How do drugs work? Mechanism of Drug Action and Drug Targets

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THE UNIVERSITY OF AUCKLAND
NEW ZEALAND

MEDICAL AND HEALTH SCIENCES

Learning Objectives

By the end of this lecture you should be able to:

1. Describe the potential drug targets within a human body
2. Describe the role of receptors, enzymes, ion channels and transporters in drug action
3. Understand how drugs bind to receptors, and define the principles of affinity, efficacy and potency
4. Understand the concentration/dose-response curve and what information can be gained from it

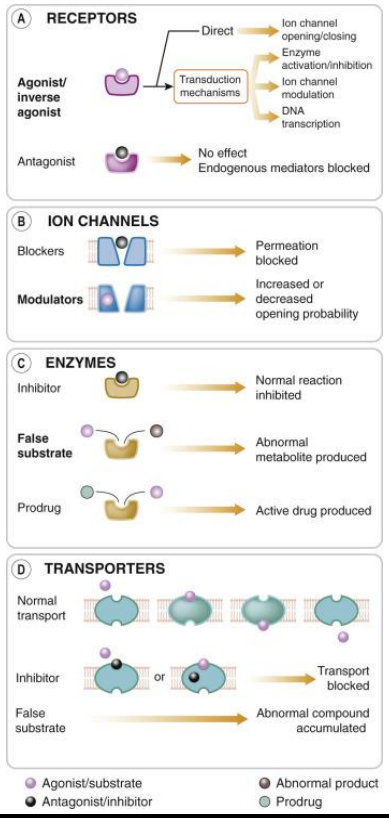
Learning Objectives

By the end of this lecture you should be able to:

5. Differentiate between inverse agonism, agonism and antagonism
6. Differentiate between different types of antagonism and understand their impact on the concentration/dose-response curve
7. Define the term selectivity and explain its relevance to drug therapy
8. Describe receptor plasticity and explain its clinical relevance

Mechanism of Drug Action

- Most drugs produce their effects by **binding** to protein molecules. Drug binding often leads to a conformational change in the protein. Four primary drug targets are:
  - Ion channels.
  - Enzymes.
  - Carrier molecules.
  - Receptors.
- Important exceptions are cytokines (ligand targeted by mAbs) and DNA (antitumour and antimicrobials)



Types of target for drug action.
How drugs act : molecular aspects
Rang, HP, MB BS MA DPhil Hon FBPharmacolS FMedSci FRS,
Rang & Dale's Pharmacology, 3, 22-49
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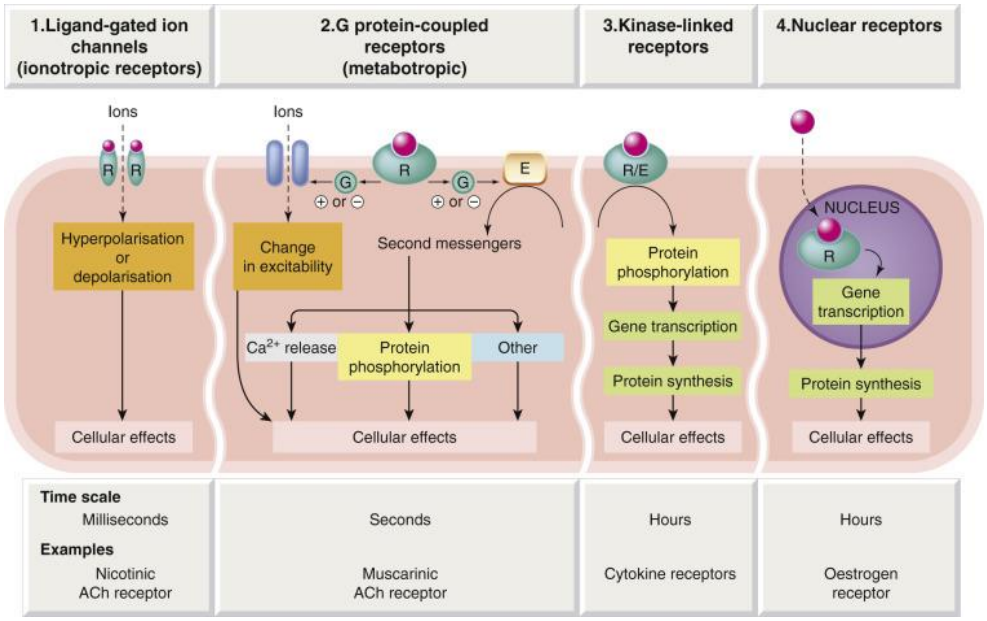
Receptors

- Proteins which specifically recognise a particular neurotransmitter/hormone and upon binding undergo a conformation change leading to activation/inhibition of cell signalling.
- Four main families of receptor:
  - Ligand Gated Ion Channels (Ionotropic receptors)
  - G-protein coupled receptors
  - Tyrosine kinase/cytokine receptors
  - Nuclear/Steroid Hormone Receptors

Receptor Terminology

- Affinity: attraction of a ligand (drug) for a receptor
- Efficacy (intrinsic activity):
  - Maximum effect = 1
  - No effect = 0
- Agonists have affinity and efficacy (mimics)
- Antagonists have affinity but no efficacy (prevents)

Signalling Pathways

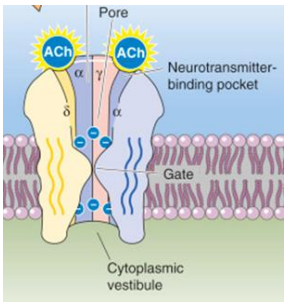


Types of receptor–effector linkage. How drugs act : molecular aspects. Rang, HP, MB BS MA DPhil Hon FBPharmacolS FMedSci FRS, Rang & Dale's Pharmacology, 3, 22-49. Accessed via Clinical Key. Copyright © 2016 © 2016, Elsevier Ltd

Ligand Gated Ion Channels

- Mediate fast signal transmission at synapses (action occurs in a fraction of a millisecond)
- All are multi-subunit complexes
- All have three important properties:
  - They are activated in response to specific ligands
  - They conduct ions through the otherwise impermeable cell membrane
  - They select among different ions

The Receptor Pore



Synaptic Transmission and the Neuromuscular Junction
Moczydlowski, Edward G., Medical Physiology, Chapter 8, 204-227.e2
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•The five TM2 helices are sharply kinked inwards halfway through the membrane forming a constriction.
•The TM2 helices are believed to snap to attention when Ach binds, opening the channel

The Receptor Pore

- Ion conductivity is highly selective, and determined by amino acids in TM2.
- Most excitatory neurotransmitters (ACh, Glutamate) cause an increase in Na+ and K+ permeability.
- The inside of the cell therefore becomes more positive (depolarised) and has an increased probability for an action potential.

Ionotropic Receptors As Drug Targets

- **$GABA_A$** – benzodiazepines and barbiturates (sedation and anxiolytic effects), flumazenil.

- **Glutamate** – ketamine (anaesthetic). Major target for neuroprotection and anti-convulsants, but to date all compounds have shown major adverse effects (predominantly hallucinations)

Fig. 45.3 Model of benzodiazepine/$GABA_A$-receptor interaction. Anxiolytic and hypnotic drugs
Ritter, James M., DPhil FRCP HonFBPhS FMedSci, Rang & Dale's Pharmacology, 45, 569-579
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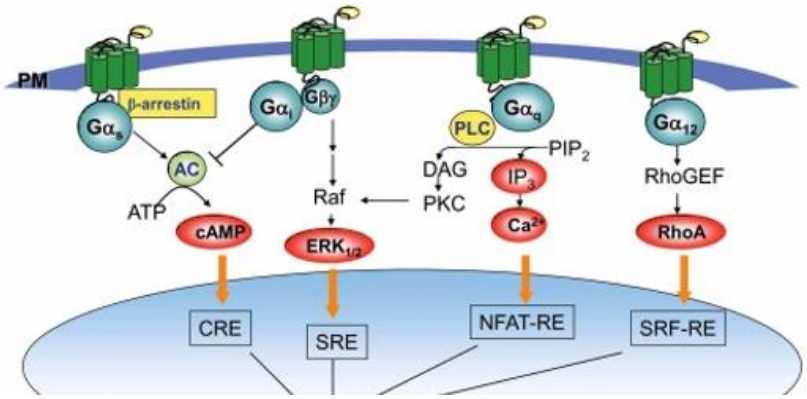
Break

- Where in the body are the ionotropic receptors we've covered?
- Why are they important in these areas?

Key GPCR Signalling Pathways



Cheng, Zhijie & Garvin, Denise & Paguio, Aileen & Stecha, Pete & Wood, Keith & Fan, Frank. (2010). Luciferase Reporter Assay System for Deciphering GPCR Pathways. Current chemical genomics. 4. 84-91. 10.2174/1875397301004010084.

Clinical condition	Drug	References‡
Alzheimer's disease Cognitive impairment	M_1, M_2 or mixed M_1/M_2 agonist; M_2 antagonist§	51,52,54–56, 58,69
Sjögren's syndrome	M_1, M_3 or mixed M_1/M_3 agonist	15,25,114
Schizophrenia	M_1, M_4 or mixed M_1/M_4 agonist	18,74,75
Parkinson's disease	M_1, M_4 or mixed M_1/M_4 antagonist	14,18,22,72
Type 2 diabetes	M_3 agonist (peripherally acting)	29
Obesity	M_3 antagonist (centrally acting)	16,129
Peptic ulcer disease‖	M_3 or mixed M_3/M_2 antagonist	116,117
OAB, COPD	M_3 antagonist	27,94,95,101
Irritable bowel syndrome Gastrointestinal spasms	M_3 or mixed M_2/M_3 antagonist	15,28,105–110
Antinociception	M_4 agonist	26
Wound healing	M_4 agonist, M_3 antagonist	121
Cerebrovascular insufficiency	M_5 agonist	19,69
Drug addiction and withdrawal	M_5 antagonist	83–85

*The proposed therapeutic uses of the indicated agents are based on gene targeting and/or pharmacological data (see text for details). ‡Note that only studies dealing with muscarinic acetylcholine receptor (*mAChR*) mutant mice have been included in this table. §Although several pharmacological studies suggest that M_2 receptor-preferring antagonists can enhance cognition in experimental animals[67,68], studies with *M2R*$^{-/-}$ mice have shown that the complete lack of M_2 receptors is associated with cognitive deficits (see text for details)[59]. ‖Note that pirenzepine and telenzepine, two M_1 receptor-preferring antagonists, are used for the treatment of peptic ulcer disease in Europe, Japan and Canada. COPD, chronic obstructive pulmonary disease; OAB, overactive bladder.

Potential therapeutic uses of mAChR subtype-selective compounds*
From: :
***Nature Reviews Drug Discovery* 6, 721-733 (2007)**
doi:10.1038/nrd2379

Tyrosine Kinase Receptors

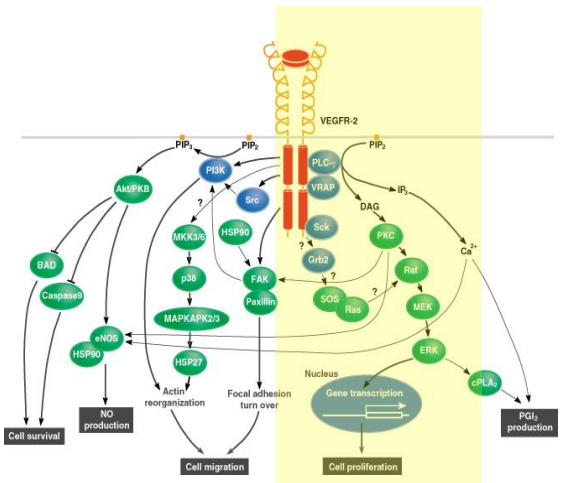
- Receptor functions as an enzyme that transfers phosphate groups from ATP to tyrosine residues on intracellular target proteins.
- Tyrosine kinase receptors mediate the actions of growth factors, cytokines and certain hormones (eg insulin).

Vascular Endothelial Growth Factor Receptors

- Essential for angiogenesis during development, pregnancy, wound healing
- Also in pathophysiological conditions eg cancer, rheumatoid arthritis, cardiovascular disease.
- Multiple receptors/multiple ligands, we will look briefly at VEGFR2

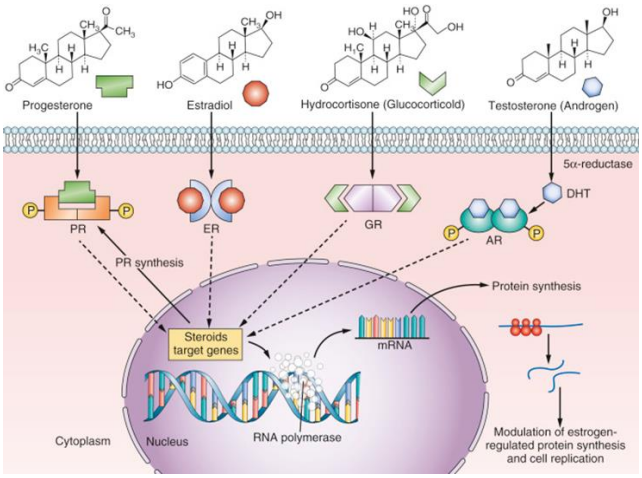
VEGFR2

- Ligand stimulated receptor dimerisation
- Autophosphorylation of tyrosine residues in cytoplasmic domain
- Associates with SH2 domain proteins
- Activation regulates a multitude of biological functions
  - Endothelial cell survival
  - Endothelial cell proliferation
  - Endothelial cell migration
  - NO and PGI2 production
  - Increase vascular permeability



http://stke.sciencemag.org/cgi/content/full/sigtrans;2001/112/re21/F3

Nuclear Receptors



Steroid Receptors in Breast Cancer
Abderrahman, Balkees, The Breast: Comprehensive Management of Benign and Malignant Diseases, 21, 272-281.e2. Accessed via ClinicalKey 23.05.19
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Nuclear Receptors

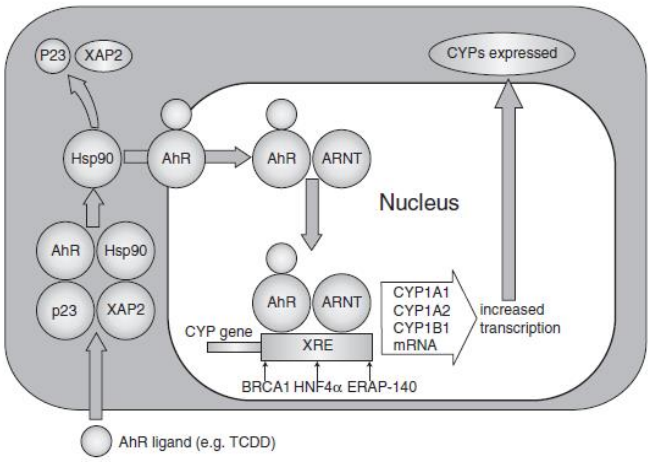

Fig 4.1, Coleman MD, Human Drug Metabolism (2nd ed). (2010). DOI:10.1002/9780470689332
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https://onlinelibrary-wiley-com.ezproxy.auckland.ac.nz/doi/book/10.1002/9780470689332

Break

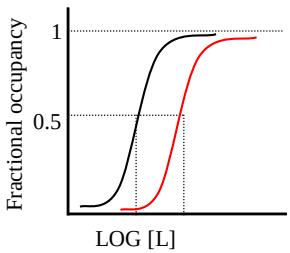
- Describe the potential drug targets within a human body
- Describe the role of receptors, enzymes, ion channels and transporters in drug action

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

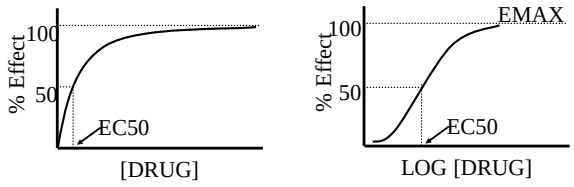
- The higher the *affinity* of the drug for the receptor, the lower the concentration at which it produces a given level of receptor occupancy.



Which drug has higher affinity?

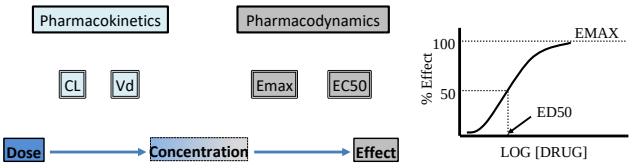
Biological Response

- 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 EC50 and Emax



Dose vs Concentration

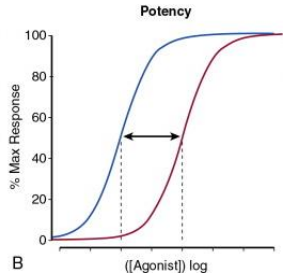
- Dose is the actual amount of medicine administered, concentration is what that dose produces in the body



Potency

- EC_{50} is used to measure the potency of an agonist.
- EC_{50} is the effective concentration of an agonist that produces 50% of the maximal response
- The ***more*** potent the agonist, the ***lower*** the EC50.
- Antagonist potencies are more complicated to determine, but a similar principle holds.

Dose Response Curve Potency



Adapted from Vascular Pharmacology
Harrison, David G., Vascular Medicine: A Companion to Braunwald's Heart Disease, Chapter 6, 75-93
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Affinity vs Biological Response

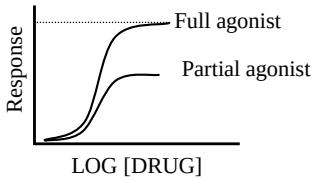
- Concentration - response curves are not a good measure of affinity because the relationship between receptor occupancy and response is not strictly proportional :
  - considerable amplification may exist - it may only take a low level of receptor occupancy to cause a maximal response in some tissues.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

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?)***

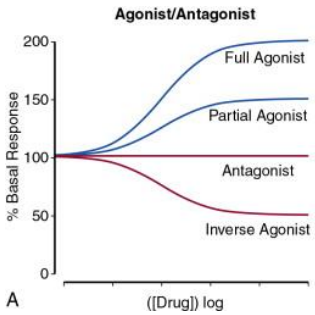
Agonism

- Drugs the 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.

Types of Agonist: Full, Partial & Inverse



Adapted from Vascular Pharmacology
Harrison, David G., Vascular Medicine: A Companion to Braunwald's Heart Disease, Chapter 6, 75-93
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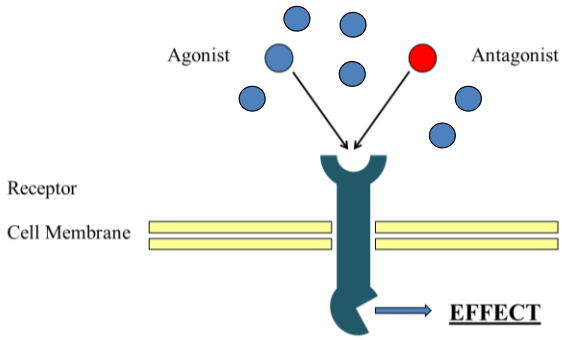
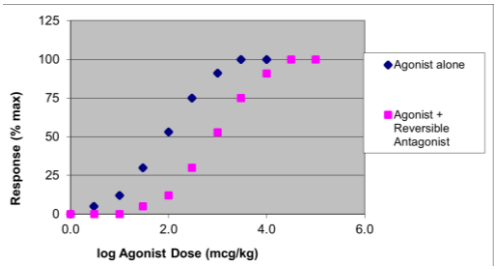
Antagonists

- 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…

Competitive Antagonism
(Reversible)

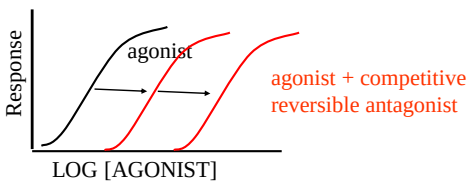
- Bind to the receptor in a reversible manner to compete directly with agonist binding.
  - What effect will this have on an agonist response curve?





Reversible Competitive Antagonism

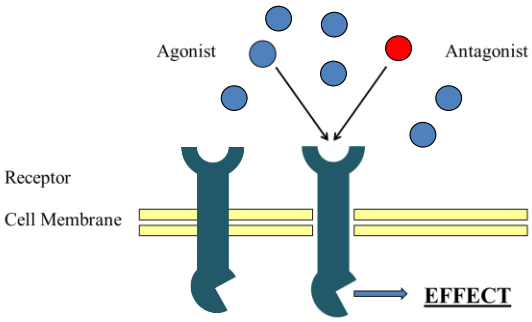
- Bind to the receptor in a reversible manner to compete directly with agonist binding.
  - What effect will this have on an agonist response curve?

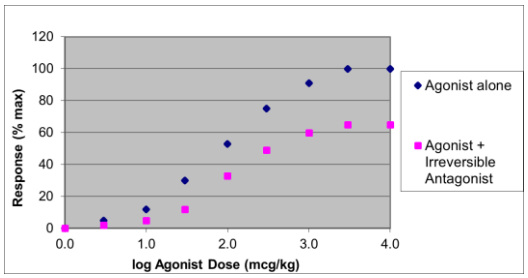


Competitive Antagonism
(Irreversible)

Drug covalently binds to the receptor. Not reversible

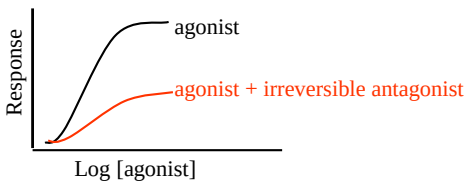
Reduces the number of receptors available to the agonist.





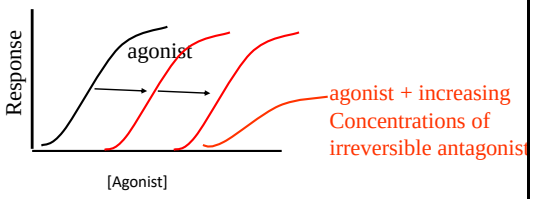
Irreversible Antagonism

- 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?



Spare Receptors?

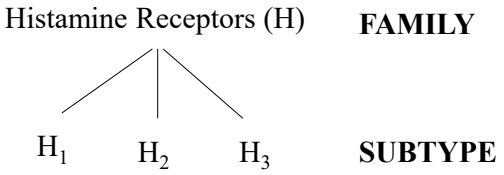
- Sometimes at low doses an irreversible antagonist looks like a reversible antagonist – what does this tell you about the number of receptors required to produce maximal response?



Break

- Construct your own summary table for the types of antagonism
  - What are their key properties?
  - How are they similar?
  - How are they different?
- Which type of antagonist is likely to be the most commonly used? Why?
- Which type of antagonist is least likely to be commonly used? Why?

Receptor Terminology



Selectivity for Subtypes

- Preferential binding to a certain subtype leads to a greater effect at that subtype than others
  e.g. salbutamol at β_2 (lungs) rather than β_1 (heart)
- Lack of selectivity can lead to unwanted drug effects
  e.g. fenoterol
- Selectivity for H1 receptors is how current antihistamines work
  e.g. fexofenadine

Antipsychotic Medications

- Typical vs atypical antipsychotics
- Typical
  - Dopamine antagonists
  - e.g. haloperidol
- Atypical
  - Dopamine and 5HT antagonists
  - e.g. quetiapine

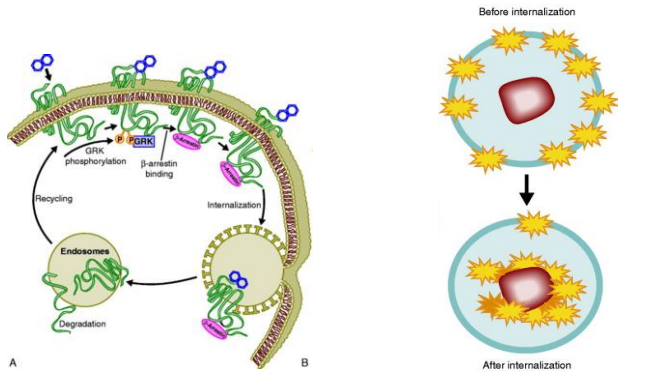
Receptor Plasticity

- Receptor states and populations do not remain constant over time
- This plasticity is largely responsible for the changes that occur in effectiveness of chronic drug (or endogenous compound) over time
  e.g. tolerance, insulin resistance

Regulation of Receptors (Receptor Plasticity)

- Changes in receptor state
  - Desensitisation / Exhaustion of mediators
- Changes in receptor populations
  - Up regulation
  - Downregulation

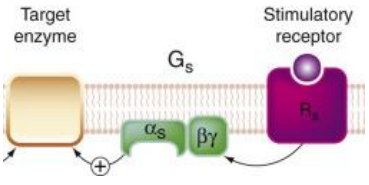
Desensitisation & Internalisation



(Adapted from A Pharmacology Primer (4th ed). T Kenakin. Elsevier. 2015 (Fig 5.7)
https://www-sciencedirect-com.ezproxy.auckland.ac.nz/science/book/9780124076631

Desensitisation

- Disconnects receptor from signalling
- Rapid
- Quickly reversible



Adapted from: Bidirectional control of a target enzyme, such as adenylyl cyclase by Gs and Gi. How drugs act : molecular aspects
Rang, HP, MB BS MA DPhil Hon FBPharmacolS FMedSci FRS, Rang & Dale's Pharmacology, 3, 22-49
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Receptor Population Changes

- Chronic agonist administration can lead to DOWN REGULATION
  - Eg chronic salbutamol can cause internalisation of receptors → less receptors available for stimulation → decreased bronchodilation
- Chronic antagonist administration can lead to UP REGULATION
  - Eg chronic propranolol can increase synthesis of β_1 receptors in the heart → less antagonism → decreased drug effect (increased HR & BP)

Clinical Significance

- Tolerance:
  - Eg morphine, salbutamol
- Adverse Effects:
  - Eg typical antipsychotics are predominantly D_2 antagonists
- Therapeutic Effects
  - Tricyclic antidepressants

Non-Receptor Protein Targets

- Not all drugs act directly at receptors
- Some drugs act at non-receptor protein targets:
  - Enzymes (COX inhibitors)
  - Carrier proteins (TCAs & SSRIs)
  - Ion channels (local anaesthetics)
- Some drugs act at non-protein targets
  - Soluble ligands e.g. inflammatory mediators
  - DNA

Enzymes As Drug Targets

- Cyclooxygenase and NSAIDs (e.g. ibuprofen) – used to treat pain and inflammation
- HMG CoA Reductase and Statins – used for lowering cholesterol

Effects of Prostanoids

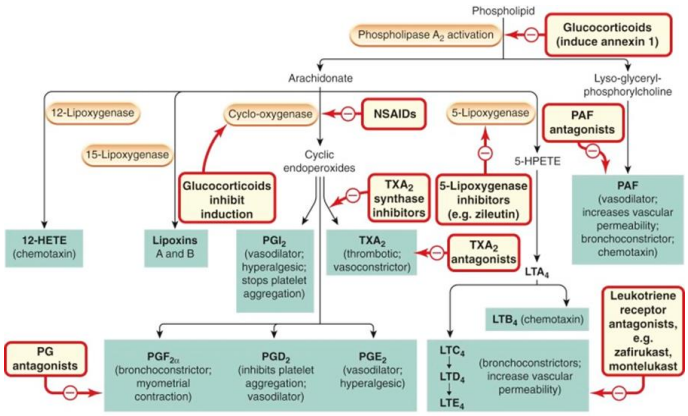

Figure 17.1 - Local hormones 1 : histamine and the biologically active lipids
Rang, HP, MB BS MA DPhil Hon FBPharmacolS FMedSci FRS, Rang & Dale's Pharmacology, 17, 212-221
Accessed via Clinical Key. Copyright © 2016 © 2016, Elsevier Ltd

Effects of Cyclooxygenase Inhibition

- NSAID inhibition of both COX 1 and COX 2 leads to:
  - ↓ inflammation
  - ↓ pain
  - ↓ fever
  - But also,
    - Reduction in homeostatic pathways involved in:
      - Kidney function (acute renal failure)
      - Maintenance of gastric mucosa (ulcers – diclofenac, aspirin)

COX 2 Selective Inhibitors

- -coxibs are selective for the inducible enzyme isoform – COX-2
- Greater safety with respect to GI adverse effects, less likelihood of GI bleeds
- Cardiovascular effects

HMG-CoA Reductase & Statins

e.g. simvastatin

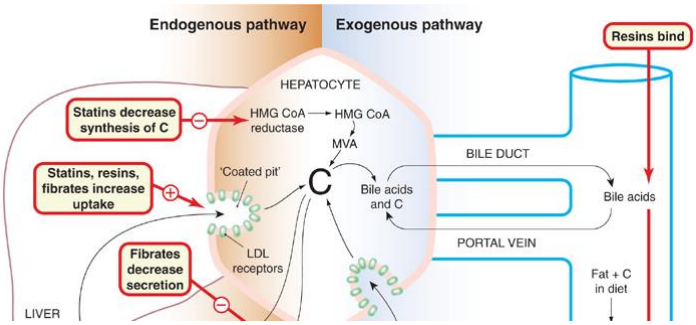

Fig. 24.1 Schematic diagram of cholesterol transport in the tissues, with sites of action of the main drugs affecting lipoprotein metabolism. Atherosclerosis and lipoprotein metabolism. Ritter, James M., DPhil FRCP HonFBPhS FMedSci, Rang & Dale's Pharmacology, 24, 310-318. Accessed via ClinicalKey
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Drugs Which Interact With Carrier Proteins

- Examples: Drugs that act on monoamine neurotransmitter uptake proteins
- Fluoxetine (Prozac) – SSRI
- SNRI – initially developed from anti-depressant research; used for weight loss and smoking cessation

Fluoxetine Mechanism

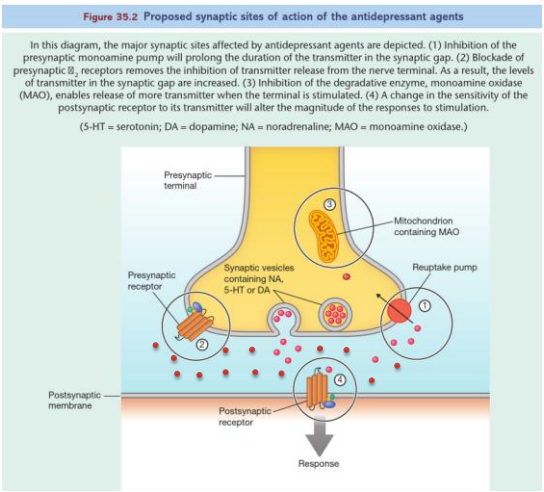
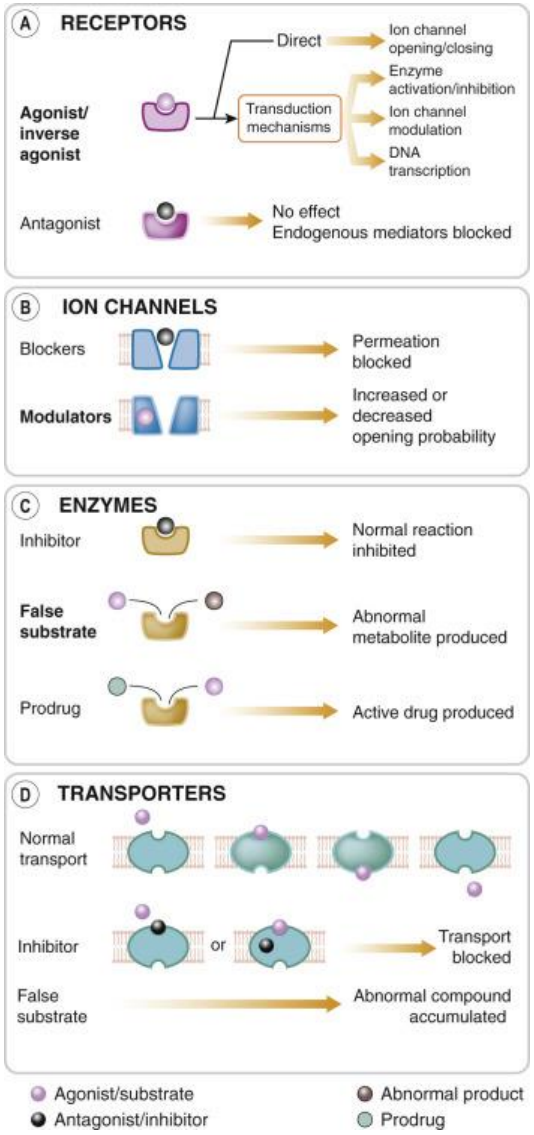


Fig 35.2. Fundamentals of Pharmacology. Bullock S & Manias E. Copyright © 2017, Pearson

Ion Channels

- Voltage-gated ion channels
  - local anaesthetics (Na^+ blockers),
  - Ca^{2+} channel blockers (verapamil, nifedipine)



Types of target for drug action.
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1. Drugs most commonly target one of four different classes of protein target. Name the types of protein below and for each type give an example of a specific drug, its target, mechanism of action and therapeutic use (the first example is completed for you)

Class of protein	Example of drug	Specific drug target	Mechanism	Therapeutic Use
1. Ion channel	lidocaine	Na channel	Blocks channel, preventing sodium entry and therefore conduction along nerves	Local anaesthetic
2.				
3.				
4.				

2. Define the following terms and draw a clearly labelled concentration response curve to illustrate each one:

a) Partial agonist :

b) Competitive reversible antagonist: