Insulin and Glucagon in regulating blood glucose levels

Describe how the effects of glucagon are achieved in the liver cell. (10)

- Ligand-receptor interactions:

1. Hormone **glucagon** binds to a **G-protein linked receptor** (GPLR) present on the cell surface membrane of liver cells
2. Ligand-receptor interaction results in formation of a **hormone-receptor complex**
3. Glucagon is the first messenger that triggers the subsequent intracellular signalling events in the target cell

- Signal Transduction:

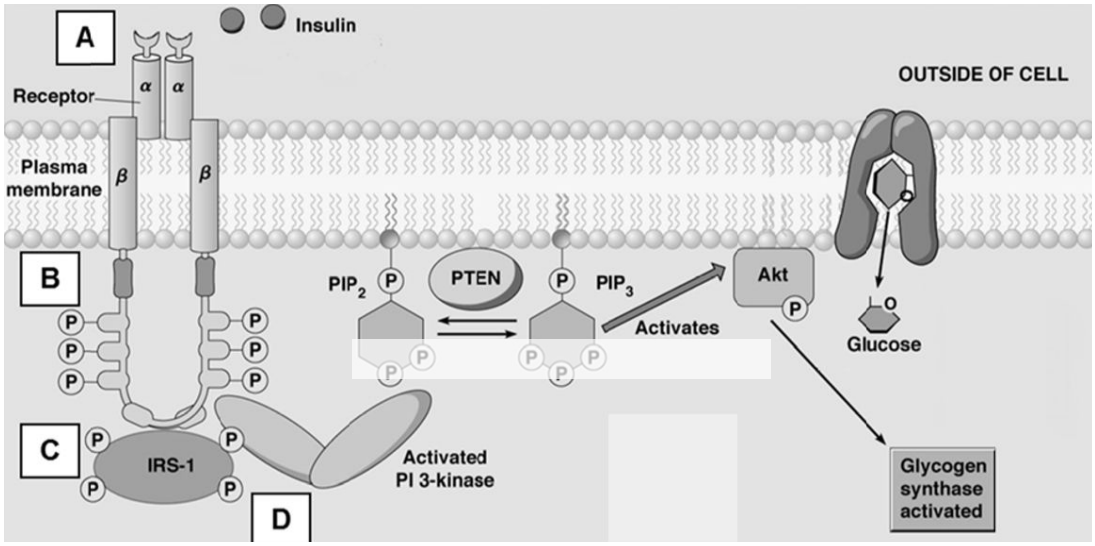
1. Binding of glucagon triggers a conformational change in GPLR, activating it
2. Activated GPLR binds to and activates **G protein** (found on the cytoplasmic side of the membrane) by replacing the GDP to a GTP (+ $\mathbf{G_a}$ subunit dissociates from β and γ subunits)
3. Activated G protein diffuses along the plasma membrane to bind to and activate **adenylyl cyclase** which in turn catalyses the conversion of many **cyclic AMP** (cAMP) molecules from **ATP**
4. cAMP acts as a second messenger (within the liver cell) to activate enzymes such as **protein kinase A**
5. protein kinase A activate **other enzymes** by phosphorylating them, triggering a **phosphorylation cascade** that serves to amplify the initial signal

- Cellular response:

1. (phosphorylation cascade) finally leads to activation of **glycogen phosphorylase** which is needed for the breakdown of glycogen stored in liver cells to glucose molecules
2. thereby bringing blood glucose levels back to the norm

Describe how regulation of blood glucose level in humans involves cAMP. (5)

1. **α cells** detects low blood glucose level & secretes **glucagon** into bloodstream
2. **Glucagon** binds to **GPLR** at the cell surface membrane, activates **GPLR**
3. Activated **GPLR** binds to **G protein**, causes **GTP** to replace **GDP**, activates **G-protein**
4. Activated G-protein activates **adenylyl cyclase** which converts **ATP** to **cAMP**
5. **cAMP** acts as second messenger, activates **protein kinase A**, phosphorylation cascade, activates **glycogen phosphorylase**
6. Glycogen phosphorylase break down glycogen to glucose (to increase blood glucose level)



With reference to figure above, describe the events occurring in stages A to D.

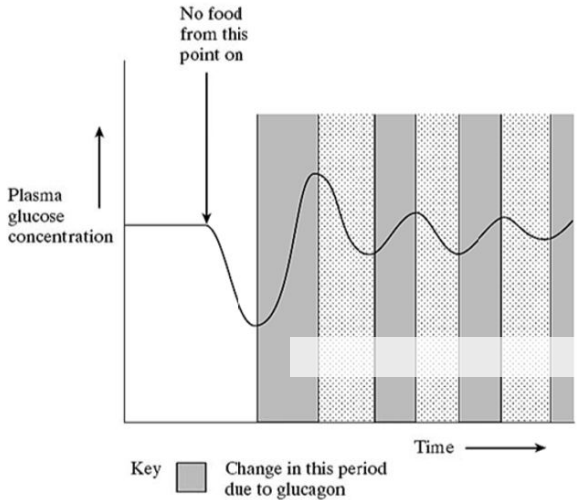
- insulin receptor **exists as a dimer** *prior* to the binding of insulin
- insulin **bind** to insulin receptor
- ref. **complementary in shape** to the ligand binding site of the insulin receptor
- results in **activation of** tyrosine kinase domains in the cytoplasmic tail
- each receptor **phosphorylates** the tyrosine residues (at the cytoplasmic tails) of the other receptor, hence **activating it**
- **auto-phosphorylation**
- receptor **phosphorylates** and **activates** IRS-1
- which in turn **phosphorylates** and **activates** PI 3-kinase

Based on your knowledge, describe the events that lead to an increase in the number of glucose transporters in the cell surface membrane. (1)

- **movement** of **vesicles** containing glucose transporters (GLUT4 transporters) towards the cell surface membrane
- **fusion** of vesicle membrane with cell surface membrane results in incorporation of glucose transporters on the cell membrane

A tissue may over time loses its responsiveness to insulin, even though insulin concentration remains unchanged. Suggest possible reasons for this decrease in responsiveness. (1)

1. Mutation in the gene coding for insulin receptor/ change in conformation of insulin receptor as cell ages
2. Mutation in the gene coding for Glut4 transporters / change in shape of Glut4 transporters as cell ages
3. Fewer insulin receptors are synthesized/ ref. Lower transcription rate of gene that codes for insulin receptor
4. Fewer Glut4 transporters are synthesized/ ref. Lower transcription rate of gene that codes for Glut4 transporters

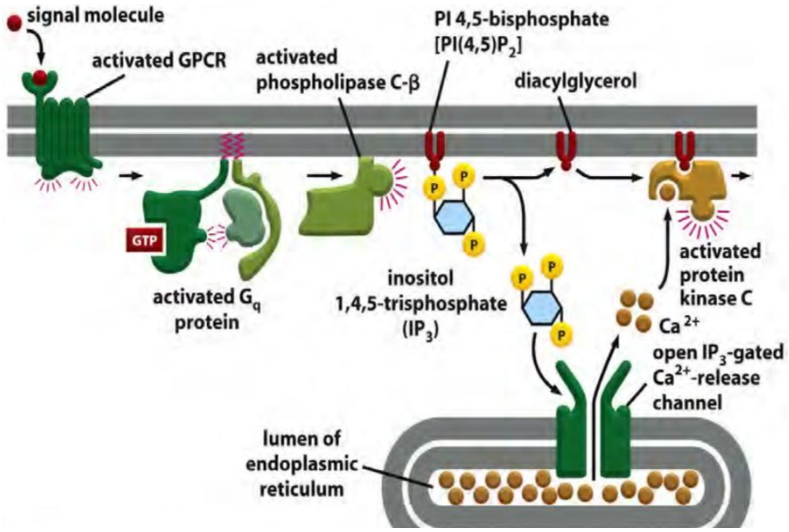


Use evidence from figure above to explain the increase in plasma glucose concentration.

No food given, thus blood glucose concentration decreases below set point of 90mg/100 ml

Detected by α-cells of islet of Langerhans in pancreas which stimulates increased secretion of glucagon into the bloodstream

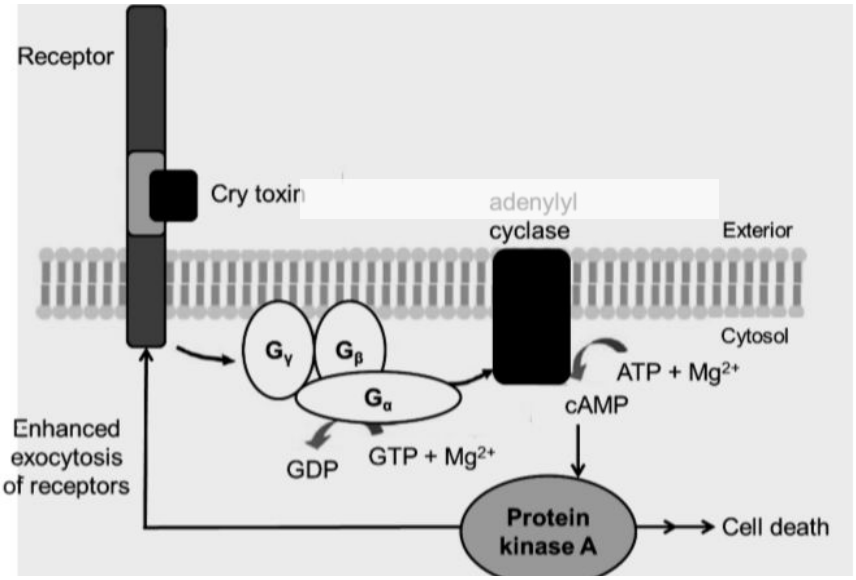
Glucagon binds to cell surface receptors of liver cells, stimulates conversion glycogen to glucose (gluconeogenesis), results in increase in blood glucose level



With ref. to fig., describe the main sequences of events that leads to the activation of protein kinase C (4)

1. **Ligand** molecule binds to **GPLR** via ligand-receptor interaction which in turn binds to and activates G-protein. G-protein is activated when it is attached to GTP nucleotide.
2. Upon activation, activated **G-protein** dissociates from the receptor, diffusing along the membrane and then binds to and activates **phospholipase C-B**.
3. **PI 4,5 bisphosphate** is cleaved into **diacylglycerol and IP3**
4. **IP3** serves as the second messenger and binds to **ligand-gated Ca^{2+} channels** in the ER membrane, causing them to open.
5. **Ca^{2+} ions** flow down concentration gradient from ER lumen to cytosol and binds to **protein kinase C**, thus activating the enzyme.

Another mechanism of action of Cry toxin in midgut epithelial cells of target insects has been proposed. Fig. 6 shows the proposed magnesium ion-dependent signalling cascade. Single-pass membrane receptors that cross the membrane only once, e.g. aminopeptidase (APN) receptors and cadherin-like receptors, are involved. Cry toxins eventually lead to destabilisation of the cytoskeleton and ion channels in the cell membrane causing cell death.



With reference to Fig 6, describe the proposed cell signalling cascade of Cry toxin.

- when **Cry toxin** binds to the **receptor**, the receptor is activated and cause the membrane-bound **G protein** to replace GDP with GTP in the presence of **Mg^{2+}** *(REJECT ref to GPLR; Receptors of Cry toxins are **not** GPLR as they only pass the membrane once unlike GPLR that are multi-pass membrane receptors which pass membrane seven times)*
- α subunit of G-protein dissociates from its β- and γ- subunits and move along the membrane to **adenylyl cyclase**
- upon binding of G_a subunit, adenylyl cyclase undergoes conformational change and catalyses conversion of **ATP** to **cyclic AMP** (cAMP) in the presence of Mg^{2+}
- **cAMP** acts as a second messenger to activate relay protein, **protein kinase A (PKA)** which phosphorylates many **downstream effector proteins and enzymes** in a phosphorylation cascade to induce incorporation of more receptors of Cry toxin at cell surface membrane and to promote cell death

NF449 is an antagonist of G protein that prevents binding of GTP. Suggest how NF449 could affect the signalling pathway of Cry toxins. (2)

- GDP of G protein not replaced by GTP, inactivating G protein and adenylyl cyclase is not activated, leading to a low level of cAMP
- PKA is inactivated causing reduced cytotoxicity of Cry toxins/ fewer cell death

Suggest why Cry toxins only kill target insects and not other organisms.

- membrane **receptors** that bind to Cry toxins are **only expressed** in the midgut epithelium cells of target insects and not that of other organisms
- conditions of gastrointestinal tract such as **pH and ion balance** that facilitate activation of Cry signalling cascade in insects are different in other organisms.

6 **Fig. 6.1** below shows the cell signalling pathway in smooth muscle cells in response to the hormone Epinephrine, resulting in smooth muscle relaxation of blood vessels (vasodilation).

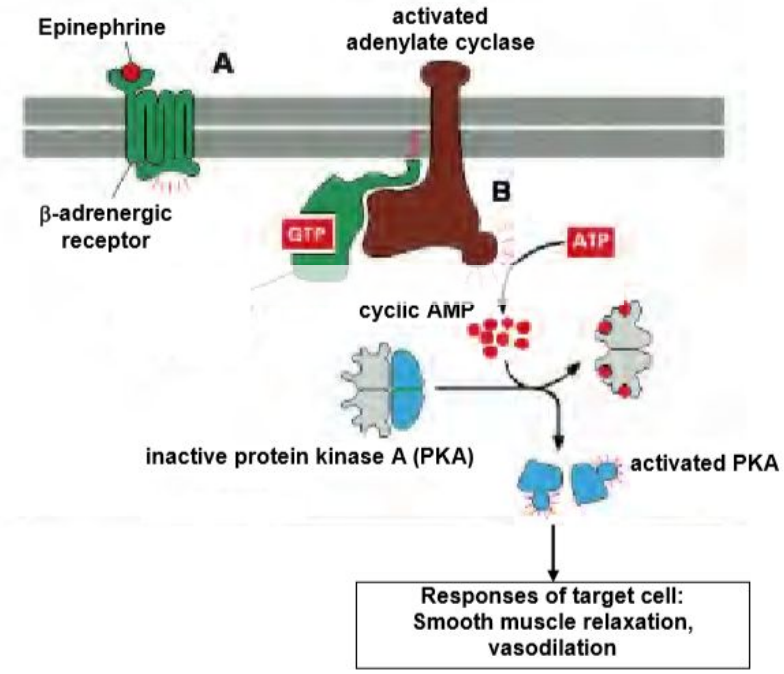


Fig. 6.1

With reference to fig 6.1,

(i) describe how adenylyl cyclase is activated.

- when epinephrine binds to the B-adrenergic receptor, a ligand-receptor complex is formed, causing the receptor to undergo a conformational change
- activated receptor bind to and activates G protein by replacing its GDP with a GTP
- the activated G-protein now translocates to bind to and activate adenylyl cyclase

(iii) describe the series of events that occur after the activation of protein kinase A that results in a cellular response. (2)

- activated **protein kinase A** passes through the nuclear pore into the nucleus
- phosphorylate **transcription factor** resulting in a conformational change/ activation thus allowing it to
- bind to a **specific regulatory DNA sequence** e.g. enhancer

Ras protein is a plasma membrane bound G-protein. Suggest how the activated G-protein can relay the signal from the ligand to activate cellular responses. (2)

- by **activating adenylyl cyclase** which will catalyse the production of second messengers, such as **cAMP**
- **cAMP will activate protein kinase A** resulting in phosphorylation cascade - activate proteins necessary for cellular responses
- recruiting **RNA polymerase** or promote assembly of **transcription initiation complex**/ result in transcription of the target gene.

Figure 5.2 below shows the sequence of events in a Ras cell cycle-stimulating pathway.

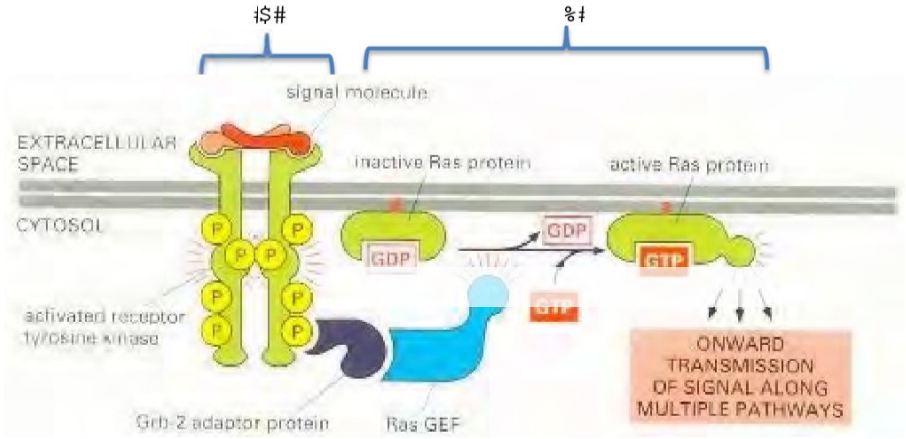


Figure 5.2

Briefly describe the stages A and B as shown in Figure 5.2. (4)

A:

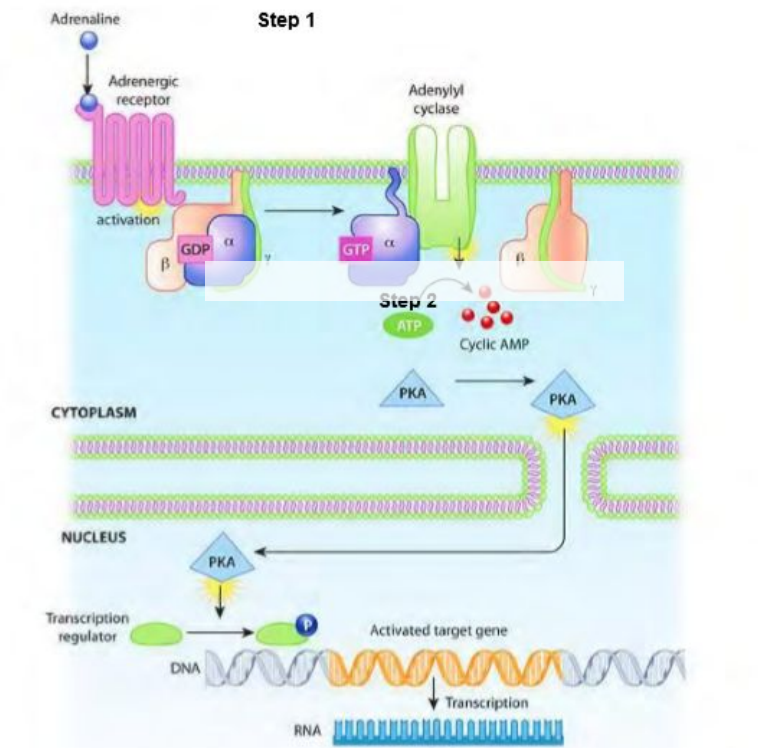
- binding of ligand molecule to the 2 receptors causes 2 receptor subunits to dimerise
- activated receptor tyrosine kinase region cross-phosphorylates tyrosine amino acids on each other's tyrosine amino acid tails (by attaching a phosphate group)
- and subsequent phosphorylation residues on their respective tail of tyrosine amino acid subunits - activates tyrosine amino acids - making them recognisable as docking sites for relay proteins

B:

- relay protein(s), Grb-2 adaptor protein recognises and binds to specific phosphorylated tyrosine(s) which acts as docking protein
- for Ras GEF, which changes conformation and becomes activated
- which activates G protein, Ras protein, by removing GDP and replacing it with GTP
- in order to activate Ras protein
- to relay signal by activating other proteins or enzymes to bring about various cellular responses (= cell proliferation)

(Other cellular responses:
Nuclear response- Regulation of the rate of transcription of genes by activating specific transcription factors (activators/silencers) E.g. stimulation of genes promoting cell division by proto-oncogenic proteins
Cytoplasmic Response- Regulation of the activity of certain proteins E.g. Adrenaline eventually triggers activation of glycogen phosphorylase which converts glycogen to glucose 1-phosphate
Cell wide responses- Apoptosis(upon extensive protein misfolding/ irreparable DNA damage); Mating (in unicellular eukaryotes))

Adrenaline is a signal molecule that stimulates similar cellular responses from liver cells and adipose cells in a way similar to that of glucagon.



Identify the stage where signal amplification is observed and explain how the signal is amplified in that stage.
- conversion of ATP to cAMP by adenylyl cyclase
- each enzyme produces many molecules of cAMP

Explain the significance of step 2 that results in effective signal transduction.
- an activated adenylyl cyclase catalyses the formation of many cAMP from many ATP
- thus amplifying the signal as
- cAMP is a second messenger that is small and diffusible and can diffuse easily in the cell to activate the next relay protein

Suggest 2 functions of signal transduction. (2)
- may transform the signal into a different form, which is suitable for passing or stimulating a cell response
- may amplify the signal it receives either by producing large amounts of a small signal intracellular mediators or by activating many copies of a downstream signalling protein

State 1 advantage and 1 disadvantage of a signalling cascade. (2)
Advantage: amplification of signalling cascade- 1 signal gives rise to multiple cellular responses
Disadvantage: prone to error: error along any part of the cascade would result in undesirable consequences

Integrin receptors are not known to have kinases associated with them. Suggest a possible problem this might present to the cell signalling pathway.
-Cannot trigger transduction directly/ requires the formation of a secondary messenger.
Outline the advantage of G-protein signalling pathway as seen in Fig 8.2

1. Glucagon is a **large, hydrophilic protein** and is unable to pass through the hydrophobic cell surface membrane to directly affect a change in its target cells.
2. However, glucagon is able to activate intracellular processes through a **second messenger** by binding to glucagon receptors on the cell surface membrane.

Explain why the signal molecule cannot act directly on the DNA in the nucleus (1)
Ligand molecule is **water soluble/ lipid-insoluble**, thus it **cannot pass through the hydrophobic lipid bilayer**/ fatty acid tails of the plasma membrane

With reference to the structure of the plasma membrane, describe how the structural features of receptor tyrosine kinase enables it to be embedded as a transmembrane protein.
- hydrophobic region of protein consist of amino acid with nonpolar R groups
- forms **hydrophobic interactions** with hydrophobic core of membrane
- consisting of non-polar fatty acid tail of phospholipid bilayer

- hydrophilic regions of protein consists of amino acids with polar R groups
- forms **hydrophilic interactions** with hydrophilic region of membrane
- consisting of polar phosphate heads of phospholipid bilayer

Fig. 2.2 shows the Ste2 receptor on another yeast cell membrane.

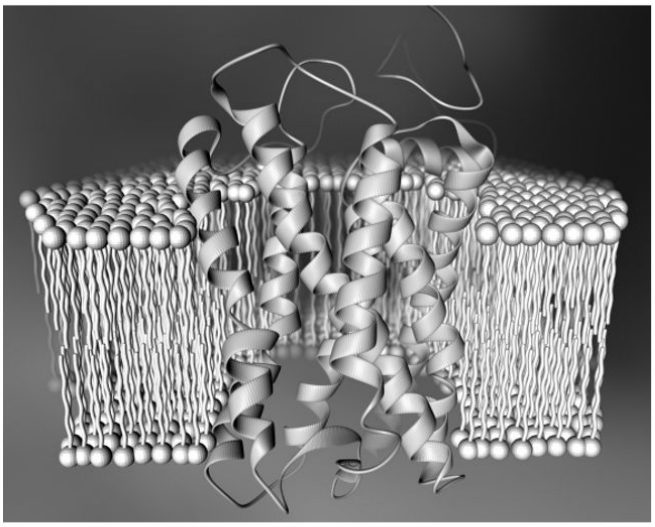


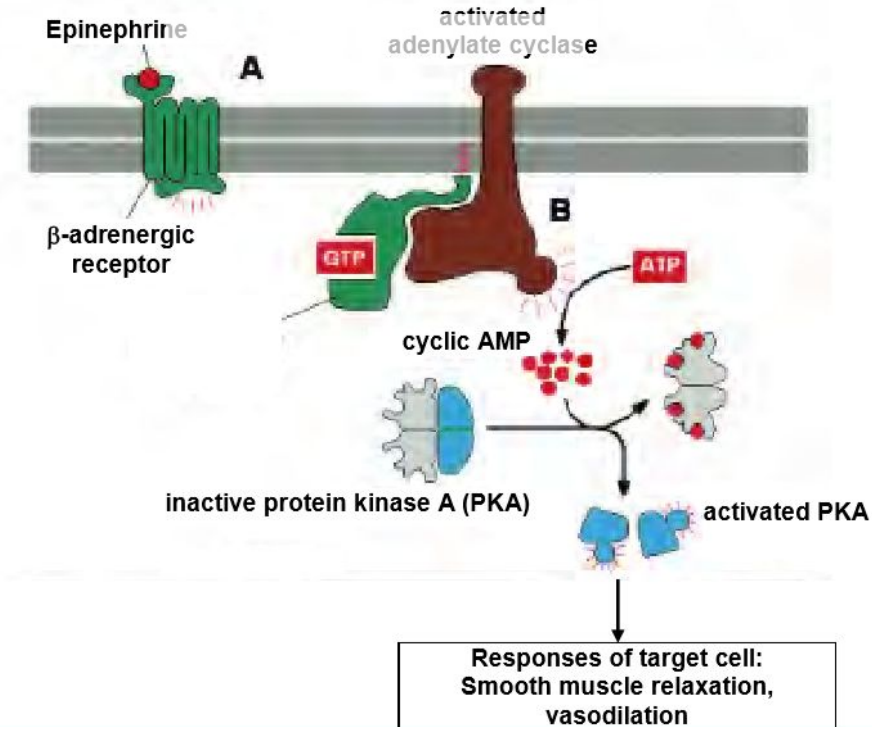
Fig. 2.2

Describe the structure of Ste2 receptor.
A single polypeptide chain
Consisting of 7 α helix segments
Folds upon itself
Such that the α helices are gathered together
Forming a cylindrical/ globular structure

State the type of receptor that glucagon binds to and explain how this receptor is fully activated. (2)

- **G-protein linked receptor**
- binding of glucagon to **complementary binding site** on receptor
- causes **conformational change** of the receptor which can now bind G-protein

6 **Fig. 6.1** below shows the cell signalling pathway in smooth muscle cells in response to the hormone Epinephrine, resulting in smooth muscle relaxation of blood vessels (vasodilation).

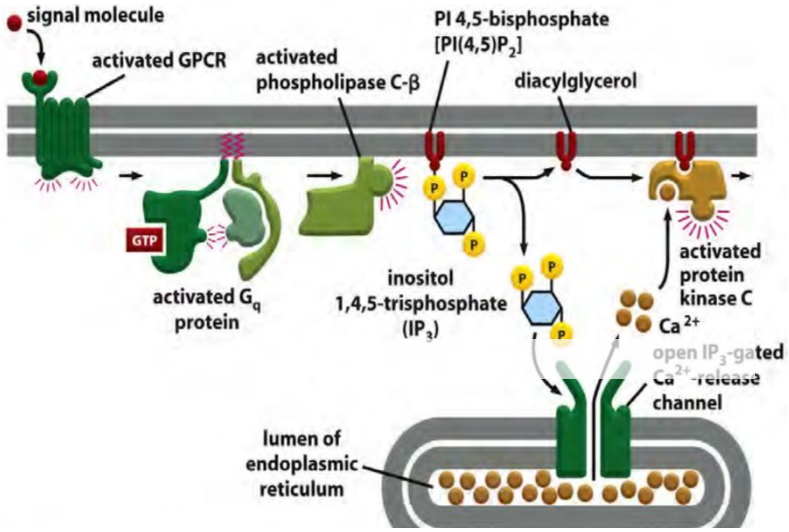


Explain how the structure of the B-adrenergic receptor allows it to carry out its function as a receptor.

- **transmembranal protein** embedded on cell surface membrane
- **extracellular binding site** is complementary to epinephrine (ligand)
- **intracellular domain** able to bind to inactive G-protein and undergo conformational changes to cause activation of G-protein

A drug was designed to ensure permanent vasodilation occurs. Suggest how this drug might work within the cell signalling pathway in Fig 6.1.

- similar structure to epinephrine and binds permanently to B-adrenergic receptor
- G-protein permanently activated, adenylate cyclase permanently activated, continuously synthesises cAMP



Suggest and explain how an IP_3-triggered response is terminated. (2)

Dephosphorylation of IP_3 by **phosphatase** which removes phosphate groups from proteins

IP_3 will no longer be able to bind to ligand-gated Ca^{2+} channels, hence leading to the inability of Ca^{2+} ions to diffuse into the cytosol

Presence of calcium ions that enter the cytosol is rapidly pumped out, mainly to the exterior of the cell- disruption to the ionic gradient of Ca^{2+}

Breakdown of signal molecule such that it is unable to bind to GPCR to form ligand-receptor interaction.

Hence, there will be no activation of G-protein and phospholipase C.

(Other ways of termination:

***phosphatases** catalyse the dephosphorylation of activated kinase proteins, thus inactivating them.*

***Intrinsic GTPase** of G protein that hydrolyzes GTP to GDP.*

***Phosphodiesterase** catalyzes the formation of AMP from cAMP*

Reversible binding of ligands to the cellular receptors)

State what is meant by an intracellular receptor.

1. Receptor proteins that are located in the cytoplasm/nucleus
2. Ligands e.g. oestrogen and testosterone that binds such receptor is hydrophobic/lipid-soluble and small enough to diffuse across the hydrophobic core of the cell membrane

Describe and explain how estrogen regulates gene expression.

1. Estrogen is lipid soluble and diffuses across phospholipid bilayer to
2. bind intracellular receptor via complementary binding
3. resulting in a change in conformation/ activation
4. Oestrogen receptor bound with oestrogens dimerises
5. And enters the nucleus
6. To bind to ERE on the enhancer sequence
7. Ref.hormone receptor complex as an activator
8. Attracting other general transcription factors and RNA polymerase to bind to promoter
9. Result in formation of stable transcription initiation complex at the promoter
10. Increases rate of transcription of cyclin and myc C which causes cell division

9(a) Discuss how signal amplification is illustrated by the effect of hormone on glycogenolysis.[8]

1 Binding of a molecule of glucagon to GPCR causes GPCR to undergo a conformational change ;;

2 to allow the activation of several G-protein by displacing GDP for GTP ;;

3 Each activated G-protein activates enzyme adenylyl cyclase, each of which is able to catalyse the conversion of large number of ATP to cAMP ;; hence amplifying the signal.

4 These cAMP in turn binds and activates large number of protein kinase A ;;

5 Each activated protein kinase A will initiate a sequential phosphorylation and activation of kinases / phosphorylation cascade ;;

6 At each phosphorylation step, each activated kinase is able to activate a large number of the next relay molecule/kinase ;; hence the signal is further amplified.

7 lead to the activation of large number of glycogen phosphorylase

8 each will catalyse for the breakdown of large amount of glycogen into glucose.

9 the number of activated products is always greater than those in the preceding step as one move down the cascade ;;

10 binding of 1 glucagon to GPLR will lead to the hydrolysis of large number of glycogen;;

9 (b) Describe the similarities between the interaction of a substrate with an enzyme and the interaction of a ligand with a receptor. [6]

1 Both bind to specific regions of protein (idea of specific region/portion is impt);
2 Substrate binds to the active site of the enzyme while ligand binds to the binding site of the receptor;
3 Both substrate and ligand are complementary in shape to sites that they bind to;
4 Both will bind to protein via H bonds, ionic bonds, hydrophobic interactions;
5 Both can induce a conformational change in the protein when they bind;
6 Ref. to induced fit hypothesis for enzymes, Ref. to activation of receptor;
7 Both interactions are not permanent;
8 Ligand dissociates from binding site of receptor ;
9 ES complex formed will be converted to product, which is released;

Describe the differences between glycogen and glucagon.

	Glycogen	**Glucagon**
Biomolecule	**Polysaccharide**	**Protein**
Monomers	**α glucose**	**Amino acids**
Bonds between monomers	**α-1,4 & α-1,6 glycosidic bonds**	**Peptide bonds**
Structure	**Highly branched structure** (α-1,6 glycosidic bonds at branch pts)	**Globular structure** (infolding of polypeptide chain; hydrophobic interactions, ionic and hydrogen bonds between R groups of amino acid residues)
Function	**Energy storage**	**Hormone, regulates blood glucose level**
Location	**Stored in muscle and liver cells**	**Stored in the α cells of the islets of Langerhans of the pancreas**
Solubility in water	**Insoluble in H_20** as polar -OH groups projected into interior of molecule + relatively large size	**Soluble in H_20** as polar amino acid residues found on exterior surface of molecule while non-polar amino acid residues buried in the interior

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