

SECOND MESSENGERS

CAMP

cGMP


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PhD Pharmacology





second MESSENGERS

Second messengers are intracellular signaling molecules released by the cell to trigger physiological changes such as proliferation, differentiation, migration, survival, and apoptosis.

- ▶ Second messengers are therefore one of the initiating components of intracellular signal transduction cascades.

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- ▶ Examples of second messengers include **cyclic AMP, cyclic GMP, Inositol trisphosphate, Diacylglycerol** and **Calcium**.
 - ▶ Releases in response to exposure to extracellular signaling molecules/ligands **the first messenger**, such as **neurotransmitters, hormones** (epinephrine, growth hormone and serotonin).

- ▶ The first messengers such as **peptide hormones**, **neurotransmitters** usually do not physically cross the phospholipid bilayers.
 - ▶ **First messengers** need to be transduced into **secondary messengers**, so that the extracellular signal may be propagated intracellularly.
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- Second messengers greatly amplify the strength of the signal.
 - Activate or inhibit the target enzymes of the pathway.

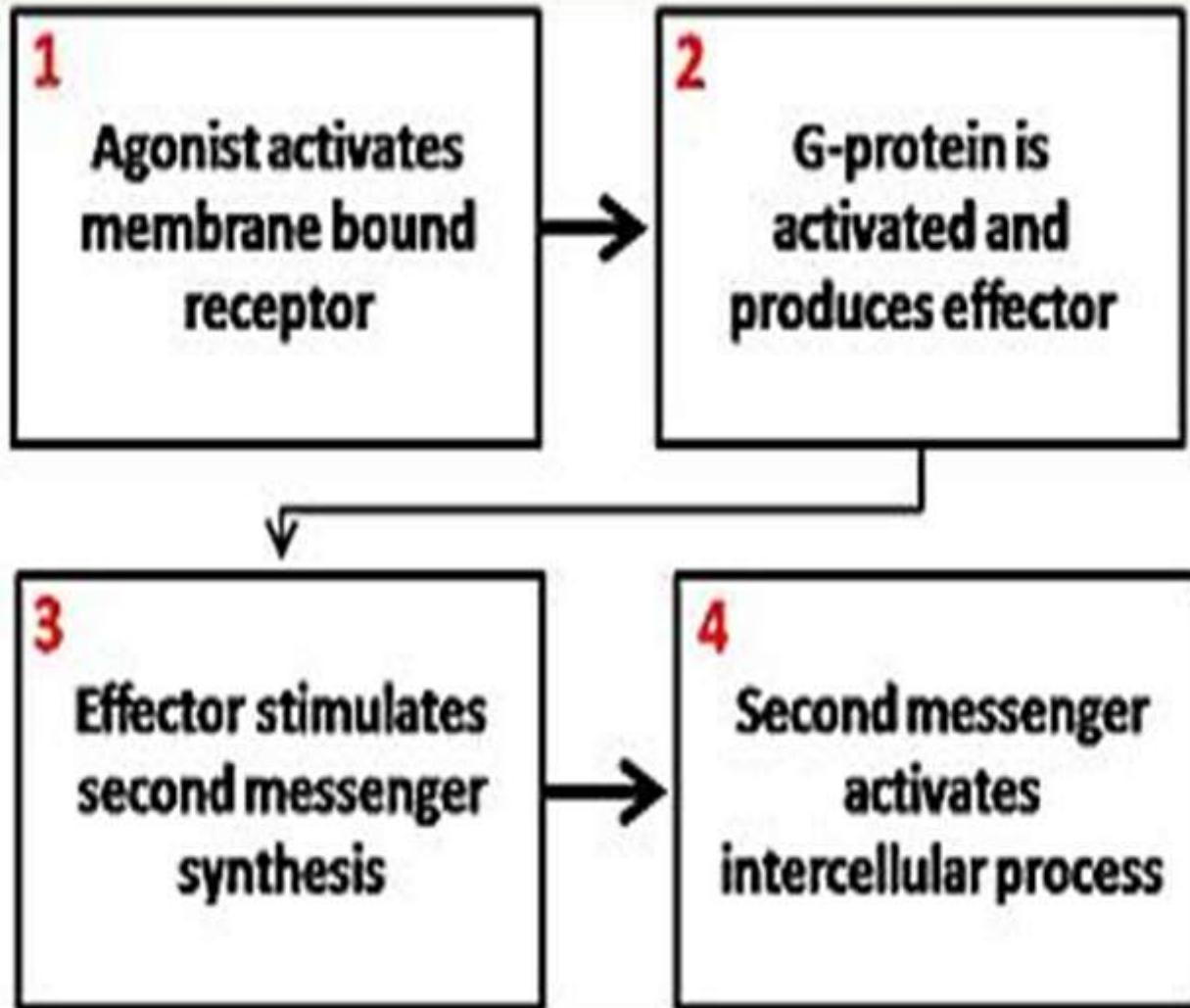
History

- Earl Wilbur Sutherland Jr. discovered secondary messengers.
- He saw that **epinephrine stimulate glycogenolysis** in liver cells, but epinephrine alone would not convert glycogen to glucose
- He found that epinephrine had to trigger a secondary messenger, **cyclic AMP** for the liver to convert glycogen to glucose. He won the 1971 Nobel Prize in Medicine.



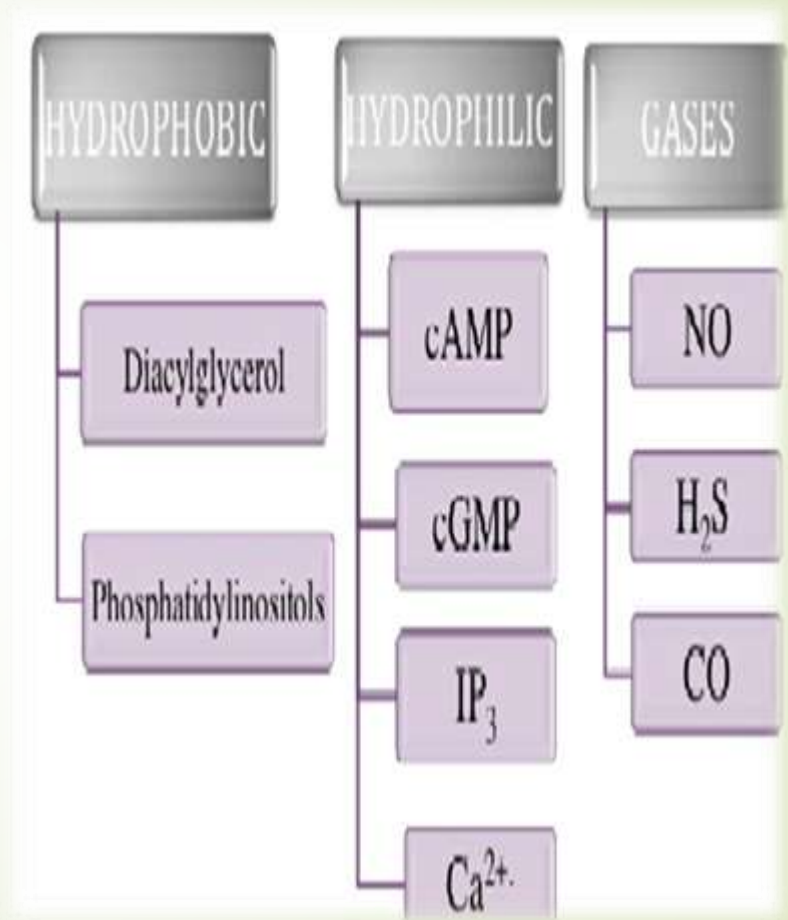
Common mechanisms of second messenger systems

There are several different secondary messenger systems (cAMP system, phosphoinositol system), but they all are quite similar in overall mechanism, although the substances involved and overall effects can vary.



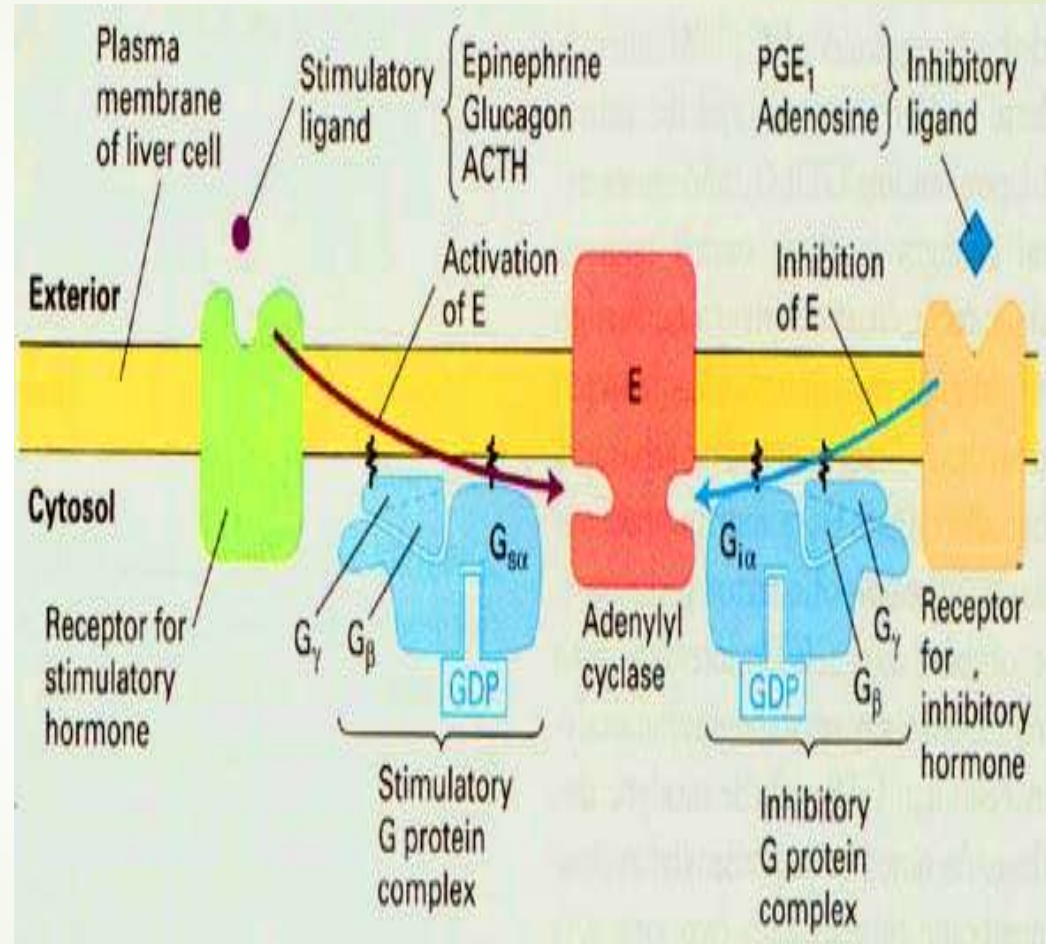
Types Of Second Messenger Molecules


- ▶ **Three basic types of second messenger molecules:**
 - ▶ **Hydrophobic molecules:** membrane-associated e.g. **diacylglycerol, phosphatidylinositol**
 - ▶ **Hydrophilic molecules:** water-soluble molecules, such as **cAMP, cGMP, IP₃**, and **Ca²⁺**, located within the cytosol.
 - ▶ **Gases:** **nitric oxide (NO)**, **carbon monoxide (CO)** and **hydrogen sulfide (H₂S)** which can diffuse both through cytosol and across cellular membranes.



1. cAMP

- cAMP is a second messenger, synthesized from ATP by enzyme **adenylyl cyclase**.
- Adenylate cyclase is activated by stimulatory G (Gs)-protein-coupled receptors.
- Inhibited by adenylate cyclase inhibitory G (Gi)-protein-coupled receptors.



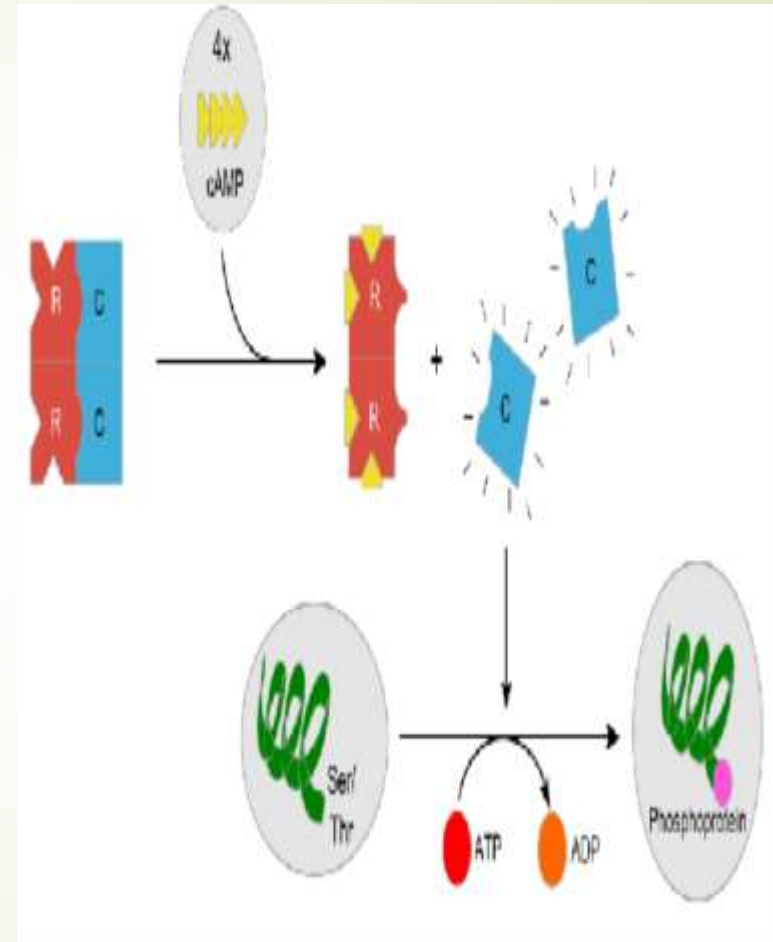



Cyclic AMP generated by adenylyl cyclases has three major targets in most cells,

- The cyclic AMP dependent protein kinase (PKA),
- cAMP-regulated guanine nucleotide exchange factors termed EPACs (exchange protein directly activated by cAMP), and
- via PKA phosphorylation, a transcription factor termed CREB (cAMP response element binding protein).

PKA REGULATION *by* cAMP

- The most common downstream effector of cAMP is **Protein kinase A (PKA)**.
- PKA is normally inactive as **tetrameric holoenzyme**(two catalytic and two regulatory units).
- The regulatory unit always block the catalytic center of catalytic unit.
- Two cAMP molecules bind to each PKA regulatory subunit.
- The regulatory subunit dissociate from the catalytic subunit.
- The free catalytic subunits interact with **proteins** to phosphorylate **Ser** or **Thr** residues, thus producing cellular response.

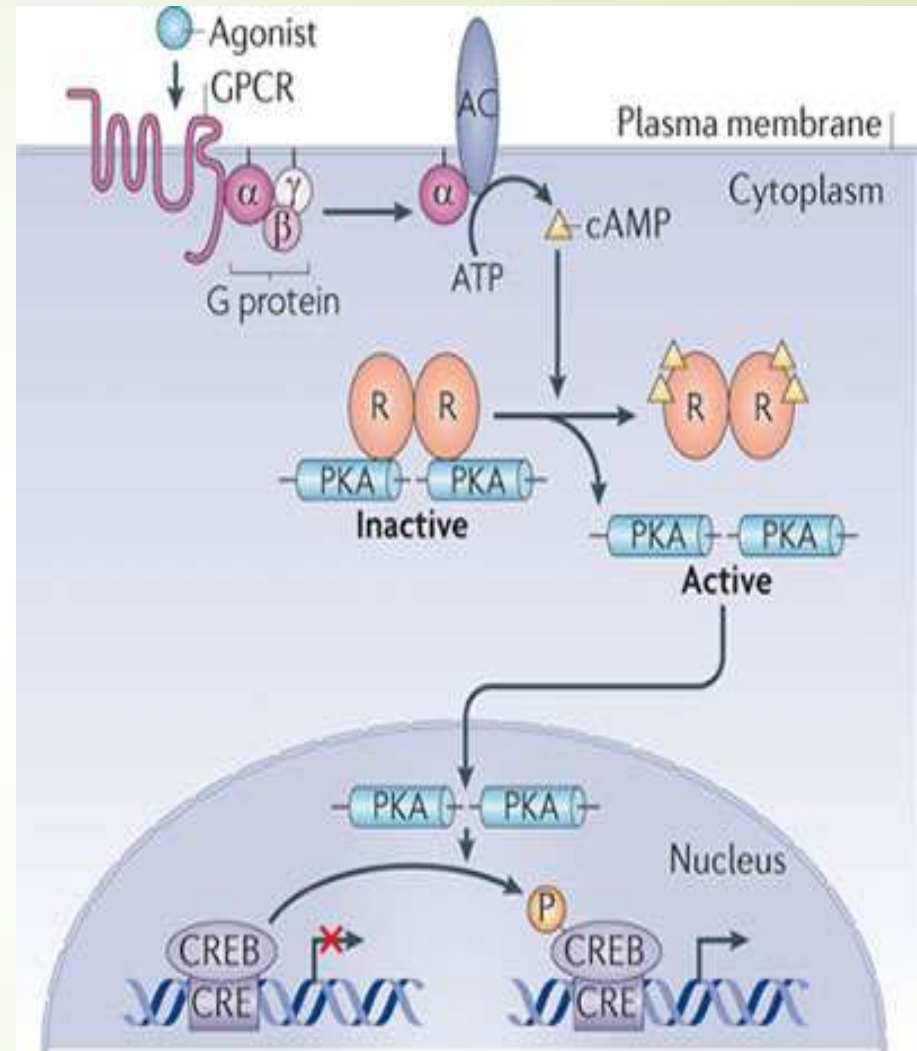



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- ▶ PKA can phosphorylate a diverse array of physiological targets such as metabolic enzymes and transport proteins, and numerous regulatory proteins including other protein kinases, regulation of glycogen, sugar, and lipid metabolism, ion channels, and transcription factors.

cAMP Response Element-Binding (CREB) Protein

Cellular transcription factor e.g. the neurotrophin BDNF, tyrosine hydroxylase & many neuropeptides binds to certain DNA sequences called cAMP response elements (CRE) thereby increasing or decreasing the transcription of the downstream genes.

➔ **Protein synthesis-PKA** directly activate CREB, which bind the cAMP response element (CRE). The activated CREB protein then binds to a CRE region, and is then bound to **CBP** (CREB binding protein), which co-activates it, allowing it to switch certain genes on or off and altering the transcription.





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- CREB has many functions in many different organs, and some of its functions have been studied in relation to the brain. CREB proteins in neurons are thought to be involved in the formation of long-term memories. CREB is also important for the survival of neurons, as studied in genetically engineered mice, where CREB and CREM were deleted in the brain. If CREB is lost in the whole developing mouse embryo, the mice die immediately after birth, again highlighting the critical role of CREB in promoting survival.



Cyclic AMP–Regulated Guanine Nucleotide Exchange Factors

The small GTP-binding proteins are monomeric GTPases and key regulators of cell function. The small GTPases operate as binary switches that exist in GTP- or GDP-liganded conformations. They integrate extracellular signals from membrane receptors with cytoskeletal changes and activation of diverse signaling pathways, regulating such processes as phagocytosis, progression through the cell cycle, cell adhesion, gene expression, and apoptosis.

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- For example, many small GTPases are regulated by GEFs (Guanine Nucleotide Exchange Factors). GEFs act by binding to the GDP-liganded GTPase and catalyzing the exchange of GDP for GTP.
 - The two GEFs regulated by cAMP are able to activate members of the Ras small GTPase family, Rap1 and Rap2; these GEFs are termed exchange proteins activated by cyclic AMP (EPAC-1 and EPAC-2). The EPAC pathway provides an additional effector system for cAMP signaling and drug action.

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- These pathways play major role in Cell differentiation/proliferation, cytoskeletal organization, vesicular trafficking & nuclear transport.
 - Potential target for cancer therapy.

Therapeutic Applications


Selected Drugs that target cAMP signalling

Drug	cAMP	Therapeutic Application
Salmeterol	↑	Asthma, COPD
Haloperidol	↑	Schizophrenia
Metoclopramide	↑	Nausea, Vomiting
Desmopressin	↑	Diabetes Insipidus

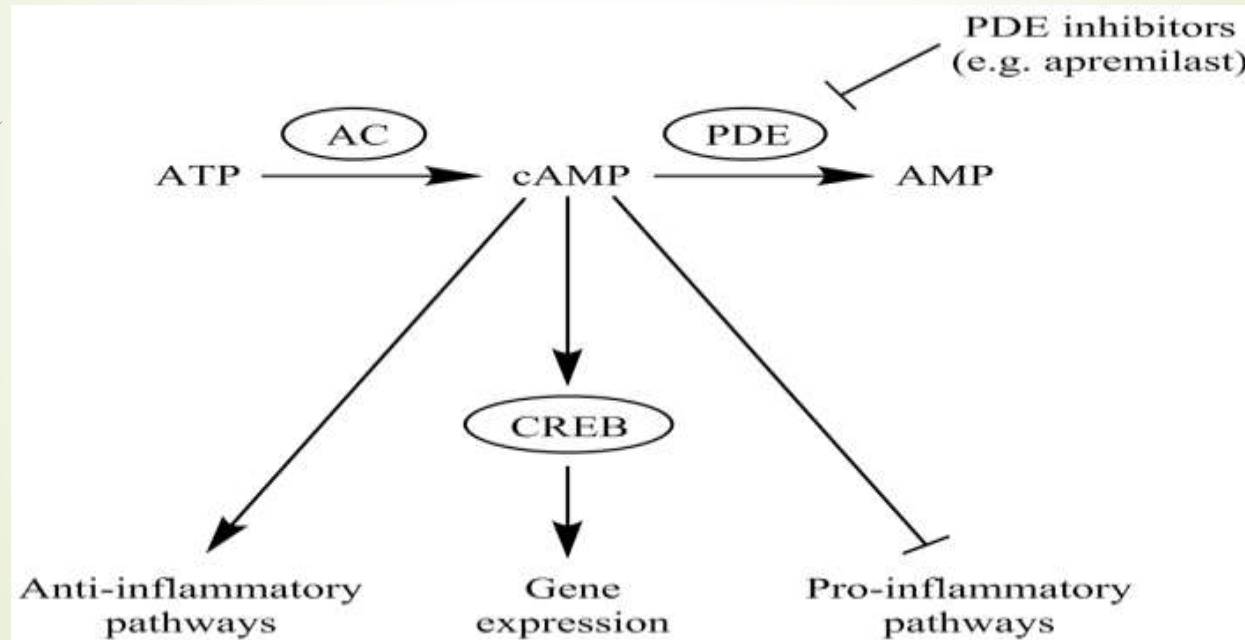
Metoprolol	↓	Angina, Hypertension, CHF
Morphine	↓	Pain
Sumatriptan	↓	Migraine
Ibuprofen	↓	Inflammation, Pain
Ranitidine	↓	Peptic ulcer, GERD
Misoprostol	↓	Prevention of NSAID ulcers
Cabergoline	↓	Parkinson's disease


Cyclic nucleotide phosphodiesterases (PDEs)

- Cyclic nucleotide phosphodiesterases form another family of important signaling proteins whose activities are regulated via the rate of gene transcription as well as by second messengers (cyclic nucleotides or Ca^{2+}) and interactions with other signaling proteins such as β arrestin and protein kinases. PDEs hydrolyze the cyclic 3',5'-phosphodiester bond in cAMP and cGMP, thereby terminating their action.

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- Phosphodiesterase 4 (PDE4) is the predominant cAMP-degrading enzyme expressed in inflammatory cells.
 - cAMP helps regulate T cell function.
 - cAMP helps maintain immune homeostasis by suppressing the release of proinflammatory mediators (eg, TNF- α , IL-17, and IFN- γ). cAMP promote the release of anti-inflammatory mediators (eg, IL-10) by immune cells.


Decrease in PDE4 increases cAMP, leads to increased transcription of genes that have CRE sites, including the gene for IL-10, which is an anti-inflammatory mediator.



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- ▶ PDEs are drug targets for treatment of diseases such as asthma, COPD, cardiovascular diseases such as heart failure, atherosclerotic coronary and peripheral arterial disease, and neurological disorders.

Recent Development

- A new Second Messenger, **c-di-AMP** was discovered in *Staphylococcus aureus* with a Role in Controlling Cell Size and Envelope Stress. This work was published in the September 2011 Issue of *PLoS Pathogens*.

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- Most of work on c-di-AMP signaling has been done in Gram-positive bacteria and actinobacteria, where c-di-AMP signaling pathways affect potassium transport, cell wall structure, and antibiotic resistance.
 - These findings greatly expand the c-di-AMP signaling role and reveal a central metabolic regulatory role for a cyclic dinucleotide.
 - However, the molecular mechanisms of c-di-AMP signaling are still poorly understood.



cGMP

The signaling pathways that regulate the synthesis of cyclic GMP in cells include hormonal regulation of transmembrane guanylate cyclases such as the atrial natriuretic peptide receptor (ANP) and the activation of soluble forms of guanylate cyclase by nitric oxide (NO).



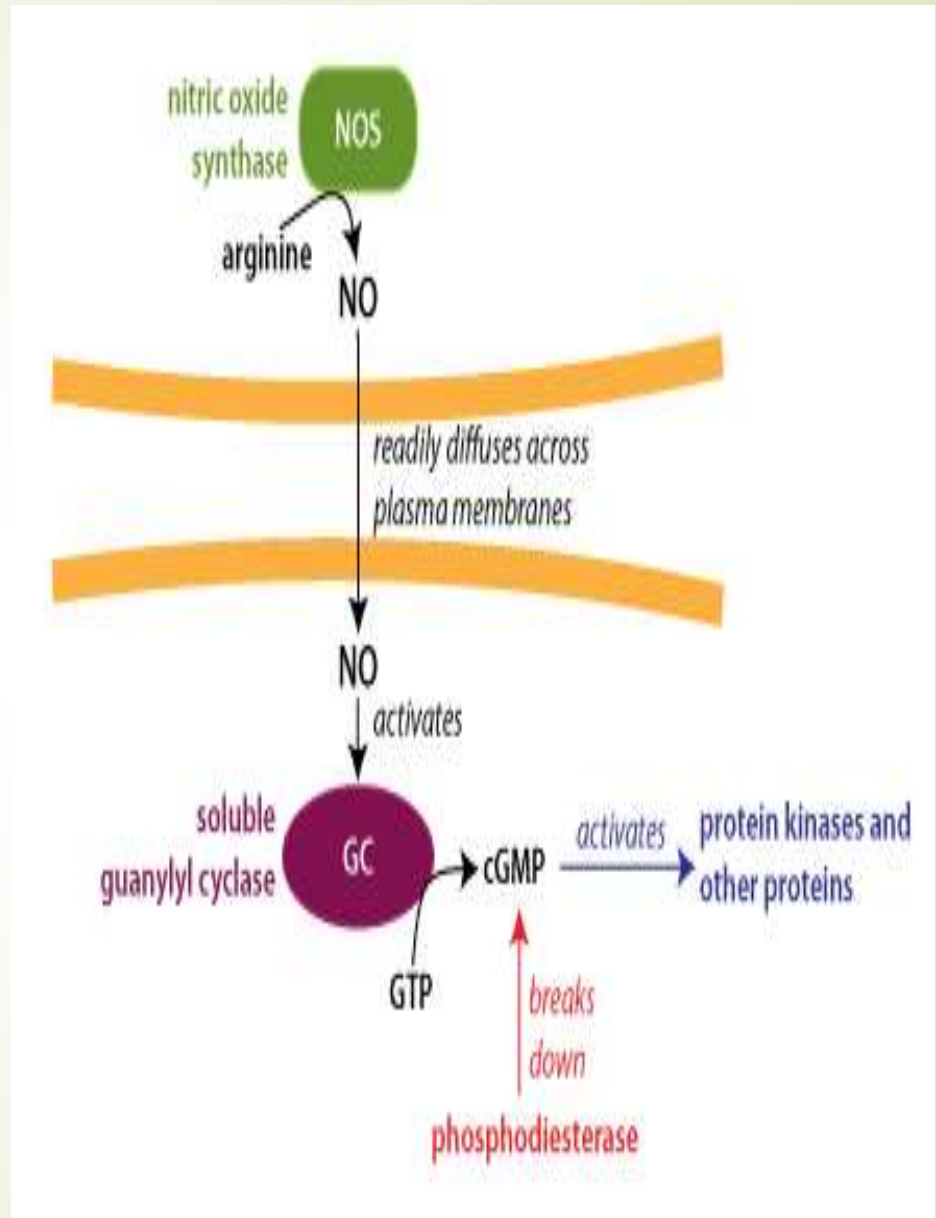
Unlike cAMP, cGMP has established signaling roles in only few cell types

Signaling pathways that regulate synthesis include:

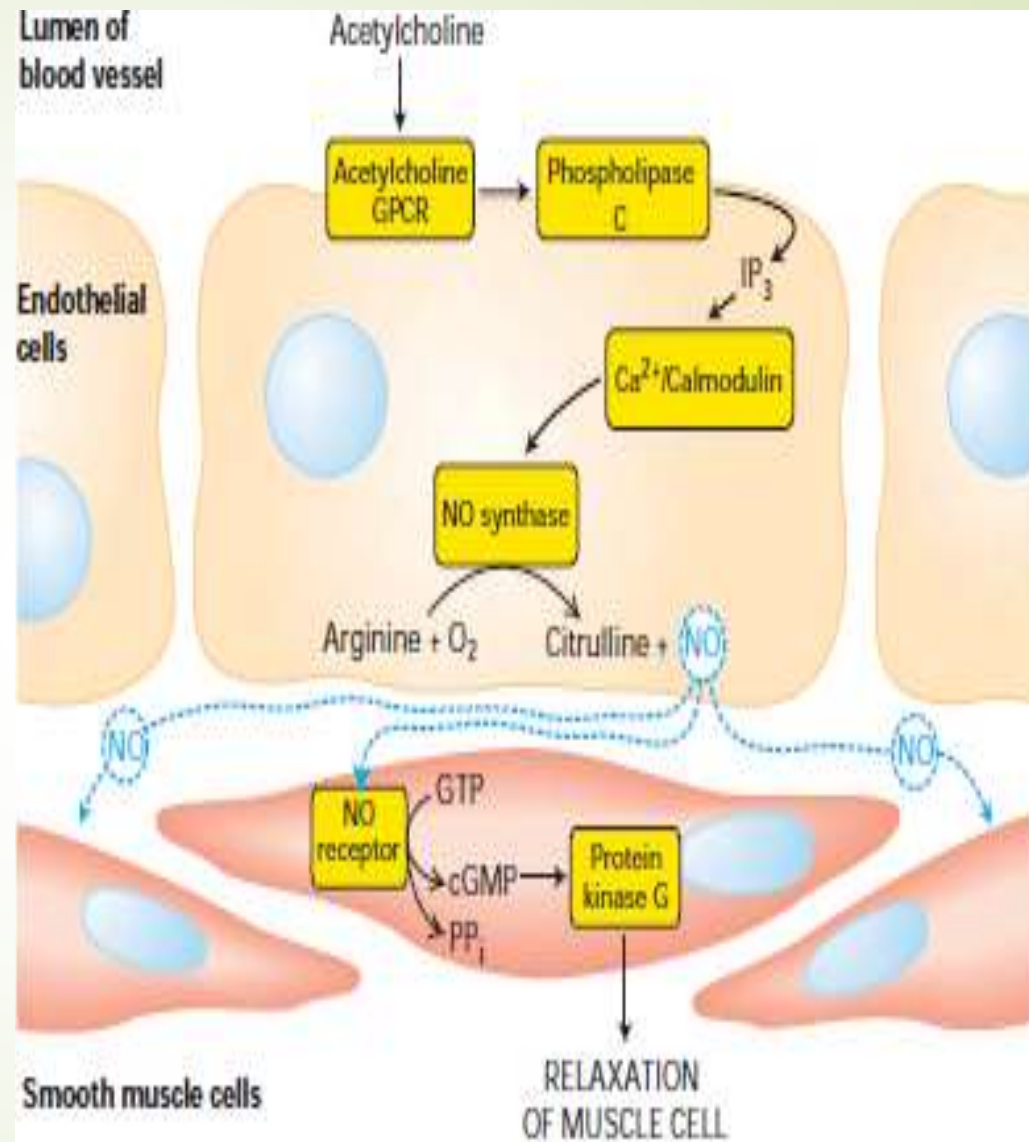
- Nitric Oxide
- Hormonal Regulation (ANP/BNP/CNP)

Nitric Oxide

- **Nitric oxide (NO)** is a **gas**, diffuse through the plasma membrane and affect nearby cells.
- Synthesized from **arginine** and **oxygen** by the **NO synthase**.
- NO then activate soluble guanylyl cyclase, to produce **cGMP**.



- The function of NO is the **dilation of blood vessels**.
- The **acetylcholine** (neurotransmitter) acts on **endothelial cells** to stimulate NO synthesis.
- NO, diffuses to **neighboring smooth muscle cells** where it interacts with the **guanylyl cyclase**.
- This increase enzymatic activity resulting in the synthesis of **cGMP**.
- The **cGMP** then **induces muscle relaxation** and **blood vessel dilation**.





Nitric Oxide Synthase (NOS) exists as 4 isoforms:

- Neuronal type I isoform (**nNOS**)
- Inducible type II isoform (**iNOS**)
- Endothelial type III isoform (**eNOS**)
- Mitochondrial isoform (**mtNOS**)



NOS constitutively expressed in:

eNOS

Endothelium, cardiac myocytes, renal mesangial cells, osteoblasts, platelets and is involved with regulating vascular function (Vasodilation).

nNOS

CNS nerves and skeletal muscles, performs a role in cell communication and is associated with plasma membranes



iNOS

Macrophages, Kupffer cells, neutrophils, fibroblasts, vascular smooth muscle cells & endothelium and involved in immune defence against pathogens.

mtNOS


NO in the mitochondrial matrix may regulate ATP synthesis.

Natriuretic Peptide


3 small peptide ligands:

- **Atrial Natriuretic Peptide (ANP)**
- **Brain Natriuretic Peptide (BNP)**
- **C-type Natriuretic Peptide (CNP)**

These peptides possess potent natriuretic, diuretic, and vasodilating activities and are implicated in body fluid homeostasis and blood pressure control.



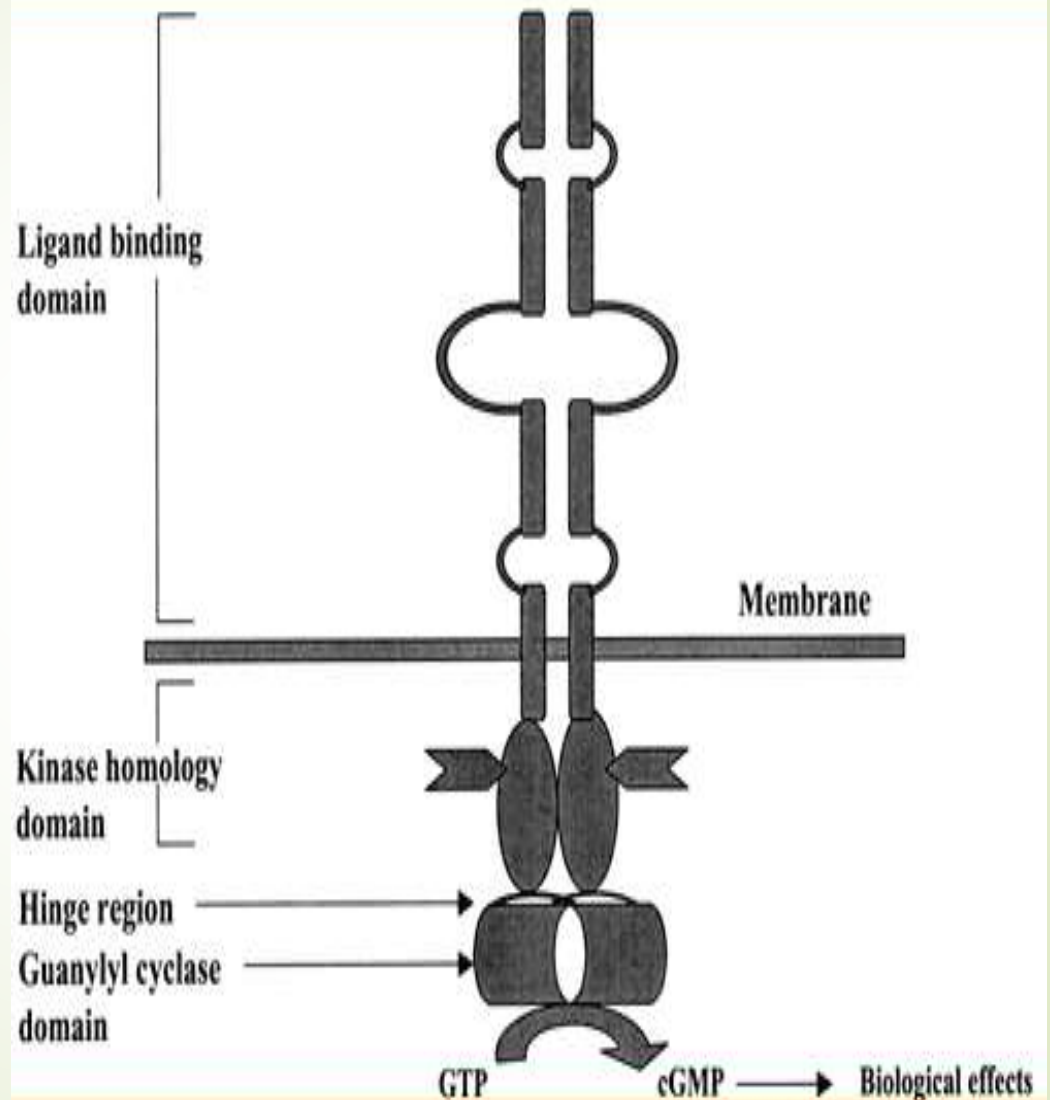
The major physiological effects of these hormones are to decrease blood pressure (ANP, BNP), to reduce cardiac hypertrophy and fibrosis (BNP), and to stimulate long bone growth (CNP).



The transmembrane receptors for ANP, BNP, and CNP are ligand-activated guanylate cyclases. The ANP and BNP receptors contain 450 amino acid extracellular domain that binds the peptide, a short 20 amino acid transmembrane domain, and large intracellular domains that contain a kinase homology region, and a C-terminal guanylate cyclase domain. Phosphorylation of serine residues in the kinase domain is important for activity; dephosphorylation of these residues leads to desensitization of the receptor.

➤ Ligand brings
juxtamembrane regions
together

➤ Phosphorylation of
serine residues →
Stimulation of guanylyl
cyclase





Downstream reactions of cGMP:

- Activation of Protein Kinase G
- cGMP gated ion channels
- cGMP-modulated Phosphodiesterase

Pharmacologically important effects of elevated cyclic GMP include modulation of platelet activation and relaxation of smooth muscle. PDEs hydrolyze the cyclic 3',5'-phosphodiester bond in cAMP and cGMP, thereby terminating their action.

Therapeutic Applications

Selected Drugs that target cGMP signalling

Drug	cGMP	Therapeutic Application
Nitroglycerine	NO Donor	Angina
Sodium Nitroprusside	NO Donor	Hypertensive Emergencies
Nesiritide	Synthetic BNP	Acutely decompensated CHF
Ecdotril	↑ BNP	Congestive Heart Failure



**BAY Series of
Compounds**

BAY 41-2272

8-pCPT-cGMP

Riociguat (Adempas)

Specific
activators &
inhibitors of
PKG

Asthma
Graft survival after liver
& lung transplantation.
Pulmonary Hypertension



THANKYOU