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Review Article

A REVIEW ON G-PROTEIN COUPLED RECEPTOR

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Abstract

G protein–coupled receptors (GPCRs), also known as seven-(pass)-transmembrane domain receptors, 7TM receptors, heptahelical receptors, serpentine receptor, and G protein–linked receptors (GPLR), constitute a large protein family of receptors that detect molecules outside the cell and activate internal signal transduction pathways and, ultimately, cellular responses. Coupling with G proteins, they are called seven-transmembrane receptors because they pass through the cell membrane seven times. G protein–coupled receptors are found only in eukaryotes, including yeast, choanoflagellates, and animals. The ligands that bind and activate these receptors include light-sensitive compounds, odors, pheromones, hormones, and neurotransmitters, and vary in size from small molecules to peptides to large proteins. G protein–coupled receptors are involved in many diseases and are also the target of approximately 34% of all modern medicinal drugs.

Keyword: -7TM receptors, heptahelical receptors, serpentine receptor, Structure.

INTRODUCTION

G protein coupled receptors (GPCRs) resemble the largest family membrane proteins in the human genome and the richest source of targets for the pharmaceutical industry. There has been remarkable progress in the field of GPCR biology during the past two decades. Notable milestones include the cloning of the first GPCR genes, and the sequencing of the human genome revealing the size of the GPCR family and the number of orphans GPCRs. Moreover, there is a growing appreciation that GPCR regulation and signaling is much more complex than originally envisioned and includes signaling through G protein independent pathways. Consequently, it has been proposed that the term GPCR be abandoned in favor of 7 transmembrane or 7TM receptors. In spite of the remarkable advances in the biology and pharmacology of GPCRs, progress in the area of protein structure has been more limited. To date, the only highresolution structures of a GPCR have been for bovine rhodopsin. In this manuscript I will briefly review the groundbreaking structural work on rhodopsin and discuss the challenges in obtaining high-resolution structures of other GPCRs. I will also discuss what we know about the structural changes associated with receptor activation.

RECEPTOR:

It is defined as a macromolecule or binding site located on cell surface or inside the effector cell that serves to recognize the signal molecule/drug and initiate the response to it, but itself has no

other function, e.g. Muscarinic (M type) and Nicotinic (N type) receptors of Cholinergic system.
Any target molecule with which a drug molecule has to combine in order to elicit its specific effect
A major group of drug receptors consists of proteins that normally serve as receptors for endogenous regulatory ligands.

Nature of Receptors:

• Not hypothesis anymore – proteins and nucleic acids
• Isolated, purified, cloned and amino acid sequencing done
• Cell surface receptors remain floated in cell membrane lipids
• Non-polar hydrophobic portion of the amino acid remain buried in membrane while polar hydrophilic remain on cell surface
• Major classes of receptors have same structural motif – pentameric etc.
• But, majority of individual receptor molecules are made up of nonidentical subunits – ligand binding brings about changes in structure or alignment of subutits
• Binding of polar drugs in ligand binding domain induces conformational changes (alter distribution of charges and transmitted to coupling domain to be transmitted to effector domain
• Many drugs act on Physiological receptors – also true drug receptors.

Receptor Subtypes:

Evaluation of receptors and subtypes – lead to discovery of various newer target molecules
Example- Acetylcholine - Muscarinic and Nicotinic, M1, M2, M3, NM, NN α (alpha) and β (beta) ….
Criteria of Classification:
Pharmacological criteria –
potencies of selective agonist and antagonists – Muscarinic, nicotinic, alpha and beta adrenergic etc.
Tissue distribution – beta 1 and beta 2
Ligand binding
Transducer pathway and Molecular cloning

Classification:-

The exact size of the GPCR superfamily is unknown, but nearly 800 different human genes (or ~ 4% of the entire protein-coding genome) have been predicted to code for them from genome sequence analysis. Although numerous classification schemes have been proposed, the superfamily was classically divided into three main classes (A, B and C) with no detectable shared sequence homology between classes.
The largest class by far is class A, which accounts for nearly 85% of the GPCR genes. Of class A GPCRs, over half of these are predicted to encode olfactory receptors, while the remaining receptors are liganded by known endogenous compounds or are classified as orphan receptors. Despite the lack of sequence homology between classes, all GPCRs have a common structure and mechanism of signal transduction. The very large rhodopsin A group has been further subdivided into 19 subgroups (A1-A19).
More recently, an alternative classification system called GRAFS (Glutamate, Rhodopsin, *Adhesion*, Frizzled/Taste2, Secretin) has been proposed. According to the classical A-F system, GPCRs can be grouped into 6 classes based on sequence homology and functional similarity:

- Class A (or 1) (Rhodopsin-like)
- Class B (or 2) (Secretin receptor family)
- Class C (or 3) (Metabotropic glutamate/pheromone)
- Class D (or 4) (Fungal mating pheromone receptors)
- Class E (or 5) (Cyclic AMP receptors)
- Class F (or 6) (Frizzled/Smoothened)

A: Rhodopsin family:

- **The largest group.**
- Receptors for most:
  - amine neurotransmitters,
  - many neuropeptides,

- purines
- prostanoids
- cannabinoids

- Short extracellular (N terminal) tail. Ligand binds to transmembrane helices (amines) or to extracellular loops (peptides).

B: Secretin/glucagon receptor family:

- Receptors for peptide hormones
  - secretin
  - glucagon
  - calcitonin
- Intermediate extracellular tail incorporating ligand-binding domain.

C: Metabotropic glutamate receptor/ calcium sensor family:

- Smallest group
  - Metabotropic glutamate receptors
  - GABAB receptors
  - Ca2+-sensing receptors
- Long extracellular tail incorporating ligand-binding domain

D: Fungal mating pheromone receptors:

- The Fungal pheromone mating factor receptors STE2 and STE3 are integral membrane proteins that may be involved in the response to mating factors on the cell membrane [PMID: 16453635, PMID: 3001640, PMID: 2836861].
- The G protein-coupled receptor repertoires of human and mouse.

E: Cyclic AMP receptors:

- Cyclic AMP receptors from slime molds are a distinct family of G-protein coupled receptors.
- These receptors control development in Dictyostelium discoideum.

F: Frizzled/Smoothened:

- Smoothened is a protein that in humans is encoded by the SMO gene.
- Smoothened is a Class Frizzled (Class F) G protein-coupled receptor.

Frizzled is a family of G protein-coupled receptor proteins that serves as receptors in the Wnt signaling pathway and other signaling pathways.

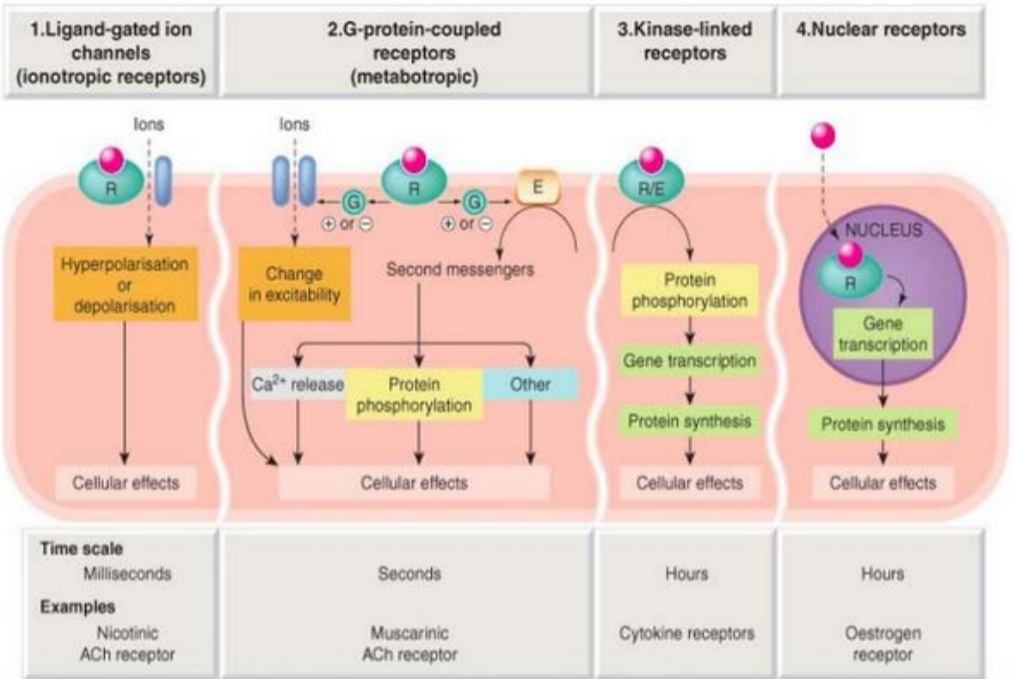

FIG:-01

G-protein coupled receptor structure

G protein–coupled receptors (GPCRs), also known as seven-(pass)-transmembrane domain receptors, 7TM receptors, heptahelical receptors, serpentine receptor, and G protein–linked receptors (GPLR), constitute a large protein family of receptors that detect molecules outside the cell and activate internal signal transduction pathways and, ultimately, cellular responses. Coupling with G proteins, they are called seven-transmembrane receptors because they pass through the cell membrane seven times.

G protein–coupled receptors are found only in eukaryotes, including yeast, choanoflagellates, and animals. The ligands that bind and activate these receptors include light-sensitive compounds, odors, pheromones, hormones, and neurotransmitters, and vary in size from small molecules to peptides to large proteins. G protein–coupled receptors are involved in many diseases and are also the target of approximately 34% of all modern medicinal drugs. There are two principal signal transduction pathways involving the G protein–coupled receptors:

- the cAMP signal pathway and
- the phosphatidylinositol signal pathway.

When a ligand binds to the GPCR it causes a conformational change in the GPCR, which allows it to act as a guanine nucleotide exchange factor (GEF). The GPCR can then activate an associated G protein by exchanging the GDP bound to the G protein for a GTP. The G protein's α subunit, together with the bound GTP, can then dissociate from the β and γ subunits to further affect intracellular signaling proteins or target functional proteins directly depending on the α subunit type ($G_{\alpha s}$, $G_{\alpha i/o}$, $G_{\alpha q/11}$, $G_{\alpha 12/13}$)

Table 01: GPC Receptors

G Protein	Receptors	Signaling Pathway
G_S	Beta adrenergic receptors, glucagon, histamine, serotonin	Increase CAMP Excitatory effects
G_i	Alpha$_2$ adrenergic receptors, mAchR, opioid, serotonin	Decrease CAMP Cardiac K^+ channel open- decrease heart rate
G_q	mAchR, H1, α1, Vasopressin type 1, 5HT$_{1C}$	PLC- IP_3 , DAG Increase Cytoplasmic Ca
G_t	Rhodopsin and colour opsins in retinal rod and cone cells	Increase cGMP phosphodiesterase. Decrease cGMP

GPCRs associate with heterotrimeric G-proteins (green), that is, G-proteins composed of three different subunits: alpha, beta, and gamma. The subunits are tethered at the membrane surface by covalently attached lipid molecules.

When a ligand binds, the receptor activates the attached G-protein by causing the exchange of GTP (yellow) for GDP (red). The activated G-protein then dissociates into an alpha (G-alpha) and a beta-gamma complex. G-alpha bound to GTP is active and can diffuse along the membrane surface to activate (and sometimes inhibit) target proteins, often enzymes that generate second messengers. Likewise, the beta-gamma complex is also able to diffuse along the inner membrane surface and affect protein activity.

Inactivation occurs because G-alpha has intrinsic GTPase activity. After GTP hydrolysis, G-alpha bound to GDP will reassociate with a beta-gamma complex to form an inactive G-protein that can again associate with a receptor. The GTPase activity of the G-alpha can be made faster by other proteins--sometimes the target protein, sometimes a separate regulatory protein. Cholera toxin causes a chemical modification that *prevents* GTP hydrolysis and leads to unregulated signaling

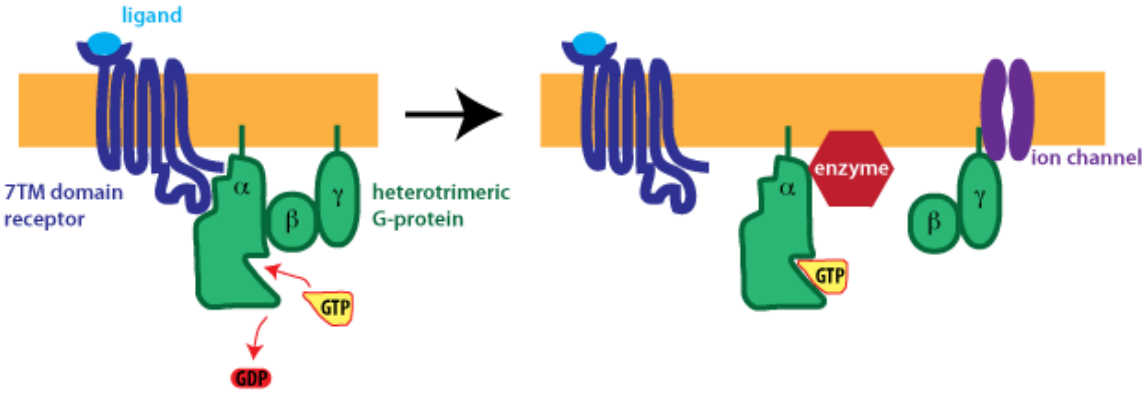

FIG:-02

The G protein cycle

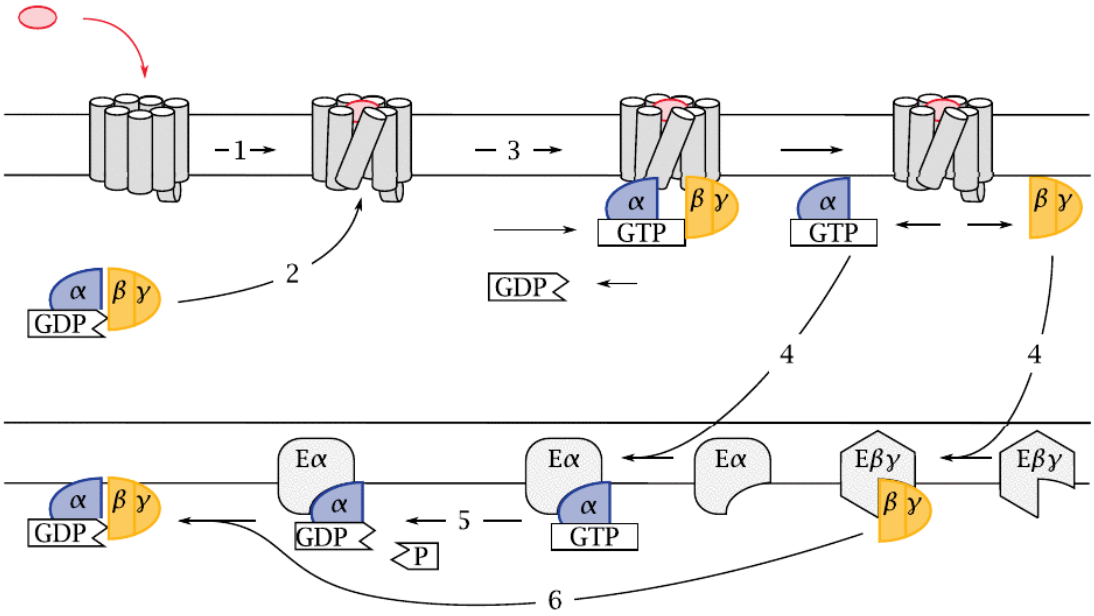

FIG:-03

GPCRs activate GTP-binding proteins, or G proteins for short, which in turn activate various effector proteins. G proteins are heterotrimers; their subunits are referred to by the Greek letters α, β and γ.

G proteins undergo repeated cycles of activation and inactivation. The cycle starts when an agonist binds to the extracellular face of a GPCR (1), which changes the conformation of the entire GPCR molecule, including its intracellular surface. In this activated conformation, the GPCR binds a G protein (2). The α-subunit of this G protein then releases a molecule of GDP, which was left behind by a previous turn of the cycle. Next, it binds

GTP and then dissociates from the βγ-dimer. The two dissociated G protein fragments are now free to seek out and bind to their respective effector proteins (4), which have various biochemical activities (see later).

After some time has elapsed, the bound GTP molecule is cleaved by the built-in GTP′ase activity of the Gα-subunit (5). This causes the α-subunit to leave its effector and to again associate with a Gβγ dimer (6). The inactive trimer then awaits another round of activation.

G protein effector mechanisms

For each of the three subunits of the heterotrimeric G proteins—α, β and γ—there are several subtypes, which may combine into heterotrimers in various permutations. However, overall, G proteins are less diverse than GPCRs; therefore, multiple types of GPCRs must converge upon the same G proteins and trigger the same intracellular responses.25 We will now look at the major intracellular signaling cascades triggered by different G proteins.

Adenylate cyclase

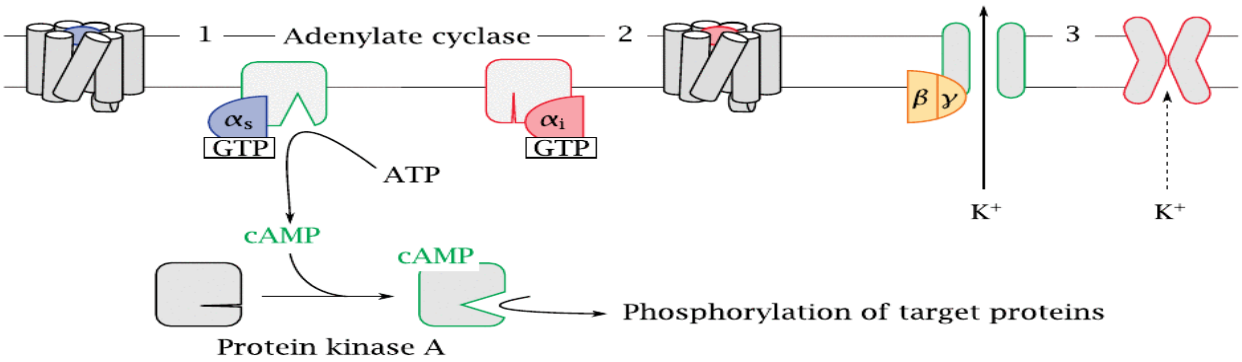

FIG:-04

One important effector protein is adenylate cyclase. This membrane-associated enzyme converts ATP to cyclic AMP (cAMP), which is an allosteric activator of protein kinase A.

Adenylate cyclase is controlled by two different Gα subunits, which are activated by different GPCRs. The stimulatory α-subunit ($G\alpha_s$, 1) activates the enzyme, whereas the inhibitory α-subunit ($G\alpha_i$, 2) inhibits it.

An important effector molecule for Gβγ dimers are K^+ channels of the K_{ir} type (3). Opening of these channels will cause hyperpolarization of the cell membrane. In excitable cells, this will tend to reduce the level of activity.

The phospholipase C cascade

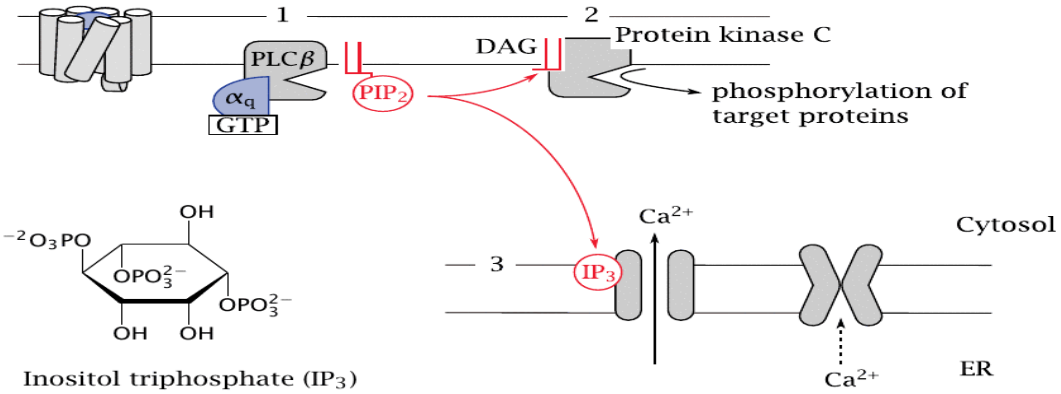

FIG:-05

Another effector pathway that is activated downstream of many pharmacologically important GPCRs is the phospholipase C cascade. For example, the contraction of vascular smooth muscle cells downstream of α-adrenergic receptors (slide 2.3.4) or angiotensin receptors (slide 1.2.5) is mediated by this pathway.

In this cascade, the α-subunit of G_q stimulates phospholipase Cβ (1). The activated enzyme then cleaves a specific membrane lipid, phosphatidylinositol-bisphosphate (PIP_2), into two secondary messengers, namely, diacylglycerol and inositoltriphosphate.

Diacylglycerol (DAG) is a very hydrophobic molecule that remains associated with the cytoplasmic membrane, where it activates protein kinase C (2). Phosphorylation of target proteins by this kinase causes various downstream effects.

The other messenger, inositoltriphosphate (IP_3), is water-soluble. It travels through the cytosol to the ER membrane, where it activates the IP_3 receptor, which is a calcium channel (3). Ca^{++} ions released from the ER into the cytosol activate calmodulin, which in turn will affect numerous calmodulin-dependent proteins. In smooth muscle, a calmodulin-dependent protein kinase phosphorylates myosin, which triggers contraction.

Structure and function of GPCRs

Much experimental effort has been devoted to studying the conformational changes that are at the heart of GPCR function. Many of these studies have been performed on rhodopsin. This molecule differs from other GPCRs in being activated not by ligand binding and dissociation, but instead by photoisomerization of its covalently bound retinal chromophore. However, it is the easiest GPCR molecule to obtain in abundance, and it was the first one to be crystallized, which made it an attractive model. Since it shares extensive homology with many GPCRs that are drug targets, it is also a credible and useful model.

Crystal structures of other GPCRs have now started to appear, and they will indubitably enhance our understanding and the precision of drug design. However, crystal structures are static, and other experimental methods remain relevant for understanding the molecular movements and interactions. We will consider a few selected examples below. The first example involves the receptor for substance P, a peptide neurotransmitter.

Orphan GPCRs

200 or so known GPCRs whose endogenous ligands and functions are not known Attempts have been made to deorphanise these receptors. Evidence that some recently deorphanised GPCRs, such as orexin receptor, may dimerise or associate with more classical GPCRs

Table 02:

Orphan receptor	*Ligand*	*Therapeutic indication*	*Action of the compounds*
ORL-1 (NOP)	Nociceptin/Orphanin FQ	Stress and pain	Agonist
Edg1, 3, 5, 6, 8	S1P	Autoimmune diseases	Agonist/antagonist
H3	Histamine	Dementia	Antagonist/inverse agonist
Orexin1, 2	OrexinA and B	Sleep disorders	Antagonist
SLC-1 (MCH1)	MCH	Obesity, anxiety and depression	Antagonist
GHSR	Ghrelin	Catabolic disorders	Agonist
GPR38	Motilin	Gastroparesis and irritable bowl syndrome	Agonist
GPRv53 (H4)	Histamine	Inflammation	Antagonist
P2Y12	ADP	Platelet aggregation	Antagonist
GPR16 (BLT1)	LTB4	Inflammation and rheumatoid arthritis	Antagonist
BLT2	LTB4	Inflammation and rheumatoid arthritis	Antagonist
HG55 (CysLT1)	LTD4	Bronchoconstriction	Antagonist
GPR40	Medium and long fatty acids	Diabetes	Agonist
HM74A, B	Nicotinic acid	Dyslipidaemia	Agonist

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