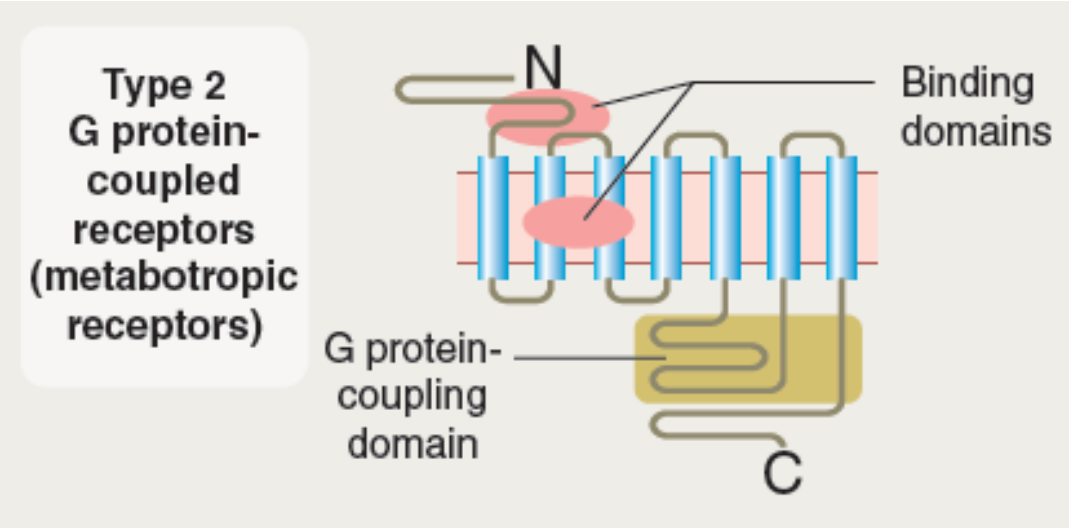
G PROTEIN-COUPLED RECEPTORS

أ.د.اسامة ايوب
كلية الصيدلة -فرع الادوية والسموم

- The abundant GPCR family comprises many of the receptors

✓ **muscarinic AChRs**
✓ **adrenoceptors**
✓ **dopamine receptors**
✓ **5-HT receptors, opioid receptors**
✓ **receptors for many peptides, purine receptors**
✓ **chemoreceptors involved in olfaction and pheromone detection, and**
✓ **many 'orphans**

- All have the characteristic heptahelical structure.



❖Many neurotransmitters can interact with both GPCRs and ligand-gated channels, allowing the same molecule to produce fast (through ligand-gated ion channels) and relatively slow (through GPCRs) effects.

❖ Individual peptide hormones generally act either on GPCRs or on kinase-linked receptors, but rarely on both,

Molecular structure

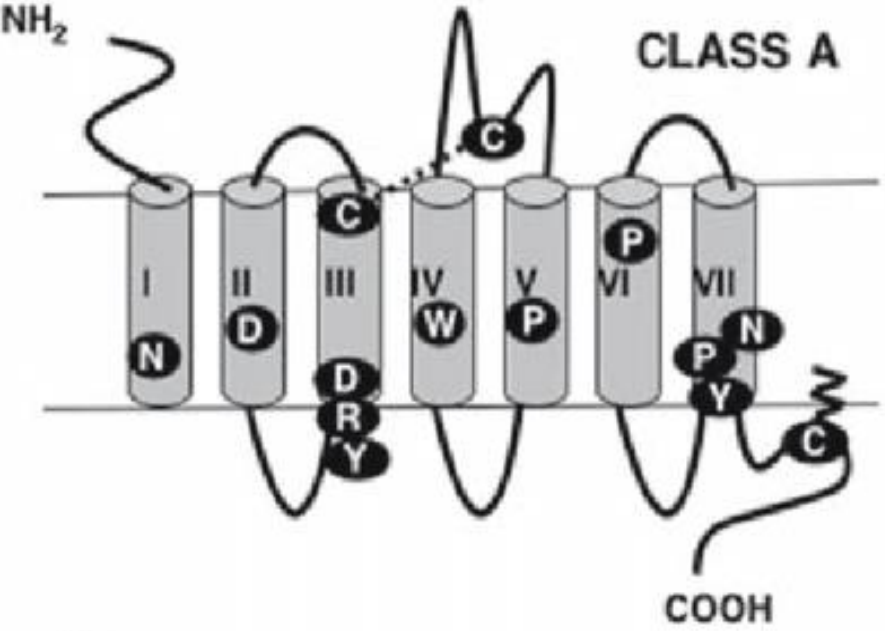
In 1986 the first pharmacologically relevant GPCR, the β2 adrenoceptor

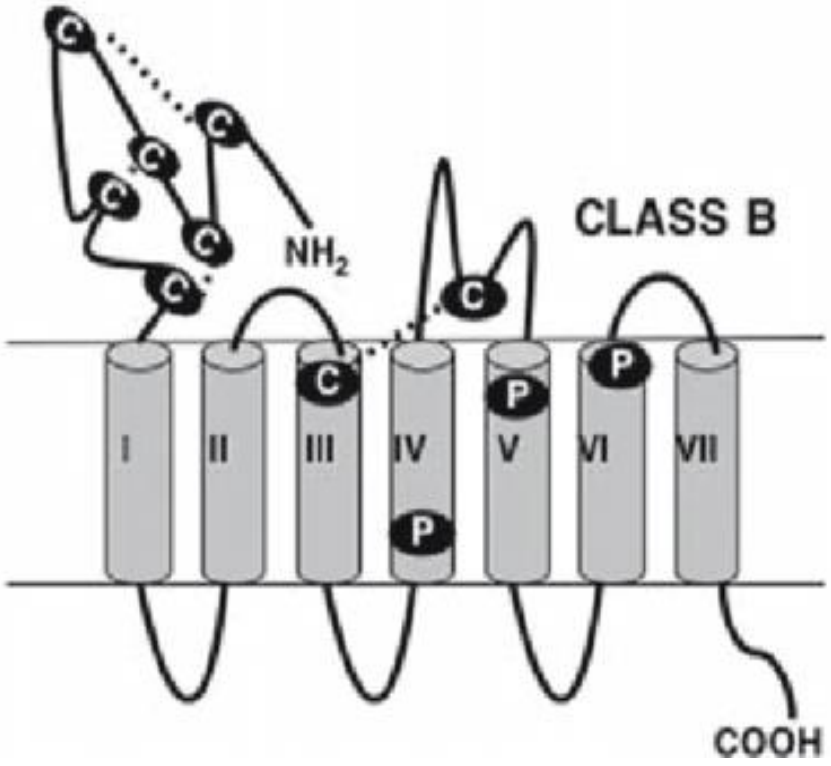
Using X-ray crystallography to study the three-dimensional molecular structure of these receptor

G protein-coupled receptors consist of a single polypeptide chain, usually of 350–400 residues, but can in some cases be up to 1100 residues

Their characteristic structure comprises seven transmembrane α-helices

GPCRs are divided into three distinct families. They share the same seven transmembrane helix (heptahelical) structure, but differ in the length of the extracellular N-terminus and the location of the agonist binding domain


NH2
CLASS A
C
C
I
II
III
IV
V
VI
VII
N
D
D
W
P
P
N
P
R
Y
Y
C
COOH


C
C
C
C
C
C
NH2
CLASS B
C
C
I
II
III
IV
V
VI
VII
P
P
P
P
COOH

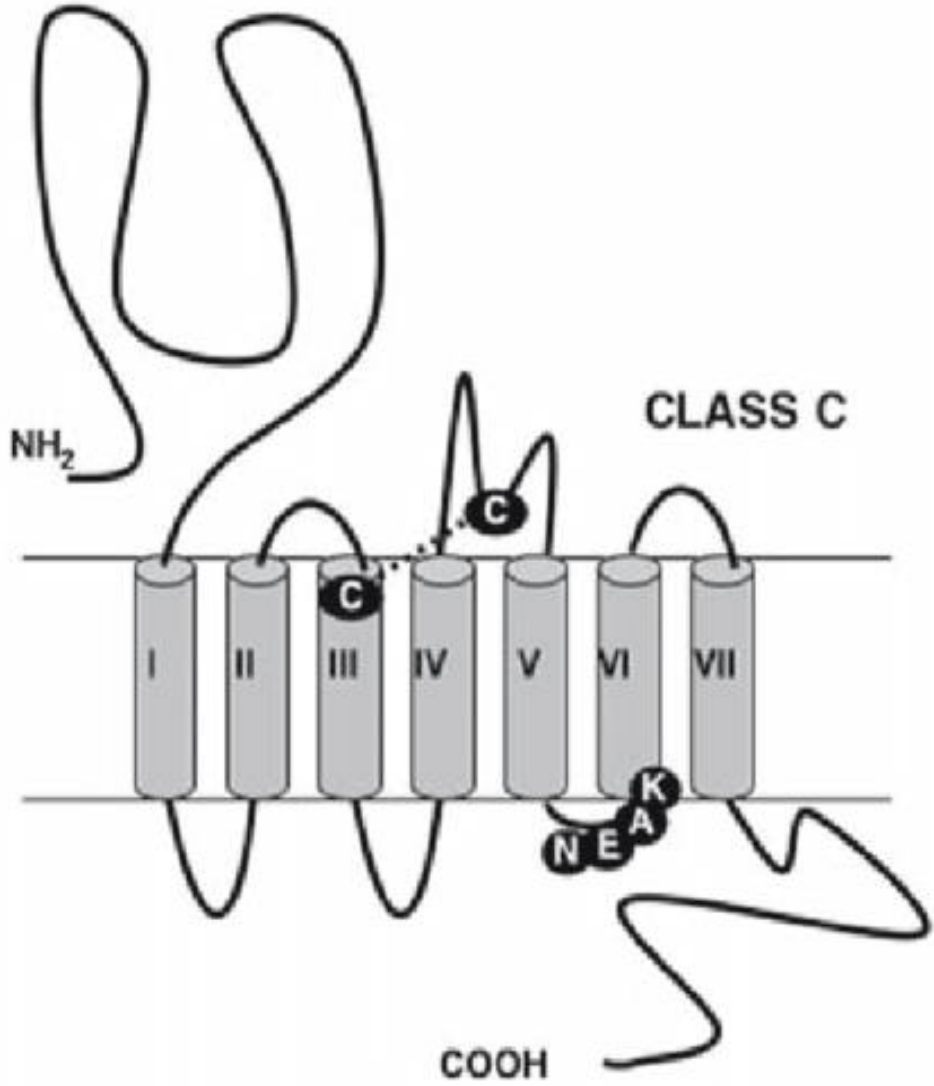

NH2
CLASS C
C
C
I
II
III
IV
V
VI
VII
K
A
N
E
COOH

Table 3.2 G protein-coupled receptor families[a]

Family	Receptors[b]	Structur
A: rhodopsin family	The largest group. Receptors for most amine neurotransmitters, many neuropeptides, purines, prostanoids, cannabinoids, etc.	Short ext Ligand bi (amines)
B: secretin/glucagon receptor family	Receptors for peptide hormones, including secretin, glucagon, calcitonin	Intermedi ligand-bi
C: metabotropic glutamate receptor/calcium sensor family	Small group. Metabotropic glutamate receptors, $GABA_B$ receptors, Ca^{2+}-sensing receptors	Long extr ligand-bi

[a]A fourth distinct family includes many receptors for pheromones but no pharmacological receptors
[b]For full lists, see www.guidetopharmacology.org.

For small molecules, such as noradrenaline (norepinephrine) and acetylcholine, the ligand-binding domain of class A receptors is buried in the cleft between the α-helical segments within the membrane

Peptide ligands, such as substance P , bind more superficially to the extracellular loops,

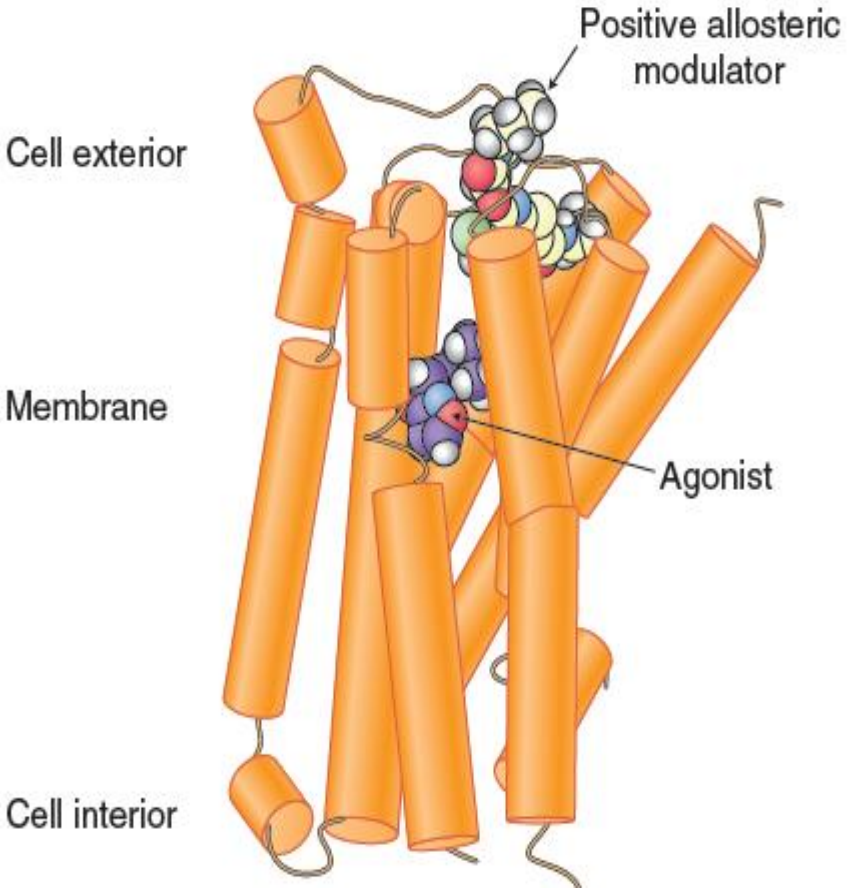


Fig. 3.7 Structure of the M_4 muscarinic receptor. High resolution image showing the conformation of the M_4 muscarinic receptor bound with both an agonist (orthosteric) and a positive allosteric modulator. The brown cylinders represent the

PROTEASE-ACTIVATED RECEPTORS

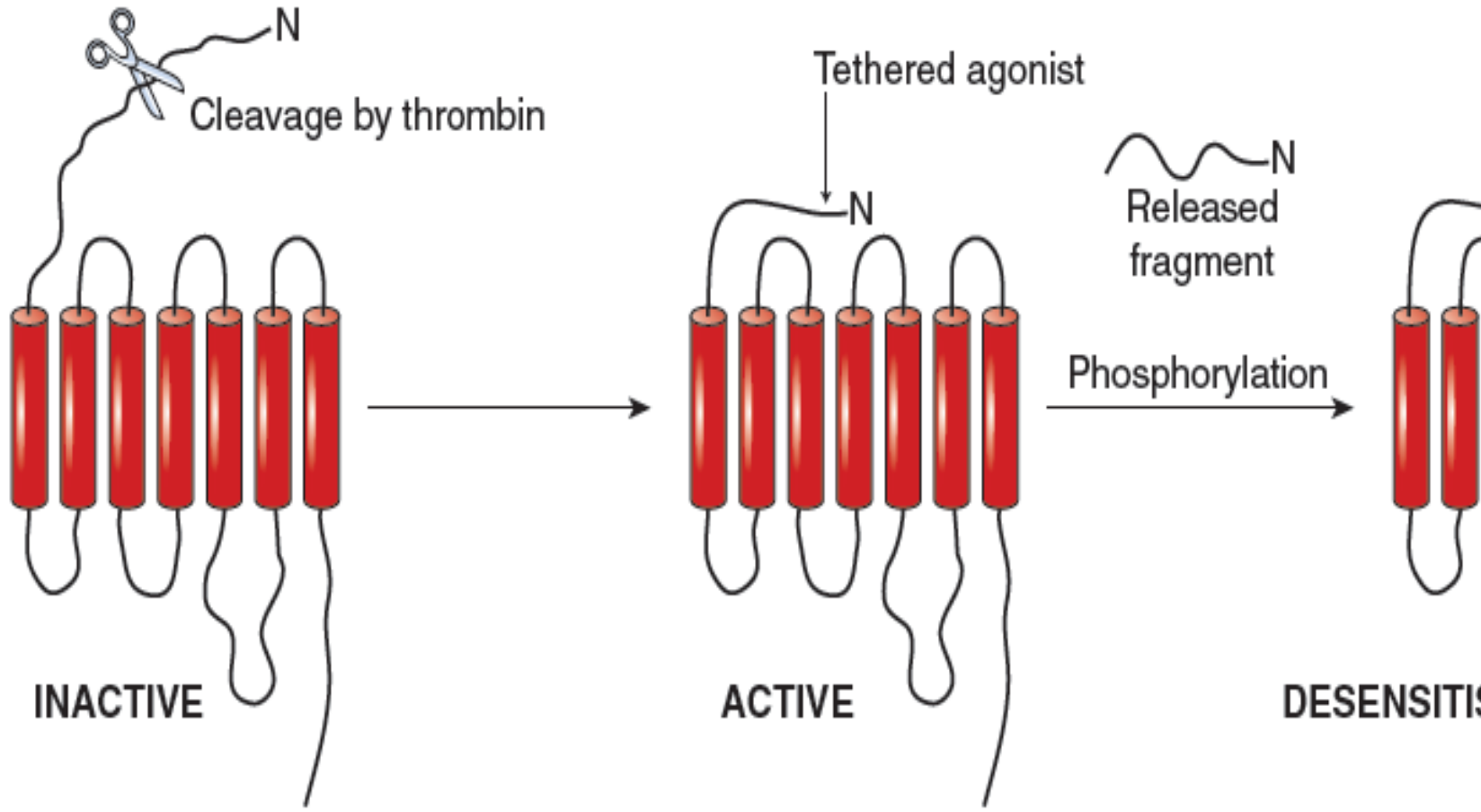


Fig. 3.8 Activation of a protease-activated receptor by cleavage of the N-terminal extracellular d
phosphorylation. Recovery requires resynthesis of the receptor.

Protease activated receptors

❖Many proteases, such as thrombin (a protease involved in the blood-clotting cascade activate PARs by snipping off the end of the extracellular N-terminal tail of the receptor

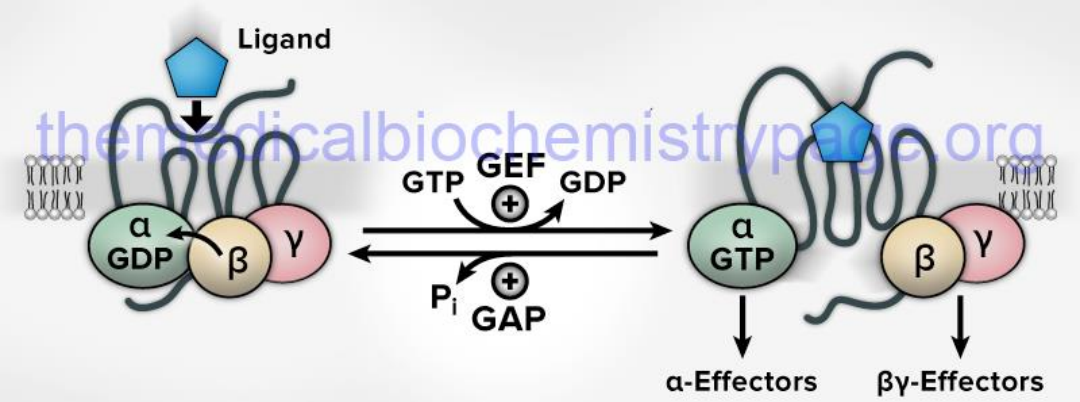
❖One of the family of PARs, PAR-2, is activated by a protease released from mast cells, and is expressed on sensory neurons. It is thought to play a role in inflammatory pain

❖A PAR molecule can be activated only once, because the cleavage cannot be reversed, so continuous resynthesis of receptor protein is necessary

❖Inactivation occurs by a further proteolytic cleavage that frees the tethered ligand, or by desensitisation, involving phosphorylation

G PROTEINS AND THEIR ROLE

❖ G proteins comprise a family of membrane-resident proteins whose function is **to recognize activated GPCRs and pass on the message to the effector systems** that generate a cellular response.



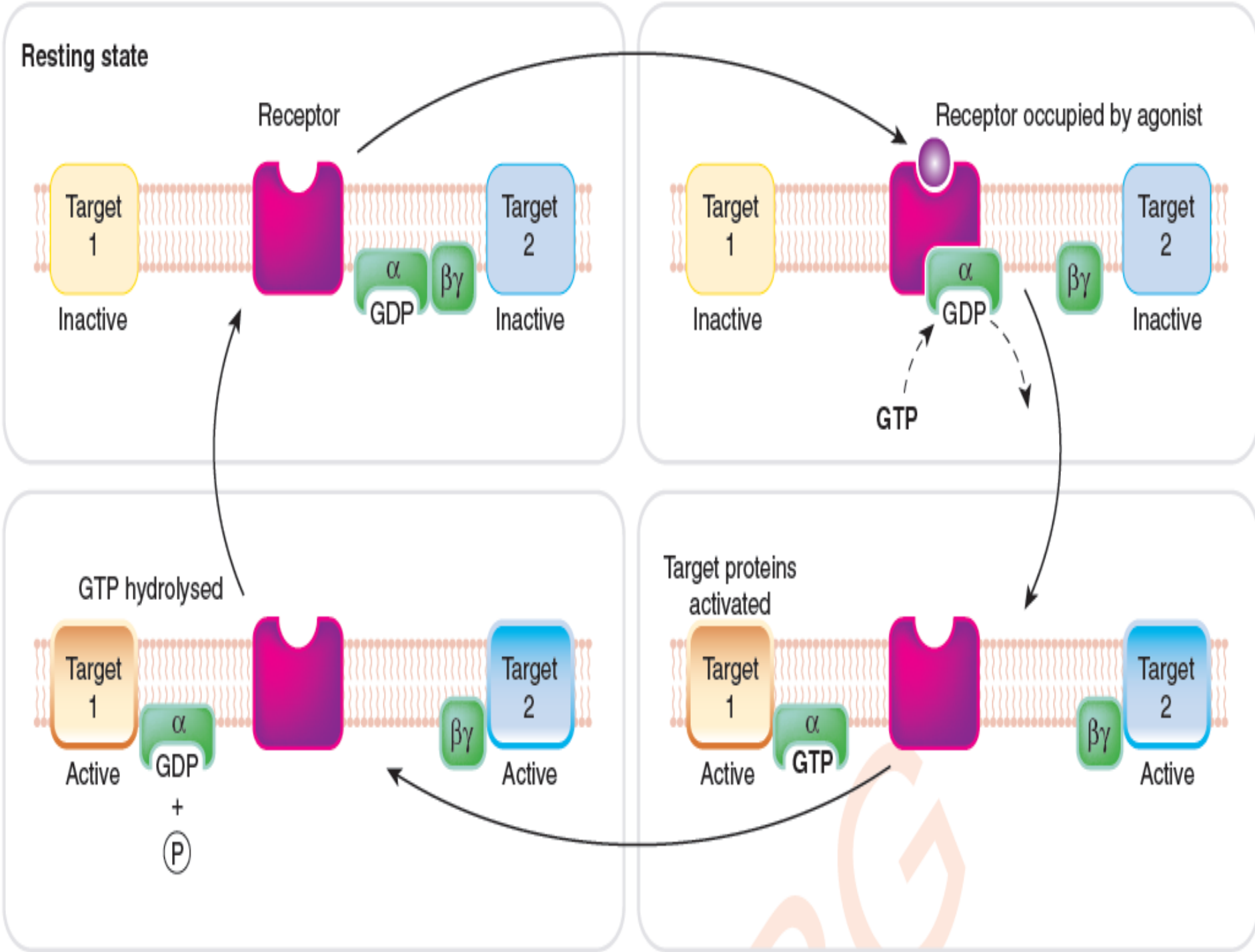
❖They are the go-between protein

❖actually called G proteins because of their interaction with the guanine nucleotides, GTP and GDP

❖G proteins consist of three subunits: α, β and γ

❖G proteins appear to be freely diffusible in the plane of the membrane, so a single pool of G protein in a cell can interact with several different receptors and effectors in an essentially promiscuous fashion

❖It was originally thought that (By) complex serving merely as chaperone


Resting state
Receptor
Target 1
Inactive
α
GDP
βγ
Target 2
Inactive
Receptor occupied by agonist
Target 1
Inactive
α
GDP
βγ
Target 2
Inactive
GTP
Target proteins activated
Target 1
Active
α
GTP
βγ
Target 2
Active
GTP hydrolysed
Target 1
Active
α
GDP
+
P
βγ
Target 2
Active

G protein activation results in amplification, because a single agonist–receptor complex can activate several G protein molecules in turn, and each of these can remain associated with the effector enzyme for long

The product is often a ‘second messenger’, and further amplification occurs before the final cellular response is produced.

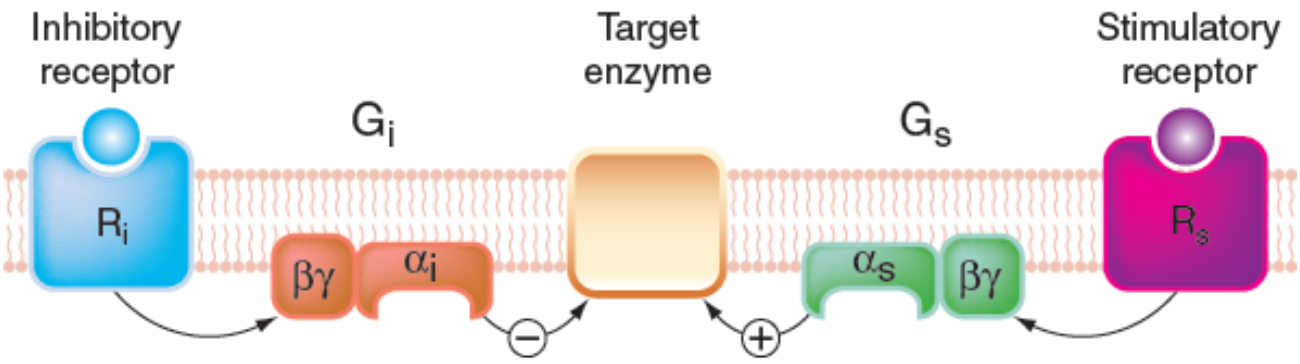
Signalling is terminated when the hydrolysis of GTP to GDP occurs through the GTPase activity of the α subunit.

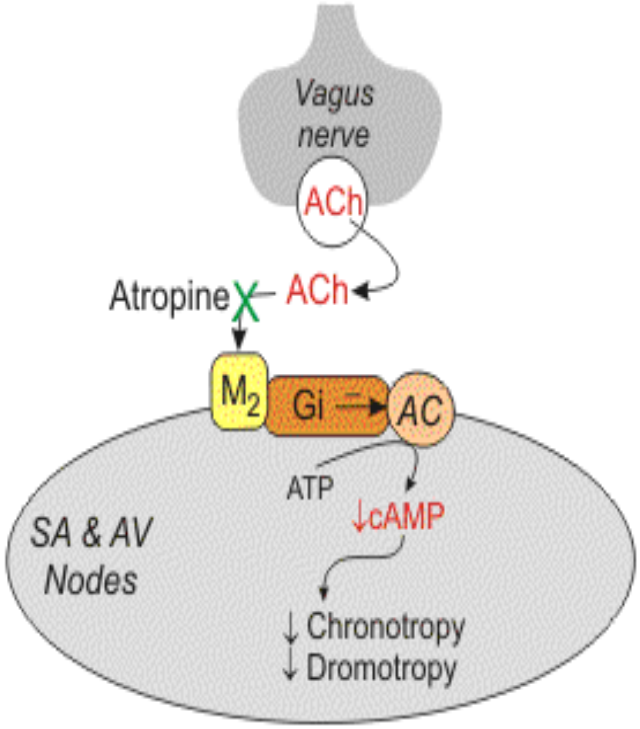
The resulting α–GDP then dissociates from the effectors, and reunites with βγ, completing the cycle.

How is specificity of GPCR function achieved so that each kind of receptor produces a distinct pattern of cellular responses?

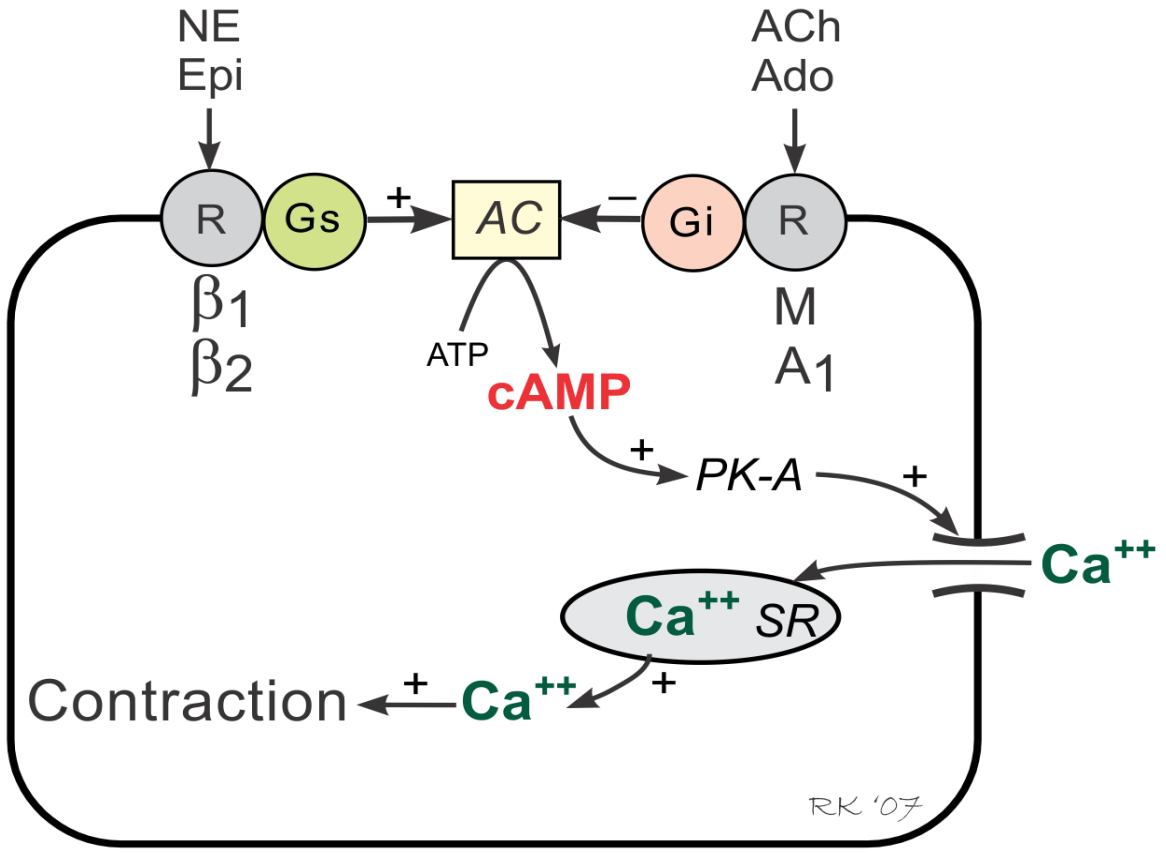
- For example, **mAChRs and β adrenoceptors**, both of which occur in cardiac muscle cells, produce opposite functional effects. The main reason is molecular variation within the α subunits, of which more than 20 subtypes have been identified

Four main classes of G protein (Gs, Gi, Go and Gq) are of pharmacological importance





Abbreviations: ACh, acetylcholine; M2, muscarinic receptor; AC, adenylate cyclase; SA, sinoatrial; AV, atrioventricular



R, receptor; Gs and Gi, stimulatory and inhibitory G-proteins; AC, adenylyl cyclase; PK-A, protein kinase A; SR, sarcoplasmic reticulum; α and β, alpha and beta-adrenoceptors; Epi, epinephrine; NE, norepinephrine; ACh, acetylcholine; M, muscarinic receptor; A_1, adenosine (Ado)

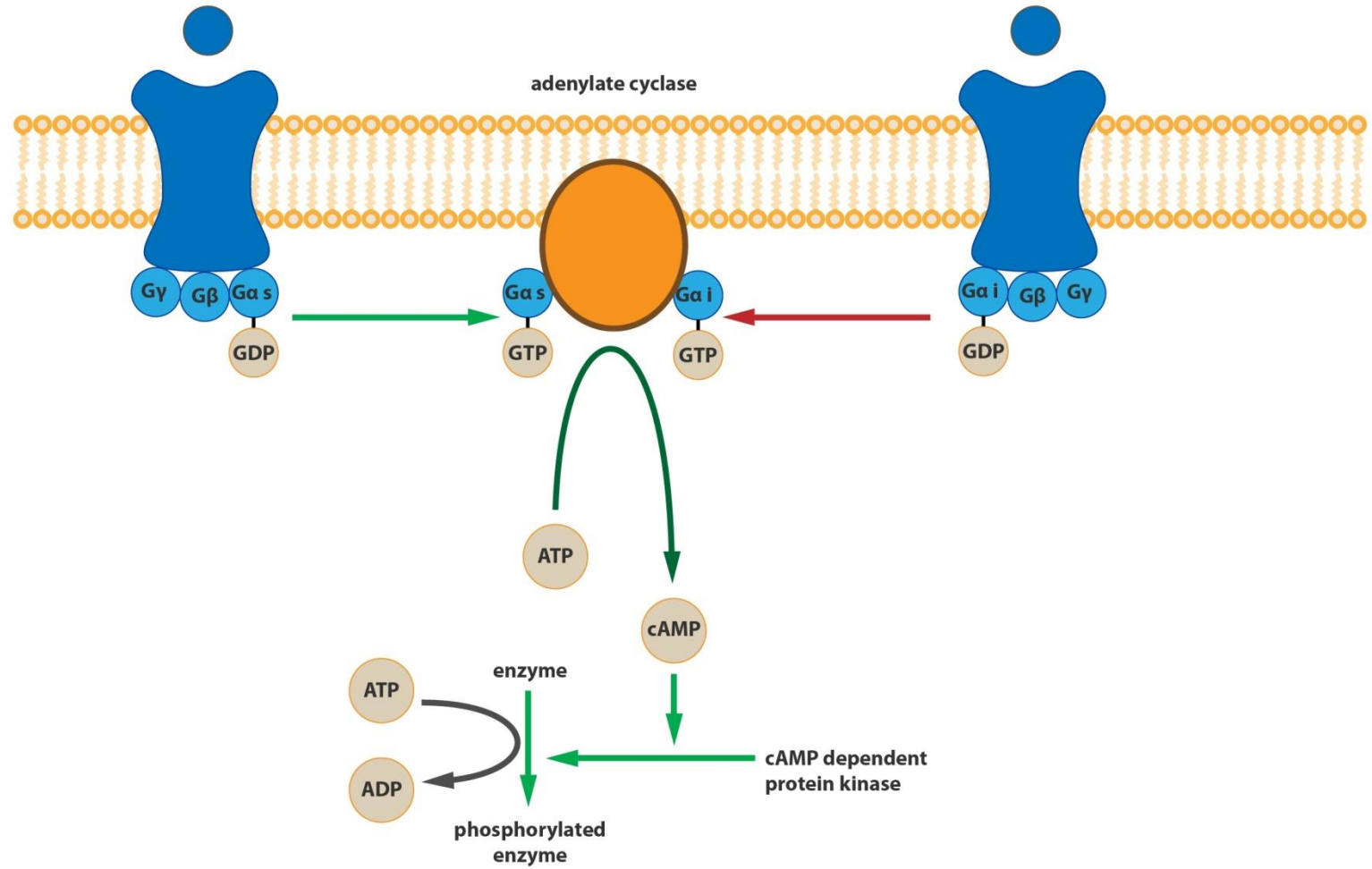

receptors coupled to stimulatory G- protien
receptors coupled to inhibitory G- protien
adenylate cyclase
Gγ
Gβ
Gα s
GDP
Gα s
GTP
Gα i
GTP
Gα i
Gβ
Gγ
GDP
ATP
cAMP
enzyme
ATP
ADP
cAMP dependent
protein kinase
phosphorylated
enzyme

Table 3.3 The main G protein subtypes and their functions[a]

Subtypes	Associated receptors	Main effectors	Notes
Gα subunits			
$G\alpha_s$	Many amine and other receptors (e.g. catecholamines, histamine, serotonin)	Stimulates adenylyl cyclase, causing increased cAMP formation	Activated by cho blocks GTPase a preventing inacti
$G\alpha_i$	As for $G\alpha_s$, also opioid, cannabinoid receptors	Inhibits adenylyl cyclase, decreasing cAMP formation	Blocked by pertu which prevents d αβγ complex
$G\alpha_o$	As for $G\alpha_s$, also opioid, cannabinoid receptors	? Limited effects of α subunit (effects mainly due to βγ subunits)	Blocked by pertu Occurs mainly in
$G\alpha_q$	Amine, peptide and prostanoid receptors	Activates phospholipase C, increasing production of second messengers inositol trisphosphate and diacylglycerol (see p. 34 and 35)	
Gβγ subunits			
	All GPCRs	Activate potassium channels inhibit voltage-gated calcium channels activate GPCR kinases (GRKs, p. 36) activate mitogen-activated protein kinase cascade Interact with some forms of adenylyl cyclase and with phospholipase Cβ	Many βγ isoforms specific functions known

● The α subunits of these G proteins differ in structure. One functional difference that has been useful as an experimental tool to distinguish which type of G protein is involved in different situations concerns the action of two bacterial toxins, *cholera toxin and pertussis toxin*

Cholera toxin acts only on Gs, and it causes persistent activation. Many of the symptoms of cholera, such as excessive secretion of fluid from the gastrointestinal epithelium, are due to the uncontrolled activation of adenylyl cyclase(which blocks GTPase activity).

Pertussis toxin specifically blocks Gi and Go by preventing dissociation of Gprotein trimer

TARGETS FOR G PROTEINS

- adenylyl cyclase, the enzyme responsible for cAMP formation

- phospholipase C, the enzyme responsible for inositol phosphate and diacylglycerol (DAG) formation

- ion channels, particularly calcium and potassium channels.
-
- Rho A/Rho kinase, a system that regulates the activity of many signalling pathways controlling cell growth and proliferation, smooth muscle contraction, etc.

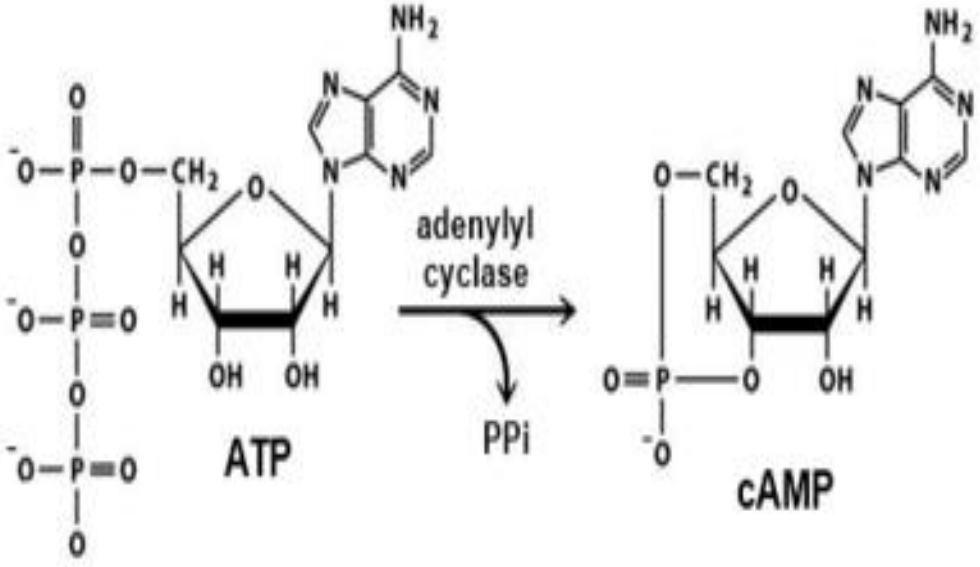
- mitogen-activated protein kinase (MAP kinase), a system that controls many cell functions, including cell division.

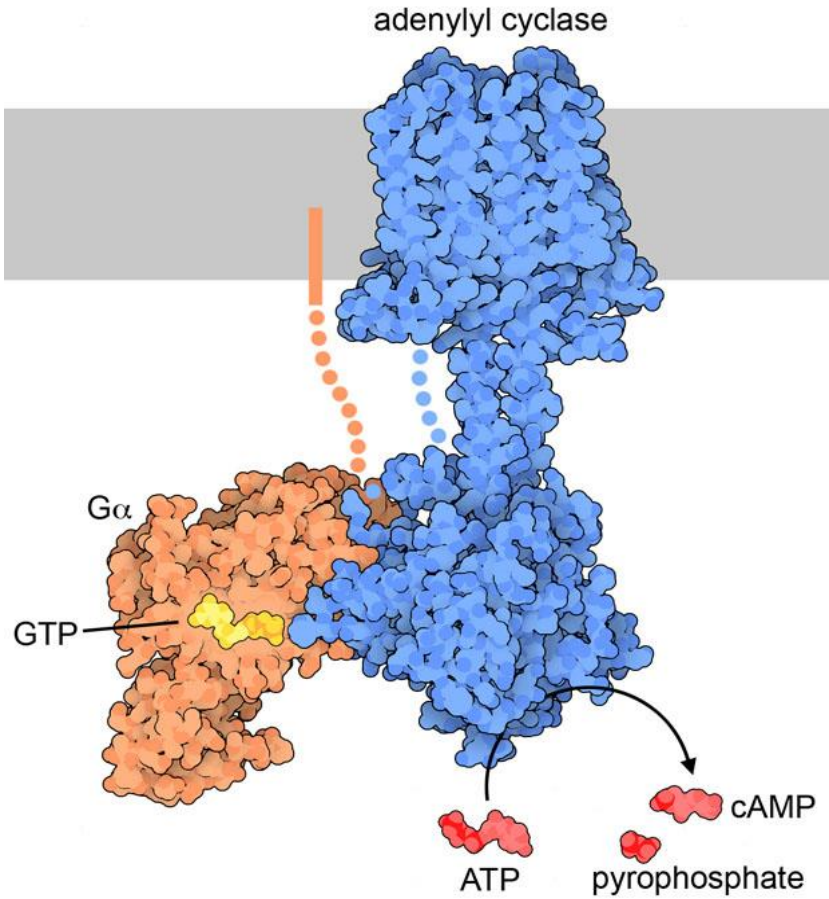
The adenylyl cyclase/cAMP system

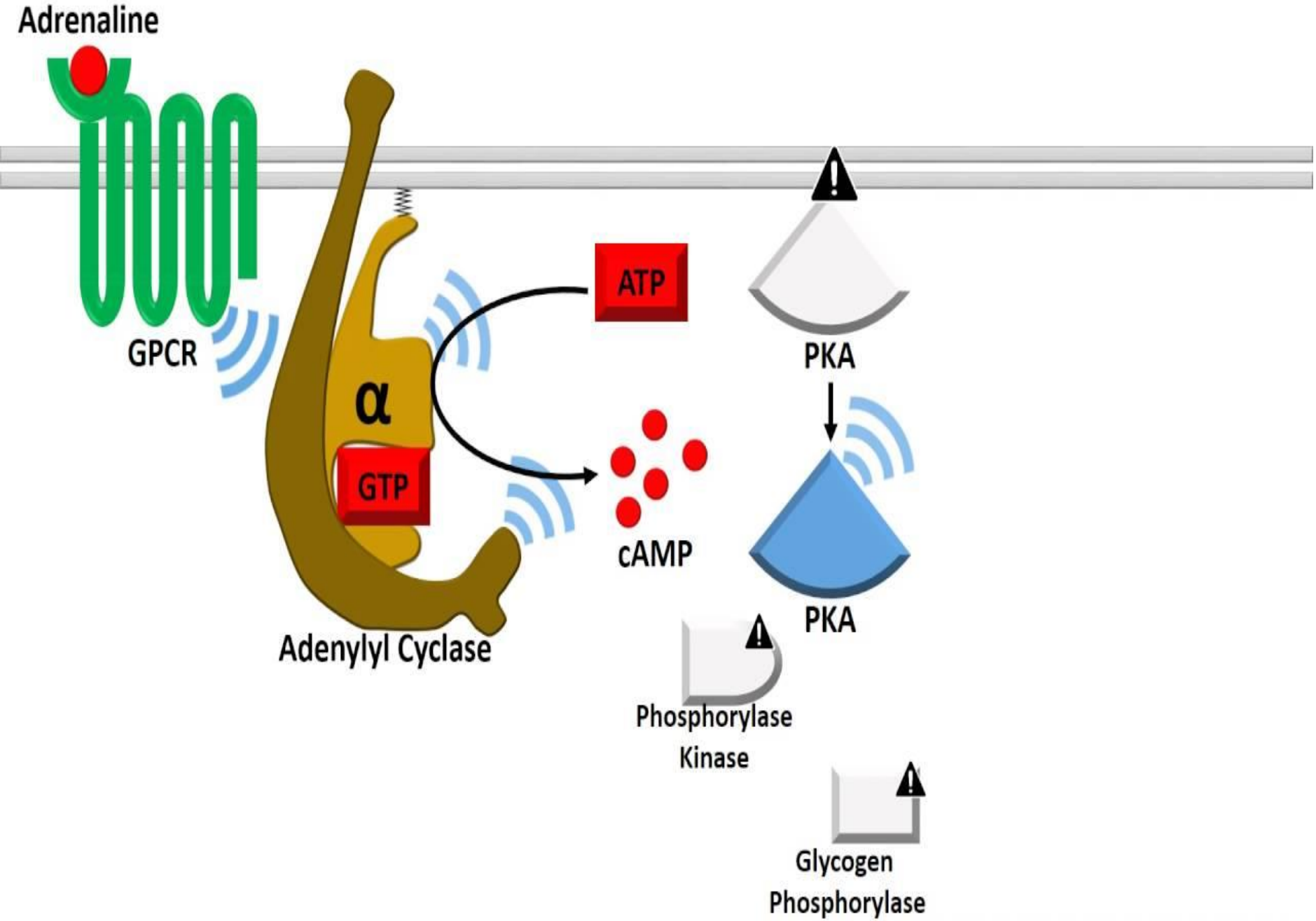
❖CAMP is a nucleotide synthesized within the cell from ATP by the action of a membrane-bound enzyme, adenylyl cyclase.

❖It is produced continuously and inactivated by hydrolysis to 5′-AMP by the action of a family of enzymes known as phosphodiesterases

❖There are 10 different isozyme





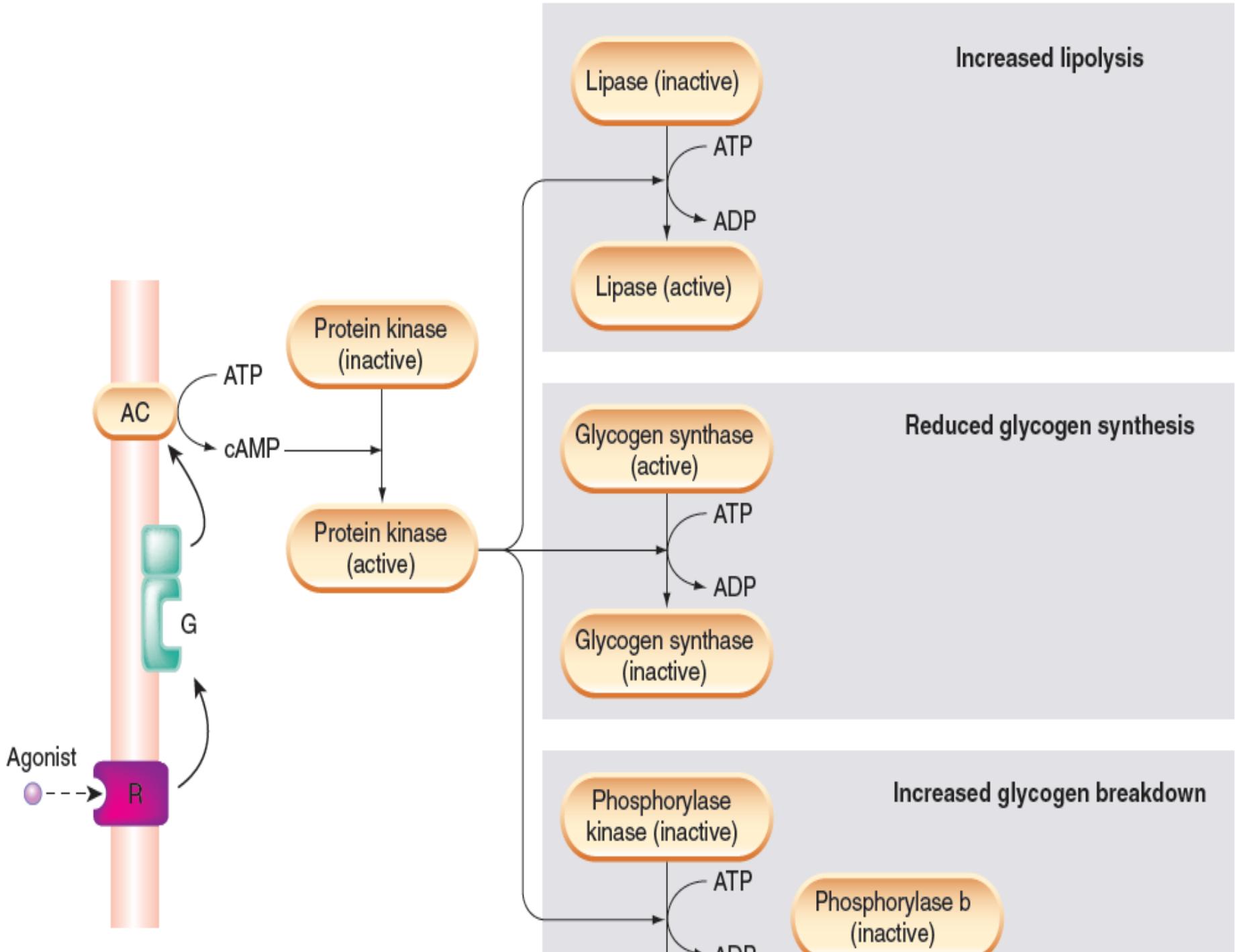

Adrenaline
GPCR
α
GTP
Adenylyl Cyclase
ATP
cAMP
PKA
PKA
Phosphorylase
Kinase
Glycogen
Phosphorylase

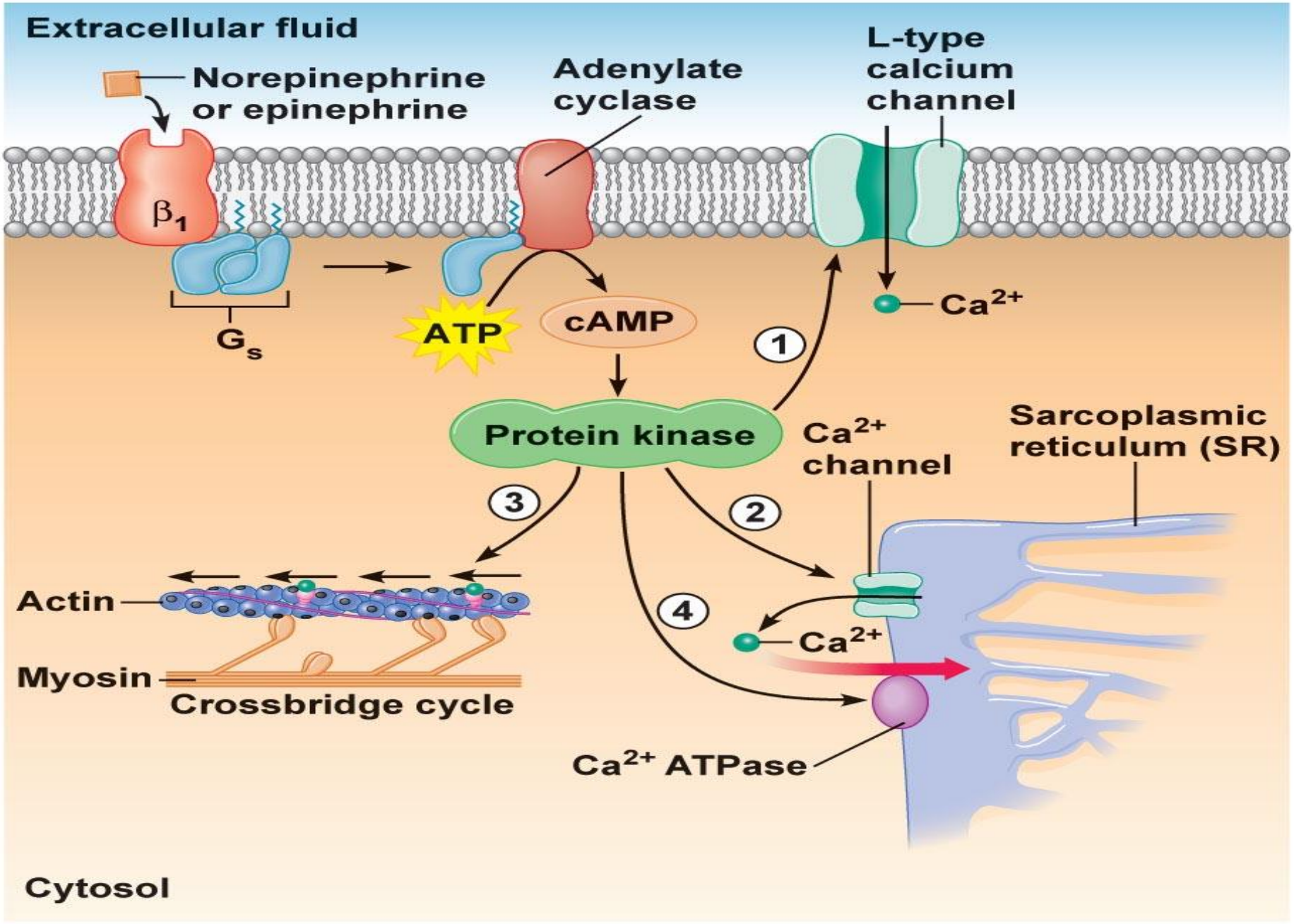
The adenylyl cyclase/cAMP system

- CAmp regulate many aspects of cellular function including, for example,

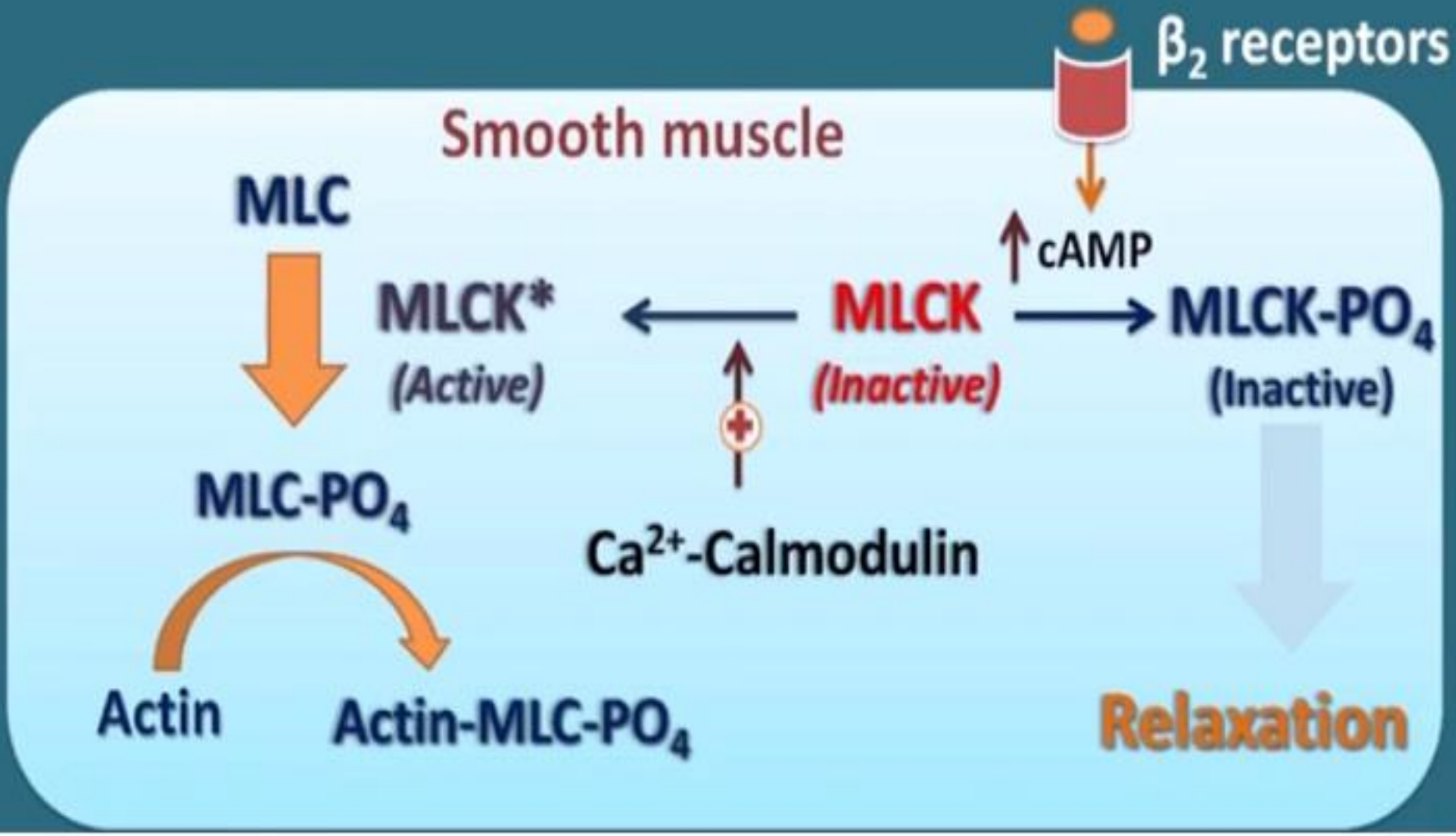
✓enzymes involved in energy metabolism
✓cell division and cell differentiation
✓ion transport, ion channels and
✓the contractile proteins in smooth muscle.

These varied effects are brought about by a common mechanism, namely the activation of *protein kinases by cAMP – primarily protein kinase A (PKA)*

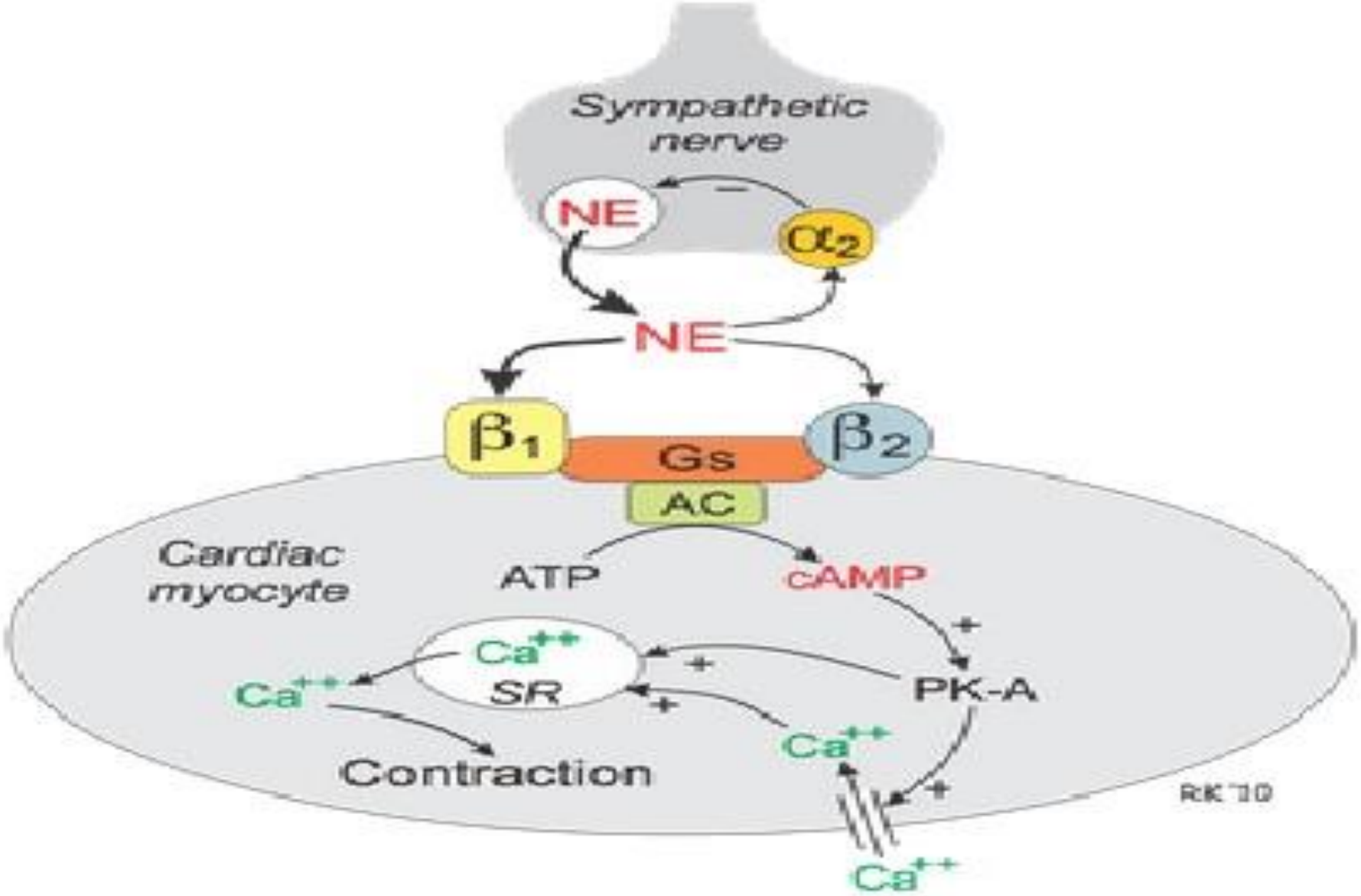

Agonist
R
G
AC
ATP
cAMP
Protein kinase (inactive)
Protein kinase (active)
Lipase (inactive)
ATP
ADP
Lipase (active)
Increased lipolysis
Glycogen synthase (active)
ATP
ADP
Glycogen synthase (inactive)
Reduced glycogen synthesis
Phosphorylase kinase (inactive)
ATP
Phosphorylase b (inactive)
Increased glycogen breakdown

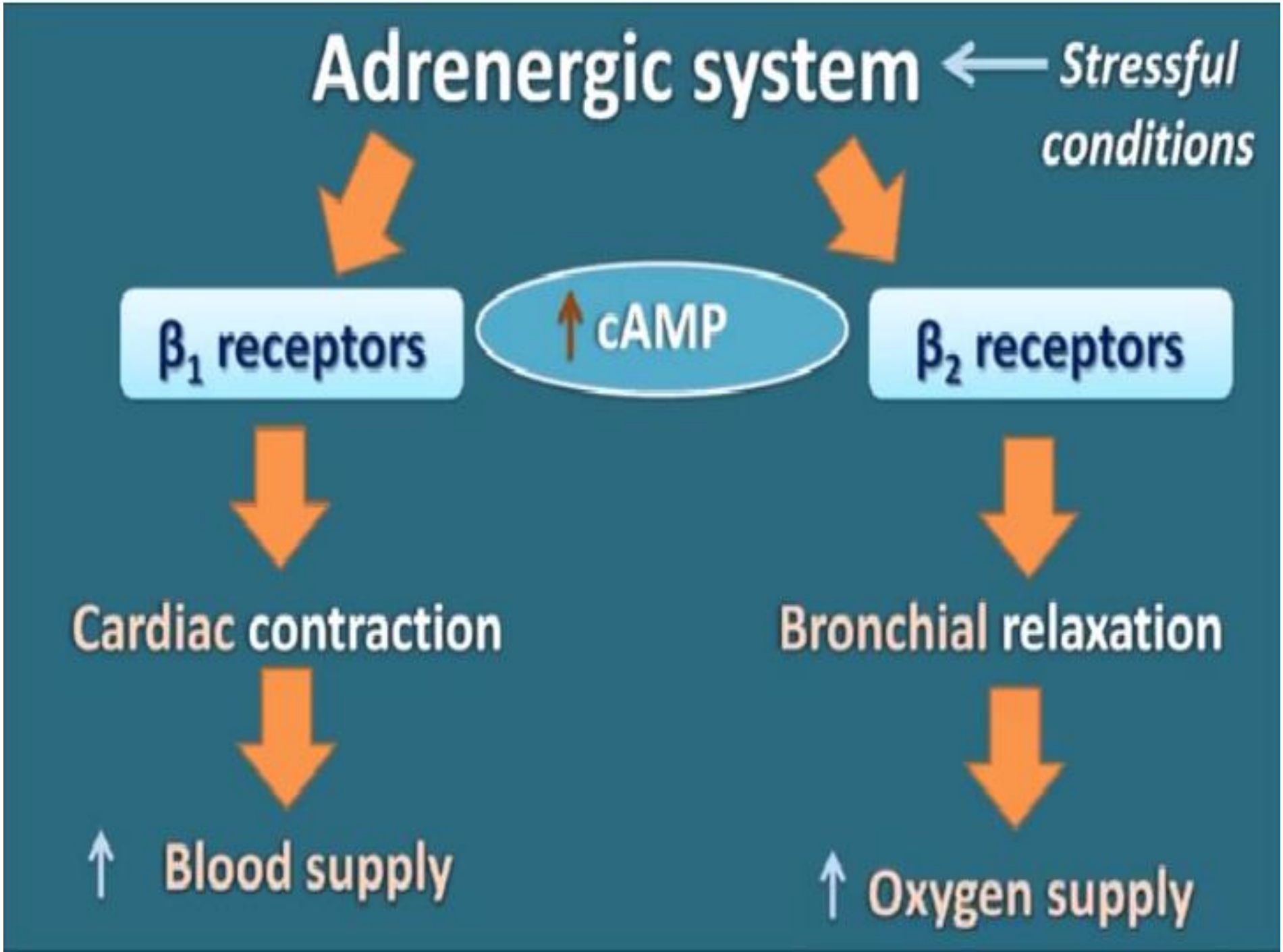


Role of cAMP in smooth muscle



S


Sympathetic nerve
NE
α2
NE
β1
Gs
β2
AC
Cardiac myocyte
ATP
cAMP
Ca++
SR
Ca++
PK-A
Ca++
Contraction
Ca++
RK '10


Adrenergic system
Stressful conditions
β1 receptors
cAMP
β2 receptors
Cardiac contraction
Bronchial relaxation
Blood supply
Oxygen supply

G-protein Linked Vascular Receptors and their Biological Agonists

G-protein	2nd Messenger	Receptor	Biological Agonist
Gs	↑cAMP	β_2	Epinephrine
		A_2	Adenosine
		IP	Prostacyclin
Gi	↓cAMP	α_2	Norepinephrine/ Epinephrine
Gq	↑IP_3 & ↑Rho-kinase	α_1	Norepinephrine/ Epinephrine
		ET_A	Endothelin-1
		AT_1	Angiotensin II
		V_1	Vasopressin

receptors linked to Gi inhibit adenylyl cyclase, and thus reduce cAMP formation.

Examples include

- ✓**mAChR (e.g. the M2 receptor of cardiac muscle)**
- ✓**α2 adrenoceptors in smooth muscle**
- ✓**opioid receptors.**

Adenylyl cyclase can be activated directly by drugs such as forskolin, which is used experimentally to study the role of the cAMP system**.**

Selective inhibitors of the various PDEs are being developed, mainly to treat cardiovascular and respiratory diseases.

Cyclic AMP is hydrolysed within cells by *phosphodiesterases (PDEs), an important and ubiquitous family of enzymes. Eleven PDE subtypes exist, of which some (e.g.* PDE3 and PDE4) are cAMP-selective, while others (e.g. PDE5) are cGMP-selective

Most are weakly inhibited by drugs such as methylxanthines (e.g. **theophylline and caffeine).**

Rolipram (used to treat asthma) selective for PDE4, expressed in inflammatory cells

milrinone (used to treat heart failure is selective for PDE3, which is expressed in heart muscle
sildenafil is selective for PDE5, and consequently enhances the vasodilator effects of nitrous oxide (NO) and drugs that release NO, whose effects are mediated by cGMP

The phospholipase C/inositol phosphate system

- The *phosphoinositide system, an important intracellular second messenger system, was first discovered in the 1950s*

PIP2 is the substrate for a membrane-bound enzyme, phospholipase Cβ (PLCβ), which splits it into *diacylglycerol (DAG) and inositol (1,4,5) trisphosphate (IP3)* both of which function as second messengers

The activation of PLCβ by various agonists is mediated through a G protein (Gq)

Lithium, an agent used in psychiatry, blocks this recycling pathway

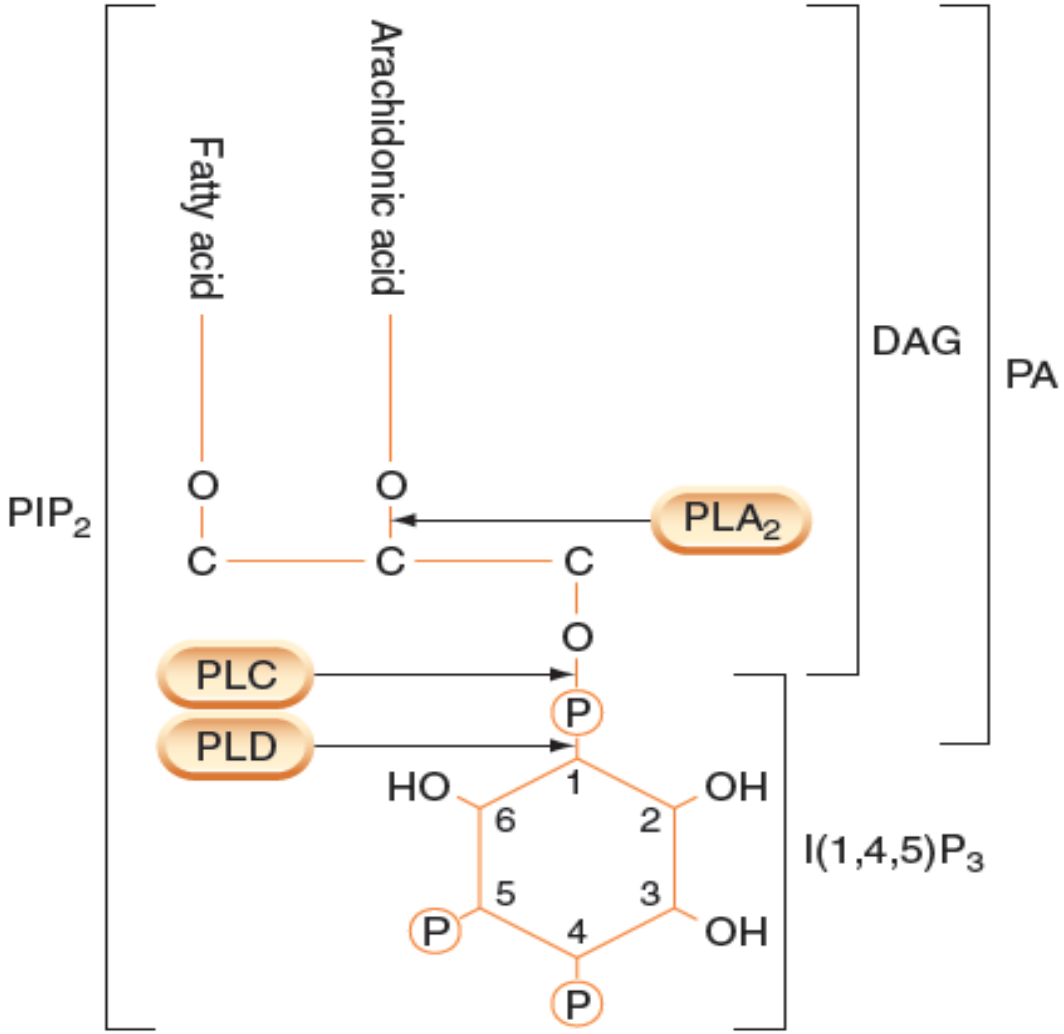
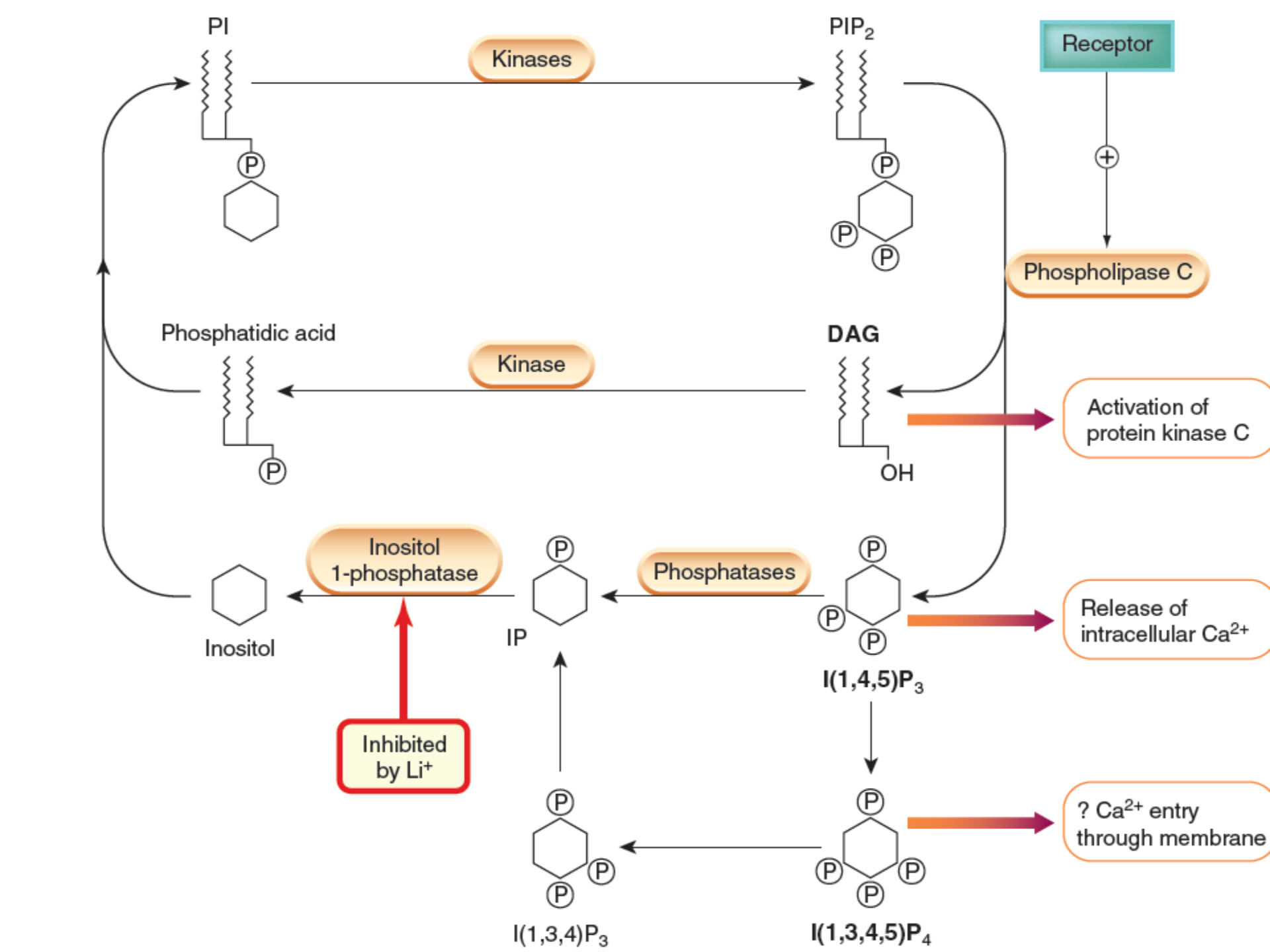


Fig. 3.12 Structure of phosphatidylinositol bisphosphate (PIP_2), showing sites of cleavage by different phospholipases to produce active mediators. Cleavage by phospholipase A2 (PLA_2) yields arachidonic acid. Cleavage by phospholipase C (PLC) yields inositol trisphosphate ($I(1,4,5)P_3$) and diacylglycerol (DAG). PA, phosphatidic acid; PLD, phospholipase D.


PI
Kinases
PIP2
Receptor
Phospholipase C
Phosphatidic acid
Kinase
DAG
OH
Activation of protein kinase C
Inositol 1-phosphatase
Phosphatases
Inositol
IP
I(1,4,5)P3
Release of intracellular Ca2+
Inhibited by Li+
? Ca2+ entry through membrane
I(1,3,4)P3
I(1,3,4,5)P4

Inositol phosphates and intracellular calcium

❖(IP3) is a water-soluble mediator that is released into the cytosol and acts on a specific receptor

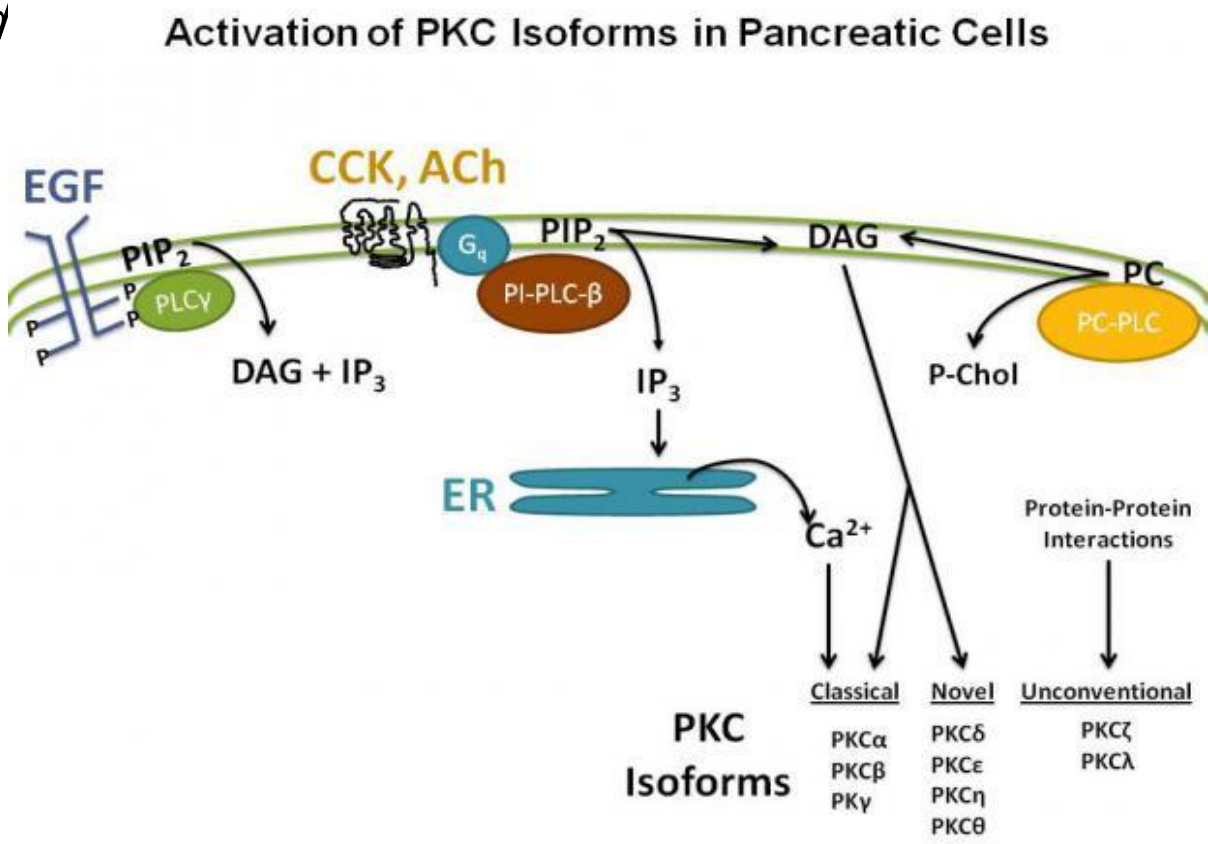
❖IP3 receptor – which is a ligand-gated calcium channel present on the membrane of the endoplasmic reticulum. The main role of IP3, is to control the release of Ca2+ from intracellular stores

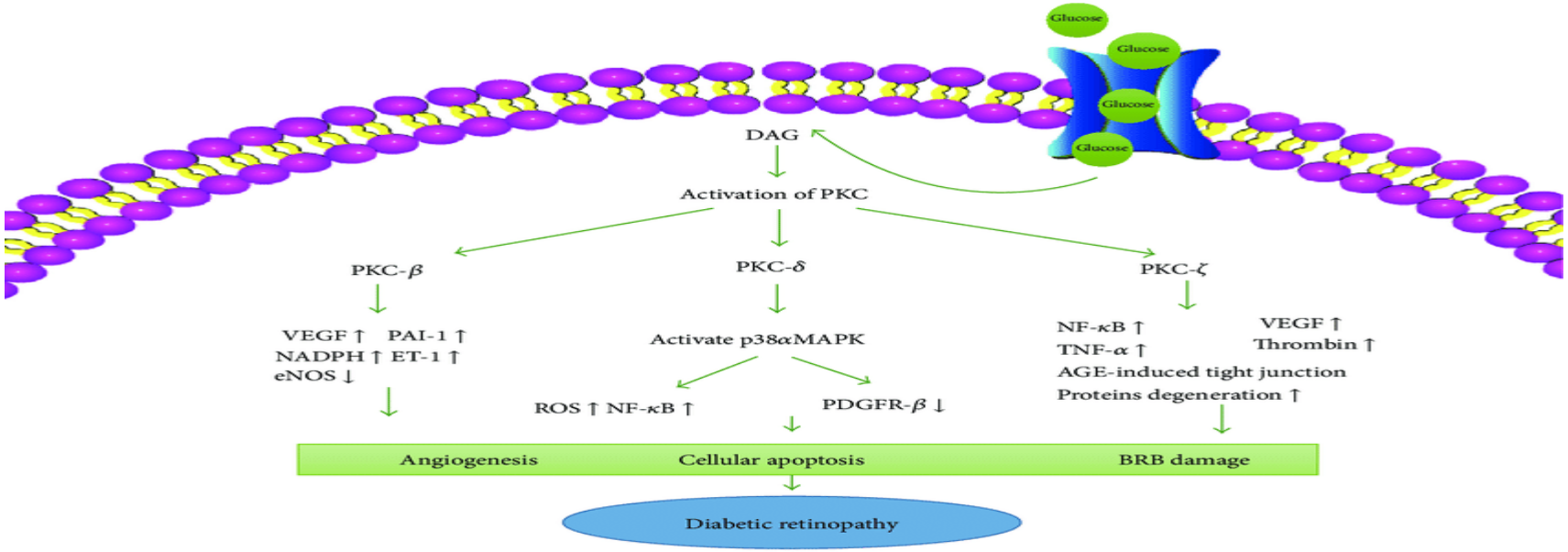
❖ IP3 can be converted inside the cell to IP4, by a specific kinase. The exact role of IP4 remains unclear, but some evidence suggests that itmay play a role in controlling gene expression.

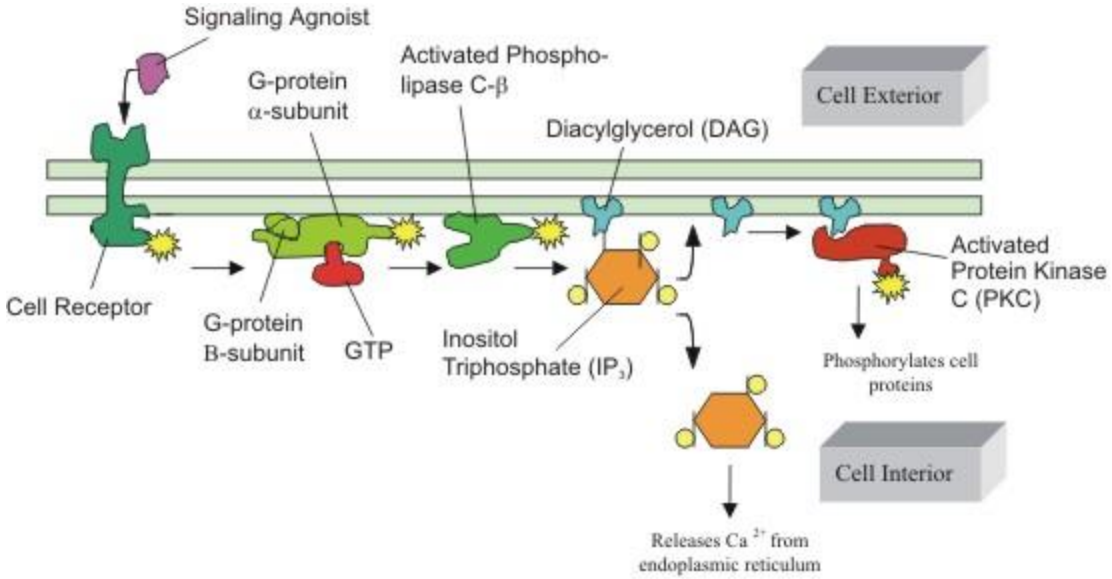
Diacylglycerol and protein kinase C

The main effect of DAG is to activate a protein kinase, *protein kinase C (PKC), which catalyses the phosphorylation of a variety of intracellular proteins.*

DAG, unlike the inositol phosphates, is highly lipophilic and remains within the membrane. It binds to a specific site on the PKC molecule, causing the enzyme to migrate from the cytosol to the cell membrane, thereby becoming activated




Glucose
Glucose
Glucose
Glucose
DAG
Activation of PKC
PKC-β
PKC-δ
PKC-ζ
VEGF ↑ PAI-1 ↑
NADPH ↑ ET-1 ↑
eNOS ↓
Activate p38αMAPK
ROS ↑ NF-κB ↑
PDGFR-β ↓
NF-κB ↑
TNF-α ↑
AGE-induced tight junction
Proteins degeneration ↑
VEGF ↑
Thrombin ↑
Angiogenesis
Cellular apoptosis
BRB damage
Diabetic retinopathy


Signaling Agnoist
G-protein
α-subunit
Activated Phospho-
lipase C-β
Diacylglycerol (DAG)
Cell Exterior
Cell Receptor
G-protein
B-subunit
GTP
Inositol
Triphosphate (IP3)
Activated
Protein Kinase
C (PKC)
Phosphorylates cell
proteins
Cell Interior
Releases Ca 2+ from
endoplasmic reticulum

Diacylglycerol and protein kinase C

There are at least 10 different mammalian PKC subtypes, which have distinct cellular distributions and phosphorylate different proteins.

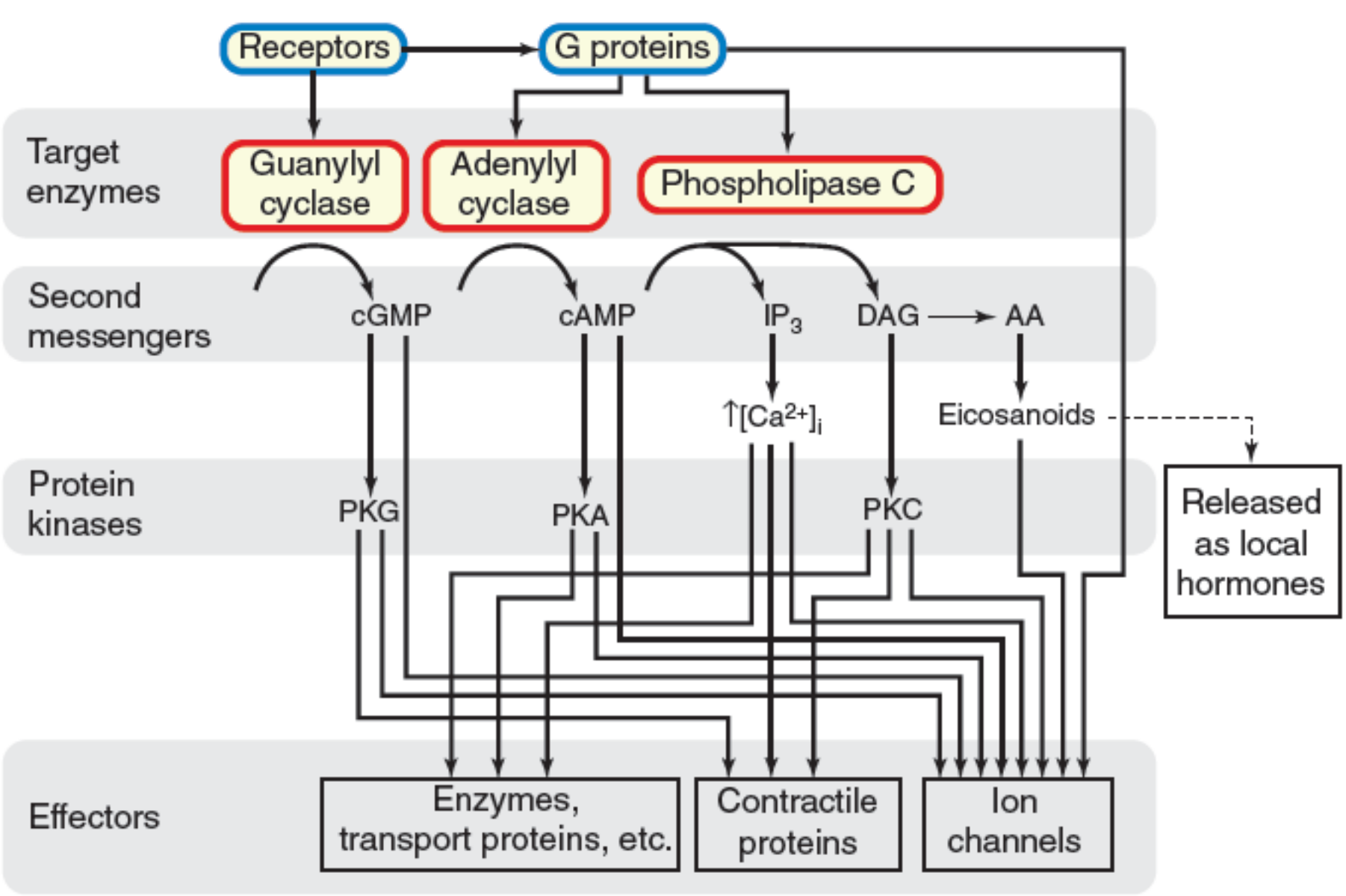
Several are activated by DAG and raised intracellular Ca2+, both of which are produced by activation of GPCRs.

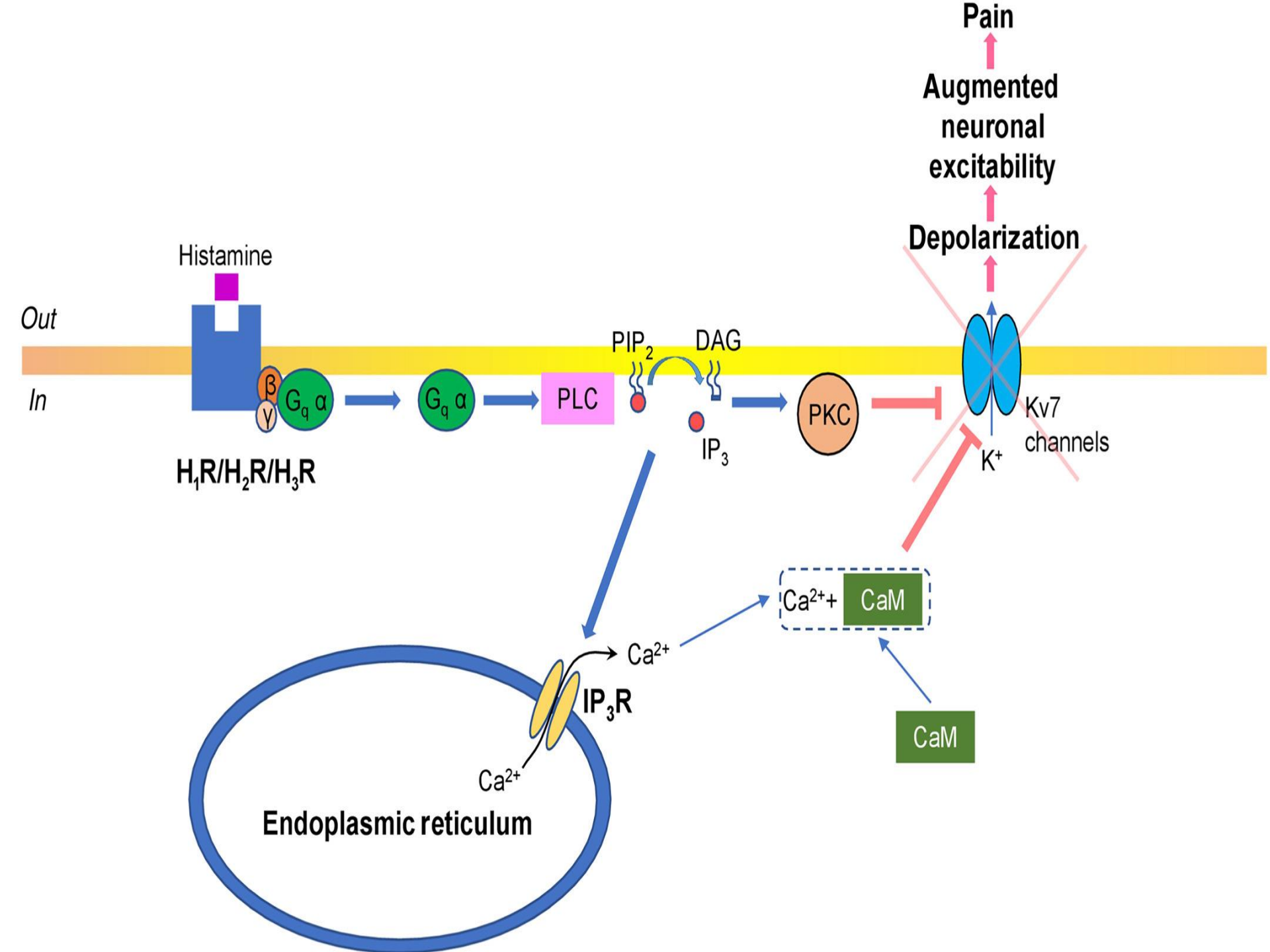
PKCs are also activated by *phorbol esters (highly irritant, tumour-promoting compounds produced by certain plants), which have been extremely useful in studying the functions of PKC.*

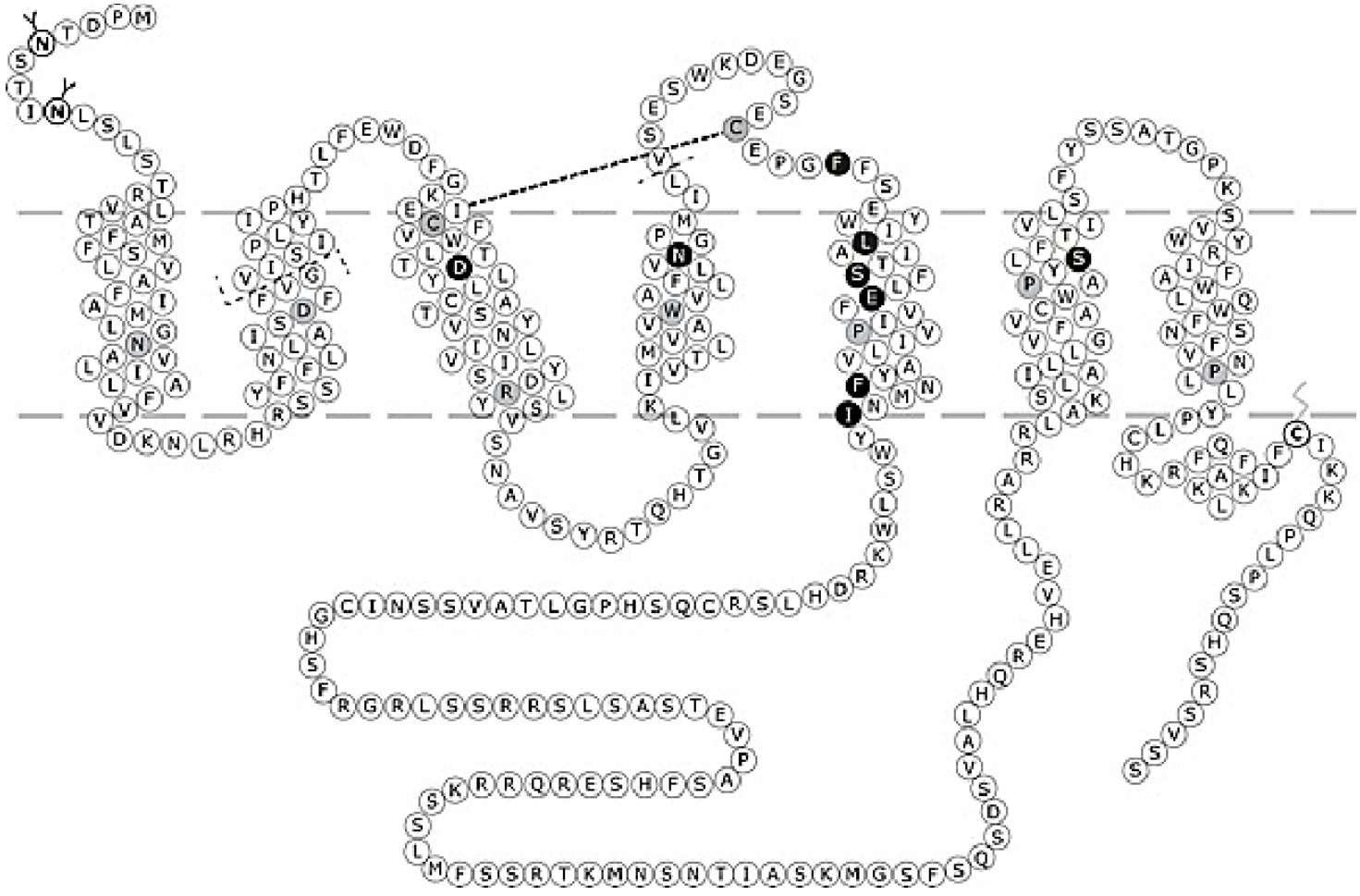
One of the subtypes is activated by the lipid mediator arachidonic acid generated by the action of phospholipase A2 on membrane phospholipids,

Table 3.2 Well-known members of the GPCR family.

Receptor family	Endogenous agonist(s)	Subtypes	G-protein coupling
5-HT	5-hydroxytryptamine	5-HT_{1A}, 5-HT_{1B},	G_i/G_o
		5-HT_{1D} 5-HT_{1F}	G_i/G_o
		5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}	G_q
		5-HT_4, 5-HT_5, 5-HT_7	G_s
Adenosine	Adenosine	A_1, A_3	G_i/G_o
		A_{2A}, A_{2B}	G_s
Adrenoceptors	Adrenaline	α_{1A}, α_{1B}, α_{1C}	G_q
	Noradrenaline	α_{2A}, α_{2B}, α_{2C}	G_i/G_q
		β_1, β_2, β_3	G_s
Angiotensin	Angiotensin II	AT_1	G_q
		AT_2	Unknown[a]
Cannabinoid	Anandamide[b]	CB_1, CB_2	G_i/G_o
Dopamine	Dopamine	D1, D5	G_s
		D2, D3, D4	G_i/G_o
$GABA_B$	γ-aminobutyric acid	$GABA_{B1}$, $GABA_{B2}$	G_i/G_o
Glutamate	L-glutamate	$mGlu_1$, $mGlu_5$	G_q
(metabotropic)	L-aspartate	$mGlu_2$, $mGlu_3$, $mGlu_4$	G_i/G_o
		$mGlu_6$, $mGlu_7$, $mGlu_8$	G_i/G_o
Histamine	Histamine	H_1	G_q
		H_2	G_s
		H_3, H_4	G_i/G_o
Muscarinic	Acetylcholine	m1, m3, m5	G_q


Receptors
G proteins
Target enzymes
Guanylyl cyclase
Adenylyl cyclase
Phospholipase C
Second messengers
cGMP
cAMP
IP3
DAG
AA
↑[Ca2+]i
Eicosanoids
Protein kinases
PKG
PKA
PKC
Released as local hormones
Effectors
Enzymes, transport proteins, etc.
Contractile proteins
Ion channels


Pain
Augmented neuronal excitability
Depolarization
Histamine
Out
In
β
γ
G_q α
G_q α
PLC
PIP_2
DAG
IP_3
PKC
Kv7 channels
K^+
$H_1R/H_2R/H_3R$
Ca^{2+}+ CaM
Ca^{2+}
IP_3R
CaM
Ca^{2+}
Endoplasmic reticulum



Opioid receptors

All four opioid receptors are seven-transmembrane spanning proteins that couple to inhibitory G proteins.

After being activated by an agonist, such as the endogenous peptide endorphin, or exogenous agonists, such as morphine and fentanyl, the G subunits dissociate from one another and subsequently act on various intracellular effector pathways.

It was later determined that GTPase activity is stimulated by opioid agonists and endogenous opioid peptides.

Agonist stimulation of opioid receptors was also shown to inhibit cyclic adenosine monophosphate (cAMP) production

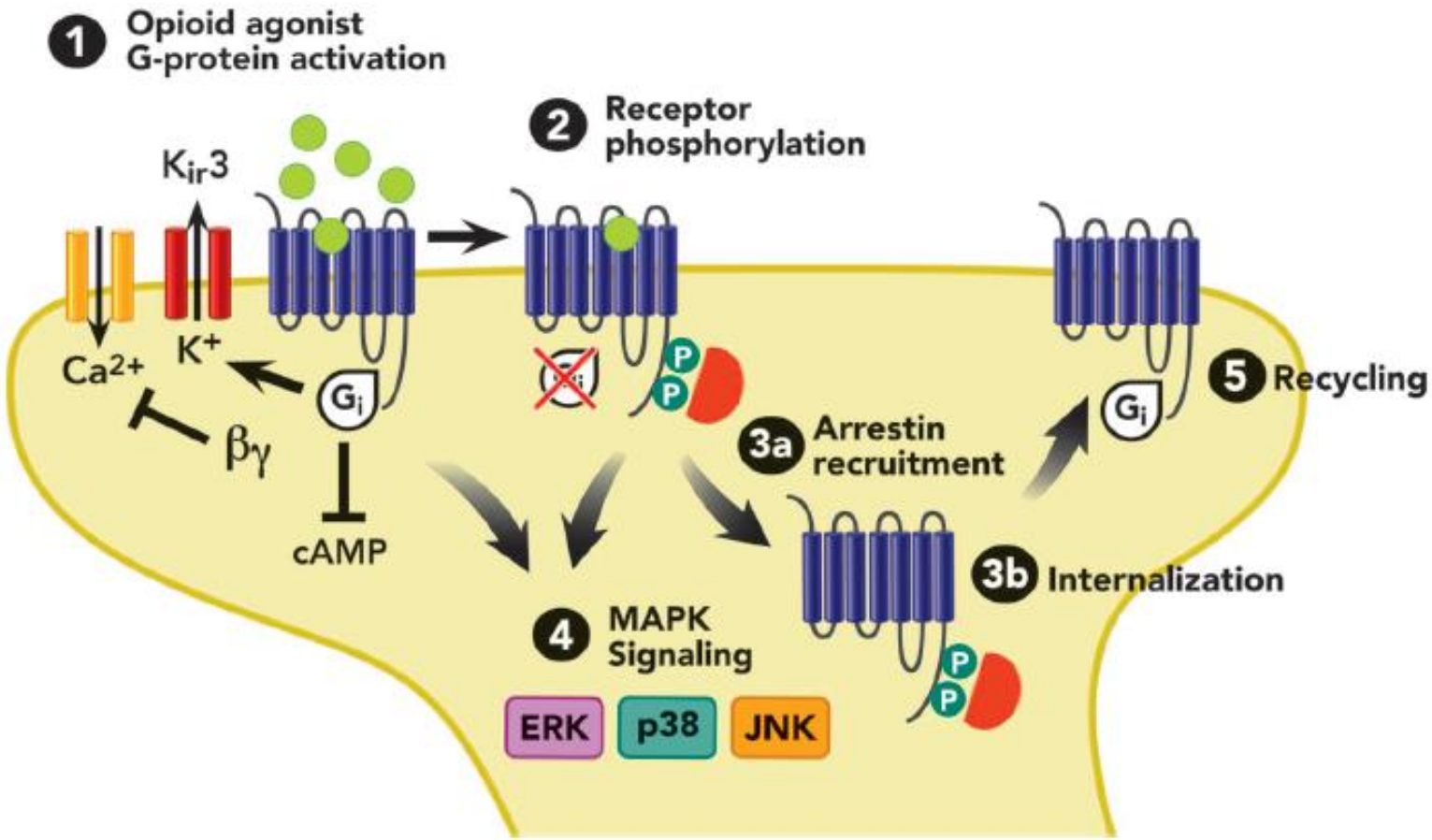
Opioid receptors

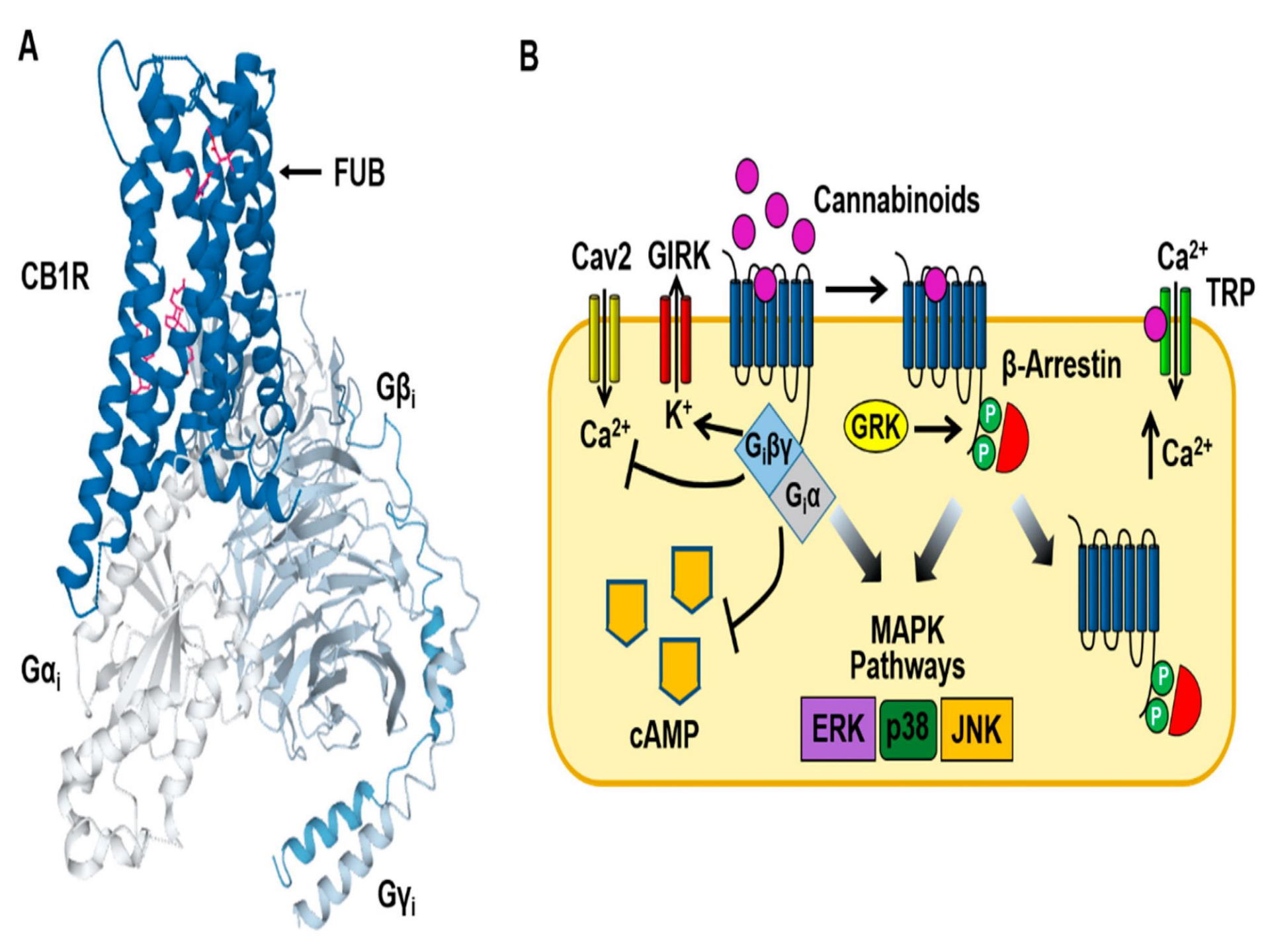
most important aspect of opioid receptor signal transduction relates to opioids' ability to modulate calcium and potassium ion channel

The inhibitory effects of opioids on neural excitability were shown to be mediated by interactions of opioid receptors with G protein-regulated inwardly rectifying potassium channel (Kir3)

Opioid receptor-induced inhibition of calcium conductance. This binding event is thought to reduce voltage activation of channel pore opening

The evidence for opioid receptors positively coupling to potassium channels while negatively modulating calcium channels has been reported in numerous model systems and cell types


1 Opioid agonist
G-protein activation
2 Receptor
phosphorylation
$K_{ir}3$
Ca^{2+}
K^+
G_i
$\beta\gamma$
cAMP
P
P
3a Arrestin
recruitment
3b Internalization
5 Recycling
4 MAPK
Signaling
ERK
p38
JNK


A
FUB
CB1R
Gβi
Gαi
Gγi
B
Cannabinoids
Cav2
GIRK
Ca2+
TRP
β-Arrestin
K+
Ca2+
GiβγGiα
GRK
P
P
Ca2+
cAMP
MAPK
Pathways
ERK
p38
JNK
P
P

Ion channels as targets for G proteins

❖control ion channel function directly by mechanisms that do not involve second messengers such as cAMP or inositol phosphates.

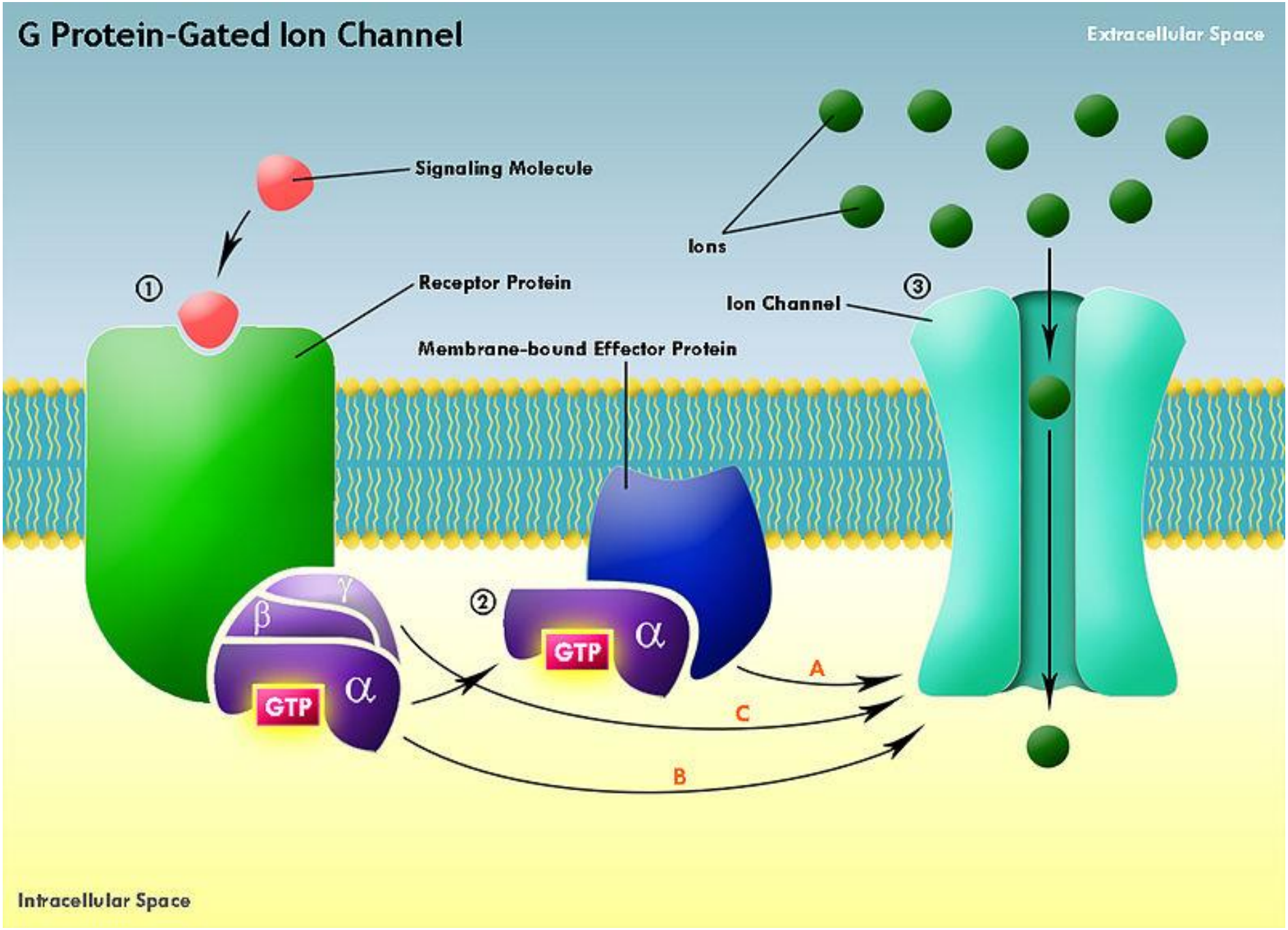
❖ Direct G protein–channel interaction, through the βγ subunits of Gi and Go proteins, appears to be a general mechanism for controlling K+ and Ca2+ channels.

✓ In cardiac muscle, for example, mAChRs enhance K+ permeability in this way (thus hyperpolarising the cells and inhibiting electrical activity

✓Similar mechanisms operate in neurons, where many inhibitory drugs such as opioid analgesics and cannabinoids reduce excitability by

1. opening certain K+ channels known as G protein-activated inwardly rectifying K+ channels

2. or by inhibiting voltage-activated N and P/Q type Ca2+ channels and thus reducing neurotransmitter release

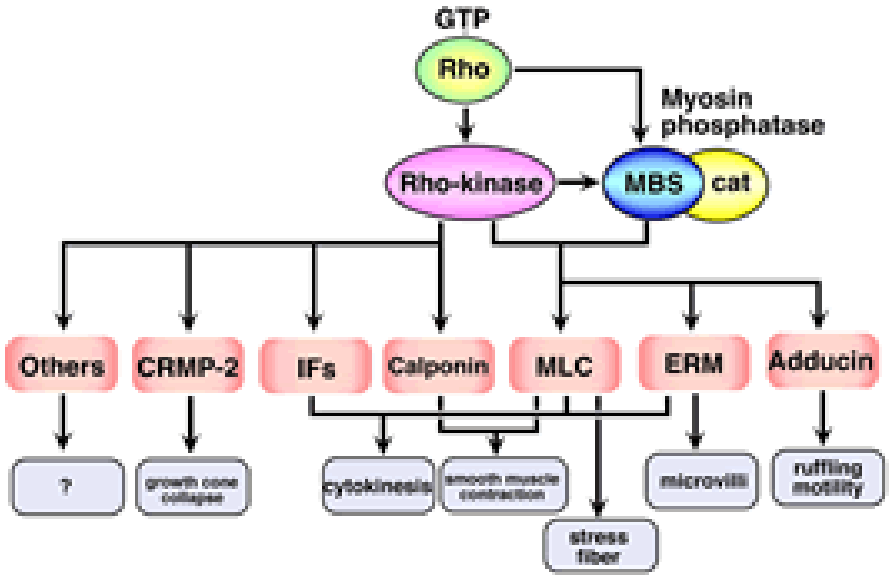

G Protein-Gated Ion Channel
Extracellular Space
Signaling Molecule
Ions
①
Receptor Protein
③
Ion Channel
Membrane-bound Effector Protein
γ
β
α
GTP
②
α
GTP
A
C
B
Intracellular Space

The Rho/Rho kinase system

❖This signal transduction pathway is couple to G proteins of the G12/13 type.

❖The free G protein α subunit interacts with a *guanosine nucleotide exchange factor, which facilitates GDP–GTP exchange at another GTPase, Rho.*

❖ *Rho–GDP, the resting form, is inactive, but when GDP–GTP exchange occurs, Rho is activated, and in turn activates Rho kinase.*



ERM; Ezrin/Radixin/Moesin, IFs; intermediate filaments

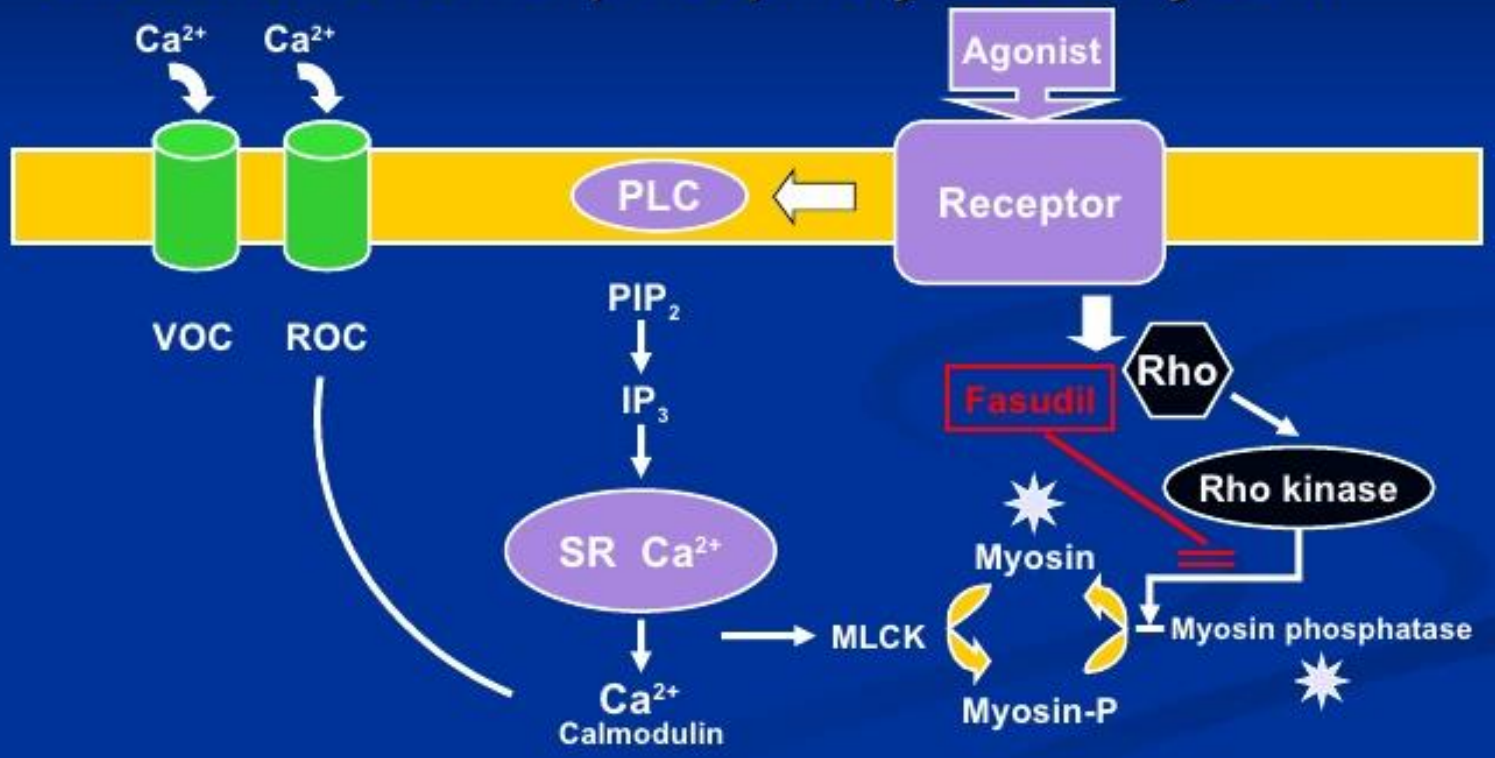
The Rho/Rho kinase system

- *Rho kinase phosphorylates many substrate proteins and controls a wide variety of cellular functions, including*
- *smooth muscle contraction and proliferation,*
- *angiogenesis and synaptic remodelling. By enhancing hypoxia-induced pulmonary artery vasoconstriction, activation of Rho kinase is thought to be important in the pathogenesis of pulmonary hypertension .*

- *Specific Rho kinase inhibitors (e.g.* ***fasudil)*** ***are in development for a wide range of clinical indications – an area to watch.***

Rho kinase inhibition: Fasudil

- Rho kinase triggers vasoconstriction through accumulation of phosphorylated myosin



Adapted from Seasholtz TM. *Am J Physiol Cell Physiol*. 2003;284:C596-8.

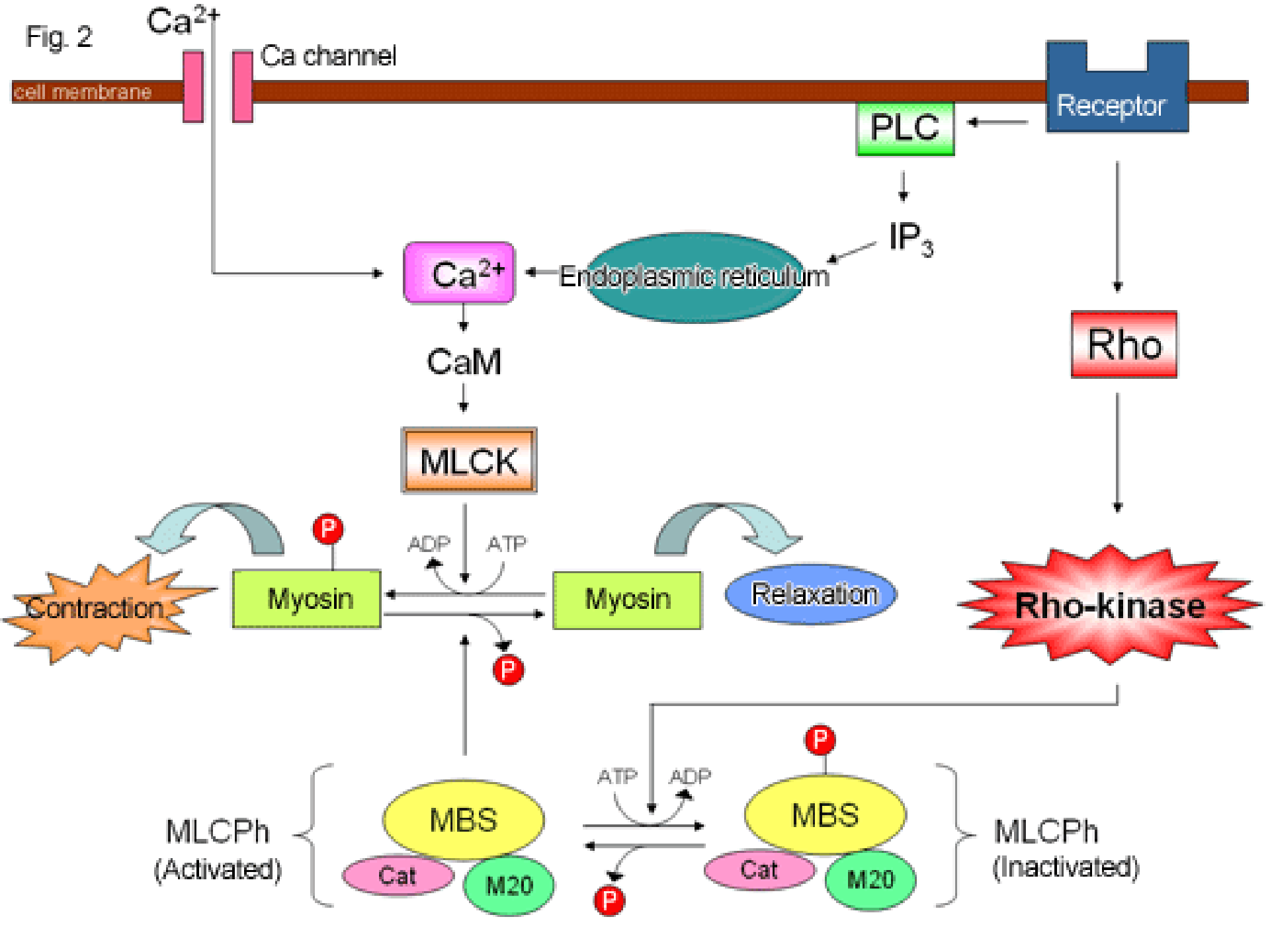
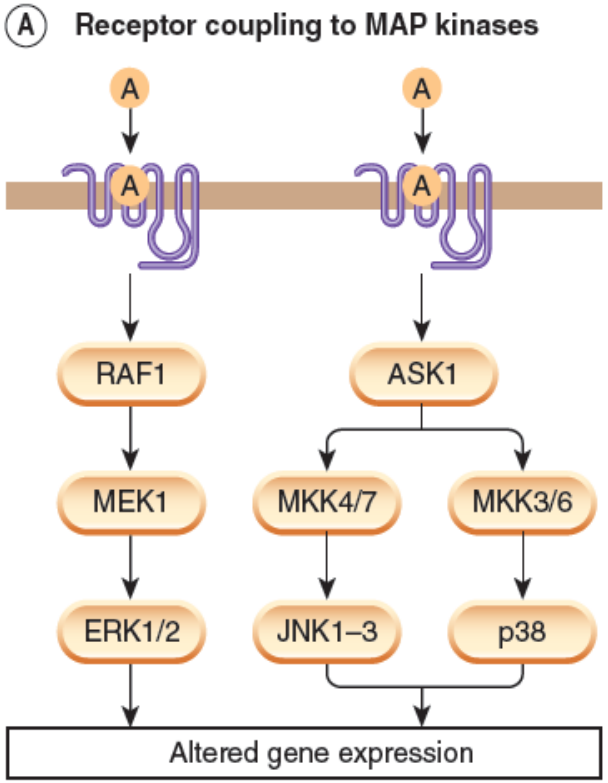


Fig. 2
Ca2+
Ca channel
cell membrane
Receptor
PLC
IP3
Ca2+
Endoplasmic reticulum
CaM
Rho
MLCK
P
ADP
ATP
Contraction
Myosin
Myosin
Relaxation
Rho-kinase
P
ATP
ADP
MLCPh
(Activated)
MBS
Cat
M20
P
MBS
Cat
M20
MLCPh
(Inactivated)

The MAP kinase system

▼ The coupling of GPCRs to different families of MAP kinases can involve G protein α and βγ subunits as well as Src and arrestins – proteins also involved in GPCR desensitisation

The MAP kinase system controls many processes involved in gene expression, cell division, apoptosis and tissue regeneration



GPCR desensitization

Indeed chronic drug (agonist) treatment often leads to a greatly reduced therapeutic response.

✓Prolonged use of β2-adrenoceptor agonists significantly decreases their effectiveness in the treatment of asthma.

✓analgesic tolerance and dependence to morphine and related opioids involves reduced responsiveness of the μ-opioid receptor.

Removal of agonist and second messenger breakdown

Lines of defence

The actions of some agonists are rapidly terminated by enzymatic degradation. For example acetylcholine is degraded by acetylcholinesterase into choline and acetate

Many agonists, such as dopamine, noradrenaline, 5-HT and glutamate, are transported back into the neuron from the synapse via specific membrane bound transporters

A second line of defence for 'switching off' GPCR signalling involves the rapid degradation of the second messengers. Inositol phosphatases and phosphodiesterases terminate the actions of IP3 and cyclic AMP, respectively.

A third line of defence is the intrinsic GTPase activity of the Gα protein subunit which hydrolyses GTP and terminates heterotrimeric G-protein function.

Role of GPCR phosphorylation in desensitisation

It is a well known phenomenon that the continuous or repeated stimulation of a GPCR reduces subsequent responsiveness to an agonist (termed **desensitisation or tachyphylaxis**).

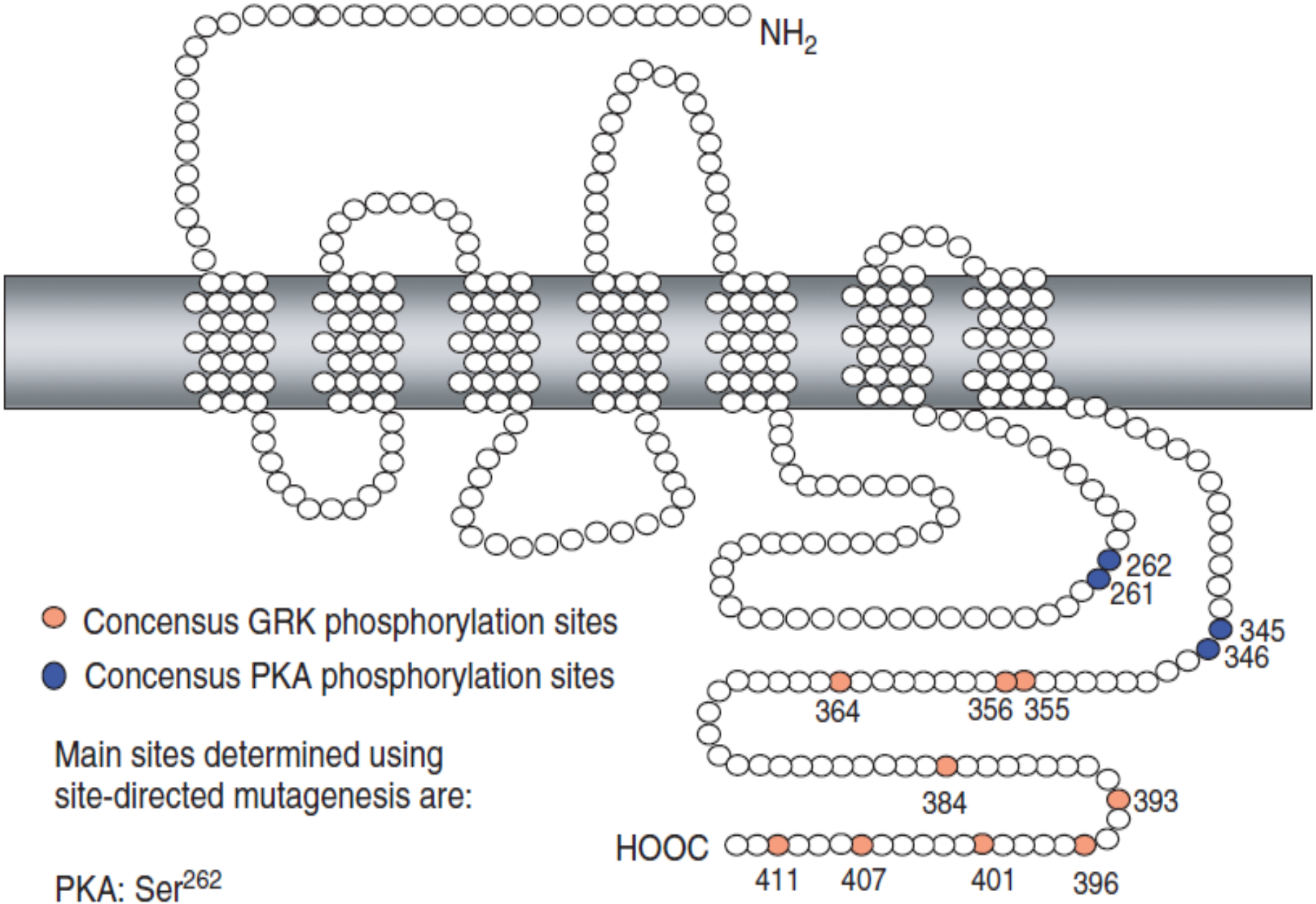
Desensitisation is a feature of most GPCRs, *Homologous desensitisation is restricted to the receptors activated by the desensitising agonist, while heterologous desensitisation affects other GPCRs in addition*

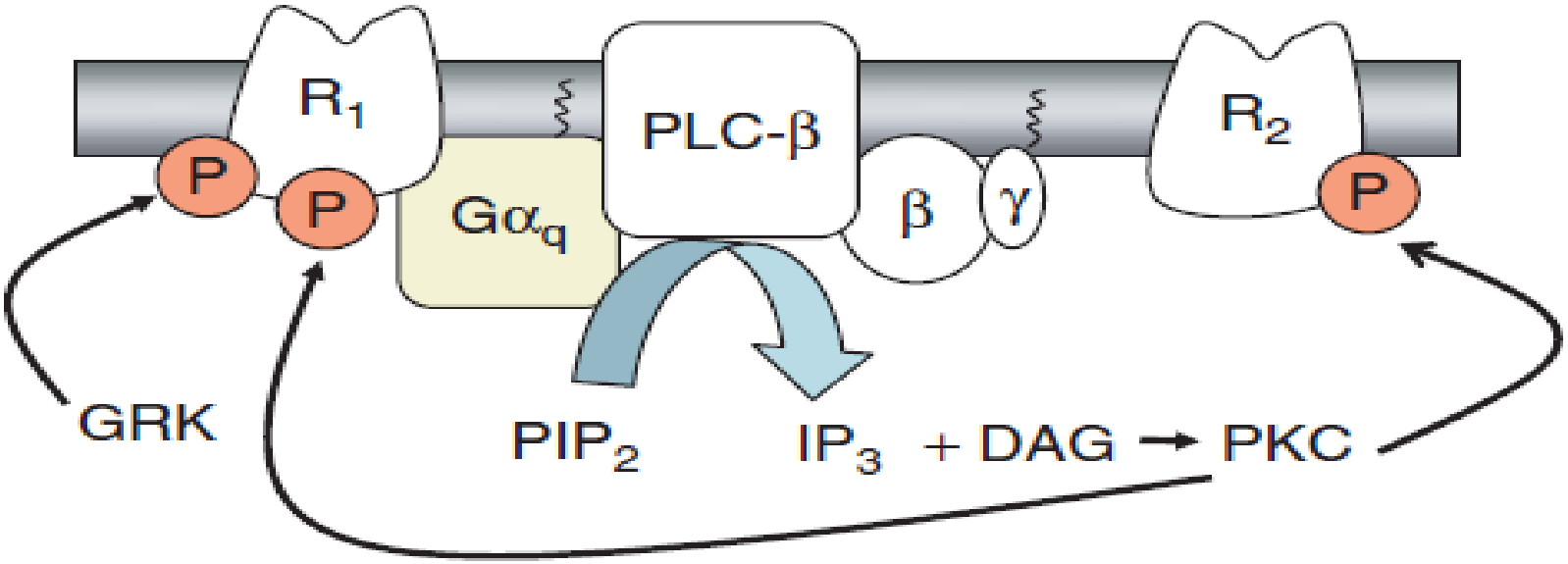
Two main processes are involved

- **Receptor phosphorylation**
- **Receptor internalization (endocytosis).**

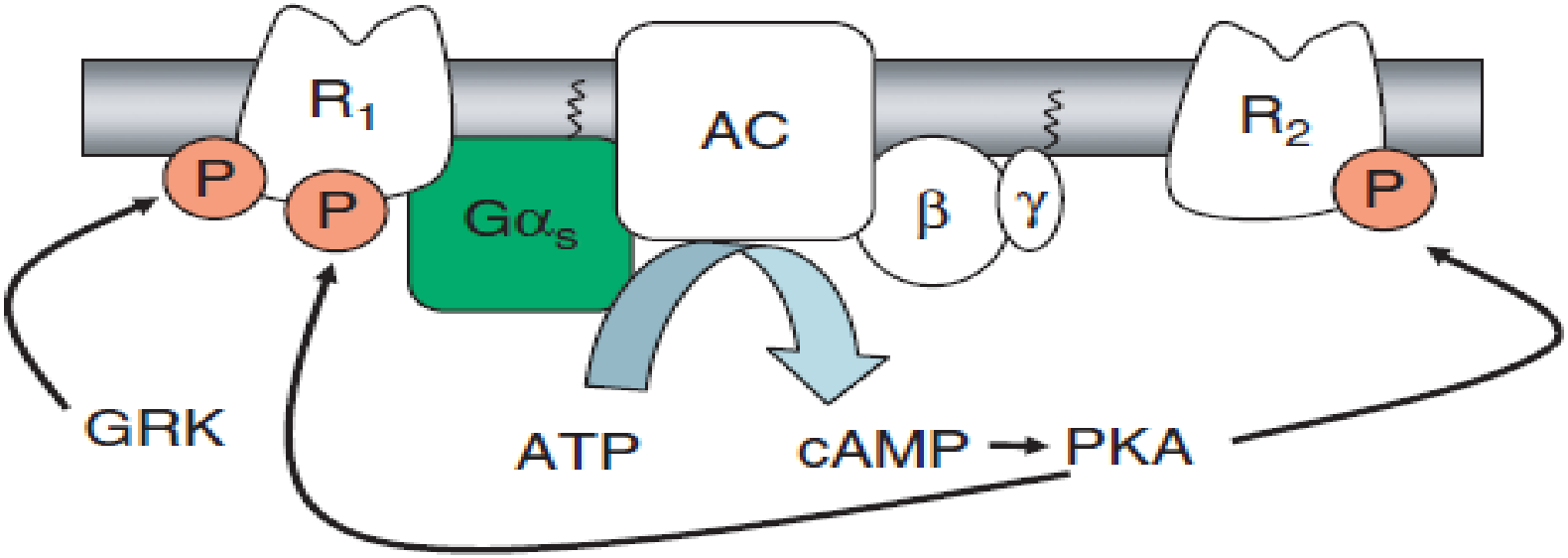
The sequence of GPCRs includes certain residues (serine and threonine), mainly in the C-terminal cytoplasmic tail, which can be phosphorylated by specific membrane-bound GPCR kinases (GRKs) and by kinases such as PKA and PKC.

There are seven GRKs in humans, named GRK1 through GRK7, and four arrestin proteins, named arrestins 1 through 4

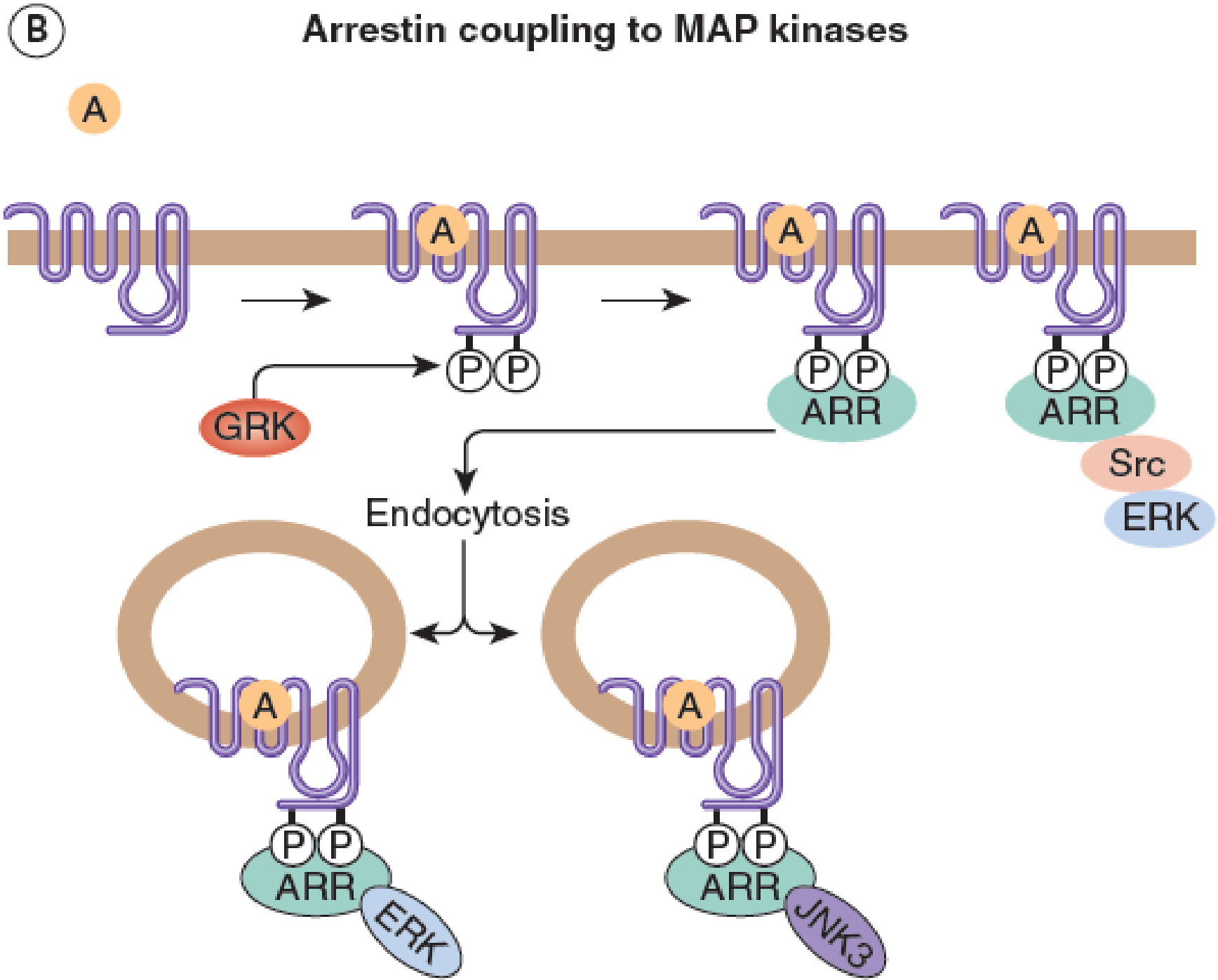

NH2
262
261
345
346
364
356 355
384
393
HOOC
411
407
401
396
Concensus GRK phosphorylation sites
Concensus PKA phosphorylation sites
Main sites determined using site-directed mutagenesis are:
PKA: Ser262
GRK: Ser355, Ser356 and Ser364


R1
PLC-β
R2
P
P
Gαq
β
γ
P
GRK
PIP2
IP3 + DAG → PKC

(a)


R1
AC
R2
P
P
Gαs
β
γ
P
GRK
ATP
cAMP → PKA

(b)


B
Arrestin coupling to MAP kinases
A
A
A
A
P
P
GRK
ARR
Src
ERK
Endocytosis
A
A
ARR
ERK
ARR
JNK3

In contrast, heterologous desensitisation mainly involves phosphorylation of specific serine and threonine residues within the third intracellular loop of the receptor

Given growing interest in the therapeutic potential of GRK inhibitors. For example, inhibition of GRK2 may help restore β1-adrenoceptor signalling in patients with chronic heart failure, who exhibit increased levels of GRK2 protein expression.

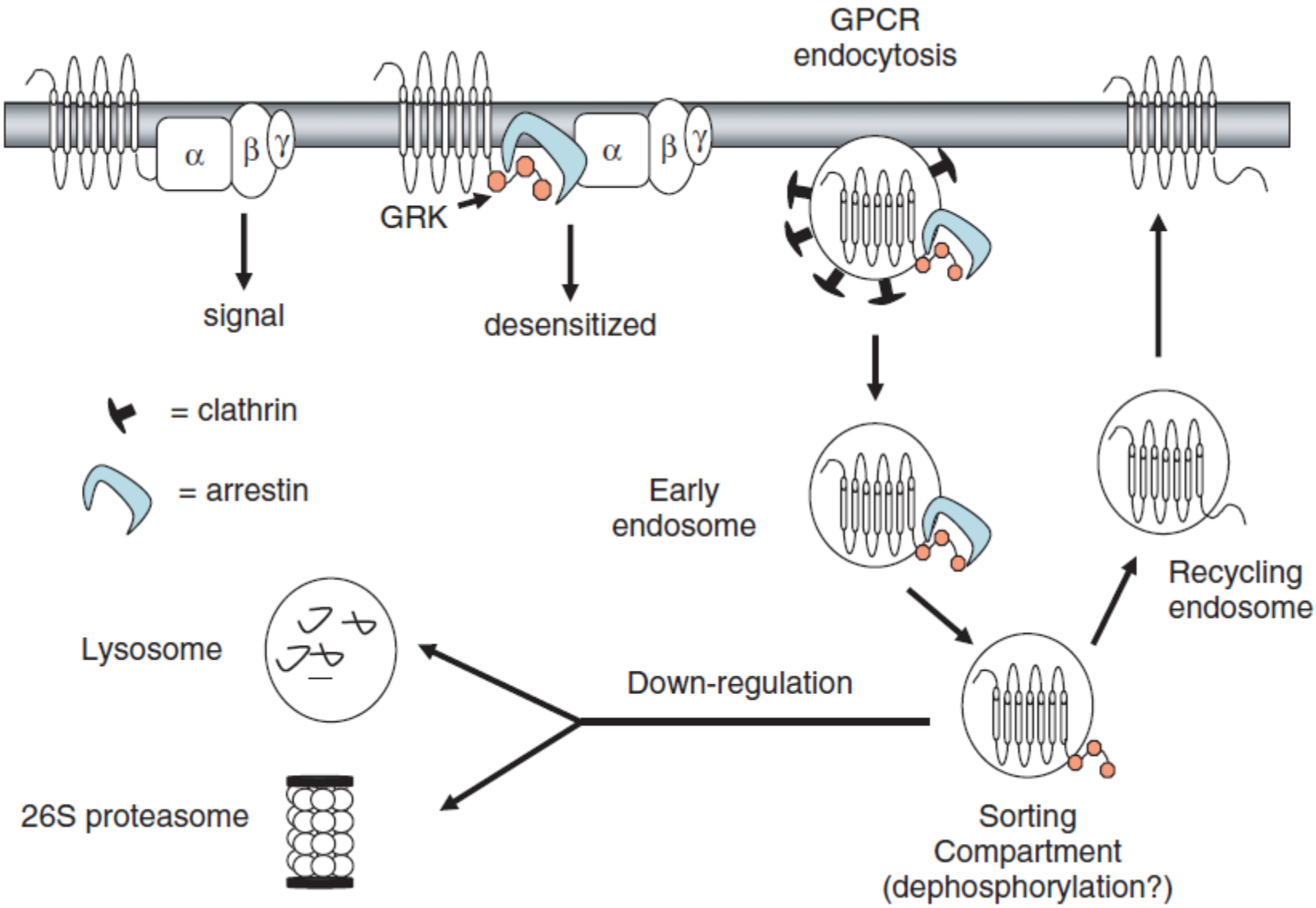
The next step in homologous desensitisation is the binding of proteins called β-arrestins to the phosphorylated GPCR.

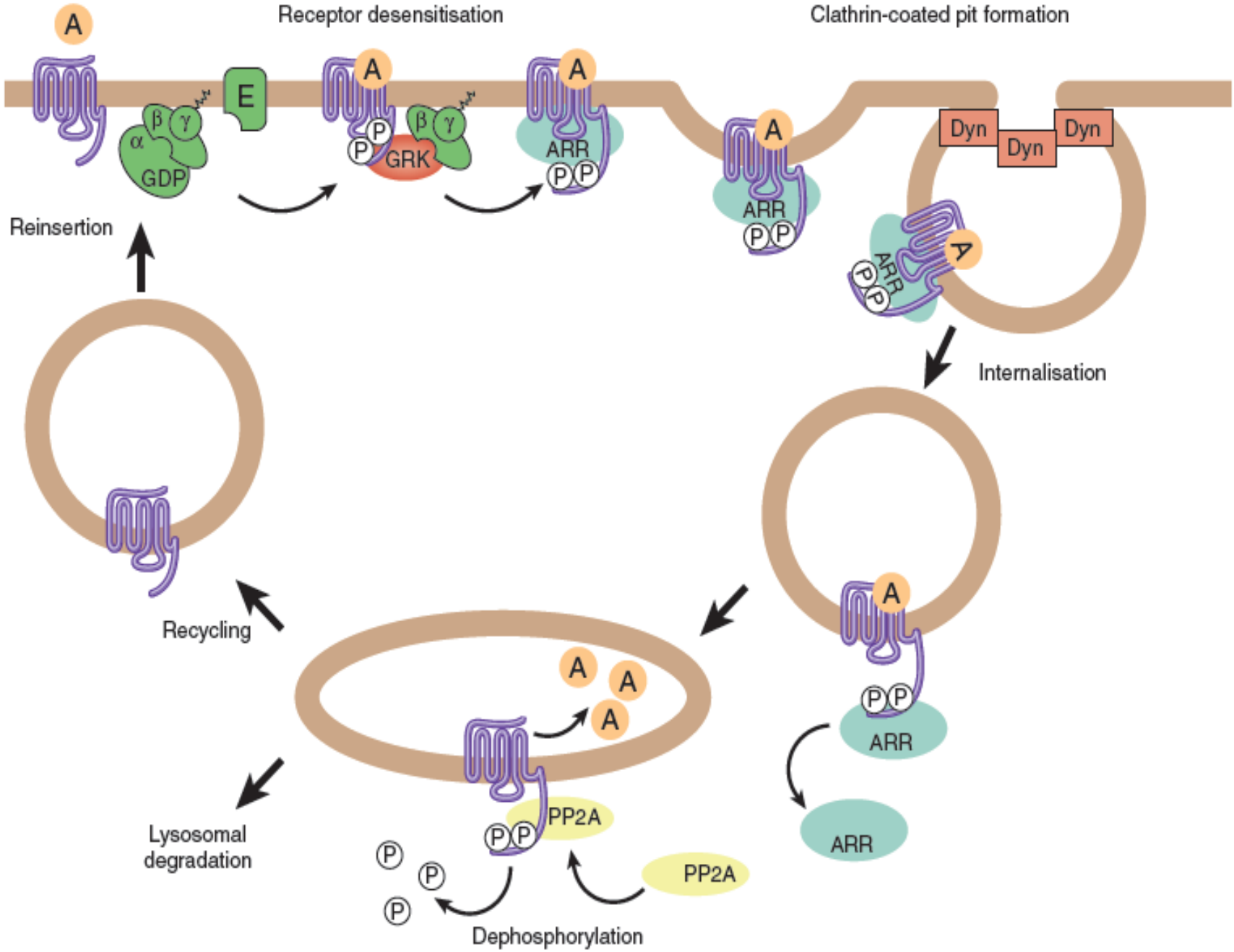
Arrestins uncouple the receptor from the G protein and initiate GPCR endocytosis via a clathrin coated pit-dependent pathway(internalisation or sequestration.)

The fate of internalised GPCRs

Internalised GPCRs are either de-phosphorylated and returned to the plasma membrane (termed re-sensitisation) or targeted for degradation leading to the loss of receptor from the cell and down-regulation.

GPCR degradation can occur either by lysosomes or via the 26S proteasome and in some cases involving ubiquitination

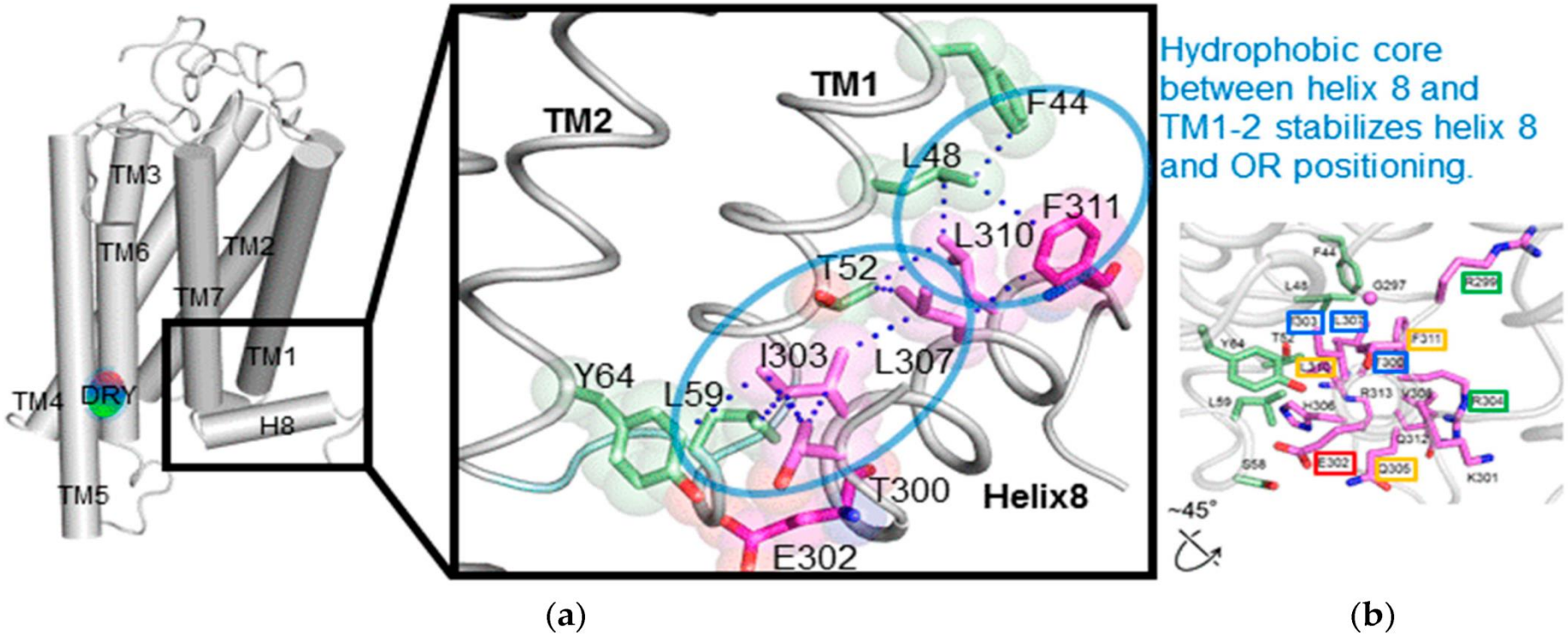

GPCR
endocytosis
α
β
γ
GRK
signal
desensitized
= clathrin
= arrestin
Early
endosome
Recycling
endosome
Lysosome
Down-regulation
26S proteasome
Sorting
Compartment
(dephosphorylation?)


A
Receptor desensitisation
Clathrin-coated pit formation
E
β
γ
α
GDP
P
GRK
ARR
Dyn
Dyn
Dyn
Reinsertion
Internalisation
Recycling
Lysosomal
degradation
PP2A
PP2A
Dephosphorylation

Constitutive GPCR activity

GPCRs exist in equilibrium between two states; an active conformation (denoted R*) and an inactive conformation (R).

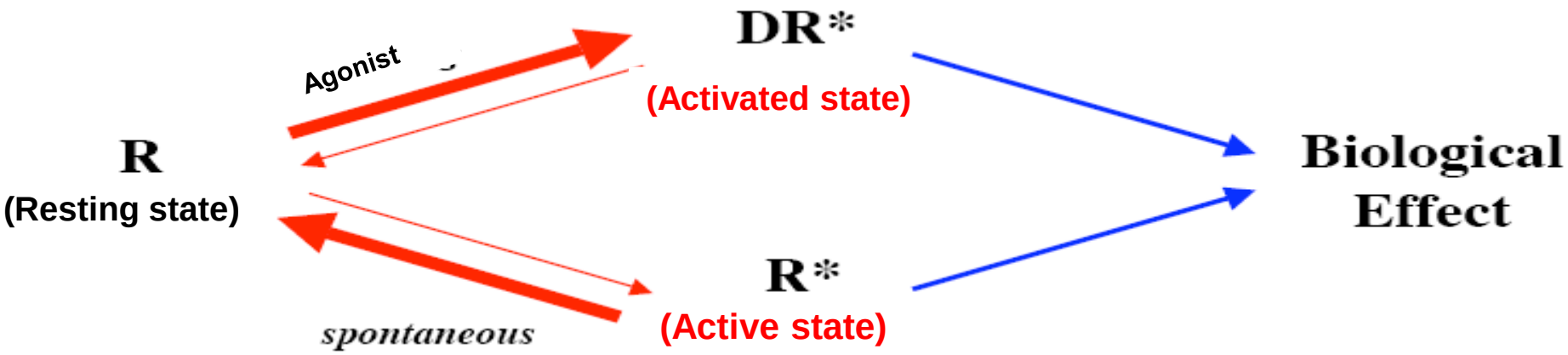
The R form does not couple to G-proteins, whereas R* can couple and activate G-proteins. Switching between R and R* states involves conformational changes within TM domains 3,6,7 and helix 8.



Constitutive GPCR activity

Agonists (A) bind preferentially to R* and stabilise the active conformation. The A/R* complex is now able to bind and activate G-proteins (G) forming the ternary complex (A/R*/G).

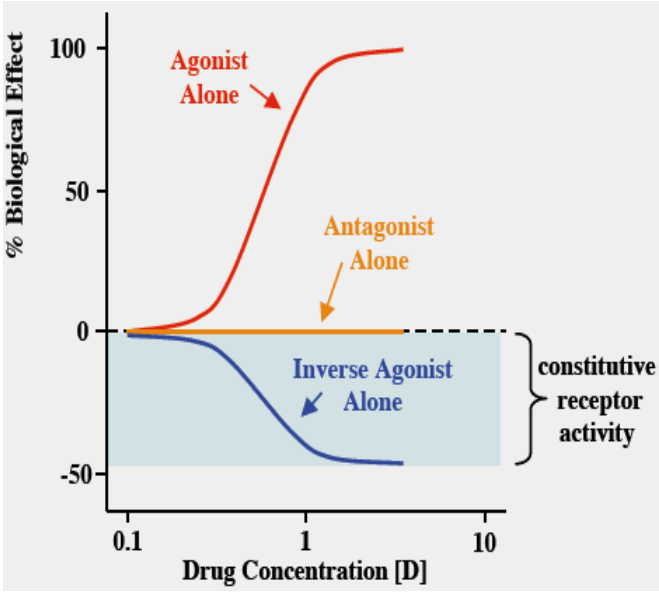
GPCRs can also switch between R and R* conformations independent of agonist binding. This property is known as constitutive activity and results in the activation of G-protein signalling independent of receptor activation



Drug Receptor Interactions, Inverse agonist

❖ Inverse agonist

"An agent which binds to the same receptor binding-site as an agonist for that receptor but exerts the opposite pharmacological effect"

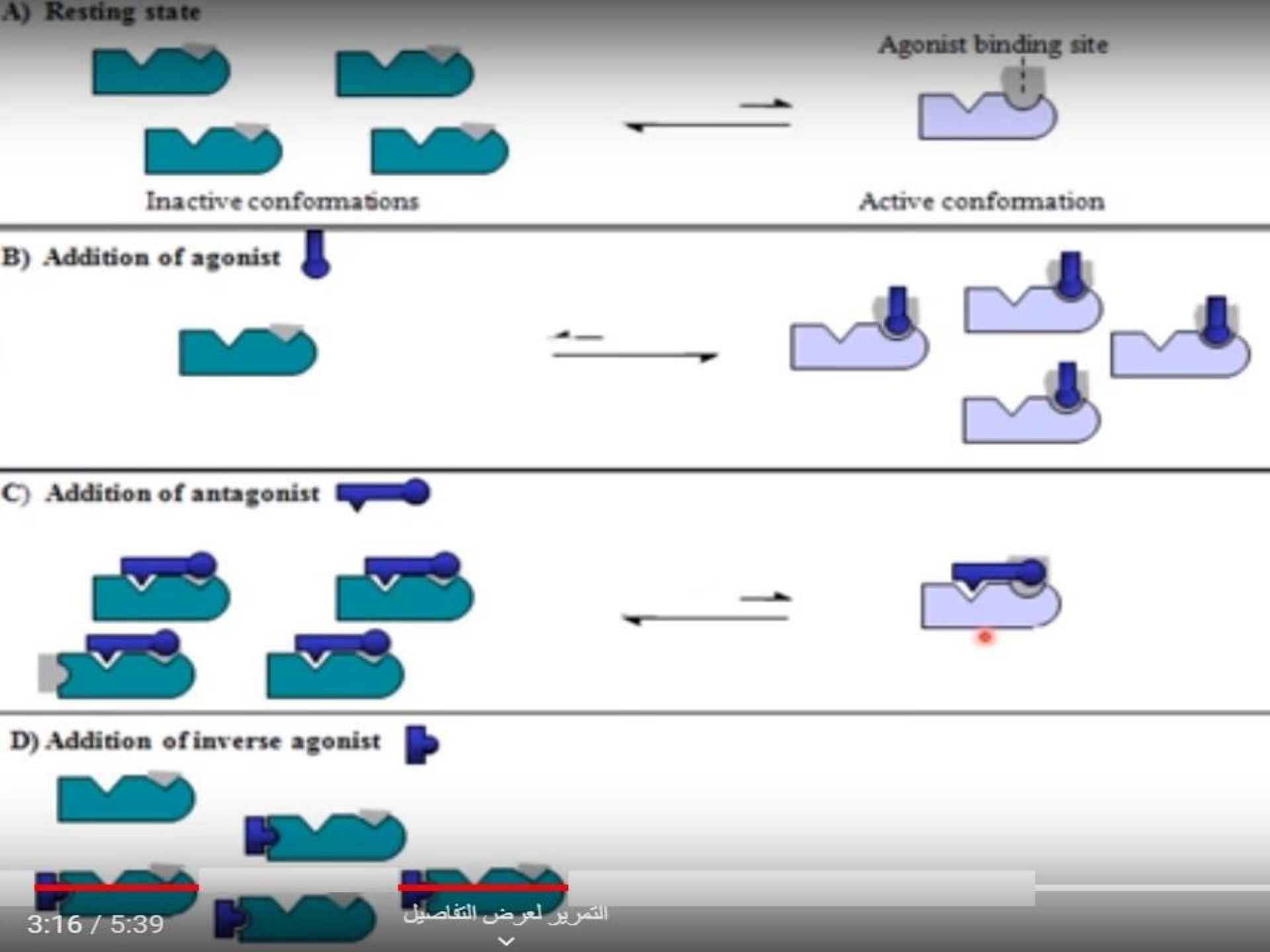


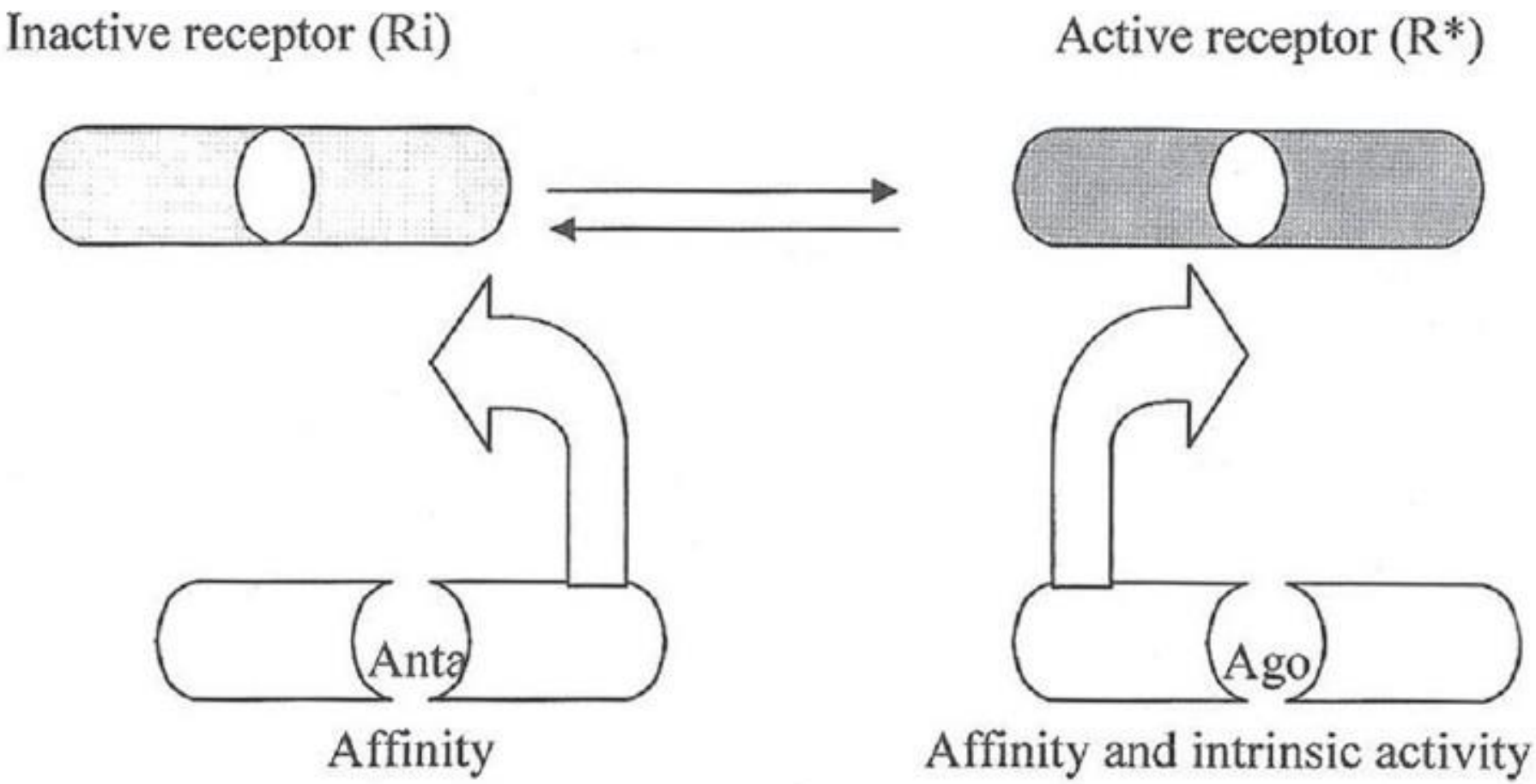
➢ Difference from Antagonist: Antagonist binds to the receptor, **but does not reduce basal activity**

- ❑ Agonist → **positive** efficacy
- ❑ Antagonist → **zero** efficacy
- ❑ Inverse agonist → **negative** efficacy

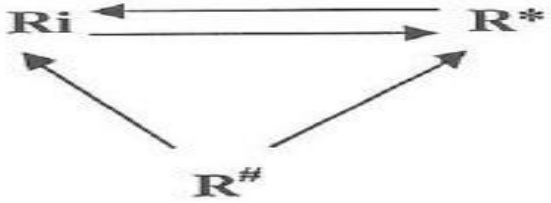
✓ The agonist action of benzodiazepines on the benzodiazepine receptor in the CNS produces sedation, muscle relaxation, and controls convulsions. β-carbolines (inverse agonists) which also bind to the same receptor cause stimulation, anxiety, increased muscle tone and convulsions

✓ Example 2: The histamine H_2 receptor has constitutive activity, which can be inhibited by the inverse agonist cimetidine. On the other hand, burimamide acts as a neutral antagonist



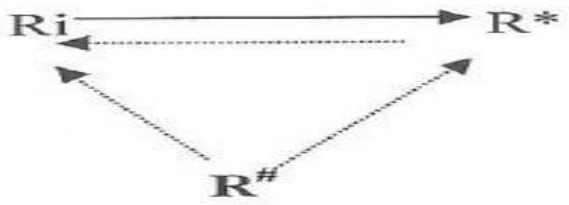


A receptor remains in dynamic equilibrium in two states Ri and R*. A ligand that binds and shifts the equilibrium to R* state is called agonist. A ligand that binds but does not shift the equilibrium to active state is called antagonist. Ligand binding is an equilibrium process.



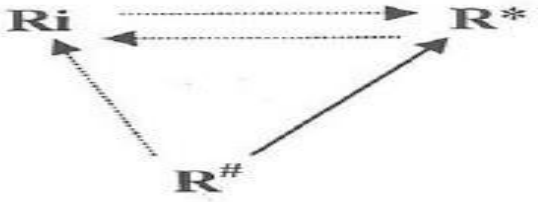
(a)

(i) Agonist

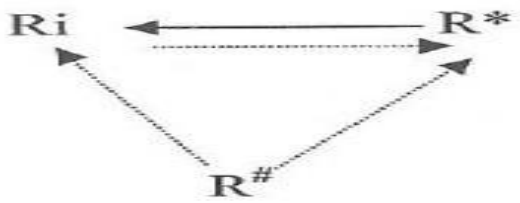


(b)

(ii) Agonist

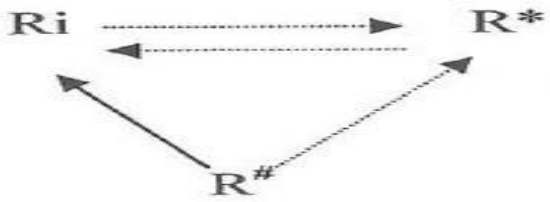


Antagonist



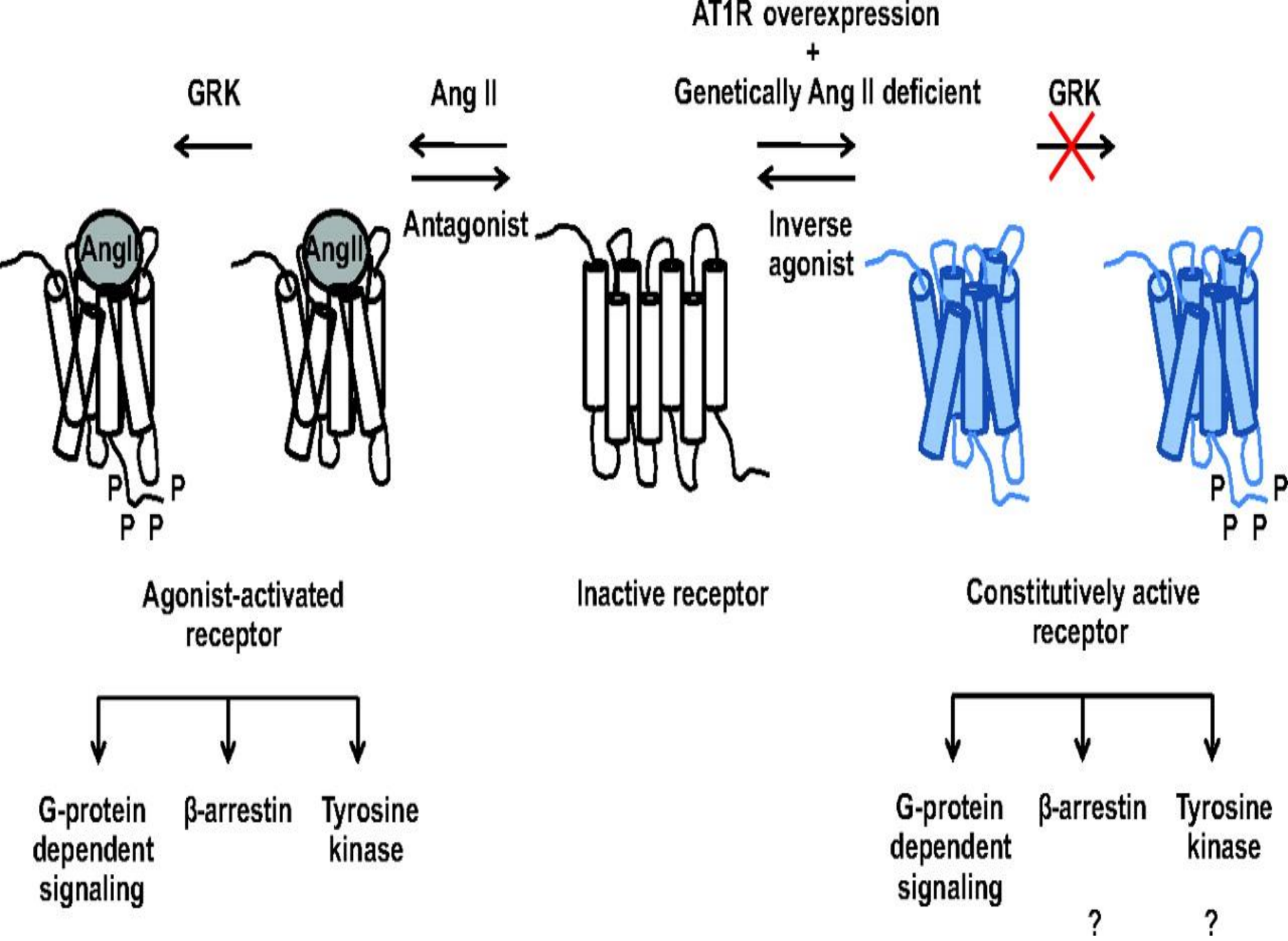
(c)

Inverse agonist



(d)

Multiple activation states of a receptor (R* Active), (Ri quiescent or inactive) and (R# Constitutionally active)
(a) Unliganded receptor state
(b- i and ii) Agonists shift equilibrium to activated state
(c) Antagonists shift equilibrium to Ri
(d) Inverse agonists act to reduce spontaneous activity of R#.


AT1R overexpression
+
Genetically Ang II deficient
GRK
Ang II
GRK
Antagonist
Inverse agonist
AngII
AngII
P
P
P
P
P
P
P
P
Agonist-activated receptor
Inactive receptor
Constitutively active receptor
G-protein dependent signaling
β-arrestin
Tyrosine kinase
G-protein dependent signaling
β-arrestin
Tyrosine kinase
?
?

CAR

The constitutive activity of a particular GPCR can increase as a consequence of genetic mutations, single nucleotide polymorphisms (SNPs) and splice variants

An activating mutation of the receptor for luteinizing hormone (LH) has been identified as the most common cause of familial male precocious puberty (FMPP). The substitution of an aspartate with glycine at position 578 of TMD VI

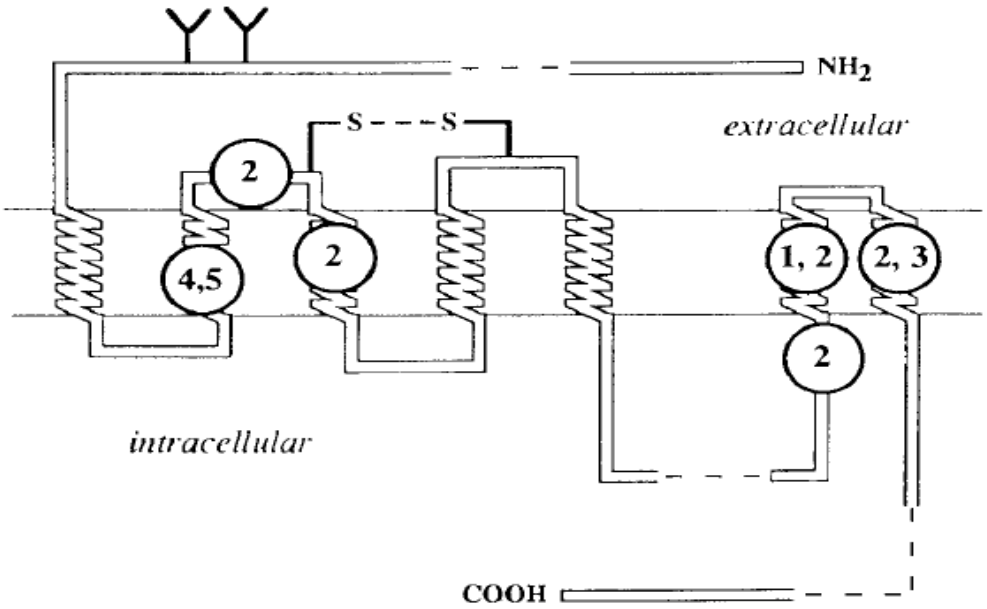
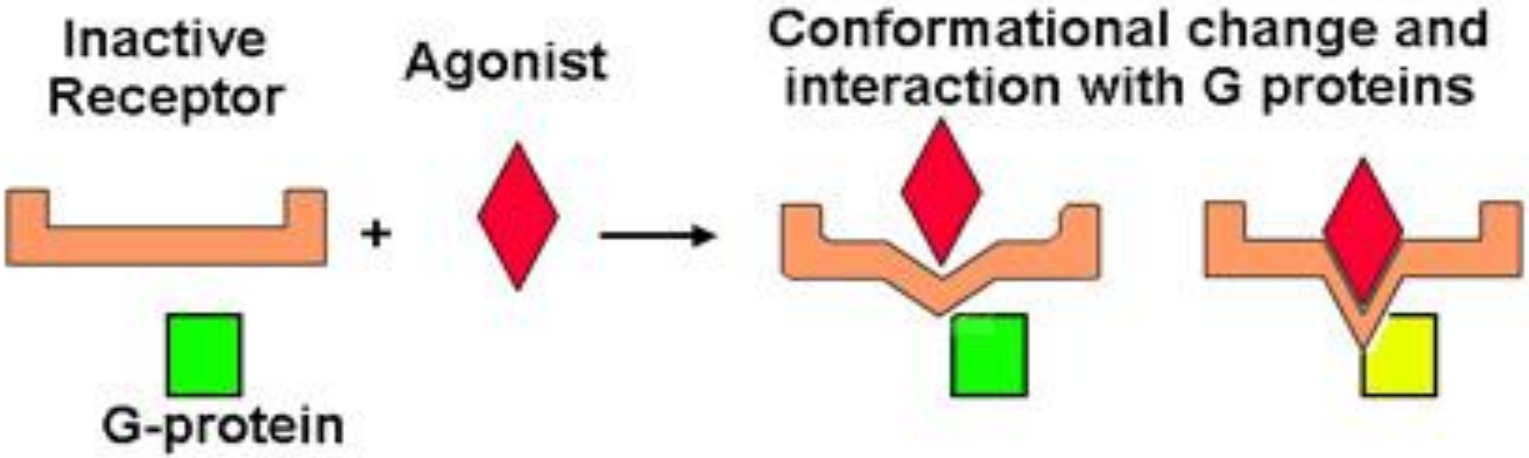
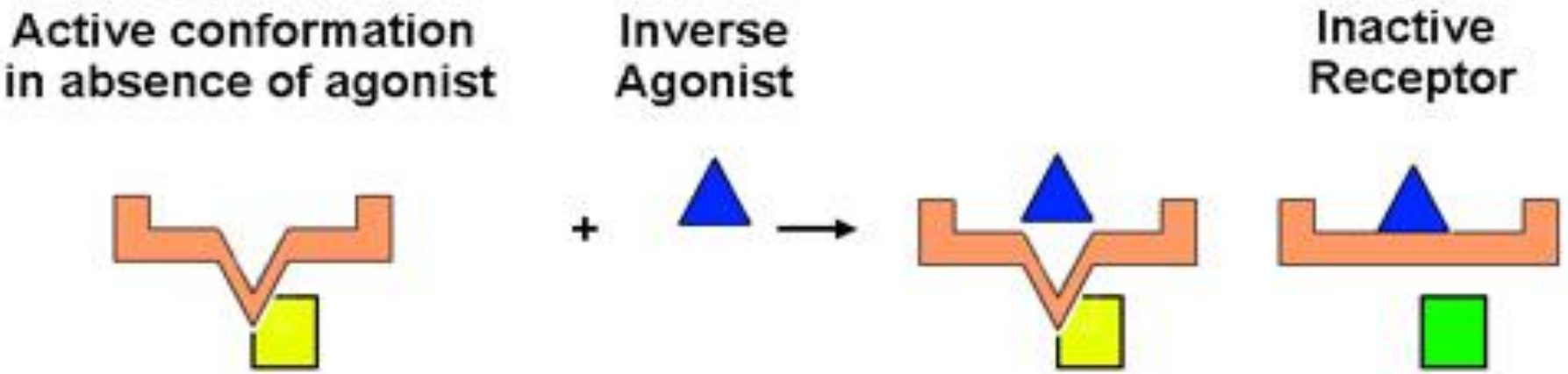


Fig. 2. Spontaneous activating mutations: (1) LH receptor, precocious puberty; (2) TSH receptor, hyperfunctioning adenomas; (3) rhodopsin, retinitis pigmentosa; (4) rhodoposin, congenital night blindness; (5) MSH receptor, inherited hyperpigmentation.

Typical Agonist Promoted Receptor Activation



Constitutively Active Receptor and an Inverse Agonist



Inverse agonists

Inverse agonists preferentially bind and stabilize the inactive R conformation which results in a decrease in constitutive activity.

In contrast, the term neutral antagonist is now used to describe an antagonist which has no inverse agonist activity and hence does not alter the R/R* equilibrium

Several clinically used drugs have been demonstrated to be inverse agonists

Table 3.5 Clinically used drugs proven to be inverse agonists.

Brand name	Generic name	Therapeutic use	GPCR target
Toprol	Metoprolol	High blood pressure	β_1-adrenoceptor
Pepcidine	Famotidine	Peptic ulcers	Histamine H_2 receptor
Cozaar	Losartan	High blood pressure and heart failure	Angiotensin AT_1 receptor

Inverse agonists may also prove useful for treatment of autoimmune diseases that involve GPCR auto-antibodies that behave as agonists.

Agonist auto-antibodies against the type 1 angiotensin receptor (AT1) are involved in allograft rejection in renal transplantation patients. Treatment with an AT1 receptor inverse agonist such as losartan may prove useful.

It is known that the prolonged treatment with an inverse agonist leads to the increased expression (upregulation) of the GPCR involved, a characteristic not observed with neutral antagonists.

β1-adrenoceptor antagonists are used to treat chronic heart failure. Their clinical success is thought to be due in part to the re-sensitisation of the β1 cardiac contractile system, which is compromised due to the elevated levels of noradrenaline associated with chronic heart failure.

The high levels of noradrenaline would cause a significant decrease in β1-adrenoceptor expression due to receptor down-regulation. inverse agonists, metoprolol and carvedilol are more effective at reducing mortality than the neutral antagonist bucindolol..

Endogenous inverse agonists

❖Melanocortin type 4 receptors (MC4), which are activated by the peptide hormone melanocyte-stimulating hormone, play a major role in regulating body weight.

❖MC4 receptor displays constitutive activity and mutations in the gene encoding this receptor have recently been discovered that reduce constitutive activity in obese patients.

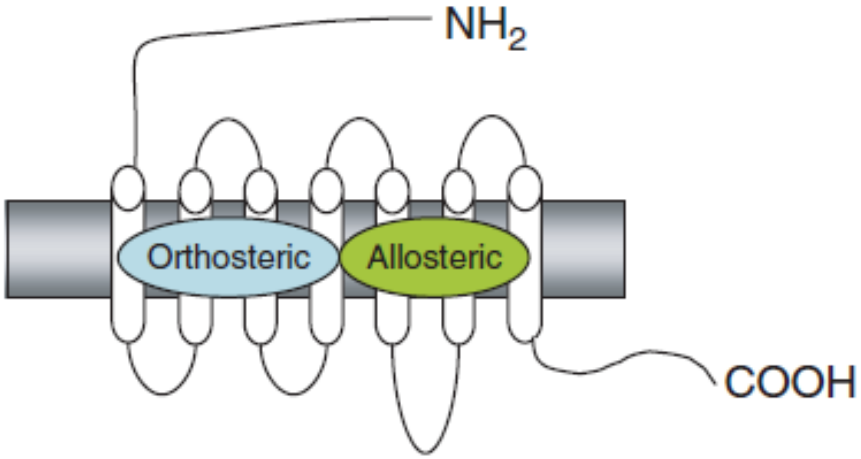
❖Infusion of MC4R agonists decreases food intake, whereas inhibition of MC receptor activity by infusion of an MC receptor antagonist or with the inverse agonist AgRP results in increased food intake.

❖MC4 receptor is regulated by an endogenous inverse agonist called agouti-related peptide

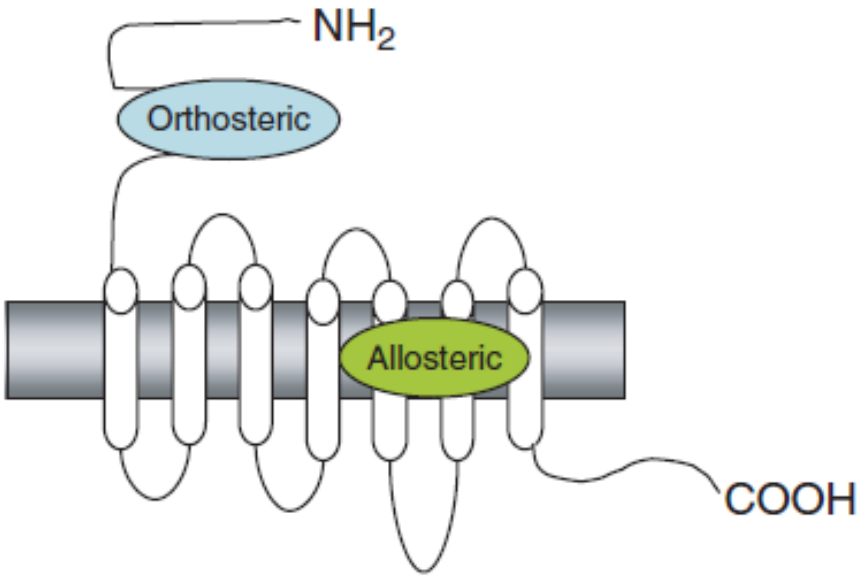
Allosteric modulators of GPCR function

The majority of synthetic ligands that target GPCRs are classified as agonists or antagonists which bind to the orthosteric site on the receptor for endogenous ligands.
More recently there has been considerable interest in the development and therapeutic use of allosteric modulators which bind to GPCRs at sites that are topographically distinct from the orthosteric binding site

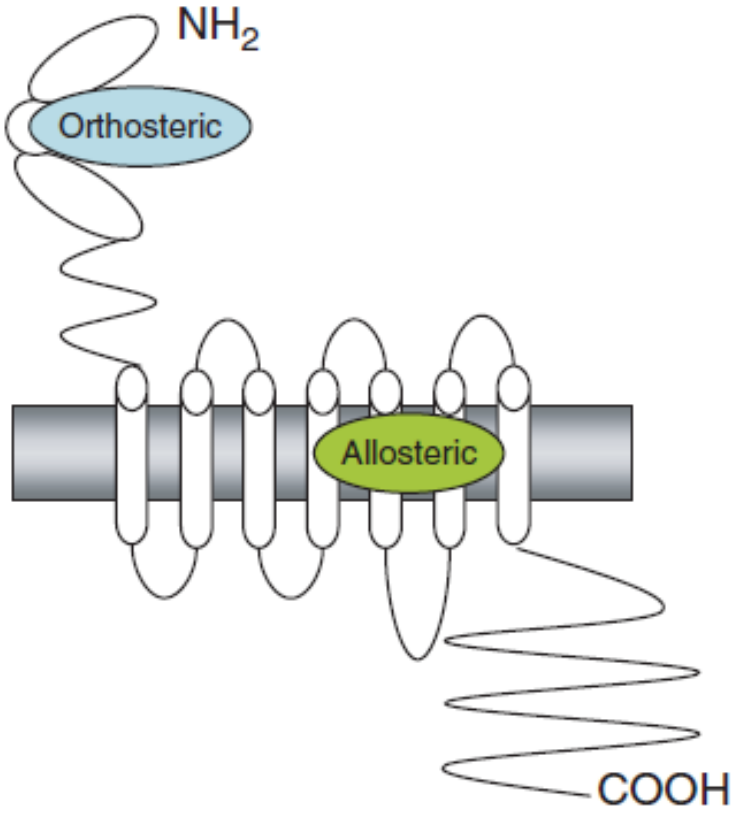
Class A (Rhodopsin-like class)



Class B (Secretin and adhesion class)



Class C (Glutamate class)



There are three categories of allosteric modulator:

1. Allosteric modulators that affect the binding affinity of orthosteric ligands (both agonists and antagonists).

2. Allosteric ligands that modulate the efficacy of orthosteric agonists.

3. Allosteric ligands which are capable of modulating GPCR activity independently of orthosteric ligand binding. Such compounds can be classed as allosteric agonists and allosteric inverse agonists.

At present two allosteric modulators are currently on the market;

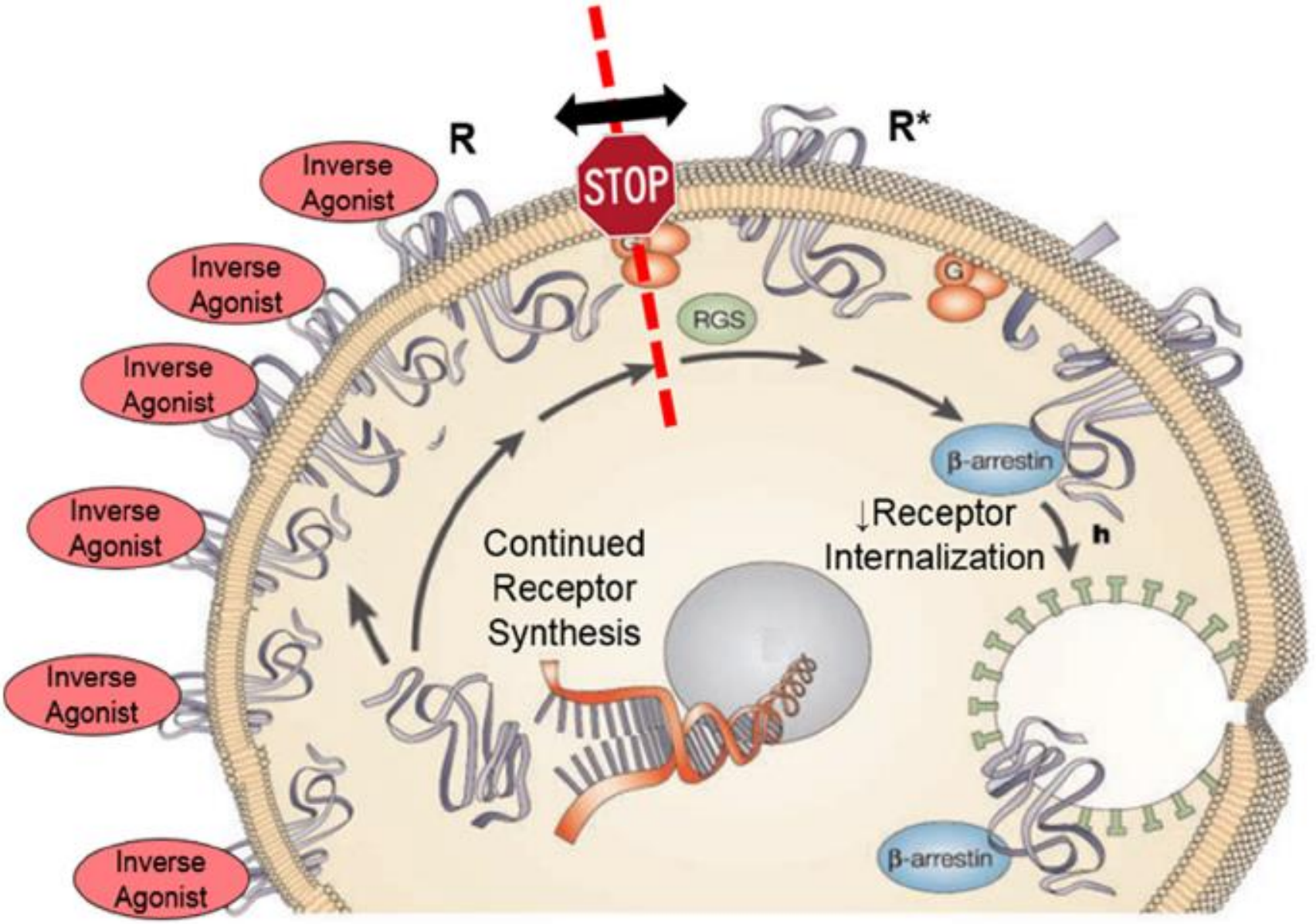
✓cinacalcet

✓maraviroc.

Cinacalcet is classed as a positive allosteric modulator of Ca^{2+} ion binding to the calcium-sensing receptor (CaSR). It is used clinically to treat hyperparathyroidism since it inhibits the release of parathyroid hormone and protects against osteoporosis

Maraviroc is an allosteric inhibitor of the chemokine receptor subtype CCR5 and is used to treat HIV infections. The CCR5 receptor functions as a co-receptor for the entry of HIV into cells and maraviroc causes conformational changes in the CCR5 receptor that block interaction with HIV.

In terms of cell signaling, this difference could take the form of increased cellular activity (positive agonism) or a decrease in elevated basal activity (inverse agonism).


R
R*
STOP
Inverse Agonist
Inverse Agonist
Inverse Agonist
Inverse Agonist
Inverse Agonist
Inverse Agonist
RGS
G
β-arrestin
↓Receptor Internalization
h
Continued Receptor Synthesis
β-arrestin