Receptors

Seminar No. 9

Q. 1

Feature	Lipophilic hormones	Hydrophilic hormones
Chemical type		
Water solubility		
Transport protein		
Plasma half-life		
Membrane penetration		
Receptor location		
2^{nd} messenger example		

Feature	Lipophilic hormones	Hydrophilic hormones
Chemical type	steroids, iodothyronines, calcitriol, retinoids	amino acid derivatives, polypeptides
Water solubility	no	yes
Transport protein	yes	no
Plasma half-life	long (hours, days)	short (minutes)
Membrane penetration	yes	no
Receptor location	intracellular	cell membrane
2^{nd} messenger example	hormone-receptor complex	cAMP, Ca^{2+} ....

Q. 2

A. 2

- Allosteric protein (in membrane or cytosol)
- It has two domains:
- **ligand-binding domain** (with binding site for signal molecule) – changes conformations of receptor
- **effector domain** – starts biological response to ligand (production of second messenger etc.)

Q. 3

A. 3

Receptor	Transporter
Common features:	
Protein, Binding selectivity to ligand, Changes in conformation	
Different features:	
Transfers **signal**	Transfers **substance**

Q. 4

A. 4

- **signal molecule (ligand)** - carries specific information into cell
- has extremely low concentration in blood (10^{-9} – 10^{-15} mol/l)
- binds to corresponding receptor
- signal molecule is usually quickly inactivated
- **agonist** – ligand which after binding to receptor transduces signal
- **antagonist** – ligand which after binding to receptor blocks signal transduction ⇒ no biological response

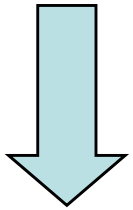
Q. 5

A. 5

The second messenger transfers information to other intracellular systems and then is quickly inactivated

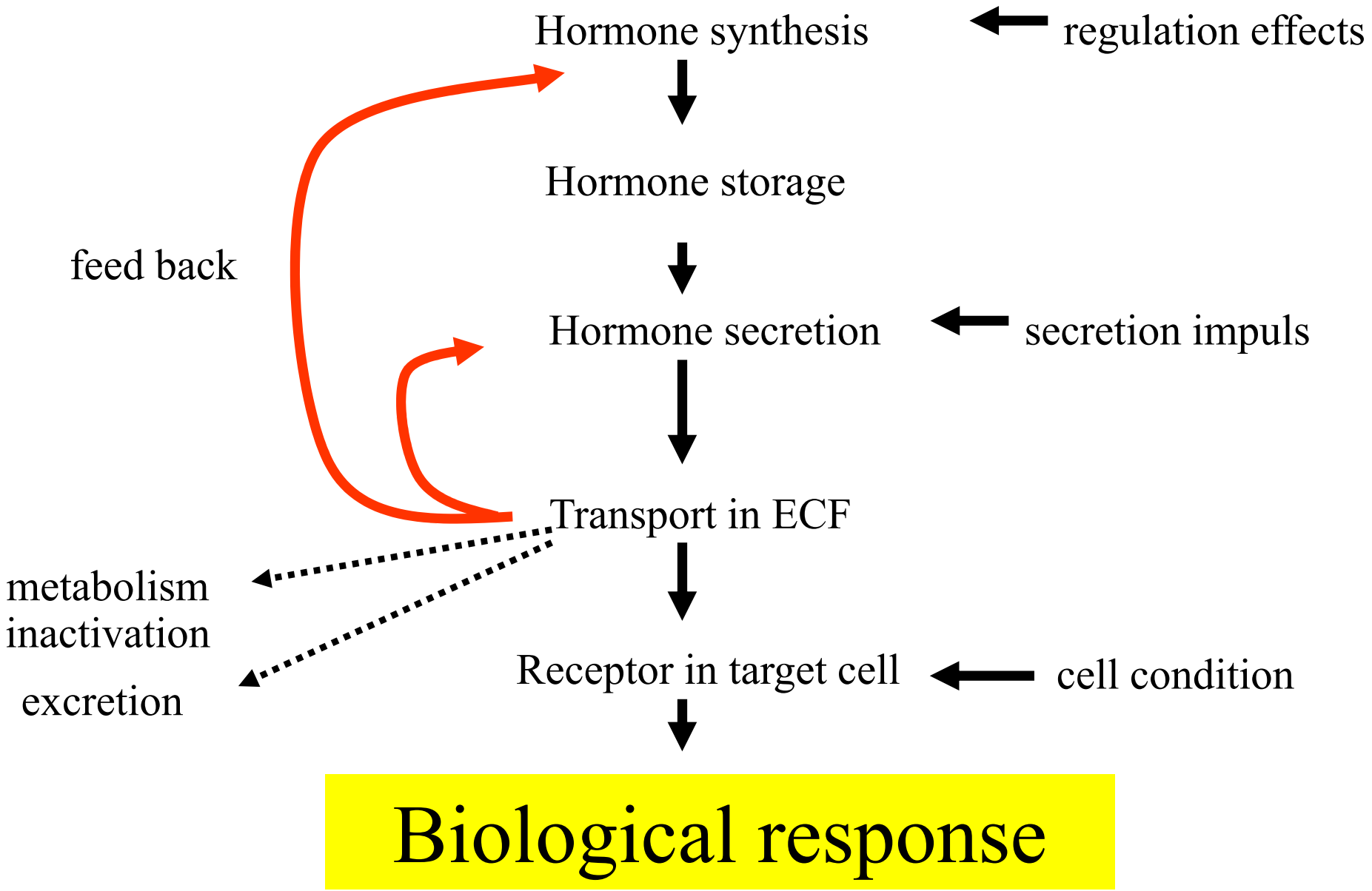
Amplification of signal:

1 signal molecule



10 000-100 000 molecules of second messenger

Factors involved in biological action of hormones



Q. 6

A. 6 Examples of second messengers

- Hydrophilic – cAMP, IP_3
- Lipophilic – diacylglycerol (DAG)
- Inorganic – Ca^{2+}, NO

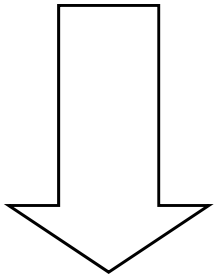
Q. 9

2nd messenger	Inactivation
cAMP	
IP_3	
Ca^{2+}	
NO	

A. 9

2nd messenger	Inactivation
cAMP	$cAMP + H_2O \rightarrow AMP$
IP_3	$IP_3 + H_2O \rightarrow IP_2 + P_i$
Ca^{2+}	$\downarrow\downarrow [Ca^{2+}]$ in cytosol
NO	oxidation to nitrate ion (NO_3^-)

Two types of receptors: membrane and intracellular



Three types of membrane receptors

Ion channels
in synapses, activated by neurotransmitters, very quick response

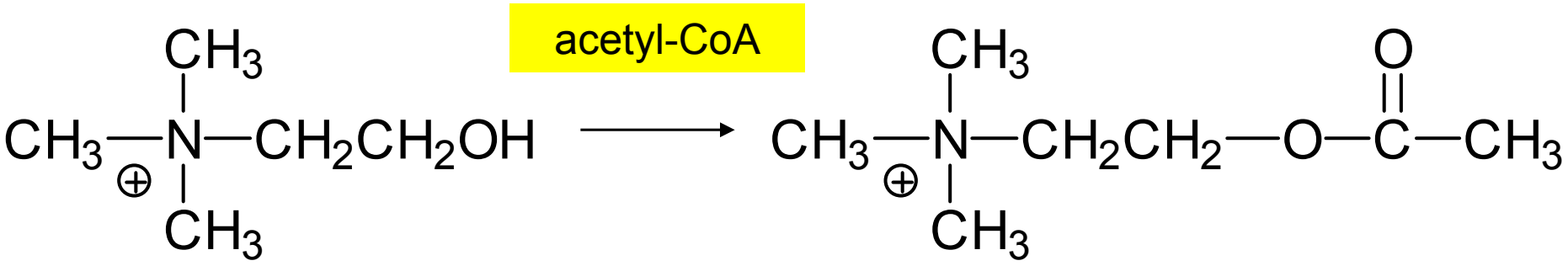
Receptors activating G-proteins
stimulate or inhibit adenylate cyclase /phospholipase C

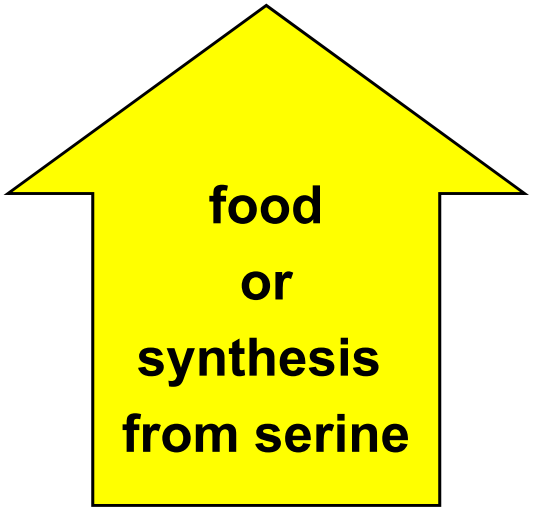
Receptors with enzyme activity
guanylate cyclase - atrial natriuretic factors
tyrosine kinase - insulin

Q. 11

Acetylcholine formation / inactivation

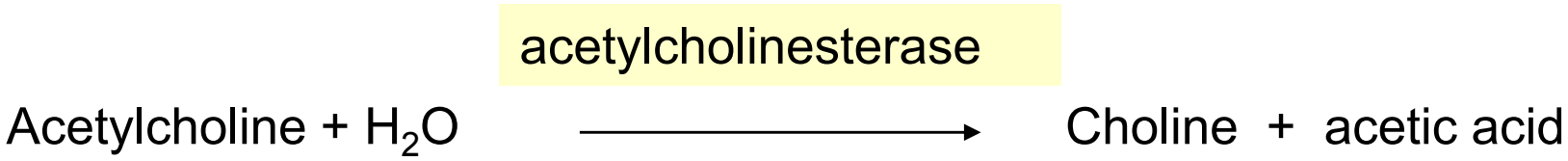
Acetylcholine formation





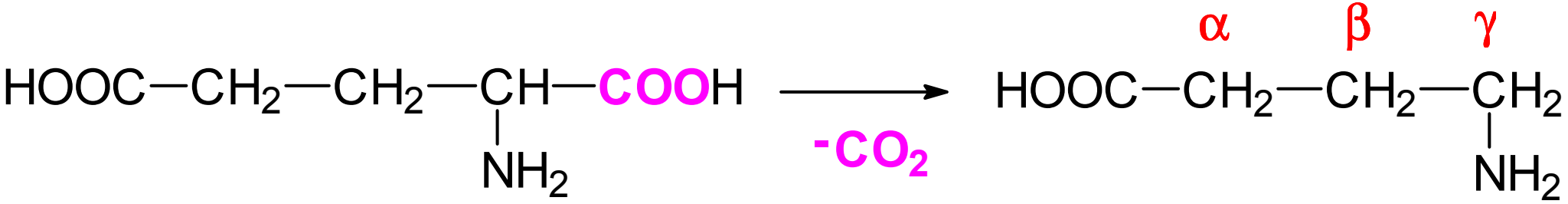
Inactivation ?

Acetylcholine inactivation



GABA formation / inactivation

GABA formation

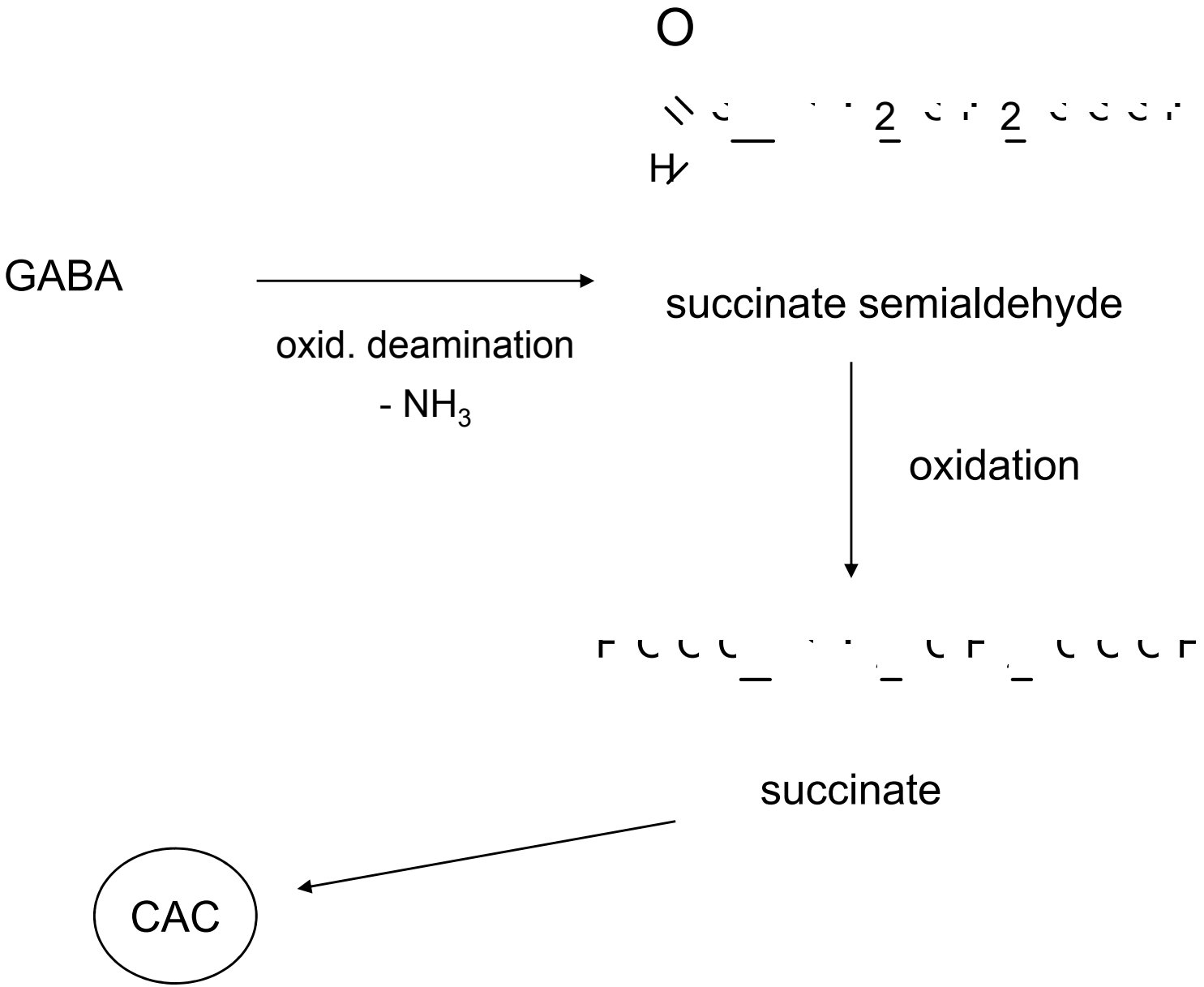


glutamate

GABA
gama-aminobutyric acid

Inactivation ?

GABA inactivation



Q. 12 + 13

A. 12 + 13

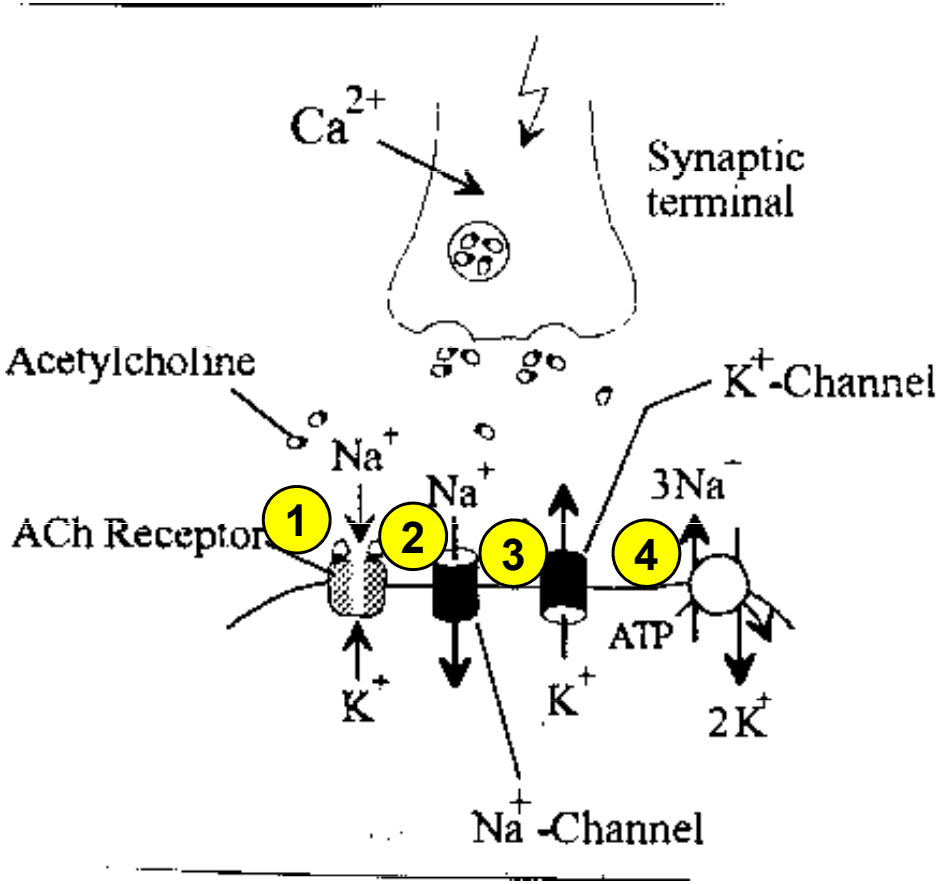
- **Excitatory** neurotransmiters open **cationic** channels ⇒ **depolarization** (more positive membrane potential)

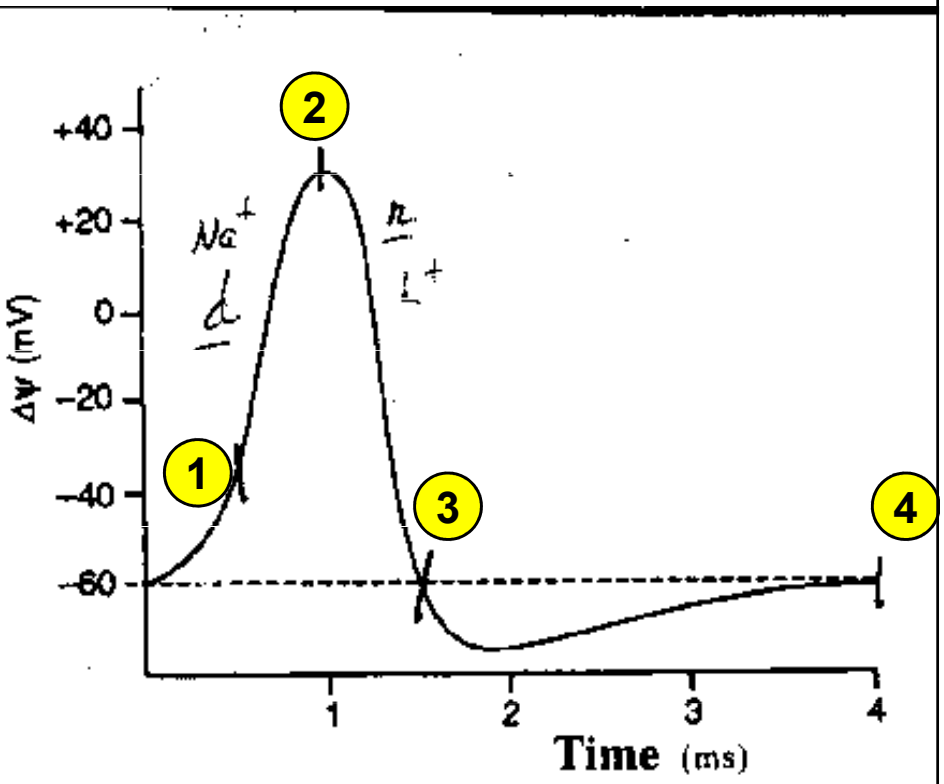
- **Inhibitory** neurotransmiters open **anionic** channels ⇒ **hyperpolarization** (more negative potential)

Nicotinic acetylcholine receptor

- transmembrane protein = channel for Na^+ and K^+
- heteropentamer ($\alpha_2\beta\gamma\delta$)
- α-subunits have two binding sites for acetylcholine (ACH)
- nicotine (= xenobiotic) is agonist of this receptor

Q. 15 – Four events on postsynaptic membrane and corresponding changes of membrane potential





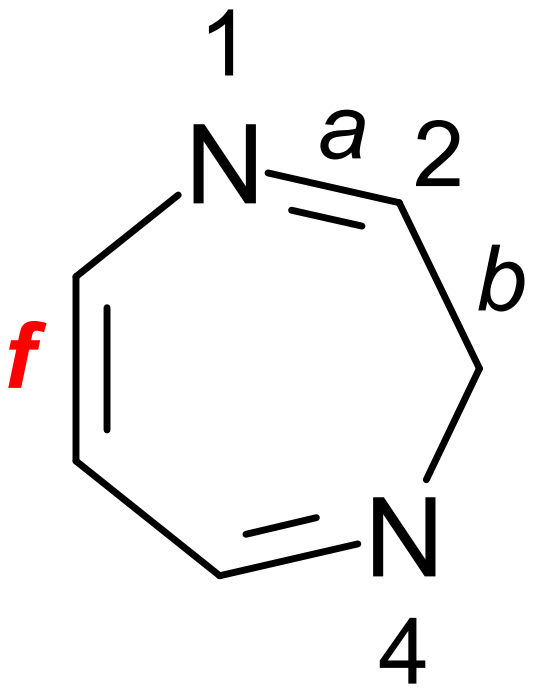
A. 15 Four events on postsynaptic membrane

1. ACH binds to receptor ⇒ channel opens ⇒ influx of Na^+ and efflux of K^+ ⇒ membr. potential changes (-60 → -40 mV)
2. partial depolarization of membrane opens voltage-dependent Na^+-channel ⇒ further influx of Na^+ ⇒ **depolarization** of postsyn. membrane (→ +20 mV)
3. this depolarization opens K^+-channel (volt. dep.) ⇒ efflux of K^+ ⇒ membrane potential returns to normal value (-60 mV) = **repolarization**
4. Na^+,K^+-ATPase gets ion distribution to normal state (Na^+ ⇒ OUT, K^+ ⇒ IN)

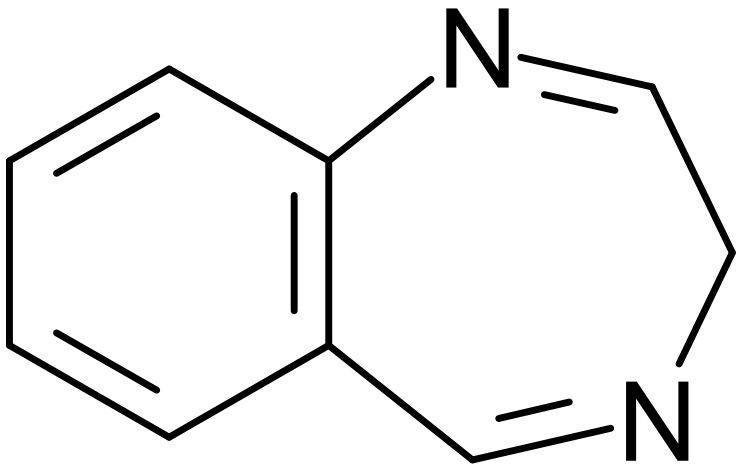
GABA receptor

- channel for chloride ion (Cl^-)
- has the binding site for GABA ⇒ channel opens ⇒ Cl^- ions get into cell ⇒ **hyperpolarization** (→ -80 mV) ⇒ decrease of excitability
- **benzodiazepines** and **barbiturates** (synthetic substances) have similar effects like GABA, they are used as anxiolytics and/or sedatives
- **endozepines** – endogenous peptides have opposite effects, close the channel (are responsible for anxiety feelings)

Diazepine

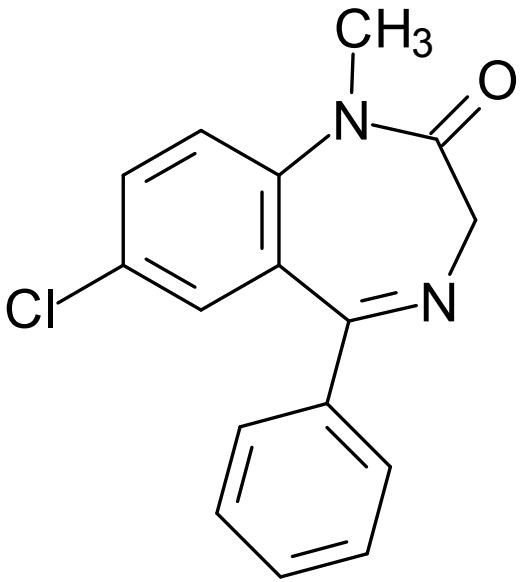


Benzo[*f*]diazepine



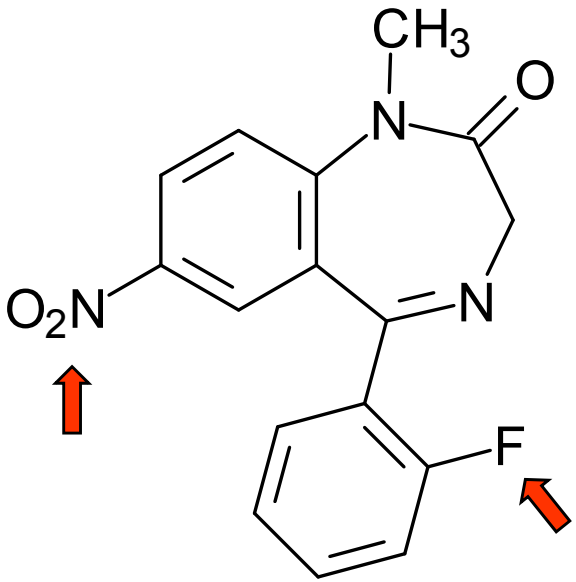
diazepine is a seven-membered unsaturated heterocycle with two nitrogen heteroatoms in the positions 1,4

Benzodiazepines



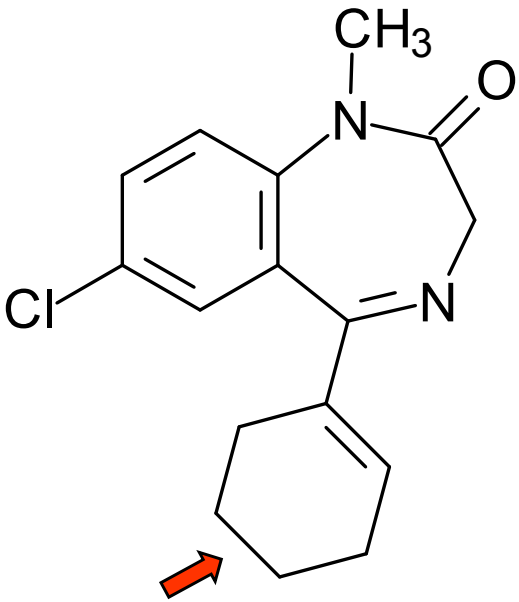
diazepam

anxiolytic / sedative



flunitrazepam

hypnotic

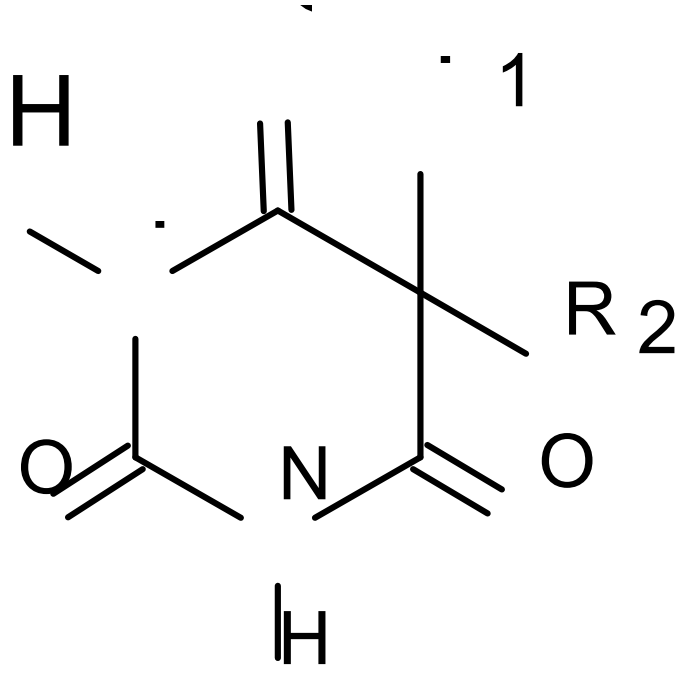


tetrazepam

myorelaxant

structural modifications lead to different pharmacological effects

Barbiturates



allobarbital:

$R_1 = R_2 = -CH_2-CH=CH_2$

derivates of 2,4,6-trioxoperhydropyrimidine

Receptors with adenylate cyclase system

(Scheme on p. 4)

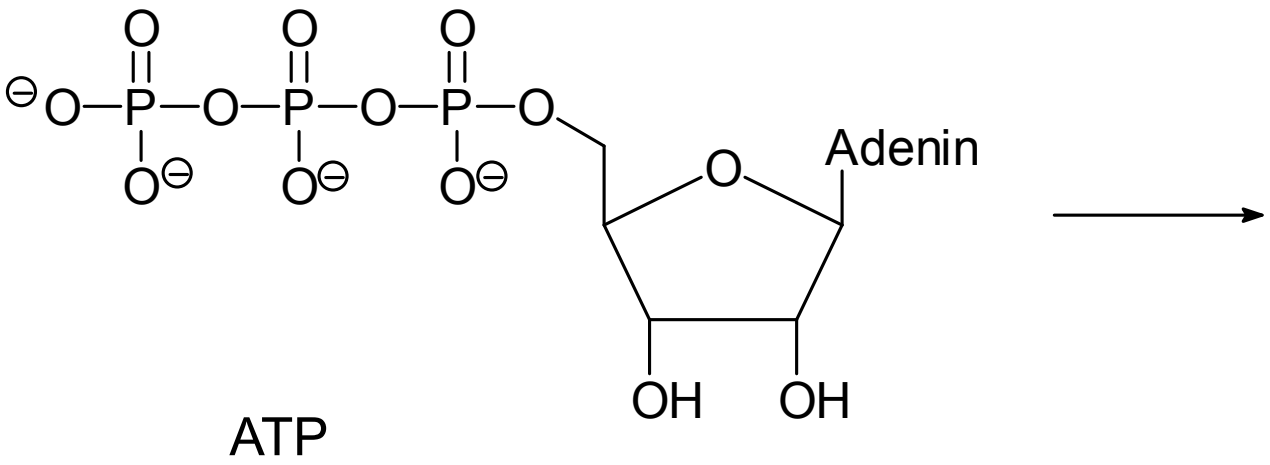
Describe the pathway of signal

Signal molecule binds to receptor
Receptor activates G-protein
Activated G-protein (α-unit with GTP) activates effector = adenylate cyclase
Adenylate cyclase produces the second messenger = cAMP
Four molecules of cAMP bind to two R(regulatory) units of protein kinase A
Two C(catalytic) units of protein kinase A catalyze protein phosphorylation
Phosphorylated protein = biological response to signal molecule
cAMP is inactivated by phosphodiesterase: $cAMP + H_2O \rightarrow AMP$

G-Protein linked receptors

- extracellular part of receptor has a binding site for hormone
- intracellular part has a binding site for G-protein
- G-proteins are heterotrimers (αβγ)
- in resting state, α-unit has GDP attached
- after binding hormone ⇒ (α-GDP)βγ makes complex with receptor ⇒ GDP is phosphorylated to GTP
- activated G-trimer dissociates: (α-GTP)βγ → α-GTP + βγ
- α-GTP interacts with **effector** (enzyme) ⇒ activated/inhibited enzyme ⇒ **second messenger** (↑ or ↓)

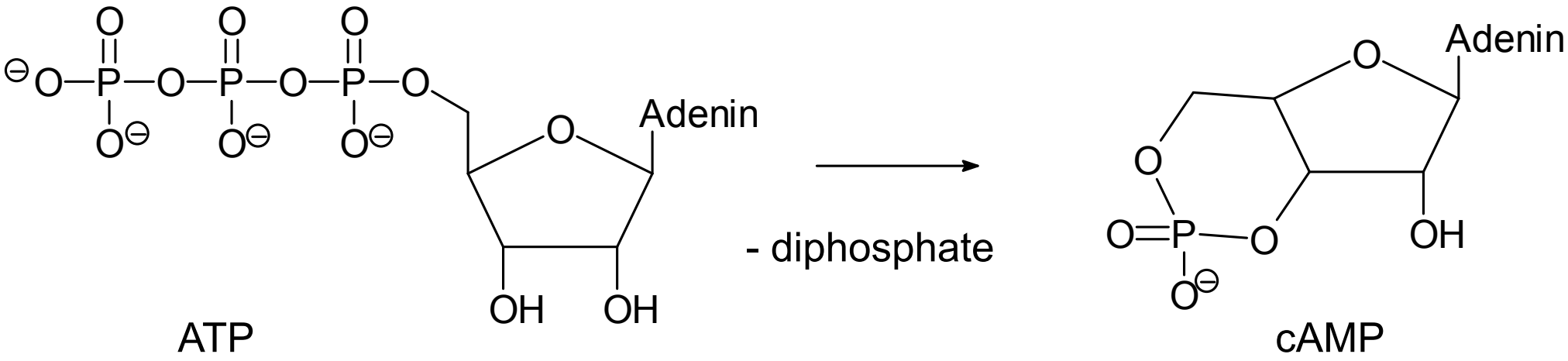
What reaction is catalyzed by adenylate (adenylyl) cyclase?



ATP

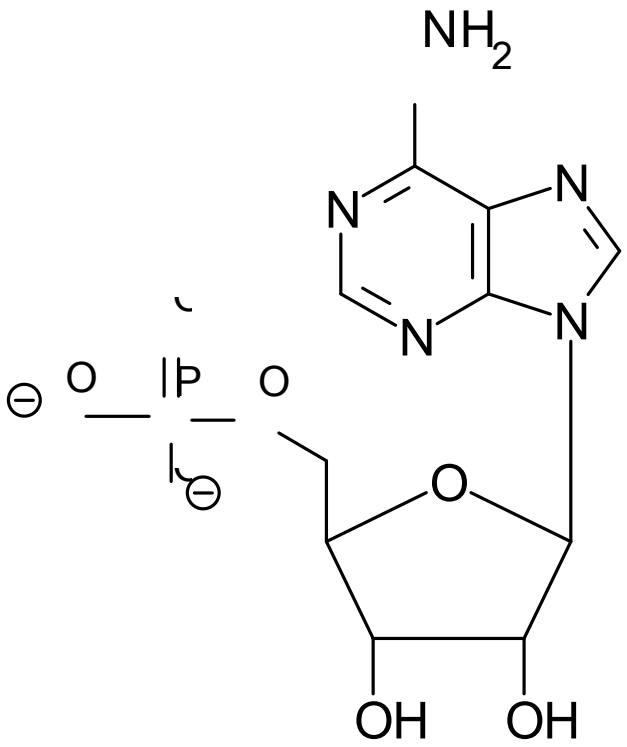
What is adenylyl ?

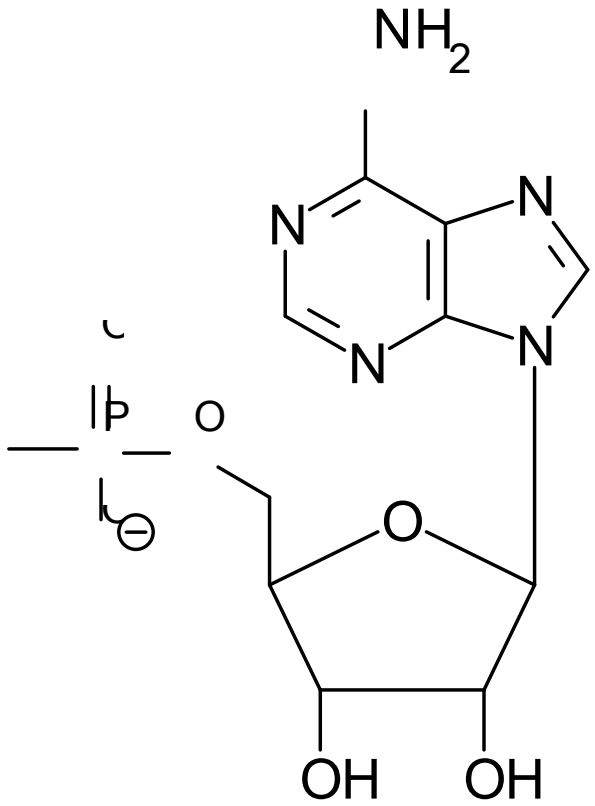
Adenylyl cyclase reaction



cAMP = **c**yclic 3',5'-**a**denosine **m**ono**p**hosphate

AMP is called also adenylic acid





adenyl<u>ate</u> is anion

adenyl<u>yl</u> is acyl

Adenylate (adenylyl) cyclase (AC)

- membrane bound receptor
- catalyzes reaction: ATP $\rightarrow$ cAMP + PP_i
- G_s protein stimulates AC $\Rightarrow$ conc. of cAMP $\uparrow$
- G_i protein inhibits AC $\Rightarrow$ conc. of cAMP $\downarrow$

Q. 19

A. 19

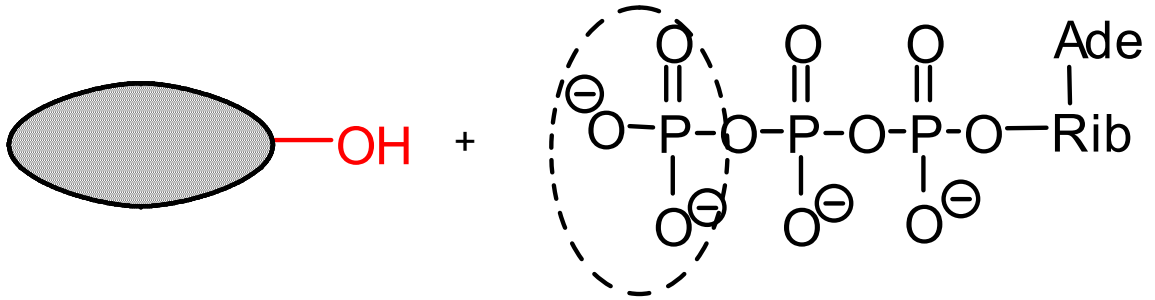
- **Protein kinase** – phosphorylation by ATP

  Protein-OH + ATP $\rightarrow$ Protein-O-P + ADP

- **Protein phosphatase** – hydrolysis of phosphate ester

  Protein-O-P + H_2O $\rightarrow$ Protein-O-H + P_i

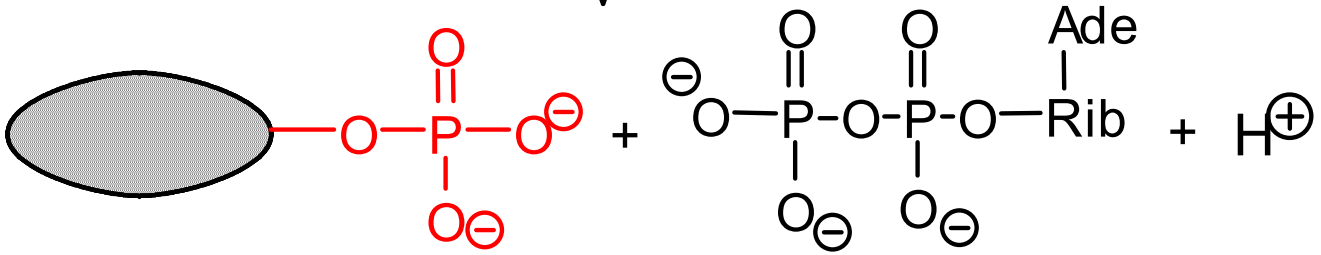
General scheme of phosphorylation



substrate (protein) ATP (4-)

Which AA are phosphorylated?

protein kinase



phosphorylated protein (2-) ADP (3-)

Three amino acids have a hydroxyl group in the side chain

- **Serine** (3 C, primary alcohol hydroxyl)
- **Threonine** (4 C, secondary alcohol hydroxyl)
- **Tyrosine** (3 + 6 C, phenolic hydroxyl)

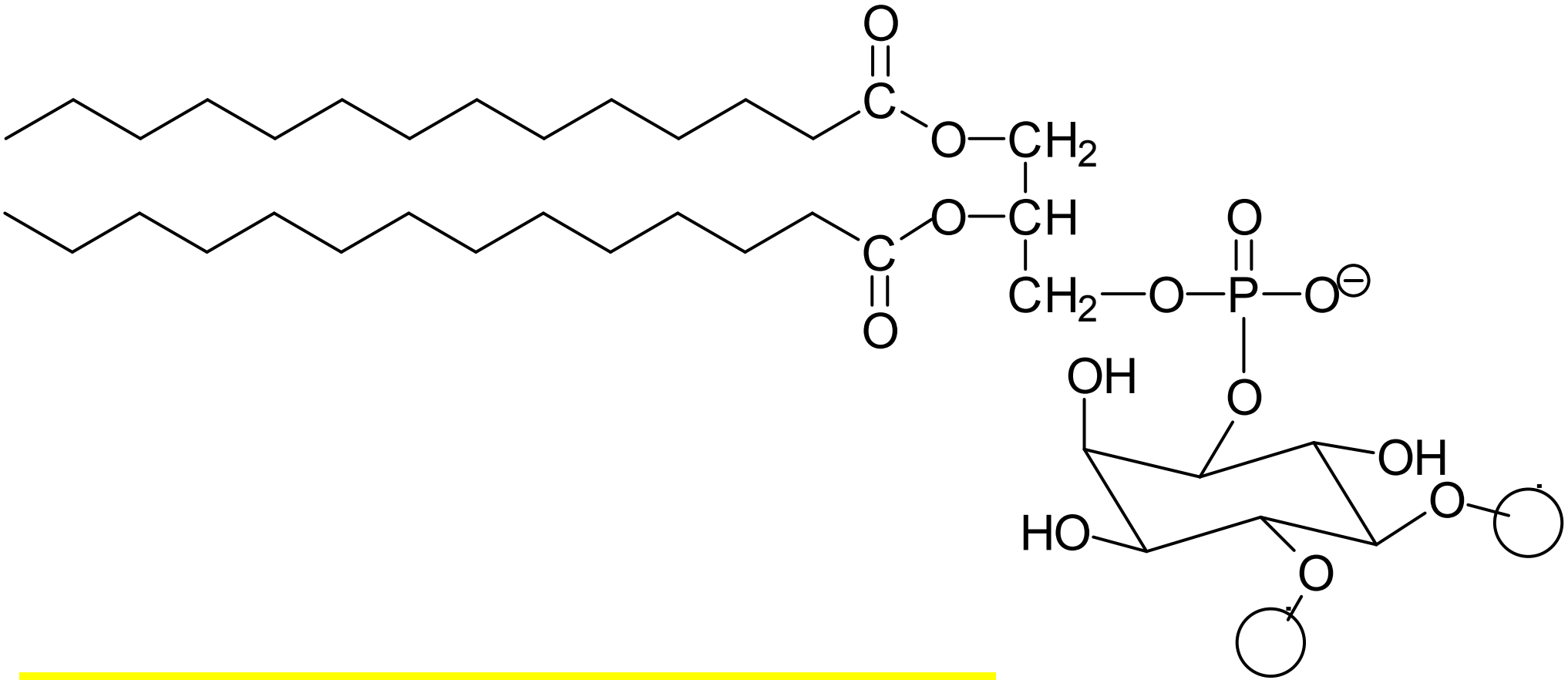
write structural formulas

Phosphatidyl inositol system
(Scheme on p. 4)

Describe the pathway of signal

Signal molecule binds to receptor
Receptor activates G-protein
Activated G-protein (α-unit with GTP) activates effector = phospholipase C
Phospholipase C catalyzes the hydrolysis of PIP_2 and produces two second messengers: DG + IP_3
DG activates protein kinase C
IP_3 opens calcium channel in ER $\Rightarrow$ Ca^{2+} concentration in cytoplasm increases and Ca^{2+} ions are associated with calmodulin (CM)
Ca^{2+}-CM complex activates caldomulin-dependent kinases
Phosphorylated intracellular proteins = biological response to signal molecule

The structure of PIP_2

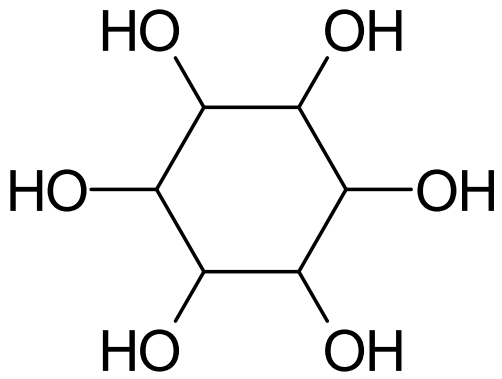


describe the structure

Q.

What is the source of inositol in human body?

The origin of inositol



Exogenous source: any plant food (inositol hexaphosphate = phytic acid)

Endogenous source: glucose-6-P (side path of metabolism)

Main types of G-proteins

G-protein	Effector	Intracell. signal	Signal change
G_s	adenylate cyclase	cAMP	
G_i	adenylate cyclase	cAMP	
G_q	phospholipase C	DG + IP_3 + Ca^{2+}	

Q. 20

A. 20

Enzyme	Activator
Protein kinase A	cAMP
Protein kinase C	DAG and Ca^{2+}
Protein kinase G	cGMP

Receptors with guanylate cyclase

- second messenger = cGMP
- activates protein kinase G (PKG)

Q. 23

A. 23

- By the action of NO
- In smooth muscle cells (chapter 14)

Insulin receptor

- has four subunits ($\alpha_2\beta_2$)
- extracellular α-units bind insulin
- intracellular β-units have **tyrosine kinase activity** ⇒ phosphorylation of tyrosine phenolic hydroxyl of intracellular proteins including **insulin receptor itself** (autophosphorylation) ⇒ cascade of further events ⇒ biological response

Intracellular receptors:

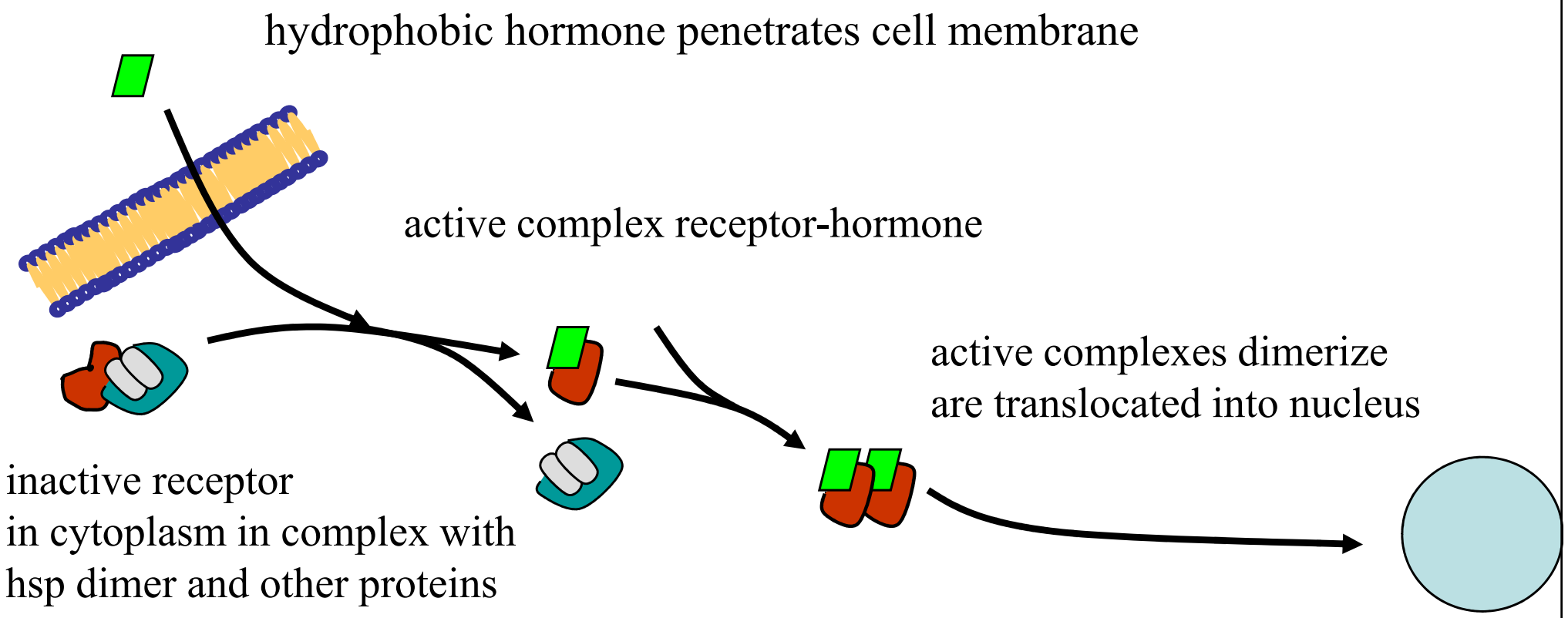
- cytoplasmatic
- nuclear

for non-polar signal molecules

steroids, iodothyronines, calcitriol, retinoids

Intracellular receptors

- make complex with hormone
- activate or repress the transcription of genes



Q. 30

A. 30

- HRE = hormone response elements
- regulatory DNA sequences, bind complexes of hydrophobic hormones with their intracellular receptors
- They act as enhancers or silencers
- Located at the beginning of regulatory DNA region
- 5’-----HRE ---Promoter-------3’

Steroid and thyroid hormones

- insoluble in water ⇒ in ECF are transported in complex with transport proteins
- hormone themselves diffuse easily across cell membrane
- they are bound to cytoplasmatic or nuclear receptors
- in nucleus, the hormone-receptor complex binds to **HRE** (hormone response element) in regulation sequence of DNA
- this leads to induction of mRNA synthesis = transcription of gene

Cholinergic synapses

- neurotransmitter: **acetylcholine**
- two types of receptors
- **nicotinic rec.** (ion channel) – e.g. neuromuscular junction
- **muscarinic rec.** (G-prot.) – e.g. smooth muscles

Cholinergic receptors

Feature	Nicotinic receptor	Muscarinic receptors	
		M_1, M_3	M_2
Receptor type	Ion channel	G_q	G_i
2nd messenger	$\Delta\psi$*	DAG, IP_3 ↑	cAMP ↓
Antagonist	tubocurarine	atropine	atropine
Locations	neuromuscular juct.	brain	myocard

* the change of membrane potential

Q. 31

A. 31

- Presynaptic membrane contains voltage-gated calcium channels
- influx of Ca^{2+} activates protein kinase which phosphorylates synapsin and other proteins
- this triggers the fusion of presynaptic vesicles (contaning acetylcholine) with cell membrane and exocytosis of acetylcholine
- acetylcholine is liberated into synapse

Q. 32

A. 32

acetylcholine + H_2O $\rightarrow$ choline + acetic acid

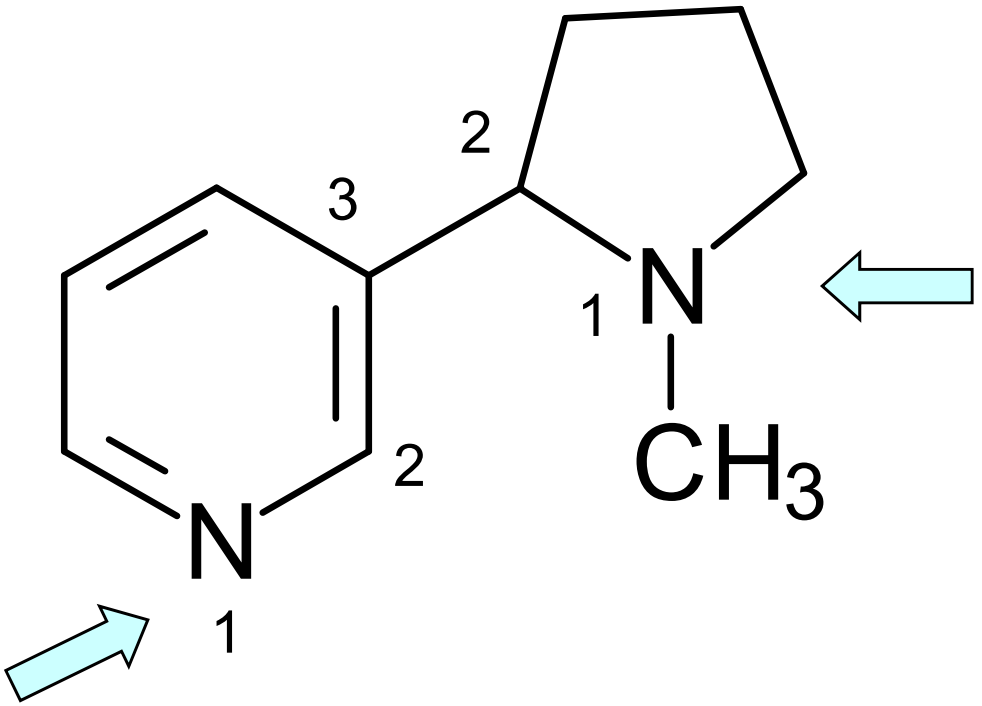
hydrolysis of ester

Q. 33

What is nicotine?

Nicotine is the main alkaloid of tobacco

(*Nicotiana tabacum*)



more basic

pK_B = 6.2

less basic

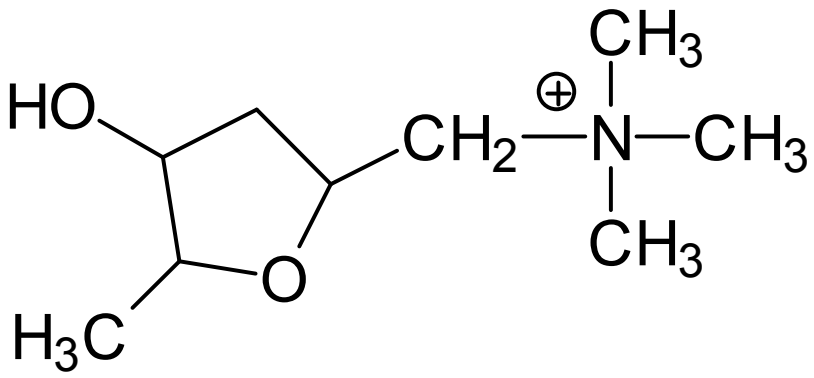
pK_B = 11

3-(1-methylpyrrolidine-2-yl)pyridine

Q. 34

What is muscarine?

A. 34 Muscarine is quaternary ammonium alkaloid in some mushrooms



muscarine

tetrahydro-4-hydroxy-N,N,N,5-tetramethyl-2-furanmethanammonium

Amanita muscaria (fly agaric)

Q. 35

A. 35

- nicotine binds to **acetylcholine nicotinic receptors** in the brain and other tissues including cells of adrenal medulla
- activation of nicotinic receptor ⇒ change of membrane potential ⇒ exocytosis of vesicles with adrenaline ⇒ secretion of adrenaline ⇒ **metabolic processes typical for acute stress** (see Seminar No. 6)

Other effects of nicotine:

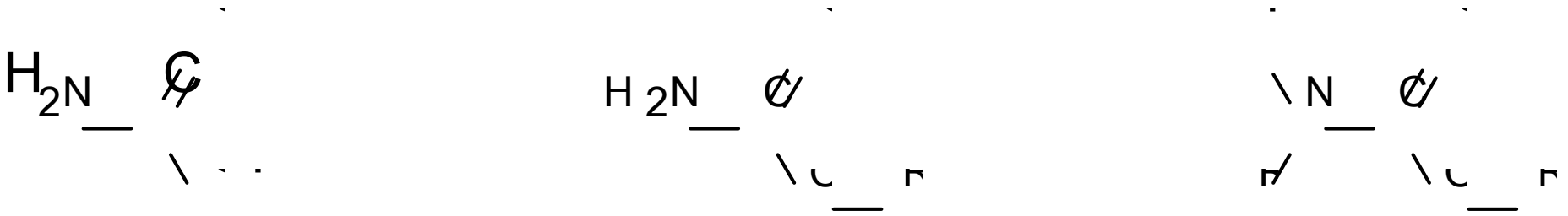
- increases the secretion of saliva and gastric juice
- increase intestinal peristalsis
- vasoconstriction

Inhibitors of acetylcholinesterase

- **Reversible** – carbamates (*N*-substituted esters of carbamic acid), e.g. fysostigmine, neostigmine
- they are used to improve muscle tone in people with myasthenia gravis and routinely in anesthesia at the end of an operation to reverse the effects of non-depolarising muscle relaxants. It can also be used for urinary retention resulting from general anaesthetia

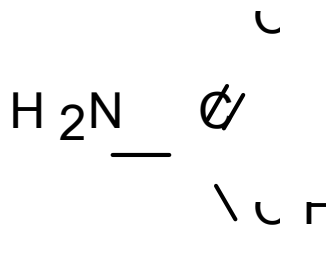
- **Irreversible** – organophosphates, very toxic compounds (chemical warfare agents)

Carbamates – General formulas



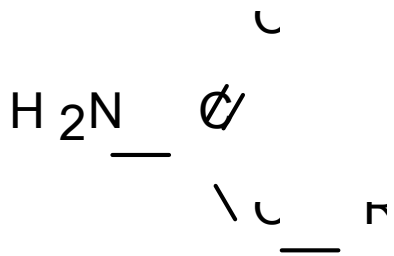
Complete the names of the structures

Carbamates – General formulas



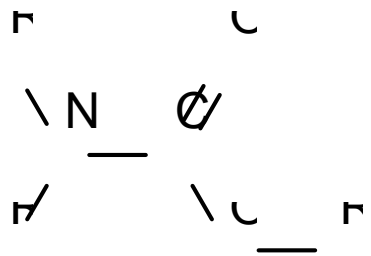
carbamic acid

(hypothetic compound)



alkyl carbamate

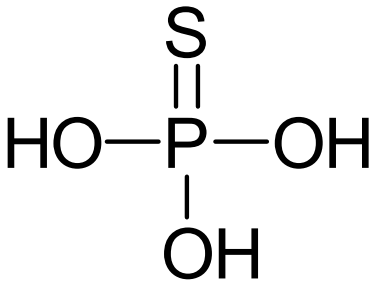
(ester)



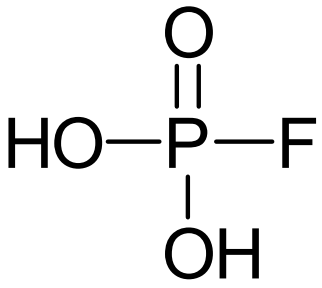
N-disubstituted

alkyl carbamate

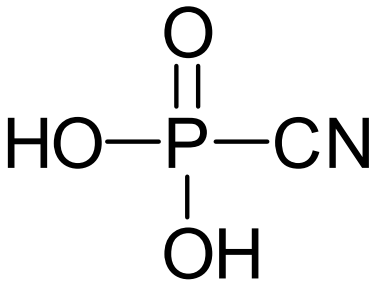
Organophosphates



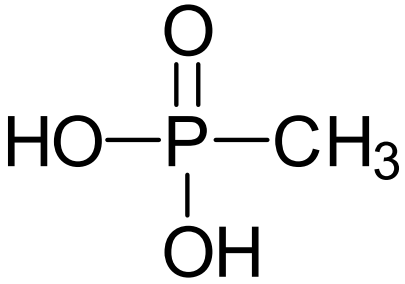
thiophosphoric acid



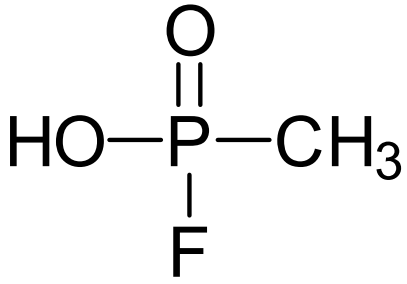
fluorophosphoric acid



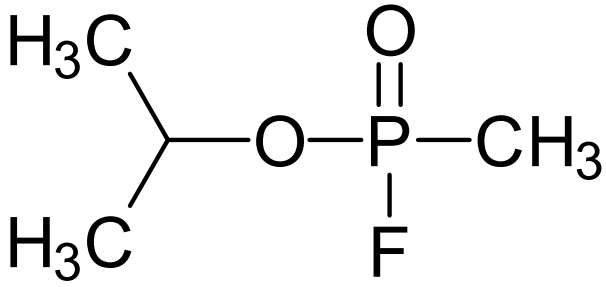
cyanophosphoric acid



methylphosphonic acid

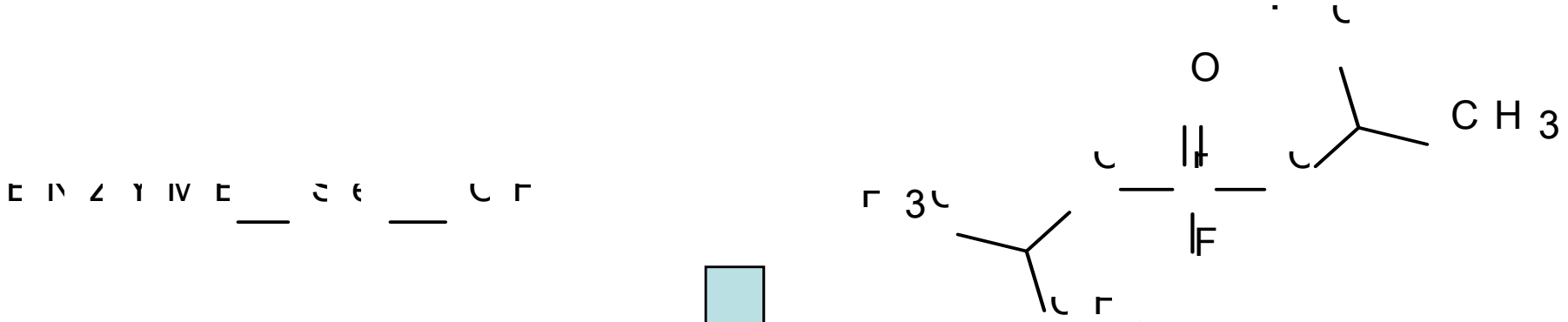


methylfluorophosphonic acid

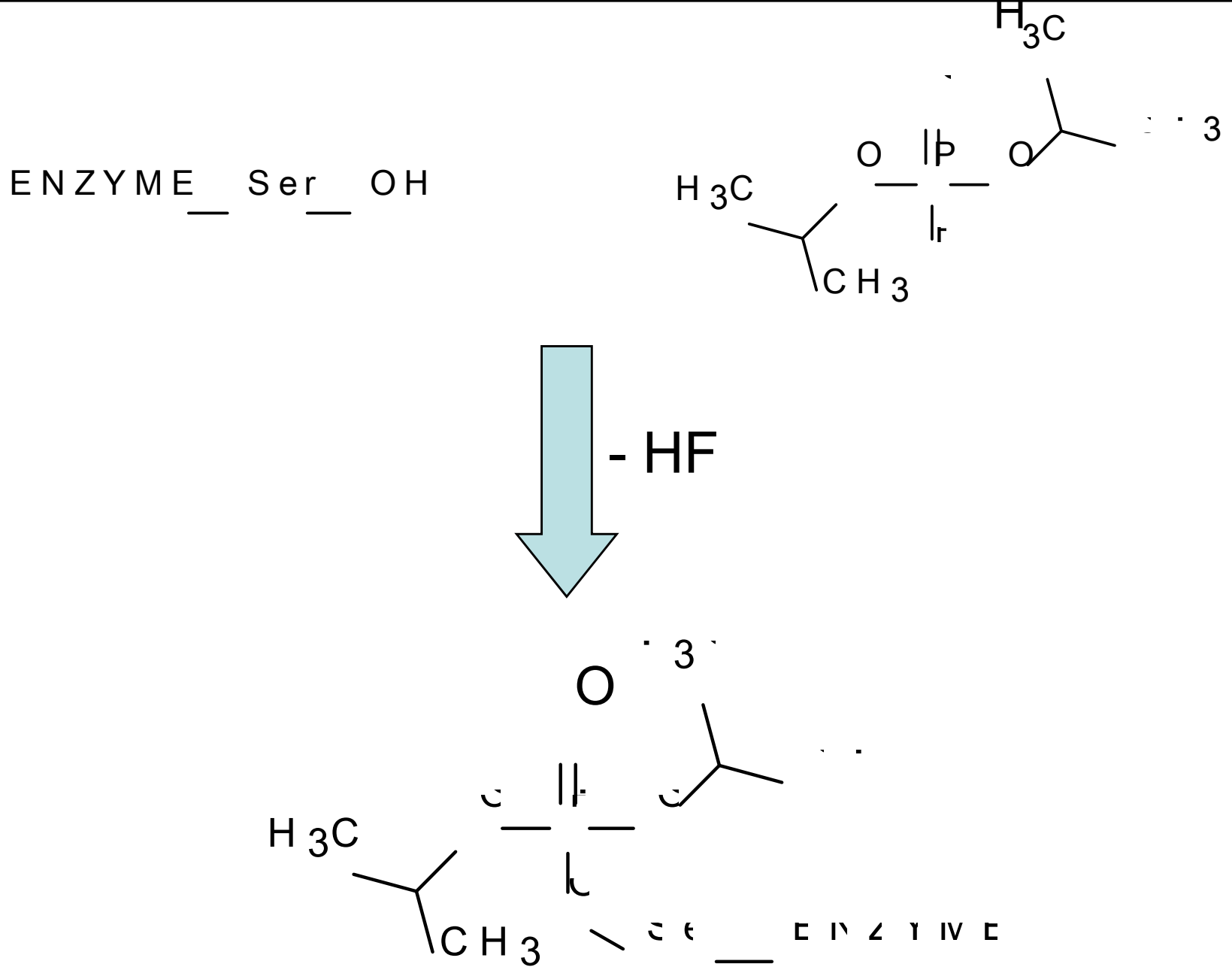


sarin

Q. 36



irreversible phosphorylation of enzyme



inactivated enzyme

Adrenergic synapses

- neurotransmitter: **noradrenaline**
- four types of receptors: α_1, α_2, β_1, β_2
- all of them are G-protein linked receptors
- occur in various cells and tissues

Adrenergic receptors

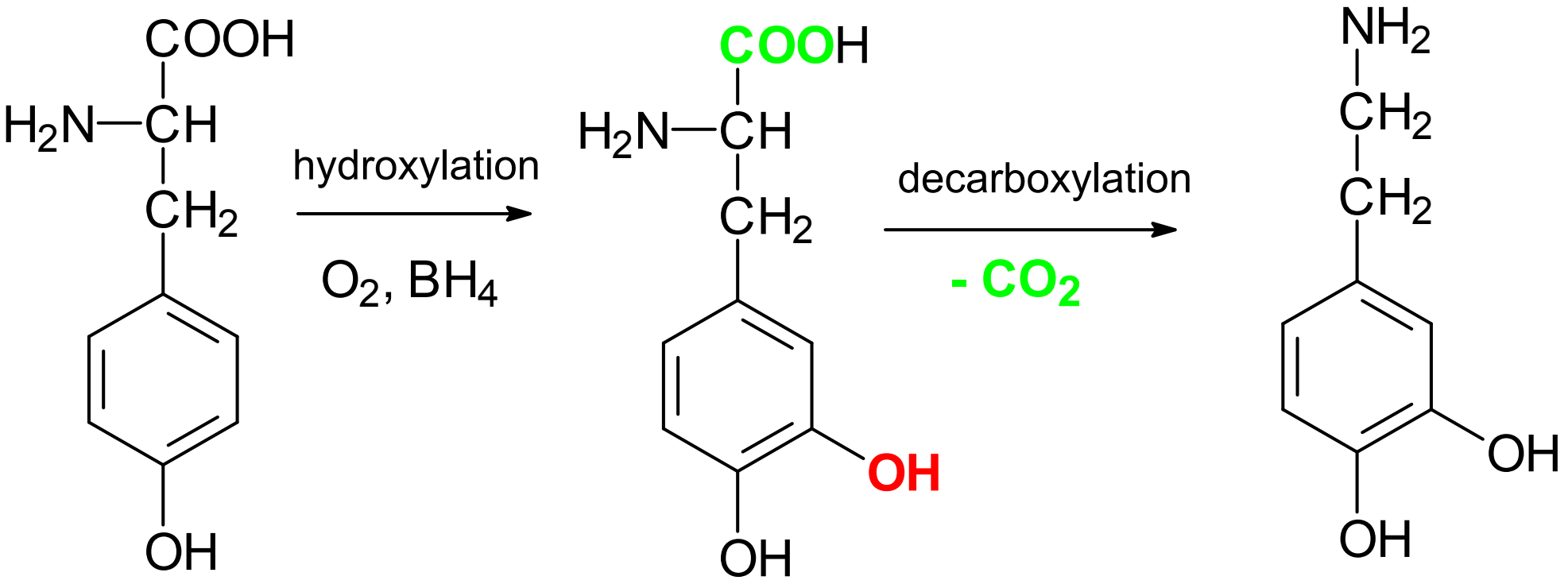
Feature	α_1	α_2	β_1	β_2
G-protein	G_q	G_i	G_s	G_s
2nd messenger	DG, IP_3	↓ cAMP	↑ cAMP	↑ cAMP
Occurence*	smooth muscle	brain	myocard	smooth m.

* Example of occurence

Q. 37

Describe the synthesis of noradrenaline.

The formation of DOPA and dopamine



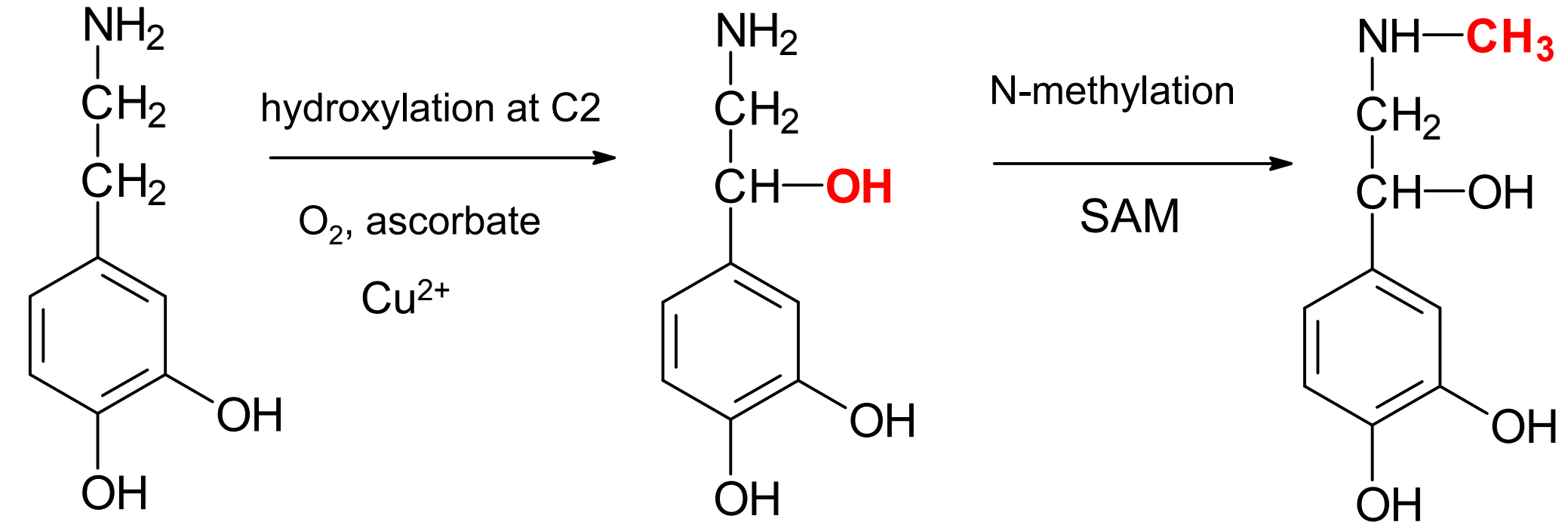
tyrosine

DOPA

3,4-dihydroxyphenylalanine

dopamine

Noradrenaline and adrenaline



dopamine noradrenaline adrenaline

prefix *nor-* means *N*-demethyl