1. Principles of Drug Action in the Nervous System

Principles of Drug Action

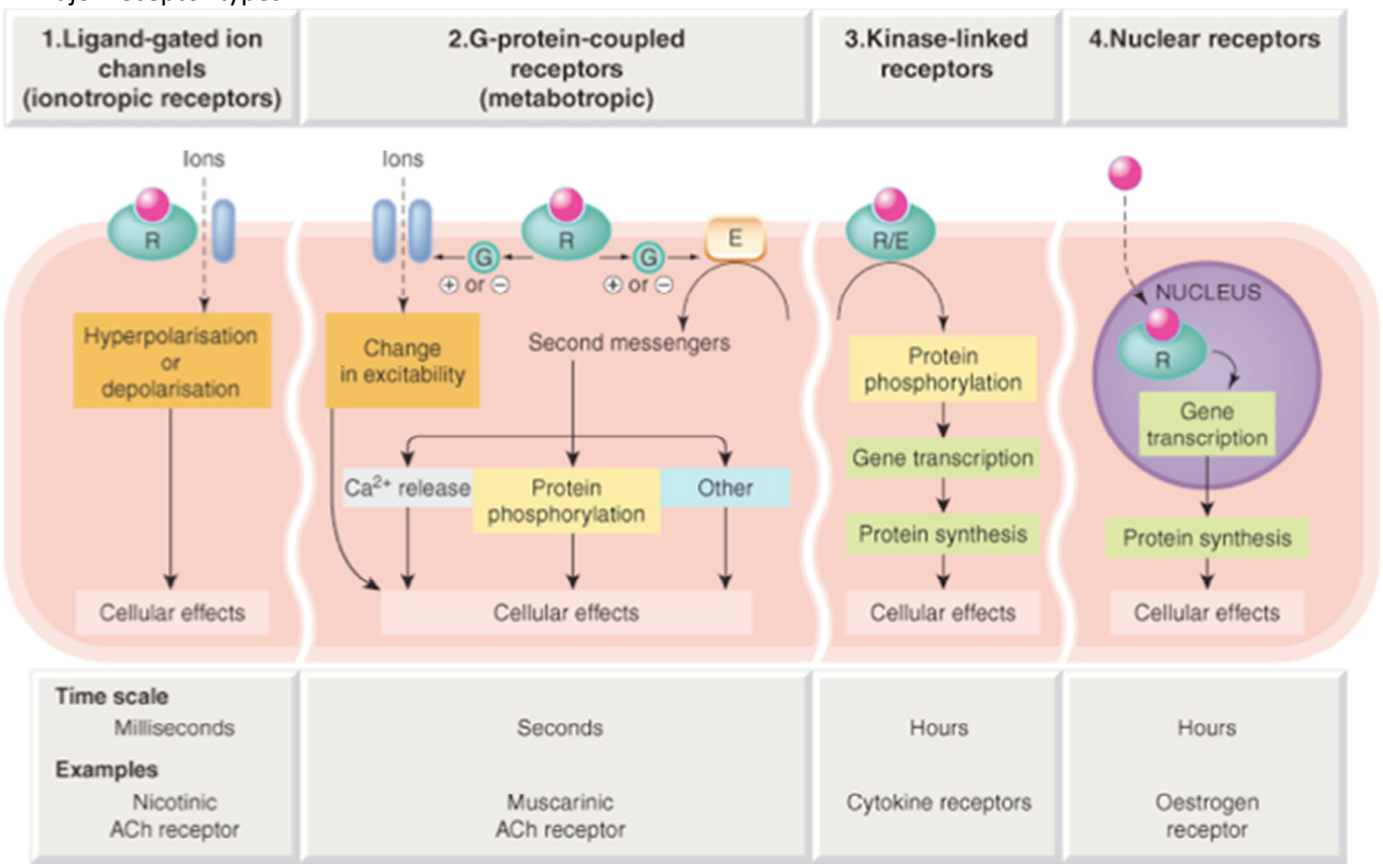
-Drugs bind to proteins (large molecules) or nucleic acids or carbohydrates/lipids
-Drug target = sites where drugs bind to produce their effects
-Drugs must show a high degree of binding site specificity in order to be useful - the higher the dose, the more likely non-specific (unwanted) effects will occur

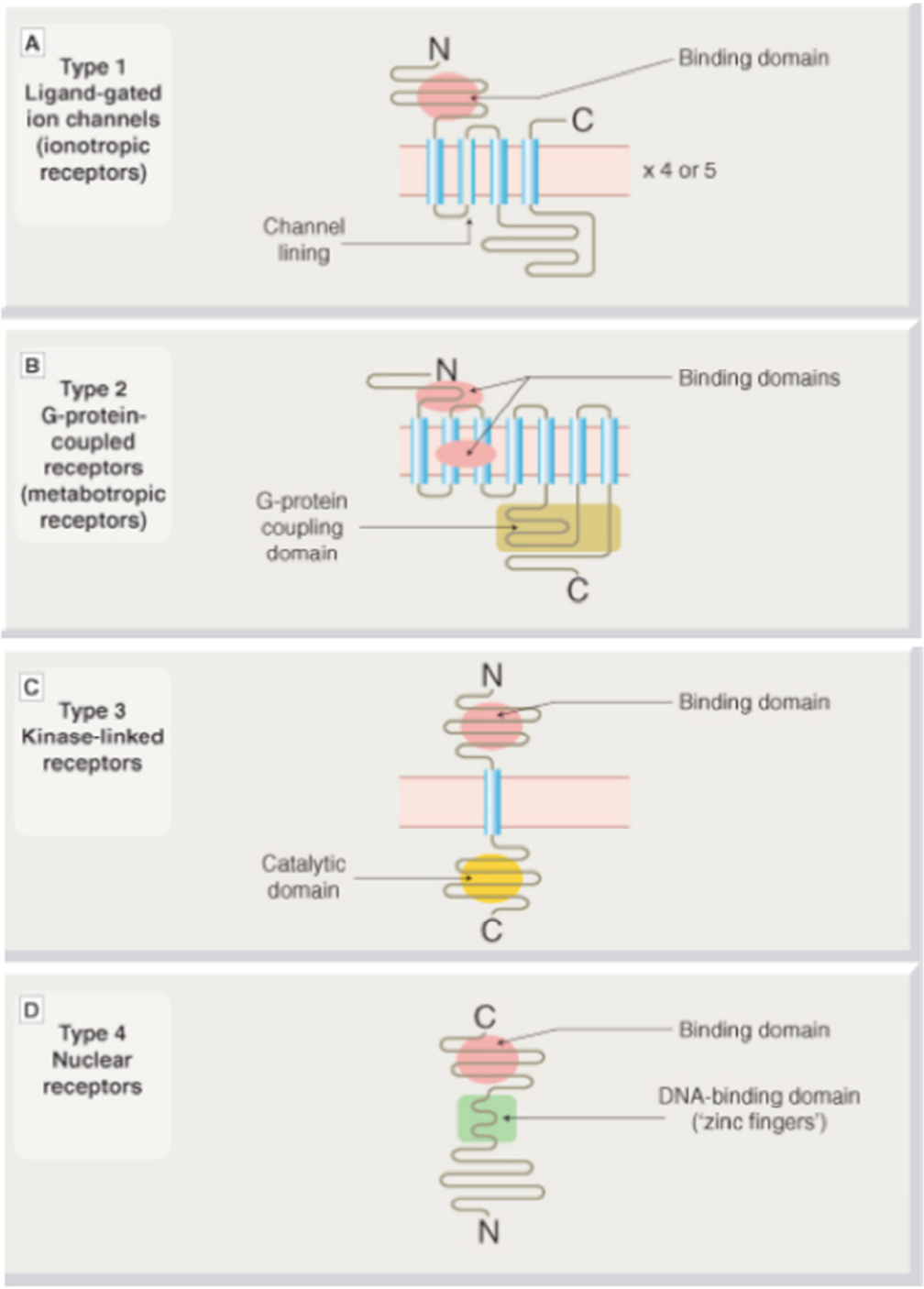
•How do drugs work?
-Drugs alter protein function by:
-Activation/ Agonism (+)
-Modulation (+/-)
-Inactivation/ Inhibition/ Antagonism/ Blocking (-)

•4 main molecular drug targets (proteins)
1) Receptor = protein molecules that recognise and respond to extracellular molecules to alter intracellular events
-Agonist = bind to and activate the receptor to produce a response directly (ion channel opening or closing) or indirectly (transduction mechanism – enzyme, ion channel, nucleus)
-Antagonist = bind to but do not activate the receptor (no effect) and block endogenous mediators

-E.g. salbutamol (ß receptor agonist, relaxes bronchial smooth muscle, opposes bronchoconstriction, which occurs in asthma)
-E.g. atenolol (ß receptor antagonist, antagonises the action of noradrenaline, decreases heart rate, reduces blood pressure)

-4 major receptor types





G-protein coupled receptor:
-Consists of three subunits (α,β,γ) anchored to the membrane
-Agonist binding to receptor causes receptor coupling with α subunit and GDP is exchanged with GTP
-α subunit(+GTP) dissociates from complex and binds to a target (e.g. enzyme, ion channel) to activate
-GTP is hydrolysed resulting in GDP binding and re-association with β,γ complex

2) Enzyme = metabolise substrates to products
-Inhibitor = prevent metabolism/ product formation
-False substrate = produce abnormal metabolites
-Pro-drug = inactive substrate for enzyme to produce active drugs

-E.g. aspirin (inhibits cyclo- oxygenase and prevents prostaglandin production, which causes analgesia and reduces inflammation)

3) Carrier molecule/ Transporter = carry substrates across membranes
-Inhibitor = prevent transport
-False substrate = cause abnormal compounds to accumulate in the cell

-E.g. fluoxetine (inhibits serotonin/5HT uptake into nerve terminals in CNS, increases synaptic concentrations of 5HT: used to treat depression)

4) Ion channel = voltage-gated channels that allow ions to cross membranes
-Ligand-gated channels are receptors
-Blocker = prevent ions from passing the channel by altering the protein conformation
-Modulator = increase or decrease the opening probability/ frequency of the channel

-E.g. lignocaine (Na+ channel blocker: prevents action potentials and sensory sensation, and causes local anaesthesia)

•Molecular binding forces
1) Reversible binding: typical of drug binding - electrostatic attraction, van der Waals' bond, hydrophobic interaction
2) Irreversible binding: applies to some drugs - covalent bond

Cholinesterase

•AChE
-ACh is a crucial neurotransmitter and its gradual loss is associated with cognitive, autonomic and neuromuscular dysfunction
-AChE hydrolyses and inactivates ACh thereby regulating the [ACh] at the synapse and limits the activity
-At the synapse, AChE is extracellular but is attached to the membrane as an insoluble tetramer

▪AChE structure
-A serine hydrolase: catalytic site contains a serine, histidine and glutamate residue
-Choline binding site, peripheral site and catalytic site
-Acetyl/Carbamyl/Phosphoryl-enzyme intermediate is formed

▪AChE inhibitor
=Enhance cholinergic neurotransmission by increasing the concentration of ACh
-To treat dementia e.g. Alzheimer's disease (e.g. tacrine): limited effectiveness
-To reverse the action of neuromuscular blocking drugs (e.g. vecuronium) postoperatively (e.g. neostigmine)
-To test for (e.g. edrophonium i.v.) or treat myasthenia gravis (e.g. neostigmine)
    -Myasthenia gravis = autoimmune disease resulting in severe loss of nAChRs from neuromuscular junction
-As pesticides, and in the treatment of head lice (e.g. malathion)
-As war gases (e.g. sarin)

Drug	Duration of action	Main site of action	Notes
Edrophonium	Short	NMJ	Diagnosis of myasthenia gravis
Neostigmine	Medium	NMJ	Treatment of myasthenia gravis and reversal of competitive nmj block
Tacrine	Medium	CNS	Alzheimer's disease: limited effectiveness
Malathion	Long	-	Insecticide + head lice (USA)
Sarin	Long	NMJ + P	Nerve gas: Tokyo 1995

•BuChE
-BuChE hydrolyses and inactivates BuCh as well as ACh
-BuChE is a soluble enzyme found in plasma
-Plasma cholinesterase = Serum cholinesterase = Pseudocholinesterase

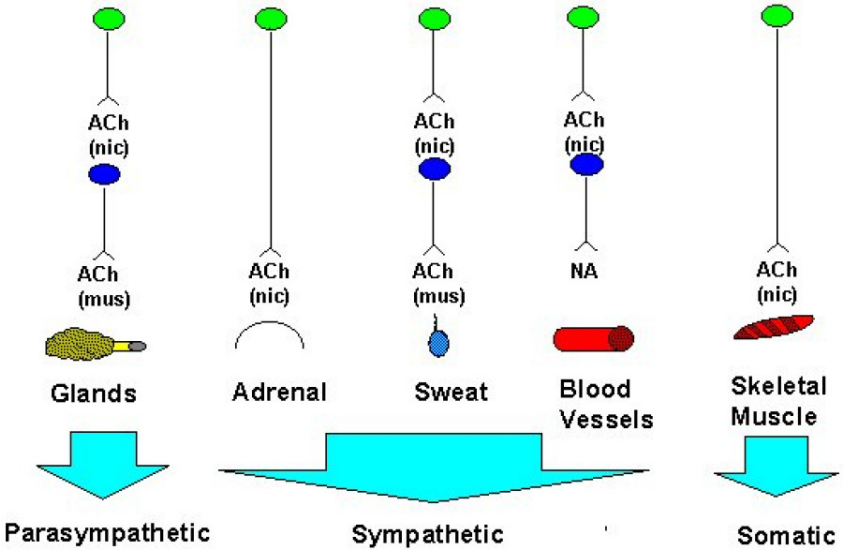
	Acetylcholinesterase (AChE)	Butyrylcholinesterase (BuChE)
Distribution	Limited: neuromuscular junction and neuronal synapse	Widespread: liver, skin, brain, GI smooth muscle; soluble form in plasma
Substrate specificity	Narrow: ACh and methacholine	Broad: BuCh, ACh, suxamethonium
Function	Hydrolyses and terminates action of ACh at synapse: also new roles	Physiological function unclear: keeps ACh levels low. Important role pharmacologically to metabolise some drugs (e.g. suxamethonium)

Anticholinesterase Poisoning

•Organophosphorus compounds
-Pro-poison or active
-Different kinetics (fat solubility and clearance) affecting half-life
-Different propensity to age
-Reversible and irreversible binding to cholinesterase
-Neuropathic

-OPs inhibit various esterase enzymes in the body – AChE inhibition induces acute effects
-AChE is important for normal communication between nerves and other nerves, glands, muscles

▪CNS



•Dose
-Carbamates fall off when the concentration drops
-OPs are irreversibly bound and the enzymes must be reactivated

•Tolerance/ Tachyphylaxis
-Due to the continuous stimulation of mAChR, the receptors are either reduced or altered

-Neuronal M2 receptor function was tested using pilocarpine
-Increasing doses of pilocarpine inhibited vagally induced bronchoconstriction in a dose-related manner in animals
-The effect of pilocarpine was shifted significantly to the right in animals 7 days after treatment with chlorpyrifos
-In the animals treated with chlorpyrifos 24h before, pilocarpine did not inhibit vagally induced bronchoconstriction, indicating neuronal M2 muscarinic receptor dysfunction after 24h

•Onset
-Variation in onset
-Skin = slow onset/ Swallowing = fast onset

2. Drug Abuse, Addiction and Analgesia

-Addiction is a brain disease that evolves through an individual's chronic use of drugs
-Primary mechanism of action of nearly all drugs of abuse is known (modulation of proteins)
-Neuroscientists have identified a common brain pathway that seems to be critically involved in addiction to most substances of abuse

•How do addictive drugs work?
-All addictive drugs produce their psychoactive effects by acting in the CNS or the brain
-They disrupt neuronal communication at the level of the synapse
-They interfere with the normal synaptic processes that underlie neuronal communication in the brain
-They short-circuit important motivational circuitry in the brain

•Symptomology of drug dependence
-Drug dependence is defined by the repetitive/ compulsive behaviour
-Repetitive behaviour becomes an addiction when this behaviour has negative health and social consequences

▪DSM-5 Criteria - Drug Dependence (2 or more in a 12 month period)
1) The substance is often taken in larger amounts or over a longer period than was intended
2) There is a persistent desire or unsuccessful efforts to cut down or control substance use
3) A great deal of time is spent in activities necessary to obtain the substance, use the substance or recover from its effects
4) Cravings and urges to use the drug
5) Important social, occupational, or recreational activities are given up or reduced because of substance use
6) The substance use is continued despite knowledge of having a persistent or recurrent physical or psychological problem that is likely to have been caused or exacerbated by the substance
7) Tolerance (diminished drug effect/use of greater doses to achieve desired effect)
8) Drug withdrawal

▪"Physical" dependence
-Abstaining from use of drugs produces physical withdrawal symptoms such as diarrhoea and convulsions
-Short-lasting
-Can be managed with appropriate medication
-E.g. heroin and alcohol
-Does not play a major role in maintaining an addiction

▪"Psychological" dependence
-Signs of "psychological dependence" include agitation, depression and most importantly craving for the drug during abstinence
-E.g. cocaine and amphetamine (they do NOT produce physical dependence during withdrawal)
-Crucial role in the chronic relapsing nature of addiction

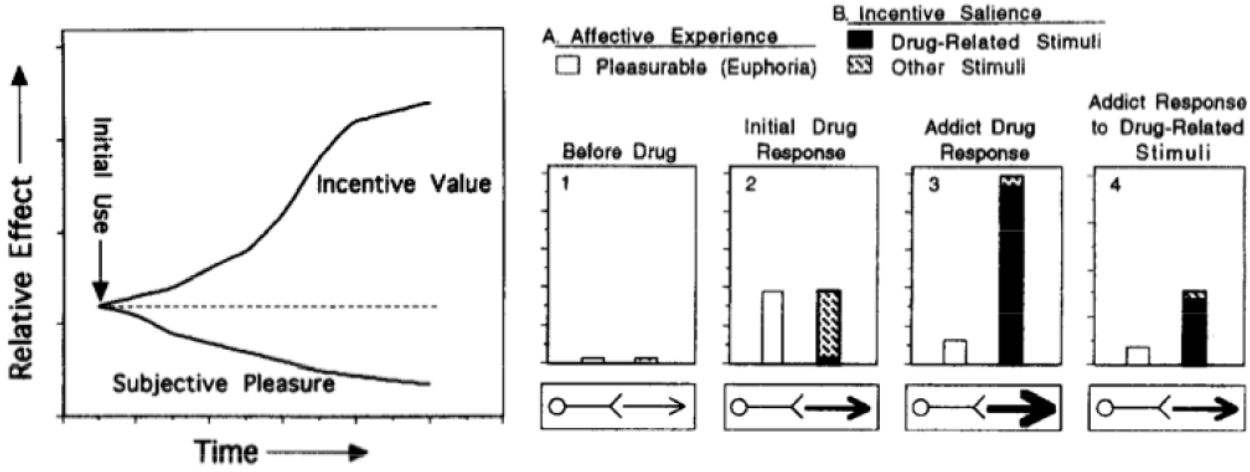
•Learning theories of addiction
1) Negative reinforcement theory
-Process that strengthens behaviour that allows escape from a negative event (=physical withdrawal syndrome)
-The behaviour of drug taking is made more likely to avoid negative psychological and physical symptoms associated with drug abstinence
-E.g. taking aspirin to alleviate the pain

▪Limitations
-Individuals will continue to self-administer compounds in the absence of unpleasant withdrawal symptoms
-An individual may be physically dependent on a drug without being addicted to it (e.g. opioids in pain management)
-A lot of drugs do not produce a physical withdrawal syndrome e.g. cocaine, amphetamine (no negative event)
-Addicts relapse long-after withdrawal symptoms have subsided
→Negative physical withdrawal symptoms/ syndromes are not central to maintaining addiction

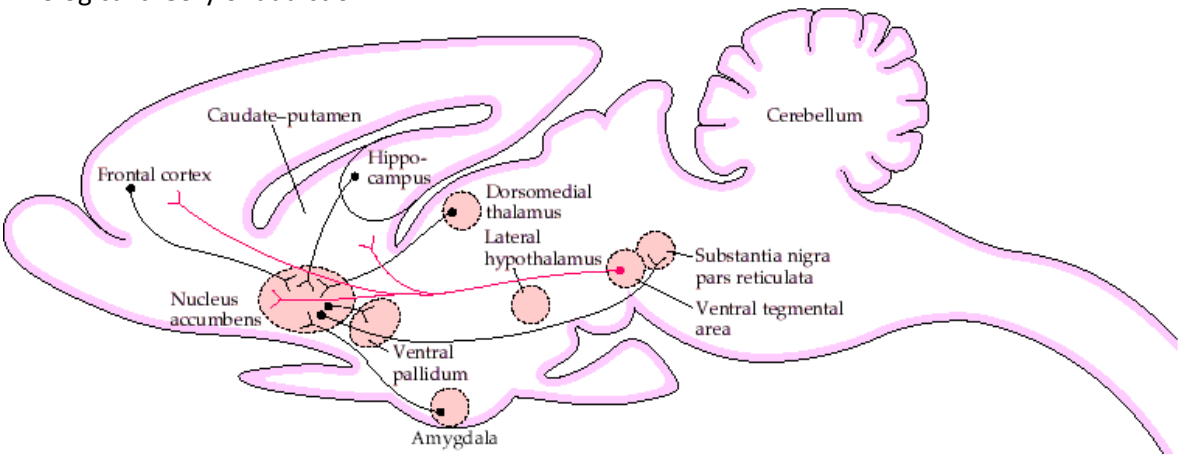
2) Positive reinforcement theory
-Process that strengthens behaviour that leads to a satisfying outcome (=pleasure)
-Drug taking behaviour is strengthened by the pleasurable consequences of psychoactive drug use
-E.g. opiate and cocaine feel like orgasm

▪Limitations
-Not all drug use is associated with pleasure e.g. cigarette smokers don't greatly enjoy the experience
-Addicts work morphine in the absence of subjective pleasure
-Study by Lamb (1993): post-addicts were given access to two levers; one lever would administer a low dose of morphine and the other a placebo control. The lever supplying morphine reinforced lever pressing behaviour, whereas the placebo lever did not. However, these addicts could not distinguish between the subjective effects of placebo and morphine → morphine was self-administered in the absence of feeling a subjective pleasure

▪Incentive-sensitisation theory of addiction: addicts feel like they want the drug more than they like it (subjective pleasure decreases but the incentive value for drug increases)



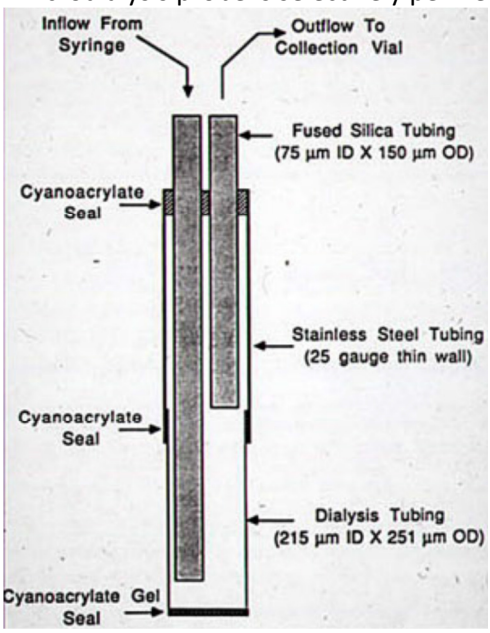
•Biological theory of addiction

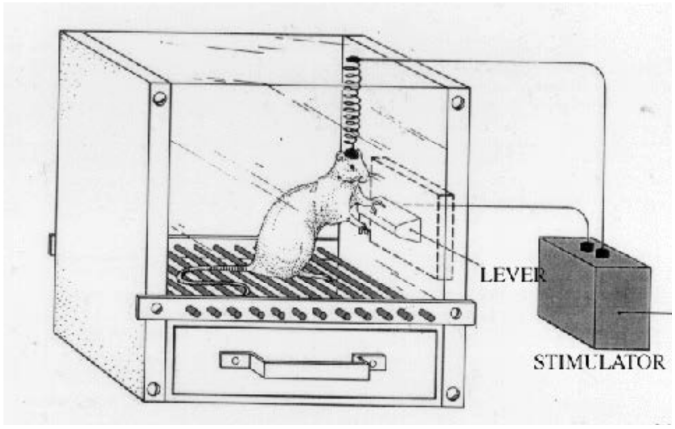


-Ventral tegmental area has the cell body that has axonal projections to the nucleus accumbens in basal forebrain
-This pathway transports dopamine (output of mesolimbic dopamine system)

1) Mesolimbic dopamine theory
-Neurotransmitter dopamine in the mesolimbic pathway of the brain (reward pathway)
-Natural rewards/ reinforcers (food, water and sex) normally activate this system to produce reinforcement and direct behaviour towards stimuli in the environment that promote survival
-Drugs of action artificially activate this system and are able to "short-circuit" this motivational system and take a strong hold over behaviour (drug-seeking)
-A "shortcircuit" in this system makes drugs take on biological significance like natural rewards
-This short-circuiting probably comes in the forms of various neuroadaptations or physical changes that occur in this system (e.g. the activity of adenylate cyclase is upregulated in the NAS after chronic administration of drugs such as cocaine and heroin). These neuroadaptations may be long-lasting → Long-lasting psychological dependence (craving and depression) and the chronic relapsing nature

▪Evidence
a) Microdialysis
-All drugs of abuse (alcohol, cannabis, opiates, and cocaine) increase levels of dopamine in the nucleus accumbens
-Microdialysis probe is selectively permeable to dopamine – it is inserted into the brain to collect dopamine





b) Dopamine antagonists such as haloperidol modulate the IV self-administration of drugs such as cocaine, nicotine and amphetamine in rats

c) Intracranial drug self-administration
-Rats will self-administer minute quantities of drugs directly into the VTA and nucleus accumbens (not cerebellum)

▪Complication
-Mesolimbic dopamine does not mediate or denote pleasure
-Aversive stimuli such as handling, electric shock, tailpinch and aggressive attacks also increase levels of dopamine in the nucleus accumbens

-Knockout mice lacking the D2 dopamine receptors still avidly self-administer cocaine (even though the level of responding is reduced)
-Cocaine also affects 5-HT re-uptake and has a greater affinity for the serotonin transporter than the dopamine transporter → cocaine's addictiveness can be from serotonin

<u>Recreational Drugs - Illicit</u>

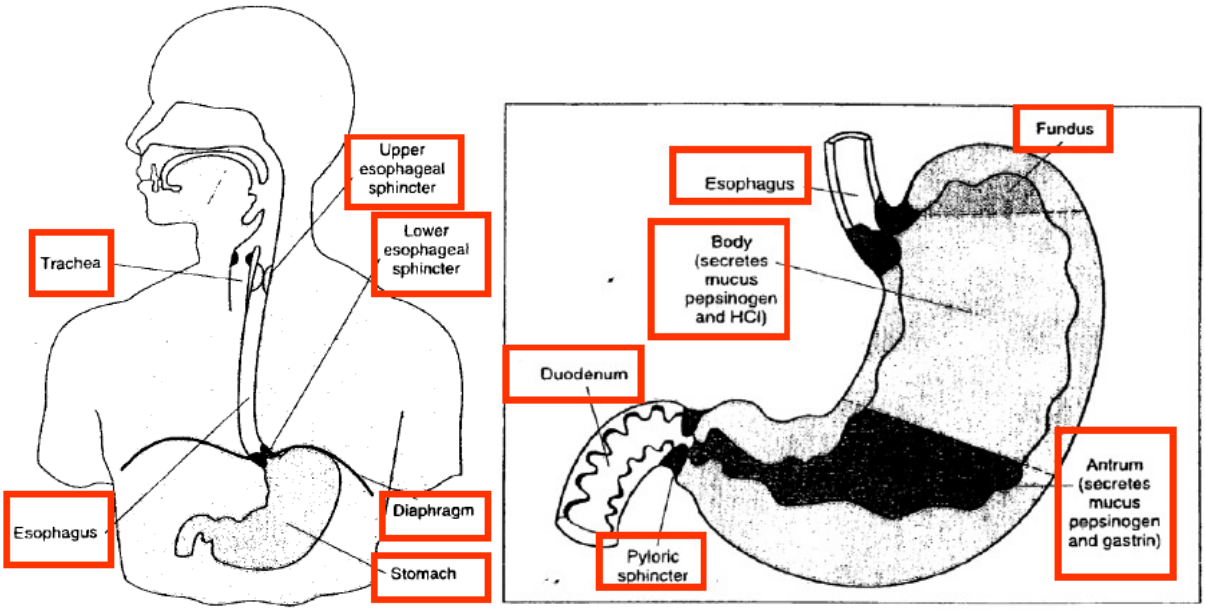
•Heroin
-Opiates are naturally occurring alkaloids such as morphine or codeine
-Heroin is the diacetyl derivative of morphine (replace hydroxyls on 3 and 6 position with 2 acetal groups)
-Increased lipophilicity → Rapidly cross the BBB and get into brain → better "rush" and is more addictive
-Heroin doesn't bind to opioid receptors but is rapidly deacetylated and metabolised into morphine in the brain
-Morphine metabolites: morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G)
-Heroin unique, active metabolite: 6 monoacetylmorphine

▪History
-Alkaloids in opium poppy: morphine, codeine and thebaine (no viable synthetic method)
-Small therapeutic window
-Bayer synthesised heroin in an effort to create a less addictive opioid analgesic - marketed as a cough suppressant, analgesic and treatment for morphine dependence
-Hedonic effects as well as analgesic

3. Drug Treatment of Allergy and Gut Disorders

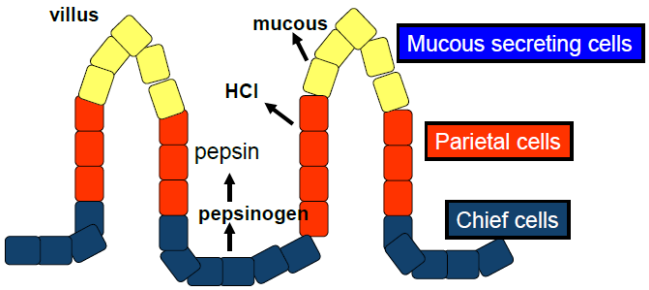
Gut Drugs

•GI Tract



-Two sphincters that regulate the GI content:
-Upper allows passage of food particles and liquids
-Low separates the stomach from the oesophagus (protected from contents of the stomach)
-Fundus: upper part of stomach
-Body of stomach: contains cells that secrete mucus, pepsinogen, HCl
-Antrum: lower part of the stomach; has <u>thicker muscle</u> (physical breakdown of food into liquid chyme); secretes mucus, pepsinogen and gastrin
-The stomach expands to allow food space but also contracts to help in digestion
-Pyloric sphincter regulates what leaves the stomach to enter the duodenum (regulate acidic contents)

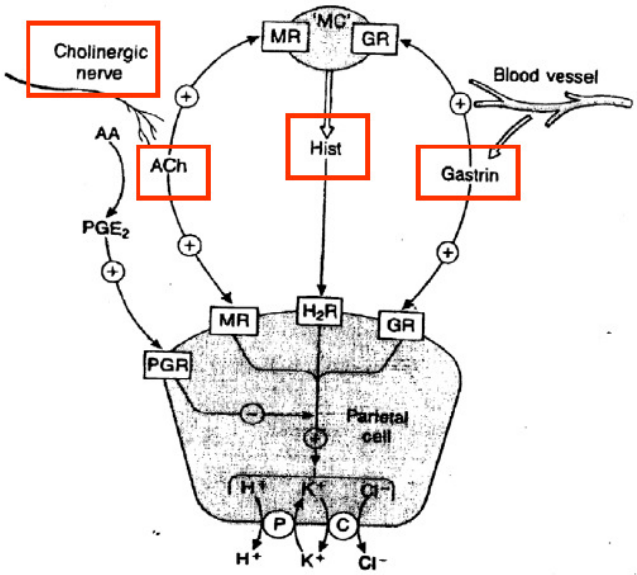
•Cells lining the stomach wall
-Stomach wall is a convoluted structure with a multitude of villi-like structures sticking out into the lumen
-2.5 L of gastric juice are secreted daily to aid mixing



-Chief cells: secrete pepsinogen (activated into pepsin at low pH only to break down proteins)
-Parietal cells: secrete HCl (pH = 1,2)
-Mucus secreting cells: secrete mucus = protective layer from the HCl for the cells

▪Acid secretion and regulation
-Two main cell types
1) Mast Cell: <u>Regulates parietal cell</u> by releasing histamine → initiates the H/K ATPase pump
2) Parietal Cell: Release HCl

-Parietal cell, gastrin, ACh and histamine stimulate acid secretion
-Prostaglandin inhibits acid secretion



▪HCl secretion
1) H_2R activation on parietal cell – Release of histamine from mast cell/ enterochromaffin-like cell
2) M3 activation on mast cell and parietal cell – Release of ACh from cholinergic nerves
3) Gastrin receptor activation on mast cell and parietal cell – Release of gastrin from the blood stream
-All these receptors are g-protein coupled

-Stimulation of potassium, proton and chloride pumps
-H-K-Cl pump: net secretion of HCl in a linked system

▪HCl inhibition
-Arachidonic acid is converted into prostaglandin E_2
-PGE_2 acts on the prostaglandin receptors on the parietal cell
-Prostaglandin inhibits proton pump and decreases acid secretion

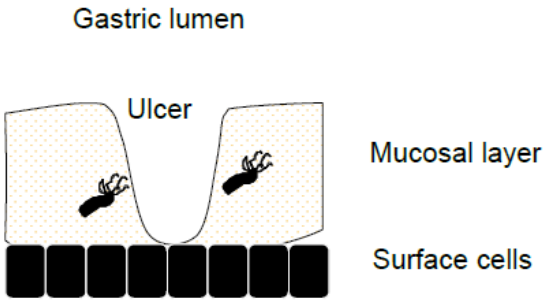
•Dyspepsia
-Upper abdominal pain or discomfort
-Acid regurgitation from stomach into the oesophagus through oesophageal sphincter
-Often but not always associated with eating
-Common - 30% adults

-Symptoms are not specific: may/may not indicate underlying pathophysiology (e.g. ulcers, reflux, gastritis v. cardiac ischaemia, cholecystitis)
-Persistent symptoms that are not alleviated by OTC agents need investigation: endoscopy required for definitive diagnosis

▪Symptoms
1) Gastro-oesophogeal reflux disease (GORD)
-Insufficient tone in the lower oesophageal sphincter → acid regurgitation into oesophagus → heartburn
-Managed by suppression of acid secretion

2) Peptic ulcers
-Chronic necrotic lesions of gastric/duodenal mucosa
-Degradation of mucosal lining that can lead to acid attacking the stomach wall
-Area of inflammation

▪Causes
1) Helicobacter pylori
-Causes 75% of stomach ulcers, 90% of duodenal ulcers
-H. pylori is a gram negative bacillus
-Cells lining the lumen are turned over every couple of days



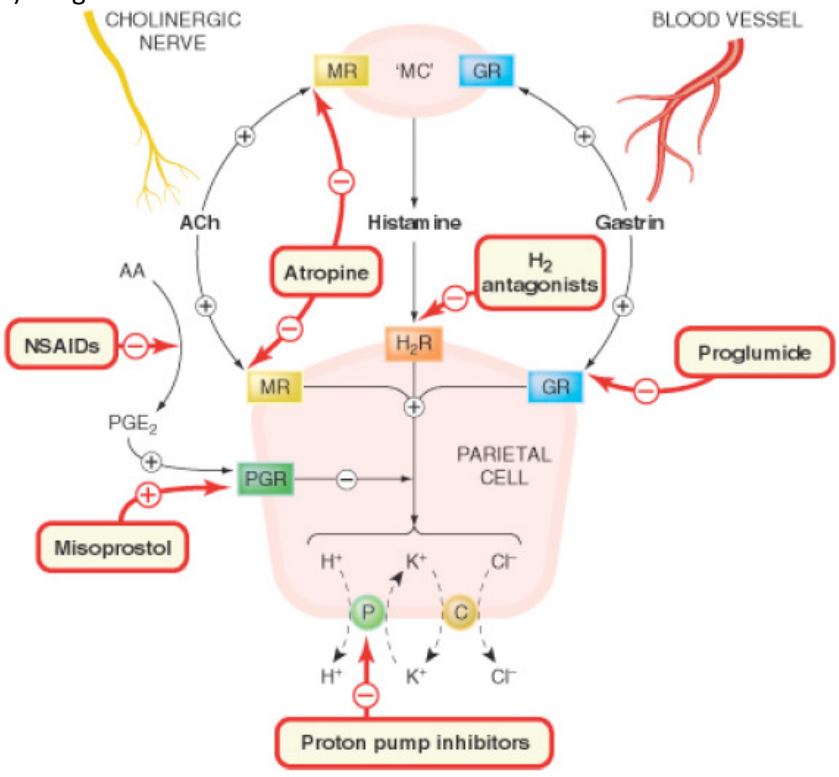
-Bacteria sit within the nutrient-rich mucosal layer and survive from low pH
-Bacteria break down and feed off the mucosal lining
-H pylori transform the cells such that there is less mucous production, elevated acid secretion, ulcer formation

2) NSAIDs/Caffeine
-NSAIDs inhibit Cyclooxygenases 1 and 2 – reduce PG production
-PGs stimulate mucous production and inhibit acid secretion
-NSAIDs reduce mucous production and increase acid secretion
-Stomach lining is susceptible to damage – ulcer

-Caffeine stimulates acid production by inhibiting phosphodiesterase (= breakdown of cAMP) → elevates cAMP → stimulates H+ secretion

▪Treatment
-Prevention of breakdown of gut mucosa:
-Acid secretion stimulated by histamine, gastrin and ACh
-Mucosal lining breakdown by H. pylori
-Protection of gut mucosa = prostaglandins stimulates:
-Mucus secretion from mucus-secreting cells
-Bicarbonate ion secretion (raise pH)
-Blood flow

1) Drugs that neutralise or inhibit acid secretion



-M3 receptor antagonists (e.g. atropine): competitively bind to M_3R and block ACh → modulates neuronal modulation of acid secretion (reduces activation of mast cell and parietal cell)
-Proglumide: reduces acid secretion by blocking GR on parietal cell and mast cell

a) Antacids
-Aluminium and magnesium hydroxide
-Neutralises acid, raises pH to ~ 5 and inhibits peptic activity

-May provide rapid symptomatic relief but limited effectiveness for peptic ulcer
-Magnesium salts cause diarrhoea, aluminium salts constipation

b) H2-receptor antagonists
-Famotidine, ranitidine, cimetidine, zantac
-Competitively inhibit actions of histamine on H_2R on parietal cell basement membrane
-Reduce histamine, gastrin and ACh stimulated acid secretion by 90%

-Treat and heal ulcers but relapse is very high
-Well tolerated

c) Proton pump inhibitors (PPI)
-Blocks acid secretion directly at the luminal membrane
-Omeprazole (activates at low pH), pantoprazole, rabeprazole

-Omeprazole is a prodrug which is converted to active form in low pH conditions. Acid attacks omeprazole and creates sulfonamide. The reactive sulfur then covalently, irreversibly binds to cysteine residues in the active site of the enzyme. The only way to recover is to synthesise new protein. Once the drug passes through the stomach it is no longer active, and hence is safer.

-Irreversible inhibition of the H+/K+ ATPase (proton pump) - acid secretion not activated until NEW proton pump proteins are made on the luminal membrane
-Markedly inhibit acid secretion; one dose can inhibit secretion for 2 - 3 days (requires new protein synthesis)

-More effective acid secretion suppression
-Well tolerated but may cause headache, nausea, diarrhoea, fatigue and dizziness

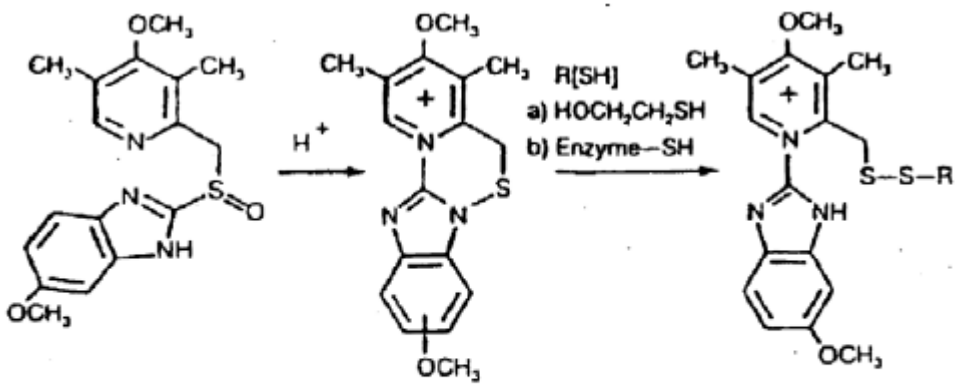


Fig. 2. Chemical reactions of omeprazole leading to inhibition of H^+/K^+-ATPase.

-Omeprazole is converted to its active form at low pH (stomach)
-Formation of mercaptan adduct
-Covalently bound to H+/K+/ATPase and inactivates the pump

2) Treatment of Helicobacter Pylori
-30 - 40% of population infected with H pylori in Australia; 90% of ulcers have H pylori
-Elimination of the bacteria can cure ulcers, however, reinfection can occur
-Triple therapy:
-PPI – reduce acid secretion to heal the ulcer
-2 antibiotics (e.g. clarithromycin and amoxycillin) – eliminate bacteria

3) Drugs that protect the gut mucosa
a) Bismuth chelate (colloidal bismuth subcitrate)
-Forms an acid- and pepsin-resistant protective coating at the ulcer base + potentially anti-bacterial

-Used after failure of first-line treatments
-Bitter tasting, causes blackening of faeces and darkening of teeth and tongue

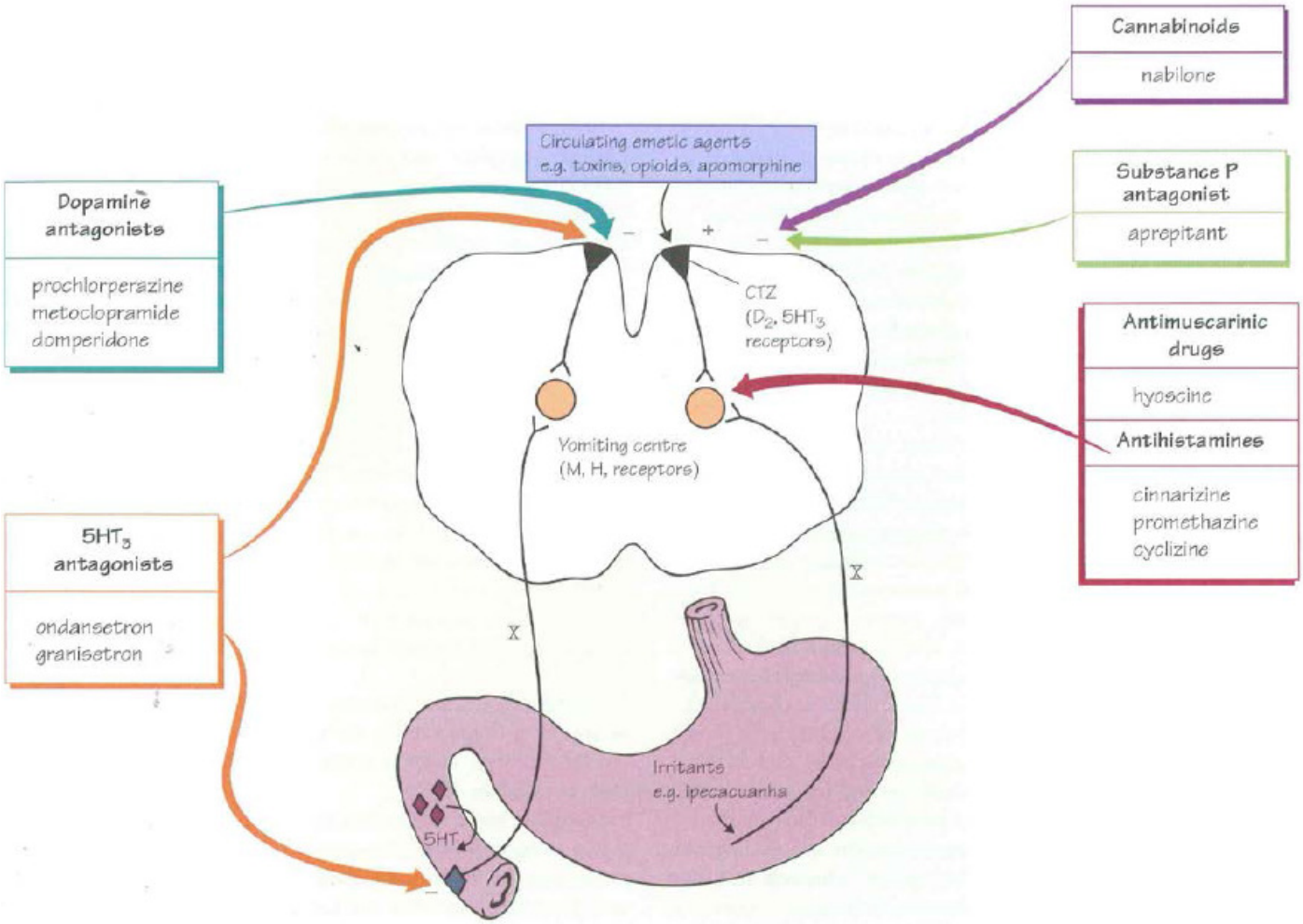
b) Sucralfate
-Forms an acid- and pepsin-resistant protective coating at the ulcer base

-Used to treat stress-induced ulcer
-Causes constipation, nausea, dry mouth, headache

c) Misoprostol
-PGE2 analogue which stimulates PGR
-Increases secretion of mucus and bicarbonate and inhibits acid secretion

-Used to treat NSAID-induced ulcer (counteracts the PG inhibition by NSAID)
-Causes diarrhoea, abdominal cramps, flatulance, nausea and vomiting, menstrual irregularities

•Vomiting
-Vomiting Centre: Located in the medulla in the brainstem
-Chemoreceptor Trigger Zone (CRT): Signals to the vomiting centre but not protected by BBB; have access to and receive inputs from toxins circulating the body



-Nerves within the stomach which send feedback up into vomiting centre
-Drugs that induce vomiting (e.g. ipecac): stimulates the nerves within the stomach and triggers vomiting reflex

▪Anti-emetics

•Mechanism of anaphylaxis
-When foreign proteins are encountered, IgE antibodies are made in response to antigenic stimulation
-The IgE antibodies have receptors that allow them to bind to cells such as mast cells and eosinophils
-With subsequent exposure to the antigen, the cell bound IgE molecules are cross linked by binding to the antigen
-This cross linking activates the cells and in the case of mast cells lead to the release of biologically active mediators such as histamine, leukotrienes and enzymes such as tryptase

-These mediators increase microvascular permeability, resulting in the escape of protein and fluid from small blood vessels into the interstitial tissues creating swelling
-These mediators lead to extensive vasodilation, which results in a fall in blood pressure
-Mediators, such as leukotrienes and histamine, constrict airway smooth muscle leading to breathlessness

▪Specificity of antigen challenge
-The synthesis of IgE is highly specific for particular antigens. This means that the IgE molecules bound to mast cells will only cross link with the specific antigen for which they have been synthesised.
-If other toxins are introduced into the body, they will not initiate a Type 1 allergic response because the specific IgE has not been synthesised
-They may produce some toxic effects because of their intrinsic properties. E.g. a toxin may not excite an antibody response but it could cause local swelling (by directly increasing microvascular permeability, thereby allowing the escape of protein and fluid form the vascular compartment into the tissues) and local redness (by dilating small blood vessels)

•Nullifying the net effect of endogenous mediators
1) Inhibiting synthesis: Non-steroidal Anti-inflammatory Drugs (NSAIDs) inhibit the enzyme responsible for the synthesis of prostaglandins from arachidonic acid to reduce inflammation

2) Specific antagonists: Leukotriene C4 exert their effects through leukotriene receptors. Drugs, such as montelukast, bind to the leukotriene receptors, thereby antagonising the actions of leukotrienes

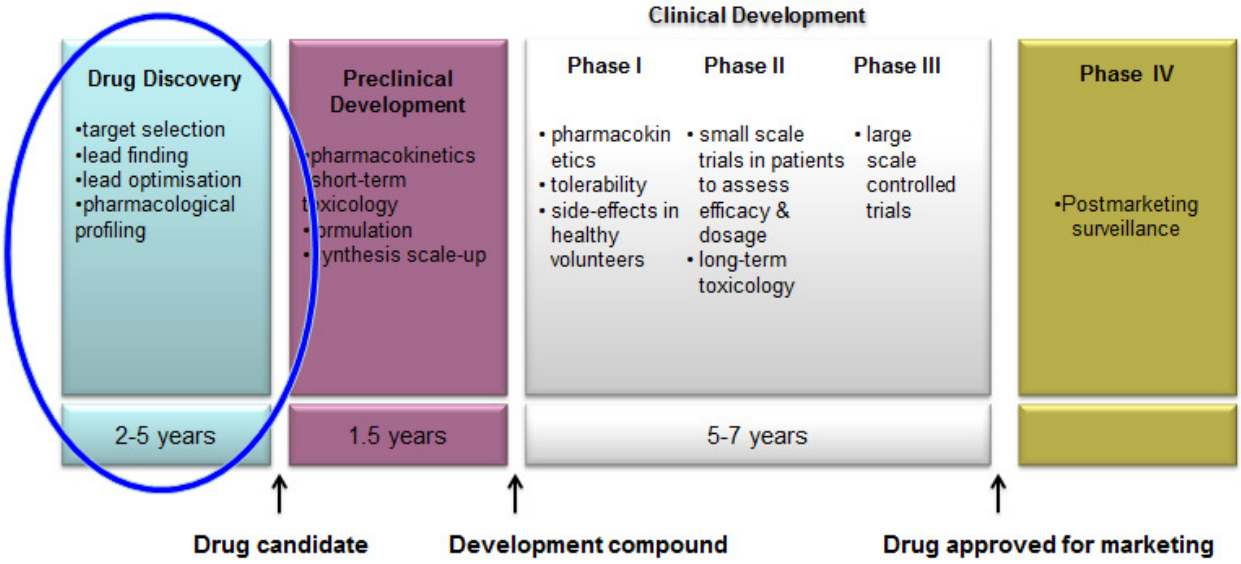
3) Functional antagonism: If an endogenous mediator causes dilatation of blood vessels, it may be possible to counter their actions by using a drug, which causes vasodilation through separate mechanisms

4) Increasing metabolism or clearance of the endogenous substance: Since most of the endogenous mediators, which are involved in allergic reactions, have quite short half-lives, there is little scope for increasing their clearance or metabolism

•Drugs that have an action against the manifestation of anaphylaxis
1) Antihistamines: Histamine produces its action by effects on: H2 receptors in the gastric mucosa, which lead to the release of hydrochloric acid into the lumen of the stomach and H1 receptors which are the predominant receptors in blood vessels and in airway smooth muscle. Thus, histamine H1 receptor antagonists will reduce the oedema that is associated with type 1 hypersensitivity.

2) Adrenaline: Adrenergic agonist activates both α and β-adrenergic receptors.
-By activating α-receptors, adrenaline leads to vasoconstriction in the peripheral circulation, thereby reversing the vasodilating properties of the mediators, which are released in type 1 hypersensitivity
-By activating β-receptors in airway smooth muscle, adrenaline leads to bronchodilation, reversing the bronchoconstriction of the mediators of type 1 hypersensitivity

4. Introduction to Drug Discovery and Development



<u>Drug Discovery</u>

-Elucidate the disease biology

•Drug target selection
-To develop a drug against a novel target costs ~$1.2 billion over 10-15 years
    -Regulatory approval for a drug against a new target is slow – a disincentive for investment
    -Approval is faster for drugs against established targets
-Validating a new target may take years
-Most will be discarded

1) Identification of drug targets
-Drug target = A cellular macromolecule that is involved in the disease process – ideally having a critical role
-Receptors
-Enzymes
-Ion channels

-Carrier molecules (drug transporter)
-Structural proteins (e.g. tubulin targeted by colchicine)
-DNA (targeted by alkylating agents e.g. busulphan)

a) Gene
-Gene arrays: utilise hybridisation to align patient genes with genes immobilised on a chip
    -Slides that have small amounts of many genes imprinted on the surface of the chip
    -Take disease and control sample – extract and tag RNA
    -Bind RNA to gene chips
    -RNA where it is complimentary to DNA will bind very tightly
    -Non-specific RNA is washed off
    -Identify genes whose expression is altered (increased or decreased)
-Recent examples: RNA-seq, protein arrays and proteomics
-These methods provide a profile of gene or protein expression in diseased versus normal samples
-Gene knock-down and knock-out/knock-in mice are used to validate target

b) Bioinformatics
-Rapid access to information in databases
-Compilations of DNA and protein sequences suitable for data mining
-Sequence alignments between new and existing genes - insight into function
    -Note similar motifs between aligned genes and categorise into family

c) 3D modelling
-Identify potential drug targets in a disease sample using arrays and bioinformatics
-3D modelling of potential drug target using a program
-Identify potential regions that could interact with chemicals → Selective targeting

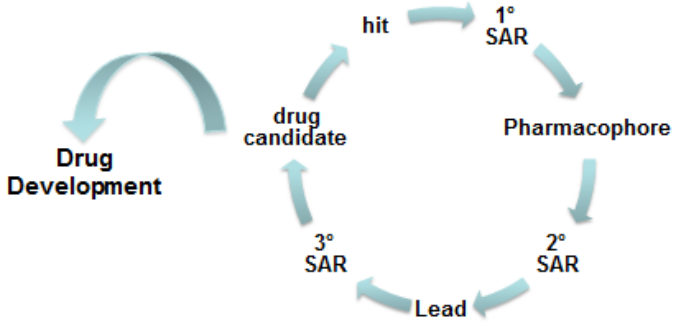
2) Lead finding
a) High-throughput assay
-Application of recombinant DNA techniques, e.g. cloned receptor or enzyme that could be a target
-Heterologous expression of human genes in cells (expression of gene to measure the protein activity)
-Direct testing of new chemical entities against these targets → select the most potent agent

b) Library screening
-High-throughput screening of chemical libraries and use of combinatorial chemistry
-Compound libraries from past projects are rescreened for new biological activities
-A disadvantage of synthetic libraries is that they are often of limited structural diversity (merely testing analogues)

c) Combinatorial chemistry
-Used to synthesise structurally analogous chemicals (libraries)
-Immobilised reactants on polystyrene bead arrays are treated with multiple reagents
-Synthesised together - Products are not isolated but used as mixtures
-Small quantities of multiple analogues tested in multi-well format
-High-throughput screens can identify potential leads
-Rapidly identifies promising leads (testing multiple chemicals at once)

d) Natural products
-Herbal extracts, fungal products, marine species can be valuable sources of new active agents
-However, problems include
    -Identification and isolation of low abundance active agents from complex mixtures of compounds
    -Sustainability of the sources of natural products
    -Chemical complexity can make purification, large-scale synthesis and formulation difficult

3) Lead identification and optimisation
-A "lead compound" with the desired biological activity emerges from screening
-Lead optimisation aims to enhance the pharmacological profile and minimise unwanted side effects
-This involves pharmacophore identification and development of structure-activity relationships (SAR)

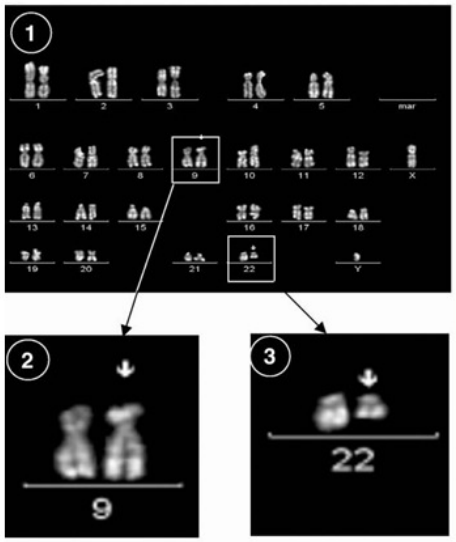


▪Drug pharmacophores
-Pharmacophore = "a molecular framework that carries (phoros) the essential features responsible for a drug's (pharmacon) biological activity" = structural features containing information necessary for pharmacological activity
-Key computational strategy in drug discovery – even if a 3D target structure is not available
-The aim is to
    -Identify critical chemical features in a set of known ligands
    -Identify essential interactions between a series of ligands and a specific macromolecular target (if known)

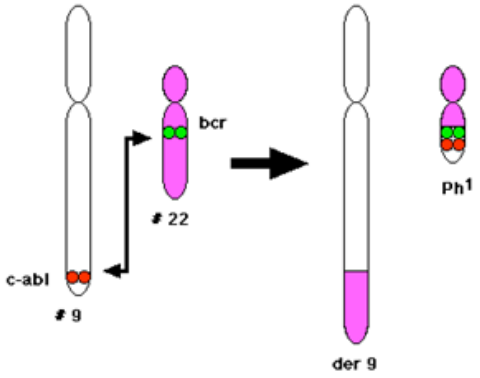
•Case study: Imatinib
-Chronic myelogenous leukaemia (CML) accounts for 15-20% of all cases of leukaemia
-CML is characterized by over-production of granulocytes (neutrophils, eosinophils, and basophils)

-These cells fight infection and disease, but in CML are abnormal and immature

-Cytogenic tests identified a characteristic defect in CML-containing cells = Philadelphia (Ph) Chromosome
-Karyotype of a patient with Ph-positive CML showing the lengthened chromosome 9 and shortened chromosome 22

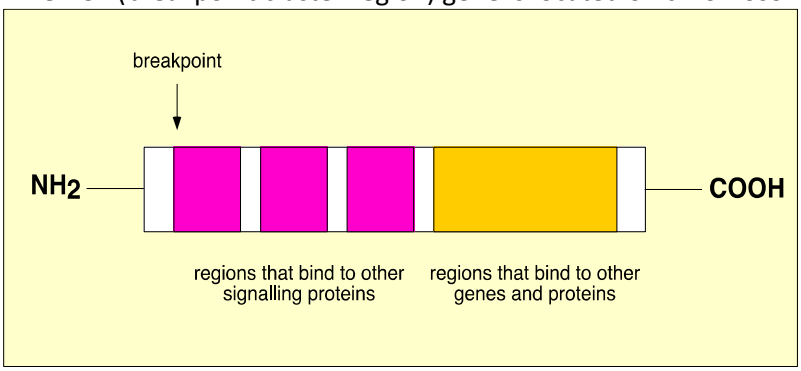


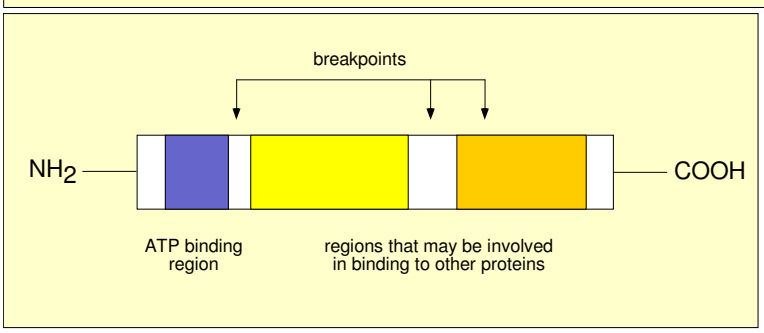
-Part of chromosome 22 has been lost and transferred to chromosome 9



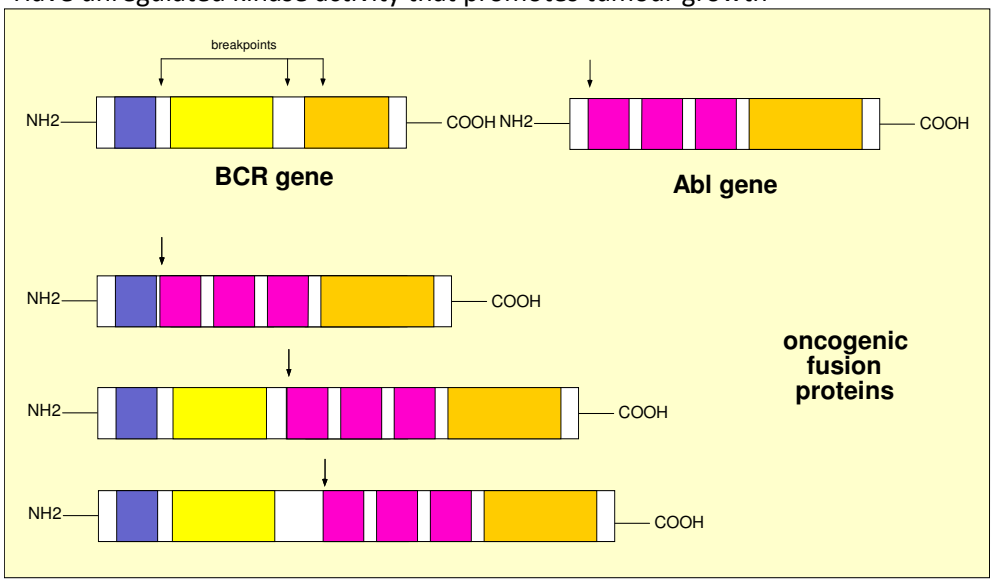
-The Ph-chromosome encodes an abnormal kinase
-Kinases mediate phosphorylation (post-translational modification) to control protein-protein interaction cascade that transduce signals within cells (e.g. gene activation)

-The Abl gene on chromosome 9 is a tyrosine kinase which is involved in cell signalling
-The BCR (breakpoint cluster region) gene is located on chromosome 22 and also has serine/threonine kinase activity



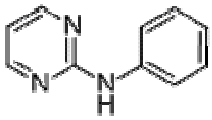


-BCR/Abl mutations - multiple fusion proteins result from chromosomal translocations (from BCR gene to Abl gene)
-Have unregulated kinase activity that promotes tumour growth

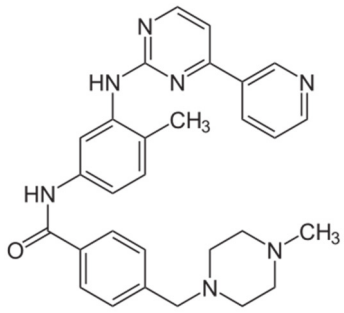


-BCR/Abl is an ideal therapeutic target
-BCR/Abl is present in 95% of CML patients but is absent from healthy cells

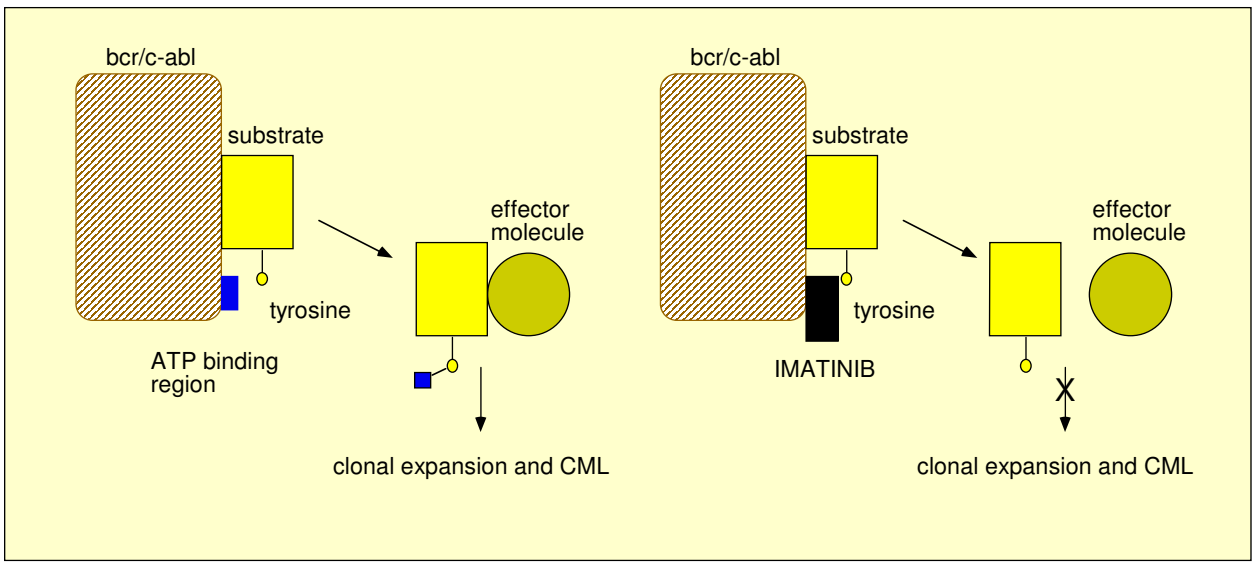
-From bioinformatics and 3D modelling, 3D structure of BCR/Abl was deduced
-From chemical libraries phenylamino-pyrimidines were identified as potential inhibitors (Selective inhibition of BCR/Abl tyrosine kinase)



-Pharmacophoric modelling to optimise structure



▪Mode of action



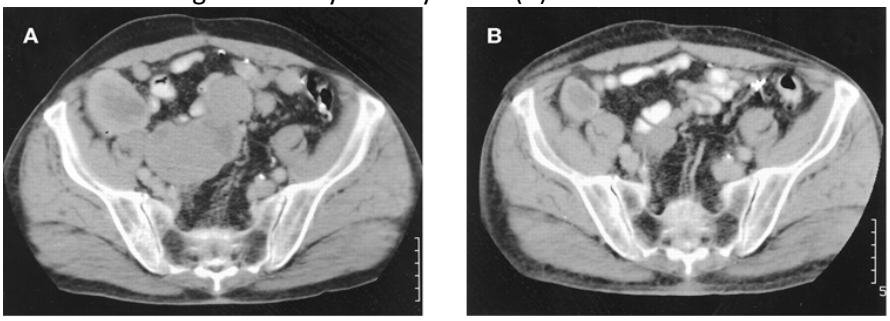
-BCR/Abl captures a protein substrate that has a suitable tyrosine residue that is exposed and can be phosphorylated by the kinase
-Tyrosine sits opposite the ATP binding region and ATP is used as a source of phosphate
-Newly phosphorylated substrate will bind to an effector molecule and set in train for clonal expansion (abnormal blood cells)

-Imatinib binds to the ATP binding site of BCR/Abl and prevents phosphorylation of tyrosine
-Prevent interaction phosphorylated substrate with downstream product
→Imatinib impedes normal phosphorylation of downstream substrates

▪Clinical experience with imatinib
-Phase I and II trials were conducted in patients with CML in whom other treatments had failed
-98% response in first phase I trial
-Low (~9%) relapse rate after 18 months of therapy
-Only 3 years between enrolment of first patient in phase I trial and FDA approval for phase III trial
-At eight years of follow-up 85% overall survival

▪Additional therapeutic applications of imatinib
-In initial high-throughput screening against a panel of recombinant kinases, c-kit was also inhibited by imatinib
-This kinase regulates haemotopoiesis and is expressed in some cells of the GI mucosa
-~60% of GI stromal tumours exhibit c-kit mutations
-Imatinib is first-line therapy for GI stromal tumours that are inoperable

-Response of a gastric tumour to imatinib
-75 year old man underwent surgery for a gastric tumour but it returned 14 months later
-MRI revealed a large mass (A, upper left)
-Imatinib 400 mg twice daily: 60 days later (B)



▪Drug resistance
-Resistance to chemotherapy
-Tumours contain different cell populations that carry a number of genetic defects
    -Susceptible cells eliminated by therapy
    -Resistant cells proliferate
-With imatinib it has emerged that resistant cells contain particular BCR/Abl variants

▪Targeting major BCR/Abl mutations
-Most significant is the “gatekeeper” mutation that has isoleucine substitution within the ATP-binding region of BCR/Abl in the place of threonine
-The isoleucine residue prevents hydrogen bonding interaction which is normally used by threonine to stabilise the drug interaction
-Newer agents are better able to treat resistant CML due to improved binding to the alternate mutation (isoleucine)

•Alternate strategies in drug design
-Activity-based drug development: insights into the activity of a chemical in a biological system is used to create improved analogues

-E.g. ω-3 polyunsaturated fatty acid metabolite was found to kill cells
-Potential anticancer properties