

Pharmacology notes

Lecture 1: Drug discovery strategies

- Modern drug discovery and development is rational (people set out to discover drugs-not serendipity anymore), coordinated (projects that have to be managed) and is multidisciplinary (biological and chemical sciences, economics and management of intellectual property)
- The timeline for drug discovery and development starts up with **basic research** where they start with 10000-30000 compounds for **synthesis, examination and screening** and then a select few of these are selected (10-20) for the **development stage** where there is **preclinical testing** on animals and then the number of compounds is further reduced as we transition into clinical trials via **phases I, II and III** until there is one drug which undergoes the process of **introduction and registration** for **phase IV** clinical trials which involves **product surveillance**. This process takes on average 10-12 years but varies. There are therefore 3 main phases' **discovery, development** (non-clinical and clinical) and **approval and clinical use**.
- Drug discovery takes place in **industry** (multinational pharmaceutical companies and biotechnology companies) which take part in **applied drug discovery research** (main reason is to discover drugs). Drug discovery also takes place in **academia** (university and research institutes) which undertake **basic research** that underpins the drug discovery process. Drug discovery is multidisciplinary from people such as chemists, pharmacologists, structural biologists, biochemists and so on.
- Drug discovery and development takes time (10-12 years if all goes well), it's risky (10s of 1000s of compounds that need to be searched through to find the therapeutic drug and this one drug could fall over at even a very late stage which is risky, can lose money and time) and it's very risky (10s to 100s of millions of dollars spent per new drug) with at least half of the time of drug discovery spent on clinical testing.
- The framework for drug discovery is to start with a disease and choose a target for the disease (a receptor or enzyme involved in the pathogenesis of the condition) and then we look for a **hit** (molecule that will interact with the target) and then we optimise the hit (making molecule better than the hit) and then we select a lead to take the drug through non-clinical and clinical development. There are many things that can impinge on this process, science and economics can have a role in the chosen disease, background science has a role in understanding roles of targets in disease and we have screening and design processes for optimising the hit.
- When choosing a disease there are scientific and economic issues. We would want to recoup the costs of developing the drug such that it's sustainable such that we can make more drugs. The ideal disease for drug discovery therefore has a lot of patients and someone that is able to pay for treatment (Rich patients and rich governments). This thus determines the types of diseases that are chosen. For example, we have multiple effective drugs to treat HIV/AIDS to economically privileged patients while there are only a few effective drugs to treat malaria (as most patients are in less economically well-off countries-lower drive to find pharmaceuticals for malaria).
- When a molecular target is found, we need to question what compounds are tested, how to find the hits and how to optimise the hits and come up with a lead. There are two main ways to base research around a target, we can have **compound centred research** where the compound is identified, its biology is explored and the properties are refined or we can have **target-centred discovery** where the target is identified, molecules are sought that interact with the target and its properties are refined.
- The things we would want to optimise of the hit include its potency, selectivity, metabolic stability, pharmacokinetic behaviour and toxicity/safety. We need to run assays for optimisation that will reflect these changes.
- The rule called **Lipinski's rule of five** says that in general for an orally active drug, it has no more than 5 H-bond donors (OH and NH groups), no more than 10 H-bond acceptors (O or N), a molecular weight under 500 and a **log P** under 5 (measure of hydrophobicity). It's a set of loose guidelines for orally bioavailable drugs (not always accurate).
- The **nonclinical development** of a lead (sometimes called preclinical-but doesn't always occur before clinical) and is when we take a lead and examine its pharmacological properties. This occurs

sometimes in vitro (such as receptor binding, cellular function and enzymatic stability) and mainly occurs in vivo (such as in an animal model of disease, pharmacokinetics-ADME-absorption, distribution, metabolism and elimination, and safety pharmacology of the drug).

- The animal models of disease are where we induce a disease or disease-like state in animals (animals don't spontaneously get human disease) such that the disease must resemble the human disease in symptoms, severity and progression and there are a range of ethical issues involved in that. The nonclinical drug testing needs to demonstrate its efficacy (works) and toxicity (safety) in animals before testing occurs in humans.
- The **clinical development** (clinical trials) of a lead is the final stage of drug discovery/development. It's the most expensive part of drug development and accounts for half the time and the risk of failure needs to be reduced (as most expensive) through judicious selection of a lead. The lead is assessed in formal clinical trials.
- The clinical drug trials are experiments in humans that are used to determine the effectiveness and safety of drugs and are carried out according to international standards (hard to get agreement to international standards) of **Good Clinical Practice** (World Medical Association's Declaration of Helsinki developed in response to post WWII Nuremberg trials where the rights, safety and well-being of patients being paramount).
- A drug that is successful in phase III clinical trials can be submitted to regulatory authority for approval (in USA it's to the FDA- food and drug administration, in Europe it's to the EMEA- European Medicines Agency and in Australia it's to the TGA- Therapeutics Goods Administration).
- Drug discovery is messy with many problems arising along the framework that could affect drug development.
- For a rational drug discovery program, clinical trials are required prior to regulatory approval.

Lecture 2: GPCRs signalling

- The basic features of signal transduction is that there is an external stimulus (first messenger) which binds with a membrane receptor which sends a signal via a transducer which goes to the effector enzyme which produces a second messenger which will then go on to cytoplasmic and nuclear effects (normally a cascade) leading to cellular responses. First messenger signalling molecules can be from the same cell (**autocrine**), from a neighbouring cell (**paracrine**) or from a distant cell (**endocrine**).
- The GPCRs are named based on the ability to couple to **G-proteins** (transducers) for the generation of second messengers. The GPCRs are the largest, most ubiquitous and most versatile of membrane receptors (play various roles in the body) and are also the most common target of therapeutic drugs. There are many different drugs that target a GPCR such as the angiotensin receptor for treatment of hypertension. Despite the GPCRs being the largest drug target family, only 26% of drugs target a GPCR with the types of GPCRs being targeting not being very variable, we have a lot of GPCRs that are likely targets but are not targeted by drugs. This is due to there being poor compounds produced from drug screening efforts (High throughput screening) and also due to a lack of structural knowledge of the GPCRs (difficult to work with as GPCR falls apart if we try to isolate it- hard to do structural analysis).
- The **β₂ adrenergic receptor** receives the endogenous catecholamines (adrenaline/noradrenaline). It has an extracellular N-terminal domain, 7 transmembrane domains, intracellular and extracellular loops and an intracellular C-terminal tail. It's coupled to the **heterotrimeric G protein** consisting of **α**, **β** and **γ** subunits. The ligand binds within the transmembrane domain.
- Although GPCRs are named based on the ability to couple to G proteins, there are many new members of this family that have been classified based on phylogeny or structural similarity and haven't been shown to be able to couple to G-proteins. Also importantly, members that do couple to G proteins can couple to non-G protein dependant second messenger generating pathways. Thus they are commonly called **seven transmembrane receptors** (7TMs).

- The **rhodopsin GPCR** is the first one cloned (as it is present in massive quantities in the eye). The rhodopsin is activated by light and turn on signalling pathways that lead to vision (responsible for night vision), light causes the protein to change its conformation and generate a second messenger.
- There are 5 main families of GPCRs: **rhodopsin, secretin, adhesion, glutamate** and **frizzled/taste2**. These families are based on phylogeny and genetic sequence homology which often relates to the function of the protein. The rhodopsin family is massive and is the largest one. Much of taste and smell comes from GPCRs.
- The **rhodopsin family** is grouped into 3 different functional categories. There is **group 1a** which contains GPCRs for small ligands including rhodopsin and β -adrenergic receptors which have binding sites in the transmembrane bundle of the receptor. There is **group 1b** which contains receptors for peptides (small proteins) whose binding site includes the N-terminal domain, the extracellular loops and the superior parts of the TMs (bind with the extracellular domain with a certain transmembrane component) and **group 1c** contains GPCRs for glycoprotein hormones, R-spondins and relaxin peptides and have a large extracellular binding domain for the large protein ligands.
- The **secretin family** show a similar morphology to the group 1c GPCRs but don't share any sequence homology. They have a large N-terminal extracellular domain containing 6 conserved **cysteine residues**. Its ligands include secretin, calcitonin, PTH and VIP (various peptide hormones). All these receptors couple to the Gs type of G-protein and only some members of the family interact with **chaperone proteins** that are called **RAMPs** (receptor activity modifying proteins) which are separate single transmembrane proteins that are expressed in particular cells. When RAMP associates with the secretin receptor at the cell surface it changes the ligands affinity to the GPCR which allows for improved diversity of GPCR targeting.
- The **adhesion** family has large extracellular domains with various different types and structures of the extracellular domains. The adhesion-GPCR nomenclature was proposed to reflect potential dual roles in cellular adhesion and cell signalling (the extracellular domains all have classic protein binding motifs within them). The adhesion name is justified as they bind to a variety of extracellular matrix molecules, however the native ligands for most of the receptors are still unknown. Although the receptors can couple to G-proteins, there is limited information on their signalling as they are difficult to study. The potential role of this family is in cell growth and the immune system which makes it an important family for future drug development.
- The **glutamate family** contain the mammalian glutamate receptors (mGluRs), the Ca^{2+} sensing receptors, the γ -aminobutyric acid GABA-B receptor and sweet and umami receptors. They have a classic extracellular domain (**venus fly trap domain**) which binds to small ligands (glutamate, calcium, GABA and taste ligands).
- The **Frizzled/Taste 2** receptors are very unique. There are 10 frizzled receptors (FZD1-10) which are receptors for the **Wnt lipoglycoproteins** that are critically involved in embryogenesis. They can couple to G-proteins but also signal via the β -catenin pathway (G-protein independent pathway). The **smoothened receptor (SMO)** seems to function in a ligand-independent manner at the signalling unit is in the patched, **sonic hedgehog (SHH)** and **SMO complex**. The **taste2** receptors contain 25 bitter taste receptors (the bitter sensation of **saccharin** has been associated with the activation of T2R43 and T2R44).
- The **orphan receptors** have been identified but have no known ligands. They are found in the rhodopsin, adhesion and glutamate family but they are part of these families based on sequence homology. There is a major focus from drug companies to understand these orphan receptors to see if they have a role in combating disease.
- There is a diversity of GPCRs and ligands with there being over 800 GPCRs and thus they have major roles in the body. There is also diversity of the G-proteins on the intracellular sides of the receptors.
- The classic structure of the heterotrimeric G-protein consists of an α , β and γ subunit with a **GDP** molecule bound to the α subunit which is important for the function of the protein.
- The basic mechanism of heterotrimeric G-protein interaction with GPCRs is normally the same but they utilise different types of G-proteins and therefore different effector proteins. When the extracellular ligand binds to the receptor, it shifts the equilibrium of receptor conformations towards

the active conformations (proteins are not static, they are dynamic-move around at physiological temperatures). This active conformation allows it to bind to the G-protein through an unmasking of the G-protein binding sites. This binding event will cause another conformational change and therefore causes the GDP from the G-protein to dissociate and a **GTP molecule** then binds to the $G\alpha$ subunit (GTP intracellular concentration is much more than the GDP). The ligand bound GPCRs are called **guanine nucleotide exchange factors (GEFs)**. The binding of GTP results in the dissociation of the heterotrimeric complex ($\beta\gamma$ goes off while the $G\alpha$ stays associated with the membrane). The $G\alpha$ with the GTP bound will bind to the effector protein in the membrane to induce signalling (modulate production of second messenger by switching effector on or off) while the $\beta\gamma$ subunit can also independently signal. Binding to the effector protein by $G\alpha$ will turn on the intrinsic **GTPase activity** of the $G\alpha$ subunit ($G\alpha$ itself is an enzyme that hydrolyses GTP) and thus GTP is hydrolysed to GDP. The $G\alpha$ will then dissociate from the effector and the signalling will be halted and the G-protein heterotrimeric complex will reform which is ready to initiate another signalling cascade if the ligand is still bound to the GPCR. These signalling processes are inherently regulated (have to be switched on or off), there are very few systems that are basally active or inactive (dynamic) all the time.

- The **second messengers** are molecules whose presence is a signal and are synthesised or released from storage. They act as intracellular ligands (initiate a chemical cascade). They are commonly used in GPCR signalling (but also other types of signalling) and are made or released by effector proteins with the ligand for the GPCR being the first messenger.
- The GTP (**guanosine triphosphate**) is at a much larger concentration than GDP (**guanosine diphosphate**).
- The GPCRs are normally in a basal state that is unlinked to G-protein but can become active without ligand (some receptors can do this more than other- **constitutively active**-locked in active conformations- can be found in mutant receptors- seen in cancers) and binding of the agonist will shift the equilibrium towards the active conformations (multiple conformations possible) while an **inverse agonist** will shift the equilibrium towards the inactive conformations.
- A **full agonist** leads to 100% biological response (fully active conformation of the receptor), a **partial agonist** produces active conformations but can't get to the maximal response, a **neutral antagonist** (rare- normally antagonists are inverse agonists) binds to the receptor and blocks the ability for an agonist to bind but doesn't influence conformational change away from the basal state while the **inverse agonist** will shift the equilibrium of the receptor towards the inactive conformations (see a decrease in response if there is basal activity in the system).
- The GPCR signalling system is very sensitive, the amount of receptor on the cell surface is extremely low and so is the concentration of ligand. There is thus **signal amplification** which can occur at multiple levels. One ligand-receptor complex can activate several G-proteins in turn (time that ligand sits there can determine how many G-proteins are activated), the G-protein can remain associated with the effector for a longer time and thus produces multiple second messengers (self-limiting due to intrinsic GTPase activity of the $G\alpha$ subunit) and there are multiple points within the signalling cascade after the second messenger where signal amplification can occur.
- There are many different types of G proteins such as the **G α s**, **G α i/G α o**, **G α q/G α 11** and **G α 12/G α 13**. There is thus a diversity of expression, subtypes and receptor affinity. The signalling diversity results from different combinations of $G\alpha$ and $\beta\gamma$ which lead to different cellular responses. This diversity is seen in the thyroid gland which activated by TSH involved for thyroid hormone synthesis and secretion while there are other roles in other areas of the body. In the heart, adrenaline will act to increase heart rate and force of contraction (cardiac output).
- A single agonist such as adrenaline can activate multiple G-proteins through different receptors and hence mediate multiple functions within the body. Adrenaline and noradrenaline activate three classes of adrenergic receptors. The **α 1 receptors** couple to **G α q** for vasoconstriction while the **α 2 receptors** couple to **G α i** for inhibitory function. The β receptors (β 1 in the heart, β 2 and β 3 in fat) couple to **G α s**.
- The **G α q** protein binds to **phospholipase C** which is bound to the membrane. This leads to the generation of two second messengers (the **inositol-phospholipid pathway**) **DAG** and **IP3**. What

happens is that phospholipase C catalyses the breakdown of **PIP2** into DAG (diacylglycerol) and IP3 (inositol triphosphate).

- The **IP3** is released into the cellular cytosol and acts on a specific receptor (the **IP3 receptor**) which is a ligand gated ion channel present on the membrane of the **endoplasmic reticulum**. IP3 hence controls the release of Ca^{2+} from intracellular stores. **DAG** will act to activate a membrane bound protein kinase (**protein kinase C**).
- A kinase is a protein that phosphorylates another protein, while a phosphatase will dephosphorylate a protein thus having effects on the activity of the various protein that has been affected. It is a common mechanism of signalling protein regulation.
- There are at least 12 different PKC subtypes which have distinct cellular distributions and phosphorylate different proteins (**serine-threonine kinases**) and consist of a regulatory and catalytic domain. The **conventional PKC** isoforms are activated by Ca^{2+} and DAG while the **unconventional** isoforms don't require Ca^{2+} . The **atypical isoforms** don't require DAG. The **C1 domain** of PKC binds DAG while the **C2 domain** binds Ca^{2+} . Domains C3 and C4 contain the ATP and substrate binding regions of the kinase core.
- Upon PLC activation there is generation of DAG (Activates PKC which phosphorylates cellular proteins) and IP3 (regulates ER channels to release Ca^{2+}).
- The ion **Ca^{2+}** is a very important second messenger. The cytosolic calcium concentrations are normally kept low while those in the extracellular space and ER are very high (200,000 fold difference in concentration-ability to rapidly change ICF calcium levels). The opening of the channels causes Ca^{2+} to rush into the cell leading to the activation of calcium dependent signals (Fast signalling). There are two major calcium pathways. Firstly in electrically excitable cells, calcium carries a charge and thus contributes to changes of membrane potential in excitable cells and in all other cells it acts a biological messenger by directly binding to proteins and modifying their functional state. Proteins directly or indirectly affected by calcium are very diverse and include enzymes, cytoskeletal proteins, ion channels and transcription factors.
- Calcium enters the cell through voltage or ligand gated channels. It is extruded rapidly by ATP-driven pumps or by sodium antiporters and hence the calcium levels are dynamically regulated. Both the mitochondria and ER serve as intracellular calcium stores.
- Ligand binding induces transient Ca^{2+} signalling in cells.
- In the classic Gq mediated pathway, angiotensin II binds to the **angiotensin type 1 receptor (AT1)** which causes Gq activation and therefore PLC activation to convert PIP2 into DAG and IP3 which will then cause a rise in Ca^{2+} and therefore contraction (through calcium activating proteins involved in contraction) leading to a rise in blood pressure. A hypertensive drug will target the AT1 receptor and doesn't let angiotensin to bind (antagonist).
- We can use Ca^{2+} signalling as a readout (it's easily measured in high-throughput format), it's easy to measure quick changes in intracellular calcium and therefore good to determine if a drug is activating a particular receptor. The pharmaceutical companies will have a bank of cells that express certain GPCRs such that they can test drugs against different receptors. This is seen in the **FLIPR machine** where we see the fluorescence from calcium to indicate that the drug if it increases calcium levels is an agonist and if it decreases calcium levels (less fluorescence) it is an antagonist. We can also examine the effect of allosteric modulators through this mechanism (allosteric modulators are important as don't want to completely block or activate the pathway) where an allosteric modulator can have positive or negative effects.

Lecture 3: GPCRs 2

- The **G α s** pathway is the first one to be identified. The Gs protein stimulates **adenylate cyclase** which causes the production of **cAMP** from **ATP**.
- The **adenylate cyclase** protein is a transmembrane protein with 12 (2 subunits of 6 transmembrane domains) transmembrane domains. It has 2 catalytic domains (**1a** and **2a**) that produce cAMP from ATP (removal of diphosphate group) and adenylyl cyclase has at least 8 mammalian isoforms. 1 G-

protein activates 1 adenylate cyclase however 1 cyclase will produce 100-1000 cAMP molecules (signal amplification- as G-protein is bound for a significant period of time to adenylate cyclase).

- An example of a Gs coupled receptor is the **relaxin receptor (RXFP1)** which is part of the rhodopsin class (**1c**) and responds to the endogenous ligand relaxin. There are multiple different types of ligands with the **H2 relaxin** being more potent than **H3** (has a higher pEC50- less drug required for 50% maximal response) but these two ligands have the same efficacy (produce same maximal agonist response).
- The intracellular messenger **cAMP** regulates ion channels (can increase calcium). This is seen in the calcium dependent signalling of the β -adrenergic receptor. This receptor activates adenylate cyclase and cAMP activates **protein kinase A**. In heart muscle, PKA will phosphorylate several Ca^{2+} transporters and channels so that the amount of Ca^{2+} available for contraction is increased. It acts on **phospholamban** to decrease calcium uptake into the ER via **SERCA** as well as activating the **ryanodine receptors** to secrete calcium into the ICF from the ER.
- The cAMP will regulate **protein kinase A** which phosphorylates many substrates with these substrates determining the effects seen in the cell such as increased glycogen breakdown, increased cardiac beat strength etc. Protein kinase A will mediate the effects of cAMP. When inactive it has 2 **regulatory subunits** and 2 **inactive catalytic subunits** where upon binding to cAMP onto the regulatory subunits, the catalytic subunits dissociate and are activated. The cAMP will activate PKA which catalyses the transfer of terminal phosphate from ATP to specific serine and threonines of selected proteins. Covalent phosphorylation of appropriate amino acids will regulate the activity of target proteins with serine-threonine phosphatases revering the effects of PKA. The substrates of PKA include **adipocyte lipolysis enzymes, hepatic glycolysis enzymes, cardiac calcium channels**, some **GPCRs** (PKA mediates desensitisation), cAMP response element binding protein-**CREB** (modulates gene transcription) and many others.
- Upon activation of PKA by cAMP, it can go translocate into the nucleus to phosphorylate transcription factors (such as CREB). CREB is inactive until it's phosphorylated such that it binds with other cofactors on specific promoter regions on genes that are cAMP responsive mediating transcription of those genes.
- We see signal amplification where 1 G-protein activates 1 adenylate cyclase and 1 cyclase will produce 100-1000 cAMP molecules. Then each catalytic subunit of PKA catalyses phosphorylation of many proteins during the life-time of the cAMP for further signal amplification. We can see multiple actions of PKA on energy metabolism in the liver where it can cause increased levels of lipolysis and glycogen breakdown while there is also a reduced glycogen synthesis (inactivating glycogen synthase). These mechanisms are caused by kinase cascades that happen in the cell.
- The cAMP levels are dynamically regulated by **cAMP phosphodiesterases** where it's degraded to AMP.
- There are 6 different phosphodiesterase families which have differing affinity for its substrates and selectivity between cAMP and cGMP as well as having differing tissue and cellular distribution. Specific phosphodiesterase inhibitors are important potential drugs or drugs that modulate the signalling pathways themselves. May want to downregulate or upregulate phosphodiesterase activity to control amount of cAMP in the cell if there is an over or under-stimulation of the certain GPCRs. Phosphodiesterase inhibitors include **xanthines** (non-selective) such as caffeine (not its only action) and **theophylline** (used for respiratory disease as it contracts bronchial smooth muscle). There is also **milrinone** which is a phosphodiesterase-3 inhibitor that improves haemodynamics and biventricular function in patients with ventricular dysfunction independent of β_1 -adrenergic receptor stimulation. The classic PDE inhibitor is **sildenafil** (Viagra) which inhibits cGMP breakdown by phosphodiesterase enzymes and thus improves erectile function.
- The **Gi protein** inhibits adenylate cyclase and activates K^+ channels (hyperpolarising effects) and inhibits Ca^{2+} channels. They do everything to counter Gs signalling. The Gi also includes **transducin** which activates cGMP phosphodiesterase (rhodopsin).
- Ligand activation of a Gi coupled GPCR is that binding of Gi to adenylate cyclase will inhibit the protein and hence cAMP production. The Gi and Gs proteins bind in regions close to each other on the

catalytic subunits of adenylate cyclase. Gs binds to the **cytoplasmic domain 2a** of adenylate cyclase while Gi binds to **cytoplasmic domain 1a**. The Gi therefore binds in a non-competitive manner, hence it doesn't compete with Gs binding but as long as Gi is bound the adenylate cyclase will stay inactive.

- The differential actions of Gs and Gi are seen in the autonomic stimulation of heart rate. Activation by the sympathetic nervous system exerts an excitatory action on the heart through catecholamines (noradrenaline or adrenaline) binding to the β_1 receptor which is coupled to Gs while parasympathetic activation (**vagus nerve**) exerts an inhibitory action on the heart through acetylcholine binding to the **muscarinic (M2)** receptor which is coupled to Gi. This differential action is also seen in pituitary endocrine cell survival where we see Gs stimulated pathways from GnRH, TRH, CRH and GnRH which elicits stimulatory signals while SST (somatostatin) and D2R are linked to the Gi pathway for inhibitory signals.
- There are bacterial toxins that modulate G-proteins. These toxins are enzymes that catalyse a conjugation reaction (**ADP-ribosylation**- transfer of ADP-ribose group from NAD⁺ onto amino acid residues in their substrate protein which alters their function) on the G α -subunit of the heterotrimeric complex.
- The **vibrio cholera** bacteria colonises the GIT and secretes **cholera toxin** which acts on cells in the GIT leading to diarrhoea. This occurs as the toxin ADP-ribosylates an arginine residue at the GTPase active site of Gs. The ADP-ribosylation prevents GTP hydrolysis by Gs and hence Gs is locked in its active GTP-bound form, thereby continually stimulating adenylate cyclase to produce cAMP. This causes massive fluid and electrolyte efflux resulting in diarrhoea. The GTP bound to Gs stays bound and continues to stimulate cAMP.
- The **Bordetella pertussis** is a very small Gram-negative aerobic bacteria that colonises the cilia of the mammalian respiratory epithelium and causes whooping cough. Whooping cough is a relatively mild disease in adults but has a significant mortality rate in infants. Until immunisation was introduced in the 30s, whooping cough was one of the most frequent and severe diseases of infants in the US (quite prevalent in Australia again). The pertussis toxin ADP-ribosylates Gi at a cysteine residue making the Gi protein incapable of exchanging GDP for GTP and hence will remain in the GDP bound state (inactive), therefore there will be an inability to inactivate adenylate cyclase or open potassium channels resulting in excess fluid in the lungs and a lot of coughing.
- The **Coleus forskohlii** is a perennially plant with a strong, camphor-like odour. It's in the mint family and grows well in warm temperate or subtropical areas. The plant is native to areas of India, Myanmar, Nepal and Sri Lanka where it's been used in traditional medicine. This plant secretes **forskolin toxin** which activates adenylate cyclase. Forskolin is a small lipid-soluble molecule that binds directly to the catalytic subunit of adenylate cyclase and stimulates cAMP production independently of the G-protein.
- GPCR signalling can be switched off (GPCR signalling is a dynamic process-want to regulate it). We can have **GPCR desensitisation** which is the waning of a response to a continuous or repeated stimulus and it's a protective mechanism where there is a turning off of the receptor-mediated signal transduction pathways. There are two mechanisms of GPCR desensitisation, there is the short term mechanism of **receptor phosphorylation and internalisation** and there is the long term mechanism of **GPCR down-regulation** (mediated at the gene level and leads to a decrease in total receptor number). GPCR downregulation is observed with medium to long term (hours to days) exposure to agonist and results in decreased synthesis and/or degradation of receptor mRNA.
- Short term desensitisation by phosphorylation and internalisation can be either homologous or heterologous. **Homologous desensitisation** is when there is a loss of cellular response to a given agonist following stimulation by that agonist alone and **heterologous desensitisation** is where there is a loss of cellular response to a given agonist following stimulation by a different agonist.
- In **homologous desensitisation** a ligand will bind to the GPCR causing activation and the G-protein cascade begins. The GPCRs will be phosphorylated by **GRKs** (G-protein coupled receptor kinases) which recognise the active site of the receptor and phosphorylates the agonist-occupied, or activated conformation of the GPCR normally on the C-terminal tail of the receptor (stopping G protein association with the receptor).

- In **heterologous desensitisation** there is a ligand bound to the GPCR and then another ligand comes along and binds to the receptor, activating **second messenger dependent kinases** (PKA or PKC). These kinases can phosphorylate the receptors in the absence of agonist switching off the receptor.
- The phosphorylation of the GPCRs occur on the serine or threonine residues in the C-terminal tail.
- The GRKs phosphorylate GPCRs only when the receptors are activated (agonist-occupied, homologous desensitisation). The GRKs interact with the receptors at sites distal from the sites of phosphorylation with GRK phosphorylation leading to the recruitment of **arrestins**.
- The arrestin proteins bind to the phosphorylated GPCRs. There are 4 families of mammalian arrestin proteins that have been identified and cloned. 2 arrestins (**visual arrestins**) have been localised to the retina while there are 2 other members (β arrestin-1 and β arrestin-2 which mediate the desensitisation of the GPCRs). The arrestins are composed of 410 amino acids with an N-terminal domain that interacts with the phosphorylated GPCRs and a C-terminal domain that is the regulatory region that interacts with **clathrin/AP2** and other proteins (G-independent signalling). Both these domains are separated by a hinge. Arrestins have important roles in internalisation and desensitisation of the GPCRs as well as with being signalling platforms in their own right.
- Internalisation of a GPCR is as follows. The GPCR will be activated and signal, causing GRK/second messenger dependent kinase to phosphorylate the GPCR causing G protein to dissociate and then β -arrestin is recruited and clathrin is then recruited. Clathrin causes a **dynamin mediated internalisation** where there is a budding off of the complex leading to the receptor being internalised into the cell and then goes into **endosomes** for either degradation or dephosphorylated and recycled. This can be visualised experimentally by adding GFP to β -arrestin.
- Latest studies suggest that there is some G protein signalling when the GPCRs are internalised where a whole new cascade of G protein signalling can occur. A paper revealed a discrete component of the acute signalling response was initiated from endosomes.
- G protein independent signalling also can occur in endosomes. B-arrestin can act as a scaffold for G-protein independent signalling. There is thus many different temporal differences in the GPCR action, with each GPCR having an ability to interact with multiple types of G proteins.
- Both the G-protein independent and dependent signalling while leading to secondary signalling (and increased signalling diversity) are still transient with the GPCR still being switched off and degraded or recycled.
- There are drugs that target the β -arrestin pathways that are in clinical development. We have drugs that are in phase II trials that target the AT1 receptor for heart failure treatment through a **β -arrestin biased ligand** (won't activated G pathways) with the β -arrestin will cause an increased cardiac contractility and a decreased cardiac cell apoptosis.