Cell signaling & communication
PART 1

Investigating Adrenergic Receptors

In a series of experiments, Awad and Anctil (1993) investigated the nature of a possible adrenergic receptor in a cnidarian. An adrenergic receptor is a membrane receptor that binds to the signaling molecule adrenaline. The following results were obtained.

Effects of several agents on rate of cAMP production in cell membrane preparations		
Treatment	cAMP (pmol min^{-1} g^{-1} protein)	Standard deviation (n=6)
Basal + ATP	0.31	0.04
Isoproterenol + ATP	0.57	0.05
Isoproterenol + ATP + GTP	0.93	0.03
Isoproterenol + ATP + GTP + GDPβs	0.29	0.03

Membrane preparation: fragments of cell membranes extracted from the animal and suspended in a buffer. Basal: no other agent is added to the membrane preparation; Isoproterenol: an agent known to mimic the effect of adrenaline; GDPβs: inhibitor of G protein. Adapted from: Awad and Anctil. *Journal of Experimental Biology* 182:131-146; 1993.

Why were the investigators interested in measuring the amount of cAMP?

A. To determine whether the AR is found on the membrane or in the cytoplasm
B. To determine whether the activation of AR is linked to the production of 2nd messenger
C. To determine whether adrenaline is the signaling molecule
D. To determine whether the activation of AR is linked to the production of RNA
E. None of the above

Why did the investigators add ATP to the reaction mixtures?

A. It acts as a source of energy for the reaction.
B. It acts as a signaling molecule.
C. It acts as a substrate for cAMP production.
D. None of the above

What is causing the amount of cAMP to increase when membranes are treated with isoproterenol?

A. The interaction of AR with ATP
B. The interaction of AR with GTP
C. The interaction of isoproterenol with ATP
D. Activation of the effector enzyme adenylate cyclase
E. All of the above

Why did the investigators look at the effect of GTP on cAMP content?

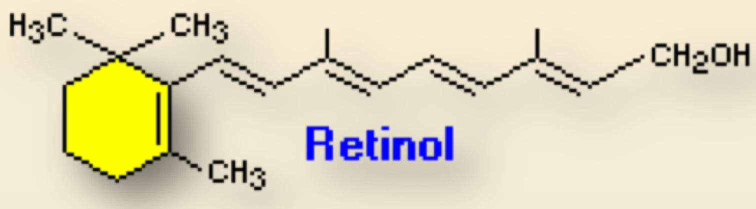
A. To determine whether isoproterenol interacts with G protein.
B. To determine whether AR is coupled to a phosphorylation cascade.
C. To determine whether AR is coupled to G protein.
D. None of the above

Why did they measure basal cAMP content?

A. As a negative control
B. As a positive control
C. To measure the basal activity of the effector enzyme
D. Both A and C are correct.
E. None of the above

Based on what you know about signaling molecules and their receptors, which of the following would you predict about the receptor of this hormone?

A. It would be an ion channel receptor.
B. It would be a protein kinase receptor.
C. It would involve a G protein.
D. It would not be connected to the plasma membrane.
E. None of the above.



Suppose you treat G protein-coupled receptors with antibody (protein) molecules that specifically bind to the ligand-binding site of these receptors. How would the signal transduction pathway of these receptors be affected?

A. Ligand would not be able to bind to receptor, thus blocking the formation of second messenger.
B. Antibody would directly block the effect of G protein on formation of second messenger.
C. Antibody would directly block the activation of the effector enzyme, thus inhibiting the formation of second messenger.
D. Both A and B are correct.

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PART 2

Part 1: Rearrange the following signal transduction events in the correct sequence:

- Inositol triphosphate (IP_3) binds to gated Ca^{++} channels.
- Large quantities of IP_3 and diacylglycerol (DAG) are produced from phosphatidyl inositol bisphosphate (PIP_2).
- G protein binds GTP.
- Norepinephrine binds to adrenergic receptor.
- Ca^{++} and DAG activate protein kinase C.
- Ca^{++} channels open.
- G protein subunit is activated.
- Phospholipase C is activated.

Identify the signal transduction components of the previous question.

Component	Identification
Primary messenger	
Receptor	
Intermediary messenger between receptor and effector protein	
Effector protein	
Second messenger	
Signal amplification	
Cellular response	

Identify the following signal transduction component of the previous question: <u>Intermediary messenger between receptor and effector protein</u>.

A. Diacylglycerol (DAG)
B. G protein
C. Phospholipase C
D. Inositol triphosphate (IP_3)
E. Protein kinase C

Identify the following signal transduction component of the previous question: Effector protein.

A. Diacylglycerol (DAG)
B. G protein
C. Phospholipase C
D. Inositol triphosphate (IP_3)
E. Protein kinase C

Identify the following signal transduction component of the previous question: Second messenger.

A. Diacylglycerol (DAG)
B. Ca++
C. Phospholipase C
D. Inositol triphosphate (IP_3)
E. Protein kinase C
F. DAG and IP_3

Identify the following signal transduction component of the previous question: Cellular response.

A. Production of DAG
B. Production of large quantities of IP_3
C. Activation of phospholipase C, resulting in protein phosphorylation
D. Activation of protein kinase C, resulting in protein phosphorylation
E. Opening of Ca++ channels

Part 2

Open the Smart Notebook file and arrange the different components of this signaling system and show how they interact with each other when the system is activated.

Questions for part 2

1. What type of receptor is this?
2. What’s the 2nd messenger?
3. Effector protein? Phospholipase C