Kirk-Othmer Encyclopedia of Chemical Technology.

PHARMACODYNAMICS

Pharmacology relates to all aspects of drug action, including drug synthesis or isolation from natural products, elucidation of physiologic and toxicologic effects, determination of therapeutic applications, and mechanisms of action (1, 2). Drugs, chemical species that affect cellular function with specificity, include hormones (qv), neurotransmitters (see Neuroregulators; Opioids, endogenous), and chemotherapeutic agents (see Antiasthmatic agents; Antibiotics; Antiparasitic agents; Chemotherapeutics, anticancer). The principles of chemical specificity are common to all of these classes of agents.

Pharmacodynamics is the study of drug action primarily in terms of drug structure, site of action, and the biochemical and physiological consequences of the drug action. The availability of a drug at its site of action is determined by several processes (Fig. 1), including absorption, metabolism, distribution, and excretion. These processes constitute the pharmacokinetic aspects of drug action. The onset, intensity, and duration of drug action are determined by these factors as well as by the availability of the drug at its receptor site(s) and the events initiated by receptor activation (see Drug delivery).

Both pharmacokinetic and pharmacodynamic processes are involved in mediating nonconstant expressions of drug action. Thus, resistance to the actions of a drug, eg, in the development of antibiotic-resistant bacteria or of barbiturate tolerance, can arise from changes in drug metabolism and/or alterations in the receptor target site. Factors controlling drug resistance may be whole-body, cellular, or individual events. Decreased absorption, increased metabolism, or increased elimination reduce circulating drug levels and affect the whole body. Increased drug metabolism, increased concentration of an agent that antagonizes drug action, decreased affinity or concentration of a drug receptor, and depletion of an agent that mediates drug action are examples of cellular events; and genetic factors controlling metabolism, receptor alterations, and disease states are examples of individual events (1). Individual variation in the susceptibility to a particular drug or class of drugs also may arise from genetically based pharmacokinetic factors as well as from specific receptor-linked changes.

For a large number of drugs, including neurotransmitters, peptide and protein hormones (qv), and their analogues and antagonists, the cell membrane is the principal locus of action. Concepts of cell membrane structure are derived from the original Davson-Danielli lipid bilayer hypothesis. More specifically, the membrane is viewed as a dynamic fluid mosaic or a matrix of fluid bilayer in which there are asymmetrically inserted proteins (qv) and glycoproteins. Phospholipids and proteins diffuse laterally and the resultant protein–protein communication is of considerable importance to the understanding of membrane-receptor function (3). Despite the dynamic nature of the membrane and the absence of global organization, local organization is possible through the local assembly of individual protein components and the attachment of membrane proteins to the subcellular structure of contractile proteins. However, the cell membrane is not the site of action of all drugs. A number of drugs, including steroid and thyroid hormones, exert their effects intracellularly at the level of the genetic material as well as at the plasma membrane (see Steroids; Thyroid and antithyroid preparations). Other agents, including polypeptide growth factors, exert their effects not only at the plasma membrane through tyrosine kinase receptors, but also on cell growth and differentiation at the genetic level.

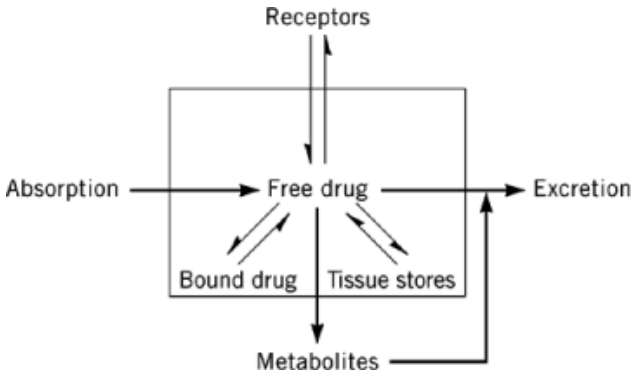


Fig. 1. Schematic representation of drug disposition following administration.

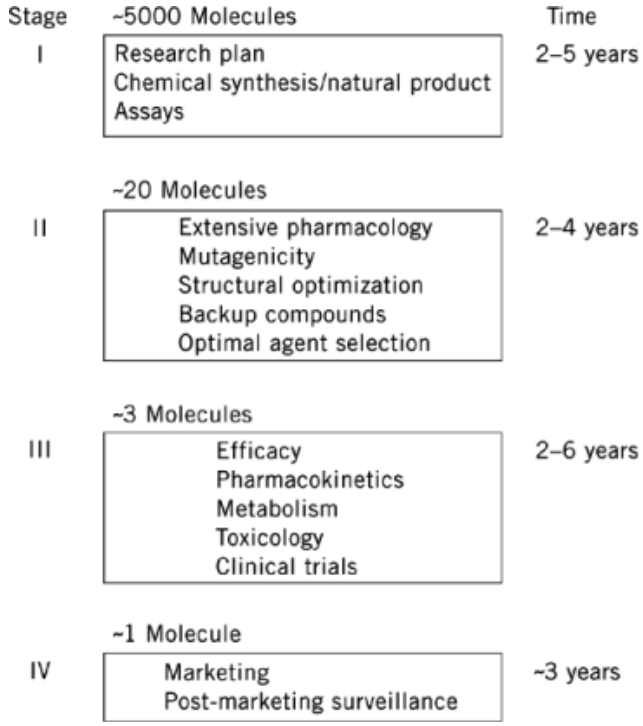


Fig. 2. Pathway for drug development.

1. Drug Discovery and Regulation

The Federal Food, Drug and Cosmetic (FDAC) Act defines drugs as "...articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in man..." and "articles (other than food) intended to affect the structure of any function of the body of man." In the United States and elsewhere, the introduction of a new drug is subject to a sequence of well-defined stages of development and approval (4). Each stage involves either scientific testing or submission and preparation of data and analysis review (Fig. 2).

An investigational new drug (IND) application usually initiates the process for drug approval. The IND derives from the concept that a specific molecule or molecules may have a particular therapeutic benefit. Preclinical data are analyzed to determine the implications of such molecules for human pharmacology, chemical composition, manufacturing processes, and the protocols for subsequent clinical work. Clinical trials are usually carried out in at least three phases. Phase one involves a small number of individuals and is designed to find information about basic safety and response issues. In phase two studies, the drug is employed on a larger number of individuals (100–200) who suffer from the condition that the drug is designed to treat. Phase three studies involve a much larger group of patients and are designed to assess safety, efficacy, and dosage regimens in a broad range of patients across lines of age, race, and gender. Phase three studies may involve several thousand patients and be carried out at several sites.

New drug application (NDA) is the process through which the U.S. Food and Drug Administration (FDA) authorizes the marketing of a new drug. In the NDA, the data are intended to demonstrate the safety and efficacy of the drug in its intended application. After approval, the drug becomes available to the public. Subsequently, dosage amounts and forms may be modified according to experience, new indications may be added, and contraindications may be noted. All of the changes require regulatory approval. A drug in human use is subject to constant surveillance.

2. The Receptor Concept

The concept of drug–receptor interaction originates in two separate lines of experimental evidence advanced in the late nineteenth and early twentieth centuries (5, 6). Work in immunology and the chemotherapy of protozoan infections led to the postulate that specific protoplasmic side chains of unique chemical and steric architecture exist, and these side chains combine in lock-and-key fashion only with the appropriate complementary groups of an antibody or a chemotherapeutic agent (7). At about the same time, the concept of a receptive substance was developed, based on the mutually antagonistic actions of atropine and pilocarpine (8). The former inhibits saliva flow; the latter stimulates it. Subsequently, on the basis of the antagonist effects of curare on nicotine-induced skeletal muscle contraction, it was concluded that a specially excitable constituent, ie, the receptor, exists (9). Thus, early attention was drawn both to the specific recognition capacity of receptors and to the ability of a drug–receptor complex to initiate a biological response. The principle of specific chemical recognition is common to ligand–macromolecule interactions, but this alone does not suffice to define a receptor in the pharmacologic sense. Rather, it is the combination of chemical specificity or recognition and the capacity to initiate biological response or transduction that define the pharmacologic receptor (1, 10, 11).

The information contained in the chemical structure of a given ligand is without value unless decoded and executed by the appropriate receptor. The pharmacologic analysis of drug–receptor interactions is based on the understanding of how the drug is recognized by the receptor, how the drug–receptor complex forms, and how the drug–receptor complex initiates its biological action (12).

Drug receptors are chemical entities which are typically, but not exclusively, small molecules that interact with cellular components, frequently at the plasma membrane level (1, 2). There are many types of receptors; heat, light, immune, hormone, ion channel, toxin, and virus are but a few that can excite a cell. The receptor concept can be applied generally to signal recognition processes where a chemical or physical signal is recognized. This recognition is translated into response (Fig. 3) and the process can be seen as a flow of information.

Elucidation of the structural requirements for drug interaction at the recognition site is by the study of structure–activity relationships (SAR), in which, according to a specific biologic response, the effects of systematic molecular modification of a parent drug structure are determined. Such studies have permitted the classification of discrete classes of pharmacological receptors. For example, the neurotransmitter acetylcholine acts at both peripheral and central receptors which are of at least three distinct types. The effects of

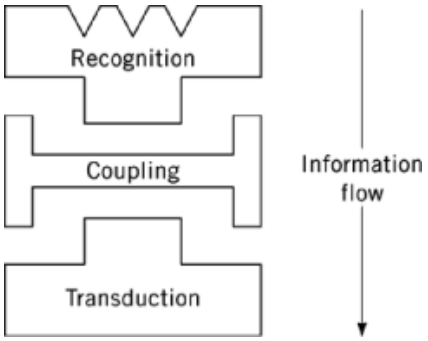


Fig. 3. Information flow at a pharmacologic receptor. The receptor is depicted as having the three components recognition, coupling, and transduction. These may be separate components or carried on a single function.

acetylcholine are mimicked in smooth and cardiac muscles and secretory glands by muscarine and in skeletal muscle and autonomic ganglia by nicotine; the effects in smooth and cardiac muscles are antagonized by atropine, in skeletal muscle by curare, and in ganglia by hexamethonium. Thus, it is recognized that there are at least two primary classes of agents acting at receptors: those that bind to the receptor and initiate its specific response, ie, agonists, and those that bind but are unable to initiate response, ie, antagonists. Additionally, selective chemical agents indicate the existence of subclasses of a primary acetylcholine-recognizing subunit.

The demonstration of the existence of strictly defined SARs, which is perhaps the most important criterion of drug action at a specific receptor site, has made possible the most important pharmacologic discoveries. For example, the analgesic actions of morphine [57-27-2] and related agents, which are indicative of specific receptors, led to the discovery of endogenous opiate peptides, ie, the leucine and methionine enkephalins and endorphins (see Opioids, endogenous) (13). Similarly, the well-defined SAR for the anxiolytic activity of the benzodiazepines, eg, diazepam [439-14-5] (Valium) and flurazepam [17617-23-1], also indicates that endogenous compounds which interact in a physiological fashion with these receptors exist (see Psychopharmacological agents; Stimulants) (14). In the 1990s, the existence of SARs for cannabinoids (Marihuana) led first to the discovery of the cannabinoid receptor, which, like the opiate receptor, is also a plasma membrane G-protein-coupled protein, and then to the discovery of an endogenous cannabinoid-receptor active factor (or factors) that may serve to mediate physiological events (15–17).

3. Pharmacokinetic Aspects of Drug Action

The receptor represents the locus of drug action. However, the pharmacokinetic processes of absorption (drug entry), distribution, metabolism, and excretion play principal roles in determining *in vivo* time courses and concentrations of drugs and thus modify actions initiated at receptors.

3.1. Drug Entry

Drugs enter the body by one of two routes. In enteral administration (sublingual, oral, rectal), the drug enters directly the gastrointestinal tract. In the parenteral route, the drug bypasses the gastrointestinal tract by, among others, subcutaneous (sc), intramuscular, intravascular (iv), inhalational, intraperitoneal (ip), intravaginal, and intranasal routes. Each route has a particular set of advantages and disadvantages. Patient convenience is high in the oral route; speed of action and ability to control concentrations are high in the iv route; and nonoral routes are best for unstable or insoluble drugs.

In light of the recognized importance of achieving stable, reproducible plasma concentrations of drugs, particular attention is given to pathways and devices, including sustained-release formulations, pumps, and transdermal entry processes that ensure such properties (see Controlled release technology, pharmaceuticals). Osmotic pumps are available for subcutaneous implants and for oral administration. These capsule-like devices contain a semipermeable membrane through which water can enter to dissolve the drug or to push the drug solution out of the system (1).

3.2. Drug Distribution

After administration, a drug may be distributed either generally or selectively in the body. The distribution pattern depends on many factors, including the pattern and time-course of blood flow, diffusion of drugs into tissues, binding of drugs to plasma proteins and cellular compartments, and elimination kinetics and mechanisms. The total body water of an average, 70-kg individual is around 42 L, which consists of 14 L of extracellular fluid which in turn includes about 3 L of plasma water. The apparent volume of distribution represents the volume of fluid in which a drug appears to be dissolved. A volume of distribution approximating that of the extracellular water indicates the locus for the drug, whereas a volume of distribution significantly higher or lower indicates a drug distribution into additional or restricted compartments, respectively (18).

Many drugs bind to plasma proteins. Such binding affects distribution and access to sites of action, metabolism, and elimination. Some drugs interact with specific plasma proteins, including the α- and β-lipoproteins for vitamins A and other carotenoids as well as the steroid hormones, the sex-steroid binding protein, and transcortin. A significant contribution to drug binding is made by albumin (mol wt ca 68,000), which constitutes some 50% of the total protein of plasma. Serum albumin contains multiple drug binding sites and is the endogenous carrier for free fatty acids. When drugs are extensively bound to albumin, competition between different drugs may underlie some drug interactions. This is well established for such drugs as warfarin [81-81-2] and the calcium antagonists, eg, verapamil [52-53-9], nifedipine [21829-25-4], and diltiazem [42399-41-7] (see Cardiovascular agents). Although albumin has a high affinity for acidic drugs, basic species tend to bind to other proteins, including alpha$_1$-acid glycoprotein, present at levels 50–100 times lower than albumin (19). Alpha$_1$-acid glycoprotein is an acute-phase reactant protein and its plasma levels are subject to great variation according to physiologic and pathologic conditions. These changes influence drug binding and the expression of drug activities.

Drug distribution into tissue reservoirs depends on the physicochemical properties of the drug. Tissue reservoirs include fat, bone, and the principal body organs. Access of drugs to these reservoirs depends on partition coefficient, charge or degree of ionization at physiological pH, and extent of protein binding. Thus, lipophilic molecules accumulate in fat reservoirs and this accumulation can alter considerably both the duration and the concentration–response curves of drug action. Some drugs may accumulate selectively in defined tissues, for example, the tetracycline antibiotics in bone (see Antibiotics, tetracyclines).

Specific barriers may serve to limit drug distribution. The placental barrier is of obvious importance to drug action in the fetus. Drug transfers across the placenta primarily by lipid solubility. Hence, this barrier is not particularly restrictive. Similarly, the lipid solubility of a drug is a primary determinant in access to the brain and cerebrospinal fluid. Generally, hydrophilic or charged drugs can also penetrate to these latter areas, but the result is slow and incomplete. The blood brain barrier is composed of cells having tight junctions which are much less permeable to solutes than are the endothelial cells of other tissues.

Drugs may also accumulate selectively in reservoirs by active processes. A number of agents, including catecholamines, choline [62-49-7] (qv), and amino acids (qv) such as glutamate and γ-aminobutyric acid [56-12-2] (GABA), are taken up into cells via a Na^+-dependent countertransport system (20). This system derives from the ion gradients established by the Na^+- and K^+-adenosinetriphosphotase (ATPase) pump. These transporters are the sites for many important therapeutic agents, including the antidepressants imipramine [50-49-7] and fluoxetine [54910-89-3], as well as drugs of abuse (see Stimulants). Drugs may also enter cells

Table 1. Biotransformation Reactions

Pathways	Reactions types	Examples
	Phase I reactions	
oxidative	aliphatic and aromatic oxidation	phenobarbital, phenytoin
	N- and *O*-dealkylation	desipramine, phenacetin
	N-oxidation	guanethidine
	oxidative deamination	amphetamine
	desulfuration	thiobarbitol
	dehalogenation	chloroform
hydrolytic	esters and amides	procaine, lidocaine
reductive	azo reduction	prontosil
	nitro reduction	chloramphenicol
nonmicrosomal oxidative	alcohol and aldehyde oxidation	ethanol
	purine oxidation	6-mercaptopurine
	oxidative deamination (monoamine oxidase)	serotonin
	Phase II reactions	
coupling	glucuronidation	acetaminophen
	acetylation	isoniazid
	glycine conjugation	salicylic acid
	sulfate conjugation	steroids, phenols
	methylation	norepinephrine

by variations on the theme of receptor-mediated endocytosis. Thus low density lipoprotein (LDL) enters cells by initial complexation to the LDL-receptor. Internalization via a specific coated-pit pathway is a key process in the metabolic control of cholesterol [57-88-5] biosynthesis and the regulation of plasma LDL receptors. This pathway and other internalization pathways are also participants in various down-regulation processes of receptors during chronic drug treatment and disease states.

3.3. Drug Metabolism

Generally, metabolism (biotransformation) of drugs increases their water solubility as well as the rate and ease of elimination, but reduces their volume of distribution. Many drug-metabolizing pathways have arisen during evolution to deal with foreign compounds present in food materials. Although metabolism generally leads to more polar and less active compounds, there are exceptions. Metabolic pathways have also been exploited to design prodrugs, materials that are converted to active species through biotransformation (1, 2).

Biotransformation reactions can be classified as phase I and phase II. In phase I reactions, drugs are converted to product by processes of functionalization, including oxidation, reduction, dealkylation, and hydrolysis. Phase II or synthetic reactions involve coupling the drug or its polar metabolite to endogenous substrates and include methylation, acetylation, and glucuronidation (Table 1).

The liver microsomal drug-metabolizing system is of particular importance. This oxidative pathway is mediated by isozymes of the cytochrome P_{450} family (Fig. 4). At least ten P_{450} enzyme families exist to accommodate the ability of humans to handle many foreign molecules (21).

The biotransforming pathways are subject to manipulation and modification in a variety of ways. Drug metabolism can be induced as in hepatic enzyme induction, whereby a variety of agents, including barbiturates, aromatic hydrocarbons, and steroids, actually increase the amount of P_{450} enzymes and thus enhance their own metabolism as well as that of other substrates. A number of agents serve as inhibitors of drug metabolism, including SKF525A (2-diethylamino-diphenylpropylacetate), the histamine H_2 antagonists cimetidine [51481-61-9] and disulfiram [97-77-8] (Antabuse). Thus, cimetidine can inhibit metabolism, potentiating the action of

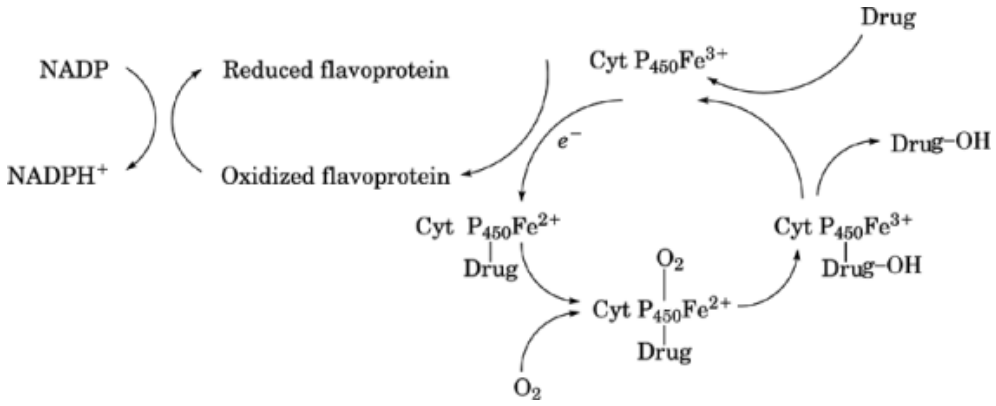
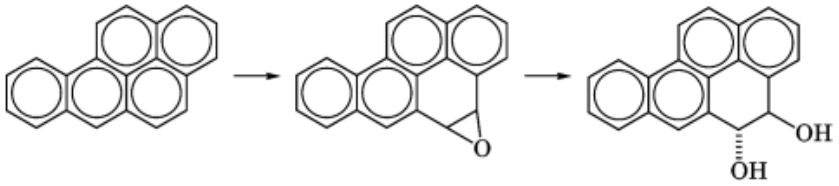


Fig. 4. The cycle of events involved in cytochrome (Cyt) P_{450} (Cyt $_{P_{450}Fe^{2+}}$) mediated drug metabolism where NADP is nicotinamide disphosphate and $_{NADPH^+}$ is the reduced form of NADP.

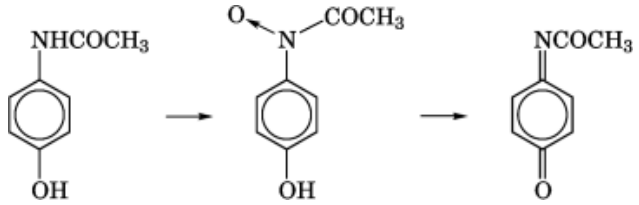
drugs handled by the P_{450} system, including benzodiazepines, phenytoin, and morphine (see Histamines and histamine antagonists).

Drug metabolism also depends on age and sex. The activities of the hepatic biotransforming enzymes are low in the neonatal (and premature) infant. Accordingly, drug toxicity can be unusually apparent, as, for example, chloramphenicol and bilirubin toxicity in newborns, owing to the low activity of conjugation pathways. During various stages of human development, drug metabolism rates can vary considerably. Young children metabolize some drugs, eg, diazoxide and phenobarbital, and eliminate theophylline, faster than adults. With old age, drug metabolism rates generally decline. Hence drug doses for older people should be generally lower than they are for younger ones.

Drug metabolism may also produce toxic materials. Thus, the aromatic hydroxylation of hydrocarbons such as benzpyrene produces the highly reactive and carcinogenic 1,2-epoxides.



Oxidation of acetaminophen yields a reactive quinone intermediate.



3.4. Drug Elimination

Drugs are removed from their sites of action through metabolism, storage, and excretion. These processes are not necessarily independent and drugs are frequently metabolized prior to excretion. Indeed, for lipophilic drugs this is virtually a necessity. Drugs are excreted via the kidneys, biliary systems, intestines, and lungs. Renal excretion is the most important. Lactating humans present another route of elimination, ie, via milk, and this can present both advantages and disadvantages to the suckled young. Fecal excretion comprises mainly unabsorbed orally administered drug or biliary-excreted material. Pulmonary excretion deals primarily with volatile anesthetics (see Anesthetic agents). Urinary excretion has three components: glomerular filtration, active tubular secretion, and tubular reabsorption. The glomerular membranes in the human kidney filter ca 200 L of fluid per day and excrete ca 1% as urine. Drugs that are not of high molecular weight are filtered in the free state. Thus, drugs tightly bound to proteins are excreted more slowly.

The process of reabsorption depends on the lipophilic–hydrophilic balance of the molecule. Charged and ionized molecules are reabsorbed slowly or not at all. Reabsorption of acidic and basic metabolites is pH-dependent, an important property in detoxification processes in drug poisoning. Both passive and active carrier-mediated mechanisms contribute to tubular drug reabsorption. The process of active tubular secretion handles a number of organic anions and cations, including uric acid, histamine, and choline. Drug metabolites such as glucuronides and organic acids such as penicillin are handled by this process.

3.5. Clinical Pharmacokinetics

Clinical pharmacokinetics attempts to define the relationship between drug concentration and therapeutic response. The underlying assumption is that response is proportional to drug concentration at the site of action. This concentration is dependent on many factors that are frequently pharmacokinetic determinants. The most important factors are defined as clearance, bioavailability, and volume of distribution.

Clearance, CL, is defined by

$$DR = CL \cdot C_{SS} \qquad (1)$$

where DR represents dosing rate and C_{SS} the steady-state concentration of the drug.

Once the steady-state concentration is known, the rate of drug clearance determines how frequently the drug must be administered. Because most drug elimination systems do not achieve saturation under therapeutic dosing regimens, clearance is independent of plasma concentration of the drug. This first-order elimination of many drugs means that a constant fraction of drug is eliminated per unit time. In the simplest case, clearance can be determined by the dose and the area under the curve (AUC) describing drug concentration as a function of total time:

$$CL_{total} = CL_{renal} + CL_{hepatic} + CL_{other} \qquad (2)$$

This concept becomes important in determining the effects of organ pathology on clearance and on the role of blood flow to individual organs in calculating clearance rate.

The half-life, $t_{1/2}$, for a drug in plasma, ie, the time it takes for the concentration of a drug to be reduced by 50%, is determined by both volume of distribution, V, and clearance:

$$t_{1/2} = 0.693 \cdot V/CL \qquad (3)$$

The bioavailability of a drug can be defined as the fraction of a dose, F, that reaches the systemic circulation. When $F < 1$,

$$F \cdot DR = CL \cdot C_{SS} \qquad (4)$$

4. Pharmacodynamic Aspects of Drug Action

Although the same general principles of chemical specificity apply to all ligand–macromolecular interactions, the term receptor is generally applied to those cellular macromolecules and macromolecular complexes with which ligands, physiological or synthetic, interact both to complex and to initiate a physiological response. Receptors are conveniently viewed as existing in several principal classes, ie, G-protein-coupled receptors, ligand-gated ion channels, voltage-gated ion channels, tyrosine kinase receptors, guanylyl cyclase receptors, and steroid hormone receptors. All of these receptors form homologous classes according to structure and mechanisms of action. G-protein-coupled receptors form a homologous class of membrane proteins characterized by seven transmembrane domains and the ability to couple to guanine (G) nucleotide-binding proteins. Examples of G-coupled receptors are as follows.

Ligand	Receptor
adenosine	A_1, A_2-adenosine
norepinephrine and epinephrine	1,2-adrenergic
dopamine	D_1, D_2-dopamine
histamine	H_2-histamine
acetylcholine	muscarinic m_1–m_5
5-hydroxytryptamine (5-HT)	5-hydroxytryptamine
prostaglandins	prostaglandin
opiates and enkephalins	opiate
polypeptide hormones	
glucagon	
vasopressin (V_1, V_2)	
thyroid-stimulating hormone (TSH)	
follicle-stimulating hormone (FSH)	
parathyroid hormone (PTH)	
somatostatin (SS)	
neuropeptide Y (NPY)	

Ligand-gated ion channels represent a significant family of ion channels that feature as an integral component of their multimeric subunit organization a receptor site for either acetylcholine (nicotine acetylcholine receptor (AChR)), amino acids including glycine and γ-aminobutyric acid (GABA) (inhibitory transmitters), or glutamic acid (excitatory transmitter). The interaction of the ligand with the endogenous receptor site causes channel opening or closing. Drugs responding to specific ligand-gated ion channels are as follows:

Channel	Drug
nicotinic AChR	curare, gallamine, decamethonium, β-erythroidine, local anesthetics
GABA	benzodiazepines, β-carbolines, avermectin, picrotoxinin
glycine	strychnine
glutamate	phencyclidine, MK 801

Voltage-gated ion channels for the cations Na^+, K^+, and Ca^{2+} are similarly multimeric structures. Openings and closings are regulated by changes in membrane potential. Drugs responding to voltage-gated ion channels are as follows:

Channel	Drug
Na^+	TTX, DDT, veratridine, scorpion toxins, procaine, lidocaine
K^+	quinidine, tolbutamide, diazoxide, glyburide, minoxidil
Ca^{2+}	nifedipine, diltiazem, verapamil, mollusc and spider toxins

An important characteristic of both classes of ion channel is that they possess multiple drug binding sites (Table 2). Many of the channel-active drugs have achieved particular therapeutic importance, including, for example, the Ca^{2+} antagonists, widely used for a number of cardiovascular disorders, such as hypertension.

Table 2. Drugs Active at Ion Channels

Drug	Property	Channel	Response
nifedipine, verapamil, diltiazem	antagonist	Ca^{2+}	cardiovascular inhibition; hypotensive agents
pinacidil, nicorandil	agonist	K^+	cardiovascular and smooth muscle inhibitors
tetrodotoxin	antagonist	Na^+	paralysis of nerve and muscle functions
glyburide	antagonist	K^+	stimulates insulin release; hypoglycemic agent

The tyrosine kinase receptors serve to recognize a variety of growth factors and growth factor-like agents, including insulin (see Insulin and other antidiabetic agents), platelet-derived growth factor (PDGF), epidermal growth factor (EGF), and colony-macrophage stimulating factor (CMSF). All of these receptors have a common mechanistic link autophosphorylating specific tyrosine residues in the intracellular domain of the receptor itself. A further characteristic of this receptor class is that, upon activation, they mediate both rapid and delayed events. Thus, metabolic responses are typically produced rapidly and within minutes whereas effects on cell growth mediated through deoxyribonucleic acid (DNA) occur according to a time scale of hours to days.

Receptors linked to guanylyl cyclase and which catalyze the formation of guanosine triphosphate (GMP) to guanosine-3′5′-cyclic monophosphate (cyclic GMP) include those for atrial natriuretic factor (ANF) and endothelial-derived relaxing factor (EDRF), mediating vasodilatation, and nitric oxide [10102-43-9], NO, or a clearly related derivative.

In marked contrast, the receptors, at which thyroid hormone (see Thyroid and antithyroid preparations) and steroids (qv) such as glucocorticoids, sex hormones, and vitamin D exert their long-term effects on cell function through the genetic machinery, are located intracellularly. These agents enter the cell by virtue of their dominantly lipophilic character and interact with a cytosolic receptor. Upon hormone binding, this heteromeric intracellular receptor is activated or converted to a form that binds to specific DNA sequences and alters transcription of a set of specific steroid-responsive genes. Discrete regions upstream of the transcriptional start sequences are known as hormone response elements (HREs). These HREs recognize the hormone–receptor complex and thus mediate transcriptional control. These receptors belong to a family possessing a region rich with carboxy-terminal cysteine and that represents the DNA binding domain. The N-terminal region, which contains the specific hormone binding site, represents the region of greater structural variability.

4.1. Structure–Activity Relationships

Until the mid-1980s, the attempted correlation of chemical structure and biological activity was the only available approach to the definition of receptor site structures. The basic assumption in the analysis of

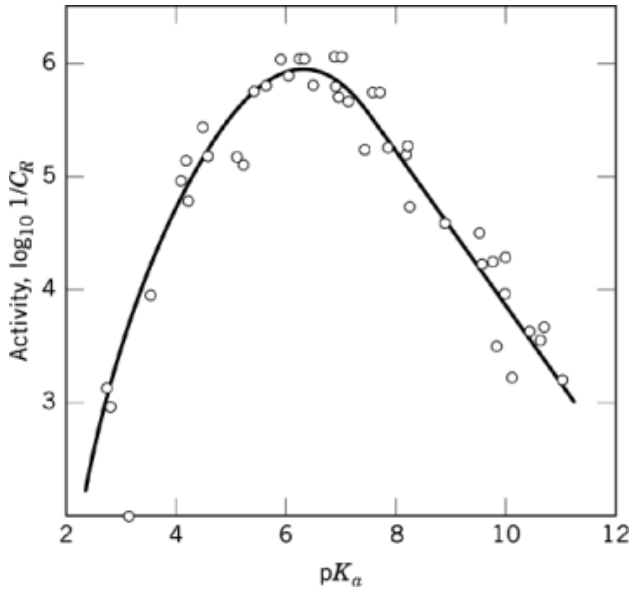


Fig. 5. Relationship between antibacterial activity of sulfonamides (log $1/C_R$) and pK_a of sulfonamide NH group where C_R represents the concentration necessary to prevent growth.(Courtesy of American Chemical Society (27).)

structure–activity relationships (SAR) is the existence of a difinable mutual complementarity between the structure of the drug and its corresponding binding site. Although this approach has been of considerable value, its application is limited when applied in empirical fashion (22, 23). Many drug molecules are flexible structures and, although conformations in the solution and solid states can be determined by spectroscopic and crystallographic methods, these bear no necessary relationship to those adopted at the receptor site. The possibility of mutual conformational adaptation of both the drug and the receptor site during the binding process adds a further complication (24). Furthermore, there may exist multiple drug-binding modes at the receptor such that transitions in binding modes occur at some point in a structurally related series. An additional problem in the quantitative interpretation of SAR is that of the relationship between biological response and drug–receptor interaction. Despite these limitations, SARs have been of great value in providing qualitative concepts of binding site geometry, classifying receptors, furnishing evidence for the existence of new classes of receptor-specific drugs, and generating new and therapeutically effective compounds.

The simplest SARs occur in homologous series of compounds. Thus a linear relationship exists between carbon chain length and biological activity in 1-alkanol-mediated anesthesia (see Anesthetics). The activity can be related to the water:cell partition coefficient (25). For other homologous series, however, such linear relationships may not be observed; for example, in the antagonistic activity of α,ω-bistrimethylammonium alkanes at acetylcholine receptors where binding to sites of defined anionic site geometry probably is involved (26).

Relatively unambiguous monotonic SARs also occur where activity depends on the ionization of a particular functional group. A classic example (Fig. 5) is that of the antibacterial sulfonamides where activity is exerted by competitive inhibition of the incorporation of *p*-aminobenzoic acid into folic acid (27). The bell-shaped relationship is consistent with the sulfonamide acting as the anion but permeating into the cell as the neutral species.

The SAR is also determined at the level of stereochemistry of interaction (28–30). In principle, three limiting situations can apply to the stereochemistry of drug–receptor interactions: the enantiomers may not differ in

activity; the species may differ quantitatively; or they may differ qualitatively. Examples of all three situations are known (28–30). Both enantiomers of dobutamine are inotropic species, but whereas both enantiomers of warfarin are anticoagulant, the activity of (*S*)-warfarin is greater than that of (*R*)-warfarin (see Blood, coagulants and anticoagulants). Additionally, (-)-propranolol is a β-blocker and (+)-propranolol is significantly less active. Finally, (-)-sotalol is a β-blocker and (+)-sotalol is a class III antiarrhythmic. The stereoselectivity of drug action is not confined to specific receptor interactions. Stereoselectivity of interaction also occurs in drug transport, protein binding, and metabolism. These stereochemical differences may contribute significantly to the observed stereoselectivity of pharmacologic or therapeutic response (31, 32). The Ca^{2+} channel antagonist verapamil is subject to stereoselective first pass metabolism, whereby the more active (*S*)-verapamil is more rapidly metabolized. The availability of this enantiomer from the clinically administered racemate is greater after intravenous than oral administration. Additionally, verapamil shows modest stereoselective binding, (*S*) $>$ (*R*), to serum albumin (see Pharmaceuticals, chiral).

The issue of drug stereoselectivity has become one of both developmental and regulatory significance. In principle, a racemic drug possesses only 50% of the active ingredient, and the rest may have other or interacting pharmacologic activities, which may contribute a metabolic burden or be inert. Over 50% of clinically available drugs have chiral centers and only about 10% of synthetic chiral drugs are marketed in homochiral (enantiomerically pure) form (33). In contrast, drugs that are naturally occurring substances, obtained from or related to naturally occurring molecules, are frequently homochiral.

There is increasing pressure to develop homochiral drugs (34). Growing demands are faced by the pharmaceutical industry in drug development to consider chiral issues in the early preclinical phases of drug design and synthesis.

Often pharmacologic agonist activity decreases and is lost with progressive structural change. A typical example is shown in Figure 6. Increasing *N*-alkyl-substitution in the basic 2-amino-1-(3,4-dihydroxyphenyl)ethanol nucleus, ie, norepinephrine, causes an ultimate loss in activity. However, although inactive as agonists, these higher homologues can interact with the receptor because they act as antagonists of the response induced by the agonists. This antagonism is competitive and, therefore, is consistent with the interaction of these homologues at the receptor, but without the capacity to initiate response. Relatively minor structural changes are frequently sufficient to produce this agonist–antagonist transition.

Increasing attention has been paid to the generation of quantitative structure–activity relationships in which the effects of molecular substitution on pharmacologic activity can be interpreted in terms of the physicochemical properties of the substituents. These approaches are based on the extrathermodynamic analysis of substituent effects (36):

$$\log\frac{k}{k_o} = p\sigma \qquad (5)$$

where k_o is the rate or equilibrium constant for an unsubstituted parent compound, k is the same for the substituted compound, σ or σ^+ is a parameter describing the electronic effect of the substituent, and p is a proportionality constant. Another substituent constant, ie, the hydrophobic constant, is defined as

$$\log P = \sum\pi; \qquad \log\frac{P_o}{P} = \pi \qquad (6)$$

where P_o and P are the partition coefficients usually of octanol:water for the unsubstituted and substituted compounds, respectively (36). The use of σ, π, and steric parameters such as E_s has made possible the analysis of multivariate quantitative SARs. A typical example where activity of 51 compounds was determined by φ alone is the ability of various organic compounds, eg, alcohols, ethers, and ketones, to produce narcosis in tadpoles:

$$\log\frac{1}{C} = 0.94\sum\pi + 0.87 \qquad (7)$$

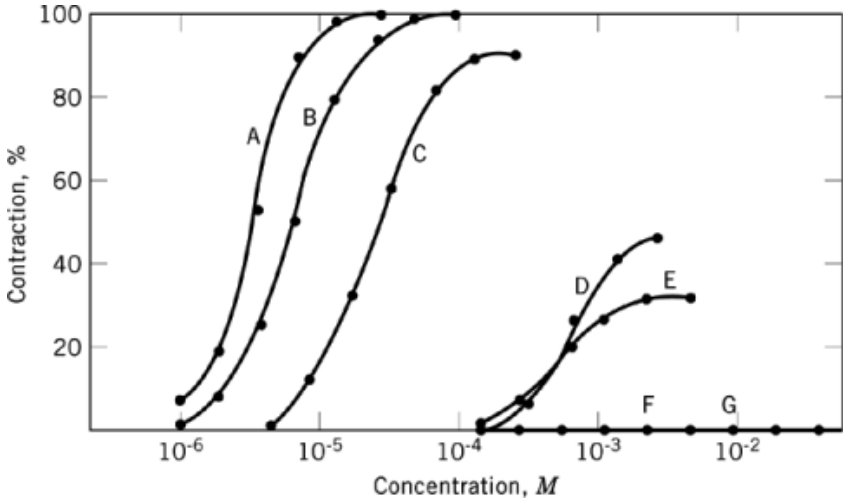
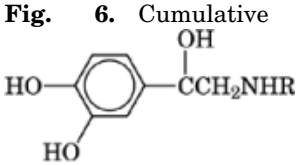


Fig. 6. Cumulative log concentration-response curves for the homologous-*N*-alkylcatecholamines,



where A–G correspond to R= CH^3, H, CH_2CH_3, $CH(CH_3)_2$, $CH_2CH_2CH_3$, $C(CH_3)_3$, and $CH_2CH_2CH_2CH_3$, respectively. There is a gradual change from active to inactive (as agonist) molecules with increasing size of the *N*-alkyl substituent (35).

where C is the biologically effective concentration (36). The correlation coefficient, r, for equation 7 was 0.97. Because of the structural diversity of the compounds in this case, it is possible that interaction at a specific receptor was not involved. There are also many examples where activity is not linearly dependent on partition coefficients. For the ability of 17 barbiturates to produce hypnosis (36):

$$\log \frac{1}{C} = 0.33\pi^2 + 1.76\pi + 0.93 \qquad (8)$$

The correlation coefficient for this equation was 0.994. Such a parabolic dependence of activity on the partition coefficient may reflect partitioning of the drug through several membrane barriers, which enabled the drug to reach its site of action.

Biological activities also may correlate with electronic substituent factors alone, eg, the inhibition of acetylcholinesterase by six diethyl phenyl phosphates (36) gave $r = 0.95$ for

$$\log \frac{1}{C} = 5.77\sigma + 2.71 \qquad (9)$$

More commonly, multiparameter correlations can be made. Thus, for the relative sweetness of nine 4-nitro-2-aminobenzenes, $r = 0.97$ (36):

$$\log(\text{relative sweetness}) = 1.03\sigma^{+} + 1.43\pi + 1.58 \qquad (10)$$

and for the local anesthetic activity of eight 2-diethylaminoethyl benzoates, $r = 0.93$(36):

$$\log \frac{1}{C} = 0.58\pi - 1.26\sigma + 0.96 \qquad (11)$$

The preceding examples of linear free-energy correlations of biological activity with the physicochemical properties of molecules may be used to deduce the types of interaction involved in biological activity and to predict new compounds (37–39). However, generally these predictions are most accurate when they are interpolative rather than extrapolative. Increasing attention is being paid to quantum mechanistic approaches to the definition of SARs. These calculations can provide measurements of nonequilibrium conformational energies, electron densities, and electrostatic potential maps of drug molecules (40).

Advancing technology permits increasing attention to the definition of the three-dimensional structure of the ligand in its bioactive conformation as it binds to the receptor or active site. This bioactive conformation is not necessarily the solution or the crystal structure of the ligand, which is often the most experimentally accessible structure. It is of further critical importance to define the three-dimensional structure of the ligand complexed with its target. This resolution permits not only the understanding of a particular ligand–macromolecule, but also the *in vivo* design of ligand homologues that may have tighter or more selective affinities for the site (40, 41).

Considerable effort must be applied to obtaining adequate quantities of the protein target and its structural solution, together with the structural solution of the complexed ligand, either by x-ray or solution nmr techniques. Alternatively, homology modeling may be possible when the structure of a homologue protein is already available. Although many examples of ligand–protein structure determinations are available, some of the most interesting targets, eg, membrane-bound receptors, defy structural solution at the necessary resolution. The examination of the real structure of ligand–receptor complexes should be an increasingly important and integral part of the drug discovery process (Fig. 7) (41).

4.2. Quantitative Aspects of Drug–Receptor Interactions

As a general rule, pharmacological responses are graded and a defined relationship exists between the concentration of a drug and the receptor response. This usually is expressed as a concentration–response (A–R) relationship in linear or semilogarithmic coordinates and usually is referred to as a dose–response curve. The shape of these curves (Fig. 8) offers a clear analogy to processes of physical adsorption but, because of the complexity of the sequence of events between drug–receptor interaction and the response, the interpretation of dose–response curves is not simple. A quantitative understanding of drug–receptor interactions is crucial both to the nontrivial interpretation of structure–activity relationships and to the determination of the mechanisms by which drug–receptor complexes initiate pharmacological response.

Reaction rates cannot be faster than collision frequencies. Such reactions are diffusion-controlled. The encounter rate, k_e, for drug binding to receptors is

$$k_e = \frac{4\pi N R_{12} D_{12}}{1000} \qquad (12)$$

where R_{12} is the sum of the radii of the drug and its binding site, D_{12} is the sum of the self-diffusion coefficients of the two molecular species, and N is Avogadro's number. Such rates frequently approximate $10^6 - 10^8 (M{\cdot}\text{s})^{-1}$ for drug–receptor interactions.

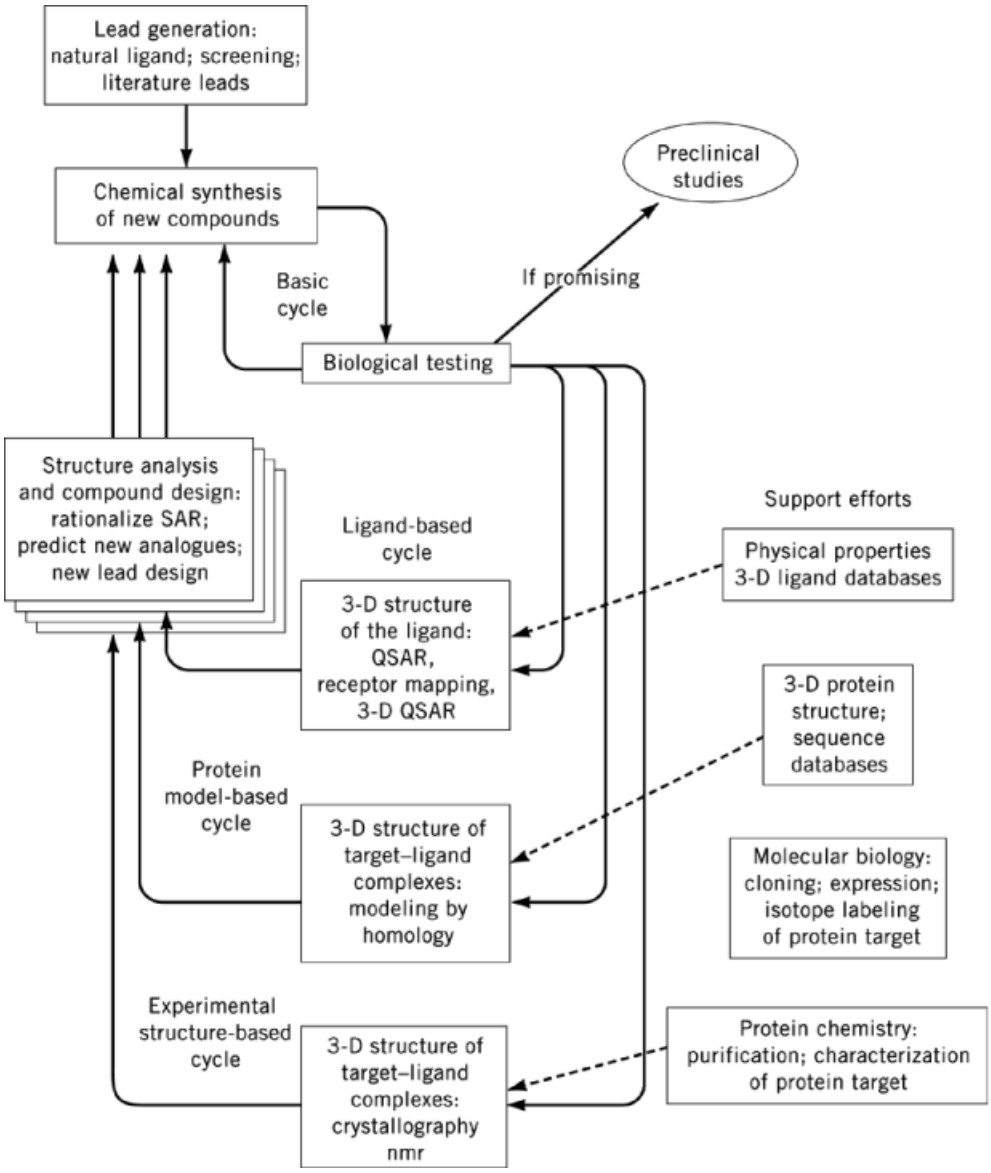


Fig. 7. The cycle of structure-based drug design. The conventional or basic cycle consists of a simple loop between chemical synthesis and biological activity. This cycle is typically initiated through a lead compound. Whereas this cycle remains the core of the drug discovery process, the resources available have been substantially amplified by the indicated developments in structural chemistry.(Courtesy of the American Chemical Society (41).)

The mechanism by which a drug binds to its specific receptor is important. Effective transmission of chemical information demands accuracy, efficiency, and rapidity. Accuracy can be achieved through the specificity of the molecular architecture of the drug and its receptor. Two extreme situations of drug binding may be visualized and both are consistent with the existence of a structurally and stereochemically defined drug binding site. The drug may interact by presentation of a single favorable binding conformation, ie, lock-and-key principle, or

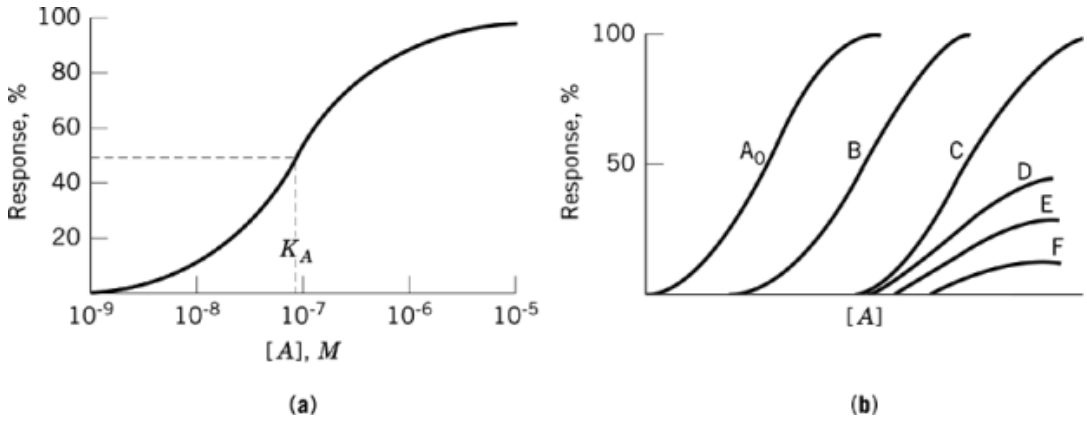


Fig. 8. Agonist *A*, dose–response curves. (**a**) For an agonist where a K_A value of 10^{-7} *M* is indicated at the concentration giving 50%response. (**b**) For an agonist alone, A_0, and in the presence of increasing amounts of irreversible receptor antagonists, B–F. There is a progressive rightward shift of the dose–response curve prior to reduction of maximum response. This pattern is consistent with the presence of a receptor reserve.

by the zipper model, ie, binding of an initial segment of the drug molecule followed by conformational alignment of the partially bound ligand (42). For drugs that are conformationally mobile, the stringent conformational and orientational demands of the lock-and-key model are likely to reduce substantially the rate of drug–receptor binding. The zipper model may permit a faster rate of drug–receptor interaction because the orientational and conformational requirements for the initial binding of a molecular component are substantially less than for the whole molecule.

In the early twentieth century, the law of mass action was applied to the basic pathway of drug–receptor interaction. Assuming that response, *R*, is proportional to the concentration of the drug–receptor complex, *RA*, and that maximum response, R_{max}, occurs when all receptors are occupied (2, 6, 10), then

$$\frac{R}{R_{max}} = \frac{[RA]}{[R_{tot}]} = \frac{[A]}{[A] + K_A} \qquad (13)$$

where K_A is the dissociation equilibrium constant for binding of the drug *A*. Here R_{max} corresponds to complete receptor occupancy. This equation is the equivalent of the Michaelis-Menten expression for enzyme–substrate interactions (1, 12). Accordingly, the K_A value of a drug for the receptor is equal to A_{50}, the concentration producing 50% response (Fig. 8**a**), and a quantitative interpretation of dose–response curves is easily provided. The limiting assumptions made in using equation 13 are (*1*) binding is reversible; (*2*) reactants exist only as bound or free species, and degradation, internalization, or other removal mechanisms are not important; and (*3*) receptor sites are assumed to be of equal affinity and independent in their binding interactions, ie, not cooperative, with ligands.

The original formalism of drug–receptor interactions assumed only two types of drugs, the agonists and the antagonists. However, this was an oversimplification. In many homologous series, such as *N*-alkyltrimethylammonium salts active at cholinergic receptors or *N*-alkylcatecholamines active at adrenergic receptors (Fig. 6), there are clearly ligands that even at saturating concentration produce only partial response (1, 10, 35). Additionally, the use of irreversibly acting receptor antagonists, eg, 2-halogeneothylamines such as phenoxybenzamine, which react covalently and eliminate receptors from response generation, did not cause the anticipated reduction of maximum response. Rather, the observed response was frequently a parallel rightward shift of the dose–response curve prior to a depression of response such as that shown in Figure 8**b** (1,

10). These observations indicate that response may not be proportional to receptor occupancy and that spare receptors, ie, receptors in excess of those necessary to generate maximum response, exist. These conclusions have been demonstrated by studies of ligand binding to receptors using radioligands (see Radioactive tracers) and studies of the coupling of receptors to the effector units.

The term intrinsic activity (ia) was defined as a measure of the ability of the drug–receptor complex to generate response. When $\text{ia} = 1$, a full agonist is defined; when $\text{ia} = 0$, an antagonist is defined. Thus, values $0 < \text{ia} < 1$ define partial agonists as follows, where R_A is the response to drug A and R_max is the maximum response achieved.

$$\frac{R_A}{R_\text{max}} = \frac{[RA]}{[R_\text{tot}]} = \frac{[A]}{[A] + K_A} \qquad (14)$$

However, maximum receptor occupancy for full agonists was still required for maximum tissue response. The efficacy, e, was introduced through the concept of a stimulus, S, defined as

$$S = \frac{e\,[RA]}{[R_\text{tot}]} = \frac{e\,[A]}{[A] + K_A} \qquad (15)$$

where e is a dimensionless parameter representing the ability of a drug, as the drug–receptor complex, to initiate response (43). Response is some undefined function of stimulus, $f(S)$, which is monotonic and continuous:

$$\frac{R_A}{R_\text{max}} = f(S) = f\left(\frac{e\,[A]}{[A] + K_A}\right) \qquad (16)$$

Thus, a drug may produce response either with low efficacy by occupying many receptors or with high efficacy by occupying few receptors. The issues of dealing with agonist–dose response relationships can be complex and reference should be made to detailed texts (44, 45).

In contrast, interactions of competitive antagonists and receptors are relatively straightforward. It can be shown by comparing the equal responses provided by an agonist, $[A]$, alone or in the presence of a competitive antagonist, $[A]_B$, so that

$$\frac{[A]_B}{[A]} = 1 + \frac{[B]}{K_B} \qquad (17)$$

The dissociation content for the competitive antagonist, K_B, can be determined without knowing the relationship between receptor occupancy and response. Equation 17 is often written in logarithmic form:

$$\log \frac{[A]_B}{[A]} - 1 = \log [B] - \log K_B \qquad (18)$$

A plot of $\log([A]_B/[A] - 1)$ versus log[B], called a Schild plot, yields a straight line of unit slope and intercept of K_B, the latter often expressed on a scale analogous to that for pH, so that $pA_2 = \log K_B$ (46–48).

Because K_B values for competitive antagonists represent true dissociation constants, these make possible quantitative interpretations of SARs. Significant use also has been made of K_B values in the quantitative comparison of receptors to determine whether receptors that respond to the same agonists are identical or whether responses produced by different agonists are initiated at the same receptors (44, 46). Thus, beta-adrenoceptors in human and guinea pig preparations can be directly compared and selective β_1 and β_2 antagonists quantitated (Table 3).

Table 3. Equilibrium Dissociation Constants for Drug–Receptor Complexes *In Vitro*[a]

	β-Adrenoceptors			
	Atria		Bronchii	
Antagonists	Guinea pig	Human	Guinea pig	Human
propranolol	8.5	8.4	8.3	8.6
pindolol	8.7	8.8	8.8	8.6
practolol[b]	6.5	6.4	4.9	4.6
atenolol[b]	7.2	7.0	5.6	5.4
acebutolol[b]	6.5	6.8	5.1	5.1
metoprolol[b]	7.4	7.4	6.1	6.4[c]

[a] Ref. 44.
[b] Cardiac selective.
[c] Whereas the agreement between the values in humans and guinea pig is close, this is not always so. For example, in human and rodent 5-$HT_{1\beta}$; receptors, significant pharmacological differences are conferred by a single amino acid residue.

Although the concept of competitive antagonism is well developed in both molecular pharmacology and clinical medicine, many antagonists act noncompetitively. A noncompetitive antagonist acts at a site distinct from the agonist ligand and prevents, by indirect mechanisms, agonist occupancy of its binding site. This concept has been particularly well developed for allosteric proteins where binding or functional behavior of a protein is controlled by equilibrium shifts between active and inactive states according to ligand binding (1, 10, 44, 49).

Direct quantitation of receptor concentrations and drug–receptor interactions is possible by a variety of techniques, including fluorescence, nmr, and radioligand binding. The last is particularly versatile and has been applied both to sophisticated receptor quantitation and to drug screening and discovery protocols (50, 51). The use of high specific activity, frequently [^{3}H]- or [^{125}I]-labeled, drugs bound to crude or purified cellular materials, to whole cells, or to tissue slices, permits the determination not only of drug–receptor saturation curves, but also of the receptor number, drug affinity, and association and dissociation kinetics either directly or by competition. Complete theoretical and experimental details are available (50, 51).

This kind of binding has been demonstrated for many receptor systems and obeys the four anticipated criteria derived from structure–activity relationships: (*1*) binding should be saturable, which is consistent with cells possessing a finite number of receptors; (*2*) binding should be reversible, which is consistent with the reversibility of action of most drugs; (*3*) binding should be observed only in systems known to be sensitive to the drug; and (*4*) there should exist a correlation between the relative binding affinities and biological activities of drugs that are active in the system (50, 51). A good correlation occurs for antagonists between K_D values for biological responses and those from binding studies. A typical example is shown in Figure 9 for atropine-like agents in intestinal smooth muscle, where binding is measured by ligand competition with the specific binding of the potent atropine-like agent, 3-quinuclidinyl benzilate, in tritiated form. Generally, however, a lack of agreement exists when a similar comparison is made for agonist molecules, as is shown in Figure 9**b** for muscarine-like agonists acting in intestinal smooth muscle (52). Such discrepancies, whereby $K_A/K_D < 1$ and K_A is the pharmacologically determined dissociation constant and K_D the dissociation constant determined from drug binding, are consistent with the existence of complex relationships between agonist concentration–response and saturation curves.

4.3. Nonreceptor-Mediated Drug Action

At least one important class of drugs, the general anesthetics (qv), has been assumed not to owe its therapeutic activities to a specific receptor process. Anesthetic potency shows an excellent linear correlation with partition

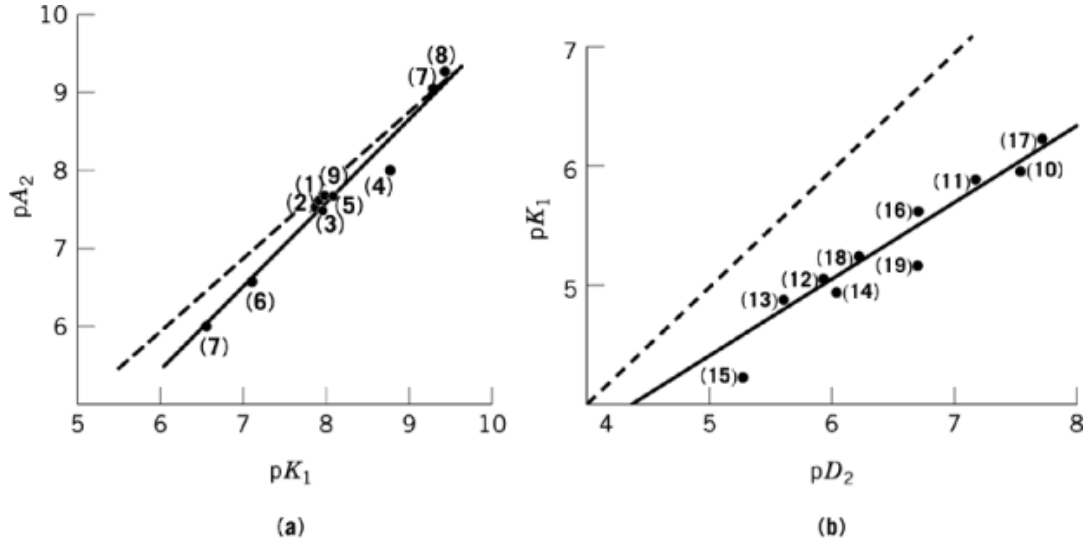


Fig. 9. Correlation between binding and pharmacologic affinities where the dashed lines correspond to the theoretical correlation of 1:1 for a series of muscarinic receptor: (**a**) antagonists, (**1**)–(**9**) and (**b**) agonists, (**10**)–(**19**). Correlation for the antagonists is essentially 1:1, deviating markedly from that relationship for the agonists. The slope is 0.63 at a correlation of 0.96.(Courtesy of the American Society for Pharmacology and Experimental Therapeutics (52). See Table 4.)

coefficient and this has been extrapolated to a definition of action at a lipid site. The phospholipids of cell membranes, particularly nerve cells, have been considered as principal targets for general anesthetic action. It has been hypothesized that anesthetics may disrupt phospholipid structure by fluidizing or expanding the cell membrane or by altering the phase relationships of the phospholipids (53, 54). However, it is possible that anesthetics bind to hydrophobic sites on proteins and thus affect directly excitable cell behavior (53–55). This latter proposal is consistent both with the activity of the gaseous general anesthetics and with the activity of structurally more complex agents, eg, 3α-hydroxy-5α-pregnane-11,20-dione, 3α-hydroxy-5-pregn-16-ene-11,20-dione, and 1,5-desmethyl-5-cyclohexenylbarbituric acid.

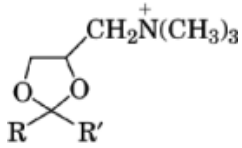
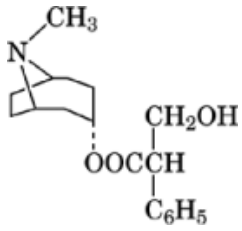
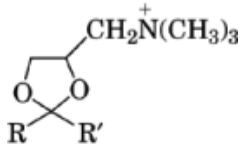
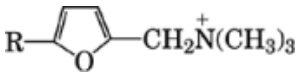
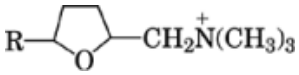
Although most anesthetics are achiral or are administered as racemic mixture, the anesthetic actions are stereoselective. This property can define a specific, rather than a nonspecific, site of action. Stereoselectivity is observed for such barbiturates as thiopental, pentobarbital, and secobarbital. The (*S*)-enantiomer is modestly more potent (56, 57). Additionally, the volatile anesthetic isoflurane also shows stereoselectivity. The (*S*)-enantiomer is the more active (58). Further evidence that proteins might serve as appropriate targets for general anesthetics come from observations that anesthetics inhibit the activity of the enzyme luciferase. The potencies parallel the anesthetic activities closely (59, 60).

It is likely that a principal target of the general anesthetics is neuronal ion channels of both voltage-gated and ligand-gated classes (61, 62). Interactions at GABA-mediated inhibitory channels is a significant, but not exclusive, target. Thus, a general anesthetic may have specific but multiple, rather than nonspecific, sites of action.

4.4. Receptor–Effector Coupling

The informational signal initiated by drug–receptor interaction must be translated to biological response. This is activated by a variety of effector-coupling processes that lead to ionic or biochemical changes, including ion channel opening and closing; the formation of second messengers such as cyclic adenosine-3′-5′-monophosphate

Table 4. Structures of Muscarinic Receptor Antagonists and Agonists

Structure number	Structure	R	R′	Stereochemistry
	Antagonists			
(1) (2) (3) (4) (5) (6)		C_6H_5 C_6H_5 C_6H_5 C_6H_{11} C_6H_{11} C_4H_9	C_6H_5 C_6H_5 C_6H_5 C_6H_5 C_6H_5 C_4H_9	(*R*),(*S*) (*R*) (*S*) (2*S*), (4*S*) (2*R*), (4*R*)
(7) (8) (9)				(*R*,*S*) (*R*) (*S*)
	Agonists			
(10) (11) (12) (13) (14) (15)		CH_3 H Cl_3C H H CH_3	H CH_3 H CCl_3 H CH_3	
(16) (17)		H CH_3		
(18) (19)		H CH_3		

(cAMP) and inositol-1,4,5-triphosphate (IP_3); and protein phosphorylation through protein kinase A (cAMP-dependent) and protein kinase C (Ca^{2+}-dependent), or through autophosphorylation (tyrosine kinase receptors). In these systems, it is increasingly clear that the individual components of a receptor system may be linked in multiple ways. The virtue of this organization lies in the multiple coupling processes permitted beyond a set of components.

These cascades serve as operational amplifiers of the initial ligand–receptor interaction. In each step of the process, amplification by several powers of 10 may occur so that an original signal may be multiplied several millionfold (63).

4.4.1. G-Protein Coupling

The heterotrimeric guanosine triphosphate (GTP) binding proteins, known as G-proteins, are a principal family of proteins serving to couple membrane receptors of the G-protein family to ionic and biochemical processes. This topic is reviewed in References (63–67). The G-proteins are heterotrimers made of three families of subunits, α, β, and γ, which can interact specifically with discrete regions on G-protein-coupled receptors. This includes most receptors for neurotransmitters and polypeptide hormones (see Neuroregulators). G-protein-coupled receptors also embrace the odorant receptor family and the rhodopsin-linked visual cascade.

The underlying coupling mechanisms are defined by the enzymatic activity of the G-protein, that of hydrolyzing GTP, ie, GTPase activity. In the inactive state, the heterotrimeric G-protein is liganded to the diphosphate GDP. Receptor activation reduces the affinity of the α-subunit for GDP and increases the affinity for GTP. The GTP-liganded complex then dissociates to the GTP-bound activated α-subunit and the β- and γ-subunits. These dissociated subunits then interact with the corresponding effectors (Fig. 10). The effectors include adenylyl cyclase, phospholipase C, cGMP phosphodiesterase, some ion channels (K^+, Ca^{2+}), and receptor kinases. These signals may be excitatory or inhibitory according to the class of G-protein, some of which are listed for G-protein-linked adenylyl cyclase:

Stimulation (Gs)	Inhibition (Gi, Go)
β-adrenergic	opiate
H_2-histamine	muscarinic
dopamine	α_1-adrenergic
polypeptide hormones (glucagon, ACTH, etc)	adenosine (fat cells) A_1, prostaglandins (fat cells)
adenosine (platelets, lymphocytes) A_2	
prostaglandins (platelets)	polypeptide hormones
serotonin (5-$HT_{1\alpha}$)	somatostatin, neuropeptide Y, atriopeptin

A critical component of the G-protein effector cascade is the hydrolysis of GTP by the activated α-subunit (GTPase). This provides not only a component of the amplification process of the G-protein cascade (63) but also serves to provide further measures of drug efficacy. Additionally, the scheme of Figure 10 indicates that the coupling process also depends on the stoichiometry of receptors and G-proteins. A reduction in receptor number should diminish the efficacy of coupling and thus reduce drug efficacy. This is seen in Figure 11, which indicates that the ability of the muscarinic drug carbachol [51-83-2] to inhibit cAMP formation and to stimulate inositol triphosphate, IP_3, formation yields different dose–response curves, and that after receptor removal by irreversible alkylation, carbachol becomes a partial agonist (68).

The ability of receptors to couple to G-proteins and initiate GTPase activity may also be independent of ligand. Thus, specific mutations in α- and β-adrenergic receptors have led to receptors that mediate agonist-independent activation of adenylyl cyclase (69, 70). These mutations presumably mimic the conformational state of the ligand-activated receptor when they are activated conventionally by ligands.

GTPase activity is also associated with the *Ras* protein family. These small proteins act as binding switches, turning GTPase on and off, and are also regulated by other proteins, including GTPase activating proteins (GAP), guanine–nucleotide exchange factors (GEF), as well as guanine–nucleotide dissociation inhibitors (GDI) and stimulators (GDS) (71). The *Ras* gene family is an extended family of proteins involved in cell growth and behavior. In mammalian cells, mutational activation of the *Ras* proteins leads to oncogene function, and constitutive activation of this pathway leads to malignant transformation (72).

The principal intracellular messengers derived from activation of G-protein-coupled receptors are cAMP and IP_3. cAMP may be degraded by phosphodiesterase (PDE) or it may activate cAMP-dependent protein kinase (PKA). The activation of this enzyme involves dissociation of the inactive form (R_2C_2) into the active form which subsequently phosphorylates specific proteins (Fig. 12). In contrast, IP_3, one of the products of receptor-

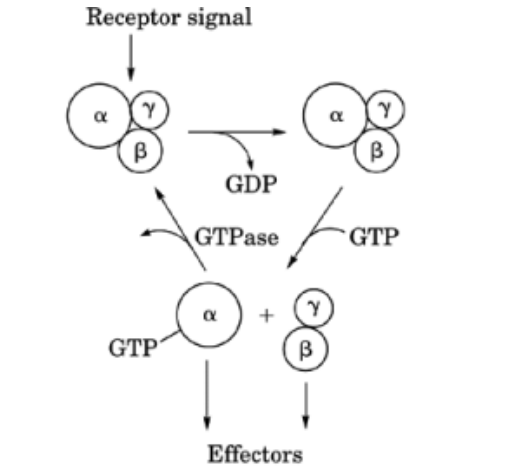


Fig. 10. The receptor–G-protein sequence. An activated receptor interacts with the trimeric GDP-ligated receptor to cause an interchange of GDP by GTP and dissociation into the activated Gα–GTP (left) and G$\beta\gamma$ (right) subunits. These then interact with a variety of effectors. The purpose of the activated receptor is to act as a switch for the G-protein complex.

mediated phospholipase C breakdown of phosphatidylinositol (PI) (Fig. 13), acts on specific receptors in the endoplasmic reticulum to release Ca^{2+} from intracellular sources. The other product of PI turnover is a 1,2-diacylglycerol that activates protein kinase C (PKC). This is also the receptor for the tumor-promoting phorbol esters (73). These diacylglycerols can be cleaved by monoacyl- or diacylglycerol kinases to yield arachidonic acid, a precursor to the prostaglandins (qv) and thromboxanes.

4.5. Ion Channels

The excitable cell maintains an asymmetric distribution across both the plasma membrane, defining the extracellular and intracellular environments, as well as the intracellular membranes which define the cellular organelles. This maintained asymmetric distribution of ions serves two principal objectives. It contributes to the generation and maintenance of a potential gradient and the subsequent generation of electrical currents following appropriate stimulation. Moreover, it permits the ions themselves to serve as cellular messengers to link membrane excitation and cellular response (74). In some instances, the current itself may be the response, as, for example, in the electric organ of electric fishes. In most instances, however, the current serves to initiate or modulate another cellular response, including propagation of impulses in nerve fibers, and alteration of the sensitivity of membranes to other stimuli or coupling to cellular responses such as contraction and secretion. In the latter examples, a role for calcium is particularly prominent because Ca^{2+} can serve as both a current-carrying and a messenger species (75).

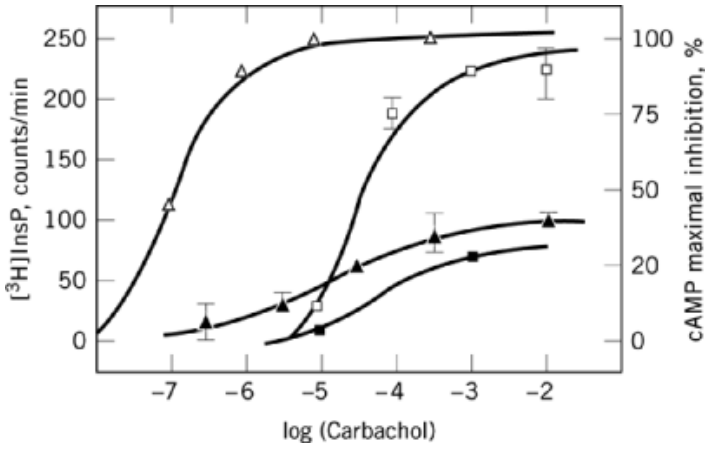


Fig. 11. Dose–response curves for (△,▲) inhibition of cyclic AMP formation and stimulation of IP_3 formation by carbachol (△,□) before and (▲,■) after reduction of receptor number by irreversible alkylation; (carbachol) is in *M*. Error bars (⌶) are shown for some studies.(Courtesy of the American Society of Pharmacology and Experimental Therapeutics (68).)

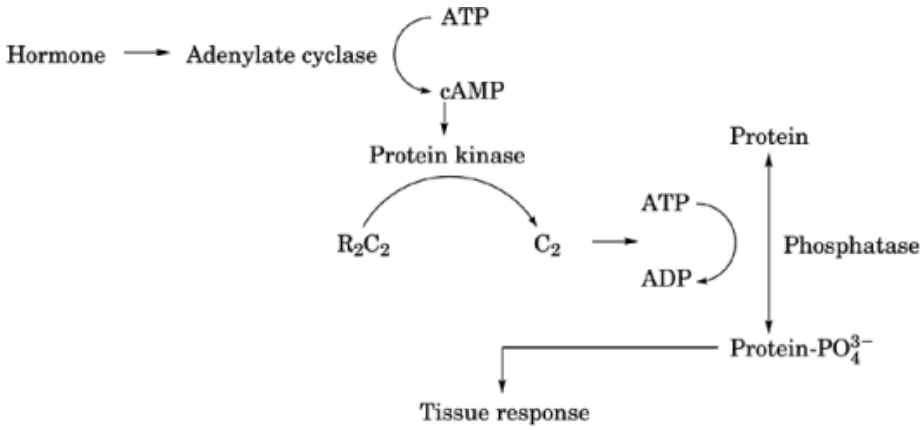


Fig. 12. The hormone-activated adenylyl cyclase cascade.

Regulation of ion channels by drugs may have excitatory or inhibitory effects according to the channels affected (Fig. 14). Thus, activation of sodium or Ca^{2+} channels represents stimulatory events, driving the membrane potential to the depolarized equilibrium potential for these ions. In contrast, activation of K^+ or Cl^- channels generally increases membrane potential representing an inhibitory signal. Conversely, antagonists at Na^+ and Ca^{2+} channels generally have inhibitory effects, whereas antagonists at K^+ and Cl^- channels are excitatory (Table 5).

Channels may be regulated exclusively by electrical or chemical signals corresponding to purely voltage-gated or ligand-gated channels, respectively. However, receptor activation may alter the operation of a potential-dependent channel, for example, by phosphorylation processes. Many ligand-sensitive ion channel processes show limited dependence on membrane potential because of the influence of electric field on the dipoles and orientations of membrane proteins or on the ligands themselves. Receptor processes may alter ion channel activity directly or indirectly. Thus, the nicotinic acetylcholine receptor consists of five subunits, two of which comprise the acetylcholine binding sites and all of which comprise the ion channel (75). This constitutes a

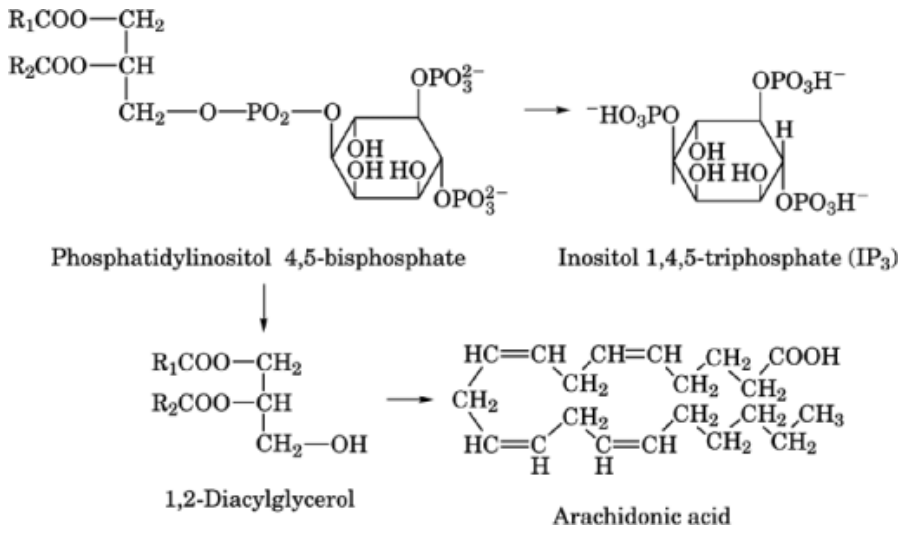


Fig. 13. The phosphatidylinositol pathway.

Table 5. Drug Activity at Ion Channels

	Drug activity	
Ion channel	Inhibitory	Stimulatory
calcium	verapamil; nifedipine; diltiazem	Bay K 8644
potassium	glibenclamide; quaternary ammonium salts; charybdotoxin and other toxins	minoxidil; pinacidil
sodium	tetrodotoxin; saxitoxin; local anesthetics; DDT	veratridine

direct linkage. In many instances, however, the link is indirect via a second messenger, such as cAMP or cGMP, derived from stimulation of adenylate and guanylate cyclases or inositol polyphosphate derived from phospholipase C-stimulated hydrolysis of inositol phospholipids (76). Regardless of regulatory mechanism, ion channels may be regarded as allosteric enzymes. The function is to accelerate the transit of ions across an essentially impermeable barrier and to be responsive to a variety of heterotropic signals (74).

Ion channels may be regarded as pharmacological receptors frequently possessing a multiplicity of drug binding sites (77, 78). These sites may be for endogenous physiological regulators or for endogenous or synthetic agents. This is illustrated in terms of the ligand-gated γ-aminobutyric acid channel (Fig. 15) and the voltage-gated Ca^{2+} channel (Fig. 16). Ion channels may be regarded, in a limiting sense, as probabilistic devices existing in two states, open (conducting) and closed (nonconducting). The current flowing is given by

$$I = N_f \cdot P_o \cdot i \qquad (19)$$

where N_f is the number of functional channels, P_o is the opening probability, and i is the unitary, ie, single channel, current. Further,

$$N_f = N_{tot} \cdot P_f \qquad (20)$$

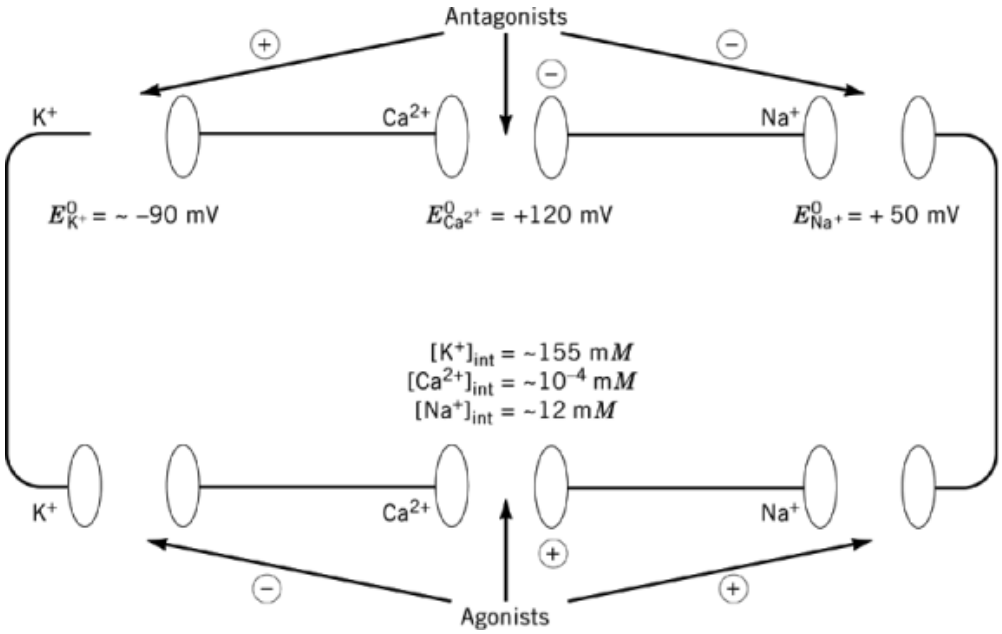


Fig. 14. The cellular ionic environment depicting representative intracellular ionic concentrations and the equilibrium potentials, E^0, for individual ions. Excitatory and inhibitory events are represented by $-$ and $+$, respectively. Thus, K^+ channel agonists and antagonists are inhibitory and excitatory, respectively; Ca^{2+} channel antagonists and activators are inhibitory and excitatory, respectively.

where N_{tot} is the total number of ion channels and P_f is the probability that a channel is available. Drugs may affect the probability of channel availability or opening either by biochemical events, including G-protein interaction and phosphorylation, or biophysically by altering the voltage dependence of activation or inactivation.

4.6. Tyrosine Kinase Receptors

The polypeptide growth factors control cell proliferation, differentiation, and survival (79). Several distinct subfamilies of receptor tyrosine kinases exist and at least nine have been characterized (80). These include families for epidermal growth factor, insulin and insulin-related factors, fibroblast growth factors, and neurotrophin receptors such as nerve growth factor and brain-derived neurotrophic factor. All of these receptors have kinetics that share certain fundamental signaling properties. Ligand binding to the extracellular domain activates a tyrosine kinase of the cytoplasmic domain. Subsequently, a variety of downstream signaling molecules are activated. These include phospholipase C, GTPase activating factor (GAP), *Ras*, and MAP kinases (79, 81, 82).

4.7. Guanylyl Cyclase Receptors

Cyclic GMP concentrations (cGMP) rise in response to a number of cell signals (83). Membrane-associated guanylyl cyclase catalyzes the conversion of guanosine triphosphate (GTP) to cGMP (84). This enzyme resembles in organization the tyrosine kinases having an intracellular protein kinase-like domain and a cyclase catalytic domain. The enzymes are activated by several distinct species that include atrial natriuretic peptide (ANF) and peptides related to the heart-stable enterotoxins.

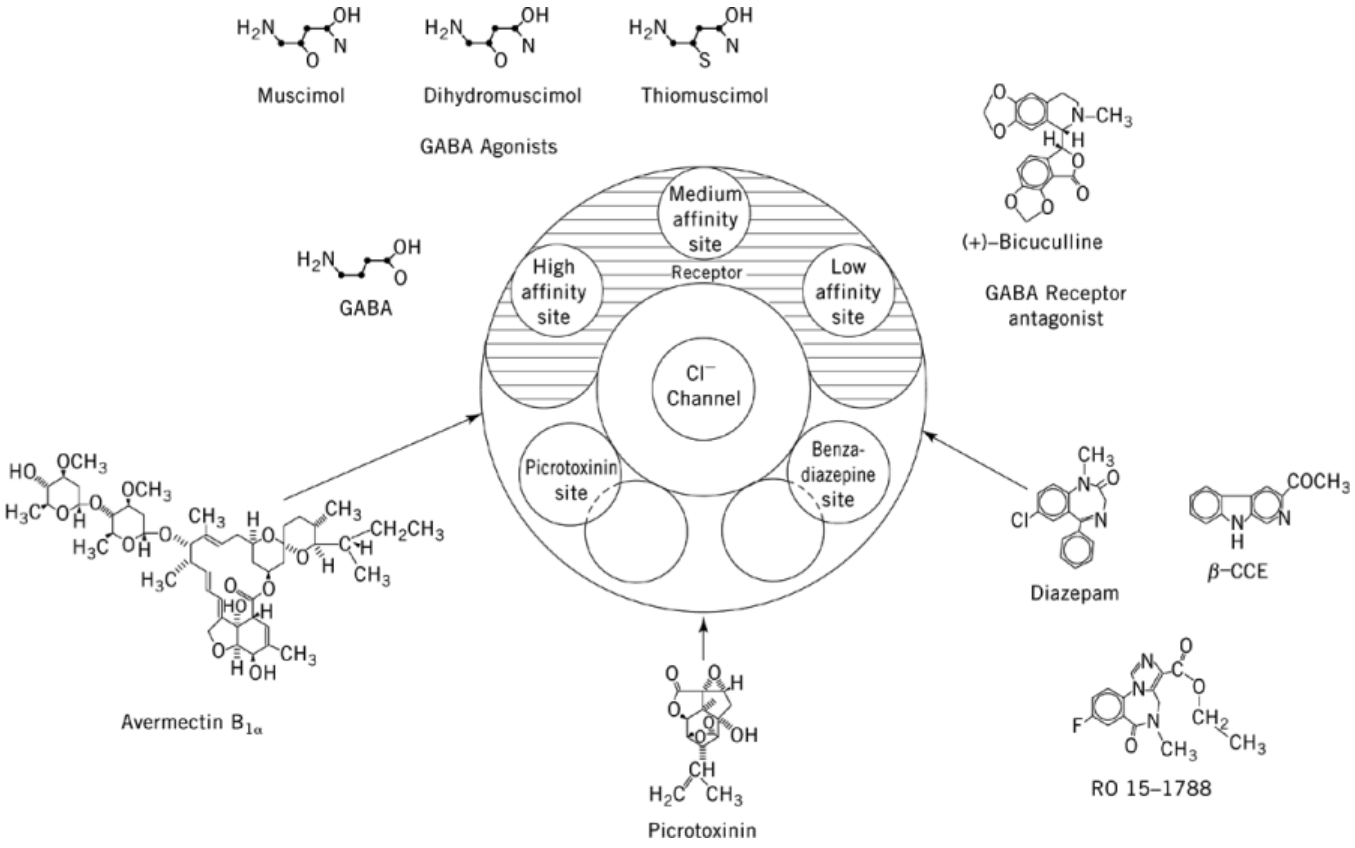


Fig. 15. Drug binding sites associated with the $GABA_A$ receptor–channel complex where (–●–) represents the carbon backbone of GABA agonists.

In contrast, the soluble guanylyl cyclases are regulated by nitric oxide and NO-forming drugs through the Ca^{2+} calmodulin-dependent nitric oxide synthase (85, 86). The soluble cGMP, derived either from the particulate or soluble forms of the enzyme, now functions as a second messenger to interact with a number of discrete pathways, including cGMP-gated ion channels, cGMP-inhibited cAMP phosphodiesterase, cGMP-stimulated cAMP phosphodiesterase, and cGMP-dependent protein kinases.

4.8. Receptor Regulation and Defects

Specific recognition and the initiation of response are the accepted attributes of the drug–receptor interaction. However, target cells can alter on both short- and long-term time scales their sensitivity to drugs. Such regulation, achieved by altering the number and/or affinity of receptors, is well established for all receptor systems and can be viewed as an integral component of the drug–receptor interaction. In this view, subsequent to the formation of the drug–receptor complex with agonist, the continued existence of the drug–receptor complex may lead to one or more phases of desensitization, according to which there may occur initially transient and subsequently prolonged phases of reduced or lost sensitivity (1, 86). Occupancy by antagonist, in contrast, leads to an increased number of receptors and increased drug sensitivity. This phenomenon may contribute to clinical rebound during abrupt withdrawal from drugs, including β-blockers (87–89). Additional

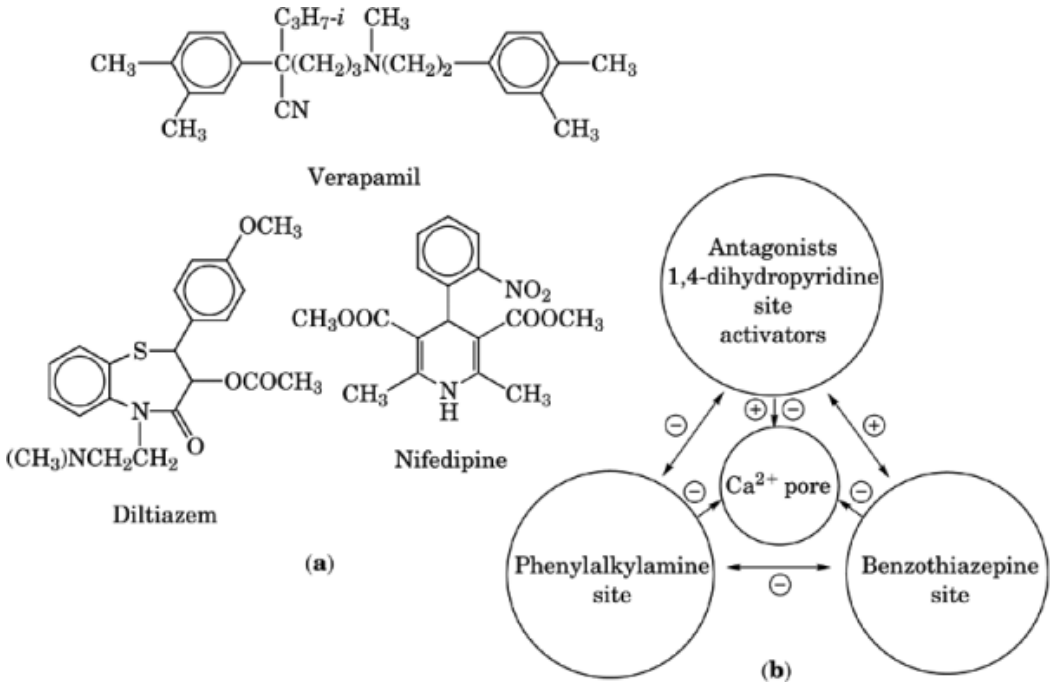


Fig. 16. (**a**) Structural formulas of the first-generation Ca^{2+} channel antagonists indicating chemical heterogeneity consistent with interaction at discrete drug binding sites associated with (**b**) the voltage-gated l-type of Ca^{2+} channel. The second-generation 1,4-dihydropyridine antagonists (amlodipine, felodipine, isradipine, nicardipine, and nimodipine) interact at the nifedipine site.

to this homologous regulation, receptor sensitivity may be controlled through heterologous influences, whereby hormones, including thyroid and corticosteroids, regulate other receptors. These regulatory events are made possible because pharmacologic receptors, in common with other cellular components, are in dynamic balance between synthesis and degradation. This balance is sensitive to a number of influences that include agonist and antagonist presence.

There are probably several processes that contribute to the total desensitization process and these may be directed homologously (to own receptor) or heterologously (to other receptor). Additionally, the influences may be directed at the receptor itself and affect only that receptor, ie, specific desensitization, or may affect other receptor processes as well, ie, nonspecific desensitization.

A number of distinct processes underlie the several receptor regulatory events and these may be distinguished in part by the time scale on which they occur. Cells frequently exhibit several desensitization events. Rapid desensitization processes frequently occur with ion channels of both the ligand-gated and voltage-gated families. On this time scale, channels may open and subsequently close in the maintained presence of the drug or stimulus in seconds or less (74). Such a process is usually rapidly reversible and involves the formation of a closed channel state. For G-protein coupled receptors, and particularly for the β-adrenergic receptor, the desensitization process has been shown to involve several stages. Both protein kinase A (PKA), activated through cAMP, and β-adrenergic receptor kinase (bARK) are involved, and the receptor phosphorylation, at different sites, uncouples the receptor and G-protein (90, 91). At low agonist concentrations, phosphorylation is principally through the PKA pathway; at high agonist concentrations, both the PKA and bARK pathways are involved. Continued occupancy by agonist leads to a second phase of desensitization in which the receptor

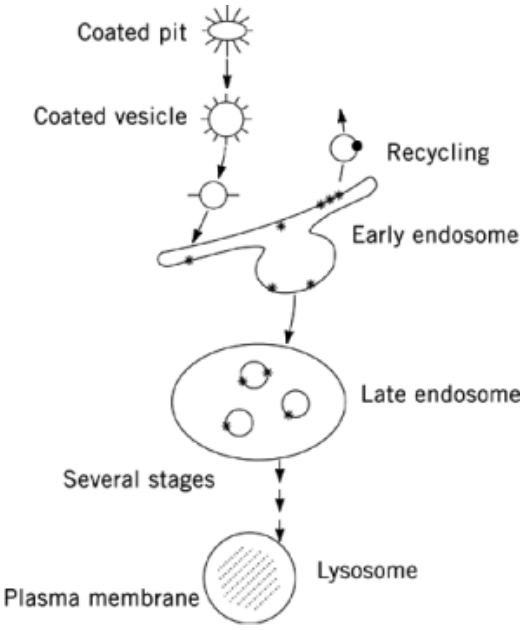


Fig. 17. The receptor-internalization process via a coated pit in which receptors are internalized and processed in a multistep pathway before being recycled or reprocessed.

is sequestered or transiently and reversibly internalized in vesicular form (Fig. 17). Further occupancy leads to down-regulation proper in which the receptors are internalized and reprocessed through the lysosomal machinery. Similar events, but differing in detail, seem likely to occur for some other receptors.

Specific processes of endocytosis occur that serve to translocate receptor, ligand, or both into the cell interior and which function also as part of the cell's physiological control mechanisms (92–95). This endocytotic process described for low density lipoprotein (LDL) entry into the cell via the LDL receptor (Fig. 18) is used by a large number of receptors and ligands, including LDL, asialoglycoproteins, transferrin, class I and II major histocompatability complex (MHC) molecules, epidermal growth factors (EGF), and immunoglobulin G (IgG). In some instances, the receptor recycles and the ligand (LDL, viruses, peptide hormones, asialoglycoproteins) is degraded. In others, both receptor and ligand (transferrin) recycle or both are degraded (EGF).

An increasing number of diseases are known to be linked to defects in receptor structure, function, or coupling. The defects may lie at several locations: in the structure of the receptor, which may alter its ability either to bind drugs, to be inserted into the membrane, or to couple to effectors (including G-proteins); in the coupling protein; or in the presence of autoantibodies, which can proceed to activate, block, or lyse the receptors and its components (96–99).

Autoantibodies are directed against nicotinic acetylcholine receptors in myasthenia gravis, resulting in receptor loss, skeletal muscle paralysis, and dysfunction (100). In addition, antibodies directed against voltage-gated Ca^{2+} channels produce similar neuromuscular dysfunction of Lambert-Eaton syndrome (101), and antibodies in amyotrophic lateral sclerosis (Lou Gehrig's disease) may contribute to muscle wastage and loss by overstimulating the Ca^{2+} channels (102).

In type A behavior in humans, an association has been suggested to correlate with the ratio of peripheral α_2- and β_2-adrenoceptor densities (103). Catecholamine receptors are well established to be altered by a variety of homologous and heterologous influences (104). Thus, in hyperthyroidism, there is an increased level of sympathetic activity associated with increased expression of α- and β-adrenoceptors.

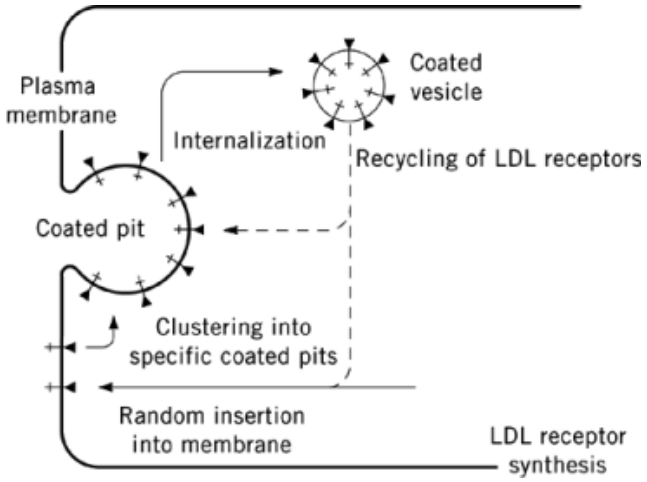


Fig. 18. Schematic representation of cycling of low density lipoprotein (LDL) receptors from the plasma membrane to the cell interior.(Courtesy of Annual Reviews Inc. (93).)

Structural defects at the receptor level are determinant for a number of receptor diseases. In nephrogenic diabetes insipidus, where patients void large volumes of dilute urine even in the presence of vasopressin (antidiuretic hormone) (105), the disease is linked to mutations in three discrete regions of the G-protein-linked vasopressin (V_2) receptor (106, 107).

Pseudohypoparathyroidism is characterized by end-organ resistance to parathyroid hormone (98, 108). This disease takes various forms, including Albright's hereditary osteodystrophy, which has unusual physical features and a generalized resistance to G-protein-linked hormones that function through cAMP as a second messenger. This defect is associated with a deficiency in the levels of the α-subunit of G_s (109). Because this defect may be generalized, such patients also have olfactory dysfunction (110).

Defects in the LDL receptor have been particularly well explored as a basis of the disease familial hypercholesterolemia (93, 111). A number of defects that collectively impair LDL receptor trafficking, binding, or delivery underlie this disease where LDL and serum cholesterol rise to levels that mediate early cardiovascular mortality. Studies of the population distribution of this defect can determine the source of the original mutation. Thus, in Quebec, about 60% of the individuals suffering from familial hypercholesterolemia have a particular 10-kilobase deletion mutation in the LDL gene (112). This may have arisen from an original founder of the French Canadian settlement in the seventeenth century.

Cystic fibrosis, a disease of the Caucasian population, is associated with defective Cl^- regulation and is essentially a disorder of epithelial cells (113, 114). The defect arises at several levels in the Cl^- ion transporter, ie, the cystic fibrosis transmembrane regulation (CFTR), and is associated with defective Cl^- transport and defective processing, whereby the protein is not correctly incorporated into the cell membrane. The most common mutation, affecting approximately 60% of patients, is termed F 608 and designates the loss of phenylalanine at this position. This mutation appears to be at least 50,000 years old, which suggests that its survival may have had evolutionary significance (115).

4.9. Components of Drug Action and Responses to Drugs

The response to a drug can vary among race, gender, and age groups. It may vary according to disease state and age, and it may vary according to the time of administration (1, 2). These factors may have several origins, including (*1*) compliance, the ability or desire of the subject to take a drug according to a specific regimen;

(*2*) pharmacokinetic, disease-, age-, race-, and gender-based factors that contribute to variable absorption, distribution, metabolism, and excretion of a drug; and (*3*) pharmacodynamic, disease-, age-, race-, and gender-based factors that contribute to variable drug–receptor interactions.

These same responses may also be classified as follows.

(*1*) Geriatric factors: a variety of factors, both pharmacokinetic and pharmacodynamic, that contribute to variable drug responses in the elderly. These responses are not seen for every class of drug. Thus, the depressant effects of the glycosides also appear to increase with aging (116, 117).

(*2*) Pharmacogenetics: the responses to drugs may be significantly different according to heritable factors that can modulate pharmacodynamic or pharmacogenetic factors (118). Atypical cholinesterase occurs in about 1 in 2000 Caucasians and is associated with a markedly reduced sensitivity to hydrolysis of the muscle-relaxant cholinesterase. Similarly, the reduced sensitivity to the anticoagulant warfarin is associated with a reduced receptor affinity.

(*3*) Racial differences: differences in racial sensitivity to drug action are quite common, although the origins of most of these differences have not been determined. In Chinese individuals, the plasma levels of α_1-glycoprotein are lower and drug binding may be lower (119). Several differences have been noted in racial sensitivity to antihypertensive drugs. Males of Chinese descent are more sensitive to the β-antagonist propranolol than are whites (120). This difference arises in part from decreased plasma protein binding of propranolol in Chinese individuals. Similarly, in a comparison of the efficacies of a group of antihypertensive drugs, significant race-related differences were observed. Whites are more sensitive to ACE inhibitors; blacks more sensitive to Ca^{2+} blockers (121).

(*4*) Hormonal factors: drug action can be significantly altered according to hormonal status. Thus, serum levels of α_1-acid glycoprotein vary according to the phase of the human menstrual cycle and set the stage for differences in drug binding (122). During pregnancy, there are significant reductions in the levels of plasma proteins and thus the binding of both basic and acidic drugs is reduced (123).

(*5*) Time factors: increasingly, it is realized that drug effects vary according to biological timing and endogenous periodicities (124). Many examples are known; eg, the effects of ranitidine, a histamine H_2 receptor antagonist, on gastric pH were greatest at night, and the anticancer drugs 5-fluouracil and adriamycin achieved different plasma levels over a period of time.

Factors such as age, gender, race, sex, disease, and time are all integral to considerations of drug efficacy and administration. These are expected to become routine determinants of the analysis of clinical drug action. The study of drug action has moved from phenomenological descriptions at the beginning of the 1900s to quantitative ones. The increasing volume of both chemical (structural) and biological (genetic) information should lead to refinements of the understanding of drug action.

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General References

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