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Review Article

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A LITERATURE REVIEW ON SECONDARY MESSENGERS

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Introduction to Receptors & Signal Transduction

Receptors are proteinaceous in nature which are in association with cells recognizes drugs, neurotransmitters and hormones.

Signaling intracellular molecules will help in transduction of information in number of ways.

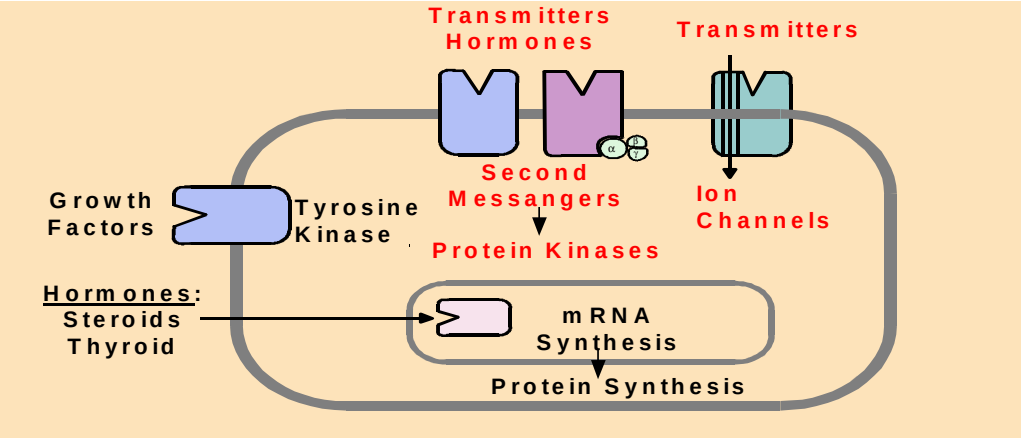

Figure-1.

Signal transduction is a phenomenon wherein the second messengers bind to a receptor extracellularly producing a chemical change inside the cell.

Signaling molecules for example Neurotransmitters acts as ion channels binding to the receptors stimulating the effector enzymes thus producing second messengers.

The neurotransmitters fall in various classes:

BIOGENIC AMINES	PEPTIDES	AMINO ACIDS	OTHER
CATECHOLAMINES	Substance P	**EXCITATORY**	Nitric oxide (NO)
Epinephrine	Neuropeptide Y	Glutamate	ATP
Norepinephrine	Enkephalins	Aspartate	Zinc

Dopamine	Somatostatin		Arachidonic acid
INDOLAMINES	VIP	**INHIBITORY**	PAF
Serotonin (5-HT)		γ-Aminobutyric acid	Carbon monoxide
Histamine		Glycine	
ESTERS			
Acetycholine			

The signal transduction at the surface of receptor occurs by three mechanisms:

1. **Ion channel systems**: The binding of a messenger to the surface of the receptor opens the ion channel, the best example being Ligand gated ion channel.
2. **Second messenger system**: The binding of first messenger on the ectoplasmic side of the receptor will activate an enzyme on the cytoplasmic side, thus increase in concentration of second messenger inside the cell. The receptor inside the cell and second messenger generating enzyme system are coupled indirectly via GTP-binding proteins(G-proteins).
3. **Receptors with integral enzyme activity**: The binding of a messenger on the ectoplasmic domain will activate an enzyme on the cytoplasmic domain of the receptor polypeptide. Thus results in producing second messenger.

The signaling processes involves G-proteins which are trimeric proteins composed of α-subunit, β-subunit and γ-subunit.

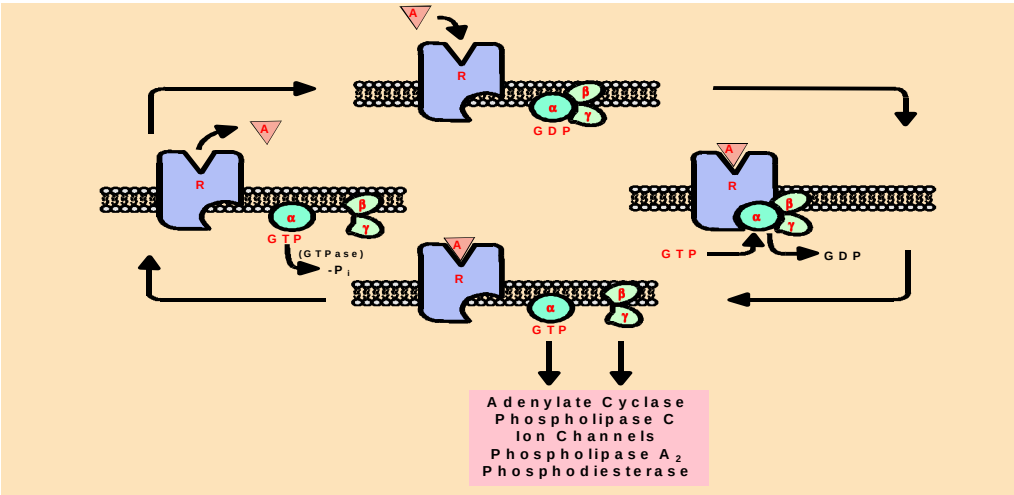

Figure 2 *"G-Protein Cycling. G-proteins are trimeric which cleave GTP (hence the name).. The alpha subunits (and probably the beta-gamma subunit as well) exerts their effects when dissociate by the binding of GTP. Activity is terminated by intrinsic GTPase activity that changes bound GTP into bound GDP. Symbols are: A = agonist; R = receptor; α,β and γ are the three subunits found in most membrane-associated G-proteins."*

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The receptor occupancy associates it with the G-protein.

1. The complex of receptor/G-protein displaces a GDP by GTP from the α-subunit.
2. GTP binding causes dissociation into free receptor, free (GTP) α-subunit, and free βγ complex.
3. The (GTP)-α-subunit produces second messenger or occasionally to open ion channels by interaction with effector protein.
4. βγ-complexes role is various signaling processes remains to be fully defined.
5. The intrinsic GTPase activity of α-subunit changes attached GTP into GDP causing inactivation of the α-subunit to rebind the βγ-complex.

The receptors to G-proteins releases a large variety of second messengers including: cAMP, Inositol triphosphate (IP_3), Diacylglycerol (DAG), cGMP, Ca^{++}, and nitric oxide (NO). These agents initiate other cellular responses.

Types of secondary messenger molecules

They are three types of second messenger molecules

- **Hydrophobic molecules**: Water insoluble molecules DAG, IP_3 Phosphatidylinositols associated with membrane and diffuse from plasma membrane into juxtamembrane space thus regulating effector proteins.
- **Hydrophillic molecules**: Water soluble molecules like cAMP, cGMP, Ca^{++} located in the cytosol.
- **Gases**: NO, CO can diffuse both through cytosol and across the membranes.

I. THE cAMP SECOND MESSENGER SYSTEM

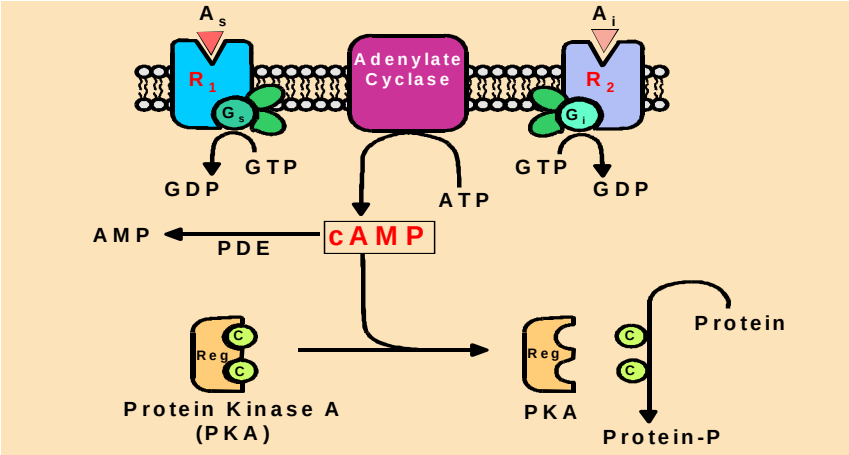

Figure-3.

Cyclic AMP is made by a membrane spanning enzymes called **Adenylate Cyclase**.

1. Two functionally important G-proteins viz G_s-type stimulate adenylate cyclase called as stimulatory G-protein and G_i-type inhibit adenylate cyclase called as inhibitory G-protein.
2. $G_{s\alpha}$ stimulates the rate of cAMP synthesis and $G_{i\alpha}$ inhibits the catalytic activity.
3. The cAMP production is regulated by **"cAMP-dependent protein kinase"** (also called **Protein Kinase C or PKC**) and modified by feedback regulation by cAMP dependant **Protein Kinase or PKA.**
4. PKA phosphorylation increases or decreases protein's activity.

Adenylate cyclase activity is modulated by factors apart from G-proteins called as multiple second messenger systems:

- Protein kinases PKA and PKC phosphorylates adenylate cyclase thus altering its activity inside the cells.
- Ca^{++}/calmodulin complexes and βγ-subunits of G-proteins alter adenylate cyclase activity.

Type	βγ-Effect	Ca^{++}/Calmodulin Effect
I	-	+
II	+	0
III	0	+
IV	+	0

First messengers which activate Adenylate cyclase

MESSENGER	TARGET CELL	PHYSIOLOGICAL EFFECTS
Glucagon	Liver	Glycogen breakdown Gluconeogenesis
Adrenaline	Adipose	Lipolysis Inhibits fatty acid synthesis
Adrenaline	Heart	Increase in heart rate Increase in contractile force
Vasopressin	Kidney	Na+/H2O reabsorption
Lutropin	Ovary	Progesterone synthesis
ACTH	Adrenal cortex	Glucocorticoid synthesis
Thyrotropin	Thyroid gland	Thyroid hormone synthesis
Parathormone	Bone	Bone resorption

First messengers which inhibit Adenylate cyclase

MESSENGER	TARGET CELL	PHYSIOLOGICAL EFFECTS
Adrenaline	Vascular smooth muscle	Contraction
Adenosine	Adipose tissue A1 purinergic receptor	Inhibits lipolysis
Acetylcholine	Heart muscle M2 receptor	Relaxation
Enkephalin	Neurons (δ receptor)	Behavioral effects
Somatostatin	Anterior Pituitary	Inhibits ACTH release

II. THE PHOSPHOINOSITOL SECOND MESSENGER SYSTEM: IP_3 AND DAG

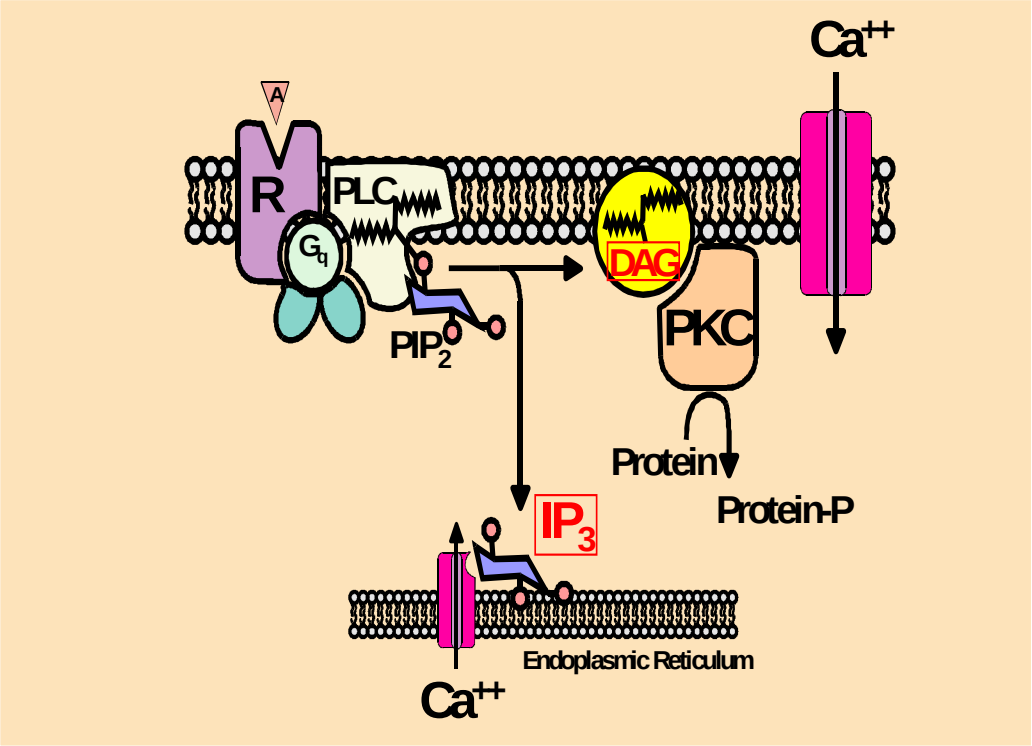

Figure 4

1. Phospholipase C (PLC) is an intracellular enzyme activated by neurotransmitters like GABA and peptide and protein hormones converting phosphatidylinositol(1,4)bis-phosphate (PIP_2) into inositol (1,4,5)tris-phosphate (IP_3) and diacylglycerol (DAG).
2. The IP_3 formed diffuse through the cytosol where it binds receptors on the endoplasmic reticulum and releases intracellular Ca^{++} in cytosol, the increased Ca^{++} triggers the response.

3. DAG remains in plasma membrane. It mediates its action through Protein kinase C (PKC). The Ca^{++} dependant activated PKC, in turn, phosphorylates many other proteins.

4. Phospholipase C activation causes influx of Ca^{++} which binds to family of Ca^{++} binding proteins which is needed for various functional activities.

Examples of responses mediated by Protein Kinase C

Tissue	Response
Blood platelets	Serotonin release
Mast cells	Histamine release
Adrenal medulla	Secretion of epinephrine
Pancreas	Secretion of insulin
Pituitary cells	Secretion of GH and LH
Thyroid	Secretion of calcitonin
Testes	Testosterone synthesis
Neurons	Dopamine release
Smooth muscle	Increased contractility
Liver	Glycogen hydrolysis
Adipose tissue	Fat synthesis

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Cellular responses elicited by adding IP_3 to either permeabilised or intact cells

Cell type	Response
Vascular smooth muscle	Contraction
Stomach smooth muscle	Contraction
Skeletal muscle	Contraction
Slime mold	Cyclic GMP formation, actin polymerisation
Blood platelets	Shape change, aggregation
Salamander rods	Modulation of light response

III. GUANYLATE CYCLASE: cGMP AND NITRIC OXIDE AS SECOND MESSENGERS

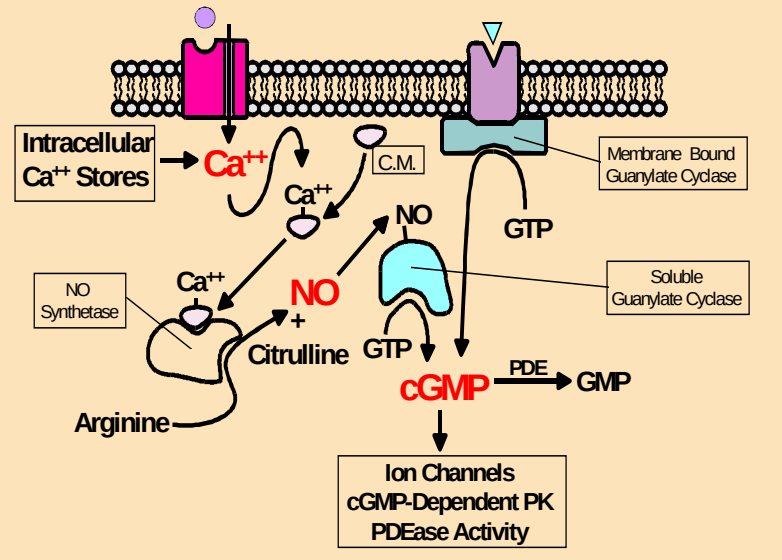


Figure 5 ***"Three second messengers: Ca^{++}, Nitric oxide (NO), and cGMP.*** *Increased intracelluar Ca^{++} can occur through receptor operated channels or by release of intracellular calcium stores. Calcium binds with a calcium binding protein such as calmodulin (C.M.), and this complex in turn activates Nitric Oxide Synthetase (NOS). NOS produces nitric oxide (NO) from the amino acid arginine. The NO that is produced activates a soluble form of guanylate cyclase to make cGMP. cGMP levels can also be increased by receptor activation of a membrane bound for of the guanylate cyclase enzyme. cGMP has a variety of tissue-specific effects".*
http://www.google.com/search?hl=en&q=signal+transduction-+membrane+function

cGMP is synthesized from GTP using an enzyme guanylyl cyclase some of the effects of cGMP are mediated through Protein kinase-G, Direct binding to ion channels and altering the activity of phosphodiesterases.

1. The phosphodiesterases break cGMP and terminate its action.
2. Arginine broken down into NO and citrulline by nitric oxide synthetase which is activated by Ca/calmodulin complex.
3. NO being membrane soluble acts by activating guanylyl cycalse and increasing cGMP in vascular endothelial cells and smooth muscles.

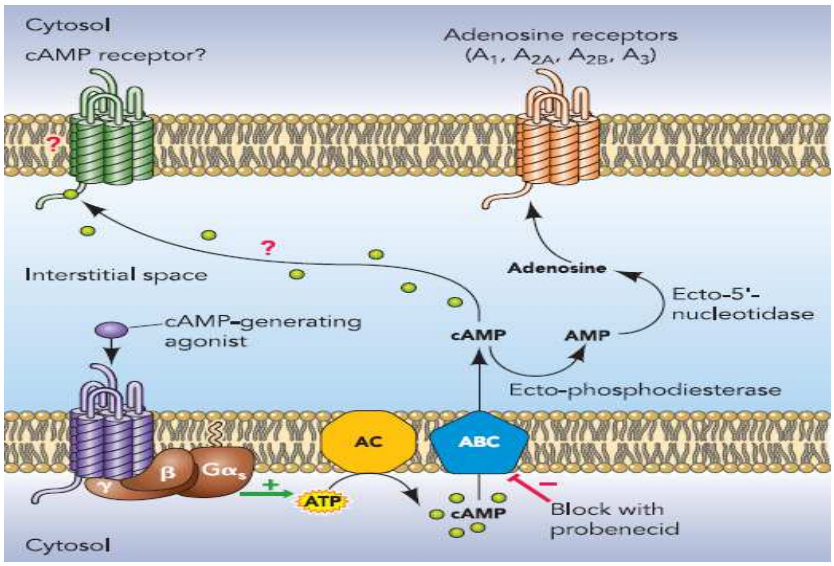
Examples

	cAMP System	**Phosphoinositol system**	**cGMP System**
Neurotransmitters (Receptor)	Epinephrine (α2, β1, β2) Acetylcholine (M2)	Epinephrine (α1) Acetylcholine (M1, M3)	-
Hormones	ACTH, ANP, CRH, CT, FSH, Glucagon, hCG, LH, MSH, PTH,	AGT, GnRH, GHRH, Oxytocin, TRH	ANP, Nitric oxide

	TSH		
Transducer	G_s (β1, β2), G_i (α2, M2)	G_p	-
Primary effector	Adenylyl cyclase	Phospholipase C	guanylate cyclase
Secondary messenger	cAMP (cyclic adenosine monophosphate)	IP_3 (inositol 1,4,5 triphosphate) and DAG (Diacylglycerol), both from PIP2	cGMP
Secondary effector	protein kinase A	Ca++ release (calcium-binding protein) and PKC (protein kinase C)	protein kinase G

Extracellular Calcium and cAMP:

Second Messengers as "Third Messengers"



"Third messenger" activity of extracellular cAMP

cAMP is formed from adenylyl cyclase by the activation of G-protein coupled receptors (GPCRs) which is linked to the stimulatory G- protein ,G_s. The cAMP thus formed can be transported actively to the extracellular space via probenecid and sulfinpyrazone through a sensitive efflux mechanism belonging to the ATP-binding cassette transporter family. The cAMP thus formed extracellularly have direct effect on the receptor effector proteins (which has not been identified yet) which are therefore expressed on the neighbouring cells.

The cAMP is sought to be metabolized by 2 enzymes

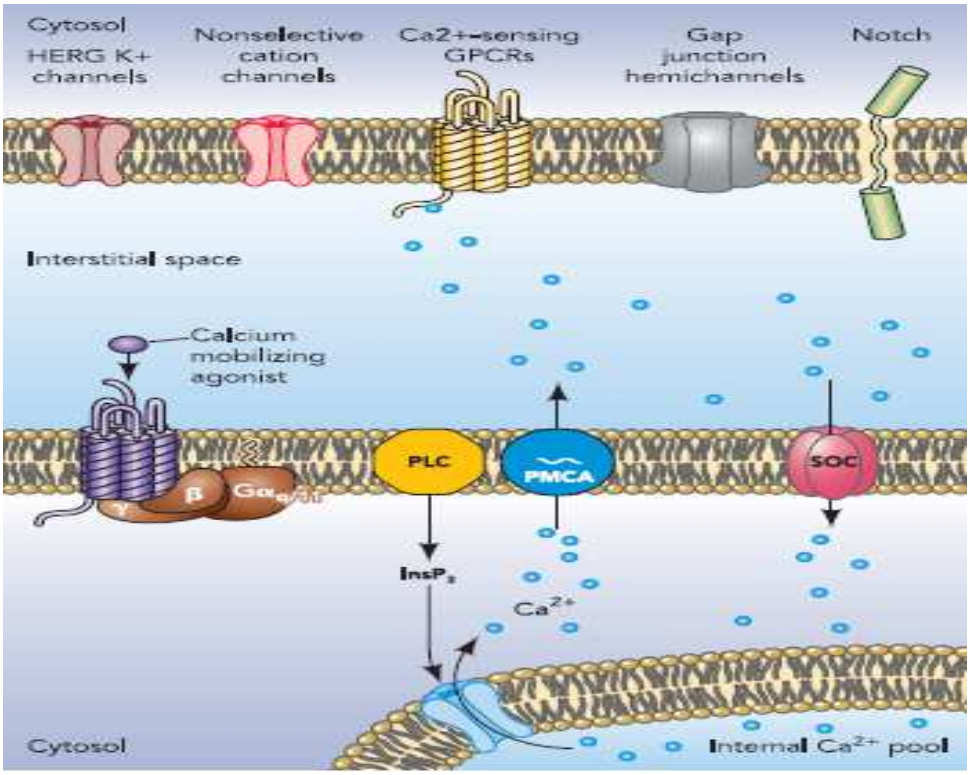
- Ecto-phosphodiesterase to adenosine monophosphate.

- Ecto-5'-nucleotidase to adenosine.

Adenosine the metabolic product of cAMP acts as paracrine or autocrine messenger to activate other transduction cascades via one or four types of adenosine receptors (A1, A2A, A2B, A3). Circulatory cAMP can be converted by ecto –enzymes rendering as a prohormone for adenosine.

"Third messenger" activity of extracellular Ca^{2+}

The [Ca^{2+}] so formed extracellularly can fluctuate due to induction by agonist intracellular Ca^{2+} signaling events. In a typical phenomenon, activation of a G_q/11-coupled receptor by a Ca^{2+}-mobilizing agonist marks an increase in the production of inositol1,4,5-trisphosphate ($InsP_3$) thus produces rise in the stored Ca2+ via $InsP_3$ receptor release channels in the intracellular Ca^{2+} pools. The fraction of calcium released in the cytoplasm is rapidly extruded by the plasma membrane Ca2+ ATPases (PMCAs), resulting in the elevation of the extracellular calcium. The emptying of store triggers influx of calcium through store-operated channels in the plasma membrane leading to depletion of calcium in the interstitial spaces. The change in the local fluctuations in calcium will influence variety of calcium sensing proteins on adjacent cells on the same cell. HERG K+ channels, various types of nonselective cation channels and host of G-protein coupled receptors modulated by extracellular calcium. Gap junction hemichannels and transmembrane protein is susceptible to alterations in extracellular calcium.



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