Bertram G. Katzung

BASIC & CLINICAL PHARMACOLOGY

14th Edition

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Basic & Clinical Pharmacology

Fourteenth Edition

Edited by

Bertram G. Katzung, MD, PhD
Professor Emeritus
Department of Cellular & Molecular Pharmacology
University of California, San Francisco

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Contents

Preface

The fourteenth edition of *Basic & Clinical Pharmacology* continues the extensive use of full-color illustrations and expanded coverage of transporters, pharmacogenomics, and new drugs of all types emphasized in prior editions. In addition, it 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. Frequent revision is necessary to keep pace with the rapid changes in pharmacology and therapeutics; the 2–3 year revision cycle of this text is among the shortest in the field, and the availability of an online version provides even greater currency. The book also offers special features that make it a useful reference for house officers and practicing clinicians.

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.

Major features that make this book particularly useful in integrated 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.

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.
- Continued expansion of the coverage of general concepts relating to newly discovered receptors, receptor mechanisms, and drug transporters.
- Descriptions of important new drugs released through May 2017.
- Many 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 & Trevor's Pharmacology: Examination & Board Review*, (Trevor AJ, Katzung BG, & Kruidering-Hall, M: McGraw-Hill). This book provides a succinct review of pharmacology with approximately one thousand 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*, second 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; 7th edition, McGraw-Hill, 2017.

This edition marks the 35th year of publication of *Basic & Clinical Pharmacology.* The widespread adoption of the first thirteen editions indicates that this book fills an important need. We believe that the fourteenth 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 editors for this edition, Caroline Define and Greg Feldman. I also wish to thank Alice Camp and Katharine Katzung for their expert proofreading contributions.

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

Bertram G. Katzung, MD, PhD
San Francisco
June 2017

Authors

Michael J. Aminoff, MD, DSc, FRCP
Professor, Department of Neurology, University of California, San Francisco

Allan I. Basbaum, PhD
Professor and Chair, Department of Anatomy and W.M. Keck Foundation Center for Integrative Neuroscience, University of California, San Francisco

Camille E. Beauduy, PharmD
Assistant Clinical Professor, School of Pharmacy, University of California, San Francisco

Neal L. Benowitz, MD
Professor of Medicine and Bioengineering & Therapeutic Science, University of California, San Francisco

Italo Biaggioni, MD
Professor of Pharmacology, Vanderbilt University School of Medicine, Nashville

Daniel D. Bikle, MD, PhD
Professor of Medicine, Department of Medicine, and Co-Director, Special Diagnostic and Treatment Unit, University of California, San Francisco, and Veterans Affairs Medical Center, San Francisco

Homer A. Boushey, MD
Chief, Asthma Clinical Research Center and Division of Allergy & Immunology; Professor of Medicine, Department of Medicine, University of California, San Francisco

Adrienne D. Briggs, MD
Clinical Director, Bone Marrow Transplant Program, Banner Good Samaritan Hospital, Phoenix

Hakan Cakmak, MD
Department of Medicine, University of California, San Francisco

Lundy Campbell, MD
Professor, Department of Anesthesiology and Perioperative Medicine, University of California San Francisco, School of Medicine, San Francisco

George P. Chrousos, MD
Professor & Chair, First Department of Pediatrics, Athens University Medical School, Athens, Greece

Edward Chu, MD
Professor of Medicine and Pharmacology & Chemical Biology; Chief, Division of Hematology-Oncology, Director, University of Pittsburgh Cancer Institute, University of Pittsburgh School of Medicine, Pittsburgh

Valerie B. Clinard, PharmD
Associate Professor, Department of Clinical Pharmacy, School of Pharmacy, University of California, San Francisco

Robin L. Corelli, PharmD
Clinical Professor, Department of Clinical Pharmacy, School of Pharmacy, University of California, San Francisco

Maria Almira Correia, PhD
Professor of Pharmacology, Pharmaceutical Chemistry and Biopharmaceutical Sciences, Department of Cellular & Molecular Pharmacology, University of California, San Francisco

Charles DeBattista, MD
Professor of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, Stanford

Cathi E. Dennehy, PharmD
Professor, Department of Clinical Pharmacy, University of California, San Francisco School of Pharmacy, San Francisco

Betty J. Dong, PharmD, FASHP, FCCP, FAPHA
Professor of Clinical Pharmacy and Clinical Professor of Family and Community Medicine, Department of Clinical Pharmacy and Department of Family and Community Medicine, Schools of Pharmacy and Medicine, University of California, San Francisco

Kenneth Drasner, MD
Professor of Anesthesia and Perioperative Care, University of California, San Francisco

Helge Eilers, MD
Professor of Anesthesia and Perioperative Care, University of California, San Francisco

Daniel E. Furst, MD
Carl M. Pearson Professor of Rheumatology, Director, Rheumatology Clinical Research Center, Department of Rheumatology, University of California, Los Angeles

Joshua M. Galanter, MD
Department of Medicine, University of California, San Francisco

Kathleen M. Giacomini, PhD
Professor of Bioengineering and Therapeutic Sciences, Schools of Pharmacy and Medicine, University of California, San Francisco

Augustus O. Grant, MD, PhD
Professor of Medicine, Cardiovascular Division, Duke University Medical Center, Durham

John A. Gray, MD, PhD
Associate Professor, Department of Neurology, Center for Neuroscience, University of California, Davis

Robert D. Harvey, PhD
Professor of Pharmacology and Physiology, University of Nevada School of Medicine, Reno

Jennifer E. Hibma, PharmD
Department of Bioengineering and Therapeutic Sciences, Schools of Pharmacy and Medicine, University of California, San Francisco

Nicholas H. G. Holford, MB, ChB, FRACP
Professor, Department of Pharmacology and Clinical Pharmacology, University of Auckland Medical School, Auckland

John R. Horn, PharmD, FCCP
Professor of Pharmacy, School of Pharmacy, University of Washington; Associate Director of Pharmacy Services, Department of Medicine, University of Washington Medicine, Seattle

John Hwa, MD, PhD
Professor of Medicine and Pharmacology, Yale University School of Medicine, New Haven

Harlan E. Ives, MD, PhD
Professor Emeritus of Medicine, Department of Medicine, University of California, San Francisco

Samie R. Jaffrey, MD, PhD
Greenberg-Starr Professor of Pharmacology, Department of Pharmacology, Cornell University Weill Medical College, New York City

John P. Kane, MD, PhD
Professor of Medicine, Department of Medicine; Professor of Biochemistry and Biophysics; Associate Director, Cardiovascular Research Institute, University of California, San Francisco

Bertram G. Katzung, MD, PhD
Professor Emeritus, Department of Cellular & Molecular Pharmacology, University of California, San Francisco

Gideon Koren, MD, FRCPC, FACMT
Consultant, Kiryat Ono, Israel

Michael J. Kosnett, MD, MPH
Associate Clinical Professor of Medicine, Division of Clinical Pharmacology and Toxicology, University of Colorado Health Sciences Center, Denver

Marieke Kruidering-Hall, PhD
Academy Chair in Pharmacology Education; Professor, Department of Cellular and Molecular Pharmacology, University of California, San Francisco

Douglas F. Lake, PhD
Associate Professor, The Biodesign Institute, Arizona State University, Tempe

Harry W. Lampiris, MD
Professor of Clinical Medicine, UCSF, Interim Chief, ID Section, Medical Service, San Francisco VA Medical Center, San Francisco

Paul W. Lofholm, PharmD
Clinical Professor of Pharmacy, School of Pharmacy, University of California, San Francisco

Roger K. Long, MD
Professor of Pediatrics, Department of Pediatrics, University of California, San Francisco

Christian Lüscher, MD
Departments of Basic and Clinical Neurosciences, Medical Faculty, University Hospital of Geneva, Geneva, Switzerland

Daniel S. Maddix, PharmD
Associate Clinical Professor of Pharmacy, University of California, San Francisco

Howard I. Maibach, MD
Professor of Dermatology, Department of Dermatology, University of California, San Francisco

Mary J. Malloy, MD
Clinical Professor of Pediatrics and Medicine, Departments of Pediatrics and Medicine, Cardiovascular Research Institute, University of California, San Francisco

Kathleen Martin, PhD
Associate Professor, Yale Cardiovascular Center, Yale University, New Haven

Umesh Masharani, MBBS, MRCP (UK)
Professor of Medicine, Department of Medicine, University of California, San Francisco

Kenneth R. McQuaid, MD
Professor of Clinical Medicine, University of California, San Francisco; Chief of Gastroenterology, San Francisco Veterans Affairs Medical Center, San Francisco

Ramana K. Naidu, MD
Department of Anesthesia and Perioperative Care, University of California, San Francisco

Ahmed A. Negm, MD
Department of Medicine, University of California, Los Angeles

Martha S. Nolte Kennedy, MD
Clinical Professor, Department of Medicine, University of California, San Francisco

Kent R. Olson, MD
Clinical Professor, Department of Medicine, Schools of Medicine and Pharmacy, University of California, San Francisco; Medical Director, San Francisco Division, California Poison Control System, San Francisco

Achilles J. Pappano, PhD
Professor Emeritus, Department of Cell Biology and Calhoun Cardiology Center, University of Connecticut Health Center, Farmington

David Pearce, MD
Professor of Medicine, University of California, San Francisco

Roger J. Porter, MD
Adjunct Professor of Neurology, University of Pennsylvania, Philadelphia; Adjunct Professor of Pharmacology, Uniformed Services University of the Health Sciences, Bethesda

Ian A. Reid, PhD
Professor Emeritus, Department of Physiology, University of California, San Francisco

David Robertson, MD
Elton Yates Professor of Medicine, Pharmacology and Neurology, Vanderbilt University; Director, Clinical & Translational Research Center, Vanderbilt Institute for Clinical and Translational Research, Nashville

Dirk B. Robertson, MD
Professor of Clinical Dermatology, Department of Dermatology, Emory University School of Medicine, Atlanta

Michael A. Rogawski, MD, PhD
Professor of Neurology, Department of Neurology, University of California, Davis

Philip J. Rosenthal, MD
Professor of Medicine, San Francisco General Hospital, University of California, San Francisco

Sharon Safrin, MD
Associate Clinical Professor, Department of Medicine, University of California, San Francisco; President, Safrin Clinical Research, Hillsborough

Ramin Sam, MD
Associate Professor, Department of Medicine, University of California, San Francisco

Mark A. Schumacher, PhD, MD
Professor, Department of Anesthesia and Perioperative Care, University of California, San Francisco

Daniel T. Teitelbaum, MD
Adjunct Professor of Occupational and Environmental Health, Colorado School of Public Health, Denver; and Adjunct Professor, Civil and Environmental Engineering, Colorado School of Mines, Golden

Anthony J. Trevor, PhD
Professor Emeritus, Department of Cellular & Molecular Pharmacology, University of California, San Francisco

Candy Tsourounis, PharmD
Professor of Clinical Pharmacy, Medication Outcomes Center, University of California, San Francisco School of Pharmacy, San Francisco

Mark von Zastrow, MD, PhD
Professor, Departments of Psychiatry and Cellular & Molecular Pharmacology, University of California, San Francisco

Lisa G. Winston, MD
Clinical Professor, Department of Medicine, Division of Infectious Diseases, University of California, San Francisco; Hospital Epidemiologist, San Francisco General Hospital, San Francisco

Spencer Yost, MD
Professor, Department of Anesthesia and Perioperative Care, University of California, San Francisco; Medical Director, UCSF-Mt. Zion ICU, Chief of Anesthesia, UCSF-Mt. Zion Hospital, San Francisco

James L. Zehnder, MD
Professor of Pathology and Medicine, Pathology Department, Stanford University School of Medicine, Stanford

SCHEDULE OF CONTROLLED DRUGS[1]

SCHEDULE I

(All nonresearch use illegal under federal law.)

Flunitrazepam (Rohypnol)

Narcotics:

Heroin and many nonmarketed synthetic narcotics

Hallucinogens:

LSD

MDA, STP, DMT, DET, mescaline, peyote, bufotenine, ibogaine, psilocybin, phencyclidine (PCP; veterinary drug only)

Marijuana

Methaqualone

SCHEDULE II

(No telephone prescriptions, no refills.)[2]

Opioids:

Opium: Opium alkaloids and derived phenanthrene alkaloids: codeine, morphine (Avinza, Kadian, MSContin, Roxanol), hydrocodone and hydrocodone combinations (Zohydro ER, Hycodan, Vicodin, Lortab), hydromorphone (Dilaudid), oxymorphone (Exalgo), oxycodone (dihydroxycodeinone, a component of Oxycontin, Percodan, Percocet, Roxicodone, Tylox)

Designated synthetic drugs: meperidine (Demerol), methadone, levorphanol (Levo-Dromoran), fentanyl (Duragesic, Actiq, Fentora), alfentanil (Alfenta), sufentanil (Sufenta), remifentanil (Ultiva), tapentadol (Nycynta)

Stimulants:

Coca leaves and cocaine

Amphetamines: Amphetamine complex (Biphetamine), Amphetamine salts (Adderall), Dextroamphetamine (Dexedrine, Procentra), Lisdexamfetamine (Vyvanse), Methamphetamine (Desoxyn), Methylphenidate (Ritalin, Concerta, Methylin, Daytrana, Medadate), Above in mixtures with other controlled or uncontrolled drugs

Cannabinoids:

Nabilone (Cesamet)

Depressants:

Amobarbital (Amytal)

Pentobarbital (Nembutal)

Secobarbital (Seconal)

SCHEDULE III

(Prescription must be rewritten after 6 months or five refills.)

Opioids:

Buprenorphine (Buprenex, Subutex)

Mixture of above Buprenorphine and Naloxone (Suboxone)

The following opioids in combination with one or more active nonopioid ingredients, provided the amount does not exceed that shown:

Codeine and dihydrocodeine: not to exceed 1800 mg/dL or 90 mg/tablet or other dosage unit

Opium: 500 mg/dL or 25 mg/5 mL or other dosage unit (paregoric)

Stimulants:

Benzphetamine (Regimex)

Phendimetrazine

Depressants:

Schedule II barbiturates in mixtures with noncontrolled drugs or in suppository dosage form

Barbiturates (butabarbital [Butisol], butalbital [Fiorinal])

Ketamine (Ketalar)

Cannabinoids:

Dronabinol (Marinol)

Anabolic Steroids:

Fluoxymesterone (Androxy), Methyltestosterone (Android, Testred), Oxandrolone (Oxandrin), Oxymetholone (Androl-50), Testosterone and its esters (Androgel)

SCHEDULE IV

(Prescription must be rewritten after 6 months or five refills; differs from Schedule III in penalties for illegal possession.)

Opioids:

Butorphanol (Stadol)

Difenoxin 1 mg + atropine 25 mcg (Motofen)

Pentazocine (Talwin)

Stimulants:

Armodafinil (Nuvigil)

Diethylpropion (Tenuate) not in USA

Modafinil (Provigil)

Phentermine (Adipex-P)

Depressants:

Benzodiazepines: Alprazolam (Xanax), Chlordiazepoxide (Librium), Clobazam (Onfi), Clonazepam (Klonopin), Clorazepate (Tranxene), Diazepam (Valium), Estazolam, Flurazepam (Dalmane), Lorazepam (Ativan), Midazolam (Versed), Oxazepam, Quazepam (Doral), Temazepam (Restoril), Triazolam (Halcion)

Carisoprodol (Soma)

Chloral hydrate

Eszopiclone (Lunesta)

Lacosamide (Vimpat)

Meprobamate

Methohexital (Brevital)

Paraldehyde not in USA

Phenobarbital

Tramadol (Ultram)

Zaleplon (Sonata)

Zolpidem (Ambien)

SCHEDULE V

(As any other nonopioid prescription drug)

Codeine: 200 mg/100 mL

Difenoxin preparations: 0.5 mg + 25 mcg atropine

Dihydrocodeine preparations: 10 mg/100 mL

Diphenoxylate (not more than 2.5 mg and not less than 0.025 mg of atropine per dosage unit, as in Lomotil)

Opium preparations: 100 mg/100 mL

Pregabalin (Lyrica)

[1]See https://www.deadiversion.usdoj.gov/schedules.

[2]Emergency prescriptions may be telephoned if followed within 7 days by a valid written prescription annotated to indicate that it was previously placed by telephone. CMEA (Combat Methamphetamine Epidemic Act of 2005) establishes regulations for ephedrine, pseudoephedrine, and phenylpropanolamine over-the-counter sales and purchases.

SECTION I BASIC PRINCIPLES

CHAPTER 1

Introduction: The Nature of Drugs & Drug Development & Regulation

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 many 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 begun. 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. The development of new drugs and their regulation by government agencies are discussed in part II.

*The author thanks Barry Berkowitz, PhD, for contributions to the second part of this chapter.

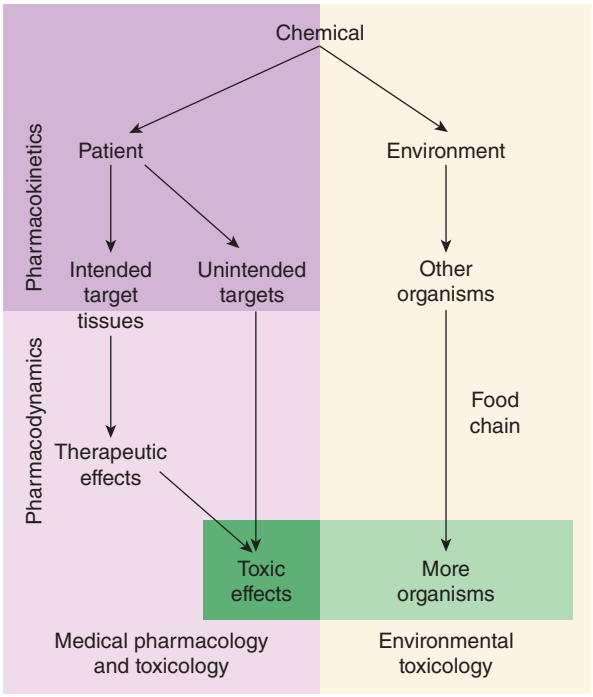


FIGURE 1–1 Major areas of study in pharmacology. The actions of chemicals can be divided into two large domains. The first (*left side*) is that of medical pharmacology and toxicology, which is aimed at understanding the actions of drugs as chemicals on individual organisms, especially humans and domestic 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.

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. In the last 1500 years, 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, reliance 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 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 tissue 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 were introduced. The last four 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.

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. 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. These intracellular targets may provide the next major wave of advances in therapeutics.

Unfortunately, the medication-consuming public is still exposed to vast amounts of inaccurate or unscientific information regarding the pharmacologic effects of chemicals. This has resulted 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 by a segment of the public and to a common 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.

I 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**. 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 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 given 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). 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, oligonucleotides, in the form of small segments of RNA, have entered clinical trials and are on the threshold of introduction into therapeutics.

A number of useful or dangerous drugs are inorganic elements, eg, lithium, iron, and heavy metals. 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). 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 drugs must be able to move within the body (eg, from the site of administration to the site of action). Drugs much larger than MW 1000 do not diffuse readily between compartments of the body (see 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, the drug 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 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 short-acting 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 receptor site via the bonds just described. Optimally, the drug's shape is complementary to that of the receptor site 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 1–1). 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 is an intravenous anesthetic. The (+) enantiomer is a more potent anesthetic and is less toxic than the (–) enantiomer. Unfortunately, the drug is still used as the racemic mixture.

TABLE 1–1 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)
R(+) enantiomer	14	45
S(–) enantiomer	16	0.4
R,S(±) enantiomers	11	0.9

[1]The 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 et al: The pharmacology of carvedilol. Eur J Clin Pharmacol 1990;38:S82.

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, only a small percentage of the chiral drugs used clinically are marketed as the active isomer—the rest are available only as racemic mixtures. As a result, most patients receive drug doses of which 50% is less active or inactive. Some 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 2015/16: Overview.

Br J Pharmacol 2015;172:5729. 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 (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 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 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 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 cholinoceptor *agonist* molecules even though cholinesterase inhibitors do not bind or only incidentally bind to cholinoceptors (see Chapter 7). Because they amplify the effects of physiologically released 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 interaction. As indicated, the receptor is postulated to exist in the inactive, nonfunctional form (R_i) and 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 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, 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 receptor, 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.

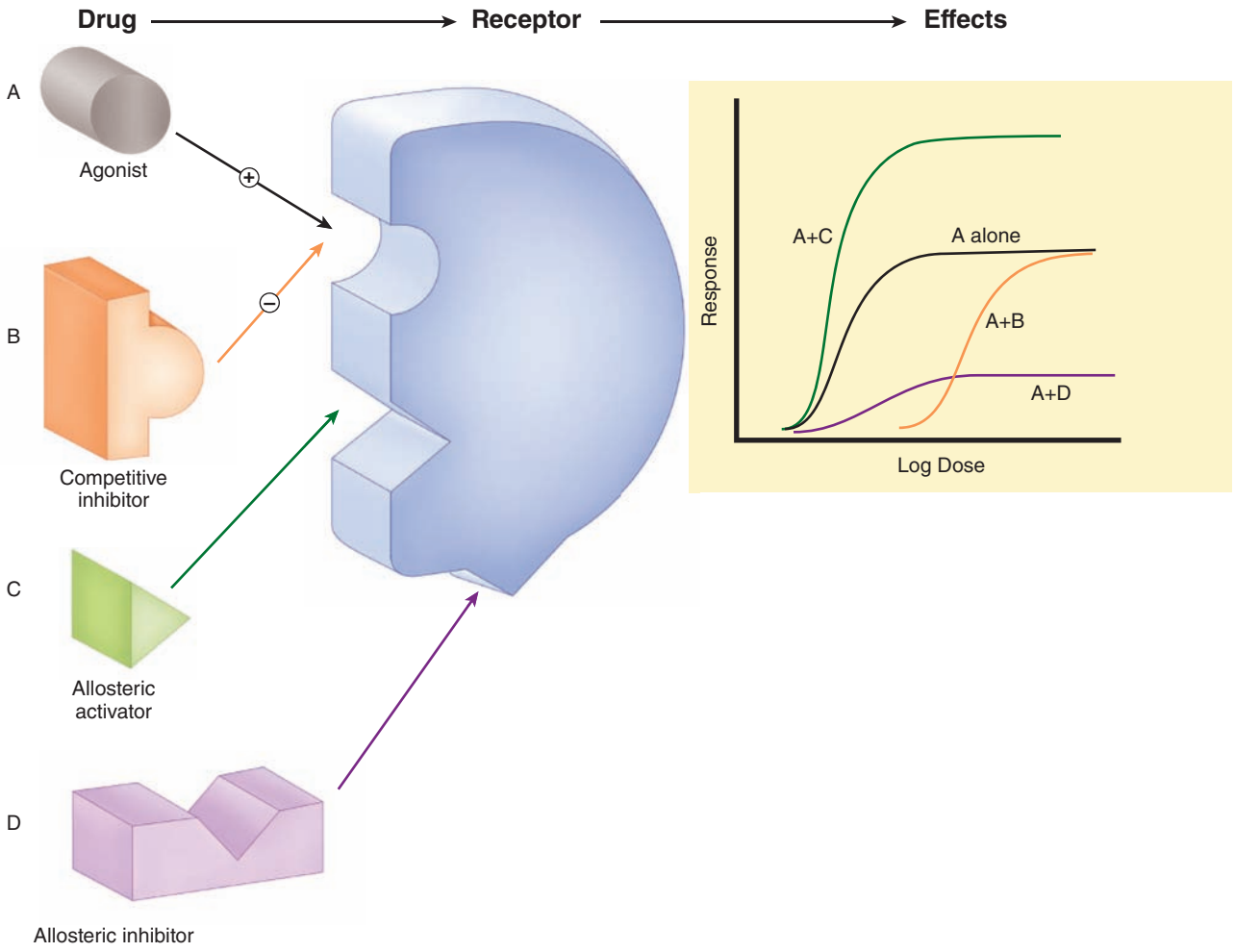


FIGURE 1–2 Drugs may interact with receptors in several ways. The effects resulting from these interactions are diagrammed in the dose-response curves at the right. Drugs that alter the agonist (**A**) response may activate the agonist binding site, compete with the agonist (competitive inhibitors, **B**), 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.

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** (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 cause GABA-like inhibition 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.

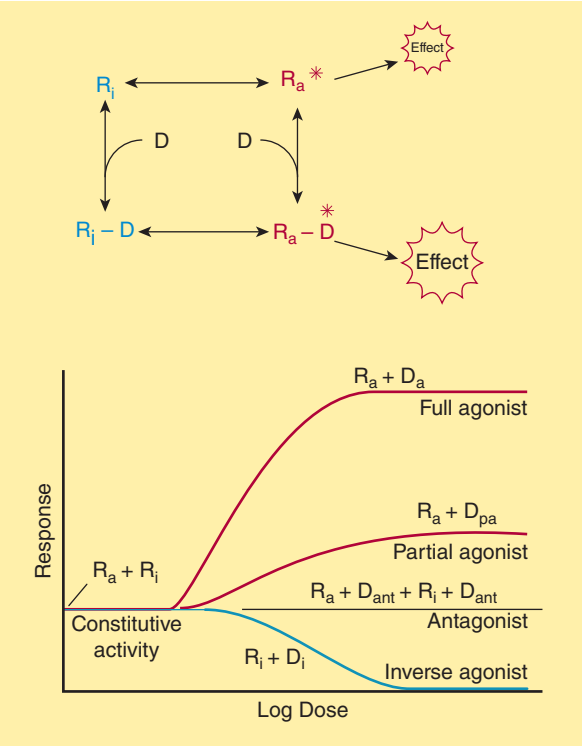


FIGURE 1–3 A model of drug-receptor interaction. The hypothetical receptor is able to assume two conformations. In the R_i conformation, it is inactive and produces no effect, even when combined with a drug molecule. In the R_a conformation, the receptor can activate downstream mechanisms that produce 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.

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. 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 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.

*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 pumps; see text). These tissues are therefore protected or "sanctuary" sites from many circulating drugs.

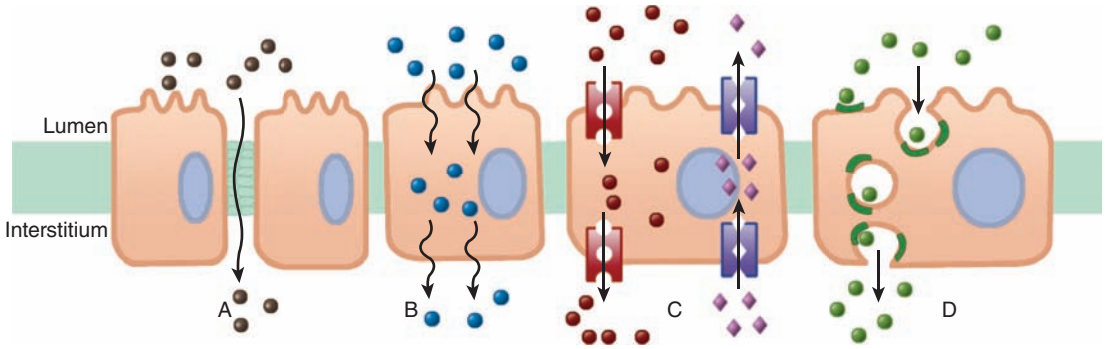


FIGURE 1–4 Mechanisms of drug permeation. Drugs may diffuse passively through aqueous channels in the intercellular junctions (eg, tight junctions, **A**), or through lipid cell membranes (**B**). Drugs with the appropriate characteristics may be transported by carriers into or out of cells (**C**). 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**).

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 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 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 1–2). 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. 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 1–2 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

MDR1, multidrug resistance protein-1; MRP1, multidrug resistance-associated protein-1; NET, norepinephrine 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, Figure 1–4D. This process is responsible for the transport of vitamin B_{12}, 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:

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

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 1–3 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:

$$\underset{\text{Neutral aspirin}}{C_8H_7O_2COOH} \rightleftharpoons \underset{\text{Aspirin anion}}{C_8H_7O_2COO^-} + \underset{\text{Proton}}{H^+}$$

A weak base can be defined as a neutral molecule that can form a cation (a positively charged molecule) by combining with a proton. For example, pyrimethamine, an antimalarial drug, undergoes the following association-dissociation process:

$$\underset{\text{Pyrimethamine cation}}{C_{12}H_{11}ClN_3NH_3^+} \rightleftharpoons \underset{\text{Neutral pyrimethamine}}{C_{12}H_{11}ClN_3NH_2} + \underset{\text{Proton}}{H^+}$$

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:

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

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 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 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 1–3, 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

TABLE 1–3 Ionization constants of some common drugs.

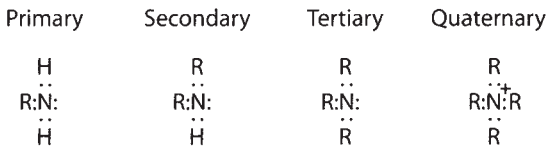
Drug	pK_a[1]	Drug	pK_a[1]	Drug	pK_a[1]
Weak acids		**Weak bases**		**Weak bases (cont'd)**	
Acetaminophen	9.5	Albuterol (salbutamol)	9.3	Isoproterenol	8.6
Acetazolamide	7.2	Allopurinol	9.4, 12.3[2]	Lidocaine	7.9
Ampicillin	2.5	Alprenolol	9.6	Metaraminol	8.6
Aspirin	3.5	Amiloride	8.7	Methadone	8.4
Chlorothiazide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

[1]The pK_a is that pH at which the concentrations of the ionized and nonionized forms are equal.

[2]More than one ionizable group.

[3]Isoelectric point.

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.

II 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

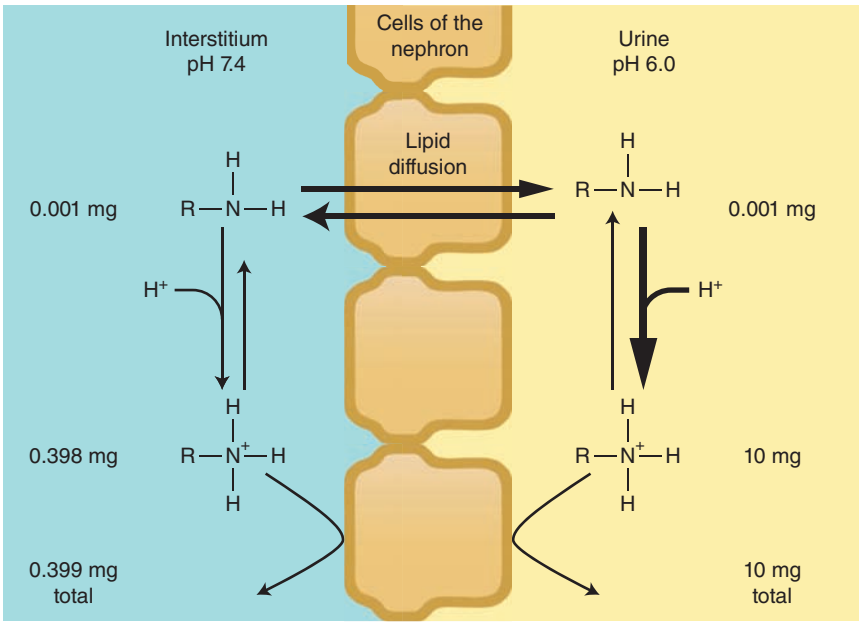


FIGURE 1–5 Trapping of a weak base (methamphetamine) in the urine when the urine is more acidic than the blood. In the hypothetical case illustrated, the diffusible uncharged form of the drug has equilibrated across the membrane, but the total concentration (charged plus uncharged) in the urine (more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

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 http://www.pharmacytimes.com/news/10-best-selling-brand-name-drugs-in-2015/).

Such breakthroughs come at a price, however, and the escalating cost of drugs has become a significant contributor to the inflationary increase in the cost of health care. Development of new drugs is enormously expensive, but considerable controversy surrounds drug pricing. Critics claim that the costs of development and marketing 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 right to an older, established drug has 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 must be characterized in vivo in animals before human drug trials can be started. 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

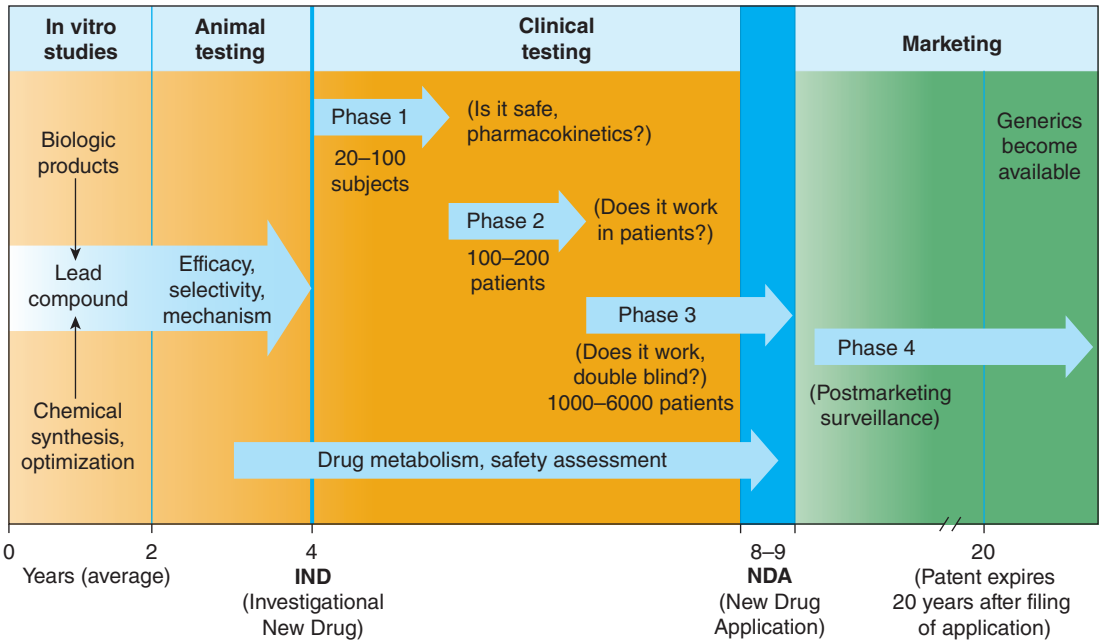


FIGURE 1–6 The development and testing process required to bring a drug to market in the USA. Some of the requirements may be different for drugs used in life-threatening diseases (see text).

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; 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 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 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. 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's disease.

At the molecular level, the 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 was 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 1–4. 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 1–4, 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 (LD_{50})**—the dose that kills approximately 50% of the animals in a test group. Presently, the LD_{50} 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.
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.

TABLE 1–4 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.

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 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 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 (**RCT**s) 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.

[*]I 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 1–5). 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.[*]

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 (Table 1–5) 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.

[*]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.

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 (Figure 1–6). The IND includes (1) information on the composition and source of the drug,

TABLE 1–5 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 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 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 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.

(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"), 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 of patients with the target disease—usually thousands—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 the new drug application 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 approval 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 (Table 1–5). Roughly 50% of drugs in phase 3 trials involve 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 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 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 65.

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 policy 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. Finally, 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 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 is hoped that greater use of electronic health records will reduce some of these risks (see Chapter 65).

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 Disease, 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 Trevor, Katzung, and Kruidering-Hall (McGraw-Hill, 2015). 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. *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://service.govdelivery.com/service/user.html?code=USFDA.

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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.

CHAPTER

2 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 physician 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 is subsequently obtained, and the medical history is remarkable only for mild hypertension that is being treated with propranolol. The physician instructs the patient to discontinue use of propranolol, and changes the patient's antihypertensive medication to verapamil. Why is the physician correct to discontinue propranolol? Why is verapamil a better choice for managing hypertension in this patient? What alternative treatment change might the physician 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.

*The author thanks Henry R. Bourne, MD, for major contributions to this chapter.

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 almost all of the receptors that we discuss 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 a number of **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,** which 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:

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

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

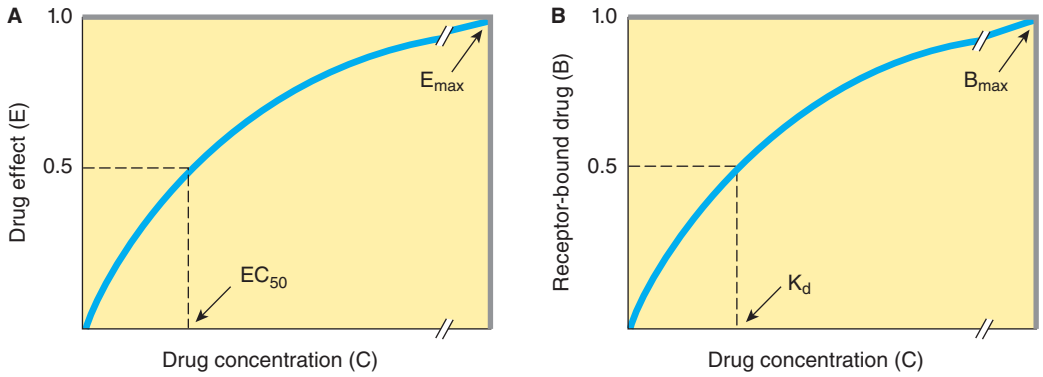

FIGURE 2–1 Relations between drug concentration and drug effect (**A**) or receptor-bound drug (**B**). The drug concentrations at which effect or receptor occupancy is half-maximal are denoted by EC_{50} and K_d, respectively.

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.

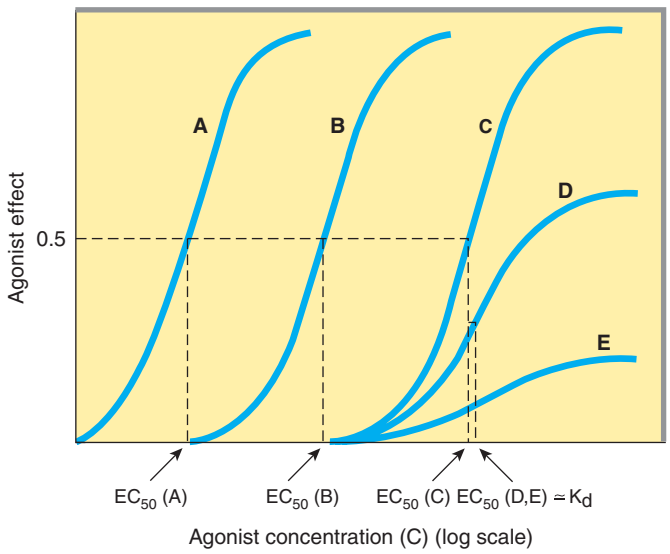

FIGURE 2–2 Logarithmic transformation of the dose axis and experimental demonstration of spare receptors, using different concentrations of an irreversible antagonist. Curve **A** shows agonist response in the absence of antagonist. After treatment with a low concentration of antagonist (curve **B**), 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.

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.

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 of 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 (Figure 2–2). For example, the same maximal inotropic response of heart muscle to catecholamines can be elicited even when 90% of β adrenoceptors to which they bind are occupied by a quasi-irreversible antagonist. Accordingly, myocardial cells are said to contain a large proportion of spare β adrenoceptors.

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-adrenoceptor 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 following section on 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 needed 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 four receptors and four effectors. Here the number of effectors does not limit the maximal response, and the receptors are *not* spare in number. Consequently, an agonist 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, two receptors stimulate two effectors). Now imagine that the number of receptors increases tenfold to 40 receptors but that 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 2 of the 40 receptors (5% of the receptors), and this same low concentration of agonist is able to elicit a half-maximal response (two of four 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 or not 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

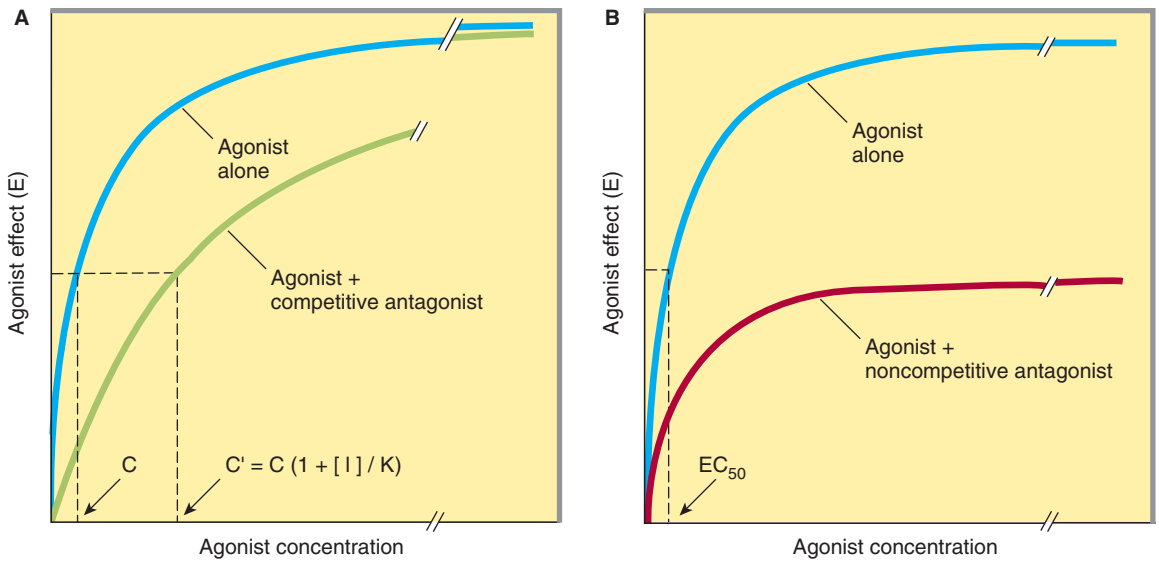

FIGURE 2–3 Changes in agonist concentration-effect curves produced by a competitive antagonist (**A**) or by an irreversible antagonist (**B**). In the presence of a competitive antagonist, higher concentrations of agonist are required to produce a given effect; thus the agonist concentration (C′) required 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}.

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) 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 (Figure 2–2B and C; see 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, Figure 1–2C and D). 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 agonist and reduce activity of the receptor. Not all allosteric modulators act as antagonists; some potentiate rather than reduce receptor activity. For example, benzodiazepines are considered *positive allosteric modulators* because they bind to an allosteric site on the ion channels activated by the neurotransmitter γ-aminobutyric acid (GABA) and potentiate the net activating effect of GABA on channel conductance. Benzodiazepines have little activating effect on their own, and this property is one reason that benzodiazepines are relatively safe in overdose; even at high doses, their ability to increase ion conductance is limited by the release of endogenous neurotransmitter. Allosteric modulation can also occur at targets lacking a known orthosteric binding site. For example, ivacaftor binds to the **cystic fibrosis transmembrane regulator (CFTR)** ion channel that is mutated in cystic fibrosis. Certain mutations that render the channel hypoactive can be partially rescued by ivacaftor, representing positive allosteric modulation of a channel for which there is no presently known endogenous ligand.

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 because it produces less respiratory depression in overdose. However, buprenorphine is effectively antianalgesic when administered in combination with more efficacious opioid drugs, and it may precipitate a drug withdrawal syndrome in opioid-dependent patients.

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 at all. For example, protamine, a protein that is positively charged at physiologic pH, can be used clinically to counteract the effects of heparin, an anticoagulant 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 for interactions with proteins involved in blood clotting.

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 sugar, an effect that is physiologically opposed by insulin. Although glucocorticoids and

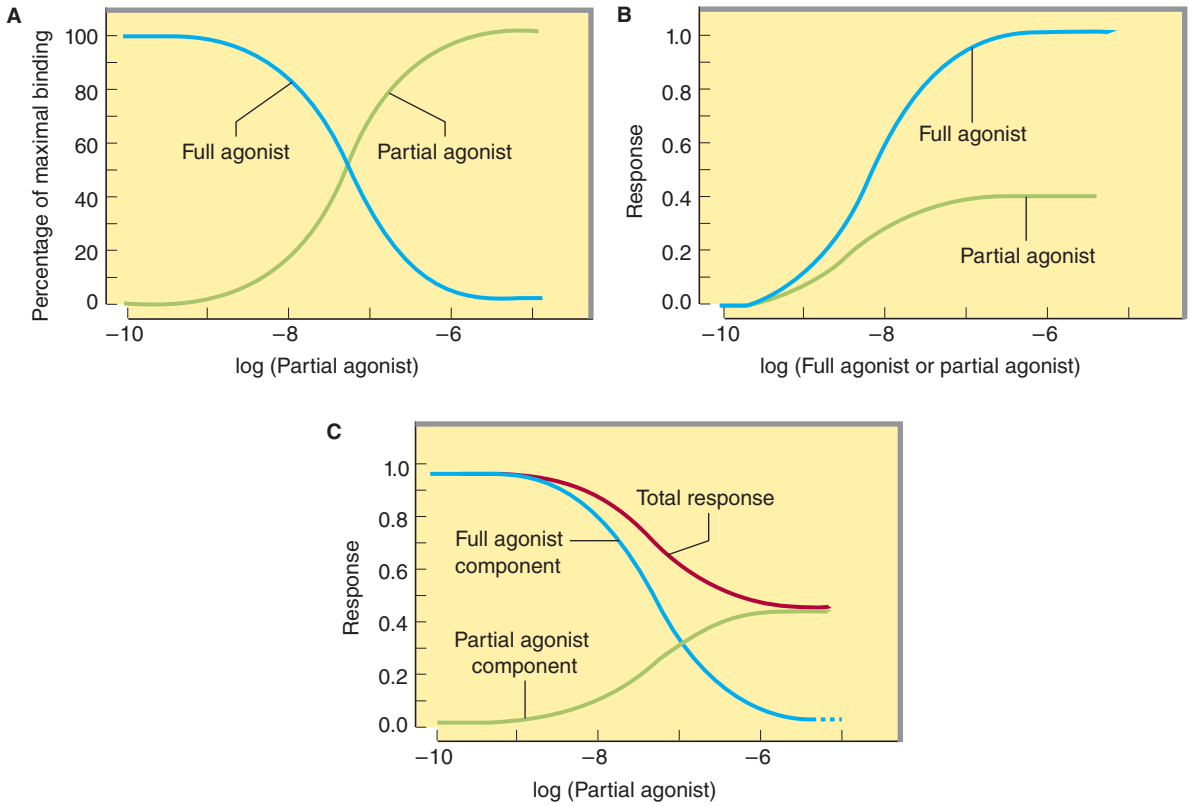

FIGURE 2–4 **A:** The percentage of receptor occupancy resulting from full agonist (present at a single concentration) binding to receptors in the presence of increasing concentrations of a partial agonist. Because the full agonist (blue line) and the partial agonist (green line) compete to bind to the 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).

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 physician 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 use of a receptor-specific antagonist such as atropine (a competitive antagonist of acetylcholine receptors that slow heart rate as the direct targets of acetylcholine released from vagus nerve endings).

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 a drug acts. We should also consider different structural families of receptor protein, 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 chemical second messengers in the cytoplasm. This section first discusses the mechanisms for carrying chemical information across the plasma membrane and then outlines key features of cytoplasmic second messengers.

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 of a ligand; or (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.

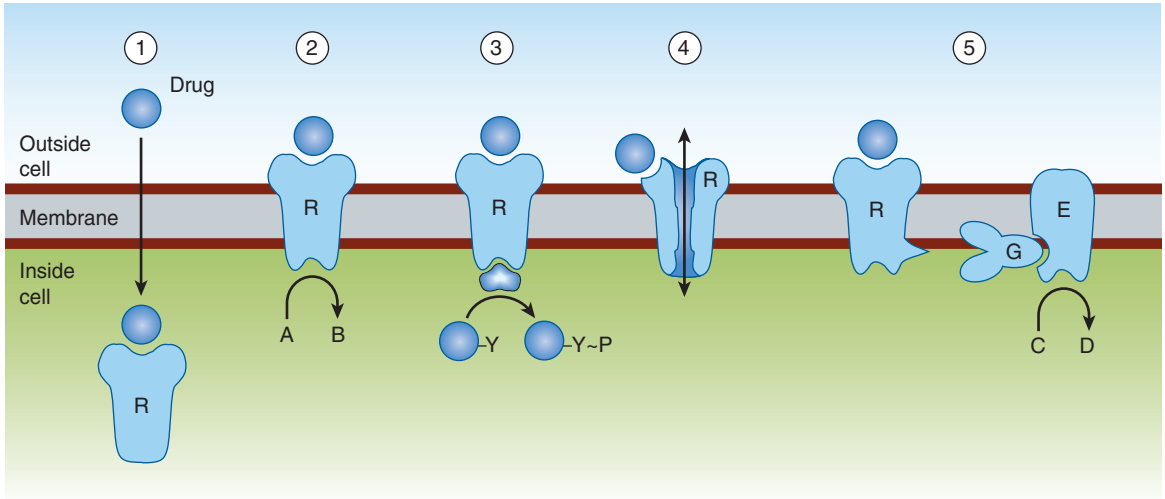

FIGURE 2–5 Known transmembrane signaling mechanisms: **1:** A lipid-soluble chemical signal crosses the plasma membrane and acts on an intracellular receptor (which may be an enzyme or a regulator of gene transcription); **2:** the signal binds to the extracellular domain of a transmembrane protein, 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.)

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. All of 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 the agonist 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.

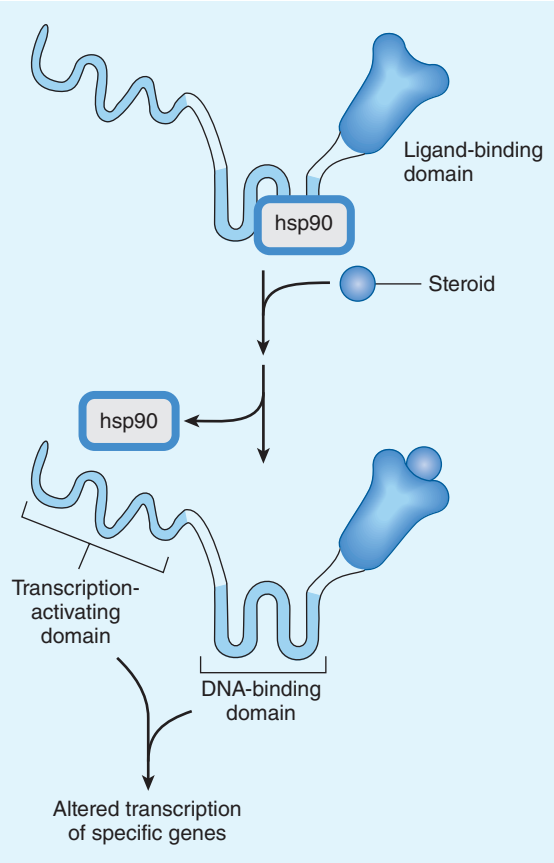

FIGURE 2–6 Mechanism of glucocorticoid action. The glucocorticoid receptor polypeptide is schematically depicted as a protein with three distinct domains. A heat-shock protein, hsp90, binds to the receptor in the absence of hormone and prevents folding into the active conformation of the receptor. Binding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

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 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 type of activated receptor to modulate a number of biochemical processes. (Some receptor tyrosine kinases form oligomeric complexes larger than dimers upon activation by ligand, but the pharmacologic significance of such higher-order complexes is presently unclear.)

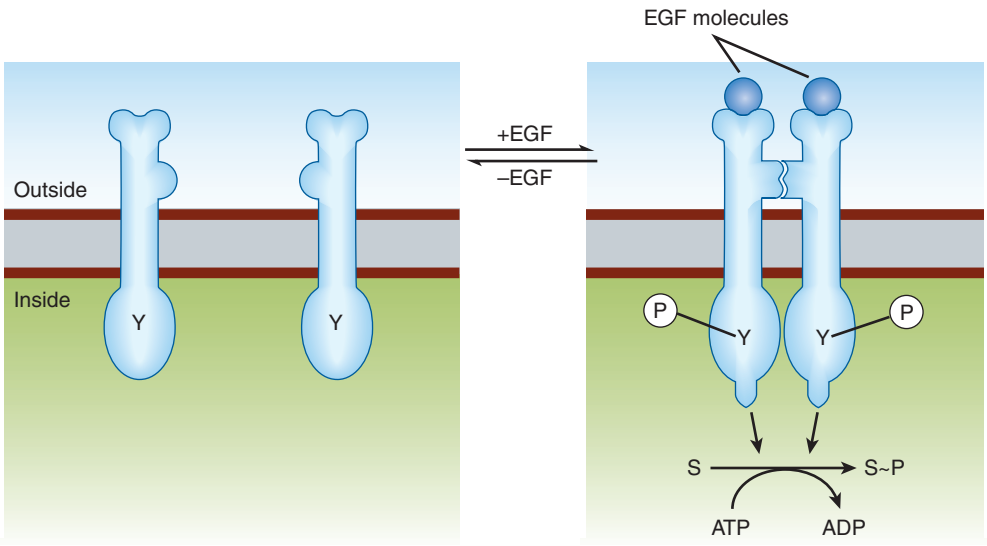

FIGURE 2–7 Mechanism of activation of the epidermal growth factor (EGF) receptor, a representative receptor tyrosine kinase. The receptor polypeptide has extracellular and cytoplasmic domains, depicted above and below the plasma membrane. Upon binding of EGF (circle), the receptor converts from its inactive 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 finding increased use in 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 **down-regulation.** Ligand binding often induces accelerated endocytosis of receptors from the cell surface, followed by the degradation of those 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, which internalizes from the plasma membrane at a greatly accelerated rate after activation by EGF and then is delivered to lysosomes and proteolyzed. This down-regulation process is essential physiologically to limit the strength and duration of the growth factor signal; genetic mutations that interfere with the down-regulation 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, serves a very different function. Internalized nerve growth factor receptors are not rapidly degraded but are translocated in endocytic vesicles from the distal axon, where receptors are activated by nerve growth factor released from the innervated tissue, to the 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 down-regulation, transports a critical survival signal from its site of agonist release 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.

A number of 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). Receptors in both groups, like the receptor tyrosine kinases, are active in their dimeric forms.

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

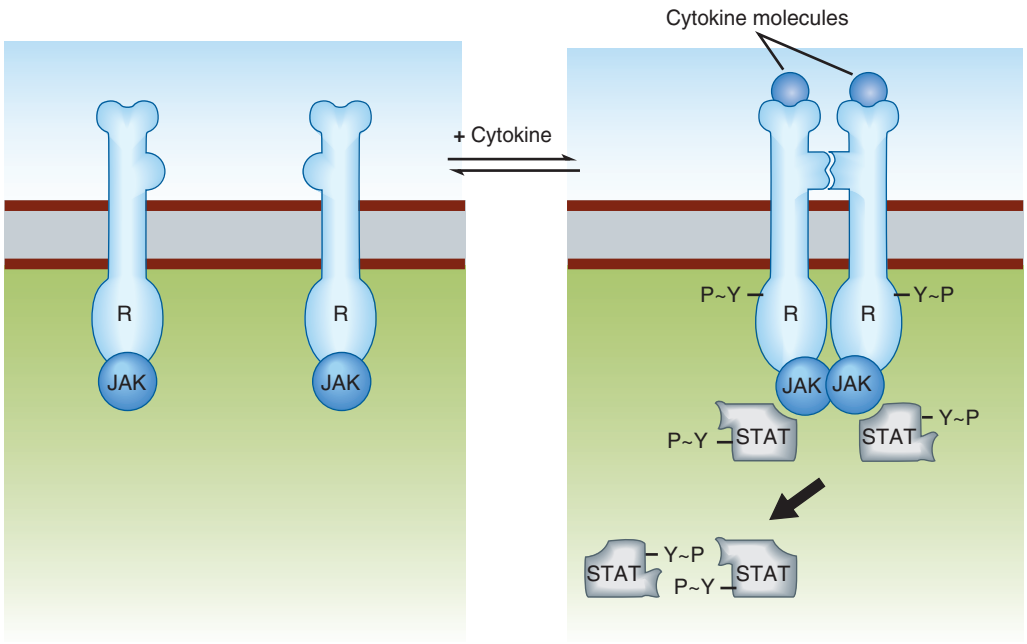

FIGURE 2–8 Cytokine receptors, like receptor tyrosine kinases, have extracellular and intracellular domains and form dimers. However, after activation by an appropriate ligand, separate mobile protein tyrosine kinase molecules (JAK) are activated, resulting in phosphorylation of signal transducers and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

dimerize after they bind the activating ligand, allowing the bound JAKs to become activated and to phosphorylate tyrosine residues on the receptor. 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, all with molecular weights ranging from 43,000–50,000). 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

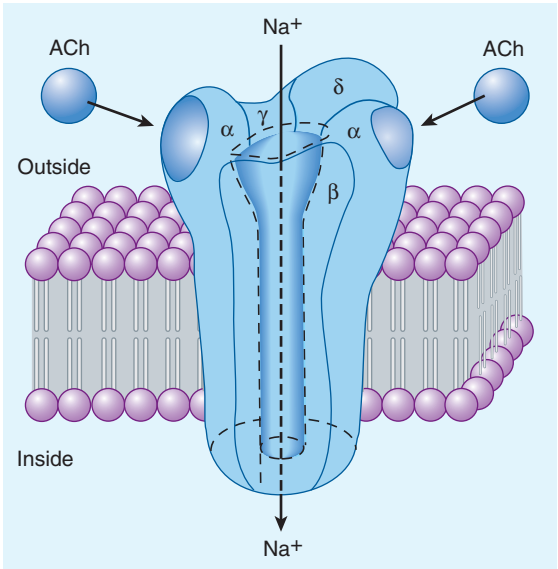

FIGURE 2–9 The nicotinic acetylcholine (ACh) receptor, a ligand-gated ion channel. The receptor molecule is depicted as embedded in a rectangular piece of plasma membrane, with extracellular fluid above and cytoplasm below. Composed of five subunits (two α, one β, one γ, and one δ), the receptor opens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

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 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 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 the central nervous system, these mechanisms 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. Other channels, such as the CFTR, although not strongly sensitive to either a known natural ligand or voltage, 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 were recently approved for 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 **(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 α and β adrenoceptors, glucagon receptors, thyrotropin receptors, and certain subtypes of dopamine and serotonin receptors.

G_s and other G proteins activate their downstream effectors when bound by GTP and also have the ability to hydrolyze GTP (Figure 2–10); this hydrolysis reaction inactivates the G protein but can occur at a relatively slow rate, effectively amplifying the transduced signal by allowing the activated (GTP-bound) G protein to have a longer lifetime in the cell than the activated receptor itself. For example, a neurotransmitter such as norepinephrine may encounter its membrane receptor for only a few 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, like other G proteins, GTP-bound G_s may remain active for tens of seconds, enormously amplifying the original signal. This mechanism also helps explain how signaling by G proteins produces the phenomenon of spare receptors. 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

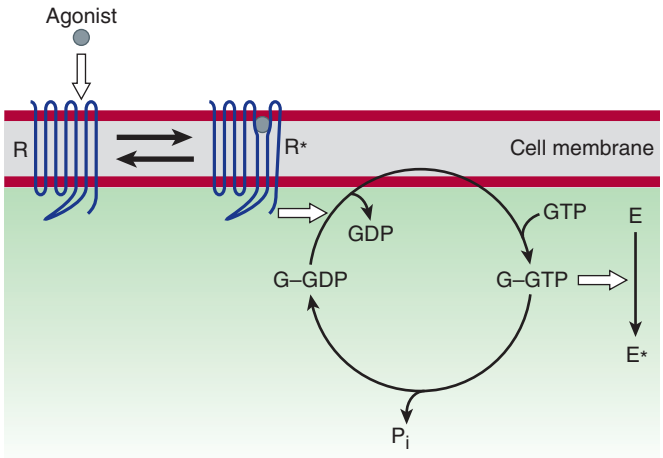

FIGURE 2–10 The guanine nucleotide-dependent activation-inactivation cycle of G proteins. The agonist activates the receptor (R→R*), which promotes release of GDP from the G protein (G), allowing entry of GTP into the nucleotide binding site. In its GTP-bound state (G-GTP), the G protein regulates activity of an effector enzyme or ion channel (E→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.)

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_{i1}, G_{i2}, G_{i3}	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.

of G proteins. The apparent promiscuity of such a ligand allows it to 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 α_1 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 even visual receptors (in retinal rod and cone cells) all belong to the GPCR family. All were derived from a common evolutionary precursor. 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, dimerization 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 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.

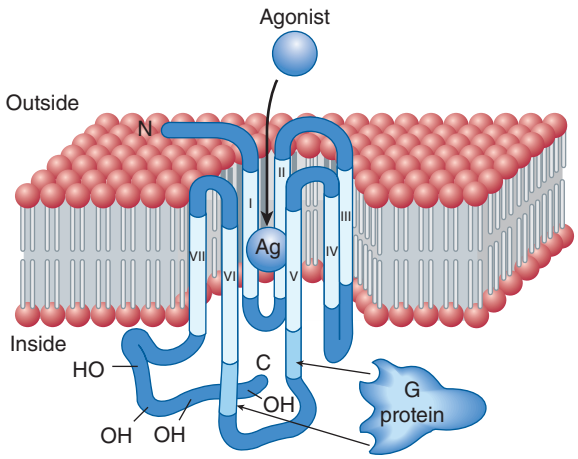

FIGURE 2–11 Transmembrane topology of a typical "serpentine" GPCR. The receptor's amino (N) terminal is extracellular (above the plane of the membrane), and its carboxyl (C) terminal intracellular, with the polypeptide chain "snaking" across the membrane seven times. The hydrophobic transmembrane segments (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.

Many high-resolution structures of GPCRs are available from the Protein Data Bank (www.rcsb.org). An animated model depicting the conformational change associated with activation is available from the Protein Data Bank in Europe (http://www.ebi.ac.uk/pdbe/quips?story=B2AR).

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 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, results in a response similar to the initial 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 also increasing the receptor's affinity for binding a third protein, 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 remove the phosphorylations, reversing the desensitized state and allowing activation to occur again upon another encounter with agonist.

For β adrenoceptors, and for many other GPCRs, β-arrestin can produce other 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, similar to down-regulation of EGF receptors, but it can also help reverse the desensitized state for those receptors returned to the plasma membrane by exposing receptors to phosphatase enzymes in endosomes (Figure 2–12B). In some cases, β-arrestin can itself act as a positive signal transducer, analogous to G proteins but through a different mechanism, by serving as a molecular scaffold to bind other signaling proteins (rather than through binding GTP). In this way, β-arrestin can confer on GPCRs a great deal of flexibility in signaling and regulation. This flexibility is still poorly understood but is presently thought to underlie the ability of some drugs to produce a different spectrum of downstream effects from other drugs, despite binding to the same GPCR. Current drug development efforts are exploring the potential of this phenomenon, called **functional selectivity** or **agonist bias**, as a means to achieve specificity in drug action beyond that presently possible using conventional agonists and antagonists. Functionally selective agonists are thought to occupy the orthosteric ligand-binding site, making their binding competitive with conventional orthosteric agonists, but differ from conventional agonists in effects on receptor conformation after binding. Allosteric ligands may also stabilize different conformational states of the receptor, but differ from functionally selective ligands by binding noncompetitively to a different site.

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 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; 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). 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

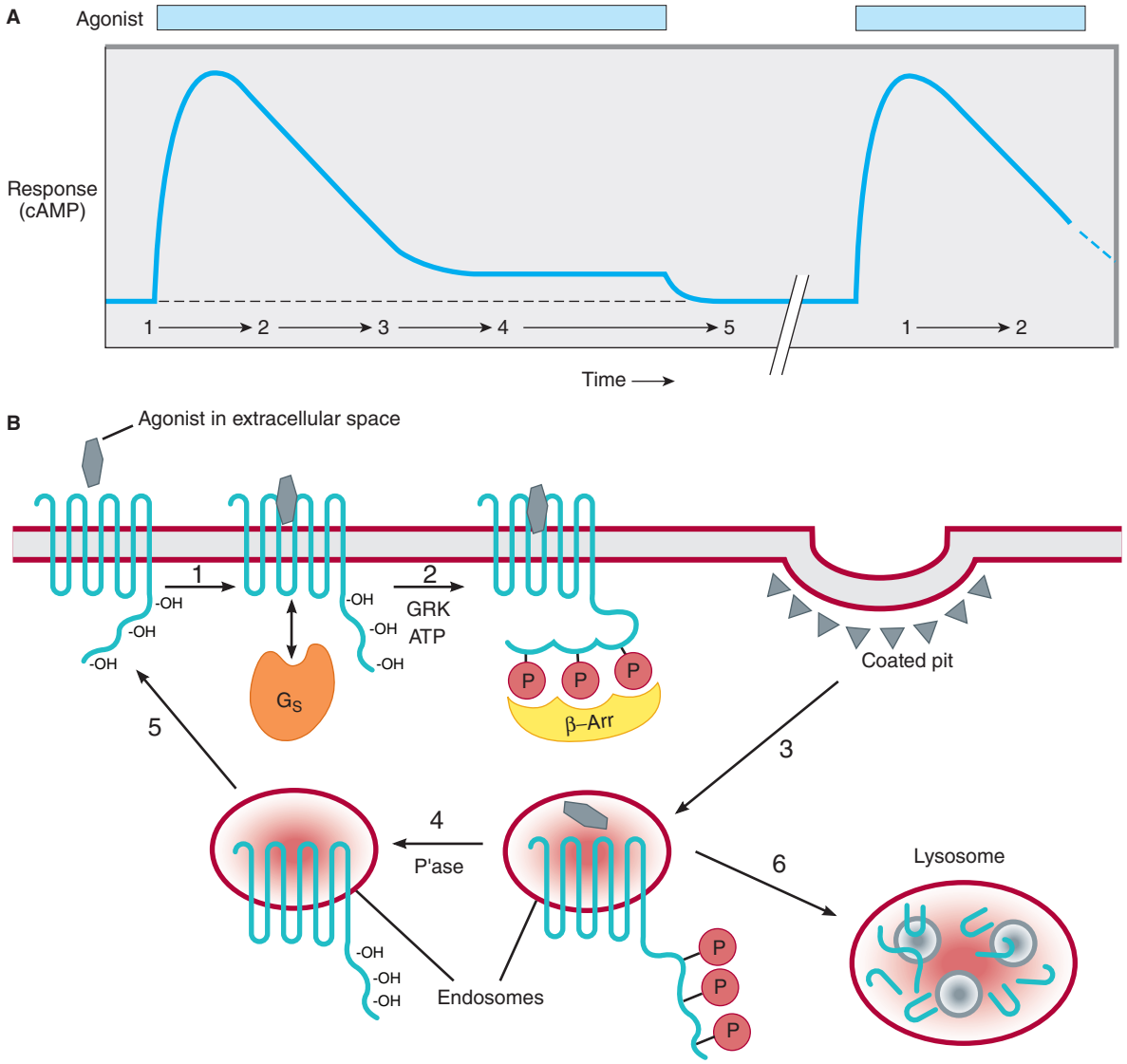

FIGURE 2–12 Rapid desensitization, resensitization, and down-regulation of β adrenoceptors. **A:** Response to a β-adrenoceptor agonist (ordinate) versus time (abscissa). (Numbers refer to the phases of receptor function in B.) Exposure of cells to agonist (indicated by the light-colored bar) produces 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) and return of receptors to the plasma membrane (step 5); together, these events result in the efficient resensitization of cellular responsiveness. Repeated or prolonged exposure of cells to agonist favors the delivery of internalized receptors to lysosomes (step 6), promoting receptor down-regulation rather than resensitization.

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

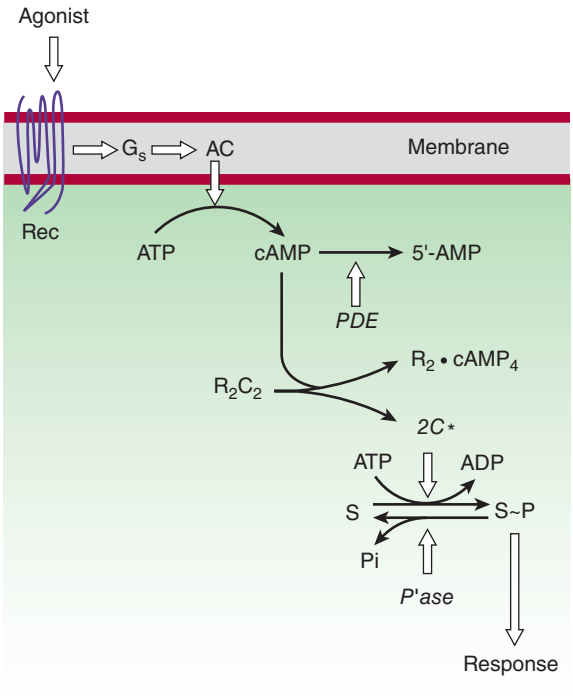

FIGURE 2–13 The cAMP second messenger pathway. Key proteins include hormone receptors (Rec), a stimulatory G protein (G_s), catalytic adenylyl cyclase (AC), phosphodiesterases (PDE) that hydrolyze cAMP, cAMP-dependent kinases, with regulatory (R) and catalytic (C) subunits, protein substrates (S) of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

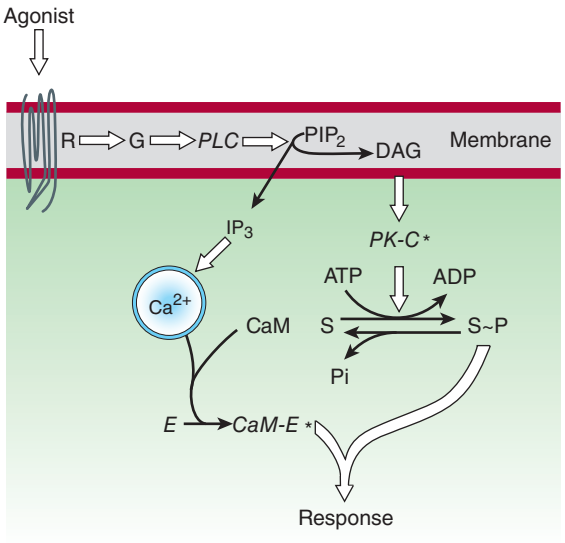

FIGURE 2–14 The Ca^{2+}-phosphoinositide signaling pathway. Key proteins include hormone receptors (R), a G protein (G), a phosphoinositide-specific phospholipase C (PLC), protein kinase C substrates of the kinase (S), calmodulin (CaM), and calmodulin-binding enzymes (E), including kinases, phosphodiesterases,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

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 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 receptor signaling (discussed earlier), is a useful therapeutic agent for breast cancer. Another example of this general approach is imatinib, a small molecule inhibitor of the cytoplasmic tyrosine kinase Abl, which is activated by growth factor signaling pathways. Imatinib is effective for treating chronic myelogenous leukemia, which is caused by a chromosomal translocation event that produces an active Bcr/Abl fusion protein in hematopoietic cells.

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 (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 are preclinical efforts under way directed at developing inhibitors of specific G proteins.

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** of 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.

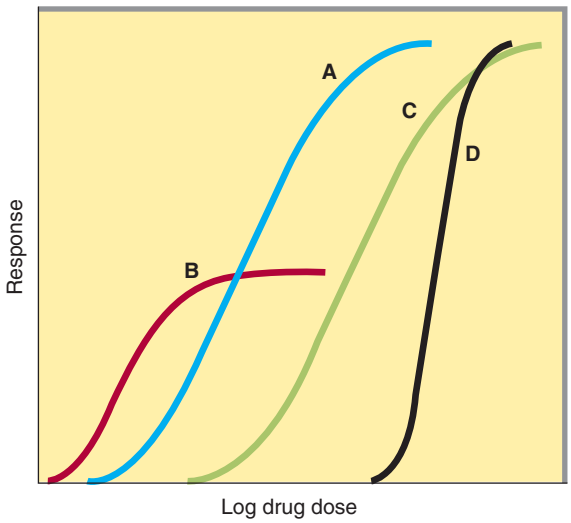

FIGURE 2–15 Graded dose-response curves for four drugs, illustrating different pharmacologic potencies and different maximal efficacies. (See text.)

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).

*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, 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.

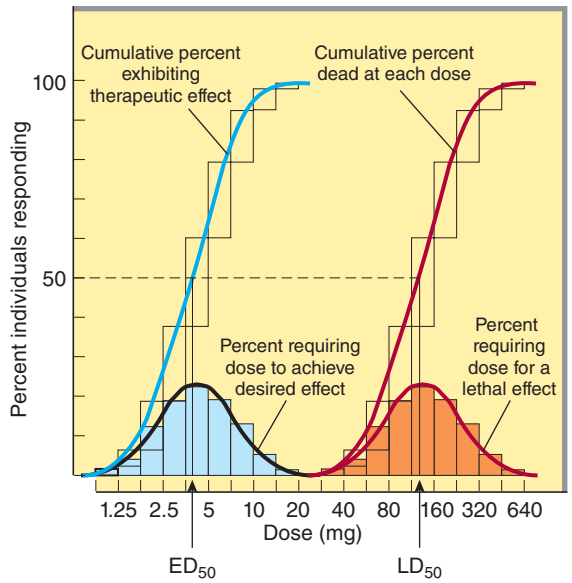

FIGURE 2–16 Quantal dose-effect plots. Shaded boxes (and the accompanying bell-shaped curves) indicate the frequency distribution of doses of drug required to produce a specified effect; that is, the percentage of animals that required a particular dose to exhibit the effect. The open boxes (and the 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 (ED_{50}),** which is the dose at which 50% of individuals exhibit the specified quantal effect. (Note that the abbreviation ED_{50} 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 (TD_{50}).** If the toxic effect is death of the animal, a **median lethal dose (LD_{50})** may be experimentally defined. Such values provide a convenient way of comparing the potencies of drugs in experimental and clinical settings: Thus, if the ED_{50}s 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 ED_{50}s 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 TD_{50} to the ED_{50} 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's 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 anti-cancer 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, down-regulation) 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 down-regulation 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 down-regulation, 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 postreceptor 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 ED_{50}s 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 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, mis-expression of microRNAs (miRNAs), small RNAs that regulate protein expression by binding to protein-coding (messenger) RNAs, was linked recently to Duchenne muscular dystrophy. Current preclinical investigations include the 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 antihypertensive 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.

CHAPTER

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 ng/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 clarify the dose-effect relationship (Figure 3–1). Pharmacodynamics governs the concentration-effect part of the interaction, whereas pharmacokinetics deals with the dose-concentration part (Holford & Sheiner, 1981). The pharmacokinetic processes of absorption, distribution, and elimination determine how rapidly and for how long the drug will appear at the target organ. 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}).

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 and Toxic Concentration columns in Table 3–1. 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 volunteers and patients with average ability to absorb, distribute, and eliminate the drug (see Clinical Trials: The IND & NDA