



Introduction to the Pharmaceutical Sciences

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Cellular processes are detected, modulated, and directed by complex systems made up of diverse biomolecules such as DNA, RNA, proteins, lipids, and carbohydrates. These molecules regulate the internal environment of living organisms and are essential to survival. Many diseases and illnesses are linked to problems in the synthesis or properties of a particular biomolecule. A **drug target** is a biomolecule that plays a role in a disease process and is the intended site of action of a drug. Proteins, particularly enzymes and cell membrane receptors, are the most common targets of existing drugs and of drug discovery research. Other less common targets are hormones, ion channels, DNA, and nuclear receptors.

Proteins are the engines of the cell, regulating activation of genes, relaying signals within and between cells, and driving metabolic processes. Proteins are required for the structure, function, and regulation of the body's cells, tissues, and organs. For cells and tissues to remain healthy they must be able to make proteins, and the proteins they make must be able to function correctly. Changes in the composition or abundance of critical proteins can lead to disease.

Drug-protein interactions play a vital role in almost all aspects of a drug's behavior and function. Many of today's drugs work by fitting into pockets, channels, or pores in proteins. Furthermore, drugs are transported, metabolized, and excreted with the help of proteins. This chapter discusses the role of proteins as important drug targets, and the nature of drug-protein interactions responsible for drug pharmacokinetics and action.

Proteins and Ligands

Proteins are folded into a highly organized tertiary structure, a three-dimensional shape with a distinct inside and outside. Many proteins self-associate into assemblies composed of two to six or more individual polypeptide chains; this represents the quaternary structure of proteins. For example, the acetylcholine receptor, a membrane protein of critical importance in neuromuscular communication, is a five-chain molecule. Only one final folded structure of the protein is functional.

Proteins are linked in networks of physical interactions and functional relationships to each other. Interactions between different proteins play pivotal roles in various aspects of the structural and functional organization of cells. The complexity of life requires proteins to be able to transfer specific signals, build tissues, control the function of enzymes, and regulate production and activities of many substances.

In general, drugs act by interaction with four kinds of regulatory protein targets:

- **Receptor proteins:** Receptors receive and process extracellular signals. An example of a drug that targets receptors is the antiallergy drug Zyrtec, which interacts with and blocks the histamine H_1 receptor.
- **Ion channel proteins:** Channel proteins control passage of solutes and

ions in and out of cells. Local anesthetics bind to and inhibit sodium ion channel proteins.

- **Enzymes:** Enzymes catalyze biochemical and metabolic reactions. The drug celecoxib binds to and inhibits the COX2 (cyclooxygenase 2) enzyme.
- **Transporters:** Transporters help to transport materials in and out of a cell. Prozac inhibits the serotonin transporter in the brain.

The term receptor is reserved mainly for those proteins that play an important role in intercellular communication via chemical messengers. As such, enzymes, ion channels, and transporters are usually not classified as receptors. The ways that these proteins interact with other molecules are similar, however, as we shall see shortly.

Receptors perform their cellular functions by binding to chemical messenger molecules called ligands. A ligand is an ion or a molecule that binds to the receptor to form a complex and consequently participate in a biological action. Ligands may be endogenous (already present in the body), or introduced into the body as drugs; examples of endogenous ligands are signaling compounds such as neurotransmitters and hormones. Most drugs take the place of endogenous ligands in exerting their action. For example, morphine and related pain-relieving drugs use the same receptors in the brain used by endorphins, compounds produced by the body to control pain. Ligands may be small molecules or macromolecules; the term small molecule generally refers to molecular weights less than 1,000. Most small molecule drugs have molecular weights in the range between 300 and 700.

The three-dimensional shape of a protein creates several pockets or active sites (also called *binding sites*) where molecules of appropriate structure may bind (see Figure 6.1). These molecules are said to have an affinity (attraction) for the protein. Protein misfolding can

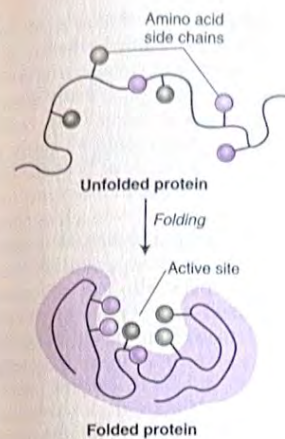


Figure 6.1. An illustration of the active site formed by the folding of a protein. Note that the unfolded protein does not have a unique binding site; such a site is created when the protein folds in a specific way.

change the active site configuration and interfere with binding of ligands, causing Alzheimer's, mad cow disease (in humans, variant Creutzfeldt-Jakob disease), cystic fibrosis, amyotrophic lateral sclerosis, and Parkinson's. Many cancers are also believed to result from protein misfolding.

Protein-Ligand Interactions

Most protein function is controlled by ligands that bind reversibly to proteins and either stimulate or inhibit their activity. The rate and extent of binding of a particular protein to a particular ligand are influenced by many factors, such as:

- structure, physicochemical properties, and concentration of the ligand
- structure and concentration of the protein
- affinity between the ligand and the protein

- competition by other substances that also bind to the protein active site

Different branches of pharmaceutical sciences use different nomenclature for ligand molecules, depending on whether the protein is a receptor or an enzyme.

Receptor Agonists and Antagonists

If the protein is a receptor, the endogenous ligand is called an **agonist**, a compound that binds to the receptor and then stimulates a cellular effect. Drugs that bind to receptors are classified as either agonists or antagonists. Agonist drugs activate their receptors, triggering a response that increases or decreases the cell's activity in a manner similar to the endogenous ligand. Antagonist drugs block the access or binding of the body's natural agonists to their receptors, and thereby prevent or reduce cell responses to natural agonists. Both the agonist and antagonist are released unchanged at the end of the process. Drugs are **receptor modulators** in that they can only influence some preexisting function of the receptor, but cannot impart any new property to it. We will explore these concepts further in Chapter 16, Mechanisms of Drug Action.

An example of an agonist drug is albuterol, which binds to **adrenergic** receptors on cells in the respiratory tract, causing relaxation of smooth muscle cells and thus opening airways in asthmatic patients. Using an appropriate antagonist may also widen constricted airways. For example, the drug ipratropium bromide binds to **cholinergic** receptors in the respiratory tract, preventing binding of the endogenous ligand acetylcholine, a neurotransmitter that causes contraction of smooth muscle cells and narrows the airways. Blocking acetylcholine binding to its cholinergic receptor prevents bronchoconstriction and makes breathing easier. Thus, a

given disease or condition may be treated in different ways.

Enzyme Inhibitors

If the protein is a soluble protein like an enzyme, the molecule that binds to it is called a substrate rather than a ligand. Enzymes catalyze chemical reactions involving substrates and turn them into products; the substrate is consumed in the reaction. Enzyme catalysis is important for most cellular metabolic processes. At any given moment, enzymes are facilitating most of the activities inside a cell and endogenous substrates are being converted into products. Many drugs have been designed to target enzymes, and most of these are enzyme inhibitors. The action of an inhibitor is to take the place of the natural substrate and prevent it from being converted into product. For example, the cholesterol-lowering drug lovastatin inhibits an enzyme called HMG-CoA reductase, critical for production of cholesterol in the body. We will discuss enzyme inhibitors further in Chapter 16, Mechanisms of Drug Action.

Enzymes are also important in drug biotransformation reactions, in which the drug (substrate) undergoes a reaction while bound to the enzyme (protein) and the reaction products (metabolites) are released in a subsequent step. A side effect of many drugs is that they are enzyme inducers; for example, one side effect of the antibiotic rifampin is that it activates enzymes involved in metabolizing oral contraceptives. When women taking an oral contraceptive also take rifampin, the contraceptive may be ineffective because it is metabolized and removed from the body more quickly than usual.

The Binding Equilibrium

Whether the protein is a receptor or enzyme, most ligands bind by a reversible process, in which bonds formed between

ligand and protein are noncovalent and weak, and can be readily broken to give back the starting materials. Irreversible protein-ligand binding can occur when a ligand attaches to a protein by a covalent bond; such binding cannot be easily reversed to give the original protein. Irreversible binding is rare and accounts for a few types of drug toxicity (such as chemical carcinogenesis) and the action of certain antibiotics on microorganisms. Many poisons and toxins also act by forming covalent bonds with enzyme or receptor proteins. An example is the nerve gas sarin, which forms an irreversible covalent bond with the enzyme acetylcholinesterase. The enzyme's active site is blocked and it becomes nonfunctional. New acetylcholinesterase molecules have to be synthesized by cells to carry out the enzyme's function.

Figure 6.2 illustrates the reversible binding of a ligand (or drug) to a protein. For simplicity, assume that the protein has only one type of binding site where the ligand can bind. In reality, proteins are quite large compared with most ligands and may contain more than one binding site, but this requires a complex analysis beyond our scope.

Consider the reversible binding between a receptor and its ligand or an enzyme and its substrate. This analysis is very similar to the discussion of enzyme catalysis and Michaelis-Menten kinetics in Chapter 4, Rates of Pharmaceutical Processes. In the present context, the

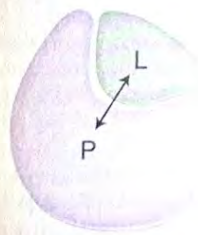
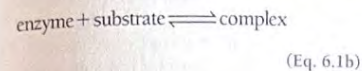
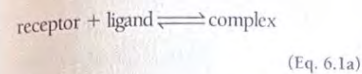


Figure 6.2. Binding of protein, P, to a ligand L at an active site. The arrow indicates that the binding is reversible.

receptor is denoted as R and the ligand as L. The same treatment applies to an enzyme, E, binding to its substrate, S. The equilibrium can be written as:



Equation 6.1a and 6.1b can be rewritten simply as:



RL represents the receptor-ligand complex, while ES is the enzyme-substrate complex.

The law of mass action defines K, the association constant for these equilibria:

$$K = \frac{[RL]}{[R][L]} \quad (\text{Eq. 6.3a})$$

$$K = \frac{[ES]}{[E][S]} \quad (\text{Eq. 6.3b})$$

The square brackets denote concentrations of the corresponding molecule at equilibrium.

Affinity

The magnitude of K gives information about the extent of ligand-receptor (or enzyme-substrate) binding, i.e., how much ligand (or substrate) is bound by the receptor (or enzyme) in the complex. A ligand that is extensively bound to a receptor has a large K, showing that there is a high affinity between receptor and ligand. The same is true for enzyme-substrate binding. Again, note the simi-

larity between this discussion and the earlier discussion of enzyme catalysis.

The strength of binding is often expressed as the inverse of the association constant K. A dissociation constant, K_d for the receptor-ligand complex or K_m for the enzyme-substrate complex, is defined as:

$$K_d = \frac{1}{K} = \frac{[R][L]}{[RL]} \quad (\text{Eq. 6.4a})$$

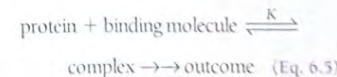
$$K_m = \frac{1}{K} = \frac{[E][S]}{[ES]} \quad (\text{Eq. 6.4b})$$

K_m is the Michaelis constant we have seen previously. K_m and K_d have units of concentration. These constants can be thought of as the concentration of ligand (or substrate) required to occupy 50% of the receptor (or enzyme) active sites. The lower the K_d , the higher is the affinity of receptor for ligand; the same holds true for K_m .

Consequences of Binding

In some situations, the only outcome of complex formation between a protein and another molecule is to tie up the molecule for some time, making it unavailable to perform its function. This occurs when proteins in the plasma (such as albumin) bind to drugs and prevent them from leaving the bloodstream and entering tissues. The complex eventually dissociates, according to the equilibrium shown in Equation 6.1, and releases the drug.

In other situations, however, the formation of the complex is only a first step in some important physiological process with a definite outcome, as indicated below.



Catalytic Outcomes

If the protein is an enzyme, the outcome in Equation 6.5 is usually catalytic in that

the substrate is transformed into a new product after initial binding to the protein. The kinetics of such a catalytic process is described by Michaelis-Menten kinetics. Figure 6.3 illustrates enzymatic catalysis of substrate(s) to product(s).

Catalytic reactions can be suppressed by enzyme inhibitors that bind to the enzyme and prevent the substrate from binding. As we saw earlier, many drugs are enzyme inhibitors, preventing the endogenous ligand from binding and performing its normal function. For example, angiotensin-converting enzyme inhibitors (also called ACE inhibitors; e.g., captopril and enalapril) are drugs that block the conversion of the substrate

angiotensin I to a substance that increases salt and water retention in the body. ACE inhibitors are used in the treatment of high blood pressure.

Noncatalytic Outcomes

The outcome of complex formation may be *noncatalytic*, in that the ligand is not chemically changed on binding to its receptors, but is eventually released unchanged. Instead, binding induces a conformational change in the receptor that triggers biochemical events in the cell as illustrated in Figure 6.4. The ligand eventually dissociates from the receptor-ligand complex. We will discuss binding

Protein-Protein Interactions

We have considered protein-small molecule interactions in detail, because a majority of drugs are small molecules. Most cellular processes, however, are mediated by protein-protein interactions, which are vital to the functioning of our body.

Enzymes perform transcription of DNA to RNA, whereas other proteins bind to specific regions of DNA to promote or inhibit its transcription, and thus, gene regulation. Nearly all the cellular machinery for synthesis, replication, manipulation, modification, and degradation involves proteins interacting with other proteins and macromolecules. Proteins are directed to the correct compartments of cells by binding to other proteins. Many endogenous ligands are proteins, which bind to receptors on cell membranes to send signals between cells. Proteins form structural connections between cells. Proteins are substrates and in-

hibitors of enzymes. Protein-protein interactions underlie large-scale movements in organisms such as muscle contraction.

Protein-protein interactions can sometimes be undesirable. Protein aggregation is associated with a number of diseases, including prion-related diseases, Alzheimer's disease, and Down's syndrome.

It is important to note that interaction forces between proteins are weak. It is through the synergism of multiple weakly interacting proteins that a cell can generate responses. If every protein had high affinity for every other protein, it would be difficult for the cell to regulate the activity of any one pathway. Thus, weak protein-protein interactions play a central role in finely tuned cellular signaling. They also provide us an opportunity to control cellular responses by using small molecule drugs to interfere with and alter protein-protein interactions.

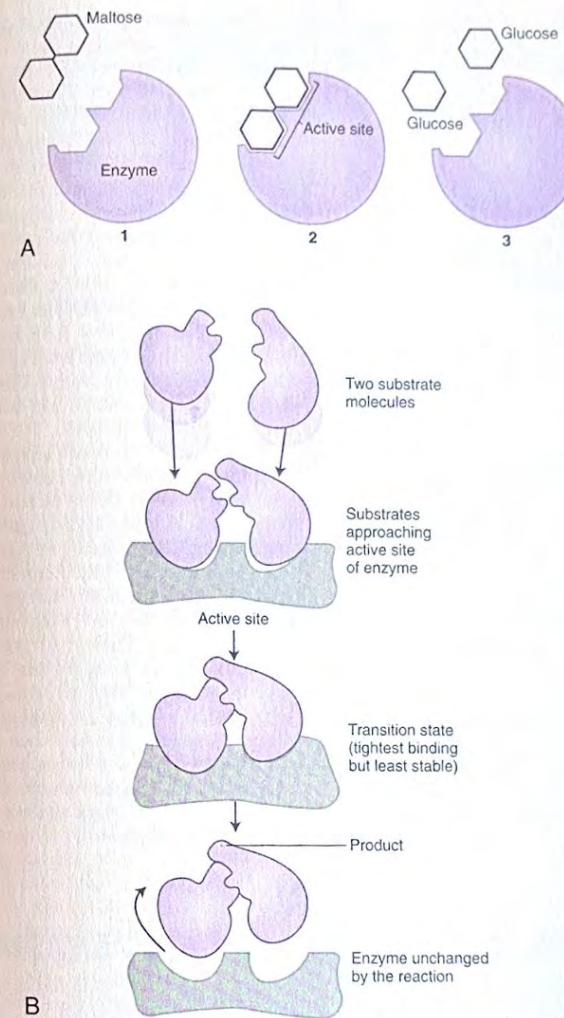


Figure 6.3. Enzymatic catalysis of substrate(s) to product(s). A. The enzyme maltase has an active site complementary to the structure of maltose and binds only to maltose. A reaction occurs when maltose binds to the enzyme, releasing two glucose molecules as products. A single maltase molecule can catalyze the reaction of more than 1,000 maltose molecules per second. B. Example of the enzyme-catalyzed synthesis of a product from two substrate molecules.

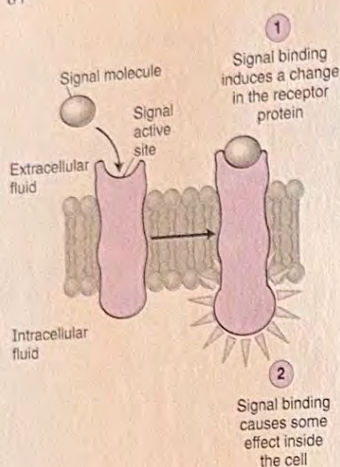


Figure 6.4. A signal molecule (an endogenous ligand or a drug) binds to a cell membrane receptor on the extracellular surface and induces a change in receptor protein that causes some effect inside the cell.

to receptors in more depth in Chapter 16, Mechanisms of Drug Action.

Similarities Between Receptors and Enzymes

Both receptor–ligand and enzyme–substrate binding are generally reversible events. The outcome depends on the

concentration of ligand–protein complex formed in the first step (Equation 6.2). Therefore, formation of the complex controls the rate and extent of the outcome. The higher the affinity of ligand for protein, the larger the concentration of the complex, and the greater the outcome of the formation of the complex is dependent on pH, temperature, and the presence of ions and other molecules. Binding can be saturated at high concentrations of the ligand or substrate. Molecules bound by enzymes and receptors may be small, such as a sugar or amino acid, or may be large, such as another protein. For example, insulin, a macromolecule, is the ligand for the insulin receptor.

The similarities between enzymes and receptors are easily recognized by direct comparison, as shown in Table 6.1. The body contains many different receptors and enzymes, and often the distinction between them is unclear. Sometimes a receptor has enzymatic activity. Many enzymes, such as those with allosteric regulation, also qualify as receptors when the broadest use of the term is applied. If we consider any ligand interacting with a macromolecule to be a receptor–ligand interaction, then any interaction of small molecules with protein, DNA, or RNA can be classified as types of receptor–ligand complexes if some response occurs.

TABLE 6.1. Similarities Between Enzymatic and Receptor Processes

	Enzyme	Receptor
Binding molecule	Substrate	Ligand
Complex	E–S	R–L
Outcome	Product formation	Complex formation, or response
Number of binding sites	One or more	One or more
Type of binding	Specific or nonspecific	Specific or nonspecific
Measurement of binding affinity	K_m	K_d
Types of binding molecules	Inhibitors, activators	Agonists, antagonists
Regulation	Allosteric activation	Gene regulation

Molecular Recognition

Protein surfaces are irregular. This irregularity enables proteins to bind specific ligands (we will use the term ligand generally to mean a substrate as well) and to associate specifically with other proteins, and it underlies the formation of quaternary structure. The primary concept behind protein–ligand binding is that of complementarity, or “fit,” between ligand and active site. This matching between a protein surface and another molecule depends on much more than shape. It extends to the weak bonds that hold complexes together; hydrogen bond donors line up with acceptors, nonpolar groups are opposite other nonpolar groups, and positive charges are opposite negative charges.

The two types of complementarity for us to consider are physicochemical complementarity, i.e., the presence of several physicochemical bonding interactions between the two molecules, and steric complementarity, i.e., whether the shape of the ligand fits the shape of the active site. Both determine the strength of the overall interaction.

Physicochemical Complementarity

Covalent bonds are not routinely formed between a protein and ligand during normal cellular processes. Therefore, several types of weaker noncovalent bonds are necessary to attract and keep the two molecules together as a complex. In most cases, the initial attraction between the two interacting species is provided by a long-range force such as an ionic interaction between opposite charges on the protein active site and the ligand. As the ligand approaches the protein, short-range forces such as hydrogen bonds provide additional attractive and orienting forces. Finally, van der Waals forces and hydrophobic interactions come into play to further orient and stabilize the complex. Thus, most protein–ligand interactions rely on many different molecular forces to form the final complex. Of the

various physicochemical interactions involved in protein–ligand binding, ionic and hydrophobic interactions are probably the most important.

Because the initial interaction between a ligand and protein is often ionic, the ionization state of weak acid and base drugs is very important. Charged atoms (from ionized amino acids) often line the protein active site, imparting a localized charge in specific regions of the pocket. Opposite charges on the active site and ligand will attract each other, beginning the complex formation process. *Electrostatic complementarity* is important in preventing inappropriate molecules from binding to the active site, as the ligand must contain correctly placed complementary charged atoms for interaction to occur.

Another critical force for ligand–protein binding is hydrophobic interaction. Nearly two thirds of the body is water, and this aqueous environment surrounds all our cells. For a ligand and protein to interact, there must be a driving force that compels the ligand to leave water and bind to the protein; hydrophobic ligands are able to accomplish this. Thus the partition coefficient of the ligand is also an important factor in protein–ligand binding.

Steric Complementarity

Although physicochemical complementarity is important for the initial attraction between ligand and protein, the ligand must also have stereochemical complementarity to sustain the ligand–protein complex. This means that the ligand must have a defined three-dimensional shape and size that fit well into the active site. Let us briefly review the basic concepts of stereochemistry as they pertain to stereochemical complementarity in protein–ligand binding. Undergraduate textbooks in chemistry are good resources for a more thorough discussion of stereochemistry.

Stereoisomers are molecules that have the same molecular formula and sequence of bonds but different spatial arrangements; the only difference between them

is the three-dimensional orientation of atoms or functional groups in space. Despite having identical chemical formulas and bonding, stereoisomers can have dramatically different chemical, physical, and biological properties. There are two main types of stereoisomers of interest in understanding stereocomplementarity: enantiomers and geometric isomers.

Enantiomers and Chirality. Chirality is the geometric property of a rigid object (like a molecule or drug) not being superimposable with its mirror image. A molecule that cannot be superimposed on its mirror image is said to be chiral (the Greek word for "handed"). This is in contrast to *achiral* molecules, which can be superimposed on their mirror images.

Chirality is analogous to our right and left hands—they are mirror images but are not superimposable. The two mirror images of a chiral molecule are termed enantiomers. Like hands, enantiomers come in pairs. Chirality usually occurs when a compound contains at least one asymmetric or chiral center in its structure. A chiral center is an atom at which the interchange of any two substituents attached to it creates a new stereoisomer. Various nomenclature systems have been

used for enantiomers; the most common ones designate the two forms in the pair as L- and D-, or as R- and S-.

Chirality is a property found in many biologically important molecules such as amino acids, carbohydrates, and lipids. For example, the natural amino acids share a common stereochemistry; they are all L-amino acids. Our bodies use only D-sugars; DNA and RNA are made up of D-sugars, resulting in a right-handed DNA double helix. Consequently, most cellular targets are chiral and can recognize differences between enantiomers of a chiral ligand. Our receptors, such as the taste receptors in our tongue, can distinguish between stereoisomers of a ligand; for example, one isomer of leucine tastes sweet and the other tastes bitter. Chemicals with different enantiomeric forms can smell different also. One isomer of limonene smells of oranges, the other of lemons.

Enantiomeric pairs have identical physicochemical properties, such as boiling point, melting point, density, and solubility. Enantiomers, however, can have marked differences in their interaction with proteins such as enzymes and receptors, and can behave very differently in biological systems as a result of their different three-dimensional shape. Figure 6.5

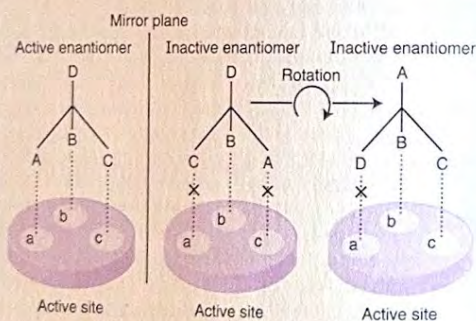


Figure 6.5. Binding of two hypothetical enantiomers of a drug to a receptor active site. The active enantiomer has a three-dimensional structure that allows drug domain A to interact with active site domain a, B to interact with b, and C to interact with c. All three binding interactions are necessary. In contrast, the inactive enantiomer cannot be aligned to have the same three interactions with the binding site simultaneously. The difference in three-dimensional structure allows the active enantiomer to bind and have a biological effect, whereas this is not possible for the inactive enantiomer.

illustrates the binding of two hypothetical enantiomers of a drug to a receptor active site. In other words, the R enantiomer of a drug will not necessarily behave the same way as the S enantiomer of the same drug when taken by a patient. In essence, the two enantiomers of a chiral drug should be considered different drugs. The ability of biomolecules to distinguish between the various steric forms of a ligand or drug is called *chiral recognition* or *chiral discrimination*.

For example, the two enantiomers of amphetamine (D-amphetamine and L-amphetamine) have the same melting points, solubilities, and pK_a values; however, the D isomer is more potent.

Geometric Isomers. Geometric isomers (or *cis-trans* isomerism) occur because of restricted rotation around a bond such as a carbon-carbon double bond, or in a ring such as cyclohexane. The *cis* and *trans* configurations are not mirror images of each other, and the two forms show significant differences in their physicochemical properties, such as ionization and lipophilicity, and in their biological activity.

Geometric *cis* and *trans* isomers can be isolated as pure substances, and mixtures of isomers are not commonly seen. However, the two *trans* isomers in a cyclic compound can exist as an enantiomeric pair. Differences in biological activity

Racemic Mixtures

A racemic mixture or racemate is a sample of a compound that contains all its possible stereoisomers in equal proportions. Thus, for a compound with one chiral center, a racemate has the two enantiomers in a 1:1 ratio. Enantiomers in a racemic mixture are difficult to separate from each other as pure stereoisomers because they have the same physicochemical properties. For this reason, the majority of synthetic drugs were produced as racemic mixtures for many years, and the properties of the individual stereoisomers were not known. More than 500 currently useful drugs are racemic mixtures containing an active drug and its enantiomer in equal proportions.

It is now clear that stereoisomers can have significantly different biological properties, and that a single stereoisomer is often therapeutically superior to a racemic mixture. The isomer with the desired activity is called the *eutomer* and the one with-

out the desired activity or with an undesired activity is the *distomer*. New methods to produce single isomers on a commercial scale are now available, removing a major obstacle in the use of single isomer drugs.

Chirality now plays a major role in the development of new pharmaceuticals; approximately 30% of marketed drugs are sold as a single isomeric form. The antiinflammatory drug naproxen is marketed as the S isomer, because the R form is a liver toxin. Similarly, L-dopa has anti-Parkinsonian activity, whereas D-dopa exhibits none of the desired anti-Parkinsonian activity and can cause granulocytopenia (loss of white blood cells that leaves patients prone to infections). There are other examples such as penicillamine, used to treat arthritis, in which the S enantiomer is active while the R form is extremely toxic. The (S, S) form of the antituberculosis drug ethambutol is active while the (R, R) form causes optical neuritis that can lead to blindness.

between *cis* and *trans* isomers may, therefore, be caused by either nonspecific physicochemical effects or stereoselectivity of receptor binding. Figure 6.6 illustrates geometric isomerism and how geometric isomers can bind differently to a target protein.

An example of *cis-trans* isomerism in drugs is found in *trans*-diethylstilbestrol, which has estrogenic activity, and *cis*-diethylstilbestrol, which has only 7% of the estrogenic activity of *trans*-diethylstilbestrol.

Selectivity and Specificity

These two terms are often used interchangeably and describe the same general concept. Selectivity refers to the ability of a ligand or drug to interact with a certain protein's active site without binding significantly to other proteins. Selectivity is generally a desirable prop-

erty in a drug, e.g., it is desirable that an antibacterial drug affect bacteria at concentrations too small to affect host cells.

Specificity is the ability of a protein to bind to a ligand with a defined structure, while having little or no interaction with other molecules of similar structure. Proteins can be specific, somewhat specific, or nonspecific in their binding to ligands. Most receptors are highly specific in their binding to other molecules.

Nonspecific or partially specific binding occurs when ligands with a variety of structures are able to bind to a given protein, and where physicochemical complementarity is most important. For example, albumin, a protein found in plasma, can bind to a variety of lipophilic drug molecules, particularly weak acids and neutral drugs. Similarly, α_1 -acid glycoprotein, another plasma protein, binds with many basic drugs, regardless of their structure. Many enzymes show group

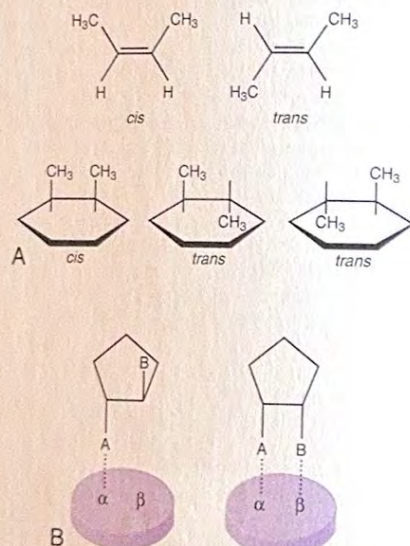


Figure 6.6. A. Geometric isomerism as a result of *cis-trans* orientation across a double bond or in a ring. B. Binding of geometric isomers to a target. On the left, the *trans* isomer does not have the B group in proper configuration for effective binding. On the right, the *cis* isomer has both the functional groups in a favorable orientation for binding to the receptor.

specificity, in which a general group of compounds can serve as substrates.

Specificity and selectivity increase when stereochemical complementarity plays a major role in protein-ligand binding, such as the binding between receptors and their ligands, or several enzymes with their substrates.

A few drugs produce their biological action without binding to a particular target. Such nonspecific activity is usually related to a physicochemical property of the drug, such as lipophilicity or pK_a . For example, antacids neutralize stomach acid because of their alkalinity, and osmotic diuretics help eliminate water by producing an osmotic gradient in the kidneys and increasing urine production. For other drugs, nonspecific activity is often manifested as a side effect or toxicity. Examples are sedation and general depression of the central nervous system by some lipophilic drugs, and bactericidal

effects of certain compounds. A wide variety of chemical structures can show the same nonspecific activity if their physicochemical properties are similar.

Models of Binding

Two simple models have been proposed to describe the binding between a ligand and a protein: the lock-and-key model and the induced-fit model. These are illustrated in Figure 6.7. Other more complex models have also been proposed but are outside the scope of this book.

Lock and Key

The lock-and-key model proposes that the surfaces of ligand (key) and protein (lock) must fit exactly or there will be no binding. The ligand and protein need to have compatible, interlocking shapes,

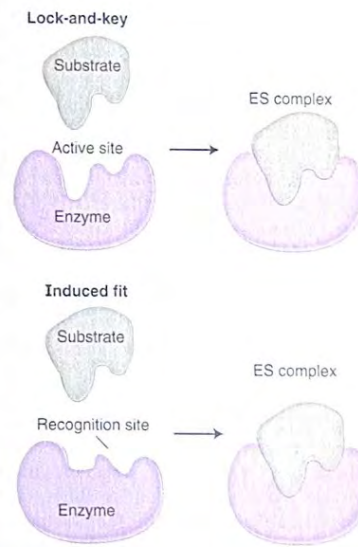


Figure 6.7. The lock-and-key and induced-fit models of protein-ligand interaction. This diagram uses an example of an enzyme binding to its substrate.

like two puzzle pieces or a lock and key. Some drugs attach to only one type of receptor and are therefore selective; others, like a master key, can bind to several types of receptors throughout the body, making them partially selective. In this simplest scenario, the protein and ligand interact with no change in their conformation on binding.

Induced Fit

Molecular interactions that involve conformational changes in one or both interacting molecules are more versatile. The induced-fit model states that when the ligand first binds, its interaction with the protein is weak. However, this initial binding causes a change in the conformation of the protein, allowing the ligand to bind more tightly. Protein conformational change is possible because proteins are flexible molecules, and because the forces that maintain secondary and tertiary protein structure are also weak. Thus, enough energy is available at body temperature to break interactions that hold the folded protein together, and allow for a different folded structure in the presence of ligand. This can be thought of as a stabilization of a particular protein conformation by ligand binding to allow for optimal binding. Induced fit also allows for binding of more than one ligand at the same active site. When the binding of a specific ligand causes a protein to change from an inactive to an active conformation, the process involves an induced fit.

Allostery and Cooperativity

Proteins often contain several active sites for different types of ligands. Until this point, we have considered ligands that bind independently to these sites, i.e., the affinity of the ligand for the protein active site remains the same regardless of ligand concentration. There are many examples, however, in which ligands do not bind independently, but a ligand bound

at a site influences the ability of another ligand to bind to a second site on the same protein.

Proteins have the ability to coordinate what is going on at these different binding sites in such a way that the binding of one ligand alters the affinity for another ligand on the same protein. There need not be a direct connection between the two ligands; they may bind to opposite sides of the protein, or even to different subunits of protein. This mechanism is called allostery; the interaction is termed allosteric binding and is illustrated in Figure 6.8.

One explanation of allostery involves protein flexibility. In some ligand-protein interactions, binding to a ligand induces conformational changes in the protein, as described by the induced-fit model discussed earlier. Conformational changes may also take place in locations far removed from the first binding site. Often, the result of the first protein-ligand interaction is to change the shape of other active sites, so that subsequent ligand binding (of a similar or different ligand) is altered. These can be relatively minor adjustments of the protein chain or can be significant changes in three-dimensional conformation. An example of drug activity caused by allostery is found in the drug nevirapine, an inhibitor of the enzyme HIV reverse transcriptase (HIV-RT). Nevirapine binds to HIV-RT at an allosteric site that is not the active site of the enzyme. This binding changes the conformation of the active site, so that HIV-RT is no longer able to function normally.

For identical ligands, the allosteric mechanism is called a cooperative effect. If ligand binding were independent, each site would exhibit the same affinity for ligand. In cooperative binding, however, the apparent individual site binding affinities differ from one another and from the independent affinity of each site. Binding may exhibit either positive cooperativity or negative cooperativity, in which the apparent ligand affinity

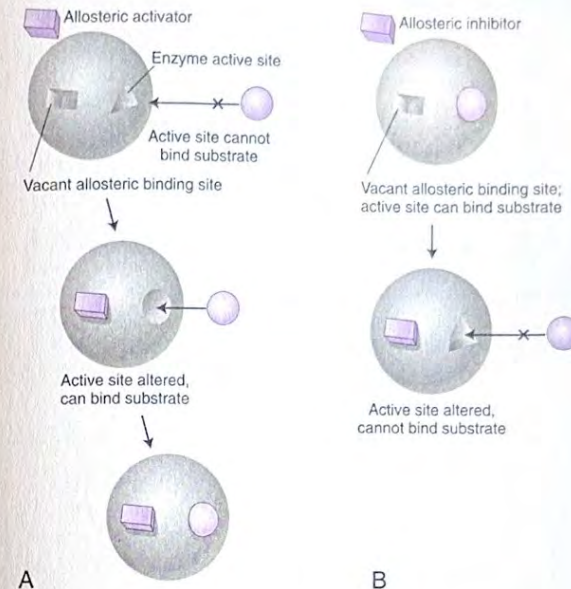


Figure 6.8. Examples of allosteric binding resulting in either an increase (A) or decrease (B) of the target's affinity for its ligand or substrate.

increases or decreases as more ligands bind, respectively. Cooperativity is evident in the binding of oxygen to hemoglobin. Hemoglobin is a protein with four subunits linked together, each subunit containing a single heme—a ringlike structure with a central iron atom that binds to an oxygen atom. Deoxyhemoglobin is relatively uninterested in oxygen, but when one oxygen attaches, the second binds more easily, and the third and fourth easier yet. The same process works in reverse: once fully oxygenated

hemoglobin lets go of an oxygen, it lets go of the next more easily, and so forth. In fact, hemoglobin is usually observed in only two states—all four subunits carrying oxygen, or completely oxygen free.

One reason that cooperative binding is widespread in biological systems is that it is an important way for cells to modulate the response to an input signal. Positive cooperativity causes the system to respond more sensitively to changes in ligand concentration. Negative cooperativity damps the response to ligand concentration.

KEY CONCEPTS

- Proteins perform their biological function by binding to endogenous compounds called ligands.
- Drugs work by binding to targets, usually receptor or enzyme proteins, and thus influencing protein-ligand binding in some way.
- Agonist drugs stimulate receptors just like the corresponding ligands. Antagonist drugs interfere with ligand binding and thus reduce its effect. Enzyme inhibitor drugs reduce the activity of enzymes.
- Ligand or drug binding to target active sites requires physicochemical and steric complementarity.
- Ligands and drugs show selectivity in binding to targets; targets show specificity in binding to ligands or drugs.
- The lock-and-key and induced-fit models are simple approaches to understanding specificity and selectivity.
- Allosteric or cooperative effects often modulate the binding of ligands or drugs to their targets.

ADDITIONAL READING

- Bohm HJ, Schneider G (eds). Protein-Ligand Interactions: From Molecular Recognition to Drug Design. John Wiley and Sons, 2003.
- Nelson DL, Cox MM. Lehninger Principles of Biochemistry, 4th ed. W.H. Freeman, 2004.
- Hardman JG, Limbird LE, Gilman AG (eds). Goodman and Gilman's The Pharmacological Basis of Therapeutics, 10th ed. McGraw-Hill Professional, 2001.
- Katzung BG (ed). Basic and Clinical Pharmacology, 8th ed. McGraw-Hill/Appleton & Lange, 2000.

REVIEW QUESTIONS

- What is meant by the term drug target?
- Explain the term affinity as it applies to ligand-protein interactions. How is affinity measured?
- Discuss the difference between a receptor agonist, a receptor antagonist, and an enzyme inhibitor.
- Differentiate between catalytic and noncatalytic outcomes of ligand-target binding.
- What is meant by complementarity? Discuss the two types of complementarity.
- Explain why chiral recognition is important in the binding of small molecule ligands to targets.
- What is a racemic mixture? Discuss the advantages and disadvantages of using single isomers of drugs instead of racemic mixtures.
- Explain the terms selectivity and specificity as they apply to ligand-protein binding.
- Discuss the lock-and-key and induced-fit models of ligand-protein binding.
- Explain the mechanisms of allosteric and cooperative binding.

Drug Discovery and Optimization



Lead Compound Versus Drug Identification of a Lead Compound

Random Screening
Natural Products Chemistry
Rational Design
De Novo Design
The Combinatorial Approach

Optimization of the Lead Compound

Selectivity and Toxicity
Drug Likeness
Bioisosterism
Structure-Activity Relationships

Key Concepts

Additional Reading

Review Questions

Most drugs on the market were not discovered in their final form but went through a process of experimentation and modification to make the best possible therapeutic agent. The starting point in drug discovery is identification of a **lead** compound, a substance that possesses the desired biological activity but that may have other undesirable characteristics. The lead compound serves as an initial prototype that is modified to retain or enhance the desired activity and to eliminate or minimize unwanted properties.

Lead Compound Versus Drug

A lead compound has the desired pharmacological activity, but may have other unfavorable properties such as high toxicity, problems with one or more absorption, distribution, metabolism, and excretion (ADME) processes, or an unusually complex or expensive manufacturing process. The lead has to be transformed into a drug by modifying its structure to impart suitable drug-like properties such as low toxicity and the ability to reach the site of action in appropriate concentrations. Other considerations such as ease and cost of synthesis must also be taken into

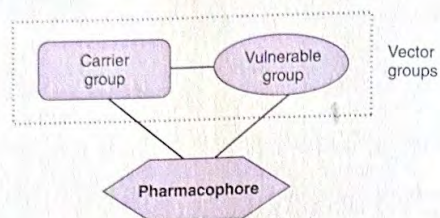


Figure 7.1. Typical components of the chemical structure of a drug molecule. The pharmacophore is needed for biological activity. The vector groups define the physicochemical properties of the molecule; carrier groups control absorption, distribution, and excretion; and vulnerable groups determine metabolism.

account. Lead-optimization is the process by which the structure of the lead compound is modified to build in these desirable properties.

We know that most drugs show a structurally specific relationship to biological activity, meaning that a definite pattern of structural features is necessary for a biological response. This is because the behavior of a majority of drugs is related to their binding to biological targets. The molecular structure of such drugs can be subdivided into various components as shown in Figure 7.1.

The pharmacophore is the part of the molecule that interacts with the target protein or receptor; a change in this portion will alter biological activity. The pharmacophore and the target must have physicochemical and stereochemical complementarity. Several parts of the molecule can together make up the pharmacophore; as an example, see Figure 7.2, which shows the pharmacophore that binds to the δ -opioid receptor.

Vector groups may not bind to the target but play a vital role in determining pharmacokinetics of the drug and may also aid in minimizing toxicity. Vector groups may be classified further as carrier groups and vulnerable groups. Carrier groups control ionization and lipophilicity of the molecule and consequently influence absorption, distribution, and excretion of the drug. Vulnerable groups are susceptible to

enzymatic action and are responsible for determining the drug's metabolism.

Identification of a Lead Compound

Several approaches are available to identify a lead compound, each one with its advantages and drawbacks. To find a lead, one generally needs a convenient laboratory test called a bioassay or a screen that can determine whether a compound has the desired biological activity.

Random Screening

Random screening is the testing of large numbers of compounds in a bioassay without regard to their chemical structure. This approach relies on luck and serendipity

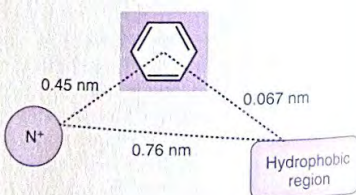


Figure 7.2. The pharmacophore recognized by the δ -opioid receptor. This pharmacophore consists of an aromatic ring, a protonated amine, and a hydrophobic region, all spatially oriented as shown.

and has been the traditional way of identifying lead compounds; in fact, many of today's important drugs were discovered this way. The use of random screening has declined as more advanced technologies have become available. However, this approach is still useful in identifying leads with unexpected or unusual structures that cannot be foreseen.

Historically, plants and other natural products were major sources of medicinal substances. For example, the foxglove plant (*Digitalis purpurea*) had been used to treat congestive heart failure. As medicinal chemistry evolved, it was found that digitalis and other related substances in foxglove are responsible for the therapeutic effect. These active compounds were isolated, synthesized, and developed into products so that dosage could be accurately controlled.

Screening in Animals

Typically, screening for new drug leads has involved *in vivo* studies in animals. This approach provides the most relevant feedback if a good animal model is available to demonstrate the desired effect. The compounds are administered to animals and screened for efficacy. A positive effect provides strong support to continue further development. However, there are several drawbacks to using animals for initial screening. The process tends to be slow and requires large quantities of the test compounds. The use of animals is also very labor intensive. Together, these disadvantages make animal screening an expensive proposition. Toxic effects of the compound may also complicate or mask the interpretation of efficacy.

Screening in Cells and Tissues

Screening in isolated tissues or cultured cells requires smaller quantities of compound than with animal screening. Such *in vitro* testing is also faster, cheaper, and less labor intensive. In addition, because the screening system is less complex than

a whole animal system, the results may allow determination of the mechanism of action, i.e., whether a compound is an agonist or an antagonist.

Screening in Binding Assays

If the target protein is known, a protein-binding assay can be used to conveniently screen compounds for affinity to the target. This screening approach requires the least amount of compound. It is also rapid, and thousands of compounds can be screened per day in a given assay. Specificity is often readily determined by testing the compound in preparations of several different related target proteins.

Tissue homogenates or intact cells containing the target protein are incubated with a radiolabeled ligand known to have affinity for the protein. The compound to be screened is added, and the amount of ligand remaining bound is determined. This assay gives a comparison of the relative affinities of the known ligand and the new compound for the target protein. Compounds that inhibit ligand binding by a certain specified percentage are evaluated further. Receptor assay screening, however, gives little information about ultimate efficacy in an animal. In addition, it is often impossible to predict whether a compound will be toxic.

Compound Diversity

Medicinal chemists often synthesize new and unusual molecules in the laboratory without any predetermined goal for biological activity. The objective is to make compounds of diverse structures to find unexpected biological activity. Random screening of such compounds in a series of biological tests can often result in identification of a lead compound. Random screening of large numbers of compounds can use up a lot of time and resources, so a narrower approach is often taken. The synthesis is restricted to compounds having a vague resemblance to known weakly active

compounds, to decrease cost of screening and increase probability of success. The keys to success with random screening are acquisition of diverse and large libraries of compounds, diversity being at least as important as number of compounds.

Natural Products Chemistry

The natural environment is a rich source of structurally diverse chemical compounds. Natural products are materials derived from nature for use as drugs or in pharmaceutical drug discovery and design. They may be extracted from tissues of terrestrial plants, marine organisms, or microorganism fermentation broths. A crude extract from any one of these typically contains many novel, structurally diverse chemical compounds. Chemical diversity in nature is based on biological and geographical diversity, so researchers travel the world obtaining samples to evaluate in drug discovery screens. This effort to search for natural products is known as bioprospecting.

Natural products have formed the basis of traditional medicine systems for thousands of years. Modern pharmaceutical science has improved on these natural medicinals by extracting, concentrating, and identifying the active compound from the natural product mixture. Many drugs on the market today were discovered from natural sources. The analgesic activity of the bark of the white willow tree (*Salix alba*) was known from folklore and gave us aspirin. The antibiotic activity of penicillin, obtained from molds of the genus *Penicillium*, was discovered serendipitously in the laboratory. Scientists have isolated dozens of natural antibiotics, such as streptomycin and vancomycin, mostly from soil microbes in the Actinomycete family.

An inherent problem with widespread use of natural products, however, lies with harvesting and processing products with low concentrations of the active

compound. Scientists are, therefore, primarily interested in natural compounds as a template or starting point for laboratory synthesis of related drug leads.

Natural products chemicals have played, and will continue to play, a key role in drug discovery. Screening natural products extracts, however, is expensive when compared with synthetic approaches, because isolation studies and structure elucidation of unknown compounds add to the expense. On the other hand, the structural diversity provided by natural products is unsurpassed by traditional synthetic chemistry. Natural products already have a function in the environment so there is a greater likelihood that they have some desired biological activity. Natural products also frequently have drug-like properties (which we will discuss later in this chapter) so that they can be absorbed, distributed, and metabolized appropriately in the body. Consequently, natural products are excellent sources of novel compounds for activity against challenging biological targets.

Rational Design

Scientists often struggle with the problem of deciding which compounds to synthesize from thousands of possible molecules. Rational design was the first approach to overcome this problem by focusing on a few promising structures. A fundamental assumption of rational design is that drug activity depends on molecular binding of the drug to a biomolecule, usually a target protein. The steps in rational design are target identification, designing a molecule to bind to the target, and then synthesizing the compound in the laboratory.

Target Identification

The first step in rational design is to understand the molecular biology of the disease or illness and to search for target proteins that play a role. If there is a

Taxol

The first plant-derived anticancer drug was Taxol (generic name paclitaxel), isolated from the bark of the Pacific yew or *Taxus brevifolia*, a slow-growing tree found in old-growth rain forests in the northwestern United States. Interest in paclitaxel revealed major supply problems, because many groups wished to conduct clinical trials and required large quantities of the drug. Because of the low concentration of paclitaxel in the bark, more than 25,000 trees were needed for early clinical trials. Once the bark is removed from the tree, the tree dies. The Pacific yew is an environmentally protected species and is also one of the slowest growing trees in the world. Isolation of the compound from the bark involved killing the tree, and the quantities available by this method were very small. It was clear that harvesting of *Taxus brevifolia* bark was not a viable long-term option for Taxol production on a large scale.

An analog of paclitaxel called baccatin III was discovered in leaves of the English yew *Taxus baccata*. Baccatin could be converted to paclitaxel in the laboratory. Removal of the leaves had no detrimental effect on the health of the tree, and the leaves regenerated relatively quickly, so it was unnecessary to cut down the trees. Although the extraction and subsequent conversion of baccatin to paclitaxel were difficult, the source was renewable and sufficient quantities were obtained to carry out clinical trials. Paclitaxel is now made by a modified version of this semisynthetic procedure.

The success of Taxol stimulated interest in preserving biodiversity and deriving value from it. It brought together several groups united in an effort to advance new drug discovery and simultaneously preserve the yew and the rain forest.

relationship between the suppression (or reinforcement) of a protein function and the symptoms of a disease, the protein is identified as a target protein for drug discovery efforts.

Once a target has been established and characterized, rational design begins. The first consideration is that the designed molecule must complement the active site of the target. Steric, electrostatic, and hydrophobic complementarity must be established, and the pharmacophore must be presented to the active site for recognition and binding to occur. The aim is to find a molecule that binds to the target protein with suitable affinity and selectivity. If reinforcement of the protein's function is desired, the mole-

cule must be an *agonist*, whereas if suppression is needed the molecule should be an *antagonist* (or *inhibitor* if the target is an enzyme).

The optimal combination of atoms and functional groups to complement the target is often the natural, endogenous ligand that binds to it. Unfortunately, this is usually an unacceptable candidate as a drug, because the natural ligand is almost always an agonist, or a compound that cannot be patented, or lacks appropriate ADME requirements for an administered drug, or has other side effects. Therefore, alternative chemical structures must be devised.

Computational tools can predict the binding of a hypothetical molecule to a

target protein and are used routinely to study drug-receptor complexes and to calculate properties of small molecule drug leads. The computer analyzes the interactions between the proposed compound structure and the target active site to design molecules that give an optimal fit.

Direct and Indirect Rational Design

Depending on whether the sequence and three-dimensional structure of the target protein is known or not known, rational design may be approached directly or indirectly. If the structure is known, medicinal chemists can directly determine an optimum ligand structure that can be placed inside the target active site in a conformation that results in a good fit. This is referred to as *docking* the relative orientation and fit of the target protein to a potential drug molecule. Docking is a function of the conformation of each partner in the interaction. Looking for a compound that can dock appropriately with a target is like searching for a key that fits a given lock. Once the computer models predict a suitable structure for a drug, the compound can be synthesized in the laboratory as a lead.

Often, the structure of the active site is unknown. In fact, the three-dimensional structure of relatively few receptors has been determined, although this number is increasing. In such cases, scientists use indirect approaches to obtain information about the target. One way is to study the binding of the target to several small molecules. Using the geometric structure and the chemical characteristics of the molecules that show strong binding, scientists can deduce some information about the structure of the active site. This is analogous to using a given key to search for other fitting keys without knowing the lock. Once the pharmacophore is identified, a suitable lead compound can be identified and further refined.

Once a promising compound structure or series of structures have been identified by computational analysis, the compounds can be synthesized in the laboratory and screened for activity in animals, cells, or binding assays, as discussed earlier.

De Novo Design

The concept of generating virtual lead compounds entirely through computer simulation is termed *de novo design*. Compound structures are both identified and screened for activity using computers. High-performance computers are first used to search for structures that fit the proposed active site of the target protein. Then automated robotic systems and advanced algorithms are used to screen compounds entirely *in silico*, meaning that experiments are carried out not in the laboratory, but on the computer by a process called virtual screening. Virtual screening is the evaluation of virtual, or hypothetical, compounds for binding to a virtual receptor protein. Virtual libraries of three-dimensional compound structures and three-dimensional protein structures are available in several computer databases. High-speed computing highlights the properties of compounds that appear to bind to a receptor and models their characteristics. As a result, researchers can identify structures that are worth investigating.

Although computers have become faster, the number of calculations needed to accurately predict the binding of a *de novo* generated structure to its target in a useful time frame still requires significant approximations. An additional difficulty is predicting how the chemical structure will behave in real life. Chemical compounds are inherently flexible structures and can assume many different conformations and orientations. Predicting how any structure will actually interact and bind with a target in the physiological environment is very uncertain. Another

major deficiency of *de novo* drug design is that the entire structure is created from scratch. There are a nearly infinite number of potential combinations of atoms, which leads to the generation of many useless chemical structures. Undesired structures are those that will have toxicity, chemical instability, or synthetic difficulty.

De novo design may not be feasible yet, but such computational techniques are valuable in optimizing hits and lead compounds into potent drugs. Although computers cannot substitute for a clear understanding of the system being studied, they are an important tool to gain better insight into the chemistry and biology of the problem at hand. Modern drug discovery combines the power of computational chemistry with other approaches to further speed up the identification of new drugs.

The Combinatorial Approach

Both random screening and rational drug design use traditional wet chemistry methods, in which a synthetic procedure is designed to make one compound at a time in the laboratory. The compound is then purified and analyzed, also one at a time. It is then tested in a biological assay to see whether it binds to a target protein, cell, or tissue. High-throughput screening and combinatorial chemistry have changed this traditional approach dramatically, and have revolutionized the capacity of pharmaceutical companies to identify potential new drug leads.

High-Throughput Screening

High-throughput screening (HTS) uses miniaturized, robotics-based technology to test large compound libraries for binding to a target to identify potential new drugs or leads. The advent of HTS allowed scientists to screen large numbers of compounds in a short time. High-throughput screening depends on the development of a quantitative, phar-

macologically relevant assay for the identified target, which can then be reproduced across a large number of samples. Recently, technological advances have allowed screening of up to 100,000 compounds per day for binding to a protein target.

Classic methods of drug synthesis, however, could not make the large numbers of compounds for which screening capability was available. A new approach was needed to significantly increasing the throughput of chemical synthesis, both in terms of the number and the diversity of compounds; this new approach is combinatorial chemistry.

Combinatorial Chemistry

Combinatorial chemistry (often called *combi*chem) is the use of a small set of chemical building blocks or reagents, combined together in multiple ways using standard synthetic chemical reactions, to make libraries of compounds. A library in this context is a mixture of several compounds made from the same building blocks. As with traditional drug design, combinatorial chemistry relies on organic synthesis. The difference is the scope—instead of synthesizing a single compound, combinatorial chemistry exploits automation and miniaturization to synthesize large compound libraries. Many different compounds are made simultaneously by assembling the same set of reagents in different ways; the resulting compound library contains all possible chemical structures that can be produced by combining the building blocks. Thus, combinatorial organic synthesis is not random but systematic and repetitive, using sets of chemical building blocks to form a diverse set of molecular entities.

The concept of combinatorial chemistry has many counterparts in nature. For example, our body is able to synthesize a variety of proteins from the same amino acid building blocks by assembling them in different sequences.

One can envision the process as shown in Figure 7.3, which depicts the library created by combining four different building blocks once. If all the reactions proceed successfully, 16 different reaction products will be present in the final mixture, with the shaded compound being active. If this mixture is screened and found active, it will indicate that at least one of the compounds has biological activity. The challenge is to identify the active compounds in the library. One way is to make each individual compound and test it for activity, but this would be very inefficient. Another is to synthesize and screen eight sets of compounds, each set representing either a row or a column from Figure 7.3. Two sets will show activity, and the only common compound in each will be the active compound β - γ .

The large number of compounds that can be generated by several such reactions is illustrated as follows. A mixture of 10 amino acids is coupled with another mixture of 10 amino acids to make a mixture of 100 possible dipeptides. These are then reacted with another 10 amino acid mixtures to create a combinatorial library of 1,000 different tripeptides. As the length of the peptide chain is increased in this manner, the number of compounds in the library increases exponentially.

Combinatorial libraries are screened as a group by HTS for binding to target proteins to identify one or more lead com-

	α	β	γ	δ
α	α - α	α - β	α - γ	α - δ
β	β - α	β - β	β - γ	β - δ
γ	γ - α	γ - β	γ - γ	γ - δ
δ	δ - α	δ - β	δ - γ	δ - δ

Figure 7.3. An example of a combinatorial library resulting from four reacting components α , β , γ , and δ . If all the reactions proceed, 16 different products will be present in the final mixture. The shaded compound, β - γ , is active.

pounds. A new compound that binds to the target is called a *hit*; when biological activity is found for a particular library, one can trace back and find the structure of the active compound as discussed above. New combinatorial libraries can then be created to focus on the specific functional groups and structural features of the initial hit, and to develop the hit into a lead.

A crucial aspect of combinatorial chemistry is being able to identify the compounds produced in a combinatorial scheme. Not all the possible reactions work, and not all possible compounds are actually present in the mixture. It is important to know not just what reactions were run, but whether the compounds are actually present in the library. Detecting the presence of an active component in a mixture of thousands of compounds and then correctly identifying its structure is a challenge and has opened up new approaches in high throughput analytical characterization.

Rational Combinatorial Chemistry. Traditional combinatorial chemistry without any preconceived biological basis results in very large libraries, with compounds that may not have any chance of being developed into a drug. In the rational combinatorial approach, the advantages of rational drug design and combinatorial chemistry are combined to reduce the number of compounds in the library and give them a greater chance of being useful drugs. The starting reagents or building blocks are selected on the basis of structural properties that are believed to have the best probability of biological activity, the result being a *targeted library*. Often, the resulting library consists of systematic variants of one chemical structure or scaffold or template. The scaffold structure contains a portion that remains constant, whereas a number of functional groups are allowed to vary using a combinatorial approach. The process of making a targeted library based on a scaffold is illustrated in Figure 7.4. Natural products are

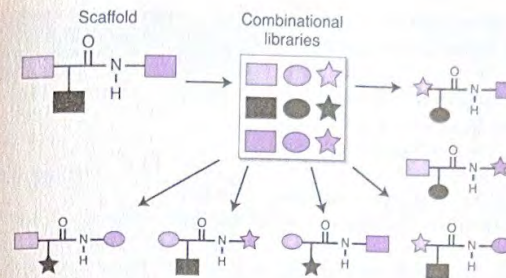


Figure 7.4. Rational combinatorial chemistry approach in making a targeted library of compounds based on a peptide scaffold.

becoming important scaffolds for building such targeted libraries, because scientists recognize that natural compound starting points have a higher degree of probability of being drug-like.

Optimization of the Lead Compound

Once a lead compound is identified, it almost always requires modification to improve its pharmacokinetic, pharmacodynamic, and safety properties. ADME properties need to be optimized for a lead

compound to become a successful drug. Although the pharmacophore is responsible for biological activity, a multitude of modifications are possible in the vector groups of the molecule. Many potential drug leads that look excellent in the laboratory can become expensive failures later because of poor ADME properties or unacceptable toxicity.

Selectivity and Toxicity

The ability of a drug compound to be selective in binding to its target is important to avoid toxicity. A lead compound may show excellent affinity to the target,

Virtual Combinatorial Chemistry

For speed and convenience, some combinatorial compound libraries begin as virtual libraries. For example, if 200 amines are reacted with 200 amino acids, the library would contain 40,000 amides, and considerable time and money would be spent in synthesizing even some of these compounds. In a virtual approach, the library is made using computational techniques. The virtual compounds are docked to their targets on the computer to identify

structural features that give the best fit. Only the compounds that meet all desired properties, such as solubility, stereochemistry, and fit into the target active site, are selected. Multiple virtual experiments can be done on the same virtual compounds using different conditions to give faster and cheaper information. A smaller, more focused combinatorial library is then synthesized in the laboratory based on the structural features identified.

but if it is not at least somewhat selective, its suitability as a drug is diminished. An important step in lead optimization is screening for selectivity against other similar proteins, to ensure that the lead compound binds to the target with much greater affinity than to nontarget proteins. A selective compound is less likely to show toxicity as development proceeds.

Other screens for general cellular toxicity are also carried out at this time to make sure that the compound is relatively safe when in contact with the body's cells.

Drug Likeness

The first step in lead optimization is to predict whether the compound has appropriate physicochemical properties to make it a good drug. Even though chemical structures of drugs differ greatly because each one is targeted to a different protein, successful drugs on the market share similarities in their physicochemical properties. This is particularly true for orally administered drugs. Druglike characteristics are those that increase the probability that the drug will have favorable ADME properties. A well-known approach is that of Lipinski, who performed statistical analyses of about 2,000 drugs. This study established a set of empirical criteria that are generally valid for the majority of the drugs. The set of criteria is known as the rule of five, shown in Table 7.1. The rule of five identifies characteristics that make a compound undesirable as a drug, particularly with regard to ADME properties.

Because all parameters can be easily calculated from the structure, the rule of five has become the most widely applied filter in selecting compounds for development. It is particularly useful in modifying structures of lead compounds to impart desirable physicochemical properties. Compounds that cannot be ruled out by the rule of five are considered

TABLE 7.1. The Rule of Five to Eliminate Leads With Undesirable ADME Properties^a

Property	Value Above Which Compound Is Undesirable as a Drug
Log P	>5
Molecular weight	>500
Number of H-bond donating groups	>5
Number of H-bond accepting groups (-N and -O)	>10

^aCompounds not ruled out are considered drug-like and are predicted to have satisfactory ADME (absorption, distribution, metabolism, and excretion) behavior after oral administration.

drug-like, meaning that they are predicted to have satisfactory ADME behavior after oral administration. Obviously, this simple rule is not always correct in identifying drug-like compounds. More complex analyses using artificial neural networks and fragment screening have been proposed and used, but these are outside the scope of our discussion.

Once the drug likeness of the lead is established, several analogs of the lead are synthesized. An analog is a compound with the same or similar pharmacophore as the lead, but with differences in other parts of the molecule. If analogs differ in structure by a simple and constant increase in one part of the molecule (such as the length of an alkyl chain), they are part of a homologous series. The objective of making analogs is to retain pharmacological activity of the lead but to minimize or eliminate unwanted properties. Analogs are then tested in the laboratory to select the compound that will proceed to animal testing and ultimately into human clinical trials.

Some important questions addressed in making analogs are as follows. Can the structure of the compound be simplified without loss of activity? Does the partition coefficient or solubility need to be

improved? Do vector groups need to be altered to prevent or change metabolism? Some logical approaches to modifying lead compounds have been more successful than others.

Bioisosterism

On the basis of the analysis of drug likeness, structural changes are often needed to improve physicochemical properties without significantly changing biological properties of a lead compound. In other words, the pharmacophore needs to be kept relatively unchanged while vector groups are modified to improve lipophilicity, water solubility, or susceptibility to metabolism.

Chemical isosterism is the similarity in physicochemical properties of ions, compounds, or elements because of similarities in their electronic structures. This concept was first introduced for atoms: elements in the same vertical row of the periodic table have similar outer shells of electrons, giving them the same electronic properties. In these rows, atoms with the same size and mass also have similar physicochemical properties. A similar trend is seen for neighboring atoms in horizontal rows of the periodic table. Thus, if one atom or group of atoms in a molecule is replaced with its isostere, the physicochemical properties of the compound do not change significantly. Chemical isosteric equivalents can

be used to synthesize different compounds with the same physicochemical properties.

Bioisosterism is an application of isosterism to biological systems and guides molecular modification of drugs without dramatic changes in their biological properties. The underlying principle is that if a modified compound is to interact with the same target as the original lead to give the same action, then the modification cannot be too drastic. Small structural changes may be achieved by replacing specific atoms or groups of atoms with their bioisosteres. The reason for making bioisosteric modifications is to synthesize similar compounds that retain biological activity, but have improved physicochemical properties and better pharmacokinetic behavior. This approach is widely used in the synthesis of improved drugs based on a lead compound.

Bioisosteric Equivalents

Classic bioisosteres are subdivided by equivalence into several categories such that atoms or functional groups within a category are interchangeable. Examples are shown in Table 7.2. Substitution with a bioisosteric equivalent usually changes one or more physicochemical properties of the drug. A few classic examples of bioisosterism are shown in Figure 7.5.

TABLE 7.2. Examples of Bioisosterically Equivalent Functional Groups^a

Category	Examples
Monovalent atoms or groups	-Cl, -Br, -I -XH _n , where X is C, N, O, or S
Divalent atoms or groups	R-O-R ₁ ; R-NH-R ₁ ; R-CH ₂ -R ₁ ; R-Si-R ₁
Trivalent atoms or groups	C and N in R-CH=R ₁ and R-N=R ₁
Tetravalent atoms or groups	=C=; =N+=; =P=
Ring equivalents	-CH=CH; -S-; -O-; -NH; and -CH ₂ -

^aThe groups in each category can be substituted for one another on a drug molecule without significant changes in biological activity.

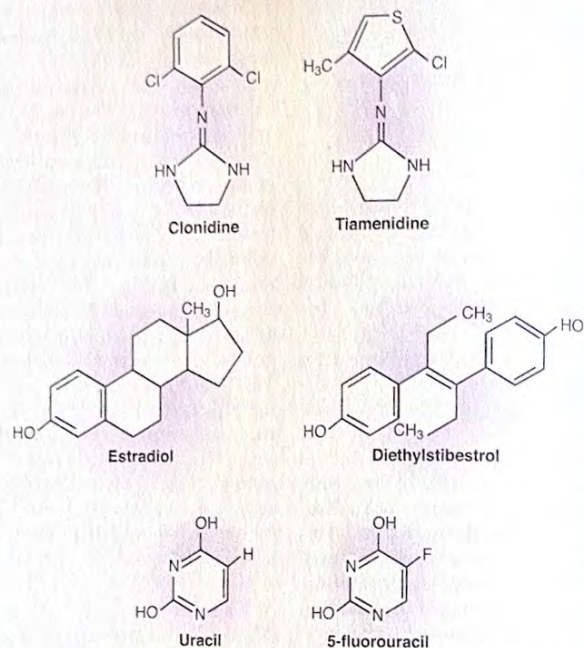


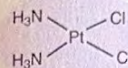
Figure 7.5. Examples of bioisosteres. On the left is the parent compound and on the right is the bioisostere.

Structure-Activity Relationships

A structure-activity relationship (SAR) is the relationship of the molecular structure of a compound with a biological property. The basic assumption behind these relationships is that different structures must give different activities or different degrees of the same activity. Relationships between structure and behavior can be found for a drug's pharmaceutical properties (solubility, stability, dissolution), its pharmacokinetic properties (absorption, distribution, metabolism, and excretion), or its pharmacodynamic properties (interaction between drug and target). These correlations may be qualitative (simple SAR) or quantitative (quantitative SAR, or

QSAR). In general, they are a set of rules that predict whether a compound will be active, and to what extent. Structure-activity relationships can then be used to predict which analogs will have the most desirable properties.

Qualitative predictions are based on a comparison of the properties of one or more analogs (i.e., structurally similar compounds) with the compound of interest. For example, terms such as "similarly active," "less active," or "more active" would be used in a qualitative SAR assessment for the biological activity of a series of analogs compared with the lead compound. An example is the SAR for analogs of the anticancer drug cisplatin, as shown in Figure 7.6.



Geometry around Platinum must be *cis*
Amine should contain at least one N-H bond
Anionic group (Cl) must be displaceable by incoming nucleophile like DNA

Figure 7.6. Structure-activity relationship rules for analogs of the anticancer drug cisplatin.

Quantitative predictions, on the other hand, are usually in the form of an equation that relates some property of the compound to specific structural features of the compound. They also give some estimation of the degree of biological activity expected. Researchers have attempted for many years to develop drugs based on QSAR, and there have been numerous attempts to mathematically correlate drug structure with pharmacological activity. Many parameters enter into the development of a QSAR as illustrated in Figure 7.7. Classic QSAR analyses consider only two-

dimensional structures whereas the newer three-dimensional QSAR approach is much more complex and takes into account three-dimensional properties.

Equations have been developed to correlate activity with physicochemical properties such as partition coefficient, pK_a , hydrogen bonding ability, or other structural features such as steric effects and electronic properties of the drug. These properties may be determined experimentally, but are increasingly being calculated by computational methods. In fact, QSAR of biological activity has evolved into what we discussed as rational drug design or computer-assisted drug design.

The QSAR equation is a model that relates variations in a biological parameter to variations in values of computed (or measured) properties for a series of molecules. QSAR relationships are often presented as graphs of variation in a compound property versus biological effect, and a systematic relationship is often observed. The relationship is usually linear but is sometimes nonlinear, as shown in Figure 7.8.

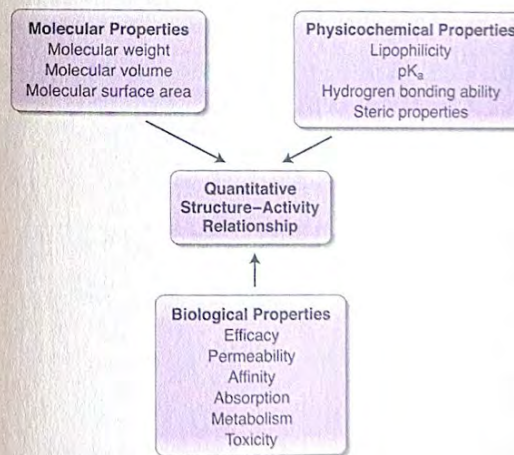


Figure 7.7. An illustration of the concept of quantitative structure-activity relationships. The relationship is established by correlating molecular or physicochemical properties of a drug with its biological or pharmacological behavior.

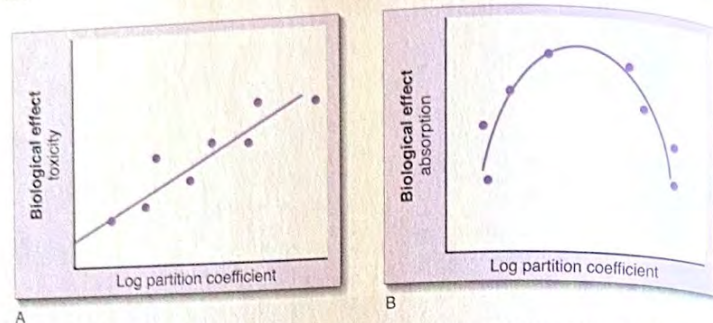


Figure 7.8. An example of linear (A) and nonlinear (B) structure–activity relationships between a biological property and a physicochemical property such as partition coefficient. The circles represent data from compounds. From such a graph, the optimal partition coefficient can be selected depending on the particular requirements.

Developing a quantitative structure–activity relationship is difficult. Molecules are typically flexible, and it is possible to compute many possibly useful properties that might relate to activity. For the method to work efficiently, compounds selected to define the equation should be diverse; the quality of any QSAR will only be as good as the quality of data used to

derive the model. Once the relationship is defined, it can be used to aid in prediction of new or unknown molecules. QSAR has been surpassed by rational drug design as a technique for lead identification, but it continues to be an important tool for lead modification and optimization to predict structures that have better ADME properties than the lead compound.

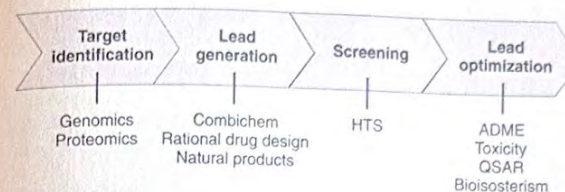


Figure 7.9. A summary of the steps involved in new drug discovery. The target is identified and selected based on information about genes or proteins involved in the disease process. Leads are generated and synthesized using several approaches. The leads are screened for biological activity, and then optimized to make the compounds more drug-like. ADME, absorption, distribution, metabolism, and excretion; HTS, high-throughput screening; QSAR, quantitative structure–activity relationship.

KEY CONCEPTS

- A drug's structure can be divided into the pharmacophore, the portion that fits into the target active site, and vector groups that control ADME properties.
- The lead compound serves as an initial prototype for drug discovery, but must be optimized to make it drug-like.
- Biological screens identify compounds that can serve as leads.
- Leads are discovered through random screening of synthetic compounds, or by screening natural products or combinatorial libraries.
- High-throughput screens are capable of rapidly testing combinatorial libraries.
- Lead compound structures are optimized by retaining the pharmacophore and modifying vector groups to make the compound more drug-like.
- Poor drug candidates can be rejected using Lipinski's rule of five.
- Bioisosteric principles and SAR are used to modify the structure of the lead compound to provide suitable ADME properties while retaining biological activity.
- The overall process of drug discovery discussed in this chapter is summarized in Figure 7.9.

ADDITIONAL READING

1. Krogsgaard-Larsen P, Liljefors T, Madsen U (eds). Textbook of Drug Design and Discovery, 3rd ed. Taylor and Francis, 2002.
2. Abraham DJ (ed). Burger's Medicinal Chemistry and Drug Discovery, 6th ed. Wiley-Interscience, 2003.
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Ligands and Receptors

The Signaling Process

Endogenous Ligands

Modes of Cell Signaling

Signal Transduction

Consequences of Ligand Signaling

Receptors

Ligand-Receptor Interactions

Noncatalytic and Catalytic Receptors

Receptor Subtypes

Receptor Structure and Classification

Modulation of Ligand-Receptor

Interactions

Concentration of Ligand

Receptor Density

Allosteric Modulation

Key Concepts

Additional Reading

Review Questions



Cells in our body live in association with other cells and must communicate and interact with each other. Cells use chemicals for communication with each other to regulate metabolism, electrical signaling, contraction, growth, ion transport, and other important processes. These chemical messengers, called ligands, are released by one set of cells as needed and bind to their receptors to cause a cellular response. Cellular communication progresses and adjusts normally in a healthy individual to maintain overall well-being. However, one or more cell communication pathways may become impaired with age or disease, and drugs may be needed to restore normal balance between cells.

Chapter 6, *Drugs and Their Targets*, introduced receptors, ligands, and concepts that underlie receptor-ligand binding. This chapter will describe common types of ligands that carry signals for cellular communication. Further, this chapter will discuss the major types of receptors that serve as important ligand and drug targets and explain further the outcomes of ligand-receptor binding.

In the broadest sense, any interaction of a chemical signal with a biological macromolecule (protein, DNA, RNA) can be

considered to be a receptor–ligand interaction if some response occurs after binding. The word “signal” in this context refers to nothing more than molecules (either ligand or drug) present in the extracellular fluid. However, we shall confine our discussion to the interaction of signal molecules with protein receptors, as generally accepted. Many enzymes, such as those with allosteric regulation, can also be considered to be receptors. Sometimes a receptor has enzymatic activity as well.

On binding to the appropriate ligand, the receptor transmits a signal that initiates biochemical changes in the target cell by a wide variety of possible pathways. Cells are exposed to many ligands from their environment, each carrying a chemical message and capable of initiating a different receptor response.

The Signaling Process

Cell signaling is the process by which cells release, transmit, receive, and respond to information from their environment and from each other. This information is carried by endogenous ligands (also called *mediators* or *signaling molecules*) made and released by cells as needed. Human cells can also detect and respond to external signals such as noxious agents, bacteria, viruses, and toxins.

There are three main players involved in the signaling process: the signaling cell, the ligand, and the recipient of the signal, the receptor. The synthesis and release of a ligand by a cell is often itself the result of a ligand–receptor interaction. The binding of the released ligand to a complementary receptor initiates a cascade of biochemical reactions that ultimately result in a physiological action. Only very small amounts of a signal are necessary to mediate body functions, cell–cell communication, and many disease processes.

The overall behavior of a cell depends on the signals it is exposed to in its environment, and each cell responds to signals according to its own specific character. A cell may respond to one set of signals by multiplying, to another set by carrying out a biochemical reaction, and to yet another set by differentiating. Different types of cells require different sets of signals for survival, and the same ligand often has different effects on different cell types.

Many drugs act by competing with or taking the place of an endogenous ligand or by modifying the signaling pathway in some manner. In this way, a drug can make a quantitative change in an existing physiological or biochemical process; however, most drugs do not qualitatively alter the nature of the process. Understanding cell signaling is a first step in understanding drug effects and enables scientists to design better drugs.

The major steps in the signaling process can be described as follows:

1. Signaling cells synthesize the appropriate ligand, usually based on a signal they themselves receive.
2. The cells release ligand molecules, usually into the extracellular fluid.
3. Ligand molecules are transported to target cells in a variety of ways, as we shall see shortly. Depending on its physicochemical properties, the ligand may remain in the extracellular fluid or enter the target cell.
4. Appropriate receptors in the target cells recognize and bind to the ligands with specificity, forming a complex. Receptors may be located either inside the target cells (intracellular receptors) or in the cell membrane (cell-surface receptors).
5. The ligand–receptor complex transforms the signal into an intracellular message and triggers a response in target cells.
6. The ligand is removed, terminating the response.

Endogenous Ligands

A simple classification of ligands secreted by the body is difficult because their chemical structures and physicochemical properties vary considerably. Some ligands are small molecules whereas others are peptides and proteins; some are charged in the physiological pH range whereas others are uncharged; some are hydrophobic whereas others are polar. Furthermore, a given ligand can bind to extracellular, membrane-bound, or intracellular components to initiate signaling cascades that result in different responses depending on the conditions.

One way to group ligands is based on their ability to cross cell membranes. The vast majority of ligands (e.g., peptides) are polar or charged and thus unable to cross the lipid bilayer of the cell membrane. Their receptor proteins must therefore reside in the cell membrane, be able to detect ligand presence in extracellular fluid, and transmit this information into the interior of the cell. These cell-surface receptors serve as conduits for transfer of information from the cell exterior to the cell interior.

Hydrophobic ligands such as steroids, some vitamins, and gases such as nitric oxide can cross the cell membrane by passive diffusion and enter cells. Their target receptors may be on the cell surface but often are located in the cytoplasm or the nucleus. Table 15.1 lists examples of common ligands classified by their hydrophilicity or hydrophobicity. Figure 15.1 illustrates the difference in signaling by hydrophilic and hydrophobic ligands.

Another method of classifying ligands is based on the type of cells that produce them. For example, neurotransmitters are produced by nerve cells and hormones are produced by endocrine glands.

Modes of Cell Signaling

Ligands are synthesized and secreted by many different types of cells and move from signaling cells to target cells in a variety of ways. The major modes of signaling are endocrine signaling by hormones and local signaling by autacoids.

Endocrine Signaling

Glands (extensions of the surface epithelium into underlying connective tissue)

TABLE 15.1. Examples of Various Types of Ligands Grouped by Their Hydrophilicity and Hydrophobicity^a

Hydrophilic ligands (cell-surface receptors)	Hydrophobic ligands (intracellular receptors)	Hydrophobic ligands (cell-surface receptors)
Small molecule hormones	Steroid hormones	Arachidonic acid derivatives
Peptide hormones	Retinoids	Prostaglandins
Neurotransmitters	Thyroxines	Leukotrienes
Growth factors	Vitamin D	Platelet activating factor
Trophic factors	Cortisol	
Cytokines	Nitric oxide	
Chemokines		
Neuromodulatory peptides		

^aHydrophilic ligands cannot cross the cell membrane and can act only on cell surface receptors. Hydrophobic ligands may have intracellular or cell surface receptors.

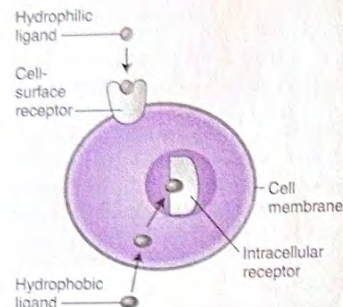


Figure 15.1. Interaction of hydrophilic ligands with cell-surface receptors and hydrophobic ligands with intracellular receptors.

contain cells whose main function is secretion. Glands that maintain contact with the surface by a system of ducts are called *exocrine* or ducted glands, such as the sweat and salivary glands. These glands release their secretions (sweat, tears, mucus, bile) into tubes or ducts that lead to internal or external body surfaces, but are not an important source of signaling molecules.

Many ligands are synthesized and secreted by *endocrine* or ductless glands that have lost their duct system and are isolated in connective tissue; examples

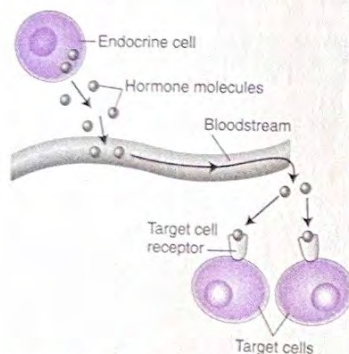


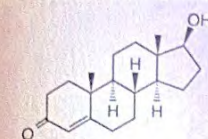
Figure 15.2. The process of endocrine signaling by hormones. Hormones released by the signaling cells enter the bloodstream and travel to the distant target cells. Here they exit the bloodstream and bind to receptors to elicit a response.

are the thyroid and adrenal glands. Endocrine glands have good blood flow, and their secretions, called *hormones*, diffuse into capillaries and are distributed by the bloodstream to target cells. Hormones may have to travel long distances to reach their target cells as illustrated in Figure 15.2, making endocrine signaling a relatively slow process. Thus, hormones are involved in the slow and steady regulation of responses all over the body.

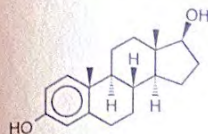
Because most hormones circulate in blood, they come into contact with essentially all cells. However, a given hormone usually affects only its target cells, i.e., those with receptors for the hormone. A few hormones, such as the thyroid hormones, are secreted in a relatively steady amount so that their concentration in the blood remains essentially constant under normal conditions. Other hormones are secreted on a specific cycle. For example, glucocorticoids are secreted on a 24-hour or diurnal rhythm, whereas female sex hormones are secreted on a 4-week cycle. Still other hormones, like insulin, antidiuretic hormone (ADH), epinephrine, and norepinephrine, are secreted on demand, depending on the physiological situation, or as a response to internal or external stimuli.

Types of Hormones. Hormones may be categorized into four structural groups, with members of each group having many properties in common.

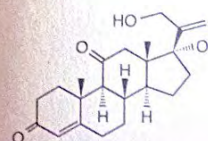
- **Steroids:** Steroids are lipids derived from cholesterol. Examples include the sex hormones, such as testosterone, and adrenal hormones, such as cortisol (Fig. 15.3).
- **Modified amino acid derivatives:** These comprise catecholamines, histamine, serotonin, and melatonin. Catecholamines (dopamine, norepinephrine, and epinephrine) are derived from tyrosine, serotonin and melatonin are synthesized from tryptophan, and histamine is derived from glutamic acid (Fig. 15.4).



Testosterone

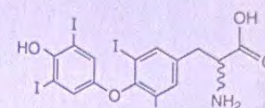


Estradiol



Cortisone

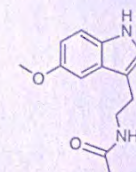
Figure 15.3. Structures of some common steroid hormones.



Thyroxine



Histamine



Melatonin

Figure 15.4. Structures of some hormones derived from amino acids.

- **Peptides and proteins:** Peptide and protein hormones are products of translation and vary considerably in size and posttranslational modifications, ranging from peptides as short as three amino acids to large, multisubunit glycoproteins. Examples are the neuropeptides (vasopressin, oxytocin), pituitary hormones (corticotropin, gonadotropins), and gastrointestinal hormones (insulin) (Fig. 15.5).
- **Eicosanoids:** This is a large group of molecules derived from polyunsaturated fatty acids, particularly arachidonic acid. The main types of hormones in this class are prostaglandins, prostacyclins, leukotrienes, and thromboxanes (Fig. 15.6).

Despite their molecular diversity, hormones can be categorized into one of two types based on the location of

their receptors as either hormones with intracellular receptors or hormones with cell-surface receptors.

Hydrophobic hormones (e.g., the steroids) can cross cell membranes by passive diffusion and carry their signal into the cell. Examples are the sex hormones (androgens, estrogens, and gestagens) and the adrenal hormones (mineralocorticoids and glucocorticoids). Thus, their receptors are usually located in the cell, either in the cytoplasm or the nucleus. Thyroid hormones (e.g., thyroxine, amino acid derivatives) enter the cell by facilitated diffusion and also bind to intracellular receptors. The primary mechanism of action of hormones with intracellular receptors is to modulate gene expression in target cells.

Most polar or charged hormones, such as the protein and peptide hormones, catecholamines, and eicosanoids, cannot cross the cell membrane, and have cell-

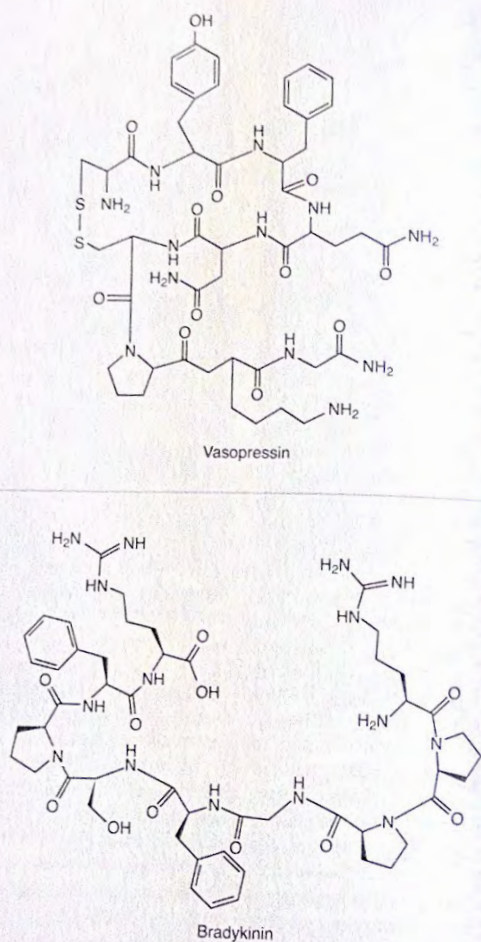


Figure 15.5. Structures of some peptide hormones.

surface receptors. Binding of hormone to its receptor initiates a series of events that leads to generation of so-called *second messengers* within the cell (the hormone is the first messenger) that transmit the signal inside the cell. We shall examine second messengers later in the chapter.

Signaling by hormones decreases and stops as hormones are eliminated from the bloodstream. Elimination takes place by biotransformation in the liver, bloodstream, and other sites. Hormones are also eliminated by excretion in the urine or bile.

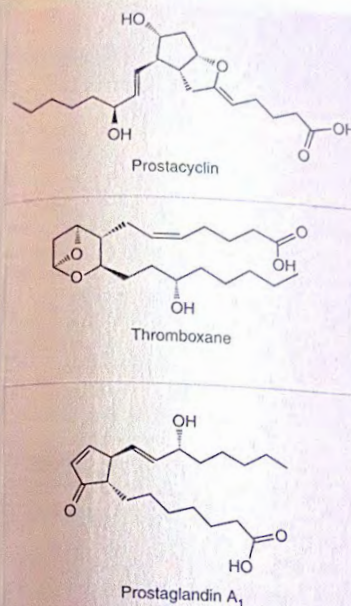


Figure 15.6. Structures of some eicosanoid hormones derived from fatty acids.

Plasma protein binding protects small hormone molecules (such as thyroid hormone) from rapid elimination. Protein binding also serves as a reservoir of hormone, so the concentration of free hormone in plasma is kept small and fairly stable.

Mechanisms of Hormone Action. Hormones exert their action by one of two fundamental mechanisms: activation of enzymes or modulation of gene expression.

Most enzymes shuttle between catalytically active and inactive conformational states. Many hormones affect their target cells by inducing such transitions, usually causing an activation of one or more enzymes. Because enzymes are catalytic and often serve to activate additional enzymes, a small change induced by

hormone-receptor binding can lead to widespread consequences within the cell.

In particular, hormones induce their biochemical changes in the cell through changes in the amount of phosphate bound to proteins. Approximately one third of the proteins present in a typical human cell may be phosphorylated (covalently bound to phosphate) at one time or another. Protein phosphorylation and dephosphorylation are controlled by two enzymes: *protein kinases* that put phosphate on a protein and *phosphatases* that remove phosphate attached to a protein. Phosphorylation can increase or decrease the biological activity of an enzyme, help move proteins between subcellular compartments, and allow interactions between proteins to occur, as well as label proteins for degradation. The variety of responses to phosphorylation is immense, and many human diseases are associated with the abnormal phosphorylation of cellular proteins.

The binding of hormones to their receptors on the surface of a cell often results in the activation of kinases and phosphatases, as illustrated in Figure 15.7. Once activated, cellular phosphorylation patterns will begin to change, with many proteins being phosphorylated or dephosphorylated. The final result will be various changes in cellular behavior.

The other major mode of action of hormones is stimulating transcription of a group of genes. This can alter a cell's phenotype by leading to synthesis of new proteins. Similarly, if transcription of a group of previously active genes is shut off, the corresponding proteins will soon disappear from the cell. When the hormone binds to its intracellular receptor, a conformational change in the receptor results in receptor activation. The major consequence of activation is that the receptor becomes capable of binding to DNA, and transcription from genes to which the receptor is bound is affected. Most commonly, receptor binding stimulates transcription, and the hormone-receptor complex functions as a transcription factor.

Antagonistic Hormone Pairs

Many hormones work in antagonistic hormone pairs with exactly opposite roles. When a body function exceeds its homeostatic level, one hormone can be released to bring the level down to normal; when that same body function falls below the homeostatic level, a completely different hormone might be released to bring the function up to normal. An example is the hormone pair of insulin and glucagon, both of which regulate blood glucose levels. Both hormones are secreted by the islets of Langerhans, a special group of cells in the pancreas; insulin is secreted by the beta cells of the islets, and glucagon by the alpha cells of the islets. Both cell types release their hormones simultaneously at a basal level. Insulin and glucagon are critical participants in glucose homeostasis and serve as acute regulators of blood glucose concentration.

When blood glucose levels rise above normal, such as after a meal, there is a rapid increase in insulin production by the beta cells. Insulin lowers blood glucose levels by mediating glucose uptake into skeletal muscle, liver, and adipose cells. When insulin binds to its receptor

on the membrane of these cells, glucose transporters are activated, allowing increased uptake of glucose. Insulin increases the use of glucose by muscle cells and its storage in the liver as glycogen and in adipose tissue as fat. This causes blood glucose levels to drop.

When blood glucose levels drop below the normal level, insulin secretion is markedly reduced to its basal level. Additionally, the alpha cells release glucagon, which stimulates the liver and muscles to break down glycogen, releasing glucose and reversing the drop in blood glucose concentration. Moreover, glucagon activates pathways in which amino acids are converted into glucose.

The brain in particular has an absolute dependence on glucose as a fuel because neurons cannot use alternative energy sources like fatty acids to any significant extent. When blood levels of glucose begin to fall below the normal range, it is critical to release additional glucose into blood. In this manner, insulin and glucagon act together to balance each other and maintain homeostasis. Type 1 diabetes is a potentially fatal disease in which insulin secretion fails.

Local Signaling

In addition to true endocrine glands and cells, there are other types of ductless glands and cells that secrete molecules for local signaling. In fact, almost every cell is capable of secreting a variety of ligands that help to maintain homeostasis. Ligands responsible for local effects are referred to as autacoids or local hormones.

Types of Autacoids. Classification of autacoids is difficult because this group encompasses a wide variety of structurally diverse compounds. In fact, many ligands can function as both endocrine and local signaling molecules; the main differences lie in the speed and selectivity with which the molecules are delivered to their targets. Thus, although the traditional definition of hormones describes them as being secreted into blood and af-

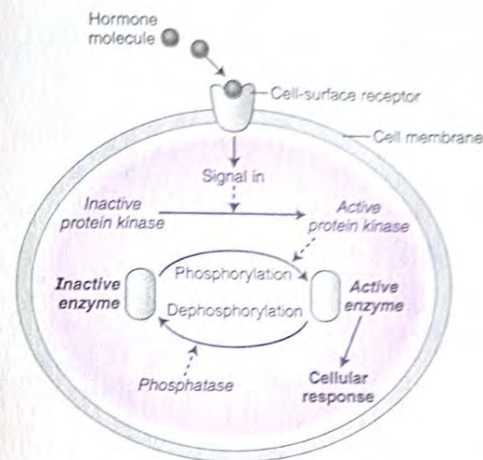


Figure 15.7. Activation and deactivation of an enzyme mediated by hormones. The binding of a hormone to its cell-surface receptor sends a signal that is transmitted into the cell interior. The signal activates a protein kinase that results in phosphorylation of another enzyme, eventually resulting in a cellular response. The active enzyme is deactivated by a phosphatase, and awaits another signal.

fecting cells at distant sites, many hormones also affect neighboring cells or even have effects on the same cells that secreted them. Figure 15.8 shows structures of some common autacoids.

Collectively, autacoids have three features in common: they do not need to be transported by the bloodstream, they exert effects near the site where they are synthesized and secreted, and the effects are typically of short duration. Most autacoids are secreted on demand at transiently high concentrations and then rapidly degraded, so their actions are local and short-lived. Even if not quickly degraded, they become diluted as they diffuse away from the site of secretion, so concentrations rapidly become low enough to be ineffective.

Autacoids are necessary for communication between cells during defense and repair processes and are involved in helping the body deal with some sort of insult or injury. They mediate the interactions between cells by either attracting or activating cells, or both.

Local signaling by autacoids can be classified, according to the way in which the target cell receives the signal, as paracrine, autocrine, or juxtacrine.

Paracrine Signaling. Paracrine signals are those that influence neighboring cells. A signaling cell releases ligands into the extracellular fluid, which diffuse to neighboring cells, bind to specific cell surface receptors, and transmit a signal into the cell. This is illustrated in Figure 15.9. An example of paracrine ligands is a group of related compounds called growth factors that stimulate neighboring cells to divide and grow.

A special type of paracrine signaling is neuronal signaling used by neurons; the ligands are called neurotransmitters. An electrical impulse triggers the release of the neurotransmitter from the neuron. The initial electrical message is sent over the length of the axon, but the neurotransmitter itself travels only the short distance of the gap, or synapse, between the signaling neuron and the target cell; an illustration is shown in Figure 15.10.

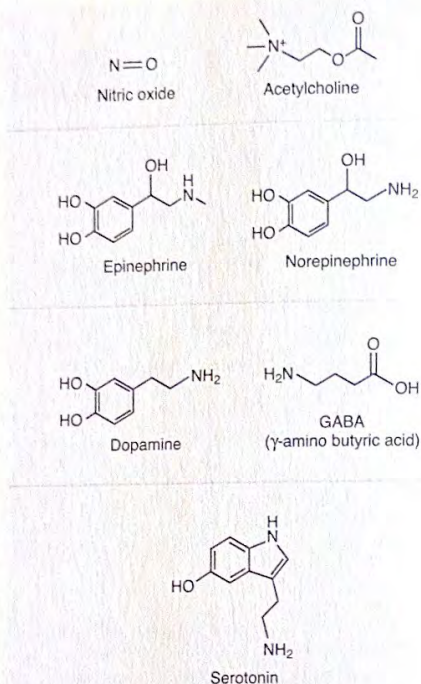


Figure 13.8. Structures of some common small-molecule autacoids.

Neuronal signaling, like all local signaling, is fast, and neurotransmitters are responsible for rapid transfer of information and quick responses at discrete sites. Several drugs act by either mimicking or blocking actions of neurotransmitters at specific locations in the body.

Autocrine Signaling. Autocrine signaling is a variation of paracrine signaling and occurs when cells respond to substances they themselves secrete, i.e., the signaling cell is also the target cell. If there is only one cell involved, the signal is weak, but a strong signal can be obtained if a group of identical cells are involved. Autocrine signaling is a mechanism to encourage a group of cells to adopt a similar behavior—a group of identical cells signal to them-

selves and each other to reinforce developmental decisions and create identical groups of cells. Autocrine signaling is illustrated in Figure 15.11.

Juxtacrine Signaling. Juxtacrine signaling, often called contact-dependent signaling, requires direct contact between the signaling cell and the target cell. The ligands are not released into the extracellular fluid but are directly transferred between the signaling and target cells, as shown in Figure 15.12. In one type of juxtacrine signaling, the ligand remains attached to the cell membrane of the signaling cell, and the signal is transmitted only when the target cell comes in direct contact with the attached ligand. Another type of juxtacrine signaling involves direct transfer of

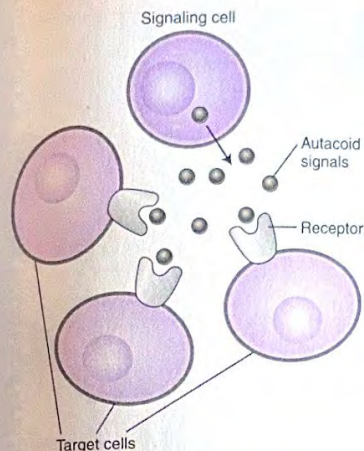


Figure 15.9. Schematic showing the process of paracrine signaling. The ligands are released by the signaling cells and bind to specific receptors on the target cells.

signaling molecules from one cell to another through gap junctions between adjacent cells. Recall that gap junctions are communicating junctions that allow small molecules and ions to pass directly from the cytoplasm of one cell to another. An example of juxtacrine signaling is electrical transduction from cardiac cell to cardiac cell via the gap junction.

Signal Transduction

We have seen that hydrophobic ligands and those capable of facilitated diffusion can enter the target cell and directly bind

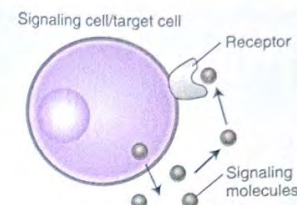


Figure 15.11. Autocrine signaling. The signaling cell is also the target cell.

to an intracellular receptor to cause an intracellular response. However, when a ligand binds to a cell-surface receptor, the signal needs to be relayed inside the cell to initiate an intracellular effect.

Signal transduction refers to the processes by which an extracellular ligand-receptor interaction causes an intracellular change without the ligand entering the cell. In other words, it is the translation of an extracellular signal into changes in cell behavior. Additional cellular molecules often become involved in transmitting the message to its ultimate destination in the cell.

The ligand is considered the *first messenger*, carrying a signal from the signaling cell to the target cell-surface receptor. Once the initial ligand-receptor binding has occurred, other compounds called second messengers play an important role in transmitting the signal to specific intracellular sites in the cytoplasm or nucleus. Second messengers are short-lived molecules that mediate the transduction of the extracellular signals, and are usually generated strictly in response to a

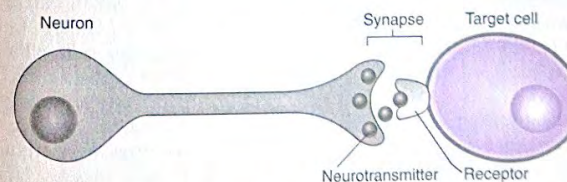
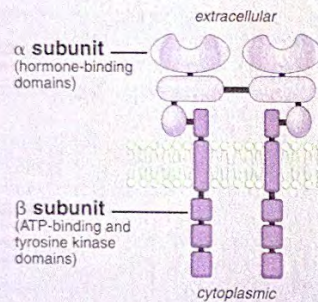


Figure 15.10. Neuronal or synaptic signaling, a special type of paracrine signaling. The signaling cell is the neuron, and the target cell may be another neuron or some other type of cell. The ligands are called neurotransmitters. The space over which the neurotransmitters travel between two neurons is the synapse.

The Insulin Receptor

Like receptors for other protein hormones, the receptor for insulin is embedded in the cell membrane of target cells (hepatocytes and myocytes). The insulin receptor is a tyrosine kinase-linked transmembrane glycoprotein with a dimeric structure capable of binding two insulin molecules at any given moment. It is composed of two α subunits and two β subunits linked by disulfide bonds. The α chains are entirely extracellular and contain the insulin binding domains. The linked β chains penetrate through the cell membrane into the



cell and contain ATP-binding and tyrosine kinase domains.

The receptor functions as an enzyme that transfers phosphate groups from ATP to tyrosine residues on intracellular target proteins. Binding of insulin to the α subunits is the initial step in a signal transduction pathway that mediates glucose uptake and metabolism. The binding causes the β subunits to phosphorylate themselves (autophosphorylation), activating the catalytic activity of the receptor. The activated receptor then phosphorylates a number of intracellular proteins, which in turn alters their activity and generates the biological response.

Signal transduction by the insulin receptor is not limited to its activation at the cell surface. The activated ligand-receptor complex initially present at the cell surface can be internalized into the cell by endocytosis, forming vesicles or endosomes. Endocytosis of activated receptors concentrates receptors within endosomes and also allows the insulin receptor tyrosine kinase to phosphorylate substrates that are further inside the cell.

specific ligand-receptor interaction. Particularly important second messengers in a variety of signaling pathways include cyclic AMP (cAMP), calcium ions, inositol 1,4,5-trisphosphate (IP_3), and diacylglycerol (DAG).

The signal transduction pathway is nonlinear such that one molecule of an agonist can produce a large signal. As an illustration, consider one molecule of an agonist activating one receptor. This activates 10 adenyl cyclases, generating 100 molecules of cAMP, which

activate 1,000 protein kinases, which phosphorylate 10,000 calcium channels, which allow 100,000 units of calcium to enter the cell, which finally causes 1 million units of contraction.

Protein phosphorylation is a common means of information transfer and many second messengers elicit responses by activating protein kinases. The use of common second messengers in multiple signaling pathways creates both opportunities and potential problems. Input from several signaling pathways, often

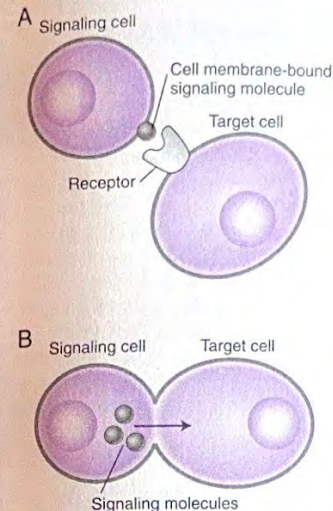


Figure 15.12. Juxtacrine signaling. A. Membrane-bound signal. B. Signal transmission through gap junctions.

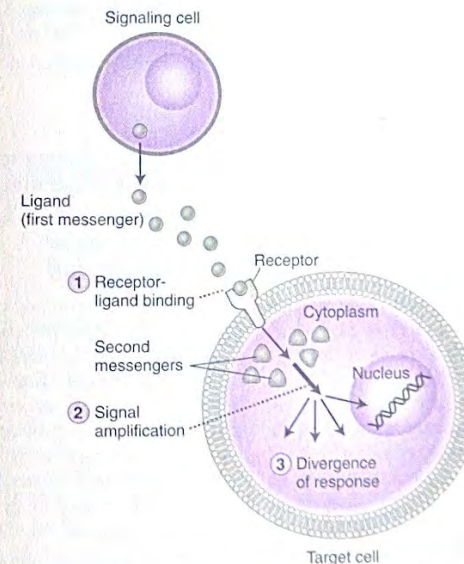


Figure 15.13. Representation of the signal-transduction process. The signaling cell releases a ligand that binds to a surface receptor on the target cell. This binding initiates signal transduction, which may involve second (intracellular) messengers. The outcome is some sort of cellular response in the target cell.

called *crosstalk*, may affect the concentrations of common second messengers. Crosstalk permits more finely tuned regulation of cell activity than would the action of individual independent pathways. However, inappropriate crosstalk can cause the signal carried by second messengers to be misinterpreted. An overview of the signal transduction process is illustrated in Figure 15.13.

After a signaling process has been initiated and the information has been transduced to affect other cellular processes, these processes must be terminated. Without such termination, cells lose their responsiveness to new signals. Moreover, signaling processes that fail to be terminated properly may lead to uncontrolled cell growth and the possibility of cancer. Protein phosphatases are one mechanism for the termination of a signaling process; the role of protein phosphorylation and dephosphorylation is shown in Figure 15.14.

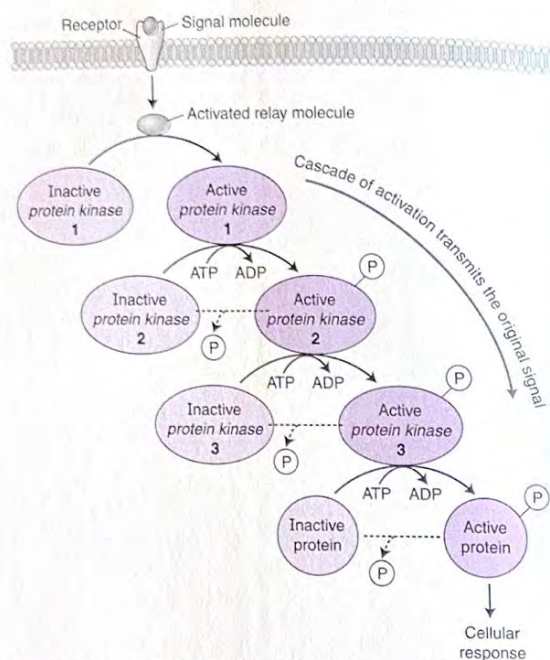


Figure 15.14. A more detailed depiction of the phosphorylation-dephosphorylation cascade that is often seen in signal transduction.

Signal transduction has several important features:

- The signal can undergo amplification, a process by which a small amount of ligand can create a large cellular response.
- The signaling pathway can diverge such that several processes in the cell can be influenced at the same time.
- Transmission of the signal can be altered according to the needs of the cell, by variation in the nature and amounts of the second messengers.
- The time course of the response can be controlled.

Several such counterbalanced signal transduction pathways control most cellular functions. Interfering with any step in the signal transduction cascade can be

potentially harmful, but may be a useful approach in the treatment of disease.

Consequences of Ligand Signaling

A ligand may act at many different cellular sites—the cell membrane, cytoplasm, or nucleus—and at several different receptors. For example, dopamine activates dopamine receptors as well as adrenergic receptors. It is amazing that a few types of endogenous signaling ligands (neurotransmitters, hormones, and autacoids) are involved in controlling and regulating a vast variety of physiological functions.

Although a ligand may be able to reach many cells, only target cells with a specific receptor for these ligands will

respond to the signal. Whether a cell responds to the signal and the manner in which it responds are dictated by what types of receptors the cell expresses and by the kind of signal transduction machinery available for responding to the signal. Variation in receptor type helps segregate the “signal” from the “noise” for various cells.

Different cells can therefore respond differently to the same signaling molecule. In other words, each cell is programmed to respond in a specific way to a signal. Additionally, the response depends on the number and type of other signals being simultaneously received by the cell. Thus, a single response may be dependent on the

presence (or absence) of several different ligands. The response of the target cell may be fast (on the order of seconds) or slow (on the order of minutes or hours).

Target cell responses to receptor–ligand interactions may include:

- Alteration in the metabolism of the cell
- Modification in the electrical charge across the cell membrane
- Readjustment of the cytoskeleton
- Secretion of substances
- Cell death
- Activation of DNA to produce cellular proteins

Some common types of cell responses to a signal are illustrated in Figure 15.15.

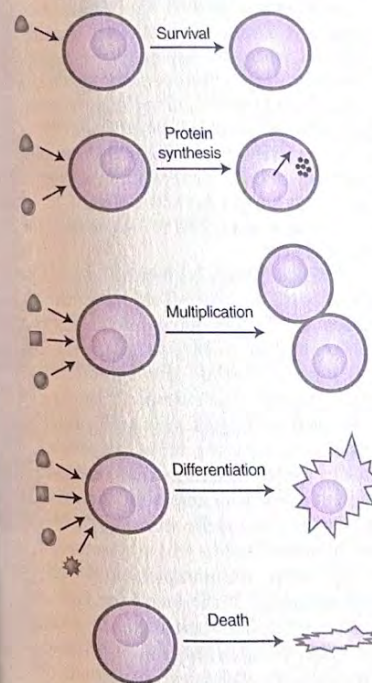


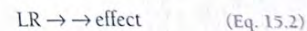
Figure 15.15. Examples of different types of responses that result after cell signaling. Each response is a consequence of a different number and type of signals.

Receptors

As we have seen, a receptor is a cellular protein that binds specifically to a ligand and responds to it, passing a signal along. A cell has numerous different receptors that can respond to various combinations of signal molecules.

Ligand-Receptor Interactions

Recall that the interaction between a receptor (R) and a ligand (L) to produce an effect can be expressed as a two-step process.



The ligand–receptor complex (LR) is formed in the first step (Eq. 15.1). In the second step, the complex becomes the stimulus for a series of events that lead to the ultimate effect. A ligand that produces a response in the second step (Eq. 15.2) is an agonist. A ligand that merely forms a complex but does not produce a subsequent response is an antagonist.

A very specific ligand structure is needed for a complex to form because receptor proteins are highly stereoselective. The ligand has to exhibit stereocomplementarity with the binding site of the receptor. Therefore, a receptor has two functions:

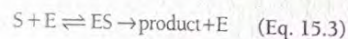
- detect a ligand and complex with it (recognition)
- conduct and translate a signal after binding to an agonist (signal transduction) to produce the desired effect

Once a ligand binds to a receptor-binding site, we say that the site is *occupied*. The number of receptors available in a particular tissue is usually limited, so that the receptors can be *saturated* if sufficient ligand is available and all the binding sites become occupied.

Noncatalytic and Catalytic Receptors

Many receptors are *noncatalytic*, meaning that ligand-receptor binding is reversible (as shown in Eq. 15.1) and the ligand dissociates intact from the receptor after the effect in Equation 15.2.

Ligands also bind to catalytic enzymes either in the cell or on the cell membrane; the ligand in this case is called a *substrate*. Enzymes interact with their substrates to form complexes, but unlike receptors, the enzyme catalytically transforms the substrate into chemically different products that are then released. The process can be viewed as follows:



where E is the enzyme and S is the substrate. Therefore, the two major characteristics of enzyme receptors are their ability to recognize a substrate (just like noncatalytic receptors) and their ability to catalyze a reaction of it.

Recent evidence suggests that some noncatalytic receptors have the potential to be active even in the absence of an ag-

onist ligand. Such receptors are referred to as *constitutively active receptors*. Their agonist-independent activity emphasizes the intrinsic regulatory activity of a receptor, i.e., the receptor (not the ligand) contains the information to modulate cell function. Many ligands inhibit this constitutive activity after binding to the receptor. These ligands act as *inverse agonists*, displaying negative intrinsic activity. The discovery has led to the concept of *inverse agonism*, which has emerged as an essential principle of receptor regulation.

Receptor Subtypes

Each agonist ligand has receptors in many different parts of the body and can stimulate a wide range of effects depending on where in the body the ligand-receptor interaction occurs. In fact, a ligand can bind with many slightly different versions of its receptor, each with a unique function, in various locations. Thus, ligands can be thought of as master keys that can fit several different locks or receptor subtypes to open different doors (effect).

For example, norepinephrine can interact with α - and β -adrenoreceptors, which are further subdivided into α_1 and α_2 and β_1 and β_2 receptors. These receptor subtypes are found in different target organs: for example, the heart contains β_1 receptors whereas β_2 receptors are found in the bronchial smooth muscle. Each receptor subtype has a different function, and the release of norepinephrine locally at each of these sites produces a different physiological effect. Another example is acetylcholine binding to *cholinergic* receptors that can be either *muscarinic* or *nicotinic*; each of these is subdivided into many different subtypes with distinct functions.

The existence of receptor subtypes allows our bodies to use just one ligand for a variety of actions by releasing it in

different locations. This phenomenon also allows scientists to design drugs that bind to only one receptor subtype of a ligand. In this way, drugs can be made very selective. Conversely, a drug that can bind to many subtypes of a receptor can cause unwanted side effects.

Receptor Structure and Classification

One way of classifying receptors is by the ligand that binds to them. In fact, many receptors are named after their signaling ligands, such as steroid, insulin, or acetylcholine (ACh) receptors.

A more useful approach to receptor classification is by their structure, function, or signal transduction pathway. On this basis, we can construct five broad categories of receptors:

Cell-surface receptors:

- Ion-channel receptors
- G protein-coupled receptors
- Enzymatic receptors

Intracellular receptors:

- Transcriptional regulation receptors
- Intracellular enzymes

Let us briefly consider each one of these structural classes and see how they work.

Cell-Surface Receptors

The primary mechanism through which a cell senses extracellular stimuli is through cell-surface receptors.

Ion-Channel Receptors. Although the cell membrane is lipidlike, we have seen that it contains proteins that form ion channels through which small ions (e.g., Na^+ , K^+ , Ca^{2+} , and Cl^-) can enter and leave the cell. The conformation and structure of the channel protein make it selective for transport of a particular ion. Conformational changes in these recep-

tor proteins can open or close these channels, regulating ion movement in and out of a cell.

Many ion channels are opened and closed by the binding of certain neurotransmitters with *ligand-gated ion-channel* receptors. These receptors are integral glycoproteins that traverse through the cell membrane and can exist in one of three states: resting (or closed), open, or desensitized. On binding with a ligand, the closed receptors rapidly change conformation to open the ion channel in a process known as *gating*. Dissociation of the ligand-receptor complex closes the ion channel. However, after prolonged exposure to the ligand, the receptors can become desensitized.

The signal ligands for these ion channels are neurotransmitters. Examples are the nicotinic ACh receptors (where the ligand is acetylcholine, which regulates the Na^+/K^+ channel) in skeletal muscle, and GABA receptors (where the ligand is γ -aminobutyric acid) in the brain. A schematic representation of a ligand-gated ion-channel receptor is shown in Figure 15.16. Signaling by ligand-gated ion channels is very fast—on the order of milliseconds—which is very important for the rapid transfer of information across synapses.

Another type of ion channel is called a *voltage-dependent ion channel*. These channels can be opened or closed by generating a voltage difference across the membrane. Such a voltage can be created as a result of a differential distribution of ions on the two sides of the membrane, resulting in a conformational change of the receptor protein and an opening and closing of the channel. There is no ligand directly involved in the transport of ions by voltage-dependent receptors.

G Protein-Coupled Receptors. The G protein-coupled receptors (GPCRs) are a superfamily of related cell-surface receptors vital for normal cellular health. GPCRs are important as targets for drug design and therapy; it is estimated that

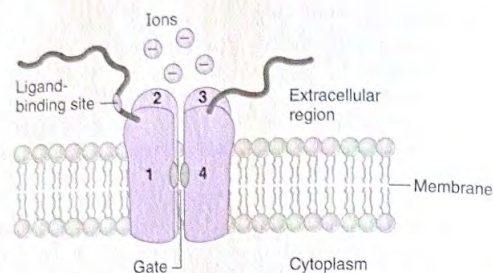


Figure 15.16. Schematic representation of a ligand-gated ion-channel receptor. The receptor is a transmembrane protein (shown here with four transmembrane segments labeled 1–4). The extracellular part of the protein contains the ligand-binding site. Ligand–receptor binding causes a change in the conformation of the receptor. This opens the gate, creating a pore through which ions can diffuse into the cell.

more than half of all the drugs on the market today target GPCRs.

The common structural feature of this class of receptors is a single polypeptide chain that has seven *transmembrane domains*, i.e., it traverses the lipid bilayer seven times. The term *G protein* in the name occurs because the signal-transduction mechanism in this class of receptors works through small intracellular proteins called *G proteins*, short for *guanine nucleotide-binding proteins*, a family of proteins involved in second messenger cascades. Their name is derived from their signaling mechanism, which uses the exchange of guanine diphosphate (GDP) for guanine triphosphate (GTP) as a molecular “switch” to allow or inhibit biochemical reactions inside the cell.

GPCRs bind to a wide spectrum of extracellular ligands, including hormones, neurotransmitters, and autacoids. Each ligand has a different mode and site of binding on the seven-transmembrane protein, resulting in a different biochemical outcome. Many of the GPCRs act through second messengers.

After a ligand binds to the active site on the cell surface, the receptor undergoes a conformational change that alters the intracellular portion of the receptor,

allowing it to further bind to the G protein in the cell. A G protein is made up of α , β , and γ subunits. Binding of the G protein with the ligand–receptor complex causes the G protein to break up into two active components—an α subunit and a β – γ complex—both of which can regulate the activity of target proteins in the cell membrane. Once the activated α -subunit has propagated its signal to its target protein, it reassociates with the β – γ complex and the signal is shut off. This “on–off” mechanism is a crucial component of controlling many signaling pathways. The receptor stays active while the signal ligand is bound to it, and can therefore catalyze the activation of many molecules of G protein in this way.

Figure 15.17 shows the structure and signal-transduction mechanism of GPCRs. Examples of this class of receptors are acetylcholine muscarinic, α - and β -adrenergic, dopamine, serotonin, and histamine receptors.

Enzymatic Receptors. This simple class of receptors has an extracellular portion that binds to the ligand, a single linear hydrophobic region that traverses the membrane lipid bilayer, and an intracellular portion that is located in the cytoplasm. This intracellular region may

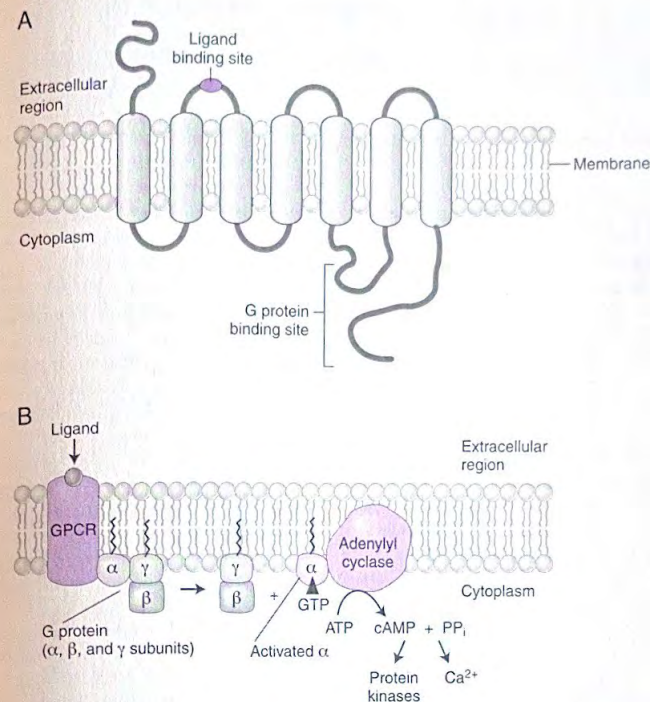


Figure 15.17. A. The structure of G protein-coupled receptors shows the seven-transmembrane (7TM) protein receptor with its seven transmembrane domains, and the binding sites for ligand and G protein. B. The signal transduction pathway after activation of a G protein-coupled receptor (GPCR) by a ligand.

have intrinsic enzyme activity that is triggered on binding to a ligand, i.e., the receptor is itself an enzyme. Note that ligand–receptor binding in this case is reversible because the signaling ligand is not the substrate. The substrate is located inside the cell and is transformed to a product on receptor activation by the ligand.

The best understood family of enzymatic receptors is the protein kinase receptor family, in which the enzyme activated is a protein kinase. For example, *tyrosine kinases* selectively attach a phos-

phate group to the amino acid tyrosine in a protein, and *serine/threonine kinases* to serine and threonine residues. Enzymatic receptors with intrinsic protein kinase activity bind to and mediate actions of most growth factors (epidermal growth factor, nerve growth factor, platelet-derived growth factor [PDGF], and so forth) and are of great interest for their role in cancer.

Alternatively, the receptor may be an enzyme-linked receptor; when activated by a ligand these receptors bind to and activate enzymes nearby. Here, too, the

signaling ligand is not the substrate. Ligand-receptor binding initiates a cascade of protein phosphorylations that result in the ultimate effect inside the cell. This class of single transmembrane (1TM) receptors is further classified according to the intracellular enzyme system that is activated after ligand binding.

Figure 15.18 illustrates an enzyme-linked receptor showing the single transmembrane protein, the extracellular ligand-binding site, and the intracellular site associated with enzyme activity. For example, the cytokine receptor is a tyrosine kinase-linked receptor. Cytokine receptors must first be activated by their signaling ligands (cytokines, interferons, and human growth factor [HGF]), and then bind to cytoplasmic tyrosine kinases before they are able to phosphorylate their target proteins. Therefore, protein kinases are involved in the process of signal transduction in these pathways as well, as they were with GPCRs.

Intracellular Receptors

Many intracellular proteins act as receptors for signaling ligands and other

compounds. Because the receptor is inside the cell, the signaling ligand has to cross the cell membrane, reach the receptor, and bind to it. Thus, the ligands must be lipophilic (like the steroid hormones) so that they can cross the cell membrane.

Transcriptional Regulation Receptors. The transcriptional regulation receptors, also known as *nuclear receptors*, constitute most of the intracellular receptor class. Transcriptional regulation receptors usually trigger an effect when the ligand-receptor complex travels from the cytoplasm into the nucleus, initiating transcription of RNA. They are further subdivided as:

- steroid receptors (e.g., receptors for corticosteroids, sex steroids, and mineralocorticoids)
- nonsteroid receptors (e.g., receptors for thyroid hormones, retinoic acid, and vitamin D)

The process by which a hormone binds to its intracellular receptor and triggers a response is illustrated in Figure 15.19. The hormone crosses the cell membrane

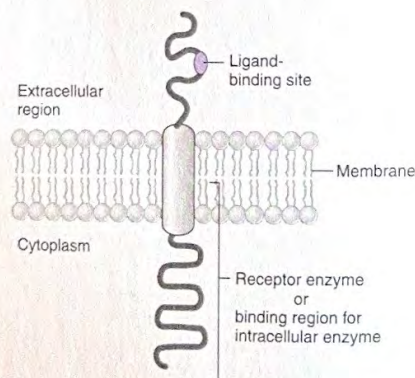


Figure 15.18. Schematic depiction of an enzymatic receptor showing the single transmembrane protein, the extracellular ligand-binding site, and the intracellular site associated with enzyme activity.

Gleevec

Disrupting messages between cells with specificity and limited side effects has become a new model for anticancer drug discovery. The success of signal-transduction inhibitors, such as Gleevec (imatinib mesylate) in first treating chronic myelogenous leukemia (CML) and then gastrointestinal stromal tumors (GISTs) has bolstered this approach to new drug discovery. Imatinib is an inhibitor of the receptor tyrosine kinases for PDGF and stem cell factor (SCF), and inhibits PDGF- and SCF-mediated cellular events.

In healthy individuals, normal receptors on stem cells respond to growth factor signals to produce new blood cells. The receptor can be turned on and off as necessary to produce the number of blood cells the body needs.

Patients with CML have a genetic defect that results in abnormal receptors that have constitutive activity and cannot be turned off, resulting in uncontrolled growth of white blood cells. The receptor needs energy provided by

ATP to carry out its kinase activity and propagate signals necessary for cellular proliferation. Imatinib fits into and inhibits the ATP-binding site on the receptor; without ATP, the receptor cannot carry out its kinase activity. Thus, imatinib is a tyrosine kinase inhibitor that prevents the constitutive cell growth signal in the abnormal, cancerous cells.

Tyrosine kinase inhibition reduces the ability of the kinase to transfer phosphate groups from ATP and phosphorylate tyrosine residues on substrate proteins, which in turn prevents the transduction of energy signals necessary for cellular proliferation. Thus, the specific signal-transduction pathway abnormally activated in the leukemic transformation process (and in PDGF-mediated pathways) is inactivated by imatinib.

Imatinib is indicated for the treatment of newly diagnosed adult patients with Philadelphia chromosome-positive CML. It is also now indicated for the treatment of patients with malignant GISTs.

by passive diffusion and binds to its receptor in the cytoplasm, thus activating the receptor. The receptor has a hormone-binding site (selective for a given hormone), a DNA-binding site, and a third site whose exact function is unknown. The hormone-receptor complex then travels to the nucleus, where it binds to DNA and initiates transcription of mRNA. The mRNA leaves the nucleus and serves as a template for synthesis of specific proteins within the cytoplasm. This process takes time, and there is generally a lag time—up to several hours—

between initial ligand-receptor binding and production of proteins. For this reason, the effect produced by hormones that act at nuclear receptors can persist long after the initial signaling event.

Figure 15.20 shows an overview of signaling by agonist binding to various types of receptors.

Intracellular Enzymes. Enzymes catalyze most cellular processes and reactions. Each cell requires more than 500 different enzymes to carry out all its functions, and the types and concentrations of

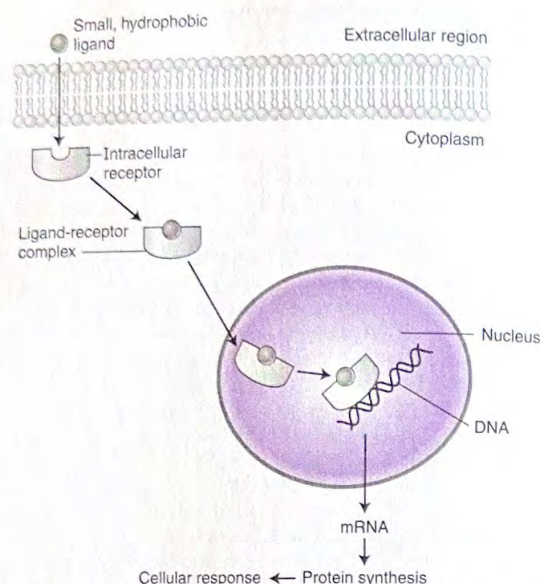


Figure 15.19. Mechanism of action of a typical intracellular hormone receptor. The receptor is located in the cytoplasm of the cell. The hormone (ligand) has to cross the lipid bilayer and enter the cell to bind to the receptor.

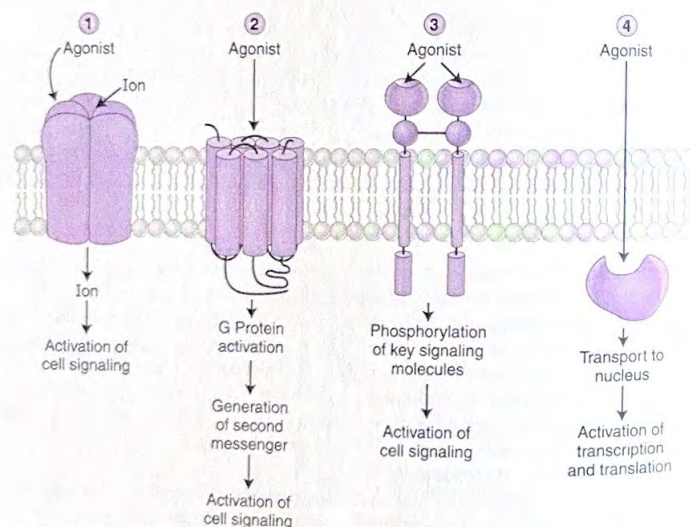


Figure 15.20. Summary of signaling by agonist-receptor binding at (1) ligand-gated ion channels, (2) G protein-coupled receptors, (3) enzyme-linked receptors, and (4) intracellular receptors.

these enzymes vary according to the needs of the cell. Enzymes may be membrane-bound as we have seen already, or may be contained in the cytosol or in various intracellular structures. Many intracellular enzymes are involved in signal transduction cascades as discussed earlier with the protein kinases, and serve as intracellular receptors.

One of the functions of enzymes is to catalyze synthesis or break down of signaling molecules according to the needs of the cell. For example, acetylcholinesterase breaks down the neurotransmitter acetylcholine, whereas dopa decarboxylase converts L-dopa to its active form dopamine.

Modulation of Ligand-Receptor Interactions

Ligand-receptor interactions are the body's way of maintaining homeostasis and defending or repairing itself. Receptors and ligands respond to external perturbations and appropriately adjust physiological processes to maintain normal functioning of our body. Therefore, the nature and extent of these interactions need to be modulated or controlled according to the situation. The body achieves this modulation by controlling or altering several parameters. When one of these modulation mechanisms does not work optimally, the result could be a disorder or disease.

Concentration of Ligand

The concentration of ligand will in part determine how much of the ligand-receptor complex is formed and therefore the strength of the signal. Ligand concentration is controlled by:

- increasing or decreasing ligand synthesis
- increasing or decreasing ligand release

- altering the destruction or removal of ligand from the receptor site

Receptor Density

Receptors also have the capacity to adapt to the changing environment to maintain homeostasis; this ability is known as receptor dynamism. The primary manifestation of this response is a constant fluctuation in receptor density or the number of receptors at a particular site. Receptor density is not fixed but is dynamic, controlled by opposing rates of receptor synthesis and receptor degeneration. The body can modify these rates by:

- Increasing the number of receptors when there is a shortage of ligand, known as receptor up-regulation
- Decreasing the number of receptors when there is an abundance of ligand, known as receptor down-regulation

Allosteric Modulation

Allosteric effects occur when the binding properties of a protein for a ligand change as a consequence of a second molecule binding to the protein; we have discussed this in an earlier chapter. The modulating molecule and ligand bind to different sites on the protein or even to different subunits; the modulator binding location is called the *allosteric* site. The structures of the ligand and modulating molecule can be very different because they bind to different sites. Binding of the modulator to the receptor alters the conformation of the receptor active site and either increases or decreases its affinity for the primary ligand. Thus, the presence and concentration of the modulating molecule can control ligand-receptor interactions, and the presence of an allosteric modulator provides one more means of controlling cell signaling.

Allosteric effects are important in the regulation of enzymatic reactions. Cells

use allosteric activators (which enhance activity) and allosteric inhibitors (which reduce activity) to control enzyme reactions. Noncatalytic receptors can also be allosterically modulated. For exam-

ple, the activation of ligand-gated ion channels is allosterically modulated by compounds such as anesthetics, barbiturates, neurosteroids, neurotoxins, and alcohol.

KEY CONCEPTS

- Cellular communication is carried out by the appropriate release and binding of ligands to their receptors.
- Hydrophilic ligands bind to cell-surface receptors, whereas hydrophobic ligands can bind to intracellular receptors after crossing the cell membrane.
- Hormones are ligands made in endocrine glands and carried by the bloodstream to their target receptors; they act by activating enzymes or modulating gene expression.
- Ductless glands and other cells secrete autacoids for local signaling; many ligands can function as both hormones and local signals.
- Paracrine signals influence neighboring cells; autocrine signaling occurs when cells respond to substances they themselves secrete; and juxtacrine signals are those passed between two cells in contact.
- Signal transduction is the translation of an extracellular signal into changes in cell behavior by a cascade of several second messenger systems.
- Receptors may be noncatalytic (act by binding reversibly to agonists), catalytic (act by catalyzing a reaction), or constitutively active (act in the absence of an agonist).
- Cell-surface receptors (e.g., ion-channel receptors, G protein-coupled receptors, and enzyme-associated receptors) bind to ligands on the cell membrane, whereas intracellular receptors (e.g., transcriptional regulator receptors and enzymes) require the ligand to enter the cell.
- Receptors of the G protein-coupled cell-surface receptor superfamily are targets for almost half of marketed drugs.
- Ligand-receptor interactions are modulated by changes in ligand or receptor concentration or by allosteric effects.

ADDITIONAL READING

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REVIEW QUESTIONS

1. What are the steps involved in cellular communication?
2. Describe the types of hormones secreted by endocrine glands and tissues.
3. Explain the process of endocrine signaling by hormones. What are the key features of endocrine signaling?
4. What are the similarities and differences between endocrine and local signaling by ligands?
5. List the features and differences between paracrine, autocrine, and juxtacrine signaling.
6. Explain the process of signal transduction. Why is transduction essential for polar signaling ligands?

Dose–Response Relationships



Concentration–Response Relationships

Agonists and Substrates

Antagonists and Inhibitors

Dose–Response Relationships

Graded Dose–Response Curves

Quantal Dose–Response Curves

Dose–Related Toxicity

Therapeutic Index

Certain Safety Factor

Risk Versus Benefit

Key Concepts

Additional Reading

Review Questions

We now understand the mechanisms by which drugs work at the molecular level in the body. This mechanistic knowledge must be translated into practical concepts for effective drug therapy. In particular, clinicians should be able to compare various drugs in terms of their effectiveness, selectivity, and safety, and to understand how these parameters are related to the dose of drug. It is also important to know how to adjust the drug dose in a particular patient to achieve efficacy without toxicity. Understanding the relationship of response to dose is one of the most challenging tasks in new drug development.

In Chapter 16, Mechanisms of Drug Action, we learned that most drugs work by binding to receptors or enzymes, and that the response is related to the concentration of drug available at the active site of the target protein. Let us proceed from there to understand the relationship of drug dose to response.

Concentration–Response Relationships

Using the concepts discussed earlier, we can describe a concentration–effect relationship for the interaction of an agonist

and antagonist (or inhibitor) with a receptor. Most of these relationships are derived from *in vitro* experiments on enzymes, cells, and isolated organs, in which it is possible to know the precise concentration of the drug at the active site.

Agonists and Substrates

Figure 17.1 shows a concentration-effect or concentration-response curve for a full and partial agonist acting at a receptor. The data to construct such a curve are obtained by placing known concentrations of drug in the *in vitro* experimental system (such as a cell, tissue, or isolated organ system) and then measuring the response of the system to the drug. Concentration-response curves are usually governed by the following equation, which was introduced in Chapter 16, Mechanisms of Drug Action:

$$\frac{E}{E_{\max}} = \frac{\alpha}{[R_1]} [DR] \quad (\text{Eq. 17.1})$$

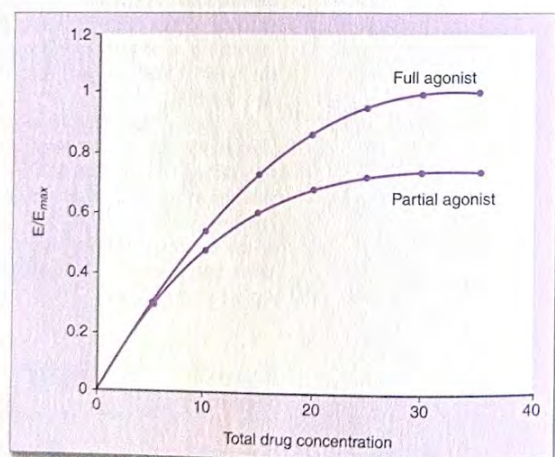


Figure 17.1. A typical concentration-response curve showing the relationship between total drug concentration and the fractional response ($E/E_{\max}$) for a full and partial agonist at a receptor site.

A plot of $E/E_{\max}$ (either as a fraction or as a percentage) on the y axis versus the $[DR]$ on the x axis should give a straight line with a slope equal to $\alpha/[R_1]$.

Although there is no direct way of measuring the concentration of the complex $[DR]$, the total concentration of drug $[D]$ used in the experiment is known. When $[D]$ is small (low drug concentrations), almost all the drug is involved in complex formation, so $[DR]$ is essentially equal to $[D]$. This approximation modifies Equation 17.1 to:

$$\frac{E}{E_{\max}} = \frac{\alpha}{[R_1]} [D] \quad (\text{Eq. 17.2})$$

Equation 17.2 shows a linear relationship between $E/E_{\max}$ and total drug concentration $[D]$; the slope of the line is $\alpha/[R_1]$.

When $[D]$ is sufficiently large (i.e., high drug concentrations), all the receptors are occupied (saturated), and maximum pharmacological response ($E/E_{\max} = 1$ or 100%) will be obtained if the drug is a full agonist ($\alpha = 1$). Any further

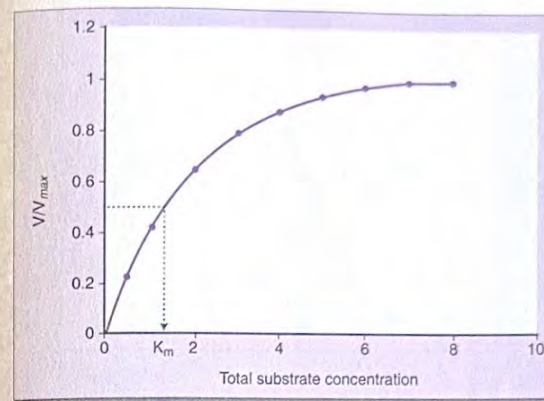


Figure 17.2. A typical concentration-response curve showing the relationship between total substrate concentration and the fractional response ($V/V_{\max}$). The Michaelis constant (K_m) is the substrate concentration at which the rate of the reaction is 50%.

increase in concentration will not give any greater effect; the biological response has reached a plateau. Thus, we obtain a hyperbolic graph when we plot relative response $E/E_{\max}$ versus total drug concentration $[D]$. For partial agonists ($0 < \alpha < 1$), the receptors will also become saturated at high concentrations, and the response will reach a plateau, but $E/E_{\max}$ will be less than 1 at the plateau.

Notice that the concentration to response relationship is not proportional, i.e., the concentration needed for 100% response is not double the concentration for 50% response, but is much larger. Initially, small changes in concentration produce a large change in response. As the response approaches maximum, large changes in concentration are needed to make small changes in response.

Note also the similarity of Figure 17.1 to Figure 17.2, which shows the catalytic response of an enzyme-substrate interaction. Thus, our discussion above about agonists applies to substrates of enzymes as well.

Concentration-response curves are better and more commonly represented with the *logarithm* of the drug concentra-

tion on the x axis, because this allows a broader range of agonist concentrations to be shown. When plotted in this manner, as in Figure 17.3 for an agonist, a sigmoid or S-shaped log concentration-response curve with a center of symmetry at 50% response is obtained. This midpoint represents the concentration that gives 50% of the maximum response and is called the EC_{50} . Another advantage of using a logarithmic concentration scale is that the curve is essentially linear around the EC_{50} (between about 20 and 80% maximal response), making mathematical analysis easier. Therefore, at intermediate agonist concentrations there is a linear relationship between relative response and log concentration. The slope is the highest in this region of the curve, and small changes in agonist concentration can produce large changes in response.

The same type of sigmoid curve is obtained for enzyme-substrate systems; here, $V/V_{\max}$ is plotted against the logarithm of the substrate concentration. The midpoint concentration of this curve, representing 50% maximum response, is K_m .

The concentrations of endogenous ligands in the body usually lie near the

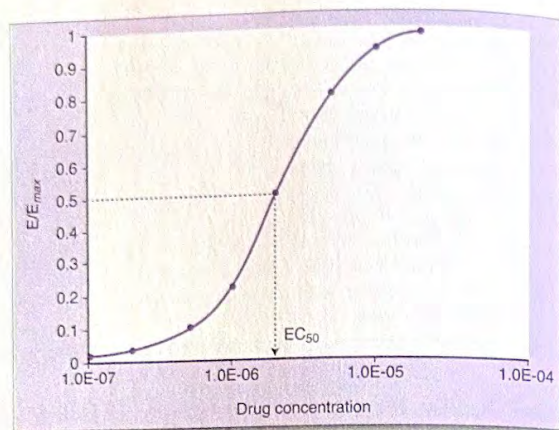


Figure 17.3. A typical logarithmic concentration-response curve for a full agonist. Note the logarithmic x axis and the characteristic sigmoid shape of the curve. The EC_{50} is the drug concentration that gives 50% maximal response ($E/E_{max} = 0.5$). Also note the linearity of the curve between approximately 20 and 80% maximal response.

EC_{50} or K_m of the drug-protein interaction, i.e., in the linear portion of the concentration-response curve. By making small changes in ligand concentration, the body can precisely control cellular behavior.

Antagonists and Inhibitors

In Chapter 16, Mechanisms of Drug Action, we discussed the mechanism of action of antagonists and inhibitors. Antagonists are drugs that reduce or prevent the effect of agonists at a receptor. Enzyme inhibitors are drugs that prevent or decrease the rate of the reaction converting substrates to products. *Competitive* antagonists or inhibitors have an affinity for and bind to the same site on the target protein as the agonist (substrate). The endogenous ligand and drug compete for the same active site, and can displace each other from the site depending on their relative concentrations and affinities. On the other hand, *noncompetitive* antagonists or inhibitors usually bind to an allosteric site on the target protein.

Competitive Binding

Figure 17.4 shows the concentration-effect curve for an agonist (or substrate) in the absence and presence of two different concentrations of a competitive antagonist (or inhibitor). To produce an effect, the agonist has to first replace the antagonist at the binding sites. Therefore, higher concentrations of agonist are needed to produce the same degree of effect when an antagonist is present. Furthermore, even higher concentrations of agonist are needed to produce the maximum effect. The result is that the concentration-response curve for the agonist is shifted to the right, i.e., to higher agonist concentrations. This is a parallel shift in curves so that the slopes of the linear portions of the curves remain the same.

Because competition between agonist and antagonist depends on their relative concentrations and affinities for the receptor, the shift to the right is greater for an antagonist present at a higher concentration, or with a greater affinity. The

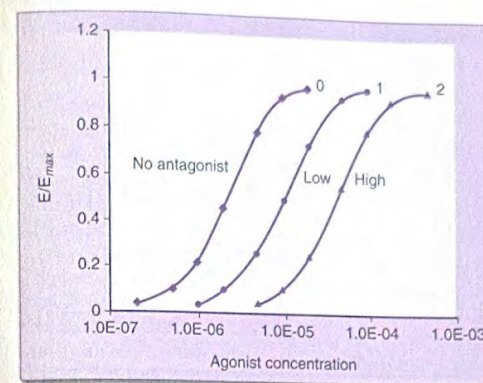


Figure 17.4. Concentration-response curve for a full agonist in the absence and presence of a competitive antagonist. Curve 0 shows the relationship when no antagonist is present. Curves 1 and 2 show the shift in the relationship in the presence of low and high concentrations of the competitive antagonist respectively.

extent of this shift is a useful way of comparing antagonists and is best expressed as a *concentration ratio*. This is the factor by which the agonist concentration must be increased to restore the original response in the presence of an antagonist. This also illustrates the concept that the effect of a competitive antagonist can be reversed by high agonist concentrations.

The same discussion holds true for enzyme substrates and their competitive inhibitors.

Noncompetitive Binding

Figure 17.5 shows concentration-response curves for an agonist in the absence and presence of different concentrations of a noncompetitive antagonist. As with competitive antagonism, the curves are shifted to the right in this case. However, the slope of the linear portion of the curves and the maximum effect are both reduced. High concentrations of antagonist may completely prevent effect, even if agonist-receptor binding takes place. Unlike

competitive antagonism, the effect of a noncompetitive antagonist cannot be reversed by high agonist concentrations. Again, the same discussion applies to enzyme substrates and their noncompetitive inhibitors.

Competitive-Noncompetitive Binding

Some antagonists demonstrate both competitive and noncompetitive properties. These drugs are competitive at low concentrations, but noncompetitive at high concentrations. Therefore, at low concentrations, these drugs cause a parallel shift of the agonist curve, and at high concentrations they decrease the slope and maximum effect. Figure 17.6 shows this graphically.

Similarly some enzyme inhibitors show both competitive and noncompetitive behavior and are often called mixed inhibitors, changing both K_m and V_{max} of the enzyme-substrate reaction. The concentration-effect curves in this case are similar to those shown in Figure 17.6.

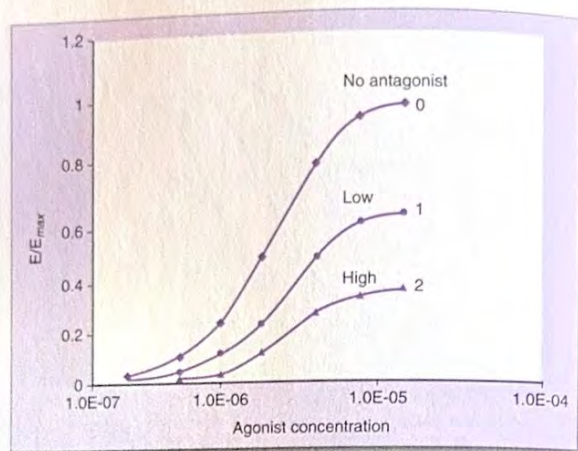


Figure 17.5. Concentration-response curve for a full agonist in the absence and presence of a noncompetitive antagonist. Curve 0 shows the profile when no antagonist is present. Curves 1 and 2 show the shift in the curve in the presence of low and high concentrations of the noncompetitive antagonist respectively.

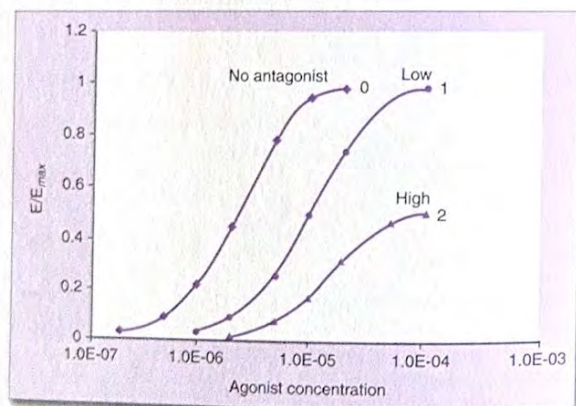


Figure 17.6. Concentration-response curve for a full agonist in the absence and presence of a competitive-noncompetitive antagonist. Curve 0 shows the profile when no antagonist is present. Curves 1 and 2 show the shift in the curve in the presence of low and high concentrations of the antagonist respectively.

Dose-Response Relationships

When dealing with animals or humans, the exact concentration of drug at the receptor is not known. However, this concentration is related to the dose of drug administered; the higher the dose, the greater the concentration at the receptor. Therefore, if the drug dose is known and the pharmacological response can be measured, a *dose-response* or *dose-effect relationship* can be established. Dose-response relationships are a common way to portray data in both experimental and clinical sciences.

From discussions in earlier chapters we know that drug dose is only one of the factors that determine concentration of drug at the receptor active site, and subsequent pharmacological response. The drug's absorption, distribution, metabolism, and excretion (ADME) behavior is also important; ADME determines drug plasma concentration, which in turn affects concentration at the receptor (site of action) and in other tissues. Thus, the same dose of drug administered by a different route, or by different formulations with the same route, can give varying pharmacological responses.

In Chapter 14, Plasma Concentration-Time Curves, we examined the relationship between plasma concentration and response. In most situations, a higher dose of drug gives a proportionately higher plasma concentration (up to a limit), all other conditions being the same. It is in this context that one should understand the discussion of dose-response relationships.

A dose-response curve (DRC) is usually constructed using the *logarithm* of the dose on the x axis. The y axis shows the response, presented either as intensity of effect in a graded dose-response curve, or as frequency of effect in a quantal dose-response curve.

Graded Dose-Response Curves

A graded dose-response curve is useful in representing the dose-effect relationship in an individual. The assumptions are that the response varies continuously with dose and that a higher dose gives a greater response. An example is shown in Figure 17.7; note that the graded DRC is sigmoid with a center of symmetry at 50% response. The threshold dose is defined as the lowest dose that produces a measurable response. The slope of the curve near its center of symmetry also has significance; a steep slope shows that a small increase in dose produces a large increase in response.

Potency and Efficacy

The potency of a drug is the dose needed to produce a certain defined response in an individual. It is a useful parameter that allows comparisons of different drugs that act at the same target site. The smaller the dose required to produce the defined effect, the more potent the drug; conversely, the more potent the drug, the less of it is required to produce a given effect. Potency is related to the affinity of a drug for the receptor; the higher the affinity, the more potent the drug.

Potency also depends on how much of the dose reaches the receptor after administration. The fraction of the dose that arrives at the receptor site depends on the drug's ADME behavior. Thus, a drug with the best activity *in vitro* may not be the most potent because less of it may reach the receptor site. Moreover, differences in ADME are often responsible for variability in potency of the same drug in different individuals.

Efficacy, on the other hand, is a measure of the maximum response a drug can produce regardless of the dose, and is often related to the intrinsic activity of the drug; drugs with high intrinsic activity usually give a greater maximal efficacy.

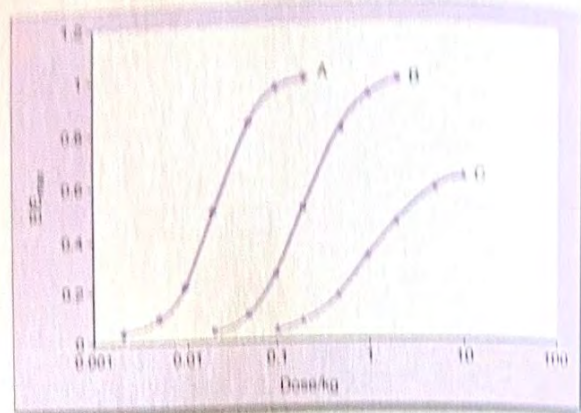


Figure 17.7. A typical graded dose-response curve for three drugs (A, B, and C) acting at the same receptor. The dose is in arbitrary units. Note the logarithmic x axis, and the sigmoid shape of the curve. Drug A is 10 times more potent than drug B. Drug C is less potent than drug A or B. Both drug A and drug B reach the same maximal effect and have the same efficacy, whereas the efficacy of drug C is lower than that of drug A or B.

This parameter is also useful in comparing two drugs with the same action.

The potency of agonists can be compared by their position on the x axis, and their efficacy can be determined from the maximum response exhibited, by the position on the y axis. For example, Figure 17.7 shows that:

- drug A has a higher potency than drugs B or C.
- drug B has the same maximal effect as drug A but is less potent.
- drug C will never achieve the efficacy of drug A, even at high doses.

Potency is useful in determining drug doses when changing from one drug to another, but does not provide any information about effectiveness or safety. Thus, the most potent drug may not necessarily be the best drug. Potency is clinically important only when drugs are very potent (difficult to administer safely) or very weak (difficult to administer conveniently). Efficacy is more important than potency for therapy because it focuses on

the effectiveness of a drug rather than on the size of the dose.

Quantal Dose-Response Curves

Not all patients respond to a drug in the same way or to the same extent. Very few individuals show the desired effect at low doses, whereas almost all show the desired effect at very high doses. This illustrates the problem of *biological variability* when dealing with individuals. The variability may be the result of differences in receptors or in the amount of drug reaching the receptor site, or some other reason. We shall discuss the various factors responsible for this behavior in Chapter 18, Therapeutic Variability.

To measure this variability in individual dose-response, the DRC can be constructed to show the frequency of a certain response (e.g., the number of individuals exhibiting a defined response to a minimum dose) on the x axis. This is useful when studying the effect of a dose on a patient population rather than on an individual patient. For such analyses, the

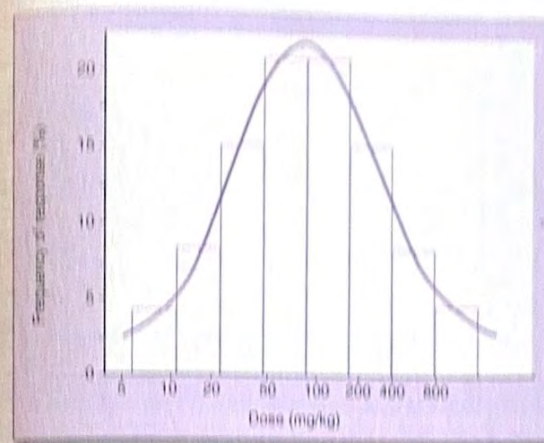


Figure 17.8. A quantal dose-response curve showing the frequency of response (number of patients responding) as a function of the minimum dose administered.

response needs to be well defined, both qualitatively and quantitatively. This is because the measurement of response is *quantal*, not continuously variable; the patient either shows the defined response or does not.

To obtain these data, the population is given increasing drug doses until virtually all patients respond. In essence, one is finding the individual threshold dose for the group. The graphs are usually bell-shaped, following a gaussian or normal distribution as shown in Figure 17.8. This type of curve, called a *quantal dose-response curve*, is useful in describing the DRC in a group of subjects.

Another way of showing quantal dose-response is to plot the data as the cumulative frequency responding to a certain dose, illustrated in Figure 17.9. As the dose is increased more individuals exhibit the defined effect; all individuals show the response at the highest dose. Notice that we again have a sigmoid curve with a center of symmetry at 50% response. The dose at the center of symmetry of a cumulative quantal DRC is called the ED_{50} or the *median effective*

dose. The ED_{50} is defined as the dose that produces the desired effect in half of the individuals studied.

One way to compare the potency of two drugs that produce the same response is to compare their ED_{50} values; the lower the value, the more potent the drug. Similarly, we can define ED_{95} as the dose at which 95% of individuals show an effect, and so on.

Drug Selectivity

The *selectivity* of a drug has been discussed in Chapter 16, Mechanisms of Drug Action; it is the ability of a drug to produce one effect (usually the desired effect) at much lower doses than other effects (such as side effects). The DRCs of all the effects of a drug will give a measure of its selectivity; the more widely spaced the DRCs, the more selective the drug.

Dose-Related Toxicity

An ideal drug would produce a therapeutically useful response without

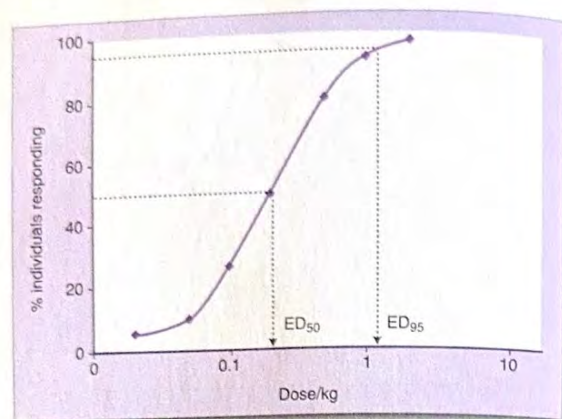


Figure 17.9. A typical quantal dose-response curve with dose per kilogram (dose has arbitrary units) on the logarithmic x axis and the cumulative percentage of individuals responding on the y axis. The ED_{50} and ED_{95} are the doses at which 50 and 95% of individuals, respectively, show the defined response.

causing any undesirable effects. Unfortunately, every drug produces unwanted side effects at a large enough dose. Side effects are pharmacological effects observed in addition to the primary drug action and vary greatly with the drug, the dose, and the patient. Sometimes these effects cause serious problems (*toxicity*), whereas in other cases they may be relatively harmless. In a few situations, a drug's side effect may even be desirable. Undesirable side effects are known as adverse drug reactions (ADRs).

Most common types of ADRs are related to the dose of the drug and are the result of one or more of the known pharmacological effects of the drug; these account for about 80% of ADRs seen in practice. Dose-related ADRs are frequently predictable and can be avoided. Examples are drowsiness after taking antihistamines or an elevated heart rate after using β -agonists. Other types of side effects are not related to the dose or even directly to the drug's biological action. These are generally called "idiosyncratic

reactions" or allergic effects. We will discuss this type of side effect in Chapter 18, Therapeutic Variability.

Just as we can construct quantal DRCs for the beneficial effects of a drug, we can also construct similar curves for the dose-dependent ADRs of drugs.

Therapeutic Index

The ultimate adverse side effect of a drug is lethality or death. In animals, we characterize this as the LD_{50} , the *median lethal dose*, the dose of the drug that kills 50% of the animals tested during a set period after an acute exposure. Ideally, the LD_{50} of a drug should be much larger than its ED_{50} because this would make the drug safer to use. The LD_{50} values may vary for the same drug given by different routes of administration because of differences in absorption and bioavailability from different administration sites.

The pharmacological therapeutic index (TI), also called the *therapeutic ratio*, of a drug is defined as:

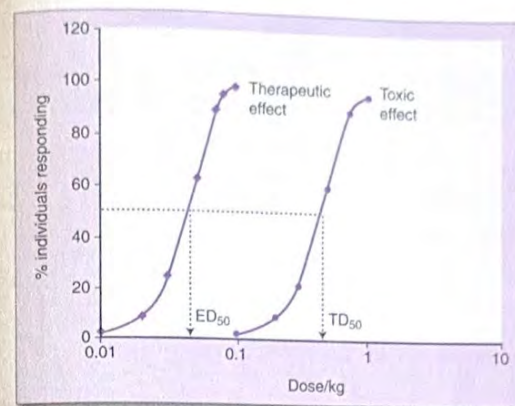


Figure 17.10. Quantal dose-response curves for the therapeutic effect and toxic effect of drug X. The ratio TD_{50}/ED_{50} gives the therapeutic index (TI) of the drug. Drug X has a TI = 10. Note that very little toxicity will be seen at a dose that gives therapeutic effect in greater than 90% of individuals.

$$\text{therapeutic index} = TI = \frac{LD_{50}}{ED_{50}} \quad (\text{Eq. 17.3})$$

A large therapeutic index implies that a drug is safe. For example, a drug with a TI of 25 is safer than a drug with a TI of 8.

Because the LD_{50} cannot be determined in human subjects, clinicians instead define the TD_{50} , the median toxic dose, as the dose that produces a defined toxic effect in 50% of individuals. In this case the clinical therapeutic index is given by:

$$TI = \frac{TD_{50}}{ED_{50}} \quad (\text{Eq. 17.4})$$

Here again the TI is a measure of the safety of the drug. A clinical TI of 5 means that the dose that produces a particular toxic effect (such as liver damage) in 50% of individuals is five times higher than the effective dose in 50% of individuals. We will use TI to mean the clinical TI for the remainder of the chapter.

Figure 17.10 illustrates the effective (therapeutic) and toxic dose-response

relationships of drug X with a TI of 10. Drug X has considerable separation between these two dose-response curves, indicating that it has good safety. A general rule of thumb is that drugs with a TI less than 2 will show significant toxicity in some patients when used clinically at therapeutic doses. Thus, such drugs should be used only if the risk-benefit analysis for a particular patient is favorable. The selectivity of a drug is very important in determining its safety profile.

A drug can have many toxic effects of different degrees of severity. Relatively mild side effects like nausea, gastrointestinal upset, or headaches may occur at low doses whereas more serious toxic effects like liver or kidney damage may occur at higher doses. Therefore, a drug has different TD_{50} and TI values for each of its toxic effects.

Certain Safety Factor

The therapeutic index is not always a good measure of the safety of a drug because it compares only the midpoints of efficacy and toxicity dose-response curves.

Digoxin

Digoxin is among the top 50 most widely prescribed drugs in the United States, and has been used therapeutically for more than 300 years. It belongs to a class of drugs known as cardiac glycosides and is widely used in management of congestive heart failure, weakened heart, and irregular heart beat (arrhythmia). The common garden plant, the foxglove or *Digitalis purpurea*, is the source of digoxin.

Few drugs in clinical medicine have a therapeutic index as low as that of digoxin, or are a more frequent cause of adverse drug reactions. Digoxin toxicity is observed in a significant number of patients. Anorexia, frequently accompanied by muscular weakness, lethargy, vomiting, diarrhea, and abdom-

inal pain, are common. The most serious and life-threatening ADRs are cardiac arrhythmias and disturbances of cardiac conduction, which may develop without warning.

One antidote for digoxin toxicity is digoxin immune Fab (Digibind, Digi-Fab), an antibody produced in sheep by immunization with a digoxin derivative. When given to patients with digoxin toxicity, the antibody binds to digoxin molecules, making them unavailable for binding at their receptors in the body. The affinity of digoxin for the antibody is higher than its affinity for its receptor. The net effect is to shift the binding equilibrium away from binding of digoxin to its receptors, thereby reversing its effects. The Fab-digoxin complex is then excreted by the kidneys.

These curves could have significant overlap, so that some patients might experience toxicity without significant therapeutic benefit even though the average patient is effectively treated.

This problem is apparent in the dose-response curves shown in Figure 17.11 for drug Y. Although the TI of drug Y is the same as that of drug X in Figure 17.8, drug Y is obviously not as safe as drug X. The reason for this is that we would need potentially toxic doses of drug Y to obtain desired effectiveness in some patients. Thus, TI alone is not useful for true determination of drug safety because it does not give any information about the slopes of the DRCs for therapeutic and toxic effects. If the therapeutic DRC is relatively flat, there may be significant overlap between the two curves even if TD_{50} and ED_{50} of a drug are quite different.

Ideally, we would like a drug that is effective in all individuals at a dose that does not produce toxic effects in any individual. This concept is characterized by a parameter called the certain safety factor (CSF) as follows:

$$TI = \frac{TD_{50}}{ED_{50}}$$

Here, TD_1 represents the *minimally toxic dose*, the dose that is toxic to only 1% of individuals, whereas ED_{99} represents the *maximally effective dose*, the dose that is effective in 99% of individuals. The percentages used here—1% and 99%—are just illustrations. In practice, one can establish the desired population percentage acceptable for both toxicity and effectiveness.

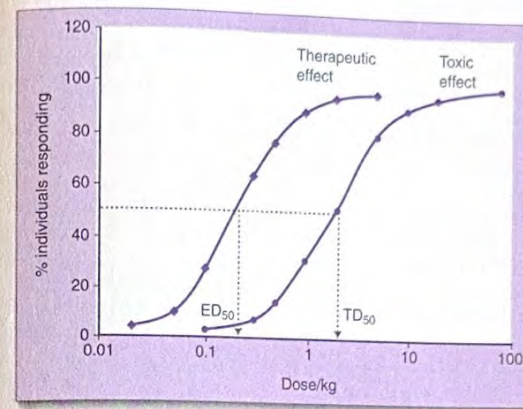


Figure 17.11. Quantal dose-response curves for the therapeutic effect and toxic effect of drug Y. The ratio TD_{50}/ED_{50} gives the therapeutic index (TI) of the drug. Drug Y has a TI = 10. Note that significant toxicity will be seen at a dose that gives therapeutic effect in greater than 90% of individuals.

The CSF is a measure of the overlap of the high end of the therapeutic DRC with the low end of the toxic DRC. A drug with a CSF of 1 can be dosed effectively to 99% of individuals while being toxic in only 1% of individuals. A higher CSF means that there is virtually no overlap between the two DRCs, and a maximally effective dose can be used with little chance of toxicity.

Risk Versus Benefit

A drug is usually considered safe if serious side effects or toxicity are unlikely

when the drug is used at its *recommended* dosage in the *average* patient. This does not mean that there are no ADRs; it only means that the risk-benefit ratio of the drug is acceptable in most patients.

The TI and CSF are often used to predict the probability of toxicity in patients. When side effects or toxicity are likely, patients are given low doses to begin with and the dose is slowly boosted while monitoring the patient for ADRs. A drug that is safe in most patients may be toxic in a patient with a specific disease, impaired physiological function, or genetic predisposition.

KEY CONCEPTS

- Drug action at receptors or enzymes can be depicted as graphical dose-response relationships.
- Response plateaus at high concentrations or doses because receptors are saturated.
- Graded dose-response curves compare efficacy, potency, and toxicity of different drugs. They also reveal whether a drug is a full or partial agonist, or a competitive or non-competitive antagonist.
- Quantal dose-response relationships are useful in illustrating the variability of drug response in a population.
- The safety of a drug can be illustrated by dose-toxic response relationships.
- The clinical therapeutic index of a drug is a ratio of the median toxic dose to the median effective dose.
- Dose-response and dose-toxic response curves are used together to define the risk-benefit of a drug and to select an appropriate dose.

ADDITIONAL READING

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3. Brunton L, Lazo J, Parker K (eds). Goodman & Gilman's The Pharmacological Basis of Therapeutics, 11th ed. McGraw-Hill Professional, 2005.
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REVIEW QUESTIONS

1. Explain the important features of a concentration-response curve for a full and partial agonist. What are the similarities of this curve and a curve of enzyme activity? How do these curves change when the concentration is plotted on a logarithmic scale?
2. How does the concentration-response curve for an agonist change in the presence of competitive and noncompetitive antagonists?
3. How does the concentration-response curve for a substrate change in the presence of competitive and noncompetitive inhibitors?
4. What are the differences between a concentration-response relationship and a dose-response relationship? Why is the latter more useful in therapeutic application?
5. Explain the difference between potency and efficacy of a drug. How is this revealed in a dose-response curve?
6. What is meant by a selective drug? How can one determine selectivity from a dose-response curve?
7. Clarify the difference between the pharmacological therapeutic index and the clinical therapeutic index. Why is the latter more relevant to drug therapy?
8. Discuss why the certain safety factor is more appropriate in the choice of a drug dose than the clinical therapeutic index.

Therapeutic Variability

Types of Therapeutic Variability

Pharmacokinetic Variability

Pharmacodynamic Variability

Immunological Variability

Drug Allergy

Drug Compliance

Individualization of Therapy

Key Concepts

Additional Reading

Review Questions



Earlier chapters have discussed what the body does to the drug (absorption, distribution, metabolism, and excretion [ADME] and pharmacokinetics) and what the drug does to the body (drug action and pharmacodynamics). Moreover, previous chapters have focused on the disposition and action of drugs in normal, relatively healthy individuals, suggesting that most patients process the drug in the same manner and that the drug works similarly in all individuals. In fact, the standard dose and dosing frequency of most drugs are based on the drug's behavior in an average patient population: approximately 18 to 65 years of age, weighing about 70 kg (150 lbs), with normal body functions.

However, patient attributes can affect the nature and degree of pharmacological response significantly. Not everyone responds to the same dose of a given drug in exactly the same manner at all times; variability in response to drug therapy is the rule rather than the exception for most medications. Even at the recommended standard doses of a drug, the intensity of the response is often different among patients because of individual differences in pharmacokinetics or pharmacodynamics. Even the nature of the response may vary in some patients, resulting in effects (such as drug allergy)

not seen in the average patient. Alteration of physiological and pathological conditions in patients further complicates this picture.

Even the most successful drugs provide optimal benefits to only a fraction of patients. Variability in patient response to drug therapy must be taken into account to adapt drug treatment to individual patient needs.

Types of Therapeutic Variability

Therapeutic variability may be *interpatient*, i.e., between different people, or *intrapatient*, in which the drug response changes for a given person at different times. Many interwoven and overlapping factors contribute to variability. Scientists are reaching the conclusion that genetic variability among individuals is the primary underlying cause of altered response to drugs; we shall discuss genetic factors in detail in Chapter 20, Pharmacogenomics. Drug interaction, in which one drug influences the behavior of another, also contributes to therapeutic variability; this too, is the subject of a separate chapter (Chapter 19, Drug Interactions).

This chapter will deal with common and generally predictable factors contributing to therapeutic variability, such as:

- Pharmacokinetic factors that produce differing concentrations of the drug at the target receptor
- Pharmacodynamic factors that cause different pharmacological responses to the same drug concentration
- Immunological factors that result in allergic response

Other non-drug-related factors such as the personality, beliefs, and attitudes of both patient and clinician have been occasionally linked to therapeutic variability. We will not explore these in this book.

Many different factors contribute to therapeutic variability, and the same factor may cause pharmacokinetic as well as pharmacodynamic variability. Some common causes of variability can be anticipated by proper evaluation of a patient. In other cases, variability is unexpected and can result in therapeutic failure or toxicity.

Pharmacokinetic Variability

Pharmacokinetic variability refers to variability in delivery of drug to or removal of drug from sites of action involved in efficacy and/or toxicity. The obvious result of this is a change in the intensity of drug effect because either too much or too little drug reaches the site of action, and side effects because of higher than normal drug concentrations. Pharmacokinetic variability is particularly problematic for drugs with a narrow therapeutic range or *therapeutic window* (or low therapeutic index) because small differences may result in therapeutic failure or toxicity, as illustrated in Figure 18.1.

A patient who does not fit the "average" patient mold can show differences in absorption, distribution, metabolism, and excretion of drugs. Much of this can be anticipated, and appropriate changes in the dose or dosing schedule made before the initiation of drug therapy.

Pharmacokinetic variability may also be attributable to genetic effects, arising from alterations in certain biomolecules such as drug-metabolizing enzymes, and drug transporters that mediate drug uptake into and efflux from intracellular sites. Such genetic effects are often unpredictable, although new research is allowing clinicians to identify patients with an abnormal ADME profile for a certain drug. The relationship between genetic characteristics, drug effects, and toxicity is called *pharmacogenetics*; we shall discuss this in greater detail in Chapter 20, Pharmacogenomics.

Let us discuss common sources of pharmacokinetic variability.

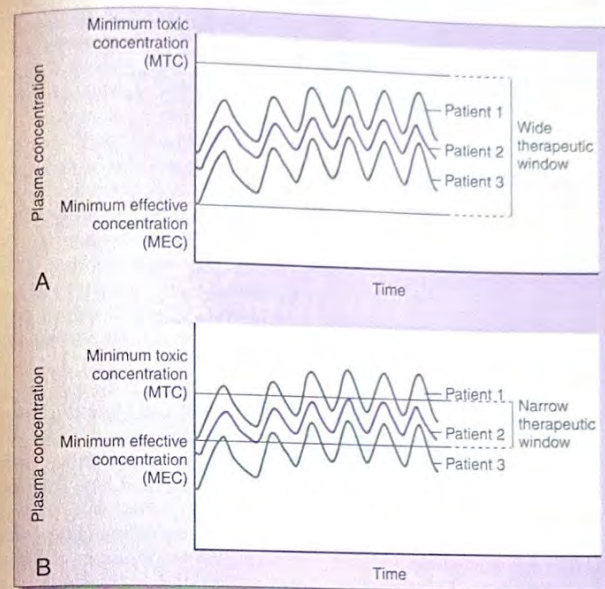


Figure 18.1. Effect of interindividual pharmacokinetic variability on the efficacy and toxicity of a drug with a wide (A) and narrow (B) therapeutic window, after multiple dosing in three patients.

Body Weight and Composition

Pharmacological effect depends on the concentration of drug reaching the site of action, which in turn depends on the drug dose and pharmacokinetics. The recommended standard dose of most drugs is typically based on a 70-kg adult male. If we assume ADME processes are functioning normally in an individual, then drug concentration at the site of action depends on dose and volume of distribution V . For a given dose, the greater the V , the lower the concentration in all tissues, including the site of action. This means that a given dose of drug gives lower tissue concentrations in large individuals compared with small ones. Thus, overweight individuals may need a higher than standard dose to achieve adequate tissue concentrations, whereas small individuals may need a lower than standard dose. Many drug products are

available in multiple strengths (e.g., 10-, 20-, and 30-mg tablets) to allow dose adjustments in individual cases.

Although larger individuals generally require a higher dose to achieve therapeutic plasma or tissue concentrations, the difficulty lies in defining what large and small mean when referring to patients. A simple approach is to adjust doses based on body weight as follows:

$$\text{dose required} = \frac{\text{average dose}}{70 \text{ kg}} \times \text{weight of individual (kg)} \quad (\text{Eq. 18.1})$$

Dose adjustments based on Equation 18.1 do not work for people who are extremely lean or obese because it does not account for dramatically altered body composition such as body fat and body

water. Recall that *V* of drugs depends on drug lipophilicity. Thus, in an obese individual, a lipophilic drug may have an unusually large *V*, whereas the *V* of a hydrophilic drug may not be changed as dramatically. Consequently, doses for lipophilic and hydrophilic drugs may have to be adjusted by different factors in obese patients. In such cases, body surface area rather than body weight is often used to adjust dosage.

Age

Many drugs behave differently in children and the elderly compared with the average adult. Some of this difference is caused by variation in body size and composition, as discussed earlier. However, even when body size is taken into account, additional factors are responsible for increased sensitivity of the very young and the very old to drugs.

Special populations—the very young (infants and pediatrics) and the very old (geriatrics)—are usually excluded in clinical testing of drugs, and their response to drugs is generally unknown when a drug is first marketed. This is changing, however, and more and more drugs are now being tested in geriatric and pediatric populations to identify appropriate doses and dosing schedules.

Infants and Children. Infants constitute a very special subgroup of patients in which drugs must be used with extreme care. In addition to a much smaller body weight, there are significant physiological differences between infants and the average adult. The most important of these are:

- Increased permeability of all tissues, including the skin and blood-brain barrier
- Proportionately greater volume of body water, and a consequently higher *V* for certain drugs
- Lower rate of blood flow to most organs
- Decreased biotransformation as a result of underdeveloped metabolic enzymes

- Decreased renal clearance attributable to reduced glomerular filtration rate

Consequently, use of any drug in an infant must be carefully considered and the dose adjusted appropriately. At an age of about 1 year, metabolic and excretion functions are better developed, and the risk of using drugs in young children is less than in infants. At about 12 years of age, adult doses are often used. However, compensation for a lower body weight and altered body composition must always be considered when using all drugs in children.

Elderly. Many organ systems show a normal decline in function with age in the elderly population. Muscle mass decreases, whereas percentage of body fat rises. Absorption efficiency is usually only slightly reduced, but there are significant decreases in efficiency of metabolism and excretion of drugs. Hepatic effects include:

- Decreased hepatic mass
- Decreased hepatic blood flow
- Decreased enzyme activity

Renal effects consist of:

- Reduced number of nephrons
- Reduced number of functioning glomeruli
- Decreased renal blood flow

The result is that plasma and tissue concentrations in geriatrics are often higher than expected, and sensitivity to the drug is increased.

Sex-Related Differences

Until the 1990s, health researchers used male subjects to determine safety and efficacy of new drugs and treatments. Reasons for this were concern about the potentially confounding effects of a woman's hormonal changes on treatment, the desire to protect a potential fetus, and fear of liability if a fetus was harmfully exposed. As a result, women of childbearing age were systematically excluded

from clinical trials. Treatment interventions, toxicity, and safety data were studied in men and then applied or assumed to be the same in women.

Sex. The discovery of differences between male and female response to disease and treatments has changed the landscape of new drug development. Women are now included in clinical trials unless the risk is unacceptable.

Sex-related differences in body weight and composition account for some pharmacokinetic variability. Women generally have a lower body weight and a higher proportion of body fat, and may therefore require a different drug dose. There is evidence that metabolic rates may also be different (usually lower) in women compared with men, requiring dosage adjustment. The issue of sex in drug safety becomes particularly important in women of childbearing age, and in pregnant and lactating women.

Pregnancy and Lactation. Many drugs administered to a mother during pregnancy and lactation may adversely influence the embryo, fetus, and infant. The placenta allows transport of many drugs from mother to embryo or fetus by passive diffusion, and drug concentrations in the embryo and in fetal circulation are often the same as those in the mother's circulation. Embryonic and fetal cells are highly sensitive to drugs, and severe toxic effects are seen when these are exposed to drugs. The type and extent of toxicity depend on the stage of embryonic or fetal development when exposure occurred.

The embryonic stage is the time between fertilization and the eighth week of pregnancy. Because major organ structures are being formed during this period, a major toxicity is teratogenicity, the ability of a drug to cause damage to a developing fetus, resulting in congenital malformation of various structures. Serious malformations result in a miscarriage. The fetal stage of development occurs after differentiation of major organ structures; here, toxic effects of

drugs can be seen on already formed organ systems.

Many drugs, particularly lipophilic drugs, distribute readily into the breast milk of the mother during lactation. Infants in their first 3 months of life do not have fully developed enzyme systems for biotransformation and renal excretion of drugs. Thus, toxic levels may accumulate in nursing infants, resulting in a variety of effects depending on the drug.

Health and Disease

The overall health of the patient is critical in determining how a drug behaves and works in the body. Drugs are often used in patients whose organ systems are not functioning normally; particularly important are organs involved in drug elimination—the liver and kidney. Patients with impaired hepatic or renal function do not eliminate drugs readily, resulting in higher plasma levels and resultant potential toxicity. A change in dose or dosing frequency is often necessary in such patients.

Impaired circulation (resulting in lower blood flow rates to organs) seen in cardiovascular disease can affect all aspects of drug pharmacokinetics. Other factors such as body temperature, hydration or dehydration, and acidosis or alkalosis may also change the pharmacokinetics of some drugs. The dosage regimen for patients with disease often needs to be individualized on the basis of assessment of hepatic, renal, and cardiovascular function.

The nutritional status of a patient can influence drug pharmacokinetics. Malnutrition, vitamin deficiencies, or decreased protein synthesis may result in altered plasma protein and metabolic enzyme levels.

Pharmacodynamic Variability

Pharmacodynamic variability refers to variable drug effects despite equivalent drug delivery to the sites of action. It may reflect differences in the structure and function of target receptors or in the

broad pathophysiological context in which a drug interacts with its target. The existence of pharmacodynamic variability means lack of a relationship between drug response and plasma concentration, and pharmacokinetic-pharmacodynamic correlations are not useful in these situations.

Sex-Related Differences

Physiological differences between men and women may result in altered pharmacodynamics of some drugs in women compared with men. For example, differences have been shown between men and women in both perception of and response to pain. Emerging scientific evidence points to sex-related pharmacological differences in opioid-receptor binding and in responses to certain opioid pain medications. These differences suggest the importance of developing sex-specific strategies for pain relief and highlight the need for further investigation of sex-related pain treatment.

Idiosyncrasy

A few individuals respond to drugs in a highly unusual and unpredictable manner, giving a response quantitatively much different than in the average patient. For example, some patients may have an intense response to a very small dose of the drug, whereas others may not respond to very high doses. The response may also be qualitatively different in some situations, with new pharmacological effects being observed. Such responses, referred to as drug idiosyncrasy or *idiosyncratic response*, are infrequent and believed to result from a genetically determined metabolic or enzyme deficiency that is not expressed under normal situations (e.g., hemolytic anemia occurring in patients with glucose-6-phosphate dehydrogenase deficiency after receiving an oxidant drug). These responses show a dose-response relationship, but not the same one shown by the average patient.

Circadian Rhythms

A circadian rhythm is the regular recurrence of a biological process or activity in cycles of about 24 hours. These cycles are set by a biological clock that seems to respond to recurring daylight and darkness. This clock, which lies in the brain, regulates organs like the liver, kidneys, and blood vessels by controlling circadian clocks within them; consequently, a daily reproducible pattern of peaks and troughs is seen in many physiological variables. The acknowledgment of circadian rhythms is philosophically different from the concept of homeostasis, which assumes that most physiological processes are in a state of equilibrium and do not change significantly with time.

It is now generally understood that there are circadian rhythms in many receptors, in signaling pathways, and in activity of enzymes. If these are targets of a drug, then pharmacodynamics of the drug can also be different at different times. There are a number of hormones that are primarily secreted in the morning, such as cortisol, catecholamines, plasma renin, aldosterone, and angiotensin. In contrast, other substances peak at the end of the day or at night, for example, gastric acid, growth hormone, prolactin, melatonin, follicle-stimulating hormone, luteinizing hormone, and adrenocorticotrophic hormone.

There are consequences to the circadian changes in these hormones, and many common diseases show significant circadian variation in onset or exacerbation of symptoms. Asthma symptoms generally worsen during the night; in fact, estimates are that symptoms of asthma occur 50 to 100 times more often at night than during the day. Many circadian-dependent factors may contribute to the worsening of nocturnal asthma. For example, researchers have reported that cortisol (an anti-inflammatory ligand) levels were lowest in the middle of the night, and histamine (a mediator of

bronchoconstriction) peaked at a level that coincided with the greatest degree of bronchoconstriction at 4 AM.

Because of its effect on physiological processes, circadian rhythm can affect all aspects of drug disposition and action and is one of the factors responsible for intraindividual variability of medications depending on time of administration. Many drugs display normal, reproducible daily variations in pharmacokinetics and pharmacodynamics. Circadian effects on drug pharmacokinetics have been related to time-dependent changes in the following processes and parameters:

- Gastrointestinal motility and intestinal absorption rates
- Intestinal enzyme activity
- Gastric acid secretion
- Hepatic drug metabolism activity and enzyme concentration
- Glomerular filtration rate
- Blood flow rate
- Urine pH

The narrower the therapeutic window for a specific drug, the more important the implication of circadian variation in plasma levels. Although most drug doses and dosing schedules have not yet taken into account such diurnal variation in drug action and disposition, mounting evidence suggests these factors must be considered in drug therapy.

For example, rhythmic changes in cell division may explain circadian variation in sensitivity of rapidly proliferating tumor tissues to chemotherapy. Many anticancer agents are most cytotoxic to normal tissues that are actively dividing during specific phases of the cell division cycle. Studies indicate the timing of cancer chemotherapy may be of practical importance in improving the therapeutic index of common cancer treatments. Corticosteroids and interferons are less toxic and no less effective when given, respectively, on awakening and just before sleep.

Drug Tolerance

Tolerance is the body's ability to adapt to the presence of a drug. The magnitude of the body's response to a particular drug depends on the concentration of drug at its site of action and the sensitivity of the target receptor to the drug. Tolerance may be defined as a state of progressively decreased responsiveness to a drug, as a result of which a larger dose of drug is needed to achieve the effect originally obtained by a smaller dose. Tolerance appears counterintuitive because addition of more of an activating ligand lessens the elicited response.

Tolerance may be pharmacokinetic in origin, but is most often related to drug pharmacodynamics. Pharmacokinetic tolerance develops when a drug induces its own metabolism and thus decreases concentration of drug at the site of action. Pharmacodynamic tolerance arises as a result of adaptive changes in receptor sensitivity in response to repeated exposure to a particular drug. The result is usually a decrease or loss of sensitivity to the drug, resulting in a decreased response. Adaptive changes may be related to receptor down-regulation, cellular adaptation, or *tachyphylaxis* (a rapidly decreasing response to a drug after administration of a few doses).

Drug Resistance

Drug resistance is defined as insensitivity or decreased sensitivity of cells to drugs that ordinarily cause growth inhibition (such as anti-tumor drugs) or cell death (such as anti-microbial drugs). It occurs despite administration of doses equal to or higher than those usually recommended but within limits of safety, and despite adequate absorption from the administration site.

Drug resistance may be classified as *intrinsic resistance*, in which the cell or organism is inherently insensitive to the drug and predisposed to respond poorly to it, or *acquired resistance*, in which

Chronopathology and Chronotherapeutics of Cardiovascular Disease

Chronopathology is the study of biological rhythms in disease processes and morbid and mortal events; a good example is seen in cardiovascular disease. Myocardial infarction, stroke, and sudden cardiac death occur with greater frequency between 6 AM and noon. These events can be explained by changes in multiple factors. With activation of the sympathetic nervous system before awakening, blood pressure and heart rate begin to increase. Blood pressure peaks between 6 AM and noon. These changes in blood pressure parallel the morning activation in catecholamines, renin, and angiotensin. Activity and sleep influence the level of blood pressure throughout the day, and blood pressure is generally lowest between midnight and 6 AM. At the same time, increase in catecholamines in the morning promotes platelet aggregation. This is especially important because fibrinogen also increases, whereas endogenous tissue plasminogen activator decreases, promoting a procoagulant state with increased blood viscosity.

The higher blood pressure and heart rate in the early morning increase shear forces in blood vessels

and myocardial oxygen consumption, creating an environment for plaque rupture. Once plaque rupture occurs, thrombosis results, exacerbated by the procoagulant state in the early morning; the resulting consequence is a clinical event.

Chronotherapeutics is the purposeful control of plasma drug concentrations to match intrinsic disease rhythms to optimize therapeutic outcomes and minimize side effects. Optimum therapy is more likely when the right amount of drug is delivered to the correct target organ at the most appropriate time. In contrast, many side effects can be minimized if a drug is not present when it is not needed.

To achieve such customized dosing, scientists have developed *chronotherapeutic formulations* of drugs. Unlike conventional controlled-release formulations, which provide relatively constant plasma drug levels for 24 hours, chronotherapeutic formulations use various release mechanisms to provide varying plasma drug levels throughout the day. Currently, there are four chronotherapeutic antihypertensive products, two using verapamil and one each containing diltiazem and propranolol.

organisms or cells initially respond to the drug, but eventually some or all fail to respond because of an acquired resistant property.

Intrinsic Resistance. Intrinsic or primary resistance may occur as a result of systemic or cellular factors:

- **Systemic factors:** factors causing low drug concentration in the organism or

tumor such as decreased absorption, increased elimination, or decreased distribution to the site of action because of functional membrane barriers (e.g., blood-brain barrier limits effective therapy of bacterial infections in the brain).

- **Cellular factors:** factors that affect ability of the drug to reach and interact with the drug target receptor or enzyme. Such factors include low intra-

cellular levels of drug (owing to efflux proteins), low affinity of receptor for drug, absence of a particular receptor, or lack of activation of a prodrug to an active metabolite in the cell.

Acquired Resistance. Acquired resistance is usually related to genetic changes that alter the sensitivity of the target to a single drug (or to a chemically similar group of drugs) through a variety of mechanisms. Acquired resistance is an inheritable trait. It may be the result of induction by the drug of DNA mutations in some cells in the population. Alternatively, it may arise as a result of a drug selectively affecting the genetically susceptible cells within a population while not affecting a small number of resistant cells in the population. In both cases, the drug will be initially effective, but will become progressively less effective as acquired resistance develops.

Once a cell has developed acquired resistance, i.e., is altered such that it is partially or completely resistant to a drug, there is a selective advantage for its survival in a drug-containing environment. Selection is the process by which drug-resistant organisms or cells are enriched in a cell population. Consequently, with continued presence of drug, eventually only drug-resistant organisms or cells will survive, resulting in a resistant population.

Different drugs may share the same biochemical mechanism leading to development of resistance. *Cross-resistance* arises when cells become resistant to drugs that are chemically related or have the same mechanism of action. More problematic is *multiple drug resistance*, or multidrug resistance, in which cells become resistant to drugs with different chemical structures and mechanisms of action.

Several clinical approaches have been implemented to avoid or minimize resistance.

- Use of high doses to achieve adequate intracellular concentrations to inhibit or kill the intended organism or cell.

Because subtherapeutic concentrations encourage development of resistance, this approach reduces the probability that drug-resistant populations will develop.

- Combination therapy with two or more drugs with different mechanisms of action. The probability that DNA changes will emerge in the same cell resulting in resistance to both drugs is small. Thus, this approach reduces the probability of selection of a population of resistant cells.
- Cells resistant to one drug often exhibit increased sensitivity to another drug that acts by a different mechanism. Thus, knowledge of the mechanism of resistance to a drug can provide an approach to specifically target drug-resistant organisms or cells.

Immunological Variability

Drug allergy is a variability in drug response among individuals that is related to an immunological reaction and is seen only after a second or subsequent exposure of the patient to the drug. The effects are qualitatively different from the normal pharmacological effects of the drug. Allergic reactions to drugs do not show the usual dose-response relationships; even a small, subtherapeutic dose may be sufficient to cause allergy.

Drug Allergy

An allergic reaction is an adverse response to a drug as a result of a previous exposure to the same drug. Drug allergy is different from drug toxicity or idiosyncrasy in that it is not dose-dependent and not drug-specific. Thus, even therapeutic doses can cause an allergic reaction in some patients. Symptoms of drug allergy are similar for all drugs that cause an allergic response, and are not related to usual pharmacological effects of the drug.

Many macromolecules (such as foreign proteins) are viewed as antigens by the body, and their presence triggers formation

Multidrug Resistance

Multidrug resistance (MDR) occurs when cells become resistant to a broad range of structurally and functionally unrelated drugs after exposure to a single cytotoxic agent. This type of resistance is a serious problem in chemotherapy and in antiviral and antimicrobial therapy.

A number of different mechanisms can cause development of MDR, including increased drug efflux from the cell by ATP-dependent transporters, decreased drug uptake into the cell, or activation of biotransformation enzymes. However, MDR has most often been linked to the overexpression of P-glycoprotein (P-gp), a 170-kilodalton ATP-dependent membrane transporter that acts as a drug efflux pump.

Clinical observation has shown that MDR may develop after a course of chemotherapy (acquired resistance) or may be present on diagnosis (intrinsic resistance). MDR in chemotherapy has been associated with overexpression of P-gp transporters that act as efflux pumps to remove cytotoxic compounds from tumor cells, thereby preventing drugs from reaching therapeutic concentrations in the cell. The cytotoxic drugs most frequently associated with MDR are hydrophobic,

amphipathic natural products, such as the taxanes (paclitaxel, docetaxel), vinca alkaloids (vinorelbine, vincristine, vinblastine), anthracyclines (doxorubicin, daunorubicin, epirubicin), epipodophyllotoxins (etoposide, teniposide), topotecan, dactinomycin, and mitomycin C.

Expression of P-gp is usually highest in tumors that are derived from tissues that normally express P-gp, such as epithelial cells of the colon, kidney, adrenal, pancreas, and liver. Such tumors may show resistance to some antitumor drugs even before chemotherapy is initiated. In other tumors, the expression of P-gp may be low at the beginning of treatment but increase after exposure to the drugs, resulting in the development of MDR.

Inhibiting P-gp as a way of reversing MDR has been extensively studied. Many P-gp inhibitors, both competitive and noncompetitive, have been identified, with the objective of administering the inhibitor along with the antitumor drug. However, clinical results have been disappointing. The inhibitors are either too toxic themselves, have a relatively low affinity for the transporter, or have other undesirable drug interactions.

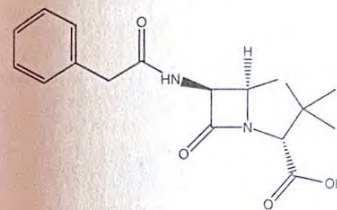
of antibodies. Thus, many biopharmaceutical protein drugs have the potential of initiating antibody formation. However, small molecule drugs can also indirectly cause sensitization. This indirect effect occurs if the drug (or its metabolite) binds covalently to an endogenous protein (*haptens*), creating an antigen complex with specificity for the drug part of the com-

plex. A subsequent exposure to the same drug then triggers an antigen-antibody response in the body, a chain reaction that is always the same regardless of drug.

The most important drug causes of immediate hypersensitivity reactions are antibiotics. Other common drugs that cause such reactions are insulin, enzymes (streptokinase and chymopapain), heterologous

Penicillin Allergy

Penicillin antibiotics are one of the most common causes of medication allergies. Penicillins are a family of antibiotics called β -lactams and include benzylpenicillin, penicillin V and penicillin G, amoxicillin, ampicillin, dicloxacillin, and nafcillin. These drugs are generally used to eradicate many common bacterial infections such as skin, ear, sinus, and upper respiratory infections.



benzylpenicillin

Common allergic reactions to penicillin include rashes, hives, itchy eyes, and swollen lips, tongue, or face. Serious and occasionally fatal hypersensitivity (anaphylaxis) has been reported in patients on penicillin therapy. The anaphylactic reaction can develop within an hour of taking penicillin. Symptoms include difficulty breathing, hives, wheezing, dizziness, loss of consciousness, rapid or weak pulse, skin turning blue, diarrhea, nausea, and vomiting. Although anaphylaxis is more frequent after parenteral therapy,

it also occurs in patients receiving oral penicillin.

Allergic reactions are more likely to occur in individuals with a history of penicillin hypersensitivity or a history of sensitivity to multiple allergens. Some people who are allergic to penicillin show cross-sensitivity to other closely related antibiotics, particularly other β -lactams. Drugs in this category include cephalosporins (e.g., cephalexin, cefprozil, and cefuroxime), carbapenems (e.g., imipenem), monobactams (e.g., aztreonam), and carbacephems.

The prevalence of penicillin hypersensitivity in the general population is not known. Before any penicillin therapy is initiated in a patient, it is important to make a careful inquiry concerning previous hypersensitivity reactions to penicillin, cephalosporins, or other antibiotics. Approximately 10% of hospitalized patients report a history of allergy to penicillin, and for this reason, many of these patients receive alternative antimicrobial drugs. The most reliable method for evaluating penicillin allergy is by skin testing to both major and minor determinants of penicillin.

If an allergic reaction occurs, the penicillin product should be discontinued immediately. Emergency treatment of penicillin allergy includes injections of epinephrine or intravenous administration of antihistamines and corticosteroids. Oxygen, intravenous steroids, and airway management, including intubation, are also administered as indicated.

antisera (equine antitoxins and antilymphocyte globulin), murine monoclonal antibodies, protamine, and heparin.

The first contact of the drug with a potentially allergic patient is called sensitization. On subsequent contacts, the body produces large amounts of histamine, an autacoid that causes potentially serious effects such as a skin rash, breathing difficulty, decrease in blood pressure and, ultimately, shock. This exaggerated immune response to a foreign agent such as an antigen or drug is called hypersensitivity. Symptoms may occur within seconds or

minutes after the second drug exposure (immediate hypersensitivity) or may begin gradually with maximum effect in days or weeks after the second exposure (delayed hypersensitivity). The most serious manifestation of such an allergic reaction is called *anaphylaxis* or *anaphylactic shock*—a severe and life-threatening allergic reaction. Structurally similar drugs with the same determinant group (group recognized by the antibody) can show cross-sensitivity, in that an allergic response to the second drug is shown even without previous contact with it.

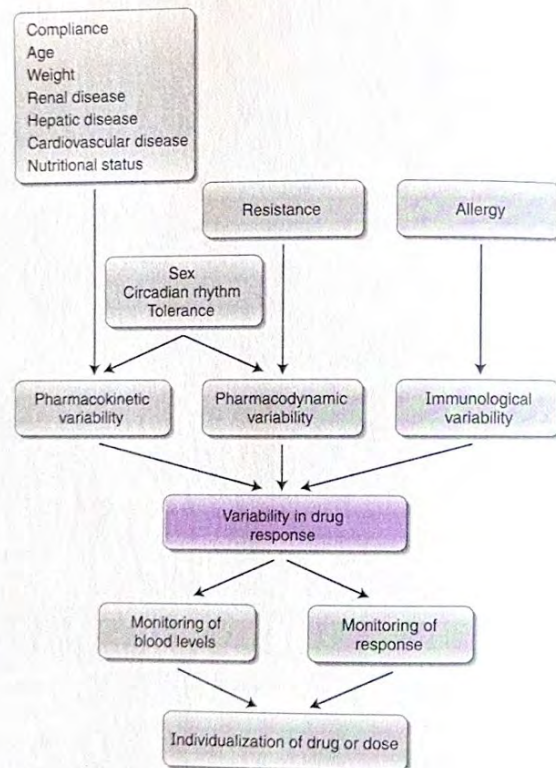


Figure 18.2. Summary of factors causing variability in drug response.

The predisposition of a patient to be allergic to a particular drug is partly genetic, but may be related to other factors such as duration of treatment or route of administration. Allergic reactions often occur without warning and cannot be predicted.

Drug Compliance

Compliance is the willingness and ability to follow a prescribed course of drug treatment. Medication noncompliance or nonadherence, i.e., the failure to take drugs on time in the doses and manner prescribed, is as dangerous and costly as many illnesses.

Noncompliance can be classified as either complete nonadherence (missing dosages) or partial adherence (prescribed dose is not completely administered) to a therapeutic regimen. Whether compliance is absent or partial, the outcome is a reduction of pharmacological effect or emergence of drug resistance. Some causes for noncompliance are:

- The patient is taking many medications (polypharmacy) on a complex dosing schedule and is unable to keep track of them.
- The cost of medications may cause the patient to miss doses or take reduced doses.

- Side effects may discourage the patient from taking the drug.
- The patient may lack understanding of a drug's benefits.

Although an obvious cause of therapeutic variability, noncompliance must be taken into account and strategies developed to encourage adherence to a dosing regimen.

Individualization of Therapy

The standard drug therapy, i.e., drug, dose, and dosing frequency, for a particular disease or condition is merely the starting point of treatment. Before the standard drug or dose is used, the patient needs to be assessed for factors discussed in this chapter to see whether this approach is suitable. Even if a patient is started on standard therapy, monitoring of response (efficacy, adverse effects) is necessary as therapy continues. For drugs with a narrow therapeutic window and in certain sensitive patients, blood levels may need to be monitored to ensure they are within the therapeutic range. The dose or the drug may have to be changed to individualize therapy to each patient. Figure 18.2 summarizes factors causing therapeutic variability and actions to be taken in these situations.

KEY CONCEPTS

- The standard dose of a drug is based on response of a majority of average patients.
- The effect of a particular dose of a particular drug is never the same in all persons, or even in the same person at different times. The standard drug or dose may need to be modified for the needs of a particular individual.
- Pharmacokinetic variability arises because of differences in ADME of the drug in patients.
- Pharmacodynamic variability is caused by differences in target receptors or in pathophysiology of the patient.
- Circadian rhythms are an important source of pharmacokinetic and pharmacodynamic variability.
- Drug tolerance and drug resistance can limit the therapeutic utility of a drug in some patients.
- Immunological variability, e.g., drug allergy, can cause serious adverse reactions in certain patients.
- Poor drug compliance is an important component of interindividual therapeutic variability.

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REVIEW QUESTIONS

1. Why do patients respond differently to the same dose of a drug?
2. Why is pharmacokinetic variability particularly problematic for drugs with a narrow therapeutic range?
3. What factors can cause pharmacodynamic variability among patients?
4. What factors can cause variability in the same patient at different times?
5. Differentiate among drug toxicity, drug idiosyncrasy, and drug allergy.
6. Differentiate between drug tolerance and drug resistance. List the clinical approaches to minimizing drug resistance.
7. Differentiate between cross-resistance and multiple-drug resistance.
8. What is an allergic reaction to a drug? How does an allergic response differ from other responses seen after administering a drug?
9. What are the clinical approaches to ensuring that each patient is given the right dose of the right drug?