Cell- Cell Communication

CellCell Communication (University College Dublin)

Cell - Cell Communication

Cell Structure and Function- 15/09/17

Common Features of all cells
- DNA
- plasma membrane
  -phospholipid bilayer separating the cell from the surrounding environment
- cytoplasm
  -material of cell within the plasma membrane excluding the nucleoid region/ nucleus
  -fluid portion called cytosol and organelles and particles suspended in it

Nucleus
- appears uniform except for neutrophils
- produces message which must be made into a protein
- nuclear envelope= highly regulated membrane barrier

Rough endoplasmic reticulum
- extensive membranous network of flattened sacs
- has ribosomal particles attached to surface
- proteins are synthesised on the attached ribosomes
  -they then enter the lumen of the reticulum where they are distributed to other organelles or secreted from the cell

Smooth endoplasmic reticulum
- highly branched tubular network with no attached chromosomes
  -may be continuos with the rough ER
- contains enzymes for fatty acid and steroid synthesis
- stores and releases calcium which controls various cell activities

Golgi apparatus
- main function= process and package macromolecules (ie proteins and lipids) that are synthesised by the cell
  -particularly important for processing of proteins for secretion

Mitochondria
- generate ATP
- no. of mitochondria in a cell varies
- involved in signalling, cell cycle, cell growth and differentiation
- mitochondrial defects cause several diseases
  -neuromuscular disease symptoms: mitochondrial myopathies (myopathy= disease of the muscle)
  -Diseases of the mitochondria appear to cause the most damage to cells of the brain, heart, liver, skeletal muscles, kidney and the endocrine and respiratory systems
  -often inherited by the mother
- round or oval shaped body surrounded by 2 membranes
  -inner membrane folds into matrix of mitochondrion, forming cristae
- O_2 utilisation and CO_2 formation
  -cellular respiration
- contains enzymes active in Krebs cycle and oxidative phosphorylation
- believed to once have been a bacteria cell

Lysosomes
- garbage disposal system
- ability to digest proteins is important for the cell
- acidic fluid within lysosome contains digestive enzymes

- important role in cells that make up defines system of the body
- Lysosomal enzyme failure: Hurler Disease, Sanfilippo Syndrome A, Tay- Sachs, Gaucher and I-Cell disease
  -irish travelling community have highest recorded frequency of lysosomal storage disorders in the world (incidence 1/371 and carrier frequency 1/10)

Peroxisomes
- generate and degrade H_2O_2 (hydrogen peroxide)
- breakdown of fatty acids
- alcohol breakdown in liver
- formed in cytoplasm
- membrane bound organelles that are similar to lysosomes but have a different set of enzymes
- contains oxidases involved in certain catabolic pathways
- Zellweger Syndrome
  -genetic disorder characterised by reduction or absence of peroxisomes
  -enlarged liver, high levels of iron and copper in blood and vision disturbances
  -symptoms at birth may include lack of muscle tone and inability to move
  -poor prognosis

Cytosol and Cytoskelton- 15/09/17

Organelles
- Protein production
  -nucleus
  -nucleolus
  -ER rough and smooth
  -golgi
- Energy
  -mitochondria
- Recycling
  -lysosome
- Detox
  -peroxisome

Cytoplasm and cytosol
- interior of cell divided into 2 sections
  -nucleus
  -cytoplasm
- cytoplasm= region outside nucleus
- intracellular fluid
  -all fluid in a cell including fluid inside organelles

Cytosol
- gelatinous, semi transparent fluid
  -appox 50% of cell volume
- water, salts and organic molecules
- organelles suspended in the cytosol
  -also contains ribsomes, enzymes, inclusions (glycogen, lipid, pigment) and components of signalling pathways (kinases, phosphatases, and transcription factors)

Cytoskeleton
- cellular scaffolding
- dynamic structure
- maintains cell shape and protects the cells

- enables cellular motion
- important for intracellular transport and cellular division

Components of cytoskeleton
- microfilaments (actin)
- intermediate filaments (several proteins)
- microtubules (tubulin)

Microfilaments
- solid rods composed of 2 strings of bead-like subunits twisted together like a rope
- found in all cells beneath the cell membrane
- functions: cell shape and support, motility, important for cytokinesis (division of cytoplasm), movement of cytoplasm in a cell, microvilli support and muscle contraction with myosin
- double chain of actin
- Globular actin= G- actin
  Filamentous actin= F- actin
- G actin subunit assemble into filaments (F- actin) and then dissociates
  -providing dynamic structural framework for cell
- dynamic framework allows for
  -phagocytosis: bacteria fuses with the membrane and phagocyte forms a vacuole and ingests the bacteria
  -attaching and effacing lesions
  -microvilli of small intestine: contain actin providing a structural framework but also allow the microvilli to shorten and elongate
  -muscle contraction: in skeletal muscle actin forms stable arrangement of bundles with another protein, myosin - contraction occurs when actin and myosin filaments slide relative to one another

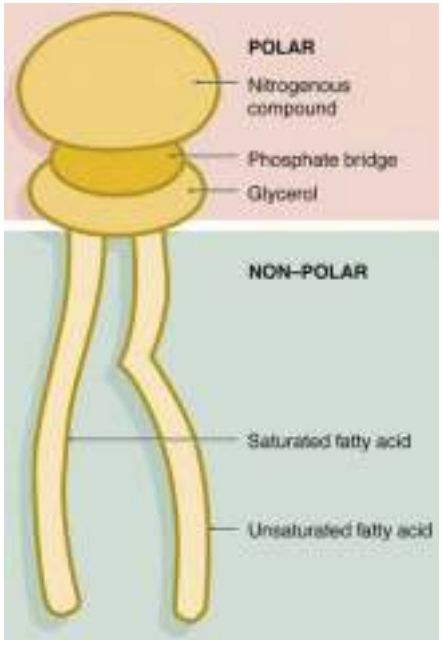
Microtubules (MTs)
- composed of 2 globular proteins which can be readily assembled and disassembled to accommodate changes in cell shape
- movement affects addition or subtraction of tubulin subunits making the microtubules longer or shorter
- 13 tubulin molecules form a hollow tube (diameter of tube made of 13 molecules)
- originate from a specialised microtubule organising centre called the centrosome
  -composed of a pair of centrioles
  -often located close to the nucleus
  -regulates formation and elongation of microtubules
- movement within a cell happens using attachment proteins dynein and kinesin
  -they move along the growing ends of microtubules in opposite directions
  -can attach to the membranes of organelles providing a mean for movement of organelles within a cell
  -microtubules act as a railway track
  -motor proteins move along microtubules using their globular heads
  -different proteins transport different cargo
- Anti- mitotc alkaloids
  -Colchicine: binds tubulin and blocks polymerisation, de-polymerisation continues resulting in breakdown of MTs
  -Taxol: forces tubulin into stable Mts and inhibits formation of mitotic spindle
  -Vinblastine: de-polymerises formed MTs and forces tubulin crystallisation
- Cilia and flagella share a common structure of a core of microtubules surrounded by a membrane
  -cilia are motile structures that project in parallel rows from some epithelial surfaces (ie respiratory tract) that beat with wave like rhythm. core contains 20 microtubles arranged in pairs with one central pair surrounded by the 9 other pairs. movement results from bending of the doublets first in one direction then the other and is fuelled by ATP

- Cilial dyskinesia
  -primary: autosomally recessive genetic defect in cilia motor proteins resulting in respiratory problems, ectopic pregnancy and male infertility
  -secondary: due to inflammation/ injury of tissue for example from smoking

Intermediate filaments
- diverse proteins- 70 family members
- great tensile strength
- they are like ropes made of long twisted strands of protein
- strengthen animal cells
- humans have over 50 types of intermediate filaments
  -cytoplasmic: keratins in epithelia, vimentin and vimentan related in connective tissue and muscle cells, neurofilaments in nerve cells
  -nuclear: nuclear lamins in all animal cells
- Epidermolysis bullosa simplex: clinical condition
  -genetic disease with mutations in gene encoding for cytokeratins 5 and 15
  -results in lack of cohesion between epithelial cells and underlying basement membrane causing blistering and fluid loss

Cell Membrane Structure and Function- 18/09/17
(look at cell bio notes from last year)

Plasma membrane
- encloses contents of the whole cell
- compartmentalise the cell
- involved in: cell communication, import and export of material and cell growth
- 3 layers of membrane
  -2 outer electron dense layers separated by electron lucent layer
- 1972 Singer and Nicholson discovered fluid mosaic model
- Contains a lipid bilayer
  -polar, hydrophilic head and non-polar facing to the outside of cell, hydrophobic tail facing inwards



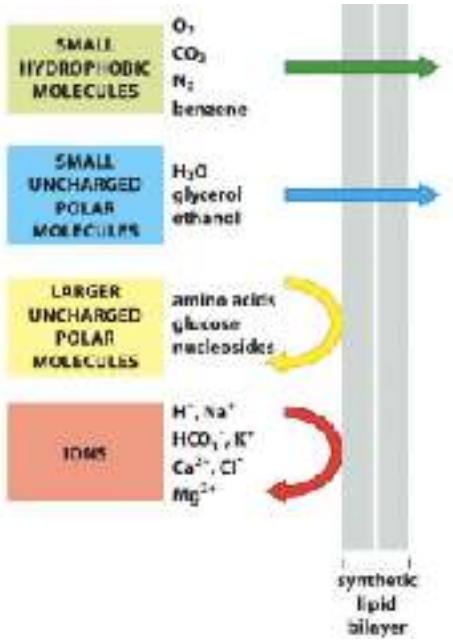
- membranes are lipid- protein assemblies
  -core of membrane consists of lipid bilayer serving as a structural backbone
  -provides barrier preventing random movement in and out of cell
  -proteins carry out more specific function
  -ratio of lipid to protein varies with cell type

Carbohydrate
- plasma membranes contain carbohydrate linked to both lipid and protein components
- carbohydrate face outwards
- display considerable variability in composition and structure

Cholesterol
- stiffens membranes
- it fits into gaps between phospholipid molecules
- negative effect because it is important for membranes to be fluid

Fluid Mosaic
- membrane is a fluid mosaic of phospholipids and proteins
- 2 main categories of membrane proteins
  -integral and peripheral proteins
- integral: permeate the surface of membrane
- peripheral: bound to the surface of membrane

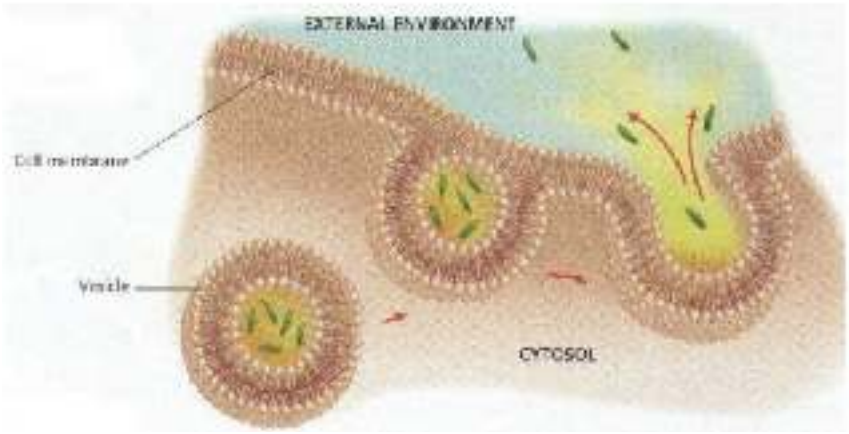
Transport across plasma membrane
- mediate the exchange of material between internal and external environment
- closely controlled
- 4 principle mechanisms
  -passive diffusion
  -facilitated diffusion
  -active transport
  -bulk transport
- rate of diffusion depends on size and solubility



- Passive diffusion
  -dependent on concentration gradient across the membrane

-lipids and lipid soluble molecules and small molecules (water, urea) can pass freely
-impermeable to large hydrophilic molecules

- Facilitated Diffusion
  -movement of large hydrophilic molecules
  -concentration dependent
  -can occur in either direction
  -strictly passive but require protein carrier molecules (gated pores)
  -glucose binds to carrier protein, protein changes shape and carries glucose to interior of cell and then regains shape
- Active Transport
  -concentration gradient does not support this process
  -often acts against extreme gradients
  -requires energy
  -transport of sodium and potassium mediated by Na+/ K+ ATPase pump
  -co-transport : it keeps high potassium, low sodium and high sugar levels within the cell
  -potassium goes into the cell at the expense of sodium which leaves the cell
  -this creates a gradient to bring sodium back into the cell
  -sodium drags sugar into the cell with it (symport)
  -constant transport of sodium in and out of the cell
  -counter transport: potassium enters the cell at expense of sodium which leaves the cell
  -creates a gradient to bring sodium back into the cell
  -calcium leaves the cell as sodium re enters the cell (antiport)
- Bulk Transport
  -transport of large molecules or small particles into cell
  -phagocytosis, endocytosis, exocytosis and pinocytosis
  -Endocytosis: an invagination is formed in the membrane of the cell, materials are taken from the external environment into the invagination, a vesicle is formed and diffuses into the cell
  -mammalian cells use receptor- mediated endocytosis to take cholesterol into cells. cholesterol usually found in low-density lipoproteins (LDLs) which bind to specific receptor proteins on the cell surface thereby triggering their uptake by receptor- mediated endocytosis
  -exocytosis is same process in the opposite way



-phagocytosis takes place in immune cells
-pinocytosis is cell gulping, on specific intake of material

Membrane function

- Compartmentalisation
  -encloses cell and intracellular spaces
  -allows for specialised activities to occur independently

- scaffold for biochemical activities
  -provide cell with extensive framework or scaffolding within which components can be ordered for effective interaction
- Selectively permeable barrier
  -prevents unrestricted exchange of material
- Transporting solutes
  -physically transport substances from one side of the membrane to the other
- respond to external signals
  -processes receptors that bind to specific molecules (ligands) with complimentary structures
  -different cells have different receptors and so can bind with different ligands
  -cell can recognise and respond
- intercellular interaction
  -allows cells to recognise each other, signal to each other, adhere to one another and exchange material and info
- energy transduction
  -intimately involved in the process by which one type of energy is converted into another type (energy transduction)

Pre-CAL tutorial - 22/09/17

Light microscope
- magnify up to 1000x
- details as small as 0.2 µm (micrometer)
- bright light focussed on specimen by lenses in the condenser and can be seen through a set of lenses (objective and eyepiece) once focussed in the eye

Haemotoxylin and Eosin (H&E stain)
- one of principle stains in histology- most widely used stain in medical diagnosis
- produced blue, violet and red colours
- haemotoxylin is basic/positive and binds to basophilic substances (DNA/RNA which are acidic and negatively charged) so they are therefore stained violet
- eosin is red or pink stain that is acidic/ negative so it binds to acidophilic substances such as positively charged amino acid side chains (proteins) so they are therefore stained pink
- so in microscopy we can observe nuclei blue/purple, cytoplasm red, muscles dark red, mitochondria and collagen in pale pink

Periodic acid- schiff stain (PAS)
- staining method to detect polysaccharides
- typically used to stain structures with high proportion of carbohydrate macromolecules typically found in connective tissues, mucus, basal laminae etc.
- aldehydes (carbohydrates) react with schiff reagent to give a purple/magenta colour
- used by treating sections with periodic acid for 5 minutes, rinsing and then covering with shiff's reagent for 5-10 min and rinsing again in a running tap. then counter stain with H&E for 15 seconds and differentiate with acid alcohol and bluing as usual

Toluidine blue stain
- basic dye with high affinity for acidic components
- stains nucleic acids blue and polysaccharides purple
- increases the sharpness of histology images
- especially useful today for staining chromosomes

Immunohistochemical techniques (IHC)
- use of antibodies for staining by using the principle of antibodies binding specifically to antigens in biological tissue

- use of enzyme to convert a colourless substrate to a coloured product
- antibody combined with enzyme reacts with protein (antigen)
- antibody reacts with antigen to stain them- cells that the antibody hasn't bound to will not be stained
- horse radish peroxidase is commonly used
- widely used in the diagnosis of abnormal cells such as those found in cancerous tumours

Cell-Cell Interations of epithelial sheets and cell junctions- 25/09/17

Epithelial tissue
- cells joined together side by side forming multicellular sheets
- cells join together and form a barrier
  -prevents fluid loss
  -prevents pathogens
  -allows molecules in and out
- tissue= group of cells working together to perform one or more specific functions
- epithelial tissue= found as the lining and covering of organs and body cavities, the secretory parts of organs and glands, the transport membranes of capillaries and alveolar sacs, and membranes which lubricate organs
- interface between biological compartments
- sheets closely bound by cell junctions
- lungs have squamous epithelial cells
  -thin flat cells
- kidney has cuboidal shapes cells
- simple= one cell layer thick , stratified= multi- layered , pseudostratified= appears to be stratified but isn't , transitional= stetchable (found in organs that can change in volume and size)

Lumen
- the inside space or lining of tubular structure
  -ie central space in artery
- cells on the luminal surface can often contain specialised projections
  -ie cilia or villi

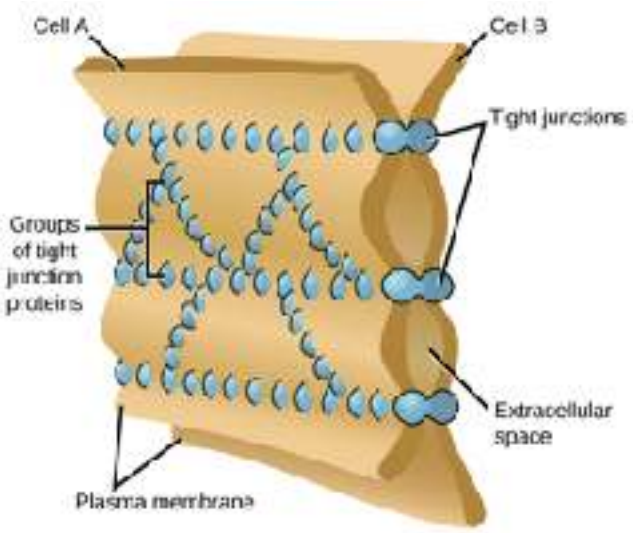
Basal surface and apical surface polarity
- epithelia are polarised with apical and basal surfaces
  -polarity of cell is critical for function
- both surfaces are functionally and biochemically different
- apical is the top of the cells which faces the external environment or limen of a tube
  -often involved in absorption or secretion
- basal surface is at the bottom
  -mediates attachment to underlying tissues/surface using integrens
- because membranes at each surface have different functions, they have different membrane proteins
  -certain proteins need to be delivered to one side or the other
  -proteins destined for apical or basal surface have signal sequences, proteins are sorted in the golgi network and then delivered in vesicles to the specific membrane
- Basal lamina supports the basal surface
  -composed of collagen and macromolecules
  -lamina provides adhesive site for integrin molecules in the plasma of membrane of epithelial cells linking the cells with extracellular membrane

Basement membrane
- basal side of the epithelial cell attaches to basement membrane
- provides some mechanical support- it holds together a sheet of epithelial cells

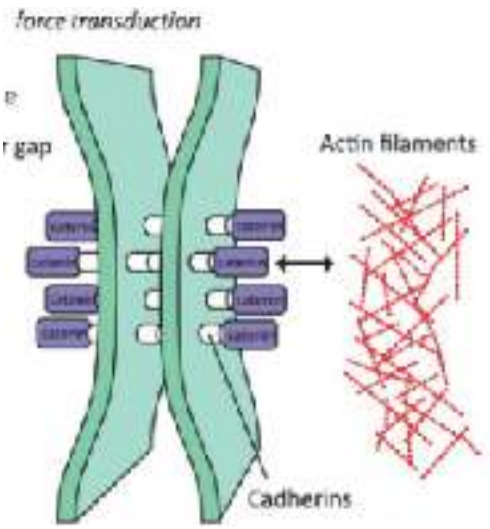
- supports the growth and survival of epithelia as it controls the access of epithelia to nutrients and oxygen from capillaries in underlying tissues
- restricts migrations of metastic cells
- Components of basement membrane
  -glycosaminoglycan
  -fibrous protein: collagen type 4
  -structural glycoproteins: fibronectin, laminin, entactin

Intercellular surfaces
- cells junctions permit cells to produce a continuous cohesive layer
- allows that to communicate and interact

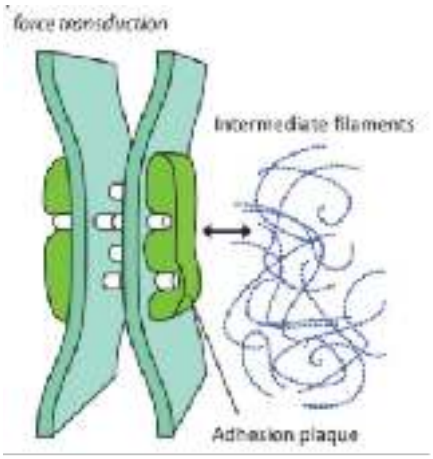
Types of cell-cell junctions
- tight junctions (can also be referred to as occluding junctions)
  -seals neighbouring cells together in epithelial sheet, prevents leakage of molecules
  -sealing strands made of occludin and claudin hold cell membranes together like a stitch



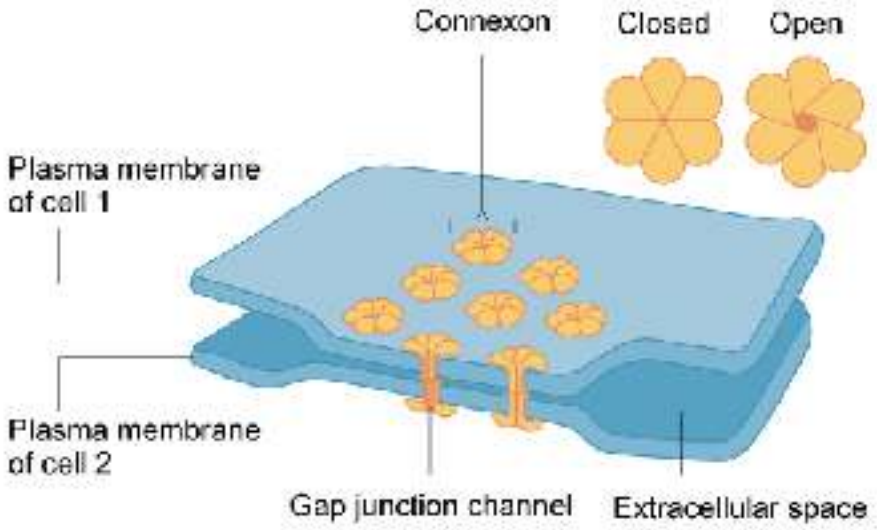
- adherens junction
  -joins an actin bundle in one cell to a similar bundle in a neighbouring cell (linking cytoskeletons)
  -proteins present in these junctions are adhering molecules: mainly cadherins which act like pieces of velcro, they stick to one another
  -linker molecules link the actin to the adhering molecules in the plasma membrane which is attached to the cytoskeleton in the other cell also by linking molecules
  -mobility above strength



- desmosome
  -joins the intermediate filaments
  -particularly abundant in tissues that undergo mechanical stress (ie cardiac muscle)
  -cadherin proteins stick them together in a different way from adherens junctions: dont act like velcro, the ones that you'd find are desmoglein and desmocollin
  -intracellular linkers are plakoglobin and desmoplakin



- gap junction
  -forms channels that allow small water-soluble molecules to pass from cell to cell
  -cleft spanned by very fine strands that act as molecular pipelines
  -communicating or nexus junctions
  -provide means of electrical coupling of visceral and cardiac muscle cells permitting synchronous contractions
  -protein connexin comes together to create pores: 6 come together to form the subunit connexon which is a very minute tubular structure
  -circular pores permit passage of molecules directly connecting cells



- hemidesmosome
- all junctions together= junctional complex

cell junctions: Permit epithelia to form a continuous cohesive layer in which all of the cells communicate and co-operate to achieve the particular functional requirements of the epithelium

Extracellular Matrix and Connective Tissue- 29/09/17

Glycocalyx
- glycoprotein and glycolipid covering of the cell
- extracellular surface of the cellular membrane
- gives fuzzy appearance when looked at under an electron microscope
- composed of carbohydrates linked to plasma membrane lipids and proteins
- they extend from cell surface into extracellular fluid
- functions: mechanical protection, barrier and important in enabling cells to identify and interact with each other

Connective tissue
- cells are more sparse but in the extracellular matrix cells are plentiful and carry mechanical load
- tough and flexible tissue in tendons
- hard and dense tissue in bone
- resiliant and shock absorbing in cartilage
- soft and transparent in the eye
- forms the extracellular matrix

Extracellular matrix
- organised network of extracellular materials
- often has role in determining shape and activity of cell
- mixture of protein and molecules
  -sometimes minerals

Proteins in the ECM
- often extended fibrous proteins
- collagen
  -most abundant protein in human body
  -main component of connective tissue
  -only found in extracellular matrix
  -fibrous glycoprotein
  -provides insoluble framework
- elastin
  -main component of elastic fibres
  -prevents tissues from tearing

Function of extracellular matrix
- provides a scaffold for cellular attachment
- transmits information in the form of chemical messengers to the cells (regulating intracellular communication)
- segregates tissues from one another
- regulates cells dynamic behaviour

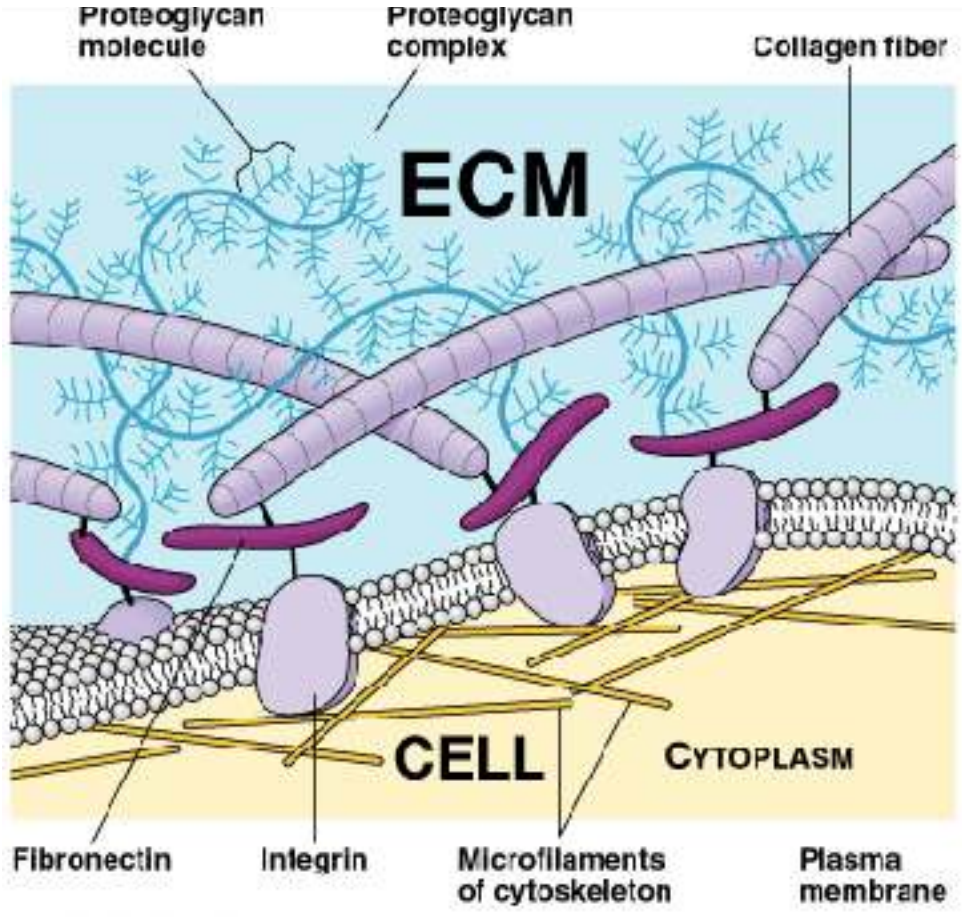
Fibroblasts
- cells which produce extracellular matrix of connective tissue
- ECM plentiful in connective tissue (ie bone)
- synthesises collagen
- most common cell of connective tissue

Collagen
- found in all animals
- many varieties
- main protein found in bone, tendon and skin
- quite a large molecule
- collagen is made in the fibroblast cell, actual fibres come together in the extracellular matrix

- 3 single collagen peptide tails come together to form triple stranded collagen molecules
  -these strands come together to form collagen fibrils which then make up the collagen fibres
- incorrect collagen assembly can cause hyper extensible skin
- cell secretion of collagen is organised: dont just secrete any type of collagen
  -creates particular arrangements
- cells move by pulling on collagen and crawling over it when bound to fibronectin

Matrix proteins
- cells need to be able to make them and break them down
- proteinases are enzymes that break down proteins
- matrix proteinases play a role in many disease processes ranging from arthritis to cancer
  -needs to be regulated

Fibronectin
- cells are attached to ECM by fibronetcin
- glycoprotein that contains binding sites for collagen and proteoglycans as well as for receptors on the cell surface
- fibronectin can link collagen to plasma membrane of the cell which will influence a cells potential for growth, differentiation and migration
- collagen does not bind directly to the cell
- cells move by pulling on collagen and crawling over it when bound to fibronectin



Wound healing - clinical application of fibronectin
- fibronectin deposited at wound interface after injury
- this promotes cell migration for cells to move over and close a gap

Integrin
- composed of alpha and beta subunits which can be different

- an inactive integrin is an integrin that is not bound to anything
- inside-out activation : signal when the integrin binds to cytoskeleton is sent from the inside- out
- outside-in activation : signal from the external environment to the cell interior
- main receptor proteins that cells use to bind to and respond to ECM
  -facilitate cell- ECM adhesion
- matrix receptors : tie ECM to cell's cytoskeleton
  -bind with collagens, fibronectin and laminas

Glycosaminoglycans (GAGS)
- help to fill space in ECM
- composed of repeating disaccharide units
  -one is very often an amino sugar
- very often negatively charged which means they can absorb a lot of water and expand
  -for example jelly in our eye is composed of a lot of GAGS so they can fill up the space
- provide the ECM with additional physical properties not provided by structural proteins alone

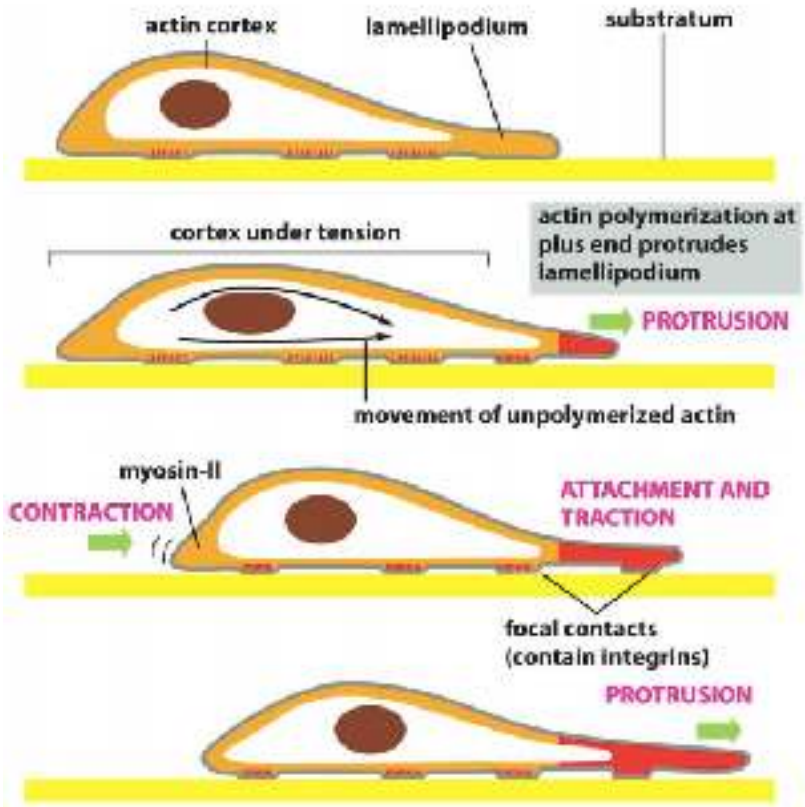
Proteoglycans
- combination proteins which GAGS are usually attached to
  -molecule of repeating disaccharide unit usually covalently linked to serine residues (precursor to amino acids) on core proteins
- normally found in the ECM but you can get some of the protein parts traversing the plasma membrane of the cell
- GAG chain composed of 2 different sugars
  -uronic acid and amino sugar
- hyaluronic acid is present in loose supporting tissue
- form porous hydrated gels which fills extracellular space
- proteoglycans and GAGs can form large aggregates (clusters)
- functions:
  1. form gels of varying pore size that act as filters to regulate the passage of molecules through ECM: if something wants to pass through ECM it must be able to fit through the pores
  2. bind secreted growth factors and other proteins that serve as signals to the cells
  3. can block, encourage or guide cell migration through ECM

2 main classes of ECM macromolecules
- GAGs
  -polysaccharide chains which are usually found covalently linked to protein in the form of proteoglycans
- Fibrous proteins
  -includes collagen, elastin, fibronectin and laminin
- members of both classes have a lot of variety

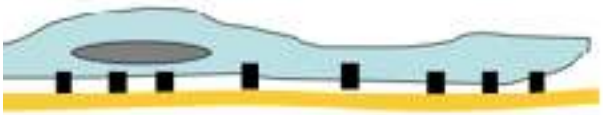
Anchoring Junctions
- from cell to the matrix
- focal adhesions and hemi-desmosomes
  -focal adhesion= type of adhesive contact between the cell and ECM through the interaction of transmembrane protein integrins with their extracellular ligands and intracellular multiprotein assemblies to the actin cytoskeleton
- hemi desomsomes connect the basal surface of an epithelial cell to the underlying basal lamina
  -stronger than focal adhesion
  -connects intracellular intermediate filament network to ECM
  -crucial for epithelial structures: connect the basal surface of an epithelial cell to the underlying basal lamina, integrin that mediates adhesion binds to laminin protein in basal lamina
  -transmembrane adhesion protein is an integrin rather than cadherin: cadherin= cell - cell , integrin= cell - ECM
- essential in providing tissue structure
  -attachment to basement membrane

Laminin
- extracellular glycoprotein
- major component of the basal lamina
- they are an important and active part of basal lamina
  -influence cell differentiation, migration and adhesion
- binds to cell surface receptors, other laminins, proteoglycans, components of basal lamina and integrin
- variability in integrin subunits dictates whether laminin can bind with it or not

Cell Crawling - movement
- crawling movement of cells across a surface represent basic form of locomotion
- three stages of movement
  1. Leading edge must extend by protrusions such as pseudopodia, lamellipodium, microspikes etc.
  2. Extension attaches to the surface across which the cell is migrating
  3. The trailing edge of the cell must dissociate from the surface from which is is attached and retract back into the cell body
- extension of leading edge involves polymerisation and cross linking of actin filaments
- the force generated at actin rich cortex of cell moves the cell forward



Focal adhesions (focal contacts)
- when cell attaches to base of a culture dish, it is not uniformly spread
  -not entire surface of cell is attached to the dish
- it is attached to dish only at scattered sites - at focal adhesions
- this allows them to be dynamic structures
  -can be rapidly disassembled
- membrane in region of focal adhesions contains large clusters of integrins

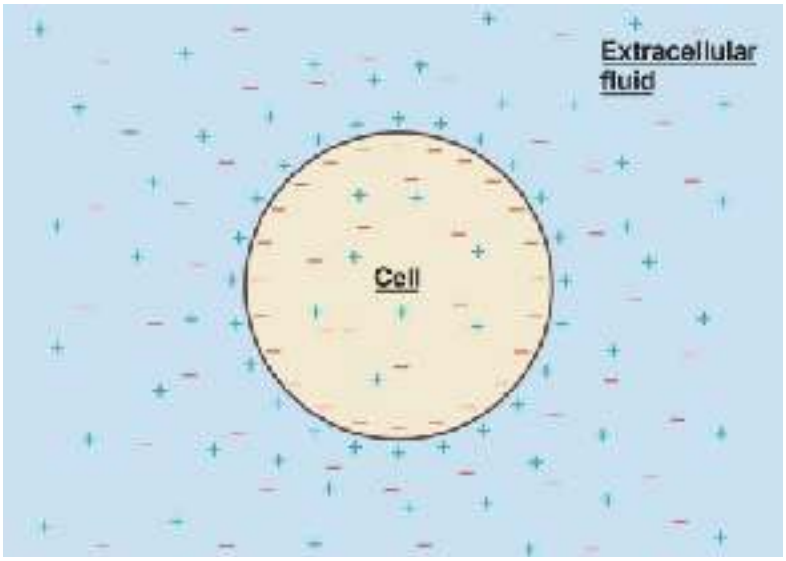


Integrins and disease
- 24 different types
- integrins on white blood cells help cells to crawl out of flood vessels at sites of infection
  -lack of this type leads to leukocyte adhesion deficiency
  -susceptibility to infection
- type of integrin found in blood platelets
  -those who lack this integrin bleed excessively as platelets cannot bind to necessary clotting factor in ECM

Membrane Potential and Cell Function- 2/10/17

Dont need to learn off nernst equation, need to understand it though and what it can tell us

Resting Membrane Potential
- cells under resting conditions have an electrical potential difference across plasma membranes
- inside of cell is negatively charged, outside is positively charged, extracellular fluid is neutral by convention
- if we allow charge to move out from the cell we can have an effect on the membrane potential
- talking about this potential it is only just right at the membrane where there is a potential difference (ie not all of the inside of the cell is negatively charged)



Resting membrane potential and disease
- hypertension: losing control of membrane potential means muscles contract
- angina: pain felt in the heart due to losing control of potential and contractibility of heart
- arrhythmia: electrical signals through the heart can cause it to spasm
- diabetes mellitus: insulin secretion is regulated by membrane potential
- pain relief and anaesthesia: if you can stop the membrane potential allowing signalling between cells you can stop pain

Membrane potential and therapeutics
- control of nerves by targeting proteins specific to maintaining membrane potential
- many drugs target ion channels: can excite or inhibit cell function
- advances in genetic manipulation allows us to take advantage of ion channels to control physiological function

How does potential difference arise

- essential to understand membrane structure/ chemistry
  -phospholipid bilayer, polar head groups, lipid tails
  -phospholipid bilayer acts as a very good insulator of charge
- need to understand location of charge in molecules
  -negative charge just inside the membrane
  -positive charge just outside the membrane
- essential to understand transport across cell membrane
- Resting membrane potentials develop due to the fact that proteins are large molecules that carry charge and cannot cross plasma membrane
  -charged proteins contribute approx. 10 mV to membrane potential

Ohms law

- effect of voltage and resistance on current
- resistance is high then current is low
- cell membrane lipid acts as an insulator (high resistance)
- cytoplasm and extracellular fluid water and ions act as conductor (low resistance)

Gibbs-Donnan Effect

- accounts for the effect of charged particles near semi-permeable membranes
  -sometimes fail to distribute evenly across the two sides
- proteins carry a lot of negative charge attracting small mobile positively charged ions
- if suddenly there are lots of small mobile ions inside the cell that are free in solution it is now a concentrated solution
- if concentration of salts inside the cell is higher than those outside then an osmotic gradient forms and water will be attracted into the cell to dilute the salt conc.
- this increases the volume of the cell and the cell may explode
- the solution to this problem is to pump out the sodium (Na+)
  -all our cells have the sodium/potassium- ATPase pump

Sodium pump (Primary Active Transport)

- allows mammalian cells to maintain osmotic equilibrium
- allows a cell to make more proteins without pulling too much water into a cell causing it to swell and explode: maintains volume of the cell
- establishes concentration gradients and membrane potentials
  -small negative potential
- uses a large amount of energy: 40% of ATP produced by the cell
  -active transport mechanism
- expels sodium, brings in potassium
  -in both cases ions are moving against their respective conc. gradients
  -for each ATP , 3 sodium out and 2 potassium in

1. The transporter with an associated ATP molecule binds three sodium ions at sites on the intracellular surface of the protein
2. Binding of Na+ results in activation of ATPase activity causing phosphorylation of the transporter and releasing ADP
3. Phosphorylation results in a conformational change in the channel exposing the Na+ ions to the extracellular fluid and the ions are released
4. The new conformation allows 2 molecules of K+ to bind to the extracellular surface of the transporter
5. Binding of K+ results in dephosphorylation returning the transporter to its original conformation and releasing K+ into the cell

- pump maintains the characteristic distribution of high intracellular K+ and low intracellular Na+
- helps establish and maintain the membrane potential of a cell

Secondary active transport

- different from primary as it uses an electrochemical gradient across the plasma membrane as its energy source rather than phosphorylation of a transport molecule by ATP
- transporters that mediate secondary active transport have 2 binding sites
  -one for an ion: typically Na+
  -one for a cotrasnported molecule: glucose, amino acid etc. (polar molecules)

Nernst equation
- allows us to calculate the voltage (membrane potent ion) at which an ion will stop moving
  -ion will always seek its equilibrium potential
- when force of electrical attraction equals that of conc gradient we have equilibrium potential for that ion
- membrane potential at which a given ion is in electrochemical equilibrium is predicted by nernst equation
- electrochemical gradient = net chemical gradient + net electrical gradient
- chemical gradient is a function of the ratio of the concentrations on either side of the membrane
- chemical gradient= RT ln([Ion A] / [Ion B])
  - R= ideal gas constant
  - T= absolute temperature
  - [Ion A / B] = ion concentrations on either side of the membrane
- electrical gradient is proportional to the potential difference across the membrane
- electrical gradient= zF(Ea- Eb)
  - z= charge number of ion
  - F= Faradays number
  - Ea- Eb = potential difference across membrane in volts
- Nernst Equation

$$RT \ln \frac{[Ion]A}{[Ion]B} + zF(Ea - Eb) = 0$$

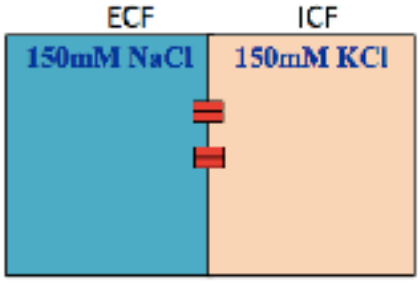
- can use it in the form

$$Vion = -\frac{0.0615 Volts}{z} \log \frac{[Ion]in}{[Ion]out}$$

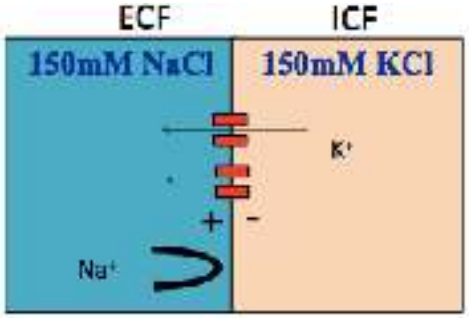
Function of Nernst Equation
- understand which way ions will flow when gates opened
- if you know intra and extracellular ion conc you can work out effect of gate opening
  -useful in pharmacology

Resting potential
- all cells under resting conditions have a potential difference across plasma membranes
  -inside of cell negatively charged with respect to the outside
- this potential is the resting membrane potential
- by convention, extracellular fluid assigned a voltage of 0 and the polarity (positive or negative) of the membrane potential is stated in terms of the sign of the excess charge on the inside of the cell
- if a cell has the potential difference has a magnitude of 70mV and the intracellular fluid has an excess negative charge so the membrane potential is -70mV
  -resting membrane potential varies from -5 to -100 mV but generally lies between -40 to -90 mV
- exists due to tiny excess of negative ions just outside the cell and execs of positive ions just outside the cell
  -bulk of intracellular and extracellular fluids remain neutral

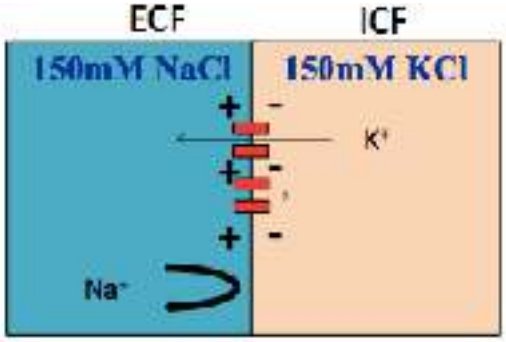
- magnitude of resting potential depends on 1) differences in specific ion concentrations and differences in membrane permeabilities



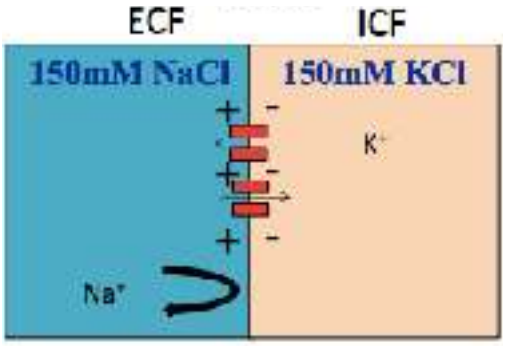
- membrane of this cell contains K+ channels and initially no ion movement occurs because the channels are closed
- there is no potential difference across the membrane because the two compartments contain equal amounts of positive and negative ions



- however, if K+ channels are opened K+ will diffuse across the membrane down its conc gradient
- sodium ions will not be able to move across the membrane so once some K+ ions have moved across into the ECF it will have an excess positive charge, leaving an excess negative charge in the ICF
- a potential difference has been created across the membrane
- potential difference= voltage difference between 2 points



- this introduces electrical potential; another factor that can cause movement of ions across a membrane
- as ECF becomes increasingly positive and ICF increasingly negative, the membrane potential difference begins to influence the movement of potassium ions
  -the negative charge of ICF tends to attract them back into their original compartment
  -as long as movement of ions due to concentration gradient is greater than movement sue to membrane potential the net movement of K+ will be from ICF to ECF

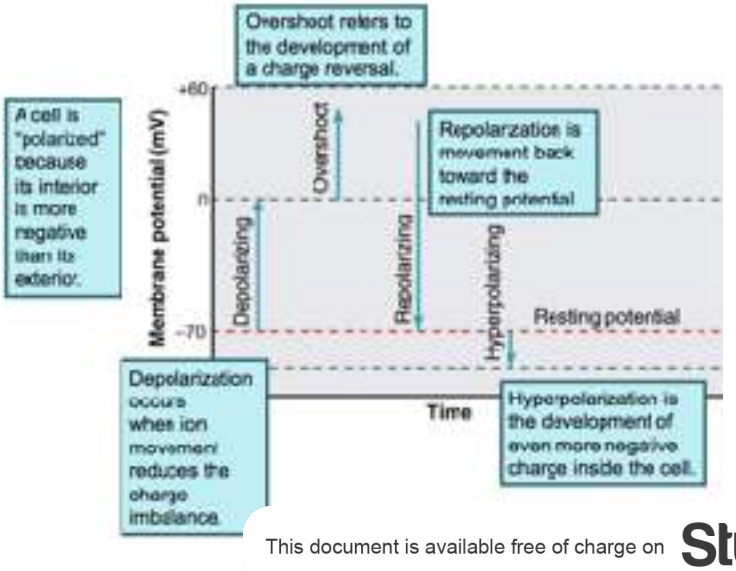


- the membrane potential at which the two influences are equal in magnitude but opposite in direction is called the equilibrium potential : there is no net movement
- summary: resting potential is generated across the plasma membrane largely because of the movement of K+ out of the cell down its concentration gradient through open K+ channels (leaky potassium channels) so that the inside of the cell becomes negative with respect to the outside
  -even though K+ flux has more impact than Na+ flux, the resting membrane potential is not equal to K+ equilibrium potential due small number of Na+ channels open in the resting state
  -some sodium ions continually move into the cell, cancelling effect of an equivalent number of potassium ions simultaneously moving out

Neuronal Signalling and Action Potentials - 6/10/17

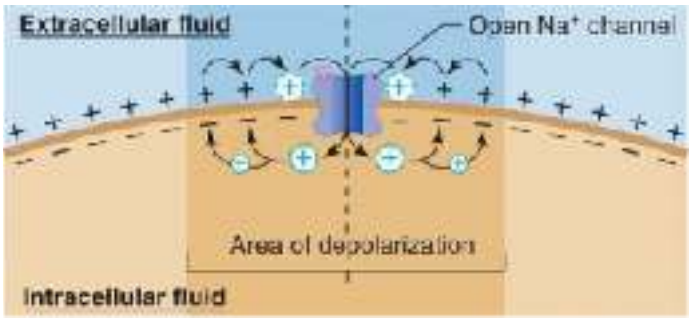
Nervous system
- trilions of neurons
- network involving CNS and PNS
  -CNS= brain and spinal cord
  -PNS= peripheral nervous system
- basic unit is a nerve cell (neuron)

Membrane potential changes
- transient changes in membrane potential from resting level produce electrical signals
- these signals occur in two forms:
  -graded potentials: important in signalling over short distances
  -action potentials: long distance signals of neuronal and muscle membranes
- terms depolarise, repolarise and hyperpolarise describe direction of changes

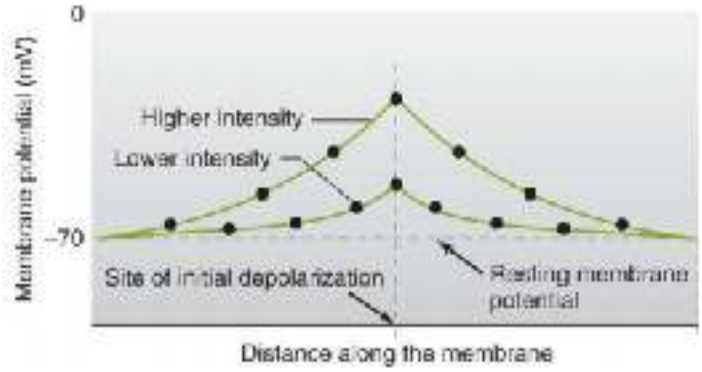


- resting potential is polarised: meaning the outside and inside of the cell have different net charge
  -depolarised when potential becomes less negative (closer to zero)
- in a neuron, the chances in membrane potential occur because of changes in the permeability of the cell membrane to ions
  -when a neuron receives a chemical signal from a neighbour some channels will open allowing greater ionic current across the membrane
  -the greater movement of ions alters the membrane potential so that it is either depolarised or hyperpolarised

Graded potentials
- changes in the membrane potential that are confined to a relatively small region of plasma membrane
- usually produced when some specific change in cell's environment acts on a specialised region of the membrane
- called graded potentials as the magnitude of the potential change can vary (is "graded")
- given various names related to the location of the potential or the function they perform
  -ie receptor potential, synaptic potential, pacemaker potential
- when graded potential occurs charge flows between the place of origin and adjacent regions of the plasma membrane which are still at resting potential
- when a region of a membrane has been depolarised and a cation (+ ion) channel is opened a potential is produced that is less negative than adjacent areas
  -positive charges in the cell will flow through the ICF away from depolarised region and toward the more negative resting regions of membrane
  -at the same time outside the cell positive charge will flow from the more positive region of the resting membrane toward the less positive regions the depolarisation just created
  -local current moves positive charges towards depolarisation site outside the cell and away from it inside the cell



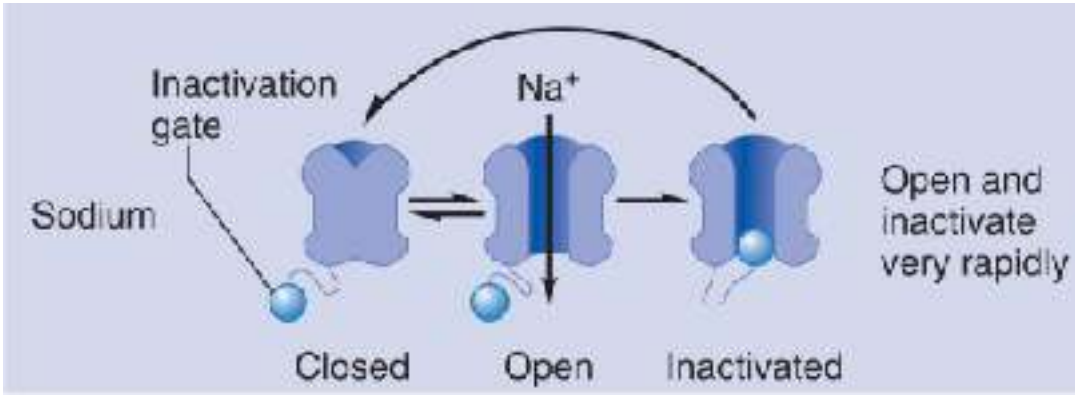
- channels close relatively quickly when the signal molecules dissociate and diffuse away
- no threshold
- different stimulus intensities result in different degrees of depolarisation



- the change in membrane potential decreases as the distance from the initial site of potential change increases
  -local current is decremental
- because of this electrical signal decreases with distance so graded potentials can only function as signals over very short distances
  -however graded potentials play a very important role in initiation of signalling over long distances

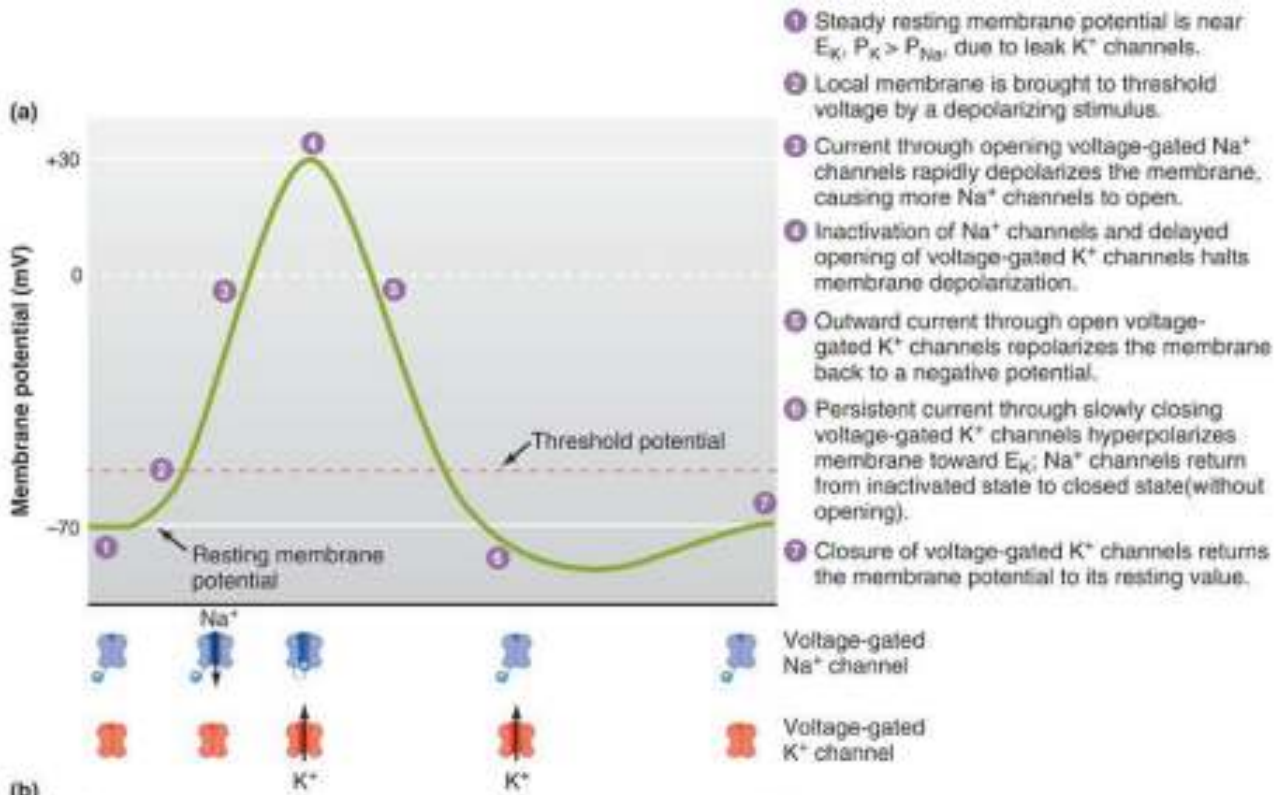
Action Potentials (APs)
- very different from graded potentials
- all cells are capable of conducting graded potentials but only excitable cells can conduct Action Potentials
  -neurons and muscle cells, endocrine, immune and reproductive cells have membranes capable of conducting graded potentials : they have excitable membranes
  -ability to generate action potentials is known as excitability
  -propagation of action potentials down the axon is the mechanism the nervous system uses to communicate over long distances (basis of neuronal signalling)
- they are large alterations in the membrane potential- may change by as much as 100 mV
- very rapid
- all or none: either reaches the threshold or does not
  -firing of a gun is used as a mechanical analogy: you either pull the trigger or not, once you do it does not matter how hard you pull it and you cannot pull it halfway
  -stimuli stronger than the threshold elicit the same response as those that just meet the threshold
- because the amplitude of a single action potential does not vary in proportion to the ampule of the stimulus an action potential cannot convey information about the magnitude of the stimulus that initiated it
  -this kind of information depends on the frequency of the membrane potential

Voltage- Gated ion channels
- types of channels that often serve as the initial stimulus for an AP
- ion channels vary by the kind of ion they conduct (Na+, K+, Cl-, etc) and in how they behave as the membrane voltage changes
- depolarisation of membrane causes Na+ channel to open and then inactivate (ball and chain mechanism) rapidly followed by opening of K+ channels
  -when membrane repolarises both channels return to closed state
- similarities between Na+ channels and K+ channels: depolarisation opens, depolarisation closes
  -sequences of charged amino acid residues in their structure make the channels reversibly change shape in response to changes in membrane potential
- 2 key differences
  1. Na+ channels are much faster to respond to changes in membrane voltage (faster to open and close)
  2. Na+ channels have an extra feature in cystolic region called an inactivation gate (ball and chain)
  -limits the flux of sodium ions by blocking the channel shortly after depolarisation opens it
  -when the membrane repolarises the channel closes, forcing the inactivation gate back out of the pore allowing the channel to return to the closed state with no Na+ influx



Action Potential Mechanism

- membrane potential depends upon the concentration gradients and membrane permeability of of different ions, particularly Na+ and K+
  -this is also true of the action potential



- STEP 1 : resting membrane potential is close to K+ equlib. potential because there are more open K+ channels than Na+ channels - these leak channels are distinct from voltage gated channels
- an action potential begins with a depolarising stimulus
  -ie when a neurotransmitter binds to a specific ion channel and allows Na+ to enter the cell
- this initial depolarisation stimulates the opening of some voltage-gated ion channels and further entry of Na+ through those channels adds to the membrane depolarisation
- STEP 2: membrane reaches a critical threshold potential and the depolarisation of the membrane becomes a positive feedback loop
  -Na+ entry causes depolarisation which opens more voltage-gated Na+ channels which causes more depolarisation and so on
- STEP 3: this process results in a large upstroke of membrane potential and it overshoots so that the membrane actually becomes positive on the inside and negative on the outside (opposite of resting state)

- STEP 4: membrane potential reaches peak value and the Na+ permeability abruptly declines as inactivation gates break the cycle of positive feedback
  -at the same time the depolarised state of membrane has begun to open the slower voltage-gated K+ channels
- STEP 5: resulting K+ flux out of the cell rapidly depolarises the membrane towards resting value
  -return of membrane to negative potential causes voltage- gated Na+ channels to go from inactivated to closed
- STEP 6: K+ channels take longer to close so there is a persistent current running through them causing hyperpolarisation of the membrane
- STEP 7: voltage K+ channels finally close and resting membrane potential is restored
- During the action potential, a second stimulus no matter how strong will not produce a second action potential as the region of the membrane will be in its absolute refractory period
  -absolute refractory period= when voltage gated Na+ channels are open
  -relative refractory period= period following the absolute refractory period where a second action potential can be produced but only if the stimulus strength is considerably greater than usual : some but not all of Na+ channels have returned to resting state and some of the K+ channels that repolarise the membrane are still open

Neurones
- the synapse is the point of communication between two neurones that operate sequentially
- the cell body integrates incoming information received at a synapse
  -signalling molecule= ligand
- if signal is sufficiently large the initial segment will fire an action potential

Action Potential Propagation and Neuro- Muscular- Junction - 9/10/17

Ion channels
- Ligand- gated and mechanically gated ion channels trigger graded potentials
  -ligand binding site not always on the outside of the cell
  -ligand sometimes closes the channel rather than opens it
  -mechanically gated ion channel: touch from finger activates neuron - when shape becomes distorted the channel open
- Voltage- gated ion channels allow propagation of action potential
  -shape of a protein is dependent on the voltage across the membrane
  -activated by depolarisation
  -fundamental important thing to remember is that every cell has a membrane potential, sodium pump etc but only excitable cells have voltage gated channels
  -channels have a threshold that needs to be exceeded for the channel to open: sufficiently large depolarisation/ graded potential

Action Potential propagation
- the propagation of the action potential from the dendritic to the axon terminal end is typically one way because the absolute refractory period follows along the “wake” of the moving action potential
- the action potential can only travel the length of a neuron if each point along the membrane is depolarised to its threshold as the action potential moves down the axon
- the membrane is depolarised at each point along the way while adjacent portions are still at resting membrane potential
  -the difference between the potentials along the axon causes the current to flow and the current depolarises adjacent section membrane causing Na+ channels there to open
  -the current entering during an AP is sufficient to easily depolarise the adjacent membrane to threshold potential
- the new AP produces local currents of its own that depolarise the region adjacent to it producing another AP at the next site and so on- action potential propagation
  -sequential opening and closing of Na+ and K+ channels along the membrane

- the AP arriving at the end of the axon is virtually the same as the initial one
  -not conducted decrementally as graded potentials are

Action Potential Velocity
- depends upon axon diameter
- large diameter= less resistance to current flow
- also depends on whether the finer is myelinated
  -myelin is an insulator that makes it more difficult for charge to flow between in intracellular and extracellular fluid compartments
  -because there is less “leakage” along a myelinated axon the local current can spread rather along an axon
- concentration of voltage-gated Na+ channels in myelinated regions is low so action potentials only occur at the nodes of ranvier where myelin coating is interrupted and conc of Na+ channels is high
  -action potentials seem to jump from one node to the next= saltatory conduction
  -propagation via saltatory conduction is faster that propagation in non myelinated fibres of same axon diameter
  -because the ions only cross the membrane at nodes of ran view the membrane pumps need to restore fewer ions ==> metabolically more efficient
- myelin adds speed, reduces metabolic cost and saves room in the nervous system because axons can be thinner

Graded Potentials	**Action Potentials**
amplitude varies with size of stimulus	all-or-none
can be summed	cannot be summed
has no threshold	has a threshold that is usually about 15 mV depolarised relative to resting potential
has no refractory period	has refractory period
conducted decrementally : amplitude decreases with distance	conducted without decrement : depolarisation is amplified at a constant volume
duration varies with initiating conditions	duration is constant for a given cell type under constant conditions
can be a depolarisation or hyperpolarisation	only a depolarisation
initiated by environmental stimulus at a receptor, by a neurotransmitter at a synapse or spontaneously	initiated by a graded potential
mechanism depends on ligand-gated channels or other chemical or physical changes	mechanism depends on voltage- gated channels

Innervation of skeletal muscle
- motor neurons also called somatic efferent neurones
- cel bodies are located in the brain or spinal cord (CNS)
- largest diameter neurones in the body and are also myelinated
  -high velocity signalling
- alpha motor neurons innervate most of the muscle
- gamma neurons innervate intrafusal muscle fibres
  -intrafusal muscle fibres= specialised sensory organs that detect the amount and rate of change in length of a muscle: proprioceptors
- upon reaching a muscle an alpha-motor neuron divides into many branches with each branch forming a single junction with a muscle fibre

-single neuron innervates many muscle fibres
-each muscle fibre innervated by one neuron

Motor Unit
- motor unit= a single motor neon and all of the muscle fibres it controls
- muscle fibres of a single motor unit are all within the same muscle but will be scattered throughout it
  -allows single motor unit to distribute signal
- when an AP occurs in motor neuron all muscle fibres in its motor unit are stimulated to contract
- delicate movement= few fibres innervated
  -eye muscle= 13 fibres
  -large skeletal muscles= 1000s

Acetylcholine - ACh
- major neurotransmitter in peripheral nervous system and the brain
- cholinergic neurons= nerve cell which mainly uses the neurotransmitter ACh to send its messages
  -Alzheimers disease: degeneration in cholinergic neurones with age causes decreased amounts of ACh in certain areas of the brain
  -these defects are related to declining language, confusion, memory loss
- ACh is synthesised from choline and acetyl coenzyme A in the cytoplasm of synaptic terminals
- when released at ACh- mediated synapses it binds to ACh receptors or is broken down by acetylcholinesterase

ACh receptors
- Nicotinic receptors: ligand gated ion channel receptor
  -permeable to both Na+ and K+
  -important in NMJs and certain regions of the brain for example the reward pathway
  -reward pathway= regulates desires and wants (addiction), motivation, reinforcement learning etc.
- Muscarinic receptors: G protein coupled receptor
  -involved in heart rate and force, contraction of smooth muscle
  -blocked by atropine which can be used as a drug to slow heart rate and decrease saliva during surgery (given IV or by injection into muscle)

NMJ
- neuromuscular junction is the point of synaptic contact between the axon terminal of a motor neurone and the muscle fibre it controls
- APs in the motor neuron cause ACh release into the NMJ
- the region of muscle fibre plasma membrane located directly under the axon terminal is the motor end plate
- muscle contraction follows the delivery of ACh to the muscle fibre
- unlike neuronal synapses, all NMJs are excitatory
  -some neuronal synapses receive inhibitory inputs

Axon Terminal of an NMJ
1. vesicles containing ACh are loosely bound to plasma membrane of the axon terminal
2. when the AP arrives it depolarises the plasma membrane opening Ca2+ channels allowing Ca2+ to flow into the axon terminal
3. Ca2+ binds to protein called synaptotagmin that allows ACh vesicles to fuse with the axon terminal membrane to release ACh into extracellular cleft towards the motor end place(exocytosis)
4. ACh diffuses across to bind to nicotinic receptors on the motor end plate opening an ion channel in each receptor
5. both Na+ and K+ ions can move across: more Na+ moves in that K+ out producing a local graded depolarisation called an end plate potential (EPP)

6. graded depolarisation typically exceed threshold for the nearby voltage-gated Na+ and K+ channels o an action potential occurs on the muscle fibre

Motor end place depolarisation
- analogous to EPSP (excitatory post-synaptic potential)
- magnitude of EPP far exceeds that of EPSP due to large surface area (200,000 channels activated)
- single EPP capable of triggering AP in muscle fibres
- AP then propagates throughout the muscle

Activation of skeletal muscle- 13/10 /17

Acetyl choline starts the process of signalling in a motor neuron
- something drives the release
  -loss of balance, pain, voluntary movement
- membrane of cell body and dendrites packed with receptors
- gradient potential dependent on the amount of acetyl choline

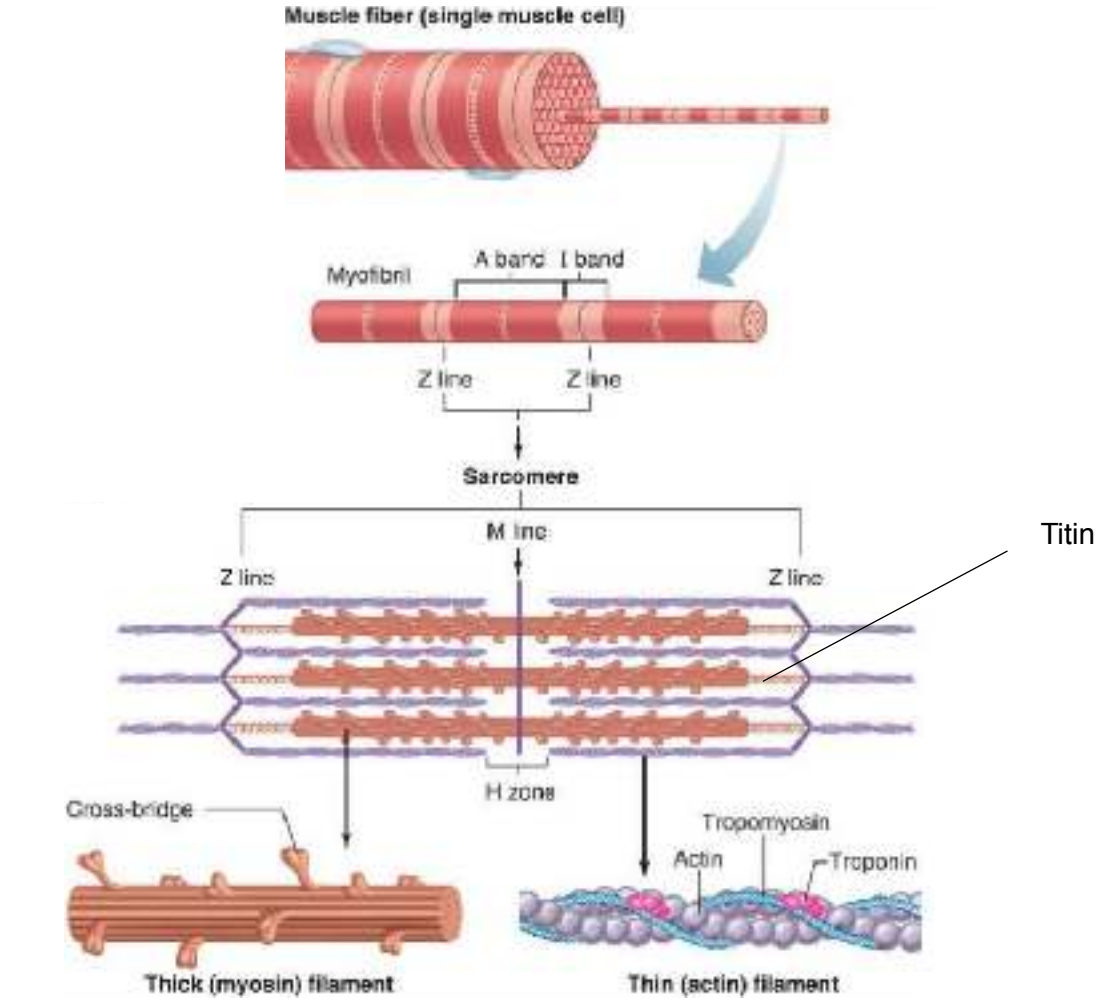
rather than this being a synapse which has fine control it is a neuro muscular junction
- means muscle will always contract when the signal reaches it
- specialised synapse
- no graded potential: except for in certain diseases
- end plate potential: excitatory graded potential

Skeletal muscle plasma membrane
- generates and propagates APs
- AP last 1-2 ms while mechanical activity it induces may last 100ms or more
  -Ca2+ drives contraction long after AP has ceased
- excitation contraction coupling is dependent upon an induction of cytosolic Ca2+
- the latent period between excitation and development of tension in a skeletal muscle includes the time needed to release Ca2+ from sarcoplasmic reticulum, move tropomyosin and cycle the cross-bridges

Skeletal Muscle
- muscle = multiple muscle fibres bound together by connective tissue
- muscle fibre= single muscle cell
  -cylindrical multi-nucleated cell formed by fusion of multiple myoblasts during development
- striated muscle: distinct series of light and dark bands when viewing through a microscope
- contains a population of undifferentiated stem cells called satellite cells
  -allows a limited capacity to regenerate as they cannot be replaced by division of existing muscle cells
  -in response to strain or injury satellite cells undergo mitotic proliferation
  -daughter cells differentiate into myoblasts which can fuse to form new fibres or fuse with damaged muscle to reinforce and repair it
- skeletal muscles attached to bone by tendons
- transmission of force from muscle to bone is like a number of people pulling on a rope, each person corresponding to a single muscle fibre and the rope corresponding to the connective tissue and tendons

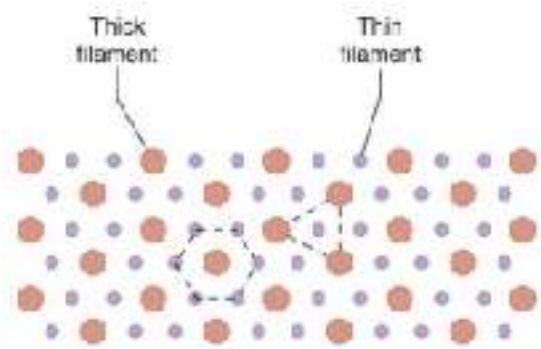
Striation
- results from arrangement of numerous thick and thin filaments in a regular pattern within the cytoplasm

- filaments = myofibrils
  -most of cytoplasm filled with myofibrils
  -myofibrils extend from one end of the fibre to the other and are linked to tendons
- thick and thin filaments are arranged in repeating pattern
  -one unit of this repeating pattern= sarcomere
- thick filaments= myosin
  -located in the middle of each sarcomere
  -orderly parallel arrangement produces a wide dark band : A band
  -H zone is a narrow lighter band in the middle of the A band that has a narrow dark M line through the centre of it
- thin filaments= actin
  -thin filaments also composed of two other proteins: troponin and tropomyosin
  -each sarcomere contains two sets of thin filaments, one at each end
  -anchored to network of interconnecting proteins : Z line
  -thin filaments from 2 adjacent sarcomeres are anchored to the two sides of each Z line
- I bands are the lighter region between A bands
  -the Z line bisects the I band
- the space between overlapping thick and thin filaments is bridged by projections called cross-bridges
- titin: one of the largest proteins, broadly described as a spring like protein that goes from the z line to the end of the myosin
  -ater a contraction it helps sarcomere go back to shape



Actin/ myosin filaments
- globular actin is linked into filaments and wrapped into a spiral

- on the actin filament there are binding sites for myosin which at resting state are blocked by tropomyosin
- along myosin filaments there are globular heads that stick out that can bind to acting sites
  -cross bridges
- to reveal binding sites tropomyosin on the actin filament needs to be pulled away which is done by calcium ions
- actin filament is teathered to the membrane by dystrophyn
  -mutations in the dystrophyn genes is muscular dystrophy (muscle weakness)
  -massive gene (0.1% of the entire genome)
- myosin does not just interact with 2 actin, each myosin is surrounded by 6 actin
  -it has many globular heads sticking out in 6 different directions
  -each actin interacts with 3 myosin

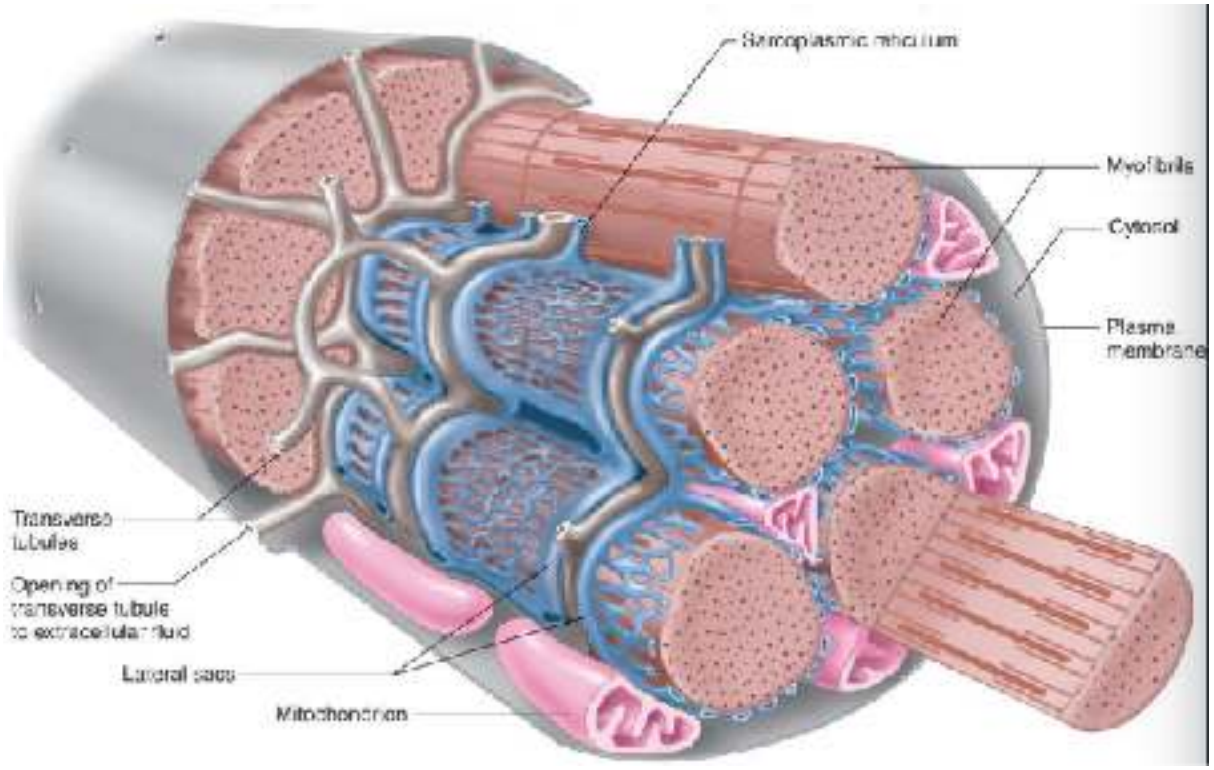


Excitation- contraction coupling
- sequence of events by which an action potential in the plasma membrane leads to the cross-bridge activity
- excite the muscle fibres all at the same time so the whole muscle contracts
- signal must rapidly move through the muscle once it reaches it
- muscle fibres have continuous membrane that are loaded with voltage gated channels
  -excitable membrane
- action potentials transmitted across NMJ via release of acetylcholine resulting in depolarisation of muscle fibre and release of calcium
  -critical link between the excitation of the muscle fibre and production of force through the cross-bridge cycle

Contraction
- globular heads of mysoin need to grab the actin filaments and bring them together to shorten the muscle
- dependent on free cystolic calcium ions (Ca^{2+})
- we need to drive the mobilisation of calcium which then will drive the contraction
  -AP does not cause the contraction, calcium does
  -calcium stored in the sarcoplasmic reticulum
- calcium is a second messenger
  -first message is the action potential arriving
  -second message is the calcium released causing something to happen
- free calcium levels in extracullular fluid are relatively high and inside the cell are low in the resting state
  -doesnt mean theres no calcium in the cell: it is just not free floating in the cytoplasm
- calcium inside the cell is stored in the sarcoplasmic reticulum in a muscle fibre
  -specialised endoplasmic reticulum
- calcium ions released into cytosol bind to troponin
  -the calcium- troponin complex “pulls” tropomyosin off the myosin- binding site of the actin
  -allows binding of the cross-bridge
  -followed by flexing of cross-bridge to slide the actin filament

Sarcoplasmic reticulum

- sleeve like segments around each myofibril
- at each end are lateral sacs which lie adjacent to T tubules
  -T tubules and lateral sacs surround the myofibrils at the region of the sarcomeres where the A bands and the I bands meet
  -the two membranes are joined by “junctional feet”
  -junctional feet are composed of DHP voltage receptors on T tubule and Ryanodine calcium channel receptor on SR
- Passage of an AP along the T tubules opens the voltage-gates Ryanodine calcium channels
- Ca2+ stored in lateral sacs and released following membrane excitation
- lumen of T tubules continuous with the extracellular fluids surrounding the muscle fibre
- depolarisation of the sarcolemma (membrane of muscle fibre) is rapidly communicated with the sarcoplasmic reticulum through the T-tubule system
- membrane of the T tubules, like the plasma membrane (all continuous), can generate APs
  -during T tubule AP Ca2+ is released from the lateral sacs of the sarcoplasmic reticulum into the cytosol activating cross bridge cycling
- t-tubule system permits synchronous contraction of all sarcomeres in the muscle fibre
  -system of tubular extensions of sarcolemma extends transversely into muscle cells to surround each myofibril at junction of A and I bands
  -calcium ion are concentrated within the sarcoplasmic reticulum so are released rapidly throughout the entire muscle allowing contraction of fibres all at the same time



End of contraction

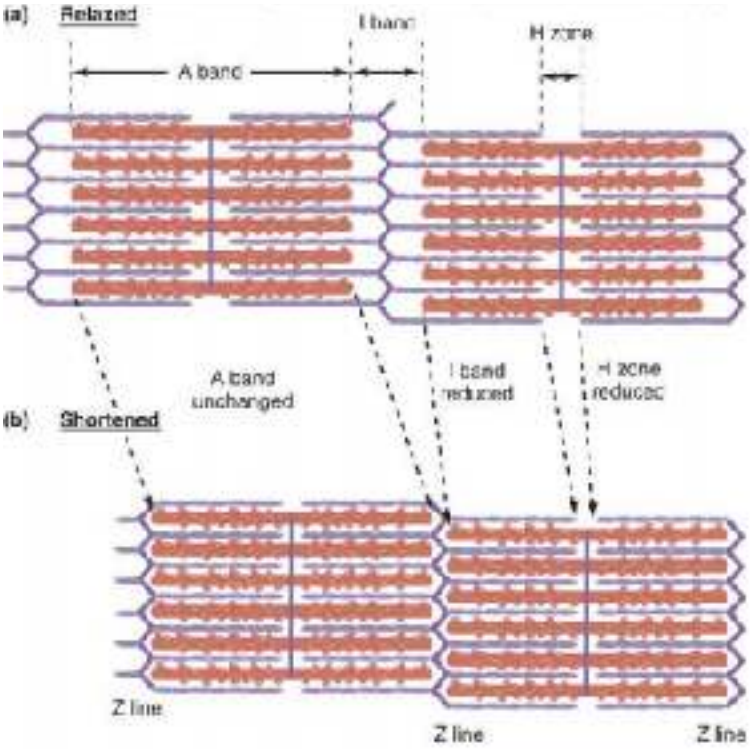
- calcium needs to be packaged away again
  -removal of Ca2+ from troponin
- uses a calcium pump to move it against its concentration gradient
  -conc of Ca in the SR is very very high
- membranes of the sarcoplasmic reticulum contain primary active-transport proteins
  - Ca2+ - ATPases
  -pump Ca2+ from the cytosol back into lumen of reticulum

- pumping of Ca2+ back into reticulum requires much longer time that it being released
  -cytosolic conc of Ca2+ remains elevated and contraction continues for some time after single AP

Mechanisms of Skeletal Muscle- 13/10/17

mechanics of skeletal muscle
- generate tone by myosin grabbing the actin and pulling it together and the muscle fibre shortens
- sliding filiament mechanism allows this to happen
  -happens in skeletal, smooth and cardiac muscle
  -smooth muscles dont need as much force to contract
  -cardiac is in the middle: needs more force than smooth muscle but less than skeletal
- basis of contraction requires filaments to slide over each other

Sliding filament mechanism
- contraction of muscle fibres is a shortening of the sarcomere
  -length of filaments does not change, they just are slide more tightly over each other propelled by movements of the cross-bridges
- myosin binds to actin and slides it over, moving Z lines closer together and reducing width of I bands



Relaxed sarcomere

Contracted sarcomere

- a common pattern of muscle shortening involves one end of the muscle remaining at a fixed position while the other end moves towards it

Action of filaments during Sliding filament mechanism
- actin molecules are single polypeptides that polymerise with other actin molecules to form a polymer made up of two intertwined, helical chais
  -each actin molecules contains binding site for myosin
- myosin molecule is composed of 2 large polypeptide heavy chains and 4 smaller light chains
  -these polypeptides combine to form a molecule that consists of 2 globular heads and a long tail

-head= heavy and light chains
-tail= 2 intertwining heavy chains

- each globular head contains two binding sites- one for actin and one for ATP
  -ATP binding site also serve as an enzyme: an ATPase that hydrolysed the bound ATP harnessing energy for contraction
- the myosin molecules in the two ends of the myosin filament are orientated in opposite directions so that their tail ends are directed towards the centre of the filament
  -this is why the actin filaments move towards the centre of the myosin filaments

Role of Troponin, tropomyosin and Calcium

- actin binding sites being blocked by tropomyosin
  -in absence of calcium, tropomyosin is in the way
- Tropomyosin= rod shaped molecule composed of two intertwined polypeptides
  -chains of tropomyosin are arranged along the actin thin filament covering the myosin binding site preventing cross-bridges from making contact with actin
- tropomyosin is held in place by troponin
  -one molecule of troponin binds to one molecule of tropomyosin regulating access to myosin-binding sites
- if there is a release of calcium it can bind to troponin Ca2+ binding site which pulls the tropomyosin out of the way
  -binding of Ca2+ produces conformational change to the troponin molecule relaxing its inhibitory grip
- when Ca2+ is bound to troponin the myosin-binding site is accessible and myosin globular heads can bind to actin

Cross Bridge Cycle

- sequence of events that occurs once cross-bridge binds to actin filament

1. attachment of cross bridge to an actin filament
   -in resting state myosin cross bridge has been spring loaded and cannot spring unless its been bound to the actin so the motor has not been fully switched on
   -uses ATP to change shape so it is not fully energies when it moves
2. movement of the cross-bridge producing tension in the actin filament
3. detachment of the cross-bridge from the actin filament
   -during movement myosin is bound very tightly to actin and the binding of a new molecule of ATP to myosin breaks the link
   -example of allosteric regulation
   - allosteric regulation is the regulation of an enzyme by binding an effector molecule at a site other than the enzyme's active site
4. energising the cross bridge by ATP hydrolysis so it can again attach to actin filament and repeat the cycle
   -hydrolysis of ATP and movement of cross bridge are not simultaneous events
   -ATP has two distinct functions:
     1. Energy from ATP hydrolysis provides energy for cross-bridge movement
     2. ATP binding to myosin breaks the link formed between actin and myosin during the cycle

Mechanics of single fibre contraction

- force exerted on object= muscle tension
- force exerted on muscle= load
  -muscle tension and load are opposite forces
- for muscle fibres to shorten and thereby move a load, muscle tension must be greater than the opposing load
  -fibres shortening depends upon relationship between load and tension

isometric= constant length

- muscle develops tension but does not shorten

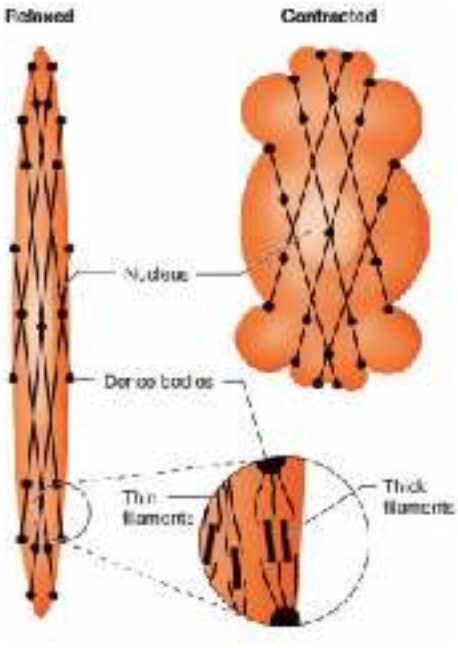
- these contractions occur when muscle supports a load in a constant position OR when a muscle tries to move a load already supported by a muscle with greater tension
- during cross bridge cycle the bound cross-bridges do exert a force but are unable to move the load

isotonic= constant tension
- contraction in which muscle changes length while the load on the muscle remains constant
- Isotonic contractions= concentric or eccentric
- concentric contraction = when tension exceeds the load
  -shortening of the muscle occurs
  -during cross bridge cycle normal shortening occurs
- eccentric contraction (lengthening contraction) = when an unsupported load is greater than the tension generated by the muscle
  -in this kind of situation the load pulls the muscle to a longer length even though the muscle is working against it
  -such contractions occur when an object being supported by muscle contraction is lowered (ie when knee extensor muscles in thigh are lengthened to lower you from standing to sitting)
  -during cross-bridge cycle the load pulls the cross bridges back toward the Z line while they are still bound to actin and exerting force
- lengthening of muscle fibres is not an active process produced by the muscle but produced by external forces
- in the absence of external lengthening forces a muscle will only ever shorten when stimulated

Activation and Mechanics of Smooth Muscle- 20/10/17

Functions of smooth muscle (squeezing of pipes)
- vascular tone
  -blood pressure
  -perfusion of organs
- airway diameter
  -regulation of ventilation
- Peristalsis of GI
- sphincters
- glandular secretions

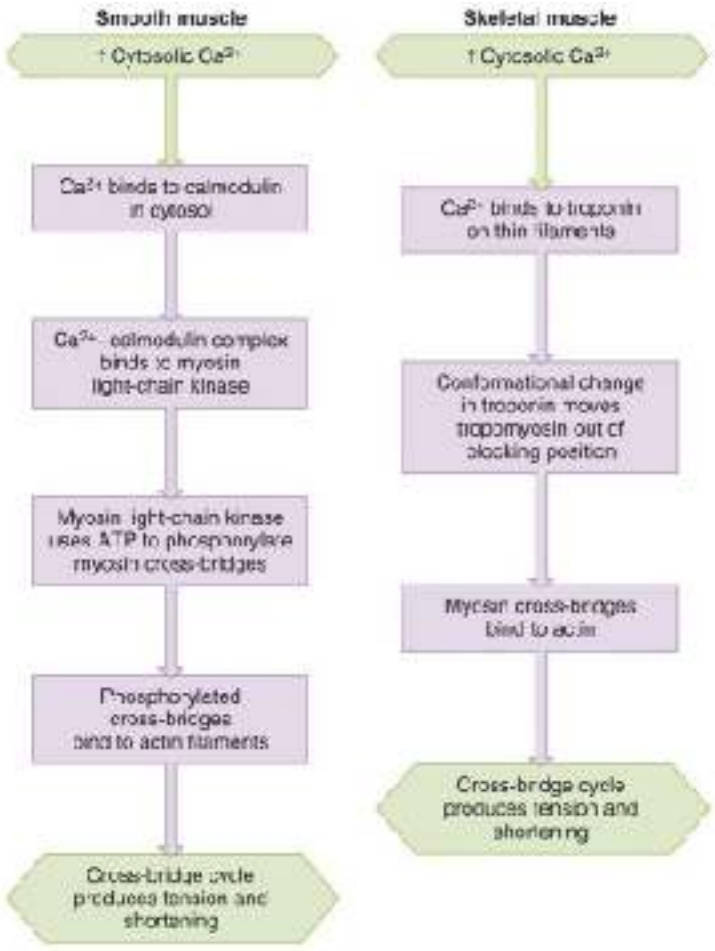
Structure of smooth muscle
- spindle shaped cells with single nucleus
- able to proliferate
- many individual smooth muscle cells interconnect to form sheetlike layers to form muscle
- not striated but still rely upon thick myosin and thin actin filaments
  -thick and thin filaments not arranged into myofibrils and no arrangement of filaments into sacromeres which accounts for absence of banding pattern
  -conc of myosin in smooth muscle is only about 1/3 of that in striated muscle whereas actin conc can be 2x as great
- no regulatory troponin even though tropomyosin is present in thin filaments
- thin filaments are anchored either to plasma membrane or to cytoplasmic structures known as dense bodies (functionally similar to Z-lines in skeletal muscle)
- filaments are oriented slightly diagonally to the long axis of cell so when the fibre shortens the regions of plasma membrane between points where actin is attached to membrane balloon out



Control of smooth muscle
- autonomic nervous system
  -sympathetic: fight or flight
  -parasympathetic: rest/digest
- hormonal secretions
- spontaneous electrical activity
  -peristalsis

Cross Bridge activation
- changes in cytosolic Ca2+ conc control the contractile activity in smooth muscle fibres as in striated muscle
- smooth muscle lacks Ca2+ binding protein, troponin
- tropomyosin is never help in a position that blocks cross-bridge access to actin
  -the thin filament cannot act as switch that regulates cross-bridge cycling
- Some smooth muscle cells respond to membrane depolarisation
  -voltage gated Ca2+ influx
  -propagates action potential and causes contraction
- some do not require depolarisation
  -hormone induced mobilisation of sarcoplasmic reticulum Ca2+
- cross bridge cycling is controlled by Ca2+ regulated enzyme that phosphorylates myosin
- sequence of events after a rise in Ca2+ in a smooth muscle fibre
  1. Ca2+ binds to calmodulin: a Ca2+ binding protein which a structure related to troponin
  2. the Ca2+ - calmodulin complex binds to myosin light-chain kinase to activate the enzyme
  3. Active enzyme uses ATP to phosphorylate myosin light chains in he globular head of myosin
  4. phosphorylation of myosin drives the cross- bridge away from the thick filament backbone allowing it to bind to actin
  5. cross- bridges go through repeated cycles of force generation as long as myosin light chains are phosphorylated
- key difference is that Ca2+ mediated changes in the thick filament turn on cross bridge activity in smooth muscle where as in striated muscle Ca2+ mediates changes in the thin filaments
- smooth muscle shortening is much slower that skeletal muscle
  -slow rate of energy usage means smooth muscle does not undergo fatigue during prolonged activity
- to relax a smooth muscle myosin must be dephosphorylated by myosin light- chain phosphatase which is continuously active during periods of rest and contraction in smooth muscle

-when Ca2+ rises in smooth muscle the rate of myosin phosphorylated by activated kinase exceeds rate of dephosphorylation by the phosphatase and amount of phosphorylated myosin increases producing a rise in tension



Sources of cytosolic Ca2+ : sarcoplasmic reticulum and extracellular

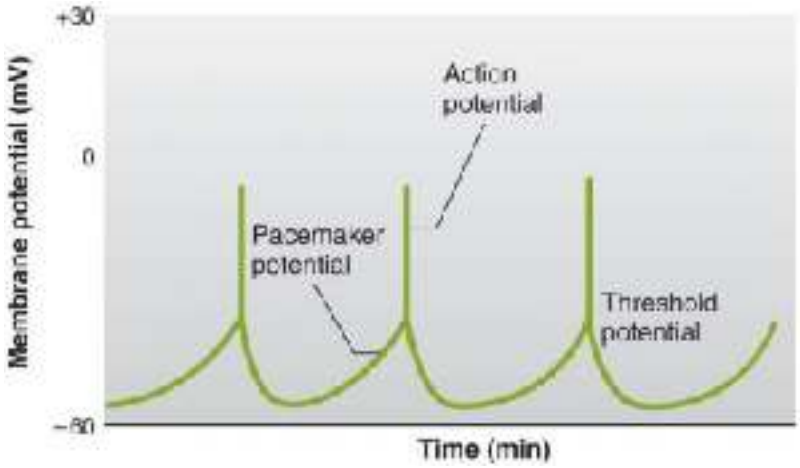
- less sarcoplasmic reticulum in smooth muscle than skeletal and it is not arranged in any specific pattern
  -no T tubules connected to the plasma membrane as the small cell diameter and slow rate of contraction do not require as rapid a mechanism for getting excitatory signal into cell
  -portions of sarcoplasmic membrane located near plasma membrane forming associations similar to the relationship with T tubules
  -action potentials in plasma membrane can be coupled to release of sarcoplasmic reticulum Ca2+ at these sites
  in some smooth muscle APs not necessary: second messengers from plasma membrane in response to binding of extracellular chemical messenger to plasma membrane receptors can trigger release of Ca2+ from more centrally located sarcoplasmic reticulum
- conc of Ca2+ in extracellular fluid is 10,000x greater than in the cytosol
  -opening of Ca2+ channels results in increased flow of it into the cell
  -due to small cell size the Ca2+ does not have far to diffuse within the cell to reach binding sites within the cell
  -removal of Ca2+ from cytosol brings about relaxation: achieved by active transport of Ca2+ back into sarcoplasmic reticulum as well as out of the cell across the cell membrane

Membrane Activation

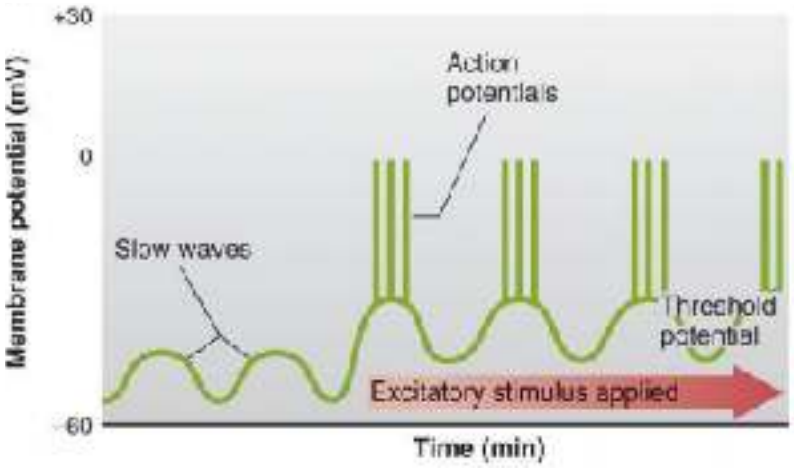
- in skeletal muscle membrane activation is dependent on single input: somatic neurons to the muscle

- in smooth muscle many inputs effect contractility (some increase contraction, some inhibit)
  -spontaneous electrical activity in plasma membrane of muscle cell
  -neurotransmitters released by autonomic neurons
  -hormones
  -locally induced changes in chemical composition of extracellular fluid surrounding cell (paracrine agents, acidity, oxygen, ion conc.)
  -stretch
- smooth muscle membrane can also receive multiple inputs at once
- some smooth muscles contract in response to membrane depolarisation and others contract in the absence of any membrane potential change
- unlike in skeletal muscle, cytosolic Ca2+ conc can be increased by graded polarisations in membrane potential which increase the number of Ca2+ channels
  -graded concentration; fine tuned response

Spontaneous Electrical Activity
- some types smooth muscle cells generate APs spontaneously in the absence of any neural or hormonal input
- plasma membranes of these cells do not maintain a constant resting potential
- they gradually depolarise until they reach the threshold potential and produce an AP
- following repolarisation the membrane again beings to depolarise causing a sequence of APs producing a rhythmic state of contractile activity
- the membrane potential change occurring is known as pacemaker potential



- other smooth pacemaker cells work differently: slow waves
  -membrane potential drifts up and own due to regular variation in ion flux across the membrane
- these fluctuations are called slow waves
- when an excitatory input is superimposed (ie food enters segment of GI) slow waves are depolarised above a threshold and APs produced lead to smooth muscle contraction



Contractile activity by nerves

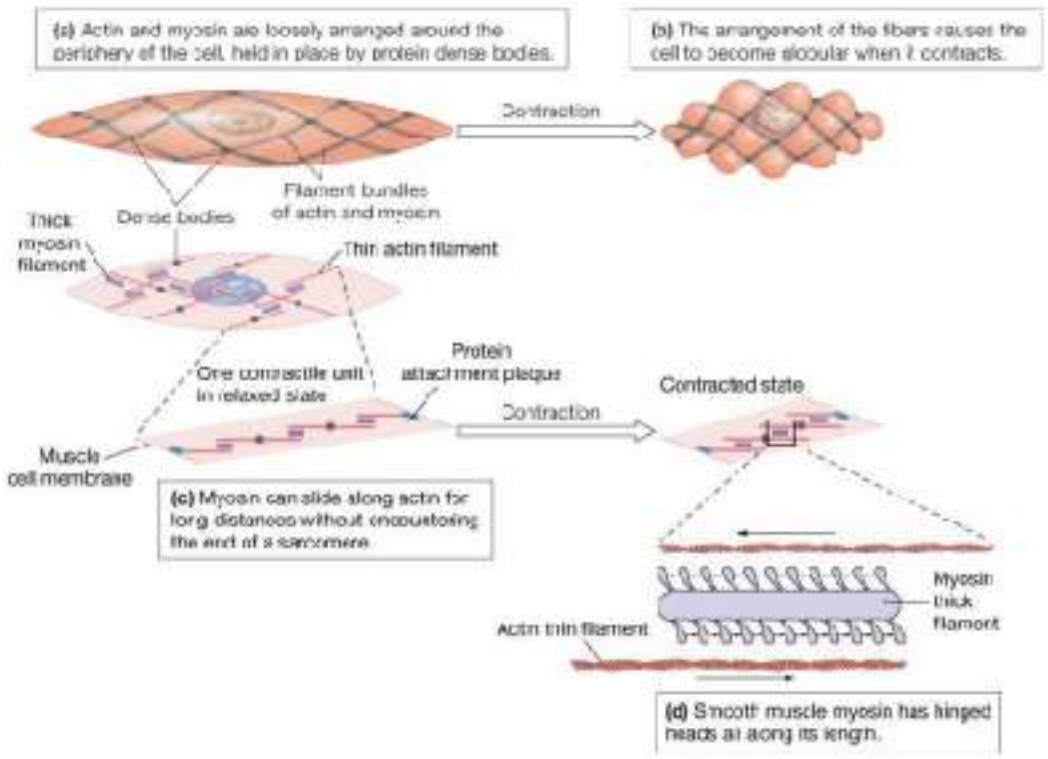
- influenced by neurotransmitters released by autonomic neurone endings
- as the axon of autonomic neuron enters region of smooth muscle cell it divides into branches with each branch containing a series of swollen regions known as varicosities
  -each varicosity contains vesicles filled with neurotransmitter some of which are released when an AP passes the varicosity
- a number of smooth muscle cells are influenced by neurotransmitters released by a single neuron and a single smooth muscle cell may be influenced by neurotransmitters from more than one neuron
- smooth muscle tension can be either increased or decreased by neural activity
  -a given neurotransmitter may produce opposite effects in different smooth muscle tissues
  -ie norepinephrine is a neurotransmitter released from most sympathetic neurons. it enhances contraction of most vascular smooth muscle and relaxes airway (bronchiolar) smooth muscle
- the type of response depends not on the chemical messenger but on the receptors the messenger binds to

Hormonal Contractile Activity

- in addition to receptors for neurotransmitters, smooth muscle membranes contain receptors for a variety of hormones
- binding of hormone may lead to either increased or decreased contractile activity

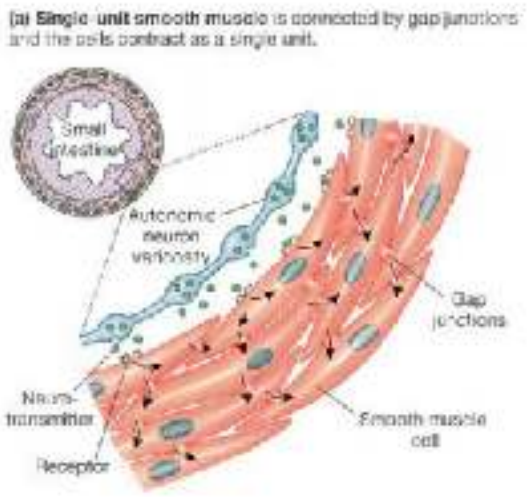
Local factors affecting contractile activity

- include paracrine signals (hormone only in direct vicinity of gland secreting it), acidity, oxygen and CO2 conc, osmolarity and ion composition of extracellular fluid
- regulation that is independent of long- distance signals from nerves and hormones
- many local factors induce smooth muscle relaxation
  -Nitric oxide (NO) is a common paracrine compound that produces muscle relaxation
  -NO released from some axon terminals and also epithelial and endothelial cells
  -reactive molecule has short lifespan causing it to act in a paracrine manner only influencing cells that are very near its release site
- some smooth muscles contract when they are stretched
  -stretching opens mechanically-gated ion channels leading to membrane depolarisation
  -the resulting contraction opposes forces acting to stretch the muscle



Types of smooth muscle

- single unit
  -intestinal tract, uterus, small blood vessels
  -synchronous activity: whole muscle responds to stimulation (both electrical and mechanical) as a single unit
  -this occurs due to gap junctions linking adjacent fibres allowing APs occurring in one cell to propagate to other cells by local currents —> electrical current occurring anywhere within a group of single-unit smooth muscle cells can be conducted to all other connected cells
  -may include pacemaker cells to initiate action potential which is passed on to other cells not capable of pacemaker activity
  -contractile response can often be induced by stretching (ie in the stomach when it is stretched due to increased volume it initiates a contractile response)



- muti unit
  -large airways/ arteries, hair attachments
  -no or few gap junctions
  -richly innervated by branches of autonomic nervous system
  -do not usually propagate action potentials
  -contractile response of whole muscle depends on the number of muscle cells that are activated and on the frequency of nerve stimulation
  -stretching does not induce contraction
- most smooth muscles do not show all the characteristics of either type: they are the 2 extremes so many smooth muscles have overlapping characteristics

Non Electrical Communication- 23/10/17

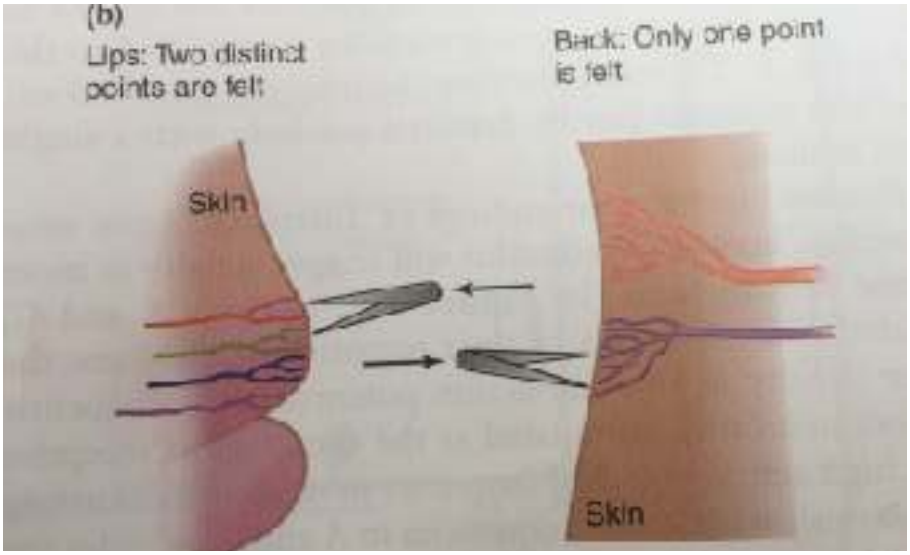
Graded potentials

- only function of graded potential is to trigger action potential
- can be exitatory or inhibitory
- neuronal signalling
  -sensory receptros
  -neuronal synapses/ NMJ
- graded potentials can activate local voltage gated ion channels
  -can either be excitatory driving a depolarisation (sodium channels)
  -inhibitory driving a hyperpolarisation (potassium channels)
- important in regulating voltage gates channels in other tissues

Excitable tissues
- CNS, interneurons, cell body for an efferant etc all things we've talked about before
- excitable cell
  -ability to generate and transmit and electrical signal
- Excitable tissues: sensory receptors, neuron cell bodies, axons, muscles

sensory receptos
- sensory receptors at peripheral ends of afferent neurons change information about external world (pressure, temperature, light, sound etc) into graded potentials that can initiate action potentials which travel into CNS
  -energy or chemical that activates sensory receptor is the stimulus
  -process in which stimulus is transformed into an electrical response is called sensory transduction
- types of receptors: mechanoreceptors (pressure/ stretch/ touch), thermoreceptors (heat/cold), photoreceptors (light at different wavelengths), chemoreceptors (smell and taste bt binding of chemicals to receptor membranes) and nociceptors (painful stimuli)
- for afferent neuron to work the graded potential needs to be large enough to activate the sensory receptor (rapid depolarisations and repolarisations)
- membranes are loaded with channels to allow for different activations
  -opening and closing of ion channels that recieve information about internal and external world either directly or through second- messenger system
  -gating of channels allows change in ion fluxes across membrane which produces a change in graded potential (receptor potential)
- as long as receptor potential keeps the afferent neuron depolarised to a level at or above threshold the action potentials continue to fire along the afferent neuron
  -increase in graded potential = increase in action potential frequency
  -frequency of graded potential does not increase mangnitude of action potential
  -stimulus strength, rate of change of stimulus strength, process of adaptation etc control magnitude of action potential
- recptive field on each neuron is very small so that we can distinguish between different senses
  -receptive field= area that when stimulated leads to activity in a particular neuron

Stimulus Location
- generally peripheral end of an afferent neuron divides into many fine branches each terminating with a receptor
- action potentials from each receptor travel along unique pathways to a specific region of the CNS associated only with that particular body location
- the accuracy with which we can locate and differentiate one stimulus from another depends on the convergence (number of smaller branches attached to a single neuron)

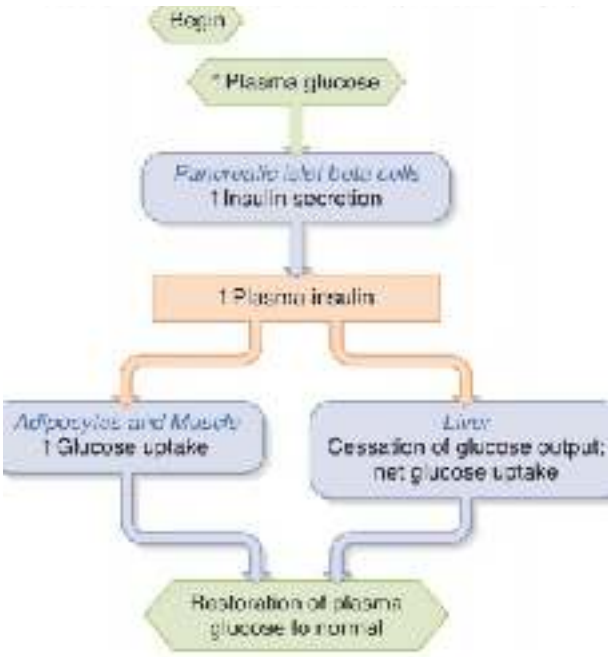


Channelrhodopsins
- subfamily of retinylidene proteins that function as light-gated ion channels
- enable light to control electrical excitability and calcium influx (muscle)
- non- specific cation channels
  -Na+, H+, K+, Ca2+
  -absorbs blue light and when all of the complex has absorbed photons it induces a conformational change opening a pore
- covalently linked light isomerisable chromophore
  -retinal
  -ability of us to see in colour is because different nerve endings respond to different wavelengths of light
- didnt finish: ask katie if she found any more info on this

Excitable Tissues
- graded potentials in sensory receptor of afferent neuron causes an electrical signal to arise
  -receptor potentials
- graded potentials in cell body of efferent neuron causes an electrical signal to arise

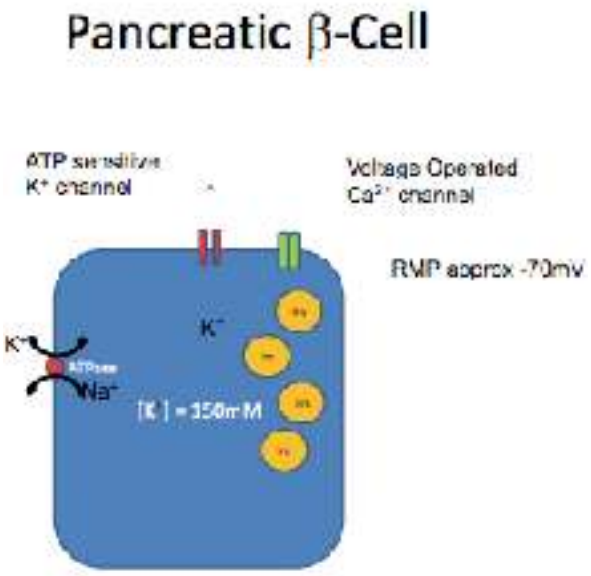
Non-excitable electrical communication
- local communication within the cell due to changes in membrane potential
  -secretion, cell poliferation, cellular differentiation
  -classic example is glucose iduced insulin secretion
- potential is always negative but not always as controlled as an excitable cell
- all cells have membrane potentials but depending on the cell there may be fluctuations in the potential so might not be as much control
- important in normal physiology
  -mitochondrial function
  -tissue responses to injury: vascular remodelling
- Mitochondrial membrane potential (MMP) essential for respiration
  -complex membranes in mitochondira (inner and outer) both with membrane potentials

Diabetes
- type I : insulin dependent
  -auto immune disease where antibodies are produced against the pancreas which influences production of insulin
  -no link with lifestyle
  -unable to make insulin
  -relatively easy to treat
- type II : adult onset
  -usually associated with life style
  -decrease in sensitivity for insulin and decreased insulin secretion
  -simple sugars cause blood sugar to rise rapidly constantly driving pancreas to produce insulin so either pancreas begins to give up in making insulin or the cells that respond to insulin give up and become insulin resistant so glucose is not stored as glycogen
  -harder to treat
- receptors for blood sugar levels make the proteins respond to produce insulin, insulin receptors in tissues allow glucose to be stored in tissues



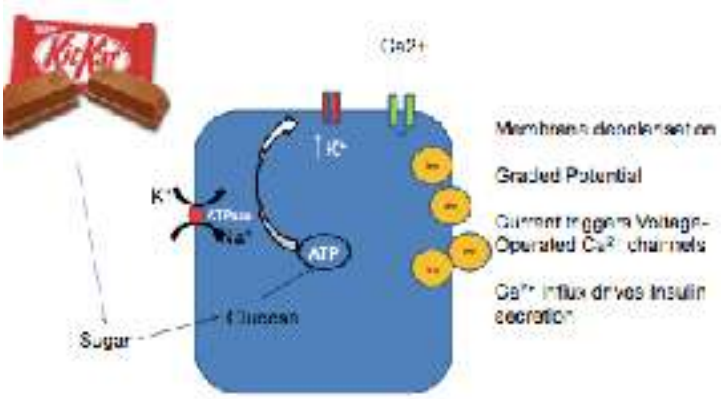
To regulate pancreatic islet beta cells use a potassium channel

- voltage regulated
- calcium regulated
- ATP regulated
- if channel is opened the potential is -90mV
- vessicles of insulin tethered to the membrane (not fused)
- sodium potassium pump in the membrane
- potassium channel
  -at rest it is allowing potassium out and keeping potential negative
  -controlling a voltage gated calcium channel that will respond to the potential



- when you eat a sugary meal and blood glucose goes up which is changed to ATP
  -ATP levels go up in the cell
  -ATP can bind to things in the cells by a whole family of ATP receptors
  -ATP binds to the ATP sensitive K+ channels and it closes to intracellular potassium builds and the membrane potential depolarises

-this causes a graded potential and the current triggers the voltage operated Ca2+ and there is a Ca2+ influx into the cell driving insulin secretion by membrane fusion using the vesicles liberating the insulin and the membrane is recycled to create new vesicles



Treatment of type II diabetes
- classes of drugs also bind to the ion channel: gilbenclamide and gliburide
  -bypasses ATP if person is not responding to glucose (no need for ATP to close potassium channel)
  -only works on patients who are not producing insulin, not patients who are insensitive to insulin
- insensitivity to insulin : PPARgamma agonist
  -increase tissue sensitivity

Cell - cell communication (autocrine, endocrine, paracrine)- 3/11/17

Types of signalling
- neuronal: long distance signalling
- autocrine: when cell is talking to itself, effect of itself
  -signalling molecule that is released by a cell and has a response back on that cell
  -immune system, inflammatory cells
  -short distance feedback
- Paracrine: releasing a signalling molecule that is effecting cells in local neighbourhood
  -can be same or different cell types
  -signalling molecule has to have a higher degree of stability to travel to other cells
- Paracrine: glad secreting signal and travelling through the blood stream to diffuse through the body
  -stable signalling molecule
  -cells have to express receptor for the signal

need for cell signalling
- control of muscle function
- control of organ function
- control of hormone secretion
- maintenance of organ structure

Evolution
- we've evolved to take advantage of physical and chemical characteristics of the building blocks from which we are composed
  -ie sodium pump
  -different receptors for same signalling molecules to produce different responses
- electrical communication
- neuronal signalling

- soluble signalling molecules
- ability to communicate is key to development of symbiotic relationships
  -communication central to multicellular organisms

Communication in a multi cellular organism
- analogy with signalling from a global perspective
  -7.5 billion people in the world with lots of different ways of communicating
  -synaptic signalling= communicating up close by yelling at someone (blasting the signal at them)
  -if you want to talk to someone far away you need a different method of communication, no matter how loud you shout they wont here you
  -neuronal signalling= phoning someone, signal gets there very quickly and get a blast from the phone
  -hormonal signalling= dropping pamphlets across Africa but only having them printed in english so that only people who read english receive the signal

The five senses
- sensory receptors to respond to a stimulus and drive a response

The human body
- cells differentiate into their functions in the tissues
- some cells can continuously differentiate
  -epithelial cells can differentiate and mature or they can transdifferentiate
  -blood cells, endothelial cells etc
- others are terminally differentiated
  -skeletal muscle, bone, nerves etc.
- controlled by environment they are in
- to maintain cell phenotypes we need to maintain gene extraction
  -when cell is terminally differentiated it has condensed all the chromatin for the genes it does not need (methylated DNA)
  -genes that are needed are in relaxed chromatin and very easy to express
- continuously differentiating cells
  -genes are accessible but not transcribed for
  -different transcription factors activated by cell signals to express or silence certain genes

Pulmonary fibrosis
- patients with this get clumps in tissue of the lungs
  -cannot have gaseous exchange as walls are too thick
- to restore lung structure you need to restore and heal epithelial cells and maintain normal collagen
- cells within an alveoli are primed to differentiate
- if cells do not stop proliferating (lost contact inhibition) a adenocarcinoma (epithelial cancer) forms
  -but without ability to proliferate there would be holes in lungs/ infection

Cell Communication Example
- important to maintain perfusion of all our tissues
- cells rely on delivery of oxygenated blood
- if cell is starved of oxygen (hypoxic) it will send out signals to form new blood vessels
  -sends out VEGF signal
- if blood supply is not brought to ischaemic tissue it will die
- when cells are dividing and growing and building a solid mass (tumour) the inner parts of the tissue becomes hypoxic
  -bad for the host because tumour can signal for blood supply formation to supply it and support it
- lungs are different: all systemic vessels dilate in hypoxia
  -blood delivered to lungs is deoxygenated so if those vessels dilated in hypoxic lungs it would not

help at all
-in lungs hypoxia drives contraction

Insulin
- endocrine signalling
- cannot cross cell membrane
- insulin receptor must stand out from the cell

Steroid
- can pass through any cell membrane
- receptors are going to be intracellular

What effects outcome of signal
- half life of signalling molecule
- distribution
  -level of stability
- type of receptors
  -speed of response: fast ion channels, moderately fast G coupled receptors, slow responses when gene expression is involved
- specificity
  -dont want a “sticky” receptor that responds to everything
- number of receptors activated
- type of intracellular signalling molecules recruited

didnt finish: missed a couple slides (ask for notes on slide with image)

General Cell signalling
- signalling molecule= ligand
  -protein, hormone, neurotransmitter, nucleotide, lipid, gases, ions
- receptor
  -membrane bound or intracellular
- signal transduction pathway
  -amplification/ second messengers
- cellular response
  -contract, live/die, differentiate

Channel Linked Receptors- 6/11/17
each receptor lecture: structure, physiology, pathology, pharmacology

Receptors
- most cases are membrane associated
- also can be intercellular
- allow cells to communicate

Signals : Tins and Ters
- Tins= prolactin, erethrpoeitin, thrombopoeitin, leptin
- Ters= interferons and Interleukin

Cell- Cell communication
- capacity of cells to communicate for movement, thought, digestion etc
  -huge complexity
- 3 Primary categories
  -paracrine signalling: 2 cells relatively close to each other communicating (local environment), signals often secreted in vesicles
  -synaptic signalling

-endocrine signalling: occurs over long distances and usually involves production of hormones in a gland to be released into the blood stream, tends to be much slower - ie sex hormones

Receptors in Biology, Medicine and Therapeutics

- biggest class of drug targets that exist: trying to give an artificial signal to enhance or block a response
- 2 types of mechanisms by which receptors can act
  -receptor is also the effector (performs the function): ion channel receptors - no need for intercellular signalling
  -other type require secondary messenger chemicals to produce a signal transduction pathway so the physiological response occurs downstream of the receptor (signal transduction pathway= can amplify response)
  -this lecture is only about first type

Cellular membrane

- fluid mosaic structure
- thousands of subclasses of proteins associated with the membrane
- many receptors embedded in the membrane
- different cells express different receptors

Receptor functions of proteins

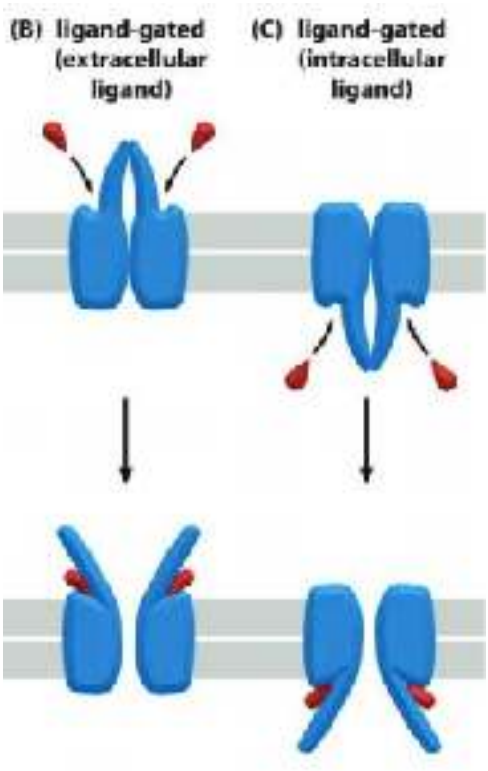
- enzymatic activity
  -tyrosine kinase receptor
  -if singlal is bound it changes enzymatic activity
- signal transduction
  -g-protein coupled receptors
  -produces signsl cascade within the cell
- transcriptional regulation
  -steroid hormone receptor
- Ion transport channel
  -focus of this lecture
  -ligand gated ion channels

Khan Academy Notes on Ligand Gated Channel Receptors

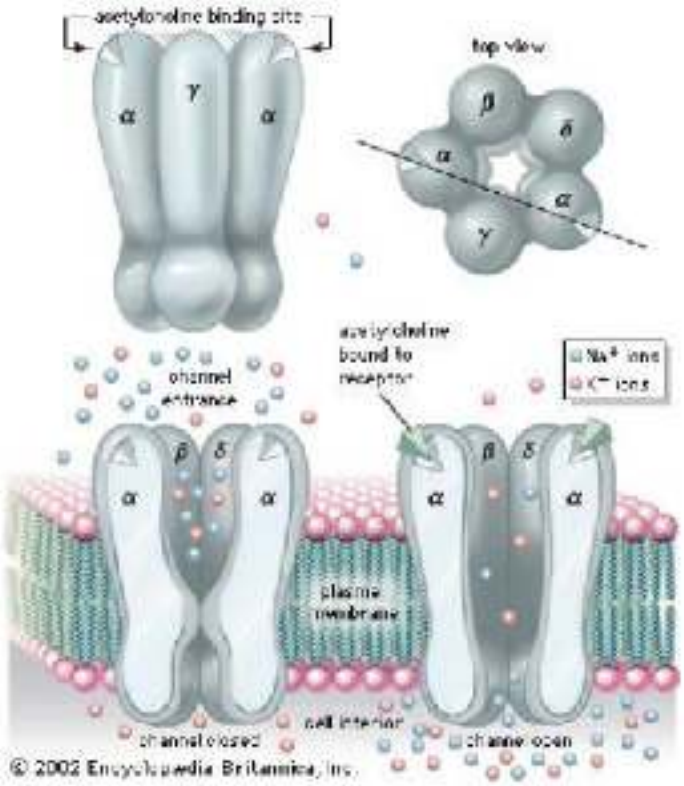
- transmembrane ion channel that open/ close in response to binding of a ligand (chemical message)
  -transmembrane proteins with a channel (hole/pore) to allow things in or out
- react extremely quickly => commonly found in cells that need to react very quickly to stimulus
- binding site can be on the intra or extracellular component of the receptor protein
  -more often extra cellular
  -proteins can have more than one binding site
  -binding site not near the actual channel
  -allosteric binding: binding at a site other than the active site (channel itself)
- shape of the ligand is complementary to the shape of the binding site so that only specific ligands can bind to specific ligands
  -called lock and key or induced fit
- once bound, it will cause closed channel to open
  -can control the opening and closing of the ion channel by changing the protein conformation of the entire protein
  -channel opens in a different place to the location of the ligand
  -the ion permeability across the entire plasma membrane can change: remember this is not just happening in one location on the membrane- when ligands bind there are many receptors scattered throughout the membrane opening and closing all at once
- ions (K+, Na+, Cl- and Ca2+ being most common) can then move through the open channel changing the electrical properties of a cell - electrical signal to tell the cell to do something
  -covert extra cellular ligand signal into and intracellular electrical signal

- do NOT get confused with voltage gated ion channels or stretch activated ion channels
  -voltage gated ion channels rely on a difference in membrane potential where LGIC rely on the binding of a ligand
  -stretch activated ion channels rely on the deformation of the cell membrane (stretch or pressure on the cell)

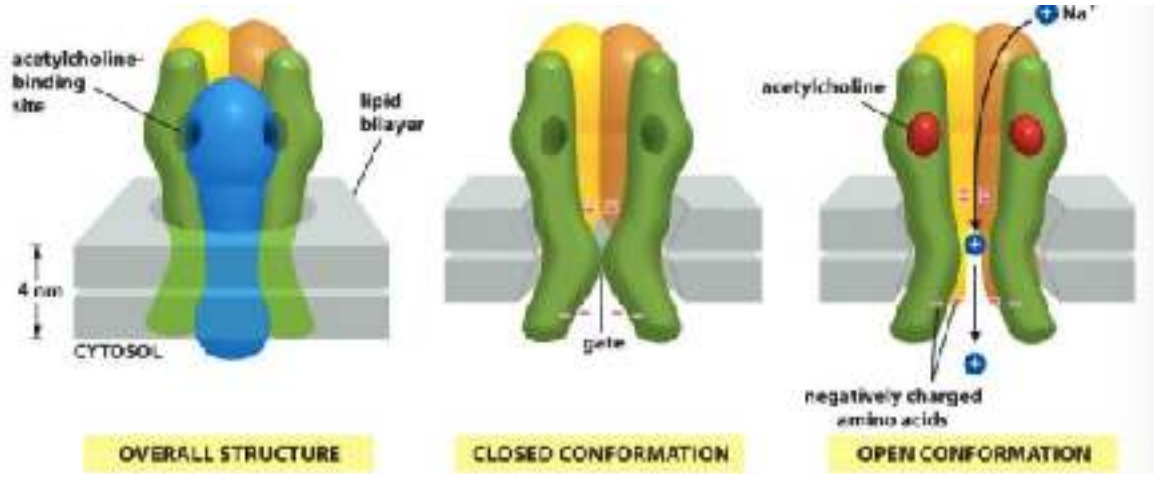
Ligand- Gated Ion Channels
- different cells express different channels
- membrane spanning, pore forming proteins
  -pore= channel
- membrane's permeability to ions can change rapidly as these channels open or close
  -process of opening and closing= channel gating
- open or close in response to an endogenous chemical messenger (ligand)
  -not designed to be drug receptors, we have manipulated them to do so
- distinct from voltage gated ion channels
- rapid responses and generation of membrane potential
- ligand signal can come from inside or outside the cell



Structure of LGIC



- composed of 5 transmembrane protein subunits (2 alpha, 1 beta, 1 delta and 1 gamma)



- 5 transmembrane alpha helices formin a hydrophillic core
- when 2 molecules of acetyl choline (example of ligand in image above) bind, a conformational change results in channel opening
- negatively charged side chains ensure charge selectivity for positive ion (Na+ and K+)
  -not absolute selectivity, more of a preference

Key features of LGICs

- can allow passage of ions in or out of cell (driven by electrochemical gradient)
  -move to equalise numbers inside and outside of the cell
- exhibit charge and size selectivity making them selective for one or two specific ions of similar size and charge

-selectivity based on the channel diameter, the charged and polar surfaces of the protein subunits and on the number of water molecules associated with the ions
- direct link between ligand binding and channel opening (no secondary messenger)- allows rapid response
- transition between closed and open in response to ligand
  -altered open probability: more likely to be closed but probability of it being open increases with interaction of ligand
- in context of neutrons can be excitatory (Acetyl choline, glutamine) or inhibitory (GABA or glycine) with respect to initiating an action potential

Measuring LGICs
- patch clamping
- allows you to isolate small section of the membrane
- can measure ionic currents in patches of the cell membrane => measuring activity
- hollow glass tube containing an electrolyte- containing solution and an amplifier is brought into contact with the cell membrane
- the current passing through the membrane is controlled by experimenter and the resulting changes in voltage are recorded generally in the form of action potentials

General Physiology
- neurotransmission
- muscle contraction
- insulin

Ligands and signals

Functional Type	Ligand*	Ion Channel
Excitatory Receptors	Acetylcholine (nicotinic receptor)	Na^+/K^+
	Glutamate (NMDA class receptors)†	Na^+/K^+ and Ca^{2+}
	Glutamate (non-NMDA class receptors)‡	Na^+/K^+
	Serotonin ($5HT_3$ class receptors)	Na^+/K^+
Inhibitory Receptors	γ-Aminobutyric acid, GABA (A-class receptors)	Cl^-
	Glycine	Cl^-

*Neurotransmitter

Inhibitory receptors might be used for tranquillisers

Functions of LGICs (rapid physiological events)
- maintain intracellular ionic homeostasis
  -right amount of fluid and ions in the cell
- play a key role in neurotransmission
  -membrane depolarisation
- regulation of contractility in cardiovascular and musculoskeletal systems
- regulation of insulin secretion from pancreatic beta cells

Non neuronal LGICs Pancreatic Beta cell
- sugar is taken up as glucose into the cell
- ATP levels in cells elevate
- ATP activates potassium ion channel to allow transport of potassium out of the membrane to allow calcium to travel into the cell
- calcium influx drives insulin secretion

Ion channelopathies
- acquired
  -myasthenia gravis: autoimmune disease against acetyl choline receptors (blocks receptors)
- genetic
  -more than 40 disorders related to channel mutations
  -cystic fibrosis: mutation in chloride channels in the lung called CFTR causing lungs to dry up : deregulation of epithelial fluid transport
  -epilepsy: mutation in Ach receptor may cause excessive or misfiring in nerve cells

Myasthenia gravis
- chronic autoimmune
- weakness of skeletal muscles
- weakness in eye muscles and slurred speech
- reduced capacity to respond to stimuli and have a contraction response
- treated with anticholinsterases to boost amount of Ach in body and immunosuppressants

LGIC as pharmacological targets
- insomnia, anxiety, depression, schizophrenia
- barbiturates and tranquillisers such as valium make channels easier to open
- tubocurarine is a skeletal muscle relaxant that antagonises the Ach receptor leading to decreased excitatory signalling
  -used in the past with an anesthetic to provide skeletal muscle relaxation during surgery or mechanical ventilation
  -problems: slow onset time for neuromuscular blocking drugs, causes histamine release which can be dangerous for asthmatics, children and those who are pregnant/lactating and significant ganglion blocking effects that manifests as hypotension
  -can cause paralysis
  -due to these problems it is now rarely used

G Protein Coupled Receptors (GPCRs)- 10/11/17

Khan Academy notes
- largest known class of membrane receptors
  -in humans more than 1000 types of receptors each with a specific function
  -ligands/effects are different but all undergo a similar process
- target of 30-50% of drugs
- huge range of ligands: light sensitive compounds, odors, pheromones, hormones, neurotransmitters etc
- can regulate immune system, growth, visuals, behaviour and mood
  -seratonin and dompamine
- many have unknown functions: huge area of research
- receptor that is coupled/ associatiated with a G protein
- structure of receptor: seven transmembrane alpha helices (squiggly tail off the binding site)
- G protein= specialised proteins with ability to bind GTP and GDP
  -all G proteins that associate with GPCRs are heterotrimeric: 3 different subunits (alpha, beta, gamma)
  -alpha and gamma subunit attached to the cell membrane by lipid anchors
  -inactive when GDP is bound to alpha subunit, active when GTP is bound
- each receptor binds to usually one or a few specific molecules fitting together like lock and key
  -complimentary binding site
- when signalling molecule is bound to the GCPR it will undergo a conformational change triggering a complex chain of events unlimitedly influencing different cell functions
  1. Ligand binds to GCPR
  2. GCPR undergoes conformational change
  3. alpha subunit of G protein is going to exchange GDP for GTP due to conformational change in

the receptor and is then activated
4. alpha subunit may dissociate or may not
5. activated G protein or dissociated active alpha subunit will find another protein in the membrane to alter and regulate its function
-target proteins can be enzymes that produce second messengers or ion channels that let ions be the second messengers
6. target protein can then relay a signal

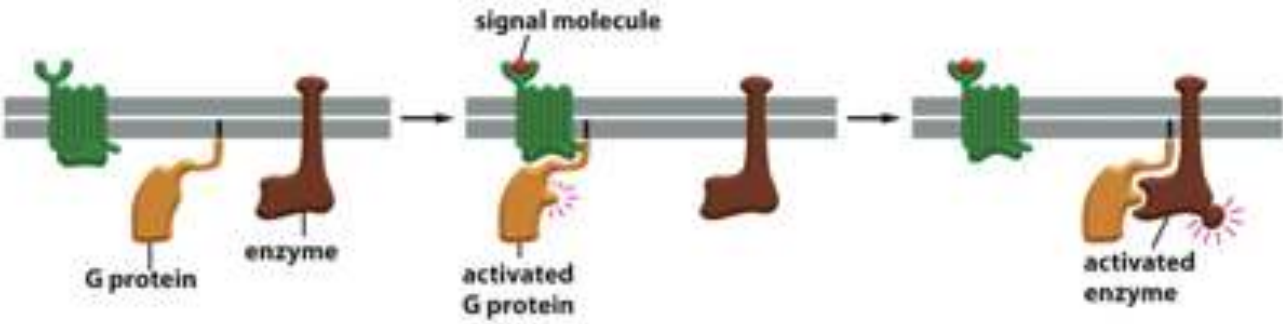
- once a ligand is bound to the receptor the whole chain of events can happen repeatedly as long as the ligand is bound
- to stop the process GTP is hydrolysised and loses a phosphate becoming GDP
  -G protein is inactive again and ligand will leave
  -receptor ready to bind again
  -usually happens on its own but our body has ways of regulating this
- common example of GCPR function involves adrenaline as ligand: fight or flight response
  -ligand = epinephrine (adrenaline)
  -GCPR = adrenergic receptor
  -effected membrane protein that G protein will activate= adenylate cyclase
  -adenylate cyclase will take ATP and produce cyclic AMP (cAMP) : taking away 2 phosphate from ATP so one is left
  -second messenger= cAMP (ligand is first messenger)
  -cAMP will tell the cell what to do for fight or flight response: increase heart rate, dilate skeletal muscle blood vessels and breakdown of glycogen to glucose for energy

General

- linked to family of large G proteins
- receptor itself is not the effector
- starts a signalling pathway- more complex
  -linked to multiple signal transduction pathways
- external hydrophilic ligand
- change in receptor activity recruits G proteins and initiates pathway
- receptors embedded in the membrane with both extra and intracellular parts
  -when ligand not present the receptor is separate from its associated G protein
- G proteins are associated with the membrane

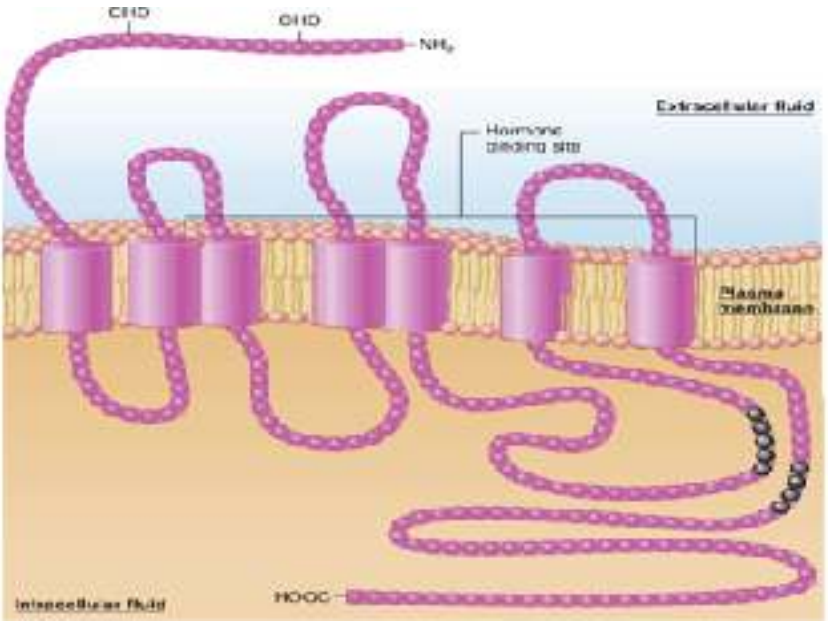
General Principles

- when ligands not bound, receptor is distinct from G protein
- when ligand becomes associated then the G protein becomes associated resulting in an activation of the G protein
- When the G protein is activated it will leave the receptor and associate with an enzyme that it then activates



Cell surface receptors
- ion channel receptors, G Protein Coupled receptors, enzyme coupled receptors
- most ligands have multiple receptors
  -Ach receptors of skeletal muscles and heart: type of receptor expressed in skeletal muscles is different than those in the heart
  ==> distribution in tissue decides the effect of the receptor
  -Histamine receptors in respiratory system or stomach
- drugs often mimic these ligands to activate these receptors to give a pharmalogical response
  -exogenous ligands= pharmacological manipulated ligands
  -endogenous ligands= natural ligands
- Examples endogenous / exogenous
  -opioid receptors: endorphins / heroin
  -nicotinic receptors: Ach / nicotine
  -GABA receptors: GABA/ barbiturates

Structure of G coupled receptor proteins
- serpentine structure : has single linear polypeptide chain that winds in and out of the cell between transmembrane domains
  -7 transmembrane domains
- over 700 types in humans : all have same kind of process
- about 50% of drugs on the market are designed to interact with these receptors
  -either block or enhance activity of
- highly evolutionarily conserved
  -over millions of years of evolution
  -shows how important they are to physiological function

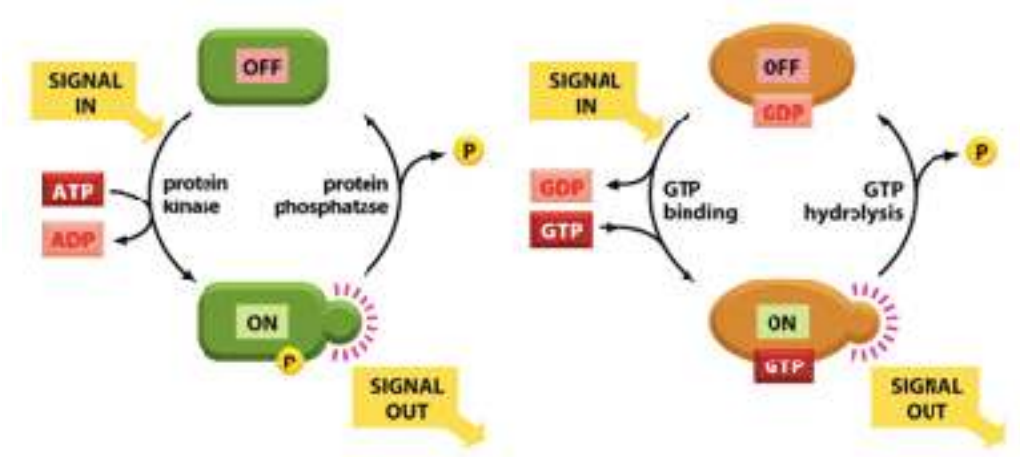


Intracellular signalling (after receptor associated with its ligand)
- ligand (first messenger) always hydrophilic and interacts with outside of cell
- receptor is linked to multiple intracellular signalling molecules
  -can react with different proteins to cause different downstream effects
- second messenger brought about by the signalling molecules carries the signal to effector proteins
- effector proteins then create the response intended by the ligand

Molecular Switches
- phosphorylation
  -phosphate added turns it on typically

-done by protein kinases (enzyme puts phosphate on protein by using ATP —> ADP)
-dephosphorylation done by protein phosphatase : turns off protein

- GTP
  -when bound to protein it is on : GTP binding
  -signal causes GDP—> GTP
  -off switch: GTP hydrolysis



Switching on G proteins

- G protein is a trimeric proteins: 3 subunits to protein
  -alpha, beta and gamma
- in inactive state it is bound to GDP
  -no activity
- when ligand binds to receptor it elicits a signal and decreases the G protein affinity for GDP so GTP binds in its place and becomes active (change in configuration
  -may or may not involve subunit dissociation
  -operates over a period of seconds (not as rapid as ion gated channels)
- G protein is then activated and can start the intracellular transduction pathway
- cholera toxin targets G-proteins in the intestine not allowing them to turn off

Switching off G-Proteins

- G protein activates target protein by the alpha subunit
- once it has activated the target protein the alpha subunit causes hydrolysis of GTP
  -formation of GDP + P
- this inactivates the alpha subunit and causes it to dissociate from the target protein
- inactive alpha subunit reassembles with beta-gamma complex to reform an inactive G protein

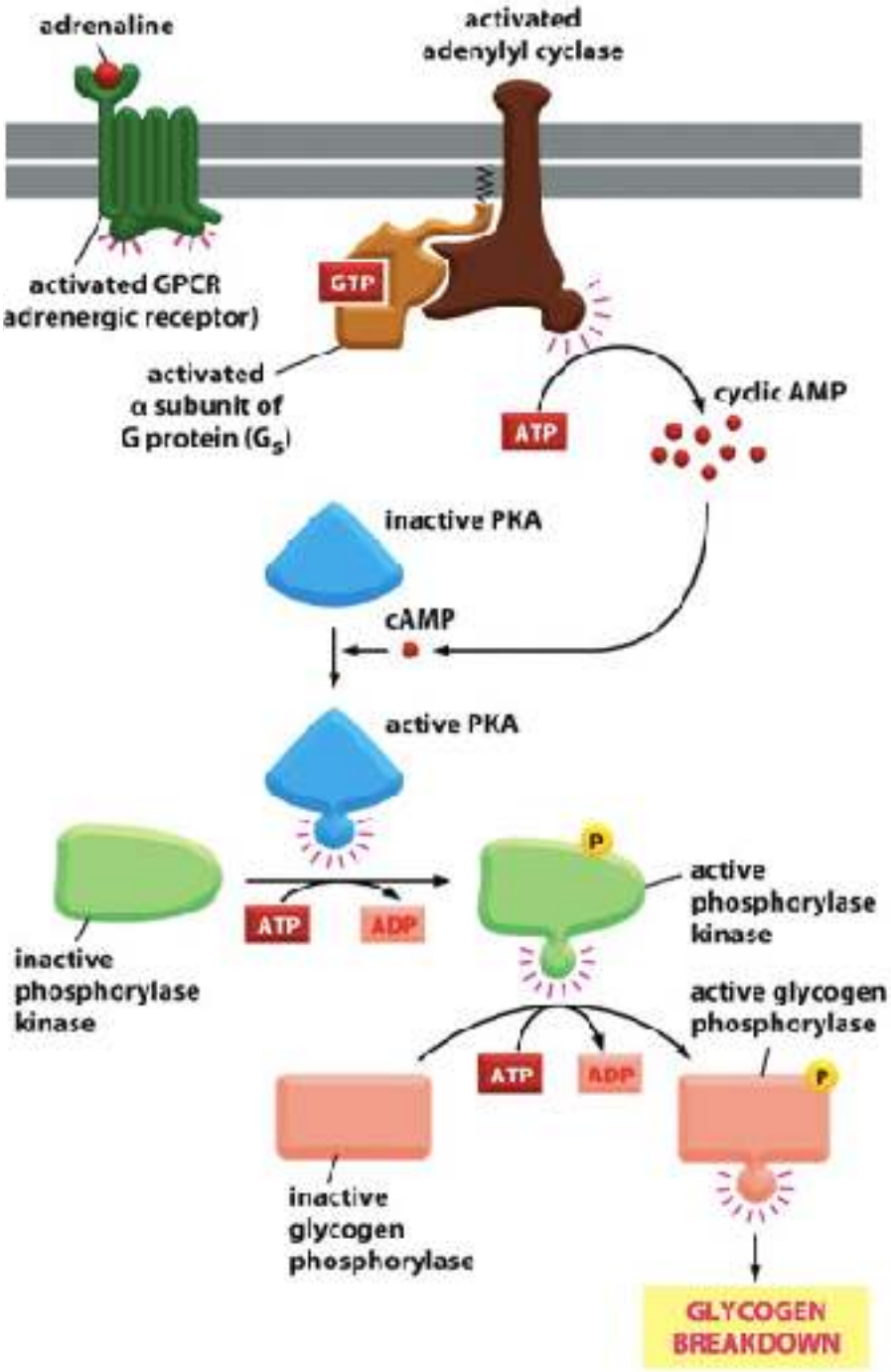
G Protein Targets

- ion channels
  -Ach can indirectly effect ion channels through G coupled protein receptors OR directly as acting as a ligand for ligand gated channel receptors
  -activated G Proteins can bind to ion channels activating them, causing them to open
- Second messengers
  -activated alpha subunit associates with another enzyme
  -this enzyme causes a response of smaller messenger molecules to disuse to act on intracellular signalling proteins

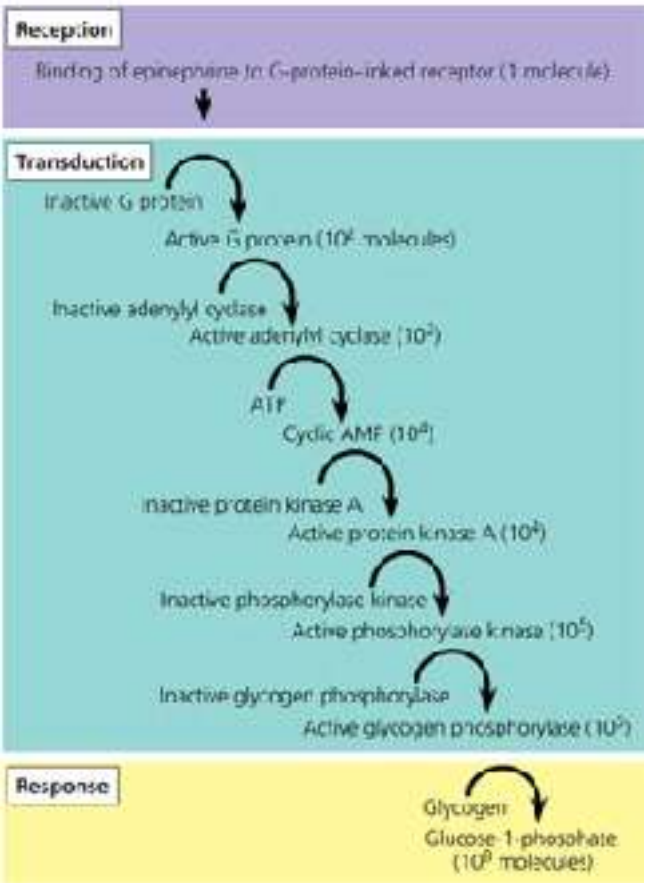
cAMP as second messenger

- adrenaline as ligand
  -activates Gs type of G Protein
- activated alpha subunit (dissosiated from beta and gamma) activates adeneylyl cyclase

- converts ATP to cyclic AMP
  -activates downstream cascade of signals
- activates Protein kinase A which activates a further downstream phosphorylase kinase
- phosphorylase kinase which goes on to cause the effect
- uses ATP to activate glycogen phosphorylase causing glycogen breakdown



Why so complex?
- signal amplification
- 1 ligand activates 1 adenylyl cyclase
- it in turn uses a lot of ATP to form a lot of cyclic AMP
- cascade continues to amplify the effect the whole way down stream
- single molecular effect causes a large effect (10^8 molecules of broken down glycogen at the end)



Lock and key

Molecular Switch

Second Messenger

Ca2+ as a second messenger
- signal molecule (ie Histamine) activates GPCR
- G protein activated is linked to an enzyme called phospholipase C
  -phospholipase C phosphorylase the lipids in the cell membrane breaking them up into IP3
- IP3 releases Ca2+ stores in the endoplasmic reticulum
- activated Protine Kinase C (PKC)

GPCRs mutations in disease
- retinitis pigmentosa : misfiring of G protein related to sight
  -affects the photoreceptor cells responsible for capturing images from the visual field
  -some mutations are so severe that the gene cannot make the protein receptor
  -other mutations produce a protein that is toxic to the cell of an abnormal protein that does not function correctly
  -leads to problems with sight
- fertility disorders
- carcinomas
  -GPCRs major component of tumour growth and metastasis
  -malignany cells often hijack normal physiological functions of GPCRs to survive, prolixerate and evade the immune system
  -tumour cell proliferation is regulated by many neuropeptides
  -activating mutations of G proteins and GPCRs drives the unregulated growth of some tumours

-overexpression of GPCRs and their activation by ligands released by tumour cells is a frequent tactic used by tumour cells to stimulate GPCRs and their signalling networks
- hypo and hyperthyroidism
  -thyroid stimulating hormone receptor (TSHR) usually only recognises their related ligands but mutations can lower this specificity

Pharmocology
- Gabapentin: anti epileptic drug
  -it is an anticonvuslant : helps to normalise the way nerve impulses travel along the nerve cell which helps prevent seizures
- Beta Blockers: treatment for hypertension and reducing blood pressure with inhibition of GPCR
  -blocks effects of epinephrine (adrenaline)
  -causes heart to beat more slowly and with less force
- Alpha receptors CNS and PNS
  -activation of these receptors by nerves system transmission or drugs will result in vasoconstriction and increase in arterial blood pressure
  -regulated by epinephrine or norepinephrine : cannot be given orally so given I.V. and in aerosol sprays
  -used in conjunction with anesthetic as vasoconstriction limits diffusion of local anaesthetic from site of injection prolonging the actions and reducing toxicity of the local anaesthetic by limiting its systematic absorption
  -also used during surgical procedures to induce vasoconstriction and reduce blood loss

Enzyme Linked Receptors: Tyrosine Kinase Receptors - 10/11/17

Khan Academy Notes
- unique because in addition to receiving signal they also function as enzymes (receptor AND effector)
- binding of signalling molecule activates the enzymatic activity
- structure: kind of Y shape with long tail going through the membrane
  -top of the Y is extracellular and where the ligand can bind
  -intracellular side is the enzymatic domain
  -ligand binding on the extracellular domain causes intracellular domain to act as an enzyme
- most common enzyme linked receptor= Receptor Tyrosine Kinases (RTKs)
  -very important because they regulate cell growth, differentiation and survival
  -respond to ligands such as growth factors
- RTKs are unique because there is tyrosine on the intracellular enzymatic section
- kinase= enzyme that has the ability to transfer phosphorous molecules usually from high energy substance like ATP
  ==> Tyrosine Kinase Receptors have the ability to transfer phosphate from ATP to intracellular proteins to activate them (enzymatic function)
- phosphorylated proteins can carry out message through signal transduction
- RTKs occur in pairs- cannot work alone
  -when ligand binds to binding site of two nearby RTKs they will come together and act together
  -this forms a cross linked dimer
- The reason why RTKs must work in pairs is because cross linking activates the tyrosine kinase activity through phosphorylation —> each RTK in the dimer phosphorylates the tyrosines on the other RTK
  -this process= cross phosphorylation
  -because of tyrosine ATP inside the cell will become ADP + Phosphate
  -tyrosine can then pick up free floating phosphate group
- once phosphorylated the enzymatic section of RTKs become a docking station for different intracellular proteins involved in signal transduction

-different proteins can come by and attach themselves to phosphorylated tyrosine
-do not have to be the same protein along the one enzymatic domain: can have different proteins binding to the same RTK at the same time
-in order for intracellular proteins to bind they have to have a specific domain called SH2 which can bind to phosphorylated tyrosine

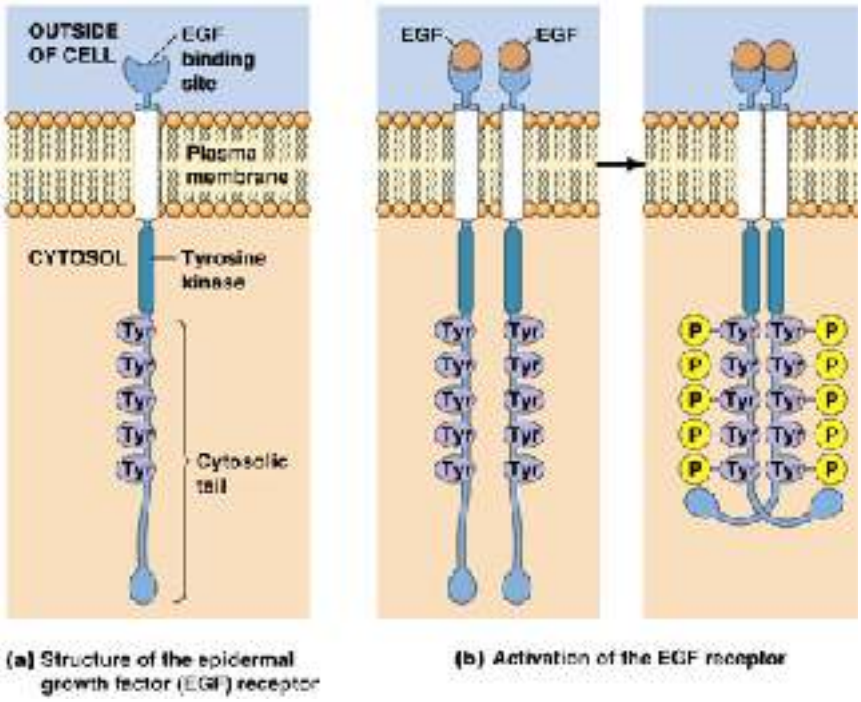
- the fact that different kinds of proteins can all bind to RTK at the same time allows for multiple different intracellular signalling pathways to happen at the same time due to the binding of ligand to 1 RTK dimer
  -signalling pathways can be really complex even ending at the nucleus effecting gene transcription
  -signal passed from bound intracellular proteins into the cytosol
- Enzyme Linked receptors have a variety of functions
  -RTKs generally known for their role with growth factors
  -ie regulating surface proteins called ephrins which can help guide developmental processes involved in tissue architecture, placement of nerve endings, and blood vessel maturation
  -nerve growth factors and platelet- derived growth factors also use RTKs
  -RTKs can bind hormones- most famously insulin
- When RTKs fail to function properly they can cause issues in growth and differentiation of cells as they are primarily known to regulate cell growth
  -many cancers involve mutations in RTKs
  -RTKs are targets of many drugs used in chemotherapy
  -ie Breast cancer drug Herceptin is antibody that binds and inhibits a particular RTK that is over expressed

General

- regulates a lot of how a cell grows and dies
  -cell death pathways
- important in drugs related to cancer
  -mutations of these pathways associated with cancer
- slow responses: hours

Structure

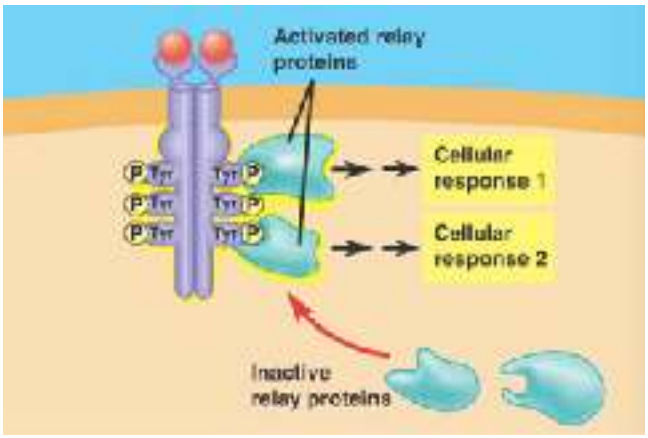
- single monomeric protein
- transmembrane: crosses it once
- binding site outside the cell (EGF binding site)
- tyrosine kinase domain domain inside the cell with a cytosolic tail with amino acids (tyrosine residues) attached
- activation of a single monomeric receptor is not capable of signalling- they have to pair up to have an effect
  -two receptors cross phosphorylate the tyrosine residues : self phosphorylation
  -this activates the receptor



(a) Structure of the epidermal growth factor (EGF) receptor

(b) Activation of the EGF receptor

Events after ligand binds
- 2 monomeric receptors form a dimer
- cross phosphorylation of tyrosine residues
  -use of ATP (one per tyrosine residue)
- in phosphorylated form the receptor becomes attractive to binding proteins that can relay the signal once activated
  -different binding proteins attached to same dimer can relay different cellular response
- switched off by tyrosine phosphates or degradation



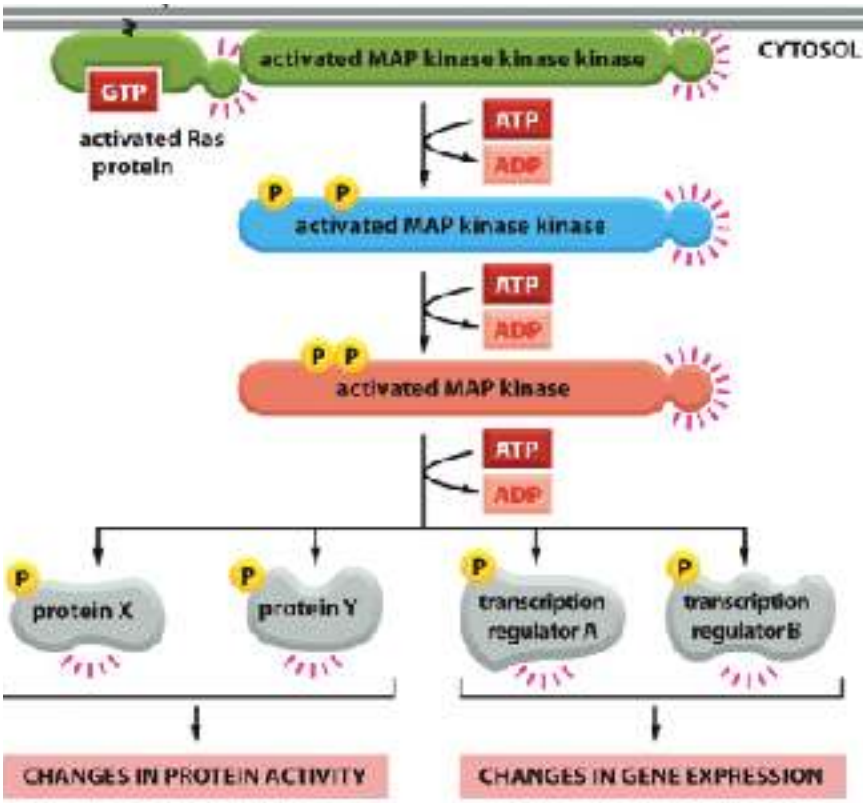
Cellular Functions
- growth
- proliferation
- movement
- survival
- differentiation

Classes of Tyrosine Kinase receptors
- receptor tyrosine kinase : receptor has intrinsic tyrosine kinase (RTKs 1 and 2)
  react to following signals:
  -insulin
  -epidermal growth factor (major cancer target)
  -platelet derived growth factor
  -fibroblast growth factor
  -vascular endothelial growth factor
- receptor- associated tyrosine kinase : has to recruit intracellular tyrosine kinase (ie Janus Kinase-JAK)
  signals:
  -prolactin
  -growth hormone
  -erythropoietin
  -many of interleukins and interferons

Receptor tyrosine kinase

Effector functions of RTKs 1: Ras
- binding protein activates Ras-activating protein
- Ras-activating protein replaces GDP with GTP
- activates Ras protein to create a response in the cell
  -small G Protein distinct from large G Proteins in GPCR
  -also require GTP to switch it on

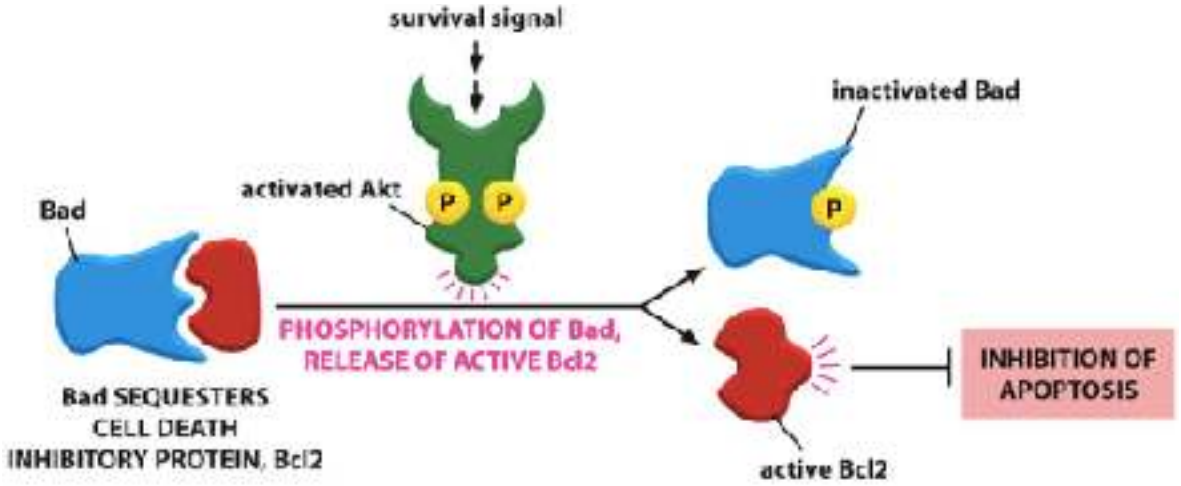
- Activated Ras proteins activate MAP kinases
  -produces a signalling cascade
  -MAP kinase kinase kinase —> MAP kinase kinase —> MAP kinase
- MAP kinase can cause changes in protein activity or gene expression



- cell growth, differentiation and survival

Mutant Ras
- dont need to be turned on: always on
- continuously transmitting signal along multiple pathways in absense of signal molecule
  -constant proliferation of cells
- huge cause of cancers
  -90% of pancreatic cancer has this mutation
  -50% of colon cancer

Effector Functions of RTKs 2 : PI3- kinase
- survival signal bound to RTK activates RTK which activates the bound PI3-kinase
  -transmembrane inositol phospholipid is activated (phosphorylated) by the kinase which in a downstream chain of events activates Akt
  -Activated Akt causes the phosphorylation of Bad releasing an active Bcl2 molecule and an inactivated Bad
  -An active Bcl2 molecule inhibits cell death
- result of pathway is it gets the cell not to die
  -Bcl2 inhibits cell death
  -Bad promotes cell death



Receptor associated tyrosine kinase

Receptor itself does not have intrinsic tyrosine kinase activity
- relies upon recruitment of a separate tyrosine kinase enzyme
  -ie JAK
- ligand induces receptor dimerisation which in turn activates associated kinase
- kinase cascade results in regulation of gene expression

Jak-STAT pathway
- three main components: receptor, JAK and STAT
- JAK = janus kinase
  -a type of tyrosine kinase
  -JAK phosphorylates the tyrosine residues on itself once active
- STAT = Signal Transducer and Activator of Transcription
- ligand binds to RTK which has JAK in place of intrinsic tyrosine kinase
- once bound the ligand activated JAK
- Activated JAK will use ATP to phosphorylate tyrosine residues on itself
- STAT protein molecules from cytoplasm will attach to phosphorylated tyrosine
- JAK will then use another ATP to phosphorylate the STAT protein
- STAT protein then dimerises (2 units of STAT) and enters the nucleus
- It binds to certain specific regions on the DNA and promotes transcription of that particular sequence (binds to promoter regions)
  -mRNA is transcribed leading to translation of a certain type of protein to take their effect
- clinical significance: disrupted or dysregulated Jak-STAT function can result in immune deficiency syndromes and cancers

Clinical Relevance ot RTKs
- diabetes- insulin- metabolism
  -insulin secreted by pancreatic beta cells= ligand
  -insulin RTK activates a complex intracellular signalling network
  -insulin receptor involved in carrying glucose into the cell (increaseing uptake) is an RTK
- cancer- VEGF- tumour growth
  -VEGF= vascular endothelial growth factor
  -VEGF is a key mediator of angiogenesis in cancer
  -angiogenesis= physiological process through which new blood vessels form from pre-existing vessels
  -VEGF binds to a VEGF tyrosine kinase receptor on a cancer cell
  -VEGFRs (vascular endothelial growth factor receptors) are a facility of RTK

- Cancer- herceptin (trastuzumab)
  -breast cancer drug treatment by herceptin
  -some breast cancer cells overexpess HER2 gene which makes a HER2 receptor (type of RTK)
  -herceptin works by attaching itself to the HER2 receptors on the surface of breast cancer cells and blocking them from receiving growth signals
  -trastuzumab= generic name for drug : herceptin= brand name
- Anaemia- EPO
  -EPO= hormone erythropoietin
  -hormone produced in the kidney to prompt bone marrow to make red blood cells to carry oxygen
  -EpoR is RTK for EPO which promotes proliferation of erythroid progenitor cells
  -defects in receptor may cause anaemia
- class of drugs that block the activation of TKRs for cancer by blocking dimerisation

Complexity exists
- integration of signalling pathways- not just 4 individual pathways
  -4 classes of receptors that communicate between each other
  -can elicit very different responses from the same ligands in the same cell by cross talking between pathways that are produced by different receptor proteins

Steroid Hormone Receptors- 13/11/17

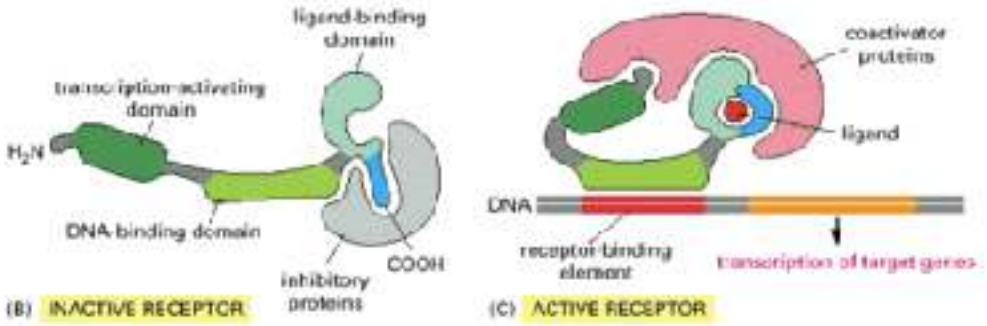
Khan Academy Notes
- kind of receptors used when the hormone is the primary messenger
  -primary messenger hormones= steroids/ thyroid hormones (often lipid based hormones)
  -other hormones can bind to an extra cellular receptor and create a signalling pathway inside the cell involving secondary messengers
  -less complex than the involvement of secondary messengers
- signals cross the cell membrane and binds to receptor thats located in the cytosol or nucleus
  -binding to a receptor is going to directly effect transcription in the nucleus or translation in the cytoplasm of the protein that is being activated by the hormone

Classes of ligands
- large and/or hydrophilic or small and/or hydrophobic
- cell surface receptors bind with hydrophilic signal molecules
- intracellular receptors bind with small hydrophobic signal molecules
  -steroid hormone receptors are in this category

Endocrine signalling
- long distance
- hormonal signalling
- when stimulus is produced in one part of the body and the target tissue is far away from the source of the signal
  -signals require delivery through the blood stream
- very different type of receptor needed
  -intracellular receptors floating around in the cytoplasm
  -ligands need to be able to cross the membrane
  -main function is to regulate expression of genes

Structure of steroid receptors
- not embedded in membrane- in cytoplasm
- number of very important domains
  -DNA binding domain
  -Transcriptional activating domain
  -Ligand binding domain
- generally transcription factor

- when ligand is not bound it is associated with and inhibitory protein to prevent it from being activated
  -sort of like a double lock to prevent activation without a ligand
  -does not allow receptor to go to the nucleus, keeps it in cytoplasms
- ligand binds to ligand binding domain and inhibitory proteins are lost to allow protein to be activated
  -receptor can travel to nucleus
- can use DNA binding domain in the nucleus to bind to DNA
- transcription domain will interact with other proteins to promote a response
  -activation of a transcriptional complex



Example
- ligand= cortisol
  -major control of inflammatory processes
- cortisol crosses the membrane
- when ligand binds the receptor looses inhibitory proteins and makes way to nucleus
- will seek out and bind to promoters of the genes that it wants to transcribe
- if body wants to stop an inflammatory response it will increase the amount of cortisol in blood stream and bind to anti inflammatory genes

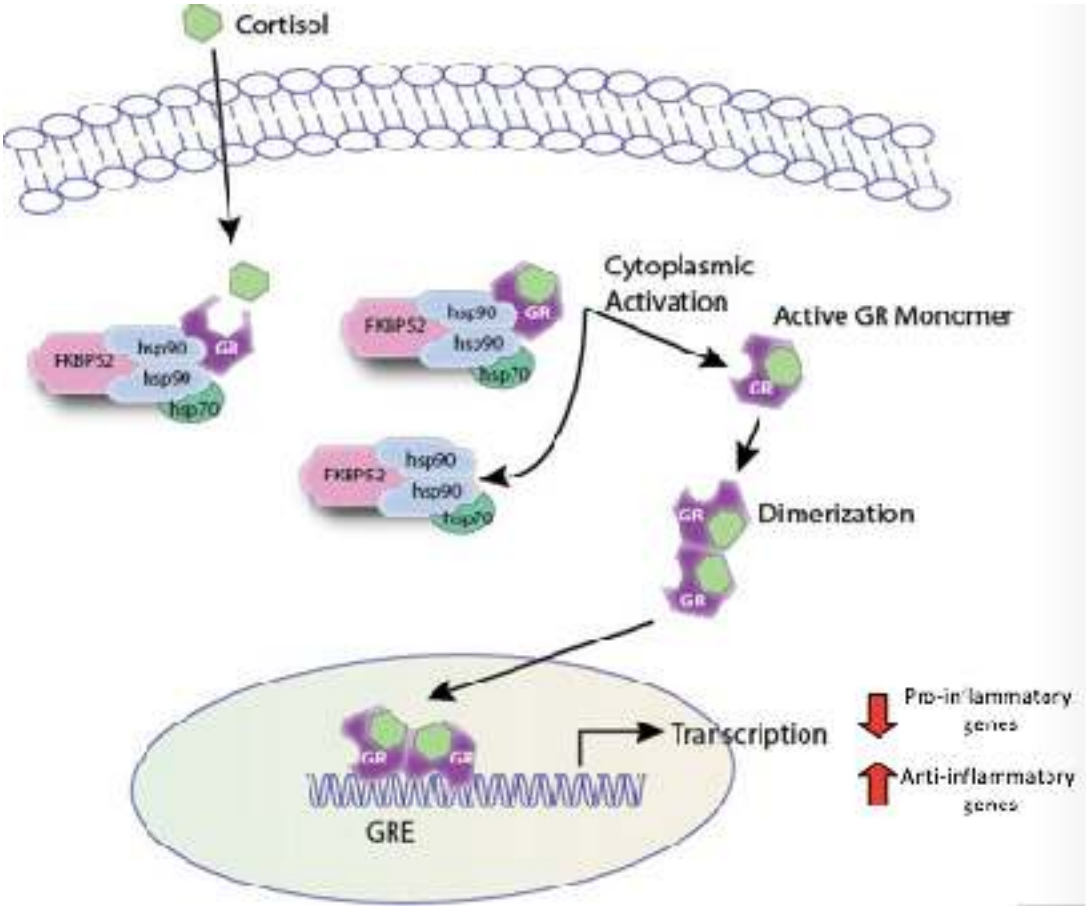
Cholesterol
- found in all cell membranes
- “mother molecule” of all steroid hormones
  -can be converted to cortisol, estradiol, testosterone, thyroxine etc
- hydrophobic molecules that can bind to nuclear receptors in cytosol or nucleus
- steroid hormones are produced in source tissues (glands)
- hormones produced in these glands have an effect very far from where they are produced

Family	Specific Hormone	Primary Site of Synthesis	Primary Receptor
Progestin	Progesterone	Ovary Placenta	Progesterone receptor
Glucocorticoid	Cortisol Corticosterone	Adrenal cortex	Glucocorticoid receptor
Mineralocorticoid	Aldosterone 11-Deoxycorticosterone	Adrenal cortex	Mineralocorticoid receptor
Androgen	Testosterone Dihydrotestosterone	Testis	Androgen receptor
Estrogen	Estradiol-17β Estriol	Ovary Placenta	Estrogen receptor

- different ligands (hormones) bind to different receptors
- same receptor can activate different gene sets in different cell types

- released into the bloodstream from endocrine organs
  -act over long distances
  -act over long time periods (puberty/ pregnancy) - slowest acting of 4 classes of receptors
  -generally bound to plasma carrier proteins to carry them from site of production to site of action

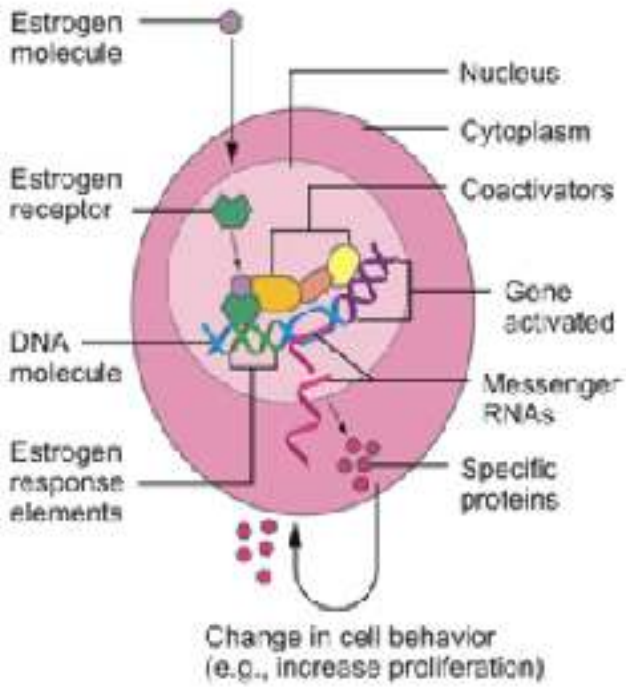
Glucocorticoids Receptors (GR)
- switching off inflammatory response
  -inhibit inflammatory gene expression or activation of anti-inflammatory gene expression
- major class of drugs: hydrocortisone is an anti inflammatory drug
- can have serious side effects - Cushings syndrome
- recognises inflammation from circulation through the adrenal cortex which will produce cortisol
  -when infection is cleared it is time to turn the response off
- when cortisol is released and enters cell to bind to receptor the receptor is liberated from inhibitory protein
  -conformational change
- usually dimerises with a second receptor and enters nucleus
- switches on anti inflammatory genes and switches of inflammatory genes



- important role in medicine : anti inflammatories
  -hydrocortisone
  -risk of side effects - if too much hydrocortisone given there is risk of cushing's syndrome

Estrogen Receptors (ER)
- activated ER binds to response element in promotor regions of genes to induce or suppress gene activation of oestrogen dependent genes
- increases cell proliferation gene expression
- over activation can result in cancer

- therapeutic target in breast caner
  -drug= tamoxifen
- estrogen typically released by ovarian cells will bind to oestrogen receptor
  -not every cell will express the receptor
- ER travels into nucleus and activated genes
- Effects of oestrogen
  -brain: regulates body temp, regulates parts of brain that prepare body for sexual and reproductive development
  -breast: stimulates development and prepares glands for future milk prod.
  -heart and liver: regulation of liver's production of cholesterol
  -ovary: stimulates maturation of the ovaries, stimulates start of a menstrual cycle
  -uterus: stimulates maturation of the uterus
  -bone: helps to preserve bone density
- Estorgen in medicine
  -overexpression of ER in many types of breast cancer : tamoxifen is ER receptor antagonist



General
- generally present in cytoplasm and bound to co factors
- steroid ligand can diffuse through plasma membrane
- steroid binding promotes translocation of steroid/ receptor complex to nucleus
- depending upon chromatin structure activated transcription factor will bind to specific response elements

Example of cell signalling: fight or flight response - 20/11/17

fight or flight response
- overwhelming activation of sympathetic nervous system
  -noradrenaline (NAd- also known as norepinephrine - NE) released from sympathetic neurones
- activation of adrenal glands
  -sphanchnic nerve
  -release of adrenaline (epinephrine) and NAd

-Ad: NAd (80: 20)
-1-3 min half life
- some tissues are innervated more densely than others
- hormone signalling
-hormone (Ad) travels through bloodstream to receptors
- adrenaline circulates in the blood as it is relatively stable and can travel long distances in the body
- NAd acts synaptically

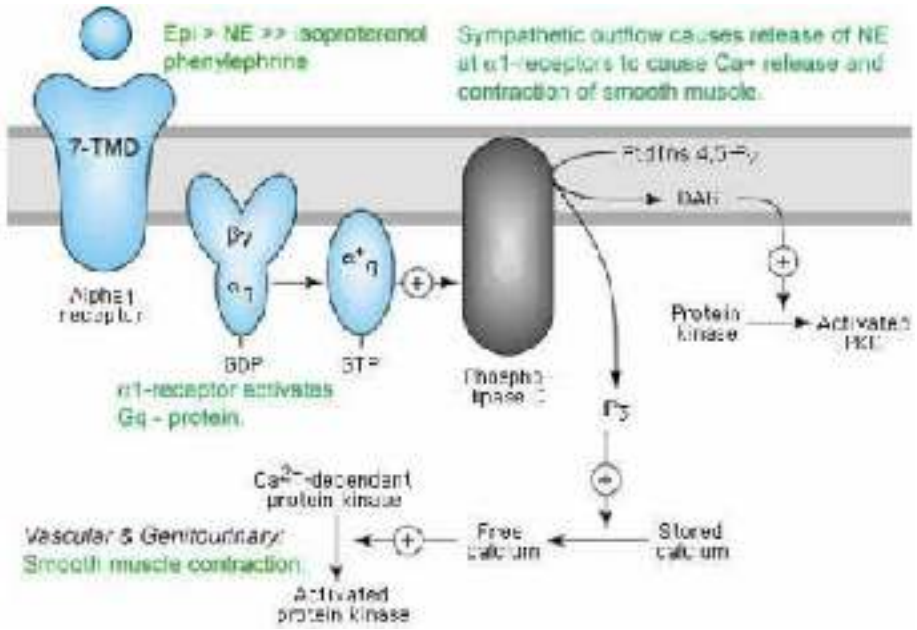
Autonomic Nervous system
- parasympathetic nervous system: acetylcholine with muscarinic acetylcholine receptors
- sympathetic nervous system: adrenaline/ noradrenaline with adrenergic receptors
- sympathetic division is the one involved with fight or flight

Sympathetic responses
- bronchial smooth muscle dilation
- contraction of vascular smooth muscle in gut
- dilation of vascular smooth muscle in skeletal muscle
- contraction of vascular smooth muscle in skin

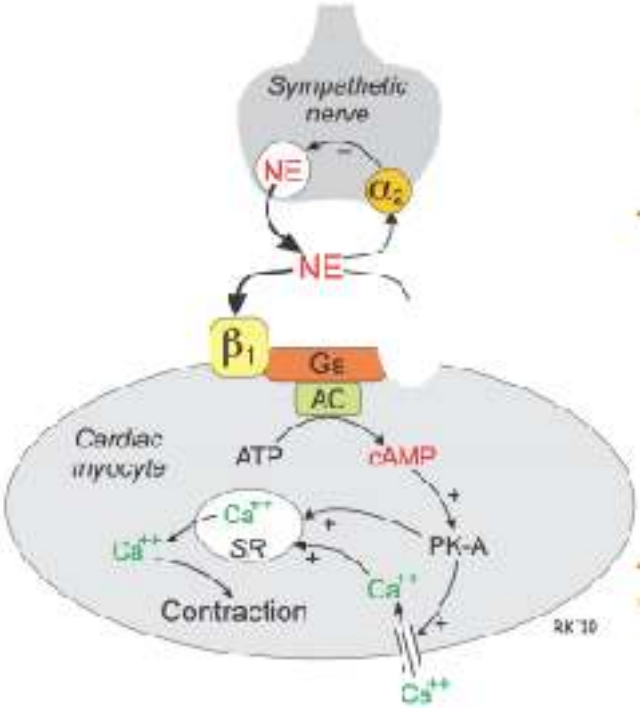
Variety of adrenaline responses
- adrenaline and noradrenaline great signalling molecules
- used to direct many responses
- heterogeneity comes from
-variety of receptors with variety of intracellular cascades
-tissue specific receptor expression
-varied affinity/potency at different receptors
- NAd and Ad cause varied responses

2 main classes of receptors
- alpha and beta
-alpha 1 and 2
-beta 1 and 2
- alpha 1: arteries and arterioles and when activated it will drive vasoconstriction
- alpha 2: gastrointestinal tract and when activated is will cause decrease muscle tone, motility and secretions
- beta 1: heart and when activated drives increased heart rate and force of contraction
- beta 2: skeletal muscle blood vessels, coronary arteries, bronchial smooth muscles and when activated causes dilation of blood vessels and relaxation of bronchial smooth muscle

Adrenoceptors
- G Protein Coupled receptors
- Alpha 1 Adrenoceptor (smooth muscle)
-preferentially activated by NAd before Ad (slightly higher affinity for NAd)
-alpha-q subunit of G Protein activates phospholipase C
-activation of this enzyme produces DAG and IP3
-activates a cascade within the cell
-drives smooth muscle contraction by regulating free calcium levels (release of calcium from sarcoplasmic reticulum)

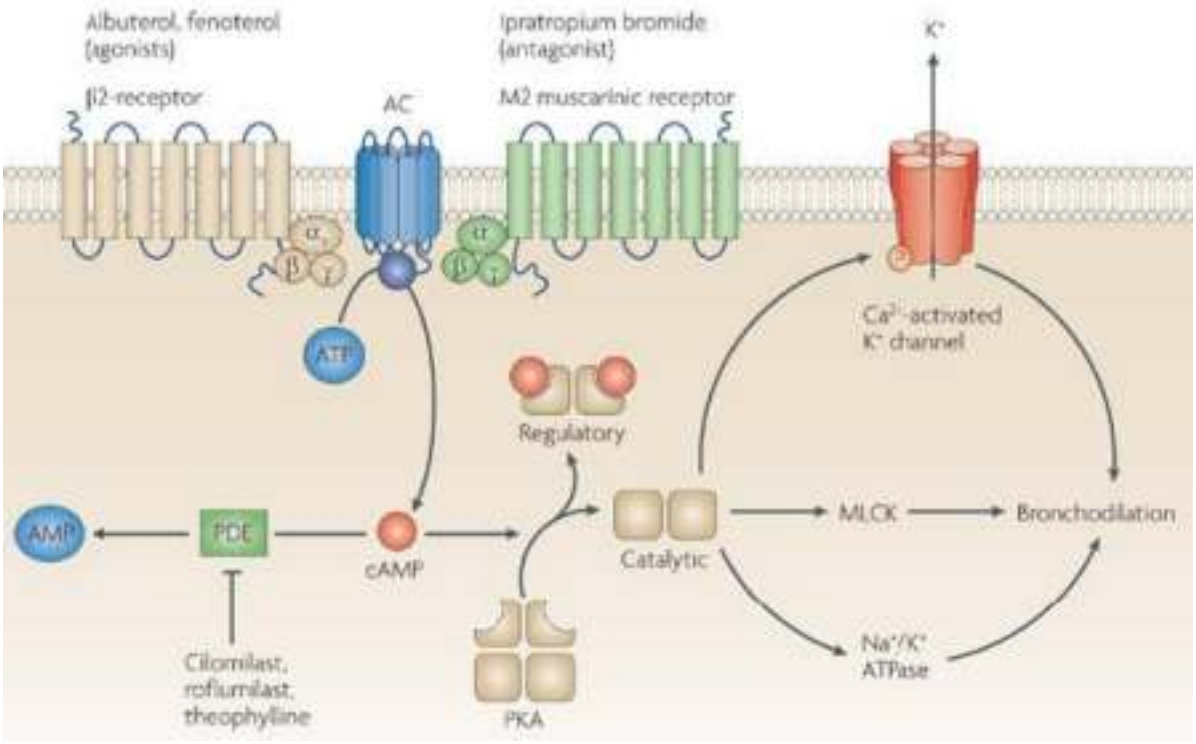


- Beta 1 adrenoreceptor (cardiac muscle)
  -response occurs in a cardiac myocyte
  -activated by NAd from a sympathetic nerve at a NMJ
  -alpha-s subunit for G protein
  -increased cAMP produced from ATP when G Protein is activated
  -this activated PKA which phosphorylates L-type calcium channel which results in an increased calcium influx from sarcoplasmic reticulum
  -increased contractility
  -also increased heart rate due to modulation of pacemaker currents in SA node



- Beta 2 adrenoceptor (vascular smooth muscle)
  -high affinity for Ad over NAd
  -also alpha-s subunit
  -increased cAMP produced activating PKA in exactly same way as with Beta 1 receptor

-different set of targets than PKA with Beta 1 adrenoceptor
-calcium phosphorylates MLCK (kinase) and causes it to become inactive (P-MLCK)
-MLCK= myosin light chain kinase
-potassium channel is activated by increased Calcium due to active PKA and potassium can leave the cell polarising the cell membrane reducing activation of the smooth muscle
-results in smooth muscle relaxation



Summary

Alpha Receptors		Beta Receptors	
1. Vasoconstriction of a. Coronary arteries b. Veins 2. ↓motility of GIT smooth muscle cells			
α1 (postsynaptic)		**β1 (postsynaptic)**	**β2 (postsynaptic)**
Gq protein coupled Activates **Phospholipase C** **PIP2 → IP3 + DAG**		**Gs protein** coupled Activates **Adenyl Cyclase** **ATP → cAMP**	
1. Vasoconstriction of blood vessels of a. Skin b. GIT c. Kidney d. Brain 2. Contraction of smooth muscles of a. Ureter b. Vas deferens c. Urethral sphincter d. Uterus e. Ciliary body (mydriasis) 3. Glucose metabolism a. Gluconeogenesis b. Glycolysis		1. The heart a. Heart rate (+ chronotropic) b. Impulse conduction (+dromotropic) c. Contraction (+ inotropic) d. Ejection fraction 2. Renin release by Juxtaglomerular cells 3. Hunger a. Ghrelin release by stomach	1. Smooth muscle relaxation of a. Bronchus b. Bronchioles c. Detrusor muscle d. Uterine muscle 2. Contraction of urethral sphincter 3. Renin release by Juxtaglomerular cells 4. Glucose metabolism a. Inhibits insulin release b. Stimulate I. Gluconeogenesis II. Glycolysis 5. Lipolysis 6. Thickened salivary secretion

Receptor affinity

- alpha 1 : NAd> Ad
- beta 1 : Ad= NAd
- beta 2: Ad >> NAd
- at low Ad concentrations the beta 2 receptor will be occupied because these receptors have a higher affinity for Ad (causing relaxation)
- at high Ad concentrations alpha 1 receptor will be occupied: because there are more of these receptors the predominant effect at high Ad conc is vascular smooth muscle contraction even though beta 2 receptors will also be occupied by Ad
- within a tissue the most numerous receptor type is likely to dominate the response to a given ligand
- however, if there are multiple receptor sub types and more than one related ligand it is likely that receptor affinity and ligand conc will dictate outcome