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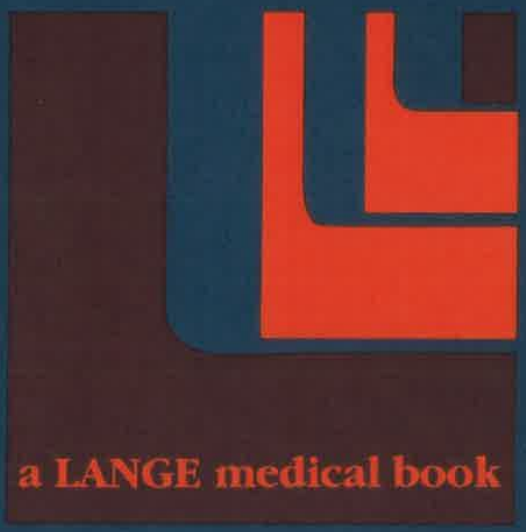
sixth edition

Basic & Clinical Pharmacology

Bertram G. Katzung



Schedule of Controlled Drugs

SCHEDULE I (All nonresearch use illegal.)
- **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.)
- **Opioids:**
  - Opium
  - Opium alkaloids and derived phenanthrene alkaloids: morphine, hydromorphone (Dilaudid), oxymorphone (Numorphan), oxycodone (dihydroxycodeinone, a component of Percodan, Percocet, Roxicodone, Tylox)
  - Designated synthetic drugs: levomethadyl (Orlaam), meperidine (Demerol), methadone, levorphanol (Levo-Dromoran), fentanyl (Sublimaze, Duragesic), alphaprodine, alfentanil (Alfenta), sufentanil (Sufenta)
- **Stimulants:**
  - Coca leaves and cocaine
  - Amphetamine
  - Amphetamine complex (Biphetamine)
  - Dextroamphetamine (Dexedrine)
  - Methamphetamine (Desoxyn)
  - Phenmetrazine (Preludin)
  - Methylphenidate (Ritalin)
  - Above in mixtures with other controlled or uncontrolled drugs
- **Depressants:**
  - Amobarbital (Amytal)
  - Pentobarbital (Nembutal)
  - Secobarbital (Seconal)
  - Mixtures of above (eg, Tuinal)
- **Cannabinoids:**
  - Dronabinol (Marinol)

SCHEDULE III (Prescription must be rewritten after 6 months or 5 refills.[1])
- **Opioids:** 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
  - Dihydrocodeinone (hydrocodone in Hycodan, Vicodin, and Lortab): not to exceed 300 mg/dL or 15 mg/tablet
  - Opium: 500 mg/dL or 25 mg/5 mL or other dosage unit (paregoric)
- **Stimulants:**
  - Benzphetamine (Didrex)
  - Phendimetrazine (Plegine)
- **Depressants:**
  - Schedule II barbiturates in mixtures with noncontrolled drugs or in suppository dosage form
  - Aprobarbital (Alurate)
  - Butabarbital (Butisol)
  - Glutethimide (Doriden)
  - Metharbital (Gemonil)
  - Talbutal (Lotusate)
  - Thiamylal (Surital)
  - Thiopental (Pentothal)

SCHEDULE IV (Prescription must be rewritten after 6 months or 5 refills; differs from Schedule III in penalties for illegal possession.)
- **Opioids:**
  - Difenoxin (Motofen)
  - Pentazocine (Talwin)
  - Propoxyphene (Darvon)
- **Stimulants:**
  - Diethylpropion (Tenuate)
  - Mazindol (Sanorex)
  - Phentermine (Ionamin)
  - Fenfluramine (Pondimin)
  - Pemoline (Cylert)
- **Depressants:**
  - Benzodiazepines
    - Alprazolam (Xanax)
    - Chlordiazepoxide (Librium)
    - Clonazepam (Klonopin)
    - Clorazepate (Tranxene)
    - Diazepam (Valium)
    - Estazolam (ProSom)
    - Flurazepam (Dalmane)
    - Halazepam (Paxipam)
    - Lorazepam (Ativan)
    - Midazolam (Versed)
    - Oxazepam (Serax)
    - Prazepam (Centrax)
    - Quazepam (Doral)
    - Temazepam (Restoril)
    - Triazolam (Halcion)
  - Chloral hydrate
  - Ethchlorvynol (Placidyl)
  - Ethinamate (Valmid)
  - Meprobamate (Equanil, Miltown, etc)
  - Mephobarbital (Mebaral)
  - Methohexital (Brevital)
  - Methyprylon (Noludar)
  - Paraldehyde
  - Phenobarbital
  - Zolpidem (Ambien)

SCHEDULE V (As any other nonopioid prescription drug; may also be dispensed without prescription unless additional state regulations apply.)
- **Opioids:**
  - Diphenoxylate (not more than 2.5 mg and not less than 0.025 mg of atropine per dosage unit, as in Lomotil)
- **The following drugs** in combination with other active nonopioid ingredients and provided the amount per 100 mL or 100 g does not exceed that shown:
  - Codeine: 200 mg
  - Dihydrocodeine: 100 mg

[1] In some states (eg, California), anabolic steroids have been classified as Schedule III.

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Basic & Clinical Pharmacology

a LANGE medical book

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sixth edition

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Notice: The authors and the publisher of this volume have taken care to make certain that the doses of drugs and schedules of treatment are correct and compatible with the standards generally accepted at the time of publication. Nevertheless, as new information becomes available, changes in treatment and in the use of drugs become necessary. The reader is advised to carefully consult the instructions and information included in the package insert of each drug or therapeutic agent before administration. This advice is especially important when using new or infrequently used drugs. The author and publisher disclaim any liability, loss, injury, or damage incurred as a consequence, directly or indirectly, of the use and application of any of the contents of this volume.

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Drug Receptors & Pharmacodynamics

2

Henry R. Bourne, MD, & James M. Roberts, MD

The therapeutic and toxic effects of drugs result from their interactions with molecules in the patient. In most instances, drugs act by associating with specific macromolecules in ways that alter their biochemical or biophysical activity. This idea, now almost a century old, is embodied in the terms **receptive substance** and **receptor:** the component of a cell or organism that interacts with a drug and initiates the chain of biochemical events leading to the drug's observed effects.

Initially, the existence of receptors was inferred from observations of the chemical and physiologic specificity of drug effects. Thus, Ehrlich noted that certain synthetic organic agents had characteristic antiparasitic effects while other agents did not, though their chemical structures differed only slightly. Langley noted that curare did not prevent electrical stimulation of muscle contraction but did block contraction triggered by nicotine. From these simple beginnings, receptors have now 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. Drug receptors are now being isolated and characterized as macromolecules, thus opening the way to precise understanding of the molecular basis of drug action.

In addition to its usefulness for explaining biology, the receptor concept has immensely important practical consequences for the development of drugs and for making therapeutic decisions in clinical practice. These consequences—explained more fully in later sections of this chapter—form the basis for understanding the actions and clinical uses of drugs described in 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 often limits 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 avidity–it will bind to a particular receptor among the vast array of chemically different binding sites available in a cell, animal, 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 antagonists. Many drugs and endogenous chemical signals, such as hormones, regulate the function of receptor macromolecules as **agonists;** ie, they change the function of a macromolecule as a more or less direct result of binding to it. Pure pharmacologic **antagonists,** however, bind to receptors without directly altering the receptors' function. Thus, the effect of a pure antagonist on a cell or in a patient depends entirely upon its preventing the binding of agonist molecules and blocking their biologic actions. Some of the most useful drugs in clinical medicine are pharmacologic antagonists.

MACROMOLECULAR NATURE OF DRUG RECEPTORS

Until recently, the chemical structures and even the existence of receptors for most drugs could only be inferred from the chemical structures of the drugs themselves. Now, however, receptors for many drugs have been biochemically purified and characterized. The accompanying box describes some of the methods by which receptors are discovered and defined. Most receptors are proteins, presumably because the structures of polypeptides provide both the necessary diversity and the necessary specificity of shape and electrical charge.

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

HOW ARE RECEPTORS DISCOVERED?

Because today's new receptor sets the stage for tomorrow's new drug, it is important to know how new receptors are discovered. The discovery process follows a few key steps, summarized in Figure 2–1. As presented in greater detail elsewhere in this chapter, the process of defining a new receptor (stage 1 in Figure 2–1) begins by studying the relations between structures and activities of a group of drugs on some conveniently measured response. Binding of radioactive ligands defines the molar abundance and binding affinities of the putative receptor and provides an assay to aid in its biochemical purification. Analysis of the pure receptor protein tells us the number of its subunits, its size, and (sometimes) provides a clue to how it works (eg, agonist-stimulated autophosphorylation on tyrosine residues, seen with receptors for insulin and many growth factors).

These "classic" steps in receptor identification now serve as a warming-up exercise for a powerful new experimental strategy aimed at molecular cloning of the segment of DNA that encodes the receptor (stages 2–5 in Figure 2–1). The core of this strategy is the ability to identify a putative receptor DNA sequence in a representative population of cDNAs (DNA sequences complementary to the messenger RNAs expressed in an appropriate cell or tissue are obtained by means of reverse transcriptase). To do so (stage 2), investigators use biochemical and functional features of the receptor protein as handles for picking out the corresponding DNA. Thus, an antibody raised against the pure receptor protein or nucleic acid sequences based on its amino acid sequence may distinguish a bacterial colony containing putative receptor cDNA from colonies containing irrelevant cDNAs, by binding to receptor antigen expressed in the bacterium (2a) or by hybridizing to receptor DNA (2b), respectively. Alternatively, the population of cDNAs may be expressed as proteins in frog oocytes or vertebrate cells, and the putative receptor cDNA can then be detected by virtue of the protein's signaling function (2c) or its ability to bind a specific ligand (2d).

Once the putative receptor cDNA has been identified, it is "validated" by carefully comparing the function and biochemical properties of the recombinant protein with those of the endogenous receptor that originally triggered the search (3a). The base sequence of the receptor DNA is also determined (3b), so that the amino acid sequence of the complete receptor protein can be deduced and compared with sequences of known receptors. Based on these criteria, it may then be possible to announce the identification of a new receptor (step 4).

A much greater quantity and quality of information flows from molecular cloning of the cDNA encoding a new receptor than from identifying a receptor in the "classic" way. The deduced amino acid sequence almost always resembles those of previously known receptors. Investigators can immediately place the new receptor into a specific class of known receptors, and the structural class tells us how the receptor works—whether it is a receptor tyrosine kinase, a seven-transmembrane region receptor coupled to G proteins, etc. The DNA sequence provides a probe to identify cells and tissues that express messenger RNA encoding the new receptor. Expression of the cDNA in cultured cells gives the pharmaceutical chemist an unlimited supply of recombinant receptor protein for precise biochemical analysis, tests of agonist and antagonist binding, and development of new drugs.

Finally (step 5), the receptor DNA itself provides a tool for identifying yet more receptors. Receptors within a specific class or subclass contain highly conserved regions of similar or identical amino acid (and therefore DNA) sequence. The DNA sequences corresponding to these conserved regions can be used as probes to find sequences of related but potentially novel receptors, either by DNA–DNA hybridization (2b) or as primers in a polymerase chain reaction (PCR) designed to amplify receptor DNA sequences (2e). These probes may lead to cloning DNA encoding a receptor whose ligand is unknown (an "orphan" receptor); the appropriate ligand is then sought by testing for functional and binding interactions with the recombinant receptor.

are described in a later section entitled Signaling Mechanisms and Drug Action.

Other classes of proteins that have been clearly identified as drug receptors include **enzymes,** which may be inhibited (or, less commonly, activated) by binding a drug (eg, dihydrofolate reductase, the receptor for the antineoplastic drug methotrexate); **transport proteins** (eg, Na^+/K^+ ATPase, the membrane receptor for cardioactive digitalis glycosides); and **structural proteins** (eg, tubulin, the receptor for colchicine, an anti-inflammatory agent).

This chapter deals with three aspects of drug receptor function, presented in increasing order of complexity: (1) The first aspect is their function as determinants of the quantitative relation between the concentration of a drug and the pharmacologic re-

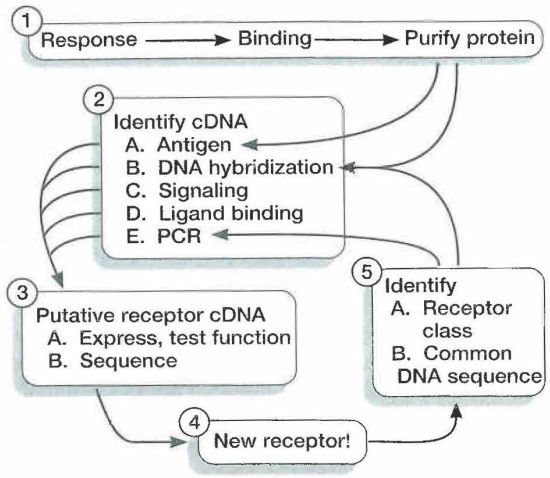

Figure 2–1. Methods used in the discovery and description of receptors. (See box: How Are Receptors Discovered?)

sponse. From this point of view, receptors are simple entities, principally characterized by their affinity for binding drug ligands and their abundance in target cells or tissues. (2) The second aspect is their function as regulatory proteins and components of chemical signaling mechanisms that provide targets for important drugs. Here receptors are considered as complex molecules whose structures and biochemical functions help to explain key features of concentration-effect relations, as well as pharmacologic selectivity. (3) The third aspect is their function as key elements of the therapeutic and toxic effects of drugs in patients. At this highest level of complexity, we discuss the crucial roles receptors play in determining selectivity of drug action, the relation between the dose of a drug and its effects, and the therapeutic usefulness of a drug (ie, therapeutic effectiveness versus toxicity).

RELATION BETWEEN DRUG CONCENTRATION & RESPONSE

The relation between dose of a drug and the clinically observed response may be quite 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. We will analyze this idealized relation first because it underlies virtually all of 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 incremental response diminishes; finally, doses may be reached at which no further increase in response can be achieved. In idealized or in vitro systems, the relation between drug concentration and effect is described by a hyperbolic curve (Figure 2–2A) according to the following equation:

$$E = \frac{E_{max} \times C}{C + EC50}$$

where E is the effect observed at concentration C, E_{max} is the maximal response that can be produced by the drug, and EC50 is the concentration of drug that produces 50% of maximal effect.

This hyperbolic relation resembles the mass action law, which predicts association between two molecules of a given affinity. This resemblance suggests

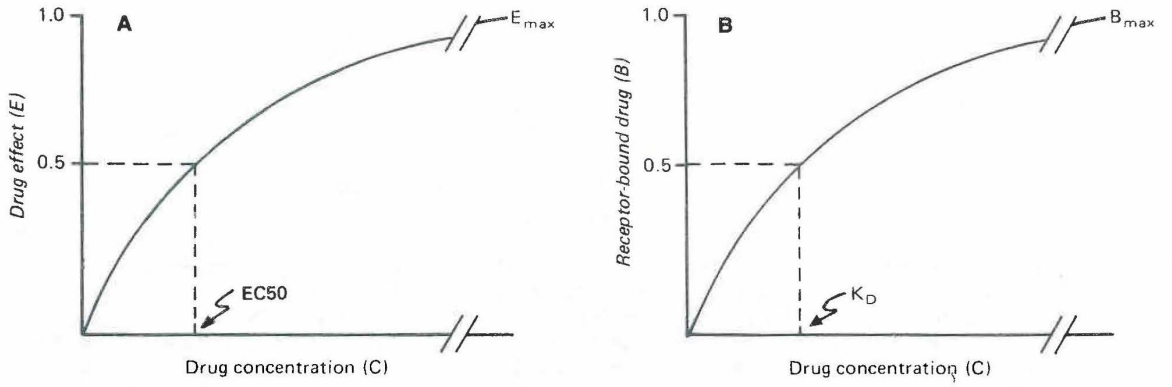

Figure 2–2. Relations between drug concentration and drug effect *(A)* or receptor-bound drug *(B)*. The drug concentrations at which effect or receptor occupancy is half-maximal are denoted EC50 and K_D, respectively.

that drug agonists act by binding to ("occupying") a distinct class of biologic molecules with a characteristic affinity for the drug receptor. With the advent of radioactive receptor ligands, including both agonists and antagonists, this occupancy assumption has been amply confirmed for a number of drug-receptor systems. In these systems, the relation between drug bound to receptors (B) and the concentration of free (unbound) drug (C) depicted in Figure 2–2B is 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). K_D (the equilibrium dissociation constant) indicates 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.

Note also that the EC50 and K_D may be identical but need not be, as discussed below.

Graphic representation of dose-response data is frequently improved by plotting the drug effect (ordinate) against the *logarithm* of the dose or concentration (abscissa). The effect of this purely mathematical maneuver is to transform the hyperbolic curve of Figure 2–2 into a sigmoid curve with a linear midportion (eg, Figure 2–3). This transformation makes it easier to compare different dose-response curves graphically 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). This transformation has no special biologic or pharmacologic significance.

Receptor-Effector Coupling & Spare Receptors

When a receptor is occupied by an agonist, the resulting conformational change is only the first of many steps usually required to produce a pharma-

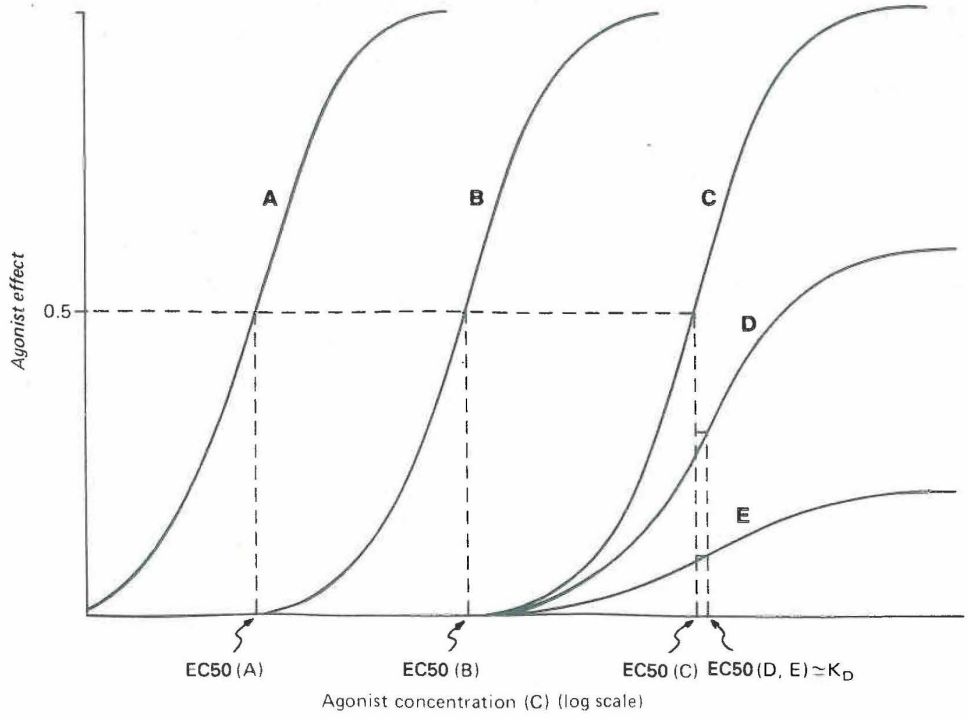

Figure 2–3. Experimental demonstration of spare receptors, using different concentrations of an irreversible antagonist. Curve *A* shows agonist response in the absence of antagonist. After treatment with a low concentration of antagonist (curve *B*), the curve is shifted to the right; maximal responsiveness 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 EC50 of the agonist in curves *D* and *E* may approximate the K_D that characterizes the binding affinity of the agonist for the receptor.

cologic response. The transduction process between occupancy of receptors and drug response is often termed coupling. The relative efficiency of occupancy-response coupling is partially determined by the initial conformational change in the receptor—thus, the effects of full agonists can be considered more efficiently coupled to receptor occupancy than can the effects of partial agonists, as described below. Coupling efficiency is also determined by the biochemical events that transduce receptor occupancy into cellular response.

High efficiency of receptor-effector interaction may also be envisioned as the result of spare receptors. Receptors are said to be "spare" for a given pharmacologic response when the maximal response can be elicited by an agonist at a concentration that does not result in occupancy of the full complement of available receptors. Spare receptors are not qualitatively different from nonspare receptors. They are not hidden or unavailable, and when they are occupied, they can be coupled to response. 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 2–3). Thus, a maximal inotropic response of heart muscle to catecholamines can be elicited even under conditions where 90% of the beta receptors are occupied by a quasi-irreversible antagonist. Accordingly, myocardium is said to contain a large proportion of spare receptors.

How can we account for the phenomenon of spare receptors? In a few cases, the biochemical mechanism is understood, such as for drugs that act on some regulatory receptors. In this situation, the effect of receptor activation—eg, binding of GTP by an intermediate—may greatly outlast the agonist-receptor interaction (see the following section called G Proteins and Second Messengers). In such a case, the "spareness" of receptors is *temporal* in that the response initiated by an individual ligand-receptor binding event persists longer in time than the binding event itself.

In other cases, where the biochemical mechanism is not understood, we imagine that the receptors are *spare in number.* If the concentration or amount of a cellular component other than the receptor limits the coupling of receptor occupancy to response, then a maximal response can occur without occupancy of all receptors. Figure 2–4 illustrates the notion of receptors that are spare in this sense and helps to explain how the sensitivity of a cell or tissue to a particular concentration of agonist may depend not only on the affinity of the receptor for binding an agonist (characterized by the K_D) but also on the total concentration of receptors. Sensitivity may be expressed in terms of EC50, the concentration of agonist that results in half-maximal response. The K_D of the agonist-receptor interaction determines what fraction (B/B_{max}) of

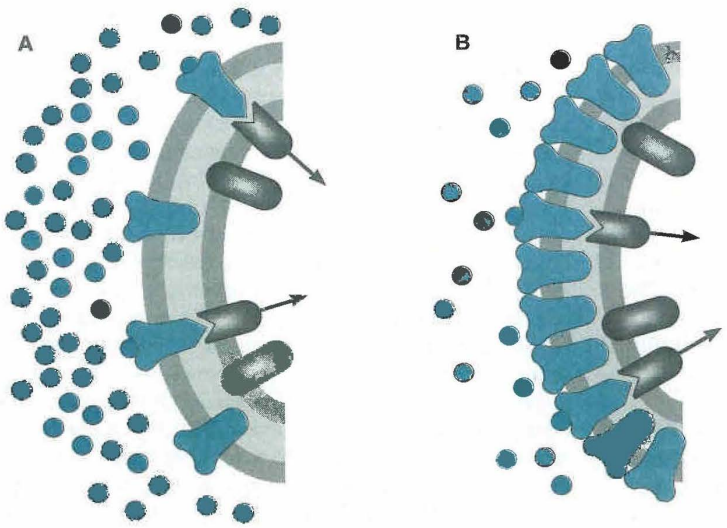

Figure 2–4. Spare receptors increase sensitivity to drug. In panel *A (left),* the free concentration of agonist is equal to the K_D concentration; this is sufficient to bind 50% of the four receptors present, resulting in the formation of two agonist-receptor complexes. (***Note:*** When the agonist concentration is equal to the K_D, half the receptors will be occupied. Remember that $B/B_{max} = C/(C + K_D)$.) Agonist occupancy of these two receptors changes their conformation so that they bind to and activate two effector molecules, resulting in a response. Because two of four effectors are stimulated by agonist-receptor complexes, the response is 50% of maximum. In membrane *B (right),* the receptor concentration has been increased tenfold (not all receptors are shown), and the K_D for binding of agonist to receptors remains unchanged. Now a very much smaller concentration of free agonist (= 0.05 times the K_D) suffices to occupy two receptors and consequently to activate two effector molecules. Thus, the response is 50% of maximum (just as in *A*), even though the agonist concentration is very much lower than the K_D.

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 (as in Figure 2–4). 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. Now most of the receptors are spare in number. As a result, a very much lower concentration of agonist suffices to occupy two 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 the receptor concentration. (***Note:*** Changing the number of receptors does not usually change the free concentration of drug achieved by administering a given dose. This is because the concentration of receptors in a tissue is usually very small relative to effective concentrations of drugs.)

An important biologic consequence of spare receptors is that they allow agonists with low affinity for receptors to produce full responses at low concentrations, to the extent that EC50 is lower than K_D. This is important because ligands with low affinity (high K_D) dissociate rapidly from receptors, allowing rapid termination of biologic responses. High binding affinity (low K_D), on the other hand, would result in slow dissociation of agonist from receptor and correspondingly slower reversal of a biologic response.

Competitive & Irreversible Antagonists

Receptor antagonists bind to the receptor but do not activate it. The effects of these antagonists result from preventing agonists (other drugs or endogenous regulatory molecules) from binding to and activating receptors. Such antagonists are divided into two classes depending on whether or not they reversibly compete with agonists for binding to receptors. The two classes of receptor antagonism produce quite different concentration-effect and concentration-binding curves in vitro and exhibit important practical differences in therapy of disease.

In the presence of a fixed concentration of agonist, increasing concentrations of a **competitive antagonist** progressively inhibit the agonist response; high antagonist concentrations prevent response completely. Conversely, sufficiently high concentrations of agonist can completely surmount the effect of a given concentration of the antagonist, ie, the E_{max} for the agonist remains the same for any fixed concentration of antagonist (Figure 2–5A). Because the antagonism is competitive, the presence of antagonist increases the agonist concentration required for a given degree of response, and so the agonist concentration-effect curve shifts 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 (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 relationship between agonist occupancy of the receptor and response, the K_I can be determined simply and accurately. As shown in Figure 2–5, 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 $[I] = K_I$. K_I values derived from such experiments agree with those determined by direct measurements of binding of radiolabeled competitive antagonists to receptors.

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

(1) The degree of inhibition produced by a competitive antagonist depends upon the concentration of antagonist. Thus, the extent and duration of action of such a drug will depend upon its concentration in plasma and will be critically influenced by the rate of its metabolic clearance or excretion. Different patients receiving a fixed dose of propranolol, for example, exhibit a wide range of plasma concentrations, owing to differences in clearance of the drug. As a result, the effects of a fixed dose of this competitive antagonist of norepinephrine may vary widely in patients, and the dose must be adjusted accordingly.

(2) The equation defines another important source of variability in clinical response to a competitive antagonist, ie, the concentration of agonist that is competing for binding to receptors. Here also propranolol provides a useful example: When this competitive beta-adrenoceptor antagonist is administered in doses sufficient to block the effect of basal levels of the neurotransmitter norepinephrine, resting heart rate is decreased. However, the increase in release of

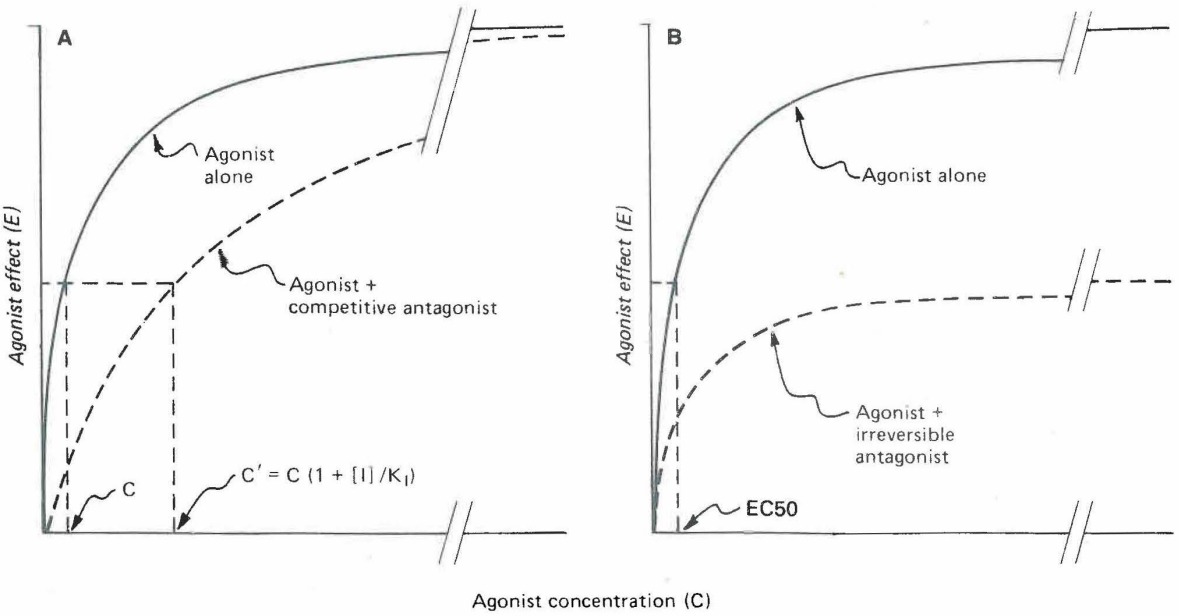

Figure 2–5. Changes in agonist concentration-effect curves produced by a competitive antagonist *(A)* or by an irreversible antagonist *(B)*. In the presence of a competitive antagonist, higher concentrations of agonist are required to produce a given effect; thus, the agonist concentration (C′) required 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 antagonist, which reduces the maximal effect the agonist can achieve, though it may not change its EC50.

norepinephrine and epinephrine that occurs with exercise, postural changes, or emotional stress may suffice to overcome competitive antagonism by propranolol and increase heart rate. Consequently, the physician who devises a dosage regimen for a competitive antagonist must always consider possible changes in endogenous agonist concentration that could influence therapeutic response.

Some receptor antagonists bind to the receptor in an **irreversible** or nearly irreversible fashion. The antagonist's affinity for the receptor may be so high that for practical purposes, the receptor is unavailable for binding of agonist. Other antagonists in this class produce irreversible effects because after binding to the receptor they form covalent bonds with it. After occupancy of a substantial proportion of receptors by such an antagonist, the number of remaining unoccupied receptors may be so low that high concentrations of agonist cannot overcome the antagonism, and a maximal agonist response cannot be obtained (Figure 2–5B). However, if spare receptors are present, a lower dose of an irreversible antagonist, however, may leave enough receptors unoccupied to allow achievement of maximum response to agonist, though a higher agonist concentration will be required (see Receptor-Effector Coupling and Spare Receptors, above).

Therapeutically, irreversible antagonists present distinctive 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 upon the rate of turnover of receptor molecules.

Phenoxybenzamine, an irreversible alpha-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 alpha-adrenoceptor blockade cannot be overcome, excess effects of the drug must be antagonized "physiologically," eg, by using a pressor agent that does not act via alpha receptors.

Partial Agonists

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

duce a lower response, at full receptor occupancy, than do **full agonists.** As compared to full agonists, partial agonists produce concentration-effect curves that resemble curves observed with full agonists in the presence of an antagonist that irreversibly blocks receptor sites (compare Figure 2–3D and 2–6B). Nonetheless, radioligand-binding experiments have demonstrated that partial agonists may occupy all receptor sites (Figure 2–6A) at concentrations that will fail to produce a maximal response comparable to that seen with full agonists (Figure 2–4B). In addition, the failure of partial agonists to produce a "full" maximal response is not due to decreased affinity for binding to receptors. Such drugs compete, frequently with high affinity, for the full complement of receptors. Indeed, the partial agonists' ability to occupy the total receptor population is indicated by the fact that partial agonists competitively inhibit the responses produced by full agonists (Figure 2–6C).

The precise molecular mechanism that accounts for blunted maximal responses to partial agonists is not known. It is simplest to imagine that the partial agonist produces an effect on receptors that is intermediate between the effect produced by a full agonist and that produced by a competitive antagonist. The full agonist changes receptor conformation in a way that initiates subsequent pharmacologic effects of receptor occupancy, while the "pure" competitive an-

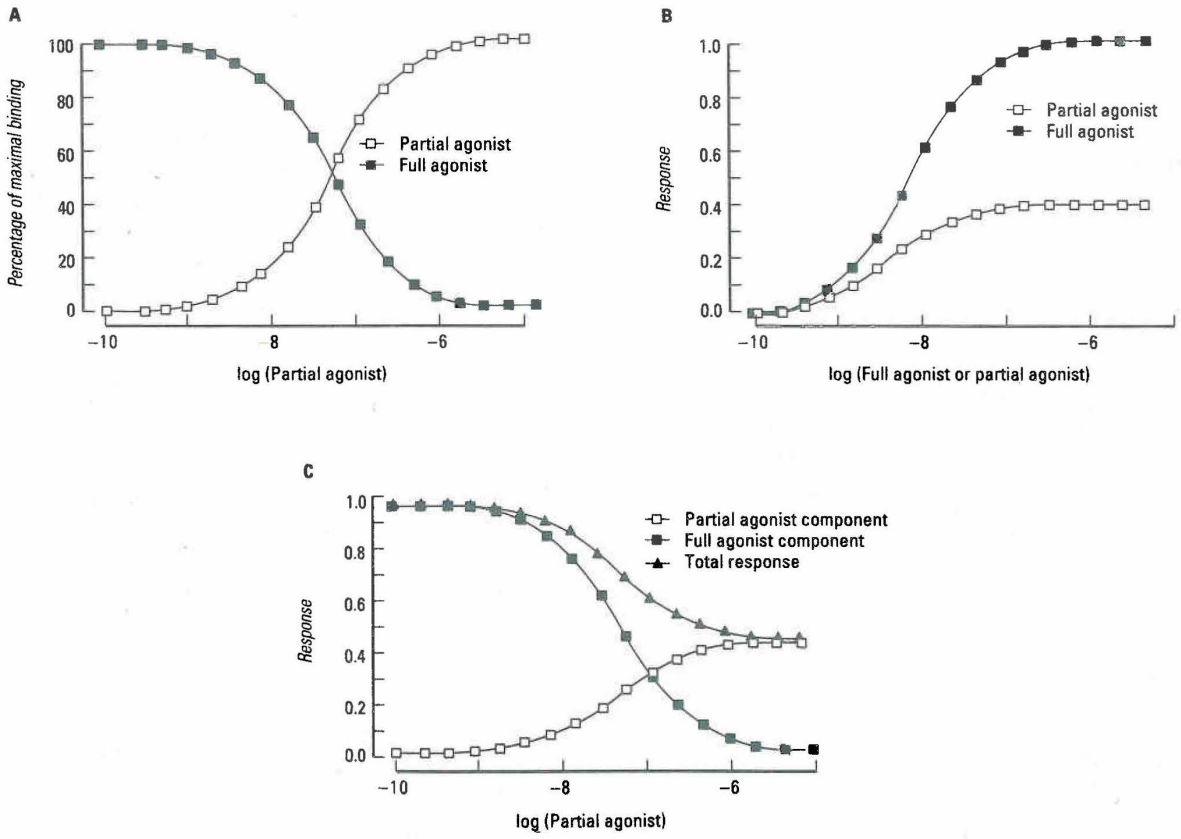

Figure 2–6. ***Panel A:*** The percentage of receptor occupancy resulting from full agonist (present at a single concentration) binding to receptors in the presence of increasing concentrations of a partial agonist. Because the full agonist (filled squares) and the partial agonist (open squares) compete to bind to the same receptor sites, when occupancy by the partial agonist increases, binding of the full agonist decreases. ***Panel 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. ***Panel 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 concentration of the full agonist (filled squares) 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 (open squares) increases, while the total response—ie, the sum of responses to the two drugs (filled triangles)—gradually decreases, eventually reaching the value produced by partial agonist alone (compare panel B).

tagonist produces no such change in receptor conformation; in this view, the partial agonist changes receptor conformation, but not to the extent necessary to result in full activation of the occupied receptor.

To express this idea, pharmacologists refer to the **efficacy** of a drug as a way of indicating the relation between occupancy of receptor sites and the pharmacologic response. A drug may have zero efficacy (ie, may be a pure antagonist) or any degree of efficacy greater than zero. Partial agonists can be viewed as drugs with such low efficacy that even occupancy of the full complement of receptors does not result in the maximal response that can be elicited by other ("full") agonists, which have higher efficacy. The reader will see that many drugs used as competitive antagonists are in fact weak partial agonists.

Other Mechanisms of Drug Antagonism

Not all of the mechanisms of antagonism involve interactions of drugs or endogenous ligands at a single type of receptor. Indeed, **chemical antagonists** need not involve a receptor at all. Thus, one drug may antagonize the actions of a second drug by binding to and inactivating the second drug. 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 antagonizes the other simply by binding it and making it unavailable for interactions with proteins involved in formation of a blood clot.

The clinician often uses drugs that take advantage of **physiologic antagonism** between endogenous regulatory pathways. Many physiologic functions are controlled by opposing regulatory pathways. 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 insulin act on quite distinct receptor-effector systems, the clinician must sometimes administer insulin to oppose the hyperglycemic effects of glucocorticoid hormone, whether the latter are 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, which may be caused by the pain of myocardial infarction, the physician could use isoproterenol, a beta-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 would use of a receptor-specific antagonist such as atropine (a competitive antagonist at the receptors at which acetylcholine slows heart rate).

SIGNALING MECHANISMS & DRUG ACTION

Until now we have considered receptor interactions and drug effects in terms of equations and concentration-effect curves. This abstract analysis explains some quantitative aspects of drug action. For a more complete explanation, we must also understand the molecular mechanisms by which a drug acts. This understanding is particularly important for drugs that mimic or block intercellular signaling by hormones and neurotransmitters.

The research of the past 10 years has revealed in considerable detail the molecular processes that transduce extracellular signals into intracellular messages that control cell function. Understanding these remarkable signaling mechanisms 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? 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? Can the signaling mechanisms explain actions of drugs that do not interact with receptors? Do these mechanisms provide targets for developing new drugs? Our new understanding allows us not only to ask these questions but also—in many cases—to answer them.

Most transmembrane signaling is accomplished by only a few 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.

Four basic mechanisms of transmembrane signaling are well understood (Figure 2–7). Each uses a different strategy to circumvent the barrier posed by the lipid bilayer of the plasma membrane. These strategies are (1) using a lipid-soluble ligand that crosses the membrane and acts on an intracellular receptor; (2) using 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) using a ligand-gated transmembrane ion channel that can be induced to open or close by the binding of a ligand; and (4) using a transmembrane receptor protein to stimulate a GTP-binding signal transducer protein (G protein) that in turn generates an intracellular second messenger.

Of course, the signaling mechanisms for many ex-

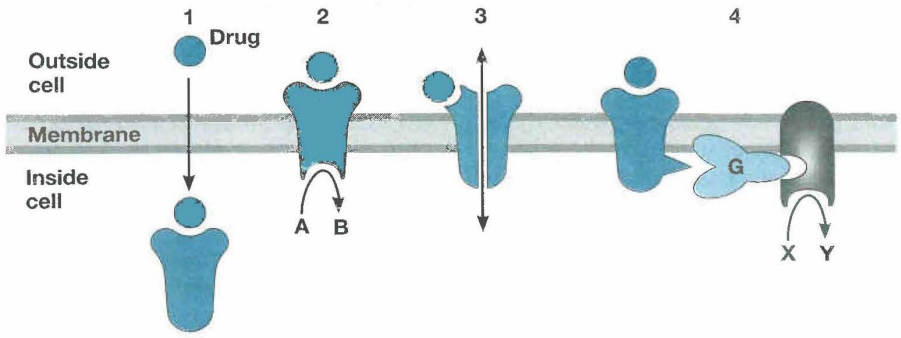

Figure 2–7. Known transmembrane signaling mechanisms: ***1:*** A lipid-soluble chemical signal crosses the plasma membrane and acts on an intracellular receptor (which may be an enzyme or a regulator of gene transcription); ***2:*** the signal binds to the extracellular domain of a transmembrane protein, thereby activating an enzymatic activity of its cytoplasmic domain; ***3:*** the signal binds to and directly regulates the opening of an ion channel; ***4:*** the signal binds to a cell-surface receptor linked to an effector enzyme by a G protein.

tracellular ligands remain unknown (eg, growth hormone, interferon, lymphokines). While the four 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 signals are sufficiently lipid-soluble to cross the plasma membrane and act on intracellular receptors. One of these is a gas, nitric oxide (NO), that acts by stimulating an intracellular enzyme, guanylyl cyclase, which produces cGMP. Signaling via cGMP is described in more detail later in this chapter. Receptors for another class of ligands—including **corticosteroids, mineralocorticoids, sex steroids, vitamin D,** and **thyroid hormone**—stimulate the transcription of genes in the nucleus by binding to specific DNA sequences near the gene whose expression is to be regulated. Many of the target DNA sequences (called **response elements**) have been identified.

The detailed molecular mechanisms used by these "gene-active" receptors are well understood. Structural features common to these receptors suggest that they 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 mechanisms. For example, removal of a carboxyl terminal segment of the glucocorticoid receptor results in a protein that binds to the DNA response element and stimulates transcription of the target gene, even in the absence of glucocorticoids. Like this experimental truncation, binding of glucocorticoid hormone to the normal receptor relieves an inhibitory constraint on the transcription-stimulating activity of the protein. Figure 2–8 schematically depicts the molecular mechanism of

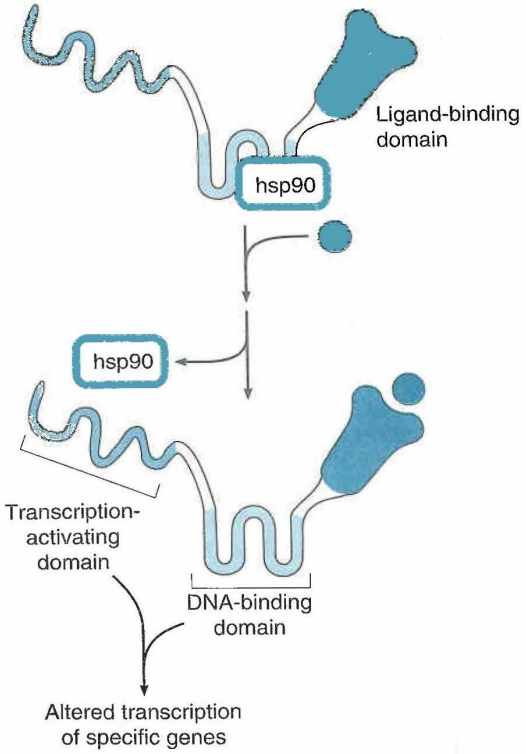

Figure 2–8. Mechanism of glucocorticoid action. The glucocorticoid receptor polypeptide is schematically depicted as a protein with three distinct domains. A heat-shock protein, **hsp90,** binds to the receptor in the absence of hormone and prevents folding into the active conformation of the receptor. Binding of a hormone ligand causes dissociation of the hsp90 stabilizer and permits conversion to the active configuration.

glucocorticoid action: In the absence of hormone, the receptor is bound to hsp90, a protein that appears to prevent 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 acute 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. (The persistence may also be partially due to the high affinity of receptors for the hormone, which results in slow dissociation of the hormone.) Therapeutically, it means that the beneficial (or toxic) effects of a gene-active hormone will usually decrease slowly when administration of the hormone is stopped and that there will be no simple temporal correlation between plasma concentration of the hormone and its effects.

Ligand-Regulated Transmembrane Enzymes Including Protein 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 factor (ANF), transforming growth factor-beta (TGFβ)**, and several 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 2–9). In all these receptors, the two domains are connected by a hydrophobic segment of the polypeptide that crosses the lipid bilayer of the plasma membrane.

The tyrosine kinase signaling pathway begins with hormone binding to the receptor's extracellular domain. The resulting change in receptor conformation causes receptor molecules to bind to one another, which in turn brings together the protein tyrosine kinase domains, which become enzymatically active. Tyrosine residues in both cytoplasmic domains become phosphorylated (each is probably phosphorylated by the other). This cross-phosphorylation can intensify or prolong the duration of allosteric regulation by the hormonal ligand. For example, the tyrosine kinase activity of the autophosphorylated insulin

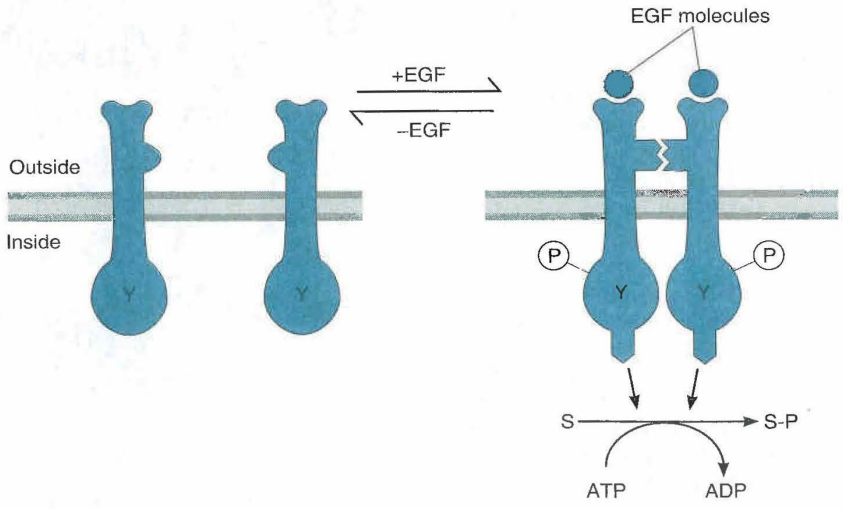

Figure 2–9. Mechanism of activation of the EGF receptor, a representative receptor tyrosine kinase. The receptor polypeptide has extracellular and cytoplasmic domains, depicted above and below the plasma membrane. Upon binding of EGF (circle), the receptor converts from its inactive monomeric state ***(left)*** to an active dimeric state ***(right),*** in which two receptor polypeptides bind noncovalently in the plane of the membrane. The cytoplasmic domains become phosphorylated *(P)* on specific tyrosine residues *(Y)* and their enzymatic activities are activated, catalyzing phosphorylation of substrate proteins *(S)*.

receptor persists after insulin is removed from the binding site. Different receptors catalyze phosphorylation of tyrosine residues on different downstream signaling proteins, but only a few of these substrate proteins have been identified. Insulin, for example, uses a single class of receptors to trigger increased uptake of glucose and amino acids and to regulate metabolism of glycogen and triglycerides in the cell. Similarly, each of the growth factors initiates in its specific target cells a complex program of cellular events ranging from altered membrane transport of protons, other ions, and metabolites to characteristic changes in the expression of many genes. Some of these responses involve phosphorylation by serine and threonine kinases, while others work via transcription factors that may themselves be kinase substrates. The tyrosine kinase receptors provide attractive targets for drug development. At present, a few compounds have been found to produce effects that may be due to inhibition of tyrosine kinase activities. It is easy to imagine therapeutic uses for specific inhibitors of growth factor receptors, especially in neoplastic disorders.

The intensity and duration of action of EGF, PDGF, and other agents that act via this class of receptors are limited by receptor **down regulation.** Ligand binding 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 growing number of regulators of growth and differentiation, including TGFβ, act on receptor serine kinases, another class of transmembrane receptor enzymes. ANF, 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 protein tyrosine kinases, are active in their dimeric forms.

Ligand-Gated Channels

Many of the most useful drugs in clinical medicine act by mimicking or blocking the actions of endogenous ligands that regulate the flow of ions through plasma membrane channels. The natural ligands include **acetylcholine, gamma-aminobutyric acid,** and the **excitatory amino acids** (glycine, aspartate, glutamate, etc). All of these agents are synaptic transmitters.

Each of these 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 (AChR), which allows Na^+ to flow down its concentration gradient into cells, producing a localized excitatory postsynaptic potential—a depolarization.

The nicotinic AChR (Figure 2–10) is one of the best characterized of all cell-surface receptors for hormones or neurotransmitters. This receptor is a pentamer made up of five polypeptide subunits (eg, two alpha chains plus one beta, one gamma, and one delta chain, all with molecular weights ranging from 43,000 to 50,000). These polypeptides, each of which crosses the lipid bilayer four times, form a cylindric structure 8 nm in diameter. When acetylcholine binds to sites on the alpha subunits, a conformational change occurs that results in the transient opening of a central aqueous channel through which sodium ions penetrate from the extracellular fluid into the cell.

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. It contrasts sharply with other molecular signaling mechanisms, which may require seconds, minutes, or even hours, as is the case with gene-active hormones.

G Proteins & Second Messengers

Many extracellular ligands act by increasing the intracellular concentrations of second messengers such

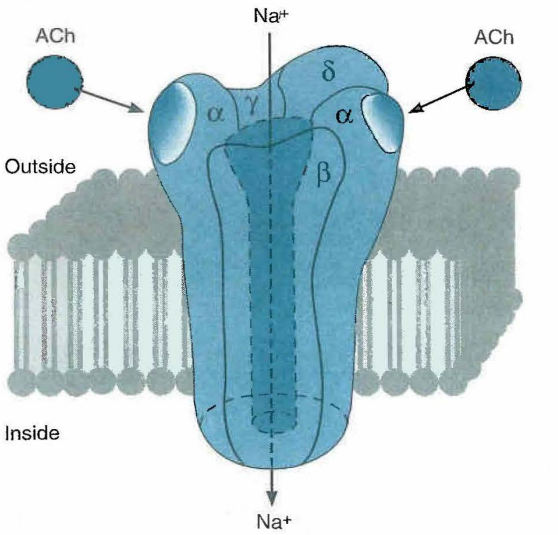

Figure 2–10. The nicotinic acetylcholine receptor, a ligand-gated ion channel. The receptor molecule is depicted as embedded in a rectangular piece of plasma membrane, with extracellular fluid above and cytoplasm below. Composed of five subunits (two α, one β, one γ, and one δ), the receptor opens a central transmembrane ion channel when acetylcholine (ACh) binds to sites on the extracellular domain of its α subunits.

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 specifically detected by a cell-surface receptor. The receptor in turn triggers the activation of a 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 transmembrane protein that converts intracellular ATP to cAMP. The corresponding G protein, called G_s, stimulates adenylyl cyclase after being activated by a host of hormones and neurotransmitters, each of which acts via a specific receptor (see Table 2–1).

G_s and other G proteins use a molecular mechanism that involves binding and hydrolysis of GTP (Figure 2–11). Significantly, this mechanism separates ligand excitation of the receptor from G protein-mediated activation of the effector, thereby allowing the transduced signal to be amplified. For example, a neurotransmitter such as norepinephrine may encounter its membrane receptor for a very short time, only a few milliseconds. When the encounter generates a GTP-bound G_s molecule, however, the duration of activation of adenylyl cyclase depends upon the longevity of GTP binding to G_s rather than upon the receptor's affinity for norepinephrine. Indeed, like other G proteins, GTP-bound G_s characteristically remains active for tens of seconds, which enormously amplifies the original signal. This mechanism explains how signaling by G proteins produces the phenomenon of spare receptors (described above). Even though one ligand-activated receptor molecule is required to initiate GTP binding by one G protein, the slow hydrolysis of GTP causes the active G protein to persist long after the receptor has dissociated from its agonist molecule. So at low concentrations of agonist the proportion of agonist-bound receptors may be much less than the proportion of G proteins in the active (GTP-bound) state; if the proportion of active G proteins correlates with pharmacologic response, receptors will appear to be spare—ie, a small fraction of receptors occupied by agonist at any given time will appear to produce a proportionately larger response.

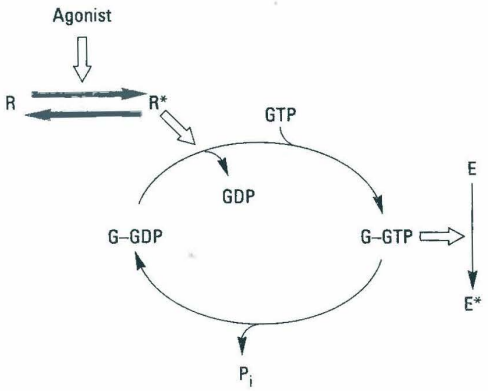

Figure 2–11. The guanine nucleotide-dependent activation-inactivation cycle of G proteins. The agonist activates the receptor *(R)*, which promotes release of GDP from the G protein *(G)*, allowing entry of GTP into the nucleotide binding site. In its GTP-bound state *(G-GTP)*, the G protein regulates activity of an effector enzyme or ion channel *(E)*. The signal is terminated by hydrolysis of GTP, followed by return of the system to the basal unstimulated state. Open arrows denote regulatory effects.

Table 2–1. A partial list of endogenous ligands and their associated second messengers.

Ligand	Second Messenger
Adrenocorticotropic hormone	cAMP
Acetylcholine (muscarinic receptors)	Ca^{2+},phosphoinositides
Angiotensin	Ca^{2+},phosphoinositides
Catecholamines (α_1-adrenoceptors)	Ca^{2+},phosphoinositides
Catecholamines (β-adrenoceptors)	cAMP
Chorionic gonadotropin	cAMP
Follicle-stimulating hormone	cAMP
Glucagon	cAMP
Histamine (H_2 receptors)	cAMP
Luteinizing hormone	cAMP
Melanocyte-stimulating hormone	cAMP
Parathyroid hormone	cAMP
Platelet-derived growth factor	Ca^{2+},phosphoinositides
Prostacyclin, prostaglandin E_2	cAMP
Serotonin (5-HT_4 receptors)	cAMP
Serotonin (5-HT_{1C} and 5-HT_2 receptors)	Ca^{2+},phosphoinositides
Thyrotropin	cAMP
Thyrotropin-releasing hormone	Ca^{2+},phosphoinositides
Vasopressin (V_1 receptors)	Ca^{2+},phosphoinositides
Vasopressin (V_2 receptors)	cAMP

The family of G proteins is quite diverse (Table 2–2); in addition to G_s, the stimulator of adenylyl cyclase, it includes other subfamilies. Members of the G_i ("i" for inhibitory) subfamily couple receptors to inhibition of adenylyl cyclase; G_i proteins also mediate receptor stimulation of the phosphoinositide second messenger system in some cells (see below) and regulation of K^+ and Ca^{2+} channels. The G_i subfamily includes two G proteins (G_{t1} and G_{t2}, also called "transducins"), that mediate phototransduction in retinal rods and cones.

Not surprisingly, receptors coupled to G proteins are structurally related to one another, comprising a family of "serpentine receptors," so called because the receptor polypeptide chain crosses the plasma membrane seven times (Figure 2–12). Receptors for adrenergic amines, serotonin, acetylcholine (muscarinic but not nicotinic), many peptide hormones,

Table 2–2. G proteins and their receptors and effectors.

G Protein	Receptors for:	Effector/Signaling Pathway
G_s	β-Adrenergic amines, glucagon, histamine, serotonin, 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 (eg, muscarinic), bombesin, serotonin (5-HT_{1C}), 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)

odorants, and even visual receptors (in retinal rod and cone cells) all belong to the serpentine family. The amino and carboxyl terminals of each of these receptors are located on the extracellular and cytoplasmic sides of the membrane, respectively. Different serpentine receptors resemble one another rather closely in amino acid sequences and in the locations of their hydrophobic transmembrane regions and hydrophilic extra- and intracellular loops, suggesting that all were derived from a common evolutionary precursor.

In parallel with these structural similarities, it appears that serpentine receptors transduce signals across the plasma membrane in essentially the same way. Often the agonist ligand—eg, a catecholamine, acetylcholine, or the photon-activated chromophore of retinal photoreceptors—is bound in a pocket enclosed by the transmembrane regions of the receptor (as in Figure 2–12). The resulting change in conformation of these regions is transmitted to cytoplasmic loops of the receptor, which in turn activate the appropriate G protein by promoting replacement of GDP by GTP, as described above. Considerable biochemical evidence indicates that the G proteins interact with amino acids in the third cytoplasmic loop of the receptor polypeptide (arrowed in Figure 2–12). The carboxyl terminal tails of these receptors, also located in the cytoplasm, can regulate the receptors' ability to interact with G proteins, as described below.

Receptor Desensitization

Receptor-mediated responses to drugs and hormonal agonists often "desensitize" with time (Figure 2–13, top). After reaching an initial high level, the response (eg, cellular cAMP accumulation, Na^+ influx, contractility, etc) gradually diminishes over seconds or minutes, even in the continued presence of the agonist. This desensitization is usually reversible. Thus, 15 minutes after removal of the agonist, a second exposure to agonist results in a response similar to the initial response. (Note that this ready reversibility distinguishes desensitization from down-regulation of the *number* of receptors, as described above for receptor tyrosine kinases.)

Although many kinds of receptors undergo desensitization, the mechanism is in most cases obscure (eg, agonist-induced desensitization of the nicotinic acetylcholine receptor). The molecular mechanism of agonist desensitization has been worked out in some detail, however, in the case of the beta adrenoceptor (Figure 2–13, bottom): Binding of agonist induces a change in conformation of the receptor's carboxyl terminal tail, making it a good substrate for phosphorylation on serine (and threonine) residues by a specific kinase, β-adrenoceptor kinase (also termed βARK). The presence of phosphoserines increases the receptor's affinity for binding a third protein, β-arrestin. Binding of β-arrestin to cytoplasmic loops of the receptor diminishes the receptor's ability to interact with G_s, thereby reducing the agonist response (ie, stimulation of adenylyl cyclase). Upon removal of agonist, however, cellular phosphatases remove phosphates from the receptor and βARK stops putting them back on, so that the receptor—and consequently the agonist response—return to normal (Figure 2–13, bottom).

Well-Established Second Messengers

A. 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 beta-adrenomimetic catecholamines), vasopressin-mediated conservation of water by the kidney, Ca^{2+} homeostasis (regulated by parathyroid hormone), and increased rate and contraction force of the heart muscle (beta-adrenomimetic catecholamines). It also regulates the production of adrenal and sex steroids (in response to corticotropin or follicle-stimulating hormone), the relaxation of smooth muscle, and many other endocrine and neural processes.

cAMP exerts most of its effects by stimulating cAMP-dependent protein kinases (Figure 2–14). These tetrameric 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, which then diffuse through the cytoplasm and nucleus, where they transfer phos-

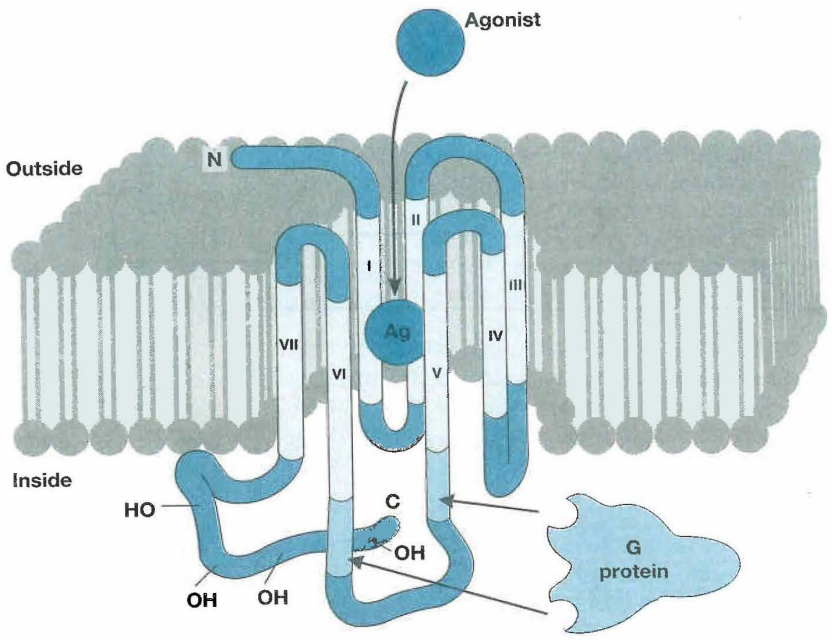

Figure 2–12. Transmembrane topology of a typical serpentine receptor. The receptor's amino *(N)* terminal is extracellular (above the plane of the membrane), and its carboxyl *(C)* terminal intracellular. The terminals are connected by a polypeptide chain that traverses the plane of the membrane seven times. The hydrophobic transmembrane segments (speckled) are designated by roman numerals *(I–VII)*. The agonist *(Ag)* approaches the receptor from the extracellular fluid and binds to a site surrounded by the transmembrane regions of the receptor protein. G proteins *(G)* interact with cytoplasmic regions of the receptor, especially with portions of the third cytoplasmic loop between transmembrane regions *V* and *VI*. The receptor's cytoplasmic terminal tail contains numerous serine and threonine residues whose hydroxyl (–OH) groups can be phosphorylated. This phosphorylation may be associated with diminished receptor-G protein interaction.

phate from ATP to appropriate substrate proteins, often enzymes.

The specificity of cAMP's regulatory effects resides in the distinct protein substrates of the kinase that are expressed in different cells. For example, liver is rich in phosphorylase kinase and glycogen synthase, enzymes whose reciprocal regulation by cAMP-dependent phosphorylation governs carbohydrate storage and release; adipocytes are rich in a lipase whose cAMP-dependent phosphorylation mediates free fatty acid release from fat cells. Similarly, phosphorylation of a kinase specific for the light chains of myosin (called myosin light chain kinase, or MLCK) is involved in relaxation of smooth muscle by beta-adrenomimetic amines. Other cell-specific responses to cAMP as a second messenger similarly depend upon the many enzymes available for regulation by phosphorylation.

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 distinct cyclic nucleotide phosphodiesterases (PDE, Figure 2–14). Competitive inhibition of cAMP degradation is one way caffeine, theophylline, and other methylxanthines produce their effects (see Chapter 19).

B. Calcium and Phosphoinositides: A second well-studied second messenger system involves hormonal stimulation of phosphoinositide hydrolysis (Figure 2–15). Some of the hormones, neurotransmitters, and growth factors that trigger this pathway (see Table 2–1) bind to receptors linked to G proteins, while others bind to tyrosine kinase receptors. In all cases, however, the crucial step is stimulation of a membrane enzyme, phospholipase C (PLC), which specifically hydrolyzes a minor phospholipid component of the plasma membrane called phosphatidylinositol-4,5-bisphosphate (PIP_2). PIP_2 is split into two second messengers, diacylglycerol (DAG) and inositol-1,4,5-trisphosphate (IP_3). The first of these messengers is confined to the membrane, where it activates a phospholipid- and calcium-sensitive protein kinase called protein kinase C. The other messenger, IP_3, is water-soluble and diffuses through the cytoplasm, where it triggers the release of Ca^{2+} from

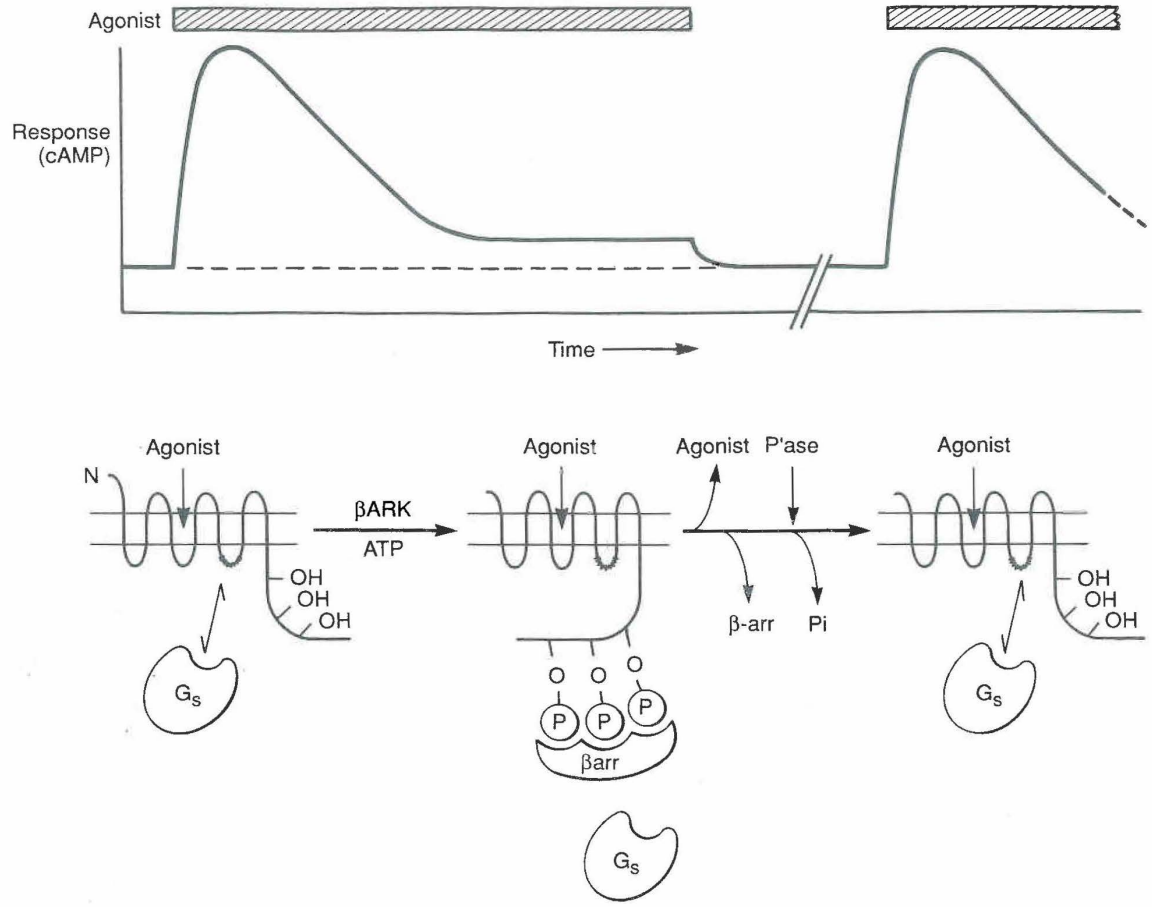

Figure 2–13. Possible mechanism for desensitization of the beta adrenoceptor. The upper part of the figure depicts the response to a β-adrenoceptor agonist (ordinate) versus time (abscissa). The break in the time axis indicates passage of time in the absence of agonist. Temporal duration of exposure to agonist is indicated by the diagonally hatched bar. The lower part of the figure schematically depicts agonist-induced phosphorylation *(P)* by β-adrenoceptor kinase (beta-adrenergic receptor kinase, βARK) of carboxyl terminal hydroxyl groups (–OH) of the beta adrenoceptor. This phosphorylation *(P)* induces binding of a protein, β-arrestin (β-arr), which prevents the receptor from interacting with G_s. Removal of agonist for a short period of time allows dissociation of β-arr, removal of phosphate *(Pi)* from the receptor by phosphatases *(P'ase)*, and restoration of the receptor's normal responsiveness to agonist.

internal storage vesicles. Elevated cytoplasmic Ca^{2+} concentration promotes the binding of Ca^{2+} to the calcium-binding protein calmodulin, which regulates activities of other enzymes, including calcium-dependent protein kinases.

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

Much of our understanding of the biologic roles of phosphoinositide second messengers comes from the use of pharmacologic agents that activate either the Ca^{2+} or the protein kinase C pathways. The concentration of cytoplasmic Ca^{2+} can be elevated by calcium ionophores, while protein kinase C is directly stimulated by binding phorbol esters or synthetic diacylglycerols. One or both of these classes of agents may reproduce the biologic response triggered by a physiologic signal using the phosphoinositide pathway.

As in the cAMP system, multiple mechanisms exist to damp or terminate signaling by this pathway. IP_3 is rapidly 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;

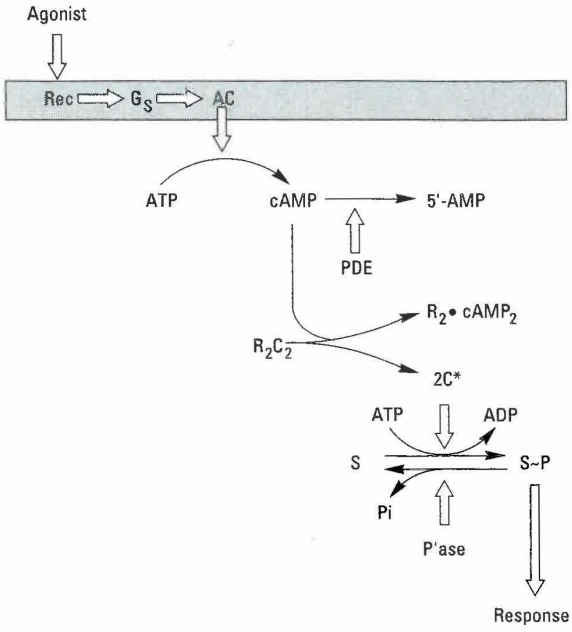

Figure 2–14. The cAMP second messenger pathway. Key proteins include hormone receptors *(Rec)*, a stimulatory G protein (G_s), catalytic adenylyl cyclase *(AC)*, phosphodiesterases *(PDE)* that hydrolyze cAMP, cAMP-dependent kinases, with regulatory *(R)* and catalytic *(C)* subunits, protein substrates *(S)* of the kinases, and phosphatases *(P'ase)*, which remove phosphates from substrate proteins. Open arrows denote regulatory effects.

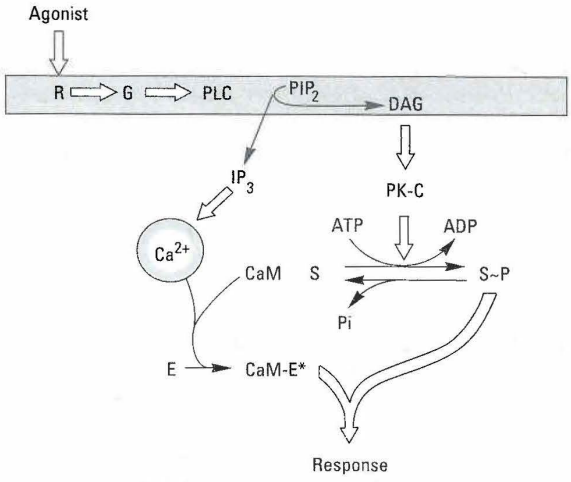

Figure 2–15. The Ca^{2+}/phosphoinositide signaling pathway. Key proteins include hormone receptors *(R)*, a G protein *(G)*, a phosphoinositide-specific phospholipase C *(PLC)*, protein kinase C *(PK-C)*, substrates of the kinase *(S)*, calmodulin *(CaM)*, and calmodulin-binding enzymes *(E)*, including kinases, phosphodiesterases, etc. (PIP_2, phosphatidylinositol-4,5-bisphosphate; DAG, diacylglycerol. Open arrows denote regulatory effects.)

Ca^{2+} is actively removed from the cytoplasm by Ca^{2+} pumps.

These and other nonreceptor elements of the calcium-phosphoinositide signaling pathway are now becoming targets for drug development. For example, the therapeutic effects of lithium ion, an established agent for treating manic-depressive illness, may be mediated by effects on the metabolism of phosphoinositides (see Chapter 28).

C. cGMP: Unlike cAMP, the ubiquitous and versatile carrier of diverse messages, cGMP (cyclic guanosine-3′,5′-monophosphate) 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. In these smooth muscle cells, cGMP synthesis can be elevated by two different transmembrane signaling mechanisms, utilizing two different guanylyl cyclases. Atrial natriuretic factor (ANF), a blood-borne peptide hormone, stimulates a transmembrane receptor by binding to its extracellular (ligand binding) domain; this binding event triggers activation of the guanylyl cyclase activity that resides in the receptor's intracellular domain. The other mechanism takes advantage of the fact that cell membranes are permeable to the stimulating ligand, nitric oxide (NO, a gas). The nitric oxide is generated in vascular endothelial cells, in response to natural vasodilator agents such as acetylcholine and histamine (nitric oxide is also called endothelium-derived relaxing factor, EDRF). After entering the cell, nitric oxide binds to and activates a cytoplasmic guanylyl cyclase. A number of useful vasodilating drugs act by generating or mimicking nitric oxide (see Chapters 11 and 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.

Phosphorylation: A Common Theme

Almost all second messenger signaling involves reversible phosphorylation. It plays a key role at every step, from regulation of receptors (autophosphorylation of tyrosine kinases and desensitization of receptors linked to G proteins) to kinases regulated by second messengers, and finally to substrates of these kinases that may themselves be kinases. These covalent modifications perform 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.

RECEPTOR CLASSES & DRUG DEVELOPMENT

As we have seen, 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, more than one class of receptor seems to have evolved for every chemical signal. For example, structure-activity studies with chemical congeners of acetylcholine, histamine, and catecholamines have identified multiple receptors for each of these endogenous ligands. Ligand-binding and molecular cloning techniques continue to reveal additional receptors eg, two classes of vasopressin receptors, five molecular species of muscarinic receptors (only three of which are distinguishable by ligand-binding techniques), and multiple classes of receptors for dopamine, opioid peptides, serotonin, and others.

Why do multiple receptors for a single ligand exist? The answer is quite straightforward: Cells use more than one signaling pathway to respond to each endogenous ligand, and therefore need more than one receptor for the same ligand. Thus, acetylcholine uses a nicotinic AChR to initiate a fast excitatory postsynaptic potential (EPSP) in the postganglionic cells of autonomic ganglia and muscarinic receptors to evoke a slow EPSP, which modulates responsiveness to the fast EPSP in the same cells.

The existence of multiple receptors for each endogenous signaling ligand creates many important opportunities for drug development. Although each endogenous ligand produces multiple clinical effects, it is often therapeutically advantageous to block or mimic one set of effects without affecting the others. Subtle structural differences in the binding sites of two receptors for a ligand can make them bind congeners of the ligand with different affinities. If the affinities are sufficiently different, it may be possible to develop a drug that acts selectively, producing its effects through one receptor and not the other. Thus, β-adrenoceptor antagonists can block cardioacceleration produced by norepinephrine without preventing norepinephrine from constricting blood vessels via α_1 adrenoceptors. Clinical uses of receptor-selective drugs are described in almost every chapter of this book.

New drug development is not confined to agents that act on receptors for extracellular chemical signals. Pharmaceutical chemists are now determining whether elements of signaling pathways distal to the receptors may also serve as targets of selective and useful drugs. For example, clinically useful agents might be developed that act selectively on specific G proteins, kinases, phosphatases, or the enzymes that degrade second messengers.

RELATION BETWEEN DRUG DOSE & CLINICAL RESPONSE

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 physician 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. Because the patient is never an idealized system, the physician will not have precise information about the physicochemical nature of the receptors in-

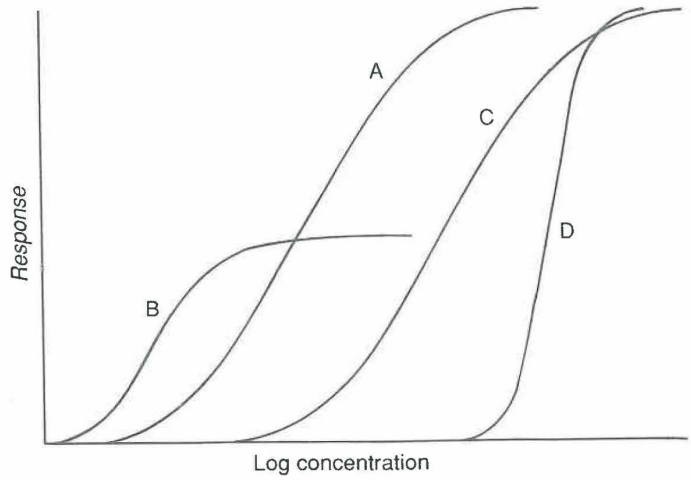

Figure 2–16. Graded dose-response curves for four drugs, illustrating different pharmacologic potencies and different maximal efficacies.

volved, the number of receptors, or their affinity for drugs. Nonetheless, in order to make rational therapeutic decisions, the physician 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 physician 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 reference to Figure 2–16, which depicts graded dose-response curves that relate dose of four different drugs to the magnitude of a particular therapeutic effect, eg, lowering of blood pressure in a hypertensive patient or increasing urinary excretion of sodium in a patient with congestive heart failure.

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 2–16. Potency refers to the concentration (EC50) or dose (ED50) of a drug required to produce 50% of that drug's maximal effect. Thus, the pharmacologic potency of drug A in Figure 2–16 is less than that of drug B, a partial agonist, because the ED50 of A is greater than the ED50 of B. Note that some doses of drug A can produce larger effects than any dose of drug B, despite the fact that we term drug B pharmacologically more potent. The reason for this is that drug A has a larger **maximal efficacy,** as described below.

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. As described above, both affinity and coupling efficiency contribute to the EC50 of a particular concentration-response relation in vitro.

For clinical use, it is helpful to distinguish between a drug's **potency** and its **efficacy.** The clinical effectiveness of a drug depends not on its potency (EC50), but on its maximal efficacy (see below) and its ability to reach the relevant receptors. This ability can depend on its route of administration, absorption, distribution through the body, and clearance from the blood or site of action. In deciding which of two drugs to administer to a patient, the physician must usually consider their relative effectiveness rather than their relative potency. However, pharmacologic potency can largely determine the administered dose of the chosen drug. In general, low potency is important only if the drug has to be administered in inconveniently large amounts.

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 μg/kg/min for an increase in heart rate of 25 beats/min). Relative potency, the ratio of equieffective 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 2–16 have equal maximal efficacy, while all have greater maximal efficacy than does 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, described above)* or by characteristics of the receptor-effector system involved. Thus, diuretics that act on one portion of the nephron may produce much greater excretion of fluid and electrolytes than diuretics that act elsewhere. In addition, the 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: While the responses depicted in curves A, B, and C of Figure 2–16 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 could 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). Such steep dose-response curves could also be produced by a receptor-effector system in which most receptors must be occupied before any effect is seen.

C. Quantal Dose-Effect Curves: Despite their usefulness for characterizing the actions of drugs, 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 relationship 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 2–17). 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 beats/min 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; ie, a frequency distribution of such responses plotted against the log of the dose produces a gaussian normal curve of variation (Figure 2–17). 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 2–17).

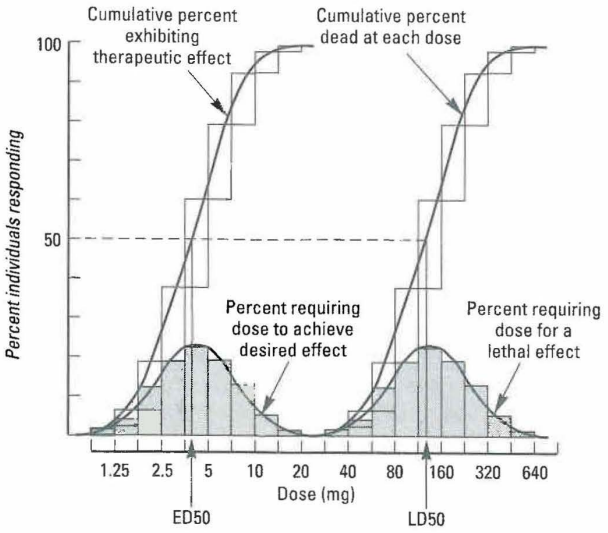

Figure 2–17. Quantal dose-effect plots. Shaded boxes (and the accompanying curves) indicate the frequency distribution of doses of drug required to produce a specified effect; ie, the percentage of animals that required a particular dose to exhibit the effect. The open boxes (and the corresponding curves) indicate the cumulative frequency distribution of responses, which are lognormally distributed.

The quantal dose-effect curve is often characterized by stating the **median effective dose** (ED50), the dose at which 50% of individuals exhibit the specified quantal effect. (Note that the abbreviation ED50 has a different meaning in this context from its meaning in relation to graded dose-effect curves, described above.) Similarly, the dose required to produce a particular toxic effect in 50% of animals is called the **median toxic dose** (TD50). If the toxic effect is death of the animal, a median lethal dose (LD50) may be experimentally defined. Such values provide a convenient way of comparing the potencies of drugs in experimental and clinical settings: Thus, if the ED50s of two drugs for producing a specified

*Note that "maximal efficacy," used in a therapeutic context, does not have exactly the meaning 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 upon a host of other factors, noted in the text.

quantal effect are 5 and 500 mg, respectively, then the first drug can be said to be 100 times more potent than the second for that particular effect. Similarly, one can obtain a valuable index of the selectivity of a drug's action by comparing its ED50s for two different quantal effects in a population (eg, cough suppression versus sedation for opiate drugs; increase in heart rate versus increased vasoconstriction for sympathomimetic amines; anti-inflammatory effects versus sodium retention for corticosteroids; etc).

Quantal dose-effect curves may also be used to generate information regarding the margin of safety to be expected from a particular drug used to produce a specified effect. One measure, which relates the dose of a drug required to produce a desired effect to that which produces an undesired effect, is the **therapeutic index.** In animal studies, the therapeutic index is usually defined as the ratio of the TD50 to the ED50 for some therapeutically relevant effect. The precision possible in animal experiments may make it useful to use such a therapeutic index to estimate the potential effectiveness 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 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 great 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 2–16 and 2–17). 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 responsiveness 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 in comparison to 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.**

The general clinical implications of individual variability in drug responsiveness are clear: The physician must be prepared to change either the dose of drug or the choice of drug, depending upon the response observed in the patient. Even before administering the first dose of a drug, the physician 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, 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. The classification described below is necessarily artificial in that most variation in clinical responsiveness is caused by more than one mechanism. Nonetheless, the classification may be useful because certain mechanisms of variation are best dealt with according to different therapeutic strategies.

A. Alteration in Concentration of Drug That Reaches the Receptor: 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 (see Chapter 3). Any of these pharmacokinetic differences may alter the concentration of drug that reaches relevant receptors and thus alter clinical response. Some differences can be predicted on the basis of age, weight, sex, disease state, or liver and kidney function of the patient, and such predictions may be used to guide quantitative decisions regarding an initial dosing regimen. Repeated measurements of drug concentrations in blood during the course of treatment are often helpful in dealing with the variability of clinical response caused by pharmacokinetic differences among individuals.

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, will markedly slow the heart rate of a patient whose endogenous catecholamines are elevated (as in pheochromocytoma) but will 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 low amounts of angiotensin.

C. Alterations in Number or Function of Receptors: Experimental studies have documented changes in drug responsiveness 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. Although such changes have not been rigorously documented in human beings, it is likely that they account for much of the individual variability in response to some drugs, particularly those that act at receptors for hormones, biogenic amines, and neurotransmitters. In some cases, the change in receptor number is caused by other hormones; for example, thyroid hormones increase both the number of beta receptors in rat heart muscle and the 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 ("down-regulation") or coupling efficiency of its receptors. Receptor-specific desensitization mechanisms presumably act physiologically to allow cells to adapt to changes in rates of stimulation by hormones and neurotransmitters in their environment. These mechanisms (discussed above, under Signaling Mechanisms and Drug Actions) 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).

Various therapeutic strategies can be used to deal with receptor-specific changes in drug responsiveness, depending on the clinical situation. Tolerance to the action of a drug may require raising the dose or substituting a different drug. The down- (or up-) regulation of receptors may make it dangerous to discontinue certain drugs. The patient may have to be weaned slowly from the drug and watched carefully for signs of a withdrawal reaction.

D. Changes in Components of Response Distal to 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 physician 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. Once treatment is begun, the most important potential cause of failure to achieve a satisfactory response is that the diagnosis is wrong or physiologically incomplete. Thus, congestive heart failure will not respond satisfactorily to agents that increase myocardial contractility if the underlying pathologic mechanism is unrecognized stenosis of the mitral valve rather than myocardial insufficiency. Conversely, drug therapy will always be 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, treatment may still not produce an optimal result. 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 response.

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. (Consider that the human genome encodes more than 10^5 different peptide gene products and that the chemical complexity of

each of these peptides is sufficient to provide many different potential binding sites.) 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 multiple cell types and would be coupled to many other biochemical functions; as a result, the patient and the physician 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. As we have seen, selectivity can be measured by comparing binding affinities of a drug to different receptors or by comparing ED50s for different effects of a drug in vivo. In drug development and in clinical medicine, selectivity is usually considered by separating effects into two categories: **beneficial** or **therapeutic effects** versus **toxic effects.** Pharmaceutical advertisements and physicians 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.

It is important to recognize that the designation of a particular drug effect as either therapeutic or toxic is a value judgment and not a statement about the pharmacologic mechanism underlying the effect. As a value judgment, such a designation depends on the clinical context in which the drug is used.

It is only because of their selectivity that drugs are useful in clinical medicine. Thus, it is important, both in the management of patients and in the development and evaluation of new drugs, to analyze ways in which beneficial and toxic effects of drugs may be related, in order to increase selectivity and usefulness of drug therapy. Figure 2–18 depicts three possible relations between the therapeutic and toxic effects of a drug based on analysis of the receptor-effector mechanisms involved.

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 (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 (eg, sedative-hypnotics ordinarily should not be used to treat patients whose complaints of insomnia are due to underlying psychiatric depression).

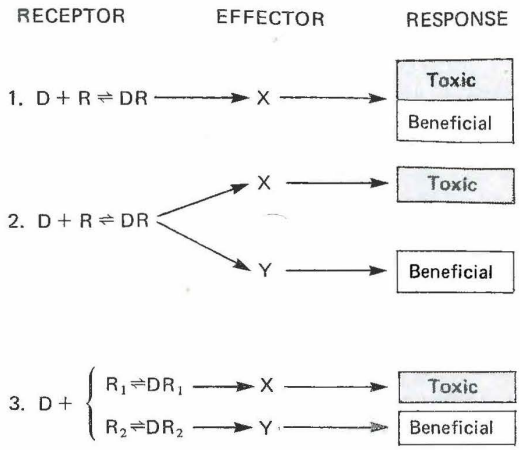

Figure 2–18. Possible relations between the therapeutic and toxic effects of a drug, based on different receptor-effector mechanisms. Therapeutic implications of these different relations are discussed in the text.

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. For example, prazosin (Chapter 11) lowers blood pressure in essential hypertension by acting as an alpha$_1$-selective antagonist on receptors in vascular smooth muscle; as an inevitable consequence, patients will suffer from symptoms of postural hypotension if the dose of drug is large enough. (Note that postural hypotension has been called a "side effect" of prazosin, though in fact it is a direct effect, closely related to the drug's principal therapeutic action.) Appropriate management of such a problem takes advantage of the fact that blood pressure is regulated by changes in blood volume and tone of arterial smooth muscle in addition to the sympathetic nerves. Thus, concomitant administration of diuretics and vasodilators may allow the dose of prazosin to be lowered, with relief of postural hypotension and continued control of blood pressure.

B. Beneficial and Toxic Effects Mediated by Identical Receptors But in Different Tissues or by Different Effector Pathways: Examples of drugs in this category include digitalis glycosides, which may be used to augment cardiac contractility but also produce cardiac arrhythmias, gastrointestinal effects, and changes in vision (all probably mediated by inhibition of Na^+/K^+ ATPase in cell membranes); methotrexate, used to treat leukemia and other neoplastic diseases, which also kills normal cells in bone marrow and gastrointestinal mucosa (all mediated by inhibition of the enzyme dihydrofolate reductase); and congeners of glucocorticoid hormones, used to treat asthma or inflammatory disorders, which also can produce protein catabolism, psychosis, and other

toxicities (all thought to be mediated by similar or identical glucocorticoid receptors). In addition to these and other well-documented examples, it is likely that adverse effects of many drugs are mediated by receptors identical to those which produce the recognized beneficial effect.

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, recognizing that complete abolition of signs or symptoms of the disease may not be achieved. Second (as described above for prazosin), 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. Such "anatomic" selectivity may be achieved, for example, by aerosol administration of a glucocorticoid to the bronchi or by selective arterial infusion of an antimetabolite into an organ containing tumor cells.

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. Such drugs include the alpha- and beta-selective adrenoceptor agonists and antagonists, the H_1 and H_2 antihistamines, nicotinic and muscarinic blocking agents, and receptor-selective steroid hormones. All of these receptors are grouped in functional families, each responsive to a small class of endogenous agonists. The receptors and their associated therapeutic uses were discovered by analyzing effects of the physiologic chemical signals—catecholamines, histamine, acetylcholine, and corticosteroids.

A number of other drugs were discovered in a similar way, although they may not act at receptors for known hormones or neurotransmitters. These drugs were discovered by exploiting toxic effects of other agents, observed in a different clinical context. Examples include quinidine, the sulfonylureas, thiazide diuretics, tricyclic antidepressants, monoamine oxidase inhibitors, and phenothiazine antipsychotics among many others.

It is likely that some of these drugs will eventually be shown to act via receptors for endogenous agonists, as is the case with morphine, a potent analgesic agent. Morphine has been shown to act on receptors physiologically stimulated by the opioid peptides. Pharmacologists have now defined several subclasses of opioid receptors in a fashion reminiscent of earlier studies of autonomic receptors.

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

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