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BIO315HF—ADVANCED CELL BIOLOGY
Midterm Test—October 15, 2008--Professor Danton H. O'Day
Length: 100 marks—50 minutes--Answer all questions—No Aids permitted
Use only the space provided for each answer, so think before writing.

1. Cells have evolved mechanism to terminate signaling events that start with the receptor and end with the intracellular signaling events themselves. Using a simple list and point form, explain what common mechanisms are used by cell to shut down signaling events? (15 marks)

- To be effective the signaling process must be able to be shut down so the cell can respond to the next stimulus

(3 marks each)

1. **Remove Ligand:** Stop production, remove ligand (e.g., acetylcholine is hydrolysed by acetylcholinesterase)

2. **Remove Receptor**: receptor is internalized via receptor-mediated endocytosis and broken down

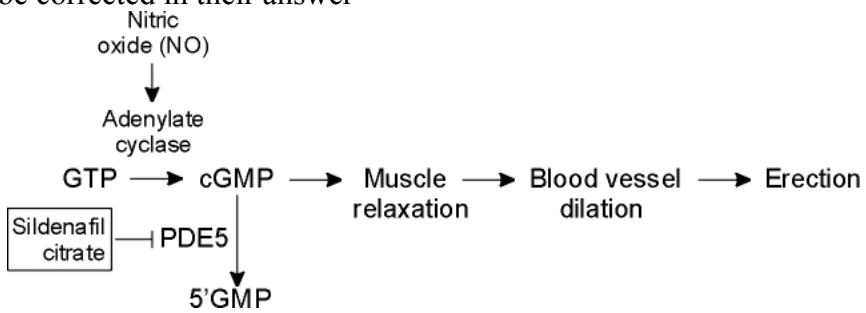
3. **Remove second messengers:** re-sequester calcium back into ER or pump out of cell (Ca2+-ATPase; break down of cAMP (phosphodiesterases)

4. **Remove phosphate groups:** dephosphorylation of activated phosphoproteins by phosphatases (ie. Calcineurin) can result in "turning off" downstream signals; phosphoform can dictate activity or nuclear translocation etc.

5. **Modify Receptor:** modification can prevent further ligand binding (ie. Posttranslational modifications – phosphates, glycosylation, sulfation etc.)

2. Using a flow chart and point form, explain how such sidenafil citrate works to help human males suffering from ED. (15 marks).

Diagram (10 marks)—note "Adenylate cyclase" is a typo—this was corrected in lecture to read "guanylate cyclase"—this must be corrected in their answer



Sildenafil citrate helps with ED (5 marks)
PDE 5 is found in corpus cavernosum of human penis—it breaks down active cGMP to inactive 5'GMP
Viagra = Sildenafil citrate inhibits PDE5 specifically; prevents breakdown of cGMP and thus sustains muscle relaxtion, blood vessel dilation and erection
-Thus Viagra and relatives (Cialis, Levitra) help with a human sexual dysfunction called Erectile Dysfunction (ED).

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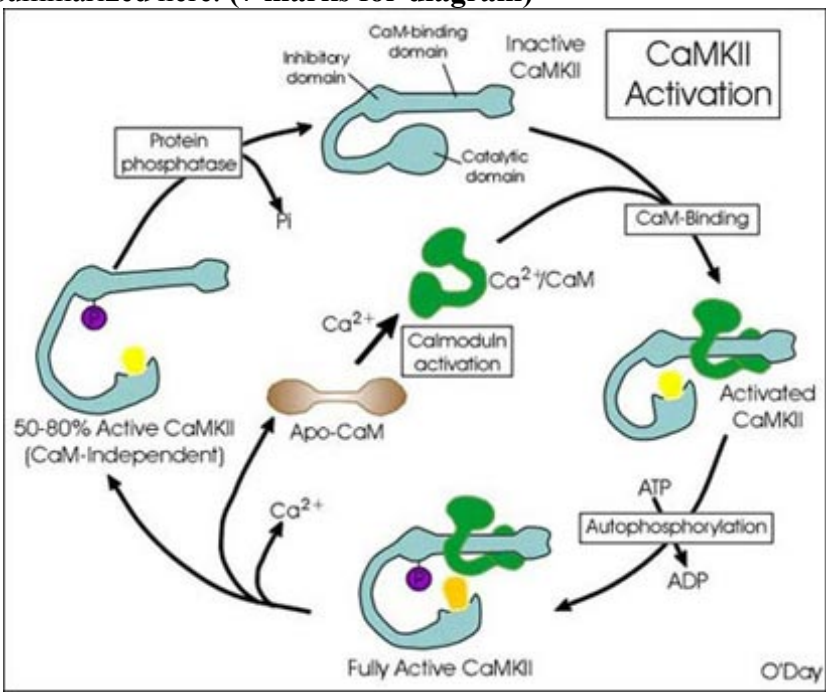
3. Using a diagram and point form, explain in detail the activation of calmodulin-dependent protein kinase II and its biological significance. (20 marks)

CaMKII has been extensively studied and since it is involved in several important cellular functions, its regulation by CaM is summarized here. **(7 marks for diagram)**



(8 marks for accurate description)

When the cell (e.g., neuron or nerve cell) is in a resting state (unstimulated), CaMKII is inactive because its autoinhibitory domain is blocking the enzymes catalytic domain. Calcium binds to CaM, allowing it to bind to the CaM-binding domain of CaMKII. This activates CaMKII allowing it to autophosphorylate its autoinhibitory domain, removing the autoinhibitory function and fully activating the enzyme. Decreases in the level of Ca2+ are followed by dissociation of CaM from CaMKII leaving the enzyme still active. Removal of the phosphate group in the autoinhibitory domain by protein phosphatase I returns the CaMKII to its inactive condition.

Biological Significance (5 marks)

CaMKII is an important component of models that are trying to explain the biochemical and cellular events underlying memory. In neurons, it was believed that CaMKII serves to store information such as memory because it goes from a CaM-dependent to a CaM-independent activated form. With prolonged stimulation, it could remain in the independent, activated state wherein it could phosphorylate target molecules important for nerve functions underlying memory storage. This model is currently less favoured than others for explaining long term potentiation (LTP) as a model of memory.

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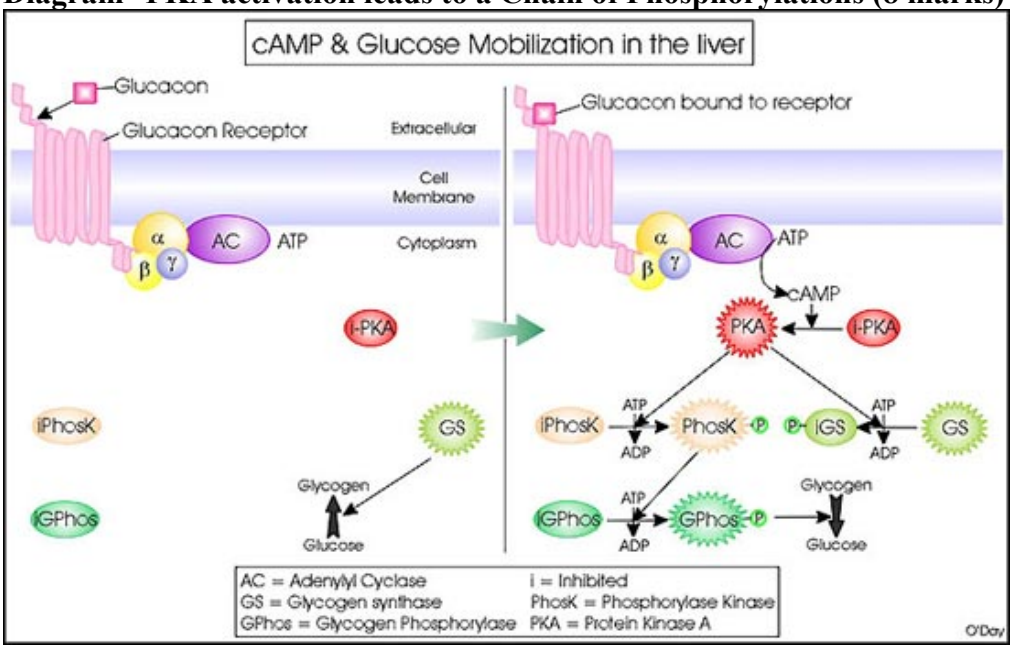
4. Starting at the initiating protein kinase, using diagrams and point form, detail a specific example of how a "chain of phosphorylations" leads to glucose mobilization in the liver. Be sure to comment on the specific effects that each phosphorylation event has. (20 marks).

The question clearly states the question should be answered from PKA—if the Glucagon/Receptor/G protein/AC sequence is detailed it get 0 marks—they must start at PKA

Diagram--PKA activation leads to a Chain of Phosphorylations (8 marks)



Description to accompany diagram (8 marks)

The cAMP binds to protein kinase A (PKA), a cAMP-dependent protein kinase, activating it. PKA in turn phosphorylates other downstream target proteins including phosphorylase kinase (PhosK) and glycogen synthase (GS). The phosphorylation of PhosK leads to its activation. Conversely, phosphorylation of GS causes its inhibition stopping the formation of glycogen. The activated PhosK then phosphorylates the next kinase in the chain, glycogen phosphorylase kinase (GPhos). Phosphorylation of GPhos activates the enzyme leading to the release of glucose subunits from glycogen.

Important point (4 marks)—this may be included as part of the above, rather than appearing as a separate statement.

Thus a chain of phosphorylations leads to the activation of some downstream signaling components while inhibiting others.

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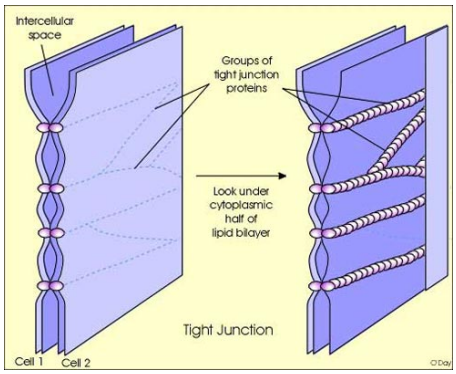
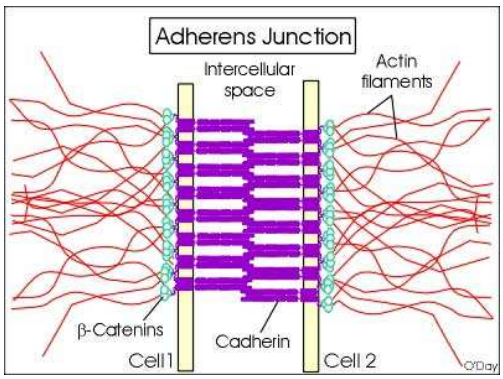
5. In proper essay format compare and contrast the structure and function of the tight junction and adherens junctions. Be sure to list their component proteins and the functions of those proteins in normal cells and when those cells are damaged. Well labeled diagrams can be used to complement your answer. (30 Marks)

Tight Junction **Adherens Junction**

-both are part of the epithelial junctional complex

Must have some description of structure or a diagram (6 marks each= 12 marks)





Diagram/description must include the following:

Proteins occludin, claudin, ZO1, 2, 3 cadherins, beta-catenins

Structure Close association of adjacent membranes belt of cadherin assoc. with β-catenins associated with actin

(1 Mark)-Tight junctions lie above adherens junctions

Functions Cell adhesion; paracellular diffusion barrier Cell adhesion

(5 marks)

-either one or both of them may play a role in defining epithelial cell polarity

(2 marks)

Cell Damage (10 marks)

NACOs -proteins Associated with the Nucleus & Adhesion COmplexes

-each have proteins that can move between junction and nucleus; reg. genes

-when epithelium is damaged:

ZO1, 2, -3 move to nucleus Beta-catenins move to nucleus

-allows cell to repair damage

-continual disruption/damage may underlie cancer?

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NOTES
Use this page for notes—it will not be marked

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