GENERAL PHARMACOLOGY

Pharmacology can be defined as the study of substances that interact with living systems through chemical processes. It concerned with drugs, their sources, appearance, chemistry, actions, and uses. **Medical pharmacology** is often defined as the science of substances used to prevent, diagnose, and treat disease.

The interactions between a drug and the body are conveniently divided into two classes. The actions of the drug on the body are termed **pharmacodynamic processes.** These properties play the major role in deciding whether that group is appropriate therapy for a particular symptom or disease. The actions of the body on the drug are called **pharmacokinetic processes**. Pharmacokinetic processes govern the absorption, distribution, and elimination of drugs. **Toxicology** is that branch of pharmacology that deals with the undesirable effects of chemicals on living systems, from individual cells to complex ecosystems.

Drug substances have different **sources**. They can be obtained from the plants, animals, minerals, bacteria, and fungi.

History. Prehistoric people undoubtedly recognized the beneficial or toxic effects of many plant and animal materials. The earliest written records from China and from Egypt list remedies of many types, including a few still recognized today as useful drugs. Most, however, were worthless or actually harmful. Around the end of the 17th century, reliance on observation and experimentation began to replace theorizing in medicine, following the example of the physical sciences. As the value of these methods in the study of disease became clear, physicians in Europe began to apply them to the effects of traditional drugs used in their own practices. Advances in chemistry and the further development of physiology in the 18th, 19th, and early 20th centuries laid the foundation needed for understanding how drugs work at the organ and tissue levels.

About 50 years ago, there also began a major expansion of research efforts in all areas of biology. As new concepts and new techniques were introduced, information accumulated about drug action and the biologic substrate of that action, the receptor. During this half-century, many fundamentally new drug groups and new members of old groups have been introduced. The last 3 decades have seen an even more rapid growth of information and understanding of the molecular basis for drug action. The molecular mechanisms of action of many drugs have now been identified, and numerous receptors have been isolated, structurally characterized, and cloned.

PHARMACOKINETICS

In practical therapeutics, a drug should be able to reach its intended site of action after administration by some convenient route. In only a few situations is it possible to directly apply a drug to its target tissue, eg, by topical application of an anti-inflammatory agent to inflamed skin or mucous membrane. In other cases, drugs may be given intravenously and circulate in the blood directly to target blood vessels in another part of the body where they bring about useful effects. Much more commonly, a drug is given into one body compartment, eg, the gut, and must move to its site of action in another compartment, eg, the brain. This requires that the drug be **absorbed** into the blood from its site of administration and **distributed** to its site of action, **permeating** through

the various barriers that separate these compartments. For a drug given orally to produce an effect in the central nervous system, these barriers include the tissues that comprise the wall of the intestine, the walls of the capillaries that perfuse the gut, and the "blood-brain barrier," the walls of the capillaries that perfuse the brain. Finally, after bringing about its effect, a drug should be **eliminated** at a reasonable rate by metabolic inactivation, by excretion from the body, or by a combination of these processes.

Principles of drug transfer.

As you know, the plasma membrane consists of a bilayer of amphipathic lipids, with their hydrocarbon chains oriented inward to form a continuous hydrophobic phase and their hydrophilic heads oriented outward. Membrane proteins embedded in the bilayer serve as receptors, ion channels, or transporters to elicit electrical or chemical signaling pathways and provide selective targets for drug actions.

Drug permeation proceeds by four primary mechanisms. Passive diffusion in an aqueous or lipid medium is most common, but active processes play a role in the movement of some drugs, especially those whose molecules are too large to diffuse readily.

1. Aqueous diffusion (filtration) - Aqueous diffusion occurs within the larger aqueous compartments of the body (interstitial space, cytosol, etc) and across epithelial membrane tight junctions and the endothelial lining of blood vessels through aqueous pores that permit the passage of molecules as large as 100 daltons.

Aqueous diffusion of drug molecules is usually driven by the concentration gradient of the permeating drug, a downhill movement described by Fick's law:

$$Flux = (C_1 - C_2) \times Area \times Permeability\ coefficient / Thickness\ of\ the\ diffusion\ path$$

Drug molecules that are bound to large plasma proteins (eg, albumin) will not permeate these aqueous pores. If the drug is charged, its flux is also influenced by electrical fields.

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

Since lipid diffusion depends on relatively high lipid solubility, ionization of drugs may markedly reduce their ability to permeate membranes. A very large fraction of the drugs in use are weak acids or weak bases. For drugs, a weak acid (aspirin) is best defined as a neutral molecule that can reversibly dissociate into an anion (a negatively charged molecule) and a proton (a hydrogen ion):

$AnH \leftrightarrow An^{--} + H^{+}$

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

$CatH^+ \leftrightarrow Cat + H^+$

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

$$log\ (Protonated)/(Unprotonated) = pKa - pH$$

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

The most important application of this principle is in the manipulation of drug excretion by the kidney. Almost all drugs are filtered at the glomerulus. If a drug is in a lipid-soluble form during its passage down the renal tubule, a significant fraction will be reabsorbed by simple passive diffusion. If the goal is to accelerate excretion of the drug, it is important to prevent its reabsorption from the tubule. This can often be accomplished by adjusting urine pH to make certain that most of the drug is in the ionized state. As a result of this pH partitioning effect, the drug will be "trapped" in the urine. Thus, weak acids are usually excreted faster in alkaline urine; weak bases are usually excreted faster in acidic urine. Other body fluids in which pH differences from blood pH may cause trapping or reabsorption are the contents of the stomach and small intestine; breast milk; aqueous humor; and vaginal and prostatic secretions (Table 1-3).

3. Special carriers

Special carrier molecules exist for certain substances that are important for cell function and too large or too insoluble in lipid to diffuse passively through membranes, eg, peptides, amino acids, glucose. These carriers bring about movement by **active transport** or **facilitated diffusion** and, unlike passive diffusion, are saturable and inhibitable. Because many drugs are or resemble such naturally occurring peptides, amino acids, or sugars, they can use these carriers to cross membranes. Active transport is characterized by a requirement for energy, movement against an electrochemical gradient. For facilitated diffusion there is no input of energy and therefore enhanced movement of the involved substance is down an electrochemical gradient. The transporter proteins can mediate both drug uptake and efflux (e.g., P-glycoprotein is important efflux transporter in intestinal and renal tubular membranes and capillary endothelium of brain capillaries).

4. Endocytosis and exocytosis-A few substances are so large that they can enter cells only by endocytosis, the process by which the substance is engulfed by the

cell membrane and carried into the cell by pinching off of the newly formed vesicle inside the membrane. The substance can then be released inside the cytosol by breakdown of the vesicle membrane. This process is responsible for the transport of iron and vitamin B_{12}, each complexed with appropriate binding proteins, across the wall of the gut into the blood. The reverse process (exocytosis) is responsible for the secretion of many substances from cells. For example, many neurotransmitter substances are stored in membrane-bound vesicles in nerve endings to protect them from metabolic destruction in the cytoplasm. Appropriate activation of the nerve ending causes fusion of the storage vesicle with the cell membrane and expulsion of its contents into the extracellular space.

Bioavailability

Bioavailability is defined as the fraction of unchanged drug reaching the systemic circulation following administration by any route. For an intravenous dose of the drug, bioavailability is equal to unity. For a drug administered orally, bioavailability may be less than 100% for two main reasons - incomplete extent of absorption and first-pass elimination.

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

B. First-pass elimination: Following absorption across the gut wall, the portal blood delivers the drug to the liver prior to entry into the systemic circulation. A drug can be metabolized in the gut wall or even in the portal blood, but most commonly it is the liver that is responsible for metabolism before the drug reaches the systemic circulation. In addition, the liver can excrete the drug into the bile. Any of these sites can contribute to this reduction in bioavailability, and the overall process is known as first-pass loss or elimination. The effect of first-pass hepatic elimination on bioavailability is expressed as the **extraction ratio** (ER):

$$ER = Cl_{hepatic} / Q\,,$$

where Q is hepatic blood flow, normally about 90 L/h in a person weighing 70 kg.
The systemic bioavailability of the drug (F) can be predicted from the extent of absorption (f) and the extraction ratio (ER):

$$F = f \times (1 - ER)$$

Example. A drug like morphine is almost completely absorbed (f = 1), so that loss in the gut is negligible. However, the hepatic extraction ratio for morphine is 0.67, so (1 - ER) is 0.33. The bioavailability of morphine is therefore expected to be about 33%.

Distribution of drugs

Following absorption or administration into the systemic blood, a drug distributes into interstitial and intracellular fluids. Cardiac output, regional blood flow, and tissue volume determine the rate of delivery and potential amount of drug distributed into tissues. Initially, liver, kidney, brain and other well-perfused organs receive most of the drug, whereas delivery to muscle, most viscera, skin, and fat is slower. This second distribution phase may require minutes to several hours before the concentration of drug in tissue is in distribution equilibrium with that in blood. Tissue distribution is determined by the concentration gradient of the drug between blood and the particular tissue; its lipid solubility; relative binding of drug to plasma proteins and tissue macromolecules.

Plasma protein binding. Many drug are bound to plasma protein, mostly to plasma albumin for acidic drugs and to $alpha_1$-acid glycoprotein for basic drugs. The binding is usually reversible. Plasma protein binding is saturable and nonlinear process. For most drugs, however, the therapeutic range of plasma concentrations is limited; thus, the extent of binding and the unbound fraction is relatively constant. The extent of plasma binding also may be affected by disease-related factors (hypoalbuminemia during liver disease or nephrotic syndrom; elevated level of $alpha_1$-acid glycoprotein during acute inflammation, eg, cancer, RA, myocardial infarction).

Importantly, binding of a drug to plasma proteins limits its concentration in tissues and at its locus of action, limits glomerular filtration of the drug. However, plasma-protein binding generally doesn't limit renal tubular secretion or biotransformation, since these processes lower the free drug concentration, and this is rapidly followed by dissociation of the drug-protein complex.

Because binding of drugs to plasma proteins is rather nonselective, many drugs with similar physicochemical characteristics can compete with each other and with endogenous substances for these binding sites (eg, displacement of unconjugated bilirubin from binding to albumin by sulfonamides or other organic anions is known to increase the risk of bilirubin encephalopathy in the newborn. Concerned for drug toxicities based on a similar competition between drugs for binding sites has, in the past, been overemphasized. Steady-state unbound concentrations will change only when either drug input (dosing rate) or clearence of unbound drug is changed.

Tissue binding. Many drugs accumulate in tissues at higher concentrations than those in the extracellular fluids and blood. Tissue binding of drugs usually occurs with cellular constituents such as proteins, phospholipids, or nuclear proteins and generally is reversible. A large fraction of drug in the body may be bound in this fashion and serve as a reservoir that prolongs drug action in that tissue or at a distant site reached through the circulation. For example, fat can be a reservoir for thiopental; bone – for tetracycline, lead, radium. In the former case, thiopental is redistributed from the CNS into other tissues, like fat and muscle; that lead to termination of thiopental action.

CNS and CSF (cerebrospinal fluid). The capillaries of the brain (as well as the testes) are characterized by an absence of the pores that permit aqueous diffusion of many drug molecules into the tissue. At the choroid plexus, a similar blood-cerebrospinal fluid barrier present except that it is epithelial cells that are joined by

tight junctions rather than endothelial cells. These tissues are therefore "protected" or "sanctuary" sites from many circulating drugs. As a result, the lipid solubility of the drug is an important determinant of its uptake by the brain. For example, nonsedating antihistamines achieve far lower brain concentrations than do other agents in this class. Drugs may penetrate into the CNS by specific uptake transporters normally involved in the transport of nutrients and endogenous compounds from blood into the brain and CSF. Another important factor are efflux carriers that present in the brain capillary endothelial cell, eg, P-glycoprotein. It is not allowing drug to even translocate across the endothelial cell and by exporting any drug that enters the brain by other means (it concerns protease inhibitors, loperamide).

Placental transfer of drugs. The fetus is to at least some extent exposed to essentially all drugs taken by the mother (placenta is not an absolute barrier to drugs). Lipid solubility, extent of plasma binding, and degree of ionization are important general determinants of drugs' transfer. The fetal plasma is slighly more acidic than that of the mother (pH 7,0-7,2 versus 7,4; pH of breast milk is 6,4-7,6), so that ion-trapping of basic drugs occurs; also present P-glycoprotein.

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

$$V_d = \text{Amount of drug in body} / C$$

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

That the V_d calculated from equation is an *apparent* volume may be appreciated by comparing the volumes of distribution of drugs such as digoxin (440 litres) or chloroquine (13000 litres). V_d can vastly exceed any physical volume in the body because the V_d is the volume necessary to contain the amount of drug *homogeneously* at the concentration found in the blood, plasma, or plasma water. Drugs with very high volumes of distribution have much higher concentrations in extravascular tissue than in the vascular compartment, ie, they are *not* homogeneously distributed. Drugs that are completely retained within the vascular compartment, on the other hand, have a minimum possible V_d equal to the blood component in which they are distributed, eg, 7 L/70 kg for a drug (tolbutamide) that is restricted to the plasma compartment.

DRUG BIOTRANSFORMATION

The lipophilic characteristics of drugs that promote their passage through biological membranes and subsequent access to their site of action hinder their excretion from the body. Renal excretion of unchanged drug play only a modest role in the overall elimination of most therapeutic agents, since lipophilic compounds filtered through the glomerulus are largely reabsorbed back into the systemic circulation during passage through the renal tubules. The metabolism of drugs and other xenobiotics as well as a number of exogenous compounds (steroids, vitamins, and fatty acids) into more hydrophilic metabolites is therefore essential for the elimination of these compounds from the body and termination of their biological activity. In general, biotransformation reactions generate more polar, inactive

metabolites that are readily excreted from the body. However, in some cases, metabolites with potent biological activity or toxic properties are generated.

In general, all of reactions of metabolic biotransformations can be assigned to one of two major categories called phase I and phase II reactions.

Phase I reactions (functionalization or metabolism) usually convert the parent drug to a more polar metabolite by introducing or unmasking a functional group (-OH, $-NH_2$, -SH). Often these metabolites are inactive, though in some instances activity is only modified.

Examples of phase I reactions:

1) Dealkylation – imipramine, diazepam, theophylline, codeine, morphine;
2) Hydroxylation – phenytoine, phenobarbital, midazolam, amphetamine, propranolol, ibuprofen, warfarin;
3) Oxidation – chlorpheniramine, quinidine, acetaminophen, meperidine;
4) Deamination – diazepam, amphetamine;
5) Hydrolysis: (i) ether – procaine, enalapril, meperidine, aspirin, clofibrate;
(ii) amide – lidocaine, procainamide

If phase I metabolites are sufficiently polar, they may be readily excreted. However, many phase I products are not eliminated rapidly and undergo a subsequent reaction in which an endogenous substrate such as glucuronic acid, sulfuric acid, acetic acid, or an amino acid combines with the newly established functional group to form a highly polar conjugate that are readily excreted and often inactive. Such **conjugation** or **biosynthetic reactions** are the hallmarks of **phase II**. Because the endogenous substrates originate in the diet, nutrition plays a critical role in the regulation of drug conjugations. Different drugs may compete for the same endogenous substrates (glucuronic acid, sulfuric acid, acetic acid, or an amino acid), and the faster-reacting drug may effectively deplete endogenous substrate levels and impair the metabolism of the slower-reacting drug.Examples of phase II reactions:

1) Glucuronidation – acetaminophen, morphine, oxazepam, lorazepam;
2) Sulfation – acetaminophen, steroids, methyldopa;
3) Acetylation – sulfonamides, isoniazid, clonazepam.

Site of biotransformations. Although every tissue has some ability to metabolize drugs, the liver is the principal organ of drug metabolism. Other tissues that display considerable activity include the gastrointestinal tract, the lungs, the skin, and the kidneys.

Following oral administration, many drugs (eg, isoproterenol, meperidine, pentazocine, morphine) are absorbed intact from the small intestine and transported first via the portal system to the liver, where they undergo extensive metabolism. This process has been called a **first-pass effect.** Some orally administered drugs (eg, clonazepam, chlorpromazine) are more extensively metabolized in the intestine than in the liver. Thus, intestinal metabolism may contribute to the overall first-pass effect. First-pass effects may so greatly limit the bioavailability of orally administered drugs that alternative routes of administration must be employed to achieve therapeutically effective blood levels. The lower gut harbors intestinal microorganisms that are capable of many biotransformation reactions. In addition, drugs may be metabolized by gastric acid

(eg, penicillin), by digestive enzymes (eg, polypeptides such as insulin), or by enzymes in the wall of the intestine (eg, sympathomimetic catecholamines).

The enzyme systems involved in phase I reactions are located primarily in the endoplasmic reticulum, while the phase II conjugation enzyme systems are mainly cytosolic. Many drug-metabolizing enzymes are located in the lipophilic membranes of the endoplasmic reticulum of the liver and other tissues. When these lamellar membranes are isolated by homogenization and fractionation of the cell, they re-form into vesicles called **microsomes.**

Cytochrome P-450 Monooxygenase System. The cytochrome P-450 enzymes are a superfamily of heme-thiolate proteins. The enzymes are involved in the metabolism of drugs, environmental chemicals, and other xenobiotics. The activity of these enzymes requires both a reducing agent (NADPH) and molecular oxygen; in a typical reaction, one molecule of oxygen is consumed (reduced) per substrate molecule, with one oxygen atom appearing in the product and the other in the form of water.

In this oxidation-reduction process, two microsomal enzymes play a key role. The first of these is a P450 reductase. The second microsomal enzyme is a hemoprotein called cytochrome P450 that serves as the terminal oxidase. The relative abundance of cytochrome P450, as compared with that of the reductase in the liver, contributes to making cytochrome P450 heme reduction a rate-limiting step in hepatic drug oxidations.

Cytochrome P-450 catalyzes many reactions, including hydroxylation, dealkylation, oxidation, deamination.

There are about 50 cytochrome P-450 that are active in human beings. These are categorized into families and many subfamilies according to amino acid-sequence similarities; the abbreviated term *CYP* is used for identification. Sequences that are greater than 40% identical belong to the same family, identified by an Arabic number; within a family, sequences greater than 55% identical are in the same subfamily, identified by a letter; and different individual isoforms within the subfamily are identified by Arabic number. For example, CYP3A4 and CYP3A5 are involved in the metabolism of about 50% of drugs.

Factors affecting drug metabolism.

1. Genetic variation. There are subpopulations with different drug-metabolizing abilities, i.e., **genetic polymorphism**. It is possible to phenotype or gentotype a person with respect to a particular genetic variant, and it is likely that such characterization will become increasingly useful in individualizing drug therapy. For instance, there is an enzyme NAT-2 (N-acetyltransferase) that metabolize isoniazid, procainamide, hydralazine, caffeine. Considerable heterogeneity is present in the worldwide population, so that the slow-acetylator phenotype frequency is about 50% in American whites and blacks, but only 5-10% in Southeast Asians.

2. Environmental deteminants.

Enzyme induction (up-regulation). An interesting feature of some of these chemically dissimilar drug substrates is their ability, on repeated administration, to "induce" cytochrome P450 by enhancing the rate of its synthesis or reducing its rate of degradation. Induction results in an acceleration of metabolism and usually in a decrease in the pharmacologic action of the inducer (autoinduction) and also of coadministered drugs. Thus, continued use of some drugs may result in a pharmacokinetic type of tolerance – progressively reduced effectiveness due to enhancement of their own metabolism. However, in the case of drugs metabolically

transformed to reactive metabolites, enzyme induction may exacerbate metabolite-mediated tissue toxicity.

Various substrates appear to induce forms of cytochrome P450 having different molecular masses and exhibiting different substrate specificities and immunochemical and spectral characteristics. The two forms that have been most extensively studied are CYP2B1, which is induced by treatment with phenobarbital; and CYP1A1, which is induced by polycyclic aromatic hydrocarbons (PAHs). In addition, glucocorticoids, macrolide antibiotics, anticonvulsants, and some steroids induce specific forms called CYP3A. Isoniazid or chronic ethanol administration induces a different form, CYP2E1, that oxidizes ethanol and activates carcinogenic nitrosamines.

Enzyme-inducing drugs include various sedative-hypnotics, tranquilizers, anticonvulsants, and insecticides. Patients who routinely ingest barbiturates, other sedative-hypnotics, or tranquilizers may require considerably higher doses of warfarin, when being treated with this oral anticoagulant, to maintain a prolonged prothrombin time. On the other hand, discontinuance of the sedative may result in reduced metabolism of the anticoagulant and bleeding – a toxic effect of the enhanced plasma levels of the anticoagulant. Similar interactions have been observed in individuals receiving various combination drug regimens such as antipsychotics or sedatives with contraceptive agents and even alcohol with hypoglycemic drugs (tolbutamide).

Environmental pollutants are capable of inducing cytochrome P450 enzymes. For example, exposure to PAHs, which are present in tobacco smoke, charcoal-broiled meat, and cruciferous vegetables are known to induce CYP1A enzymes and to alter the rates of drug metabolism in both experimental animals and in humans. Whereas grapefruit juice is known to inhibit the CYP3A metabolism of coadministered drug substrates

Enzyme inhibition. Certain drug substrates may inhibit cytochrome P450 enzyme activity. Imidazole-containing drugs such as cimetidine and ketoconazole bind tightly to the heme iron of cytochrome P450 and effectively reduce the metabolism of endogenous substrates (testosterone) or other coadministered drugs (theophylline, lidocaine, nifedipine) through competitive inhibition. However, macrolide antibiotics are metabolized, apparently by CYP3A, to metabolites that complex the cytochrome heme-iron and render it catalytically inactive. The antibiotic chloramphenicol is metabolized by cytochrome P450 to a species that alkylates its protein and thus also inactivates the enzyme. A growing list of inactivators that attack the heme moiety includes the steroids ethinyl estradiol, norethindrone, and spironolactone; and propylthiouracil. With CYP2D6, quinidine and selective serotonin reuptake inhibitors are potent inhibitors that may produce **phenocopying** (convertion of genotypic extensive metabolizer into a phenotypic poor metabolizer).

Thus, simultaneous administration of two or more drugs may result in impaired elimination of the more slowly metabolized drug and prolongation or potentiation of its pharmacologic effects. Both competitive substrate inhibition and irreversible substrate-mediated enzyme inactivation may augment plasma drug levels and lead to toxic effects from drugs with narrow therapeutic indices. For example, allopurinol both prolongs the duration and enhances the chemotherapeutic action of mercaptopurine by competitive inhibition of xanthine oxidase. Consequently, to avoid bone marrow toxicity, the dose of mercaptopurine is usually reduced in patients receiving allopurinol. Cimetidine, a drug used in the treatment of peptic ulcer, has been shown to potentiate the pharmacologic actions of anticoagulants and sedatives.

Disease factors. The severity of the liver damage determines the extent of reduced metabolism. Drugs that undergo substantial hepatic first-pass metabolism,

oral bioavailability may be increased two- to fourfold in liver disease. Severe cardiac failure increases some drugs (eg, lidocaine) level.

Age and sex. Newborns and infants are able to metabolize drugs relatively efficiently but generally at a slower rate than are adults (exception: impairment of bilirubin glucuronidation at birth). Drug use in the elderly requires moderate reductions in drug dose and awareness of the possibility of exaggerated pharmacodynamic responsiveness. Also, during pregnancy there is induction of certain drug-metabolizing enzymes in the second and third trimesters. A few clinical reports suggest that sex-dependent differences in drug metabolism also exist in humans for ethanol, propranolol, benzodiazepines, estrogens, and salicylates.

So, drug (phenytoin) dosage may have to be increased during this period. Finally, oral contraceptive agents also are potent irreversible inhibitors of CYP isoforms.

Drug conjugations were once believed to represent terminal inactivation events and as such have been viewed as "true detoxification" reactions. However, this concept must be modified, since it is now known that certain conjugation reactions (acyl glucuronidation of nonsteroidal anti-inflammatory drugs and *N*-acetylation of isoniazid) may lead to the formation of reactive species responsible for the hepatotoxicity of the drug. An example is acetaminophen (paracetamol)-induced hepatotoxicity. This analgesic antipyretic drug is quite safe in therapeutic doses (1.2 g/d for an adult). It normally undergoes glucuronidation and sulfation to the corresponding conjugates, which together comprise 95% of the total excreted metabolites. The alternative cytochrome P450-dependent glutathione (GSH) conjugation pathway accounts for the remaining 5%. When acetaminophen intake far exceeds therapeutic doses, the glucuronidation and sulfation pathways are saturated, and the cytochrome P450-dependent pathway becomes increasingly important. Little or no hepatotoxicity results as long as glutathione is available for conjugation. However, with time, hepatic glutathione is depleted faster than it can be regenerated, and accumulation of a reactive and toxic metabolite occurs. In the absence of intracellular nucleophiles such as glutathione, this reactive metabolite (thought to be an *N*-hydroxylated product or an *N*-acetylbenzoiminoquinone) reacts with nucleophilic groups present on cellular macromolecules such as protein, resulting in hepatotoxicity.

The chemical and toxicologic characterization of the electrophilic nature of the reactive acetaminophen metabolite has led to the development of effective antidote - *N*-acetylcysteine. Administration of *N*-acetylcysteine (the safer of the two) within 8-16 hours following acetaminophen overdosage has been shown to protect victims from fulminant hepatotoxicity and death.

EXCRETION OF DRUGS

Drugs are eliminated from the body either inchanged by the process of excretion or coverted to metabolites. Excretory organs, the lung exluded, eliminate polar compounds more effeciently than substances with high lipid solubility.

The kidney is the most important organ for excreting drugs and their metabolites. Substances excreted in the feces are mainly unabsorbed, orally ingested drugs or metabolites excreted either in the bile or secreted directly into the intestinal tract and, subsequently, not absorbed. Excretion of drugs in breast milk is important, not because of the amount of eliminated, but because the excreted drugs are potential sources of unwanted pharmacological effects in the nursing infants. Pulmonary excretion is important mainly for the elimination of anesthetic gases and vapors; occasionally, small quantities of other drugs or metabolites are excreted by this route.

Renal excretion. Excretion of drugs and metabolities in the urine invoves three processes: glomerular filtration, active tubular secretion, and passive tubular

reabsorption. The amount of drug entering the tubular lumen by filtration is dependent on the glomerular filtration rate and the extent of plasma binding of the drug. In the proximal renal tubule, active, carrier-mediated tubular secretion also may add drug to the tubular fluid. Tranporters such as P-glycoprotein and the multidrug resistance-associated protein-type 2 (MRP-2) localized in the apical, brush-border membrane are lagerly responsible for the secretion of amphipathic anions and conjugated metabolites, respectively. Transport systems that are similar but more selective for organic cationic drugs (OCDs) are involved in the secretion of organic bases.

In the proximal and distal tubules, the nonionized forms of weak acids and bases undergo net passive reabsorption. Since the tubular cells are less permeable to the ionized forms of weak electrolytes, passive reabsorption of these substances is pH-dependent. In the treatment of drug poisoning, the excretion of some drugs can be hastened by appropriate alkalinization or acidification of the urine.

Biliary and fecal excretion. Transport system analogous to those in the kindney also are present in the canalicular membrane of the hepatocyte, and these actively secrete drugs and metabolites into bile. P-glycoprotein transports a plethora of amphipathic, lipid-soluble drugs, where as MRP2 is mainly involved in the secretion of conjugated metabolites and endogenous compounds. Active biliary secretion of organic cations also involves transporters. Because secretory transporters such as P-glycoprotein also are expressed on the apical membrane of enterocytes, direct secretion of drugs and metabolites may occur from the systemic circulation into the intestinal lumen. Subsequently, drugs and metabolites can be reabsorbed back into the body from the intestine.which in the case of conjugated metabolites. Such enterohepatic recycling, if extensive, may prolong significantly the presence of a drug and its effects within the body prior to elimination by other pathway..

THEORETICAL PHARMACOKINETICS.

The "standard" dose of a drug is based on trials in healthy volunteers and patients with average ability to absorb, distribute, and eliminate the drug. This dose will not be suitable for every patient. Several physiologic (eg, maturation of organ function in infants) and pathologic processes (eg, heart failure, renal failure) dictate dosage adjustment in individual patients. These processes modify specific pharmacokinetic parameters. The four basic parameters are **clearance,** the measure of the ability of the body to eliminate the drug; **volume of distribution,** the measure of the apparent space in the body available to contain the drug; **elimination half-life,** a measure of the rate of removal of drug from the body; and **bioavailability,** the fraction of drug absorbed as such into the systemic circulation. .

Clearance. Drug clearance principles are similar to the clearance concepts of renal physiology, in which creatinine clearance is defined as the rate of elimination of creatinine in the urine relative to its serum concentration. At the simplest level, clearance of a drug is the ratio of the rate of elimination by all routes to the concentration of drug in a biologic fluid (C):

$$\mathbf{Cl = \text{rate of elimination} / C} \qquad \mathbf{(1)}$$

Clearance, like volume of distribution, may be defined with respect to blood (CL_b) or plasma (CL_p), depending on the concentration measured.

It is important to note the additive character of clearance. Elimination of drug from the body may involve processes occurring in the kidney, the lung, the liver, and other organs. Dividing the rate of elimination at each organ by the concentration of drug (eg, plasma concentration) yields the respective clearance at that organ. Added together, these separate clearances equal total systemic clearance:

$$\mathbf{Cl_{renal} + Cl_{hepatic} + Cl_{other} = Cl} \quad \textbf{(2)}$$

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

The two major sites of drug elimination are the kidneys and the liver. For most drugs, clearance is constant over the plasma or blood concentration range encountered in clinical settings, ie, elimination is not saturable, and the rate of drug elimination is directly proportionate to concentration (rearranging equation [1]):

$$\textbf{Rate of elimination} = \mathbf{Cl \times C} \quad \textbf{(3)}$$

This is sometimes referred to as "first-order" elimination. It means that a constant **fraction** of drug in the body is eliminated per unit of time. When clearance is first-order, it can be measured by calculating the area under the curve (AUC) of the time-concentration curve after a dose. Clearance is proportionate to the dose divided by AUC.

$$\mathbf{Cl = Dose / AUC} \quad \textbf{(4)}$$

Capacity-limited elimination. For drugs that exhibit capacity-limited elimination (eg, phenytoin, ethanol), clearance will vary depending on the concentration of drug that is achieved. Capacity-limited elimination is also known as saturable, dose-dependent, nonlinear, zero-order, and Michaelis-Menten elimination. It means that a constant **amount** of drug in the body is eliminated per unit of time.

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

$$\textbf{Rate of elimination} = \mathbf{V_{msx} \times C / (K_{max} + C)} \quad \textbf{(5)}$$

where K_{max} is the drug concentration at which the rate of elimination is 50% of the maximal rate; V_{max} is equal to the maximal rate of elimination This equation is similar to the Michaelis-Menten statement of enzyme kinetics. It is important to note that in the nonlinear region, the increment in elimination rate becomes less as concentration increases. At concentrations that are high relative to the K_{max} the elimination rate is almost independent of concentration. If dosing rate exceeds elimination capacity, steady state cannot be achieved: The concentration will keep on rising as long as dosing continues. This pattern of capacity-limited elimination is important for such drugs as ethanol, phenytoin, theophylline, and aspirin. Design of dosage regimens for such drugs is more complex than when elimination is first-order and clerance is independent of drug’s concentration.

Flow-dependent elimination. In contrast to capacity-limited drug elimination, some drugs are cleared very readily by the organ of elimination, so that at any clinically realistic concentration of the drug, most of the drug in the blood perfusing the organ is eliminated on the first pass of the drug through it. The elimination of these drugs will thus depend primarily on the blood flow through the organ of elimination. Such drugs (amitriptyline, isoniazid, meperidine, morphine, propranolol, etc) can be called "high-extraction" drugs, since they are almost completely extracted from the blood by the organ. These drugs possess high **intrinsic clearance** – it means strong intrinsic ability of the determined organ (kidney, liver) to eliminate a drug in the absence of limitations imposed by blood flow). In contrast, when the metabolic capability is small in comparison to the rate of drug presentation, clearence will be proportional to the unbound fraction of drug in blood and the drug's intrinsic clearence.

Volume of distribution (V_d) as it was mention before relates the amount of drug in the body to the concentration of drug (C) in blood or plasma:

$$\mathbf{V_d = Amount\ of\ drug\ in\ body / C} \qquad \mathbf{(6)}$$

The volume of distribution may vary widely depending on the relative degrees of binding to plasma and tissue proteins, the partition coefficient of the drug in fat, and so forth. As might be expected, the V_d for a given drug can differ according to patient's age, gender, body composition, and presence of disease.

The V_d defined in equation (6) considers the body as a single homogeneous compartment. In this one-compartment model, all drug administration occurs directly into the central compartment and distribution of drug instantaneous throughout the volume (V).

The multipley exponential decay observed for a drug that is eliminated from the body with first–order kinetics results from differences in the rates at which the drug equilibrates with tissues. The rate of equilibration will depend upon the ration of the perfusion of the tissue to the partition of drug into the tissue. It is as though the drugs starts in a "central" volume, which consists of plasma and tissue reservoirs that are in rapid equilibrium with it, and distributes to a "final" volume, at which point concentrations in plasma decrease in a log-linear fashion with a rate constant of k.

Two different terms have been used to desribe the V_d for drugs that follow multiple exponential decay. The first, designated V_{area}, is calculated as the ratio of clearence to the rate of decline of concentration during the elimination (final) phase of the logarithmic concentration versus time curve:

$$\mathbf{V_{area} = Cl / k = dose / k \times AUC} \qquad \mathbf{(7)}$$

The estimation of this parameter may be done after administration of a single dose of a drug. However, another multicompartment volume of distribution may be more useful, especially when the effect of disease states on pharmacokinetics is to be determined. The volume of distribution at steady state (V_{ss}) represents the volume in which a drug would appear to be distributed during steady state if the drug existed throughout that volume at the same concentration as that in the measured fluid (plasma or blood).

Half-life ($t_{1/2}$) is the time required to decrease the amount of drug in the body by one-half during elimination (or during a constant infusion). In the simplest case – and the most useful in designing drug dosage regimens – the body may be considered as a single compartment of a size equal to the volume of distribution (V_d). While the organs of elimination can only clear drug from the blood or plasma in direct contact with the organ, this blood or plasma is in equilibrium with the total volume of

distribution. Thus, the time course of drug in the body will depend on both the volume of distribution and the clearance:

$$\mathbf{t_{1/2} = 0{,}7^{*} \times V_{ss} / Cl^{**}} \qquad \mathbf{(8)}$$

*The constant 0.7 in this equation is an approximation to 0.693, the natural logarithm of 2. Because drug elimination can be described by an exponential process, the time taken for a twofold decrease can be shown to be proportionate to ln(2);
**$k = Cl / V_{ss}$, where k – is a fractional elimination constant

However, many drugs will exhibit multicompartment pharmacokinetics. Under these conditions, where more than one half-life term may apply to a single drug, the "true" terminal half-life will be greater than that calculated from equation (8). As the rule, the lower concentrations measured appeared to yield longer and longer terminal half-life. Nevertheless, half-life is a useful kinetic parameter in that it indicates the time required to attain 50% of steady state, or to decay 50% from steady-state conditions, after a change (ie, starting or stopping) in a particular rate of drug administration (the dosing regimen).

As an indicator of either drug elimination or distribution, half-life alone can be misleading. Disease states can affect both of the physiologically related parameters, volume of distribution, and clearance; thus, the derived parameter, $t_{1/2}$, will not necessarily reflect the expected change in drug elimination. For example, patients with chronic renal failure have a decreased renal clearance of digoxin and also a decreased volume of distribution; the increase in digoxin half-life is not as great as might be expected based on the change in renal function. The decrease in V_d is due to the decreased renal and skeletal muscle mass and consequent decreased tissue binding of digoxin.

Steady State. Equation (9) indicates that a steady-state concentration eventually will be achieved when a drug is administered at a constant rate.

$$\mathbf{Dosing\ rate = Cl \times C_{ss}} \qquad \mathbf{(9)}$$

Steady state is a point, when drug intake per unit of time is the same as the rate of elimination, with the fluctuation between peak and trough plasma levels remaining constant. For drugs with 1st order kinetic steady state is attained after 4 half-lifes (94%); time to steady state independent of dosage. Fluctuation of C_{ss} is proportional to dosage interval/half-time; it blunted by slow absorption.

$$\mathbf{C_{ss} = F \times dose / Cl \times T} \qquad \mathbf{(10),}$$

where F – fractional bioavailability of the dose, T – dosage interval (time).

Bioavailability

Bioavailability, F, as it was mentioned before, is defined as the fraction of unchanged drug reaching the systemic circulation following administration by any route and escapes any first-pass elimination). The rate of absorption may influence drug therapy. If a drug is absorbed rapidly and has a small “central” volume, the concentration of drug initially will be high. It will then fall as the drug is distributed to its “final” (larger) volume. If the same drug is absorbed more slowly, it will be

distributed while it is being given, and peak concentrations will be lower and will occur later. Controlled-release preparations are designed to provide a slow and sustained rate of absorption in order to produce a less fluctuating plasma concentration-time profile during the dosage interval compared to more immediate-release formulations.

THE TARGET CONCENTRATION APPROACH TO DESIGNING A RATIONAL DOSAGE REGIMEN

Introduction. A rational dosage regimen is based on the assumption that there is a *target concentration* that will produce the desired therapeutic effect. By considering the pharmacokinetic factors that determine the dose-concentration relationship, it is possible to individualize the dose regimen to achieve the target concentration. The effective concentration ranges are a guide to the concentrations measured when patients are being effectively treated. The initial target concentration should usually be chosen from the lower end of this range. In some cases, the target concentration will also depend on the specific therapeutic objective – eg, the control of atrial fibrillation by digoxin often requires a target concentration of 2 ng/mL, while heart failure is usually adequately managed with a target concentration of 1 ng/mL.

Following administration of a dose of drug, its effects usually show a characteristic temporal pattern. Onset of the effect is preceded by a lag period, after which the magnitude of the effect increases to a maximum and then declines; if a further dose is not administered, the effect eventually disappears. Therapeutic window reflects a concentration range that provides efficacy without unacceptable toxicity.

Maintenance Dose

In most clinical situations, drugs are administered in such a way as to maintain a steady state of drug in the body associated with the therapeutich window, ie, just enough drug is given in each dose to replace the drug eliminated since the preceding dose. Thus, calculation of the appropriate maintenance dose is a primary goal. Clearance is the most important pharmacokinetic term to be considered in defining a rational steady state drug dosage regimen. At steady state (SS), the dosing rate ("rate in") must equal the rate of elimination ("rate out"). Substitution of the target concentration (TC) for concentration (C) in equation (9) predicts the maintenance dosing rate:

$$\textbf{Dosing rate} = \textbf{TC} \times \textbf{Cl} \qquad \textbf{(11)}$$

Thus, if the desired target concentration is known, the clearance in that patient will determine the dosing rate. If the drug is given by a route that has a bioavailability less than 100%, then the dosing rate predicted by equation (11) must be modified. For oral dosing:

$$\textbf{Dosing rate} = \textbf{TC} \times \textbf{Cl} / \textbf{F}_{\textbf{oral}} \qquad \textbf{(11)}$$

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

$$\textbf{Maintenance dose} = \textbf{Dosing rate} \times \textbf{Dosing interval} \qquad \textbf{(12)}$$

Note that the steady state concentration achieved by continuous infusion or the *average* concentration following intermittent dosing depends only on clearance. The

volume of distribution and the half-life need not be known in order to determine the average plasma concentration expected from a given dosing rate or to predict the dosing rate for a desired target concentration.

Estimates of dosing rate and average steady state concentrations, which may be calculated using clearance, are independent of any specific pharmacokinetic model. In contrast, the determination of maximum and minimum steady state concentrations requires assumptions about the pharmacokinetic model. The accumulation factor

Accumulation factor = 1 / Fractional lost in one dosing interval (13)

assumes that the drug follows a one-compartment body model, and the peak concentration prediction assumes that the absorption rate is much faster than the elimination rate. For the calculation of estimated maximum and minimum concentrations in a clinical situation, these assumptions are usually reasonable.

Loading Dose

When the time to reach steady state is appreciable, as it is for drugs with long half-lives, it may be desirable to administer a loading dose that promptly raises the concentration of drug in plasma to the target concentration. In theory, only the amount of the loading dose need be computed – not the rate of its administration – and, to a first approximation, this is so:

$$\textbf{Loading dose} = TC_p \times V_{ss} / F \quad (14)$$

Loading dose = 100 / percent of drug eliminated per dosage interval (15)

Up to this point, we have ignored the fact that some drugs follow more complex multicompartment pharmacokinetics, eg, the distribution process. This is justified in the great majority of cases. However, in some cases the distribution phase may not be ignored, particularly in connection with the calculation of loading doses. If the rate of absorption is rapid relative to distribution (this is always true for intravenous bolus administration), the concentration of drug in plasma that results from an appropriate loading dose – calculated using the apparent V_d – can initially be considerably higher than desired. Severe toxicity may occur, albeit transiently. This may be particularly important, for example, in the administration of antiarrhythmic drugs such as lidocaine, where an almost immediate toxic response may occur. Thus, while the estimation of the *amount* of a loading dose may be quite correct, the *rate of administration* can sometimes be crucial in preventing excessive drug concentrations, and slow administration of an intravenous drug (over minutes rather than seconds) is almost always prudent practice.

Nonlinear pharmacokinetics.

Nonlinearity in pharmacokinetics (i.e., changes in Cl, V_d, and $t_{1/2}$ as a function of dose or concentration of drug) usually is due to saturation of protein binding, hepatic metabolism, or active renal transport of the drug.

As the molar concentrationof drug increases, the unbound fraction eventually also must increase. For drugs with low intrinsic clearance/extraction ratio, saturation associated with raising of Cl and V_d as drug concentration increases; $t_{1/2}$ – is constant, C_{ss} won't increase linearly. For drugs with high clearance/extraction ratio, saturation is associated with constant Cl; increasing of V_d leads to raising of $t_{1/2}$ by reducing the fraction of the total drug in the body that is delivered to the liver per unit of time; C_{ss} can remain linearly proportional to the rate of drug administration.

Saturation of metabolism usually decreases oral first-pass metabolism, C_{ss}, and F. There is a greater fractional increase in C_{ss} than the corresponding fractional increase in the rate of drug administration. In the same time V_d is constant; Cl and rate of drug elimination decrease as the concentration increases.

PHARMACODYNAMICS

Drug receptors

Most drugs act by associating with specific macromolecules in ways that alter the macromolecules' biochemical or biophysical activities. This idea, now almost a century old, is embodied in the terms **receptive substance** and **receptor:** the component of a cell or organism that interacts with a drug and initiates the chain of biochemical events leading to the drug's observed effects. The statement that the receptor for a drug can be any functional macromolecular component of the organism has several fundamental corollaries. One is that a drug potentially is capable of altering the rate at which any bodily function proceeds. Another is that drugs do not create effects, but instead modulate intrinsic physiological functions.

Receptors largely determine the quantitative relations between dose or concentration of drug and pharmacologic effects: The receptor's affinity for binding a drug determines the concentration of drug required to form a significant number of drug-receptor complexes, and the total number of receptors often limits the maximal effect a drug may produce.

Many drugs act on physiological receptors and are often particularly selective, because physiological receptors are specialized to recognize and respond to individual signaling molecules with great selectivity. Drugs that bind to physiological receptors and mimic the regulatory effects of the endogenous signaling compounds are termed **agonists**. Other that bind to receptors without regulatory effect, but their binding blocks the binding of endogenous agonist. Such compounds, which may still produce useful effects by inhibiting the action of an agonist (e.g,, by competition for agonist binding sites and blocking it biologic actions), are termed **antagonists**. Some of the most useful drugs in clinical medicine are pharmacologic antagonists. Agents that are only partly as effective as agonists are termed **partial agonists**, and those that stabilize the receptor in its inactive conformation are termed **inverse agonists.**

The binding of drugs to receptors can involve all known types of interactions – ionic, hydrogen bonding, hydrophobic, van der Waals, and covalent. In most interactions between drugs and receptors, it is likely that bonds of multiple type are important. If binding is covalent, the duration of drug action is frequently, but not necessarily, prolonged. Noncovalent interactions of high affinity also may appear to be essentially irreversible.

Structure-activity relationship. Both the affinity of a drug for its receptor and its intrinsic activity are determined by its chemical structure (molecular size, shape, and electrical charge). This relation is often quite stringent. The molecular chemical structure of a drug determine whether – and with what avidity – it will bind to a particular receptor among the vast array of chemically different binding sites available in a cell, animal, or patient. Relatively minor modification in the drug molecule may result in major changes in pharmacological properties.

Eploitation of structure-activity relationships has on many occasions led to the synthesis of valuable therapeutic agents (structure-based approach). Because changes in molecular configuration need not alter all actions and effects of a drug equally, it is sometimes possible to develop a congener with a more favorable ratio of therapeutic to toxic effects, enhanced selectivity among different cells or tissues, or

more acceptible secondary characteristics than those of the parent drug. Therapeutically useful antagonists of hormones or neurotransmitters have been developed by chemical modification of the structure of the physiological agonist. Minor modifications of structure also can have profound effects on the pharmakinetic properties of drugs.

Macromolecular nature of drug receptors. Most receptors are proteins, presumably because the structures of polypeptides provide both the necessary diversity and the necessary specificity of shape and electrical charge.

The best-characterized drug receptors are **regulatory proteins,** which mediate the actions of endogenous chemical signals such as neurotransmitters, autacoids, and hormones. This class of receptors mediates the effects of many of the most useful therapeutic agents.

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

Signaling mechanisms & drug action

Four basic mechanisms of transmembrane signaling are well understood.

1) Intracellular receptors for lipid-soluble agents. Several biologic signals are sufficiently lipid-soluble to cross the plasma membrane and act on intracellular receptors. One of these is a gas, nitric oxide (NO), that acts by stimulating an intracellular enzyme, guanylyl cyclase, which produces cGMP. Receptors for another class of ligands including **corticosteroids, mineralocorticoids, sex steroids, vitamin D,** and **thyroid hormones** stimulate the transcription of genes in the nucleus by binding to specific DNA sequences near the gene whose expression is to be regulated. Many of the target DNA sequences (called **response elements**) have been identified.

The mechanism used by hormones that act by regulating gene expression has two therapeutically important consequences: (1) All of these hormones produce their effects after a characteristic lag period of 30 minutes to several hours - the time required for the synthesis of new proteins. This means that the gene-active hormones cannot be expected to alter a pathologic state within minutes, eg, glucocorticoids will not immediately relieve the symptoms of acute bronchial asthma. (2) The effects of these agents can persist for hours or days after the agonist concentration has been reduced to zero. The persistence of effect is primarily due to the relatively slow turnover of most enzymes and proteins, which can remain active in cells for hours or days after they have been synthesized. (The persistence may also be partially due to the high affinity of receptors for the hormone, which results in slow dissociation of the hormone.) Therapeutically, it means that there will be no simple temporal correlation between plasma concentration of the hormone and its effects.

2) Ligand-regulated transmembrane enzymes including receptor tyrosine kinases

This class of receptor molecules mediates the first steps in signaling by **insulin, epidermal growth factor (EGF), atrial natriuretic factor (ANF),** and several other trophic hormones. These receptors are polypeptides consisting of an

extracellular hormone-binding domain and a cytoplasmic enzyme domain, which may be a protein tyrosine kinase, a serine kinase, or a guanylyl cyclase. In all these receptors, the two domains are connected by a hydrophobic segment of the polypeptide that crosses the lipid bilayer of the plasma membrane.

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

The intensity and duration of action of EGF, PDGF, and other agents that act via this class of receptors are limited by receptor down-regulation. Ligand binding induces accelerated endocytosis of receptors from the cell surface, followed by the degradation of those receptors (and their bound ligands). When this process occurs at a rate faster than de novo synthesis of receptors, the total number of cell-surface receptors is reduced **(down-regulated)** and the cell's responsiveness to ligand is correspondingly diminished.

3) **Ligand-gated channels**

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

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

The nicotinic Ach receptor is one of the best characterized of all cell-surface receptors for hormones or neurotransmitters. This receptor is a pentamer made up of five polypeptide subunits (eg, two alpha chains plus one beta, one gamma, and one delta chain, all with molecular weights ranging from 43,000 to 50,000). When acetylcholine binds to sites on the alpha subunits, a conformational change occurs that results in the transient opening of a central aqueous channel through which sodium ions penetrate from the extracellular fluid into the cell.

The time elapsed between the binding of the agonist to a ligand-gated channel and the cellular response can often be measured in milliseconds. The rapidity of this signaling mechanism is crucially important for moment-to-moment transfer of information across synapses. It contrasts sharply with other molecular signaling mechanisms, which may require seconds, minutes, or even hours, as is the case with gene-active hormones.

4) **G proteins & second messengers.**

Many extracellular ligands act by increasing the intracellular concentrations of second messengers such as cyclic adenosine-3',5'-monophosphate (cAMP), calcium ion, or the phosphoinositides (described below). In most cases they use a transmembrane signaling system with three separate components. First, the extracellular ligand is specifically detected by a cell-surface receptor. The receptor in turn triggers the activation of a G protein (nucleotide-binding protein) located on the cytoplasmic face of the plasma membrane. The activated G protein then changes the activity of an effector element, usually an enzyme or ion channel. This element then changes the concentration of the intracellular second messenger.

For cAMP, the effector enzyme is adenylyl cyclase, a transmembrane protein that converts intracellular ATP to cAMP. The corresponding G protein, called Gs, stimulates adenylyl cyclase.

Gs and other G proteins use a molecular mechanism that involves binding and hydrolysis of GTP. Significantly, this mechanism separates ligand excitation of the receptor from G protein-mediated activation of the effector, thereby allowing the transduced signal to be amplified (prolonged).

.**Receptor desensitization (down-regulation, refractoriness).** Receptor-mediated responses to drugs and hormonal agonists often "desensitize" with time. After reaching an initial high level, the response (eg, cellular cAMP accumulation, Na+ influx, contractility, etc) gradually diminishes over seconds or minutes, even in the continued presence of the agonist. This desensitization is usually reversible. If the drug is removed for a brief period, the state of desensitization is maintained. Removal of the drug for a more extended period (15 min) allows the cell to “reset” its capacity to respond, and *recovery* of response usually is complete. (Note that this ready reversibility distinguishes desensitization from down-regulation of the *number* of receptors, as described above for receptor tyrosine kinases.)

Well-established second messengers

1. cAMP: Follow ligands act through cAMP pathway: beta-adrenomimetic catecholamines, vasopressin, parathyroid hormone, corticotropin, and follicle-stimulating hormone.

For cAMP, the effector enzyme is adenylyl cyclase, a transmembrane protein that converts intracellular ATP to cAMP. The corresponding G protein, called Gs, stimulates adenylyl cyclase. cAMP exerts most of its effects by stimulating cAMP-dependent protein kinases. These tetrameric kinases are composed of a cAMP-binding regulatory (R) dimer and two catalytic (C) chains. When cAMP binds to the R dimer, active C chains are released which then diffuse through the cytoplasm and nucleus, where they transfer phosphate from ATP to appropriate substrate proteins (phosphorilation of proteins), often enzymes. The specificity of cAMP's regulatory effects resides in the distinct protein substrates of the kinase that are expressed in different cells.

When the hormonal stimulus stops cAMP is degraded to 5'-AMP by several distinct cyclic nucleotide phosphodiesterases (PDE). Competitive inhibition of cAMP degradation is one way caffeine, theophylline, and other methylxanthines produce their effects.

2. Calcium and phosphoinositides: Follow ligands act through this signaling pathway: M-cholinergic agonists, alpha$_1$-adrenergic agnonists, angiotensin). The effector enzyme in this pathway is a membrane enzyme, phospholipase C (PLC), which specifically hydrolyzes a minor phospholipid component of the plasma membrane called phosphatidylinositol-4,5-bisphosphate (PIP_2). PIP_2 is split into two

second messengers, diacylglycerol (DAG) and inositol-1,4,5-trisphosphate (IP_3). The first of these messengers is confined to the membrane, where it activates a protein kinase called protein kinase C. The other messenger, IP_3, is water-soluble and diffuses through the cytoplasm, where it triggers the release of Ca^{2+} from internal storage vesicles. Elevated cytoplasmic Ca^{2+} concentration promotes the binding of Ca^{2+} to the calcium-binding protein calmodulin (CaM), which regulates activities of other enzymes, including calcium-dependent protein kinases.

C. cGMP (cyclic guanosine-3',5'-monophosphate) has established signaling roles in only a few cell types (in intestinal mucosa and vascular smooth muscle). Ligands detected by cell surface receptors stimulate membrane-bound guanylyl cyclase to produce cGMP, and cGMP acts by stimulating a cGMP-dependent protein kinase. Increased cGMP concentration causes relaxation of vascular smooth muscle by a kinase-mediated mechanism that results in dephosphorylation of myosin light chains.

In these smooth muscle cells, cGMP synthesis can be elevated by two different transmembrane signaling mechanisms utilizing two different guanylyl cyclases. Atrial natriuretic factor (ANF), a blood-borne peptide hormone, stimulates a transmembrane receptor by binding to its extracellular (ligand-binding) domain; this binding event triggers activation of the guanylyl cyclase activity that resides in the receptor's intracellular domain. The other mechanism takes advantage of the fact that cell membranes are permeable to the stimulating ligand, nitric oxide (NO, a gas). The nitric oxide is generated in vascular endothelial cells in response to natural vasodilator agents such as acetylcholine and histamine (nitric oxide is also called endothelium-derived relaxing factor [EDRF]). After entering the cell, nitric oxide binds to and activates a cytoplasmic guanylyl cyclase. A number of useful vasodilating drugs act by generating or mimicking nitric oxide. The actions of cGMP are terminated by enzymatic degradation of the cyclic nucleotide and by dephosphorylation of kinase substrates.

A framework for considering agonist activity. A receptor, by definition, exists in at least two conformational states, active (a) and inactive (i)

$$\begin{array}{ccc} R_i & \leftrightarrow & R_a \\ \updownarrow & & \updownarrow \\ D{\times}R_i & \leftrightarrow & D{\times}R_a \end{array}$$

These conformations might correspond to open and closed states of an ion channel, the active and inactive states of a protein kinase, or the productive *versus* nonproductive conformations of a receptor for coupling to G proteins. If these states are in equilibrium and the inactive state predominates in the absence of drug, then the basal signal output will be low. The *extent* to which the equilibrium is shifted toward the active state is determined by the *relative* affinity of the drug for the two conformation. A drug that has a higher affinity for the active conformation than for the inactive conformation will drive the equilibrium to the active state and thereby activate receptors. Such drug will be an *agonist*. A *full agonist* is sufficiently selective for the active conformation that, a saturating concentration, it will drive the receptors essentially completely to the active state. If a different but perhaps structurally compounds binds to the same site on R but with only moderately greater affinity for R_a than for R_i, its effect will be less, even at saturating concentration. A drug that display such intermediate effetiveness is referred to as a *partial agonist*. Note that in absolute sense, all agonists are partial; selectivity for R_a over R_i cannot be total. A drug that binds with equall affinity to either confotmation will not alter the activation equilibrium and will act as a *competitive antagonist*. Last, a drug with preferential affinity to R_i will actually produce an effect opposite to that of an agonist; examples of such *inverse agonists* do exist (beta-carbolines)

$$R_i \overset{A}{\leftrightarrow} AR_a$$ Full and partial agonists

.

$$AR_i \overset{A}{\leftrightarrow} AR_a$$ Antagonist

$$AR_i \overset{A}{\leftrightarrow} R_a$$ Inverse agonist

Classification of receptors. Traditioanlly, drug receptors have been identified and classified primarily on the basis of the effect and relative potency of selective agonists and antagonists. E.g., the effects of acetylcholine that are mimicked by the alkaloid muscarine and that are selectively antagonized by atropine are termed muscarinic effects. Other effects of acetylcholine that are mimicked by nicotine are described as nicotinic effects. By extension, these two types of cholinergic effects are said to be mediated by muscarinic or nicotinic receptors. As the diversity and selectivity of drug increased, it became clear that multiple subtypes of drugs exist within many previously defined classses of receptors. If selective ligands are not known, the receptors are more commonly referred to as isoforms rather than as subtypes.

N.B. If one restricts the definition of receptors to macromolecules, then several drugs may be said not to act upon receptors as such (antacids, mesna, mannitol, antiherpes and antimetabolites).

RELATION BETWEEN DRUG CONCENTRATION AND RESPONSE

A. Graded dose-response relations. Responses to low doses of a drug usually increase in direct proportion to dose. As doses increase, however, the response increment diminishes; finally, doses may be reached at which no further increase in response can be achieved. In idealized or in vitro systems, the relation between drug concentration and effect is described by a hyperbolic curve **(grade response curve)** according to the following equation:

$$E = E_{max} \times C / (C + EC_{50})$$

where E is the effect observed at concentration C, E_{max} is the maximal response that can be produced by the drug, and EC_{50} is the concentration of drug that produces 50% of maximal effect. Graded responses (y axis as a % of maximal response) expressed as a function of the concentration of drug A present at the receptors.

This hyperbolic relation resembles the mass action law, which predicts association between two molecules of a given affinity. This resemblance suggests that drug agonists act by binding to ("occupying") a distinct class of biologic molecules with a characteristic affinity for the drug receptor. This occupancy assumption has been amply confirmed for a number of drug-receptor systems. In these systems, the relation between drug bound to receptors (B) and the concentration of free (unbound) drug (C) is described by an analogous equation:

$$B = B_{max} \times C / (C + K_d)$$

in which B_{max} indicates the total concentration of receptor sites (ie, sites bound to the drug at infinitely high concentrations of free drug). K_d (the equilibrium dissociation

constant) represents the concentration of free drug at which half-maximal binding is observed. This constant characterizes the receptor's affinity for binding the drug in a reciprocal fashion: If the K_d is low, binding affinity is high, and vice versa. Note also that the EC50 and K_d may be identical but need not be, as discussed below.

Graphic representation of dose-response data is frequently improved by plotting the drug effect (ordinate) against the *logarithm* of the dose or concentration (abscissa). The effect of this mathematical maneuver is to transform the hyperbolic curve into a sigmoid curve with a linear midportion. This transformation makes it easier to compare dose-response curves graphically because it expands the scale of the concentration axis at low concentrations (where the effect is changing rapidly) and compresses it at high concentrations (where the effect is changing slowly). This transformation has no special biologic or pharmacologic significance.

Classical receptor theory. Receptor occupancy theory, in which it is assumed that response emanates from a receptor occupied by a drug, has its basis in the law of mass action, with modifying constants added to accommodate experimental findings (see below). *Agonism* is described by modification of this model; *stimulus* is the initial effect of drug upon the receptor itself. Stimulus is then processed by the system to yield the observable response. The basic components of drug receptor-mediated response are shown:

$$A + R \underset{}{\overset{K_a}{\leftrightarrow}} AR \rightarrow \text{Stimulus} \rightarrow \text{Response}$$

Affinity is measured by the equilibrium dissociation constant of the drug-receptor complex; the fraction of receptors occupied by the drug is determined by the drug is determined by the concentration of the drug and K_d, as shown below. Intrinsic efficacy is a proportionality constant (denoted ε) that defines the power of the drug to induce response. The product of occupancy, intrinsic efficacy, and receptor number yields the total receptor-mediated stimulus given to the system.

$$\text{Stimulus} = \text{Binding}\ ([A] / ([A] + K_d)) + \varepsilon\ (\text{efficacy}) \times [R]\ \text{receptor number},$$

Stimulus is conveyed to physiological effectors by biochemical reactions to produce the response. Efficacy is a function of occupancy and the stimulus-response function (comprising all of the biochemical reactions that take place to translate agonist binding into response) and amplifies stimulus. Therefore, the location of the dose-response curves for response is shifted to the left of the receptor occupancy curve.

The activation of a receptor by a drug can be thought of as an initial signal that is then amplified by the cell. Different cells have different **amplification properties**; thus a weak receptor signal may produce no visible response in one cell type and a powerful signal in another. The amplification properties of the cell (referred to as the **stimulus-response capability**) control the observed outcome of drug-receptor interaction. One drug may act as full agonist in one cell (highly efficient coupling), partial agonist in second cell (moderate coupling), or antagonist (inefficient coupling).

To choose among drugs and to determine appropriate doses of a drug, the prescriber must know the relative **pharmacologic potency** and **maximal efficacy** of the drugs in relation to the desired therapeutic effect.

1. Potency-Drug A is said to be more potent than drug B because of the relative positions of their dose-response curves along the dose axis. Potency refers to the concentration (EC_{50}) or dose (ED_{50}) of a drug required to produce 50% of that

drug's maximal effect. Thus, the pharmacologic potency of drug B is less than that of drug A, because the EC_{50} of B is greater than the EC_{50} of A. Note that some doses of drug B can produce larger effects than any dose of drug A, despite the fact that we term drug A pharmacologically more potent. The reason for this is that drug B has a larger **maximal efficacy,** as described below.

Potency of a drug depends in part on the affinity (K_d) of receptors for binding the drug and in part on the efficiency with which drug-receptor interaction is coupled to response. As described above, both affinity and coupling efficiency contribute to the EC_{50} of a particular concentration-response relation in vitro.

For clinical use, it is helpful to distinguish between a drug's **potency** and its **efficacy.** The clinical effectiveness of a drug depends not on its potency (EC_{50}), but on its maximal efficacy (see below) and its ability to reach the relevant receptors.

This ability can depend on its route of administration, absorption, distribution through the body, and clearance from the blood or site of action. In deciding which of two drugs to administer to a patient, the prescriber must usually consider their relative effectiveness rather than their relative potency. However, pharmacologic potency can largely determine the administered dose of the chosen drug. In general, low potency is important only if the drug has to be administered in inconveniently large amounts. Relative potency, the ratio of equieffective doses (0.2, 10, etc), may be used in comparing one drug with another.

2. Maximal efficacy-This parameter reflects the limit of the dose-response relation on the **response axis.** Drug B has maximal efficacy greater than does drug B. The maximal efficacy (sometimes referred to simply as efficacy) of a drug is obviously crucial for making clinical decisions when a large response is needed. It may be determined by the drug's mode of interactions with receptors (as with partial agonists) or by characteristics of the receptor-effector system involved (see intrinsic efficicy above).

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

*Note that therapeutic efficacy (unlike scientific efficacy) may be affected by the characteristics of a particular drug-receptor interaction, but it also depends upon a host of other factors as noted in the text.

B. Quantal dose-effect curves.

Despite their usefulness for characterizing the actions of drugs, graded dose-response curves may be impossible to construct if the pharmacologic response is an either-or (quantal) event, such as prevention of convulsions, arrhythmia, or death. Furthermore, the clinical relevance of a quantitative dose-response relationship in a single patient may be limited in application to other patients, owing to the great potential variability among patients in severity of disease and responsiveness to drugs.

Some of difficulties of graded dose-response curves may be avoided by determining the dose of drug required to produce a specified magnitude of effect in a large number of individual patients or experimental animals and plotting the cumulative frequency distribution of responders versus the log dose. The specified quantal effect may be chosen on the basis of clinical relevance (eg, relief of headache) or for preservation of safety of experimental subjects (eg, using low doses

of a cardiac stimulant and specifying an increase in heart rate of 20 beats/min as the quantal effect), or it may be an inherently quantal event (eg, death of an experimental animal). For most drugs, the doses required to produce a specified quantal effect in individuals are lognormally distributed; ie, a frequency distribution of such responses plotted against the log of the dose produces a gaussian normal curve of variation. When these responses are summated, the resulting cumulative frequency distribution constitutes a quantal dose-effect curve (or dose-percent curve) of the proportion or percentage of individuals who exhibit the effect plotted as a function of log dose.

The quantal dose-effect curve is often characterized by stating the **median effective dose (ED_{50})**, the dose at which 50% of individuals exhibit the specified quantal effect. (Note that the abbreviation ED_{50} has a different meaning in this context from its meaning in relation to graded dose-effect curves, described above.) Similarly, the dose required to produce a particular toxic effect in 50% of animals is called the **median toxic dose (TD_{50})**. If the toxic effect is death of the animal, a **median lethal dose (LD50)** may be experimentally defined.

Such values provide a convenient way of comparing the potencies of drugs in experimental and clinical settings. Thus, if the ED_{50}s of two drugs for producing a specified quantal effect are 5 and 500 mg, respectively, then the first drug can be said to be 100 times more potent than the second for that particular effect. Similarly, one can obtain a valuable index of the selectivity of a drug's action by comparing its ED_{50}s for two different quantal effects in a population (eg, cough suppression versus sedation for opiate drugs; increase in heart rate versus increased vasoconstriction for sympathomimetic amines; anti-inflammatory effects versus sodium retention for corticosteroids; etc).

Quantal dose-effect curves may also be used to generate information regarding the margin of safety to be expected from a particular drug used to produce a specified effect. One measure, which relates the dose of a drug required to produce a desired effect to that which produces an undesired effect, is the **therapeutic index.** In animal studies, the therapeutic index is usually defined as the ratio of the TD_{50} to the ED_{50} for some therapeutically relevant effect. The precision possible in animal experiments may make it useful to use such a therapeutic index to estimate the potential benefit of a drug in humans.

Of course, the therapeutic index of a drug in humans is almost never known with real precision; instead, drug trials and accumulated clinical experience often reveal a range of usually effective doses and a different (but sometimes overlapping) range of possibly toxic doses. The clinically acceptable risk of toxicity depends critically on the severity of the disease being treated. For example, the dose range that provides relief from an ordinary headache in the great majority of patients should be very much lower than the dose range that produces serious toxicity, even if the toxicity occurs in a small minority of patients. However, for treatment of a lethal disease such as Hodgkin's lymphoma, the acceptable difference between therapeutic and toxic doses may be smaller.

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

Variation in drug responsiveness among individuals

1) idiosyncratic drug response, one that is infrequently observed in most patients. The idiosyncratic responses are usually caused by genetic differences in metabolism of the drug or by immunologic mechanisms, including allergic reactions;

2) hyporeactive or **hyperreactive** to a drug in that the intensity of effect of a given dose of drug is diminished or increased in comparison to the effect seen in most individuals. (***Note:*** The term **hypersensitivity** usually refers to allergic or other immunologic responses to drugs.);

3) **tolerance** means that the intensity of response to a given dose may decreases during the course of therapy;

4) **tachyphylaxis** is a rapid diminish of responsiveness after administration of a drug**.**

The general clinical implications of individual variability in drug responsiveness are clear: The prescriber must be prepared to change either the dose of drug or the choice of drug, depending upon the response observed in the patient. Even before administering the first dose of a drug, the prescriber should consider factors that may help in predicting the direction and extent of possible variations in responsiveness. These include the propensity of a particular drug to produce tolerance or tachyphylaxis as well as the effects of age, sex, body size, disease state, and simultaneous administration of other drugs.

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

A. Alteration in concentration of drug that reaches the receptor. Patients may differ in the rate of absorption of a drug, in distributing it through body compartments, or in clearing the drug from the blood.

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

B. Variation in concentration of an endogenous receptor ligand. This mechanism contributes greatly to variability in responses to pharmacologic antagonists. Thus, propranolol, a β-adrenoceptor antagonist, will markedly slow the heart rate of a patient with hyperadrenalinemia (as in pheochromocytoma) but will not affect the resting heart rate of a well-trained marathon runner (low level of adrenaline). Saralasin, a weak partial agonist at angiotensin II receptors, lowers blood pressure in patients with hypertension caused by increased angiotensin II production and raises blood pressure in patients who produce low amounts of angiotensin.

C. Alterations in number or function of receptors. In some cases, the change in receptor number is caused by other hormones; for example, thyroid hormones increase both the number of beta receptors in rat heart muscle and cardiac sensitivity to catecholamines.

In other cases, the agonist ligand itself induces a decrease in the number ("down-regulation") or coupling efficiency of its receptors. These mechanisms may contribute to tachyphylaxis or tolerance to the effects of some drugs.

Second, is the "overshoot" phenomena that follow withdrawal of certain drugs. For example, the withdrawal of clonidine (a drug whose a_2-adrenoceptor agonist activity reduces blood pressure) can produce hypertensive crisis, probably because the drug down-regulates a_2 adrenoceptors.

An antagonist may increase the number of receptors in a critical cell or tissue. When the antagonist is withdrawn, the elevated number of receptors can produce an exaggerated response to physiologic concentrations of agonist "overshoot" phenomena.

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

D. Changes in components of response distal to receptor. The response observed in a patient depends not only on binding to receptors, but also on the functional integrity of biochemical processes in the responding cell and physiologic regulation by interacting organ systems. Compensatory increases in sympathetic nervous tone and fluid retention by the kidney, for example, can contribute to tolerance to antihypertensive effects of a vasodilator drug. In such cases, additional drugs may be required to achieve a useful therapeutic response.

Clinical selectivity: beneficial versus toxic effects of drugs.

In drug development and in clinical medicine, selectivity is usually considered by separating effects into two categories: **beneficial** or **therapeutic effects** versus **toxic or side effects.**

A. Beneficial and toxic effects mediated by the same receptor-effector mechanism. Much of the serious drug toxicity in clinical practice represents a **direct pharmacologic extension** of the therapeutic actions of the drug. In some of these cases (bleeding caused by anticoagulant therapy; hypoglycemic coma due to insulin), toxicity may be avoided by judicious management of the dose of drug administered, guided by careful monitoring of effect (measurements of blood coagulation or serum glucose) and aided by ancillary measures (avoiding tissue trauma that may lead to hemorrhage; regulation of carbohydrate intake).

In still other cases, the toxicity may be avoided by not administering the drug at all, if the therapeutic indication is weak or if other therapy is available (eg, sedative-hypnotics ordinarily should not be used to treat patients whose complaints of insomnia are due to underlying psychiatric depression).

In certain situations, a drug is clearly necessary and beneficial but produces unacceptable toxicity when given in doses that produce optimal benefit. In such situations, it may be necessary to add another drug to the treatment regimen. For example, prazosin cam cause postural hypotension. Concomitant administration of diuretics and vasodilators may allow the dose of prazosin to be lowered, with relief of postural hypotension and continued control of blood pressure.

B. Beneficial and toxic effects mediated by identical receptors but in different tissues or by different effector pathways. Examples of drugs in this category include digitalis glycosides, which may be used to augment cardiac contractility but also produce cardiac arrhythmias, gastrointestinal effects, and changes in vision (all probably mediated by inhibition of Na^+/K^+ ATPase in cell

membranes); methotrexate, used to treat leukemia and other neoplastic diseases, which also kills normal cells in bone marrow and gastrointestinal mucosa (all mediated by inhibition of the enzyme dihydrofolate reductase).

Three therapeutic strategies are used to avoid or mitigate this sort of toxicity. First, the drug should always be administered at the lowest dose that produces acceptable benefit. Second (as described above for prazosin), adjunctive drugs that act through different receptor mechanisms and produce different toxicities may allow lowering the dose of the first drug. Third, selectivity of the drug's actions may be increased by manipulating the concentrations of drug available to receptors in different parts of the body (selective arterial infusion of an antimetabolite into an organ containing tumor cells).

C. Beneficial and toxic effects mediated by different types of receptors.

The propensity of drugs to bind to different classes of receptor sites is not only a potentially vexing problem in treating patients, it also presents a continuing challenge to pharmacology and an opportunity for developing new and more useful drugs. A number of drugs were discovered by exploiting toxic effects of other agents, observed in a different clinical context. Examples include quinidine, the sulfonylureas, thiazide diuretics, tricyclic antidepressants, monoamine oxidase inhibitors, and phenothiazine antipsychotics among many others.

Competitive irreversible antagonists.

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

In the presence of a fixed concentration of agonist, increasing concentrations of a **competitive antagonist** progressively inhibit the agonist response; high antagonist concentrations prevent response completely. Conversely, sufficiently high concentrations of agonist can completely surmount the effect of a given concentration of the antagonist; ie, the E_{max} for the agonist remains the same for any fixed concentration of antagonist.. So the agonist concentration-effect curve shifts to the right.

Some receptor antagonists bind to the receptor in an **irreversible** or nearly irreversible fashion, ie, not competitive. The antagonist's affinity for the receptor may be so high that for practical purposes, the receptor is unavailable for binding of agonist. Other antagonists in this class produce irreversible effects because after binding to the receptor they form covalent bonds with it. After occupancy of some proportion of receptors by such an antagonist, the number of remaining unoccupied receptors may be too low to permit the previous maximal agonist response to be obtained. However, if spare receptors are present, a lower dose of an irreversible antagonist may leave enough receptors unoccupied to allow achievement of maximum response to agonist, though a higher agonist concentration will be required.

Therapeutically, irreversible antagonists present distinctive advantages and disadvantages. Tthe duration of action of such an irreversible antagonist is relatively independent of its own rate of elimination and more dependent upon the rate of turnover of receptor molecules.

Partial agonists

Based on the maximal pharmacologic response that occurs when all receptors are occupied, agonists can be divided into two classes: **Partial agonists** produce a lower response, at full receptor occupancy, than do **full agonists.** As compared with full agonists, partial agonists produce concentration-effect curves that resemble those observed with full agonists in the presence of an antagonist that irreversibly blocks receptor sites. Nonetheless, radioligand-binding experiments have demonstrated that partial agonists may occupy all receptor sites at concentrations that will fail to produce a maximal response comparable to that seen with full agonists. In addition, the failure of partial agonists to produce a "full" maximal response is not due to decreased affinity for binding to receptors. Such drugs compete, frequently with high affinity, for the full complement of receptors. Indeed, the partial agonists' ability to occupy the total receptor population is indicated by the fact that they competitively inhibit the responses produced by full agonists.

Other mechanisms of drug antagonism

Chemical antagonists. One drug may antagonize the actions of a second drug by binding to and inactivating the second drug. For example, protamine, a protein that is positively charged at physiologic pH, can be used clinically to counteract the effects of heparin, an anticoagulant that is negatively charged.

Physiologic antagonism. For example, several catabolic actions of the glucocorticoid hormones lead to increased blood sugar, an effect that is physiologically opposed by insulin. Although glucocorticoids and insulin act on quite distinct receptoreffector systems, the clinician must sometimes administer insulin to oppose the hyperglycemic effects of glucocorticoid hormone.

In general, use of a drug as a physiologic antagonist produces effects that are less specific and less easy to control than are the effects of a receptorspecific antagonist. Thus, for example, to treat bradycardia caused by increased release of acetylcholine from vagus nerve endings, which may be caused by the pain of myocardial infarction, the physician could use isoproterenol, a beta-adrenoceptor agonist that increases heart rate by mimicking sympathetic stimulation of the heart. However, use of this physiologic antagonist would be less rational and potentially more dangerous than would use of a receptorspecific antagonist such as atropine (a competitive antagonist at the receptors at which acetylcholine slows heart rate).

ADDITION

Most transmembrane signaling is accomplished by only a few different molecular mechanisms. Each type of mechanism has been adapted, through the evolution of distinctive protein families, to transduce many different signals. These protein families include receptors on the cell surface and within the cell, as well as enzymes and other components that generate, amplify, coordinate, and terminate postreceptor signaling by chemical second messengers in the cytoplasm.

The receptor concept, extended to endocrinology, immunology, and molecular biology, has proved essential for explaining many aspects of biologic regulation. Drug receptors are now being isolated and characterized as macromolecules, thus opening the way to precise understanding of the molecular basis of drug action.

In addition to its usefulness for explaining biology, the receptor concept has important practical consequences for the development of drugs and for arriving at therapeutic decisions in clinical practice. These consequences form the basis for understanding the actions and clinical uses of drugs.

How are new receptors discovered?

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

These "classic" steps in receptor identification serve as a warming-up exercise for a powerful new experimental strategy aimed at molecular cloning of the segment of DNA that encodes the receptor (stages 2-5). The core of this strategy is the ability to identify a putative receptor DNA sequence in a representative population of cDNAs (DNA sequences complementary to their RNAs expressed in an appropriate cell or tissue, obtained by using reverse transcriptase). To do so (stage 2), investigators use biochemical and functional features of the receptor protein as handles for picking out the corresponding DNA. Thus, an antibody raised against the pure receptor protein or nucleic acid sequences based on the receptor's amino acid sequence may distinguish a bacterial colony containing putative receptor cDNA from colonies containing irrelevant DNA, by binding to receptor antigen expressed in the bacterium (2A), or by hybridizing to receptor DNA (2B), respectively. Alternatively, the population of cDNAs may be expressed as proteins in frog oocytes or vertebrate cells, and the putative receptor cDNA can then be detected by virtue of the protein's signaling function (2C) or its ability to bind a specific ligand (2D). Once the putative receptor cDNA has been identified, it is "validated" by carefully comparing the function and biochemical properties of the recombinant protein with those of the endogenous receptor that originally triggered the search (3A). The base sequence of the receptor DNA is also determined (3B), so that the amino acid sequence of the complete receptor protein can be deduced and compared with sequences of known receptors. Based on these criteria, it may then be possible to announce the identification of a new receptor (step 4).

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

receptor protein for precise biochemical analysis, tests of agonist and antagonist binding, and development of new drugs.

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

Tyrosine kinase receptors provide attractive targets for drug development. At present, a few compounds have been found to produce effects that may be due to inhibition of tyrosine kinase activities. It is easy to imagine therapeutic uses for specific inhibitors of growth factor receptors, especially in neoplastic disorders.

ANF, an important regulator of blood volume and vascular tone, acts on a transmembrane receptor whose intracellular domain, a guanylyl cyclase, generates cGMP. Receptors in both groups, like the protein tyrosine kinases, are active in their dimeric forms.

Cytokine receptors respond to a heterogeneous group of peptide ligands that includes growth hormone, erythropoietin, several kinds of interferon, and other regulators of growth and differentiation. These receptors use a recently discovered mechanism that closely resembles that of receptor tyrosine kinases, but with a difference. In this case, the protein tyrosine kinase activity is not intrinsic to the receptor molecule; instead, a separate protein tyrosine kinase, from the Janus-kinase (JAK) family, binds noncovalently to the receptor. As in the case of the EGF-receptor, cytokine receptors dimerize after they bind the activating ligand, allowing the bound JAKs to become activated and to phosphorylate tyrosine residues on the receptor. Tyrosine phosphates on the receptor then set in motion a complex signaling dance by binding another set of proteins, called STATs (signal transducers and activators of transcription). Then the bound STATs are themselves phosphorylated by the JAKs, and two STAT molecules dimerize (attaching to one another's tyrosine phosphates). Finally, the STAT/STAT dimer dissociates from the receptor and travels to the nucleus, where it regulates transcription of specific genes. This multistep signaling process furnishes several attractive targets for potential drugs presently undergoing active investigation.

The family of G proteins is quite diverse; in addition to Gs, the stimulator of adenylyl cyclase, it includes other subfamilies. Members of the Gi ("i" for inhibitory) subfamily couple receptors to inhibition of adenylyl cyclase; Gi proteins also mediate receptor stimulation of the phosphoinositide second messenger system in some cells (see below) and regulation of K^+ and Ca^{2+} channels.

Receptors coupled to G proteins are structurally related to one another, comprising a family of "serpentine receptors," so called because the receptor polypeptide chain crosses the plasma membrane seven times.

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

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

As in the cAMP system, multiple mechanisms exist to damp or terminate signaling by this pathway. IP_3 is rapidly inactivated by dephosphorylation; diacylglycerol is either phosphorylated to yield phosphatidic acid, which is then converted back into phospholipids, or it is deacylated to yield arachidonic acid; Ca^{2+} is actively removed from the cytoplasm by Ca^{2+} pumps.

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

Receptor-effector coupling spare receptors.

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

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

In other cases, where the biochemical mechanism is not understood, we imagine that the receptors are *spare in number.* If the concentration or amount of a cellular component other than the receptor limits the coupling of receptor occupancy to response, then a maximal response can occur without occupancy of all receptors (different number of receptors and effectors).

An important biologic consequence of spare receptors is that they allow agonists with low affinity for receptors to produce full responses at low concentrations, to the extent that EC_{50} is lower than K_d.

The concentration (C') of an agonist required to produce a given effect in the presence of a fixed concentration ([I]) of competitive antagonist is greater than the agonist concentration (C) required to produce the same effect in the absence of the antagonist. The ratio of these two agonist concentrations (the "dose ratio") is related to the dissociation constant (K_I) of the antagonist by the Schild equation:

$$C' / C = 1 + [I]/K_I$$

Pharmacologists often use this relation to determine the K_I of a competitive antagonist. Even without knowledge of the relationship between agonist occupancy of the receptor and response, the K_I can be determined simply and accurately.

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

(1) Thus, the extent and duration of action of a competitive antagonist will depend upon its concentration in plasma and will be critically influenced by the rate of its metabolic clearance or excretion. Different patients receiving a fixed dose of propranolol, for example, exhibit a wide range of plasma concentrations, owing to differences in clearance of the drug.

(2) The equation defines another important source of variability in clinical response to a competitive antagonist, ie, the concentration of agonist that is competing for binding to receptors. Here also provides a useful example: When propranolol is administered in doses sufficient to block the effect

of basal levels of the neurotransmitter norepinephrine, resting heart rate is decreased. However, the increase in release of norepinephrine and epinephrine that occurs with exercise, postural changes, or emotional stress may suffice to overcome competitive antagonism by propranolol and increase heart rate.

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

Partial agonists are easier to understand, however, by envisioning the receptor as capable of taking on either of two shapes: R, the inactive form, and R_a , the active form, *in the absence as well as the presence of bound ligand (L).* This implies that the receptor oscillates in an equilibrium between the two conformations (although the concentration of R_a is low) even in the absence of any ligand whatever..

Now imagine that a receptor ligand L is added to the system. An equilibrium will now exist between four states of the receptor – free (unbound) R and R_a, as well as LR and LR_a. If L is a full agonist, it will bind to the active form, R_a, very much more tightly than to R, for which its affinity is negligible. This will result in a shift of the equilibrium, strongly favoring formation of LR_a. Conversely, a pure antagonist will bind well to R, but with negligible affinity to R_a, resulting in formation of a preponderance of the inactive LR form. In this context it is easy to imagine a partial agonist as a ligand that can bind reasonably well to both R and R_a. Thus, at any concentration of such a ligand some of the receptor will be in the LR_a form – and therefore capable of promoting (to some degree) the pharmacologic effect characteristic of the active receptor. As the concentration of partial agonist rises to the point that all receptors are occupied, however, some receptors will be in the LR form, so that the effect will be less than the maximum that might be achieved if all receptors were in the LR_a form. Thus, the relative efficacy of a partial agonist depends upon its relative affinities for R and R_a.

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3. Pharmacokinetics Pharmacodynamics: Dose Selection the Time Course of Drug Action

INTRODUCTION

The goal of therapeutics is to achieve a desired beneficial effect with minimal adverse effects. When a medicine has been selected for a patient, the clinician must determine the dose that most closely achieves this goal. A rational approach to this objective combines the principles of pharmacokinetics with pharmacodynamics to clarify the dose-effect relationship (Figure 3-1). Pharmacodynamics governs the concentration-effect part of the interaction, whereas pharmacokinetics deals with the dose-concentration part (Holford et al, 1981). The pharmacokinetic processes of absorption, distribution, and elimination determine how rapidly and in what concentration and for how long the drug will appear at the target organ. The pharmacodynamic concepts of maximum response and sensitivity determine the magnitude of the effect at a particular concentration (see E_{max} and EC50, Chapter 2).

Figure 3-1 illustrates a fundamental hypothesis of pharmacology, namely, that a relationship exists between a beneficial or toxic effect of a drug and the concentration of the drug in a readily accessible site of the body (eg, blood). This hypothesis has been documented for many drugs, as indicated by the Effective Concentrations and Toxic Concentrations columns in Table 3-1. The apparent lack of such a relationship for some drugs does not weaken the basic hypothesis but points to the need to consider the time course of concentration at the actual site of pharmacologic effect (see below).

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

Drug Accumulation

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

Accumulation is inversely proportionate to the fraction of the dose lost in each dosing interval. The fraction lost is 1 minus the fraction remaining just before the

next dose. The fraction remaining can be predicted from the dosing interval and the half-life. A convenient index of accumulation is the **accumulation factor.**

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

THE TIME COURSE OF DRUG EFFECT

Introduction

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

Immediate Effects

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

Consider the effect of an angiotensin-converting enzyme inhibitor, such as enalapril, on plasma angiotensin-converting enzyme (ACE). The half-life of enalapril is about 3 hours. After an oral dose of 10 mg, the peak plasma concentration at 3 hours is about 64 ng/mL. Enalapril is usually given once a day, so seven half-lives will elapse from the time of peak concentration to the end of the dosing interval. The concentration of enalapril after each half-life and the corresponding extent of ACE inhibition are shown in Figure 3-6. The extent of inhibition of ACE is calculated using the E_{max} model, where E_{max}, the maximum extent of inhibition, is 100% and the EC50 is about 1 ng/mL.

Note that plasma concentrations of enalapril change by a factor of 16 over the first 12 hours (four half-lives) after the peak, but ACE inhibition has only decreased by 20%. Because the concentrations over this time are so high in relation to the EC50, the effect on ACE is almost constant. After 24 hours, ACE is still 33% inhibited. This explains why a drug with a short half-life can be given once a day and still maintain its effect throughout the day. The key factor is a high initial concentration in relation

to the EC50. Even though the plasma concentration at 24 hours is less than 1% of its peak, this low concentration is still half the EC50. This is very common for drugs that act on enzymes, eg, ACE inhibitors, or compete at receptors, eg, propranolol.

When concentrations are in the range between one-fourth and four times the EC50, the time course of effect is essentially a linear function of time□13% of the effect is lost every half-life over this concentration range. At concentrations below one-fourth the EC50, the effect becomes almost directly proportionate to concentration and the time course of drug effect will follow the exponential decline of concentration. It is only when the concentration is low in relation to the EC50 that the concept of a "half-life of drug effect" has any meaning.

Delayed Effects

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

A common reason for more delayed drug effects□especially those that take many hours or even days to occur□is the slow turnover of a physiologic substance that is involved in the expression of the drug effect. For example, warfarin works as an anticoagulant by inhibiting vitamin K epoxidase in the liver. This action of warfarin occurs rapidly, and inhibition of the enzyme is probably closely related to plasma concentrations of warfarin. The clinical effect of warfarin, eg, on the prothrombin time, reflects a decrease in the concentration of the prothrombin complex of clotting factors (Figure 34-7). Inhibition of vitamin K epoxidase decreases the synthesis of these clotting factors, but the complex has a long half-life (about 14 hours), and it is this half-life that determines how long it takes for the concentration of clotting factors to reach a new steady state and for a drug effect to become manifest reflecting the warfarin plasma concentration.

Cumulative Effects

Some drug effects are more obviously related to a cumulative action than to a rapidly reversible one. The renal toxicity of aminoglycoside antibiotics (eg, gentamicin) is greater when administered as a constant infusion as compared with intermittent dosing. It is the accumulation of aminoglycoside in the renal cortex that is thought to cause renal damage. Even though both dosing schemes produce the same average steady state concentration, the intermittent dosing scheme produces much higher peak concentrations, which saturate an uptake mechanism into the cortex; thus, total aminoglycoside accumulation is less. The difference in toxicity is a predictable

consequence of the different patterns of concentration and the saturable uptake mechanism.

The effect of many drugs used to treat cancer also reflects a cumulative action, eg, the extent of binding of a drug to DNA is proportionate to drug concentration and is usually irreversible. The effect on tumor growth is therefore a consequence of cumulative exposure to the drug. Measures of cumulative exposure, such as area under the concentration time curve of the drug (AUC), have shown promise as predictors of response and as a target AUC provide a means to individualize treatment.