugs may diffuse passively through aqueous channels in the intercellular junctions (eg, tight junctions, **A**), or through lipid cell membranes (**B**). Drugs with the appropriate characteristics may be transported by carriers into or out of cells (**C**). Very impermeant drugs may also bind to cell surface receptors (dark binding sites), be engulfed by the cell membrane (endocytosis), and then be released inside the cell or expelled via the membrane-limited vesicles out of the cell into the extracellular space (exocytosis, **D**).

distribution of drug within the body and determines the amount of free drug in the circulation. Both of these factors are of pharmacokinetic importance (see also Chapter 3).

Pharmacokinetic Principles

In practical therapeutics, a drug should be able to reach its intended site of action after administration by some convenient route. In many cases, the active drug molecule is sufficiently lipid-soluble and stable to be given as such. In some cases, however, an inactive precursor chemical that is readily absorbed and distributed must be administered and then converted to the active drug by biologic processes—inside the body. Such a precursor chemical is called a **prodrug**.

In only a few situations is it possible to apply a drug directly to its target tissue, eg, by topical application of an anti-inflammatory agent to inflamed skin or mucous membrane. Most often, a drug is administered into one body compartment, eg, the gut, and must move to its site of action in another compartment, eg, the brain in the case of an antiseizure medication. This requires that the drug be **absorbed** into the blood from its site of administration and **distributed** to its site of action, **permeating** through the various barriers that separate these compartments. For a drug given orally to produce an effect in the central nervous system, these barriers include the tissues that make up the wall of the intestine, the walls of the capillaries that perfuse the gut, and the blood-brain barrier, the walls of the capillaries that perfuse the brain. Finally, after bringing about its effect, a drug should be **eliminated** at a reasonable rate by metabolic inactivation, by excretion from the body, or by a combination of these processes.

A. Permeation

Drug permeation proceeds by several mechanisms. Passive diffusion in an aqueous or lipid medium is common, but active processes play a role in the movement of many drugs, especially those whose molecules are too large to diffuse readily (Figure 1-4). Drug **vehicles** can be very important in facilitating transport and permeation, eg, by encapsulating the active agent in liposomes and in regulating release, as in slow release preparations. Newer methods of facilitating transport of drugs by coupling them to **nanoparticles** are under investigation.

1. Aqueous diffusion—Aqueous diffusion occurs within the larger aqueous compartments of the body (interstitial space, cytosol, etc) and across epithelial membrane tight junctions and the endothelial lining of blood vessels through aqueous pores that—in some tissues—permit the passage of molecules as large as MW 20,000–30,000.* See Figure 1-4A.

Aqueous diffusion of drug molecules is usually driven by the concentration gradient of the permeating drug, a downhill movement described by Fick's law (see below). Drug molecules that are bound to large plasma proteins (eg, albumin) do not permeate most vascular aqueous pores. If the drug is charged, its flux is also influenced by electrical fields (eg, the membrane potential and—in parts of the nephron—the transtubular potential).

2. Lipid diffusion—Lipid diffusion is the most important limiting factor for drug permeation because of the large number of lipid barriers that separate the compartments of the body. Because these lipid barriers separate aqueous compartments, the **lipid:aqueous partition coefficient** of a drug determines how readily the molecule moves between aqueous and lipid media. In the case of weak acids and weak bases (which gain or lose electrical charge-bearing protons, depending on the pH), the ability to move from aqueous to lipid or vice versa varies with the pH of the medium, because charged molecules attract water molecules. The ratio of lipid-soluble form to water-soluble form for a weak acid or weak base is expressed by the Henderson-Hasselbalch equation (described in the following text). See Figure 1-4B.

3. Special carriers—Special carrier molecules exist for many substances that are important for cell function and are too large or too insoluble in lipid to diffuse passively through membranes, eg, peptides, amino acids, and glucose. These carriers bring about movement by active transport or facilitated diffusion and, unlike passive diffusion, are selective, saturable, and inhibitable. Because

*The capillaries of the brain, the testes, and some other tissues are characterized by the absence of pores that permit aqueous diffusion. They may also contain high concentrations of drug export pumps (MDR pump molecules; see text). These tissues are therefore protected or “sanctuary” sites from many circulating drugs.

many drugs are or resemble such naturally occurring peptides, amino acids, or sugars, they can use these carriers to cross membranes. See Figure 1–4C.

Many cells also contain less selective membrane carriers that are specialized for expelling foreign molecules. One large family of such transporters binds adenosine triphosphate (ATP) and is called the ABC (ATP-binding cassette) family. This family includes the **P-glycoprotein** or **multidrug resistance type 1 (MDR1) transporter** found in the brain, testes, and other tissues and in some drug-resistant neoplastic cells (Table 1–3). Similar transport molecules from the ABC family, the **multidrug resistance-associated protein (MRP)** transporters, play important roles in the excretion of some drugs or their metabolites into urine and bile and in the resistance of some tumors to chemotherapeutic drugs. Genomic variation in the expression of these transporters has a profound effect on sensitivity to these drugs (see Chapter 5). Several other transporter families have been identified that do not bind ATP but use ion gradients to drive transport. Some of these (the solute carrier [SLC] family) are particularly important in the uptake of neurotransmitters across nerve-ending membranes. The latter carriers are discussed in more detail in Chapter 6.

TABLE 1–3 Some transport molecules important in pharmacology.

Transporter	Physiologic Function	Pharmacologic Significance
NET	Norepinephrine reuptake from synapse	Target of cocaine and some tricyclic antidepressants
SERT	Serotonin reuptake from synapse	Target of selective serotonin reuptake inhibitors and some tricyclic antidepressants
VMAT	Transport of dopamine and norepinephrine into adrenergic vesicles in nerve endings	Target of reserpine and tetrabenazine
MDR1	Transport of many xenobiotics out of cells	Increased expression confers resistance to certain anticancer drugs; inhibition increases blood levels of digoxin
MRP1	Leukotriene secretion	Confers resistance to certain anticancer and antifungal drugs
OCT	Organic cationic compound uptake	Target for uptake of organic cationic compounds (acetylcholine, dopamine, norepinephrine, metformin, etc) into many tissues including the brain, liver, heart, kidney
OATP	Organic anionic and neutral compound uptake	Target for uptake of organic anionic compounds (prostaglandins, hormones, bile salts, statins, etc) into many tissues including the brain, liver, kidney, intestine

MDR1, multidrug resistance protein-1; MRP1, multidrug resistance-associated protein-1; NET, norepinephrine transporter; OATP, organic anionic transporter polypeptide; OCT, organic cationic transporter; SERT, serotonin reuptake transporter; VMAT, vesicular monoamine transporter.

4. Endocytosis and exocytosis—A few substances are so large or impermeant that they can enter cells only by endocytosis, the process by which the substance is bound at a cell-surface receptor, engulfed by the cell membrane, and carried into the cell by pinching off of the newly formed vesicle inside the membrane. The substance can then be released into the cytosol by breakdown of the vesicle membrane (see Figure 1–4D). This process is responsible for the transport of vitamin B₁₂, complexed with a binding protein (intrinsic factor) across the wall of the gut into the blood. Similarly, iron is transported into hemoglobin-synthesizing red blood cell precursors in association with the protein transferrin. Specific receptors for the binding proteins must be present for this process to work.

The reverse process (exocytosis) is responsible for the secretion of many substances from cells. For example, many neurotransmitter substances are stored in membrane-bound vesicles in nerve endings to protect them from metabolic destruction in the cytoplasm. Appropriate activation of the nerve ending causes fusion of the storage vesicle with the cell membrane and expulsion of its contents into the extracellular space (see Chapter 6).

B. Fick's Law of Diffusion

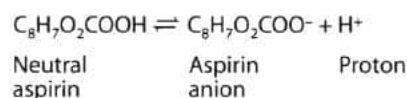
The passive flux of molecules down a concentration gradient is given by Fick's law:

$$\text{Flux (moles per unit time)} = (C_1 - C_2) \times \frac{\text{Area} \times \text{Permeability coefficient}}{\text{Thickness}}$$

where C_1 is the higher concentration, C_2 is the lower concentration, area is the cross-sectional area of the diffusion path, permeability coefficient is a measure of the mobility of the drug molecules in the medium of the diffusion path, and thickness is the length of the diffusion path. In the case of lipid diffusion, the lipid:aqueous partition coefficient is a major determinant of mobility of the drug because it determines how readily the drug enters the lipid membrane from the aqueous medium.

C. Ionization of Weak Acids and Weak Bases; the Henderson-Hasselbalch Equation

The electrostatic charge of an ionized molecule attracts water dipoles and results in a polar, relatively water-soluble and lipid-insoluble complex. Because lipid diffusion depends on relatively high lipid solubility, ionization of drugs may markedly reduce their ability to permeate membranes. A very large percentage of the drugs in use are weak acids or weak bases; Table 1–4 lists some examples. For drugs, a weak acid is best defined as a neutral molecule that can reversibly dissociate into an anion (a negatively charged molecule) and a proton (a hydrogen ion). For example, aspirin dissociates as follows:



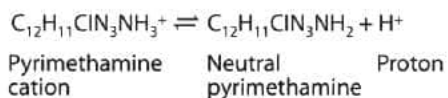
A weak base can be defined as a neutral molecule that can form a cation (a positively charged molecule) by combining with a

TABLE 1-4 Ionization constants of some common drugs.

Drug	pK _a ¹	Drug	pK _a ¹	Drug	pK _a ¹
Weak acids		Weak bases		Weak bases (cont'd)	
Acetaminophen	9.5	Albuterol (salbutamol)	9.3	Isoproterenol	8.6
Acetazolamide	7.2	Allopurinol	9.4, 12.3 ²	Lidocaine	7.9
Ampicillin	2.5	Alprenolol	9.6	Metaraminol	8.6
Aspirin	3.5	Amiloride	8.7	Methadone	8.4
Atorvastatin	4.3	Amiodarone	6.6	Methamphetamine	10.0
Chlorothiazide	6.8, 9.4 ²	Amphetamine	9.8	Methyldopa	10.6
Chlorpropamide	5.0	Atropine	9.7	Metoprolol	9.8
Ciprofloxacin	6.1, 8.7 ²	Bupivacaine	8.1	Morphine	7.9
Cromolyn	2.0	Chlordiazepoxide	4.6	Nicotine	7.9, 3.1 ²
Ethacrynic acid	2.5	Chloroquine	10.8, 8.4	Norepinephrine	8.6
Furosemide	3.9	Chlorpheniramine	9.2	Pentazocine	7.9
Ibuprofen	4.4, 5.2 ²	Chlorpromazine	9.3	Phenylephrine	9.8
Levodopa	2.3	Clonidine	8.3	Physostigmine	7.9, 1.8 ²
Methotrexate	4.8	Cocaine	8.5	Pilocarpine	6.9, 1.4 ²
Methyldopa	2.2, 9.2 ²	Codeine	8.2	Pindolol	8.6
Penicillamine	1.8	Cyclizine	8.2	Procainamide	9.2
Pentobarbital	8.1	Desipramine	10.2	Procaine	9.0
Phenobarbital	7.4	Diazepam	3.0	Promethazine	9.1
Phenytoin	8.3	Diphenhydramine	8.8	Propranolol	9.4
Propylthiouracil	8.3	Diphenoxylate	7.1	Pseudoephedrine	9.8
Salicylic acid	3.0	Ephedrine	9.6	Pyrimethamine	7.0–7.3 ³
Sulfadiazine	6.5	Epinephrine	8.7	Quinidine	8.5, 4.4 ²
Sulfapyridine	8.4	Ergotamine	6.3	Scopolamine	8.1
Theophylline	8.8	Fluphenazine	8.0, 3.9 ²	Strychnine	8.0, 2.3 ²
Tolbutamide	5.3	Hydralazine	7.1	Terbutaline	10.1
Warfarin	5.0	Imipramine	9.5	Thioridazine	9.5

¹The pK_a is that pH at which the concentrations of the ionized and nonionized forms are equal.²More than one ionizable group.³Isoelectric point.

proton. For example, pyrimethamine, an antimalarial drug, undergoes the following association-dissociation process:



Note that the protonated form of a weak acid is the neutral, more lipid-soluble form, whereas the unprotonated form of a weak base is the neutral form. The law of mass action requires that these reactions move to the left in an acid environment (low pH, excess protons available) and to the right in an alkaline environment. The Henderson-Hasselbalch equation relates the ratio of protonated to unprotonated weak acid or weak base to the molecule's pK_a and the pH of the medium as follows:

$$\log \frac{(\text{Protonated})}{(\text{Unprotonated})} = \text{pK}_a - \text{pH}$$

This equation applies to both acidic and basic drugs. Inspection confirms that the lower the pH relative to the pK_a, the greater will be the fraction of drug in the protonated form. Because the uncharged form is the more lipid-soluble, more of a weak acid will be in the lipid-soluble form at acid pH, whereas more of a basic drug will be in the lipid-soluble form at alkaline pH.

Application of this principle is made in the manipulation of drug excretion by the kidney (see Case Study). Almost all drugs are filtered at the glomerulus. If a drug is in a lipid-soluble form during its passage down the renal tubule, a significant fraction will be reabsorbed back into the tissue by simple passive diffusion. If the goal is to accelerate excretion of the drug (eg, in a case of drug overdose), it is important to prevent its reabsorption from the tubule. This can often be accomplished by adjusting urine pH to make certain that most of the drug is in the ionized state, as shown in Figure 1-5. As a result of this partitioning effect, the drug is “trapped” in the urine. Thus, weak acids are usually excreted faster in alkaline urine; weak bases are

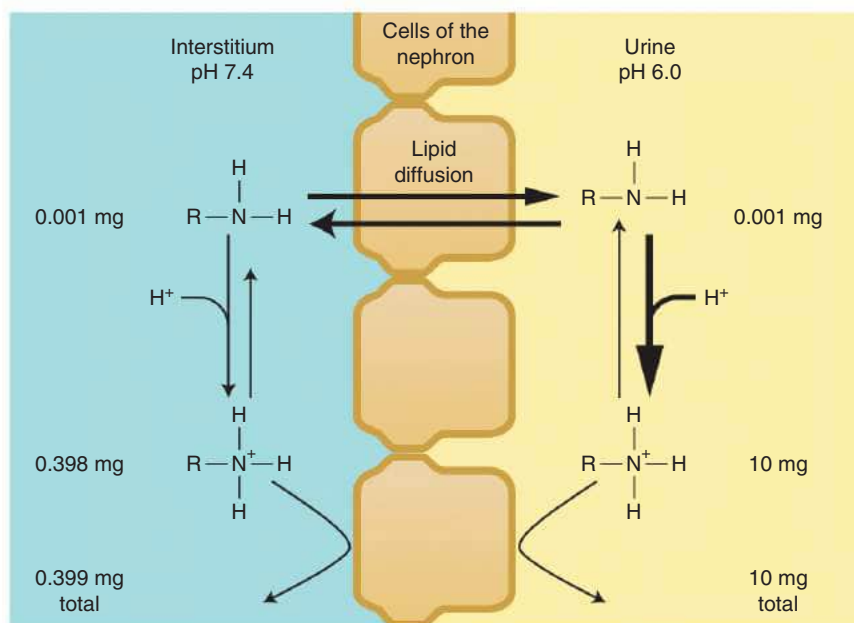
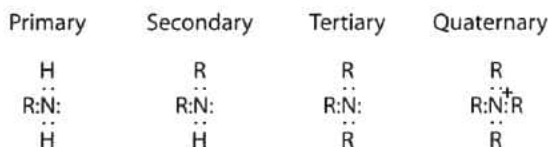


FIGURE 1–5 Trapping of a weak base (methamphetamine) in the urine when the urine is more acidic than the blood. In the hypothetical case illustrated, the diffusible uncharged form of the drug has equilibrated across the membrane, but the total concentration (charged plus uncharged) in the urine (more than 10 mg) is 25 times higher than in the blood (0.4 mg).

usually excreted faster in acidic urine. Other body fluids in which pH differences from blood pH may cause trapping or reabsorption are the contents of the stomach (normal pH 1.9–3) and small intestine (pH 7.5–8), breast milk (pH 6.4–7.6), aqueous humor (pH 6.4–7.5), and vaginal and prostatic secretions (pH 3.5–7).

As indicated by Table 1–4, a large number of drugs are weak bases. Most of these bases are amine-containing molecules. The nitrogen of a neutral amine has three atoms associated with it plus a pair of unshared electrons (see the display that follows). The three atoms may consist of one carbon or a chain of carbon atoms (designated “R”) and two hydrogens (a **primary amine**), two carbons and one hydrogen (a **secondary amine**), or three carbon atoms (a **tertiary amine**). Each of these three forms may reversibly bind a proton with the unshared electrons. Some drugs have a fourth carbon-nitrogen bond; these are **quaternary amines**. However, the quaternary amine is permanently charged and has no unshared electrons with which to reversibly bind a proton. Therefore, primary, secondary, and tertiary amines may undergo reversible protonation and vary their lipid solubility with pH, but quaternary amines are always in the poorly lipid-soluble charged form.



DRUG GROUPS

To learn each pertinent fact about each of the many hundreds of drugs mentioned in this book would be an impractical goal and, fortunately, is unnecessary. Almost all the several thousand drugs

currently available can be arranged into about 70 groups. Many of the drugs within each group are very similar in pharmacodynamic actions and in their pharmacokinetic properties as well. For most groups, one or two **prototype drugs** can be identified that typify the most important characteristics of the group. This permits classification of other important drugs in the group as variants of the prototype, so that only the prototype must be learned in detail and, for the remaining drugs, only the differences from the prototype.

■ DRUG DEVELOPMENT & REGULATION

A truly new drug (one that does not simply mimic the structure and action of previously available drugs) requires the discovery of a new drug target, ie, the pathophysiologic process or substrate of a disease. Such discoveries are usually made in public sector institutions (universities and research institutes), and molecules that have beneficial effects on such targets are often discovered in the same laboratories. However, the development of new drugs usually takes place in industrial laboratories because optimization of a class of new drugs requires painstaking and expensive chemical, pharmacologic, and toxicologic research. In fact, much of the recent progress in the application of drugs to disease problems can be ascribed to the pharmaceutical industry including “big pharma,” the multibillion-dollar corporations that specialize in drug development and marketing. These companies are uniquely skilled in translating basic findings into successful therapeutic breakthroughs and profit-making “blockbusters” (see <https://clincalc.com/DrugStats/Top200Drugs.aspx>).

Such breakthroughs come at a price, however, and the escalating cost of drugs has become a significant contributor to the increase in the cost of health care. Development of new drugs is enormously

expensive, but considerable controversy surrounds drug pricing. Drug prices in the United States have increased many-fold faster than overall price inflation. Critics claim that the costs of development and marketing drugs are grossly inflated by marketing activities, advertising, and other promotional efforts, which may consume as much as 25% or more of a company's budget. Furthermore, profit margins for big pharma are relatively high. Recent drug-pricing scandals have been reported in which the rights to an older, established drug that requires no costly development have been purchased by a smaller company and the price increased by several hundred or several thousand percent. This "price gouging" has caused public outrage and attracted regulatory attention that may result in more legitimate and rational pricing mechanisms. Finally, pricing schedules for many drugs vary dramatically from country to country and even within countries, where large organizations can negotiate favorable prices and small ones cannot. Some countries have already addressed these inequities, and it seems likely that all countries will have to do so during the next few decades.

NEW DRUG DEVELOPMENT

The development of a new drug usually begins with the discovery or synthesis of a potential new drug compound or the elucidation of a new drug target. After a new drug molecule is synthesized or extracted from a natural source, subsequent steps seek an understanding of the drug's interactions with its biologic targets. Repeated application of this approach leads to synthesis of related compounds with increased efficacy, potency, and selectivity (Figure 1–6). In the United States, the safety and efficacy of drugs must be established before marketing can be legally carried out. In addition to *in vitro* studies, relevant biologic effects,

drug metabolism, pharmacokinetic profiles, and relative safety of the drug are recommended to be characterized *in vivo* in animals before human drug trials can be started but not necessary if studies can be carried out in cells or alternative approaches. With regulatory approval, human testing may then go forward (usually in three phases) before the drug is considered for approval for general use. A fourth phase of data gathering and safety monitoring is becoming increasingly important and follows after approval for marketing. Once approved, the great majority of drugs become available for use by any appropriately licensed practitioner. Highly toxic drugs that are nevertheless considered valuable in lethal diseases may be approved for restricted use by practitioners who have undergone special training in their use and who maintain detailed records.

DRUG DISCOVERY

Most new drugs or drug products are discovered or developed through the following approaches: (1) screening for biologic activity of large numbers of natural products, banks of previously discovered chemical entities, or large libraries of peptides, nucleic acids, and other organic molecules; (2) chemical modification of a known active molecule, resulting in a "me-too" analog; (3) identification or elucidation of a new drug target, which suggests molecules that will hit that target; and (4) rational design of a new molecule based on an understanding of biologic mechanisms and drug receptor structure. Steps (3) and (4) are often carried out in academic research laboratories and are more likely to lead to breakthrough drugs, but the costs of steps (1) and (2) usually ensure that industry carries them out.

Once a new drug target or promising molecule has been identified, the process of moving from the basic science laboratory to the clinic begins. This **translational research** involves the preclinical and clinical

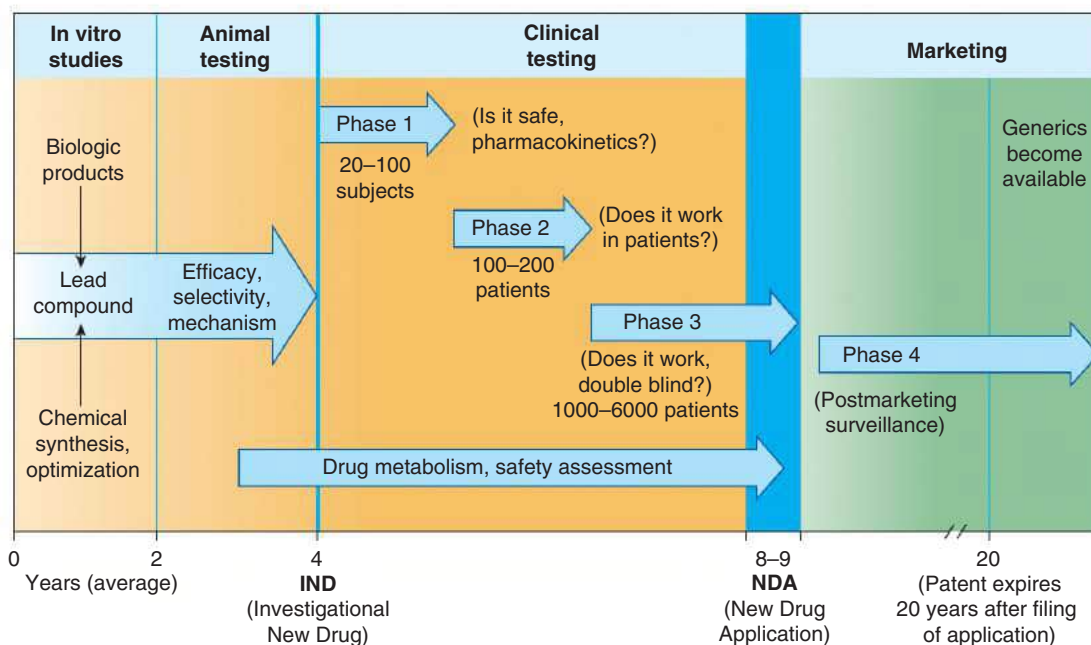


FIGURE 1–6 The development and testing process required to bring a drug to market in the USA. Some of the requirements may be different for drugs used in life-threatening diseases (see text).

steps described next. While clinical trials in humans are required only for drugs to be used in humans, all of the other steps described apply to veterinary drugs as well as drugs for human diseases.

Drug Screening

Drug screening involves a variety of assays at the molecular, cellular, organ system, and whole animal levels to define the **pharmacologic profile**, ie, the activity and selectivity of the drug. The type and number of initial screening tests depend on the pharmacologic and therapeutic goal. For example, anti-infective drugs are tested against a variety of infectious organisms, some of which are resistant to standard agents; hypoglycemic drugs are tested for their ability to lower blood sugar, etc.

The candidate molecule is also studied for a broad array of other actions to determine the mechanism of action and selectivity of the drug. This can reveal both expected and unexpected toxic effects. Occasionally, an unexpected therapeutic action is serendipitously discovered by a careful observer; for example, the era of modern diuretics was initiated by the observation that certain antimicrobial sulfonamides caused metabolic acidosis in patients. The selection of compounds for development is most efficiently conducted in animal models of human disease. Where good predictive preclinical models exist (eg, infection, hypertension, or thrombotic disease), we generally have good or excellent drugs. Good drugs or breakthrough improvements are conspicuously lacking and slow for diseases for which preclinical models are poor or not yet available, eg, autism and Alzheimer disease.

At the molecular level, the candidate compound would be screened for activity on the target, for example, receptor binding affinity to cell membranes containing the homologous animal receptors (or if possible, on the cloned human receptors). Early studies would be done to predict effects that might later cause undesired drug metabolism or toxicologic complications. For example, studies on liver cytochrome P450 enzymes would be performed to determine whether the molecule of interest is likely to be a substrate or inhibitor of these enzymes or to alter the metabolism of other drugs.

Effects on cell function determine whether the drug is an agonist, partial agonist, inverse agonist, or antagonist at relevant receptors. Isolated tissues would be used to characterize the pharmacologic activity and selectivity of the new compound in comparison with reference compounds. Comparison with other drugs would also be undertaken in a variety of *in vivo* studies. At each step in this process, the compound would have to meet specific performance and selectivity criteria to be carried further.

Whole animal studies are generally necessary to determine the effect of the drug on organ systems and disease models. Cardiovascular and renal function studies of new drugs are generally first performed in normal animals. Studies on disease models, if available, are then performed. For a candidate antihypertensive drug, animals with hypertension would be treated to see whether blood pressure was lowered in a dose-related manner and to characterize other effects of the compound. Evidence would be collected on duration of action and efficacy after oral and parenteral administration. If the agent possessed useful activity, it would be further

studied for possible adverse effects on other organs, including the respiratory, gastrointestinal, renal, endocrine, and central nervous systems.

These studies might suggest the need for further chemical modification (compound optimization) to achieve more desirable pharmacokinetic or pharmacodynamic properties. For example, oral administration studies might show that the drug was poorly absorbed or rapidly metabolized in the liver; modification to improve bioavailability might be indicated. If the drug is to be administered long term, an assessment of tolerance development would be made. For drugs related to or having mechanisms of action similar to those known to cause physical or psychological dependence in humans, ability to cause dependence in animals would also be studied. Drug interactions would be examined.

The desired result of this screening procedure (which may have to be repeated several times with congeners of the original molecule) is a **lead compound**, ie, a leading candidate for a successful new drug. A patent application would be filed for a novel compound (a *composition of matter* patent) that is efficacious, or for a new and nonobvious therapeutic use (a *use* patent) for a previously known chemical entity.

PRECLINICAL SAFETY & TOXICITY TESTING

All chemicals are toxic in some individuals at some dose. Candidate drugs that survive the initial screening procedures must be carefully evaluated for potential risks before and during clinical testing. Depending on the proposed use of the drug, preclinical toxicity testing includes most or all of the procedures shown in Table 1–5. Although no chemical can be certified as completely “safe” (free of risk), the objective is to estimate the risk associated with exposure to the drug candidate and to consider this in the context of therapeutic needs and likely duration of drug use.

The goals of preclinical toxicity studies include identifying potential human toxicities, designing tests to further define the toxic mechanisms, and predicting the most relevant toxicities to be monitored in clinical trials. In addition to the studies shown in Table 1–5, several quantitative estimates are desirable. These include the **no-effect dose**—the maximum dose at which a specified toxic effect is not seen; the **minimum lethal dose**—the smallest dose that is observed to kill any experimental animal; and, if necessary, the **median lethal dose (LD₅₀)**—the dose that kills approximately 50% of the animals in a test group. Presently, the LD₅₀ is estimated from the smallest number of animals possible. These doses are used to calculate the initial dose to be tried in humans, usually taken as one hundredth to one tenth of the no-effect dose in animals.

It is important to recognize the limitations of preclinical testing. These include the following:

1. Toxicity testing is time-consuming and expensive. Two to 6 years may be required to collect and analyze data on toxicity before the drug can be considered ready for testing in humans.

TABLE 1-5 Safety tests.

Type of Test	Approach and Goals
Acute toxicity	Usually two species, two routes. Determine the no-effect dose and the maximum tolerated dose. In some cases, determine the acute dose that is lethal in approximately 50% of animals.
Subacute or subchronic toxicity	Three doses, two species. Two weeks to 3 months of testing may be required before clinical trials. The longer the duration of expected clinical use, the longer the subacute test. Determine biochemical, physiologic effects.
Chronic toxicity	Rodent and at least one nonrodent species for ≥ 6 months. Required when drug is intended to be used in humans for prolonged periods. Usually run concurrently with clinical trials. Determine same end points as subacute toxicity tests.
Effect on reproductive performance	Two species, usually one rodent and rabbits. Test effects on animal mating behavior, reproduction, parturition, progeny, birth defects, postnatal development.
Carcinogenic potential	Two years, two species. Required when drug is intended to be used in humans for prolonged periods. Determine gross and histologic pathology.
Mutagenic potential	Test effects on genetic stability and mutations in bacteria (Ames test) or mammalian cells in culture; dominant lethal test and clastogenicity in mice.

2. Large numbers of animals may be needed to obtain valid preclinical data. Scientists are properly concerned about this situation, and progress has been made toward reducing the numbers required while still obtaining valid data. Cell and tissue culture in vitro methods and computer modeling are increasingly being used, but their predictive value is still limited. Nevertheless, some segments of the public attempt to halt all animal testing in the unfounded belief that it has become unnecessary.
3. Extrapolations of toxicity data from animals to humans are reasonably predictive for many but not for all toxicities.
4. For statistical reasons, rare adverse effects are unlikely to be detected in preclinical testing.

EVALUATION IN HUMANS

A very small fraction of lead compounds reach clinical trials, and less than one-third of the drugs studied in humans survive clinical trials and reach the marketplace. Federal law in the USA and ethical considerations require that the study of new drugs in humans be conducted in accordance with stringent guidelines. Scientifically valid results are not guaranteed simply by conforming to government regulations, however, and the design and execution of a good clinical trial require interdisciplinary personnel including basic scientists, clinical pharmacologists, clinician specialists, statisticians, and others. The current standard for clinical trials is known as the randomized controlled trial (RCT) and is described below. The need for careful design and execution is based on three major confounding factors inherent in the study of any drug in humans.

Confounding Factors in Clinical Trials

A. The Variable Natural History of Most Diseases

Many diseases tend to wax and wane in severity; some disappear spontaneously, even, on occasion, cancer. A good experimental design takes into account the natural history of the disease by evaluating a large enough population of subjects over a sufficient period of time. Further protection against errors of interpretation caused by disease fluctuations is sometimes provided by using a **crossover design**, which consists of alternating periods of administration of test drug, placebo preparation (the control), and the previous standard treatment (positive control), if any, in each subject. These sequences are systematically varied so that different subsets of patients receive each of the possible sequences of treatment.

B. The Presence of Other Diseases and Risk Factors

Known and unknown diseases and risk factors (including lifestyles of subjects) may influence the results of a clinical study. For example, some diseases alter the pharmacokinetics of drugs (see Chapters 3 through 5). Other drugs and some foods alter the pharmacokinetics of many drugs. Concentrations of blood or tissue components being monitored as a measure of the effect of the new agent may be influenced by other diseases or other drugs. Attempts to avoid this hazard usually involve the crossover technique (when feasible) and proper selection and assignment of patients to each of the study groups. This requires obtaining accurate diagnostic tests and medical and pharmacologic histories (including use of recreational drugs, over-the-counter drugs, and “supplements”) and the use of statistically valid methods of randomization in assigning subjects to particular study groups. There is growing interest in analyzing genetic variations as part of the trial that may influence whether a person responds to a particular drug. It has been shown that age, gender, and pregnancy influence the pharmacokinetics of some drugs, but these factors have not been adequately studied because of legal restrictions and reluctance to expose these populations to unknown risks.

C. Subject and Observer Bias and Other Factors

Most patients tend to respond in a positive way to any therapeutic intervention by interested, caring, and enthusiastic medical personnel. The manifestation of this phenomenon in the subject is the **placebo response** (Latin, “I shall please”) and may involve objective physiologic and biochemical changes as well as changes in subjective complaints associated with the disease. The placebo response is usually quantitated by administration of an inert material with exactly the same physical appearance, odor, consistency, etc., as the active dosage form. The magnitude of the response varies considerably from patient to patient and may also be influenced by the duration of the study. In some conditions, a positive response may be noted in as many as 30–40% of subjects given placebo. Placebo adverse effects and “toxicity” also occur but usually involve subjective effects: stomach upset, insomnia, sedation, and so on.

Subject bias effects can be quantitated—and minimized relative to the response measured during active therapy—by the **single-blind** design. This involves use of a placebo as described above, administered to the same subjects in a crossover design, if possible, or to a separate control group of well-matched subjects.

Observer bias can be taken into account by disguising the identity of the medication being used—placebo or active form—from both the subjects and the personnel evaluating the subjects' responses (**double-blind** design). In this design, a third party holds the code identifying each medication packet, and the code is not broken until all the clinical data have been collected.

Drug effects seen in clinical trials are obviously affected by the patient taking the drugs at the dose and frequency

prescribed. In a recent phase 2 study, one-third of the patients who said they were taking the drug were found by blood analysis to have not taken the drug. Confirmation of **compliance** with protocols (also known as **adherence**) is a necessary element to consider.

The various types of studies and the conclusions that may be drawn from them are described in the accompanying text box. (See Box: Drug Studies—The Types of Evidence.)

Drug Studies—The Types of Evidence*

As described in this chapter, drugs are studied in a variety of ways, from 30-minute test tube experiments with isolated enzymes and receptors to decades-long observations of populations of patients. The conclusions that can be drawn from such different types of studies can be summarized as follows.

Basic research is designed to answer specific, usually single, questions under tightly controlled laboratory conditions, eg, does drug x inhibit enzyme y? The basic question may then be extended, eg, if drug x inhibits enzyme y, what is the concentration-response relationship? Such experiments are usually reproducible and often lead to reliable insights into the mechanism of the drug's action.

First-in-human studies include phase 1–3 trials. Once a drug receives FDA approval for use in humans, *case reports* and *case series* consist of observations by clinicians of the effects of drug (or other) treatments in one or more patients. These results often reveal unpredictable benefits and toxicities but do not generally test a prespecified hypothesis and cannot prove cause and effect. *Analytic* epidemiologic studies consist of observations designed to test a specified hypothesis, eg, that thiazolidinedione antidiabetic drugs are associated with adverse cardiovascular events. *Cohort* epidemiologic studies utilize populations of patients that have (exposed group) and have not (control group) been exposed to the agents under study and ask whether the exposed groups show a higher or lower incidence of the effect. *Case-control* epidemiologic studies utilize populations of patients that have displayed the end point under study and ask whether they

have been exposed or not exposed to the drugs in question. Such epidemiologic studies add weight to conjectures but cannot control all confounding variables and therefore cannot conclusively prove cause and effect.

Meta-analyses utilize rigorous evaluation and grouping of similar studies to increase the number of subjects studied and hence the statistical power of results obtained in multiple published studies. While the numbers may be dramatically increased by meta-analysis, the individual studies still suffer from their varying methods and end points, and a meta-analysis cannot prove cause and effect.

Large randomized controlled trials (RCTs) are designed to answer specific questions about the effects of medications on clinical end points or important surrogate end points, using large enough samples of patients and allocating them to control and experimental treatments using rigorous randomization methods. Randomization is the best method for distributing all foreseen confounding factors, as well as unknown confounders, equally between the experimental and control groups. When properly carried out, such studies are rarely invalidated and are considered the gold standard in evaluating drugs.

A critical factor in evaluating the data regarding a new drug is *access to all the data*. Unfortunately, many large studies are never published because the results are negative, ie, the new drug is *not* better than the standard therapy. This *missing data* phenomenon falsely exaggerates the benefits of new drugs because negative results are hidden.

*We thank Ralph Gonzales, MD, for helpful comments.

The Food & Drug Administration

The FDA is the administrative body that oversees the drug evaluation process in the USA and grants approval for marketing of new drug products. To receive FDA approval for marketing, the originating institution or company (almost always the latter) must submit evidence of safety and effectiveness. Outside the USA, the regulatory and drug approval process is generally similar to that in the USA.

As its name suggests, the FDA is also responsible for certain aspects of food safety, a role it shares with the US Department of Agriculture (USDA). Shared responsibility results in complications when questions arise regarding the use of drugs, eg, antibiotics, in

food animals. A different type of problem arises when so-called food supplements are found to contain active drugs, eg, sildenafil analogs in “energy food” supplements.

The FDA's authority to regulate drugs derives from specific legislation (Table 1–6). If a drug has not been shown through adequately controlled testing to be “safe and effective” for a specific use, it cannot be marketed in interstate commerce for this use.*

*Although the FDA does not directly control drug commerce within states, a variety of state and federal laws control interstate production and marketing of drugs.

TABLE 1–6 Some major legislation pertaining to drugs in the USA.

Law	Purpose and Effect
Pure Food and Drug Act of 1906	Prohibited mislabeling and adulteration of drugs.
Opium Exclusion Act of 1909	Prohibited importation of opium.
Amendment (1912) to the Pure Food and Drug Act	Prohibited false or fraudulent advertising claims.
Harrison Narcotic Act of 1914	Established regulations for use of opium, opiates, and cocaine (marijuana added in 1937).
Food, Drug, and Cosmetic Act of 1938	Required that new drugs be safe as well as pure (but did not require proof of efficacy). Enforcement by the FDA.
Durham-Humphrey Act of 1952	Vested in the FDA the power to determine which products could be sold without prescription.
Kefauver-Harris Amendments (1962) to the Food, Drug, and Cosmetic Act	Required proof of efficacy as well as safety for new drugs and for drugs released since 1938; established guidelines for reporting of information about adverse reactions, clinical testing, and advertising of new drugs.
Comprehensive Drug Abuse Prevention and Control Act (1970)	Outlined strict controls in the manufacture, distribution, and prescribing of habit-forming drugs; established drug schedules and programs to prevent and treat drug addiction.
Orphan Drug Amendment of 1983	Provided incentives for development of drugs that treat diseases with fewer than 200,000 patients in the USA.
Drug Price Competition and Patent Restoration Act of 1984	Abbreviated new drug applications for generic drugs. Required bioequivalence data. Patent life extended by amount of time drug delayed by FDA review process. Cannot exceed 5 extra years or extend to more than 14 years post-NDA approval.
Prescription Drug User Fee Act (1992, reauthorized 2007, 2012)	Manufacturers pay user fees for certain new drug applications. “Breakthrough” products may receive special category approval after expanded phase 1 trials (2012).
Dietary Supplement Health and Education Act (1994)	Established standards with respect to dietary supplements but prohibited full FDA review of supplements and botanicals as drugs. Required the establishment of specific ingredient and nutrition information labeling that defines dietary supplements and classifies them as part of the food supply but allows unregulated advertising.
Bioterrorism Act of 2002	Enhanced controls on dangerous biologic agents and toxins. Seeks to protect safety of food, water, and drug supply.
Food and Drug Administration Amendments Act of 2007	Granted the FDA greater authority over drug marketing, labeling, and direct-to-consumer advertising; required post-approval studies, established active surveillance systems, made clinical trial operations and results more visible to the public.
Biologics Price Competition and Innovation Act of 2009	Authorized the FDA to establish a program of abbreviated pathways for approval of “biosimilar” biologics (generic versions of monoclonal antibodies, etc).
FDA Safety and Innovation Act of 2012	Renewed FDA authorization for accelerated approval of urgently needed drugs; established new accelerated process, “breakthrough therapy,” in addition to “priority review,” “accelerated approval,” and “fast-track” procedures.
Drug Quality and Security Act of 2013	Granted the FDA more authority to regulate and monitor the manufacturing of compounded drugs.

Unfortunately, “safe” can mean different things to the patient, the physician, and society. Complete absence of risk is impossible to demonstrate, but this fact may not be understood by members of the public, who frequently assume that any medication sold with the approval of the FDA should be free of serious “side effects.” This confusion is a major factor in litigation and dissatisfaction with aspects of drugs and medical care.

The history of drug regulation in the USA (see Table 1–6) reflects several health events that precipitated major shifts in public opinion. For example, the Federal Food, Drug, and Cosmetic Act of 1938 was largely a reaction to deaths associated with the use of a preparation of sulfanilamide marketed before it and its vehicle were adequately tested. Similarly, the Kefauver-Harris Amendments of 1962 were, in part, the result of a teratogenic drug disaster involving thalidomide. This agent was introduced in Europe in 1957–1958 and was marketed as a “nontoxic” hypnotic and promoted as being especially useful as a sleep aid during pregnancy. In 1961, reports

were published suggesting that thalidomide was responsible for a dramatic increase in the incidence of a rare birth defect called phocomelia, a condition involving shortening or complete absence of the arms and legs. Epidemiologic studies provided strong evidence for the association of this defect with thalidomide use by women during the first trimester of pregnancy, and the drug was withdrawn from sale worldwide. An estimated 10,000 children were born with birth defects because of maternal exposure to this one agent. The tragedy led to the requirement for more extensive testing of new drugs for teratogenic effects and stimulated passage of the Kefauver-Harris Amendments of 1962, even though the drug was not then approved for use in the USA. Despite its disastrous fetal toxicity and effects in pregnancy, thalidomide is a relatively safe drug for humans other than the fetus. Even the most serious risk of toxicities may be avoided or managed if understood, and despite its toxicity, thalidomide is now approved by the FDA for limited use as a potent immunoregulatory agent and to treat certain forms of leprosy.

Clinical Trials: The IND & NDA

Once a new drug is judged ready to be studied in humans, a Notice of Claimed Investigational Exemption for a New Drug (IND) must be filed with the FDA (see Figure 1–6). The IND includes (1) information on the composition and source of the drug, (2) chemical and manufacturing information, (3) all data from animal studies, (4) proposed plans for clinical trials, (5) the names and credentials of physicians who will conduct the clinical trials, and (6) a compilation of the key preclinical data relevant to study of the drug in humans that have been made available to investigators and their institutional review boards.

It often requires 4–6 years of clinical testing to accumulate and analyze all required data. Testing in humans is begun only after sufficient acute and subacute animal toxicity studies have been completed. Chronic safety testing in animals, including carcinogenicity studies, is usually done concurrently with clinical trials. In each phase of the clinical trials, volunteers or patients must be informed of the investigational status of the drug as well as the possible risks and must be allowed to decline or to consent to participate and receive the drug. In addition to the approval of the sponsoring organization and the FDA, an interdisciplinary institutional review board (IRB) at each facility where the clinical drug trial will be conducted must review and approve the scientific and ethical plans for testing in humans.

In **phase 1**, the effects of the drug as a function of dosage are established in a small number (20–100) of healthy volunteers. If the drug is expected to have significant toxicity, as may be the case in cancer and AIDS therapy, volunteer patients with the disease participate in phase 1 rather than normal volunteers. Phase 1 trials are done to determine the probable limits of the safe clinical dosage range. These trials may be nonblind or “open”; that is, both the investigators and the subjects know what is being given. Alternatively, they may be “blinded” and placebo controlled. Many predictable toxicities are detected in this phase. Pharmacokinetic measurements of absorption, half-life, and metabolism are often done. Phase 1 studies are usually performed in research centers by specially trained clinical pharmacologists.

In **phase 2**, the drug is studied in patients with the target disease to determine its efficacy (“proof of concept” studies), and the doses to be used in any follow-on trials. A modest number of patients (100–200) are studied in detail. A single-blind design may be used, with an inert placebo medication and an established active drug (positive control) in addition to the investigational agent. Phase 2 trials are usually done in special clinical centers (eg, university hospitals). A broader range of toxicities may be detected in this phase. Phase 2 trials have the highest rate of drug failures, and only 25% of innovative drugs move on to phase 3.

In **phase 3**, the drug is evaluated in much larger numbers—usually thousands—of patients with the target disease to further establish and confirm safety and efficacy. Using information gathered in phases 1 and 2, phase 3 trials are designed to minimize errors caused by placebo effects, variable course of the disease, etc. Therefore, double-blind and crossover techniques are often used. Phase 3 trials are usually performed in settings similar to those anticipated for the ultimate use of the drug. Phase 3 studies can be difficult to

design and execute and are usually expensive because of the large numbers of patients involved and the masses of data that must be collected and analyzed. The drug is formulated as intended for the market. The investigators are usually specialists in the disease being treated. Certain toxic effects, especially those caused by immunologic processes, may first become apparent in phase 3.

If phase 3 results meet expectations, application is made for permission to market the new agent. Marketing approval requires submission of a New Drug Application (NDA)—or for biologicals, a Biological License Application (BLA)—to the FDA. The application contains, often in hundreds of volumes, full reports of all preclinical and clinical data pertaining to the drug under review. The number of subjects studied in support of new drug applications has been increasing and currently averages more than 5000 patients for new drugs of novel structure (new molecular entities). The duration of the FDA review leading to approval (or denial) of the new drug application may vary from months to years. If problems arise, eg, unexpected but possibly serious toxicities, additional studies may be required and the review process may extend to several additional years.

Many phase 2 and phase 3 studies attempt to measure a new drug’s “noninferiority” to the placebo or a standard treatment. Interpretation of the results may be difficult because of unexpected confounding variables, loss of subjects from some groups, or realization that results differ markedly between certain subgroups within the active treatment (new drug) group. Older statistical methods for evaluating drug trials often fail to provide definitive answers when these problems arise. Therefore, new “adaptive” statistical methods are under development that allow changes in the study design when interim data evaluation indicates the need. Preliminary results with such methods suggest that they may allow decisions regarding superiority as well as noninferiority, shortening of trial duration, discovery of new therapeutic benefits, and more reliable conclusions regarding the results (see Bhatt & Mehta, 2016).

In cases of urgent need (eg, cancer chemotherapy), the process of preclinical and clinical testing and FDA review may be accelerated. For serious diseases, the FDA may permit extensive but controlled marketing of a new drug before phase 3 studies are completed; for life-threatening diseases, it may permit controlled marketing even before phase 2 studies have been completed. “Fast track,” “priority approval,” and “accelerated approval” are FDA programs that are intended to speed entry of new drugs into the marketplace. In 2012, an additional special category of “breakthrough” products (eg, for cystic fibrosis) was approved for restricted marketing after expanded phase 1 trials (see Table 1–6). Roughly 50% of drugs in phase 3 trials enter early, controlled marketing. Such accelerated approval is usually granted with the requirement that careful monitoring of the effectiveness and toxicity of the drug be carried out and reported to the FDA. Unfortunately, FDA enforcement of this requirement has not always been adequate.

Once approval to market a drug has been obtained, **phase 4** begins. This constitutes monitoring the safety of the new drug under actual conditions of use in large numbers of patients. The importance of careful and complete reporting of toxicity by physicians after marketing begins can be appreciated by noting that

many important drug-induced effects have an incidence of 1 in 10,000 or less and that some adverse effects may become apparent only after chronic dosing. The sample size required to disclose drug-induced events or toxicities is very large for such rare events. For example, several hundred thousand patients may have to be exposed before the first case is observed of a toxicity that occurs with an average incidence of 1 in 10,000. Therefore, low-incidence drug effects are not generally detected before phase 4 no matter how carefully phase 1, 2, and 3 studies are executed. Phase 4 has no fixed duration. As with monitoring of drugs granted accelerated approval, phase 4 monitoring has often been lax.

The time from the filing of a patent application (which usually precedes clinical trials) to approval for marketing of a new drug may be 5 years or considerably longer. Since the lifetime of a patent is 20 years in the USA, the owner of the patent (usually a pharmaceutical company) has exclusive rights for marketing the product for only a limited time after approval of the new drug application. Because the FDA review process itself can be lengthy (300–500 days for evaluation of an NDA), the time consumed by the review is sometimes added to the patent life. However, the extension (up to 5 years) cannot increase the total life of the patent to more than 14 years after approval of a new drug application. The Patient Protection and Affordable Care Act of 2010 provides for 12 years of patent protection for new drugs. After expiration of the patent, any company may produce the drug, file an abbreviated new drug application (ANDA), demonstrate required equivalence, and, with FDA approval, market the drug as a **generic** product without paying license fees to the original patent owner. Currently, more than half of prescriptions in the USA are for generic drugs. Even biotechnology-based drugs such as antibodies and other proteins are now qualifying for generic (“biosimilar”) designation, and this has fueled regulatory concerns. More information on drug patents is available at the FDA website at <http://www.fda.gov/Drugs/DevelopmentApprovalProcess/ucm079031.htm>.

A **trademark** is a drug’s proprietary trade name and is usually registered; this registered name may be legally protected as long as it is used. A generically equivalent product, unless specially licensed, cannot be sold under the trademark name and is often designated by the official generic name. Generic prescribing is described in Chapter 66.

Conflicts of Interest

Several factors in the development and marketing of drugs result in conflicts of interest. Use of pharmaceutical industry funding to support FDA approval processes raises the possibility of conflicts of interest within the FDA. Supporters of this practice point out that chronic FDA underfunding by the government allows for few alternatives. Another important source of conflicts of interest is the dependence of the FDA on outside panels of experts who are recruited from the scientific and clinical community to advise the government agency on questions regarding drug approval or withdrawal. Such experts are often recipients of grants from the companies producing the drugs in question. The need for favorable data in the new drug application leads to phase 2 and 3 trials in which the new agent is compared only to placebo, not to older, effective

drugs. As a result, data regarding the efficacy and toxicity of the new drug *relative to a known effective agent* may not be available when the new drug is first marketed.

Manufacturers promoting a new agent may pay physicians to use it in preference to older drugs with which they are more familiar. Manufacturers sponsor small and often poorly designed clinical studies after marketing approval and aid in the publication of favorable results but may retard publication of unfavorable results. The need for physicians to meet continuing medical education (CME) requirements in order to maintain their licenses encourages manufacturers to sponsor conferences and courses, often in highly attractive vacation sites, and new drugs are often featured in such courses. The common practice of distributing free samples of new drugs to practicing physicians has both positive and negative effects. The samples allow physicians to try out new drugs without incurring any cost to the patient. On the other hand, new drugs are usually much more expensive than older agents, and when the free samples run out, the patient (or insurance carrier) may be forced to pay much more for treatment than if the older, cheaper, and possibly equally effective drug were used. Finally, when the patent for a drug is nearing expiration, the patent-holding manufacturer may try to extend its exclusive marketing status by paying generic manufacturers to *not* introduce a generic version (“pay to delay”).

Adverse Drug Reactions

An adverse drug event (ADE) or reaction to a drug (ADR) is a harmful or unintended response. Adverse drug reactions are claimed to be the fourth leading cause of death, higher than pulmonary disease, AIDS, accidents, and automobile deaths. The FDA has further estimated that 300,000 preventable adverse events occur annually in hospitals, many as a result of confusing medical information or lack of information (eg, regarding drug incompatibilities). Adverse reactions occurring only in certain susceptible patients include intolerance, idiosyncrasy (frequently genetic in origin), and allergy (usually immunologically mediated). During IND studies and clinical trials before FDA approval, all adverse events (serious, life-threatening, disabling, reasonably drug related, or unexpected) must be reported. After FDA approval to market a drug, surveillance, evaluation, and reporting must continue for any adverse events that are related to use of the drug, including overdose, accident, failure of expected action, events occurring from drug withdrawal, and unexpected events not listed in labeling. Events that are both serious and unexpected must be reported to the FDA within 15 days. The ability to predict and avoid adverse drug reactions and optimize a drug’s therapeutic index is an increasing focus of pharmacogenetic and personalized (also called “precision”) medicine. It has been suggested that greater use of electronic health records will reduce some of these risks (see Chapter 66). This hope has not yet been realized.

Orphan Drugs & Treatment of Rare Diseases

Drugs for rare diseases—so-called orphan drugs—can be difficult to research, develop, and market. Proof of drug safety and efficacy

in small populations must be established, but doing so is a complex process. Furthermore, because basic research in the pathophysiology and mechanisms of rare diseases receives relatively little attention or funding in both academic and industrial settings, recognized rational targets for drug action may be few. In addition, the cost of developing a drug can greatly influence priorities when the target population is relatively small. Funding for development of drugs for rare diseases or ignored diseases that do not receive priority attention from the traditional industry has received increasing support via philanthropy or similar funding from not-for-profit foundations such as the Cystic Fibrosis Foundation, the Michael J. Fox Foundation for Parkinson's Research, the Huntington's Disease Society of America, and the Gates Foundation.

The Orphan Drug Amendment of 1983 provides incentives for the development of drugs for treatment of a rare disease or condition defined as "any disease or condition which (a) affects less than 200,000 persons in the USA or (b) affects more than 200,000 persons in the USA but for which there is no reasonable expectation that the cost of developing and making available in the USA a drug for such disease or condition will be recovered from sales in the USA of such drug." Since 1983, the FDA has approved for marketing more than 300 orphan drugs to treat more than 82 rare diseases.

■ SOURCES OF INFORMATION

Students who wish to review the field of pharmacology in preparation for an examination are referred to *Pharmacology: Examination and Board Review*, by Katzung, Kruidering-Hall, Lalchandani Tuan, Vanderah, and Trevor (McGraw Hill, 2021). This book provides approximately 1000 questions and explanations in USMLE format. A short study guide is *USMLE Road Map: Pharmacology*, by Katzung and Trevor (McGraw Hill, 2006). *Road Map* contains numerous tables, figures, mnemonics, and USMLE-type clinical vignettes.

The references at the end of each chapter in this book were selected to provide reviews or classic publications of information specific to those chapters. More detailed questions relating to basic or clinical research are best answered by referring to the journals covering general pharmacology and clinical specialties. For the student and the physician, three periodicals can be recommended as especially useful sources of current information about drugs: *The New England Journal of Medicine*, which publishes much original drug-related clinical research as well as frequent reviews of topics in pharmacology; *The Medical Letter on Drugs and Therapeutics*, which publishes brief critical reviews of new and old therapies; and *Prescriber's Letter*, a monthly comparison of new and older drug therapies with much useful advice. On the Internet/World Wide Web, two sources can be particularly recommended: the Cochrane Collaboration and the FDA site (see reference list below).

Other sources of information pertinent to the United States should be mentioned as well. The "package insert" is a summary of information that the manufacturer is required to place in the prescription sales package; *Physicians' Desk Reference (PDR)* is a compendium of package inserts published annually with supplements twice a year. It is sold in bookstores and distributed to licensed

physicians. The package insert consists of a brief description of the pharmacology of the product. This brochure contains much practical information, but also lists every toxic effect ever reported, no matter how rare, thus shifting responsibility for adverse drug reactions from the manufacturer to the prescriber. A black box warning, if included, is of medicolegal importance, because it indicates a potentially lethal adverse effect. *UpToDate.com* is a large subscription website containing detailed descriptions of current standards of care, including drug therapy, for a wide variety of clinical conditions. *Micromedex* and *Lexi-Comp* are extensive subscription websites. They provide downloads for personal digital assistant devices, online drug dosage and interaction information, and toxicologic information. A useful and objective quarterly handbook that presents information on drug toxicity and interactions is *Drug Interactions: Analysis and Management*. Finally, the FDA maintains an Internet website that carries news regarding recent drug approvals, withdrawals, warnings, etc. It can be accessed at <http://www.fda.gov>. The MedWatch drug safety program is a free e-mail notification service that provides news of FDA drug warnings and withdrawals. Subscriptions may be obtained at <https://www.fda.gov/safety/medwatch-fda-safety-information-and-adverse-event-reporting-program>.

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CASE STUDY ANSWER

Aspirin overdose commonly causes a mixed respiratory alkalosis and metabolic acidosis. Because aspirin is a weak acid, serum acidosis favors entry of the drug into tissues (increasing toxicity), and urinary acidosis favors reabsorption of excreted drug back into the blood (prolonging the effects of the

overdose). Sodium bicarbonate, a weak base, is an important component of the management of aspirin overdose. It causes alkalosis, reducing entry into tissues, and increases the pH of the urine, enhancing renal clearance of the drug. See the discussion of the ionization of weak acids and weak bases in the text.

Drug Receptors & Pharmacodynamics

Mark von Zastrow, MD, PhD

CASE STUDY

A 51-year-old man presents to the emergency department due to acute difficulty breathing. The patient is afebrile and normotensive but anxious, tachycardic, and markedly tachypneic. Auscultation of the chest reveals diffuse wheezes. The clinician provisionally makes the diagnosis of bronchial asthma and administers epinephrine by intramuscular injection, improving the patient's breathing over several minutes. A normal chest x-ray and electrocardiogram are subsequently obtained, and

the medical history is remarkable only for mild hypertension that is being treated with propranolol. The clinician instructs the patient to discontinue use of propranolol and changes the patient's antihypertensive medication to verapamil. Why is the clinician correct to discontinue propranolol? Why is verapamil likely to be a more suitable choice for managing hypertension in this patient? Are there alternative pharmacotherapies that the clinician should also consider?

Therapeutic and toxic effects of drugs result from their interactions with molecules in the patient. Most drugs act by associating with specific macromolecules in ways that alter the macromolecules' biochemical or biophysical activities. This idea, more than a century old, is embodied in the term **receptor**: the component of a cell or organism that interacts with a drug and initiates the chain of events leading to the drug's observed effects.

Receptors have become the central focus of investigation of drug effects and their mechanisms of action (pharmacodynamics). The receptor concept, extended to endocrinology, immunology, and molecular biology, has proved essential for explaining many aspects of biologic regulation. Many drug receptors have been isolated and characterized in detail, thus opening the way to precise understanding of the molecular basis of drug action.

The receptor concept has important practical consequences for the development of drugs and for arriving at therapeutic decisions in clinical practice. These consequences form the basis for understanding the actions and clinical uses of drugs described in almost every chapter of this book. They may be briefly summarized as follows:

1. **Receptors largely determine the quantitative relations between dose or concentration of drug and pharmacologic effects.**

The receptor's affinity for binding a drug determines the concentration of drug required to form a significant number of drug-receptor complexes, and the total number of receptors may limit the maximal effect a drug may produce.

2. **Receptors are responsible for selectivity of drug action.** The molecular size, shape, and electrical charge of a drug determine whether—and with what affinity—it will bind to a particular receptor among the vast array of chemically different binding sites available in a cell, tissue, or patient. Accordingly, changes in the chemical structure of a drug can dramatically increase or decrease a new drug's affinities for different classes of receptors, with resulting alterations in therapeutic and toxic effects.
3. **Receptors mediate the actions of pharmacologic agonists and antagonists.** Some drugs and many natural ligands, such as hormones and neurotransmitters, regulate the function of receptor macromolecules as **agonists**; this means that they activate the receptor to signal as a direct result of binding to it. Some agonists activate a single kind of receptor to produce all their biologic functions, whereas others selectively promote one receptor function more than another.

Other drugs act as pharmacologic **antagonists**; that is, they bind to receptors but do not activate generation of a signal; consequently, they interfere with the ability of an agonist to activate the receptor. Some of the most useful drugs in clinical medicine are pharmacologic antagonists. Still other drugs bind to a different site on the receptor than that bound by endogenous ligands; such drugs can produce useful and quite different clinical effects by acting as so-called **allosteric modulators** of the receptor.

MACROMOLECULAR NATURE OF DRUG RECEPTORS

Most receptors for clinically relevant drugs, and nearly all of those discussed in this chapter, are proteins. Traditionally, drug binding was used to identify or purify receptor proteins from tissue extracts; consequently, receptors were discovered after the drugs that bind to them. Advances in molecular biology and genome sequencing made it possible to identify receptors by predicted structural homology to other (previously known) receptors. This effort revealed that many known drugs bind to a larger diversity of receptors than previously anticipated and motivated efforts to develop increasingly selective drugs. It also identified **orphan receptors**, so-called because their natural ligands are presently unknown; these may prove to be useful targets for future drug development.

The best-characterized drug receptors are **regulatory proteins** that mediate the actions of endogenous chemical signals such as neurotransmitters, autacoids, and hormones. This class of receptors mediates the effects of many of the most useful therapeutic agents. The molecular structures and biochemical mechanisms of these regulatory receptors are described in a later section entitled Signaling Mechanisms & Drug Action.

Other classes of proteins have been clearly identified as drug receptors. **Enzymes** may be inhibited (or, less commonly, activated) by binding a drug. Examples include dihydrofolate reductase, the receptor for the antineoplastic drug methotrexate; 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the receptor for statins; and various protein and lipid kinases. **Transport proteins** can be useful drug targets. Examples include Na^+/K^+ -ATPase, the membrane receptor for cardioactive digitalis

glycosides; norepinephrine and serotonin transporter proteins that are membrane receptors for antidepressant drugs; and dopamine transporters that are membrane receptors for cocaine and a number of other psychostimulants. **Structural proteins** are also important drug targets, such as tubulin, the receptor for the anti-inflammatory agent colchicine.

This chapter deals with three aspects of drug receptor function, presented in increasing order of complexity: (1) receptors as determinants of the quantitative relation between the concentration of a drug and the pharmacologic response, (2) receptors as regulatory proteins and components of chemical signaling mechanisms that provide targets for important drugs, and (3) receptors as key determinants of the therapeutic and toxic effects of drugs in patients.

RELATION BETWEEN DRUG CONCENTRATION & RESPONSE

The relation between dose of a drug and the clinically observed response may be complex. In carefully controlled *in vitro* systems, however, the relation between concentration of a drug and its effect is often simple and can be described with mathematical precision. It is important to understand this idealized relation in some detail because it underlies the more complex relations between dose and effect that occur when drugs are given to patients.

Concentration-Effect Curves & Receptor Binding of Agonists

Even in intact animals or patients, responses to low doses of a drug usually increase in direct proportion to dose. As doses increase, however, the response increment diminishes; finally, doses may be reached at which no further increase in response can be achieved. This relation between drug concentration and effect is traditionally described by a hyperbolic curve (Figure 2-1A) according to the following equation:

$$E = \frac{E_{\max} \times C}{C + EC_{50}}$$

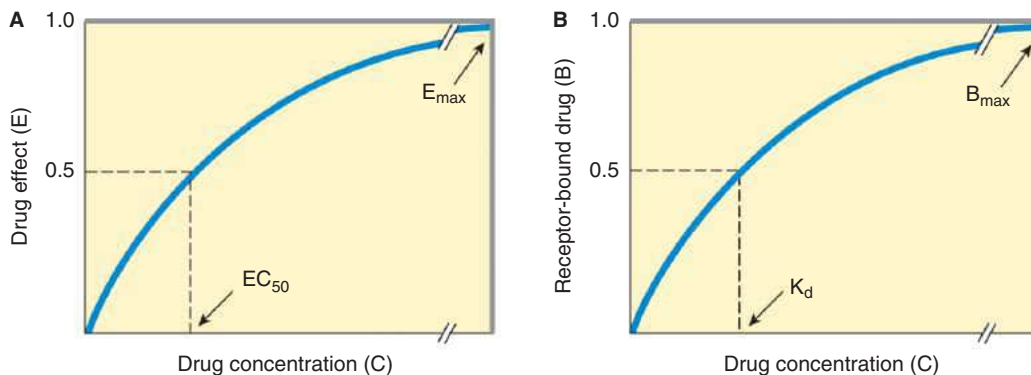


FIGURE 2-1 Relations between drug concentration and drug effect (A) or receptor-bound drug (B). The drug concentrations at which effect or receptor occupancy is half-maximal are denoted by EC_{50} and K_d , respectively.

where E is the effect observed at concentration C , $E_{\max}$ is the maximal response that can be produced by the drug, and EC_{50} is the concentration of drug that produces 50% of maximal effect.

This hyperbolic relation resembles the mass action law that describes the association between two molecules of a given affinity. This resemblance suggests that drug agonists act by binding to (“occupying”) a distinct class of biologic molecules with a characteristic affinity for the drug. Radioactive receptor ligands have been used to confirm this occupancy assumption in many drug-receptor systems. In these systems, drug bound to receptors (B) relates to the concentration of free (unbound) drug (C) as depicted in Figure 2–1B and as described by an analogous equation:

$$B = \frac{B_{\max} \times C}{C + K_d}$$

in which $B_{\max}$ indicates the total concentration of receptor sites (ie, sites bound to the drug at infinitely high concentrations of free drug) and K_d (the equilibrium dissociation constant) represents the concentration of free drug at which half-maximal binding is observed. This constant characterizes the receptor’s affinity for binding the drug in a reciprocal fashion: If the K_d is low, binding affinity is high, and vice versa. The EC_{50} and K_d may be identical but need not be, as discussed below. Dose-response data are often presented as a plot of the drug effect (ordinate) against the *logarithm* of the dose or concentration (abscissa), transforming the hyperbolic curve of Figure 2–1 into a sigmoid curve with a linear midportion (eg, Figure 2–2). This transformation is convenient because it expands the scale of the concentration axis at low concentrations (where the effect is changing rapidly) and compresses it at high concentrations (where the effect is changing slowly), but otherwise has no biologic or pharmacologic significance.

Receptor-Effector Coupling & Spare Receptors

When an agonist occupies a receptor, conformational changes occur in the receptor protein that represent the fundamental basis of receptor activation and the first of often many steps required to produce a pharmacologic response. The overall transduction process that links drug occupancy of receptors and pharmacologic response is called **coupling**. The relative efficiency of occupancy-response coupling is determined, in part, at the receptor itself; full agonists tend to shift the conformational equilibrium of receptors more strongly than partial agonists (described in the text that follows). Coupling is also determined by “downstream” biochemical events that transduce receptor occupancy into cellular response. For some receptors, such as ligand-gated ion channels, the relationship between drug occupancy and response can be simple because the ion current produced by a drug is often directly proportional to the number of receptors (ion channels) bound. For other receptors, such as those linked to enzymatic signal transduction cascades, the occupancy-response relationship is often more complex because the biologic response reaches a maximum before full receptor occupancy is achieved.

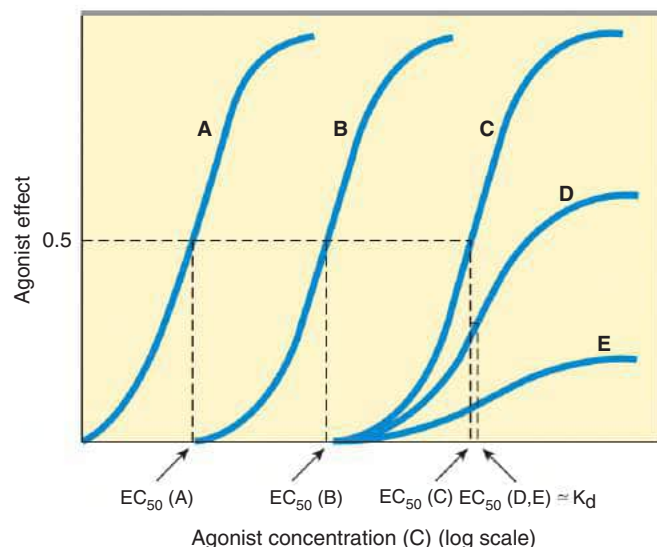


FIGURE 2–2 Logarithmic transformation of the dose axis and experimental demonstration of spare receptors, using different concentrations of an irreversible antagonist. Curve **A** shows agonist response in the absence of antagonist. After treatment with a low concentration of antagonist (curve **B**), the curve is shifted to the right. Maximal responsiveness is preserved, however, because the remaining available receptors are still in excess of the number required. In curve **C**, produced after treatment with a larger concentration of antagonist, the available receptors are no longer “spare”; instead, they are just sufficient to mediate an undiminished maximal response. Still higher concentrations of antagonist (curves **D** and **E**) reduce the number of available receptors to the point that maximal response is diminished. The apparent EC_{50} of the agonist in curves **D** and **E** may approximate the K_d that characterizes the binding affinity of the agonist for the receptor.

Many factors can contribute to nonlinear occupancy-response coupling, and often these factors are only partially understood. A useful concept for thinking about this is that of **receptor reserve** or **spare receptors**. Receptors are said to be “spare” for a given pharmacologic response if it is possible to elicit a maximal biologic response at a concentration of agonist that does not result in occupancy of all the available receptors. Experimentally, spare receptors may be demonstrated by using irreversible antagonists to prevent binding of agonist to a proportion of available receptors and showing that high concentrations of agonist can still produce an undiminished maximal response (see Figure 2–2). For example, the same maximal inotropic response of heart muscle to catecholamines can be elicited even when 90% of β adrenoreceptors to which they bind are occupied by a quasi-irreversible antagonist. Accordingly, myocardial cells are said to contain a large proportion of spare β adrenoreceptors.

What accounts for the phenomenon of spare receptors? In some cases, receptors may be simply *spare in number* relative to the total number of downstream signaling mediators present in the cell, so that a maximal response occurs without occupancy of all receptors. In other cases, “spareness” of receptors appears to be *temporal*. For example, β -adrenoreceptor activation by an agonist promotes binding of guanosine triphosphate (GTP) to a trimeric

G protein, producing an activated signaling intermediate whose lifetime may greatly outlast the agonist-receptor interaction (see also the section G Proteins & Second Messengers). Here, maximal response is elicited by activation of relatively few receptors because the response initiated by an individual ligand-receptor-binding event persists longer than the binding event itself. Irrespective of the biochemical basis of receptor reserve, the sensitivity of a cell or tissue to a particular concentration of agonist depends not only on the *affinity* of the receptor for binding the agonist (characterized by the K_d) but also on the *degree of spareness*—the total number of receptors present compared with the number actually necessary to elicit a maximal biologic response.

The concept of spare receptors is very useful clinically because it allows one to think precisely about the effects of drug dosage without having to consider (or even fully understand) biochemical details of the signaling response. The K_d of the agonist-receptor interaction determines what fraction ($B/B_{\max}$) of total receptors will be occupied at a given free concentration (C) of agonist regardless of the receptor concentration:

$$\frac{B}{B_{\max}} = \frac{C}{C + K_d}$$

Imagine a responding cell with 100 receptors and 100 effectors. Here the number of effectors does not limit the maximal response, and the receptors are *not* spare in number. Consequently, an agonist that is present at a concentration equal to the K_d will occupy 50% of the receptors, and half of the effectors will be activated, producing a half-maximal response (ie, 50 receptors stimulate 50 effectors). Now imagine that the number of receptors increases tenfold to 1000 receptors but the total number of effectors remains constant. Most of the receptors are now spare in number. As a

result, a much lower concentration of agonist suffices to occupy 50 of the 1000 receptors (5% of the receptors), and this same low concentration of agonist elicits a half-maximal response (50 of 100 effectors activated). Thus, it is possible to change the sensitivity of tissues with spare receptors by changing receptor number.

Competitive & Irreversible Antagonists

Receptor antagonists bind to receptors but do not activate them; the primary action of antagonists is to reduce the effects of agonists (other drugs or endogenous regulatory molecules) that normally activate receptors. While antagonists are traditionally thought to have no functional effect in the absence of an agonist, some antagonists exhibit “inverse agonist” activity (see Chapter 1) because they also reduce receptor activity below basal levels observed in the absence of any agonist at all. Antagonist drugs are further divided into two classes depending on whether they act *competitively* or *noncompetitively* relative to an agonist present at the same time.

In the presence of a fixed concentration of agonist, increasing concentrations of a **competitive antagonist** progressively inhibit the agonist response; high antagonist concentrations prevent the response almost completely. Conversely, sufficiently high concentrations of agonist can surmount the effect of a given concentration of the antagonist; that is, the $E_{\max}$ for the agonist remains the same for any fixed concentration of antagonist (Figure 2–3A). Because the antagonism is competitive, the presence of antagonist increases the agonist concentration required for a given degree of response, and so the agonist concentration-effect curve is shifted to the right.

The concentration (C') of an agonist required to produce a given effect in the presence of a fixed concentration ($[I]$) of competitive antagonist is greater than the agonist concentration (C)

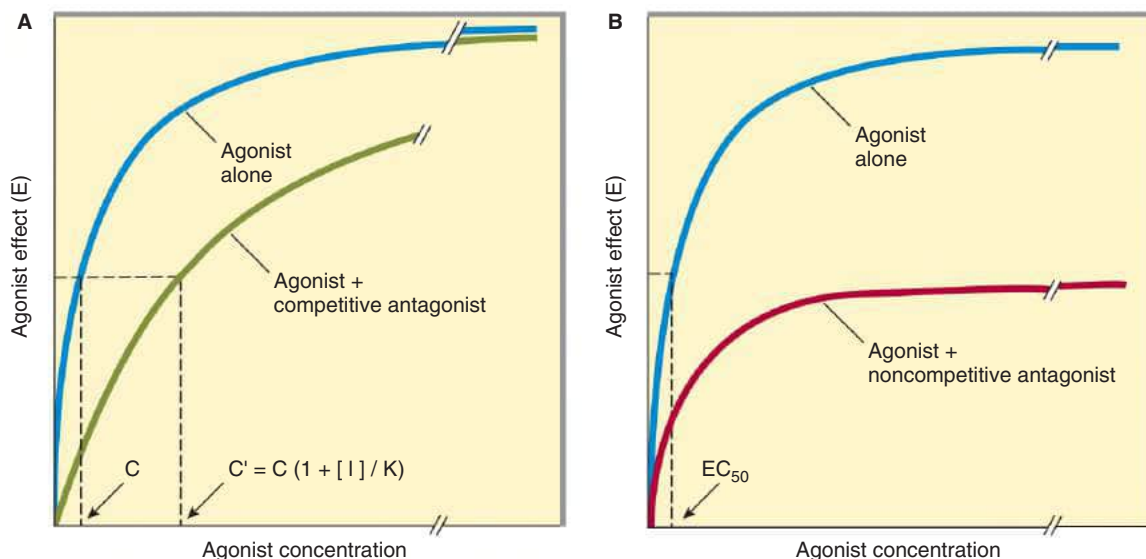


FIGURE 2–3 Changes in agonist concentration-effect curves produced by a competitive antagonist (A) or by an irreversible antagonist (B). In the presence of a competitive antagonist, higher concentrations of agonist are required to produce a given effect; thus the agonist concentration (C') required for a given effect in the presence of concentration $[I]$ of an antagonist is shifted to the right, as shown. High agonist concentrations can overcome inhibition by a competitive antagonist. This is not the case with an irreversible (or noncompetitive) antagonist, which reduces the maximal effect the agonist can achieve, although it may not change its EC_{50} .

required to produce the same effect in the absence of the antagonist. The ratio of these two agonist concentrations (called the dose ratio) is related to the dissociation constant (K_i) of the antagonist by the **Schild equation**:

$$\frac{C'}{C} = 1 + \frac{[I]}{K_i}$$

Pharmacologists often use this relation to determine the K_i of a competitive antagonist. Even without knowledge of the relation between agonist occupancy of the receptor and response, the K_i can be determined simply and accurately. As shown in Figure 2–3, concentration-response curves are obtained in the presence and in the absence of a fixed concentration of competitive antagonist; comparison of the agonist concentrations required to produce identical degrees of pharmacologic effect in the two situations reveals the antagonist's K_i . If C' is twice C , for example, then $[I] = K_i$.

For the clinician, this mathematical relation has two important therapeutic implications:

1. The degree of inhibition produced by a competitive antagonist depends on the concentration of antagonist. The competitive β -adrenoceptor antagonist propranolol provides a useful example. Patients receiving a fixed dose of this drug exhibit a wide range of plasma concentrations, owing to differences among individuals in the clearance of propranolol. As a result, inhibitory effects on physiologic responses to norepinephrine and epinephrine (endogenous adrenergic receptor agonists) may vary widely, and the dose of propranolol must be adjusted accordingly.
2. Clinical response to a competitive antagonist also depends on the concentration of agonist that is competing for binding to receptors. Again, propranolol provides a useful example: When this drug is administered at moderate doses sufficient to block the effect of basal levels of the neurotransmitter norepinephrine, resting heart rate is decreased. However, the increase in the release of norepinephrine and epinephrine that occurs with exercise, postural changes, or emotional stress may suffice to overcome this competitive antagonism. Accordingly, the same dose of propranolol may have little effect under these conditions, thereby altering therapeutic response. Conversely, the same dose of propranolol that is useful for treatment of hypertension in one patient may be excessive and toxic to another, based on differences between the patients in the amount of endogenous norepinephrine and epinephrine that they produce.

The actions of a **noncompetitive antagonist** are different because, once a receptor is bound by such a drug, agonists cannot surmount the inhibitory effect irrespective of their concentration. In many cases, noncompetitive antagonists bind to the receptor in an **irreversible** or nearly irreversible fashion, sometimes by forming a covalent bond with the receptor. After occupancy of some proportion of receptors by such an antagonist, the number of remaining unoccupied receptors may be too low for the agonist (even at high concentrations) to elicit a response comparable to the previous maximal response (Figure 2–3B). If spare receptors are present, however, a lower dose of an irreversible antagonist may

leave enough receptors unoccupied to allow achievement of maximum response to agonist, although a higher agonist concentration will be required (Figures 2–2B and 2–2C; see section Receptor-Effector Coupling & Spare Receptors).

Therapeutically, such irreversible antagonists present distinct advantages and disadvantages. Once the irreversible antagonist has occupied the receptor, it need not be present in unbound form to inhibit agonist responses. Consequently, the duration of action of such an irreversible antagonist is relatively independent of its own rate of elimination and more dependent on the rate of turnover of receptor molecules.

Phenoxybenzamine, an irreversible α -adrenoceptor antagonist, is used to control the hypertension caused by catecholamines released from pheochromocytoma, a tumor of the adrenal medulla. If administration of phenoxybenzamine lowers blood pressure, blockade will be maintained even when the tumor episodically releases very large amounts of catecholamine. In this case, the ability to prevent responses to varying and high concentrations of agonist is a therapeutic advantage. If overdose occurs, however, a real problem may arise. If the α -adrenoceptor blockade cannot be overcome, excess effects of the drug must be antagonized “physiologically,” ie, by using a pressor agent that does not act via α adrenoceptors.

Antagonists can function noncompetitively in a different way; that is, by binding to a site on the receptor protein separate from the agonist binding site; in this way, the drug can modify receptor activity without blocking agonist binding (see Chapter 1, Figures 1–2C and 1–2D). Although these drugs act noncompetitively, their actions are often reversible. Such drugs are called *negative allosteric modulators* because they act through binding to a different (ie, “allosteric”) site on the receptor relative to the classical (ie, “orthosteric”) site bound by the endogenous agonist to reduce activity of the receptor. Other allosteric modulators have the opposite effect: they increase receptor activity. Benzodiazepine drugs like diazepam, for example, bind to an allosteric site on ion channels that are physiologically activated by the neurotransmitter γ -aminobutyric acid (GABA). Benzodiazepines are considered *positive allosteric modulators* of GABA receptors because they potentiate (rather than inhibit) the ability of the orthosteric agonist (GABA) to increase channel conductance. A useful feature of this allosteric mechanism is that benzodiazepines have little activating effect on their own. This contributes to making benzodiazepines relatively safe in overdose unless combined with other sedating drugs. The concept of allosteric modulators also applies to proteins that lack any known orthosteric binding site. For example, ivacaftor binds to the **cystic fibrosis transmembrane regulator (CFTR)** ion channel that is mutated in cystic fibrosis. Some mutations that cause disease by rendering this channel hypoactive can be partially rescued by ivacaftor, despite CFTR having no known physiological agonist—and thus no (presently recognized) orthosteric binding site.

Partial Agonists

Based on the maximal pharmacologic response that occurs when all receptors are occupied, agonists can be divided into two classes:

partial agonists produce a lower response, at full receptor occupancy, than do **full agonists**. Partial agonists produce concentration-effect curves that resemble those observed with full agonists in the presence of an antagonist that irreversibly blocks some of the receptor sites (compare Figures 2–2 [curve D] and 2–4B). It is important to emphasize that the failure of partial agonists to produce a maximal response is not due to decreased affinity for binding to receptors. Indeed, a partial agonist's inability to cause a maximal pharmacologic response, even when present at high concentrations that effectively saturate binding to all receptors, is indicated by the fact that partial agonists competitively inhibit the responses produced by full agonists (Figure 2–4). This mixed “agonist-antagonist” property of partial agonists can have both beneficial and deleterious effects in the clinic. For example, buprenorphine, a partial agonist of μ -opioid receptors, is a generally safer analgesic drug than morphine, a more efficacious partial agonist, and much safer than fentanyl which is a full agonist. This is because buprenorphine suppresses breathing upon binding opioid receptors (in ventilatory pacemaker neurons of the brainstem) less strongly than morphine, and much less strongly than fentanyl; breathing stops

if these receptors are activated too strongly. On the other hand, buprenorphine is effectively antianalgesic when administered in combination with more efficacious opioid drugs, due to the mixed agonist-antagonist effect. In addition, buprenorphine may precipitate a severe drug withdrawal reaction in opioid-dependent individuals, due to physiologic adaptations of the nervous system that are induced after prolonged or repeated drug exposure and effectively change the “set point” for sensitivity to opioids.

Other Mechanisms of Drug Antagonism

Not all mechanisms of antagonism involve interactions of drugs or endogenous ligands at a single type of receptor, and some types of antagonism do not involve a receptor protein at all. For example, protamine, a protein that is positively charged at physiologic pH, can be used clinically as a drug to counteract the effects of heparin, a nonprotein (glycosaminoglycan) anticoagulant drug that is negatively charged. In this case, one drug acts as a **chemical antagonist** of the other simply by ionic binding that makes the other drug unavailable to interact with proteins involved in blood clotting.

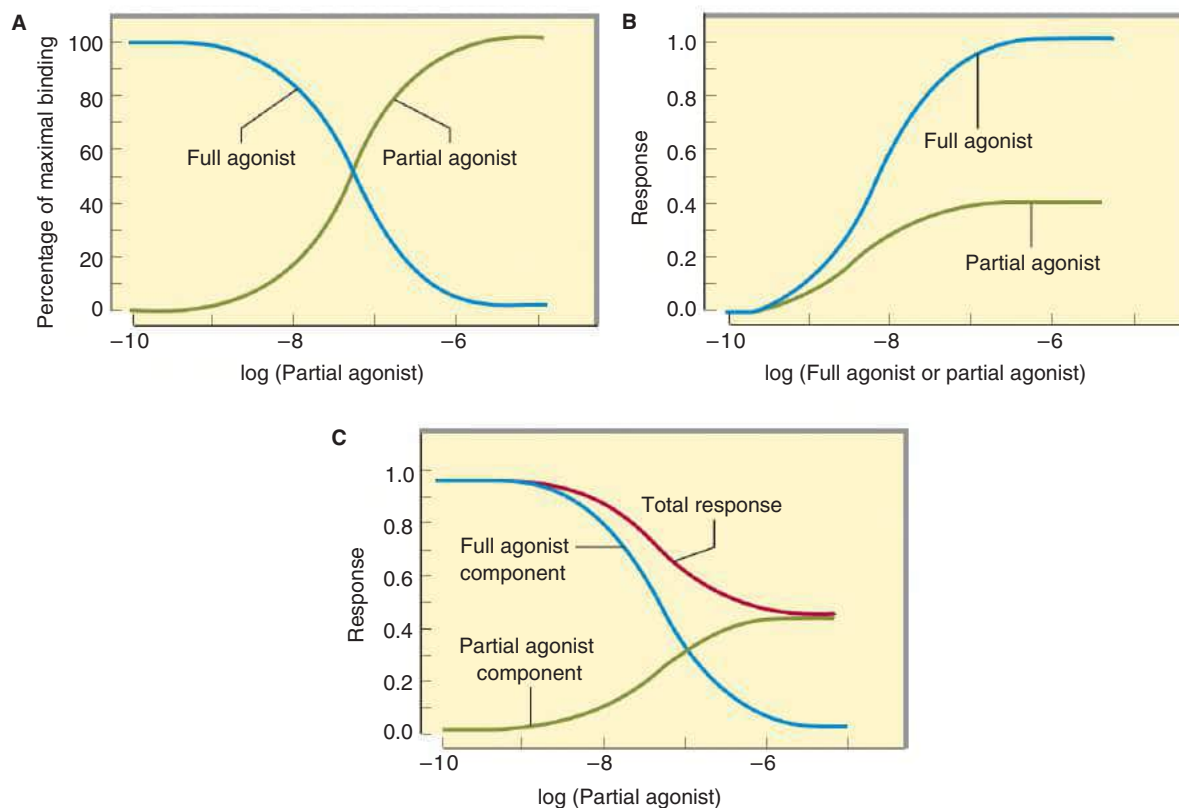


FIGURE 2–4 **A:** The percentage of receptor occupancy resulting from full agonist (present at a single concentration) binding to receptors in the presence of increasing concentrations of a partial agonist. Because the full agonist (blue line) and the partial agonist (green line) compete to bind to the same receptor sites, when occupancy by the partial agonist increases, binding of the full agonist decreases. **B:** When each of the two drugs is used alone and response is measured, occupancy of all the receptors by the partial agonist produces a lower maximal response than does similar occupancy by the full agonist. **C:** Simultaneous treatment with a single concentration of full agonist and increasing concentrations of the partial agonist produces the response patterns shown in the bottom panel. The fractional response caused by a single high concentration of the full agonist decreases as increasing concentrations of the partial agonist compete to bind to the receptor with increasing success; at the same time, the portion of the response caused by the partial agonist increases, while the total response—ie, the sum of responses to the two drugs (red line)—gradually decreases, eventually reaching the value produced by partial agonist alone (compare with B).

Another type of antagonism is **physiologic antagonism** between endogenous regulatory pathways mediated by different receptors. For example, several catabolic actions of the glucocorticoid hormones lead to increased blood glucose levels, an effect that is physiologically opposed by insulin. Although glucocorticoids and insulin act on quite distinct receptor-effector systems, the clinician must sometimes administer insulin to oppose the hyperglycemic effects of a glucocorticoid hormone, whether the latter is elevated by endogenous synthesis (eg, a tumor of the adrenal cortex) or as a result of glucocorticoid therapy.

In general, use of a drug as a physiologic antagonist produces effects that are less specific and less easy to control than are the effects of a receptor-specific antagonist. Thus, for example, to treat bradycardia caused by increased release of acetylcholine from vagus nerve endings, the clinician could use isoproterenol, a β -adrenoceptor agonist that increases heart rate by mimicking sympathetic stimulation of the heart. However, use of this physiologic antagonist would be less rational—and potentially more dangerous—than a receptor-specific antagonist such as atropine.

SIGNALING MECHANISMS & DRUG ACTION

Until now we have considered receptor interactions and drug effects in terms of equations and concentration-effect curves. We must also understand the molecular mechanisms by which drugs act. We should also consider different structural families of receptor proteins, and this allows us to ask basic questions with important clinical implications:

- Why do some drugs produce effects that persist for minutes, hours, or even days after the drug is no longer present?
- Why do responses to other drugs diminish rapidly with prolonged or repeated administration?
- How do cellular mechanisms for amplifying external chemical signals explain the phenomenon of spare receptors?
- Why do chemically similar drugs often exhibit extraordinary selectivity in their actions?
- Do these mechanisms provide targets for developing new drugs?

Most transmembrane signaling is accomplished by a small number of different molecular mechanisms. Each type of mechanism has been adapted, through the evolution of distinctive protein families, to transduce many different signals. These protein families include receptors on the cell surface and within the cell, as well as enzymes and other components that generate, amplify, coordinate, and terminate postreceptor signaling by nonprotein chemical mediators in the cell that are collectively called **second messengers**. This section first discusses mechanisms for carrying chemical information across the plasma membrane and then discusses some examples of second messenger chemicals.

Five basic mechanisms of transmembrane signaling are well understood (Figure 2–5). Each represents a different family of receptor protein and uses a different strategy to circumvent the barrier posed by the lipid bilayer of the plasma membrane. These strategies use (1) a lipid-soluble ligand that crosses the membrane and acts on an intracellular receptor; (2) a transmembrane receptor protein whose intracellular enzymatic activity is allosterically regulated by a ligand that binds to a site on the protein's extracellular domain; (3) a transmembrane receptor that binds and stimulates an intracellular protein tyrosine kinase; (4) a ligand-gated transmembrane ion channel that can be induced to open or close by the binding

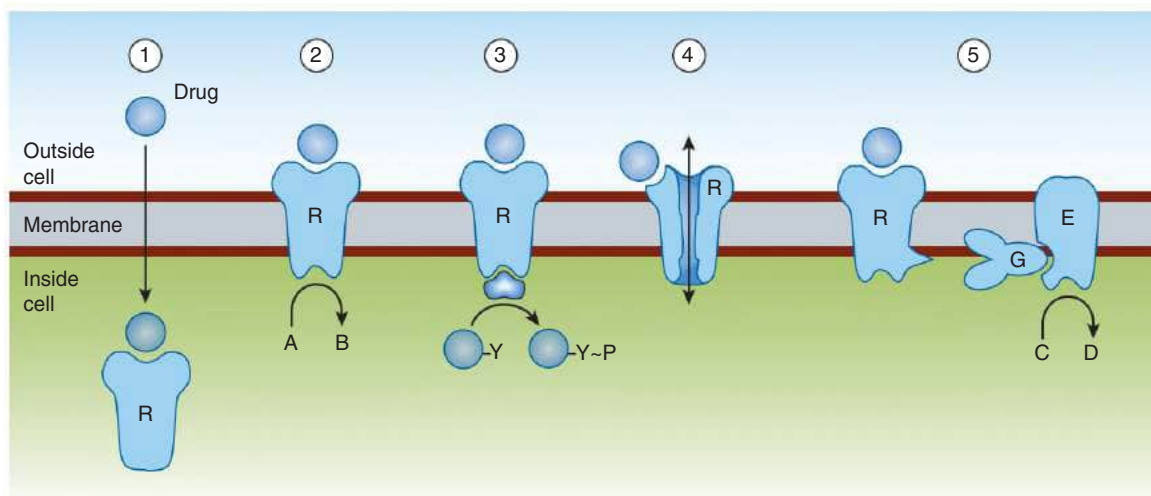


FIGURE 2–5 Known transmembrane signaling mechanisms: **1:** A lipid-soluble chemical signal crosses the plasma membrane and acts on an intracellular receptor (which may be an enzyme or a regulator of gene transcription); **2:** the signal binds to the extracellular domain of a transmembrane protein, thereby activating an enzymatic activity of its cytoplasmic domain; **3:** the signal binds to the extracellular domain of a transmembrane receptor bound to a separate protein tyrosine kinase, which it activates; **4:** the signal binds to and directly regulates the opening of an ion channel; **5:** the signal binds to a cell-surface receptor linked to an effector enzyme by a G protein. (A, C, substrates; B, D, products; R, receptor; G, G protein; E, effector [enzyme or ion channel]; Y, tyrosine; P, phosphate.)

of a ligand; and (5) a transmembrane receptor protein that stimulates a GTP-binding signal transducer protein (G protein), which in turn modulates production of an intracellular second messenger.

Although the five established mechanisms do not account for all the chemical signals conveyed across cell membranes, they do transduce many of the most important signals exploited in pharmacotherapy.

Intracellular Receptors for Lipid-Soluble Agents

Several biologic ligands are sufficiently lipid-soluble to cross the plasma membrane and act on intracellular receptors. One class of such ligands includes steroids (corticosteroids, mineralocorticoids, sex steroids, vitamin D) and thyroid hormone, whose receptors stimulate the transcription of genes by binding to specific DNA sequences (often called **response elements**) near the gene whose expression is to be regulated.

These “gene-active” receptors belong to a protein family that evolved from a common precursor. Dissection of the receptors by recombinant DNA techniques has provided insights into their molecular mechanism. For example, binding of glucocorticoid hormone to its normal receptor protein relieves an inhibitory constraint on the transcription-stimulating activity of the protein. Figure 2–6 schematically depicts the molecular mechanism of glucocorticoid action: In the absence of hormone, the receptor is bound to hsp90, a protein that prevents normal folding of several structural domains of the receptor. Binding of hormone to the ligand-binding domain triggers release of hsp90. This allows the DNA-binding and transcription-activating domains of the receptor to fold into their functionally active conformations, so that the activated receptor can initiate transcription of target genes.

The mechanism used by hormones that act by regulating gene expression has two therapeutically important consequences:

1. These hormones produce their effects after a characteristic lag period of 30 minutes to several hours—the time required for the synthesis of new proteins. This means that the gene-active hormones cannot be expected to alter a pathologic state within minutes (eg, glucocorticoids will not immediately relieve the symptoms of bronchial asthma).
2. The effects of these agents can persist for hours or days after their concentration has been reduced to zero. The persistence of effect is primarily due to the relatively slow turnover of most enzymes and proteins, which can remain active in cells for hours or days after they have been synthesized. Consequently, it means that the beneficial (or toxic) effects of a gene-active hormone usually decrease slowly when administration of the hormone is stopped.

Ligand-Regulated Transmembrane Enzymes Including Receptor Tyrosine Kinases

This class of receptor molecules mediates the first steps in signaling by insulin, epidermal growth factor (EGF), platelet-derived

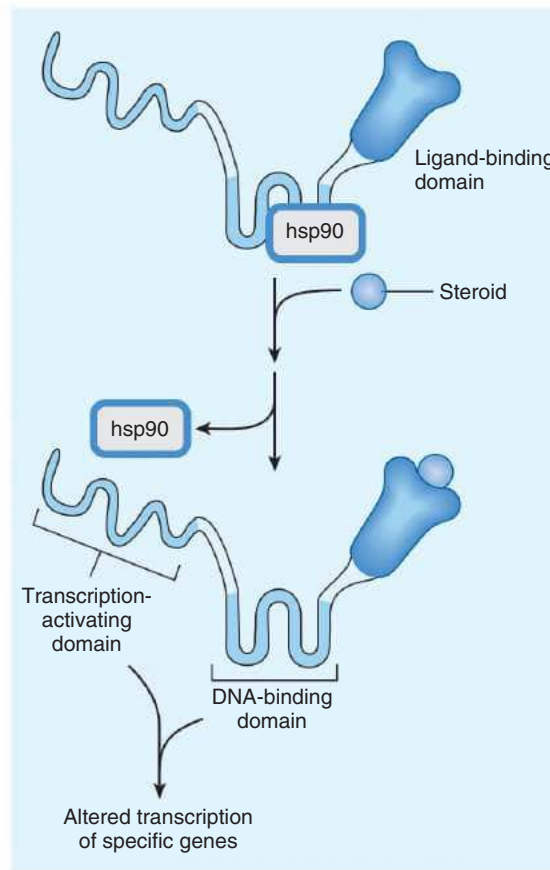


FIGURE 2–6 Mechanism of glucocorticoid action. The glucocorticoid receptor polypeptide is schematically depicted as a protein with three distinct domains. A heat-shock protein, hsp90, binds to the receptor in the absence of hormone and prevents folding into the active conformation of the receptor. Binding of a hormone ligand (steroid) causes dissociation of the hsp90 stabilizer and permits conversion to the active configuration.

growth factor (PDGF), atrial natriuretic peptide (ANP), transforming growth factor- β (TGF- β), and many other trophic hormones. These receptors are polypeptides consisting of an extracellular hormone-binding domain and a cytoplasmic enzyme domain, which may be a protein tyrosine kinase, a serine kinase, or a guanylyl cyclase (Figure 2–7). In all these receptors, the two domains are connected by a hydrophobic segment of the polypeptide that resides in the lipid bilayer of the plasma membrane.

The receptor tyrosine kinase signaling function begins with binding of ligand, typically a polypeptide hormone or growth factor, to the receptor’s extracellular domain. The resulting change in receptor conformation causes two receptor molecules to bind to one another (*dimerize*). This activates the tyrosine kinase enzyme activity present in the cytoplasmic domain of the dimer, leading to phosphorylation of the receptor as well as additional downstream signaling proteins. Activated receptors catalyze phosphorylation of tyrosine residues on different target signaling proteins, thereby allowing a single activated receptor complex to modulate a large number of biochemical processes.

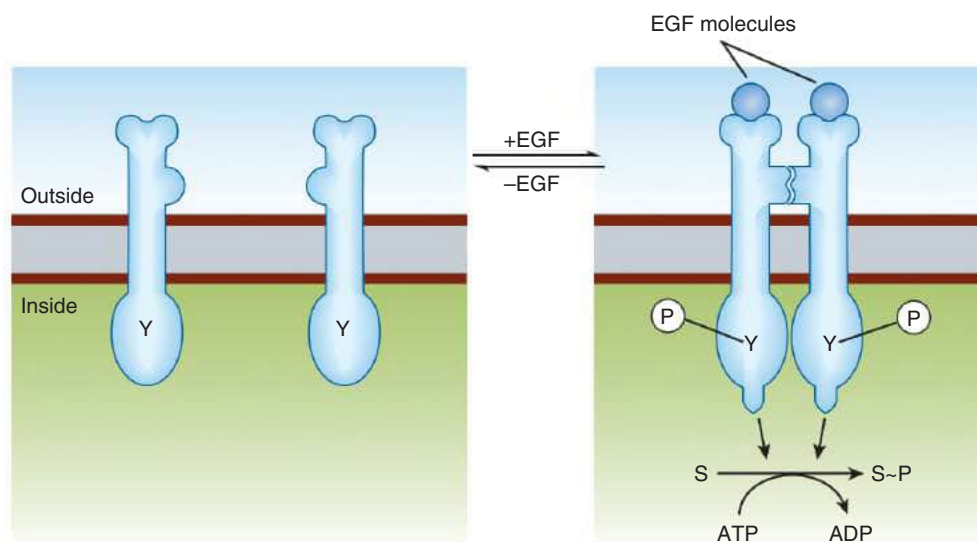


FIGURE 2-7 Mechanism of activation of the epidermal growth factor (EGF) receptor, a representative receptor tyrosine kinase. The receptor polypeptide has extracellular and cytoplasmic domains, depicted above and below the plasma membrane. Upon binding of EGF (circle), the receptor converts from its inactive monomeric state (*left*) to an active dimeric state (*right*), in which two receptor polypeptides bind noncovalently. The cytoplasmic domains become phosphorylated (P) on specific tyrosine residues (Y), and their enzymatic activities are activated, catalyzing phosphorylation of substrate proteins (S).

Insulin, for example, uses a single class of tyrosine kinase receptors to trigger increased uptake of glucose and amino acids and to regulate metabolism of glycogen and triglycerides in the cell. Activation of the receptor in specific target cells drives a complex program of cellular events ranging from altered membrane transport of ions and metabolites to changes in the expression of many genes.

Inhibitors of particular receptor tyrosine kinases are used in treating neoplastic disorders in which excessive growth factor signaling is often involved. Some of these inhibitors are monoclonal antibodies (eg, trastuzumab, cetuximab), which bind to the extracellular domain of a particular receptor and interfere with binding of growth factor. Other inhibitors are membrane-permeant small molecule chemicals (eg, gefitinib, erlotinib), which inhibit the receptor's kinase activity in the cytoplasm.

The intensity and duration of action of EGF, PDGF, and other agents that act via receptor tyrosine kinases are often limited by a process called **receptor downregulation**. This typically occurs by endocytosis of receptors from the cell surface followed by the degradation of internalized receptors (and their bound ligands). When this process occurs at a rate faster than *de novo* synthesis of receptors, the total number of cell-surface receptors is reduced (down-regulated), and the cell's responsiveness to ligand is correspondingly diminished. A well-understood example is the EGF receptor tyrosine kinase, whose rate of internalization from the plasma membrane is greatly accelerated after activation by EGF; internalized receptors are subsequently delivered to lysosomes and proteolyzed. This downregulation process is essential physiologically to limit the strength and duration of the endogenous growth factor signal, and genetic mutations that interfere with the downregulation process cause excessive and prolonged responses that underlie or contribute to many forms of cancer. Endocytosis of

other receptor tyrosine kinases, most notably receptors for nerve growth factor, can serve a very different function. Internalized nerve growth factor receptors are not rapidly degraded but remain active in endocytic vesicles that move from the distal axon, where receptors are initially activated by nerve growth factor (released from the innervated tissue), into the neuron's cell body. In the cell body, the growth factor signal is transduced to transcription factors regulating the expression of genes controlling cell survival. This process, effectively opposite to downregulation, transports a critical survival signal from its site of initiation to the site of a critical downstream signaling effect, and can do so over a remarkably long distance—up to a meter in some neurons.

Other regulators of growth and differentiation, including TGF- β , act on another class of transmembrane receptor enzymes that phosphorylate serine and threonine residues. Atrial natriuretic peptide (ANP), an important regulator of blood volume and vascular tone, acts on a transmembrane receptor whose intracellular domain, a guanylyl cyclase, generates cGMP (see below).

Cytokine Receptors

Cytokine receptors respond to a heterogeneous group of peptide ligands, which include growth hormone, erythropoietin, several kinds of interferon, and other regulators of growth and differentiation. These receptors use a mechanism (Figure 2-8) closely resembling that of receptor tyrosine kinases, except that in this case, the protein tyrosine kinase activity is not intrinsic to the receptor molecule. Instead, a separate protein tyrosine kinase, from the Janus-kinase (JAK) family, binds noncovalently to the receptor. As in the case of the EGF receptor, cytokine receptors dimerize after they bind the activating ligand, allowing the bound JAKs to become activated and to phosphorylate tyrosine residues on the receptor.

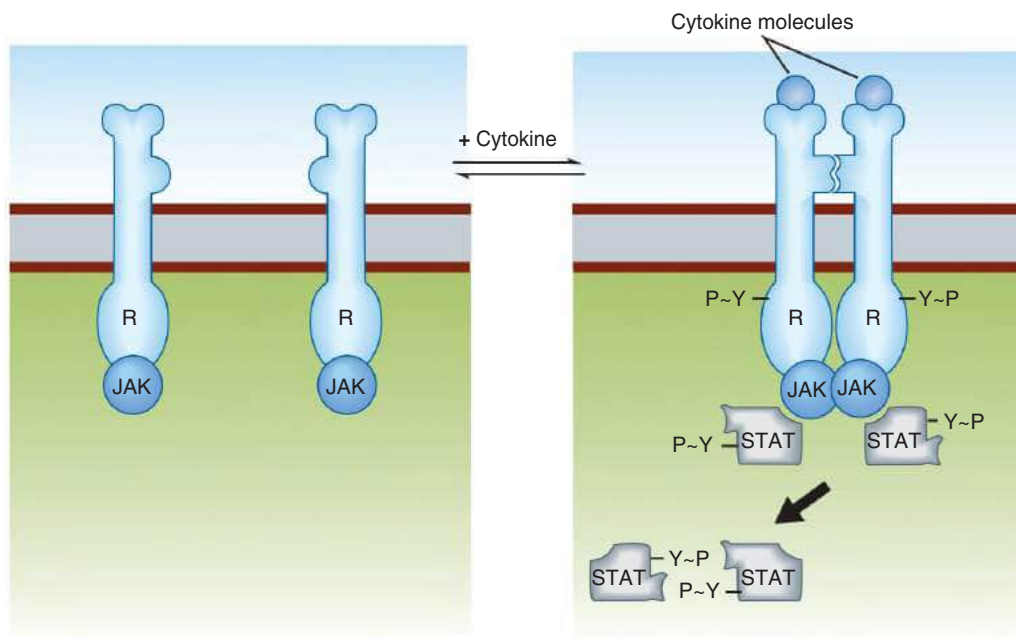


FIGURE 2-8 Cytokine receptors, like receptor tyrosine kinases, have extracellular and intracellular domains and form dimers. However, after activation by an appropriate ligand, separate mobile protein tyrosine kinase molecules (JAK) are activated, resulting in phosphorylation of signal transducers and activation of transcription (STAT) molecules. STAT dimers then travel to the nucleus, where they regulate transcription.

Phosphorylated tyrosine residues on the receptor's cytoplasmic surface then set in motion a complex signaling dance by binding another set of proteins, called STATs (signal transducers and activators of transcription). The bound STATs are themselves phosphorylated by the JAKs, two STAT molecules dimerize (attaching to one another's tyrosine phosphates), and finally the STAT/STAT dimer dissociates from the receptor and travels to the nucleus, where it regulates transcription of specific genes.

Ion Channels

Many of the most useful drugs in clinical medicine act on ion channels. For ligand-gated ion channels, drugs often mimic or block the actions of natural agonists. Natural ligands of such receptors include acetylcholine, serotonin, GABA, and glutamate; all are synaptic transmitters.

Each of their receptors transmits its signal across the plasma membrane by increasing transmembrane conductance of the relevant ion and thereby altering the electrical potential across the membrane. For example, acetylcholine causes the opening of the ion channel in the nicotinic acetylcholine receptor (nAChR), which allows Na^+ to flow down its concentration gradient into cells, producing a localized excitatory postsynaptic potential—a depolarization.

The nAChR is one of the best characterized of all cell-surface receptors for hormones or neurotransmitters (Figure 2-9). One form of this receptor is a pentamer made up of four different polypeptide subunits (eg, two α chains plus one β , one γ , and one δ chain). These polypeptides, each of which crosses the lipid bilayer four times, form a cylindrical structure that is approximately 10 nm in diameter but is impermeable to ions. When acetylcholine binds

to sites on the α subunits, a conformational change occurs that results in the transient opening of a central aqueous channel, approximately 0.5 nm in diameter, through which sodium ions penetrate from the extracellular fluid to cause electrical depolarization of the cell. The structural basis for activating other

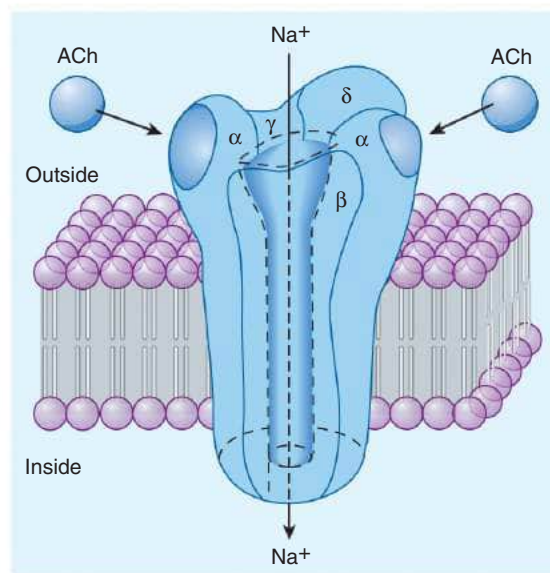


FIGURE 2-9 The nicotinic acetylcholine (ACh) receptor, a ligand-gated ion channel. The receptor molecule is depicted as embedded in a rectangular piece of plasma membrane, with extracellular fluid above and cytoplasm below. Composed of five subunits (two α , one β , one γ , and one δ), the receptor opens a central transmembrane ion channel when ACh binds to sites on the extracellular domain of its α subunits.

ligand-gated ion channels has been determined recently, and similar general principles apply, but there are differences in key details that may open new opportunities for drug design and action. For example, receptors that mediate excitatory neurotransmission at central nervous system synapses bind glutamate, a major excitatory neurotransmitter, through a large appendage domain that protrudes from the receptor and has been called a “flytrap” because it physically closes around the glutamate molecule; the glutamate-loaded flytrap domain then moves as a unit to control pore opening. Drugs can regulate the activity of such glutamate receptors by binding to the flytrap domain, to surfaces on the membrane-embedded portion around the pore, or within the pore itself.

The time elapsed between the binding of the agonist to a ligand-gated channel and the cellular response can often be measured in milliseconds. The rapidity of this signaling mechanism is crucially important for moment-to-moment transfer of information across synapses. Ligand-gated ion channels can be regulated by multiple mechanisms, including phosphorylation and endocytosis. In some cases, such as the nicotinic synapse controlling the diaphragmatic muscles mediating ventilation, these mechanisms maintain a uniform response (ie, they are homeostatic). In other cases, such as at many synapses in the central nervous system, these mechanisms can produce long-term changes in the magnitude of the response and contribute to synaptic plasticity involved in learning and memory.

Voltage-gated ion channels do not bind neurotransmitters directly but are controlled by membrane potential; such channels are also important drug targets. Drugs that regulate voltage-gated channels typically bind to a site of the receptor different from the charged amino acids that constitute the “voltage sensor” domain of the protein used for channel opening by membrane potential. For example, verapamil binds to a region in the pore of voltage-gated calcium channels that are present in the heart and in vascular smooth muscle, inhibiting the ion conductance separately from the voltage sensor, producing antiarrhythmic effects, and reducing blood pressure without mimicking or antagonizing any known endogenous transmitter. Local anesthetics such as procaine work by inhibiting voltage-sensitive sodium channels expressed in sensory neurons, probably by binding to multiple sites on the channel. Other channels, such as the CFTR, are not strongly sensitive to either a known natural ligand or voltage but are still important drug targets. Lumacaftor binds CFTR and promotes its delivery to the plasma membrane after biosynthesis. Ivacaftor binds to a different site and enhances channel conductance. Both drugs act as allosteric modulators of the CFTR and are used in the treatment of cystic fibrosis, but each has a different effect.

G Proteins & Second Messengers

Many extracellular ligands act by increasing the intracellular concentrations of second messengers such as **cyclic adenosine-3', 5'-monophosphate (cAMP)**, **calcium ion**, or the **phosphoinositides** (described below). In most cases, they use a transmembrane signaling system with three separate components. First, the extracellular ligand is selectively detected by a cell-surface receptor. The receptor in turn triggers the activation of a GTP-binding protein

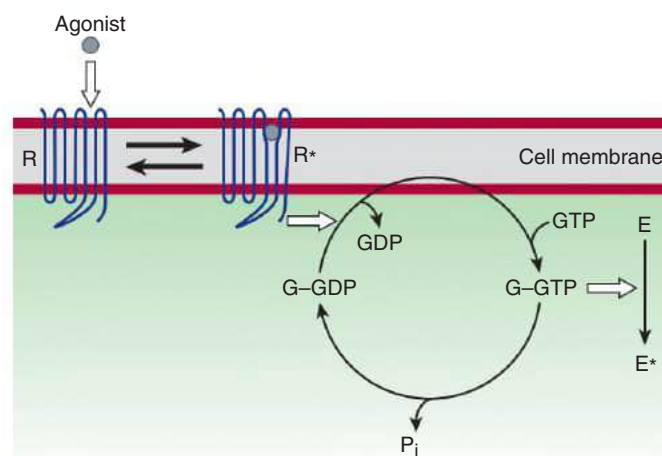


FIGURE 2-10 The guanine nucleotide-dependent activation-inactivation cycle of G proteins. The agonist activates the receptor ($R \rightarrow R^*$), which promotes release of GDP from the G protein (G), allowing entry of GTP into the nucleotide binding site. In its GTP-bound state (G-GTP), the G protein regulates activity of an effector enzyme or ion channel ($E \rightarrow E^*$). The signal is terminated by hydrolysis of GTP, followed by return of the system to the basal unstimulated state. Open arrows denote regulatory effects. (P_i , inorganic phosphate.)

(**G protein**) located on the cytoplasmic face of the plasma membrane. The activated G protein then changes the activity of an effector element, usually an enzyme or ion channel. This element then changes the concentration of the intracellular second messenger. For cAMP, the effector enzyme is adenylyl cyclase, a membrane protein that converts intracellular adenosine triphosphate (ATP) to cAMP. The corresponding G protein, G_s , stimulates adenylyl cyclase after being activated by hormones and neurotransmitters that act via specific G_s -coupled receptors. There are many examples of such receptors, including β adrenoceptors, glucagon receptors, thyrotropin receptors, and certain subtypes of dopamine and serotonin receptors.

G_s and other G proteins rapidly activate their downstream effectors when bound by GTP, but they also have the ability to hydrolyze GTP (Figure 2-10); this hydrolysis reaction inactivates the G protein, and the rate of GTP hydrolysis is a major determinant of both the duration and amount of downstream responses. For example, a neurotransmitter such as norepinephrine may encounter its membrane receptor for only a few tens of milliseconds. When the encounter generates a GTP-bound G_s molecule, however, the duration of activation of adenylyl cyclase depends on the longevity of GTP binding to G_s rather than on the duration of norepinephrine's binding to the receptor. Indeed, GTP-bound G_s may remain active for tens of seconds before it is inactivated by hydrolysis of the bound GTP to GDP, prolonging and enormously amplifying the original signal. The family of G proteins contains several functionally diverse subfamilies (Table 2-1), each of which mediates effects of a particular set of receptors to a distinctive group of effectors. Note that an endogenous ligand (eg, norepinephrine, acetylcholine, serotonin, many others not listed in Table 2-1) may bind and stimulate receptors that couple to different subsets of G proteins. The apparent promiscuity of such a ligand allows it to

TABLE 2–1 G proteins and their receptors and effectors.

G Protein	Receptors for	Effector/Signaling Pathway
G _s	β-Adrenergic amines, histamine, serotonin, glucagon, and many other hormones	↑ Adenylyl cyclase → ↑ cAMP
G ₁₁ , G ₁₂ , G ₁₃	α ₂ -Adrenergic amines, acetylcholine (muscarinic), opioids, serotonin, and many others	Several, including: ↓ Adenylyl cyclase → ↓ cAMP Open cardiac K ⁺ channels → ↓ heart rate
G _{olf}	Odorants (olfactory epithelium)	↑ Adenylyl cyclase → ↑ cAMP
G _o	Neurotransmitters in brain (not yet specifically identified)	Not yet clear
G _q	Acetylcholine (muscarinic), bombesin, serotonin (5-HT ₂), and many others	↑ Phospholipase C → ↑ IP ₃ , diacylglycerol, cytoplasmic Ca ²⁺
G _{t1} , G _{t2}	Photons (rhodopsin and color opsins in retinal rod and cone cells)	↑ cGMP phosphodiesterase → ↓ cGMP (phototransduction)

cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; IP₃, inositol-1,4,5-trisphosphate.

elicit different G protein–dependent responses in different cells. For instance, the body responds to danger by using catecholamines (norepinephrine and epinephrine) both to increase heart rate and to induce constriction of blood vessels in the skin, by acting on G_s-coupled β adrenoceptors and G_q-coupled α₁ adrenoceptors, respectively. Ligand promiscuity also offers opportunities in drug development (see Receptor Classes & Drug Development in the following text).

Receptors that signal via G proteins are often called “G protein–coupled receptors” (**GPCRs**). GPCRs make up the largest receptor family and are also called “seven-transmembrane” (7TM) or “serpentine” receptors because the receptor polypeptide chain “snakes” across the plasma membrane seven times (Figure 2–11). Receptors for adrenergic amines, serotonin, acetylcholine (muscarinic but not nicotinic), many peptide hormones, odorants, and light receptors (in retinal rod and cone cells) all belong to the GPCR family. A few GPCRs (eg, GABA_B and metabotropic glutamate receptors) require stable assembly into *homodimers* (complexes of two identical receptor polypeptides) or *heterodimers* (complexes of different isoforms) for functional activity. However, in contrast to tyrosine kinase and cytokine receptors, oligomerization is not universally required for GPCR activation, and many GPCRs are thought to function as monomers.

GPCRs can bind agonists in a variety of ways, but they all appear to transduce signals across the plasma membrane in a similar way. Agonist binding (eg, a catecholamine or acetylcholine) stabilizes a conformational state of the receptor in which the cytoplasmic ends of the transmembrane helices spread apart by about 1 nm, opening a cavity in the receptor’s cytoplasmic surface that binds a critical regulatory surface of the G protein. This reduces nucleotide affinity

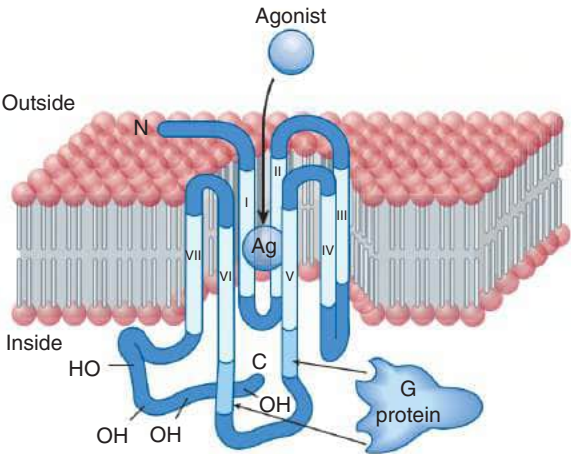


FIGURE 2–11 Transmembrane topology of a typical “serpentine” GPCR. The receptor’s amino (N) terminal is extracellular (above the plane of the membrane), and its carboxyl (C) terminal intracellular, with the polypeptide chain “snaking” across the membrane seven times. The hydrophobic transmembrane segments (light color) are designated by Roman numerals (I–VII). Agonist (Ag) approaches the receptor from the extracellular fluid and binds to a site surrounded by the transmembrane regions of the receptor protein. G protein interacts with cytoplasmic regions of the receptor, especially around the third cytoplasmic loop connecting transmembrane regions V and VI. Lateral movement of these helices during activation exposes an otherwise buried cytoplasmic surface of the receptor that promotes guanine nucleotide exchange on the G protein and thereby activates the G protein, as discussed in the text. The receptor’s cytoplasmic terminal tail contains numerous serine and threonine residues whose hydroxyl (–OH) groups can be phosphorylated. This phosphorylation is associated with diminished receptor–G protein coupling and can promote receptor endocytosis.

for the G protein, allowing GDP to dissociate and GTP to replace it (this occurs because GTP is normally present in the cytoplasm at much higher concentration than GDP). The GTP-bound form of G protein then dissociates from the receptor and can engage downstream mediators. Thus GPCR–G protein coupling involves coordinated conformational change in both proteins, allowing agonist binding to the receptor to effectively “drive” a nucleotide exchange reaction that “switches” the G protein from its inactive (GDP-bound) to active (GTP-bound) form. Figure 2–11 shows the main components schematically. Many high-resolution structures of GPCRs, and several of active-conformation GPCRs in complex with G protein, are available from the Protein Data Bank (www.rcsb.org). Such information provides deep insight into the actions and basis for specificity of existing GPCR-directed drugs, and it is beginning to be used to discover new drug leads through computational chemistry approaches.

Receptor Regulation

G protein–mediated responses to drugs and hormonal agonists often attenuate with time (Figure 2–12A). After reaching an initial high level, the response (eg, cellular cAMP accumulation, Na⁺ influx, contractility, etc) diminishes over seconds or minutes, even

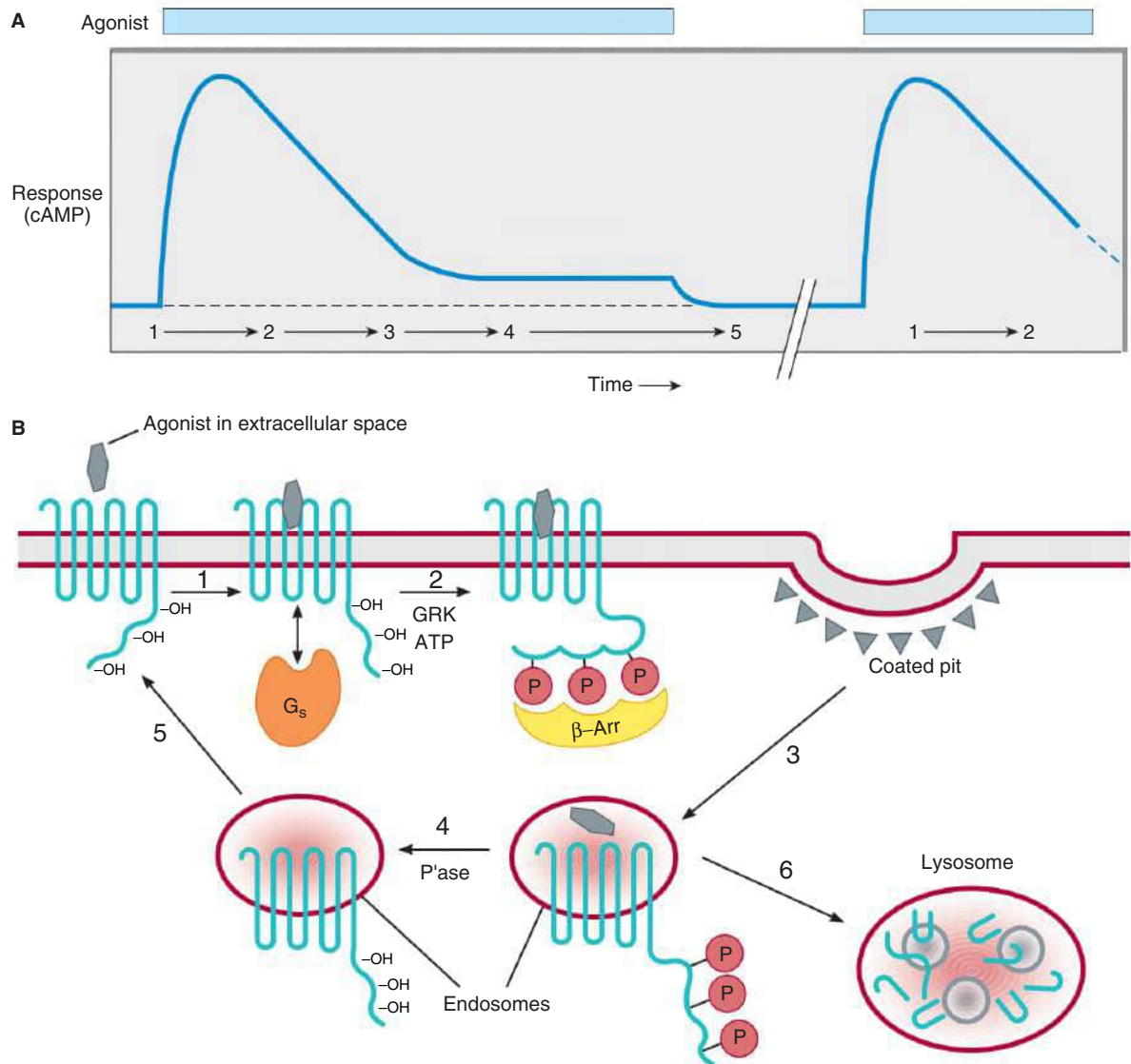


FIGURE 2-12 Rapid desensitization, resensitization, and downregulation of β adrenoceptors. **A:** Response to a β -adrenoceptor agonist (ordinate) versus time (abscissa). (Numbers refer to the phases of receptor function in B.) Exposure of cells to agonist (indicated by the light-colored bar) produces a cyclic AMP (cAMP) response. A reduced cAMP response is observed in the continued presence of agonist; this “desensitization” typically occurs within a few minutes. If agonist is removed after a short time (typically several to tens of minutes, indicated by broken line on abscissa), cells recover full responsiveness to a subsequent addition of agonist (second light-colored bar). This “resensitization” fails to occur, or occurs incompletely, if cells are exposed to agonist repeatedly or over a more prolonged time period. **B:** Agonist binding to receptors initiates signaling by promoting receptor interaction with G proteins (G_s) located in the cytoplasm (step 1 in the diagram). Agonist-activated receptors are phosphorylated by a G protein–coupled receptor kinase (GRK), preventing receptor interaction with G_s and promoting binding of a different protein, β -arrestin (β -Arr), to the receptor (step 2). The receptor–arrestin complex binds to coated pits, promoting receptor internalization (step 3). Dissociation of agonist from internalized receptors reduces β -Arr binding affinity, allowing dephosphorylation of receptors by a phosphatase (P’ase, step 4). Depending on the receptor, and on the agonist concentration achieved in the endosome, a second phase of G protein activation can occur internally before receptors recycle to the plasma membrane (step 5). Recycling of receptors serves to “resensitize” cellular responsiveness by restoring functional receptors at the cell surface, where they can initiate signaling again in response to agonist. The efficiency and rate of this recycling process depends on the GPCR, the physiological state of the cell, and on pharmacological variables including agonist concentration and duration of exposure. In general, repeated or prolonged agonist exposure favors the delivery of internalized receptors to lysosomes rather than their recycling to the cell surface (step 6), causing receptors to down-regulate and reducing, rather than recovering, cellular signaling responsiveness to agonist.

in the continued presence of the agonist. In some cases, this **desensitization** phenomenon is rapidly reversible; a second exposure to agonist, if provided a few minutes after termination of the first exposure, can produce a response similar to that produced initially. In other cases, the desensitized state is more persistent, resulting in a longer-lasting suppression of the cell or tissue response.

Multiple mechanisms contribute to desensitization of GPCRs. One well-understood mechanism involves phosphorylation of the receptor. The agonist-induced change in conformation of the β -adrenoceptor causes it not only to activate G protein, but also to recruit and activate a family of protein kinases called G protein–coupled receptor kinases (GRKs). GRKs phosphorylate serine and threonine residues in the receptor's cytoplasmic tail (Figure 2–12B), diminishing the ability of activated β adrenoceptors to activate G_s and increasing the receptor's affinity for binding a third protein, arrestin or β -arrestin. Binding of β -arrestin to the receptor further diminishes the receptor's ability to interact with G_s , attenuating the cellular response (ie, stimulation of adenylyl cyclase as discussed below). Upon removal of agonist, phosphorylation by the GRK is terminated, β -arrestin can dissociate, and cellular phosphatases reverse the desensitized state and allow activation to occur again upon another encounter with agonist.

For β adrenoceptors, and for many other GPCRs, β -arrestin can produce additional effects. One effect is to accelerate endocytosis of β adrenoceptors from the plasma membrane. This can down-regulate β adrenoceptors if receptors subsequently travel to lysosomes, by a process similar to the downregulation of EGF receptors. However, endocytosis of β adrenoceptors can also accelerate dephosphorylation of receptors by exposing them to phosphatase enzymes in endosomes (see Figure 2–12B). This enables receptors to return to the plasma membrane by recycling to signal again and, in some cases, it also enables receptors to initiate a second round of G protein activation from endosomes. In addition, β -arrestin may itself promote signaling by serving as a molecular scaffold that binds to other signaling proteins. Accordingly, β -arrestin can confer on GPCRs a great deal of flexibility in overall signaling and regulation. This may provide an opportunity to achieve additional selectivity in drug action through GPCRs, a concept called **functional selectivity** or **agonist bias**, whose clinical utility is presently under investigation.

Well-Established Second Messengers

A. Cyclic Adenosine Monophosphate (cAMP)

Acting as an intracellular second messenger, cAMP mediates such hormonal responses as the mobilization of stored energy (the breakdown of carbohydrates in liver or triglycerides in fat cells stimulated by β -adrenomimetic catecholamines), conservation of water by the kidney (mediated by vasopressin), Ca^{2+} homeostasis (regulated by parathyroid hormone), and increased rate and contractile force of heart muscle (β -adrenomimetic catecholamines). It also regulates the production of adrenal and sex steroids (in response to corticotropin or follicle-stimulating hormone), relaxation of smooth muscle, and many other endocrine and neural processes.

cAMP exerts most of its effects by stimulating cAMP-dependent protein kinases (Figure 2–13). These kinases are composed of a

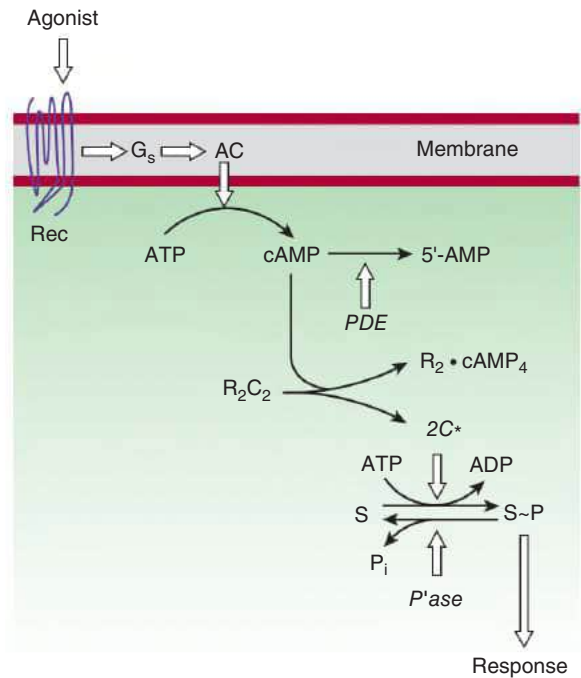


FIGURE 2–13 The cAMP second messenger pathway. Key proteins include hormone receptors (Rec), a stimulatory G protein (G_s), catalytic adenylyl cyclase (AC), phosphodiesterases (PDE) that hydrolyze cAMP, cAMP-dependent kinases, with regulatory (R) and catalytic (C) subunits, protein substrates (S) of the kinases, and phosphatases (P'ase), which remove phosphates from substrate proteins. Open arrows denote regulatory effects.

cAMP-binding regulatory (R) dimer and two catalytic (C) chains. When cAMP binds to the R dimer, active C chains are released to diffuse through the cytoplasm and nucleus, where they transfer phosphate from ATP to appropriate substrate proteins, often enzymes. The specificity of the regulatory effects of cAMP resides in the distinct protein substrates of the kinases that are expressed in different cells. For example, the liver is rich in phosphorylase kinase and glycogen synthase, enzymes whose reciprocal regulation by cAMP-dependent phosphorylation governs carbohydrate storage and release.

When the hormonal stimulus stops, the intracellular actions of cAMP are terminated by an elaborate series of enzymes. cAMP-stimulated phosphorylation of enzyme substrates is rapidly reversed by a diverse group of specific and nonspecific phosphatases. cAMP itself is degraded to 5'-AMP by several cyclic nucleotide phosphodiesterases (PDEs; see Figure 2–13). Milrinone, a selective inhibitor of type 3 phosphodiesterases that are expressed in cardiac muscle cells, has been used as an adjunctive agent in treating acute heart failure. Competitive inhibition of cAMP degradation is one way that caffeine, theophylline, and other methylxanthines produce their effects (see Chapter 20).

B. Phosphoinositides and Calcium

Another well-studied second messenger system involves hormonal stimulation of phosphoinositide hydrolysis (Figure 2–14).

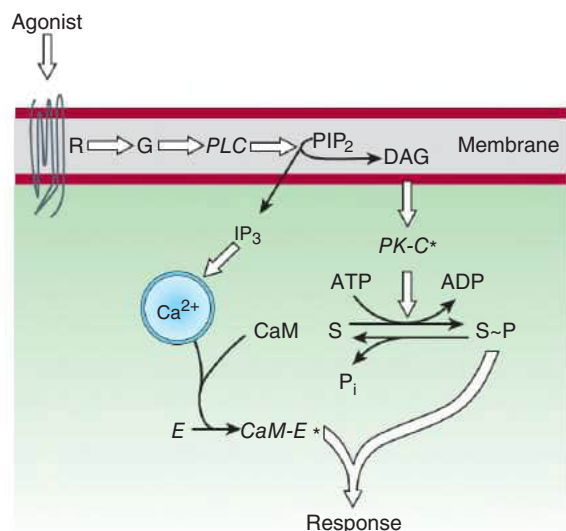


FIGURE 2-14 The Ca^{2+} -phosphoinositide signaling pathway. Key proteins include hormone receptors (R), a G protein (G), a phosphoinositide-specific phospholipase C (PLC), protein kinase C substrates of the kinase (S), calmodulin (CaM), and calmodulin-binding enzymes (E), including kinases, phosphodiesterases, etc. (PIP_2 , phosphatidylinositol-4,5-bisphosphate; DAG, diacylglycerol; IP_3 , inositol trisphosphate. Asterisk denotes activated state. Open arrows denote regulatory effects.)

Some of the hormones, neurotransmitters, and growth factors that trigger this pathway bind to receptors linked to G proteins, whereas others bind to receptor tyrosine kinases. In all cases, the crucial step is stimulation of a membrane enzyme, phospholipase C (PLC), which splits a minor phospholipid component of the plasma membrane, phosphatidylinositol-4,5-bisphosphate (PIP_2), into two second messengers, **diacylglycerol (DAG)** and **inositol-1,4,5-trisphosphate (IP_3 or InsP_3)**. Diacylglycerol is confined to the membrane, where it activates a phospholipid- and calcium-sensitive protein kinase called protein kinase C. IP_3 is water-soluble and diffuses through the cytoplasm to trigger release of Ca^{2+} by binding to ligand-gated calcium channels in the limiting membranes of internal storage vesicles. Elevated cytoplasmic Ca^{2+} concentration resulting from IP_3 -promoted opening of these channels promotes the binding of Ca^{2+} to the calcium-binding protein calmodulin, which regulates activities of other enzymes, including calcium-dependent protein kinases.

With its multiple second messengers and protein kinases, the phosphoinositide signaling pathway is much more complex than the cAMP pathway. For example, different cell types may contain one or more specialized calcium- and calmodulin-dependent kinases with limited substrate specificity (eg, myosin light-chain kinase) in addition to a general calcium- and calmodulin-dependent kinase that can phosphorylate a wide variety of protein substrates. Furthermore, at least nine structurally distinct types of protein kinase C have been identified.

As in the cAMP system, multiple mechanisms damp or terminate signaling by this pathway. IP_3 is inactivated by dephosphorylation;

diacylglycerol is either phosphorylated to yield phosphatidic acid, which is then converted back into phospholipids, or it is deacylated to yield arachidonic acid; Ca^{2+} is actively removed from the cytoplasm by Ca^{2+} pumps.

These and other nonreceptor elements of the calcium-phosphoinositide signaling pathway are of considerable importance in pharmacotherapy. For example, lithium ion, used in treatment of bipolar (manic-depressive) disorder, affects the cellular metabolism of phosphoinositides (see Chapter 29).

C. Cyclic Guanosine Monophosphate (cGMP)

Unlike cAMP, the ubiquitous and versatile carrier of diverse messages, cGMP has established signaling roles in only a few cell types. In the retina, cGMP mediates vision and is destroyed by hydrolysis in response to light, mediated by a phosphodiesterase stimulated by a G protein coupled to the light-activated GPCR rhodopsin. In intestinal mucosa and vascular smooth muscle, the cGMP-based signal transduction mechanism closely parallels the cAMP-mediated signaling mechanism. Ligands detected by cell-surface receptors stimulate membrane-bound guanylyl cyclase to produce cGMP, and cGMP acts by stimulating a cGMP-dependent protein kinase. The actions of cGMP in these cells are terminated by enzymatic degradation of the cyclic nucleotide and by dephosphorylation of kinase substrates.

Increased cGMP concentration causes relaxation of vascular smooth muscle by a kinase-mediated mechanism that results in dephosphorylation of myosin light chains (see Figure 12-2). In these smooth muscle cells, cGMP synthesis can be elevated by two transmembrane signaling mechanisms utilizing two different guanylyl cyclases. Atrial natriuretic peptide, a blood-borne peptide hormone, stimulates a transmembrane receptor by binding to its extracellular domain, thereby activating the guanylyl cyclase activity that resides in the receptor's intracellular domain. The other mechanism mediates responses to nitric oxide (NO; see Chapter 19), which is generated in vascular endothelial cells in response to natural vasodilator agents such as acetylcholine and histamine. After entering the target cell, nitric oxide binds to and activates a cytoplasmic guanylyl cyclase (see Figure 19-2). A number of useful vasodilating drugs, such as nitroglycerin and sodium nitroprusside used in treating cardiac ischemia and acute hypertension, act by generating or mimicking nitric oxide. Other drugs produce vasodilation by inhibiting specific phosphodiesterases, thereby interfering with the metabolic breakdown of cGMP. One such drug is sildenafil, used in treating erectile dysfunction and pulmonary hypertension (see Chapter 12).

Interplay Among Signaling Mechanisms

The calcium-phosphoinositide and cAMP signaling pathways oppose one another in some cells and are complementary in others. For example, vasopressor agents that contract smooth muscle act by IP_3 -mediated mobilization of Ca^{2+} , whereas agents that relax smooth muscle often act by elevation of cAMP. In contrast, cAMP and phosphoinositide second messengers act together to stimulate glucose release from the liver.

Isolation of Signaling Mechanisms

The opposite of signal interplay is seen in some situations—an effective isolation of signaling according to location in the cell. For example, calcium signaling in the heart is highly localized because calcium released into the cytoplasm is rapidly sequestered by nearby calcium-binding proteins and is locally pumped from the cytoplasm into the sarcoplasmic reticulum. Even the second messenger cAMP can have surprisingly local effects, with signals mediated by the same messenger effectively isolated according to location. Here, it appears that signal isolation occurs by local hydrolysis of the second messenger by phosphodiesterase enzymes and by physical scaffolding of signaling pathway components into organized complexes that allow cAMP to transduce its local effects before hydrolysis. One mechanism by which phosphodiesterase inhibitor drugs produce toxic effects may be through “scrambling” local cAMP signals within the cell.

Phosphorylation: A Common Theme

Almost all second messenger signaling involves reversible phosphorylation, which performs two principal functions in signaling: amplification and flexible regulation. In **amplification**, rather like GTP bound to a G protein, the attachment of a phosphoryl group to a serine, threonine, or tyrosine residue powerfully amplifies the initial regulatory signal by recording a molecular memory that the pathway has been activated; dephosphorylation erases the memory, taking a longer time to do so than is required for dissociation of an allosteric ligand. In **flexible regulation**, differing substrate specificities of the multiple protein kinases regulated by second messengers provide branch points in signaling pathways that may be independently regulated. In this way, cAMP, Ca^{2+} , or other second messengers can use the presence or absence of particular kinases or kinase substrates to produce quite different effects in different cell types. Inhibitors of protein kinases have great potential as therapeutic agents, particularly in neoplastic diseases. Trastuzumab, an antibody that antagonizes growth factor signaling by the HER2 tyrosine kinase receptor (discussed earlier), is used for treating breast cancer. Imatinib, a small molecule inhibitor of the cytoplasmic tyrosine kinase Abl, is used for treating chronic myelogenous leukemia caused by a chromosomal translocation event that produces an inappropriate Bcr/Abl fusion protein with unregulated kinase activity.

RECEPTOR CLASSES & DRUG DEVELOPMENT

The existence of a specific drug receptor is usually inferred from studying the **structure-activity relationship** of a group of structurally similar congeners of the drug that mimic or antagonize its effects. Thus, if a series of related agonists exhibits identical relative potencies in producing two distinct effects, it is likely that the two effects are mediated by similar or identical receptor molecules. In addition, if identical receptors mediate both effects, a competitive antagonist will inhibit both responses with the same K_i ; a second competitive antagonist will inhibit both responses with its own

characteristic K_i . Thus, studies of the relation between structure and activity of a series of agonists and antagonists can identify a species of receptor that mediates a set of pharmacologic responses.

Exactly the same experimental procedure can show that observed effects of a drug are mediated by *different* receptors. In this case, effects mediated by different receptors may exhibit different orders of potency among agonists and different K_i values for each competitive antagonist.

Wherever we look, evolution has created many different receptors that function to mediate responses to any individual chemical signal. In some cases, the same chemical acts on completely different structural receptor classes. For example, acetylcholine uses ligand-gated ion channels (nicotinic AChRs) to initiate a fast (in milliseconds) excitatory postsynaptic potential (EPSP) in postganglionic neurons. Acetylcholine also activates a separate class of G protein-coupled receptors (muscarinic AChRs), which mediate slower (seconds to minutes) modulatory effects on the same neurons. In addition, each structural class usually includes multiple subtypes of receptor, often with significantly different signaling or regulatory properties. For example, many biogenic amines (eg, norepinephrine, acetylcholine, histamine, and serotonin) activate more than one receptor, each of which may activate a different G protein, as previously described (see also Table 2–1). The existence of many receptor classes and subtypes for the same endogenous ligand has created important opportunities for drug development. For example, propranolol, a selective antagonist of β adrenoceptors, can reduce an accelerated heart rate without preventing the sympathetic nervous system from causing vasoconstriction, an effect mediated by α_1 adrenoceptors.

The principle of drug selectivity may even apply to structurally identical receptors expressed in different cells, eg, receptors for steroids (see Figure 2–6). Different cell types express different accessory proteins, which interact with steroid receptors and change the functional effects of drug-receptor interaction. For example, tamoxifen is a drug that binds to steroid receptors naturally activated by estrogen. Tamoxifen acts as an *antagonist* on estrogen receptors expressed in mammary tissue but as an *agonist* on estrogen receptors in bone. Consequently, tamoxifen may be useful not only in the treatment of breast cancer but also in the prevention of osteoporosis by increasing bone density (see Chapters 40 and 42). Tamoxifen may create complications in postmenopausal women, however, by exerting an agonist action in the uterus, stimulating endometrial cell proliferation.

New drug development is not confined to agents that act on receptors for extracellular chemical signals. Increasingly, pharmaceutical chemists are determining whether elements of signaling pathways distal to the receptors may also serve as targets of selective and useful drugs. We have already discussed drugs that act on phosphodiesterase and some intracellular kinases. Several new kinase inhibitors and modulators are presently in therapeutic trials, and there has been recent progress in developing inhibitors of GTP-binding transducer proteins that function downstream of tyrosine kinase growth factor receptors. This includes selective inhibitors for an activated form of K-Ras (G12C mutation) that is produced (due to somatic mutation) in many tumors and that causes a pathologic stimulation of growth factor signaling

pathways in the absence of upstream receptor activation. These drugs are now used in the therapy of lung cancer.

RELATION BETWEEN DRUG DOSE & CLINICAL RESPONSE

In this chapter, we have dealt with receptors as molecules and shown how receptors can quantitatively account for the relation between dose or concentration of a drug and pharmacologic responses, at least in an idealized system. When faced with a patient who needs treatment, the prescriber must make a choice among a variety of possible drugs and devise a dosage regimen that is likely to produce maximal benefit and minimal toxicity. To make rational therapeutic decisions, the prescriber must understand how drug-receptor interactions underlie the relations between dose and response in patients, the nature and causes of variation in pharmacologic responsiveness, and the clinical implications of selectivity of drug action.

Dose & Response in Patients

A. Graded Dose-Response Relations

To choose among drugs and to determine appropriate doses of a drug, the prescriber must know the relative **pharmacologic potency** and **maximal efficacy** of the drugs in relation to the desired therapeutic effect. These two important terms, often confusing to students and clinicians, can be explained by referring to Figure 2–15, which depicts graded dose-response curves that relate the dose of four different drugs to the magnitude of a particular therapeutic effect.

1. Potency—Drugs A and B are said to be more potent than drugs C and D because of the relative positions of their dose-response curves along the **dose axis** in Figure 2–15. Potency

refers to the concentration (EC_{50}) or dose (ED_{50}) of a drug required to produce 50% of that drug's maximal effect. Thus, the pharmacologic potency of drug A in Figure 2–15 is less than that of drug B, a partial agonist because the EC_{50} of A is greater than the EC_{50} of B. Potency of a drug depends in part on the affinity (K_d) of receptors for binding the drug and in part on the efficiency with which drug-receptor interaction is coupled to response. Note that some doses of drug A can produce larger effects than any dose of drug B, despite the fact that we describe drug B as pharmacologically more potent. The reason for this is that drug A has a larger maximal efficacy (as described below).

For therapeutic purposes, the potency of a drug should be stated in dosage units, usually in terms of a particular therapeutic end point (eg, 50 mg for mild sedation, 1 mcg/kg/min for an increase in heart rate of 25 bpm). Relative potency, the ratio of equi-effective doses (0.2, 10, etc), may be used in comparing one drug with another.

2. Maximal efficacy—This parameter reflects the limit of the dose-response relation on the **response axis**. Drugs A, C, and D in Figure 2–15 have equal maximal efficacy, and all have greater maximal efficacy than drug B. The maximal efficacy (sometimes referred to simply as efficacy) of a drug is obviously crucial for making clinical decisions when a large response is needed. It may be determined by the drug's mode of interactions with receptors (as with partial agonists)* or by characteristics of the receptor-effector system involved.

Thus, diuretics that act on one portion of the nephron may produce much greater excretion of fluid and electrolytes than diuretics that act elsewhere. In addition, the *practical* efficacy of a drug for achieving a therapeutic end point (eg, increased cardiac contractility) may be limited by the drug's propensity to cause a toxic effect (eg, fatal cardiac arrhythmia) even if the drug could otherwise produce a greater therapeutic effect.

B. Shape of Dose-Response Curves

Although the responses depicted in curves A, B, and C of Figure 2–15 approximate the shape of a simple Michaelis–Menten relation (transformed to a logarithmic plot), some clinical responses do not. Extremely steep dose-response curves (eg, curve D) may have important clinical consequences if the upper portion of the curve represents an undesirable extent of response (eg, coma caused by a sedative-hypnotic). Steep dose-response curves in patients can result from cooperative interactions of several different actions of a drug (eg, effects on brain, heart, and peripheral vessels, all contributing to lowering of blood pressure).

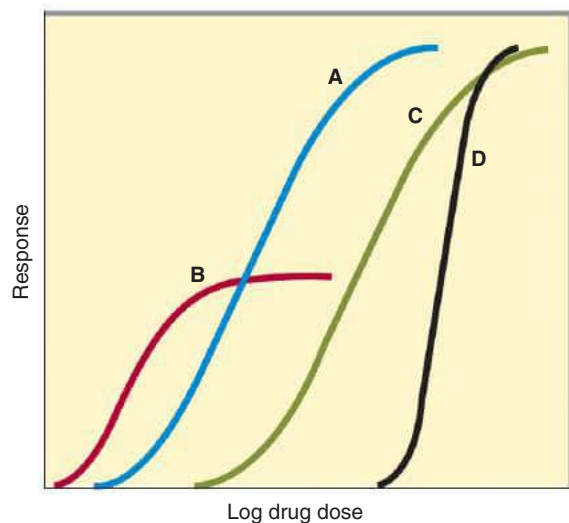


FIGURE 2–15 Graded dose-response curves for four drugs, illustrating different pharmacologic potencies and different maximal efficacies. (See text.)

*Note that “maximal efficacy,” used in a therapeutic context, does not have exactly the same meaning that the term denotes in the more specialized context of drug-receptor interactions described earlier in this chapter. In an idealized *in vitro* system, efficacy denotes the relative maximal efficacy of agonists and partial agonists that act via the same receptor. In therapeutics, efficacy denotes the extent or degree of an effect that can be achieved in the intact patient. Thus, therapeutic efficacy may be affected by the characteristics of a particular drug-receptor interaction, but it also depends on a host of other factors as noted in the text.

C. Quantal Dose-Effect Curves

Graded dose-response curves of the sort described above have certain limitations in their application to clinical decision making. For example, such curves may be impossible to construct if the pharmacologic response is an either-or (quantal) event, such as prevention of convulsions, arrhythmia, or death. Furthermore, the clinical relevance of a quantitative dose-response relation in a single patient, no matter how precisely defined, may be limited in application to other patients, owing to the great potential variability among patients in severity of disease and responsiveness to drugs.

Some of these difficulties may be avoided by determining the dose of drug required to produce a specified magnitude of effect in a large number of individual patients or experimental animals and plotting the cumulative frequency distribution of responders versus the log dose (Figure 2–16). The specified quantal effect may be chosen on the basis of clinical relevance (eg, relief of headache) or for preservation of safety of experimental subjects (eg, using low doses of a cardiac stimulant and specifying an increase in heart rate of 20 bpm as the quantal effect), or it may be an inherently quantal event (eg, death of an experimental animal). For most drugs, the doses required to produce a specified quantal effect in individuals are lognormally distributed; that is, a frequency distribution of such responses plotted against the log of the dose produces a gaussian normal curve of variation (colored areas; see Figure 2–16). When these responses are summated, the resulting cumulative frequency distribution constitutes a quantal dose-effect curve (or dose-percent curve) of the proportion or percentage of individuals who exhibit the effect plotted as a function of log dose.

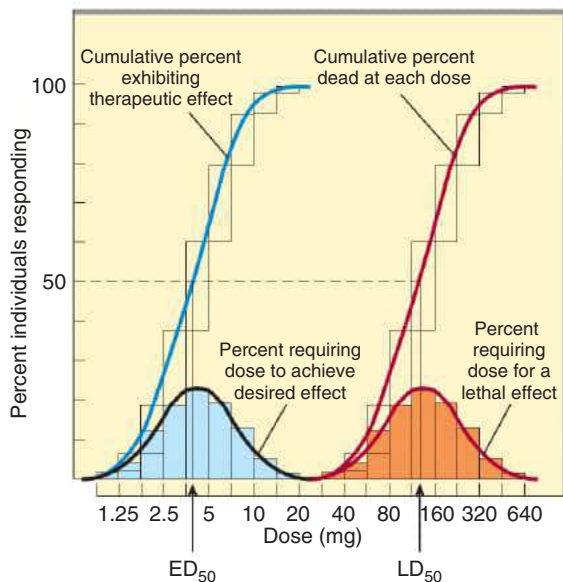


FIGURE 2–16 Quantal dose-effect plots. Shaded boxes (and the accompanying bell-shaped curves) indicate the frequency distribution of doses of drug required to produce a specified effect; that is, the percentage of animals that required a particular dose to exhibit the effect. The open boxes (and the corresponding colored curves) indicate the cumulative frequency distribution of responses, which are lognormally distributed.

The quantal dose-effect curve is often characterized by stating the **median effective dose (ED₅₀)**, which is the dose at which 50% of individuals exhibit the specified quantal effect. (Note that the abbreviation ED₅₀ has a different meaning in this context from its meaning in relation to graded dose-effect curves, described in previous text.) Similarly, the dose required to produce a particular toxic effect in 50% of animals is called the **median toxic dose (TD₅₀)**. If the toxic effect is death of the animal, a **median lethal dose (LD₅₀)** may be experimentally defined. Such values provide a convenient way of comparing the potencies of drugs in experimental and clinical settings: Thus, if the ED₅₀s of two drugs for producing a specified quantal effect are 5 and 500 mg, respectively, then the first drug can be said to be 100 times more potent than the second for that particular effect. Similarly, one can obtain a valuable index of the selectivity of a drug's action by comparing its ED₅₀s for two different quantal effects in a population (eg, cough suppression versus sedation for opioid drugs).

Quantal dose-effect curves may also be used to generate information regarding the margin of safety to be expected from a particular drug used to produce a specified effect. One measure, which relates the dose of a drug required to produce a desired effect to that which produces an undesired effect, is the **therapeutic index**. In animal studies, the therapeutic index is usually defined as the ratio of the TD₅₀ to the ED₅₀ for some therapeutically relevant effect. The precision possible in animal experiments may make it useful to use such a therapeutic index to estimate the potential benefit of a drug in humans. Of course, the therapeutic index of a drug in humans is almost never known with real precision; instead, drug trials and accumulated clinical experience often reveal a range of usually effective doses and a different (but sometimes overlapping) range of possibly toxic doses. The range between the minimum toxic dose and the minimum therapeutic dose is called the **therapeutic window** and is of greater practical value in choosing the dose for a patient. The clinically acceptable risk of toxicity depends critically on the severity of the disease being treated. For example, the dose range that provides relief from an ordinary headache in the majority of patients should be very much lower than the dose range that produces serious toxicity, even if the toxicity occurs in a small minority of patients. However, for treatment of a lethal disease such as Hodgkin lymphoma, the acceptable difference between therapeutic and toxic doses may be smaller.

Finally, note that the quantal dose-effect curve and the graded dose-response curve summarize somewhat different sets of information, although both appear sigmoid in shape on a semilogarithmic plot (compare Figures 2–15 and 2–16). Critical information required for making rational therapeutic decisions can be obtained from each type of curve. Both curves provide information regarding the **potency** and **selectivity** of drugs; the graded dose-response curve indicates the **maximal efficacy** of a drug, and the quantal dose-effect curve indicates the potential **variability** of responsiveness among individuals.

Variation in Drug Responsiveness

Individuals may vary considerably in their response to a drug; indeed, a single individual may respond differently to the same

drug at different times during the course of treatment. Occasionally, individuals exhibit an unusual or **idiosyncratic** drug response, one that is infrequently observed in most patients. The idiosyncratic responses are usually caused by genetic differences in metabolism of the drug or by immunologic mechanisms, including allergic reactions.

Quantitative variations in drug response are, in general, more common and more clinically important. An individual patient is **hyporeactive** or **hyperreactive** to a drug in that the intensity of effect of a given dose of drug is diminished or increased compared with the effect seen in most individuals. (*Note:* The term **hypersensitivity** usually refers to allergic or other immunologic responses to drugs.) With some drugs, the intensity of response to a given dose may change during the course of therapy; in these cases, responsiveness usually decreases as a consequence of continued drug administration, producing a state of relative **tolerance** to the drug's effects. When responsiveness diminishes rapidly after administration of a drug, the response is said to be subject to **tachyphylaxis**.

Even before administering the first dose of a drug, the prescriber should consider factors that may help in predicting the direction and extent of possible variations in responsiveness. These include the propensity of a particular drug to produce tolerance or tachyphylaxis as well as the effects of age, sex, body size, disease state, genetic factors, and simultaneous administration of other drugs.

Four general mechanisms may contribute to variation in drug responsiveness among patients or within an individual patient at different times.

A. Alteration in Concentration of Drug That Reaches the Receptor

As described in Chapter 3, patients may differ in the rate of absorption of a drug, in distributing it through body compartments, or in clearing the drug from the blood. By altering the concentration of drug that reaches relevant receptors, such pharmacokinetic differences may alter the clinical response. Some differences can be predicted on the basis of age, weight, sex, disease state, and liver and kidney function, and by testing specifically for genetic differences that may result from inheritance of a functionally distinctive complement of drug-metabolizing enzymes (see Chapters 4 and 5). Another important mechanism influencing drug availability is active transport of drug from the cytoplasm, mediated by a family of membrane transporters encoded by the so-called multidrug resistance (*MDR*) genes. For example, up-regulation of *MDR* gene-encoded transporter expression is a major mechanism by which tumor cells develop resistance to anticancer drugs.

B. Variation in Concentration of an Endogenous Receptor Ligand

This mechanism contributes greatly to variability in responses to pharmacologic antagonists. Thus, propranolol, a β -adrenoceptor antagonist, markedly slows the heart rate of a patient whose endogenous catecholamines are elevated (as in pheochromocytoma) but does not affect the resting heart rate of a well-trained marathon runner. A partial agonist may exhibit even more dramatically

different responses: Saralasin, a weak partial agonist at angiotensin II receptors, lowers blood pressure in patients with hypertension caused by increased angiotensin II production and raises blood pressure in patients who produce normal amounts of angiotensin.

C. Alterations in Number or Function of Receptors

Experimental studies have documented changes in drug response caused by increases or decreases in the number of receptor sites or by alterations in the efficiency of coupling of receptors to distal effector mechanisms. In some cases, the change in receptor number is caused by other hormones; for example, thyroid hormones increase both the number of β adrenoceptors in rat heart muscle and cardiac sensitivity to catecholamines. Similar changes probably contribute to the tachycardia of thyrotoxicosis in patients and may account for the usefulness of propranolol, a β -adrenoceptor antagonist, in ameliorating symptoms of this disease.

In other cases, the agonist ligand itself induces a decrease in the number (eg, downregulation) or coupling efficiency (eg, desensitization) of its receptors. These mechanisms (discussed previously under Signaling Mechanisms & Drug Action) may contribute to two clinically important phenomena: first, tachyphylaxis or tolerance to the effects of some drugs (eg, biogenic amines and their congeners), and second, the "overshoot" phenomena that follow withdrawal of certain drugs. These phenomena can occur with either agonists or antagonists. An antagonist may increase the number of receptors in a critical cell or tissue by preventing downregulation caused by an endogenous agonist. When the antagonist is withdrawn, the elevated number of receptors can produce an exaggerated response to physiologic concentrations of agonist. Potentially disastrous withdrawal symptoms can result for the opposite reason when administration of an agonist drug is discontinued. In this situation, the number of receptors, which has been decreased by drug-induced downregulation, is too low for endogenous agonist to produce effective stimulation. For example, the withdrawal of clonidine (a drug whose α_2 -adrenoceptor agonist activity reduces blood pressure) can produce hypertensive crisis, probably because the drug down-regulates α_2 adrenoceptors (see Chapter 11).

The study of genetic factors determining drug response is called **pharmacogenetics**, and the use of gene sequencing or expression profile data to tailor therapies specific to an individual patient is called **personalized** or **precision medicine**. For example, somatic mutations affecting the tyrosine kinase domain of the epidermal growth factor receptor in lung cancers can confer enhanced sensitivity to kinase inhibitors such as gefitinib. This effect enhances the antineoplastic effect of the drug, and because the somatic mutation is specific to the tumor and not present in the host, the therapeutic index of these drugs can be significantly enhanced in patients whose tumors harbor such mutations. Genetic analysis can also predict drug resistance during treatment or identify new targets for therapy based on rapid mutation of the tumor in the patient.

D. Changes in Components of Response Distal to the Receptor

Although a drug initiates its actions by binding to receptors, the response observed in a patient depends on the functional integrity of

biochemical processes in the responding cell and physiologic regulation by interacting organ systems. Clinically, changes in these post-receptor processes represent the largest and most important class of mechanisms that cause variation in responsiveness to drug therapy.

Before initiating therapy with a drug, the prescriber should be aware of patient characteristics that may limit the clinical response. These characteristics include the age and general health of the patient and—most importantly—the severity and pathophysiologic mechanism of the disease. The most important potential cause of failure to achieve a satisfactory response is that the diagnosis is wrong or physiologically incomplete. Drug therapy is most successful when it is accurately directed at the pathophysiologic mechanism responsible for the disease.

When the diagnosis is correct and the drug is appropriate, an unsatisfactory therapeutic response can often be traced to compensatory mechanisms in the patient that respond to and oppose the beneficial effects of the drug. Compensatory increases in sympathetic nervous tone and fluid retention by the kidney, for example, can contribute to tolerance to antihypertensive effects of a vasodilator drug. In such cases, additional drugs may be required to achieve a useful therapeutic result.

Clinical Selectivity: Beneficial Versus Toxic Effects of Drugs

Although we classify drugs according to their principal actions, it is clear that *no drug causes only a single, specific effect*. Why is this so? It is exceedingly unlikely that any kind of drug molecule will bind to only a single type of receptor molecule, if only because the number of potential receptors in every patient is astronomically large. Even if the chemical structure of a drug allowed it to bind to only one kind of receptor, the biochemical processes controlled by such receptors would take place in many cell types and would be coupled to many other biochemical functions; as a result, the patient and the prescriber would probably perceive more than one drug effect. Accordingly, drugs are only *selective*—rather than *specific*—in their actions, because they bind to one or a few types of receptor more tightly than to others and because these receptors control discrete processes that result in distinct effects.

It is only because of their selectivity that drugs are useful in clinical medicine. Selectivity can be measured by comparing binding affinities of a drug to different receptors or by comparing ED₅₀s for different effects of a drug in vivo. In drug development and in clinical medicine, selectivity is usually considered by separating effects into two categories: **beneficial** or **therapeutic effects** versus **toxic** or **adverse effects**. Pharmaceutical advertisements and prescribers occasionally use the term **side effect**, implying that the effect in question is insignificant or occurs via a pathway that is to one side of the principal action of the drug; such implications are frequently erroneous.

A. Beneficial and Toxic Effects Mediated by the Same Receptor-Effector Mechanism

Much of the serious drug toxicity in clinical practice represents a direct pharmacologic extension of the therapeutic actions of the drug. In some of these cases (eg, bleeding caused by anticoagulant

therapy; hypoglycemic coma due to insulin), toxicity may be avoided by judicious management of the dose of drug administered, guided by careful monitoring of effect (measurements of blood coagulation or serum glucose) and aided by ancillary measures (avoiding tissue trauma that may lead to hemorrhage; regulation of carbohydrate intake). In still other cases, the toxicity may be avoided by not administering the drug at all, if the therapeutic indication is weak or if other therapy is available.

In certain situations, a drug is clearly necessary and beneficial but produces unacceptable toxicity when given in doses that produce optimal benefit. In such situations, it may be necessary to add another drug to the treatment regimen. In treating hypertension, for example, administration of a second drug often allows the prescriber to reduce the dose and toxicity of the first drug (see Chapter 11).

B. Beneficial and Toxic Effects Mediated by Identical Receptors but in Different Tissues or by Different Effector Pathways

Many drugs produce both their desired effects and adverse effects by acting on a single receptor type in different tissues. Examples discussed in this book include digitalis glycosides, which act by inhibiting Na⁺/K⁺-ATPase in cell membranes; methotrexate, which inhibits the enzyme dihydrofolate reductase; and glucocorticoid hormones.

Three therapeutic strategies are used to avoid or mitigate this sort of toxicity. First, the drug should always be administered at the lowest dose that produces acceptable benefit. Second, adjunctive drugs that act through different receptor mechanisms and produce different toxicities may allow lowering the dose of the first drug, thus limiting its toxicity (eg, use of other immunosuppressive agents added to glucocorticoids in treating inflammatory disorders). Third, selectivity of the drug's actions may be increased by manipulating the concentrations of drug available to receptors in different parts of the body, for example, by aerosol administration of a glucocorticoid to the bronchi in asthma.

C. Beneficial and Toxic Effects Mediated by Different Types of Receptors

Therapeutic advantages resulting from new chemical entities with improved receptor selectivity were mentioned earlier in this chapter and are described in detail in later chapters. Many receptors, such as those for catecholamines, histamine, acetylcholine, and corticosteroids, and their associated therapeutic uses were discovered by analyzing effects of the physiologic chemical signals. This approach continues to be fruitful. For example, microRNAs (miRNAs), small RNAs that regulate protein expression by binding to protein-coding (messenger) RNAs, were linked recently to Duchenne muscular dystrophy. Current preclinical investigations include the potential utility of RNA-based therapy for this and other diseases.

Other drugs were discovered by exploiting therapeutic or toxic effects of chemically similar agents observed in a clinical context. Examples include quinidine, the sulfonylureas, thiazide diuretics, tricyclic antidepressants, opioid drugs, and phenothiazine antipsychotics. Often such agents turn out to interact with receptors for endogenous substances (eg, opioids and phenothiazines for endogenous opioid and dopamine receptors, respectively). This approach

is evolving toward understanding the structural details of how chemically similar agents differ in binding to receptors. For example, x-ray crystallography of β_1 and β_2 adrenoceptors shows that their orthosteric binding sites are identical; drugs discriminate between subtypes based on differences in traversing a divergent “vestibule” to access the orthosteric site. Many GPCRs have such passages, revealing a new basis for improving the selectivity of GPCR-targeted drugs.

Thus, the propensity of drugs to bind to different classes of receptor sites is not only a potentially vexing problem in treating patients, but it also presents a continuing challenge to pharmacology and an opportunity for developing new and more useful drugs.

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CASE STUDY ANSWER

Propranolol, a β -adrenoceptor antagonist, is a useful anti-hypertensive agent because it reduces cardiac output and probably vascular resistance as well. However, it also prevents β -adrenoceptor-induced bronchodilation and therefore may precipitate bronchoconstriction in susceptible individuals. Calcium channel blockers such as verapamil also reduce blood pressure but, because they act on a different target, rarely cause bronchoconstriction or prevent bronchodilation. An alternative approach in this patient

would be to use a more highly selective adrenoceptor antagonist drug (such as metoprolol) that binds preferentially to the β_1 subtype, which is a major β adrenoceptor in the heart, and has a lower affinity (ie, higher K_d) for binding the β_2 subtype that mediates bronchodilation. Selection of the most appropriate drug or drug group for one condition requires awareness of the other conditions a patient may have and the receptor selectivity of the drug groups available.

3

Pharmacokinetics & Pharmacodynamics: Rational Dosing & the Time Course of Drug Action

Nicholas H. G. Holford, MB, ChB, FRACP

CASE STUDY

An 85-year-old, 60-kg woman with a serum creatinine of 1.8 mg/dL has atrial fibrillation. A decision has been made to use digoxin to control the rapid heart rate. The target concentration of digoxin for the treatment of atrial

fibrillation is 1 mg/mL. Tablets of digoxin are available that contain 62.5 micrograms (mcg) and 250 mcg. What maintenance dose would you recommend?

The goal of therapeutics is to achieve a desired beneficial effect with minimal adverse effects. When a medicine has been selected for a patient, the clinician must determine the dose that most closely achieves this goal. A rational approach to this objective combines the principles of pharmacokinetics with pharmacodynamics to understand the dose-effect relationship (Figure 3–1). Pharmacodynamics governs the concentration-effect part of the relationship, whereas pharmacokinetics deals with the dose-concentration part (Holford & Sheiner, 1981). The pharmacodynamic concepts of maximum response and sensitivity determine the magnitude of the effect at a particular concentration (see $E_{\max}$ and C_{50} , Chapter 2; C_{50} is also known as EC_{50}). The pharmacokinetic processes of input, distribution, and elimination determine how rapidly and for how long the target organ will be exposed to the drug.

Figure 3–1 illustrates a fundamental hypothesis of pharmacology, namely, that a relationship exists between a beneficial or toxic effect of a drug and the concentration of the drug. This hypothesis has been documented for many drugs, as indicated by the target concentration column in Table 3–1. The target concentration is

the concentration that reflects a balance between the beneficial and adverse effects. The apparent lack of such a relationship for some drugs does not weaken the basic hypothesis but points to the need to consider the time course of concentration at the actual site of pharmacologic effect (see below).

Knowing the relationship between dose, drug concentration, and effects allows the clinician to take into account the various pathologic and physiologic features of a particular patient that make him or her different from the average individual in responding to a drug. The importance of pharmacokinetics and pharmacodynamics in patient care thus rests upon the improvement in therapeutic benefit and reduction in toxicity that can be achieved by application of these principles.

PHARMACOKINETICS

The “standard” dose of a drug is based on trials in healthy subjects and patients with average ability to absorb, distribute, and eliminate the drug (see section Clinical Trials: The IND & NDA in Chapter 1).

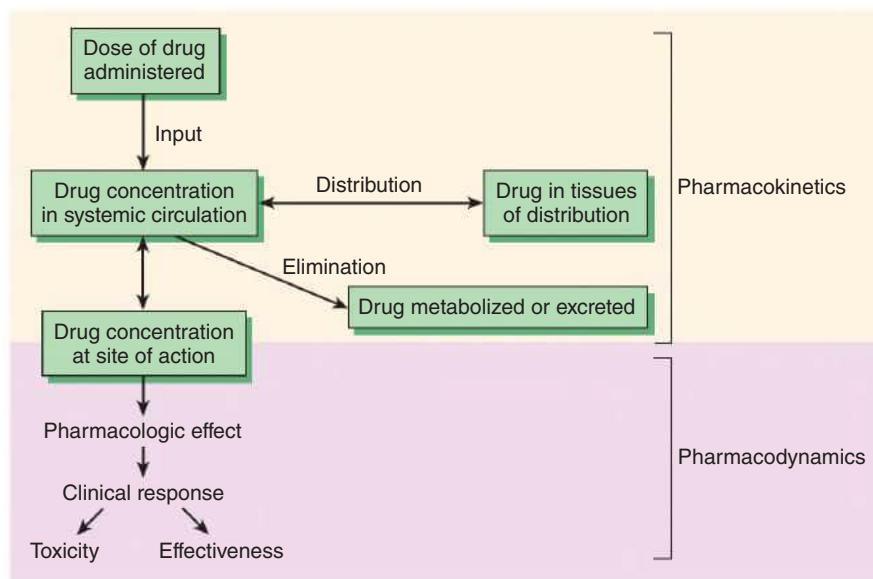


FIGURE 3-1 The relationship between dose and effect can be separated into pharmacokinetic (dose-concentration) and pharmacodynamic (concentration-effect) components. Concentration provides the link between pharmacokinetics and pharmacodynamics and is the focus of the target concentration approach to rational dosing. The three primary processes of pharmacokinetics are input, distribution, and elimination.

This dose will not be suitable for every patient. Several physiologic processes (eg, body size, maturation of organ function in neonates and infants) and pathologic processes (eg, heart failure, renal failure) may be used for dosage adjustment in individual patients. Individual differences in these physiological and pathological processes are associated with specific pharmacokinetic and pharmacodynamic properties (usually referred to as parameters) of the drug. The two basic pharmacokinetic parameters are **volume of distribution**, the measure of the apparent space in the body available to contain the drug, and **clearance**, the measure of the ability of the body to eliminate the drug. These parameters are illustrated schematically in Figure 3-2, where the volume of the beakers into which the drugs diffuse represents the volume of distribution, and the size of the outflow “drain” in Figures 3-2B and 3-2D represents the clearance.

Volume of Distribution

Volume of distribution (V) relates the amount of drug in the body to the concentration of drug (C) in blood or plasma:

$$V = \frac{\text{Amount of drug in body}}{C} \quad (1)$$

The volume of distribution may be defined with respect to blood, plasma, or water (unbound drug), depending on the concentration used in equation (1) ($C = C_b$, C_p , or C_u).

That the V calculated from equation (1) is an *apparent* volume may be appreciated by comparing the volumes of distribution of drugs such as digoxin or chloroquine (see Table 3-1) with some

of the physical volumes of the body (Table 3-2). Volume of distribution often exceeds any physical volume in the body because it is the volume *apparently* necessary to contain the amount of drug *homogeneously* at the concentration found in the blood, plasma, or water. Drugs with very high volumes of distribution have much higher concentrations in extravascular tissue than in the vascular compartment, ie, they are *not* homogeneously distributed. Drugs that are completely retained within the vascular compartment, on the other hand, would have a minimum possible volume of distribution equal to the plasma component in which they are distributed, eg, 0.04 L/kg body weight or 2.8 L/70 kg (see Table 3-2) for a drug that is restricted to the plasma compartment.

Clearance

Drug clearance concepts are similar to clearance concepts of renal physiology. Clearance of a drug is the factor that predicts the rate of elimination in relation to the drug concentration (C):

$$CL = \frac{\text{Rate of elimination}}{C} \quad (2)$$

Clearance, like volume of distribution, may be defined with respect to blood (CL_b), plasma (CL_p), or unbound in water (CL_u), depending on where and how the concentration is measured.

It is important to note the additive character of clearance. Elimination of drug from the body may involve processes occurring in the kidney, the lung, the liver, and other organs. Dividing the rate of elimination at each organ by the concentration of drug yields

TABLE 3–1 Pharmacokinetic and pharmacodynamic parameters for selected drugs in adults. (See Holford et al, 2013, for parameters in neonates and children.)

Drug	Oral Availability (F) (%)	Urinary Excretion (%) ¹	Bound in Plasma (%)	Clearance (L/h/70 kg) ²	Volume of Distribution (L/70 kg)	Half-Life (h)	Target Concentration	Toxic Concentration
Acetaminophen	88	3	0	21	67	2	15 mg/L	>300 mg/L
Acyclovir	23	75	15	19.8	48	2.4	...	...
Amikacin	...	98	4	5.46	19	2.3	10 mg/L ³ ...	...
Amoxicillin	93	86	18	10.8	15	1.7	...	...
Amphotericin	...	4	90	1.92	53	18	...	...
Ampicillin	62	82	18	16.2	20	1.3	...	...
Aspirin	68	1	49	39	11	0.25	...	...
Atenolol	56	94	5	10.2	67	6.1	1 mg/L	...
Atropine	50	57	18	24.6	120	4.3	...	...
Captopril	65	38	30	50.4	57	2.2	50 ng/mL	...
Carbamazepine	70	1	74	5.34	98	15	6 mg/L	>9 mg/L
Cephalexin	90	91	14	18	18	0.9	...	...
Cephalothin	...	52	71	28.2	18	0.57	...	...
Chloramphenicol	80	25	53	10.2	66	2.7	...	...
Chlordiazepoxide	100	1	97	2.28	21	10	1 mg/L	...
Chloroquine	89	61	61	45	13,000	214	20 ng/mL	250 ng/mL
Chlorpropamide	90	20	96	0.126	6.8	33	...	...
Cimetidine	62	62	19	32.4	70	1.9	0.8 mg/L	...
Ciprofloxacin	60	65	40	25.2	130	4.1	...	...
Clonidine	95	62	20	12.6	150	12	1 ng/mL	...
Cyclosporine	30	1	98	23.9	244	15	200 ng/mL	>400 ng/mL
Diazepam	100	1	99	1.62	77	43	300 ng/mL	...
Digoxin	70	67	25	9	500	39	1 ng/mL	>2 ng/mL
Diltiazem	44	4	78	50.4	220	3.7	...	...
Disopyramide	83	55	2	5.04	41	6	3 mg/mL	>8 mg/mL
Enalapril	95	90	55	9	40	3	>0.5 ng/mL	...
Erythromycin	35	12	84	38.4	55	1.6	...	...
Ethambutol	77	79	5	36	110	3.1	...	>10 mg/L
Fluoxetine	60	3	94	40.2	2500	53	...	...
Furosemide	61	66	99	8.4	7.7	1.5	...	>25 mg/L
Gentamicin	...	76	10	4.7	20	3	3 mg/L ³	...
Hydralazine	40	10	87	234	105	1	100 ng/mL	...
Imipramine	40	2	90	63	1600	18	200 ng/mL	>1 mg/L
Indomethacin	98	15	90	8.4	18	2.4	1 mg/L	>5 mg/L
Labetalol	18	5	50	105	660	4.9	0.1 mg/L	...
Lidocaine	35	2	70	38.4	77	1.8	3 mg/L	>6 mg/L
Lithium	100	95	0	1.5	55	22	0.7 mEq/L	>2 mEq/L
Meperidine	52	12	58	72	310	3.2	0.5 mg/L	...

(continued)

TABLE 3–1 Pharmacokinetic and pharmacodynamic parameters for selected drugs in adults. (See Holford et al, 2013, for parameters in neonates and children.) (Continued)

Drug	Oral Availability (F) (%)	Urinary Excretion (%) ¹	Bound in Plasma (%)	Clearance (L/h/70 kg) ²	Volume of Distribution (L/70 kg)	Half-Life (h)	Target Concentration	Toxic Concentration
Methotrexate	70	48	34	9	39	7.2	750 $\mu\text{M}\cdot\text{h}$ ^{4,5}	>950 $\mu\text{M}\cdot\text{h}$
Metoprolol	38	10	11	63	290	3.2	25 ng/mL	...
Metronidazole	99	10	10	5.4	52	8.5	4 mg/L	...
Midazolam	44	56	95	27.6	77	1.9	...	...
Morphine	24	8	35	60	230	1.9	15 ng/mL	...
Nifedipine	50	0	96	29.4	55	1.8	50 ng/mL	...
Nortriptyline	51	2	92	30	1300	31	100 ng/mL	>500 ng/mL
Phenobarbital	100	24	51	0.258	38	98	15 mg/L	>30 mg/L
Phenytoin	90	2	89	Conc dependent ⁵	45	Conc dependent ⁶	10 mg/L	>20 mg/L
Prazosin	68	1	95	12.6	42	2.9	...	...
Procainamide	83	67	16	36	130	3	5 mg/L	>14 mg/L
Propranolol	26	1	87	50.4	270	3.9	20 ng/mL	...
Pyridostigmine	14	85	...	36	77	1.9	75 ng/mL	...
Quinidine	80	18	87	19.8	190	6.2	3 mg/L	>8 mg/L
Ranitidine	52	69	15	43.8	91	2.1	100 ng/mL	...
Rifampin	?	7	89	14.4	68	3.5	...	...
Salicylic acid	100	15	85	0.84	12	13	200 mg/L	>200 mg/L
Sulfamethoxazole	100	14	62	1.32	15	10	...	...
Tacrolimus	20	...	98 ⁷	3 ⁸	133 ⁸	28	10 mcg/L	...
Terbutaline	14	56	20	14.4	125	14	2 ng/mL	...
Tetracycline	77	58	65	7.2	105	11	...	...
Theophylline	96	18	56	2.8	35	8.1	10 mg/L	>20 mg/L
Tobramycin	...	90	10	4.62	18	2.2	...	...
Tocainide	89	38	10	10.8	210	14	10 mg/L	...
Tolbutamide	93	0	96	1.02	7	5.9	100 mg/L	...
Trimethoprim	100	69	44	9	130	11	...	...
Tubocurarine	...	63	50	8.1	27	2	0.6 mg/L	...
Valproic acid	100	2	93	0.462	9.1	14	75 mg/L	>150 mg/L
Vancomycin	...	79	30	5.88	27	5.6	20 mg/L ³	...
Verapamil	22	3	90	63	350	4	...	...
Warfarin	93	3	99	0.192	9.8	37	...	...
Zidovudine	63	18	25	61.8	98	1.1	...	...

¹Assuming creatinine clearance 100 mL/min/70 kg.²Convert to mL/min by multiplying the number given by 16.6.³Average steady-state concentration.⁴Target area under the concentration-time curve after a single dose.⁵Can be estimated from measured C using $CL = V_{\text{max}}/(K_m + C)$; $V_{\text{max}} = 415 \text{ mg/d}$, $K_m = 5 \text{ mg/L}$. See text.⁶Varies because of concentration-dependent clearance.⁷Bound in whole blood (%).⁸Based on whole blood standardized to hematocrit 45%.

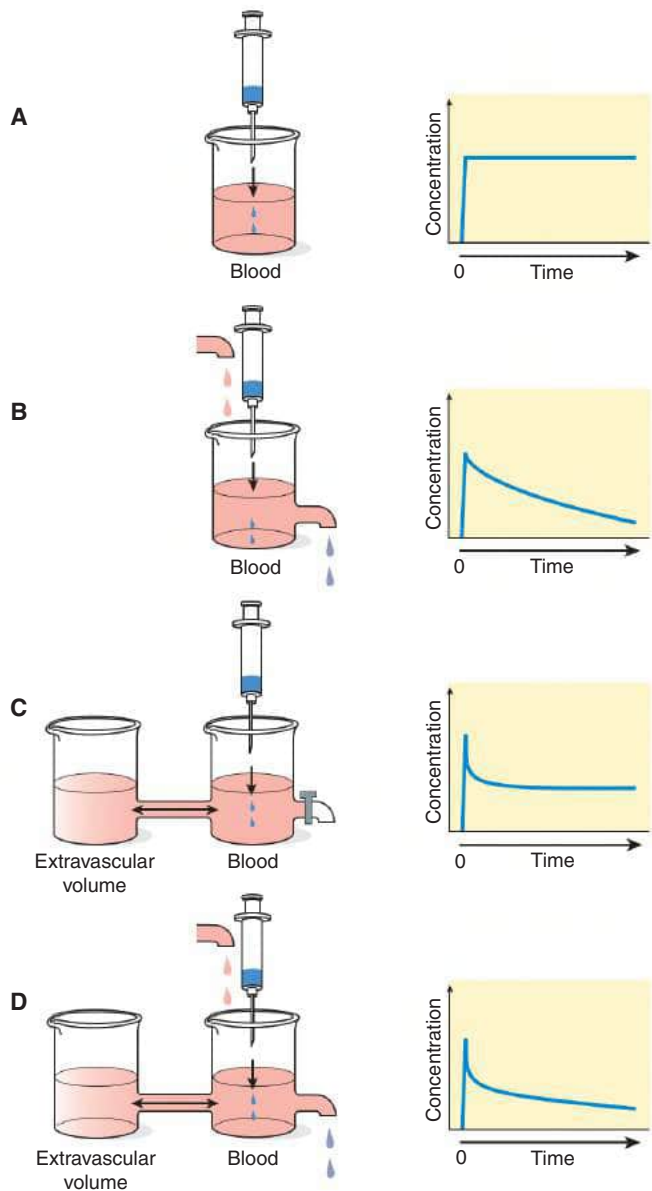


FIGURE 3–2 Models of drug distribution and elimination. The effect of adding drug to the blood by rapid intravenous injection is represented by injecting a known amount of the agent into a beaker. The time course of the amount of drug in the beaker is shown in the graphs at the right. In the first example (A), there is no movement of drug out of the beaker, so the graph shows only a steep rise to a maximum followed by a plateau. In the second example (B), a route of elimination is present, and the graph shows a slow decay after a sharp rise to a maximum. Because the amount of agent in the beaker falls, the “pressure” driving the elimination process also falls, and the slope of the curve decreases. This is an exponential decay curve. In the third model (C), drug placed in the first compartment (“blood”) equilibrates rapidly with the second compartment (“extravascular volume”) and the amount of drug in “blood” declines exponentially to a new steady state. The fourth model (D) illustrates a more realistic combination of elimination mechanism and extravascular equilibration. The resulting graph shows an early distribution phase followed by the slower elimination phase. Note that the volume of fluid remains constant because of a fluid input at the same rate as elimination in (B) and (D).

TABLE 3–2 Physical volumes (in L/kg body weight) of some body compartments into which drugs may be distributed.

Compartment and Volume	Examples of Drugs
Water	
Total body water (0.6 L/kg ¹)	Small water-soluble molecules: eg, ethanol
Extracellular water (0.2 L/kg)	Larger water-soluble molecules: eg, gentamicin
Plasma (0.04 L/kg)	Large protein molecules: eg, antibodies
Fat (0.2–0.35 L/kg)	Highly lipid-soluble molecules: eg, diazepam
Bone (0.07 L/kg)	Certain ions: eg, lead, fluoride

¹An average figure. Total body water in a young lean person might be 0.7 L/kg; in an obese person, 0.5 L/kg.

the respective clearance at that organ. Added together, these separate clearances equal total systemic clearance:

$$CL_{\text{kidney}} = \frac{\text{Rate of elimination}_{\text{kidney}}}{C} \tag{3a}$$

$$CL_{\text{liver}} = \frac{\text{Rate of elimination}_{\text{liver}}}{C} \tag{3b}$$

$$CL_{\text{other}} = \frac{\text{Rate of elimination}_{\text{other}}}{C} \tag{3c}$$

$$CL_{\text{systemic}} = CL_{\text{kidney}} + CL_{\text{liver}} + CL_{\text{other}} \tag{3d}$$

“Other” tissues of elimination could include the lungs and additional sites of metabolism, eg, blood or muscle.

The two major sites of drug elimination are the kidneys and the liver. Measurement of unchanged drug in the urine may be used to determine renal clearance. Within the liver, drug elimination occurs via biotransformation of parent drug to one or more metabolites, or excretion of unchanged drug into the bile, or both. Elimination of drug by the liver is difficult to measure directly, unlike renal elimination, so hepatic clearance is often assumed to be the difference between total systemic clearance and renal clearance. The pathways of biotransformation are discussed in Chapter 4. For most drugs, clearance is constant over the concentration range encountered in clinical settings, ie, elimination is not saturable, and the rate of drug elimination is directly proportional to concentration (rearranging equation [2]):

$$\text{Rate of elimination} = CL \times C \tag{4}$$

When elimination is directly proportional to C, this is called first-order elimination. When clearance is first-order, it can be estimated by calculating the **area under the curve (AUC)** of the time-concentration profile after a dose. Clearance is calculated from the dose divided by the AUC. Note that this is a convenient form of calculation—not the definition of clearance.

A. Capacity-Limited Elimination

For drugs that exhibit capacity-limited elimination (eg, phenytoin, ethanol), clearance does not remain constant but will vary depending on the concentration of drug that is achieved (see Table 3–1). Capacity-limited elimination is also known as mixed-order, saturable, nonlinear, and Michaelis-Menten elimination. It is associated with dose- or concentration-dependent clearance.

Most drug elimination pathways by metabolism will become saturated if the dose and therefore the concentration are high enough. When blood flow to an organ does not limit elimination (see below), the relation between elimination rate and concentration (C) is expressed mathematically in equation (5):

$$\text{Rate of elimination} = \frac{V_{\max} \times C}{K_m + C} \quad (5)$$

The maximum elimination capacity is $V_{\max}$, and K_m is the drug concentration at which the rate of elimination is 50% of $V_{\max}$. At concentrations that are high relative to the K_m , the elimination rate is almost independent of concentration—a state of “pseudo-zero order” elimination. If dosing rate exceeds elimination capacity, steady state cannot be achieved. The concentration will keep on rising as long as dosing continues. This pattern of capacity-limited elimination is important for three drugs in common use: ethanol, phenytoin, and aspirin. Clearance has no real meaning for drugs with capacity-limited elimination because it varies with concentration, and AUC should not be used to calculate clearance of such drugs.

B. Flow-Dependent Elimination

In contrast to capacity-limited drug elimination, some drugs are cleared very readily by the organ of elimination, so that at any clinically realistic concentration of the drug, most of the drug in the blood perfusing the organ is eliminated on the first pass of the drug through the organ. The elimination of these drugs will thus depend primarily on the rate of drug delivery to the organ of elimination. Such drugs (see Table 4–7) can be called “high-extraction” drugs since they are almost completely extracted from the blood by the organ. Blood flow to the organ is the main determinant of drug delivery, but plasma protein binding and blood cell partitioning may also be important for extensively bound drugs that are highly extracted.

C. Large Molecules

There are two aspects to the pharmacokinetics of proteins, often referred to as large molecules, when used as therapeutic agents. The first is that they all have much the same pharmacokinetics with a half-life of a couple of weeks. The second is that for some, but not all, the effect of the molecule is produced by binding to the target site. Elimination of the molecule is to some extent determined by the elimination of the target (eg, T cells). This is called target-mediated drug disposition. When target-mediated disposition occurs, the clearance of the molecule is increased and the half-life gets shorter. The time course of the effect of the molecule often follows the resulting changes in the time course of drug concentration.

Half-Life

Half-life ($t_{1/2}$) is the time required to change the amount of drug in the body by one-half during elimination (or during a constant infusion). In the simplest case—and the most useful in designing drug dosage regimens—the body may be considered as a single compartment (as illustrated in Figure 3–2B) of a size equal to the volume of distribution (V). The time course of drug in the body will depend on both the volume of distribution and the clearance:

$$t_{1/2} = \frac{0.7 \times V}{CL} \quad (6)$$

Because drug elimination can be described by an exponential process, the time taken for a twofold decrease can be shown to be proportional to the natural logarithm of 2. The constant 0.7 in equation (6) is an approximation to the natural logarithm of 2.

The elimination half-life is useful because it indicates the time required to attain 50% of steady state—or to decay 50% from steady-state conditions—after a change in the rate of drug input. Figure 3–3 shows the time course of drug accumulation during a constant-rate drug infusion and the time course of drug elimination after stopping an infusion that has reached steady state.

Disease states can affect both of the physiologically related primary pharmacokinetic parameters: volume of distribution and clearance. A change in elimination half-life will not necessarily reflect a change in drug elimination. For example, patients with chronic renal failure have both decreased renal clearance of digoxin and a decreased volume of distribution; the increase in digoxin elimination half-life is not as great as might be expected based on the change in renal function. The decrease in volume of distribution is due to the decreased renal and skeletal muscle mass and consequent decreased tissue binding of digoxin to Na^+/K^+ -ATPase.

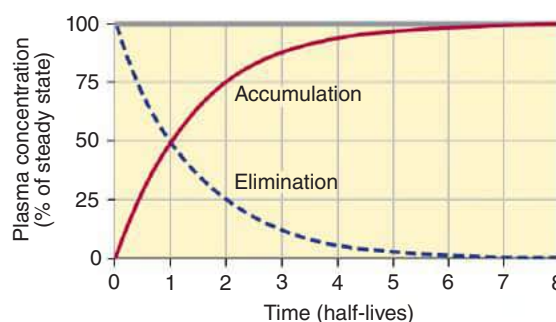


FIGURE 3–3 The time course of drug accumulation and elimination. **Solid line:** Plasma concentrations reflecting drug accumulation during a constant-rate infusion of a drug. Fifty percent of the steady-state concentration is reached after one half-life, 75% after two half-lives, and over 90% after four half-lives. **Dashed line:** Plasma concentrations reflecting drug elimination after a constant-rate infusion of a drug had reached steady state. Fifty percent of the drug is lost after one half-life, 75% after two half-lives, etc. The “rule of thumb” that four half-lives must elapse after starting a drug-dosing regimen before full effects will be seen is based on the approach of the accumulation curve to over 90% of the final steady-state concentration.

Many drugs will exhibit multicompartment pharmacokinetics (as illustrated in Figures 3–2C and 3–2D). Under these conditions, the “half-life” describing drug accumulation, as given in Table 3–1, will be greater than that calculated from equation (6).

Drug Accumulation

Whenever drug doses are repeated, the drug will accumulate in the body until dosing stops. This is because it takes an infinite time (in theory) to eliminate all of a given dose. In practical terms, this means that if the dosing interval is shorter than four half-lives, accumulation will be detectable.

Accumulation is inversely proportional to the fraction of the dose lost in each dosing interval. The fraction lost is 1 minus the fraction remaining just before the next dose. The fraction remaining can be predicted from the dosing interval and the half-life. A convenient index of accumulation is the **accumulation factor**:

Accumulation factor = $\frac{1}{\text{Fraction lost in one dosing interval}}$

Accumulation factor = $\frac{1}{1 - \text{Fraction remaining}}$ (7)

Accumulation factor = $\frac{1}{1 - e^{-0.7 \times \text{Dosing Interval} / \text{Half-Life}}}$

For a drug given once every half-life, the accumulation factor is 1/0.5, or 2. The accumulation factor predicts the ratio of the steady-state concentration to that seen at the same time following the first dose. Thus, the peak concentrations after intermittent doses at steady state will be equal to the peak concentration after the first dose multiplied by the accumulation factor.

Bioavailability

Bioavailability is defined as the fraction of unchanged drug reaching the systemic circulation following administration by any route (Table 3–3). The area under the blood concentration-time curve (AUC) is proportional to the dose and the extent of bioavailability for a drug if its elimination is first-order (Figure 3–4). For an intravenous dose, bioavailability is assumed to be equal to unity. For a drug administered orally, bioavailability may be less than 100% for two main reasons—incomplete extent of absorption across the gut wall and first-pass elimination by the liver (see below).

A. Extent of Absorption

After oral administration, a drug may be incompletely absorbed, eg, only 70% of a dose of digoxin reaches the systemic circulation. This is mainly due to lack of absorption from the gut. Other drugs are either too hydrophilic (eg, atenolol) or too lipophilic (eg, acyclovir) to be absorbed easily, and their low bioavailability is also due to incomplete absorption. If too hydrophilic, the drug cannot cross the lipid cell membrane; if too lipophilic, the drug is not soluble enough to cross the water layer adjacent to the cell. Drugs may not be absorbed because of a reverse transporter associated

TABLE 3–3 Routes of administration, bioavailability, and general characteristics.

Route	Bioavailability (%)	Characteristics
Intravenous (IV)	100 (by definition)	Most rapid onset
Intramuscular (IM)	75 to ≤100	Large volumes often feasible; may be painful
Subcutaneous (SC)	75 to ≤100	Smaller volumes than IM; may be painful
Oral (PO)	5 to <100	Most convenient; first-pass effect may be important
Rectal (PR)	30 to <100	Less first-pass effect than oral
Inhalation	5 to <100	Often very rapid onset
Transdermal	80 to ≤100	Usually very slow absorption; used for lack of first-pass effect; prolonged duration of action

with P-glycoprotein. This process actively pumps drug out of gut wall cells back into the gut lumen. Inhibition of P-glycoprotein and gut wall metabolism, eg, by grapefruit juice, may be associated with substantially increased drug absorption.

B. First-Pass Elimination

Following absorption across the gut wall, the portal blood delivers the drug to the liver prior to entry into the systemic circulation. A drug can be metabolized in the gut wall (eg, by the CYP3A4

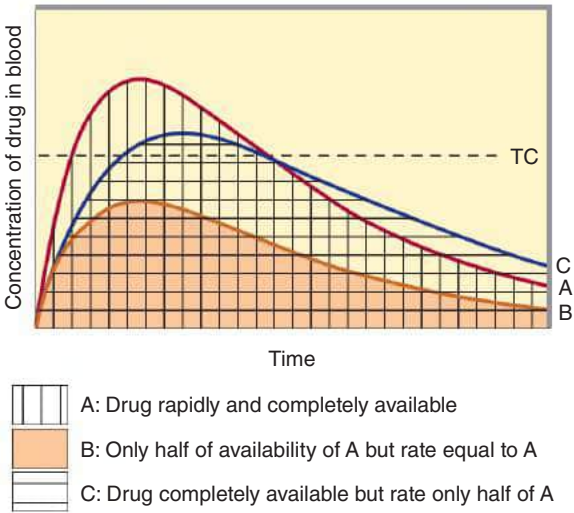


FIGURE 3–4 Blood concentration-time curves illustrating how changes in the rate of absorption and extent of bioavailability can influence both the duration of action and the effectiveness of the same total dose of a drug administered in three different formulations. The dashed line indicates the target concentration (TC) of the drug in the blood.

enzyme system) or even in the portal blood, but most commonly it is the liver that is responsible for metabolism before the drug reaches the systemic circulation. In addition, the liver can excrete the drug into the bile. Any of these sites can contribute to this reduction in bioavailability, and the overall process is known as first-pass elimination. The effect of first-pass hepatic elimination on bioavailability is expressed as the extraction ratio (ER):

$$ER = \frac{CL_{\text{liver}}}{Q} \quad (8a)$$

where CL_{liver} is the hepatic blood clearance and Q is hepatic blood flow, normally about 90 L/h in a person weighing 70 kg.

The systemic bioavailability of the drug (F) can be predicted from the extent of absorption (f) and the extraction ratio (ER):

$$F = f \times (1 - ER) \quad (8b)$$

A drug such as morphine is almost completely absorbed ($f = 1$), so that loss in the gut is negligible. However, the hepatic extraction ratio for morphine is morphine blood clearance (60 L/h/70 kg) divided by hepatic blood flow (90 L/h/70 kg) or 0.67. Its oral bioavailability ($1 - ER$) is therefore expected to be about 33%, which is close to the observed value (see Table 3–1).

Rate of Absorption

The distinction between rate and extent of absorption is shown in Figure 3–4. The rate of absorption is determined by the site of administration and the drug formulation. Both the rate of absorption and the extent of input can influence the clinical effectiveness of a drug. For the three different dosage forms depicted in Figure 3–4, differences in the intensity of clinical effect are expected. Dosage form B would require twice the dose to attain blood concentrations equivalent to those of dosage form A. Differences in rate of absorption may become important for drugs given as a single dose, such as a hypnotic used to induce sleep. In this case, drug from dosage form A would reach its target concentration earlier than drug from dosage form C; concentrations from A would also reach a higher value and remain above the target concentration for a longer period. In a multiple dosing regimen, dosage forms A and C would yield the same average blood concentrations, although dosage form A would show somewhat greater maximum and lower minimum concentrations.

The mechanism of drug absorption is said to be zero-order when the rate is independent of the amount of drug remaining in the gut, eg, when it is determined by the rate of gastric emptying or by a controlled-release drug formulation. In contrast, when the dose is dissolved in gastrointestinal fluids, the rate of absorption is usually proportional to the gastrointestinal fluid concentration and thus is first-order. The first-order rate of absorption can be described by the absorption half-life. After four absorption half-lives almost all of the dose will have been absorbed.

Extraction Ratio & the First-Pass Effect

Systemic clearance is not affected by bioavailability. However, clearance can markedly affect the extent of availability because

it determines the extraction ratio (equation [8a]). Of course, therapeutic blood concentrations may still be reached by the oral route of administration if large enough doses are given. However, in this case, the concentrations of the drug *metabolites* may be increased compared with those that would occur following intravenous administration. Lidocaine and verapamil are both used to treat cardiac arrhythmias and have bioavailability less than 40%, but lidocaine is never given orally because its metabolites are believed to contribute to central nervous system toxicity. Other drugs that are highly extracted by the liver include morphine (see above), isoniazid, propranolol, and several tricyclic antidepressants (see Table 3–1).

Drugs with high extraction ratios will show marked variations in bioavailability between subjects because of differences in hepatic function and blood flow. These differences can explain some of the variation in drug concentrations that occurs among individuals given similar doses. For drugs that are highly extracted by the liver, bypassing hepatic sites of elimination (eg, in hepatic cirrhosis with portosystemic shunting) will result in substantial increases in drug availability, whereas for drugs that are poorly extracted by the liver (for which the difference between entering and exiting drug concentration is small), shunting of blood past the liver will cause little change in availability. Drugs in Table 3–1 that are poorly extracted by the liver include warfarin, diazepam, phenytoin, theophylline, tolbutamide, and chlorpropamide.

Alternative Routes of Administration & the First-Pass Effect

There are several reasons for different routes of administration used in clinical medicine (see Table 3–3)—for convenience (eg, oral), to maximize concentration at the site of action and minimize it elsewhere (eg, topical), to prolong the duration of drug absorption (eg, transdermal), or to avoid the first-pass effect (sublingual or rectal).

The hepatic first-pass effect can be avoided to a great extent by use of sublingual tablets and transdermal preparations and to a lesser extent by use of rectal suppositories. Sublingual absorption provides direct access to systemic—not portal—veins. The transdermal route offers the same advantage. Drugs absorbed from suppositories in the lower rectum enter vessels that drain into the inferior vena cava, thus bypassing the liver. However, suppositories tend to move upward in the rectum into a region where veins that lead to the liver predominate. Thus, only about 50% of a rectal dose can be assumed to bypass the liver.

Although drugs administered by inhalation bypass the hepatic first-pass effect, the lung may also serve as a site of first-pass loss by excretion and possibly metabolism for drugs administered by nongastrointestinal (“parenteral”) routes.

THE TIME COURSE OF DRUG EFFECT

The principles of pharmacokinetics (discussed in this chapter) and those of pharmacodynamics (discussed in Chapter 2 and Holford & Sheiner, 1981) provide a framework for understanding the time course of drug effect.

Immediate Effects

In the simplest case, drug effects are immediately related to plasma concentrations, but this does not necessarily mean that effects simply parallel the time course of concentrations. Because the relationship between drug concentration and effect is not linear (recall the $E_{\max}$ model described in Chapter 2), the effect will not be linearly proportional to the concentration.

Consider the effect of an angiotensin-converting enzyme (ACE) inhibitor, such as enalapril, on ACE. The elimination half-life that explains the time course of ACE inhibition is about 4 hours. Enalapril is usually given once a day, so more than four of these half-lives will elapse from the time of peak concentration to the end of the dosing interval. The concentration of enalapril explaining the effect and the corresponding extent of ACE inhibition with an initial peak concentration of 100 ng/mL are shown in Figure 3–5. The extent of inhibition of ACE is calculated using the $E_{\max}$ model, where $E_{\max}$, the maximum extent of inhibition, is 100% and the C_{50} , the concentration of enalapril associated with 50% of maximum effect, is 5 ng/mL.

Note that plasma concentrations of enalapril change by a factor of eight over the first 12 hours (three half-lives), but ACE inhibition has only decreased by about 30%. Because the concentrations over this time are so high in relation to the C_{50} , the effect on ACE is almost constant. After 24 hours (six half-lives), the concentration is about 2% of the initial peak which is just under half of the C_{50} , so ACE is still about 25% inhibited. This explains why a drug with a short half-life can be given once a day and still maintain its effect throughout the day. The key factor is a high initial concentration in relation to the C_{50} . Once-a-day dosing is common for drugs with minimal adverse effects related to peak concentrations

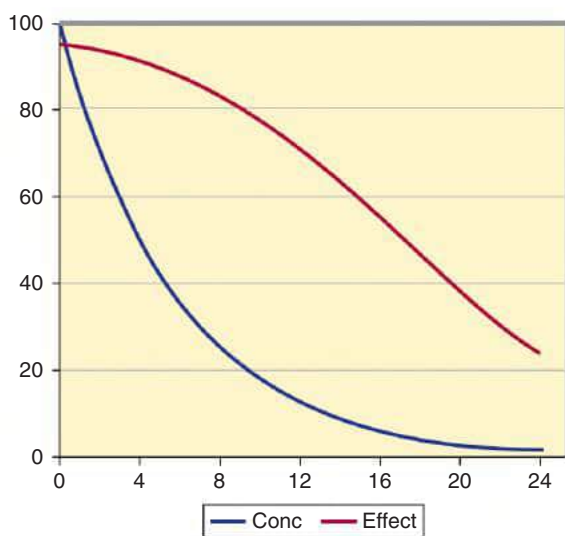


FIGURE 3–5 Time course (hours) of angiotensin-converting enzyme (ACE) inhibitor concentrations and effects. The blue line shows the plasma enalapril concentrations in nanograms per milliliter after a single oral dose. The red line indicates the percentage inhibition of its target, ACE. Note the different shapes of the concentration-time course (exponentially decreasing) and the effect-time course (linearly decreasing in its central portion).

that act on enzymes (eg, ACE inhibitors) or compete at receptors (eg, propranolol).

When concentrations are in the range between four times and one fourth of the C_{50} , the time course of effect is essentially a linear function of time. It takes four half-lives for concentrations to drop from an effect of 80% to 20% of $E_{\max}$ —15% of the effect is lost every half-life over this concentration range. At concentrations below one-fourth the C_{50} , the effect becomes almost directly proportional to concentration, and the time course of drug effect will follow the exponential decline of concentration. It is only when the concentration is low in relation to the C_{50} that the concept of a “half-life of drug effect” has any meaning.

Delayed Effects

Changes in drug effects are often delayed in relation to changes in plasma concentration. This delay may reflect the time required for the drug to distribute from plasma to the site of action. This will be the case for almost all drugs. The delay due to distribution is a pharmacokinetic phenomenon that can account for delays of a few minutes. This distributional process can account for the short delay of effects after rapid intravenous injection of central nervous system (CNS)–active agents such as thiopental.

Some drugs bind tightly to receptors, and it is the half-life of dissociation that determines the delay in effect, eg, for digoxin. Note that it is the dissociation process that controls the time to receptor equilibrium. This is exactly the same principle as the elimination process controlling the time to accumulate to steady state with a constant rate infusion (see Figure 3–3).

A common reason for more delayed drug effects—especially those that take many hours or even days to occur—is the slow turnover of a physiologic substance that is involved in the expression of the drug effect. For example, warfarin works as an anti-coagulant by inhibiting vitamin K epoxide reductase (VKOR) in the liver. This action of warfarin occurs rapidly, and inhibition of the enzyme is closely related to plasma concentrations of warfarin. The clinical effect of warfarin, eg, on the international normalized ratio (INR), reflects a decrease in the concentration of the prothrombin complex of clotting factors. Inhibition of VKOR decreases the synthesis of these clotting factors, but the complex has a long elimination half-life (about 14 hours), and it is this half-life that determines how long it takes for the concentration of clotting factors to reach a new steady state and for a drug effect to reflect the average warfarin plasma concentration.

Schedule-Dependent Effects

The renal toxicity of aminoglycoside antibiotics (eg, gentamicin) is greater when administered as a constant infusion than with intermittent dosing. Even though both dosing schemes may produce the same average steady-state concentration, the intermittent dosing scheme produces much higher peak concentrations, which saturate an uptake mechanism into the renal cortex; thus, total aminoglycoside accumulation is less. The difference in toxicity is a predictable consequence of the different patterns of concentration and the saturable uptake mechanism. This is an example of schedule dependence of drug effect. It is associated with reversible drug

action and a nonlinear relationship between systemic concentration and eventual response.

Cumulative Effects

Some drug effects are more obviously related to a cumulative action than to a rapidly reversible one. The effect of many drugs used to treat cancer also reflects a cumulative action—eg, the extent of binding of a drug to DNA is proportional to drug concentration and is usually irreversible. The effect on tumor growth is therefore a consequence of cumulative exposure to the drug. Measures of cumulative exposure, such as AUC, provide a means to individualize treatment.

THE TARGET CONCENTRATION APPROACH TO DESIGNING A RATIONAL DOSAGE REGIMEN

A rational dosage regimen is based on the assumption that there is a **target concentration** that will produce the desired therapeutic effect. By considering the pharmacokinetic factors that determine the dose-concentration relationship, it is possible to individualize the dose regimen to achieve the target concentration. The target concentrations shown in Table 3-1 are a guide to the concentrations measured when patients are being effectively treated. In some cases, the target concentration will also depend on the specific therapeutic objective—eg, the control of atrial fibrillation by digoxin may require a target concentration of 2 ng/mL, while heart failure is usually adequately managed with a target concentration of 1 ng/mL.

Maintenance Dose

In most clinical situations, drugs are administered in such a way as to maintain a steady state of drug in the body, ie, the amount given with each dose replaces the drug eliminated since the preceding dose.

Thus, calculation of the appropriate maintenance dose is a primary goal. Clearance is the most important pharmacokinetic parameter to be considered in defining a rational steady-state drug dosage regimen. At steady state, the dosing rate (“rate in”) must equal the rate of elimination (“rate out”). Substitution of the target concentration (TC) for concentration (C) in equation (4) predicts the maintenance dosing rate:

$$\begin{aligned}\text{Dosing rate}_{ss} &= \text{Rate of elimination}_{ss} \\ &= CL \times TC\end{aligned}\quad (9)$$

Thus, if the desired target concentration is known, the clearance in that patient will determine the dosing rate. If the drug is given by a route that has a bioavailability less than 100%, then the dosing rate predicted by equation (9) must be modified. For oral dosing:

$$\text{Dosing rate}_{\text{oral}} = \frac{\text{Dosing rate}}{F_{\text{oral}}}\quad (10)$$

If intermittent doses are given, the maintenance dose is calculated from:

$$\text{Maintenance dose} = \text{Dosing rate} \times \text{Dosing interval}\quad (11)$$

(See Box: Example: Maintenance Dose Calculations.)

Note that the steady-state concentration achieved by continuous infusion or the average concentration following intermittent dosing depends only on clearance. The volume of distribution and the half-life need not be known in order to determine the average plasma concentration expected from a given dosing rate or to predict the dosing rate for a desired target concentration. Figure 3-6 shows that at different dosing intervals, the concentration-time curves will have different maximum and minimum values even though the average concentration will always be 10 mg/L.

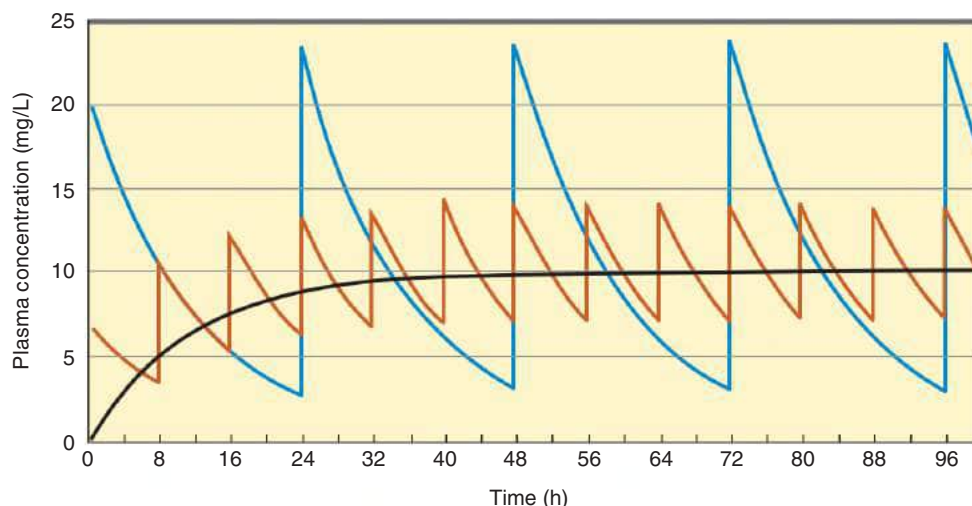


FIGURE 3-6 Relationship between frequency of dosing and maximum and minimum plasma concentrations when a steady-state theophylline plasma concentration of 10 mg/L is desired. The smoothly rising black line shows the plasma concentration achieved with an intravenous infusion of 28 mg/h. The dose for 8-hour administration (orange line) is 224 mg and for 24-hour administration (blue line) 672 mg. In each of the three cases, the mean steady-state plasma concentration is 10 mg/L.

Example: Maintenance Dose Calculations

A target plasma theophylline concentration of 10 mg/L is desired to relieve acute bronchial asthma in a patient. If the patient is a nonsmoker and otherwise normal except for asthma, we may use the mean clearance given in Table 3–1, ie, 2.8 L/h/70 kg. Since the drug will be given as an intravenous infusion, $F = 1$.

$$\begin{aligned}\text{Dosing rate} &= \text{CL} \times \text{TC} \\ &= 2.8 \text{ L/h/70 kg} \times 10 \text{ mg/L} \\ &= 28 \text{ mg/h/70 kg}\end{aligned}$$

Therefore, in this patient, the infusion rate would be 28 mg/h/70 kg.

If the asthma attack is relieved, the clinician might want to maintain this plasma concentration using oral theophylline,

which might be given every 12 hours using an extended-release formulation to approximate a continuous intravenous infusion. According to Table 3–1, F_{oral} is 0.96. When the dosing interval is 12 hours, the size of each maintenance dose would be:

$$\begin{aligned}\text{Maintenance dose} &= \text{Dosing Rate}/F \times \text{Dosing interval} \\ &= 28 \text{ mg/h}/0.96 \times 12 \text{ h} \\ &= 350 \text{ mg}\end{aligned}$$

A tablet or capsule size close to the ideal dose of 350 mg would then be prescribed at 12-hour intervals. If an 8-hour dosing interval was used, the ideal dose would be 233 mg; and if the drug was given once a day, the dose would be 700 mg. In practice, F could be omitted from the calculation since it is so close to 1.

Estimates of dosing rate and average steady-state concentrations, which may be calculated using clearance, are independent of any specific pharmacokinetic model. In contrast, the determination of maximum and minimum steady-state concentrations requires further assumptions about the pharmacokinetic model. The accumulation factor (equation [7]) assumes that the drug distribution is a one-compartment model (see Figure 3–2B), and the peak concentration prediction assumes that the absorption rate is much faster than the elimination rate. For the calculation of estimated maximum and minimum concentrations in a clinical situation, these assumptions are usually reasonable.

Loading Dose

When the time to reach steady state is appreciable, as it is for drugs with long half-lives, it may be desirable to administer a loading dose that promptly raises the concentration of drug in plasma to the target concentration. In theory, only the amount of the loading dose needs to be computed—not the rate of its administration—and, to a first approximation, this is so. The volume of distribution is the proportionality factor that relates the total amount of drug in the body to the concentration; if a loading dose is to achieve the target concentration, then it can be calculated from equation (12):

$$\begin{aligned}\text{Loading dose} &= \frac{\text{Amount in the body}}{\text{the loading dose}} \\ &= V \times \text{TC}\end{aligned} \quad (12)$$

For the theophylline example given in the box, Example: Maintenance Dose Calculations, the loading dose would be 350 mg ($35 \text{ L} \times 10 \text{ mg/L}$) for a 70-kg person. For most drugs, the loading dose can be given as a single dose by the chosen route of administration.

Up to this point, we have ignored the fact that some drugs follow more complex multicompartment pharmacokinetics,

eg, the distribution process illustrated by the two-compartment model in Figure 3–2. This is reasonable in the great majority of cases. However, in some cases the distribution phase may not be ignored, particularly in connection with the calculation of loading doses. If the rate of input is rapid relative to distribution (this is always true for rapid intravenous administration), the concentration of drug in plasma that results from an appropriate loading dose—calculated using the apparent volume of distribution—will initially be considerably higher than desired. Severe toxicity may occur, albeit transiently. This may be particularly important, eg, in the administration of an anticonvulsant drug such as phenytoin, where an almost immediate toxic response may occur. Thus, while the estimation of the *amount* of a loading dose may be quite correct, the *rate of administration* can sometimes be crucial in preventing excessive drug concentrations, and slow administration of an intravenous drug (eg, over an hour rather than seconds) is almost always prudent practice.

When intermittent doses are given, the loading dose calculated from equation (12) will only reach the average steady-state concentration and will not match the peak steady-state concentration (see Figure 3–6). To match the peak steady-state concentration, the loading dose can be calculated from equation (13):

$$\text{Loading dose} = \text{Maintenance dose} \times \text{Accumulation factor} \quad (13)$$

TARGET CONCENTRATION INTERVENTION: APPLICATION OF PHARMACOKINETICS & PHARMACODYNAMICS TO DOSE INDIVIDUALIZATION

The basic principles outlined above can be applied to the interpretation of clinical drug concentration measurements on the basis of three major pharmacokinetic variables: rate and extent of input,

clearance, and volume of distribution (and the derived variable, half-life). In addition, it may be necessary to consider two pharmacodynamic variables: maximum effect attainable in the target tissue ($E_{\max}$) and the sensitivity (C_{50}) of the tissue to the drug.

Diseases may modify all of these parameters, and the ability to predict the effect of disease states on these parameters is important in properly adjusting dosage in such cases. (See Box: The Target Concentration Strategy.)

The Target Concentration Strategy

Recognition of the essential role of concentration in linking pharmacokinetics and pharmacodynamics leads naturally to the target concentration strategy. Pharmacodynamic principles can be used to predict the concentration required to achieve a particular degree of therapeutic effect. This target concentration can then be achieved by using pharmacokinetic principles to arrive at a suitable dosing regimen (Holford, 1999). The target concentration strategy is a process for optimizing the dose in an individual on the basis of a measured surrogate response such as drug concentration:

1. Choose the target concentration, TC.
2. Predict volume of distribution (V) and clearance (CL) based on standard population values (eg, Table 3–1) with adjustments for factors such as weight and renal function.
3. Give a loading dose or maintenance dose calculated from TC, V, and CL.
4. Measure the patient's response (including, usually, drug concentration).
5. Revise V and/or CL based on the measured response.
6. Repeat steps 3–5, adjusting the predicted dose to achieve TC.

Pharmacokinetic Variables

A. Input

The amount of drug that enters the body depends on the patient's adherence to the prescribed regimen and on the rate and extent of transfer from the site of administration to the blood.

Overdosage and underdosage relative to the prescribed dosage—both aspects of failure of adherence—can frequently be detected by concentration measurements when gross deviations from expected values are obtained. If adherence is found to be adequate, absorption abnormalities in the small bowel may be the cause of abnormally low concentrations. Variations in the extent of bioavailability are rarely caused by irregularities in the manufacture of the particular drug formulation. More commonly, variations in bioavailability are due to metabolism during absorption.

B. Clearance

Abnormal clearance may be anticipated when there is major impairment of the function of the kidney, liver, or heart. Creatinine clearance is a useful quantitative indicator of renal function. Conversely, drug clearance may be a useful indicator of the functional consequences of heart, kidney, or liver failure, often with greater precision than clinical findings or other laboratory tests. For example, when renal function is changing rapidly, estimation of the clearance of aminoglycoside antibiotics may be a more accurate indicator of glomerular filtration than serum creatinine.

Hepatic disease has been shown to reduce the clearance and prolong the half-life of many drugs. However, for many other drugs known to be eliminated by hepatic processes, no changes in clearance or half-life have been noted with similar hepatic disease. This reflects the fact that hepatic disease does not always affect the

hepatic intrinsic clearance. At present, there is no reliable marker of hepatic drug-metabolizing function that can be used to predict changes in liver clearance in a manner analogous to the use of creatinine clearance as a marker of renal drug clearance.

C. Volume of Distribution

The apparent volume of distribution reflects a balance between binding to tissues, which decreases plasma concentration and makes the apparent volume larger, and binding to plasma proteins, which increases plasma concentration and makes the apparent volume smaller. Changes in either tissue or plasma binding can change the apparent volume of distribution determined from plasma concentration measurements. Older people have a relative decrease in skeletal muscle mass and tend to have a smaller apparent volume of distribution of digoxin (which binds to muscle proteins). The volume of distribution may be overestimated in obese patients if based on body weight and the drug does not enter fatty tissues well, as is the case with digoxin. In contrast, theophylline has a volume of distribution similar to that of total body water. Adipose tissue has almost as much water in it as other tissues, so that the apparent total volume of distribution of theophylline is proportional to body weight even in obese patients.

Abnormal accumulation of fluid—edema, ascites, pleural effusion—can markedly increase the volume of distribution of drugs such as gentamicin that are hydrophilic and have small volumes of distribution.

D. Elimination Half-Life

The differences between clearance and elimination half-life are important in defining the underlying mechanisms for the effect

of a disease state on drug disposition. For example, the elimination half-life of diazepam increases with patient age. However, in the same patients it is found that clearance of diazepam does not change with age. The increasing elimination half-life for diazepam actually results from changes in the volume of distribution with age; the metabolic processes responsible for eliminating diazepam do not change with age.

Pharmacodynamic Variables

A. Maximum Effect

All pharmacologic responses must have a maximum effect ($E_{\max}$). No matter how high the drug concentration goes, a point will be reached beyond which no further increment in response is achieved.

If increasing the dose in a particular patient does not lead to a further clinical response, it is possible that the maximum effect has been approached. Recognition of a maximum effect is helpful in avoiding ineffectual increases of dose with the attendant risk of toxicity.

B. Sensitivity

The sensitivity of the target organ to drug concentration is reflected by the concentration required to produce 50% of maximum effect, the C_{50} . Diminished sensitivity to the drug can be detected by measuring drug concentrations that are usually associated with therapeutic response in a patient who has not responded. This may be a result of abnormal physiology—eg, hyperkalemia diminishes responsiveness to digoxin—or drug antagonism—eg, calcium channel blockers impair the inotropic response to digoxin.

Increased sensitivity to a drug is usually signaled by exaggerated responses to small or moderate doses. The pharmacodynamic nature of this sensitivity can be confirmed by measuring drug concentrations that are low in relation to the observed effect.

INTERPRETATION OF DRUG CONCENTRATION MEASUREMENTS

Clearance

Clearance is the single most important factor determining drug concentrations. The interpretation of measurements of drug concentrations depends on a clear understanding of three factors that may influence concentrations reflecting clearance: the dose, the organ blood flow, and the intrinsic function of the liver or kidneys. Each of these factors should be considered when interpreting clearance estimated from a drug concentration measurement.

It must also be recognized that changes in protein binding may lead the unwary to believe there is a change in clearance when in fact drug elimination is not altered (see Box: Plasma Protein Binding:

Is It Important?). Factors affecting protein binding include the following:

1. **Albumin concentration:** Drugs such as phenytoin, salicylates, and disopyramide are extensively bound to plasma albumin. Albumin concentrations are low in many disease states, resulting in lower total drug concentrations.
2. **Alpha₁-acid glycoprotein concentration:** α_1 -Acid glycoprotein is an important binding protein with binding sites for drugs such as quinidine, lidocaine, and propranolol. It is increased in acute inflammatory disorders and causes major changes in total plasma concentration of these drugs even though drug elimination is unchanged.
3. **Capacity-limited protein binding:** The binding of drugs to plasma proteins is capacity-limited. Therapeutic concentrations of salicylates and prednisolone show concentration-dependent protein binding. Because unbound drug concentration is determined by dosing rate and unbound clearance—which is not altered by protein binding, in the case of these low-extraction-ratio drugs—increases in dosing rate will cause corresponding changes in the pharmacodynamically important unbound concentration. In contrast, total drug concentration will increase less rapidly than the dosing rate would suggest as protein binding approaches saturation at higher concentrations.
4. **Binding to red blood cells:** Drugs such as cyclosporine and tacrolimus bind extensively inside red blood cells. Typically, whole blood concentrations are measured, and they are about 50 times higher than plasma concentration. A decrease in red blood cell concentration (reflected in the hematocrit) will cause whole blood concentration to fall without a change in pharmacologically active concentrations. Standardization of concentrations to a standard hematocrit helps to interpret the concentration-effect relationship.

Dosing History

An accurate dosing history is essential if one is to obtain maximum value from a drug concentration measurement. In fact, if the dosing history is unknown or incomplete, a drug concentration measurement loses all predictive value.

Timing of Samples for Concentration Measurement

Information about the rate and extent of drug absorption in a particular patient is rarely of great clinical importance. Absorption usually occurs during the first 2 hours after a drug dose and varies according to food intake, posture, and activity. Therefore, it is important to avoid drawing blood until absorption is complete (about 2 hours after an oral dose). Attempts to measure peak concentrations early after oral dosing are usually unsuccessful and compromise the validity of the measurement, because one cannot be certain that absorption is complete.

Plasma Protein Binding: Is It Important?

Plasma protein binding is often mentioned as a factor playing a role in pharmacokinetics, pharmacodynamics, and drug interactions. However, there are no clinically relevant examples of changes in drug disposition or effects that can be clearly ascribed to changes in plasma protein binding (Benet & Hoener, 2002). The idea that if a drug is displaced from plasma proteins it would increase the unbound drug concentration and increase the drug effect and, perhaps, produce toxicity seems a simple and obvious mechanism. Unfortunately, this simple theory, which is appropriate for a test tube, does not work in the body, which is an open system capable of eliminating unbound drug.

A seemingly dramatic change in the unbound fraction from 1% to 10% releases less than 5% of the total amount of drug in the body into the unbound pool because less than one third of the drug in the body is bound to plasma proteins even in the most extreme cases, eg, warfarin. Drug displaced from plasma protein will of course distribute throughout the volume of distribution,

so that a 5% increase in the amount of unbound drug in the body produces at most a 5% increase in pharmacologically active unbound drug at the site of action.

If the amount of unbound drug in plasma increases due to displacement from plasma proteins, the rate of elimination will increase, and after four half-lives the unbound concentration will return to its previous steady-state value (if unbound clearance is unchanged). When drug interactions associated with protein binding displacement and clinically important effects have been studied, it has been found that the displacing drug is also an inhibitor of clearance, and it is the change in *clearance* of the *unbound* drug that is the relevant mechanism explaining the interaction.

The clinical importance of plasma protein binding is only to help interpretation of measured drug concentrations. When plasma proteins are lower than normal, total drug concentrations will be lower but unbound concentrations will not be affected.

Some drugs, such as digoxin and lithium, take several hours to distribute to tissues. Digoxin samples should be taken at least 6 hours after the last dose and lithium just before the next dose (usually 24 hours after the last dose). Aminoglycosides distribute quite rapidly, but it is still prudent to wait 1 hour after giving the dose before taking a sample.

Clearance is readily estimated from the dosing rate and mean steady-state concentration. Blood samples should be appropriately timed to estimate steady-state concentration. Provided steady state has been approached (at least three half-lives of constant dosing), a sample obtained near the midpoint of the dosing interval will usually be close to the mean steady-state concentration and is a better choice than a trough concentration just before the next dose.

Initial Predictions of Volume of Distribution & Clearance

A. Volume of Distribution

Volume of distribution is commonly calculated for a particular patient using body weight (70-kg body weight is assumed for the values in Table 3–1). If a patient is obese, drugs that do not readily penetrate fat (eg, gentamicin, digoxin, tacrolimus, gemcitabine) should have their volumes calculated from fat-free mass (FFM) as shown below. Total body weight (WT) is in kilograms and height (HTM) is in meters:

$$\text{For women: FFM (kg)} = \frac{37.99 \times \text{HTM}^2 \times \text{WT}}{35.98 \times \text{HTM}^2 + \text{WT}} \quad (14a)$$

$$\text{For men: FFM (kg)} = \frac{42.92 \times \text{HTM}^2 \times \text{WT}}{30.93 \times \text{HTM}^2 + \text{WT}} \quad (14b)$$

Patients with edema, ascites, or pleural effusions offer a larger volume of distribution to the aminoglycoside antibiotics (eg, gentamicin) than is predicted by body weight. In such patients, the weight should be corrected as follows: Subtract an estimate of the weight of the excess fluid accumulation from the measured weight. Use the resultant “normal” body weight to calculate the normal volume of distribution. Finally, this normal volume should be increased by 1 L for each estimated kilogram of excess fluid. This correction is important because of the relatively small volumes of distribution of these water-soluble drugs.

B. Clearance

Drugs cleared by the renal route often require adjustment of clearance in proportion to renal function. This can be conveniently estimated from the creatinine clearance, calculated from a single serum creatinine measurement and the predicted creatinine production rate.

The predicted creatinine production rate in women is 85% of the calculated value because they have a smaller muscle mass per kilogram, and it is muscle mass that determines creatinine production. Muscle mass as a fraction of body weight decreases with age, which is why age appears in the Cockcroft-Gault equation for estimating creatinine clearance.* The decrease of renal function with age is caused not by the decrease in creatinine production but by the progressive loss of nephrons. The loss of nephrons will reduce creatinine clearance and increase serum creatinine. This increase in serum creatinine will be reflected in estimated creatinine clearance.

*The Cockcroft-Gault equation is given in Chapter 60.

Because of the difficulty of obtaining complete urine collections, creatinine clearance calculated from serum creatinine is usually more reliable than estimates based on urine collections. The fat-free mass (equation [14]) may be considered rather than total body weight for obese patients, and correction should be made for muscle wasting in severely ill patients.

Revising Individual Estimates of Volume of Distribution & Clearance

The commonsense approach to the interpretation of drug concentrations compares predictions of pharmacokinetic parameters and expected concentrations to measured values. If measured concentrations differ by more than 20% from expected values, revised estimates of V or CL for that patient should be calculated using equation (1) or equation (2). If the change calculated is more than a 100% increase or 50% decrease in either V or CL , the assumptions made about the timing of the sample and the dosing history should be critically examined.

For example, if a patient is taking 0.25 mg of digoxin a day, a clinician may expect the digoxin concentration to be about 1 ng/mL. This is based on typical values for bioavailability of 70% and total clearance of about 7 L/h (CL_{renal} 4 L/h, CL_{nonrenal} 3 L/h). If the patient has heart failure, the nonrenal (hepatic) clearance might be halved because of hepatic congestion and hypoxia, so

the expected clearance would become 5.5 L/h. The concentration is then expected to be about 1.3 ng/mL. Suppose that the concentration actually measured is 2 ng/mL. Common sense would suggest halving the daily dose to achieve a target concentration of 1 ng/mL. This approach implies a revised clearance of 3.5 L/h. The smaller clearance compared with the expected value of 5.5 L/h may reflect additional renal functional impairment due to heart failure.

This technique will often be misleading if steady state has not been reached. At least a week of regular dosing (four half-lives) must elapse before the implicit method will be reliable. Dosing tools using pharmacokinetic models developed from representative patient populations should be considered for more reliable prediction of individual doses based on measured patient response (eg, www.nextdose.org).

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CASE STUDY ANSWER

Sixty-seven percent of total standard digoxin clearance is renal, so the standard renal clearance is $0.67 \times 9 \text{ L/h} = 6 \text{ L/h/70 kg}$ with creatinine clearance of 100 mL/min and nonrenal clearance is $(1 - 0.67) \times 9 \text{ L/h} = 3 \text{ L/h/70 kg}$ (see Table 3–1 for standard pharmacokinetic parameters). Her predicted creatinine clearance is 22 mL/min (Cockcroft and Gault), so for digoxin, her renal

clearance is $6 \times 22/100 \times 60/70 = 1.1 \text{ L/h}$, nonrenal clearance $2.7 \times 60/70 = 2.6 \text{ L/h}$, and total clearance 3.7 L/h. The parenteral maintenance dose rate is $1 \text{ mcg/L} \times 3.7 \text{ L/h} = 3.7 \text{ mcg/h}$. Once-a-day oral dosing with bioavailability of 0.7 would require a daily maintenance dose of $3.7/0.7 \times 24 = 127 \text{ mcg/day}$. A practical dose would be two 62.5-mcg tablets per day.

4

Drug Biotransformation

Maria Almira Correia, PhD

CASE STUDY

A 40-year-old woman presents to the emergency department of her local hospital somewhat disoriented, complaining of midsternal chest pain, abdominal pain, shaking, and vomiting for 2 days. She admits to having taken a “handful” of Lorcet (hydrocodone/acetaminophen, an opioid/nonopioid analgesic combination), Soma (carisoprodol, a centrally acting muscle relaxant), and Cymbalta (duloxetine HCl, an antidepressant/antifibromyalgia agent) 2 days earlier. On physical examination, the sclera of her eyes shows yellow discoloration. Laboratory analyses of blood drawn within an hour of her admission reveal abnormal liver function

*Normal values are in parentheses.

as indicated by the increased indices: alkaline phosphatase 302 (41–133),* alanine aminotransferase (ALT) 351 (7–56),* aspartate aminotransferase (AST) 1045 (0–35),* bilirubin 3.33 mg/dL (0.1–1.2),* and prothrombin time of 19.8 seconds (11–15).* In addition, plasma bicarbonate is reduced, and she has ~45% reduced glomerular filtration rate from the normal value at her age, elevated serum creatinine, and blood urea nitrogen, markedly reduced blood glucose of 35 mg/dL, and a plasma acetaminophen concentration of 75 mcg/mL (10–20).* Her serum titer is significantly positive for hepatitis C virus (HCV). Given these data, how would you proceed with the management of this case?

Humans are exposed daily to a wide variety of foreign compounds called **xenobiotics**—substances absorbed across the lungs or skin or, more commonly, ingested either unintentionally as compounds present in food and drink or deliberately as drugs for therapeutic or “recreational” purposes. Exposure to environmental xenobiotics may be inadvertent and accidental or—when they are present as components of air, water, and food—inescapable. Some xenobiotics are innocuous, but many can provoke biologic responses. Such biologic responses often depend on conversion of the absorbed substance into an active metabolite. The discussion that follows is applicable to xenobiotics in general (including drugs) and to some extent to endogenous compounds.

WHY IS DRUG BIOTRANSFORMATION NECESSARY?

The mammalian drug biotransformation systems are thought to have first evolved from the need to detoxify and eliminate plant and bacterial bioproducts and toxins, which later extended to

drugs and other environmental xenobiotics. Renal excretion plays a pivotal role in terminating the biologic activity of some drugs, particularly those that have small molecular volumes or possess polar characteristics, such as functional groups that are fully ionized at physiologic pH. However, many drugs do not possess such physicochemical properties. Pharmacologically active organic molecules tend to be lipophilic and remain un-ionized or only partially ionized at physiologic pH; these are readily reabsorbed from the glomerular filtrate in the nephron. Certain lipophilic compounds are often strongly bound to plasma proteins and may not be readily filtered at the glomerulus. Consequently, most drugs would have a prolonged duration of action if termination of their action depended solely on renal excretion.

An alternative process that can lead to the termination or alteration of biologic activity is metabolism. In general, lipophilic xenobiotics are transformed to more polar and hence more readily excreted products. The role that metabolism plays in the inactivation of lipid-soluble drugs can be quite dramatic. For example, lipophilic barbiturates such as thiopental and pentobarbital would

have extremely long half-lives if it were not for their metabolic conversion to more water-soluble compounds.

Metabolic products are often less pharmacodynamically active than the parent drug and may even be inactive. However, some biotransformation products have *enhanced* activity or toxic properties. It is noteworthy that the synthesis of endogenous substrates such as steroid hormones, cholesterol, active vitamin D congeners, and bile acids involves many pathways catalyzed by enzymes associated with the metabolism of xenobiotics. Finally, drug-metabolizing enzymes have been exploited in the design of pharmacologically inactive prodrugs that are converted to active molecules in the body.

THE ROLE OF BIOTRANSFORMATION IN DRUG DISPOSITION

Most metabolic biotransformations occur at some point between absorption of the drug into the circulation and its renal elimination. A few transformations occur in the intestinal lumen or intestinal wall. In general, all of these reactions can be assigned to one of two major categories called **phase I** and **phase II reactions** (Figure 4–1).

Phase I reactions usually convert the parent drug to a more polar metabolite by introducing or unmasking a functional group ($-\text{OH}$, $-\text{NH}_2$, $-\text{SH}$). Often these metabolites are inactive, although in some instances, activity is only modified or even enhanced.

If phase I metabolites are sufficiently polar, they may be readily excreted. However, many phase I products are not eliminated rapidly and undergo a subsequent reaction in which an endogenous substrate such as glucuronic acid, sulfuric acid, acetic acid, or an amino acid combines with the newly incorporated functional group to form a highly polar conjugate. Such conjugation or synthetic reactions are the hallmarks of phase II metabolism. A great variety of drugs undergo these sequential biotransformation reactions, although in some instances, the parent drug may already possess a functional group that may form a conjugate directly. For

example, the hydrazide moiety of isoniazid is known to form an *N*-acetyl conjugate in a phase II reaction. This conjugate is then a substrate for a phase I type reaction, namely, hydrolysis to isonicotinic acid (Figure 4–2). Thus, phase II reactions may actually precede phase I reactions.

WHERE DO DRUG BIOTRANSFORMATIONS OCCUR?

Although every tissue has some ability to metabolize drugs, the liver is the principal organ of drug metabolism. Other tissues that display considerable activity include the gastrointestinal tract, the lungs, the skin, the kidneys, and the brain. After oral administration, many drugs (eg, isoproterenol, meperidine, pentazocine, morphine) are absorbed intact from the small intestine and transported first via the portal system to the liver, where they undergo extensive metabolism. This process is called the **first-pass effect** (see Chapter 3). Some orally administered drugs (eg, clonazepam, chlorpromazine, cyclosporine) are more extensively metabolized in the intestine than in the liver, while others (eg, midazolam) undergo significant (~50%) intestinal metabolism. Thus, intestinal metabolism can contribute to the overall first-pass effect, and individuals with compromised liver function may rely increasingly on such intestinal metabolism for drug elimination. Compromise of intestinal metabolism of certain drugs (eg, felodipine, cyclosporine A) can also result in significant elevation of their plasma levels and clinically relevant drug-drug interactions (**DDIs**, see below). First-pass effects may limit the bioavailability of orally administered drugs (eg, lidocaine) so greatly that alternative routes of administration must be used to achieve therapeutically effective blood levels. Furthermore, the lower gut harbors intestinal microorganisms that are capable of many biotransformation reactions. In addition, drugs may be metabolized by gastric acid (eg, penicillin), by digestive enzymes (eg, polypeptides such as insulin), or by

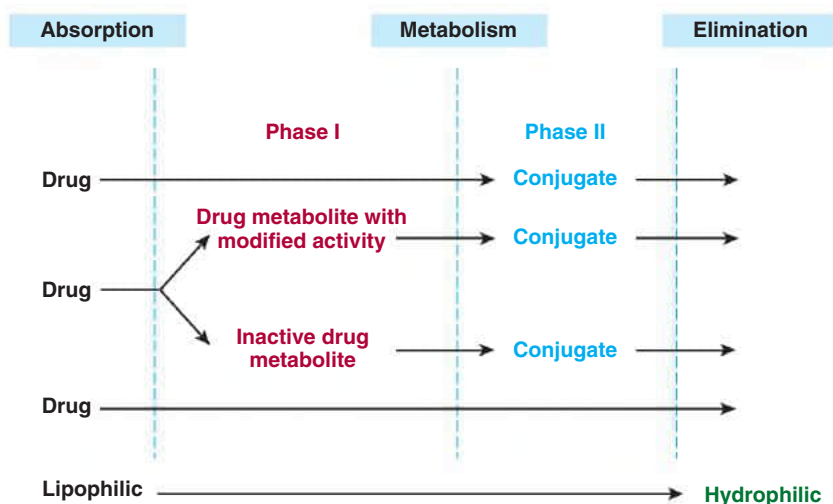


FIGURE 4–1 Phase I and phase II reactions and direct elimination, in drug biodisposition. Phase II reactions may also precede phase I reactions.

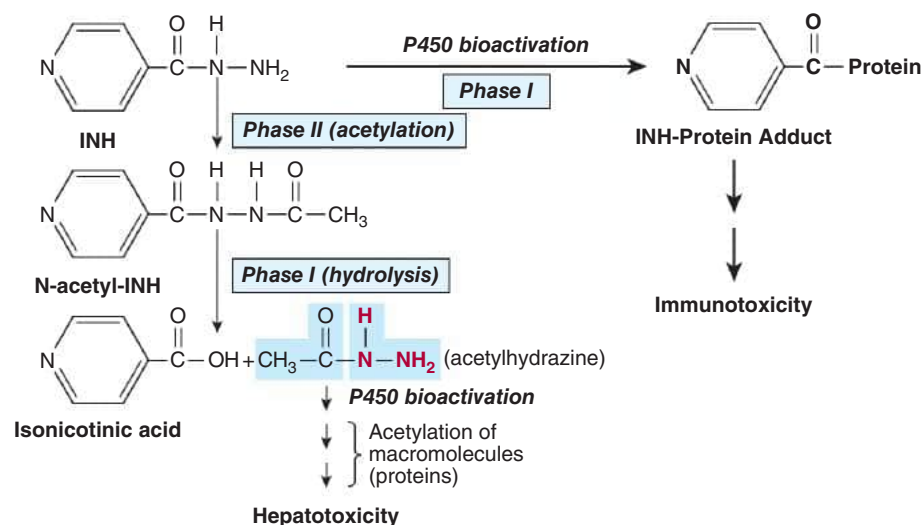


FIGURE 4-2 Phase II activation of isoniazid (INH) to a hepatotoxic metabolite. Note that INH can also undergo P450-mediated bioactivation to an isonicotinic acyl radical that can subsequently attack hepatic proteins including the P450s that metabolize it. In certain individuals, this results in idiosyncratic immunotoxicity as the INH-adducted proteins are proteolytically degraded into isonicotinic acylated peptides that act as autoantigens engendering pathogenic immunoreactive autoantibodies. Such INH-P450 autoantibodies can interact with the small fraction of the intracellular P450s that is integrated in the cell membrane but exposed extracellularly, triggering liver cell injury.

enzymes in the wall of the intestine (eg, sympathomimetic catecholamines).

Although drug biotransformation *in vivo* can occur by spontaneous, noncatalyzed chemical reactions, most transformations are catalyzed by specific cellular enzymes. At the subcellular level, these enzymes may be located in the endoplasmic reticulum, mitochondria, cytoplasm, lysosomes, or even the nuclear envelope or plasma membrane.

MICROSOMAL MIXED FUNCTION OXIDASE SYSTEM & PHASE I REACTIONS

Many drug-metabolizing enzymes are located in the lipophilic endoplasmic reticulum membranes of the liver and other tissues. When these lamellar membranes are isolated by homogenization and fractionation of the cell, they re-form into vesicles called **microsomes**. Microsomes retain most of the morphologic and functional characteristics of the intact membranes, including the rough and smooth surface features of the rough (ribosome-studded) and smooth (no ribosomes) endoplasmic reticulum. Whereas the rough microsomes tend to be dedicated to protein synthesis, the smooth microsomes are relatively rich in enzymes responsible for oxidative drug metabolism. In particular, they contain the important class of enzymes known as the **mixed function oxidases** (MFOs), or **monooxygenases**. The activity of these enzymes requires both a reducing agent (nicotinamide adenine dinucleotide phosphate [**NADPH**]) and molecular oxygen; in a typical reaction, one molecule of oxygen is consumed (reduced) per substrate molecule, with one oxygen atom appearing in the product and the other in the form of water.

In this oxidation-reduction process, two microsomal enzymes play a key role. The first of these is a flavoprotein, **NADPH-cytochrome P450 oxidoreductase** (CPR, or POR). One mole of this enzyme contains 1 mol each of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). The second microsomal enzyme is a hemoprotein called **cytochrome P450**, which serves as the terminal oxidase. In fact, the microsomal membrane harbors multiple forms of this hemoprotein, and this multiplicity is increased by repeated administration of or exposure to exogenous chemicals (see text that follows). The name cytochrome P450 (abbreviated as **P450** or **CYP**) is derived from the spectral properties of this hemoprotein. In its reduced (ferrous) form, it binds carbon monoxide to give a complex that absorbs light maximally at 450 nm. The relative abundance of P450s, compared with that of the reductase in the liver, contributes to making P450 heme reduction a rate-limiting step in hepatic drug oxidations.

Microsomal drug oxidations require P450, P450 reductase, NADPH, and molecular oxygen. A simplified scheme of the oxidative cycle is presented in Figure 4-3. Briefly, oxidized (Fe^{3+}) P450 combines with a drug substrate to form a binary complex (*step 1*). NADPH donates an electron to the flavoprotein P450 reductase, which in turn reduces the oxidized P450-drug complex (*step 2*). A second electron is introduced from NADPH via the same P450 reductase (or even microsomal cytochrome b_5), which serves to reduce molecular oxygen and to form an “activated oxygen”—P450–substrate complex (*step 3*). This complex in turn transfers activated oxygen to the drug substrate to form the oxidized product (*step 4*).

The potent oxidizing properties of this activated oxygen permit oxidation of a large number of substrates. Substrate specificity is very low for this enzyme complex. High lipid solubility is the only common structural feature of the wide variety of structurally

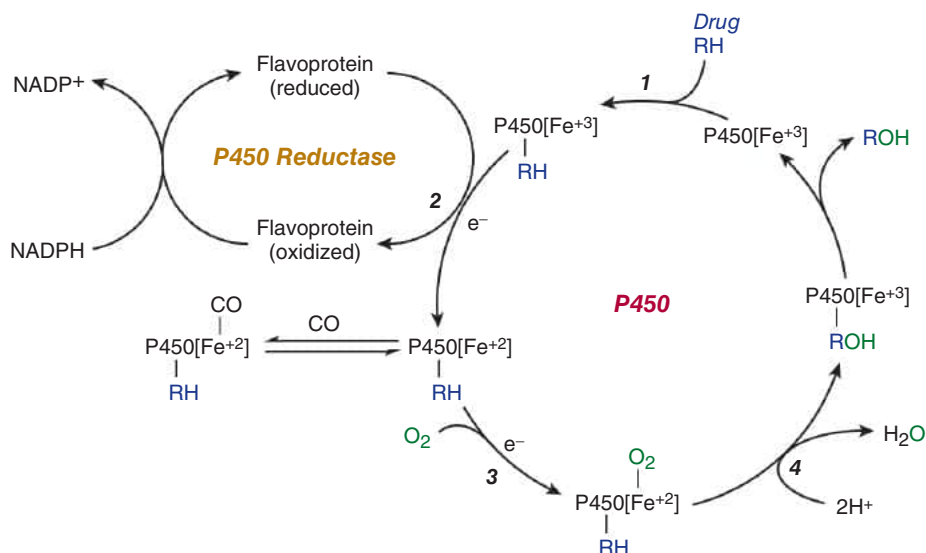


FIGURE 4-3 Cytochrome P450 cycle in drug oxidations. e⁻, electron; RH, parent drug; ROH, oxidized metabolite.

unrelated drugs and chemicals that serve as substrates in this system (Table 4-1). However, compared with many other enzymes including phase II enzymes, P450s are remarkably sluggish catalysts, and their drug biotransformation reactions are slow.

HUMAN LIVER P450 ENZYMES

Gene arrays combined with immunoblotting analyses of microsomal preparations, as well as the use of relatively selective functional markers and selective P450 inhibitors, have identified numerous P450 isoforms (CYPs: 1A2, 2A6, 2B6, 2C8, 2C9, 2C18, 2C19, 2D6, 2E1, 3A4, 3A5, 4A11, and 7) in the human liver. Of these, **CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2D6, CYP2E1, and CYP3A4** appear to be the most important forms, accounting for approximately 15%, 4%, 1%, 20%, 5%, 10%, and 30%, respectively, of the total human liver P450 content. Together, they are responsible for catalyzing the bulk of the hepatic drug and xenobiotic metabolism (Table 4-2, Figure 4-4).

It is noteworthy that CYP3A4 alone is responsible for the metabolism of over 50% of the prescription drugs metabolized by the liver. The involvement of individual P450s in the metabolism of a given drug may be screened *in vitro* by means of selective functional markers, selective chemical P450 inhibitors, and P450 antibodies. *In vivo*, such screening may be accomplished by means of relatively selective noninvasive markers, which include breath tests or urinary analyses of specific metabolites after administration of a P450-selective substrate probe.

Enzyme Induction

Some of the chemically dissimilar P450 substrate drugs, on repeated administration, *induce* P450 expression by enhancing the rate of its synthesis or reducing its rate of degradation (see Table 4-2). Induction results in accelerated substrate metabolism and usually in a decrease in the pharmacologic action of the inducer and also

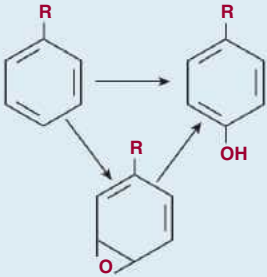
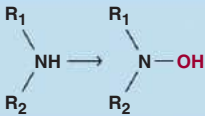
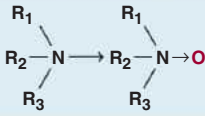
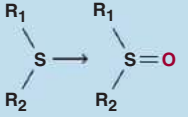
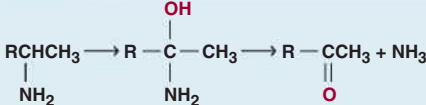
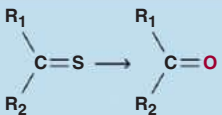
of co-administered drugs. However, in the case of drugs metabolically transformed to reactive metabolites, enzyme induction may exacerbate metabolite-mediated toxicity.

Various substrates induce P450 isoforms having different molecular masses and exhibiting different substrate specificities and immunochemical and spectral characteristics.

Environmental chemicals and pollutants are also capable of inducing P450 enzymes. Exposure to benzo[*a*]pyrene and other polycyclic aromatic hydrocarbons, which are present in tobacco smoke, charcoal-broiled meat, and other organic pyrolysis products, is known to induce CYP1A enzymes and to alter the rates of drug metabolism. Other environmental chemicals known to induce specific P450s include the polychlorinated biphenyls (PCBs), which were once used widely in industry as insulating materials and plasticizers, and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (dioxin, TCDD), a trace byproduct of the chemical synthesis of the defoliant 2,4,5-T (see Chapter 56).

Increased P450 synthesis requires enhanced transcription and translation along with increased synthesis of heme, its prosthetic cofactor. A cytoplasmic receptor (termed AhR) for polycyclic aromatic hydrocarbons (eg, benzo[*a*]pyrene, dioxin) has been identified. The translocation of the inducer-receptor complex into the nucleus, followed by ligand-induced dimerization with Arnt, a closely related nuclear protein, leads to subsequent activation of regulatory elements of *CYP1A* genes, resulting in their induction. This is also the mechanism of CYP1A induction by cruciferous vegetables, and the proton pump inhibitor, omeprazole. A pregnane X receptor (PXR), a member of the steroid-retinoid-thyroid hormone receptor family, has recently been shown to mediate CYP3A induction by various chemicals (dexamethasone, rifampin, mifepristone, phenobarbital, atorvastatin, and hyperforin, a constituent of St. John's wort) in the liver and intestinal mucosa. A similar receptor, the constitutive androstane receptor (CAR), has been identified for the relatively large and structurally diverse phenobarbital class of inducers of CYP2B6, CYP2C9,

TABLE 4–1 Phase I reactions. (Continued)

Reaction Class	Structural Change	Drug Substrate
Oxidations		
<i>Cytochrome P450-dependent oxidations:</i>		
Aromatic hydroxylations		Acetanilide, propranolol, phenobarbital, phenytoin, phenylbutazone, amphetamine, warfarin, 17 α -ethinyl estradiol, naphthalene, benzpyrene
Aliphatic hydroxylations	$\begin{aligned} \text{RCH}_2\text{CH}_3 &\longrightarrow \text{RCH}_2\text{CH}_2\text{OH} \\ \text{RCH}_2\text{CH}_3 &\longrightarrow \text{RCH}(\text{OH})\text{CH}_3 \end{aligned}$	Amobarbital, pentobarbital, secobarbital, chlorpropamide, ibuprofen, meprobamate, glutethimide, phenylbutazone, digitoxin
Epoxidation	$\text{RCH}=\text{CHR} \longrightarrow \text{R}-\text{C}(\text{H})\text{O}(\text{H})-\text{C}(\text{H})-\text{R}$	Aldrin
Oxidative dealkylation		
N-Dealkylation	$\text{RNHCH}_3 \longrightarrow \text{RNH}_2 + \text{CH}_2\text{O}$	Morphine, ethylmorphine, benzphetamine, aminopyrine, caffeine, theophylline
O-Dealkylation	$\text{ROCH}_3 \longrightarrow \text{ROH} + \text{CH}_2\text{O}$	Codeine, <i>p</i> -nitroanisole
S-Dealkylation	$\text{RSCH}_3 \longrightarrow \text{RSH} + \text{CH}_2\text{O}$	6-Methylthiopurine, methitural
N-Oxidation		
Primary amines	$\text{RNH}_2 \longrightarrow \text{RNHOH}$	Aniline, chlorphentermine
Secondary amines		2-Acetylaminofluorene, acetaminophen
Tertiary amines		Necotine, methaqualone
S-Oxidation		Thioridazine, cimetidine, chlorpromazine
Deamination		Amphetamine, diazepam
Desulfuration		Thiopental

(continued)

TABLE 4–1 Phase I reactions. (Continued)

Reaction Class	Structural Change	Drug Substrate
Cytochrome P450-dependent oxidations: (continued)		
	$\begin{array}{ccc} \text{R}_1 & & \text{R}_1 \\ & \diagdown & \diagup \\ & \text{P}=\text{S} & \longrightarrow & \text{P}=\text{O} \\ & \diagup & \diagdown \\ \text{R}_2 & & \text{R}_2 \end{array}$	Parathion
Dechlorination	$\text{CCl}_4 \longrightarrow [\text{CCl}_3^\cdot] \longrightarrow \text{CHCl}_3$	Carbon tetrachloride
Cytochrome P450-independent oxidations:		
Flavin monooxygenase (Ziegler's enzyme)	$\text{R}_3\text{N} \longrightarrow \text{R}_3\text{N}^+ \xrightarrow{\text{O}^-} \text{R}_3\text{N}^+\text{OH}$	Chlorpromazine, amitriptyline, benzphetamine
	$\begin{array}{ccc} \text{RCH}_2\text{N}-\text{CH}_2\text{R} & \longrightarrow & \text{RCH}_2-\text{N}-\text{CH}_2\text{R} \\ & & \\ \text{H} & & \text{OH} \end{array}$ $\begin{array}{ccc} \text{RCH}=\text{N}-\text{CH}_2\text{R} & & \\ & & \\ \text{O}^- & & \end{array}$	Desipramine, nortriptyline
	$\begin{array}{ccc} \text{—N} & & \text{—N} & & \text{—N} \\ & \diagdown & & \diagdown & \diagdown \\ & \text{C}=\text{SH} & \longrightarrow & \text{C}=\text{SOH} & \longrightarrow & \text{C}=\text{SO}_2\text{H} \\ & \diagup & & \diagup & \diagup \\ \text{—N} & & \text{—N} & & \text{—N} \end{array}$	Methimazole, propylthiouracil
Amine oxidases	$\text{RCH}_2\text{NH}_2 \longrightarrow \text{RCHO} + \text{NH}_3$	Phenylethylamine, epinephrine
Dehydrogenations	$\text{RCH}_2\text{OH} \longrightarrow \text{RCHO}$	Ethanol
Reductions		
Azo reductions	$\text{RN}=\text{NR}_1 \longrightarrow \text{RNH}-\text{NHR}_1 \longrightarrow \text{RNH}_2 + \text{R}_1\text{NH}_2$	Prontosil, tartrazine
Nitro reductions	$\text{RNO}_2 \longrightarrow \text{RNO} \longrightarrow \text{RNHOH} \longrightarrow \text{RNH}_2$	Nitrobenzene, chloramphenicol, clonazepam, dantrolene
Carbonyl reductions	$\begin{array}{ccc} \text{RCR}' & \longrightarrow & \text{RCHR}' \\ & & \\ \text{O} & & \text{OH} \end{array}$	Metyrapone, methadone, naloxone
Hydrolyses		
Esters	$\text{R}_1\text{COOR}_2 \longrightarrow \text{R}_1\text{COOH} + \text{R}_2\text{OH}$	Procaine, succinylcholine, aspirin, clofibrate, methylphenidate
Amides	$\text{RCONHR}_1 \longrightarrow \text{RCOOH} + \text{R}_1\text{NH}^1$	Procainamide, lidocaine, indomethacin

¹Hydrolytic reactions are generally classified as Phase I reactions, because although intracellular H₂O is utilized, it is split into its constituent H⁺ and OH[−] that are each singly incorporated into one or the other of the ensuing products. However, when the intact “endogenous” H₂O molecule is incorporated into a single product, ie, “water-added” arene-dihydrodiol generated from arene-epoxide, they are considered as Phase II reactions (Table 4–3).

and CYP3A4. Peroxisome proliferator receptor α (PPAR- α) is yet another nuclear receptor highly expressed in liver and kidneys, which uses lipid-lowering drugs (eg, fenofibrate and gemfibrozil) as ligands. Consistent with its major role in the regulation of fatty acid metabolism, PPAR- α mediates the induction of CYP4A enzymes, responsible for the metabolism of fatty acids such as arachidonic acid and its physiologically relevant derivatives. It is noteworthy that on binding of its particular ligand, PXR, CAR, and PPAR- α each form heterodimers with another nuclear receptor, the retinoid X-receptor (RXR). This heterodimer in turn binds to response elements within the promoter regions of specific *P450* genes to induce gene expression.

P450 enzymes may also be induced by **substrate stabilization**, eg, decreased degradation, as is the case with troleandomycin- or clotrimazole-mediated induction of CYP3A enzymes, the ethanol-mediated induction of CYP2E1, and the isosafrole-mediated induction of CYP1A2.

Enzyme Inhibition

Certain drug substrates inhibit cytochrome P450 enzyme activity (see Table 4–2). Imidazole-containing drugs such as cimetidine and ketoconazole bind tightly to the P450 heme iron and effectively reduce the metabolism of endogenous substrates (eg, testosterone) or other co-administered drugs through competitive inhibition.

TABLE 4–2 Human liver P450s (CYPs), and some of the drugs metabolized (substrates), inducers, and selective inhibitors. Note: Some P450 substrates can be potent competitive inhibitors and/or mechanism-based inactivators. (Continued)

CYP	Substrates	Inducers	Inhibitors
1A2	Acetaminophen, alosetron, antipyrine, caffeine, clomipramine, clozapine, duloxetine, flutamide, frovatriptan, melatonin, mexiletine, mirtazapine, olanzapine, phenacetin, ramelteon, rasagiline, ropinirole, tacrine, tamoxifen, theophylline, tizanidine, triamterene, warfarin, zolmitriptan	Charcoal-broiled foods, cruciferous vegetables, grilled meat, lansoprazole, omeprazole, primidone, rifampin, smoking	Artemisinin, atazanavir, cimetidine, ciprofloxacin, enoxacin, ethinyl estradiol, fluvoxamine, furafylline, galangin, mexiletene, tacrine, thiabendazole, zileuton
2A6	Coumarin, dexmedetomidine, tobacco nitrosamines, nicotine (to cotinine and 2'-hydroxynicotine)	Efavirenz, rifampin, phenobarbital	Clotrimazole, isoniazid, ketoconazole, letrozole, menthofuran, methimazole, methoxsalen, miconazole, tranlycypromine
2B6	Artemisinin, bupropion, clopidogrel, cyclophosphamide, efavirenz, ifosfamide, irinotecan, ketamine, S-mephobarbital, S-mephenytoin (<i>N</i> -demethylation to nirvanol), methadone, nevirapine, promethazine, propofol, selegiline, sertraline, ticlopidine	Carbamazepine, cyclophosphamide, fosphenytoin, nevirapine, phenobarbital, primidone, rifampin	Amiodarone, amlodipine, clopidogrel, clotrimazole, desipramine, disulfiram, doxorubicin, ethinyl estradiol, fluoxetine, fluvoxamine, isoflurane, ketoconazole, mestranol, methimazole, nefazodone, nelfinavir, orphenadrine, paroxetine, phenacyclidine, sertraline, thiotepa, ticlopidine
2C8	Amiodarone, cabazitaxel, carbamazepine, chloroquine, diclofenac, ibuprofen, paclitaxel, all- <i>trans</i> -retinoic acid, repaglinide, rosiglitazone, treprostinil	Rifampin, barbiturates	Deferasirox, gemfibrozil, lapatinib, montelukast, pioglitazone, quercetin, rosiglitazone, trimethoprim
2C9	Alosetron, bosentan, celecoxib, chlorpropamide, diclofenac, dronabinol, flurbiprofen, fluvastatin, glimepiride, glipizide, glyburide, hexobarbital, ibuprofen, indomethacin, irbesartan, losartan, meloxicam, montelukast, naproxen, nateglinide, phenobarbital, phenytoin, piroxicam, rosiglitazone, rosuvastatin, sulfamethoxazole, sulfaphenazole, ticrynafen, tolbutamide, torsemide, trimethadione, valsartan, S-warfarin	Aminoglutethimide, barbiturates, bosentan, carbamazepine, phenytoin, primidone, rifabutin, rifampin, rifapentine, St. John's wort	Amiodarone, clopidogrel, delavirdine, disulfiram, doxifluridine, efavirenz, fluconazole, fluvoxamine, fluorouracil, imatinib, leflunomide, metronidazole, miconazole, phenytoin, sulfamethoxazole, sulfaphenazole, sulfinpyrazone, tienilic acid, valproic acid, voriconazole
2C18	Tolbutamide, phenytoin	Phenobarbital	
2C19	Aripiprazole, carisoprodol, citalopram, clomipramine, clopidogrel, clozapine, desipramine, diazepam, diphenhydramine, doxepin, escitalopram, fluoxetine, imipramine, lansoprazole, S-mephenytoin, methadone, moclobemide, naproxen, nelfinavir, nirvanol, olanzapine, omeprazole, pantoprazole, phenobarbital, phenytoin, proguanil, propranolol, rabeprazole, sertraline, thalidomide, voriconazole, <i>R</i> -warfarin	Aminoglutethimide, artemisinin, barbiturates, carbamazepine, phenytoin, primidone, rifampin, rifapentine, St. John's wort	<i>N</i> 3-Benzylnirvanol, <i>N</i> 3-benzylphenobarbital, chloramphenicol, cimetidine, clopidogrel, delavirdine, efavirenz, esomeprazole, felbamate, fluconazole, fluoxetine, fluvoxamine, isoniazid, moclobemide, modafinil, nootkatone, omeprazole, ticlopidine, voriconazole
2D6	Amitriptyline, atomoxetine, buprinalol, bupranolol, carvedilol, chlorpheniramine, chlorpromazine, clomipramine, clozapine, codeine, debrisoquine, desipramine, dextromethorphan, dihydrocodeine, encainide, flecainide, fluoxetine, fluvoxamine, guanoxan, haloperidol, hydrocodone, imipramine, maprotiline, 4-methoxyamphetamine, metoclopramide, metoprolol, mexiletine, nebivolol, nortriptyline, oxycodone, palonosetron, paroxetine, perhexiline, perphenazine, phenformin, propafenone, propoxyphene, propranolol, risperidone, selegiline (deprenyl), sparteine, tamoxifen, thioridazine, timolol, tolterodine, tricyclic antidepressants, tramadol, trazodone, venlafaxine	Unknown	Bupropion, cinacalcet, chloroquine, diphenhydramine, fluoxetine, haloperidol, imatinib, paroxetine, propafenone, propoxyphene, quinidine, terbinafine, thioridazine
2E1	Acetaminophen, chlorzoxazone, dacarbazine, enflurane, ethanol (a minor pathway), halothane, isoflurane, isoniazid, sevoflurane, theophylline, trimethadione	Ethanol, isoniazid	Amitriptyline, chlorpromazine, cimetidine, clomethiazole, clotrimazole, clozapine, disulfiram, diethylthiocarbamate, diallyl sulfide, econazole, methimazole, methoxsalen, 4-methylpyrazole, miconazole, modafinil, ritonavir, selegiline, sildenafil, sulconazole, ticlopidine, tioconazole

(continued)

TABLE 4–2 Human liver P450s (CYPs), and some of the drugs metabolized (substrates), inducers, and selective inhibitors. Note: Some P450 substrates can be potent competitive inhibitors and/or mechanism-based inactivators. (Continued)

CYP	Substrates	Inducers	Inhibitors
3A4 ¹	Acetaminophen, alfentanil, alfuzosin, almotriptan, alprazolam, amiodarone, amlodipine, aprepitant, astemizole, atazanavir, atorvastatin, bepridil, bexarotene, bosentan, bromocriptine, budesonide, buspirone, carbamazepine, cisapride, clarithromycin, clonazepam, clopidogrel, cocaine, colchicine, conivaptan, cortisol, cyclosporine, dapsone, darunavir, dasatinib, delavirdine, dexamethasone, diazepam, dihydroergotamine, dihydropyridines, diltiazem, disopyramide, doxorubicin, droperidol, dutasteride, ebastine, efavirenz, eletriptan, eplerenone, ergotamine, erlotinib, erythromycin, estazolam, eszopiclone, ethinyl estradiol, ethosuximide, etoposide, everolimus, exemestane, felodipine, fentanyl, finasteride, flurazepam, fluticasone, fosamprenavir, galantamine, gefitinib, gestodene, granisetron, halofantrine, ifosfamide, imatinib, indinavir, irinotecan, isradipine, itraconazole, ixabepilone, lapatinib, lidocaine, loperamide, lopinavir, loratadine, lovastatin, macrolides, maraviroc, mefloquine, methadone, methylprednisolone, miconazole, midazolam, mifepristone, modafinil, nefazodone, nevirapine, nicardipine, nifedipine, nimodipine, nisoldipine, paclitaxel, paricalcitol, pimozide, pioglitazone, praziquantel, prednisolone, prednisone, progesterone, quetiapine, quinine, quinidine, quinine, ranolazine, rapamycin, repaglinide, rifabutin, ritonavir, saquinavir, sibutramine, sildenafil, simvastatin, sirolimus, solifenacin, spironolactone, sufentanil, sulfamethoxazole, sunitinib, tacrolimus, tadalafil, tamoxifen, tamsulosin, teniposide, terfenadine, testosterone, tetrahydrocannabinol, tiagabine, tinidazole, tipranavir, tolvaptan, topiramate, triazolam, troleandomycin, vardenafil, verapamil, vinblastine, vincristine, ziprasidone, zolpidem, zonisamide, zopiclone	Aminoglutethimide, avasimibe, barbiturates, carbamazepine, efavirenz, glucocorticoids, nevirapine, pioglitazone, phenytoin, primidone, rifampin, rifapentine, St. John's wort	Amprenavir, azamulin, boceprevir, clarithromycin, conivaptan, diltiazem, erythromycin, fluconazole, grapefruit juice (furanocoumarins), indinavir, itraconazole, ketoconazole, lopinavir, mibefradil, nefazodone, nelfinavir, posaconazole, ritonavir, saquinavir, telaprevir, telithromycin, troleandomycin, verapamil, voriconazole

¹CYP3A5 has similar substrate and inhibitor profiles but, except for a few drugs, is generally less active than CYP3A4.

Macrolide antibiotics such as troleandomycin, erythromycin, and erythromycin derivatives are metabolized, apparently by CYP3A, to metabolites that complex the cytochrome P450 heme iron and render it catalytically inactive. Another compound that acts through this mechanism is the inhibitor proadifen (SKF-525-A, used in research), which binds tightly to the heme iron and quasi-irreversibly inactivates the enzyme, thereby inhibiting the metabolism of potential substrates.

Some substrates irreversibly inhibit P450s via covalent interaction of a metabolically generated reactive intermediate that may react with the P450 apoprotein or heme moiety or even cause the heme to fragment and irreversibly modify the apoprotein. The antibiotic chloramphenicol is metabolized by CYP2B1 to a species that modifies the P450 protein and thus also inactivates the enzyme. A growing list of such **suicide inhibitors**—inactivators that attack the heme or the protein moiety—includes certain steroids (ethinyl estradiol, norethindrone, and spironolactone); fluorene; allobarbitol; the analgesic sedatives allylisopropylacetylurea, diethylpentenamide, and ethchlorvynol; carbon disulfide; grapefruit furanocoumarins; selegiline; phencyclidine; ticlopidine and clopidogrel; ritonavir; and propylthiouracil. On the other hand, the barbiturate secobarbital is found to inactivate CYP2B1 by modification of *both* its heme and protein moieties. Other

metabolically activated drugs whose P450 inactivation mechanism is not fully elucidated are mifepristone, troglitazone, raloxifene, and tamoxifen.

PHASE II REACTIONS

Parent drugs or their phase I metabolites that contain suitable chemical groups often undergo coupling or conjugation reactions with an endogenous substance to yield **drug conjugates** (Table 4–3). In general, conjugates are polar molecules that are readily excreted and often inactive. Conjugate formation involves high-energy intermediates and specific transfer enzymes. Such enzymes (**transferases**) may be located in microsomes or in the cytoplasm. Of these, uridine 5′-diphosphate (UDP)-glucuronosyl transferases (**UGTs**) are the most dominant enzymes (see Figure 4–4). These microsomal enzymes catalyze the coupling of an activated endogenous substance (such as the UDP derivative of glucuronic acid) with a drug (or endogenous compound such as bilirubin, the end product of heme metabolism). Nineteen *UGT* genes (*UGT1A1* and *UGT2*) encode UGT proteins involved in the metabolism of drugs and xenobiotics. Similarly, 11 human sulfotransferases (**SULTs**) catalyze the sulfation of substrates using 3′-phosphoadenosine 5′-phosphosulfate (**PAPS**)

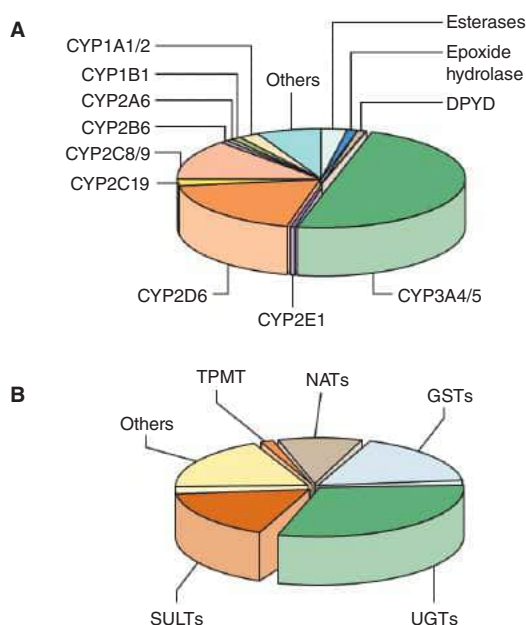


FIGURE 4-4 Relative contributions of various cytochrome P450 isoforms (**A**) and different phase II pathways (**B**) to metabolism of drugs in clinical use. Many drugs are metabolized by two or more of these pathways. Note that two pathways, CYP3A4/5 and UGT, are involved in the metabolism of more than 75% of drugs in use. DPYD, dihydropyrimidine dehydrogenase; GST, glutathione-S-transferase; NAT, *N*-acetyltransferase; SULT, sulfotransferase; TPMT, thiopurine methyltransferase; UGT, UDP-glucuronosyltransferase. (Reproduced with permission from Brunton LL, Chabner BA, Knollman BC: *Goodman & Gilman's The Pharmacological Basis of Therapeutics*, 12th ed. New York, NY: McGraw Hill; 2011.)

as the endogenous sulfate donor. Cytosolic and microsomal glutathione (**GSH**) transferases (**GSTs**) are also engaged in the metabolism of drugs and xenobiotics, and in that of leukotrienes and prostaglandins, respectively. Chemicals containing an aromatic amine or a hydrazine moiety (eg, isoniazid) are substrates of cytosolic *N*-acetyltransferases (**NATs**), encoded by *NAT1* and *NAT2* genes, which utilize **acetyl-CoA** as the endogenous cofactor.

S-Adenosyl-L-methionine (**SAME**; AdoMet)-mediated *O*-, *N*-, and *S*-methylation of drugs and xenobiotics by methyltransferases (**MTs**) also occurs. Finally, endobiotic, drug, and xenobiotic epoxides generated via P450-catalyzed oxidations can also be hydrolyzed by microsomal or cytosolic epoxide hydrolases (**EHs**). Conjugation of an activated drug such as the *S*-CoA derivative of benzoic acid, with an endogenous substrate, such as glycine, also occurs. Because the endogenous substrates originate in the diet, nutrition plays a critical role in the regulation of drug conjugations.

Phase II reactions are relatively faster than P450-catalyzed reactions, thus effectively accelerating drug biotransformation.

Drug conjugations were once believed to represent terminal inactivation events and as such have been viewed as “true detoxification” reactions. However, this concept must be modified, because it is now known that certain conjugation reactions (acyl glucuronidation of nonsteroidal anti-inflammatory drugs, *O*-sulfation of *N*-hydroxyacetylaminofluorene, and *N*-acetylation of isoniazid) may lead to the formation of reactive species responsible for the toxicity of the drugs. Furthermore, sulfation is known to activate the orally active prodrug minoxidil into a very efficacious vasodilator, and morphine-6-glucuronide is more potent than morphine itself.

TABLE 4-3 Phase II reactions.

Type of Conjugation	Endogenous Reactant	Transferase (Location)	Types of Substrates	Examples
Glucuronidation	UDP glucuronic acid (UDPGA)	UDP glucuronosyl-transferase (microsomes)	Phenols, alcohols, carboxylic acids, hydroxylamines, sulfonamides	Nitrophenol, morphine, acetaminophen, diazepam, <i>N</i> -hydroxydapsone, sulfathiazole, meprobamate, digitoxin, digoxin
Acetylation	Acetyl-CoA	<i>N</i> -Acetyltransferase (cytoplasm)	Amines	Sulfonamides, isoniazid, clonazepam, dapsone, mescaline
Glutathione conjugation	Glutathione (GSH)	GSH-S-transferase (cytoplasm, microsomes)	Epoxides, arene oxides, nitro groups, hydroxylamines	Acetaminophen, ethacrynic acid, bromobenzene
Glycine conjugation	Glycine	Acyl-CoA glycintransferase (mitochondria)	Acyl-CoA derivatives of carboxylic acids	Salicylic acid, benzoic acid, nicotinic acid, cinnamic acid, cholic acid, deoxycholic acid
Sulfation	Phosphoadenosyl phosphosulfate (PAPS)	Sulfotransferase (cytoplasm)	Phenols, alcohols, aromatic amines	Estrone, aniline, phenol, 3-hydroxycoumarin, acetaminophen, methyl dopa
Methylation	<i>S</i> -Adenosylmethionine (SAM)	Transmethylases (cytoplasm)	Catecholamines, phenols, amines	Dopamine, epinephrine, pyridine, histamine, thiouracil
Water conjugation	Water	Epoxide hydrolase (microsomes)	Arene oxides, <i>cis</i> -disubstituted and monosubstituted oxiranes	Benzopyrene 7,8-epoxide, styrene 1,2-oxide, carbamazepine epoxide
		(cytoplasm)	Alkene oxides, fatty acid epoxides	Leukotriene A ₄

METABOLISM OF DRUGS TO TOXIC PRODUCTS

Metabolism of drugs and other foreign chemicals may not always be an innocuous biochemical event leading to detoxification and elimination of the compound. Indeed, as previously noted, several compounds have been shown to be metabolically transformed to reactive intermediates that are toxic to various organs. Such toxic reactions may not be apparent at low levels of exposure to parent compounds when alternative detoxification mechanisms are not yet overwhelmed or compromised and when the availability of endogenous detoxifying cosubstrates (GSH, glucuronic acid, sulfate) is not limited. However, when these resources are exhausted, the toxic pathway may prevail, resulting in overt organ toxicity or carcinogenesis. The number of specific examples of such drug-induced toxicity is expanding rapidly. An example is acetaminophen (APAP; paracetamol)-induced hepatotoxicity (Figure 4–5). Acetaminophen, an analgesic antipyretic drug, is quite safe in therapeutic doses (1.2 g/d for an adult). It normally undergoes glucuronidation and sulfation to the corresponding conjugates, which together make up 95% of the total excreted metabolites. The alternative P450-dependent GSH conjugation pathway accounts for the remaining 5%. When acetaminophen intake far exceeds therapeutic doses, the glucuronidation and sulfation pathways are saturated, and the P450-dependent pathway becomes increasingly important. Little or no hepatotoxicity results as long as hepatic GSH is available for conjugation. However, with time, hepatic GSH is depleted faster than it can be regenerated, and a reactive, toxic metabolite accumulates. In the absence of intracellular nucleophiles such as GSH, this reactive metabolite (*N*-acetylbenzoiminoquinone) not only reacts with nucleophilic groups of cellular proteins resulting in direct hepatocellular damage, but also participates in redox cycling, thereby generating reactive O_2 species (ROS) and consequent oxidative stress that greatly enhance acetaminophen-induced hepatotoxicity.

The chemical and toxicologic characterization of the electrophilic nature of the reactive acetaminophen metabolite has led to the development of effective antidotes—cysteamine and *N*-acetylcysteine (NAC; Acetadote; Mucomyst). Administration of *N*-acetylcysteine (the safer of the two) within 8–16 hours after acetaminophen overdose has been shown to protect victims from fulminant hepatotoxicity and death (see Chapter 58). Administration of GSH is not effective because it does not cross cell membranes readily.

CLINICAL RELEVANCE OF DRUG METABOLISM

The dose and frequency of administration required to achieve effective therapeutic blood and tissue levels vary in different patients because of individual differences in drug distribution and rates of drug metabolism and elimination. These differences are determined by genetic factors as well as nongenetic variables, such as commensal gut microbiota, age, sex, liver size, liver function, circadian rhythm, body temperature, and nutritional and

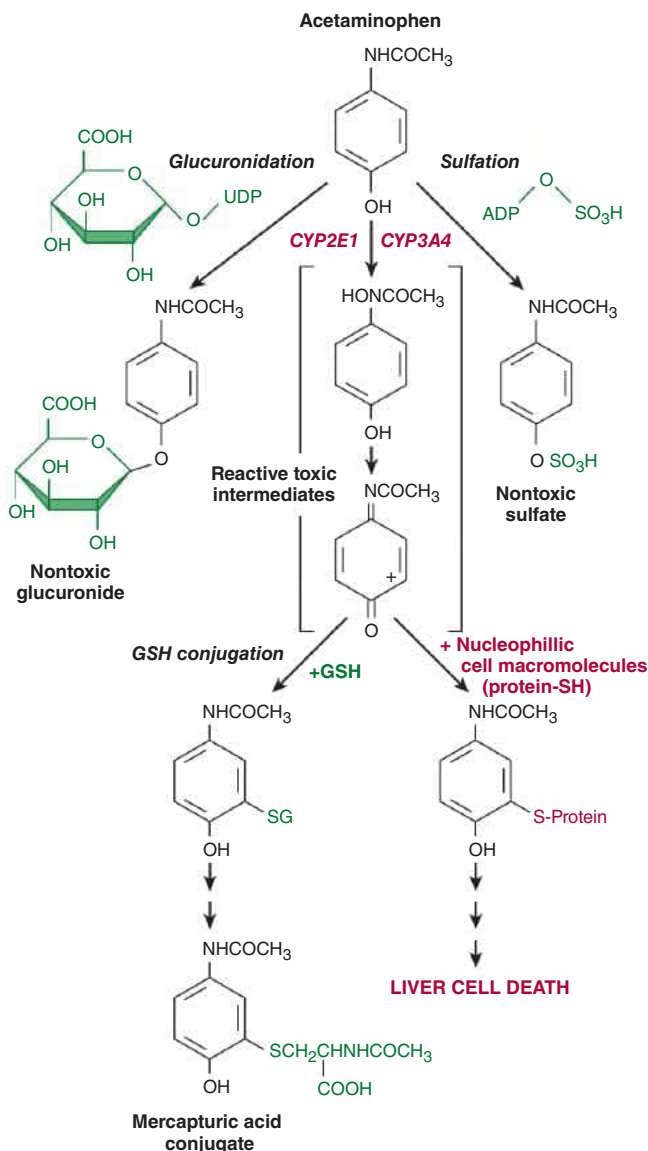


FIGURE 4–5 Metabolism of acetaminophen (top center) to hepatotoxic metabolites. GSH, glutathione; SG, glutathione moiety.

environmental factors such as concomitant exposure to inducers or inhibitors of drug metabolism. The discussion that follows summarizes the most important of these variables.

Individual Differences

Individual differences in metabolic rate depend on the nature of the drug itself. Thus, within the same population, steady-state plasma levels may reflect a 30-fold variation in the metabolism of one drug and only a twofold variation in the metabolism of another.

Genetic Factors

Genetic factors that influence enzyme levels account for some of these differences, giving rise to “genetic polymorphisms” in drug metabolism (see also Chapter 5). The first examples of drugs found to be subject to genetic polymorphisms were the muscle relaxant

succinylcholine, the antituberculosis drug isoniazid, and the anti-coagulant warfarin. A true genetic polymorphism is defined as the occurrence of a variant allele of a gene at a population frequency of $\geq 1\%$, resulting in altered expression or functional activity of the gene product, or both. Well-defined and clinically relevant genetic polymorphisms in both phase I and phase II drug-metabolizing enzymes exist that result in altered efficacy of drug therapy or adverse drug reactions (**ADRs**). The latter frequently necessitate dose adjustment (Table 4-4), a consideration particularly crucial for drugs with low therapeutic indices.

A. Phase I Enzyme Polymorphisms

Genetically determined defects in the phase I oxidative metabolism of several drugs have been reported (see Table 4-4; see also Chapter 5). These defects are often transmitted as autosomal recessive traits and may be expressed at any one of the multiple metabolic transformations that a chemical might undergo. Human liver P450s 3A4, 2C9, 2D6, 2C19, 1A2, and 2B6 are responsible for about 75% of all clinically relevant phase I drug metabolism (see Figure 4-4), and thus for about 60% of all physiologic drug biotransformation and elimination. Thus, genetic polymorphisms of these enzymes, by significantly influencing phase I drug metabolism, can alter their pharmacokinetics and the magnitude or the duration of drug response and associated events.

Three P450 genetic polymorphisms have been particularly well characterized, affording some insight into possible underlying molecular mechanisms, and are clinically noteworthy, as they require therapeutic dosage adjustment. The first is the **debrisoquin-sparteine oxidation** type of polymorphism, which apparently occurs in 3–10% of Caucasians and is inherited as an autosomal recessive trait. In affected individuals, the **CYP2D6**-dependent oxidations of debrisoquin and other drugs (see Table 4-2; Figure 4-6) are impaired. These defects in oxidative drug metabolism are probably co-inherited. The precise molecular basis for the defect appears to be faulty expression of the P450 protein due to either defective mRNA splicing or protein folding, resulting in little or no isoform-catalyzed drug metabolism and thereby conferring a **poor metabolizer (PM)** phenotype. This PM phenotype correlates with a higher risk of relapse in patients with breast cancer treated with tamoxifen, an anticancer drug that relies on its CYP2D6-dependent metabolic activation to endoxifen for its efficacy. More recently, however, another polymorphic genotype has been reported that results in **ultrarapid metabolism** of relevant drugs due to the presence of CYP2D6 allelic variants with up to 13 gene copies in tandem. This ultrarapid metabolizer (**UM**) genotype is most common in Ethiopians and Saudi Arabians, populations that display it in up to one third of individuals. As a result, these subjects require twofold to threefold higher daily doses of nortriptyline (an antidepressant and a CYP2D6 substrate) to achieve therapeutic plasma levels. The poor responsiveness to antidepressant therapy of the UM phenotype also clinically correlates with a higher incidence of suicides relative to that of deaths due to natural causes in this patient population. Conversely, in these UM populations, the prodrug codeine (another CYP2D6 substrate) is metabolized much faster to morphine, often resulting in undesirable adverse effects of morphine, such as abdominal

pain. Indeed, intake of high doses of codeine by a mother of the ultrarapid metabolizer type was held responsible for the morphine-induced death of her breast-fed infant.

The second well-studied genetic drug polymorphism involves the stereoselective **aromatic (4)-hydroxylation** of the anticonvulsant mephenytoin, catalyzed by **CYP2C19**. This polymorphism, which is also inherited as an autosomal recessive trait, occurs in 3–5% of Caucasians and 18–23% of Japanese populations. It is genetically independent of the debrisoquin-sparteine polymorphism. In normal **extensive metabolizers (EMs)** (*S*)-mephenytoin is extensively hydroxylated by CYP2C19 at the 4-position of the phenyl ring before its glucuronidation and rapid excretion in the urine, whereas (*R*)-mephenytoin is slowly *N*-demethylated to nirvanol, an active metabolite. PMs, however, appear to totally lack the stereospecific (*S*)-mephenytoin hydroxylase activity, so both (*S*)- and (*R*)-mephenytoin enantiomers are *N*-demethylated to nirvanol, which accumulates in much higher concentrations. Thus, PMs of mephenytoin show signs of profound sedation and ataxia after doses of the drug that are well tolerated by normal metabolizers. Two defective CYP2C19 variant alleles (*CYP2C19**2 and *CYP2C19**3), the latter predominant in Asians, are largely responsible for the PM genotype. The molecular bases include splicing defects resulting in a truncated, nonfunctional protein. CYP2C19 is responsible for the metabolism of various clinically relevant drugs (see Table 4-4). Thus, it is clinically important to recognize that the safety of each of these drugs may be severely reduced in persons with the PM phenotype. On the other hand, the PM phenotype can notably increase the therapeutic efficacy of omeprazole, a proton-pump inhibitor, in gastric ulcer and gastroesophageal reflux diseases (see Chapter 5 for additional discussion of the CYP2C19 polymorphism).

Another CYP2C19 variant allele (*CYP2C19**17) exists that is associated with increased transcription and thus higher CYP2C19 expression and even higher functional activity than that of the wild type CYP2C19-carrying EMs. Individuals carrying this *CYP2C19**17 allele exhibit higher metabolic activation of prodrugs such as the breast cancer drug tamoxifen, the antimalarial chlorproguanil, and the antiplatelet drug clopidogrel. The former event is associated with a lower risk of breast cancer relapse, and the latter event with an increased risk of bleeding. Carriers of the *CYP2C19**17 allele are also known to enhance the metabolism and thus the elimination of drugs such as the antidepressants escitalopram and imipramine, as well as the antifungal voriconazole. This consequently impairs the therapeutic efficacy of these drugs, thus requiring clinical dosage adjustments.

The third relatively well-characterized genetic polymorphism is that of **CYP2C9**. Two well-characterized variants of this enzyme exist, each with amino acid mutations that result in altered metabolism. The *CYP2C9**2 allele encodes an Arg144Cys mutation, exhibiting impaired functional interactions with CPR. The other allelic variant, *CYP2C9**3, encodes an enzyme with an Ile359Leu mutation that has lowered affinity for many substrates. For example, individuals displaying the *CYP2C9**3 phenotype have greatly reduced tolerance for the anticoagulant warfarin. The warfarin clearance in *CYP2C9**3-homozygous individuals is about 10% of normal values, and these people have a much lower tolerance for the drug than those who are homozygous for the normal wild type

TABLE 4–4 Some examples of genetic polymorphisms in phase I and phase II drug metabolism. (Continued)

Enzyme Involved	Defect	Genotype	Drug and Therapeutic Use	Clinical Consequences ¹
CYP1A2	N-Demethylation	EM	Caffeine (CNS stimulant)	Reduced CNS stimulation due to increased gene inducibility and thus increased metabolism/clearance in cigarette smokers and frequent ingesters of omeprazole.
	N-Demethylation	PM	Caffeine (CNS stimulant)	Enhanced CNS stimulation.
CYP2A6	Oxidation	PM	Nicotine (cholinoceptorstimulant)	Nicotine toxicity. Lesser craving for frequent cigarette smoking.
	Oxidation	EM	Nicotine (cholinoceptorstimulant)	Increased nicotine metabolism. Greater craving for frequent cigarette smoking.
	Oxidation	PM	Coumarin (anticoagulant)	Increased risk of bleeding.
	Oxidation	EM	Coumarin (anticoagulant)	Increased clearance. Greater risk of thrombosis.
CYP2B6	Oxidation, N-Dechloroethylation	PM	Cyclophosphamide, ifosfamide (anti-cancer)	Reduced clearance. Increased risk of ADRs.
	Oxidation	PM	Efavirenz, nevirapine (anti-HIV)	Reduced clearance. Increased risk of ADRs.
CYP2C8	Hydroxylation	PM	Repaglinide, rosiglitazone, pioglitazone (antidiabetic)	Reduced clearance. Increased risk of ADRs.
	Hydroxylation	PM	Paclitaxel (anti-cancer)	Reduced clearance. Increased risk of ADRs (myelosuppression).
	N-Deethylation/ N-Dealkylation	PM	Amodiaquine, chloroquine (antimalarial)	Reduced clearance. Increased risk of ADRs.
	N-Deethylation	PM	Amiodarone (antiarrhythmic)	Reduced clearance. Increased risk of ADRs.
CYP2C9	Hydroxylation	PM	Celecoxib, diclofenac, flurbiprofen, S-ibuprofen (NSAIDs)	Reduced clearance. Increased risk of ADRs.
	Hydroxylation	PM	S-Warfarin, S-acenocoumarol (anticoagulants)	Enhanced bleeding risk. Clinically highly relevant. Dose adjustment required.
	Hydroxylation	PM	Tolbutamide (antidiabetic)	Cardiotoxicity.
	Hydroxylation	PM	Phenytoin (antiepileptic)	Nystagmus, diplopia, and ataxia.
CYP2C19	N-Demethylation	PM	Amitriptyline, clomipramine (antidepressants)	Reduced clearance. Increased risk of ADRs. Dose adjustment required.
	Oxidation	PM	Moclobemide (MAOI)	
	N-Demethylation	PM	Citalopram (SSRI)	Increased risk of gastrointestinal side effects. Increased risk of QT-prolongation and Torsades de Pointes arrhythmias.
	O-Demethylation	PM	Omeprazole (PPI)	Increased therapeutic efficacy.
	Hydroxylation	PM	Mephenytoin (antiepileptic)	Overdose toxicity.
	N-Demethylation	EM	Escitalopram (antidepressants)	Increased gene transcription resulting in increased activity and thus reduced therapeutic efficacy.
	O-Demethylation	EM	Omeprazole (PPI)	Reduced therapeutic efficacy.
	Hydroxylation	EM	Tamoxifen (anti-cancer)	Increased metabolic activation, increased therapeutic efficacy; reduced risk of relapse. Dose adjustment required.
	Oxidative cyclization	EM	Chlorproguanil (antimalarial)	Increased metabolic activation, increased therapeutic efficacy. Dose adjustment required.
	Oxidation	EM	Clopidogrel (antiplatelet)	Increased metabolic activation, increased therapeutic efficacy. Dose adjustment required.
CYP2D6	Oxidation	PM	Bufuralol (β -adrenoceptor blocker)	Exacerbation of β blockade, nausea.
	O-Demethylation	PM	Codeine (analgesic)	Reduced metabolic activation to morphine and thus reduced analgesia.

(continued)

TABLE 4–4 Some examples of genetic polymorphisms in phase I and phase II drug metabolism. (Continued)

Enzyme Involved	Defect	Genotype	Drug and Therapeutic Use	Clinical Consequences ¹
	Oxidation	PM	Debrisoquin (antihypertensive)	Orthostatic hypotension.
	<i>N</i> -Demethylation	PM	Nortriptyline (antidepressant)	Reduced clearance. Increased risk of ADRs.
	Oxidation	PM	Sparteine	Oxytotic symptoms.
	<i>O</i> -Demethylation	PM	Dextromethorphan (antitussive)	Reduced clearance. Increased risk of ADRs.
	<i>O</i> -Demethylation	PM	Tramadol (analgesic)	Increased risk of seizures.
	Hydroxylation	PM	Tamoxifen (anti-cancer)	Reduced metabolic activation to the therapeutically active endoxifen and thus reduced therapeutic efficacy.
	<i>O</i> -Demethylation	UM	Codeine (analgesic)	Increased metabolic activation to morphine and thus increased risk of respiratory depression.
	<i>N</i> -Demethylation	UM	Nortriptyline (antidepressant)	Reduced therapeutic efficacy due to increased clearance.
	<i>O</i> -Demethylation	UM	Tramadol (analgesic)	Reduced therapeutic efficacy due to increased clearance.
CYP3A4		PM?	All drugs metabolized by this enzyme would be potentially affected	Reduced clearance. Dose adjustment may be required to avoid drugdrug interactions.
CYP3A5		PM?	Saquinavir, and other CYP3A substrates	Usually less catalytically active than CYP3A4. A higher frequency of a functional CYP3A5*1 allele is seen in Africans than in Caucasians; the latter most often carry the defective CYP3A5*3 allele. This may significantly affect therapeutics of CYP3A substrates in CYP3A5*1 or CYP3A5*3 homozygous individuals.
ALDH	Aldehyde dehydrogenation	PM	Ethanol (recreational drug)	Facial flushing, hypotension, tachycardia, nausea, vomiting.
BCHE	Ester hydrolysis	PM	Succinylcholine (muscle relaxant)	Prolonged apnea.
			Mivacurium (neuromuscular blocker)	Prolonged muscle paralysis.
			Cocaine (CNS stimulant)	Increased blood pressure, tachycardia, ventricular arrhythmias.
GST	GSH-conjugation	PM	Acetaminophen (analgesic), Busulfan (anticancer)	Impaired GSH conjugation due to gene deletion.
NAT2	<i>N</i> -Acetylation	PM	Hydralazine (antihypertensive)	Lupus erythematosus-like syndrome.
	<i>N</i> -Acetylation	PM	Isoniazid (antitubercular)	Peripheral neuropathy.
TPMT	<i>S</i> -Methylation	PM	6-Thiopurines (anti-cancer)	Myelotoxicity.
UGT1A1	Glucuronidation	PM	Bilirubin (heme metabolite)	Hyperbilirubinemia.
			Irinotecan (anti-cancer)	Reduced clearance. Dose adjustment may be required to avoid toxicity (GI dysfunction, immunosuppression).

¹Observed or predictable.

ADR, adverse drug reaction; EM, extensive metabolizer; PM, poor metabolizer; UM, ultrarapid metabolizer.

allele. These individuals also have a much higher risk of adverse effects with warfarin (eg, bleeding) and with other CYP2C9 substrates such as phenytoin, losartan, tolbutamide, and some non-steroidal anti-inflammatory drugs (see Table 4–4). Note, however, that despite the predominant role of CYP2C9 in warfarin clearance (particularly that of its pharmacologically more potent *S*-isomer), warfarin maintenance doses are largely dictated by polymorphisms

in the *VKORC1* gene responsible for the expression of vitamin K epoxide reductase, the specific cellular target of warfarin, rather than by *CYP2C9**2/*3 polymorphisms alone (see Chapter 5).

Allelic variants of CYP3A4 have also been reported, but their contribution to the well-known interindividual variability in drug metabolism apparently is limited. On the other hand, the expression of CYP3A5, another human liver isoform, is markedly

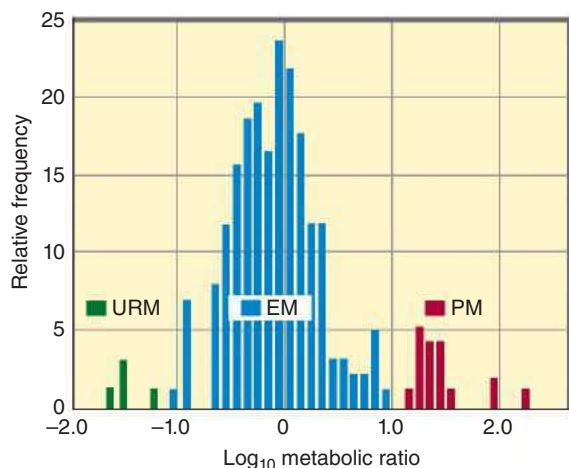


FIGURE 4-6 Genetic polymorphism in debrisoquin 4-hydroxylation by CYP2D6 in a Caucasian population. The semilog frequency distribution histogram of the metabolic ratio (MR; defined as percent of dose excreted as unchanged debrisoquin divided by the percent of dose excreted as 4-hydroxydebrisoquin metabolite) in the 8-hour urine collected after oral ingestion of 12.8 mg debrisoquin sulfate (equivalent to 10 mg free debrisoquin base). Individuals with MR values >12.6 were phenotyped as poor metabolizers (PM, red bars), and those with MR values <12.6 but >0.2 were designated as extensive metabolizers (EM, blue bars). Those with MR values <0.2 were designated as ultrarapid metabolizers (URM, green bars) based on the MR values (0.01–0.1) of individuals with documented multiple copies of CYP2D6 allelic variants resulting from inherited amplification of this gene. (Adapted with permission from Woolhouse NM, Andoh B, Mahgoub A, et al: Debrisoquin hydroxylation polymorphism among Ghanaians and Caucasians. *Clin Pharmacol Ther* 1979 Nov;26(5):584-591.)

polymorphic, ranging from 0% to 50% of the total hepatic CYP3A content. Robust CYP3A5 expression requires at the least, one normally spliced CYP3A5*1 allele. This CYP3A5 protein polymorphism is now known to result from a single nucleotide polymorphism (SNP) within intron 3 or 6, resulting in alternative splicing, as well as protein truncation and loss, thus enabling normally spliced CYP3A5 transcripts in just 5% of Caucasians, 29% of Japanese, 27% of Chinese, and 30% of Koreans, but 73% of African Americans. Thus, it can significantly contribute to interindividual differences in the metabolism of preferential CYP3A5 substrates such as midazolam. Two other CYP3A5 allelic variants that result in a PM phenotype also are known.

Polymorphisms in the *CYP2A6* gene have also been recently characterized, and their prevalence is apparently racially linked. CYP2A6 is responsible for nicotine oxidation, and tobacco smokers with low CYP2A6 activity consume less and have a lower incidence of lung cancer. CYP2A6 1B allelic variants associated with faster rates of nicotine metabolism have been recently discovered. It remains to be determined whether patients with these faster variants will fall into the converse paradigm of increased smoking behavior and lung cancer incidence.

Additional genetic polymorphisms in drug metabolism are being discovered. Of these, the gene for **CYP2B6** has become noteworthy as one of the most polymorphic P450 genes, with a 20- to 250-fold variation in interindividual CYP2B6 expression.

Despite its low (1–5%) contribution to the total liver P450 content, these CYP2B6 polymorphisms may have a significant impact on the CYP2B6-dependent metabolism of several clinically relevant drugs such as cyclophosphamide, *S*-methadone, efavirenz, nevirapine, bupropion, selegiline, and propofol. Of clinical relevance, women (particularly Hispanic American women) express considerably higher hepatic levels of CYP2B6 protein than men.

Studies of theophylline metabolism in monozygotic and dizygotic twins that included pedigree analysis of various families have revealed that a distinct polymorphism may exist for this drug and may be inherited as a recessive genetic trait. Genetic drug metabolism polymorphisms also appear to occur for aminopyrine and carbocysteine oxidations. Regularly updated information on human P450 polymorphisms is available at <https://www.pharmvar.org/>.

Although genetic polymorphisms in drug oxidations often involve specific P450 enzymes, such genetic variations can also occur in other enzymes. Recently, genetic polymorphisms in CPR/POR, the essential P450 electron donor, have been reported. In particular, an allelic variant (at a 28% frequency) encoding a CPR A503V mutation (eg, *POR*10*) has been reported to result in impaired CYP17-dependent sex steroid synthesis and impaired CYP3A4- and CYP2D6-dependent drug metabolism in vitro. However, an associated impairment in drug metabolism was not always found clinically. By contrast, several other CPR/POR allelic variants (eg, *POR*5*, *POR*16*) have now been established with an associated decreased drug metabolism as their plausible clinical phenotype. Descriptions of a polymorphism in the oxidation of trimethylamine, believed to be metabolized largely by the **flavin monooxygenase (Ziegler's enzyme)**, result in the “fish-odor syndrome” in slow metabolizers, thus suggesting that genetic variants of other non-P450-dependent oxidative enzymes may also contribute to such polymorphisms.

Genetic polymorphisms also exist in other Phase I enzymes, ie, esterases. Thus, succinylcholine is metabolized only half as rapidly in persons with genetically determined deficiency in pseudocholinesterase (now generally referred to as butyrylcholinesterase [**BCHE**]) as in persons with normally functioning enzyme. Different mutations, inherited as autosomal recessive traits, account for the enzyme deficiency. Deficient individuals treated with succinylcholine as a muscle relaxant may become susceptible to prolonged respiratory paralysis (succinylcholine apnea); see Chapter 27.

B. Phase II Enzyme Polymorphisms

Pharmacogenetic differences are seen in the acetylation of isoniazid. The defect in slow acetylators (of isoniazid and similar amines) appears to be caused by the synthesis of less of the NAT2 enzyme rather than of an abnormal form of it. Inherited as an autosomal recessive trait, the **slow acetylator phenotype** occurs in about 50% of blacks and whites in the USA, more frequently in Europeans living in high northern latitudes, and much less commonly in Asians and Inuit (Eskimos). The slow acetylator phenotype is also associated with a higher incidence of isoniazid-induced peripheral neuritis, drug-induced autoimmune disorders, and bicyclic aromatic amine-induced bladder cancer.

A clinically important polymorphism of the *TPMT* (thiopurine *S*-methyltransferase) gene is encountered in Europeans

(frequency, 1:300), resulting in a rapidly degraded mutant enzyme and consequently deficient *S*-methylation of aromatic and heterocyclic sulfhydryl compounds including the anti-cancer thiopurine drugs 6-mercaptopurine, thioguanine, and azathioprine, required for their detoxification. Patients inheriting this polymorphism as an autosomal recessive trait are at high risk of thiopurine drug-induced fatal hematopoietic toxicity.

Genetic polymorphisms in the expression of other phase II enzymes (UGTs and GSTs) also occur. Thus, UGT polymorphisms (*UGT1A1**28) are associated with hyperbilirubinemic diseases (Gilbert syndrome) as well as toxic effects due to impaired drug conjugation and/or elimination (eg, the anticancer drug irinotecan). Similarly, genetic polymorphisms (*GSTM1*) in GST (mul isoform) expression can lead to significant adverse effects and toxicities of drugs dependent on its GSH conjugation for elimination.

C. Role of Pharmacogenomic Testing in Clinically Safe & Effective Drug Therapy

Despite our improved understanding of the molecular basis of pharmacogenetic defects in drug-metabolizing enzymes, their impact on drug therapy and ADRs, and the availability of validated pharmacogenetic biomarkers to identify patients at risk, this clinically relevant information has not been effectively translated to patient care. Thus, the much-heralded potential for personalized medicine, except in a few instances of drugs with a relatively low therapeutic index (eg, warfarin), has remained largely unrealized. This is so even though 98% of US physicians are apparently aware that such genetic information may significantly influence therapy. This is partly due to the lack of adequate training in translating this knowledge to medical practice, and partly due to the logistics of genetic testing and the issue of cost-effectiveness. Severe ADRs are known to contribute to 100,000 annual US deaths, about 7% of all hospital admissions, and an increased average length of hospital stay. Genotype information could greatly enhance safe and efficacious clinical therapy through dose adjustment or alternative drug therapy, thereby curbing much of the rising ADR incidence and its associated costs. (See Chapter 5 for further discussion.)

Commensal Gut Microbiota

It is increasingly recognized that the human gut microbiome can also significantly influence drug responses. It thus serves as another relevant source of therapeutic misadventures and adverse drug-drug interactions. More than 1000 species of intestinal microorganisms have been identified, including obligate anaerobic bacteria and various yeasts that coexist in a dynamic, often symbiotic, ecological equilibrium. Their biotransformation repertoire is non-oxidative, albeit highly versatile, extending from predominantly reductive and hydrolytic reactions to decarboxylation, dehydroxylation, dealkylation, dehalogenation, and deamination. Notably, such bacterially mediated reduction of the cardiac drug digoxin significantly contributes to its metabolism and elimination. Co-treatment with antibiotics such as erythromycin or tetracycline increases digoxin serum levels twofold, increasing the risk of cardiotoxicity. Similarly, drugs that are primarily glucuronidated in

the liver are excreted into the gut via the bile, whereupon they are subjected to de-glucuronidation by gut microbial β -glucuronidases (hydrolases). The pharmacologically active parent aglycone is subsequently reabsorbed into the portal circulation with consequent extension of its pharmacologic action and hepatic phase II re-conjugation and subsequent enterohepatic recycling. Thus, if the parent drug is dosage limited or has a low therapeutic index, this may mean increased toxicity. For example, under normal dosage, the analgesic acetaminophen is largely metabolized via glucuronidation and sulfation, as discussed earlier, and eliminated into the hepatic sinusoidal plasma. However, upon overdosage, the increased production of these metabolites is quite likely to saturate their normal excretory transport process. Their consequently enhanced biliary excretion would subject a greater fraction of the acetaminophen-glucuronide to de-glucuronidation by intestinal microbial β -glucuronidases, which may further contribute to the toxic acetaminophen burden. This possibility is even more relevant for glucuronides of parent drugs of noted gastrointestinal toxicity. Accordingly, selective inhibition of microbial β -glucuronidases has been documented to alleviate the gastrointestinal toxicity of anticancer drugs such as irinotecan, as well as the enteropathies induced by nonsteroidal anti-inflammatory drugs (NSAIDs) such as indomethacin, ketoprofen or diclofenac, that incur substantial enterohepatic circulation. This possibility has fueled the pharmaceutical design and development of even more selective inhibitors targeted against microbial β -glucuronidases.

Diet & Environmental Factors

Diet and environmental factors contribute to individual variations in drug metabolism. Charcoal-broiled foods and cruciferous vegetables are known to induce CYP1A enzymes, whereas grapefruit juice is known to inhibit the CYP3A metabolism of co-administered drug substrates (see Table 4–2; also see below). Cigarette smokers metabolize some drugs more rapidly than nonsmokers because of enzyme induction (see previous section). Industrial workers exposed to some pesticides metabolize certain drugs more rapidly than unexposed individuals. Such differences make it difficult to determine effective and safe doses of drugs that have narrow therapeutic indices.

Age & Sex

Increased susceptibility to the pharmacologic or toxic activity of drugs has been reported in very young and very old patients compared with young adults (see Chapters 59 and 60). Although this may reflect differences in absorption, distribution, and excretion, differences in drug metabolism also play a role. Slower metabolism could be due to reduced activity of metabolic enzymes or reduced availability of essential endogenous cofactors.

Sex-dependent variations in drug metabolism have been well documented in rats but not in other rodents. Young adult male rats metabolize drugs much faster than mature female rats or pre-pubertal male rats. These differences in drug metabolism have been clearly associated with androgenic hormones. Clinical reports suggest that similar sex-dependent differences in drug metabolism also exist in humans for ethanol, propranolol, some benzodiazepines, estrogens, and salicylates.

TABLE 4–5 Partial list of drugs that enhance drug metabolism in humans.

Inducer	Drugs Whose Metabolism Is Enhanced
Benzo[<i>a</i>]pyrene	Theophylline
Carbamazepine	Carbamazepine, clonazepam, itraconazole
Chlorcyclizine	Steroid hormones
Ethchlorvynol	Warfarin
Glutethimide	Antipyrine, glutethimide, warfarin
Griseofulvin	Warfarin
Phenobarbital and other barbiturates ¹	Barbiturates, chloramphenicol, chlorpromazine, cortisol, coumarin anticoagulants, desmethyl imipramine, digitoxin, doxorubicin, estradiol, itraconazole, phenylbutazone, phenytoin, quinine, testosterone
Phenylbutazone	Aminopyrine, cortisol, digitoxin
Phenytoin	Cortisol, dexamethasone, digitoxin, itraconazole, theophylline
Rifampin	Coumarin anticoagulants, digitoxin, glucocorticoids, itraconazole, methadone, metoprolol, oral contraceptives, prednisone, propranolol, quinidine, saquinavir
Ritonavir ²	Midazolam
St. John's wort ³	Alprazolam, cyclosporine, digoxin, indinavir, oral contraceptives, ritonavir, simvastatin, tacrolimus, warfarin

¹Secobarbital is an exception. See Table 4–6 and text.

²With chronic (repeated) administration; acutely, ritonavir is a potent CYP3A4 inhibitor/inactivator.

³For a more comprehensive list of drugs whose metabolism is enhanced by St. John's wort, see Rahimi and Abdollahi, 2012; Russo et al, 2014; and Tsai et al, 2012.

Drug-Drug Interactions (DDIs) During Metabolism

Many substrates, by virtue of their relatively high lipophilicity, are not only retained at the active site of the enzyme but remain non-specifically bound to the lipid endoplasmic reticulum membrane. In this state, they may induce microsomal enzymes, particularly after repeated use. Acutely, depending on the residual drug levels at the active site, they also may competitively inhibit metabolism of a simultaneously administered drug.

Enzyme-inducing drugs include various sedative-hypnotics, antipsychotics, anticonvulsants, the antitubercular drug rifampin, and insecticides (Table 4–5). Patients who routinely ingest barbiturates, other sedative-hypnotics, or certain antipsychotic drugs may require considerably higher doses of warfarin to maintain a therapeutic effect. On the other hand, discontinuance of the sedative inducer may result in reduced metabolism of the anticoagulant and bleeding—a toxic effect of the ensuing enhanced plasma levels of the anticoagulant. Similar interactions have been observed in individuals receiving various combinations of drug regimens such as rifampin, antipsychotics, or sedatives with contraceptive agents, sedatives with anticonvulsant drugs, and even alcohol with hypoglycemic drugs (tolbutamide). One inducer of note is St. John's wort, a popular over-the-counter herbal medicine ingested

as treatment for mild to severe depression. Because of its marked induction of hepatic CYP3A4 and, to a lesser extent, CYP2C9 and CYP2C19, St. John's wort has been linked to a large number of DDIs. Most of such DDIs stem from P450 induction by St. John's wort and entail accelerated P450-dependent metabolism of the co-ingested drug (eg, alprazolam, contraceptive estrogens, warfarin, lovastatin, delavirdine, ritonavir). In contrast, St. John's wort-mediated CYP2C19 induction may enhance the activation of the antiplatelet prodrug clopidogrel by accelerating its conversion to the active metabolite. Finally, some St. John's wort-elicited DDIs may entail decreased P450-dependent metabolism due to competitive inhibition and consequently increased plasma levels and clinical effect (eg, meperidine, hydrocodone, morphine, oxycodone). Other DDIs entail synergistic increases in serotonin levels (due to monoamine oxidase inhibition) and correspondingly increased serotonergic tone and adverse effects (eg, paroxetine, sertraline, fluoxetine, fenfluramine).

It must also be noted that an inducer may enhance not only the metabolism of other drugs but also its own metabolism. Thus, continued use of some drugs may result in a pharmacokinetic type of **tolerance**—progressively reduced therapeutic effectiveness due to enhancement of their own metabolism.

Conversely, simultaneous administration of two or more drugs may result in impaired elimination of the more slowly metabolized drug and prolongation or potentiation of its pharmacologic effects (Table 4–6). Both competitive substrate inhibition and irreversible substrate-mediated enzyme inactivation may augment plasma drug levels and lead to toxic effects from drugs with narrow therapeutic indices. Indeed, such acute interactions of terfenadine (a second-generation antihistamine) with a CYP3A4 substrate-inhibitor (ketoconazole, erythromycin, or grapefruit juice) resulted in fatal cardiac arrhythmias (torsades de pointes) requiring its withdrawal from the market. Similar DDIs with CYP3A4 substrate-inhibitors (such as the antibiotics erythromycin and clarithromycin, the antidepressant nefazodone, the antifungals itraconazole and ketoconazole, and the HIV protease inhibitors indinavir and ritonavir) and consequent cardiotoxicity led to withdrawal or restricted use of the 5-HT₄ agonist cisapride. Similarly, allopurinol both prolongs the duration and enhances the chemotherapeutic and toxic actions of mercaptopurine by competitive inhibition of xanthine oxidase. Consequently, to avoid bone marrow toxicity, the dose of mercaptopurine must be reduced in patients receiving allopurinol. Cimetidine, a drug used in the treatment of peptic ulcer, has been shown to potentiate the pharmacologic actions of anticoagulants and sedatives. The metabolism of the sedative chlordiazepoxide has been shown to be inhibited by 63% after a single dose of cimetidine; such effects are reversed within 48 hours after withdrawal of cimetidine.

Impaired metabolism may also result if a simultaneously administered drug irreversibly inactivates a common metabolizing enzyme. These inhibitors, in the course of their metabolism by cytochrome P450, inactivate the enzyme and result in impairment of their own metabolism and that of other cosubstrates. This is the case of the furanocoumarins in grapefruit juice, eg, 6',7'-dihydroxybergamottin and bergamottin, which inactivate CYP3A4 in the intestinal mucosa and consequently enhance its

TABLE 4–6 Partial list of drugs that inhibit drug metabolism in humans.

Inhibitor ¹	Drug Whose Metabolism Is Inhibited
Allopurinol, chloramphenicol, isoniazid	Antipyrine, dicumarol, probenecid, tolbutamide
Chlorpromazine	Propranolol
Cimetidine	Chlordiazepoxide, diazepam, warfarin, others
Dicumarol	Phenytoin
Diethylpentenamide	Diethylpentenamide
Disulfiram	Antipyrine, ethanol, phenytoin, warfarin
Ethanol	Chlordiazepoxide (?), diazepam (?), methanol
Grapefruit juice ²	Alprazolam, atorvastatin, cisapride, cyclosporine, midazolam, triazolam
Itraconazole	Alfentanil, alprazolam, astemizole, atorvastatin, buspirone, cisapride, cyclosporine, delavirdine, diazepam, digoxin, felodipine, indinavir, loratadine, lovastatin, midazolam, nisoldipine, phenytoin, quinidine, ritonavir, saquinavir, sildenafil, simvastatin, sirolimus, tacrolimus, triazolam, verapamil, warfarin
Ketoconazole	Astemizole, cyclosporine, terfenadine
Nortriptyline	Antipyrine
Oral contraceptives	Antipyrine
Phenylbutazone	Phenytoin, tolbutamide
Ritonavir	Amiodarone, cisapride, itraconazole, midazolam, triazolam
Saquinavir	Cisapride, ergot derivatives, midazolam, triazolam
Secobarbital	Secobarbital
Spironolactone	Digoxin
Troleandomycin	Theophylline, methylprednisolone

¹While some inhibitors are selective for a given P450 enzyme, others are more general and can inhibit several P450s concurrently.

²Active components in grapefruit juice include furanocoumarins such as 6',7'-dihydroxybergamottin (which inactivates both intestinal and liver CYP3A4) as well as other unknown components that inhibit P-glycoprotein-mediated intestinal drug efflux and consequently further enhance the bioavailability of certain drugs such as cyclosporine. For a more comprehensive list of drugs whose metabolism is inhibited by grapefruit juice furanocoumarins, see Bailey et al, 2013.

proteolytic degradation. This impairment of intestinal first-pass CYP3A4-dependent metabolism significantly enhances the bioavailability of drugs such as ergotamine, felodipine, nifedipine, terfenadine, verapamil, ethinylestradiol, lovastatin, saquinavir, and cyclosporine A and is associated with clinically relevant DDIs and food-drug interactions. The list of drugs subject to DDIs involving grapefruit juice is extensive and includes many drugs with a very narrow therapeutic index and a high potential for lethal adverse reactions. However, it must be borne in mind that not all commercially available grapefruit juices are equally potent, as the CYP3A4 inactivation potency is totally dependent on the amount of furanocoumarins extracted into the juice from the zest (highest), pith,

and pulp of the grapefruit. Furthermore, recovery from these interactions is dependent on CYP3A4 resynthesis and thus may be slow.

Interactions Between Drugs & Endogenous Compounds

Some drugs require conjugation with endogenous substrates such as GSH, glucuronic acid, or sulfate for their inactivation. Consequently, different drugs may compete for the same endogenous substrates, and the faster-reacting drug may effectively deplete endogenous substrate levels and impair the metabolism of the slower-reacting drug. If the latter has a steep dose-response curve or a narrow margin of safety, potentiation of its therapeutic and toxic effects may result.

Diseases Affecting Drug Metabolism

Acute or chronic diseases that affect liver architecture or function markedly affect hepatic metabolism of some drugs. Such conditions include alcoholic hepatitis, active or inactive alcoholic cirrhosis, hemochromatosis, chronic active hepatitis, biliary cirrhosis, and acute viral or drug-induced hepatitis. Depending on their severity, these conditions may significantly impair hepatic drug-metabolizing enzymes, particularly microsomal oxidases, and thereby markedly affect drug elimination. For example, the half-lives of chlordiazepoxide and diazepam in patients with liver cirrhosis or acute viral hepatitis are greatly increased, with a corresponding increase in their effects. Consequently, these drugs may cause coma in patients with liver disease when given in ordinary doses.

Some drugs are metabolized so readily that even marked reduction in liver function does not significantly prolong their action. However, cardiac disease, by limiting blood flow to the liver, may impair disposition of those drugs whose metabolism is flow-limited (Table 4–7). These drugs are so readily metabolized by the liver that hepatic clearance is essentially equal to liver blood flow. The impaired enzyme activity or defective formation of enzymes associated with heavy metal poisoning or porphyria also results in reduced hepatic drug metabolism. Pulmonary disease may also affect drug metabolism, as indicated by the impaired hydrolysis of procainamide and procaine in patients with chronic respiratory insufficiency and the increased half-life of antipyrine (a P450 functional probe) in patients with lung cancer.

TABLE 4–7 Rapidly metabolized drugs whose hepatic clearance is blood flow-limited.

Alprenolol	Lidocaine
Amitriptyline	Meperidine
Clomethiazole	Morphine
Desipramine	Pentazocine
Imipramine	Propoxyphene
Isoniazid	Propranolol
Labetalol	Verapamil

Although the effects of endocrine dysfunction on drug metabolism have been well explored in experimental animal models, corresponding data for humans with endocrine disorders are scanty. Thyroid dysfunction has been associated with altered metabolism of some drugs and of some endogenous compounds as well. Hypothyroidism increases the half-life of antipyrine, digoxin, methimazole, and some β blockers, whereas hyperthyroidism has the opposite effect. A few clinical studies in diabetic patients indicate no apparent impairment of drug metabolism, although impairment has been noted in diabetic rats. Malfunctions of the pituitary, adrenal cortex, and gonads markedly reduce hepatic drug metabolism in rats. On the basis of these findings, it may be supposed that such disorders could significantly affect drug metabolism in humans. However, until sufficient evidence is obtained from clinical studies in patients, such extrapolations must be considered tentative.

Finally, the release of inflammatory mediators, cytokines, and nitric oxide associated with bacterial or viral infections, cancer, or inflammation are known to impair drug metabolism by inactivating P450s and enhancing their degradation.

Metabolism of Therapeutic Biologics

In recent decades, in addition to small chemical molecules (SMs, MW <1000 Da), large biological molecules have enriched the therapeutic armamentarium targeted to various diseases such as cancer, rheumatoid arthritis, autoimmune conditions, infections, and genetic and orphan diseases (see Chapters 36 and 55 for various pertinent examples). These include monoclonal antibodies (mAbs), cytokines (Interferons [IFN], TNF α), recombinant proteins, fusion proteins, growth factors, hormones (insulin, recombinant growth hormone), enzymes, vaccines, antibody fragments (Fabs that endow specificity and diversity), antibody-drug conjugates (ADCs), etc. The metabolism and clearance of these therapeutic agents, designed to exhibit high selectivity/affinity for their cellular targets, but possessing complex pharmacokinetic properties quite distinct from those of small molecules (SM), involve none of the drug-metabolizing/elimination pathways so far discussed for small drugs. Because of their proteinaceous nature in full (eg, mAbs) or in part (eg, PEGylated [polyethylene glycol polymer adducted] antibodies, ADCs), these biologics, much like native endogenous proteins, incur intracellular protein degradation to their constitutive amino acids, usually by lysosomes, or cellular proteases of circulating phagocytes (macrophages and monocytes), target-antigen bearing cells, or various tissue cells. However, irrespective of the cell type involved in the metabolic breakdown of the biologics, the constitutive amino acid residues are recycled into their common intracellular pool and re-utilized for *de novo* protein synthesis.

Biologic protein degradation requires their intracellular absorption for delivery to the target, but due to their relatively greater hydrophilicity and much larger size (~150 kDa for immunoglobulins G [IgGs]), their cellular permeability is poor and poses a major challenge. They cannot be delivered orally as they would be denatured in the acidic stomach lumen or degraded by the gastrointestinal proteases. They are therefore usually delivered parenterally

via IV, IM or SC routes. Their distribution is generally limited to plasma and extracellular fluids such as the interstitial fluid and lymph. While the IV route for biologics is optimally suited for the treatment of blood cell disorders, its major impediment is the poor diffusion of such large hydrophilic molecules through the capillary endothelial cell walls to sites of cellular targets and/or protein degradation. For these reasons, large biologics (>19 kDa) are often administered via the IM or SC routes to enable them to access the tissue interstitial spaces, from where they can access the more permeable lymphatic capillaries and be delivered, albeit very slowly (120 mL/kg/h), via lymphatic convective transport into the general circulation. Thus, it is not surprising that depending on the size of the biologics in question, this relatively slow absorption means that they typically require 2–8 days to reach maximal plasma concentrations. The distribution of biologics from the systemic circulation into peripheral tissues is also very slow, involving convective transport across capillary pores as well as transcytosis from the extracellular/interstitial spaces. Such transcytosis involves receptor-mediated endocytosis, phagocytosis, and fluid-phase pinocytosis (ability of cells to take up proteins and other macromolecules from the surrounding fluid spaces through endocytosis).

Biologic protein degradation (catabolism) can occur pre-systemically upon IM and SC injection by extracellular proteases in the interstitial spaces or during their blood or lymph circulation. From the vascular or interstitial spaces, the biologics are predominantly taken up into endothelial cells or tissues by fluid-phase pinocytosis. Biologics with high affinity antigen-binding fragments (Fabs) for their pharmacological targets, eg, mAbs directed to membrane-bound antigens, can also undergo target-mediated absorption (endosomal internalization) and subsequent lysosomal catabolism within the target cell.

Partly due to the well-characterized catabolic fate of circulating endogenous IgGs, the protein degradation process is best understood for therapeutic biologics such as mAbs and IgGs, ADCs, Fabs, and antibody fusion proteins, most of which include the Fc (stem) domain of the IgGs (IgG-Fc; Chapter 55). Upon parenteral administration, IgG-Fc interacts with the Fc receptors such as the Fc γ -receptor (Fc γ R) or neonatal Fc-receptor (FcRn) ubiquitously expressed on the extracellular surface of various immune and nonimmune cell types, endothelial, and epithelial cells. Upon this IgG-Fc/FcRn interaction, the cell surface is endocytosed, the integral FcRn receptors sequestered within the early endosome are saturated with the available IgG-Fc molecules, and the pH drops below physiological pH (6.0–6.5), which enhances the affinity of this IgG-Fc/FcRn binding. The excess, unbound IgG-Fc fraction is degraded by the lysosome, while the bound IgG-Fc molecules are protected from lysosomal degradation. The endosome with the firmly bound IgG-Fc/FcRn complexes is then recycled to the cell surface. Upon re-exposure to the physiological pH of the extracellular fluid, the IgG-Fc/FcRn complexes dissociate, enabling the therapeutic salvage of the intact undegraded therapeutic IgGs. This FcRn-mediated recycling of endogenous and therapeutic IgGs is thought to be responsible for their extended half-life of approximately 21 days. This FcRn recycling and consequent salvage have led to the structural incorporation of this Fc-feature in the design of therapeutic Fc-fusion proteins (eg, Interferon α - or

β -Fc, or factor IX-Fc fusion proteins). Fc-domains of biologics (eg, mAbs, endogenous Abs, and fusion proteins) can also interact with the similarly ubiquitously expressed cell surface Fc γ R, whereupon they are endosomally internalized and degraded by the lysosomes. This target-mediated degradation pathway generally favors soluble mAb-antigen complexes.

Biliary and renal excretion of biologics is also negligible due to the same issues that hinder their absorption. In contrast to their important role in SM-elimination, the kidneys play a largely metabolic rather than excretory role in the clearance of smaller (<60 kDa) biologics that can adequately incur glomerular filtration, and subsequent intraluminal catabolism, tubular reabsorption followed by intracellular lysosomal degradation, or even peritubular endocytosis with subsequent lysosomal proteolysis. To circumvent this glomerular clearance and prolong their therapeutic duration, small sized biologics are often PEGylated to increase their molecular size.

Often, for maximal therapeutic benefit, biologics are co-administered with SM drugs and although in principle inconceivable given their differential metabolism, bona fide drug-interactions (DIs) with SMs occur. Thus, certain biologics that have the potential as “foreign proteins” to trigger the release of pro-inflammatory cytokines (IL-1 β , IL-6, IFN- γ , and TNF α) from circulating peripheral polymorphonuclear cells could influence the transport and metabolism of co-administered SM drugs. Such pro-inflammatory cytokines are known to influence various physiological processes by up- or down-regulating the expression of drug-transporters, transcription factors, nuclear factors (CAR, PXR, and FXR), receptors, and various drug-metabolizing P450 enzymes, thereby influencing the metabolism of co-ingested SMs and triggering DIs. Alternatively, DIs can also occur when the therapeutic proteins are aimed at targets involved in disease states that alter cytokine levels or even when these biologics are targeted to the cytokines themselves that in turn affect the P450-dependent metabolism and transport of a given SM drug. Thus, tocilizumab (rheumatoid arthritis [RA] therapy) can influence the pharmacokinetics of co-administered simvastatin by reversing the RA-associated enhanced cytokine levels. Basiliximab (B-cell lymphoma therapy) and muromonab-CD3 (immunosuppressant) increase the plasma levels of cyclosporine, through IL-2-mediated down-regulation of CYP3A4, the dominant cyclosporine-metabolizing P450. Conversely, an SM drug such as methotrexate is known to reduce the clearance of adalimumab (Humira; RA therapy), and in fact is employed clinically to extend the duration of adalimumab by as much as 19% after a single dose and by 44% after multiple doses. Similarly, mycophenolate can prolong the response of basiliximab by reducing its clearance by 50%. Many other examples of DIs exist.

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CASE STUDY ANSWER

Acetaminophen (APAP) is a relatively safe drug, provided it is taken at the recommended therapeutic doses. As discussed in the text, at normally ingested dosages, 95% of APAP is converted by phase II enzymes into much less toxic and more water-soluble APAP-glucuronide and APAP-sulfate, both of which are eliminated in the urine (see Figure 4–5). Five percent of parent APAP is converted by phase I P450 enzymes into a reactive toxic product that is conjugated by GSH, excreted in the urine, and thus detoxified. However, APAP's safety may be greatly compromised in mixed drug overdoses, eg, when ingested with other drugs such as hydrocodone, duloxetine, and carisoprodol, which compete with APAP for phase II-dependent elimination or for cellular cofactors (GSH, UDPGA, PAPS) involved in these processes. Accordingly, more APAP is diverted into its hepatotoxic reactive metabolite pathway,

resulting in liver cell damage. Moreover, HCV infection could indeed have further compromised liver function including drug metabolism. APAP's half-life is 2 hours, and therapeutic and toxic blood levels are 15 mcg/mL and >300 mcg/mL, respectively (Chapter 3). Given that at 48 hours after ingestion (ie, 24 half-lives later), the patient's APAP blood level is 75 mcg/mL, it is obvious that her initial APAP levels were dangerously above the toxic range, and thus upon ED admission, her liver function tests are consistent with ongoing liver failure. She should be given N-acetylcysteine, the APAP-specific antidote (Acetadote, Mucomyst; see Chapter 58) and continuous intravenous glucose infusion to provide the precursor (glucose) for generating the UDPGA cofactor required for APAP glucuronidation, as well as the fluid to induce urine output and accelerate APAP-metabolite elimination.

Pharmacogenomics

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CASE STUDY

A 35-year-old man with newly diagnosed human immunodeficiency virus (HIV) infection was prescribed an antiretroviral regimen that included the protease inhibitor atazanavir 300 mg to be taken by mouth once daily, along with ritonavir, a pharmacokinetic enhancer, and two nucleoside analog antiretroviral agents. Liver function and renal function were normal. After 1 year of treatment, the patient experienced visible

yellow discoloration of the skin and eyes. Blood samples were drawn, and grade 4 hyperbilirubinemia was documented. When atazanavir was discontinued and the antiretroviral regimen was modified to include lopinavir, the plasma levels of bilirubin returned to the normal range, and skin and eye color were cleared. Could a *UGT1A1**28 polymorphism have led to the adverse effects?

Pharmacogenomics, the study of genetic factors that underlie variation in drug response, is a modern term for **pharmacogenetics**. Pharmacogenomics implies a recognition that more than one genetic variant may contribute to variation in drug response. Historically, the field began with observations of severe adverse drug reactions in certain individuals, who were found to harbor genetic variants in drug-metabolizing enzymes. As a scientific field, pharmacogenomics has advanced rapidly since the sequencing of the human genome. In the last decade, powerful genome-wide association (GWA) studies, in which hundreds of thousands of genetic variants across the genome are tested for association with drug response, led to the discovery of many other important polymorphisms that underlie variation in both therapeutic and adverse drug responses. In addition to polymorphisms in genes that encode drug-metabolizing enzymes, it is now known that polymorphisms in genes that encode transporters, human leukocyte antigen (HLA) loci, cytokines, and various other proteins also are predictive of variation in therapeutic and adverse drug responses. In addition to the new discoveries that have been made, the past decade has ushered in **precision medicine**, also known as **stratified** or **personalized medicine**,

in which genetic information is used to guide drug and dosing selection for subgroups of patients or individual patients in medical practice. The Clinical Pharmacogenetics Implementation Consortium (CPIC) published a series of guidelines for using genetic information in selecting medications and in dosing (<https://www.pharmgkb.org/>). These highly informative guidelines are being used by practitioners in prescribing drugs to more effectively treat patients. In this chapter, we begin with a case study and then describe genetic variants that are determinants of drug response. Where appropriate, CPIC recommendations are included to provide information on how to use genetic variant data appropriately in therapeutic medicine. Other expert recommendations such as those from the Dutch Pharmacogenetics Working Group (DPWG) also are available (see <https://www.knmp.nl/dossiers/farmacogenetica>).

The description in this chapter of DNA sequence variations in germline DNA involves a number of terms that describe the nature of the variations and their locations within the genome. A glossary of commonly used terms is presented in the Glossary Table. Some of the more common and important variations are described in the text that follows.

GLOSSARY

Term	Definition
Allele	One of two or more alternative forms of a gene that arise by mutation and are found at the same genetic locus. Example: <i>CYP2D6</i> *3 is an important variant allele for a drug-metabolizing enzyme, <i>CYP2D6</i> .
Allele frequency	The fraction or percentage that a specific allele is observed in proportion to the total of all possible alleles in a population.
Coding single nucleotide polymorphisms (cSNPs)	A single base-pair substitution that occurs in the coding region.
Copy number variations (CNVs)	A segment of DNA in which a variable number of that segment has been found.
Haplotype	A series of alleles found in a linked locus on a chromosome.
Hardy-Weinberg equilibrium	The principle that allele frequencies will remain constant from generation to generation in the absence of evolutionary influences.
Insertion/deletion (indel)	Insertion or deletion of base pairs, which may occur in coding and noncoding regions.
Linkage disequilibrium	The nonrandom association of alleles at two or more loci that descend from a single ancestral chromosome.
Noncoding region polymorphism	Polymorphisms that occur in the 3' and 5' untranslated regions, intronic regions, or intergenic regions.
Nonsynonymous SNPs (nsSNPs)	A single base-pair substitution in the coding region that results in an amino acid change.
Polymorphism or variant	Any genetic variation in the DNA sequence; the terms can be used interchangeably.
PM, IM, EM, or UM	Poor, intermediate, extensive, or ultrarapid metabolizer phenotype.
SNPs	Single nucleotide polymorphisms: base-pair substitutions that occur in the genome.
Synonymous SNPs	Base-pair substitutions in the coding region that do not result in an amino acid change.

■ GENETIC VARIATIONS IN ENZYMES

PHASE I ENZYMES

As described in Chapter 4, biotransformation reactions mediated by P450 phase I enzymes typically modify functional groups ($-\text{OH}$, $-\text{SH}$, $-\text{NH}_2$, $-\text{OCH}_3$) of endogenous and xenobiotic compounds, resulting in an alteration of the biological activity of the compound. Phase I enzymes are involved in the biotransformation of more than 75% of prescription drugs; therefore, polymorphisms in these enzymes may significantly affect blood levels, which in turn may alter response to many drugs. Polymorphisms in drug-metabolizing enzymes dominated the field of pharmacogenomics for many years, and for some years, metabolic phenotypes such as extensive metabolizer (EM), reflecting an individual's metabolic rate of a particular drug that is a known substrate of a specific enzyme, were used to describe genetic effects on drug metabolism. After genotypic information became available, a new nomenclature was used to characterize an individual's metabolic rate. In particular, diploypes, consisting of one maternal and one paternal allele, using star (*) allele nomenclature, have been used. Each star (*) allele is defined by specific sequence variation(s) within the gene locus, eg, single nucleotide polymorphisms (SNPs), and may be assigned a functional activity score when the functional characterization is known, eg, 0 for nonfunctional, 0.5 for reduced function, and 1.0 for fully functional. Some genes, such as *CYP2D6*, are subject to whole gene deletions, eg, *CYP2D6**5, and whole gene duplications or multiplications, eg, *1 $\times$ N, *2 $\times$ N, where N is the number of copies. If more than one copy of the gene is detected, the

activity score is then multiplied by the number of copies observed. Enzyme activity is generally a co-dominant or additive trait. For example, if an individual carries one normal function allele and one nonfunctional allele, he will have an intermediate metabolic activity or be considered an intermediate metabolizer (IM). The sum of allelic activity scores typically ranges between 0 and ≥ 3.0 and is most often used to define phenotypes as follows: 0 = PM (poor metabolizer), $0 < x < 1.25$ = IM, $1.25 < x < 2.25$ = EM, and > 2.25 = UM (ultrarapid metabolizer). For more information regarding nomenclature, see <https://www.pharmvar.org/genes>.

CYP2D6

As described in Chapter 4, cytochrome P450 2D6 (*CYP2D6*) is involved in the metabolism of up to one-quarter of all drugs used clinically, including predominantly basic compounds such as β blockers, antidepressants, antipsychotics, and opioid analgesics. Similar to other polymorphic enzymes, four clinically defined metabolic phenotypes, ie, PMs, IMs, EMs, and UMs, are used to predict therapeutic and adverse responses following the administration of *CYP2D6* substrates.

The gene encoding *CYP2D6* is highly polymorphic, with more than 100 alleles defined (<https://www.pharmvar.org/gene/CYP2D6>); however, greater than 95% of phenotypes can be accounted for with just nine alleles, ie, *CYP2D6* alleles *3, *4, *5, and *6 are nonfunctional; alleles *10, *17, and *41 have reduced function; and alleles *1 and *2 are fully functional. As with many polymorphisms, allele frequencies vary across populations (Table 5–1). Some genetic variants are shared among populations at similar allele frequencies, whereas others vary considerably.

TABLE 5–1 Major alleles and frequencies in African, Asian, and European populations.

Gene	Allele(s) ¹	dbSNP ² Number	Amino Acid	Function	Activity	Fraction in African Populations	Fraction in Asian Populations	Fraction in European Populations
CYP2D6								
	*1	Reference	—	Normal	1.0	0.39	0.34	0.54
	*1 × N	Gene duplication or multiplication	Increased expression	Increased	1.0 × N	0.015	0.0028	0.0080
	*2	rs16947, rs1135840	R296C, S486T	Normal	1.0	0.20	0.13	0.27
	*2 × N	Duplication or multiplication	Increased expression	Increased	1.0 × N	0.016	0.0038	0.013
	*3	rs35742686	Frameshift	None	0.0	0.00030	0.00	0.013
	*4	rs1065852, rs3892097	P34S, Splicing defect	None	0.0	0.034	0.0042	0.19
	*5	—	No enzyme	None	0.0	0.061	0.056	0.027
	*6	rs5030655	Frameshift	None	0.0	0.031	0.0002	0.0095
	*10	rs1065852, rs1135840	P34S, S486T	Decreased	0.25	0.068	0.42	0.032
	*17	rs28371706, rs16947, rs1135840	T107I, R296C, S486T	Decreased	0.5	0.20	0.0001	0.0032
	*41	rs16947, rs1135840, rs28371725	R296C, S486T, Splicing defect	Decreased	0.5	0.11	0.020	0.086
CYP2C19								
	*1	Reference	—	Normal	—	0.68	0.60	0.63
	*2	rs4244285	Splicing defect	None	—	0.15	0.29	0.15
	*3	rs4986893	W212X	None	—	0.0052	0.089	0.0042
	*17	rs12248560	Increased expression	Increased	—	0.16	0.027	0.21
CYP2C9								
	*1	Reference	—	Normal	—			
	*2	rs1799853	R144C	Decreased	—	0.03	0.00	0.13
	*3	rs1057910	I359L	Decreased	—	0.02	0.04	0.07
CYP2B6								
	*1			Normal		0.36	0.58	0.53
	*4	rs2279343	K262R	Increased		0.0093	0.090	0.040
	*6	rs2279343, rs3745274	K262R, Q172H	Decreased		0.34	0.20	0.20
	*9	rs3745274	Q172H	Decreased		0.084	0.060	0.015
	*18	rs28399499	I328T	None		0.052	0.00	0.00
DPYD								
	*1	Reference	—	Normal	1.0			
	*2A	rs3918290	Splicing defect	None	0.0	0.0006	0.0037	0.0080
	*13	rs55886062	I560S	None	0.0	0.00	0.00	0.0006
	—	rs67376798	D949V	Decreased	0.5	0.0006	0.0005	0.00370
	HapB3	rs56038477	E412E	Decreased	0.5	0.0018	0.0094	0.0204
UGT1A1								
	*1	Reference	TA ₆	Normal	—	0.50	0.85	0.68
	*28	rs8175347	TA ₇	Decreased	—	0.39	0.15	0.32
	*36	rs8175347	TA ₅	Increased	—	0.066	0.00	0.00
	*37	rs8175347	TA ₈	Decreased	—	0.036	0.00	0.0010

(continued)

TABLE 5-1 Major alleles and frequencies in African, Asian, and European populations. (Continued)

Gene	Allele(s) ¹	dbSNP ² Number	Amino Acid	Function	Activity	Fraction in African Populations	Fraction in Asian Populations	Fraction in European Populations
TPMT								
	*1	Reference	—	Normal	—	0.94	0.98	0.96
	*2	rs1800462	A80P	None	—	0.00079	0.00	0.0019
	*3A	rs1800460, rs1142345	A154T, Y240C	None	—	0.0020	0.00012	0.036
	*3B	rs1800460	A154T	None	—	0.00	0.00	0.00046
	*3C	rs1142345	Y240C	None	—	0.050	0.016	0.0042
G6PD								
	B	Reference	—	Normal	IV	—	—	—
	A	rs1050829	N126D	Normal	III–IV	0.31–0.35	0.00	0.00–0.060
	A- (rs1050829, rs1050828)		(N126D, V68M)	Decreased (5–10%)	III	0.117	n/a	0.00
	A- (rs1050829, rs137852328)		(N126D, R227L)					
	A- (rs1050829, rs76723693)		(N126D, L323P)					
	Mediterranean (rs5030868)		S188P	Decreased (<1%)	II	0.00–0.052	0.00	0.00–0.074
	Canton (rs72554665), Kaiping		R459L/R463H	Decreased	II	0.00	0.017	0.00
	Mahidol (rs137852314)		G163S	Decreased (5–32%)	III			
	Chinese-5 (rs137852342), Gaohe (rs72554664)		L342F H32R	Decreased	III			
SLCO1B1								
	*1a	Reference	—	Normal	—	0.17	0.27	0.50
	*1b	rs2306283	N130D	Normal	—	0.78	0.60	0.22
	*5	rs4149056	V174A	Decreased	—	0.00	0.00	0.01
	*15, *17	rs4149056, others	V174A others	Decreased	—	0.03	0.13	0.14
ABCG2								
		rs2231142	Q141K	Decreased	—	0.01	0.29	0.09
SLC22A1								
	—	rs12208357	R61C	Decreased	—	0.004	0.00	0.063
	—	rs202220802	Mdel	Decreased	—	0.045	0.005	0.184
	—	rs34130495	G401S	Decreased	—	0.003	0.001	0.02
	—	rs34059508	G465R	Decreased	—	0.00	0.00	0.02
HLA-B								
	*57:01	—	—	positive	—	0.010	0.016	0.068
IFNL3								
	TT/CT	Reference	—	Unfavorable	—	—	—	—
	CC	rs12979860	—	Favorable	—	0.39	0.87	0.63
VKORC1								
	–1639G	Reference	—	Normal	—			
	–1639A	rs9923231	Reduced expression	Decreased	—	0.11	0.91	0.39

¹For the official nomenclature for alleles of drug-metabolizing enzymes, see the Pharmacogene Variation Consortium (<https://www.pharmvar.org/genes>).²The Single Nucleotide Polymorphism Database (dbSNP) is an online public repository of genomic variation established by the National Center for Biotechnology Information (NCBI).

For example, a common nonfunctional allele, *CYP2D6**4, is observed at a frequency of approximately 20% in Europeans and is nearly absent (<1%) in Asians (see Table 5–1). Based on Hardy-Weinberg principles (see Glossary), the percentage of Europeans who are homozygous for the *CYP2D6**4 allele, ie, who carry the *4 allele on both maternal and paternal chromosomes, would be 4%, whereas that of those who are heterozygotes would be 32%. This parallels the lower number of PMs (defined as having two nonfunctional alleles, eg, PMs are homozygous for *3, *4, *5, *6, or any combination of nonfunctional alleles such as *4/*5), observed in Asian populations (~1%) compared with European populations (~5–10%) (see Table 5–1). In contrast, the *5 gene deletion is found at similar frequencies (~3–5%) across European, African, and Asian populations, suggesting that this mutation likely took place prior to the separation of these three populations more than 100,000 years ago. *CYP2D6**17, which consists of three SNPs in a single haplotype, constitutes a common reduced-function haplotype in populations of African ancestry. Clinically, since some genotyping platforms are specific to a single ethnicity, it is important to ensure that alleles applicable to the patient population being treated are tested. Of note, rare or previously undiscovered variants are typically not included in commercial tests, and thus novel or rare polymorphisms, which may exhibit altered function, will be missed.

Example: Codeine is a phenanthrene derivative prodrug opioid analgesic indicated for the management of mild to moderately severe pain (Chapter 31). Codeine, like its active metabolite morphine, binds to μ -opioid receptors in the central nervous system (CNS). Morphine is 200 times more potent as an agonist than codeine, and conversion of codeine into morphine is essential for codeine's analgesic activity. The enzyme responsible for the *O*-demethylation conversion of codeine into morphine is *CYP2D6*. Patients with normal *CYP2D6* activity (ie, EMs) convert sufficient codeine to morphine (~5–10% of an administered dose) to produce the desired analgesic effect. PMs and IMs are more likely to experience insufficient pain relief, while UMs are at an increased risk for side effects, eg, drowsiness and respiratory depression, due to higher systemic concentrations of morphine. Interestingly, gastrointestinal adverse effects, eg, constipation, are decreased in PMs, whereas the central side effects, eg, sedation and dizziness, do not differ between PMs and EMs. The antitussive properties associated with codeine are not affected by *CYP2D6* activity. According to CPIC guidelines, standard starting doses are recommended in EMs and IMs with close monitoring, especially in IMs, and CPIC recommends use of an alternative agent in PMs and UMs (Table 5–2).

CYP2C19

Cytochrome P450 *CYP2C19* (*CYP2C19*) is known to preferentially metabolize acidic drugs including proton-pump inhibitors, antidepressants, antiepileptics, and antiplatelet drugs (Chapter 4). Four clinical phenotypes related to *CYP2C19* activity (PM, IM, EM, and UM) are closely associated with genetic biomarkers that may assist in guiding individualized therapeutic dosing strategies. The gene that encodes *CYP2C19* is highly polymorphic, with more than 35 alleles defined (<https://www.pharmvar.org/>

genes/*CYP2C19*), yet just four alleles can account for the majority of phenotypic variability—ie, *CYP2C19* alleles *2 and *3 are nonfunctional, *CYP2C19* allele *1 is fully functional, and *CYP2C19**17 has increased function. Phenotypes range from PMs who have two deficient alleles, eg, *2/*3, *2/*2, or *3/*3, to UMs who have increased hepatic expression levels of the *CYP2C19* protein due to *1/*17 or *17/*17 alleles (see Table 5–2). Of note, the *17 increased function allele is unable to fully compensate for nonfunctional alleles, and therefore the presence of a *17 allele in combination with a nonfunctional allele would be considered an IM phenotype (see Table 5–2). The PM phenotype is more common in Asians (~16%) than in Europeans and Africans (~2–5%), which can be expected based on the inheritance patterns of variant alleles across populations—eg, the most common nonfunctional allele, *CYP2C19**2, is observed approximately twice as frequently in Asians (~30%) compared with Africans and Europeans (~15%), while the apparent gain-of-function *17 allele is observed rarely in Asians (<3%) but more frequently in Europeans and Africans (16–21%) (see Table 5–1).

Example: Clopidogrel is a thienopyridine antiplatelet prodrug indicated for the prevention of atherothrombotic events. Active metabolites selectively and irreversibly inhibit adenosine diphosphate–induced platelet aggregation (Chapter 34). Clopidogrel is metabolized in the body via one of two main mechanisms; approximately 85% of an administered dose is rapidly hydrolyzed by hepatic esterases to its inactive carboxylic acid derivative, while the remaining ~15% is converted via two sequential CYP-mediated oxidation reactions (predominantly *CYP2C19*) to the active thiol metabolite responsible for antiplatelet activity.

Genetic polymorphisms in the *CYP2C19* gene that decrease active metabolite formation and consequently reduce the drug's antiplatelet activity are associated with variability in response to clopidogrel. Carriers of the nonfunctional *CYP2C19**2 alleles taking clopidogrel are at increased risk for serious adverse cardiovascular events, particularly in acute coronary syndrome managed with percutaneous coronary intervention (PCI); the hazard ratios (HRs) are 1.76 for *2/*2 genotype and 1.55 for *2 heterozygotes compared with noncarriers. The risk associated with stent thrombosis is even greater (HR 3.97 for *2/*2 genotype and 2.67 for *2 heterozygotes compared with *1 homozygotes). However, for other indications, eg, atrial fibrillation and stroke, the effects of the *CYP2C19**2 allele are less dramatic. Thus, current clinical recommendations from CPIC are specific for acute coronary syndrome with PCI: standard starting doses are recommended in EMs and UMs, and CPIC recommends use of an alternative antiplatelet agent, eg, prasugrel or ticagrelor, in PMs and IMs (see Table 5–2). The US Food and Drug Administration (FDA)-approved label for clopidogrel recommends alternative antiplatelet drugs for patients who are poor metabolizers of clopidogrel.

CYP2C9

Found in the same cluster on chromosome 10q24 as *CYP2C19*, *CYP2C9* is another highly polymorphic drug-metabolizing enzyme. *CYP2C9* is discussed later in this chapter in the context of polygenic effects along with *VKORC1*.

TABLE 5-2 Gene-based dosing recommendations for selected drugs.

Gene	Drug	Diplotype ¹	Likely Phenotype (Activity Score)	Dosing Recommendation	Source of Recommendation
CYP2D6					
	Codeine	*1/*1 × N, *1/*2 × N	UM (>2.0)	• Alternative analgesic, eg, morphine or nonopioid; increased formation of morphine following codeine administration leads to higher risk of toxicity.	CPIC ² , DPWG ³
		*1/*1, *1/*2, *2/*2, *1/*41, *2/*5	EM (1.0–2.0)	• Standard starting dose.	
		*4/*10, *5/*41	IM (0.5)	• Standard starting dose; monitor closely for lack of analgesic response due to reduced morphine formation. Consider alternate analgesic, eg, morphine or nonopioid.	
		*3/*4, *4/*4, *4/*5, *5/*5, *4/*6	PM (0.0)	• Alternative analgesic, eg, morphine or nonopioid analgesic; greatly reduced morphine formation following codeine administration, leading to insufficient pain relief. Avoid higher doses, as central side effects do not differ in PMs.	
CYP2C19					
	Clopidogrel	*1/*17, *17/*17 (UM), and *1/*1 (EM)	UM, EM	• Standard dose.	CPIC, DPWG
		*1/*2, *1/*3, *2/*17	IM	• Alternative antiplatelet agent, eg, prasugrel or ticagrelor.	
		*2/*2, *2/*3, *3/*3	PM	• Alternative antiplatelet agent, eg, prasugrel or ticagrelor.	
CYP2B6					
	Efavirenz	*4/*4, *22/*22, *4/*22	UM	• Initiate efavirenz with standard dose (600 mg/day)	CPIC, DPWG
		*1/*4, *1/*22	RM	• Initiate efavirenz with standard dose (600 mg/day)	
		*1/*1	EM	• Initiate efavirenz with standard dose (600 mg/day)	
		*1/*6, *1/*18, *4/*6, *4/*18, *6/*22, *18/*22	IM	• Consider initiating efavirenz with decreased dose (400 mg/day)	
		*6/*6, *18/*18, *6/*18	PM	• Consider initiating efavirenz with decreased dose (400 or 200 mg/day)	
DPYD					
	Fluoropyrimidines	*1/*1	Normal	• Standard dose.	CPIC
		*1/*2A, *1/*13, *1/rs67376798A	Reduced activity	• Reduce initial dose 50% and titrate based on toxicity or on pharmacokinetic test results (if available).	
		*2A/*2A, *2A/*13, *13/*13, rs67376798A/rs67376798A	Complete deficiency	• Different non-fluoropyrimidine anticancer agent.	
UGT1A1					
	Irinotecan	*1/*1, *1/*28	Normal	• Standard starting dose.	
		*28/*28	Reduced	• Reduce starting dose by at least one dose level. Or, Start with 70% of the standard dose. If the patient tolerates this initial dose, the dose can be increased, guided by the neutrophil count.	Drug label DPWG
	Atazanavir	*1/*1, *1/*36, *36/*36, rs887829 C/C	Normal	No reason to avoid prescribing atazanavir. Inform patient of risks. Based on this genotype, there is a less than 1 in 20 chance of stopping atazanavir for jaundice.	CPIC

(continued)

TABLE 5–2 Gene-based dosing recommendations for selected drugs. (Continued)

Gene	Drug	Diplotype ¹	Likely Phenotype (Activity Score)	Dosing Recommendation	Source of Recommendation
		*1/*28, *1/*37, *36/*28, *36/*37, rs887829 C/T, *1/*6	Intermediate	No reason to avoid prescribing atazanavir. Inform patient of risks. Based on this genotype, there is a less than 1 in 20 chance of stopping atazanavir for jaundice.	
		*28/*28, *28/*37, *37/*37, rs887829 T/T (*80/*80), *6/*6	Reduced	Consider alternative agent. Based on this genotype, there is a high (20–60%) likelihood of developing jaundice that will result in discontinuation of atazanavir.	
TPMT					
	Thiopurines	*1/*1	Normal, high activity	• Standard starting dose.	CPIC, DPWG
		*1/*2, *1/*3A, *1/*3B, *1/*3C, *1/*4	Intermediate activity	• Start at 30–70% of target dose and titrate every 2–4 weeks with close clinical monitoring of tolerability, eg, white blood cell counts and liver function tests.	
		3A/*3A, *2/*3A, *3C/*3A, *3C/*4, *3C/*2, *3A/*4	Low activity	• Malignant disease: Drastic reduction of thiopurine doses, eg, tenfold given thrice weekly instead of daily. • Nonmalignant conditions: Alternative nonthiopurine immunosuppressive agent.	
G6PDX-linked trait					
	Rasburicase	B, A	Normal	• Standard dose.	Drug label/CPIC
		A-, Mediterranean, Canton	Deficient	• Alternative agent, eg, allopurinol: Rasburicase is contraindicated in patients with G6PD deficiency.	
		Variable	Unknown risk of hemolytic anemia	• Enzyme activity must be measured to determine G6PD status. An alternative is allopurinol.	
SLCO1B1					
	Simvastatin 40 mg	*1a/*1a, *1a/*1b, *1b/*1b	Normal activity	• Standard dose.	CPIC
		*1a/*5, *1a/*15, *1a/*17, *1b/*5, *1b/*15, *1b/*17	Intermediate activity	• Prescribe a lower dose or consider an alternative statin, eg, pravastatin or rosuvastatin; consider routine CK monitoring.	
		*5/*5, *5/*15, *5/*17, *15/*15, *15/*17, *17/*17	Low activity	• Prescribe a lower dose or consider an alternative statin, eg, pravastatin or rosuvastatin; consider routine CK monitoring.	
HLA					
	Abacavir	*Other/*Other	Negative	• Standard dose.	CPIC, DPWG
		*Other/*57:01, *57:01/*57:01	Positive	• Alternative agent: abacavir is contraindicated in HLA-B*57:01-positive patients.	
IFNL3					
	PEG-IFN- α /RBV	rs12979860/rs12979860	Favorable	• PEG-IFN- α /RBV: Consider cure rates before initiating regimen; ~70% chance for SVR ⁴ after 48 weeks of therapy. • PEG-IFN- α /RBV + protease inhibitor combinations: Regimen recommended; ~90% chance for SVR after 24–48 weeks of therapy, with 80–90% chance for shortened duration of therapy.	CPIC

(continued)

TABLE 5-2 Gene-based dosing recommendations for selected drugs. (Continued)

Gene	Drug	Diplotype ¹	Likely Phenotype (Activity Score)	Dosing Recommendation	Source of Recommendation
		Reference/reference or reference/rs12979860	Unfavorable	• PEG-IFN-α/RBV: Consider cure rates before initiating regimen; ~30% chance for SVR after 48 weeks of therapy. • PEG-IFN-α/RBV + protease inhibitor combinations: Consider cure rates before initiating regimen; ~60% chance for SVR after 24–48 weeks of therapy, with 50% chance for shortened duration of therapy.	
CYP2C9, VKORC1					
	Warfarin	*1/*1, *1/*2, *2/*2, *2/*3, *1/*3, *3/*3, 1639GG, 1639GA, 1639AA	Various	• Apply validated dosing algorithm, eg, www.warfarindosing.org (or IWPC⁵) for international normalized ratio target 2–3) or FDA-approved dosing table per manufacturer's labeling.	CPIC

¹Diplotypes are shown as the two members of a chromosome pair, eg, *1/*1 indicates both chromosomes contain the *1 allele for that gene, whereas *1/*17 denotes a heterozygote with one *1 allele and one *17 allele.

²CPIC: Clinical Pharmacogenetics Implementation Consortium: Full drug-specific recommendations are available online at <http://www.pharmgkb.org/page/cpic>, <https://cpicpgx.org/genes-drugs/>.

³DPWG: Dutch Pharmacogenetics Working Group: Full drug-specific recommendations are available online at <https://www.knmp.nl/patientenzorg/medicatiebewaking/farmacogenetica/pharmacogenetics-1/pharmacogenetics>.

⁴SVR: sustained viral response.

⁵IWPG: International Warfarin Pharmacogenetics Consortium.

CYP2B6

CYP2B6 is highly polymorphic, with 38 known variant alleles and multiple suballeles identified (www.pharmvar.org/gene/CYP2B6). Allele frequencies vary substantially across ancestrally diverse groups (see Table 5-1). Alleles are categorized into functional groups as follows: normal function (eg, *CYP2B6**1), decreased function (eg, *CYP2B6**6 and *9), no function (eg, *CYP2B6**18), and increased function (eg, *CYP2B6**4), and phenotypes are defined based on the individual diplotype, as shown in Table 5-1. Interestingly, allele *6, which is the most frequent (from 15% in East Asian ancestry to 62% in Oceania ancestry) and most studied allele, has demonstrated substrate-specific phenotype-diplotype associations.

Example: Efavirenz is a nonnucleoside HIV type-1 reverse transcriptase inhibitor. Efavirenz is a potent suppressor of HIV-1 replication that is indicated for treatment in HIV-positive, treatment-naïve individuals, in combination with tenofovir disoproxil fumarate and emtricitabine. Efavirenz was part of the first “one pill, once a day” treatment, and efavirenz-based regimens were “recommended” by the US Department of Health and Human Services until the year 2015. In 2015, efavirenz was designated to the “alternative” therapy category due to the more favorable tolerability profile and less drug-drug interaction potential for integrase-inhibitor-based regimens. Efavirenz remains extensively prescribed worldwide, especially in resource-limited settings, pregnant patients, patients desiring pregnancy, and patients co-infected with tuberculosis.

Efavirenz has a relatively narrow therapeutic index (suggested plasma concentration range of 1–4 mcg/mL) and large interindividual pharmacokinetic variability, due in part to variants in

CYP2B6. CYP2B6 is responsible for generating efavirenz major inactive metabolite, 8-hydroxy-efavirenz. Minor metabolic pathways include other CYP-mediated hydroxylation (CYP2A6, CYP3A4, and CYP1A2) and UGT glucuronidation (UGT2B7), which may play a larger role in CYP2B6 poor metabolizers. Chronic dosing of efavirenz leads to increased expression of CYP2B6, which enhances its own metabolism in most patients, ie, *1/*1 or *1/*6 genotypes. However, patients with *6/*6 genotype are largely unaffected by CYP2B6 autoinduction.

Substantial evidence connects CYP2B6 genotype, plasma efavirenz concentration, and efavirenz adverse effects. Toxic effects of efavirenz are mainly related to the CNS—eg, sleep disorders, impaired concentration, psychosis, suicidal ideation, and depression—and typically resolve after the first few days of treatment. However, adverse effects may persist in some patients, resulting in impaired quality of life and treatment discontinuation. CPIC recommendations are provided in Table 5-2.

Dihydropyrimidine Dehydrogenase (DPD)

Dihydropyrimidine dehydrogenase (DPD, encoded by the *DPYD* gene) is the first and rate-limiting step in pyrimidine catabolism, as well as a major elimination route for fluoropyrimidine chemotherapy agents (Chapter 54). Considerable intergroup and intragroup variation exists in DPD enzyme activity. While many of the alleles identified in the *DPYD* gene either are too rare to sufficiently characterize or have shown conflicting associations with DPD activity, four alleles have been recognized clinically, ie, *DPYD* *2A, *13, rs67376798, and HapB3. Of these variants, the *2A and *13 allele are nonfunctional (activity score = 0) while rs67376798 and HapB3 have reduced function (activity score = 0.5). All four

alleles are rare, with frequencies less than 1% in most European, African, and Asian populations, with the most common alleles being HapB3, which ranges up to 2% in Asian and European populations, and *2A, which ranges up to 3.5% in a Swedish population (see Table 5–1).

Example: Three fluoropyrimidine drugs are used clinically, namely 5-fluorouracil (5-FU), capecitabine, and tegafur (approved only in Europe). 5-FU is the pharmacologically active compound of each drug, and all are approved to treat solid tumors including colorectal and breast cancer (Chapter 54). 5-FU must be administered intravenously, while both capecitabine and tegafur are oral prodrugs that are rapidly converted to 5-FU in the body. Only 1–3% of an administered dose of the prodrug is converted to the active cytotoxic metabolites, ie, 5-fluorouridine 5′-monophosphate (5-FUMP) and 5-fluoro-2′-deoxyuridine-5′-monophosphate (5-FdUMP), which effectively target rapidly dividing cancer cells and inhibit DNA synthesis. The majority of an administered dose (~80%) is subjected to pyrimidine catabolism via DPD and is excreted in the urine. Complete or partial deficiency of DPD can lead to dramatically reduced clearances of 5-FU, increased levels of toxic metabolites 5-FUMP and 5-FdUMP, and consequently an increased risk for severe dose-dependent fluoropyrimidine toxicities, eg, myelosuppression, mucositis, neurotoxicity, hand-and-foot syndrome, and diarrhea. In a recent genotype-driven dosing study, more than 1100 patients were treated with fluoropyrimidine-based chemotherapy, including 85 (8%) heterozygous variant allele carriers of *DPYD* who received an initial dose reduction of 25% (c.2846A>T and c.1236G>A) or 50% (*DPYD**2A and c.1679T>G) compared with *DPYD* wild-type patients. The relative risk for severe fluoropyrimidine-related toxicity was reduced with genotype-guided dosing, eg, 1.31 (95% CI 0.63–2.73) compared with 2.87 (2.14–3.86) in the historic cohort for *DPYD**2A carriers. CPIC recommendations for therapeutic regimens are shown in Table 5–2.

PHASE II ENZYMES

As described in Chapter 4, phase II enzyme biotransformation reactions typically conjugate endogenous molecules, eg, sulfuric acid, glucuronic acid, and acetic acid, onto a wide variety of substrates in order to enhance their elimination from the body. Consequently, polymorphic phase II enzymes may diminish drug elimination and increase risks for toxicities. In this section, we describe key examples of polymorphic phase II enzymes and the pharmacologic consequence for selected prescription drugs.

Uridine 5′-Diphosphoglucuronosyl Transferase 1 (*UGT1A1*)

The uridine 5′-diphospho- (UDP) glucuronosyltransferase 1A1 (*UGT1A1*) enzyme, encoded by the *UGT1A1* gene, conjugates glucuronic acid onto small lipophilic molecules, eg, bilirubin, and a wide variety of therapeutic drug substrates so that they may be more readily excreted into bile (Chapter 4). The *UGT1A1* gene locus has more than 30 defined alleles, some of which lead to reduced or completely abolished *UGT1A1* function. Most

reduced function polymorphisms within the *UGT1A1* gene locus are quite rare; however, the *28 allele is common across three major ethnic groups (see Table 5–1). Approximately 10% of European populations are homozygous carriers of the *28 allele, ie, *UGT1A1* *28/*28 genotype, and are recognized clinically to have Gilbert syndrome. The *28 allele is characterized by an extra TA repeated in the proximal promoter region and is associated with reduced expression of the *UGT1A1* enzyme. Clinically, Gilbert syndrome is generally benign; however, affected individuals may have 60–70% increased levels of circulating unconjugated bilirubin due to a ~30% reduction in *UGT1A1* activity. Individuals with the *UGT1A1**28/*28 genotype are thus at an increased risk for adverse drug reactions with *UGT1A1* drug substrates due to reduced biliary elimination.

Example: Irinotecan is a topoisomerase I inhibitor prodrug and is indicated as first-line chemotherapy in combination with 5-FU and leucovorin for treatment of metastatic carcinoma of the colon or rectum (Chapter 54). Irinotecan is hydrolyzed by hepatic carboxylesterase enzymes to its cytotoxic metabolite, SN-38, which inhibits topoisomerase I and eventually leads to termination of DNA replication and cell death. The active SN-38 metabolite is responsible for the majority of therapeutic action as well as the dose-limiting bone marrow and gastrointestinal toxicities. Inactivation of SN-38 occurs via the polymorphic *UGT1A1* enzyme, and carriers of the *UGT1A1**6 and *UGT1A1**28 polymorphisms are consequently at increased risk for severe life-threatening toxicities, eg, neutropenia and diarrhea, due to decreased clearance of the SN-38 metabolite.

Thiopurine S-Methyltransferase (TPMT)

Thiopurine S-methyltransferase (TPMT) covalently attaches a methyl group onto aromatic and heterocyclic sulfhydryl compounds and is responsible for the pharmacologic deactivation of thiopurine drugs (Chapter 4). Genetic polymorphisms in the gene encoding TPMT may lead to three clinical TPMT activity phenotypes, ie, high, intermediate, and low activity, which are associated with differing rates of inactivation of thiopurine drugs and altered risks for toxicities. While the majority (86–97%) of the population inherits two functional *TPMT* alleles and has high TPMT activity, around 10% of Europeans and Africans inherit only one functional allele and are considered to have intermediate activity. Furthermore, about 0.3% of Europeans inherit two defective alleles and have very low to no TPMT activity (see Table 5–1). More than 90% of the phenotypic TPMT variability across populations can be accounted for with just three point mutations that are defined by four nonfunctional alleles, ie, *TPMT**2, *3A, *3B, and *3C (see Table 5–2). Most commercial genotyping platforms test for these four common genetic biomarkers and are therefore able to identify individuals with reduced TPMT activity.

Example: Three thiopurine drugs are used clinically, ie, azathioprine, 6-mercaptopurine (6-MP), and 6-thioguanine (6-TG). All share similar metabolic pathways and pharmacology. Azathioprine (a prodrug of 6-MP) and 6-MP are used for treating immunologic disorders, while 6-MP and 6-TG are important anticancer agents (Chapter 54). 6-MP and 6-TG may be activated by the salvage pathway enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPRTase) to form 6-thioguanine nucleotides (TGNs),

which are responsible for the majority of therapeutic efficacy as well as bone marrow toxicity. Alternatively, 6-MP and 6-TG may be inactivated by enzymes such as polymorphic TPMT and xanthine oxidase, leaving less available substrate to be activated by HGPRTase. The *TPMT* gene is a major determinant of thiopurine metabolism and exposure to cytotoxic 6-TGN metabolites and thiopurine-related toxicities. See Table 5–2 for recommended dosing strategies. Recent GWA studies have also implicated variants in the enzyme NUDT15, which catalyzes the hydrolysis of nucleotide diphosphates, as being associated with thiopurine intolerance in children from Japan, Singapore, and Guatemala.

OTHER ENZYMES

G6PD

Glucose 6-phosphate dehydrogenase (G6PD) is the first and rate-limiting step in the pentose phosphate pathway and supplies a significant amount of reduced NADPH in the body. In red blood cells (RBCs), where mitochondria are absent, G6PD is the exclusive source of NADPH and reduced glutathione, which play a critical role in the prevention of oxidative damage. Under normal conditions, G6PD in RBCs is able to detoxify unstable oxygen species while working at just 2% of its theoretical capacity. Following exposure to exogenous oxidative stressors, eg, infection, fava beans, and certain therapeutic drugs, G6PD activity in RBCs increases proportionately to meet NADPH demands and ultimately to protect hemoglobin from oxidation. Individuals with G6PD deficiency, defined as less than 60% enzyme activity, according to World Health Organization classification (Table 5–3), are at increased risk for abnormal RBC destruction, ie, hemolysis, due to reduced antioxidant capacity under oxidative pressures.

The gene that encodes the G6PD enzyme is located on the X chromosome and is highly polymorphic, with more than 180 genetic variants identified that result in enzyme deficiency. Greater than 90% of variants are single-base substitutions in the coding region that produce amino acid changes, which result in unstable proteins with reduced enzyme activity. As with most X-linked traits, males with one reference X chromosome and females with

two reference X chromosomes will have equivalent “normal” G6PD activity. Similarly, hemizygous-deficient males (with a deficient copy of the *G6PD* gene on their single X chromosome) and homozygous-deficient females (with two deficient copies) express reduced activity phenotypes (see Table 5–1). However, for heterozygous females (with one deficient allele and one normal allele), genotype-to-phenotype predictions are less reliable due to the X-chromosome mosaicism, ie, where one X chromosome in each female cell is randomly inactivated, leading to G6PD activity that may range from fully functional to severely deficient. G6PD enzyme activity phenotype estimations for heterozygous females therefore may be improved with complementary G6PD activity testing.

G6PD enzyme deficiency affects more than 400 million people worldwide, and the World Health Organization has categorized G6PD activity into five classes (see Table 5–3). The majority of polymorphic *G6PD*-deficient genotypes are associated with class II for severe deficiency (<10% enzyme activity) and class III for moderate deficiency (10–60% enzyme activity). Most individuals with reduced function alleles of *G6PD* have ancestries in geographic areas of the world corresponding to areas with high malaria prevalence. Polymorphic alleles gained in frequency over time as they offered some benefit against death from malaria. The estimated frequency of G6PD deficiency is approximately 8% in malaria-endemic countries, with the milder *G6PD-A*(–) allele prevalent in Africa, and the more severe *G6PD*-Mediterranean allele widespread across western Asia (Saudi Arabia and Turkey to India). There is a much more heterogeneous distribution of variant alleles in East Asia and Asia Pacific, which complicates G6PD risk predictions; however, the most frequently identified forms in Asia include the more severe class II alleles, eg, Mediterranean, Kaiping, and Canton, as well as some class III alleles, eg, Mahidol, Chinese-5, and Gaohe (see Table 5–1).

Example: Rasburicase, a recombinant urate-oxidase enzyme, is indicated for the initial management of high uric acid levels in cancer patients receiving chemotherapy. Rasburicase alleviates the uric acid burden that often accompanies tumor-lysing treatments by converting uric acid into allantoin, a more soluble and easily excreted molecule. During the enzymatic conversion of uric acid to allantoin, hydrogen peroxide, a highly reactive oxidant, is formed. Hydrogen peroxide must be reduced by glutathione to prevent free radical formation and oxidative damage. Individuals with G6PD deficiency receiving rasburicase therapy are at greatly increased risk for severe hemolytic anemia and methemoglobinemia. The manufacturer recommends that patients at high risk (individuals of African or Mediterranean ancestry) be screened prior to the initiation of therapy and that rasburicase not be used in patients with G6PD deficiency (see Table 5–2).

TABLE 5–3 Classification of G6PD deficiency (WHO Working Group, 1989).

World Health Organization Class	Level of Deficiency	Enzyme Activity	Clinical phenotype
I	Severe	<10%	Chronic (non-spherocytic) hemolytic anemia
II	Severe	<10%	Risk of acute hemolytic anemia; intermittent hemolysis
III	Moderate	10–60%	Risk of acute hemolytic anemia; hemolysis with stressors
IV	None	60–150%	Normal
V	None	>150%	Enhanced activity

GENETIC VARIATIONS IN TRANSPORTERS

Plasma membrane transporters, located on epithelial cells of many tissues, eg, intestinal, renal, and hepatic membranes, mediate selective uptake and efflux of endogenous compounds and xenobiotics

including many drug products. Transporters, which often work in concert with drug-metabolizing enzymes, play important roles in determining plasma and tissue concentrations of drugs and their metabolites. Genetic differences in transporter genes can dramatically alter drug disposition and response and thus may increase risk for toxicities. In this section, a key example of a polymorphic uptake transporter and its pharmacologic impact on statin toxicity are described.

ORGANIC ANION TRANSPORTER (OATP1B1)

The OATP1B1 transporter (encoded by the *SLCO1B1* gene) is located on the sinusoidal membrane (facing the blood) of hepatocytes and is responsible for the hepatic uptake of mainly weakly acidic drugs and endogenous compounds, eg, statins, methotrexate, and bilirubin. More than 40 nonsynonymous variants (nsSNPs) have been identified in this transporter, some of which result in decreased transport function. A common reduced-function polymorphism, rs4149056, has been shown to reduce transport of OATP1B1 substrates in vitro as well as to alter pharmacokinetic and clinical outcomes in vivo. The variant results in an amino acid change, Val174Ala, and is associated with reduced membrane expression, likely as a result of impaired trafficking capability. Allele *5 is relatively rare (rs4149056 alone; ~1%), but various other reduced-function alleles (*15 and *17; haplotypes containing rs4149056) are common in most European and Asian populations (between 5% and 15%) (see Table 5–1).

Example: HMG-coenzyme A (CoA) reductase inhibitors (statins) are highly effective medications that are widely prescribed to reduce serum lipids for the prevention of cardiovascular events (Chapter 35). Seven statins in use currently are generally safe and well-tolerated, but skeletal muscle toxicity can limit their use. Known risk factors include high statin dose, interacting medications, advanced age, and metabolic comorbidities. Furthermore, the common variant, rs4149056 in *SLCO1B1*, increases systemic exposure of simvastatin (221% increase in plasma area under the curve for patients homozygous for the rs4149056 variant, eg, *SLCO1B1**5/*5; *5/*15 or *17; or [*15 or *17]/[*15 or *17]) and was identified to have the single strongest association with simvastatin-induced myopathy in a GWA analysis. For individuals receiving simvastatin with reduced OATP1B1 function (at least one nonfunctional allele), CPIC recommends a lower simvastatin dose or an alternative statin (see Table 5–2).

BREAST CANCER RESISTANCE PROTEIN (BCRP, ABCG2)

BCRP (encoded by the *ABCG2* gene), an efflux transporter in the ATP binding cassette (ABC) superfamily, is located on epithelial cells of the kidney, liver, and intestine as well as on the endothelial cells of the blood-brain barrier. Recent studies have implicated a reduced-function variant in *ABCG2*, which encodes an amino acid change from glutamine to lysine at position 141 of the protein (rs2231142), as a determinant of the pharmacokinetics,

response, and toxicity of several drugs. The variant has a low frequency in individuals of African ancestry but is found at an allele frequency of about 30% in East Asians including Chinese and Japanese (see Table 5–1). Notably, the variant has been associated with changes in response to the xanthine oxidase inhibitor, allopurinol, and the statin rosuvastatin. In fact, the approved label of rosuvastatin, a substrate of BCRP, suggests that a lower dose (half) be given to East Asians, who have higher levels of the drug. Although still controversial, many studies speculate that the reason for the higher levels of rosuvastatin is the high allele frequency of rs2231142 in this population leading to increased bioavailability of the drug. In addition, the variant has been associated with toxicity to various anticancer drugs. Because of its high allele frequency, particularly in Asian populations, and the fact that the transporter is a determinant of the pharmacokinetics of many drugs, it is likely that this variant will become increasingly important in precision medicine.

ORGANIC CATION TRANSPORTER 1 (OCT1, SLC22A1)

Similar to OATP1B1, OCT1 is a transporter that is highly expressed in the liver and plays a role in drug disposition. Its substrates include many basic drugs from various pharmacologic classes such as morphine, tramadol, sumatriptan, and ondansetron. The transporter has many common reduced-function polymorphisms that are found at allele frequencies greater than 5%, particularly in Hispanics and European populations. For drugs that are metabolized in the liver, studies indicate that individuals who are compound heterozygotes for these reduced-function alleles (ie, carrying two reduced-function alleles) have on the order of twofold higher drug levels than either individuals who are heterozygous for a reduced-function allele or those who do not carry a reduced-function allele (see Table 5–1).

■ GENETIC VARIATIONS IN IMMUNE SYSTEM FUNCTION

Genetic predispositions to drug response and toxicities are not limited to genes related to pharmacokinetic processes, eg, drug-metabolizing enzymes and drug transporters. Additional genetic sources of variation may include genes involved in pharmacodynamic processes such as drug receptors and drug targets. For example, a polymorphism in HLA loci is associated with a predisposition to drug toxicity.

DRUG-INDUCED HYPERSENSITIVITY REACTIONS

Hypersensitivity reactions to various drugs can range from mild rashes to severe skin toxicities. The most severe hypersensitivity reactions are liver injury, toxic epidermal necrosis (TEN), and Stevens-Johnson syndrome (SJS), in which drugs or their metabolites form antigens.