MACQUARIE University

Biochemical Signalling - 2
G proteins and GPCRs

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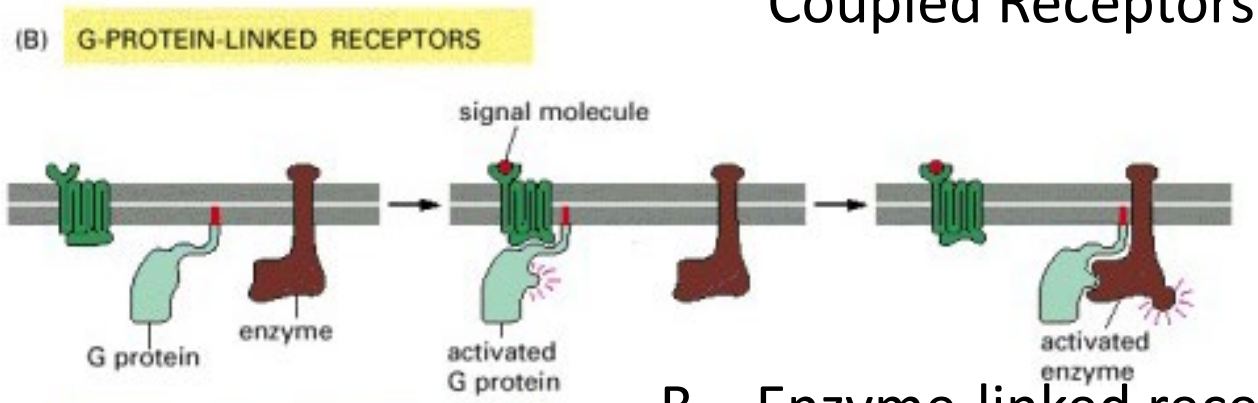
Objectives

- Receptors that bind hormones (continued)
  - G Proteins and their receptors, GPCRs
    - cAMP Pathway
    - Phosphoinositide Pathway
  - Drugs and Toxins affecting Cell Signalling
- Complexity of Hormonal Signalling

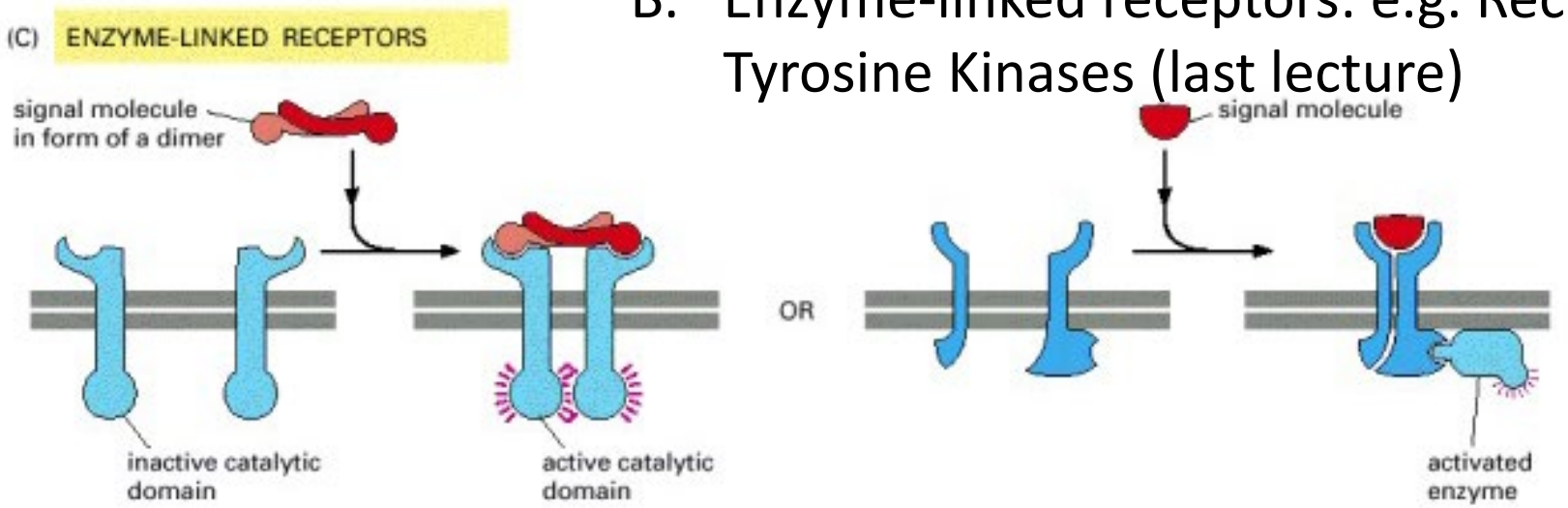
Textbook Chap.20

Main classes of hormonal receptors

A. G-Protein-linked receptors or G-Protein Coupled Receptors (GPCRs) – this lecture



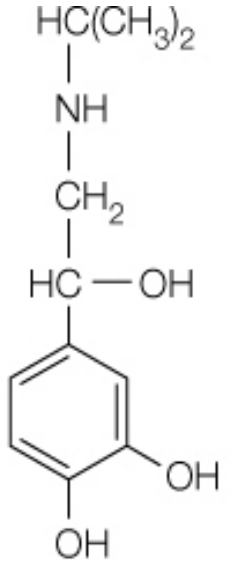
B. Enzyme-linked receptors: e.g. Receptor Tyrosine Kinases (last lecture)



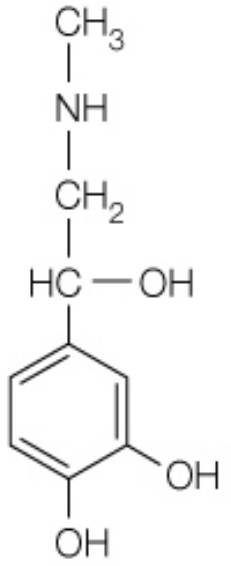
What are GPCRs?

- Largest known protein family with >800 members in the human genome.
- Essential sensory functions: sight, smell and taste.
- Ligands include:
  - nucleosides, nucleotides,
  - Ca^{2+},
  - catecholamines (adrenalin and nonadrenalin), other bioactive amines (e.g., histamine and serotonin) and a variety of peptide and protein hormones, and
  - Lipids.
- Target of >30% of pharmaceutical drugs.

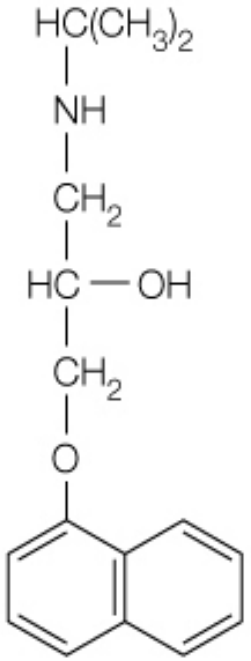
Receptors historically defined by Interactions with Drugs (from last lecture)



Isoproterenol



Epinephrine



Propranolol

- Before the structure of hormone receptors were known, tissues and cells had been categorized pharmacologically in terms of their response to epinephrine analogs
- The epinephrine (adrenaline) analogs such as isoproterenol and propranolol acted either as:
  - agonists – agents that act similarly to epinephrine
  - antagonists – agents that block the action of epinephrine
- Propranolol antagonizes β_2-adrenergic receptors (controls blood pressure)
- Isoproterenol agonizes β_2-adrenergic receptors (used to treat asthma)

Adrenergic Receptors are GPCRs

- Adrenergic is based on **adrenalin**, an older term for **epinephrine**
- Adrenergic receptors were originally thought to be adenylate cyclases
- It is now known that hormones that act through second messengers involve a three-protein module:
  - **Receptor**
  - **Transducer** (G-protein)
  - **Effector** (adenylate cyclase or a related enzyme)

TABLE 20.1 Some biological actions associated with adrenergic receptors

Receptor Class	Action
α_1	Smooth muscle contraction in blood vessels and skin, gastrointestinal system, kidney, and urethral sphincter Increased sweat gland secretion
α_2	Decreased glucagon and insulin release from pancreas Contraction of sphincters in the gastrointestinal tract
β_1	Increased heart rate, contraction, and expelled fluid volume Increased renin secretion from kidney
β_2	Smooth muscle relaxation in gastrointestinal tract, lung bronchia Increased lipolysis Increased glycogenolysis and gluconeogenesis Decreased histamine release from mast cells Increased anabolism in skeletal muscle

Signalling by G Proteins – 3 components

1. ***Receptor:*** The extracellular signalling molecule binds to **G-protein-coupled receptors (GPCRs),** which are transmembrane proteins.
2. ***Transducer:*** G proteins are heterotrimers that are anchored to the cytoplasmic side of the membrane.
3. ***Effector*** - Second messenger system: Adenylate cyclase is a transmembrane enzyme that generates the secondary messenger, cAMP (cyclic AMP).

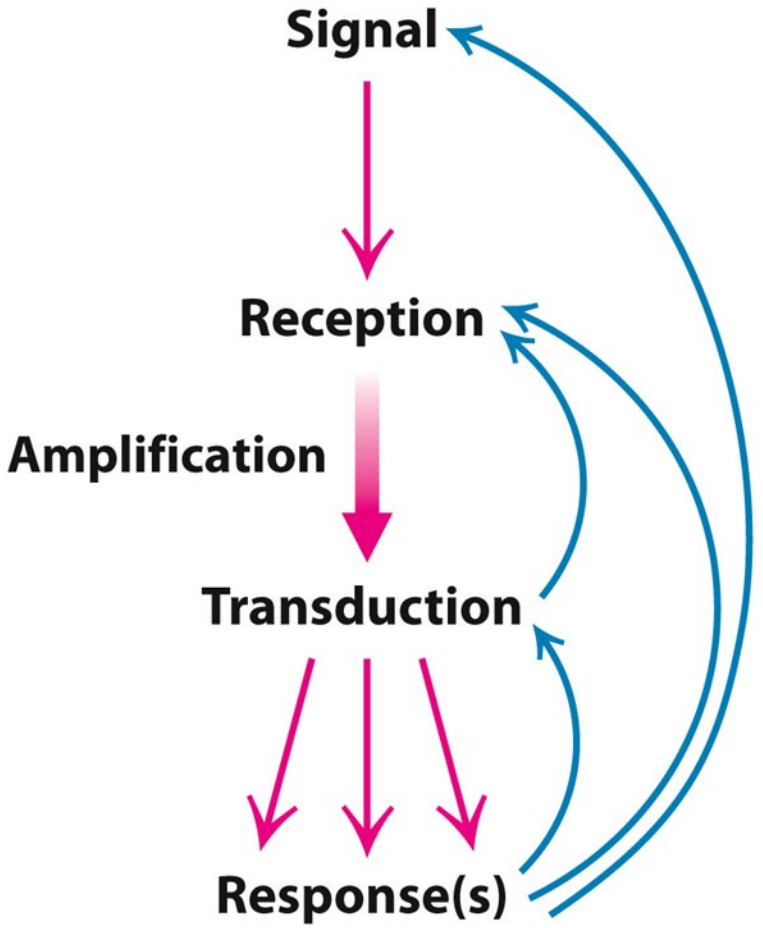


Figure 14.2
Biochemistry, Eighth Edition

Amino Acid Sequence of the Human β_2-Adrenergic Receptor

1. Like all G protein-coupled receptors (GPCRs), the human β_2-adrenergic receptor has seven transmembrane (TM) helices
2. GPCRs are the largest family of membrane receptors in humans.
   - N-terminus: extracellular
   - 7 hydrophobic sequence segments embedded in the membrane as α-helices
   - Loops in between
   - Long cytoplasmic tail, with the C-terminus
3. Ligand-binding site is located between the TM helices.
   - TM helices twist when ligand is bound.

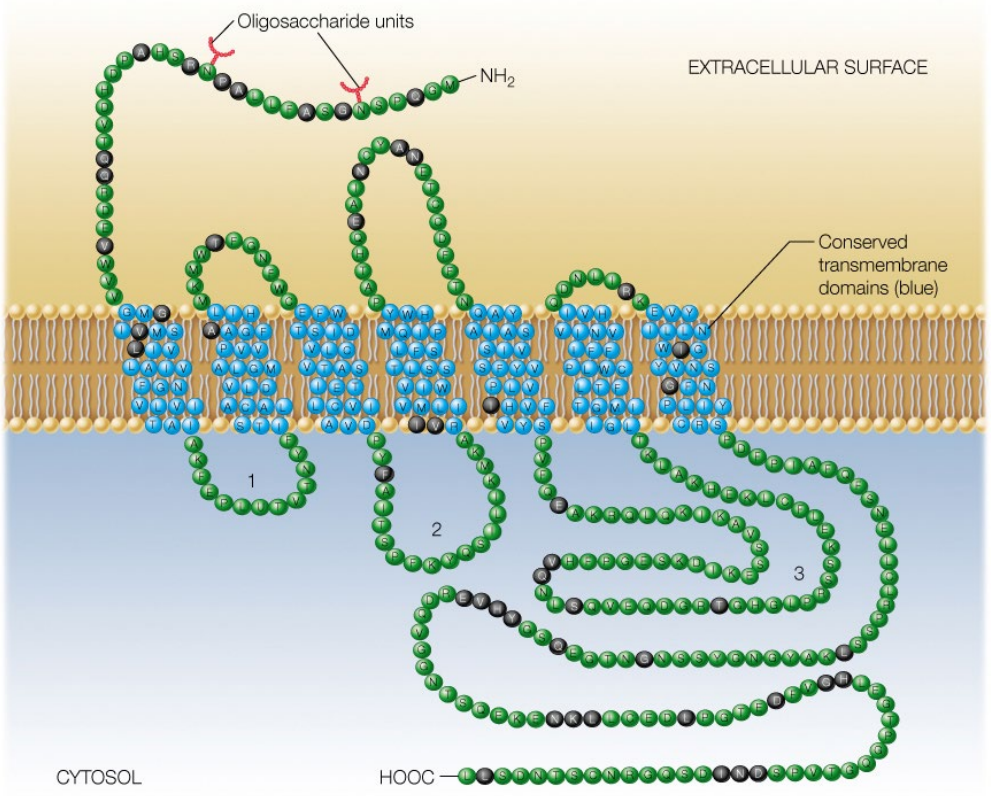


FIGURE 20.3 Amino acid sequence of the human β_2-adrenergic receptor.

GPCR Structure

- The β_2-adrenergic receptor (β_2AR) and rhodopsin are G-protein coupled receptors (GPCRs)
- The structure of β_2AR in complex with carazolol (a β_2 antagonist) is shown at top
- The bottom panel shows striking similarity between β_2AR (bottom right) and the vision protein rhodopsin (bottom left)

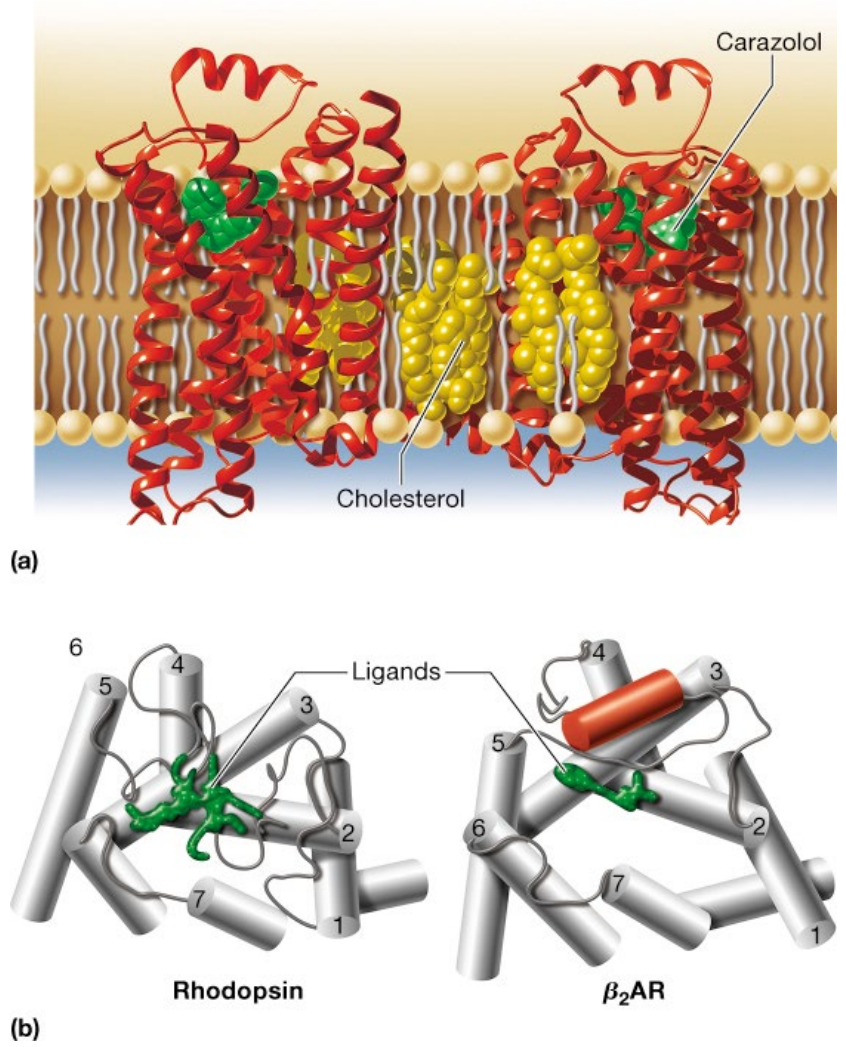
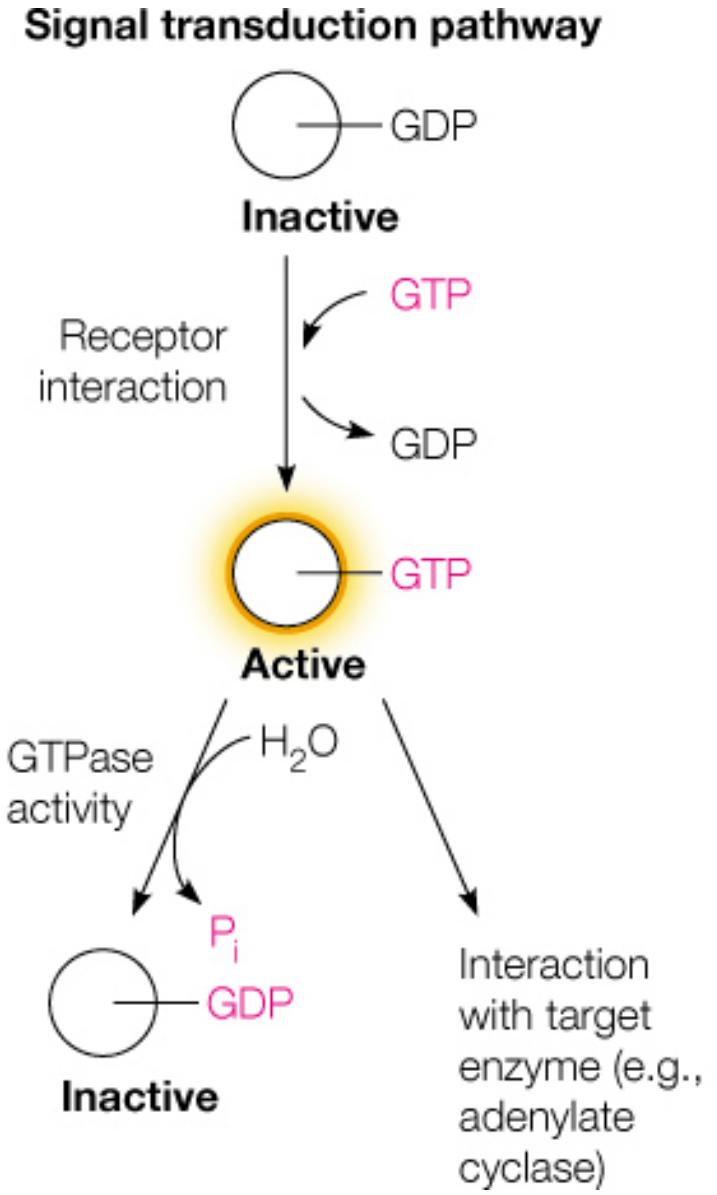


FIGURE 20.4 Structure of the human β_2 adrenergic receptor.

G Proteins

- are heterotrimeric proteins (α, β, γ) that can bind GTP and GDP via the α subunit
- The binding of extracellular hormone or agonist to a GPCR causes a conformational change that stimulates the receptor to interact with a G protein
- This interaction of GPCR with G protein stimulates an exchange of bound GDP to GTP in the α subunit of the G protein
- When G proteins bind GTP, some (G_s) activate adenylate cyclase; others (G_i) inhibit adenylate cyclase
- The α subunit possesses weak GTPase activity, slowly converting GTP to GDP



The α-subunit of G protein binds to the intracellular part of GPCR

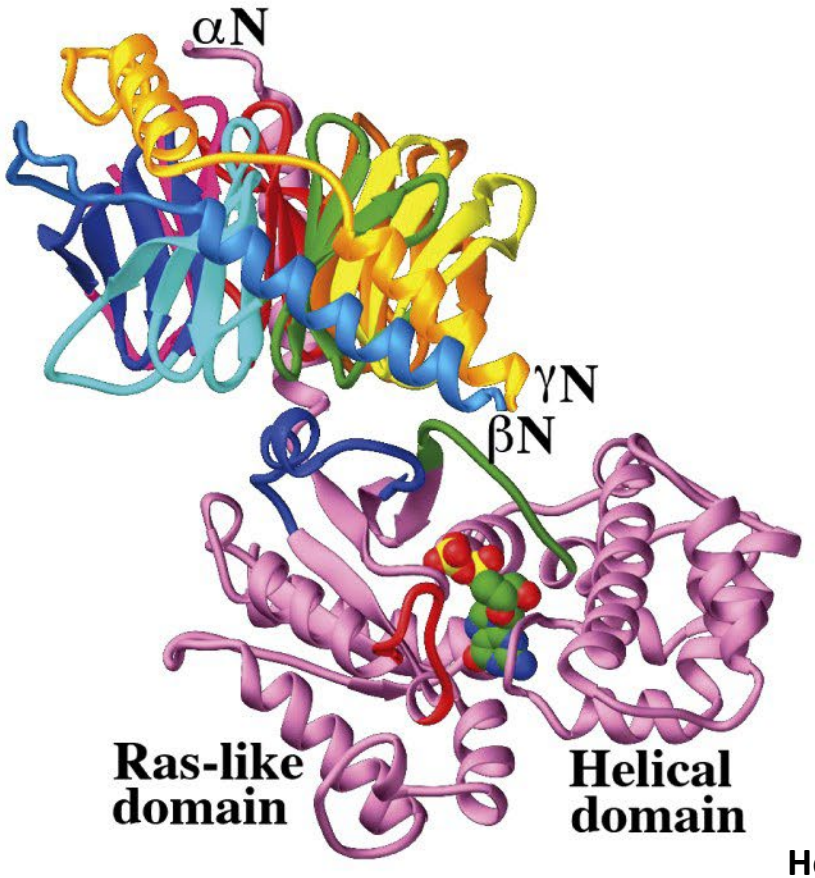
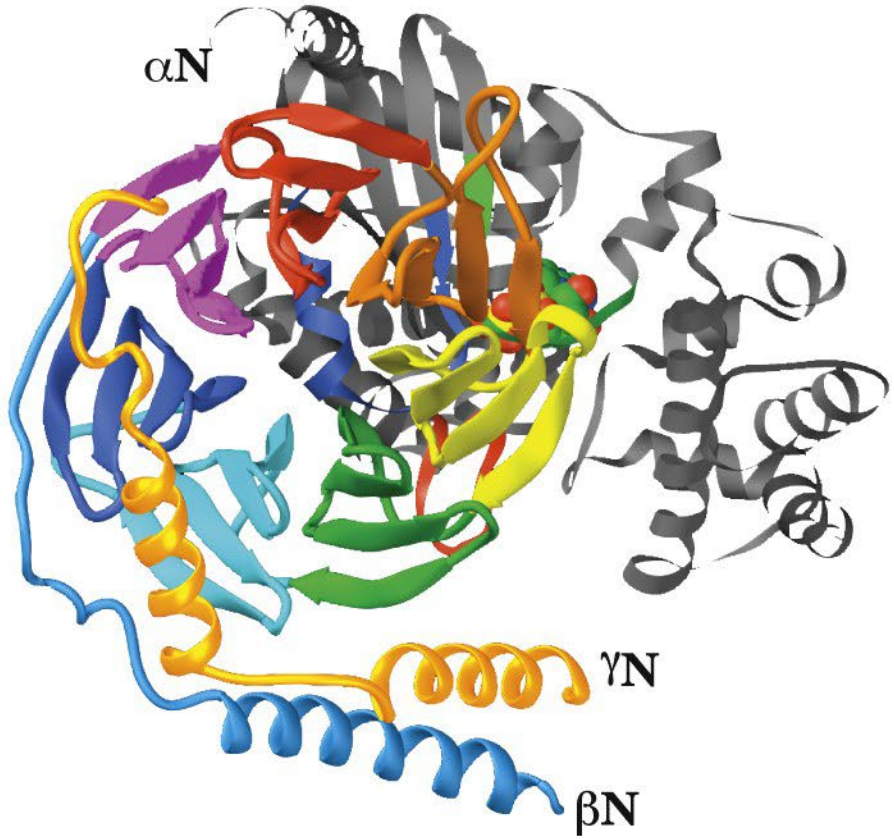


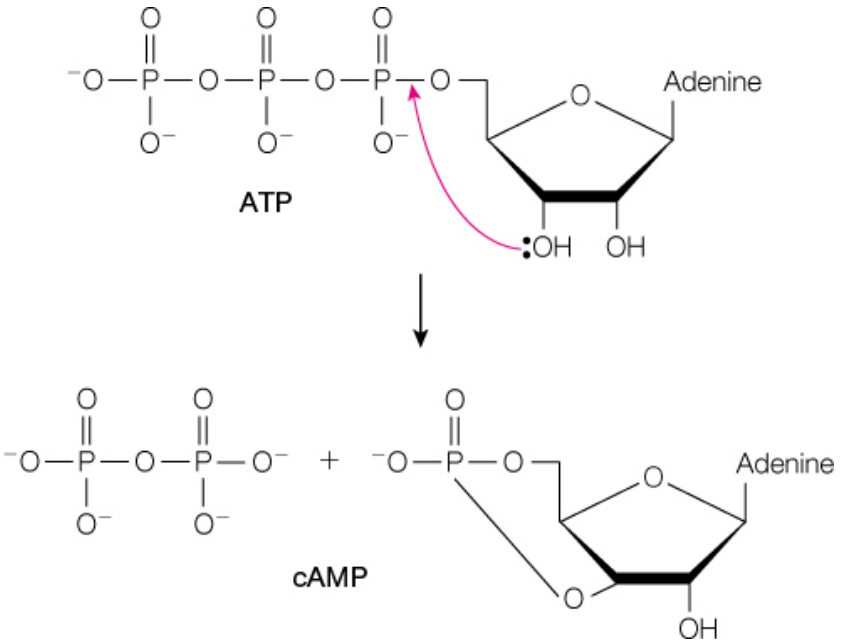
Figure 13-19

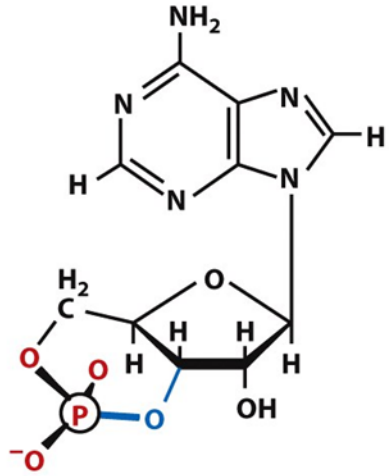


Heterotrimeric G protein
PDBid 1GP2

Effector Protein Adenylate Cyclase (AC)

- G-proteins have several targets but **AC is the target in adrenergic signaling**
- AC catalyzes the conversion of ATP to the second messenger **cyclic AMP (cAMP)** and pyrophosphate
- **GAP, the GTPase-activating protein** helps to turn on the GTPase activity of the α subunit, **initiating the release from AC, thereby deactivating AC**
- **cAMP targets soluble protein kinases** and **activates them to phosphorylate other proteins** – this is the end result of GPCR signalling.
- Active second **cAMP** is eventually **converted to the normal (inactive) AMP** by **cAMP phosphodiesterase**

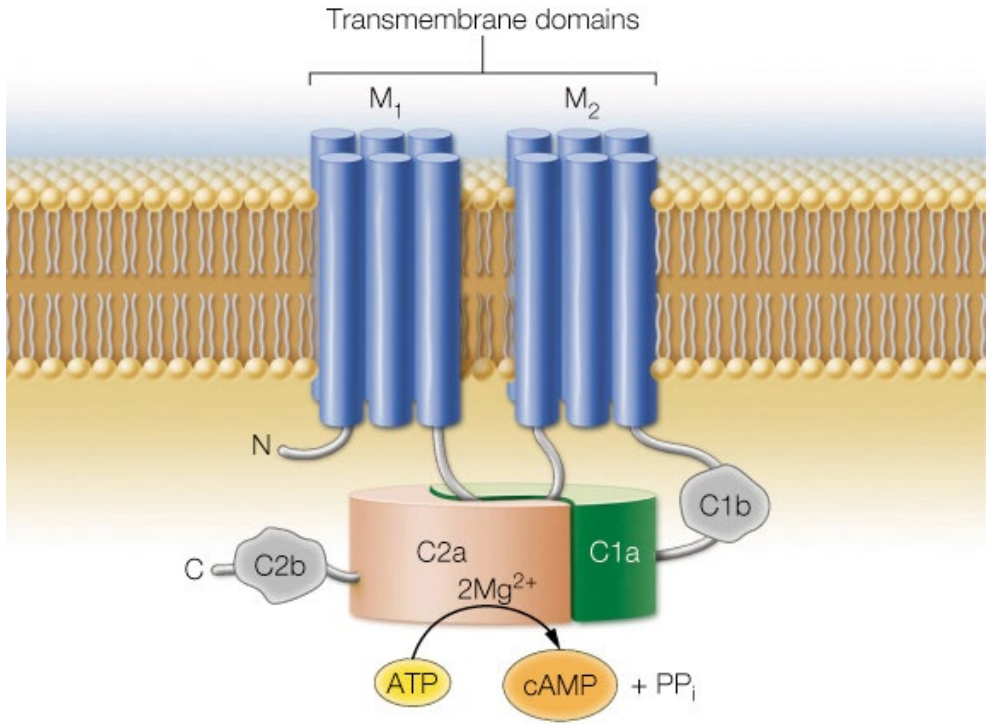




3′,5′-Cyclic AMP (cAMP)

Effector Protein Adenylate Cyclase (AC)

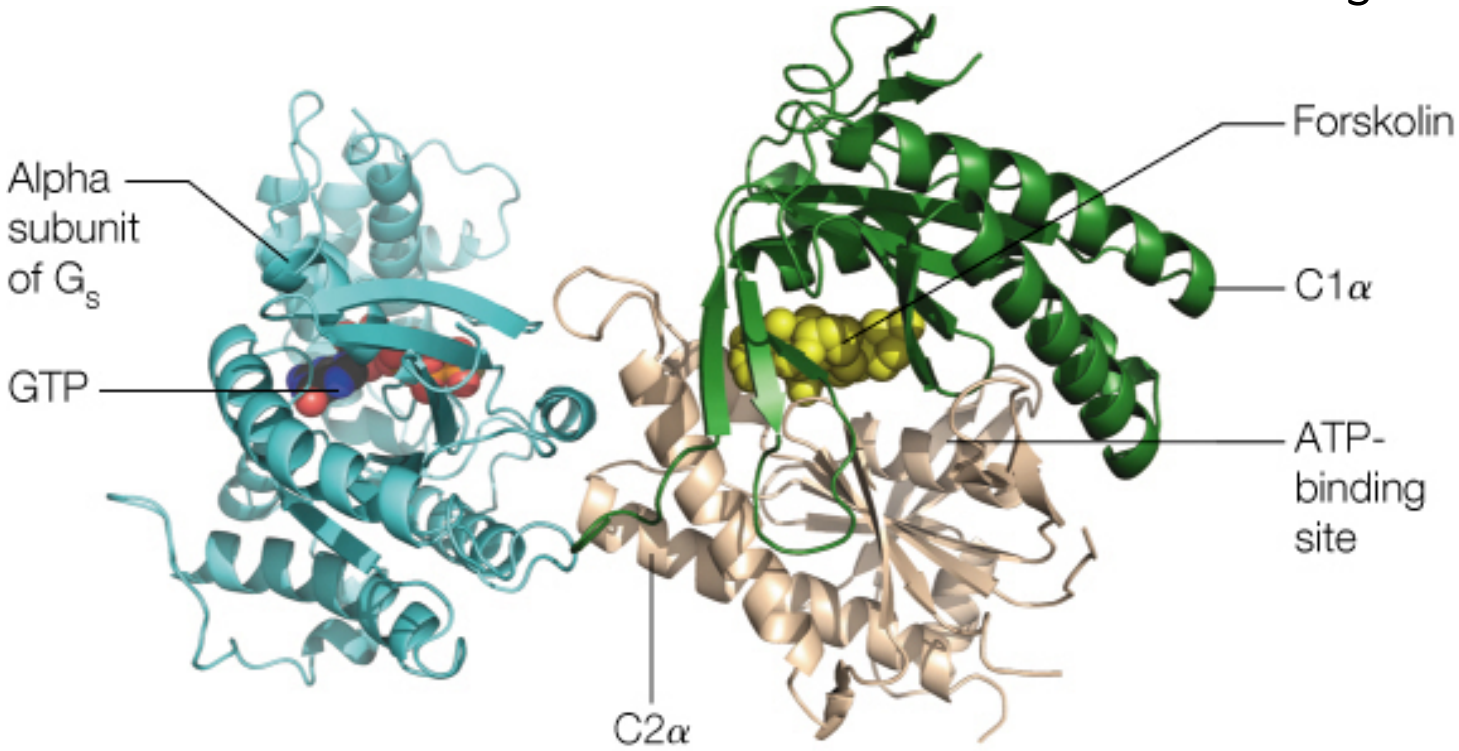
- AC consists of two transmembrane domains (M_1 and M_2) and two homologous cytoplasmic domains (C1α and C2α) that come together to form the active site



(b) Schematic diagram showing relationships of the catalytic domains to the transmembrane helical regions.

FIGURE 20.7 Crystal structure of an adenylate cyclase catalytic domain.

Crystal Structure of the AC Catalytic Domains Bound the α Subunit of G_s



(a) The C1α and C2α catalytic domains (tan and green) were crystallized as a complex with forskolin (yellow) and α_s, the α subunit of G_s (turquoise). The catalytic site where ATP is bound consists of residues from both domains. GTP bound to α_s is shown as well (blue and red colored atoms).

FIGURE 20.7 Crystal structure of an adenylate cyclase catalytic domain.

Steps in G protein signalling

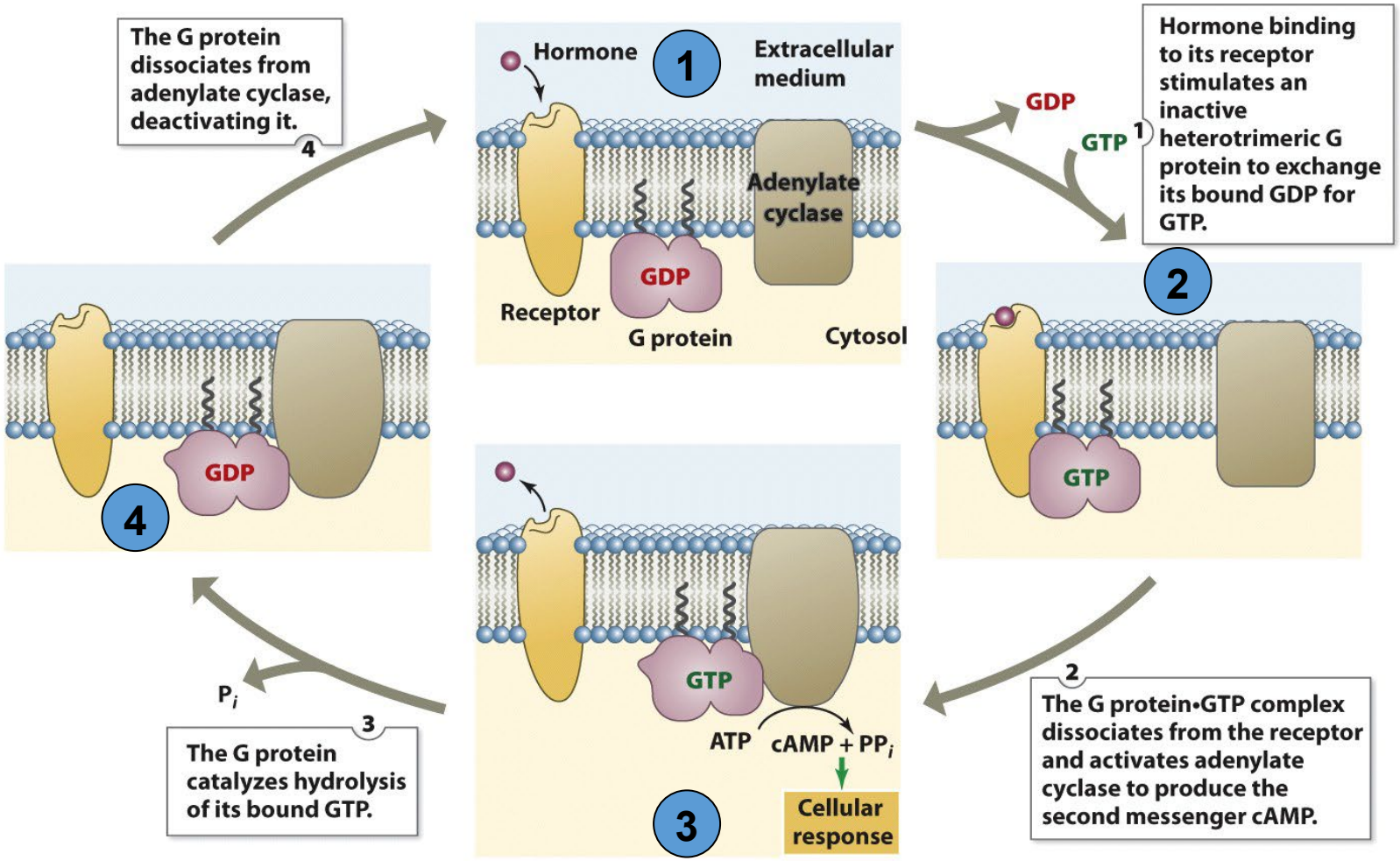


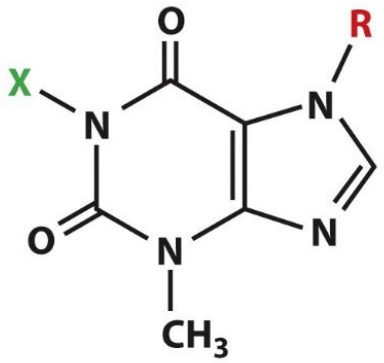
Figure 13-17

Ending cAMP signalling

- The secondary messenger, cAMP, has to be hydrolysed to AMP, to end the signalling process.
- Enzymes, cAMP-phosphodiesterases (PDEs), step in to do this. PDEs are activated by
  - Ca^{2+}
  - Phosphorylation by PKA and insulin-stimulated PKA.
- PDEs provide crosstalk between cAMP-based and other signalling systems.
- Drugs that are PDE inhibitors used for asthma, congestive heart failure, depression, inflammation, retinal degeneration and erectile dysfunction (Viagra).

Drugs affect Cell Signalling

- Adenylate cyclase system affected by adenosine analogues in coffee, tea and chocolate (xanthine alkaloids).



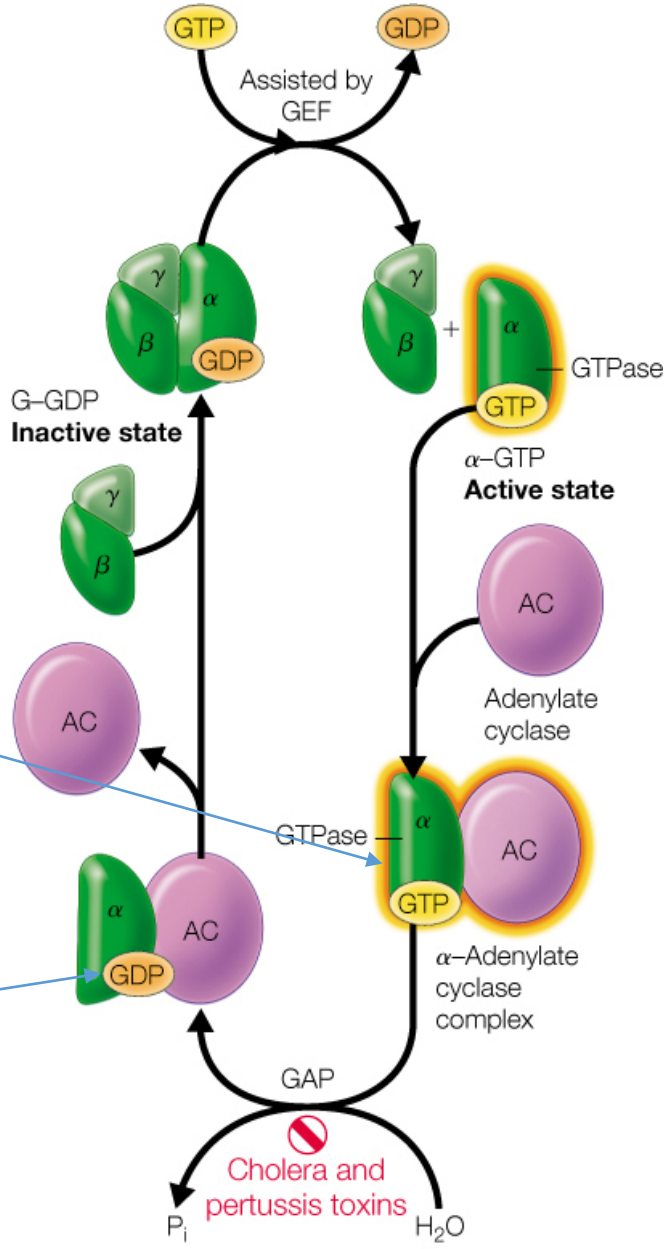
$R = CH_3$	$X = CH_3$	**Caffeine (1,3,7-trimethylxanthine)**
$R = H$	$X = CH_3$	**Theophylline (1,3-dimethylxanthine)**
$R = CH_3$	$X = H$	**Theobromine (1,7-dimethylxanthine)**

Box 13-4 part 1

- Antagonise adenosine receptors, through inhibitory G proteins
- Lead to excess cAMP production
- Results in downstream PKA activation.

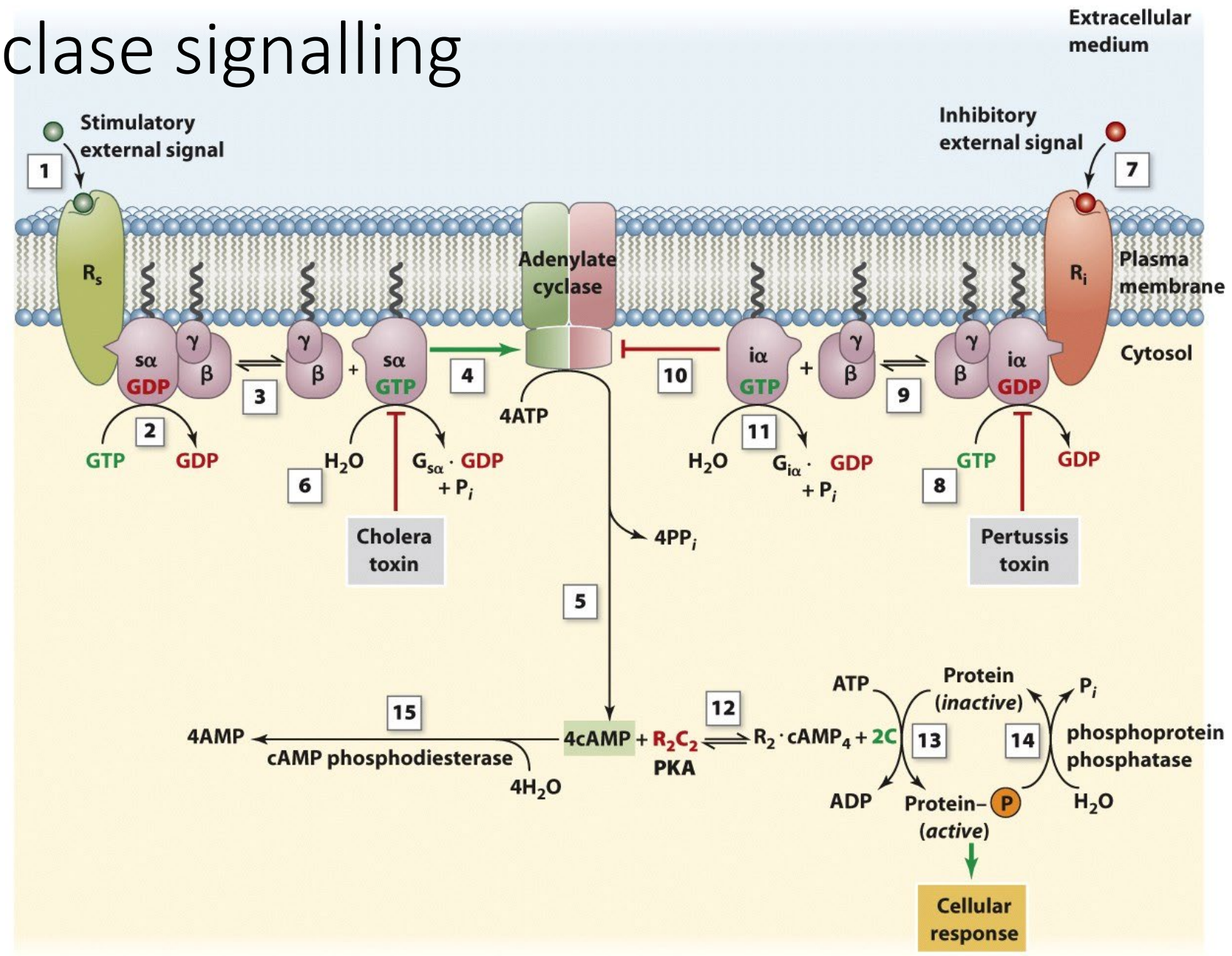
Toxins affect Cell Signalling

- Bacterial toxins affect G protein function
- A fragment of **cholera toxin** causes a side chain modification on Gα, forming Gsα.
  - cAMP is produced.
  - GTP cannot be hydrolysed by Gsα.
  - Fluid influx in intestines, due to uncontrolled cAMP levels.
  - Dehydration.
- Similar but less dramatic effect from ***E. coli*** through heat-labile enterotoxins produced.
- **Pertussis toxin** (from the whooping cough agent) causes a different side chain modification on Gα, forming Giα.
  - Giα cannot exchange bound GDP for GTP
  - Once again cAMP levels go unchecked.
  - ~400,000 infant deaths worldwide.
  - (prevented by triple antigen, DTP vaccine).



The adenylate cyclase signalling system

- Toxins affect G protein function



Module

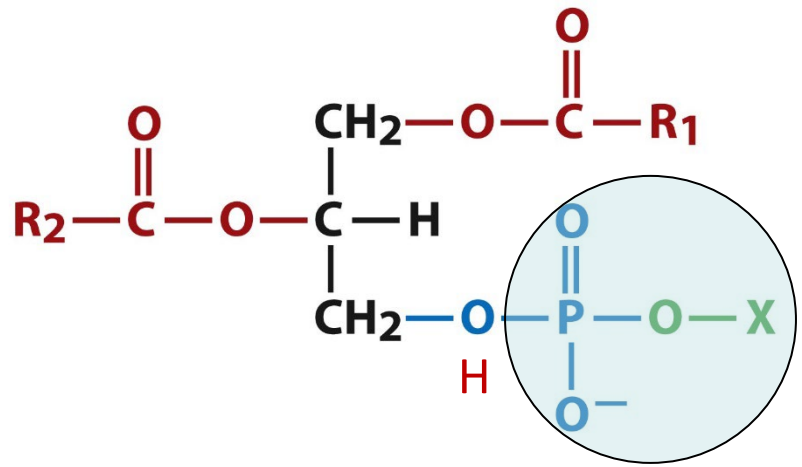
Figure 13-23

Alternative to cAMP Signalling: the Phosphoinositide Pathway

- Ingredients: GPCR, heterotrimeric G protein, a specific phospholipase (PLC) and a phosphorylated glycerophospholipid.
- Three secondary messengers generated:
  - IP3: inositol-1,4,5-triphosphate
  - Ca^{2+} and
  - **DAG: diacyl-glycerol**. This could differ, based on the phospholipid that is hydrolysed.
    - Lipid soluble and activates membrane bound PKC, which triggers a cascade.

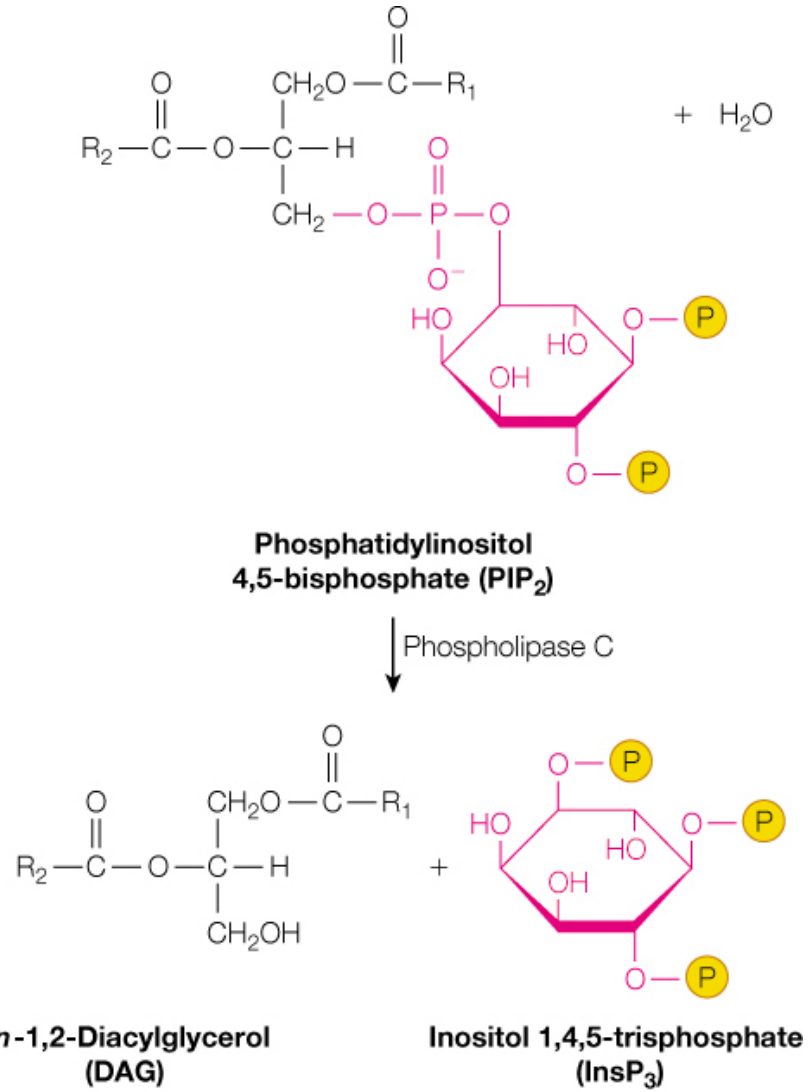
Phosphoinositide Signalling

- DAGs are produced from glycerophospholipids by hydrolysis (Lecture 9)
- Important for lipid synthesis and breakdown (later lectures)
- Implications for **obesity** and fat burning
- Involved in several **cancers**, especially in melanoma.

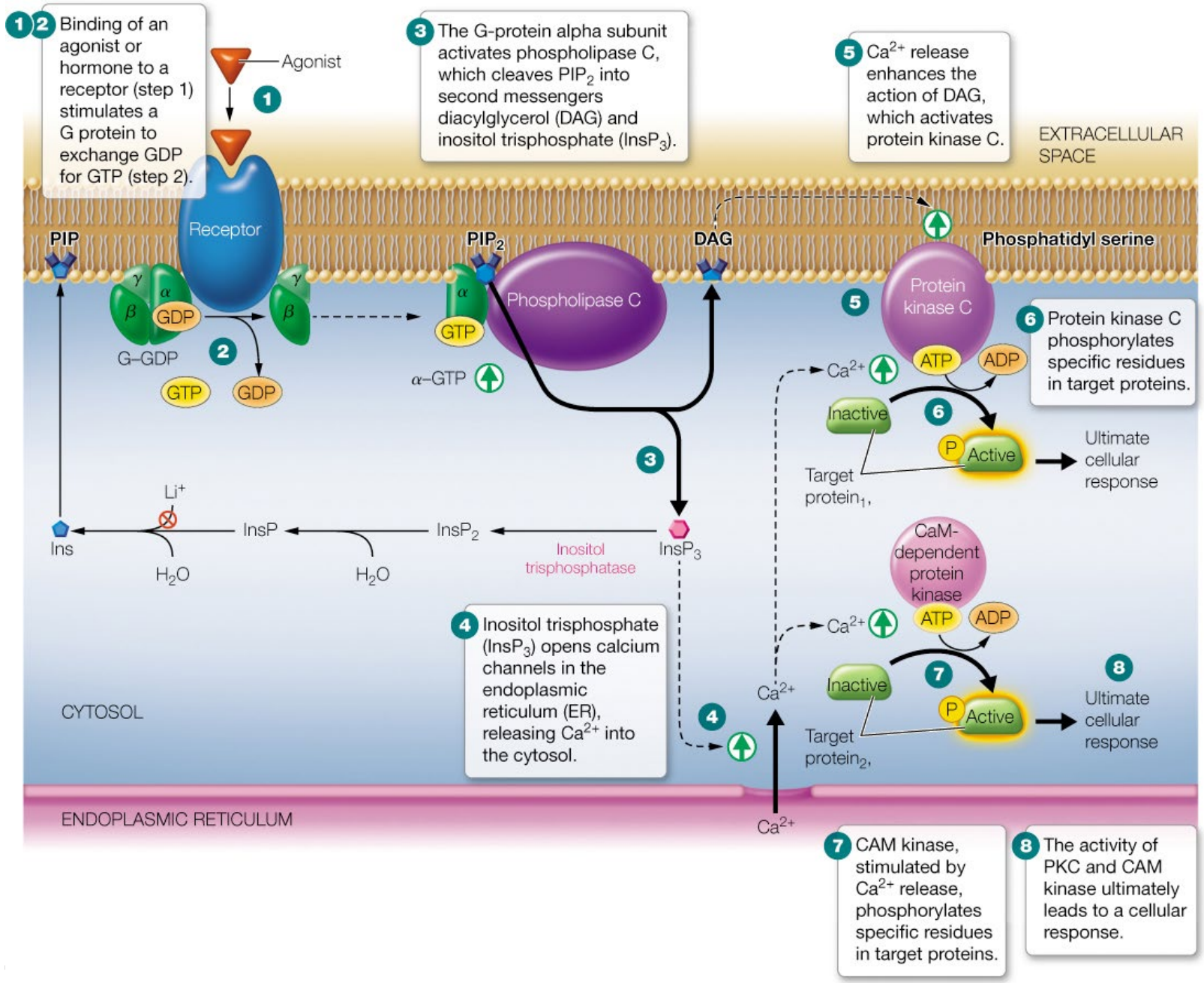


Phosphatidylinositol 4,5-Bisphosphate (PIP_2) as a Source of Two Second Messengers

- In the phosphoinositide system, G protein activates **phospholipase C** (instead of adenylate cyclase)
- **Phospholipase C** cleaves PIP_2 into the second messengers inositol 1,4,5-trisphosphate (**$InsP_3$**) and *sn*-1,2-diacylglycerol (**DAG**)
- $InsP_3$ binds and opens calcium channels, releasing Ca^{2+} from its intracellular ER stores
- **Protein kinase C** is activated by DAG and Ca^{2+}, which phosphorylates target proteins such as calmodulin



Signal Transduction Pathways Involving Phospho-Inositide Turnover



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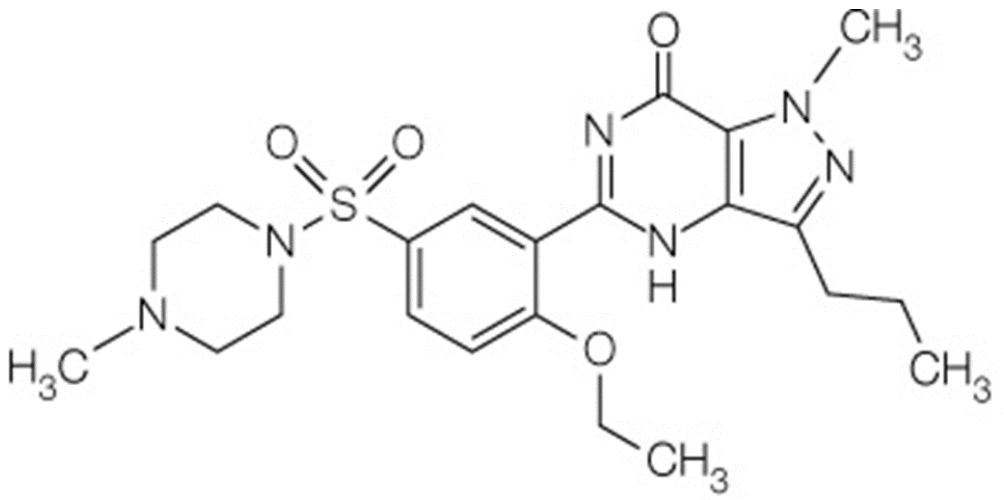
Cellular Processes Controlled by Phosphoinositide

TABLE 20.2 Some cellular processes controlled by the phosphoinositide second-messenger system

Extracellular Signal	Target Tissue	Cellular Response
Acetylcholine	Pancreas Pancreas (islet cells) Smooth muscle	Amylase secretion Insulin release Contraction
Vasopressin	Liver	Glycogenolysis
Thrombin	Blood platelets	Platelet aggregation
Antigens	Lymphoblasts Mast cells	DNA synthesis Histamine secretion
Growth factors	Fibroblasts	DNA synthesis
Spermatozoa	Eggs (sea urchin)	Fertilization
Light	Photoreceptors (*Limulus*)	Phototransduction
Thyrotropin-releasing hormone	Pituitary anterior lobe	Prolactin secretion

Nitric Oxide (NO) as a Second Messenger

- Actions of nitric oxide:
  - Regulation of neurotransmission
  - Stimulating defense to microbial infection
  - Vasodilation
- NO acts by stimulating guanylate cyclase, which converts GTP to cGMP
- Action of cGMP is terminated by cGMP phosphodiesterase, which converts cGMP to GMP
- Viagra is a cGMP phosphodiesterase inhibitor, so its action sustains levels of cGMP needed to stimulate sustained blood flow.



Sildenafil (Viagra)

GP/GPCR Signalling Summary

- G protein–coupled receptors (GPCRs) are integral membrane proteins, containing seven membrane-spanning helices and undergo conformational changes when a hormone binds.
- A hormone binds at a specific site of a GPCR located on the extracellular side of the plasma membrane, which triggers the interaction of a G protein on the cytosolic side of the membrane
- This interaction activates the G protein, which then initiates the synthesis of second messengers (such as cyclic AMP) to affect target metabolic processes
- Ligand binding to a GPCR induces the α subunit of the associated G protein to exchange GDP for GTP and dissociate from the β and y subunits.
- Adenylate cyclase is activated to produce cAMP, which in turn activates protein kinase A.
- Signalling activity is limited through the action of phosphodiesterases that act on cAMP and cGMP.
- GPCRs can also signal through the phosphoinositide pathway.

Complexity of hormone signalling

- Hormones have multiple roles in the cell
- They can trigger multiple signalling pathways, involving
  - metabolism as well as
  - cellular growth and differentiation
- Signalling pathways are complex and involve multiple enzymes and second messengers in the case of GPCRs
- Example: insulin signals via an RTK receptor:
  - Glucose transport
  - Glycogen synthesis
  - DNA/RNA/Protein synthesis

Insulin signalling cascade