Pharmacology I

Lecture 3 and 4

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Drug–Receptor Interactions and Pharmacodynamics

Pharmacodynamics describes the actions of a drug on the body and the influence of drug concentrations on the magnitude of the response. Most drugs exert their effects, both beneficial and harmful, by interacting with receptors (that is, specialized target macromolecules) present on the cell surface or within the cell. The drug–receptor complex initiates alterations in biochemical and/or molecular activity of a cell by a process called signal transduction.

Most drug targets (receptors) are protein molecules. Even general anesthetics, which were long thought to produce their effects by an interaction with membrane lipid, now appear to interact mainly with membrane proteins.

All rules need exceptions, and many antimicrobial and antitumor drugs, as well as mutagenic and carcinogenic agents, interact directly with DNA rather than protein.

Types of Receptors:

Pharmacology defines a receptor as any biologic molecule to which a drug binds and produces a measurable response. Thus, enzymes, nucleic acids, and structural proteins can act as receptors for drugs or endogenous agonists. However, the richest sources of therapeutically relevant pharmacologic receptors are proteins that transduce extracellular signals into intracellular responses. These receptors may be divided into four families:

1) ligand-gated ion channels.
2) G protein- coupled receptors.
3) enzyme-linked receptors.
4) intracellular receptors .

The type of receptor a ligand interacts with depends on the chemical nature of the ligand. Hydrophilic ligands interact with receptors that are found on the cell surface. In

contrast, hydrophobic ligands enter cells through the lipid bilayers of the cell membrane to interact with receptors found inside cells.

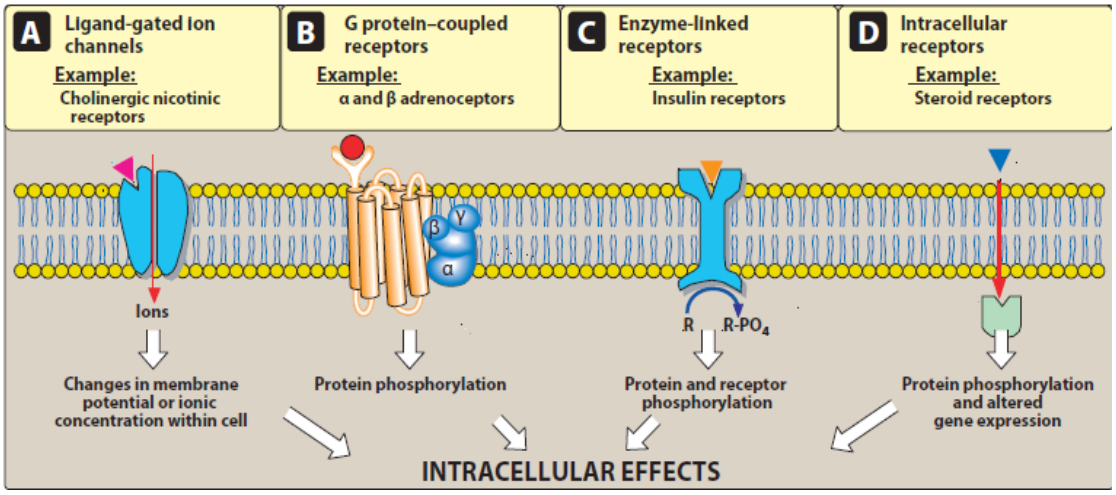


Figure 4: Transmembrane signaling mechanisms.

1. **Transmembrane ligand-gated ion channels:**

The extracellular portion of ligand-gated ion channels usually contains the ligand binding site. This site regulates the shape of the pore through which ions can flow across cell membranes (Figure 4A). The channel is usually closed until the receptor is activated by an agonist, which opens the channel briefly for a few milliseconds. Depending on the ion conducted through these channels, these receptors mediate diverse functions, including neurotransmission, and cardiac or muscle contraction. For example, stimulation of the nicotinic receptor by acetylcholine results in sodium influx and potassium outflux, generating an action potential in a neuron or contraction in skeletal muscle. On the other hand, agonist stimulation of the γ-aminobutyric acid (GABA) receptor increases chloride influx and hyperpolarization of neurons. Voltage-gated ion channels may also possess ligand-binding sites that can regulate channel function. For example, local anesthetics bind to the voltage-gated sodium channel, inhibiting sodium influx and decreasing neuronal conduction.

2. **Transmembrane G protein–coupled receptors:**

The extracellular domain of this receptor contains the ligand-binding area, and the intracellular domain interacts (when activated) with a G protein or effector molecule (Figure 4B). There are many kinds of G proteins (for example, Gs, Gi, and Gq), but they all are composed of three protein subunits. The α subunit binds guanosine triphosphate

(GTP), and the β and γ subunits anchor the G protein in the cell membrane. Binding of an agonist to the receptor increases GTP binding to the α subunit, causing dissociation of the α-GTP complex from the βγ complex. These two complexes can then interact with other cellular effectors, usually an enzyme, a protein, or an ion channel, that are responsible for further actions within the cell. These responses usually last several seconds to minutes (Figure 5).

Sometimes, the activated effectors produce second messengers that further activate other effectors in the cell, causing a signal cascade effect.

A common effector, activated by Gs and inhibited by Gi, is adenylyl cyclase, which produces the second messenger cyclic adenosine monophosphate (cAMP). Gq activates phospholipase C, generating two other second messengers: inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG). DAG and cAMP activate different protein kinases within the cell, leading to a myriad of physiological effects. IP3 regulates intracellular free calcium concentrations, as well as some protein kinases.

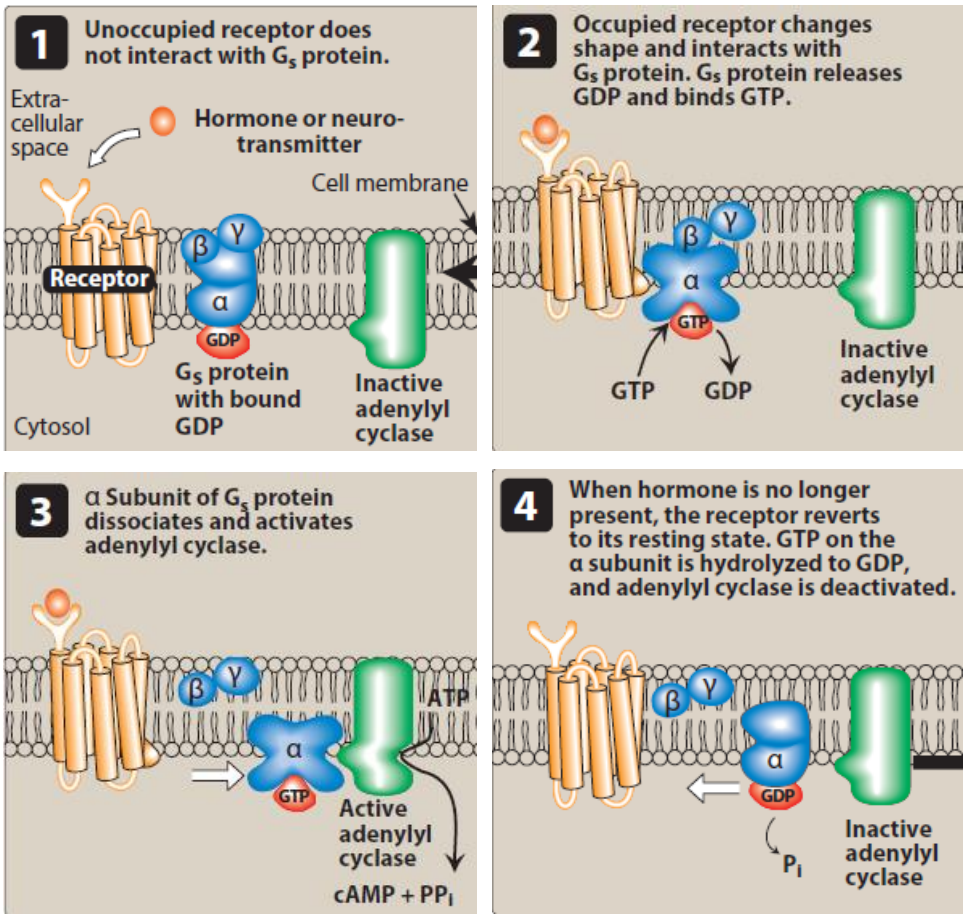


Figure 5: The recognition of chemical signals by G protein–coupled membrane receptors affects the activity of adenylyl cyclase.

3. **Enzyme-linked receptors:**

This family of receptors consists of a protein that may form dimers or multisubunit complexes. When activated, these receptors undergo conformational changes resulting in increased cytosolic enzyme activity, depending on their structure and function (Figure 6). This response lasts on the order of minutes to hours. The most common enzyme-linked receptors (epidermal growth factor, platelet-derived growth factor, atrial natriuretic peptide, insulin, and others) possess tyrosine kinase activity as part of their structure. The activated receptor phosphorylates tyrosine residues on itself and then other specific proteins (Figure 6). Phosphorylation can substantially modify the structure of the target protein, thereby acting as a molecular switch. For example, when the peptide hormone insulin binds to two of its receptor subunits, their intrinsic tyrosine kinase activity causes auto phosphorylation of the receptor itself. In turn, the phosphorylated receptor phosphorylates other peptides or proteins that subsequently activate other important cellular signals. This cascade of activations results in a multiplication of the initial signal, much like that with G protein–coupled receptors.

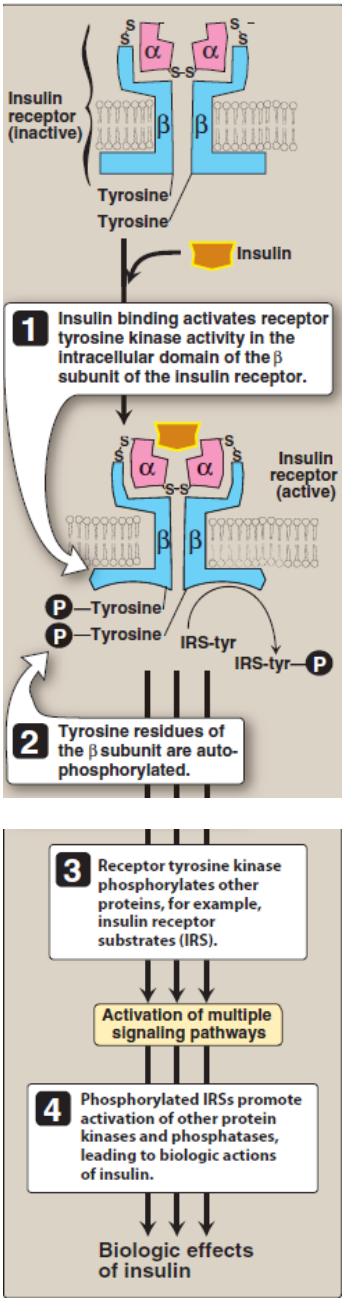
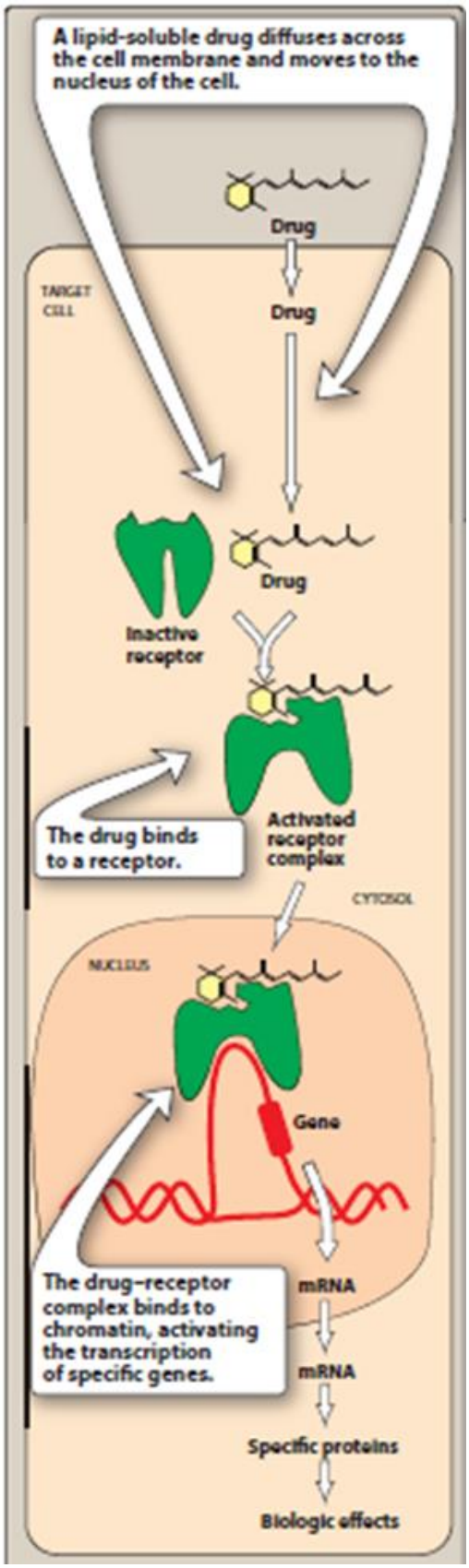


Figure 6: Insulin receptor.

4. **Intracellular receptors:**

The fourth family of receptors differs considerably from the other three in that the receptor is entirely intracellular, and, therefore, the ligand must diffuse into the cell to interact with the receptor. In order to move across the target cell membrane, the ligand must have sufficient lipid solubility. The primary targets of these ligand– receptor complexes are transcription factors in the cell nucleus.

Binding of the ligand with its receptor generally activates the receptor via dissociation from a variety of binding proteins. The activated ligand–receptor complex then translocates to the nucleus, where it often dimerizes before binding to transcription factors that regulate gene expression. The activation or inactivation of these factors causes the transcription of DNA into RNA and translation of RNA into an array of proteins. The time course of activation and response of these receptors is on the order of hours to days. For example, steroid hormones exert their action on target cells via intracellular receptors. Other targets of intracellular ligands are structural proteins, enzymes, RNA, and ribosomes. For example, tubulin is the target of antineoplastic agents such as *paclitaxel*, the enzyme dihydrofolate reductase is the target of antimicrobials such as *trimethoprim*, and the 50S subunit of the bacterial ribosome is the target of macrolide antibiotics such as *erythromycin*.


A lipid-soluble drug diffuses across the cell membrane and moves to the nucleus of the cell.
Drug
TARGET CELL
Drug
Drug
Inactive receptor
The drug binds to a receptor.
Activated receptor complex
CYTOSOL
NUCLEUS
Gene
The drug–receptor complex binds to chromatin, activating the transcription of specific genes.
mRNA
mRNA
Specific proteins
Biologic effects

Drug–Receptor Interactions and Pharmacodynamics

Dose-Response Relationship:

Occupation of a receptor by a drug molecule may or may not result in *activation* of the receptor. By activation, we mean that the receptor is affected by the bound molecule in such a way as to alter the receptor's behavior towards the cell and elicit a tissue response.

Binding and activation represent two distinct steps in the generation of the receptor-mediated response by an agonist (Fig. 1). If a drug binds to the receptor without causing activation and thereby prevents the agonist from binding, it is termed a *receptor antagonist*. The tendency of a drug to bind to the receptors is governed by its *affinity*, whereas the tendency for it, once bound, to activate the receptor is denoted by its *efficacy*.

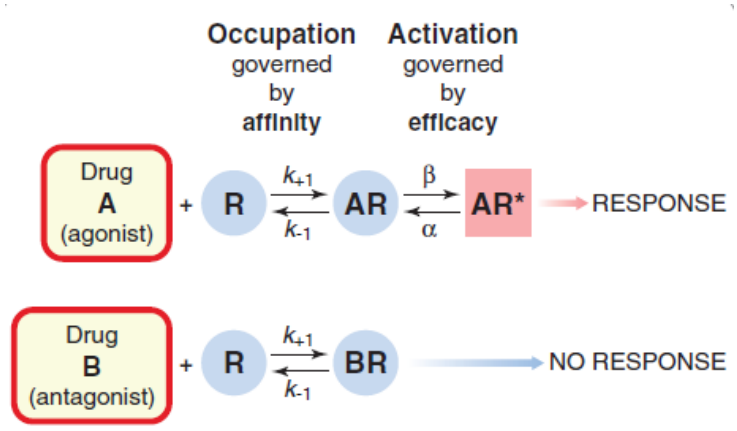


Figure 1: *The distinction between drug binding and receptor activation. Ligand A is an agonist, because when it is bound, the receptor (R) tends to become activated, whereas ligand B is an antagonist, because binding does not lead to activation. It is important to realise that for most drugs, binding and activation are reversible, dynamic processes. The rate constants k+1, k−1, α and β for the binding, unbinding and activation steps vary between drugs. For an antagonist, which does not activate the receptor, β = 0.*

Drugs of high potency generally have a high affinity for the receptors and thus occupy a significant proportion of the receptors even at low concentrations. Agonists also possess significant efficacy, whereas antagonists, in the simplest case, have zero efficacy. Drugs with intermediate levels of efficacy, such that even when 100% of the receptors are occupied the tissue response is submaximal, are known as *partial agonists*, to distinguish them from *full agonists*, the efficacy of which is sufficient that they can elicit a maximal tissue response.

Agonist drugs mimic the action of the original endogenous ligand for the receptor (for example, *isoproterenol* mimics norepinephrine on β1 receptors of the heart). The magnitude of the drug effect depends on the drug concentration at the receptor site,

which, in turn, is determined by both the dose of drug administered and by the drug's pharmacokinetic profile, such as rate of absorption, distribution, metabolism, and elimination.

THE BINDING OF DRUGS TO RECEPTORS

The *binding curve* (Fig. 2 A, B) defines the relationship between concentration and the amount of drug bound as well as the *binding capacity* (B_{max}), representing the density of receptors in the tissue.

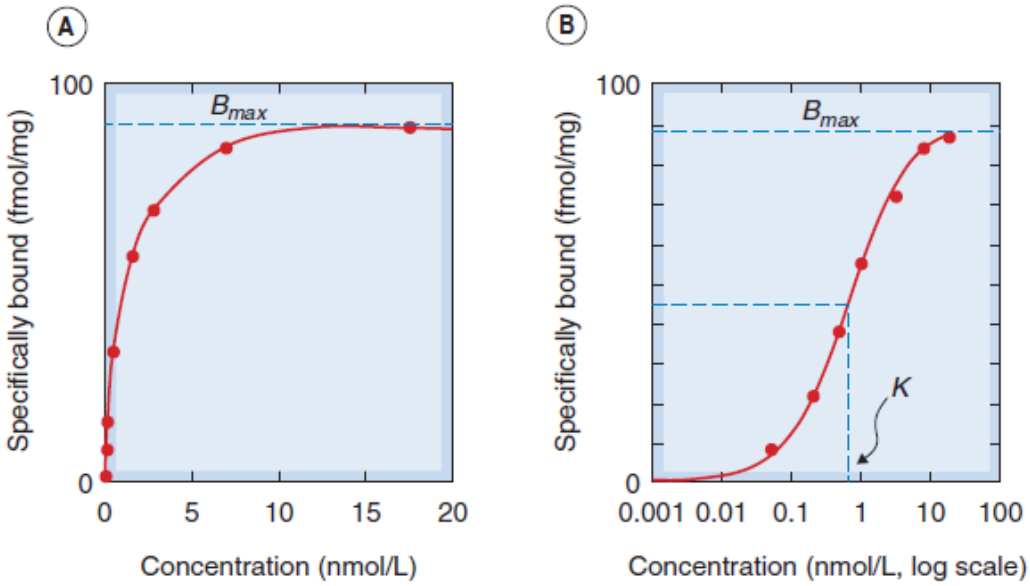


Figure 2: Measurement of receptor binding (β adrenoceptors in cardiac cell membranes). [A] Specific binding plotted against concentration. The curve is a rectangular hyperbola. [B] Specific binding as in [A] plotted against the concentration on a log scale. The sigmoid curve is a *logistic curve* representing the logarithmic scaling of the rectangular hyperbola plotted in panel [A] from which the binding parameters *K* and *B*max can be determined.

A. Graded dose–response relations

As the concentration of a drug increases, its pharmacologic effect also gradually increases until all the receptors are occupied (the maximum effect). Plotting the magnitude of response against increasing doses of a drug produces a graded dose–response curve that has the general shape depicted in Figure 3A. The curve can be described as a rectangular hyperbola, which is a familiar curve in biology because it can be applied to diverse biological events, such as enzymatic activity, and responses to

pharmacologic agents. Two important properties of drugs, potency and efficacy, can be determined by graded dose–response curves.

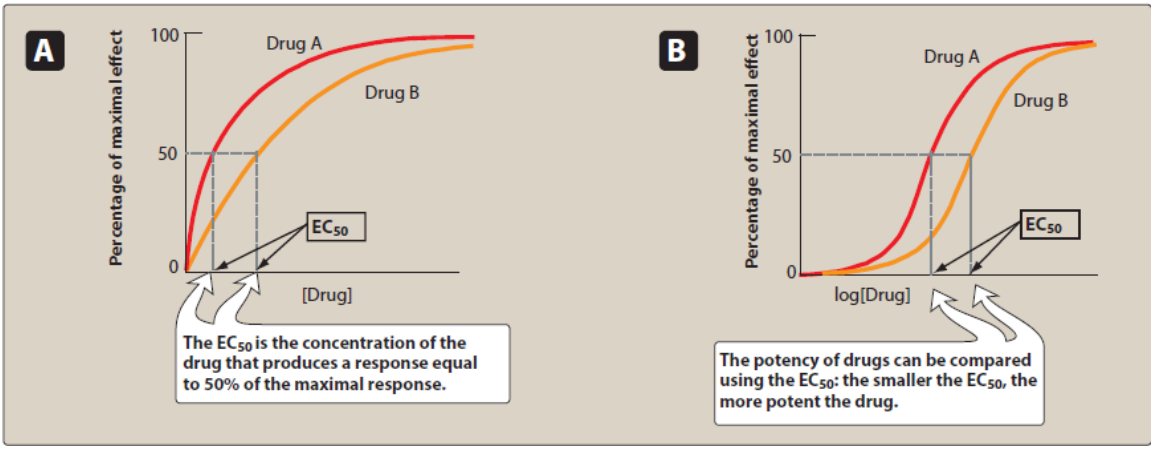


Figure 3: *The effect of dose on the magnitude of pharmacologic response. Panel A is a linear graph. Panel B is a semilogarithmic plot of the same data. EC50 = drug dose causing 50% of maximal response.*

1. **Potency:** Potency is a measure of the amount of drug necessary to produce an effect of a given magnitude. The concentration of drug producing 50% of the maximum effect (EC_{50}) is usually used to determine potency. In Figure 3, the EC_{50} for Drugs A and B indicate that Drug A is more potent than Drug B, because a lesser amount of Drug A is needed when compared to Drug B to obtain 50-percent effect. Therapeutic preparations of drugs reflect their potency. For example, *candesartan* and *irbesartan* are angiotensin receptor blockers that are used to treat hypertension. The therapeutic dose range for *candesartan* is 4 to 32 mg, as compared to 75 to 300 mg for *irbesartan.* Therefore, *candesartan* is more potent than is *irbesartan* (it has a lower EC_{50} value, similar to Drug A in Figure 3). Since the range of drug concentrations (from 1% to 99% of the maximal response) usually spans several orders of magnitude, semilogarithmic plots are used so that the complete range of doses can be graphed. As shown in Figure 3B, the curves become sigmoidal in shape, which simplifies the interpretation of the dose– response curve.
2. **Efficacy:** Efficacy is the magnitude of response a drug causes when it interacts with a receptor. Efficacy is dependent on the number of drug–receptor complexes formed and the intrinsic activity of the drug (its ability to activate the receptor and cause a cellular response).

Maximal efficacy of a drug (E_{max}) assumes that all receptors are occupied by the drug, and no increase in response is observed if a higher concentration of drug is obtained. Therefore, the maximal response differs between full and partial agonists, even when 100% of the receptors are occupied by the drug. Similarly, even though an antagonist occupies 100% of the receptor sites, no receptor activation results and E_{max} is zero. Efficacy is a more clinically useful characteristic than is drug potency, since a drug with greater efficacy is more therapeutically beneficial than is one that is more potent. Figure 4 shows the response to drugs of differing potency and efficacy.

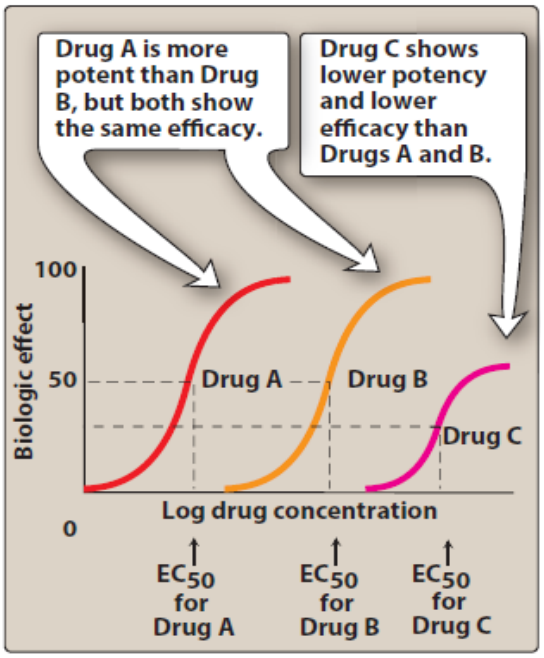


Figure 4: *Typical dose–response curve for drugs showing differences in potency and efficacy. EC50 = drug dose that shows 50% of maximal response.*

Thus, even if the concentration–effect curves, as in Figure 3B, looks just like a facsimile of the binding curve (Fig. 2B), it cannot be used directly to determine the affinity of the agonist for the receptors.

Spare receptors:

In the studying the actions of acetylcholine analogues in isolated tissues, found that many full agonists were capable of eliciting maximal responses at very low occupancies, often less than 1%. This means that the mechanism linking the response to receptor occupancy has a substantial reserve capacity. Such systems may be said to possess *spare receptors*, or a receptor reserve

Intrinsic Activity:

As mentioned above, an agonist binds to a receptor and produces a biologic response based on the concentration of the agonist and the fraction of activated receptors. The intrinsic activity of a drug determines its ability to fully or partially activate the receptors. Drugs may be categorized according to their intrinsic activity and resulting Emax values.

A. Full agonists

If a drug binds to a receptor and produces a maximal biologic response that mimics the response to the endogenous ligand, it is a full agonist (Figure 5). Full agonists bind to a receptor, stabilizing the receptor in its active state and are said to have an intrinsic activity of one. All full agonists for a receptor population should produce the same E_{max}. For example, *phenylephrine* is a full agonist at α1-adrenoceptors, because it produces the same Emax as does the endogenous ligand, norepinephrine.

Upon binding to α1-adrenoceptors on vascular smooth muscle, *phenylephrine* stabilizes the receptor in its active state. This leads to the mobilization of intracellular Ca^{2+}, causing interaction of actin and myosin filaments and shortening of the muscle cells. The diameter of the arteriole decreases, causing an increase in resistance to blood flow through the vessel and an increase in blood pressure. As this brief description illustrates, an agonist may have many measurable effects, including actions on intracellular molecules, cells, tissues, and intact organisms.

All of these actions are attributable to interaction of the drug with the receptor. For full agonists, the dose–response curves for receptor binding and each of the biological responses should be comparable.

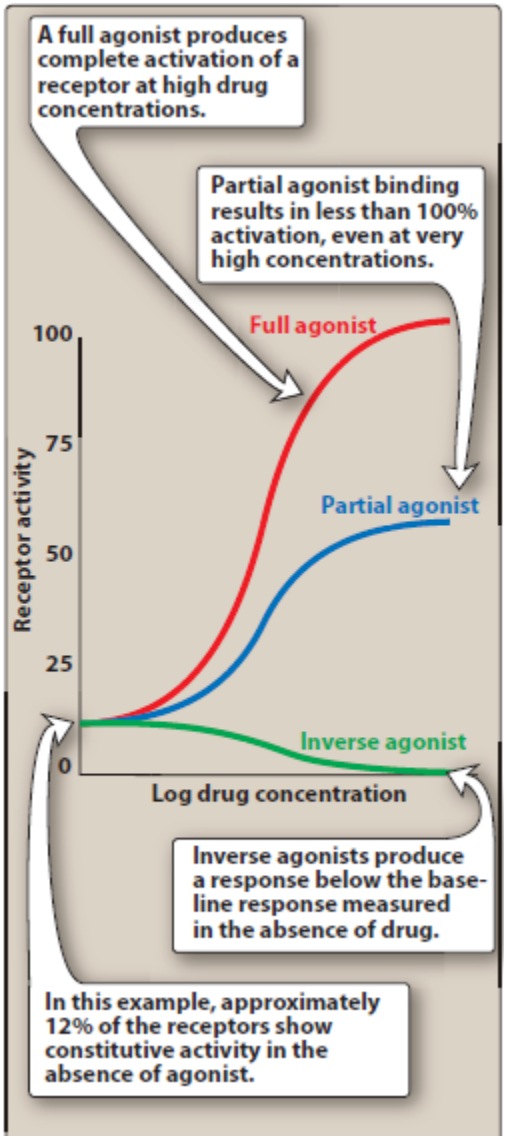


Figure 5: *Effects of full agonists, partial agonists, and inverse agonists on receptor activity.*

B. Partial agonists

Partial agonists have intrinsic activities greater than zero but less than one (Figure 5). Even if all the receptors are occupied, partial agonists cannot produce the same E_{max} as a full agonist. However, a partial agonist may have an affinity that is greater than, less than, or equivalent to that of a full agonist. When a receptor is exposed to both a partial agonist

and a full agonist, the partial agonist may act as an antagonist of the full agonist. Consider what would happen to the E_{max} of a receptor saturated with an agonist in the presence of increasing concentrations of a partial agonist (Figure 6). As the number of receptors occupied by the partial agonist increases, the E_{max} would decrease until it reached the E_{max} of the partial agonist. This potential of partial agonists to act as both an agonist and antagonist may be therapeutically utilized. For example, *aripiprazole*, an atypical antipsychotic, is a partial agonist at selected dopamine receptors.

Dopaminergic pathways that are overactive tend to be inhibited by *aripiprazole*, whereas pathways that are underactive are stimulated. This might explain the ability of *aripiprazole* to improve symptoms of schizophrenia, with a small risk of causing extrapyramidal adverse effects.

C. Inverse agonists

Typically, unbound receptors are inactive and require interaction with an agonist to assume an active conformation. However, some receptors show a spontaneous conversion from R to R* in the absence of an agonist (constitutive activation). Inverse agonists, unlike full agonists, stabilize the inactive R form and cause R* to convert to R. This decreases the number of activated receptors to below that observed in the absence of drug (Figure 5). Thus, inverse agonists have an intrinsic activity less than zero, reverse the activity of receptors, and exert the opposite pharmacological effect of agonists.

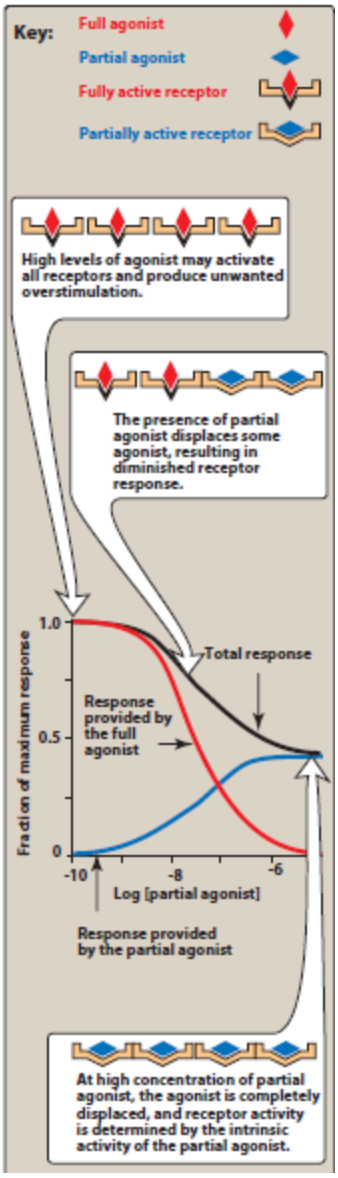


Figure 6: Effects of partial agonists.

Drug–Receptor Interactions and Pharmacodynamics

Antagonist:

Antagonists bind to a receptor with high affinity but possess zero intrinsic activity. An antagonist has no effect in the absence of an agonist but can decrease the effect of an agonist when present.

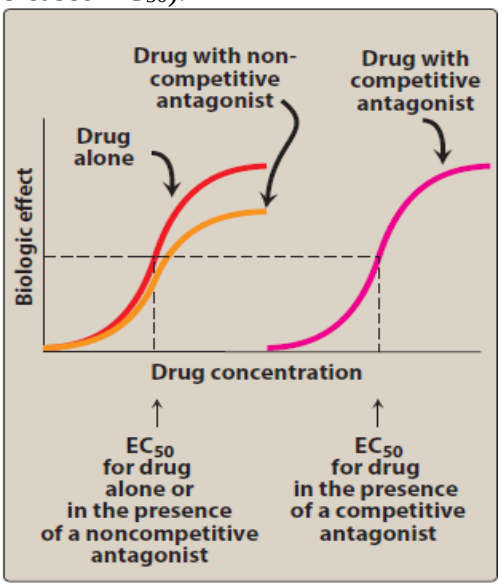
Antagonism may occur either by blocking the drug's ability to bind to the receptor or by blocking its ability to activate the receptor.

Receptor antagonists bind to receptors but do not activate them; the primary action of antagonists is to reduce the effects of agonists (other drugs or endogenous regulatory molecules) that normally activate receptors. While antagonists are traditionally thought to have no functional effect in the absence of an agonist, some antagonists exhibit "inverse agonist" activity because they also reduce receptor activity below basal levels observed in the absence of any agonist at all. Antagonist drugs are further divided into two classes depending on whether or not they act *competitively* or *noncompetitively* relative to an agonist present at the same time.

In the presence of a fixed concentration of agonist, increasing concentrations of a **competitive antagonist** progressively inhibit the agonist response; high antagonist concentrations prevent response completely. Conversely, sufficiently high concentrations of agonist can surmount the effect of a given concentration of the antagonist; that is, the E_{max} for the agonist remains the same for any fixed concentration of antagonist (Figure 7). Because the antagonism is competitive, the presence of antagonist increases the agonist concentration required for a given degree of response, and so the agonist concentration-effect curve is shifted to the right (increased EC_{50}).

The concentration of an agonist required to produce a given effect in the presence of a fixed concentration of competitive antagonist is greater than the agonist concentration required producing the same effect in the absence of the antagonist.

The ratio of these two agonist concentrations (dose ratio) is related to the dissociation constant (Ki) of the antagonist.



The salient features of competitive antagonism are:

- Shift of the agonist log concentration–effect curve to the right, without change of slope or maximum (i.e. antagonism can be overcome by increasing the concentration of the agonist).
- Linear relationship between agonist dose ratio and antagonist concentration
- Evidence of competition from binding studies.

Competitive antagonism is the most direct mechanism by which one drug can reduce the effect of another (or of an endogenous mediator).

The actions of a **noncompetitive antagonist** are different because, once a receptor is bound by such a drug; agonists cannot surmount the inhibitory effect irrespective of their concentration.

In many cases, noncompetitive antagonists bind to the receptor in an **irreversible** or nearly irreversible fashion, sometimes by forming a covalent bond with the receptor. After occupancy of some proportion of receptors by such an antagonist, the number of remaining unoccupied receptors may be too low for the agonist (even at high concentrations) to elicit a response comparable to the previous maximal response.

If spare receptors are present, however, a lower dose of an irreversible antagonist may leave enough receptors unoccupied to allow achievement of maximum response to agonist, although a higher agonist concentration will be required (figure 8).

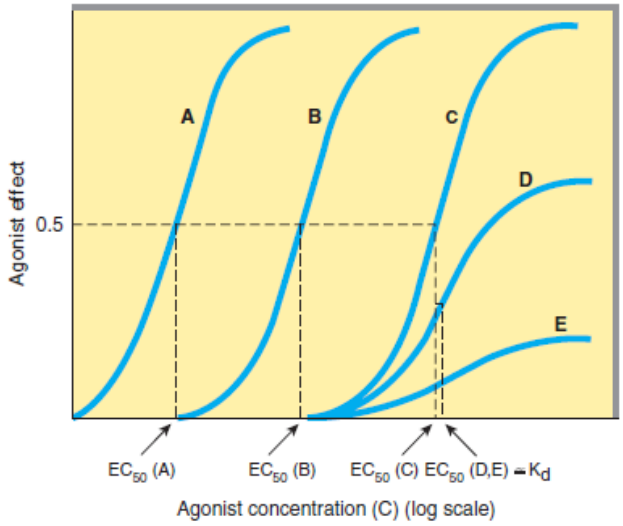


Figure 8: Logarithmic transformation of the dose axis and experimental demonstration of spare receptors, using different concentration of an irreversible antagonist. Curve **A** shows agonist response in the absence of antagonist. After treatment with a low concentration of antagonist (curve **B**), the curve is shifted to the right. Maximal responsiveness is preserved, however, because the remaining available receptors are still in excess of the number required. In curve **C,** produced after treatment with a larger concentration of antagonist, the available receptors are no longer "spare"; instead, they are just sufficient to mediate an undiminished maximal response. Still higher concentrations of antagonist (curves **D** and **E**) reduce the number of available receptors to the point that maximal response is diminished. The apparent EC_{50} of the agonist in curves **D** and **E** may approximate the Kd that characterizes the binding affinity of the agonist for the receptor.

Antagonists can function noncompetitively in a different way; that is, by binding to a site on the receptor protein separate from the agonist binding site; in this way, the drug can modify receptor activity without blocking agonist binding (Figure 9C and D).

Although these drugs act noncompetitively, their actions are often reversible. Such drugs are called *negative allosteric modulators* because they act by binding to a different (ie, "allosteric") site on the receptor relative to the classical ("orthosteric") site bound by the agonist. Not all allosteric modulators act as antagonists; some bind an allosteric site but, instead of inhibiting receptor activation, potentiate it. For example, benzodiazepines are considered *positive allosteric modulators* because they bind noncompetitively to ion channels activated by the neurotransmitter γ-aminobutyric acid (GABA), thereby enhancing the net activating effect of GABA on channel conductance.

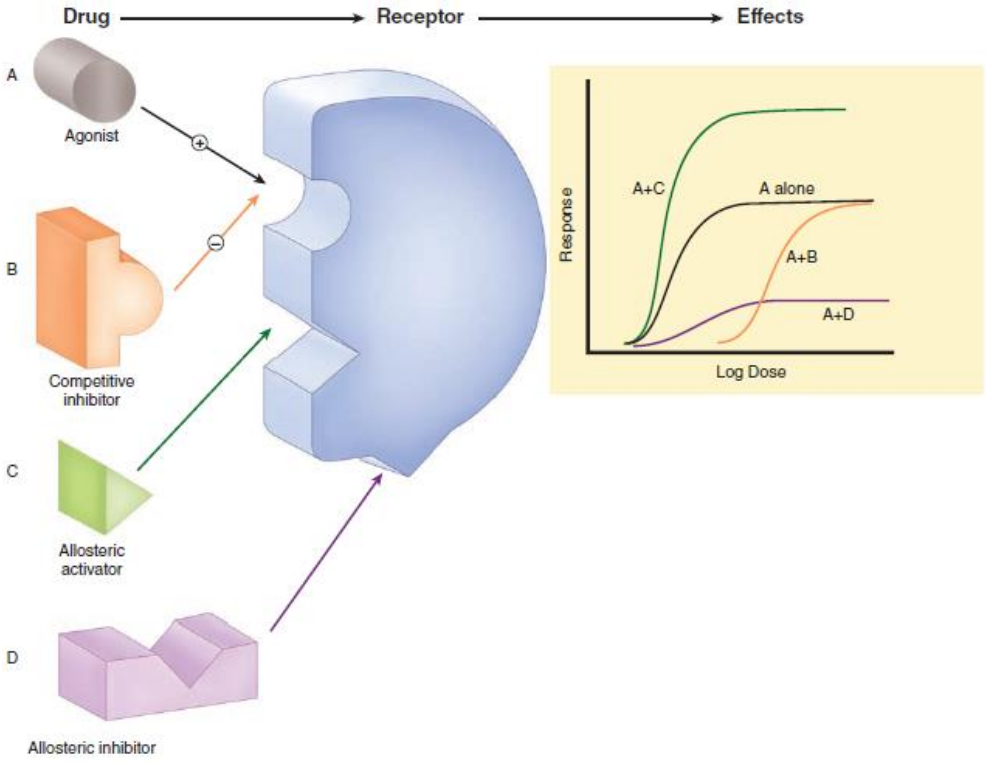


Figure 9: *Drugs may interact with receptors in several ways. The effects resulting from these interactions are diagrammed in the dose-response curves at the right. Drugs that alter the agonist (**A**) response may activate the agonist binding site, compete with the agonist (competitive inhibitors, **B**), or act at separate (allosteric) sites, increasing (**C**) or decreasing (**D**) the response to the agonist. Allosteric activators (**C**) may increase the efficacy of the agonist or its binding affinity. The curve shown reflects an increase in efficacy; an increase in affinity would result in a leftward shift of the curve.*

Functional antagonism: An antagonist may act at a completely separate receptor, initiating effects that are functionally opposite those of the agonist. A classic example is the functional antagonism by epinephrine to histamine-induced bronchoconstriction. Histamine binds to H1 histamine receptors on bronchial smooth muscle, causing bronchoconstriction of the bronchial tree. Epinephrine is an agonist at β2-adrenoceptors on bronchial smooth muscle, which causes the muscles to relax. This functional antagonism is also known as "physiologic antagonism."