Receptors of Hormones & Neurotransmitters

Seminar No. 8

- Chapter 22 -

Q.

What are general features of signal molecule?

(see scheme on p. 130)

Signal molecule (e.g. hormone)

- carries information into cell
- has extremely low concentration in blood (10^{-9} – 10^{-15} mol/l)
- specifically binds to receptor
- signal molecule is quickly inactivated

- **agonist** – (external) molecule which acts the same way as physiological signal molecule
- **antagonist** – (external) molecule which blocks receptor
  $\Rightarrow$ no biological response

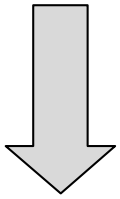
Q.

What is the amplification of signal?

Amplification of signal

= to make it more powerful

1 molecule of hormone



~ **100-1000** molecules of second messenger

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

Two classes of hormones

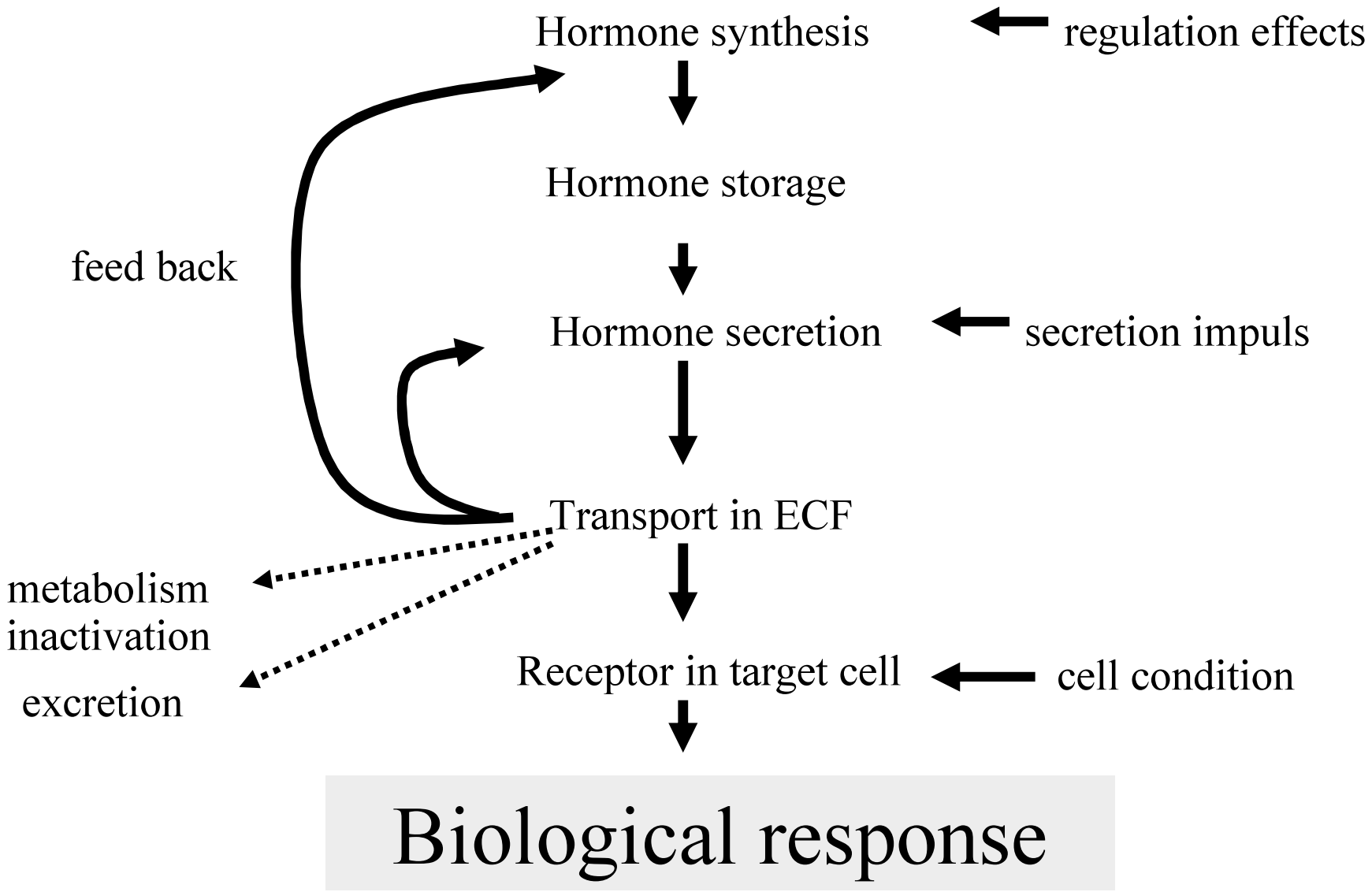
Feature	Lipophilic hormone	Hydrophilic hormone
Chemical type	steroids, iodothyronines, calcitriol, retinoids	aminoacid derivatives, polypeptides, proteins
Water solubility	no	yes
Transport protein*	yes	no
Plasma half-time	long (hours, days)	short (minutes)
Membrane penetration	yes	no
Receptor	intracellular	in cell membrane**
Second messenger	hormone-receptor complex	cAMP, Ca^{2+} ....

* in blood ** hormone acts without entering the cell

Concentration of hormone in blood generally does not correlate with its biological effects

More factors are involved (transport systems, chemical modifications, activity of receptors etc.)

Factors involved in biological action of hormones



Two principal types of receptors

- membrane receptors
- intracellular receptors

The main types of membrane receptors

Ligand gated ion channels

- in synapses, activated by neurotransmitters, very quick response

Receptors activating G-proteins

- stimulate or inhibit adenylate cyclase /phospholipase C

Receptors with guanylate cyclase activity

- atrial natriuretic factors

Receptors with tyrosine kinase activity

- insulin

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 is agonist of this receptor

Four events on postsynaptic membrane

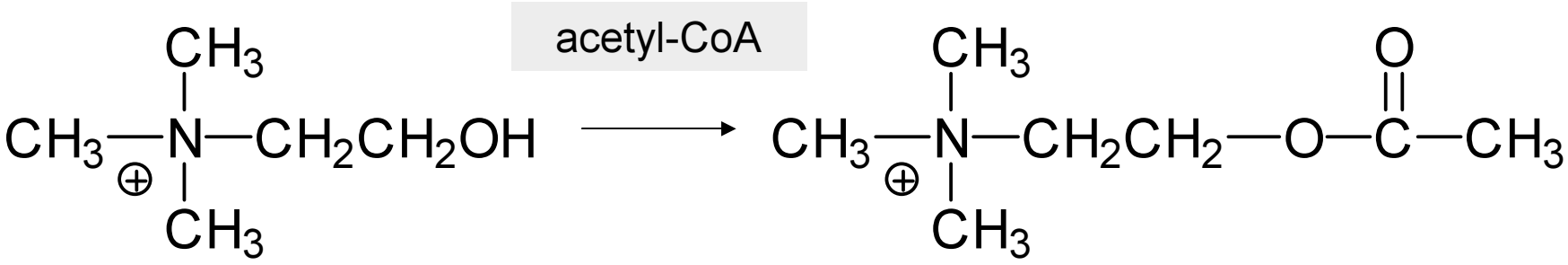
(see the scheme and the graph on p. 131)

1. ACH binds to receptor ⇒ channel opens ⇒ influx of Na^+ and efflux of K^+
2. partial depolarization of membrane (-60 → -40 mV) opens other type of 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)

Q.

Describe the formation of acetylcholine in the body.

Acetylcholine



choline → acetylcholine

food

free choline or phosphatidylcholine

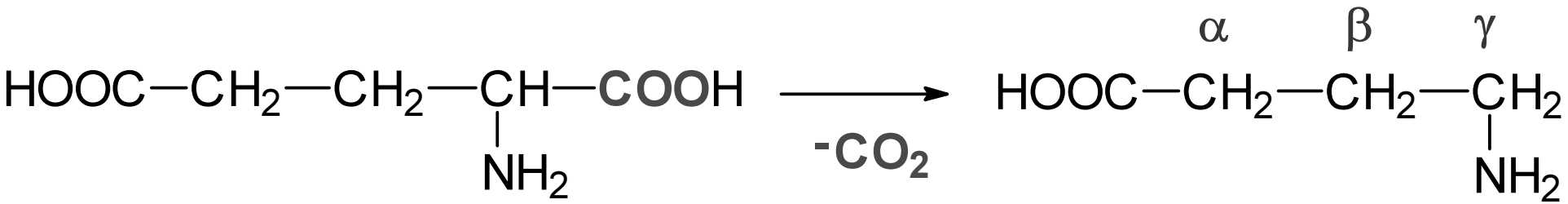
GABA receptor

- channel for chloride ion (Cl^-)
- has the binding site for GABA $\Rightarrow$ channel opens $\Rightarrow$ Cl^- ions get into cell $\Rightarrow$ **hyperpolarization** ($\rightarrow$ -80 mV) $\Rightarrow$ 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)

Q.

Describe the synthesis of GABA.

GABA formation



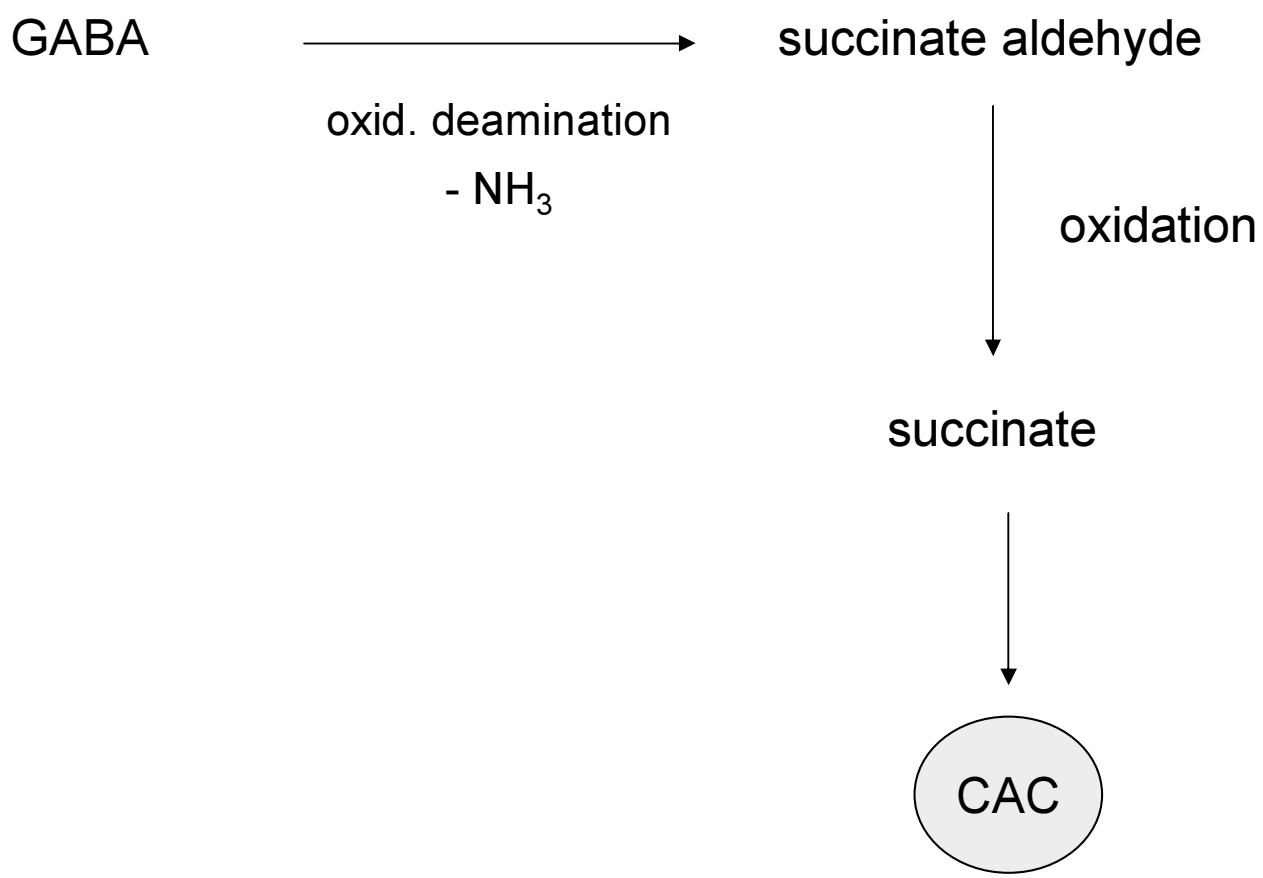
glutamate

GABA
gama-aminobutyric acid

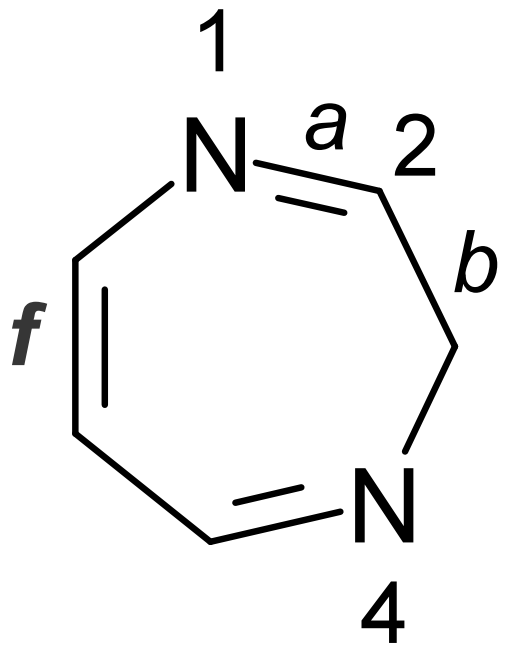
the reaction requires:

enzyme (decarboxylase) + cofactor (pyridoxal phosphate)

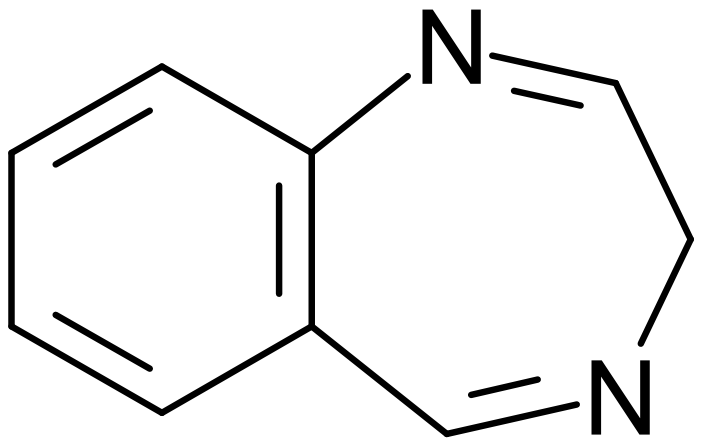
GABA inactivation



Diazepine

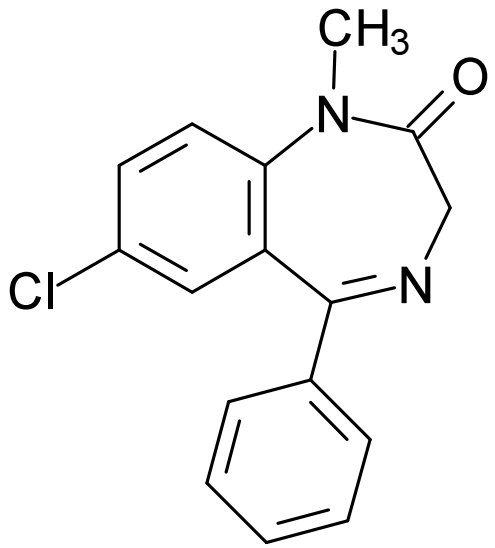


Benzo[*f*]diazepine



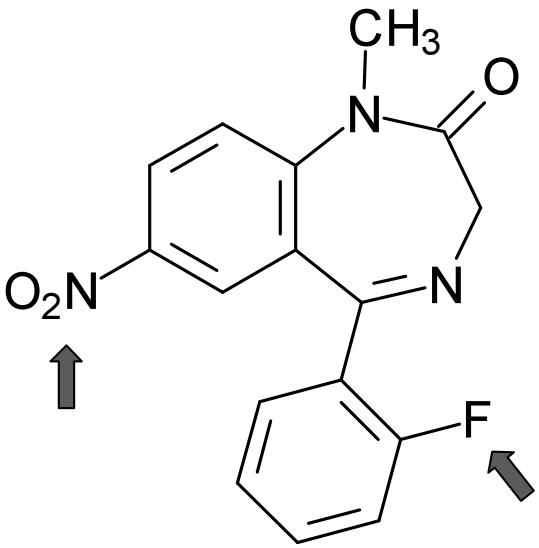
diazepine is a seven-membered unsaturated heterocycle with two nitrogen heteroatoms in the positions 1,4

Benzodiazepines



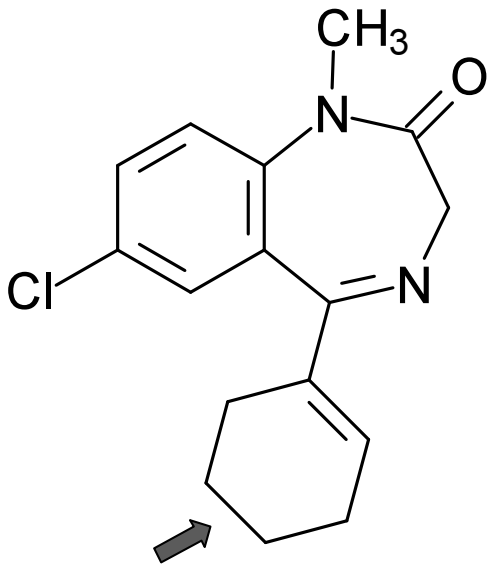
diazepam

anxiolytic / sedative



flunitrazepam

hypnotic

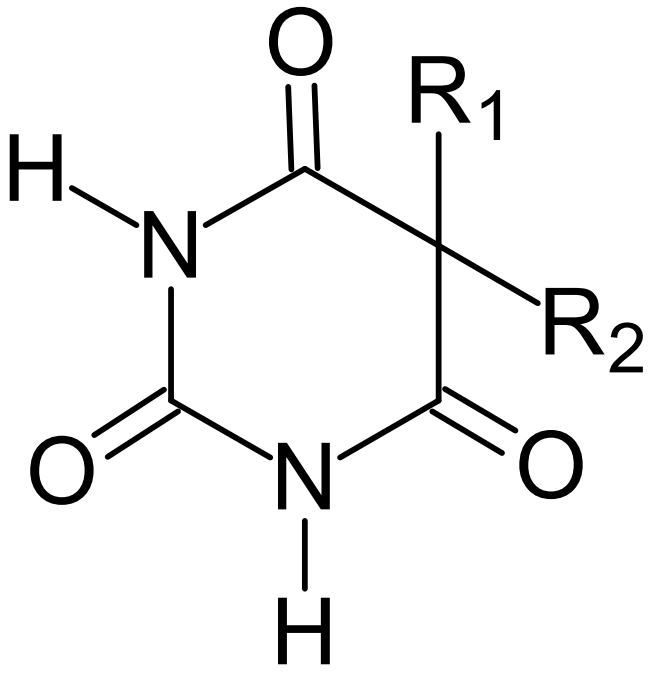


tetrazepam

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

G-Protein linked receptors (scheme, p. 132)

- 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 ↓)

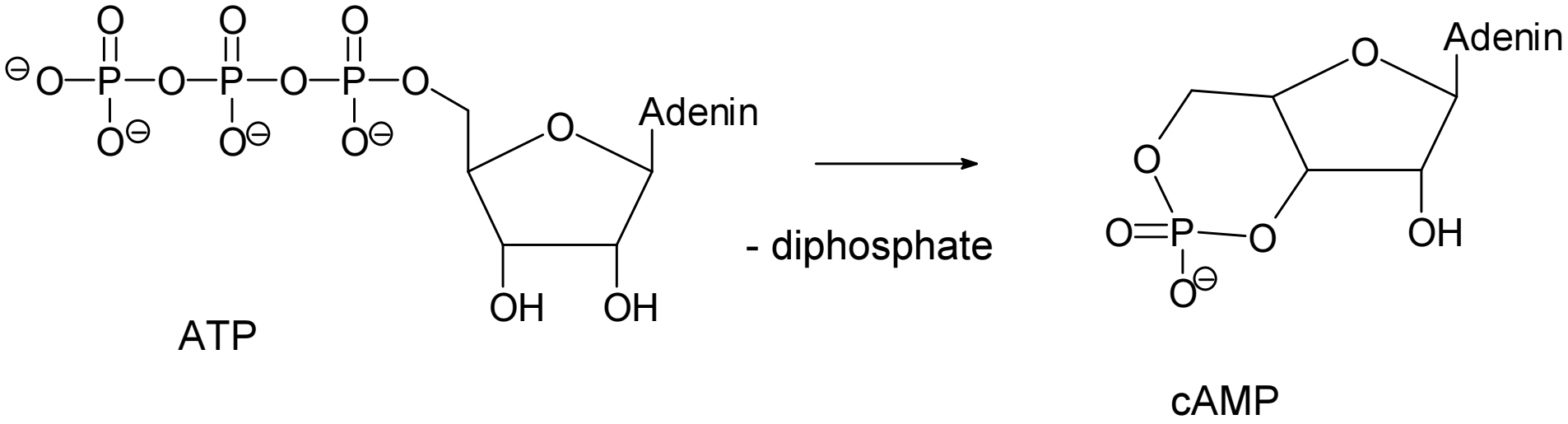
Main types of G-proteins

- G_s (stimulatory)
- G_i (inhibitory)
- G_p (phospholipid)
- and other ...
- **see table on p. 132 !!**

Q.

What reaction is catalyzed by adenylate cyclase?

Adenylate cyclase reaction



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

Adenylate cyclase (AC)

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

Q.

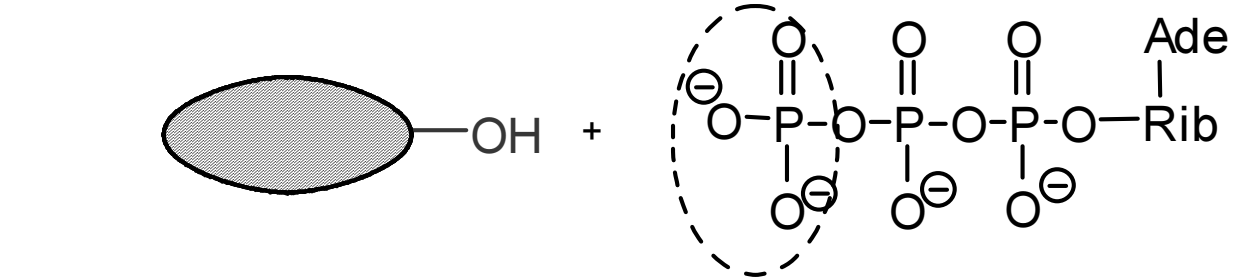
What is the function of cAMP?

cAMP is the second messenger

- cAMP activates protein kinase A $\Rightarrow$ phosphorylation of cell proteins:
- Protein-OH + ATP $\rightarrow$ **Protein-O-P** + ADP

- the second messenger cAMP is quickly inactivated
- cAMP is removed by hydrolysis, catalyzed by phosphodiesterase:
- cAMP + H_2O $\rightarrow$ AMP

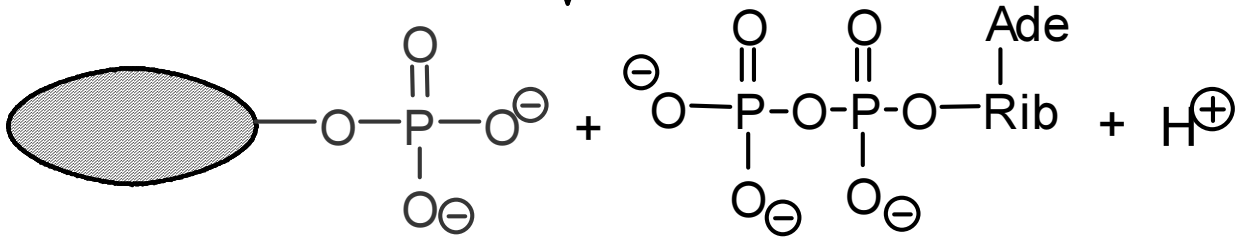
General scheme of phosphorylation



substrate (protein)

ATP (4-)

protein kinase



phosphorylated protein (2-)

ADP (3-)

Q.

Which aminoacids can be phosphorylated?

A.

- AA with a hydroxyl group in a side chain

- serine
- threonine
- tyrosine

write the structural formulas

Q.

What reaction is catalyzed by protein phosphatase?

A.

$$\text{Protein-O-P} + H_2O \rightarrow \text{Protein-O-H} + P_i$$

hydrolysis

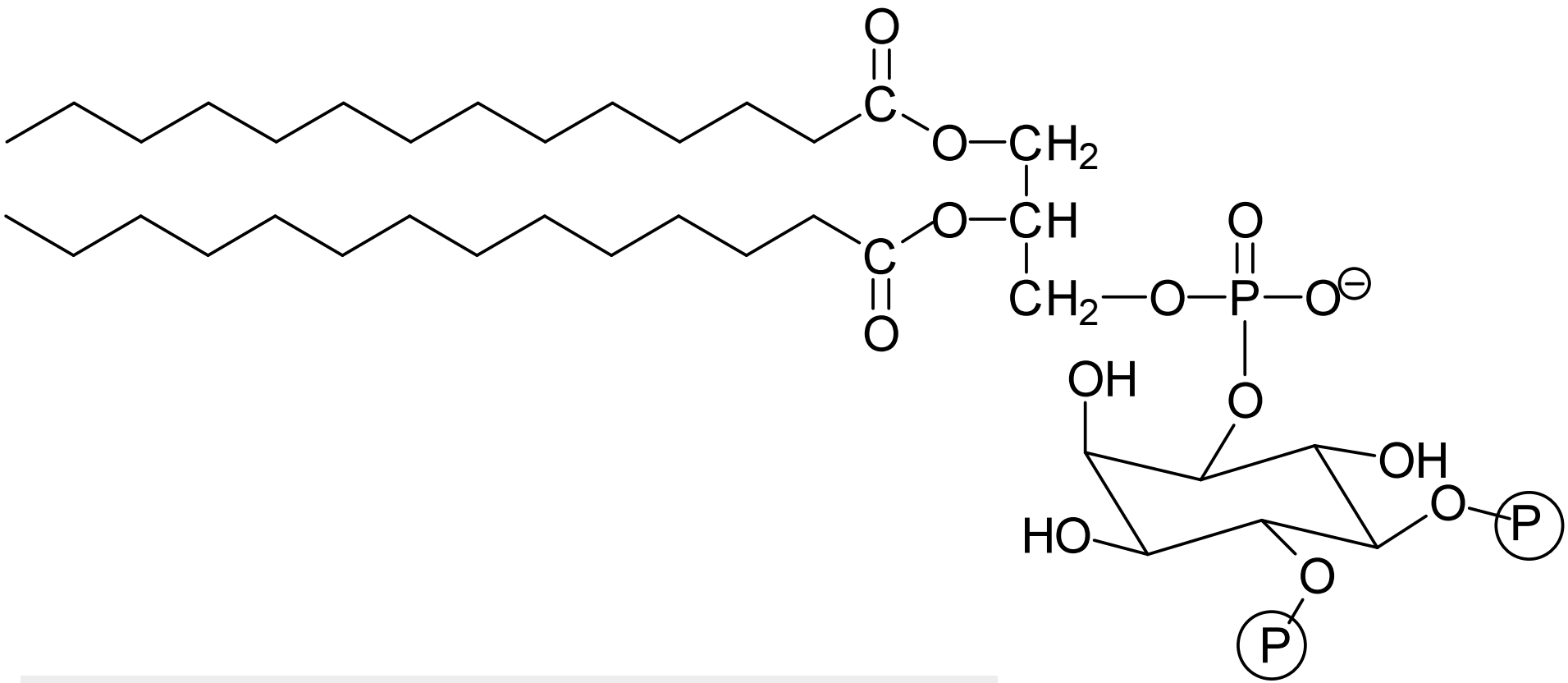
(dephosphorylation)

Phosphatidyl inositol system (p. 133)

- G_p protein activates phospholipase C (PL-C)
- PL-C catalyzes the hydrolysis of phosphatidyl inositol bis phosphate (PIP_2):
- $PIP_2 + H_2O \rightarrow IP_3 + DG$

- **both products (IP_3, DG) are second messengers**

The structure of PIP_2

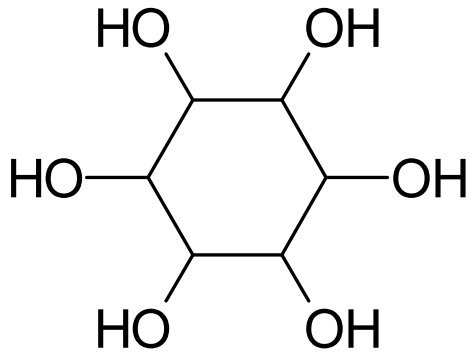


describe the structure

Q.

What is the source of inositol in human body?

The origine of inositol



Exogenous source: food

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

DG and IP_3 as second messengers

- DG activates protein kinase C $\Rightarrow$ phosphorylation of intracellular proteins
- IP_3 opens calcium channel in ER $\Rightarrow$ Ca^{2+} concentration in cytoplasm increases $\Rightarrow$ **Ca^{2+}** ions are associated with special protein **calmodulin** (CM) $\Rightarrow$ Ca^{2+}-CM complex activates certain types of kinases $\Rightarrow$ biological response

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

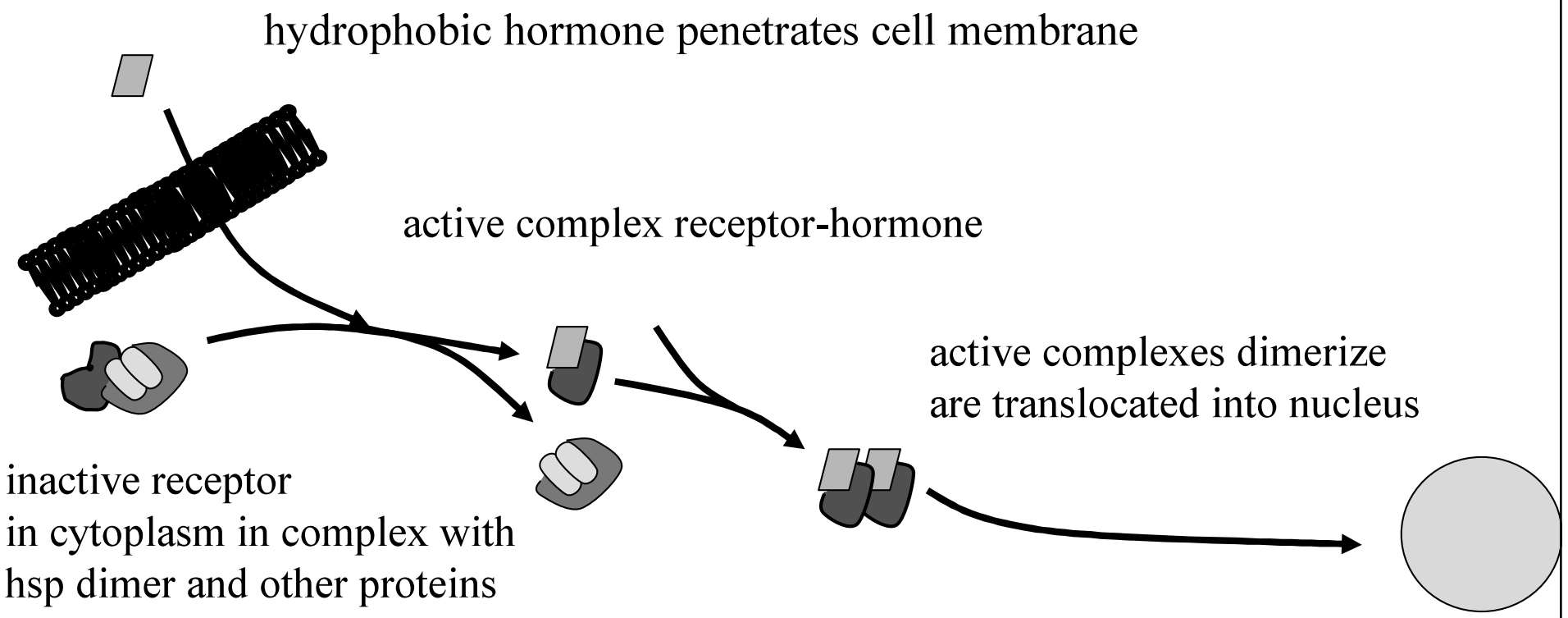
Intracellular receptors:

- cytoplasmatic
- nucleic

for steroids, iodothyronines, calcitriol, retinoids

Intracellular receptors

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



Steroid and thyroid hormones

- insoluble in water $\Rightarrow$ 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

Events on synapses

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_p	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.

How is acetylcholine released from presynaptic terminal?

A.

- influx of Ca^{2+} triggers the fusion of presynaptic vesicles (contaning acetylcholine) with cell membrane and exocytosis of acetylcholine
- acetylcholine is liberated into synapse

Q.

What reaction is catalyzed by acetylcholinesterase?

A.

acetylcholine + $H_2O \rightarrow$ choline + acetic acid

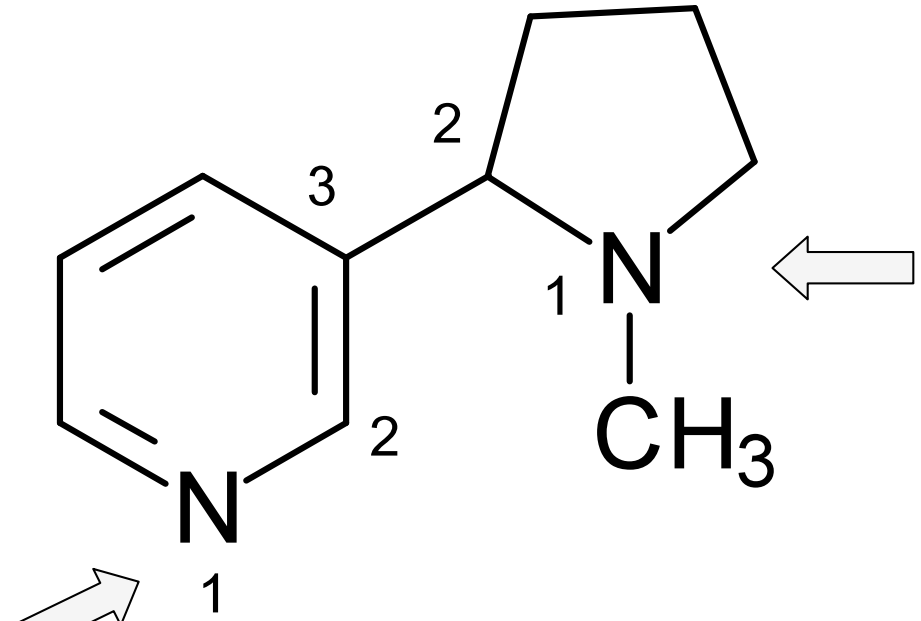
hydrolysis of ester

Q.

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.

Why nicotine triggers the release of adrenaline?

Main effects of nicotine

- nicotine binds to **acetylcholine nicotinic receptors** in brain and other tissues including cells of adrenal medula
- stimulates the secretion of adrenaline – because it binds to receptors in adrenal medula (p. 135 !) - **silent stress**

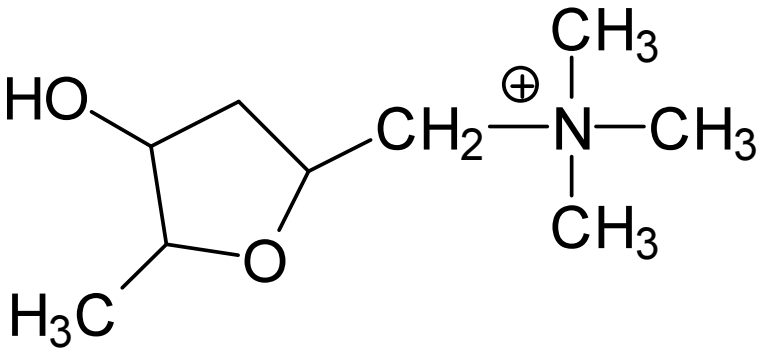
other effects:

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

Q.

What is muscarine?

Muscarine is an alkaloid in some mushrooms



muscarine

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

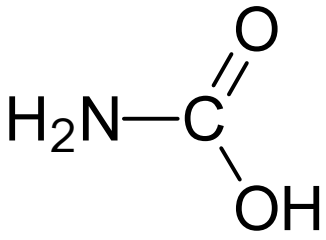
Amanita muscaria (fly agaric)

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

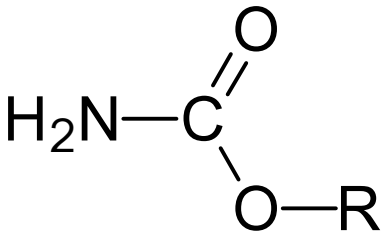
- **Irreversible** – organophosphates, very toxic compounds

Carbamates – General formulas



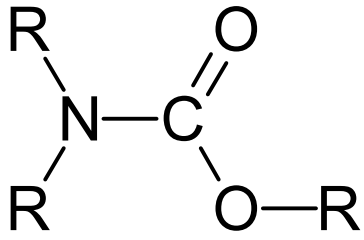
carbamic acid

(hypothetic compound)



alkyl carbamate

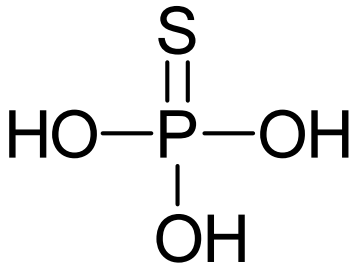
(ester)



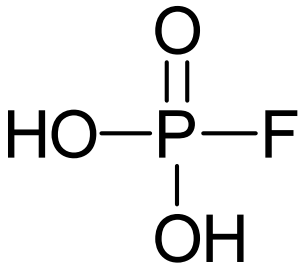
N-disubstituted

alkyl carbamate

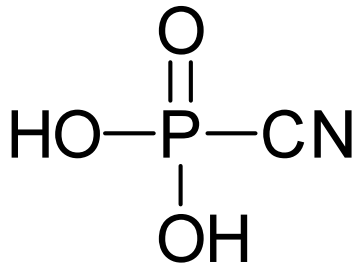
Organophosphates



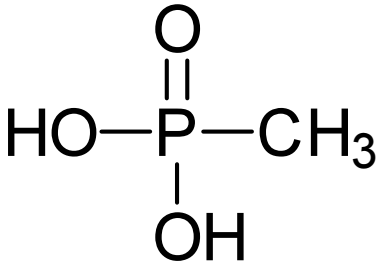
thiophosphoric acid



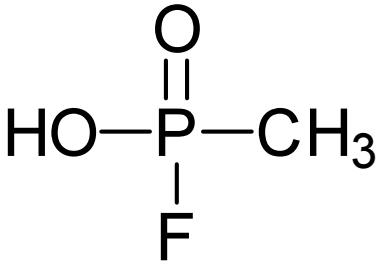
fluorophosphoric acid



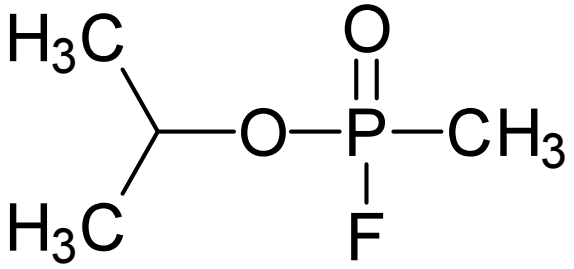
cyanophosphoric acid



methylphosphonic acid



methylfluorophosphonic acid



sarin

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

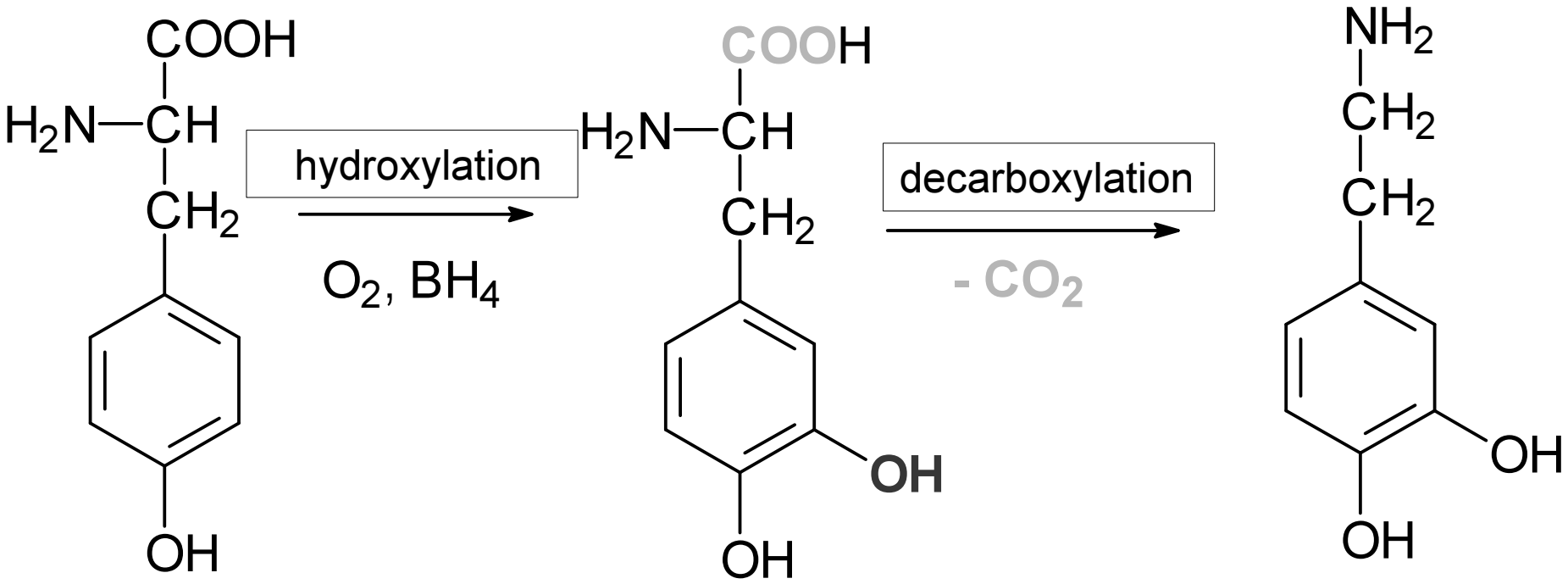
Feature	**α_1**	**α_2**	**β_1**	**β_2**
G-protein	G_p	G_i	G_s	G_s
2^{nd} messenger	DG, IP_3	cAMP	cAMP	cAMP
Occurence*	smooth muscle	brain	myocard	smooth m.

* Example of occurence

Q.

Describe the synthesis of noradrenaline.

The formation of DOPA and dopamine



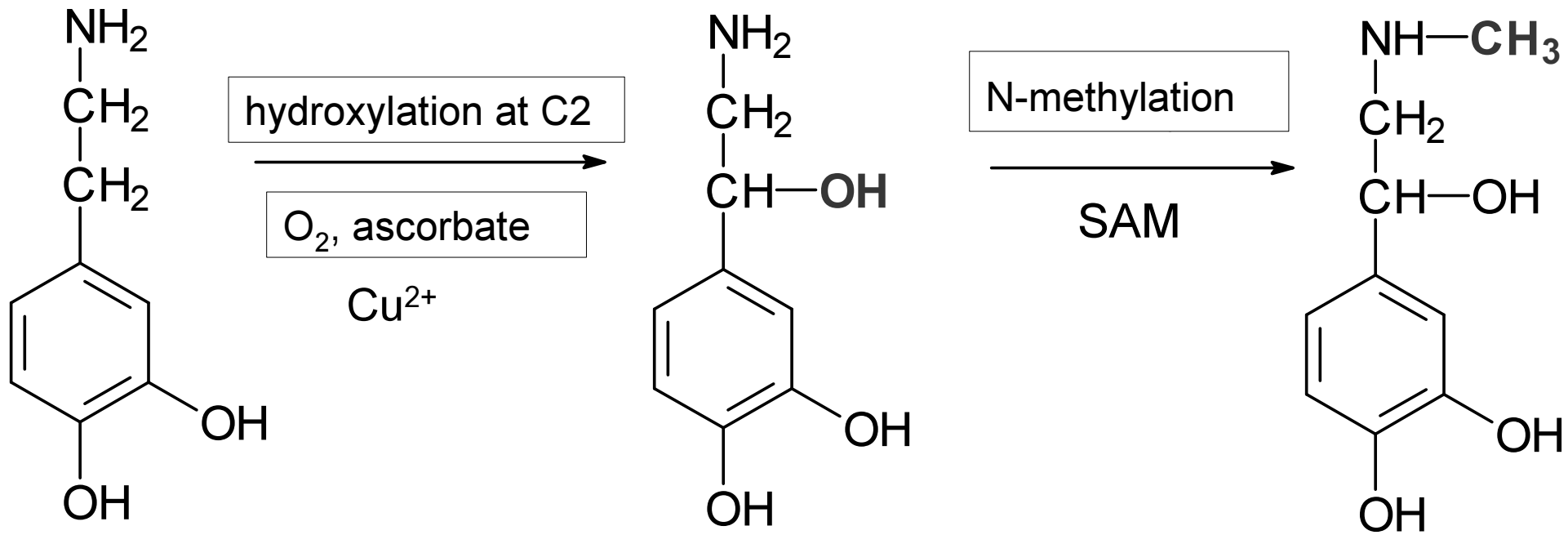
tyrosine

DOPA

3,4-dihydroxyphenylalanine

dopamine

Noradrenaline and adrenaline



dopamine

noradrenaline

adrenaline

prefix ***nor-*** means ***N*-demethyl**

The next seminar

April 24, 2006

Chapter 21 - I. part