

Drug Receptor Interactions

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Introduction

Drugs : any agents that change living processes.

The effects of a drug, the response you observe after administration, usually result from changes in physiological functions that lie well “downstream” from the primary site of drug action. In addition, the signaling pathways are making network in human body, so changing one system can affect the function of many other systems. Therefore the observed responses may represent both “direct” effects of the drug and compensatory changes that may results from these primary effects. The time course of both the direct and the compensatory changes may be immediate or delayed.

When you choose drugs for treatment, you always have to consider direct effect and indirect effect, desirable effect and undesirable effect, tissue specificity, or time course of drug action.

The understanding of drug-receptor interaction will be critically important to make these decisions.

In this lecture we will focus on the following points:

- 1) The general principles of drug action.
- 2) The mechanism which the drug action is translated into effects.
- 3) The basis for classification of receptors. And an overview of receptor mechanism.

1) General Principles of drug action.

A few drugs act by simple mechanisms related to their chemical or physical properties.

Example:

EDTA is a metal chelating agent with high affinity for Pb^{2+} . It is used for treatment of lead intoxication.

Antacids such as $\text{Mg}(\text{OH})_2$ and $\text{Al}(\text{OH})_3$ are bases and act by neutralizing gastric acid after oral administration

Mannitol is used as an osmotic diuretic. This is biologically inert and penetrates membrane very poorly. When administered intravenously, it is filtered at the glomerulus and is not reabsorbed. The osmotic property of mannitol then induces diuresis

Most drugs produce their effects by interacting with specific macromolecular components of the organism. The macromolecule to which a drug binds to modify biological function is called its "receptor".

Drug + Receptor \longrightarrow Drug-Receptor Complex \longrightarrow Altered function

A drug is useful if the produced effect is beneficial for therapeutic purpose and if the effect is sufficiently selective to offer an acceptable balance between wanted and unwanted effects.

Dose Response Curves

A pharmacological response is a function of the dose or concentration of a drug. Dose response curves are graphical representation of the relationship between dose and response. Usually, it is convenient to have log scale on axis of drug concentration.

There are two types of responses: quantal or "all-or-none" and graded.

Quantal response:

Example of quantal or "all-or-none" responses

analgesia for headache
digitalis to stop heart
sleep or lethal dose for anesthetics

The response is represented as cumulative percentage of subjects exhibiting a defined effect. Quantal relationship can be defined for both toxic and therapeutic effects. This allows the calculation of therapeutic index.

A safe drug has large therapeutic index.

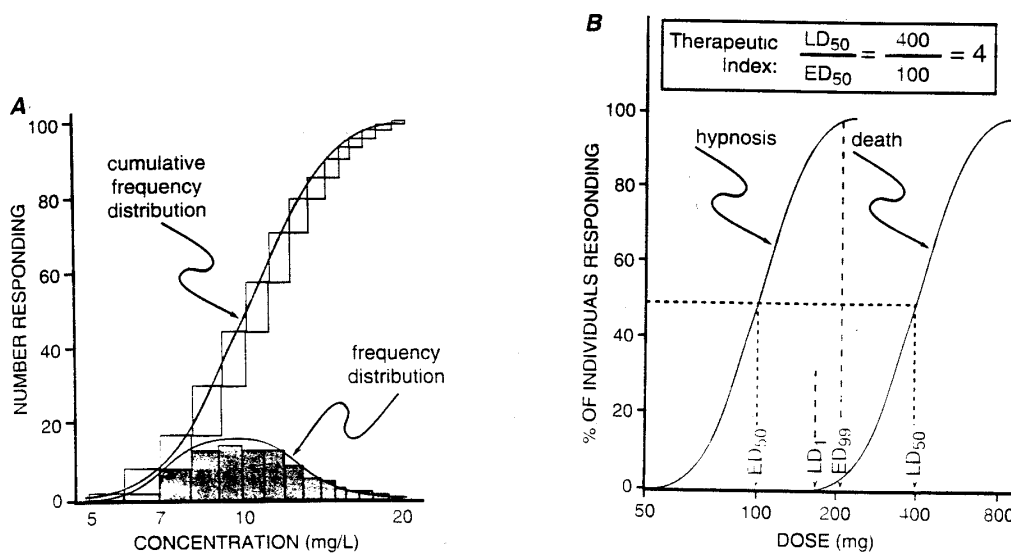
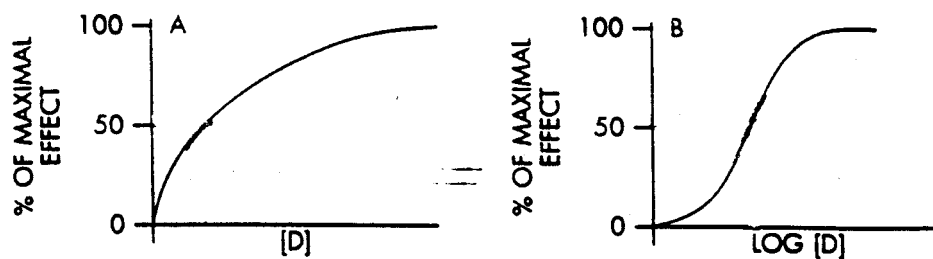


Figure 3-3. Frequency distribution curves and quantal concentration-effect and dose-effect curves.

Graded response:

Responses are often described as a percentage of maximal response.

- A. A linear concentration scale yields the rectangular hyperbolic curve.
- B. Plotting using logarithm of concentration yields a sigmoidal curve. (Most common)



Drug receptors

Almost any macromolecule in an organism is a potential drug receptor. (Hence a potential drug target.)

Most receptors are proteins, but nucleic acids (particularly DNA) are receptors for several drugs.

Drugs are potentially capable of changing any functions by modulating the rates of ongoing process. However, they do not add new functions.

Drug receptors can be classified into two different groups.

1. The usual macromolecules without endogeneous ligand. Receptors for these drugs are enzymes, transporters, channels, structural proteins, nucleic acids etc.

Example:

Digitalis glycoside (digoxin) bind to and inhibit Na^+/K^+ -ATPase. Secondary effect of inhibition of Na^+/K^+ -ATPase cause increase of intracellular Ca^{2+} which results in positive inotropic effects on the heart.

Ca^{2+} channel blockers are widely used to treat hypertension.

Drugs that cross-link DNA block DNA and cell replication are used to treat malignant tumor.

Inhibitors of angiotensin converting enzyme (ACE inhibitors) are used to treat hypertension.

Colchicine is used to treat gouty arthritis attacks, binds to and causes depolymerization of microtubules.

2. "Real receptors". Receptors for endogeneous regulatory ligands. i.e. Hormones, neurotransmitters, autacoids, growth factors, cytokines, etc. The function of these receptors is to sense the presence of these ligands in the environment and to initiate the appropriate responses. These receptors function as signal transducers. These receptors will be the subject of today's lecture.

Important general properties of signal-transducing receptors.

- 1) These receptors have two functions --- (1) sensing the ligand and (2) transmitting the message into cell.
- 2) Most signal-transducing receptors are integral components of the cellular membrane, with extracellular ligand binding domain and intracellular signal transmitting domain. This orientation permits chemical diversity in signaling molecules.
- 3) Several signal-transducing receptors are found in the nucleus or are translocated to the nucleus after ligand binding. The ligands are lipid-soluble in order to cross plasma membrane. Examples: steroids hormones, thyroid hormone, retinoids, and vitamine D.
- 4) Most signal-transducing receptors are present in small numbers -- a few thousand per cell.
- 5) There are often many isoforms of an "individual" receptor. Different receptor isoforms may have different mechanism of signal transduction. This permits a great deal of regulatory flexibility.

Since signal-transducing receptors have specific ligand binding sites -- usually located extracellularly -- and since their job is to regulate crucial cellular functions, they are obvious sites for drug action. Furthermore, novel receptors and novel ligands of receptors are discovered. A great deal of drug development is focused on such receptors and ligands.

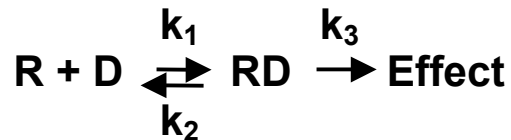
Many drugs act by mimicking or blocking the actions of natural regulators of signal transducing receptors.

Basic knowledge of receptor theory and its principles of action will greatly help you to organize information about many drugs.

2) The mechanism of drug-receptor interaction.

"Occupational theory of drug action"

The magnitude of effect is proportional to the concentration of the drug-receptor complex that is formed. This is the most simple assumption.



[R] and [D] are concentration of free drug and receptor at equilibrium and k_1 , k_2 , and k_3 are the rate constants. The occupancy model states that the magnitude of effect is proportional to [RD]. Thus the fraction of the maximum possible response that is observed is equal to fractional occupancy of the receptor, f_b

$$(1) \quad f_b = \frac{[\text{RD}]}{[\text{R}_T]} = \frac{[\text{RD}]}{[\text{R}] + [\text{RD}]}$$

The rate of the forward reaction is equal to the product of the rate constant, k_1 , and the concentrations of the reactions, [R] and [D]:

$$\text{Forward rate} = k_1[\text{R}][\text{D}]$$

And similarly

$$\text{Reverse rate} = k_2[\text{RD}]$$

At binding equilibrium, forward rate = reverse rate. Therefore,

$$k_1[\text{R}][\text{D}] = k_2[\text{RD}]$$

Hence

$$[\text{R}][\text{D}] / [\text{RD}] = k_2 / k_1 = K_d,$$

K_d = "equilibrium dissociation constant" unit is concentration. or

$$(2) \quad [\text{RD}] = [\text{R}][\text{D}] / K_d$$

If you use (2) for (1), you will get an important equation:

$$(3) \quad f_b = \frac{[\text{D}]}{K_d + [\text{D}]}$$

When $[D]$ is equal to K_d , $fb=0.5$. Thus, K_d is equal to the concentration of free drug required to occupy half of the receptors. In other words, the concentration of the drug when the half of the receptor is occupied, equals to K_d .

K_d represents the affinity of the drug to its receptor.

k_1 is usually limited by the rates of diffusion of the molecule. Therefore, k_1 has little to do with affinity. Thus, K_d is dependent on k_2 . High affinity ligand has lower k_2 , therefore lower K_d .

Low K_d = high affinity = difficult to dissociate, nM to pM, hours to dissociate
 High K_d = low affinity = dissociate quickly, mM to uM, seconds to dissociate

According to occupancy model,

$$fb = \text{Effect} / \text{Max. Effect}$$

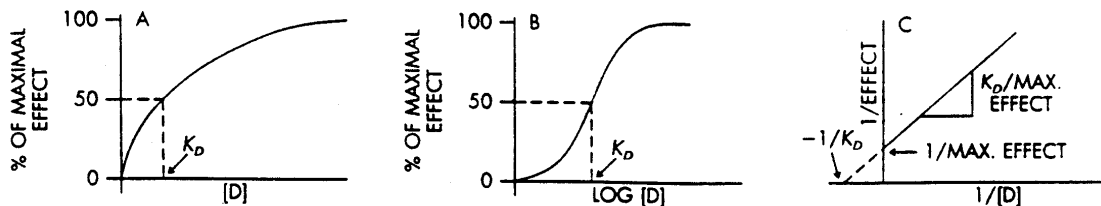
Therefore

$$\text{Effect} / \text{Max. effect} = [D] / (K_d + [D])$$

or

$$\text{Effect} = \text{Max.Effect} \frac{[D]}{K_d + [D]}$$

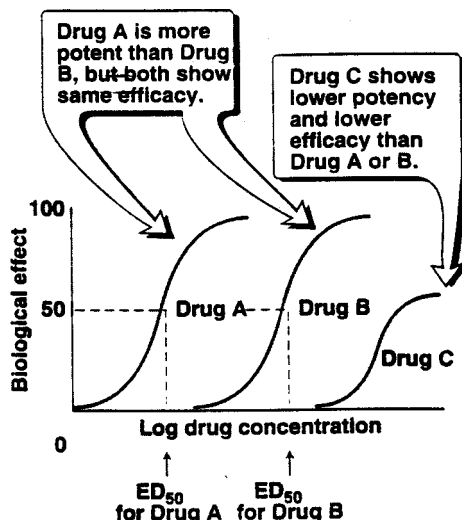
When the occupancy assumption is true, a half-maximal effect is observed when $[D]$ is equal to the K_d of the drug-receptor interaction.



Two important terms of dose-response curve

Potency: The location of the concentration curve along the concentration axis. Potency is related to the dose of a drug required to produce a given effect.

Efficacy : The magnitude of effect that can be produced by a drug. Maximal efficacy is reflected in the plateau of the dose-response curve.



Agonist and Antagonist

Agonist is a drug that interacts with a receptor and produces effects by "activating" receptor.

Certain drugs interact with receptors but do not initiate an effect. However, they can compete with an agonist for binding to the same effector and that will eventually inhibit the action of agonist. Such drugs are called antagonists. Most antagonists act by binding reversibly at the receptor's ligand binding site. The inhibitory effects of antagonists that act reversibly at the agonist-binding site can be overcome by increasing the concentration of agonist. They are called competitive antagonist. A few antagonist act non-competitively. This usually results from an irreversible interaction between the receptor and the antagonist. The effect of an irreversible antagonist is the equivalent of removing receptors from the system.

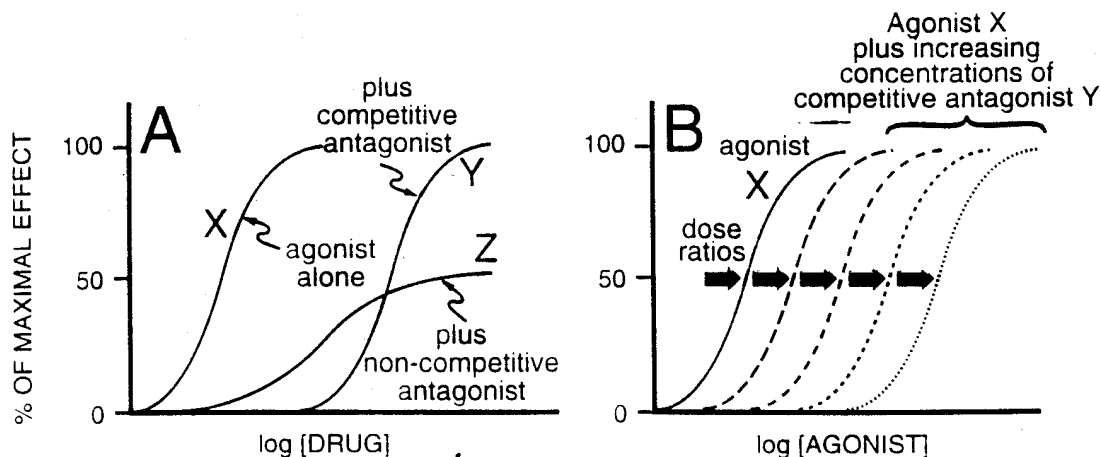
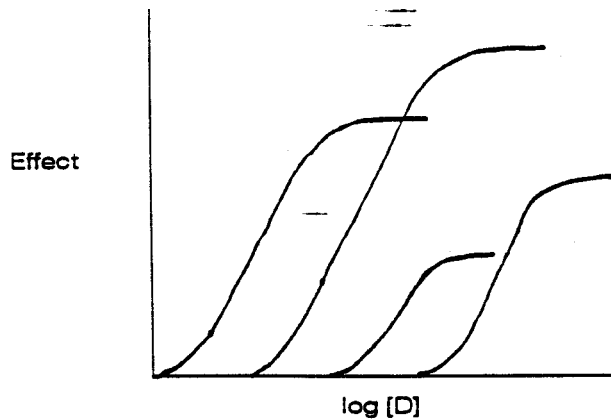


Figure 2-6. Properties of antagonist blockade of response.

Partial agonist

A drug that displays efficacy that is intermediate between that of an agonist and an antagonist is called a partial agonist. Partial agonist has intermediate efficacy of full agonist and antagonist. Partial agonists are common, and their responses are also dependent on tissue or specific effect. Also please note that potencies vary independently of efficacy.

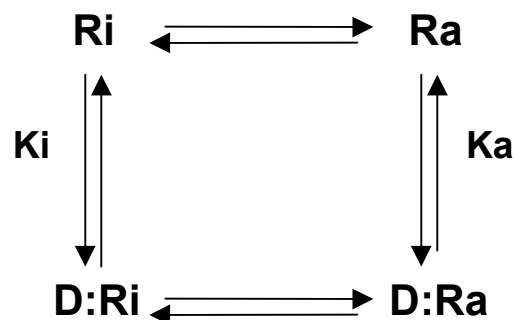


Responses to a Series of Partial and Full Agonists: Note that potencies vary independently of efficacy.

What is the mechanism of action of partial agonist?

Allosteric Theory of Drug Action

Suppose that a receptor can exist in two conformations -- active (R_a) or inactive (R_i). These conformations might represent, for example, open and closed states of an ion channel, or the active and inactive forms of an enzyme. And also assume that the drug can bind to both conformations but with different affinities. In this case, the effect is function of the amount of receptor in active state and not a function of drug-receptor complex $[DR]$. A drug will change this equilibrium.



In resting state (without agonist), inactive state usually predominate. With binding of an agonist, the equilibrium shifts to active state. A full agonist will be sufficiently selective for the active state that it will drive the receptor "completely" to the active state.

If a different drug binds to the same receptor with only slightly greater affinity for R_a than for R_i , the effect observed will be less than that of full agonist even at the maximally effective concentration of the agent. This type of drug is a partial agonist. The relative affinities for the R_i and R_a determines the efficacy of a drug.

A compound that has equal affinity for R_a and R_i will not change the equilibrium of the resting state. However such compound will interfere the action of agonist by occupying the binding sites. Such compound is antagonist.

Then, there is a compound that has higher affinity for R_i than R_a . This is called inverse agonist (or negative antagonist).

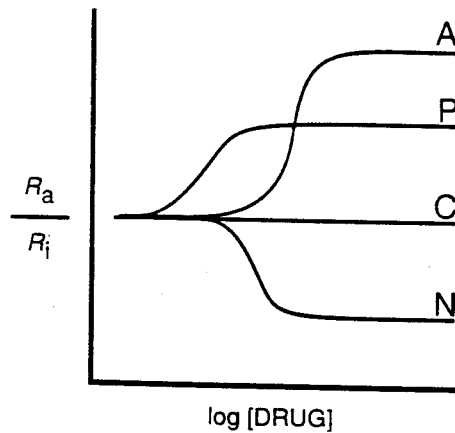
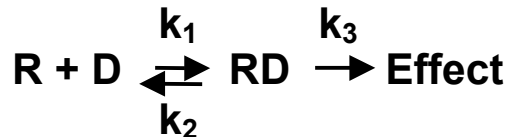


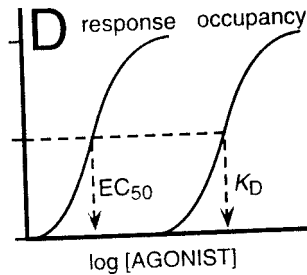
Figure 2-7. A working model for receptor-mediated response.

Modified occupational theory

- 1) A maximum effect can be produced by an agonist when occupying only a small proportion of the receptors -- "spare receptor"
- 2) The response is not linearly proportional to the number of receptors occupied.
- 3) Different drugs have varying capacity to initiate the response. Or the occupancy of a receptor to the same extent by different compounds can produce responses of different magnitudes. Different compounds which interact at the same site have different efficacy.



k_3 can be taken as a measure of efficacy. Antagonist has zero efficacy. Partial agonist has lower efficacy compared with full agonist.



Because of spare receptor and signal amplification, dose-response curves often fall to the left of receptor occupancy curve.

Characterization and classification of receptors.

How receptors were identified?

1) Structure-activity relationship : This is a classical approach. A small change of a ligand can result in dramatic alteration of effect with a receptor. For example, epinephrine and norepinephrine differ only by one methylgroup. Both can stimulate the heart with roughly equal potency. However, epinephrine is a much better bronchodilator than norepinephrine. Cardiac cells contain predominantly β_1 -adrenergic receptors, whereas bronchial smooth muscle cells contain β_2 -adrenergic receptors. Epinephrine and norepinephrine activate β_1 receptors with roughly equal potency and efficacy. Norepinephrine has lower affinity for β_2 receptors.

2) Binding of radioactive ligand: The binding of a highly selective, high affinity, and radioactive ligand permits characterization of tissue distribution, numbers, subtypes, and affinity of receptors. It was also used as an assay for receptor purification.

3) Molecular cloning : The availability of purified receptors made it possible to clone cDNAs for receptor. Expression cloning with radioactive ligand was also used to identify receptor cDNA. cDNA cloning permits us to analyze sequence, tissue distribution and detailed function of the receptor. It also made it possible to analyze the change of structure or function of the receptor in diseases. Subtypes of the receptor or homologous receptors were cloned by low stringency cloning or PCR.

4) Genome project (Reverse Pharmacology): It starts from a novel receptor cDNA with unknown ligand (Orphan receptor). Identify its endogenous ligand using various methods.

Example: Orexin -- hypothalamic hormone that affects sleep or appetite

Bile acid -- identified as a ligand for nuclear orphan receptor FXR

Receptors for endogeneous regulatory ligands

Membrane-bound receptors

G protein-coupled receptors

Ligand regulated ion channels

Tyrosine kinase and phosphatases

Gualyllyl Cyclase

Cytosolic Receptors

DNA binding proteins/transcription factors

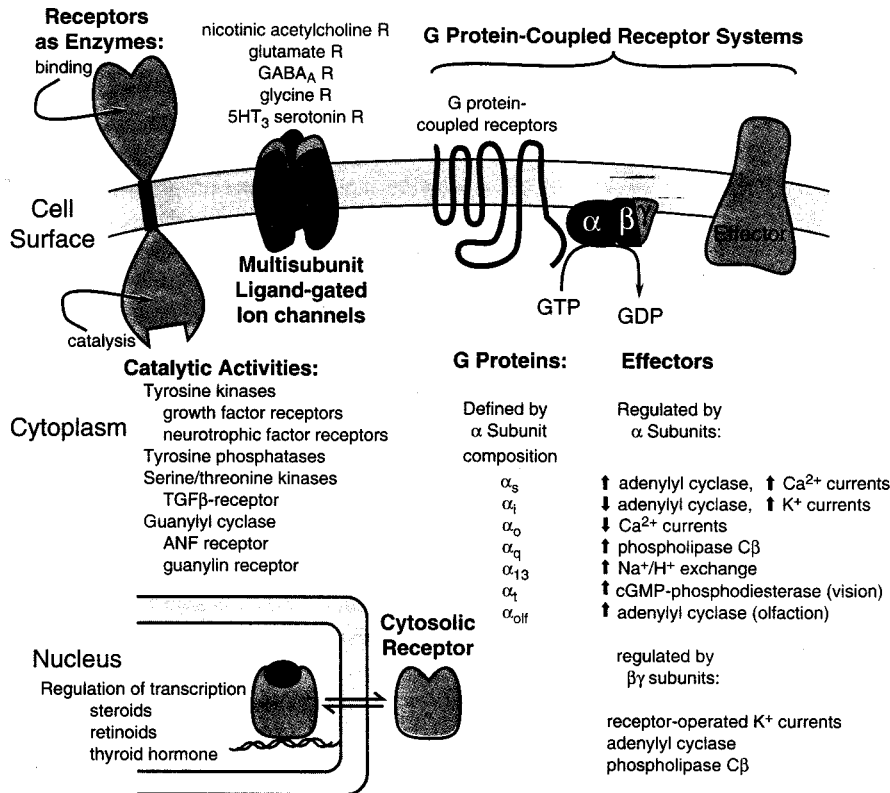


Figure 2-1. Structural motifs of physiological receptors and their relationships to signaling pathways.

TABLE 3-2. Examples of Drug Receptors and Signal Transduction Mechanisms*

Family and Type of Receptor	Mechanism of Signal Transduction	Drug Ligands
G protein-coupled receptors		
α ₁ -Adrenergic receptors	Activation of phospholipase	Phenylephrine (Ag)
α ₂ -Adrenergic receptors	Inhibition of adenylyl cyclase	Clonidine (Ag)
β-Adrenergic receptors	Stimulation of adenylyl cyclase	Isoproterenol (Ag) and propranolol (Ant)
Muscarinic receptors	Activation of phospholipase	Atropine (Ant)
Ligand-gated ion channels		
GABA _A receptors	Chloride flux	Benzodiazepines (Ag) and flumazenil (Ant)
Nicotinic ACh receptors	Sodium flux	Carbachol (Ag) and tubocurarine (Ant)
Membrane-bound enzymes		
Atrial natriuretic factor receptors	Activation of guanylyl cyclase	Nitroglycerin
Insulin receptors	Activation of tyrosine kinase	Insulin
Nuclear receptors		
Steroid receptors	Activation of gene transcription	Adrenal and gonadal steroids
Thyroid hormone receptors	Activation of gene transcription	Thyroxin

*Ag = agonist; Ant = antagonist; GABA = gamma-aminobutyric acid; and ACh= acetylcholine.

G protein-coupled receptors

Example : adrenergic receptors, muscarinic cholinergic receptors

The most common mechanism of signal transduction.

Broad range of signal such as mating in yeast, vision, smell, taste, neurotransmission, or cell growth control are mediated using the same mechanism.

Receptors have seven transmembrane structure.

About 300 receptors for regulatory ligands . We only know ligands for about 100 of them. Also there are about 1000 odorant receptors.

Ligands are biogenic amines, eicosanoids, opioid, bioactive lipids, peptides, or proteins.

Ligand binding site is extracellular or transmembrane region. Binding of ligand is transmitted to intracellular domain through unknown mechanism. This activates G protein which is coupling with the receptor

G proteins are heterotrimers : GTP, GDP-binding α subunit and a high affinity complex of β and γ subunits.

About 20 α subunits are known. They are unique to each G protein.

G protein is in GDP bound in resting state. Receptor catalyzes exchange of GTP to GDP. GTP induces conformational change of α subunit and it dissociates from $\beta\gamma$. Both the GTP- α and $\beta\gamma$ can regulate downstream effectors. GTP is hydrolysed by intrinsic GTPase activity of $G\alpha$, which turns off the signal to return to resting state.

Time course of signal is seconds to hours.

Effectors -- adenylyl cyclases, phospholipase C- β s, K^+ channels, Ca^{2+} channels, cyclic GMP phosphodiesterases

Major Systems of current interest:

$R \longrightarrow G_s \longrightarrow \text{Adenylyl Cyclase} \longrightarrow \text{CyclicAMP} \longrightarrow \text{Protein kinase A}$

Metabolic effects (glycogenolysis, glucogenolysis, lipolysis)

Positive inotropic and chronotropic effects on heart

Relaxation of smooth muscle

Stimulation of secretion

$R \longrightarrow G_i/o \longrightarrow \text{Adenylyl Cyclase, } K^+ \text{ channel, } Ca^{2+} \text{ channels}$
(inhibition)

Inhibition of secretion

Hyperpolarization

Negative inotropic, chronotropic effect on heart

$R \rightarrow G_q \rightarrow PLC-\beta$

Smooth muscle contraction
Secretion

Advantage of this system

Signal Amplification

Signal Integration

G proteins are switches and timers of signals.

If you know what G protein/effector system mediates the action of a given receptor and the effect of the second messengers involved, you can predict the effects of agonists or antagonists that work on these receptor.

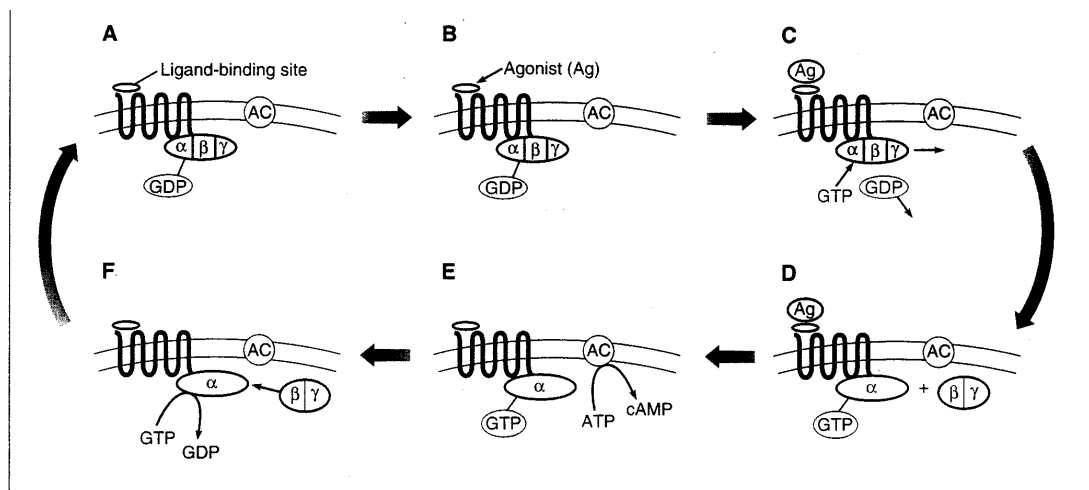


FIGURE 3-2. Signal transduction with a G protein-coupled receptor. (A) A typical G protein-coupled receptor contains a ligand-binding site on the external surface of the plasma membrane and a G protein-binding site on the internal surface. In the inactive state, guanosine diphosphate (GDP) is bound to the α subunit of the G protein. (B) and (C) When the agonist (Ag) binds to the receptor, guanosine triphosphate (GTP) binds to the G protein and causes the dissociation of GDP. (D) Activation of the α subunit by GTP causes the dissociation of the β and γ subunits. (E) The α subunit is then able to activate adenylyl cyclase (AC) and thereby stimulate the conversion of adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cyclic AMP, or cAMP). (F) GTP hydrolysis, catalyzed by α subunit GTPase, leads to reassociation of the β and γ subunits.

Ligand-regulated ion channels

Examples : nicotinic cholinergic receptor, GABA_A , glycine, glutamate receptors. Receptors for several neurotransmitters form agonist-regulated, ion-selective channels in the plasma membrane.

Channel activation results in alterations of membrane potential or ionic composition.

These receptors can transmit signals very quickly, in the order of msec to sec.

Receptor Protein Kinases

Common mechanism for growth factors, including EGF, PDGF, NGF and insulin. Most are tyrosine kinases with extracellular ligand binding domain, intracellular catalytic domain.

The binding of the agonist activates kinase activity of catalytic domain, which usually phosphorylate the receptor itself. The phosphorylated receptor interacts with other signaling molecules.

These are slow systems involved in cell growth cell death, or differentiation, usually acting over hours to days.

Receptor guanylyl cyclases

Extracellular peptide binding domain and intracellular guanylyl cyclase domain. Cytosolic guanylate cyclases are receptors for NO

Cytosolic transcription factor receptors

Ligand binding induces gene transcription.

Ligand binding domain, DNA binding domain, and regulatory domain.

Receptors for glucocorticoid, mineral corticoid, androgen, estrogen, thyroid hormone, retinoid, and vitamin D.

Regulation of Receptors

Receptors are themselves subject to feedback regulatory controls.

Desensitization (refractoriness or downregulation) often follows continued stimulation with agonists.

Desensitization may be homologous or heterologous.

Mechanism involve receptor phosphorylation, relocalization (internalization), alteration in rate of synthesis.

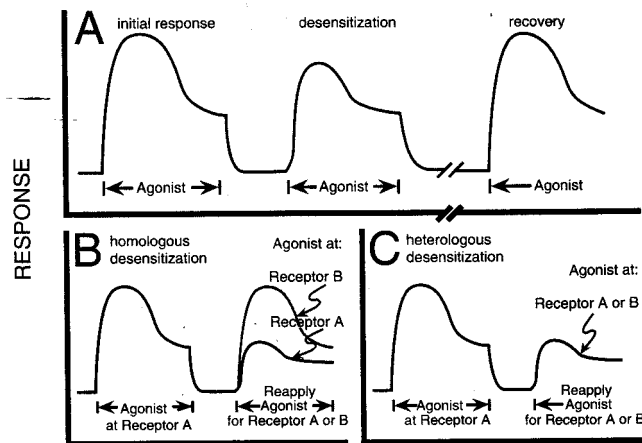


Figure 2-4. Desensitization in response to an agonist.

Examples of receptor- based diseases

Myasthenia Gravis --- Autoimmune disease, antibody for nicotinic Ach receptor.

Familial hypercholesterolemia --- polymorphism in LDL receptor heterozygote with one mutation is about one out of 500 population. Mutations affect several different steps; receptor synthesis, transport, binding of LDL, or recycling of receptor.

Diabetes Insipidus --- Some of the congenital forms of DI have mutation in vasopressin receptor.

Hirschprung disease (aganglionosis) --- enteric neurons in the distal colon and rectum is missing, resulting in megacolon. Endothelin-B receptor mutation.

Pseudohypoparathyroidism (Albright osteodystrophy) --- one defective allele in Gs alpha subunit. Homozygote is probably lethal. Low serum calcium and high phosphate. Low response to other ligands that increase cAMP. Short stature, abnormal calcification, cataract.