

How do drugs work? Mechanisms of Drug Action

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Learning Objectives

- Describe the potential drug targets within a human body.
- Describe the role of receptors, enzymes, ion channels and transporters in drug action.
- Understand how drugs bind to receptors, and define the principles of affinity, efficacy and potency and be aware of the influence of the tissue on these properties.
- Understand the concentration response curve and what information can be gained from it.
- Differentiate between inverse agonism, agonism and antagonism and explain them using the two state model of receptor activity.
- Differentiate between different types of antagonism and understand their impact on the concentration response curve.

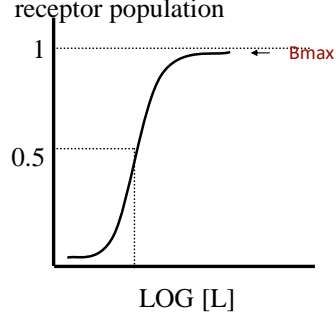
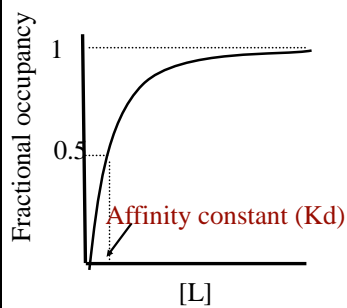
How do drugs bind to receptors

- Van der Waals forces – weak forces
- Hydrogen binding – stronger
- Ionic interactions – between atoms with opposite charges, stronger than hydrogen, weaker than covalent.
- Covalent binding – essentially irreversible

Affinity Constants

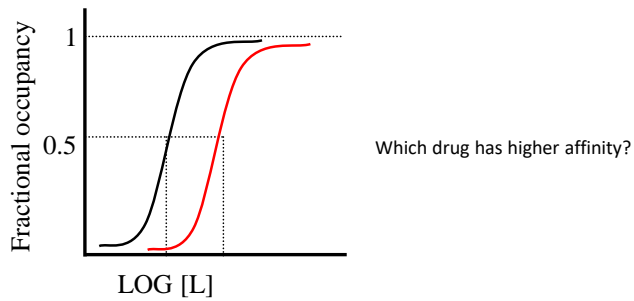
$$FO = \frac{[L]}{K_d + [L]}$$

If you solve the equation for K_d , you will find that $K_d = [L]$ when $FO = 0.5$, thus K_d can be defined as the concentration of drug $[L]$ which occupies 50% of the existing receptor population



Affinity

- The higher the *affinity* of the drug for the receptor, the lower the concentration at which it produces a given level of receptor occupancy.

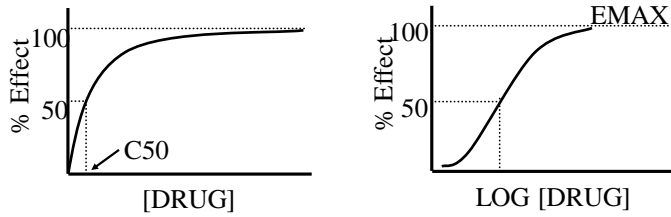


Affinity vs Biological Response

- Concentration - response curves are not a good measure of affinity because the relationship between receptor occupancy and response is not strictly proportional :
 - considerable amplification may exist - it may only take a low level of receptor occupancy to cause a maximal response in some tissues.
 - Many factors downstream from the receptor binding may interact to produce the final response.

Biological Response

- Biological response, eg a rise in blood pressure, contraction of a strip of smooth muscle in an organ bath, or the activation of an enzyme can be measured and plotted as a concentration response curve.
- In any system the response to a drug can be classified by its C_{50} and E_{max}



Potency

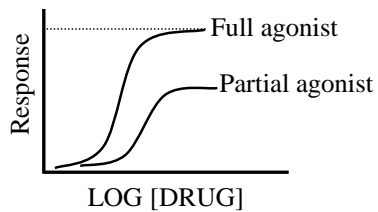
- C_{50} is used to measure the potency of an agonist.
- C_{50} is the effective concentration of an agonist that produces 50% of the maximal response
- The *more* potent the agonist, the *lower* the C_{50} .
- Antagonist potencies are more complicated to determine, but a similar principle holds.

Efficacy

- The ability of a drug to bind to a receptor and cause a change in the receptor's action is termed "efficacy" and measured by Emax
 - A drug with positive efficacy will activate a receptor to promote cellular response - **AGONISTS**
 - A drug with negative efficacy will bind to receptors to decrease basal receptor activity - **INVERSE AGONIST**
 - A drug with no efficacy will bind to the receptors but have no effect on activity –**ANTAGONIST (what would the effect of this be?)**

Agonism

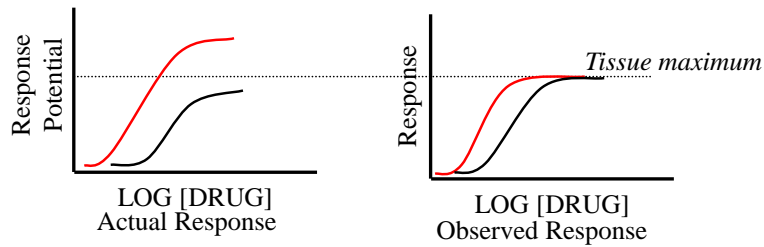
- Drugs that elicit the maximum tissue response are *full agonists*, drugs that produce less than the maximum response are *partial agonists*.



- *Partial agonists* can not produce maximal response even at 100% receptor occupancy.

Agonism

- While affinity is thought to be a function of only the drug and the receptor, the maximum response of a tissue to a drug is determined by *efficacy* and *tissue properties*. A drug may appear to be a partial agonist in one tissue and a full agonist in another

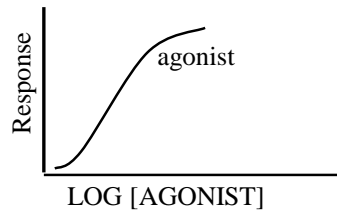


Antagonists

- A compound that binds to but does not activate (or inactivate) the receptor.
- Antagonists have affinity but NO efficacy.
- Defined by how they bind to the receptor...

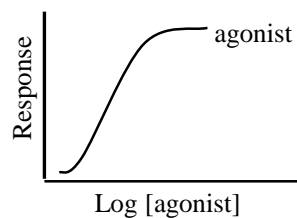
Reversible Competitive Antagonism

- Bind to the receptor in a reversible manner to compete directly with agonist binding.
 - What effect will this have on an agonist response curve?



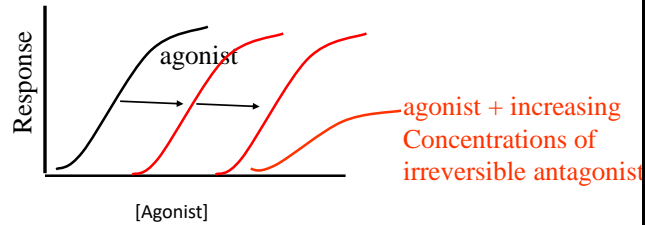
Irreversible Antagonism

- Drug covalently binds to the receptor. Not reversible
- Reduces the number of receptors available to the agonist.
What would this do to an agonist dose response curve?

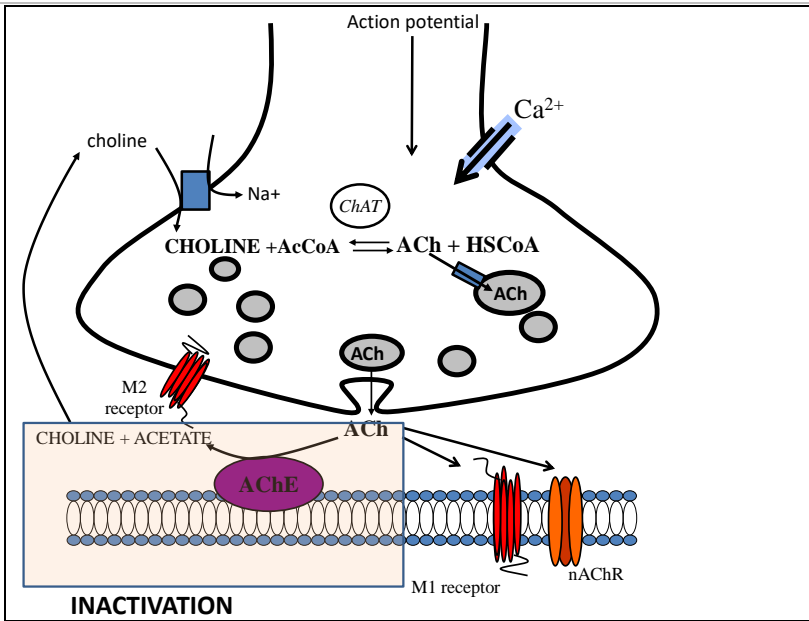


Irreversible antagonist

- Sometimes at low doses an irreversible antagonist looks like a reversible antagonist – what does this tell you about the number of receptors required to produce maximal response?



In contrast to drugs that act on receptors which can activate or inactivate, most drugs acting on enzymes or transporters only inhibit – why is this?



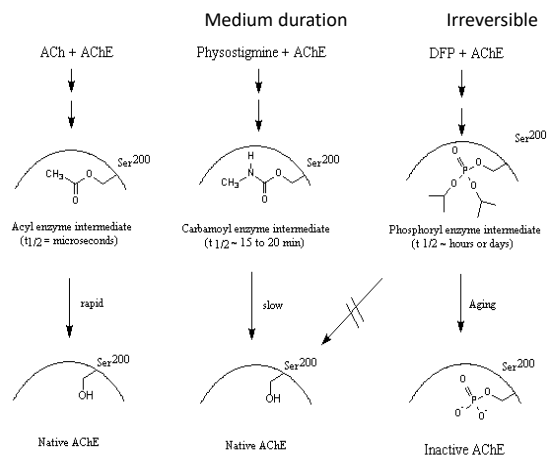
Inactivation

- Once in the synapse neurotransmitter must be quickly removed or chemically inactivated in order to prevent constant stimulation of the post-synaptic cell
- There are two key mechanisms of inactivation
 - Transport back into the pre-synaptic synapse (eg dopamine, serotonin)
 - Enzymic degradation (eg Acetylcholine)

Inhibitors of Acetylcholine esterase

- The effect that a specific AChE inhibitor can have on the body depends largely on the chemical properties of the molecule and the strength of the bond it forms with AChE.
- Irreversible AChE inhibitors are highly toxic –
 - organophosphorus compounds or nerve gases
 - form incredibly stable phosphorus bonds with AChE. Which resist hydrolytic cleavage for up to several hours inhibiting all normal AChE activity and leading to an excessive build-up of ACh at cholinergic synapses.
 - Lead to profuse sweating, dimmed vision, uncontrollable vomiting and defecation, convulsions, filling of the bronchial passages with mucus, bronchial constriction, and at last, paralysis and asphyxiation from respiratory failure.

Acetylcholinesterase inhibitors.

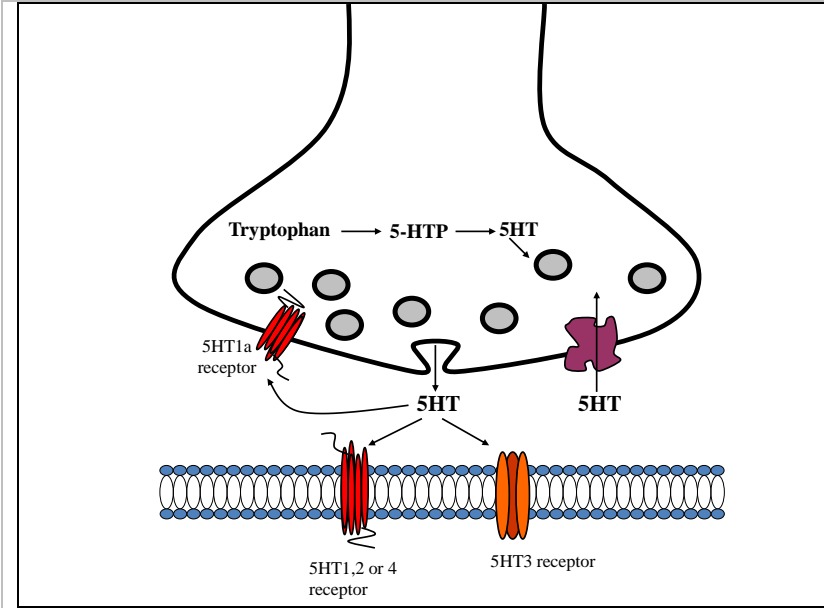


Reversible AChE inhibitors

- Bind to AChE for a short time.
- Used in disorders characterized by a decrease in cholinergic function – increase the duration that Ach is available in synapse, so one molecule can activate more receptors.
- Used to treat myasthenia gravis
 - an autoimmune disorder characterized by debilitating muscle weakness.
 - weakness is caused by a progressive breakdown of ACh receptor sites on the muscular endplate, making muscle movement difficult.
 - Pyridostigmine bromide, (Mestinon or Regonol), is the most widely used. Cannot pass the blood-brain barrier, so their action is limited to the inactivation of AChE at the neuromuscular junction., so no psychotropic effects.
- Used in the treatment of Alzheimer's disease
 - brain cell loss results in a progressive and significant loss of cognitive and behavioral function.
 - Drugs must readily cross the blood-brain barrier.
 - Tetrahydroaminoacridine (Tacrine or Cognex), and Donepezil (Aricept)
 - can help to ease some of the memory and language deficits.

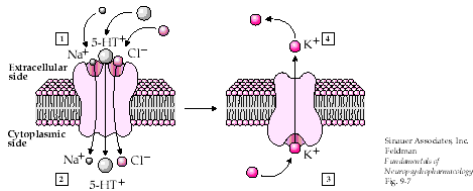
Serotonin (5HT) Transporter

- 5HT is involved in sleep, appetite, memory, sexual behaviour, neuroendocrine function and mood.
- It is synthesized from the amino acid precursor tryptophan, packaged in vesicles, and released into synapses following an action potential.
- Reuptake, determines the extent and duration of receptor activation.



Serotonin Transporter

- Part of a family of sodium and chloride dependent transporters.
- Twelve transmembrane domains.



- Na⁺ binds first to the carrier, followed by 5-HT (1). Cl⁻ is needed for transport but not for transmitter binding.
- The molecules are translocated in the membrane and dissociate (2). K⁺ then binds (3), and is translocated to the outside of the membrane (4), and dissociates to complete the cycle.

SSRI's

- What does this stand for?
- Can you think of an example?
- What will the effect be?

Revision

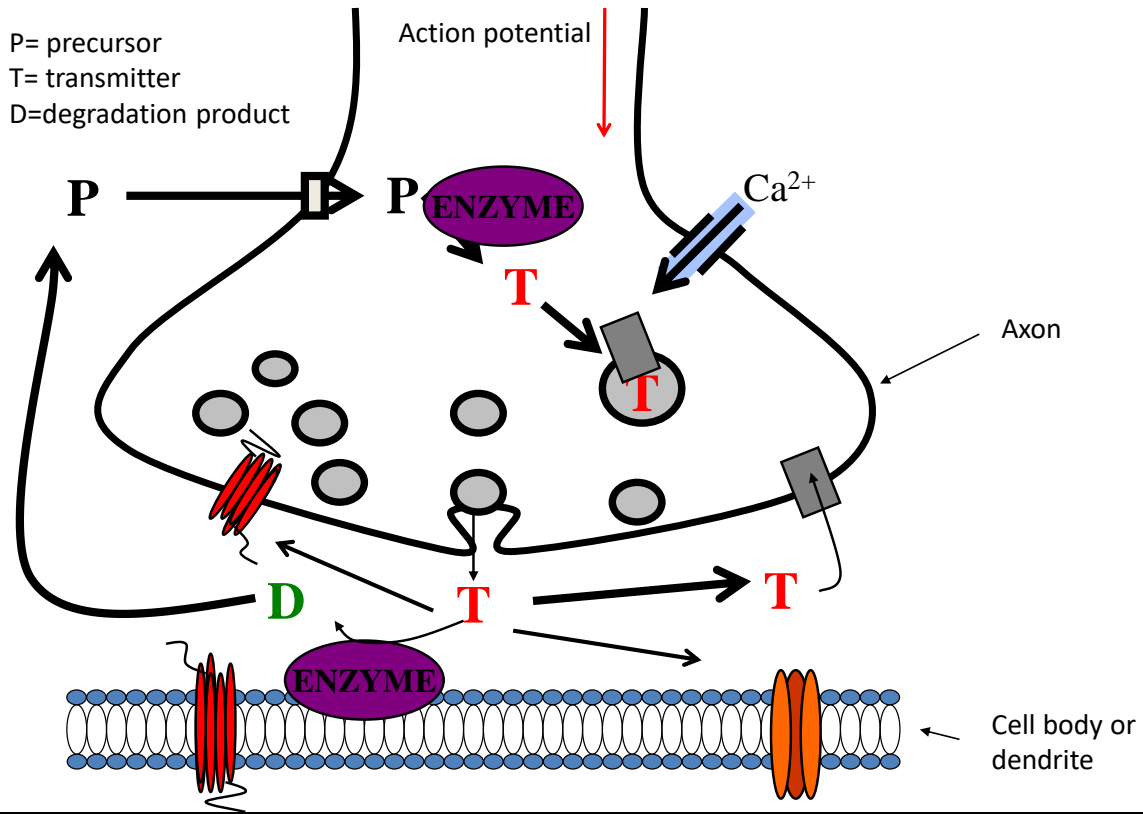
- On the following “stylised” synapse find at least ten sites of drug action.
- Describe what the likely effect of the drug is on neurotransmission
- For five give an example of a drug that acts in this way.

A "typical" nerve terminal

P= precursor

T= transmitter

D=degradation product



Mechanism of Drug Action and Drug Targets

Receptors

Neurotransmitter receptors in the plasma membrane of postsynaptic cells fall into two broad classes: ligand-gated ion channels and G protein – coupled receptors. Synapses containing either type can be excitatory or inhibitory, but the two types vary greatly in the speed of their response.

Ligand-Gated Ion Channels (Fast synaptic signalling)

Binding of the neurotransmitter to the extracellular binding domain on a ligand gated ion channel causes an immediate conformational change that opens the channel portion of the protein, allowing ions to cross the membrane and causing the membrane potential to change within 0.1 – 2 milliseconds. Binding of ligand to excitatory receptors opens cation channels that allow passage of both Na⁺ and K⁺ ions, leading to rapid depolarization of the postsynaptic membrane; in contrast, binding of ligand to inhibitory receptors opens Cl⁻ channels, leading to hyperpolarization of the postsynaptic membrane.

G Protein coupled receptors (GPCRs) (Slow synaptic transmission)

Many functions of the nervous system operate with time course of seconds or minutes; regulation of the heart rate, for instance, requires that action of neurotransmitters extend over several beating cycles measured in seconds. In general, the neurotransmitter receptors utilized in slow synapses are coupled to G proteins. GPCR signalling is also common in non-neuronal cells. Binding of the neurotransmitter leads to a conformational change in the receptor, that results in activation of a specific G protein. The G-protein may directly binds to and activate or inhibit ion-channels. In other cases, the receptor-activated G protein activates (or inhibits) adenylate cyclase or phospholipase C, triggering a rise in cytosolic cAMP or Ca²⁺, respectively; these second messengers in turn affect the ion conductance of a linked ion channel, or trigger kinase cascades that result in phosphorylation/dephosphorylation of target proteins. The postsynaptic responses

induced by neurotransmitter binding to GPCR are intrinsically slower and longer lasting than those induced by ligand-gated channels.

In this lecture, the diversity of receptors for and responses to a single kind of neurotransmitter is illustrated by acetylcholine. Synapses in which acetylcholine is the neurotransmitter are termed cholinergic synapses. Acetylcholine receptors that cause excitatory responses lasting only milliseconds are called nicotinic acetylcholine receptors. They are so named because nicotine, like acetylcholine, causes a rapid depolarization. These receptors are ligand-gated channels for Na⁺ and K⁺ ions. Other acetylcholine receptors are called muscarinic acetylcholine receptors because muscarine (a mushroom alkaloid) causes the same response as acetylcholine. There are several subtypes of muscarinic acetylcholine receptors present in different cell types; all are coupled to G proteins, but they induce different responses. The M₂ receptor present in heart muscle activates a G_i protein that causes the opening of a K⁺ channel and thus a hyperpolarization lasting seconds when post-synaptic. Presynaptic M₂ receptors, in addition to inhibiting adenylate cyclase, inhibit the opening of voltage gated calcium channels, leading to decreased ACh release from the nerve terminal. The M₁, M₃, and M₅ subtypes are coupled to G_q and activate phospholipase C; the M₄ subtype activates G_i and inhibits adenylate cyclase. Thus, a single neurotransmitter induces very different responses in different nerve and muscle cells, depending on the type of receptor found in the target cells.

How drugs act at receptors

Affinity: a measure of how tightly a drug binds to the receptor. If the drug does not bind well, then the action of the drug will be shorter and the chance of binding will also be less. This can be measured numerically by using the dissociation constant K_D . The K_D is the concentration of drug when 50% of receptors are occupied. Thus, the higher the K_D the lower the affinity of the drug (the more drug it'll take to bind to the receptors).

Potency is a measure of how much a drug is required in order to produce a particular effect. This is defined in a concentration response curve by the EC50 – the concentration of drug required to produce 50% of maximal response. If a drug has high potency drug only a small amount is required to induce a large response.

Efficacy is the ability of a drug produce a response. Agonists are described as having affinity and efficacy, partial agonists are drugs which bind with affinity, but do not produce maximal response – *even at 100% receptor occupancy*, thus they are less efficacious than full agonists. Antagonists are drugs which have no intrinsic efficacy – they can bind to the receptor (i.e. they have affinity), but do not induce a conformational change in the receptor. They produce their effects by preventing agonist from binding.

Enzymes

Many enzymes form useful drug targets eg acetylcholinesterase (neostigmine); cyclo-oxygenase (asprin), angiotensin converting enzyme (captopril). Often the drug mimics the natural substrate acting as a competitive inhibitor of the enzyme (eg captopril); in other cases the binding is non-competitive and irreversible (eg asprin). Drugs may also act as false substrates, where the drug molecule undergoes transformation to form an abnormal product that subverts the normal metabolic pathway. An example of this is the anticancer drug fluorouracil, which replaces uracil as an intermediate in purine biosynthesis, but cannot be converted into thymidylate, thus blocking DNA synthesis and preventing cell division. Some drugs require enzymic degradation to convert them from a prodrug into an active form. Other drugs may be converted into a toxic or reactive metabolite. We will consider the example of acetylcholine esterase inhibitors in the lecture.

Carrier/Transporter Molecules

Transportation of polar molecules across lipid membranes often requires a carrier protein. The carrier proteins embody a recognition site that makes them specific for a particular

permeating species, and these sites can also be targets for drugs, whose effect is generally to block the transporter. We will consider the example of the serotonin transporter, which are the drug target for selective serotonin reuptake inhibitors (SSRIs), for depression.

Ion Channels

Ion channels are protein molecules that span the cell membrane, and can switch between open and closed states, allowing the controlled entry or exit of specific ions across the cell membrane in their open state. Ion channels are highly selective for the ions that they control. The channels are all gated i.e they have particular triggers that allow for their opening. You are already familiar with two forms of gating – ligand gated ion channels, which are an integral part of a receptor, and second messenger gated ion channels, which are opened either by G-proteins directly, or downstream effector molecules such as cAMP. The third major class of gated-ion channel are the voltage gated ion channels, which open in response to voltage changes across cell membrane. These channels open when the membrane becomes depolarized, and are critical to the mechanism of membrane excitability (development of action potentials). Channel opening is usually short lasting. Drugs may bind directly within the ion channel and prevent the flow of ions, or may bind elsewhere on the molecule and increase or decrease the probability of the channel opening in response to a change in voltage. Local anaesthetics are a class of drugs which block pain pathways by blocking voltage gated sodium channels and therefore preventing the action potential that carries the pain signal from the site of injury to the brain.