G proteins and Adenylate Cyclase /cAMP

In this pathway, the alpha subunit interacts with a membrane enzyme called adenylate cyclase (AC).

- If the alpha subunit is a stimulatory subunit, it will activate (turn on) Adenylate cyclase.
- If the alpha subunit is an inhibitory subunit, it will de-activate (turn off) Adenylate cyclase.

What does AC enzyme do?

It produces cyclic AMP from ATP (See Fig. 16-2). cAMP is a second messenger that diffuses into the cytosol and in turn activates cAMP dependent protein kinases within the cell.

The G-protein signal has a self-limiting activity and turns itself off as follows.

- Binding to AC also activates the GTPase activity of the α subunit, turning GTP into GDP
- This causes the alpha subunit to dissociate from the AC enzyme and results in cessation of the AC enzyme activity
- Re-association of alpha with beta-gamma subunits follows.

Now binding of a new hormone can start the cycle over.

The generation of cAMP within the cell is referred to as the second messenger (the hormone being the first messenger).

**cAMP and Protein Kinases**

cAMP usually triggers the activation of specific enzymes called cAMP dependent Protein Kinases. Activated Protein Kinases in turn phosphorylate many other proteins, many of which are enzymes. This phosphorylation, depending on the target protein, can activate or inactivate the function of that specific protein. (follow the red arrows in the figure on next page).

Phosphorylation is usually reversed by specific enzymes called Phosphatases (see arrow (A)). However, phosphorylation and de-phosphorylation cannot go on at the same time since it would result in a futile cycle and wasting of ATP. To prevent this, cAMP also results in activation of a Phosphatase Inhibitor (arrow (B)), which will de-activate phosphatases and thus prevent unwanted cycles of phosphorylation and dephosphorylation (see figure next page).
The equilibrium of phosphorylated proteins versus unphosphorylated proteins (and thus active proteins and inactive proteins) is determined by the actual concentration of c-AMP in the cell. An increase in c-AMP shifts the equilibrium in the figure to the right, due to activation of protein kinases. This thus results not only in activation of key enzymes through phosphorylation, but also maintains the activation state by simultaneously activation of phosphatase inhibitors.

**cAMP amplification effect**

A few molecules of cAMP can activate many Protein Kinases, each of which in turn can activate many proteins, each of which will execute their function (see figure below). The result is thus an amplification effect. To prevent unwanted long lasting effects, cAMP is kept in check by a rapid destruction mechanism. This is accomplished by the enzyme phosphodiesterase (PDE). This enzyme breaks cAMP down to the inactive form AMP. The reduction in cAMP will shift the equilibrium in the figure above back to the left (in the direction of the black arrows and de-phosphorylated proteins).

PDE can also be activated by specific Hormones coupled to G-proteins (see Fig. 16-2)!
G proteins and Phospholipase C / IP3, DAG

This mechanism uses a receptor and G-protein complex as well. The membrane target enzyme is different. (see Fig. 16-3)

- Binding of hormone to the receptor initiates the release of the alpha subunit from the G-protein complex
- The alpha subunit now activates a membrane bound Phospholipase (PL) enzyme
- The PL enzyme acts on Phosphatidyl inositol Bi Phosphate (PIP2), an inositol phospholipid in the plasma membrane of every cell
- PL breaks PIP2 into a molecule of inositol triphosphate (IP3) and a molecule of diacylglycerol (these are the second messengers).
  - IP3 is water soluble and is released into the cytoplasm, where it triggers the release of calcium from the ER and other Calcium stores
  - Calcium acts as a third messenger
    - It can directly alter enzyme activities
    - It can bind to calmodulin which in turn activates other enzymes, amplifying the response
  - Diacylglycerol
    - remains bound to the membrane where it activates a membrane bound Protein Kinase C enzyme
    - Protein kinase C phosphorylates cell proteins, which in most instances renders them more active

![Diagram of G protein signaling](image_url)
Regulation of Receptors

The effect of the hormone-receptor interaction depends on several factors:

- Chemical messenger level in the blood
- the number of receptors on the target cell
- affinity of the receptor for the messenger

A higher affinity causes a hormone to stay on the receptor longer and thus keep the triggering action going. When a hormone dissociates from a receptor it can jump on a new receptor and start the trigger again. This lasts until the hormone is destroyed or removed.

Once the desired effect is obtained, hormone concentration is reduced via:

- inhibition or attenuation of production of the hormone through negative feedback
- breakdown via degrading enzymes or removal via diffusion
- the hormone can be removed by the action of kidney and/or liver

Receptors are dynamic entities in that their numbers are not always constant but regulated according to the desired/needed effect.

When there is an abundance of certain hormone, the number of cellular receptors for that hormone tend to decrease over time = **downregulation**. The result is a less sensitive response to a similar level of hormone (desensitizing effect).

When a cell is not being targeted by a lot of messengers due to a shortage of hormones, the receptor numbers tend to increase = **upregulation**. Having more receptors will allow the cell to “catch” the limited number of hormones better.

Up and down regulation of receptors is regulated by physiological feedback systems. Some disease processes may however result in similar receptor modification, causing unusual small or large responses to certain hormones. In addition, certain hormones may induce up- or down regulation of receptors for other hormones.

Control of Hormonal Secretion

Release of hormones is usually not constant but triggered by certain stimuli. These stimuli can fall into 3 categories. (see Fig. 16-5)

1. **Control by Blood Plasma Agents** (Humoral stimuli)

   - The release of the hormone is induced by **changes** in the plasma concentration of ions and nutrients (the stimulating agents)
   - The effect of the released hormone is usually a negative feedback, bringing the deviation in the concentration of the stimulating agent back to normal
   - The restoration of the blood plasma value of the agent takes away the stimulus, and thus controls in turn the secretion of the hormone (turns it off)
2. **Hormonal stimulation**
   - The secretion of one hormone is induced by the presence of another hormone
   - Such hormone that stimulates the release of another hormone is called a tropic hormone
   - Tropic hormones have the additional effect that they also stimulate the growth of the stimulated gland tissue

3. **Neuronal stimulation**
   - Direct stimulation of a gland by means of nerve connection
   - The nerve signal causes the gland to release the hormones

Keep in mind that the control of many hormones can be controlled by a combination of the 3 input levels shown above, the end results being an integrated response of all inputs involved. In addition, the secretion of many hormones show burst effects or cyclical variations that depend on diurnal patterns (24 hr cycles), indicating integration with other neural pathways.

**Aspects of Endocrine disorders**

Many endocrine disorders can be categorized in four ways

**Definition**: a tropic hormone is a hormone that induces the release of another hormone.

1. **Hypo-secretion**
   - Gland secretes too little hormone
   - If the gland itself is not functioning properly = primary hypo-secretion
   - If the gland is normal but there is a problem with the tropic hormone that stimulates this gland = secondary hypo-secretion
   - If the tropic-hormone releasing gland is normal but something is missing that results in secretion of the tropic hormone = tertiary hypo-secretion

2. **Hyper-secretion**
   - Primary hyper-secretion: gland is secreting too much hormone on its own
   - Secondary hyper-secretion: over-stimulation by the tropic hormone

Most hyper-secretions result from endocrine-cell tumors

3. **Hypo- and hyper- responsiveness**

There is nothing wrong with the secretion of the hormones but the response is abnormal

   - Hypo-responsiveness can be due to
     - Deficient receptors or lack of receptors
     - Deficient membrane proteins that interact with an otherwise normal receptor
     - Lack or deficiency of enzymes that turn pro-hormones into active hormones
     - Hyper-responsiveness is most often due to an abnormal upregulation of receptors for the hormone.