



CHAPTER 4

Pharmacodynamics: Mechanism of Drug Action; Receptor Pharmacology

Pharmacodynamics is the study of drug effects. It attempts to elucidate the complete action-effect sequence and the dose-effect relationship. Modification of the action of one drug by another drug is also an aspect of pharmacodynamics.

PRINCIPLES OF DRUG ACTION

Drugs (except those gene based) do not impart new functions to any system, organ or cell; they only alter the pace of ongoing activity. The basic types of drug action can be broadly classed as:

1. Stimulation It refers to selective enhancement of the level of activity of specialized cells, e.g. adrenaline stimulates heart, pilocarpine stimulates salivary glands. However, excessive stimulation is often followed by depression of that function, e.g. high dose of picrotoxin, a central nervous system (CNS) stimulant, produces convulsions followed by coma and respiratory depression.

2. Depression It means selective diminution of activity of specialized cells, e.g. barbiturates depress CNS, quinidine depresses heart.

Certain drugs stimulate one type of cells but depress the other, e.g. acetylcholine stimulates intestinal smooth muscle but depresses SA node in heart. Thus, most drugs cannot be simply classed as stimulants or depressants.

3. Irritation This connotes a nonselective, often noxious effect and is particularly applied to less specialized cells (epithelium, connective tissue). Mild irritation may stimulate associated function, e.g. bitters increase salivary and gastric secretion, counterirritants increase blood flow to the site. But strong irritation results in inflammation, corrosion, necrosis and morphological damage. This may result in diminution or loss of function.

4. Replacement This refers to the use of natural metabolites, hormones or their congeners in deficiency states, e.g. levodopa in parkinsonism, insulin in diabetes mellitus, iron in anaemia.

5. Cytotoxic action Selective cytotoxic action for invading parasites or cancer cells, attenuating them without significantly affecting the host cells is utilized for cure/palliation of infections and neoplasms, e.g. penicillin, chloroquine, zidovudine, cyclophosphamide, etc.

MECHANISM OF DRUG ACTION

Only a handful of drugs act by virtue of their simple physical or chemical property; examples are:

- Bulk laxatives (ispaghula)—physical mass
- Dimethicone, petroleum jelly—physical form, opacity

- Paraamino benzoic acid—absorption of UV rays
- Activated charcoal—adsorptive property
- Mannitol, mag. sulfate—osmotic activity
- ^{131}I and other radioisotopes—radioactivity
- Antacids—neutralization of gastric HCl
- Pot. permanganate—oxidizing property
- Chelating agents (EDTA, dimercaprol)—chelation of heavy metals.
- Cholestyramine—sequestration of cholesterol in the gut
- Mesna—Scavenging of vasicotoxic reactive metabolites of cyclophosphamide

Majority of drugs produce their effects by interacting with a discrete target biomolecule, which usually is a protein. Such mechanism confers selectivity of action to the drug. Functional proteins that are targets of drug action can be grouped into *four* major categories, *viz.* enzymes, ion channels, transporters and receptors (See Fig. 4.1). However, a few drugs do act on other proteins (e.g. colchicine, vinca alkaloids, taxanes bind to the structural protein tubulin) or on nucleic acids (alkylating agents).

I. ENZYMES

Almost all biological reactions are carried out under catalytic influence of enzymes; hence, enzymes are a very important target of drug action. Drugs can either increase or decrease the rate of enzymatically mediated reactions. However, in physiological systems enzyme activities are often optimally set. Thus, stimulation of enzymes by drugs, that are truly foreign substances, is unusual. Enzyme stimulation is relevant to some natural metabolites only, e.g. pyridoxine acts as a cofactor and increases decarboxylase activity. Several enzymes are stimulated through receptors and second messengers, e.g. adrenaline stimulates hepatic glycogen phosphorylase through β receptors and cyclic AMP. Stimulation of an enzyme increases its affinity for the substrate so that rate constant (k_M) of the reaction is lowered (Fig. 4.2).

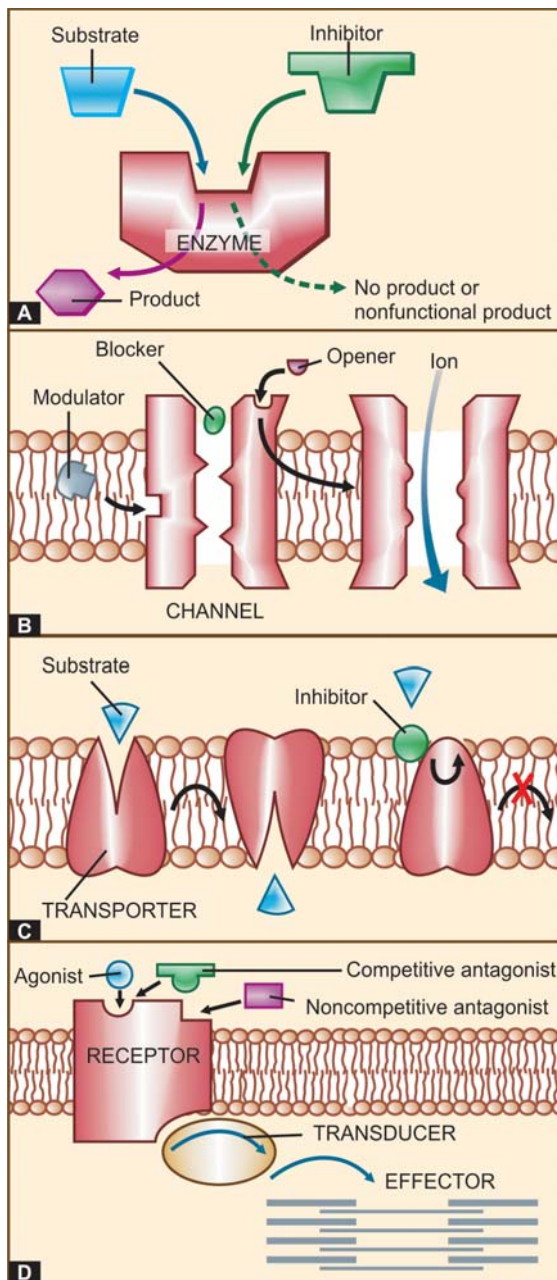


Fig. 4.1: Four major types of biomacromolecular targets of drug action.

(A) Enzyme; (B) Transmembrane ion channel; (C) Membrane bound transporter; (D) Receptor (see text for description)

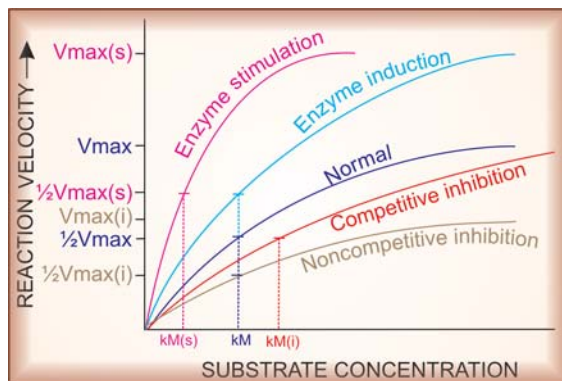


Fig. 4.2: Effect of enzyme induction, stimulation and inhibition on kinetics of enzyme reaction

V_{max} —Maximum velocity of reaction; $V_{max}(s)$ of stimulated enzyme; $V_{max}(i)$ —in presence of noncompetitive inhibitor; kM —rate constant of the reaction; $kM(s)$ —of stimulated enzyme; $kM(i)$ —in presence of competitive inhibitor

Note: Enzyme induction and noncompetitive inhibition don't change the affinity of the enzyme (kM is unaltered), whereas enzyme stimulation and competitive inhibition respectively decrease and increase the kM .

Apparent increase in enzyme activity can also occur by *enzyme induction*, i.e. synthesis of more enzyme protein. This cannot be called stimulation because the kM does not change. Many drugs induce microsomal enzymes (see p. 27).

Inhibition of enzymes is a common mode of drug action.

A. Nonspecific inhibition Many chemicals and drugs are capable of denaturing proteins. They alter the tertiary structure of any enzyme with which they come in contact and thus inhibit it. Heavy metal salts, strong acids and alkalis, alcohol, formaldehyde, phenol inhibit enzymes nonspecifically. Such inhibitors are too damaging to be used systemically.

B. Specific inhibition Many drugs inhibit a particular enzyme without affecting others. Such inhibition is either competitive or noncompetitive.

(i) Competitive (equilibrium type) The drug being structurally similar competes with the normal substrate for the catalytic binding site of the enzyme so that the product is not formed or a

nonfunctional product is formed (Fig. 4.1A), and a new equilibrium is achieved in the presence of the drug. Such inhibitors increase the kM but the V_{max} remains unchanged (Fig. 4.2), i.e. higher concentration of the substrate is required to achieve $\frac{1}{2}$ maximal reaction velocity, but if substrate concentration is sufficiently increased, it can displace the inhibitor and the same maximal reaction velocity can be attained.

- Physostigmine and neostigmine compete with acetylcholine for cholinesterase.
- Sulfonamides compete with PABA for bacterial folate synthetase.
- Moclobemide competes with catecholamines for monoamine oxidase-A (MAO-A).
- Captopril competes with angiotensin 1 for angiotensin converting enzyme (ACE).
- Finasteride competes with testosterone for 5 α -reductase
- Letrozole competes with androstenedione and testosterone for the aromatase enzyme.
- Allopurinol competes with hypoxanthine for xanthine oxidase; is itself oxidized to alloxanthine (a non competitive inhibitor).
- Carbidopa and methyldopa compete with levodopa for dopa decarboxylase.

A *nonequilibrium type* of enzyme inhibition can also occur with drugs which react with the same catalytic site of the enzyme but either form strong covalent bonds or have such high affinity for the enzyme that the normal substrate is not able to displace the inhibitor, e.g.

- Organophosphates react covalently with the esteretic site of the enzyme cholinesterase.
- Methotrexate has 50,000 times higher affinity for dihydrofolate reductase than the normal substrate DHFA.

In these situations, kM is increased and V_{max} is reduced.

(ii) Noncompetitive The inhibitor reacts with an adjacent site and not with the catalytic site, but alters the enzyme in such a way that it loses its catalytic property. Thus, kM is unchanged but V_{max} is reduced. Examples are given in the box.

Noncompetitive inhibitor	Enzyme
Acetazolamide	— Carbonic anhydrase
Aspirin, indomethacin	— Cyclooxygenase
Disulfiram	— Aldehyde dehydrogenase
Omeprazole	— H ⁺ K ⁺ ATPase
Digoxin	— Na ⁺ K ⁺ ATPase
Theophylline	— Phosphodiesterase
Propylthiouracil	— Peroxidase in thyroid
Lovastatin	— HMG-CoA reductase
Sildenafil	— Phosphodiesterase-5

II. ION CHANNELS

Proteins which act as ion selective channels participate in transmembrane signaling and regulate intracellular ionic composition. This makes them a common target of drug action (Fig. 4.1B). Drugs can affect ion channels either through specific receptors (ligand gated ion channels, G-protein operated ion channels, *see* Fig. 4.4 and p. 48), or by directly binding to the channel and affecting ion movement through it, e.g. local anaesthetics which physically obstruct voltage sensitive Na⁺ channels (*See* Ch 26). In addition, certain drugs modulate opening and closing of the channels, e.g.:

- Quinidine blocks myocardial Na⁺ channels.
- Dofetilide and amiodarone block myocardial delayed rectifier K⁺ channel.
- Nifedipine blocks L-type of voltage sensitive Ca²⁺ channel.
- Nicorandil opens ATP-sensitive K⁺ channels.
- Sulfonylurea hypoglycaemics inhibit pancreatic ATP-sensitive K⁺ channels.
- Amiloride inhibits renal epithelial Na⁺ channels.
- Phenytoin modulates (prolongs the inactivated state of) voltage sensitive neuronal Na⁺ channel.
- Ethosuximide inhibits T-type of Ca²⁺ channels in thalamic neurones

III. TRANSPORTERS

Several substrates are translocated across membranes by binding to specific transporters

(carriers) which either facilitate diffusion in the direction of the concentration gradient or pump the metabolite/ion against the concentration gradient using metabolic energy (*see* p. 13–15; Fig. 2.5). Many drugs produce their action by directly interacting with the solute carrier (SLC) class of transporter proteins to inhibit the ongoing physiological transport of the metabolite/ion (Fig. 4.1C). Examples are:

- Desipramine and cocaine block neuronal reuptake of noradrenaline by interacting with norepinephrine transporter (NET).
- Fluoxetine (and other SSRIs) inhibit neuronal reuptake of 5-HT by interacting with serotonin transporter (SERT).
- Amphetamines selectively block dopamine reuptake in brain neurons by dopamine transporter (DAT).
- Reserpine blocks the granular reuptake of noradrenaline and 5-HT by the vesicular amine transporter.
- Hemicholinium blocks choline uptake into cholinergic neurones and depletes acetylcholine.
- The anticonvulsant tiagabine acts by inhibiting reuptake of GABA into brain neurones by GABA transporter GAT 1.
- Furosemide inhibits the Na⁺K⁺2Cl⁻ cotransporter in the ascending limb of loop of Henle.
- Hydrochlorothiazide inhibits the Na⁺Cl⁻ symporter in the early distal tubule.
- Probenecid inhibits active transport of organic acids (uric acid, penicillin) in renal tubules by interacting with organic anion transporter (OAT).

IV. RECEPTORS

The largest number of drugs do not bind directly to the effectors, *viz.* enzymes, channels, transporters, structural proteins, template biomolecules, etc. but act through specific regulatory macromolecules which control the above listed effectors. These regulatory macromolecules or the sites on them which bind and interact with the drug are called 'receptors'.

Receptor: It is defined as a macromolecule or binding site located on the surface or inside the effector cell that serves to recognize the signal molecule/drug and initiate the response to it, but itself has no other function.

Though, in a broad sense *all types* of target biomolecules, including the effectors (enzymes, channels, transporters, etc.) with which a drug can bind to produce its action have been denoted as 'receptors' by some authors, such designation tends to steal the specific meaning of this important term. If so applied, xanthine oxidase would be the 'receptor' for allopurinol, L-type Ca^{2+} channel would be the 'receptor' for nifedipine, serotonin transporter (SERT) would be the 'receptor' for fluoxetine; a connotation not in consonance with the general understanding of the term. It is therefore better to reserve the term 'receptor' for purely regulatory macromolecules which combine with and mediate the action of signal molecules including drugs.

The following terms are used in describing drug-receptor interaction:

Agonist An agent which activates a receptor to produce an effect similar to that of the physiological signal molecule.

Inverse agonist An agent which activates a receptor to produce an effect in the opposite direction to that of the agonist.

Antagonist An agent which prevents the action of an agonist on a receptor or the subsequent response, but does not have any effect of its own.

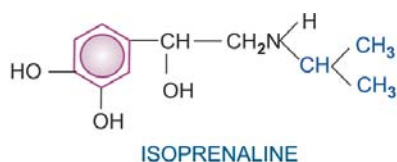
Partial agonist An agent which activates a receptor to produce submaximal effect but antagonizes the action of a full agonist.

Ligand (Latin: *ligare*—to bind) Any molecule which attaches selectively to particular receptors or sites. The term only indicates affinity or binding without regard to functional change: agonists and competitive antagonists are both ligands of the same receptor.

The overall scheme of drug action through receptors is depicted in Fig. 4.1D.

Basic evidences for drug action through receptors

(i) Many drugs exhibit structural specificity of action, i.e. specific chemical configuration is associated with a particular action, e.g. isopropyl substitution on the ethylamine side chain of sympathetic drugs produces compounds with marked cardiac and bronchial activity—most β adrenergic agonists and antagonists have this substitution. A 3 carbon internitrogen separation in the side chain of phenothiazines



results in antidopaminergic-antipsychotic compounds, whereas 2 carbon separation produces anticholinergic-antihistaminic compounds. Further, chiral drugs show stereospecificity in action, e.g. *levo* noradrenaline is 10 times more potent than *dextro* noradrenaline; *d*-propranolol is about 100 times less potent in blocking β receptors than the *l*-isomer, but both are equipotent local anaesthetics.

Thus, the cell must have some mechanism to recognize a particular chemical configuration and three dimensional structure.

(ii) Competitive antagonism is seen between specific agonists and antagonists. Langley in 1878 was so impressed by the mutual antagonism among two alkaloids pilocarpine and atropine that he proposed that both reacted with the same 'receptive substance' on the cell. Ehrlich (1900) observed quantitative neutralization between toxins and antitoxins and designated 'receptor' to be the anchoring group of the protoplasmic molecule for the administered compound.

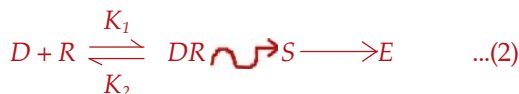
(iii) It was calculated by Clark that adrenaline and acetylcholine produce their maximal effect on frog's heart by occupying only 1/6000th of the cardiac cell surface—thus, special regions of reactivity to such drugs must be present on the cell.

Receptor occupation theory

After studying quantitative aspects of drug action, Clark (1937) propounded a theory of drug action based on occupation of receptors by specific drugs and that the pace of a cellular function can be altered by interaction of these receptors with drugs which, in fact, are small molecular ligands. He perceived the interaction between the two molecular species, *viz.* drug (D) and receptor (R) to be governed by the law of mass action, and the effect (E) to be a direct function of the drug-receptor complex (DR) formed:



Subsequently, it has been realized that occupation of the receptor is essential but not itself sufficient to elicit a response; the agonist must also be able to activate (induce a conformational change in) the receptor. The ability to bind with the receptor designated as *affinity*, and the capacity to induce a functional change in the receptor designated as *intrinsic activity (IA)* or *efficacy* are independent properties. Competitive antagonists occupy the receptor but do not activate it. Moreover, certain drugs are partial agonists which occupy and submaximally activate the receptor. An all or none action is not a must at the receptor. A theoretical quantity (S) denoting strength of stimulus imparted to the cell was interposed in the Clark's equation:



Depending on the agonist, DR could generate a stronger or weaker S, probably as a function of the conformational change brought about by the agonist in the receptor. Accordingly:

Agonists have both affinity and maximal intrinsic activity (IA = 1), e.g. adrenaline, histamine, morphine.

Competitive antagonists have affinity but no intrinsic activity (IA = 0), e.g. propranolol, atropine, chlorpheniramine, naloxone.

Partial agonists have affinity and submaximal intrinsic activity (IA between 0 and 1), e.g. dichloroisoproterenol (on β adrenergic receptor), pentazocine (on μ opioid receptor).

Inverse agonists have affinity but intrinsic activity with a minus sign (IA between 0 and -1), e.g. DMCM (on benzodiazepine receptor).

It has also been demonstrated that many full agonists can produce maximal response even while occupying <1% of the available receptors.

A large receptor reserve exists in their case, or a number of *spare receptors* are present.

The two-state receptor model

A very attractive alternative model for explaining the action of agonists, antagonists, partial agonists and inverse agonists has been proposed.

The receptor is believed to exist in two interchangeable states: *R_a* (active) and *R_i* (inactive) which are in equilibrium. In the case of majority of receptors, the *R_i* state is favoured at equilibrium—no/very weak signal is generated in the absence of the agonist—the receptor exhibits no constitutive activation (Fig. 4.3I). The agonist (A) binds preferentially to the *R_a* conformation and shifts the equilibrium $\rightarrow R_a$ predominates and a response is generated (Fig. 4.3II) depending on

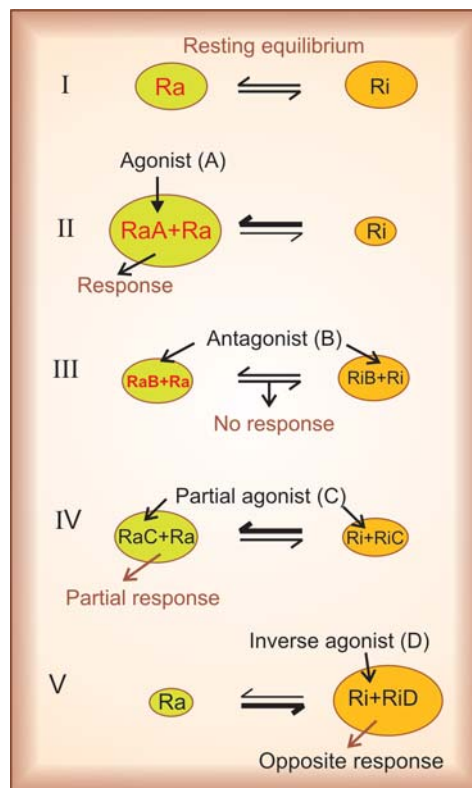


Fig. 4.3: Illustration of the two-state receptor model (see text for explanation)

the concentration of A. The competitive antagonist (B) binds to R_a and R_i with equal affinity \rightarrow the equilibrium is not altered \rightarrow no response is generated (Fig. 4.3 III), and when the agonist is applied fewer R_a are available to bind it—response to agonist is decreased. If an agonist has only slightly greater affinity for R_a than for R_i , the equilibrium is only modestly shifted towards R_a (Fig. 4.3 IV) even at saturating concentrations \rightarrow a submaximal response is produced and the drug is called a partial agonist (C). The inverse agonist (D) has high affinity for the R_i state (Fig. 4.3 V), therefore it can produce an opposite response, provided the resting equilibrium was in favour of the R_a state. Certain receptors (mainly G-protein coupled ones) such as benzodiazepine, histamine H_2 , angiotensin AT_1 , adrenergic β_1 and cannabinoid receptors exhibit constitutive activation, i.e. an appreciable intensity signal is generated even in the basal state (no agonist present). In their case the inverse agonist stabilizes the receptor in the inactive conformation resulting in an opposite response. Only few inverse agonists are known at present, but as more receptors with constitutive activation are found, more inverse agonists are likely to be discovered.

This model has gained wide acceptance because it provides an explanation for the phenomenon of positive cooperativity often seen with neurotransmitters, and is supported by studies of conformational mutants of the receptor with altered equilibrium.

Nature of receptors

Receptors are regulatory macromolecules, mostly proteins, though nucleic acids may also serve as receptors. They are no longer hypothetical. Hundreds of receptor proteins have been isolated, purified, cloned and their primary amino acid (AA) sequence has been worked out. Molecular cloning has also helped in obtaining the receptor protein in larger quantity to study its structure and properties, and in subclassifying receptors. The cell surface receptors with their coupling and

effector proteins are considered to be floating in a sea of membrane lipids; the folding, orientation and topography of the system being determined by interactions between the lipophilic and hydrophilic domains of the peptide chains with solvent molecules (water on one side and lipids on the other). Nonpolar portions of the AA chain tend to bury within the membrane, while polar groups tend to come out in the aqueous medium. In such a delicately balanced system, it is not difficult to visualize that a small molecular ligand binding to one site in the receptor molecule could be capable of tripping the balance (by altering distribution of charges, etc.) and bringing about conformational changes at distant sites. Each of the four major families of receptors (described later) have a well defined common structural motif, while the individual receptors differ in the details of amino acid sequencing, length of intra/extracellular loops, etc. Majority of receptor molecules are made up of several non-identical subunits (heteropolymeric), and agonist binding has been shown to bring about changes in their quaternary structure or relative alignment of the subunits, e.g. on activation the subunits of nicotinic receptor move apart opening a centrally located cation channel.

Radioligand binding studies have helped in characterizing and classifying receptors even when they have been dissociated from the effector system.

Many drugs act upon *physiological receptors* which mediate responses to transmitters, hormones, autacoids and other endogenous signal molecules; examples are cholinergic, adrenergic, histaminergic, steroid, leukotriene, insulin and other receptors. In addition, now some truly *drug receptors* have been described for which there are no known physiological ligands, e.g. benzodiazepine receptor, sulfonylurea receptor, cannabinoid receptor.

Receptor subtypes

The delineation of multiple types and subtypes of receptors for signal molecules has played an important role in the development of a number of

targeted and more selective drugs. Even at an early stage of evolution of receptor pharmacology, it was observed that actions of acetylcholine could be grouped into 'muscarinic' and 'nicotinic' depending upon whether they were mimicked by the then known alkaloids muscarine or nicotine. Accordingly, they were said to be mediated by two types of cholinergic receptors, *viz.* muscarinic (M) or nicotinic (N); a concept strengthened by the finding that muscarinic actions were blocked by atropine, while nicotinic actions were blocked by curare. In a landmark study, Ahlquist (1948) divided adrenergic receptors into ' α ' and ' β ' on the basis of two distinct rankorder of potencies of adrenergic agonists. These receptors have now been further subdivided ($M_1, M_2 \dots M_5$), (N_M, N_N) (α_1, α_2) ($\beta_1, \beta_2, \beta_3$). Multiple subtypes of receptors for practically all transmitters, autacoids, hormones, etc. are now known and have paved the way for introduction of numerous clinically superior drugs. In many cases, receptor classification has provided sound explanation for differences observed in the actions of closely related drugs.

The following criteria have been utilized in classifying receptors:

- Pharmacological criteria** Classification is based on relative potencies of selective agonists and antagonists. This is the classical and oldest approach with direct clinical bearing; was used in delineating M and N cholinergic, α and β adrenergic, H_1 and H_2 histaminergic receptors, etc.
- Tissue distribution** The relative organ/tissue distribution is the basis for designating the subtype, e.g. the cardiac β adrenergic receptors as β_1 , while bronchial as β_2 . This division was confirmed by selective agonists and antagonists as well as by molecular cloning.
- Ligand binding** Measurement of specific binding of high affinity radio-labelled ligand to cellular fragments (usually membranes) *in vitro*, and its displacement by various selective agonists/antagonists is used to delineate receptor subtypes. Multiple 5-HT receptors were distinguished by this approach. Autoradiography has helped in mapping distribution of receptor subtypes in the brain and other organs.
- Transducer pathway** Receptor subtypes may be distinguished by the mechanism through which their activation is linked to the response, e.g. M cholinergic receptor acts through G-proteins, while N cholinergic receptor gates influx of Na^+ ions; α adrenergic receptor

acts *via* IP_3 -DAG pathway and by decreasing cAMP, while β adrenergic receptor increases cAMP; GABA_A receptor is a ligand gated Cl^- channel, while GABA_B receptor increases K^+ conductance through a G-protein.

- Molecular cloning** The receptor protein is cloned and its detailed amino acid sequence as well as three dimensional structure is worked out. Subtypes are designated on the basis of sequence homology. This approach has in the recent years resulted in a flood of receptor subtypes and several isoforms (which do not differ in ligand selectivity) of each subtype. The functional significance of many of these subtypes/isoforms is dubious. Even receptors without known ligands (orphan receptors) have been described.

Application of so many approaches has thrown up several detailed, confusing and often conflicting classifications of receptors. However, a consensus receptor classification is now decided on a continuing basis by an expert group of the International Union of Pharmacological Sciences (IUPHAR).

Silent receptors These are sites which bind specific drugs but no pharmacological response is elicited. They are better called *drug acceptors* or *sites of loss*, e.g. plasma proteins which have binding sites for many drugs. To avoid confusion, the term receptor should be restricted to those regulatory binding sites which are capable of generating a response.

ACTION-EFFECT SEQUENCE

'Drug action' and 'drug effect' are often loosely used interchangeably, but are not synonymous.

Drug action It is the initial combination of the drug with its receptor resulting in a conformational change in the latter (in case of agonists), or prevention of conformational change through exclusion of the agonist (in case of antagonists).

Drug effect It is the ultimate change in biological function brought about as a consequence of drug action, through a series of intermediate steps (transducer).

Receptors subserve two essential functions, *viz.* *recognition* of the specific ligand molecule and *transduction* of the signal into a response. Accordingly, the receptor molecule has a *ligand binding domain* (spatially and energetically suitable for binding the specific ligand) and an *effector domain* (Fig. 4.4) which undergoes a functional conformational change. These domains have

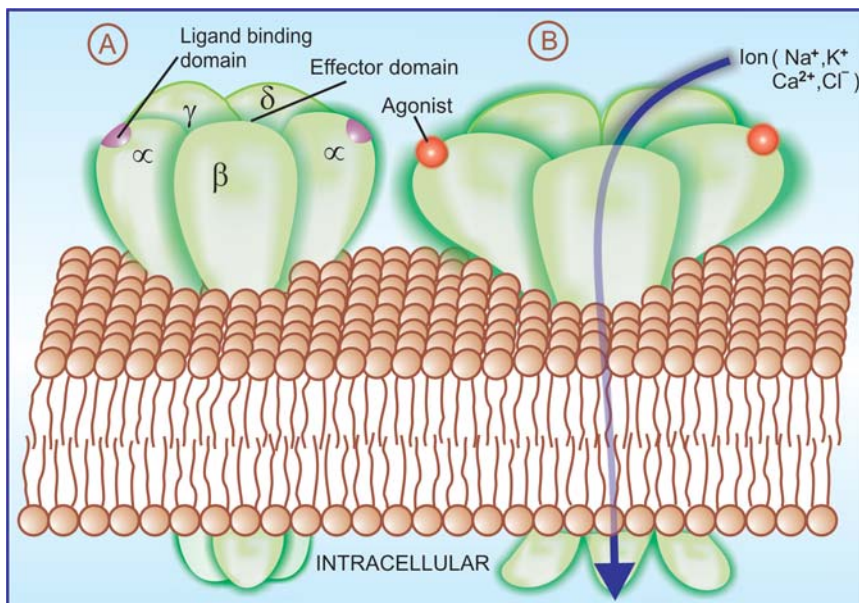


Fig. 4.4: Diagrammatic representation of receptor mediated operation of membrane ion channel.

In case of nicotinic cholinergic receptor, the molecule (8 nm in diameter) is composed of 5 subunits ($2\alpha + \beta + \gamma + \delta$) enclosing a cylindrical ion channel. Normally the channel is closed (A). When two molecules of acetylcholine bind to the two α subunits (B), all subunits move apart opening the central pore to 0.7 nm, enough to allow passage of partially hydrated Na⁺ ions. Anions are blocked from passage through the channel by positive charges lining it. In other cases, K⁺, Ca²⁺ or Cl⁻ ions move through the channel depending on its ion selectivity.

now actually been identified in some receptors. The perturbation in the receptor molecule is variously translated into the response. The sequential relationship between drug action, transducer and drug effect can be seen in Fig. 4.1D and 4.6.

TRANSDUCER MECHANISMS

Considerable progress has been made in the understanding of transducer mechanisms which in most instances have been found to be highly complex multistep processes that provide for amplification and integration of concurrently received extra- and intra-cellular signals at each step. Because only a handful of transducer pathways are shared by a large number of receptors, the cell is able to generate an integrated response reflecting the sum total of diverse signal input. The transducer mechanisms can be grouped into 4 major categories. Receptors falling in one

category have also been found to possess considerable structural homology, and belong to one super family of receptors.

1. G-protein coupled receptors (GPCR)

These are a large family of cell membrane receptors which are linked to the effector (enzyme/channel/carrier protein) through one or more GTP-activated proteins (G-proteins) for response effectuation. All such receptors have a common pattern of structural organization (Fig. 4.5). The molecule has 7 α -helical membrane spanning hydrophobic amino acid (AA) segments which run into 3 extracellular and 3 intracellular loops. The agonist binding site is located somewhere between the helices on the extracellular face, while another recognition site formed by cytosolic segments binds the coupling G-protein. The G-proteins float in the membrane with their exposed

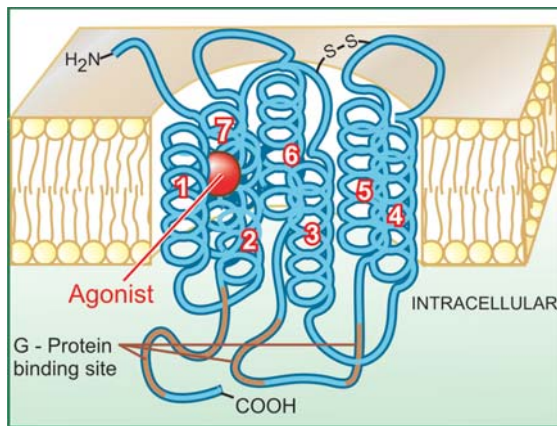


Fig. 4.5: Diagrammatic representation of G-protein coupled receptor molecule

The receptor consists of 7 membrane spanning helical segments of hydrophobic amino acids. The intervening segments connecting the helices form 3 loops on either side of the membrane. The amino terminus of the chain lies on the extracellular face, while the carboxy terminus is on the cytosolic side. The approximate location of the agonist and G-protein binding sites is indicated

domain lying in the cytosol, and are heterotrimeric in composition (α , β and γ subunits). In the inactive state GDP is bound to their exposed domain; activation through the receptor leads to displacement of GDP by GTP. The active α -subunit carrying GTP dissociates from the other two subunits and either activates or inhibits the effector. The $\beta\gamma$ subunits have also been shown to modulate certain effectors like receptor-operated K^+ channels, adenylylcyclase (AC) and phospholipase C.

A number of G proteins distinguished by their α subunits have been described. The important ones with their action on the effector are:

- Gs : Adenylyl cyclase \uparrow , Ca^{2+} channel \uparrow
- Gi : Adenylyl cyclase \downarrow , K^+ channel \uparrow
- Go : Ca^{2+} channel \downarrow
- Gq : Phospholipase C \uparrow
- G₁₃ : Na^+/H^+ exchange \uparrow

In addition Gn, Gk, Gt and G_{olf} have been distinguished. A limited number of G-proteins are shared between different receptors and one receptor can utilize more than one G-protein (agonist pleotropy), e.g. the following couplers have been associated with different receptors.

Receptor	Coupler
Muscarinic	Gi, Go, Gq
Dopamine D2	Gi, Go
β -adrenergic	Gs, Gi
α_1 -adrenergic	Gq
α_2 -adrenergic	Gi, Gs, Go
GABA _B	Gi, Go
5-HT	Gi, Gq, Gs, Gk

In addition, a receptor can utilize different biochemical pathways in different tissues.

The α -subunit has GTPase activity: the bound GTP is slowly hydrolysed to GDP: the α -subunit then dissociates from the effector to rejoin its other subunits, but not before the effector has been activated/inhibited for a few seconds and the signal has been amplified. The onset time of response through this type of receptors is also in seconds.

There are three major effector pathways (Table 4.1) through which GPCRs function.

(a) Adenylylcyclase: cAMP pathway Activation of AC results in intracellular accumulation of second messenger cAMP (Fig. 4.6) which functions mainly through cAMP-dependent protein kinase (PK_A). The PK_A phosphorylates and alters the function of many enzymes, ion channels, transporters and structural proteins to manifest as increased contractility/impulse generation (heart), relaxation (smooth muscle), glycogenolysis, lipolysis, inhibition of secretion/mediator release, modulation of junctional transmission, hormone synthesis, etc. In addition, cAMP directly opens a specific type of membrane Ca^{2+} channel called *cyclic nucleotide gated channel* (CNG) in the heart, brain and kidney. Responses opposite to the above are produced when AC is inhibited through inhibitory Gi-protein.

(b) Phospholipase C: IP₃-DAG pathway Activation of phospholipase C (PLC) hydrolyses the membrane phospholipid phosphatidylinositol 4, 5-bisphosphate (PIP₂) to generate the second messengers inositol 1,4,5-trisphosphate (IP₃) and diacylglycerol (DAG). The IP₃ mobilises Ca^{2+} from intracellular organellar depots and DAG enhances protein kinase C (PKc) activation by Ca^{2+} (Fig. 4.7). Cytosolic Ca^{2+} (third messenger in this

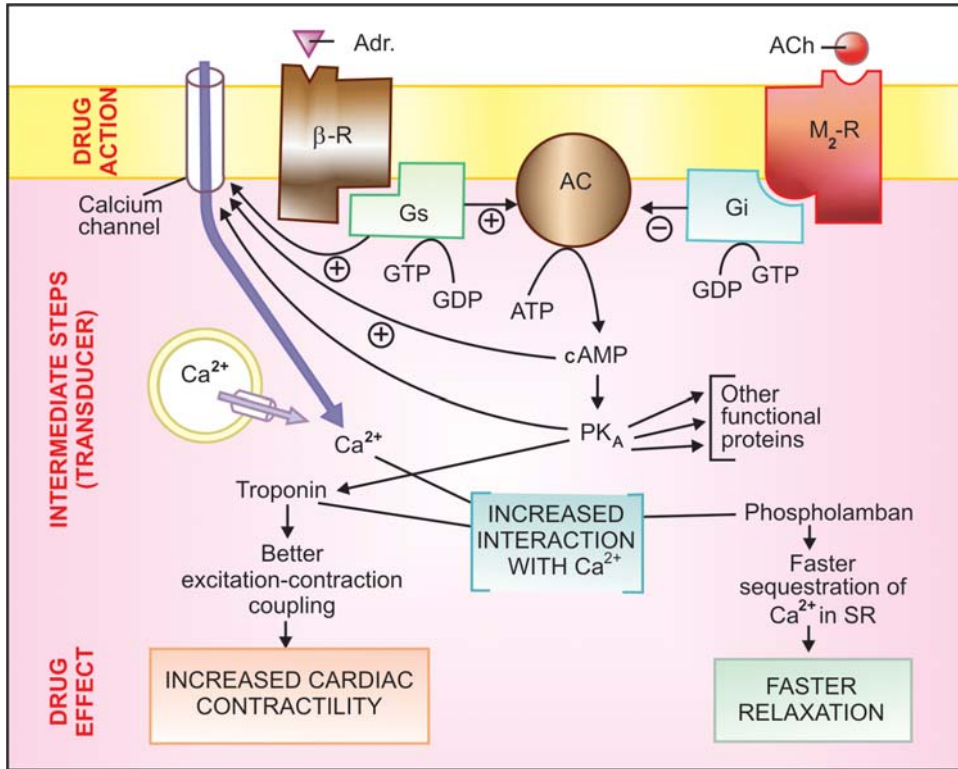


Fig. 4.6: The action-effect sequence of two G-protein coupled (β adrenergic and muscarinic M_2) receptor activation in myocardial cell

Adrenaline (Adr) binds to β -adrenergic receptor (β -R) on the cell surface inducing a conformational change which permits interaction of the G-protein binding site with the stimulatory G-protein (Gs). The activated Gs now binds GTP (in place of GDP), causing its active subunit to dissociate and in turn activate the enzyme adenyl cyclase (AC) located on the cytosolic side of the membrane: ATP is hydrolysed to cAMP which phosphorylates and thus activates cAMP dependent protein kinase (PK_A). The PK_A phosphorylates many functional proteins including troponin and phospholamban, so that they interact with Ca^{2+} , respectively resulting in increased force of contraction and faster relaxation. Calcium is made available by entry from outside (direct activation of myocardial membrane Ca^{2+} channels by Gs and through their phosphorylation by PK_A) as well as from intracellular stores.

One of the other proteins phosphorylated by cAMP is phosphorylase kinase which then activates the enzyme phosphorylase resulting in breakdown of glycogen to be utilized as energy source for increased contractility.

Action of acetylcholine (ACh) on muscarinic M_2 receptor (M_2 -R), also located in the myocardial membrane, can similarly activate an inhibitory G-protein (Gi) which then opposes the activation of AC by Gs.

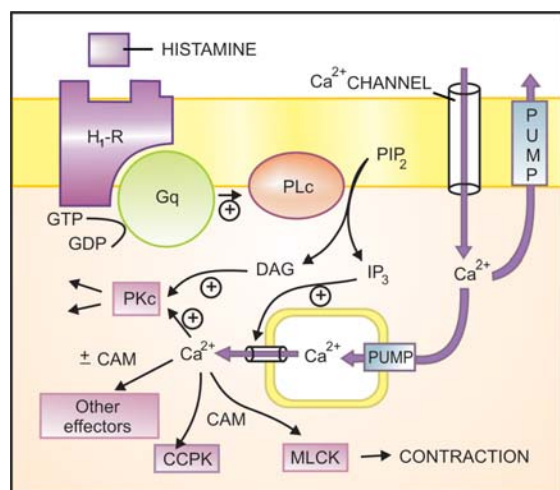
setting) is a highly versatile regulator acting through calmodulin (CAM), PKc and other effectors—mediates/modulates contraction, secretion/transmitter release, eicosanoid synthesis, neuronal excitability, intracellular movements, membrane function, metabolism, cell proliferation, etc. Like AC, the PLC can also be inhibited through inhibitory G-protein when directionally opposite responses would be expected.

Intracellular Ca^{2+} release has been found to occur in waves (Ca^{2+} mediated Ca^{2+} release from successive pools facilitated by inositol 1, 3, 4, 5-tetrakisphosphate— IP_4) and exhibits a variety of agonist and concentration dependent oscillatory patterns. The activation of different effectors may depend on the amplitude and pattern of these oscillations. Thus, the same intracellular messenger can trigger different responses depending on the nature and strength of the extracellular signal.

(c) Channel regulation The activated G-proteins can also open or close ionic channels

Table 4.1: Major functional pathways of G-protein coupled receptor transduction

Adenylyl cyclase: cAMP		Phospholipase IP ₃ -DAG	Channel regulation		
↑	↓		Ca ²⁺ ↑	Ca ²⁺ ↓	K ⁺ ↑
Adrenergic-β	Adrenergic-α ₂	Adrenergic-α ₁	Adrenergic-β ₁	Dopamine-D2	Adrenergic-α ₂
Histamine-H ₂	Muscarinic-M ₂	Histamine-H ₁	(Heart,	GABA _B	Muscarinic-M ₂
Dopamine-D1	Dopamine-D2	Muscarinic-M ₁ , M ₃	Sk.muscle)	Opioid-κ	Dopamine-D2
Glucagon	5-HT ₁	5-HT ₂		Adenosine-A ₁	5-HT _{1A}
FSH & LH	GABA _B	Vasopressin-Oxytocin		Somatostatin	GABA _B
ACTH	Opioid-μ, δ	Bradykinin-B ₂			Opioid-μ, δ
TSH	Angiotensin-AT ₁	Angiotensin-AT ₁			Adenosine-A ₁
Prostaglandin-EP ₂	Prostaglandin-EP ₃	Prostaglandin-FP, EP ₁ , EP ₃			
Prostacyclin-IP	Somatostatin	Thromboxane-TP			
Adenosine-A ₂	Adenosine-A ₁	Leukotriene			
		Cholecystokinin-Gastrin			
		PAF			

**Fig. 4.7:** The important steps of phospholipase C (PLC) pathway of response effectuation (in smooth muscle)

The agonist, e.g. histamine binds to its H₁ receptor (H₁ R) and activates the G-protein G_q, which in turn activates membrane bound phospholipase C (PLC) that hydrolyses phosphatidylinositol 4, 5-bisphosphate (PIP₂), a membrane bound phospholipid. The products inositol 1, 4, 5-trisphosphate (IP₃) and diacylglycerol (DAG) act as second messengers. The primary action of IP₃ is facilitation of Ca²⁺ mobilization from intracellular organellar pools, while DAG in conjunction with Ca²⁺ activates protein kinase C (PKC) which phosphorylates and alters the activity of a number of functional and structural proteins. Cytosolic Ca²⁺ is a veritable messenger: combines with calmodulin (CAM) to activate myosin light chain kinase (MLCK) inducing contraction, and another important regulator calcium-calmodulin protein kinase (CCPK). Several other effectors are regulated by Ca²⁺ in a CAM dependent or independent manner.

specific for Ca²⁺, K⁺ or Na⁺, without the intervention of any second messenger like cAMP or IP₃, and bring about hyperpolarization/depolarization/changes in intracellular Ca²⁺. The G_s opens Ca²⁺ channels in myocardium and skeletal muscles, while G_i and G_o open K⁺ channels in heart and smooth muscle as well as close neuronal Ca²⁺ channels. Physiological responses like changes in inotropy, chronotropy, transmitter release, neuronal activity and smooth muscle relaxation follow. Receptors found to regulate ionic channels through G-proteins are listed in Table 4.1.

2. Receptors with intrinsic ion channel

These cell surface receptors, also called *ligand gated ion channels*, enclose ion selective channels (for Na⁺, K⁺, Ca²⁺ or Cl⁻) within their molecules. Agonist binding opens the channel (Fig. 4.4) and causes depolarization/hyperpolarization/changes in cytosolic ionic composition, depending on the ion that flows through. The nicotinic cholinergic, GABA_A, glycine (inhibitory), excitatory AA (kainate, NMDA or N-methyl-D-aspartate, quisqualate) and 5HT₃ receptors fall in this category.

The receptor is usually a pentameric protein; all subunits, in addition to large intra- and extracellular segments, generally have four membrane spanning domains in each of which the AA chain traverses the width of the membrane six times. The subunits are thought to be arranged round the channel like a rosette and the α subunits usually bear the agonist binding sites.

Certain receptor-operated (or ligand-gated) ion channels also have secondary ligands which bind to an allosteric site and modulate the gating of the channel by the primary ligand, e.g. the benzodiazepine receptor modulates GABA_A gated Cl channel.

Thus, in these receptors, agonists directly operate ion channels, without the intervention of any coupling protein or second messenger. The onset and offset of responses through this class of receptors is the fastest (in milliseconds).

3. Enzyme-linked receptors

This class of receptors have a subunit with enzymatic property or bind a JAK (Janus-Kinase) enzyme on activation. The agonist binding site and the catalytic site lie respectively on the outer and inner face of the plasma membrane (Fig. 4.8). These two domains are interconnected through a single transmembrane stretch of peptide chain. There are two major subgroups of such receptors.

- Those that have intrinsic enzymatic activity.

- Those that lack intrinsic enzymatic activity, but bind a JAK-STAT kinase on activation.

a. Intrinsic enzyme receptors The intracellular domain is either a protein kinase or guanylyl cyclase.

In most cases the protein kinase specifically phosphorylates tyrosine residues on substrate proteins (Fig. 4.8A), e.g. insulin, epidermal growth factor (EGF), nerve growth factor (NGF) receptors, but in few it is a serine or threonine protein kinase. In the monomeric state, the kinase remains inactive. Agonist binding induces dimerization of receptor molecules and activates the kinase to autophosphorylate tyrosine residues on each other, increasing their affinity for binding substrate proteins and carrying forward the cascade of tyrosine phosphorylations. Intracellular events are triggered by phosphorylation of relevant proteins, many of which carry a SH₂ domain. A general feature of this class of receptors

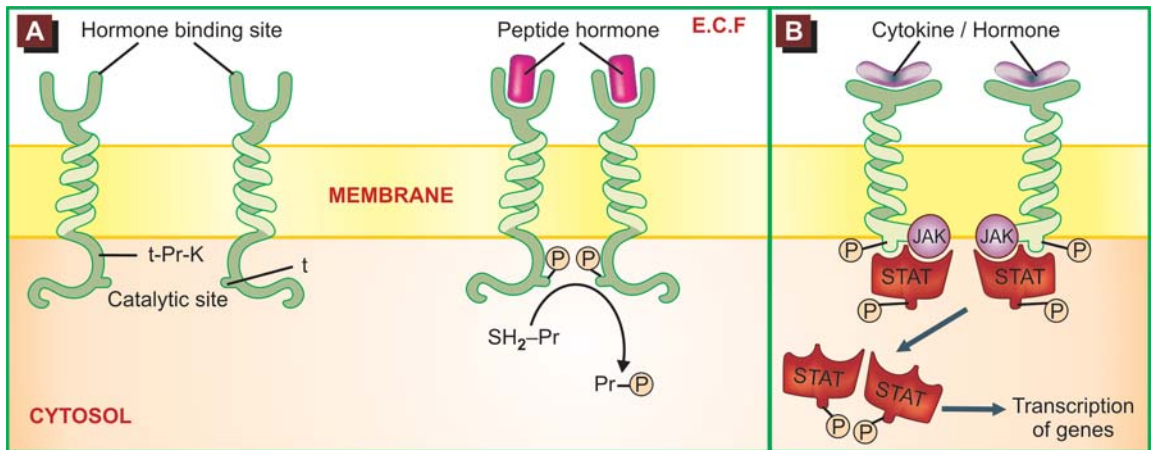


Fig. 4.8: Models of enzyme-linked receptors.

- Intrinsic tyrosine protein kinase receptor:** On binding the peptide hormone to the extracellular domains, the monomeric receptors move laterally in the membrane and form dimers. Dimerization activates tyrosine-protein kinase (t-Pr-K) activity of the intracellular domains so that they phosphorylate tyrosine (t) residues on each other, as well as on several SH₂ domain substrate proteins (SH₂-Pr). The phosphorylated substrate proteins then perform downstream signaling function.
- JAK-STAT kinase binding receptor:** The intracellular domain of these receptors lacks intrinsic protein kinase activity. Signal molecule binding to the extracellular domain induces receptor dimerization which activates the intracellular domain to bind free moving JAK (Janus Kinase) molecules. The activated JAK phosphorylate tyrosine residues on the receptor which then binds another protein STAT (signal transducer and activator of transcription). Tyrosine residues of STAT also get phosphorylated by JAK. The phosphorylated STAT dimerize, dissociate from the receptor and move to the nucleus to regulate transcription of target genes.

is that their dimerization also promotes receptor internalization, degradation in lysosomes and down regulation.

The enzyme can also be guanylyl cyclase (GC), as in the case of atrial natriuretic peptide (ANP). Agonist activation of the receptor generates cGMP in the cytosol as a second messenger which in turn activates cGMP-dependent protein kinase and modulates cellular activity.

b. JAK-STAT-kinase binding receptors These receptors differ in not having any intrinsic catalytic domain. Agonist induced dimerization alters the intracellular domain conformation to increase its affinity for a cytosolic tyrosine protein kinase JAK. On binding, JAK gets activated and phosphorylates tyrosine residues of the receptor, which now binds another free moving protein STAT (signal transducer and activator of transcription) which is also phosphorylated by JAK. Pairs of phosphorylated STAT dimerize and translocate to the nucleus to regulate gene transcription resulting in a biological response. Many cytokines, growth hormone, interferons, etc. act through this type of receptor.

The enzyme-linked receptors transduce responses in a matter of few minutes to a few hours.

4. Receptors regulating gene expression (Transcription factors)

In contrast to the above 3 classes of receptors, these are intracellular (cytoplasmic or nuclear) soluble proteins which respond to lipid soluble chemical messengers that penetrate the cell (Fig. 4.9). The receptor protein (specific for each hormone/regulator) is inherently capable of binding to specific genes, but is kept inhibited till the hormone binds near its carboxy terminus and exposes the DNA binding regulatory segment located in the middle of the molecule. Attachment of the receptor protein to the genes facilitates their expression so that specific mRNA is synthesized on the template of the gene. This mRNA moves to the ribosomes and directs synthesis of specific proteins which regulate the activity of target cells.

All steroidal hormones (glucocorticoids, mineralocorticoids, androgens, estrogens, progesterone), thyroxine, vit D and vit A function in this manner. Different steroidal hormones affect different target cells and produce different effects because each one binds to its own receptor and directs a unique pattern of synthesis of specific proteins. The specificity as to which hormone will be bound is provided by the hormone binding domain, while that as to which gene will be activated or repressed is a function of the DNA binding/N-terminus domain. Chimeric receptors have been produced which respond to one hormone, but produce the effects of the other hormone.

This transduction mechanism is the slowest in its time course of action (takes hours).

Receptor regulation

Receptors exist in a dynamic state; their density and efficacy is subject to regulation by the level of on going activity, feedback from their own signal output and other physiopathological influences. In tonically active systems, prolonged deprivation of the agonist (by denervation or continued use of an antagonist or a drug which reduces input) results in supersensitivity of the receptor as well as the effector system to the agonist. This has clinical relevance in clonidine/CNS depressant/opioid withdrawal syndromes, sudden discontinuation of propranolol in angina pectoris, etc. The mechanisms involved may be unmasking of receptors or their proliferation (*up regulation*) or accentuation of signal amplification by the transducer.

Conversely, continued/intense receptor stimulation causes desensitization or refractoriness: the receptor becomes less sensitive to the agonist. This can be easily demonstrated experimentally (Fig. 4.10); clinical examples are bronchial asthma patients treated continuously with β adrenergic agonists and parkinsonian patients treated with high doses of levodopa. The changes may be brought about by:

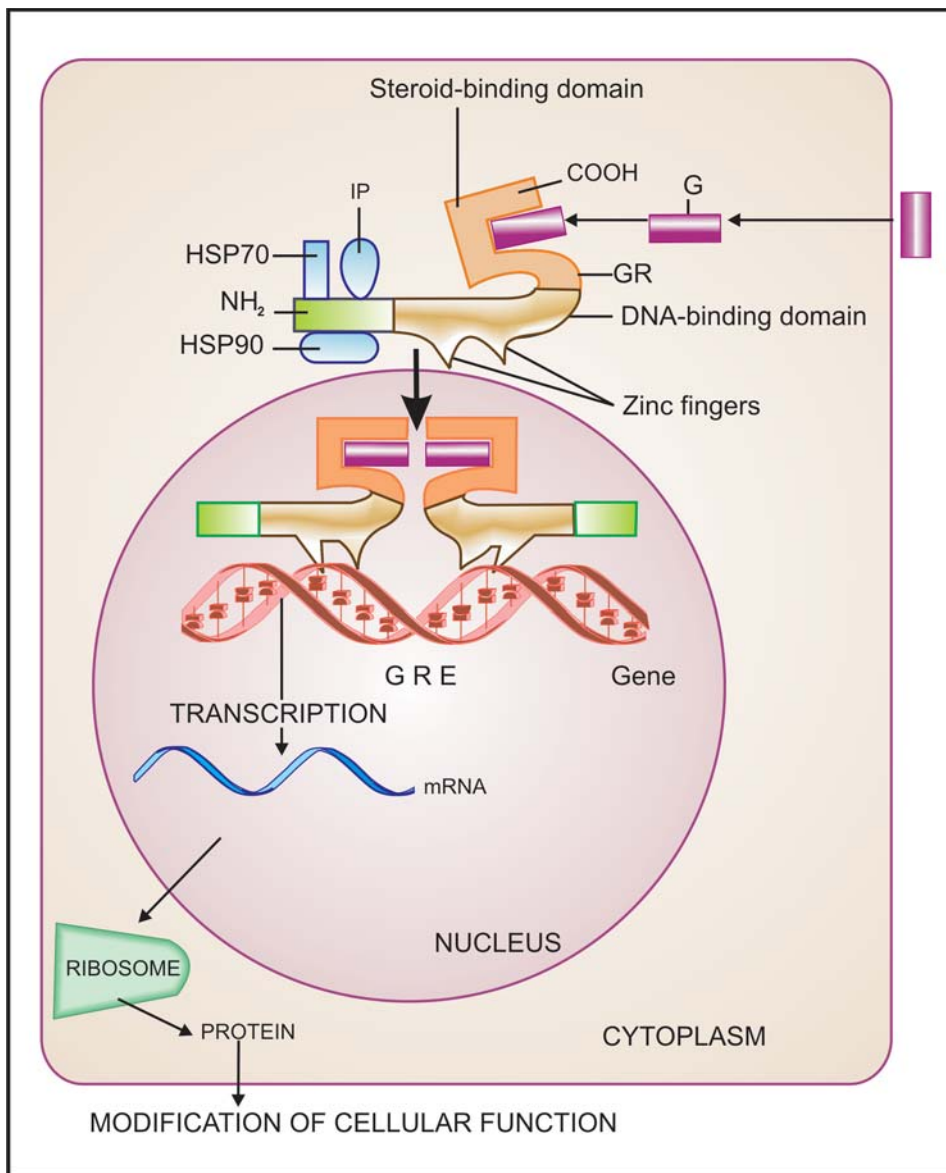


Fig. 4.9: Operational scheme of intracellular (glucocorticoid) receptor

The glucocorticoid (G) penetrates the cell membrane and binds to the glucocorticoid receptor (GR) protein that normally resides in the cytoplasm in association with 3 other proteins, viz. heat shock protein 90 (HSP90), HSP70 and immunophilin (IP). The GR has a steroid binding domain near the carboxy terminus and a mid region DNA binding domain having two 'zinc fingers', each made up of a loop of amino acids with chelated zinc ion. Binding of the steroid to GR dissociates the complexed proteins (HSP90, etc) removing their inhibitory influence on it. A dimerization region that overlaps the steroid binding domain is exposed, promoting dimerization of the occupied receptor. The steroid bound receptor dimer translocates to the nucleus and interacts with specific DNA sequences called 'glucocorticoid responsive elements' (GREs) within the regulatory region of appropriate genes. The expression of these genes is consequently altered resulting in promotion (or suppression) of their transcription. The specific mRNA thus produced is directed to the ribosome where the message is translated into a specific pattern of protein synthesis, which in turn modifies cell function.

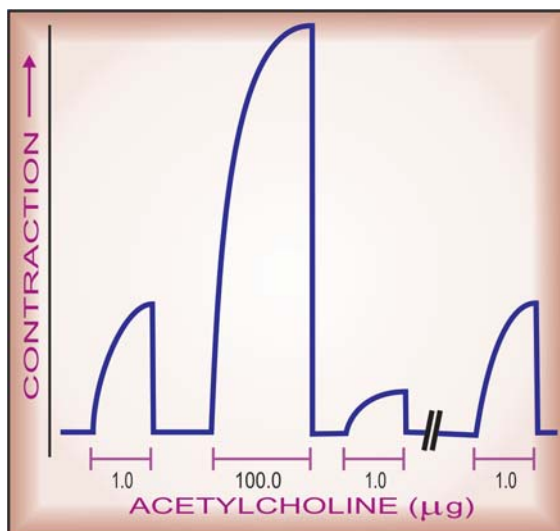


Fig. 4.10: Illustration of the phenomenon of desensitization

Contractile responses of frog's rectus abdominis muscle to acetylcholine. Note that shortly after exposure to a high (100 fold) dose of the agonist, the response is markedly attenuated, but is regained if sufficient time is allowed to elapse.

(i) Masking or internalization of the receptor (it becomes inaccessible to the agonist). In this case refractoriness develops as well as fades quickly.

In the case of β adrenergic receptor, it has been found that agonist binding promotes phosphorylation of its serine residues near the intracellular carboxy terminus by an enzyme β adrenergic receptor kinase (β ARK), allowing it to bind a protein called β -arrestin which hinders its interaction with Gs \rightarrow receptor transduction is impaired. When the β -agonist is removed, the serine residues are dephosphorylated and receptor mediated activation of Gs is restored.

(ii) Decreased synthesis/increased destruction of the receptor (*down regulation*): refractoriness develops over weeks or months and recedes slowly. Receptor down regulation is particularly exhibited by the tyrosine protein kinase receptors.

Some times response to all agonists which act through different receptors but produce the same overt effect (e.g. histamine and acetylcholine both contract intestinal smooth muscle) is decreased by exposure to any one of these agonists (heterologous desensitization), showing that mecha-

nisms of response effectuation have become less efficient. However, often desensitization is limited to agonists of the receptor being repeatedly activated (homologous desensitization).

Both homologous and heterologous desensitization has been observed in the case of GPCRs. The BARK- β arrestin mechanism described above produces homologous desensitization. The GPCRs transduce many responses by activating PK_A and PK_C. These kinases phosphorylate the GPCRs as well rather nonselectively (at a site different from that of BARK) and hinder their interaction with G-proteins, resulting in heterologous desensitization.

Functions of receptors These can be summarized as:

- To propagate regulatory signals from outside to within the effector cell when the molecular species carrying the signal cannot itself penetrate the cell membrane.
- To amplify the signal.
- To integrate various extracellular and intracellular regulatory signals.
- To adapt to short term and long term changes in the regulatory milieu and maintain homeostasis.

Nonreceptor-mediated drug action

This refers to drugs which do not act by binding to specific regulatory macromolecules. Drug action by purely physical or chemical means, interactions with small molecules or ions (antacids, chelating agents, cholestyramine, etc.), as well as direct interaction with enzymes, ionic channels and transporters has already been described. In addition, there are drugs like alkylating agents which react covalently with several critical biomolecules, especially nucleic acids, and have cytotoxic property useful in the treatment of cancer. Another important class of drugs are the antimetabolites (purine/pyrimidine analogues) which lead to production of non-functional or dysfunctional cellular components that exert antineoplastic, antiviral and immunosuppressant activity.

DOSE-RESPONSE RELATIONSHIP

When a drug is administered systemically, the dose-response relationship has two components:

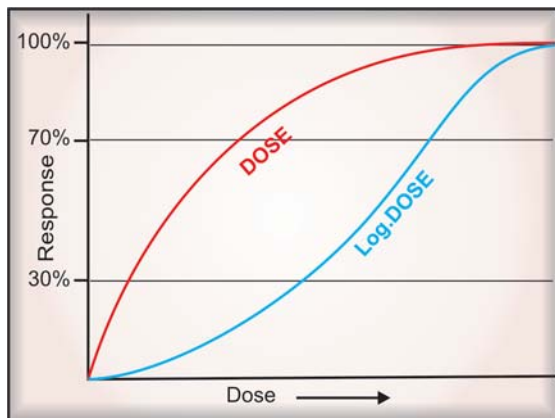


Fig. 4.11: Dose-response curves and log dose-response curves

dose-plasma concentration relationship and plasma concentration-response relationship. The former is determined by pharmacokinetic considerations and ordinarily, descriptions of dose-response relationship refer to the latter, which can be more easily studied *in vitro*.

Generally, the intensity of response increases with increase in dose (or more precisely concentration at the receptor) and the dose-response curve is a rectangular hyperbola (Fig. 4.11). This is because drug-receptor interaction obeys law of mass action, accordingly—

$$E = \frac{E_{max} \times [D]}{K_D + [D]} \quad \dots(3)$$

Where E is the observed effect at a dose [D] of the drug, E_{max} is the maximal response, K_D is the dissociation constant of the drug-receptor complex, which is equal to the dose of the drug at which half maximal response is produced. If the dose is plotted on a logarithmic scale, the curve becomes sigmoid and a linear relationship between log of dose and the response is seen in the intermediate (30–70% response) zone, as can be predicted from equation (3). This is not peculiar to drugs. In fact all stimuli are graded biologically by the fractional change in stimulus intensity, e.g. 1 kg and 2 kg weights held in two hands can be easily differentiated, but not 10 kg and 11 kg weights. Though the absolute difference remains

1kg, there is a 100% fractional change in the former case but only 10% change in the latter case. In other words, response is proportional to an exponential function (log) of the dose.

Other advantages of plotting log dose-response curves (DRC) are:

- (i) A wide range of drug doses can be easily displayed on a graph.
- (ii) Comparison between agonists and study of antagonists becomes easier.

Therapeutic window phenomenon

This is an unusual feature seen with certain drugs: optimal therapeutic effect is exerted only over a narrow range of plasma drug concentrations or drug doses; both below and above this range, beneficial effects are suboptimal, i.e., the effect declines if the doses are increased beyond a certain level. Examples are:

- Tricyclics (imipramine etc.) exert maximal antidepressant effect when their plasma concentration is maintained between 50–150 ng/ml.
- Clonidine lowers BP over a plasma concentration range of 0.2–2.0 ng/ml; BP may rise at concentrations above 2 ng/ml.
- Glipizide exerts poorer glycaemia control at doses > 25 mg/day.

The pharmacological basis of this phenomenon is not well understood, but may be due to dual or complex actions of the drug—different facets of which become prominent at different concentrations.

The log dose-response curve (DRC) can be characterized by its shape (slope and maxima) and position on the dose axis.

Drug potency and efficacy

The position of DRC on the dose axis is the index of *drug potency* which refers to the amount of drug needed to produce a certain response. A DRC positioned rightward indicates lower potency (Fig. 4.12). Relative potency is often more meaningful than absolute potency, and is generally defined by comparing the dose (concentration) of the two agonists at which they elicit half maximal response (EC_{50}). Thus, if 10 mg of morphine = 100 mg of pethidine as analgesic, morphine is 10 times more potent than pethidine. However, a higher potency, in itself, does not confer clinical

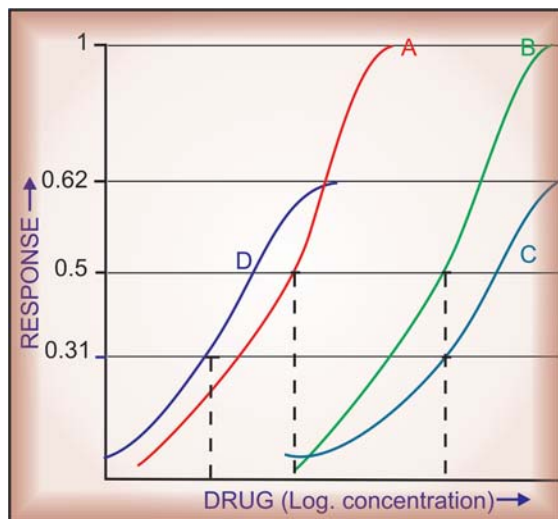


Fig. 4.12: Illustration of drug potency and drug efficacy. Dose-response curve of four drugs producing the same qualitative effect

Note:

Drug B is less potent but equally efficacious as drug A.

Drug C is less potent and less efficacious than drug A.

Drug D is more potent than drugs A, B, & C, but less efficacious than drugs A & B, and equally efficacious as drug C.

superiority unless the potency for therapeutic effect is selectively increased over potency for adverse effect.

The upper limit of DRC is the index of *drug efficacy* and refers to the maximal response that can be elicited by the drug, e.g. morphine produces a degree of analgesia not obtainable with any dose of aspirin—morphine is more efficacious than aspirin. Efficacy is a more decisive factor in the choice of a drug.

Often the terms 'drug potency' and 'drug efficacy' are used interchangeably, but these are not synonymous and refer to different characteristics of the drug. The two can vary independently:

- Aspirin is less potent as well as less efficacious analgesic than morphine.
- Pethidine is less potent but equally efficacious analgesic as morphine.
- Furosemide is less potent but more efficacious diuretic than metolazone.
- Diazepam is more potent but less efficacious CNS depressant than pentobarbitone.

Depending on the type of drug, both higher efficacy (as in the case of furosemide conferring utility in renal failure) or lower efficacy (as in the case of diazepam conferring safety in over-dose) could be clinically advantageous.

The slope of the DRC is also important. A steep slope indicates that a moderate increase in dose will markedly increase the response (dose needs individualization), while a flat one implies that little increase in response will occur over a wide dose range (standard doses can be given to most patients). Hydralazine has a steep, while hydrochlorothiazide has a flat DRC of antihypertensive effect (Fig. 4.13).

Selectivity

Drugs seldom produce just one action: the DRCs for different effects of a drug may be different. The extent of separation of DRCs of a drug for different effects is a measure of its selectivity, e.g. the DRCs for bronchodilatation and cardiac stimulation (Fig. 4.14) are quite similar in case of isoprenaline, but far apart in case of salbutamol—the latter is a more selective drug.

The gap between the therapeutic effect DRC and the adverse effect DRC defines the *safety margin* or the *therapeutic index* of a drug. In

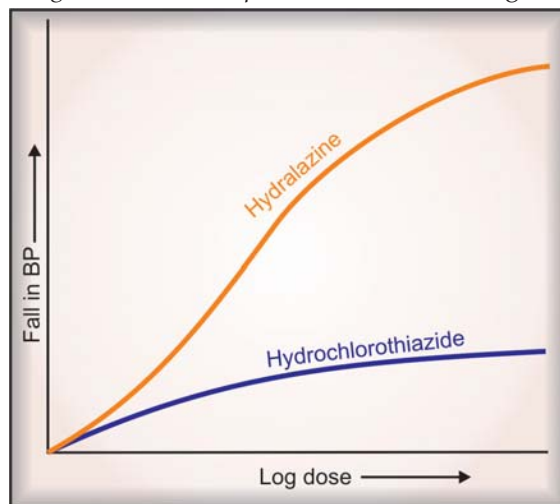


Fig. 4.13: Steep and flat dose-response curves illustrated by antihypertensive effect of hydralazine and hydrochlorothiazide

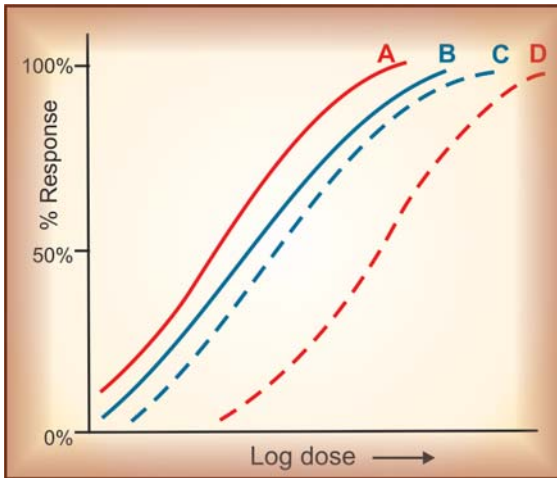


Fig. 4.14: Illustration of drug selectivity. Log dose-response curves of salbutamol for bronchodilatation (A) and cardiac stimulation (D) Log dose-response curves of isoprenaline for bronchodilatation (B) and cardiac stimulation (C)

experimental animals, therapeutic index is often calculated as:

$$\text{Therapeutic index} = \frac{\text{median lethal dose}}{\text{median effective dose}}$$

or

$$\frac{LD_{50}}{ED_{50}}$$

But this is irrelevant in the clinical set up where the *therapeutic range* is bounded by the dose which produces minimal therapeutic effect and the dose which produces maximal acceptable adverse effect (Fig. 4.15). Because of individual variability, the effective dose for some subjects may be toxic for others; defining the therapeutic range for many drugs is a challenging task. A drug may be capable of inducing a higher therapeutic response (have higher efficacy) but development of intolerable adverse effects may preclude use of higher doses, e.g. prednisolone in bronchial asthma.

Risk-benefit ratio This term is very frequently used, and conveys a judgement on the estimated harm (adverse effects, cost, inconvenience) *vs* expected advantages (relief of symptoms, cure, reduction of complications/mortality, improve-

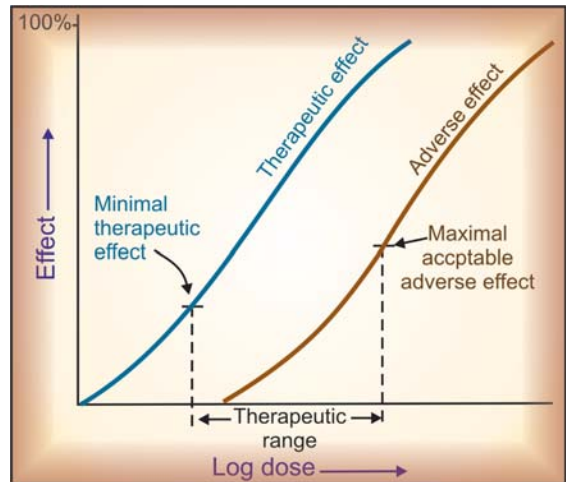


Fig. 4.15: Illustrative dose-response curves for therapeutic effect and adverse effect of the same drug

ment in quality of life). A drug should be prescribed only when the benefits outweigh the risks. However, risk-benefit ratio can hardly ever be accurately measured for each instance of drug use, because 'risk' is the probability of harm; and harm has to be qualified by its nature, quantum, time-course (transient to life-long) as well as the value that the patient attaches to it. None of these can be precisely ascertained. As such, the physician has to rely on data from use of drugs in large populations (pharmacoepidemiology) and his own experience of the drug and the patient.

COMBINED EFFECT OF DRUGS

When two or more drugs are given simultaneously or in quick succession, they may be either indifferent to each other or exhibit *synergism* or *antagonism*. The interaction may take place at pharmacokinetic level (see Ch. 2 and 3) or at pharmacodynamic level.

SYNERGISM

(Greek: *Syn*—together; *ergon*—work)

When the action of one drug is facilitated or increased by the other, they are said to be synergistic. In a synergistic pair, both the drugs can have action in the same direction or given alone

one may be inactive but still enhance the action of the other when given together. Synergism can be:

(a) Additive The effect of the two drugs is in the same direction and simply adds up:

$$\text{effect of drugs A + B} = \text{effect of drug A} + \text{effect of drug B}$$

Additive drug combinations

Aspirin + paracetamol	as analgesic/antipyretic
Nitrous oxide + halothane	as general anaesthetic
Amlodipine + atenolol	as antihypertensive
Glibenclamide + metformin	as hypoglycaemic
Ephedrine + theophylline	as bronchodilator

Side effects of the components of an additive pair may be different—do not add up. Thus, the combination is better tolerated than higher dose of one component.

(b) Supraadditive (potentiation) The effect of combination is greater than the individual effects of the components:

$$\text{effect of drug A + B} > \text{effect of drug A} + \text{effect of drug B}$$

This is always the case when one component is inactive as such.

Supraadditive drug combinations

<i>Drug pair</i>	<i>Basis of potentiation</i>
Acetylcholine + physostigmine	Inhibition of breakdown
Levodopa + carbidopa/benserazide	Inhibition of peripheral metabolism
Adrenaline + cocaine/desipramine	Inhibition of neuronal uptake
Sulfamethoxazole + trimethoprim	Sequential blockade
Antihypertensives (enalapril+ hydrochlorothiazide)	Tackling two contributory factors
Tyramine + MAO inhibitors	Increasing releasable CA store

ANTAGONISM

When one drug decreases or abolishes the action of another, they are said to be antagonistic:

$$\text{effect of drugs A + B} < \text{effect of drug A} + \text{effect of drug B}$$

Usually in an antagonistic pair one drug is inactive as such but decreases the effect of the other. Depending on the mechanism involved, antagonism may be:

(a) Physical antagonism Based on the physical property of the drugs, e.g. charcoal adsorbs alkaloids and can prevent their absorption—used in alkaloidal poisonings.

(b) Chemical antagonism The two drugs react chemically and form an inactive product, e.g.

- KMnO_4 oxidizes alkaloids—used for gastric lavage in poisoning.
- Tannins + alkaloids—insoluble alkaloidal tannate is formed.
- Chelating agents (BAL, Cal. disod. edetate) complex toxic metals (As, Pb).
- Nitrites form methaemoglobin which reacts with cyanide radical.

Drugs may react when mixed in the same syringe or infusion bottle:

- Thiopentone sod. + succinylcholine chloride
- Penicillin-G sod. + succinylcholine chloride
- Heparin + penicillin/tetracyclines/streptomycin/hydrocortisone

(c) Physiological/functional antagonism The two drugs act on different receptors or by different mechanisms, but have opposite overt effects on the same physiological function, i.e. have pharmacological effects in opposite direction, e.g.

- Histamine and adrenaline on bronchial muscles and BP.
- Hydrochlorothiazide and triamterene on urinary K^+ excretion.
- Glucagon and insulin on blood sugar level.

(d) Receptor antagonism One drug (antagonist) blocks the receptor action of the other (agonist). This is a very important mechanism of drug action, because physiological signal molecules act

through their receptors, blockade of which can produce specific and often profound pharmacological effects. Receptor antagonists are selective (relatively), i.e. an anticholinergic will oppose contraction of intestinal smooth muscle induced by cholinergic agonists, but not that induced by histamine or 5-HT (they act through a different set of receptors). Receptor antagonism can be competitive or noncompetitive.

Competitive antagonism (equilibrium type) The antagonist is chemically similar to the agonist, competes with it (Fig. 4.16 A, D) and binds to the same site to the exclusion of the agonist molecules. Because the antagonist has affinity but no intrinsic activity (*see p. 42*), no response is produced and the log DRC of the agonist is shifted to the right. Since antagonist binding is reversible and depends on the relative concentration of the agonist and antagonist molecules, higher concentration of the agonist progressively overcomes the block—a parallel shift of the agonist DRC with no suppression of maximal response is obtained (Fig. 4.17a). The extent of shift is dependent on the affinity and concentration of the antagonist.

A partial agonist (Fig. 4.16 C), having affinity for the same receptor, also competes with and antagonizes a full agonist, while producing a submaximal response of its own.

Noncompetitive antagonism The antagonist is chemically unrelated to the agonist, binds to a different *allosteric site* altering the receptor in such a way that it is unable to combine with the agonist (Fig. 4.16E), or unable to transduce the response, so that the downstream chain of events are uncoupled. Because the agonist and the antagonist are combining with different sites, there is no competition between them—even high agonist concentration is unable to reverse the block completely. Increasing concentrations of the antagonist progressively flatten the agonist DRC (Fig. 4.17b). Noncompetitive antagonists have been produced experimentally, but are not in clinical use.

Nonequilibrium (competitive) antagonism Certain antagonists bind to the receptor with strong

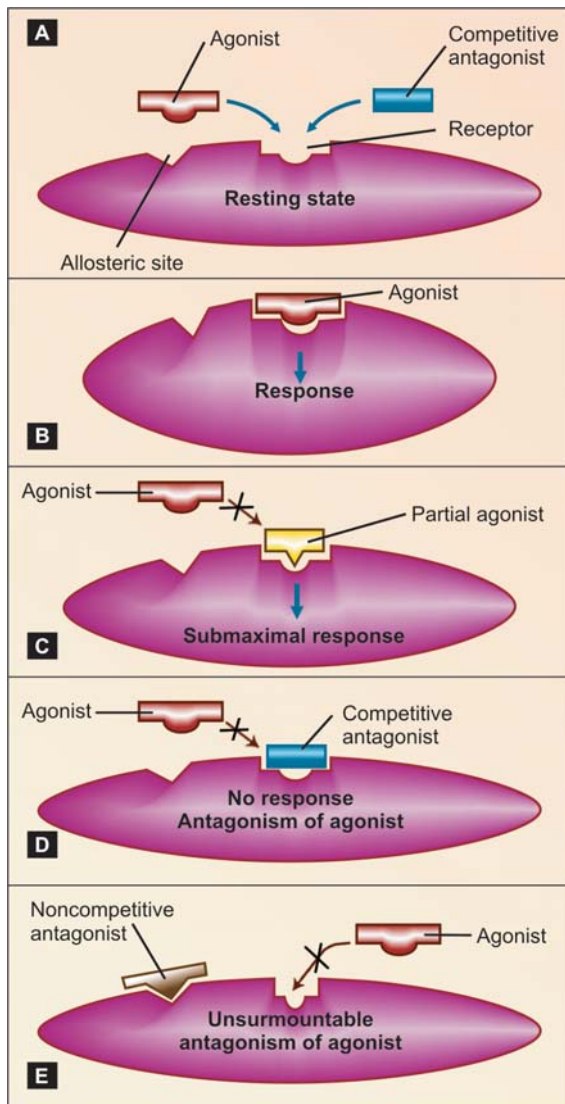


Fig. 4.16: Illustration of sites of action of agonists and antagonists (A), and the action of full agonist (B), partial agonist (C), competitive antagonist (D) and noncompetitive antagonist (E) on the receptor

(covalent) bonds or dissociate from it slowly so that agonist molecules are unable to reduce receptor occupancy of the antagonist molecules—law of mass action cannot apply—an irreversible or nonequilibrium antagonism is produced. The agonist DRC is shifted to the right and the maximal response is lowered (if spare receptors

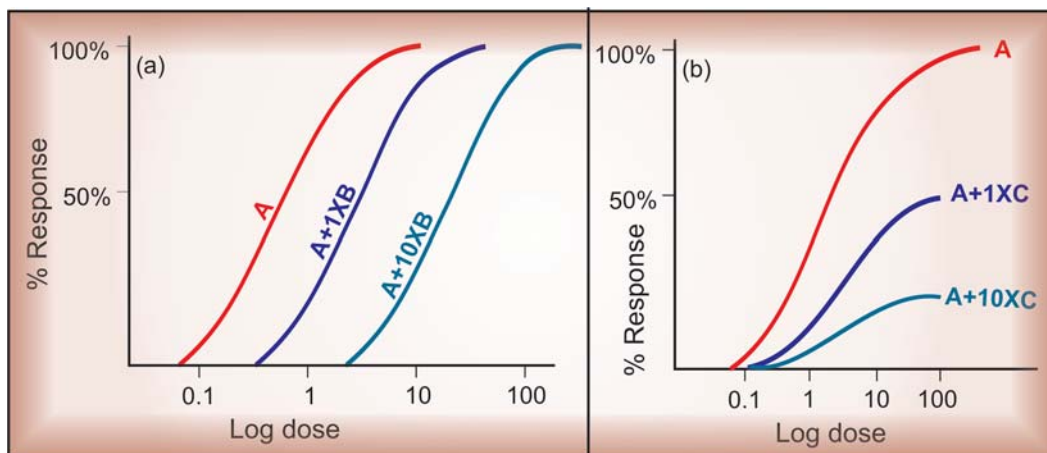


Fig. 4.17: Dose-response curves showing competitive (a) and noncompetitive (b) antagonism
 A—agonist, B—competitive antagonist, C—noncompetitive antagonist

are few). Since flattening of agonist DRC is a feature of noncompetitive antagonism; nonequilibrium antagonism has also been called 'a type of noncompetitive antagonism'. This appears inappropriate because the antagonist is binding to the

same site as the agonist. Phenoxybenzamine is a nonequilibrium antagonist of adrenaline at the α adrenergic receptors.

Features of competitive and noncompetitive antagonism are compared below:

Competitive (equilibrium type)	Noncompetitive
1. Antagonist binds with the same receptor as the agonist	Binds to another site of receptor
2. Antagonist resembles chemically with the agonist	Does not resemble
3. Parallel rightward shift of agonist DRC	Flattening of agonist DRC
4. The same maximal response can be attained by increasing dose of agonist (surmountable antagonism)	Maximal response is suppressed (unsurmountable antagonism)
5. The antagonist appears to have inactivated a certain number of agonist molecules	The antagonist appears to have inactivated a certain number of receptors
6. Intensity of response depends on the concentration of both agonist and antagonist	Maximal response depends only on the concentration of antagonist
7. Examples: ACh—Atropine Morphine—Naloxone	Diazepam—Bicuculline