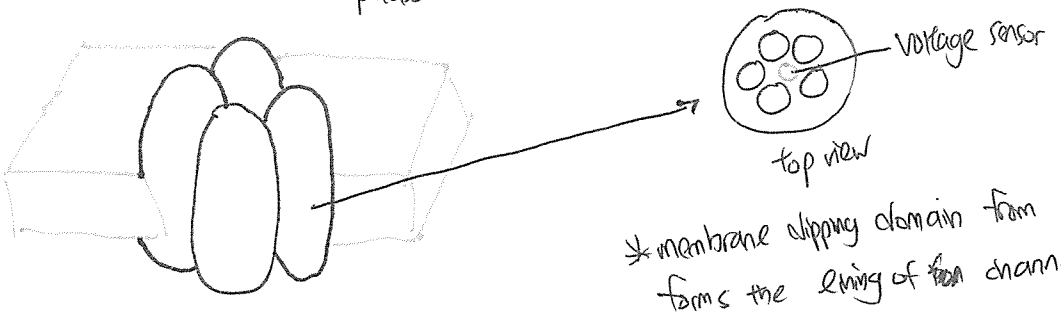
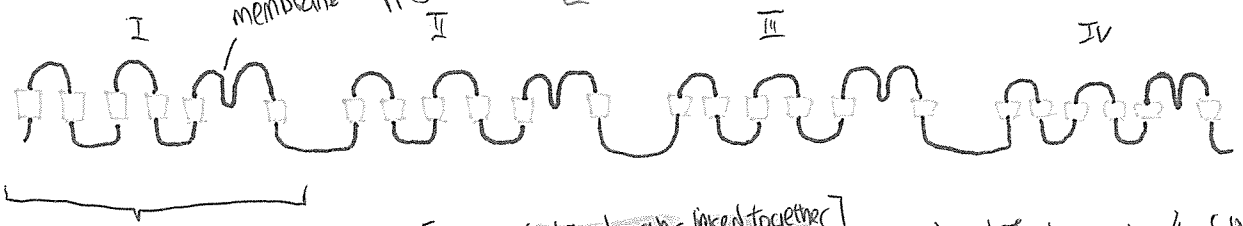
Voltage-gated channels ← calcium channels, sodium channels, potassium channels } have similar structure



* membrane dipping domain from each pseudosubunit forms the lining of the channel

Structure of Ca^{2+} & Na^+ channels (1) [each subunit – 6 transmembrane domains]



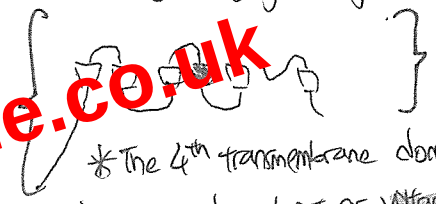
↳ Consists of 4 pseudosubunits [4 very similar domains linked together]

~ α (channel forming) + β (accessory) subunit structure

~ α subunit very large (> 2000 aa), β subunit smaller

~ Evolutionarily related to K^+ channel

→ In K^+ channel, 4 of these subunits join together



* The 4th transmembrane domain is charged and act as voltage sensor. When membrane potential changes, the 4th TM domain moves, opening & closing the channel.

Molecular Diversity ..... Diversity is the rule, not the exception

- many diff subunits
- distinct tissue distributions
- Come together to form receptor / channel subtypes
- pharmacology determined by subunit composition

- Calcium channels, L, N, P, Q, R, T-types
  - L-type antagonists — useful CV drugs & heart drugs
  - N-, P-type antagonist — neurotoxins
- Benzodiazepine agonists (GABA$_A$ receptors)
  - α1 selective : hypnotics
  - α2, α3, α5 selective : anxiolytics
    - ↳ anti anxiety drugs

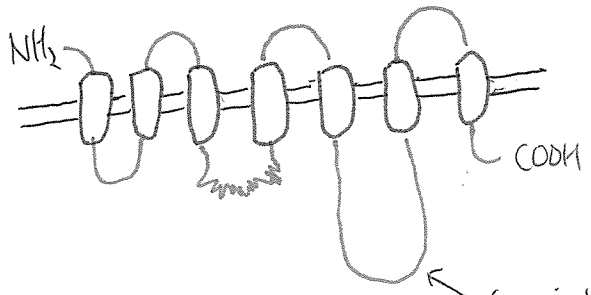
G-protein coupled receptors: also 7TM receptor

- Historically, most important drug class
- 31 of top 100 drugs target GPCR
- Most diverse receptor class
- 1500 GPCR sequences in human genome
- 600 suitable drug targets, only 200 have know ligands

Family C → 3 (metabotropic family)

→ mostly monomeric, some dimeric though

G protein → membrane associated protein
→ sits on the inner phase of plasma membrane



* Agonist binding site in the centre of G-protein coupled receptor
↳ located extracellularly

↖ G protein binding domain - allow binding of G protein

Diversity... → mostly caused by diversity in α subunit of G-protein

→ As with ion channels, multiple subtypes

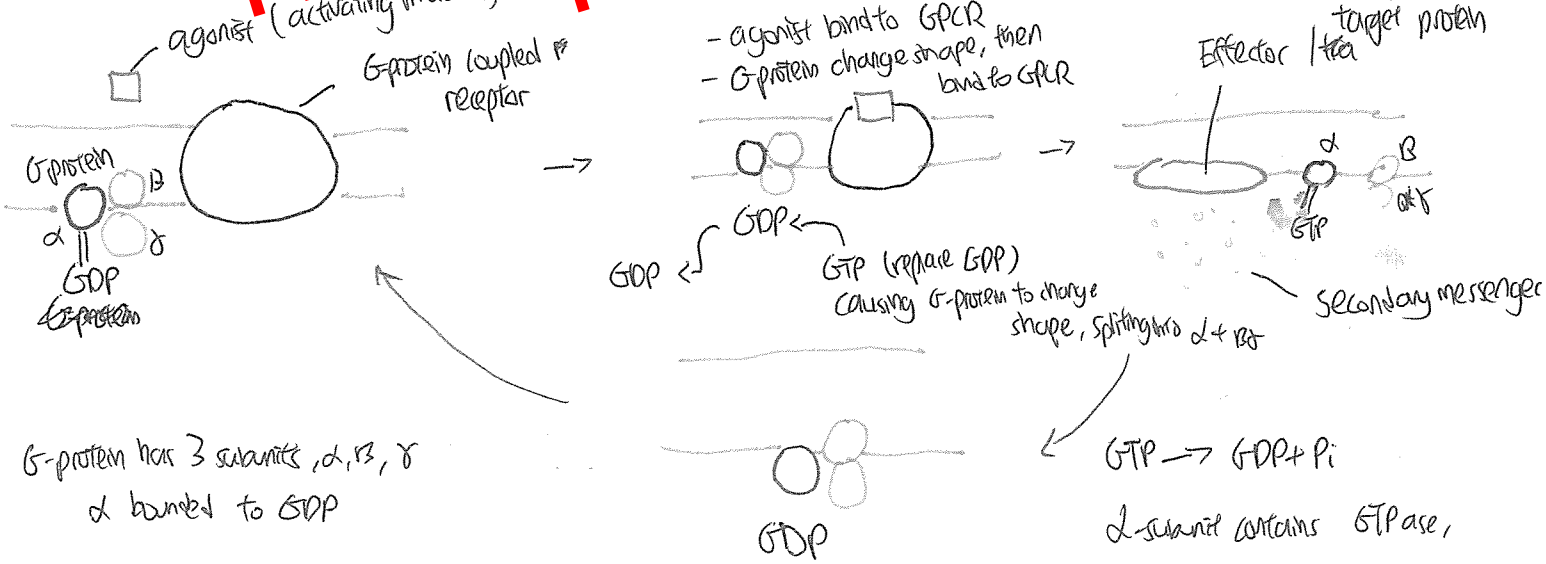
→ muscarinic acetylcholine receptor M1-M5

→ Adrenergic receptors α1, α2, β1, β2... (at least)

different protein sequence

α-subunit bounded to GTP can now activate other target protein

G-protein activation cycle:



G-protein has 3 subunits, α, β, γ
α bounded to GDP

GDP

GTP → GDP + Pi

α-subunit contains GTPase, hydrolyzing GTP into GDP + Pi

α-subunit without GTP has no activation effect, hence binds to β & γ again.

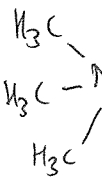
Affinity & Efficacy of a Drug:

- Affinity (binding) (how tight)
- Efficacy: what it does once bound
  - Agonist: full efficacy
  - Antagonist: zero efficacy

- Partial agonists lies between (Ariens & Stephenson (1950)
- Homologous series - (similar drugs, but differ in length:)
  - Maximum less than full agonist
  - can antagonise an agonist
    - ↳ Still activate the receptor but NOT as much as full agonist
    - ↳ same mechanism as competitive antagonism

↓
Alkyltrimethylammonium drugs

Alkyltrimethylammonium drugs (agonist at muscarinic acetylcholine receptor)



R - butyl
- heptyl
- Decyl

* Partial agonists also produce a rightward shift in conc. response curve to a full agonist

- responses require affinity & efficacy
- agonists have both
- competitive antagonist have affinity but no efficacy
- Partial agonists have affinity & some efficacy

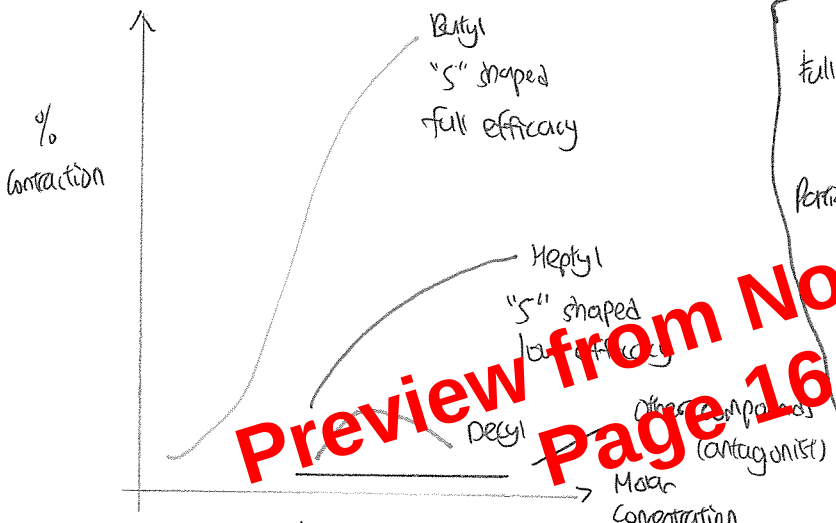
IMPORTANT

Full Agonist
- high affinity to active receptors (stabilizes it)
- low affinity to inactive receptors

Partial Agonist
- high affinity to active receptors
- low affinity
- moderate affinity to inactive receptors

Antagonist
- equal affinity to active receptors
- equal affinity to inactive receptors

Contraction of Guinea Pig Ileum



* ↑ Carbon chain length, ↓ response
→ no response

* receptors capable of spontaneous activation
* agonist stabilizes active receptor

Tone and Antagonists:

↝ Competitive antagonists alone do not have effects (no intrinsic efficacy)

↝ tone: basal activity of a system

↝ antagonists antagonize endogenous mediators responsible for tone

* Atropine ↑HR by blocking mAchR

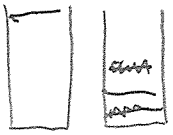
↝ At receptor level, antagonist bind and do NOTHING

Salivary Secretion:

(1) Low secretion (tone)

(2) High secretion (tone)

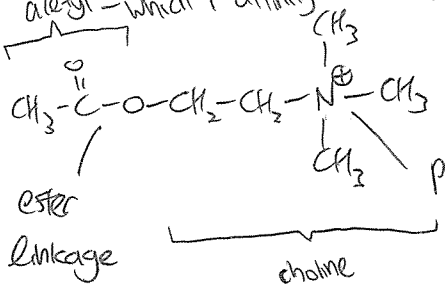
Control Atropine

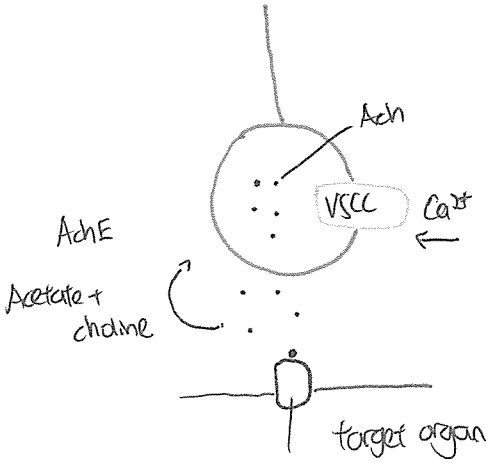


Control Atropine

Cholinergic transmission

- cholinergic = via acetylcholine



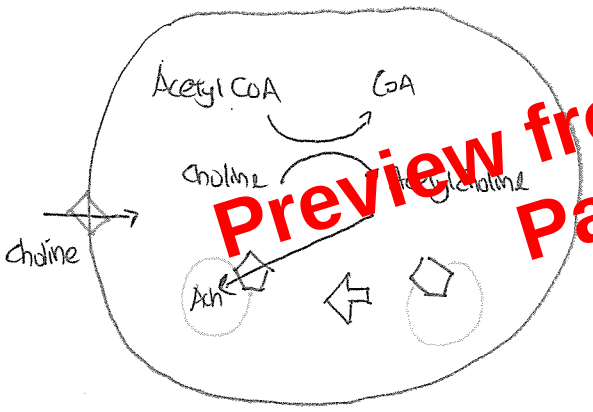


VSCC - voltage sensitive Ca^{2+} channels

AchE - Acetylcholinesterase
↳ cleaves ester bond of Ach into acetyl + choline

Ach receptor – can be nicotinic / muscarinic

- choline is recycle back into [nerve terminal] via a specific transporter [→ Sodium dependent choline transporter]
- choline acetyltransferase synthesis Ach from choline + acetate
Acetyl from Acetyl CoA



Acetylcholine receptors: (metabotropic)
- muscarinic: G-protein coupled, slow (s-ms)
- nicotinic: ligand-gated ion channel, fast transmission (ms-μs)
(ionotropic)

Xenopus oocytes : lifespan 20 years

- unfertilized eggs from Xenopus laevis (African clawed frog)
- injection w DNA / RNA coding for protein of interest, it will be synthesised
- reliable : most proteins properly expressed
- very large (1mm), easy to handle, versatile, equipment relatively simple
- Every oocyte needs to be injected
- High-throughput screening difficult
- some endogenous proteins (proteins of the Xenopus)

optimum temperature for mammalian → 37°C

" " Xenopus → 18°C

Hence, Xenopus not 100% reliable for protein production

Mammalian cell lines :

↳ usually best for fidelity of expression

↳ can be cultured in large batches - good for high-throughput

↳ versatile: binding studies, ion flux experiments, imaging studies, electrophysiology

↳ difficult to culture (expensive media)

↳ require aseptic lab conditions

↳ possible problems w endogenous proteins as they may interfere w desired proteins

↳ Typically an immortalized cell

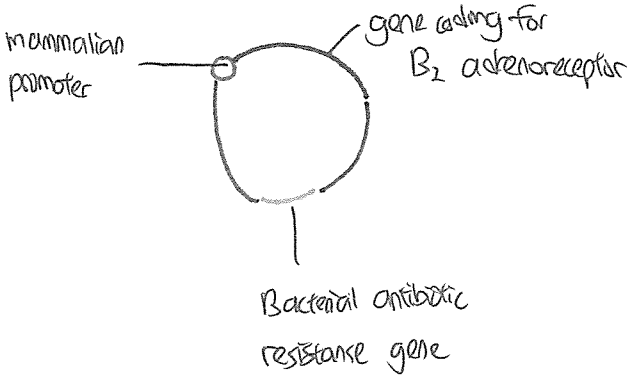
- cancer cell / cell w mutation / viral infection
- normal restrictions on cell division lost
- cell can divide & reproduce indefinitely

Eg: HEK 293, CHO cell, COS-7 (monkey) — African green monkey kidney cells immortalized with SV40 virus

↳ derived from human embryonic kidney transformed w adenovirus

↳ chinese hamster ovary

Expression vector (Plasmid)



- plasmid is placed w a strong promoter
- plasmid would be grown up in bacteria and also contains an antibiotic resistance gene - this allows selection for desired bacteria in culture.

Typical bacterial culture might yield several mg of plasmid DNA

Blood Pressure — 120/80 mmHg

Hypertension — ↑ diastolic, systolic, PP

Quantifying hypertension: (diastolic)
- severe >120mmHg
- moderate 105–120 mmHg
- mild 90–105 mmHg

* many CV diseases ⇆ renal diseases related to hypertension

white coat hypertension: only exhibit high BP in a clinical setting but not at normal conditions

Classification of Hypertension by aetiology:
1) Primary (essential / idiopathic) hypertension — unknown cause
2) Secondary hypertension — identified cause eg: polycystic renal disease
renal artery stenosis [narrowing of renal artery]
↳ hypertension arises as symptoms of other disorders
phaeochromocytoma — tumour of adrenal medulla secretes adrenaline, causing ↑BP

Consequences of Hypertension:
- Coronary artery disease (myocardial infarction)
- Stroke
  - ↳ cerebral haemorrhage
  - ↳ thrombosis ⇆ thromboembolism

thrombosis - abnormal blood clot in blood vessels

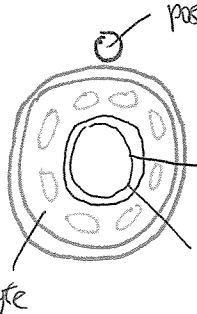
Lifestyle modifications
1) lose weight
2) limit alcohol intake
3) regular exercise / activity
4) reduce sodium intake
5) maintain potassium intake
6) maintain calcium and magnesium intake
7) stop smoking!
8) Reduce dietary fat ⇆ cholesterol

Principles of Treatment: Blood pressure control by drugs

BP = CO × peripheral resistance

Basic principle: interfere w/ control mechanisms that do not compromise cardiovascular efficiency

Typical Resistance Arteriole:



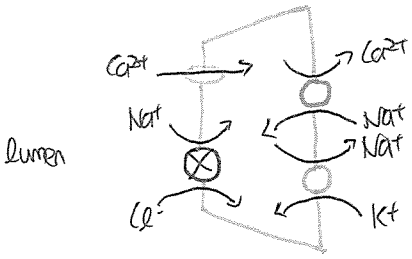
↗ can change its diameter due to contraction and ↘ relaxation of smooth muscle

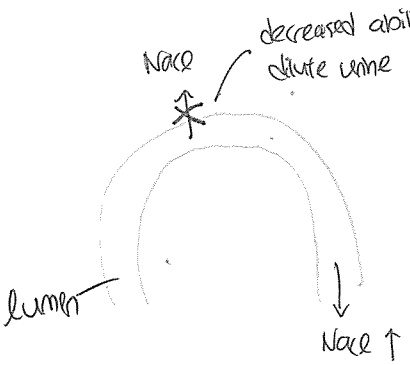
adrenaline ⇆ vascular tone:

noradrenaline
↘ α_1- adrenoreceptor
phospholipase C activation
↓
IP3 release (inositol trisphosphate)
↘ Ca^{2+} store
↑ $[Ca^{2+}_i]$ intracellular stores eg: mitochondria, ER
Sarcoplasmic reticulum
↓
Ca^{2+} sensitive Cl^- channels open
↓
Cl^- efflux from myocyte
↓
membrane depolarisation

Thiazide Diuretics: main eg: bendroflumethiazide, ~~indapa~~ chlortalidone (thiazide related)

↳ Act by inhibiting Na^+/Cl^- cotransporter in distal convoluted tubule





↳ ↑ NaCl passed to more distal segments

↳ ↓ difference in osmolality between urine & plasma

↳ ↓ less ability to reabsorb water in more distal portions

↳ similar effects as loop diuretics but milder effects & act at diff. locations of the kidney

Clinical use

↝ oedema due to heart failure → oedema shows up as cloudiness over the lungs on fluid absorbs X-ray

↝ hypertension (lower doses)

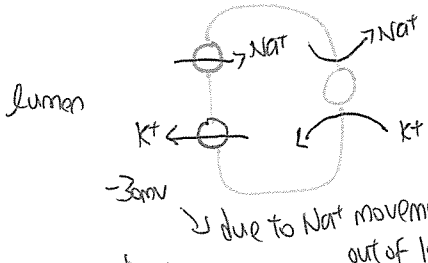
↝ initial effects due to diuresis

↝ later, get vascular effects

↝ mild diuretics

Hypokalaemia: low K^+ levels

- major problem ∵ loop diuretics, thiazides
- ↑ NaCl passed onto collecting duct increases reabsorption across principal cells
- ↑ lumen -ve potential leading to K^+ loss ~~function~~ to reabsorb K^+
- ↑ volume of urine: ↑ flushing of K^+ as it is sucked by -ve membrane potential of lumen



↳ due to Na^+ movement ~~into~~ out of lumen

Potassium sparing diuretics:

↝ weak diuretic action on their own

↝ usually used in combination ∵ other diuretics

↝ spironolactone used in hyperaldosteronism

↳ cirrhosis (failure to metabolize ALD

↳ Conn's syndrome (adrenal tumour)

Potassium sparing diuretics:

General mechanism: decreases trans-principal cell Na^+ movement, decreases -ve lumen potential

main eg: spironolactone, amiloride, triamterene

Amiloride, Triamterene } Block Sodium channels in luminal membrane

Spironolactone

↳ aldosterone antagonist (competitive)

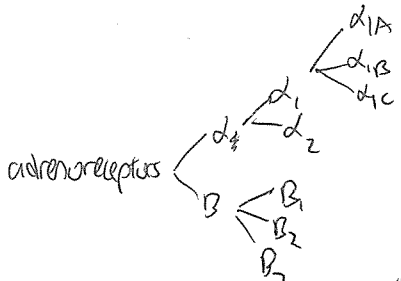
↳ aldosterone ↑ sodium absorption by { ↑ no. of Na^+/K^+ ATPase, ↑ no. of Na^+ channels; stimulating Na^+ channels via protein mediator

Antihypertensive drugs: Antagonists at adrenoreceptors

Fight or flight response:

Sympathetic nerves – noradrenaline
adrenal medulla – adrenaline

} blood vessels, heart, lungs, eye, bladder, GI tract, genitalia, salivary glands, sweat glands



β_3 – coupled to adenylyl cyclase through $G\alpha_s$
– present on fat cells, stimulate breakdown of FAT

Function of Adrenergic Receptors:

α_1
$G\alpha_q$
phospholipase C
↓
- vasoconstriction, ↑BP
- contract visceral smooth muscle
  - ↳ bladder sphincter
  - ↳ uterus
  - ↳ iris radial muscle
  - ↳ seminal tract
  - ↳ pilomotor muscles
- relax GI tract

α_2
$G\alpha_i$
adenylyl cyclase
↓
- transmitter release
- insulin release

β_1
$G\alpha_s$
adenylyl cyclase
↓
- Heart rate & force
- release of renin
- lypolysis
- glycogenolysis

β_2
$G\alpha_s$
adenylyl cyclase
↓
- Bronchodilation
- vasodilation (↓BP)
- relax visceral smooth muscle
- lypolysis
- glycogenolysis
- muscle tremor

Agonist

α_1	α_2	β_1	β_2
phenylephrine	Clonidine	dobutamine	salbutamol
oxymetazoline	α2 agonist		
	α-methylnoradrenaline		

methoxamine

isoprenaline

noradrenaline

adrenaline

Antagonist

Drug — no therapeutic effects

α_1	α_2	β_1	β_2
Prazosin	Yohimbine	Atenolol	Butoxamine
Doxazosin		Metoprolol	
Phentolamine (competitive)		Propranolol	
Chlorpromazine			
Phenoxybenzamine (insurmountable / non-competitive)			
Labetalol			

Uses of α-adrenoreceptor antagonists:

Treatment of phaeochromocytoma

Phaeochromocytoma — tumour of adrenal medulla
- Secretes adrenaline erratically
- episodes of severe hypertension

→ Phentolamine — used as treatment

→ phenoxybenzamine used during surgery as precaution when removing adrenal tumour that may lead to release of excess adrenaline at a lethal dose

α-adrenoreceptor antagonists as antihypertensive agents

Phenoxybenzamine — non-selective α-adrenoreceptor antagonists
- unsuitable for treatment of hypertension

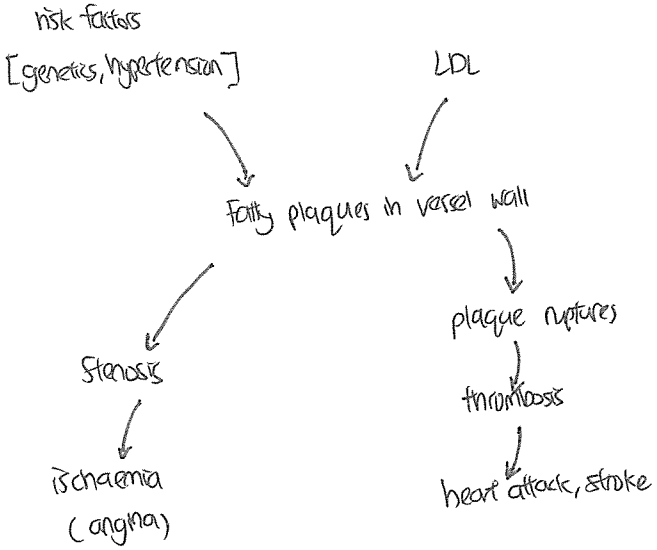
Prazosin — α_1-selective adrenoreceptor antagonist
- used for treatment of hypertension

→ α_2 acts as inhibitory autoreceptors

→ α_2 provide negative feedback to release of NA @ the heart

→ Simultaneous blockade of α_1 & α_2 adrenoreceptors by phentolamine leads to unacceptable reflex tachycardia as autoinhibition of noradrenaline prevented [no negative feedback as α_2 blocked]

→ Selective blockade of α_1-adrenoreceptors by prazosin leads to acceptable reflex tachycardia



Hyperlipidaemias : high blood lipid content

- number of diff. forms
- diff disturbances in LDL, VLDL, cholesterol
- treated differently due to diversity of hyperlipidaemias

Lipid lowering drugs — Aim < reduce LDL / increase HDL

↳ Approach varies in hyperlipidaemia

↳ 'statins'
↳ ezetimibe
↳ fibrates
↳ exchange resins
↳ nicotinic acid

dietary changes :

- ↓ saturated fat
- ↑ unsaturated fat
- ↑ fibre
- avoid trans fat & hydrogenated fats
- ↑ exercise level

statins → Atorvastatin, Simvastatin

- competitively inhibits HMG-CoA reductase, rate limiting enzyme in production of cholesterol
  ↳ similar in structure to HMG-CoA

- reduces liver production of cholesterol
- lowered cholesterol leads to ↑ LDL receptors, ↑ removal LDL from plasma
- also ↑ HDL & ↓ triglycerides

HMG-CoA reductase → inhibited by statins, causing decrease in cholesterol

Acetyl Coenzyme A → (⊕) mevalonate → (↓) cholesterol

Problems

- Myositis → muscle inflammation
- Rhabdomyolysis → muscle breakdown
- altered liver function tests
- Some cases, may cause liver failure

Ezetimibe

- Inhibits intestinal cholesterol absorption by inhibiting specific cholesterol transporter in gut
- results in ↓ LDL, ↓ total cholesterol
- Co-administered with "statins" → gives dual inhibition of cholesterol absorption & synthesis
  - absorption: intestinal tract
  - synthesis: liver

Cholesterol Absorption in Intestine:

Anion Exchange Resins [Colestyramine]

↳ sequester bile acids in GT, bind to -ve charged bile acids
- bile acids can't be recycled to liver
- bile acids can't emulsify fats

- reduce their absorption
- reduce exogenous cholesterol absorption
- increase conversion of endogenous cholesterol to bile acids
- increase LDL receptors expression in liver cells → removal of LDL from plasma

Problems
- Anion exchange resins need to be taken into body in large quantities to obtain desired effect
  ↳ now rarely used

GIT upsets
- nausea, bloating, constipation or diarrhoea are common
- ↓ absorption of fat soluble vitamins (A, D, E, K), fat soluble drugs
  ↳ eg: warfarin

Heart as an Electrically Controlled Pump

- SAN initiates cycle
- AVN relays to ventricles
- Purkinje fibres } Bundle of His spread impulse through ventricles

Surface electrocardiogram
- Compound AP from individual APs
- basis for diagnosis } treatment

P- atrial depolarisation
QRS- ventricular depolarisation
T- Ventricular repolarisation

- malfunctioning electrical system leads to poor pumping:
inadequate supply of blood to body
- lack of coordination of pumping or very fast rate leads to turbulence (risk factor for blood clots, stroke, heart attack)
↳ blood being pumped back } forth between atria } ventricles

cardiac arrhythmias / Dysrhythmias: any disorder of heart rate / rhythm
↳ disruption of the normal electrical conduction system of the heart

dysrhythmia- disorganised rhythm
arrhythmia – absence of rhythm (more serious)

Classification of Dysrhythmias
- Site of origin
  - atrial
  - ventricular
  - junctional (supraventricular) [AV node having something wrong]
- True arrhythmias – a very disorganized rhythms or completely absent rhythm
- Tachycardia
- Bradycardia

Dysrhythmic Mechanisms
- Ectopic pacemaker – cardiac tissue other than SAN initiates heart beat
- Delayed after-hyperpolarisation – build up of calcium in cells lead to train of AP
- Re-entry circuits – tissue damage / abnormality causes AP to travel in circles
- Congenital abnormalities – additional conducting pathways between atria } ventricles
- Heart block – damage to conducting pathways disrupt atrial-ventricular signalling
  - ↓ slow down pumping of heart
  - atria contract at a higher rate than ventricle
  - primarily leads to bradycardia

Verapamil in Angina of Effort

- blockade of Ca^{2+} channels is use dependent
- more potent on heart than vascular smooth muscle
- Ca^{2+} channel blockade in myocardium reduces heart rate & cardiac output
- Dilation of arterioles reduces cardiac afterload
- cardiac work & O_2 demand are reduced

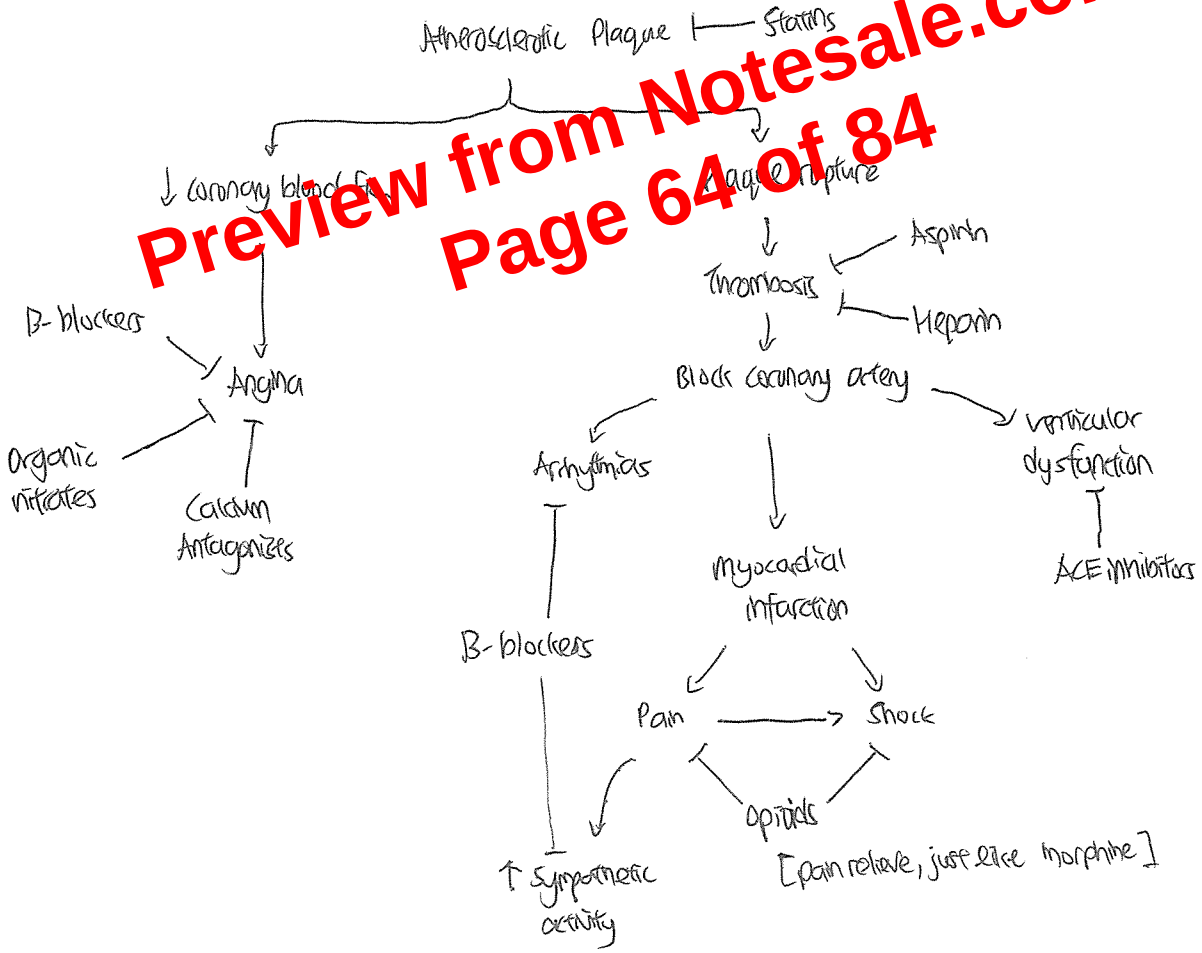
Nifedipine in Angina of Effort

- blockade of Ca^{2+} channels is NOT use-dependent
- more potent on vascular smooth muscle than heart
- dilation of arterioles reduce cardiac workload
- dilation of capacitance veins reduces cardiac preload

} similar to GTN

- Cardiac work and O_2 demand ↓

Drugs used to treat Angina and Myocardial Infarction:



Leukotriene Antagonists

Phospholipids

↓ phospholipase A_2

Arachidonic Acid

↓ lipoxygenase → Leukotrienes → action open!

↓ cyclo-oxygenase → Prostanoids (prostaglandins, prostacyclins)

Leukotriene, member of Eicosanoids Family — 20 carbon — derived from fatty acid (arachidonic acid)

Consequence of taking aspirin → can lead to all the arachidonic acids are channelled for leukotriene production

[if aspirin taken in excess, there may be excess leukotriene production → asthma; asthma aspirin is very dangerous for asthma]

Leukotrienes

- produced by mast cells, eosinophils, basophils and macrophages
- important mediator in asthma
- agonist at cysteinyl ~~cysteine~~ - leukotriene (cysLT) receptors
- contracts bronchial smooth muscle
- stimulates mucus secretion
- increases microvascular permeability (walls of blood vessel open up, allow entry of leucocytes) infiltration

Competitive antagonists at cysLT receptors

- montelukast and ~~zafirlukast~~
- given orally
- can be relievers / preventers
- Add-on, prophylactic therapy in mild to moderate asthma
- reduces exercise-induced asthma
- reduces aspirin-induced asthma

Unwanted Effects

↳ abdominal pain, nausea

↳ headache

↳ hepatotoxicity (zafirlukast)