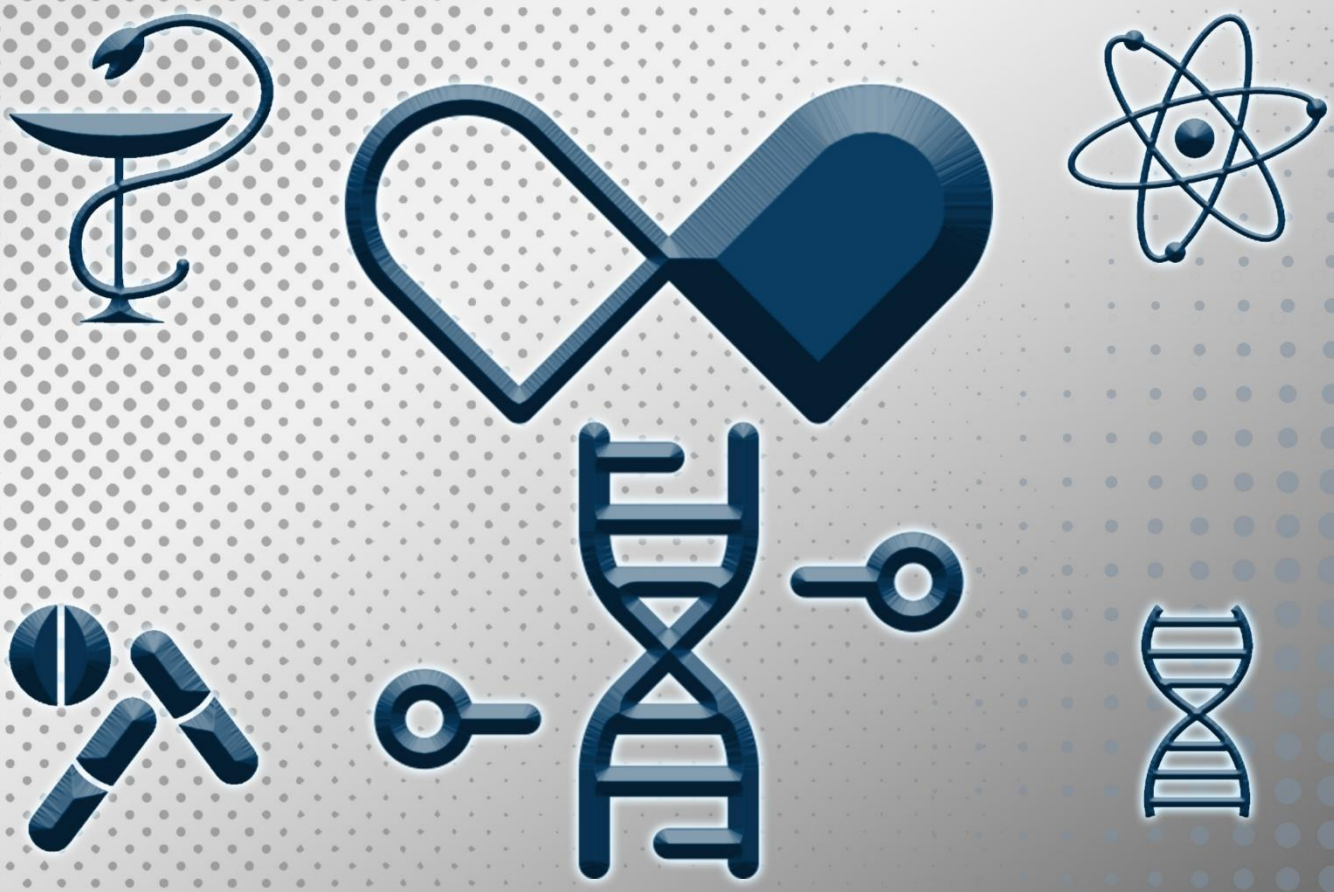
# Pharmacology



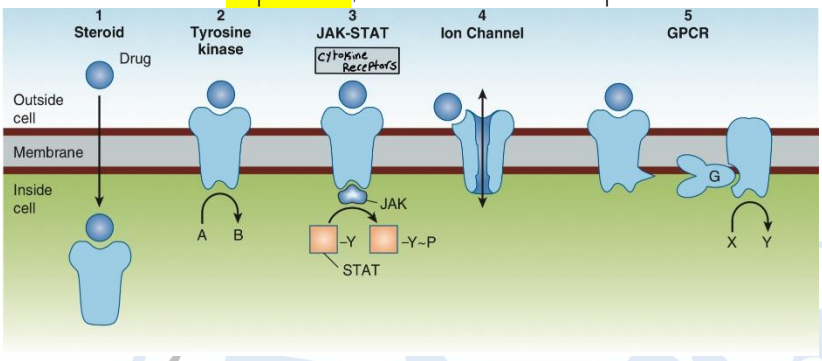

**Done By:**

Mohammad Alomari

0789791324

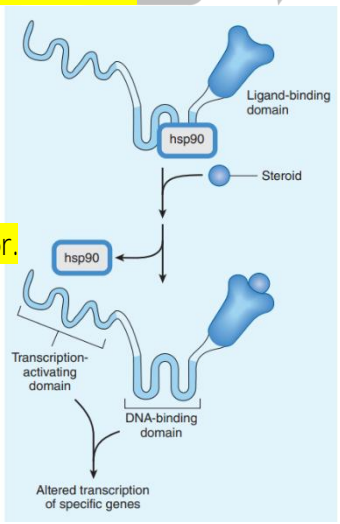
# Signaling Mechanisms and Drug Action

- Main categories of receptors:
  - **Intracellular receptor:**
    - ➔ Lipid-soluble ligand: Steroids & L-Dopa & Vit-D.
  - **Extracellular receptor:**
    - ➔ Regulated allosterically by binding of ligand on the extracellular domain.
    - ➔ Ligand binds & stimulates an intracellular protein tyrosine kinase.
    - ➔ Ligand gated ion channel that can be induced to open or close by the binding of a ligand.
    - ➔ The receptor stimulates a G protein, which modulates production of second messengers.




## 1) Receptors for Lipid-Soluble Agents "gene-active" receptors

- Steroids: corticosteroids, mineralocorticoids, sex steroids, vitamin D & Thyroid Hormone.
  - Those Receptors Stimulate genes transcription by binding to DNA response elements near the gene that its expression is regulated.



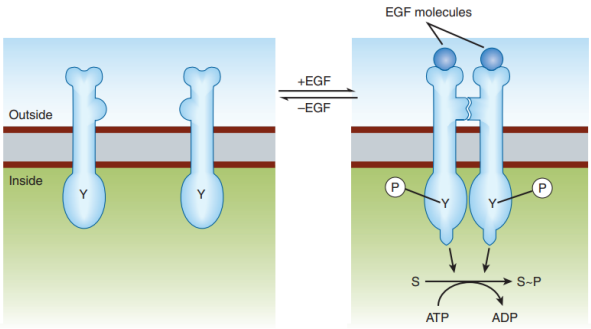

- Mechanism of Glucocorticoid action:
  - In the absence of hormone, the receptor is bound to hsp90,
    - ➔ hps90: prevent normal folding of several structural domains of receptor.
  - Glucocorticoid (GC) diffuses into the cell membrane.
  - GC binds to an intracellular receptor and hsp90 protein dissociates.
  - DNA-binding and transcription-activating domains of the receptor.
    - ➔ Fold into their functionally active conformations.
  - Alterations in target genes transcription.

- Important Therapeutic Consequences:
  - Their effect produced after **a lag period** of 30m-hour.
    - ➔ Due to the time needed to produce proteins.
    - ➔ **We don't expect to alter pathology (symptoms of bronchial asthma) within minutes.**
  - Their effect can persist for hours or days after agonist concentration reaches zero; due to **slow turnover** (long-half-life of proteins).
    - ➔ Most enzymes and proteins remain active in cells for hours or days after synthesis.
    - ➔ Their effects decrease slowly when administration stopped.

## 2) Tyrosine kinase Receptors.

- Mediates the actions of:
  - Insulin, Epidermal growth factor (EGF), Platelet-derived growth factor (PDGF), Atrial natriuretic peptide (ANP), Transforming growth factor-β (TGFβ) & Other trophic hormones.

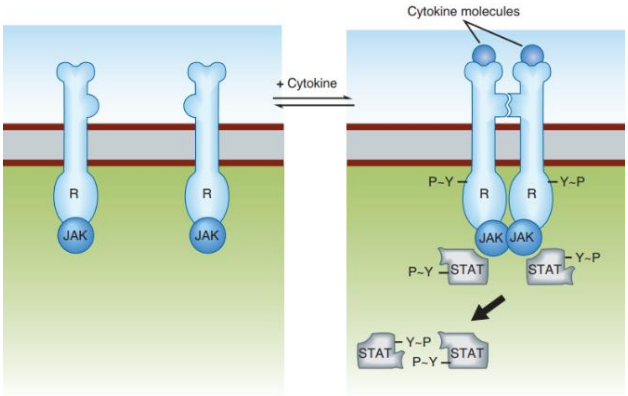
- Have 3 domains:
  - **Extracellular hormone binding domain.**
  - **Connected by a hydrophobic segment.**
  - **Cytoplasmic enzyme domain: initiate the response (intrinsic part of receptor).**
    - ➔ **Tyrosine kinase protein**
    - ➔ **Serine kinase, or a guanylyl cyclase**




- Binding of the ligand will stimulate conformational change, of the receptor to change from monomeric to dimeric form.
- This dimerization will stimulate intracellular cascade of events, including:
  - Activation of **Tyrosine kinase protein.**
    - ➔ **Phosphorylation of the dimeric form** (at tyrosine) & the downstream signaling proteins.
  - Then, activate a cascade of events in the cell.
- Duration of action:
  - The effect is limited by down-regulation.
    - ➔ Binding of ligand enhances endocytosis & degradation of the ligand & its receptor.
    - ➔ If occurred at high rate than de novo synthesis of receptors this will leads to:
      - Reduce the number of receptors & cell responsiveness.
      - Example:
        - ⇨ EGF receptor tyrosine kinase, which undergoes rapid endocytosis followed by proteolysis in lysosomes after EGF binding.
          - ↳ Genetic mutations that interfere with this process cause excessive growth factor-induced cell proliferation and are associated with cancer.

- **Tyrosine kinase protein inhibitors:**
  - Used in neoplastic disorders.
  - Binds to the extracellular domain:
    - ➔ Interfere with binding of growth factor.
    - ➔ Monoclonal antibodies (eg, trastuzumab, cetuximab).
  - Inhibit the receptor's kinase activity in the cytoplasm:
    - ➔ Membrane-permeant molecules (eg, gefitinib, erlotinib).

## 3) Cytokine Receptors

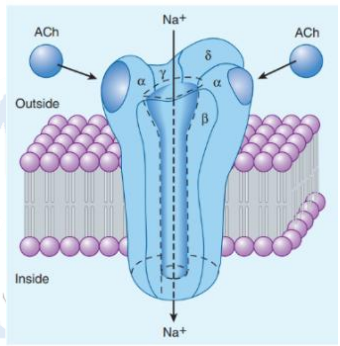



- Endogenous Ligands:
  - Regulators of growth & differentiation.
  - Growth hormone
  - Erythropoietin
  - Interferon
- Have 3 domains:
  - **Extracellular hormone binding domain.**
  - **Connected by a hydrophobic segment.**
  - **Cytoplasmic enzyme domain: bounded to JAKs.**
- Cytokine Kinase Signaling
  - Ligand binds to a receptor and causes two receptor molecules to dimerize.
  - Activation of JAKs protein & Phosphorylation of tyrosine residues on the receptor.
  - Phosphorylation of STAT molecules by JAKs.
  - STAT dimerization then travels to the nucleus, where they regulate gene transcription.

☒ **These receptors work like previous type, except that the tyrosine-kinase activity is not intrinsic to the receptor molecule, Instead, a separate tyrosine-kinase (JAK) binds receptor.**

## 4) Ion Channels

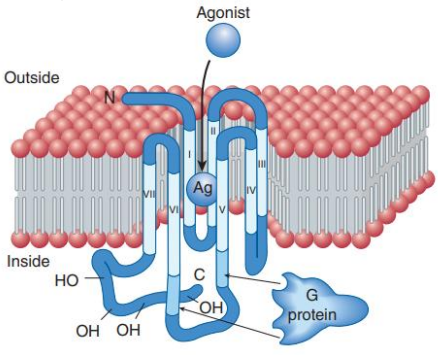
- Regulate influx or efflux of ions, thus altering the electrical potential.
- Transmits its signal across the membrane by altering the electrical potential across the membrane.
- Ligand-Gated Ion Channels:
  - Those ligands: Acetylcholine, Serotonin, GABA & Glutamate.
- Voltage-Gated Ion Channels: controlled by membrane potential.
- Cholinergic receptors:
  - Muscarinic receptors.
  - Nicotinic receptors.
    - ➔ Found in the ganglion & muscles & CNS.
    - ➔ Composed of five subunits (2α, 1β, 1γ, 1δ).
    - ➔ When ACh binds to sites on the extracellular domain of its α subunits, the receptor opens allowing Na+ ions to go inside the cell.



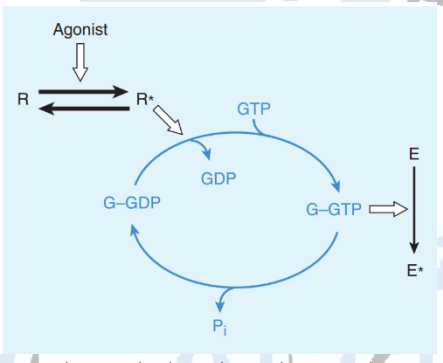

## 5) G-Protein Coupled Receptors – GPCR

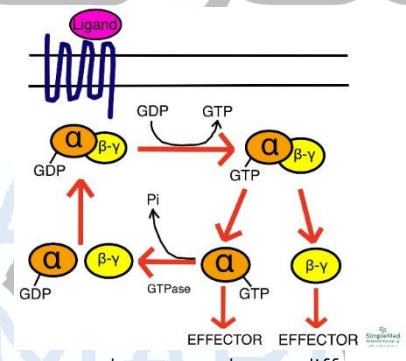
- Receptors for: Monoamines, serotonin, Ach, peptide hormones, odorants, visual.
- Most common & most important receptors in the body.
- 7 Transmembrane domain Receptor (serpentine receptors).
- Its components:

1. The Extracellular Ligand and Receptor.
   - ➔ 7 Transmembrane domains:
     - Loops numbered (I-VII).
       - ❖ Agonist will bind between I & III.
       - ❖ G-Protein binds to V & VI.
     - C-terminus: Contains serine & threonine residues whose (-OH) can be phosphorylated.
     - N-terminus binds to ligand.



2. GTP-binding protein (G Protein)
   - ➔ Separate part of the receptor.
   - ➔ Consists of:
     - o **αβγ complex**.
     - o When it is inactive: It binds to GDP.
     - o When it is active: It binds to GTP.
       - ❖ The **αβγ complex** will dissociate ➔ **βγ** will stay stationary.
       - ❖ **α** will activate or inhabit Effectors to produce Second Messengers.
   - ➔ G proteins activate their downstream effectors when bound by GTP and also have the ability to hydrolyze GTP (**يعني بحفزوا حالهم لحالهم وبثبطوا حالهم لحالهم**).
     - o This hydrolysis reaction inactivates the G protein.
       - ❖ But can occur at a relatively **slow rate**.
         - ⇨ Amplifying the transduced signal.
           - ↳ By allowing the activated G protein to have a longer lifetime in the cell than the activated receptor itself.
         - ⇨ Active G-protein may remain active for seconds, enormously amplifying the signal.
         - ⇨ This mechanism also helps explain how signaling by G proteins produces the phenomenon of spare receptors.
           - ↳ GTP-bound Gs molecule, however, the duration of effector activation depends on the longevity of GTP binding to Gs rather than on receptor's affinity to NTs.




   - ➔ The endogenous ligands bind and stimulate receptors that couple to different G proteins.
     - o The apparent promiscuity of such a ligand allows it to elicit different G protein-dependent responses in different cells.
     - o Example: NE stimulates (Gq-coupled **α** receptors) in cutaneous blood vessels, & stimulates (Gs-coupled **β** receptors) in the heart.

## G-protein Signaling

1) Signaling Molecule binds to the Receptor & stabilizes its conformational state.
2) Opening a cavity in the receptor's cytoplasmic surface that binds the G protein.
3) Reduces nucleotide affinity for the G protein, allowing GDP to dissociate and GTP to replace it.
4) Active form of G protein dissociates from the receptor and can engage downstream mediators.
   - Activate or inhabit Effectors to produce Second Messengers
5) Signal is terminated by hydrolysis of GTP, returning the system to the basal unstimulated state.

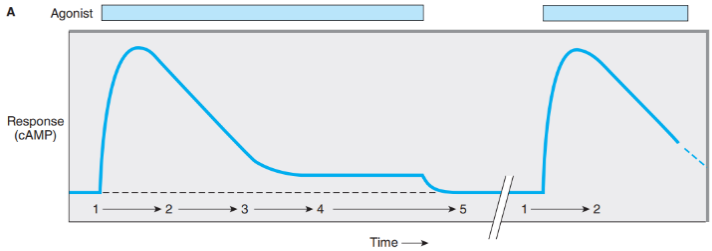
- Summary:
  - GPCR-G protein coupling involves conformational change in both proteins,
  - Allowing agonist binding to the receptor to effectively "drive" a nucleotide exchange reaction that "switches" the G protein from its inactive (GDP-bound) to active (GTP-bound) form.

**TABLE 2–1 G proteins and their receptors and effectors.**

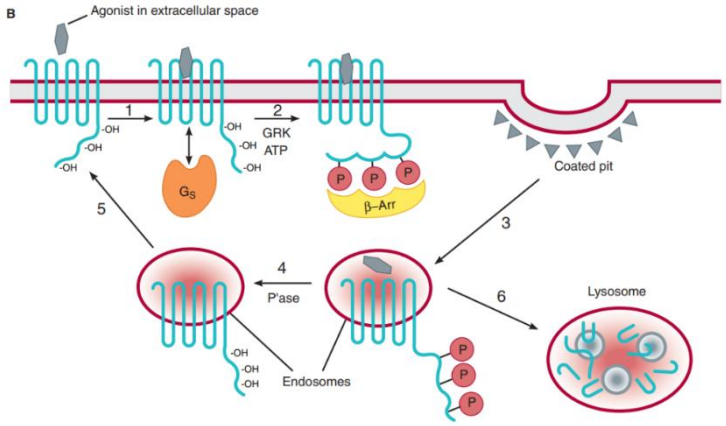
| G Protein | Receptors for | Effector/Signaling Pathway |
|---|---|---|
| $G_s$ | β-Adrenergic amines, histamine, serotonin, glucagon, and many other hormones | ↑ Adenylyl cyclase →↑ cAMP ⊕ Stimulation |
| $G_{i1}$, $G_{i2}$, $G_{i3}$ | $\alpha_2$-Adrenergic amines, acetylcholine (muscarinic), opioids, serotonin, and many others | Several, including: ↓ Adenylyl cyclase →↓ cAMP; Open cardiac $K^+$ channels →↓ heart rate ⊖ Inhibition |
| $G_{olf}$ | Odorants (olfactory epithelium) | ↑ Adenylyl cyclase →↑ cAMP ⊕ Stimulation |
| $G_o$ | Neurotransmitters in brain (not yet specifically identified) | Not yet clear |
| $G_q$ | Acetylcholine (muscarinic), bombesin, serotonin (5-$HT_2$), and many others | ↑ Phospholipase C →↑ $IP_3$, diacylglycerol, cytoplasmic $Ca^{2+}$ ⊕ Stimulation |
| $G_{t1}$, $G_{t2}$ Retina (Eye) | Photons (rhodopsin and color opsins in retinal rod and cone cells) | ↑ cGMP phosphodiesterase →↓ cGMP (phototransduction) ⊕ Stimulation |

## Receptor Regulation and Desensitization:

- Many GPCRs are regulated by phosphorylation.
  - **Done By Kinases (esp. GRK) and reversed by phosphatases**.
- **Desensitization:** G protein-mediated responses attenuate with time after reaching a high level, the response diminishes over seconds or minutes, even in the continued presence of the agonist.
  - It is often reversible.
  - 2nd exposure, after first exposure termination, leads to similar response (Resensitization).




- GPCRs regulation (Resensitization):
  1) Agonist binding to receptors initiates signaling by G proteins (Gs) located in cytoplasm.
  2) GRK will phosphorylate the receptor at C-terminal serine residues:
     - Increases the receptor's affinity to β-arrestin to binding to the receptor.
       - ➔ ↓Receptor's ability to interact with Gs, (reducing the agonist response).
       - ➔ β-arrestin binding also accelerates endocytosis of receptors.



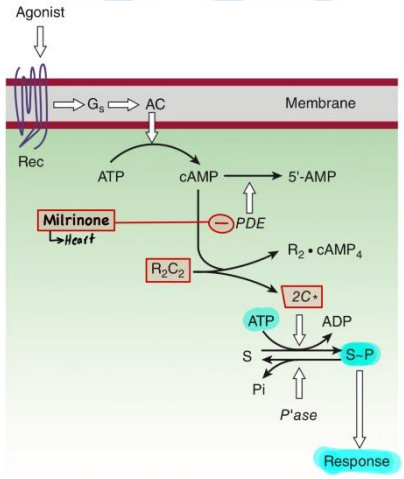

  3) The receptor-arrestin complex binds to coated pits, promoting receptor internalization.
     - Endocytosis of receptors promotes their **dephosphorylation** by phosphatases.
     - Dissociation of agonists from internalized receptors; thus ↓β-Arrestin binding affinity.
  4) Return of receptors to the plasma membrane.

☒ **Internalized receptors may go to lysosomes & Promoting receptor down-regulation.**

- Alterations in Number or Function of Receptors
  - The agonist itself induces a:
    - ➔ Decrease in the number (eg, down-regulation) of its receptors.
    - ➔ Decrease in the coupling efficiency (eg, desensitization) of its receptors.

  - An antagonist may increase the number of receptors.
    - ➔ By preventing down-regulation this may lead to up-regulation.
    - ➔ When the antagonist is withdrawn, the elevated number of receptors can produce an exaggerated response to physiologic concentrations of agonist.

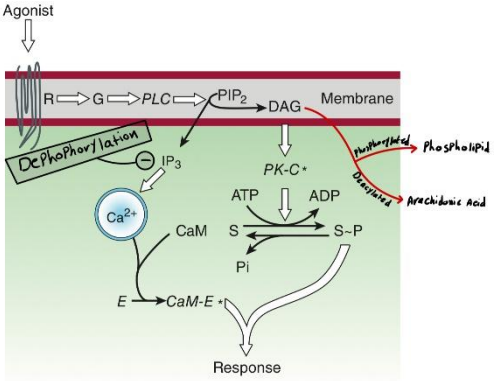
- Types of desensitization:
  - Homologous: related to occupation of receptor by agonist & occupied receptor only is altered.
  - Heterologous: related to excess production of cAMP & other adenylate cyclase-coupled receptors are altered.




## Second Messengers

- Cyclic Adenosine Monophosphate (cAMP)
  - Mediates many hormonal responses:
    - ➔ PTH, ADH, HR, heart contractility, insulin, steroids.
  - cAMP exerts most of its effects by stimulating cAMP-dependent protein kinases, that composed of:
    - ➔ cAMP-binding regulatory (R) dimer & two catalytic (C) chains.
    - ➔ When cAMP binds to R dimer, active C chains are released.
    - ➔ C chains diffuse through the cytoplasm and nucleus,
      - Transfer (Pi) from ATP to appropriate substrate proteins, often enzymes.
        - ❖ Depending on the organ & the action.
  - When the hormonal stimulus stops, the intracellular actions of cAMP are **terminated** by:
    - ➔ **cAMP degradation into 5'-AMP** by cyclic nucleotide phosphodiesterases (PDEs).
      - Milrinone:
        - ❖ Selective inhibitor of type 3 phosphodiesterases
        - ❖ Used as an adjunctive agent in treating acute heart failure.
      - Caffeine, theophylline, and methylxanthines:
        - ❖ Competitive inhibition of cAMP degradation.

- Phosphoinositides and Calcium
  - Mediates many other hormonal responses.
  - The crucial step is stimulation of phospholipase C (PLC)
    - ➔ Which splits (PIP2), into two second messengers:
      - Diacylglycerol (DAG):
      - Inositol-1,4,5-trisphosphate (IP3).



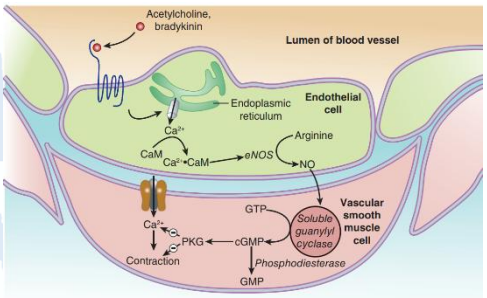
- Diacylglycerol (DAG):
  ➔ Activates a phospholipid & calcium sensitive protein kinase (protein kinase C).
- Inositol-1,4,5-trisphosphate (IP3).
  ➔ Trigger release of Ca2+ from storage vesicles ➔ Elevated cytoplasmic $Ca^{2+}$.
    - Promotes the binding of $Ca^{2+}$ to calmodulin, which regulates activities of other enzymes, including calcium-dependent protein kinases.

- Mechanisms to damp or terminate signaling:
  **➔ IP3 is inactivated by dephosphorylation.**
  **➔ DAG is either:**
    - **Phosphorylated** to yield phosphatidic acid, which is then converted into phospholipids,
    - Deacylated to yield arachidonic acid;
  ➔ Ca2+ is actively removed from the cytoplasm by Ca2+ pumps.

## - Cyclic Guanosine Monophosphate (cGMP)

- Has established signaling roles in only a few cell types (Unlike cAMP).
  ➔ In intestinal mucosa and vascular smooth muscle.
  ➔ Atrial natriuretic peptide (ANP).
- cGMP-based signal transduction mechanism closely parallels the cAMP signaling mechanism.

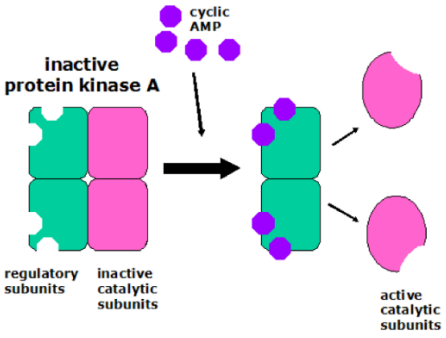
- Ligands detected by receptors stimulate guanylyl cyclase to produce cGMP,
  ➔ cGMP acts by stimulating a cGMP-dependent protein kinase (protein kinase G).
  ➔ Increased cGMP concentration
    - Caused by:
      - ❖ Activating the guanylyl cyclase activity that resides in the receptor's intracellular domain (TKR).
      - ❖ Mediates responses to nitric oxide that activates guanylyl cyclase activity
    - Causes:
      - ❖ Relaxation of vascular smooth muscle.
      - ❖ Dephosphorylation of myosin light chains




- Mechanisms to damp or terminate signaling:
  ➔ Enzymatic degradation of the cyclic nucleotide
  ➔ Dephosphorylation of kinase substrates

| ☒ Name | ☒ Produced by | ☒ Downstream proteins |
|---|---|---|
| ☒ cAMP | ☒ Adenylyl cyclase | ☒ Protein kinase A |
| ☒ Phosphoino-sitides (IP3) | ☒ Phospholipase C (PLC) | ☒ Endoplasmic reticulum receptors |
| ☒ Diacylglycerol (DAG) | | ☒ Protein kinase C |
| ☒ Calcium ($Ca^{2+}$) | ☒ Its release is stimulated by IP3 | ☒ --- |
| ☒ cGMP | ☒ Guanylyl cyclase | ☒ Protein kinase G |

- Effector: **Adenylate cyclase** & Second Messenger: **cAMP**.
  - **Gs → Adenylate cyclase → (ATP → cAMP)**
  - **cAMP** will stimulate Protein Kinase A.
    - ➔ 2catalytic subunits, normally inhibited by regulatory ones.
    - ➔ 2regulatory subunits, will dissociate from catalytic subunits by binding of 4cAMP.
    - ➔ This will increase the PKA activity (not levels).
      - Increase the phosphorylation to do the response.




# THE END OF CHAPTER 2-2