

# Regulation of the metabolism

## **PRINCIPLES OF THE REGULATION OF THE METABOLISM:** **THEORETIC BASES**

**ENZYMES -BIOCATALYSATORS**

**HORMONES and NEUROTRANSMITTERS (Common mechanisms of the effect of hormones and neurotransmitters)**

**RECEPTORS (Type of membrane receptors and intracellular receptors)**

**ENZYMES**

**VITAMINS**

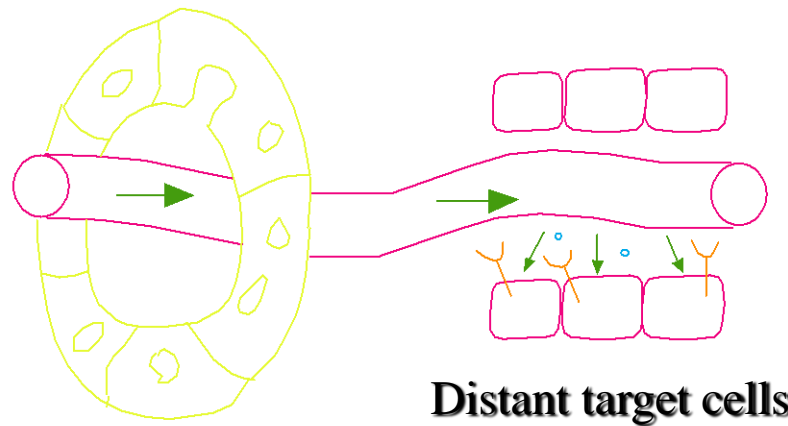
**METABOLIC REGULATIONS**

# Definitions

- Endocrine** - Refers to the internal secretion of biologically active substances.
- Exocrine** - Refers to secretion outside the body, for example, through sweat glands, mammary glands, or ducts lead to the gastrointestinal.
- Hormone** - Substances released by an endocrine gland and transported through the bloodstream to another tissue where it acts to regulate functions in the target tissue (classic definition).
- Paracrine** - Hormones that act locally on cells that did not produce them.
- Autocrine** - Hormones that act on cells that produced them.
- Receptors** - Hormones bind to receptors molecules on cells. A receptor must specifically recognize the hormone from the numerous other molecules in the blood and transmit the hormone binding information into a cellular specific action.

# Endocrine

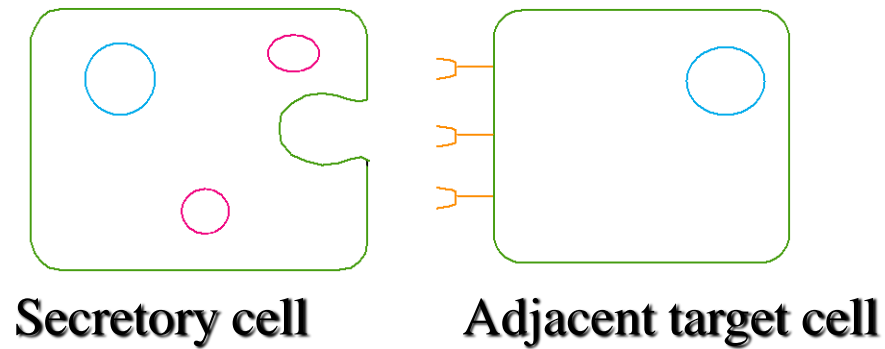
Hormone secretion into blood by endocrine gland



Blood vessel

Distant target cells

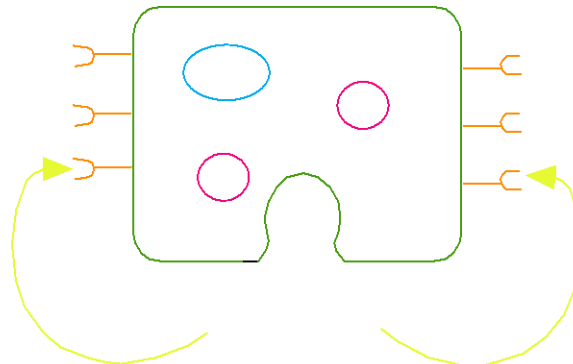
# Paracrine



Secretory cell

Adjacent target cell

# Autocrine



Receptor

Hormone or other extra cellular signal

Target sites on same cell

# Communication between cells

**The signal transduction** is the process, when signal molecules carry specific information over membranes from the outside of the target cell into the inside, where cause relevant biological answer.

In the higher organisms there are **three main signal systems**, which regulate and integrate activity of the cells.

Except for them other signal molecules (they aren't in these systems) also play a role in the metabolism.

## Main signal systems in the higher organisms

System	Source of the signal molecules	Signal molecules
Endocrine	Endocrine glands, scattered glandular cells	Hormones
Nervous	Nervous cell	Neurotransmitters, neurohormones
Imununity	Cells of the imunity system	Cytokines
Other types	Different cells	Eicosanoids, growth factors

## Effects of signal molecules

Name of the effect	Character of the effect
endocrine	The signal molecule is carried by blood into the target cell, which is usually distant from the place of the synthesis. Typically hormones
paracrine	The signal molecule is secreted into the ambient surroundings of the cell (local mediators). The signal molecule influences only the cells of the nearest surroundings.
autocrine	The cell secretes the signal molecule and it is also a target. Features are similar like paracrine effect.

**Endocrine** – the signal molecule is carried by blood into the target cell, which is usually distant from the place of the synthesis. It is typical for hormones.

The concentration of the signal molecule in blood is very low ( $10^{-12}$ – $10^{-9}$  mol/l) – so target cell has a big affinity to the signal molecule – the binding hormone to receptor is very strong, hormone doesn't dissociate easy. Other feature is that it takes definite time that the concentration of the hormone in blood will rise and the concentration of the hormone in blood stays for definite time (a few minutes or hours) raised.

**Paracrine** – The signal molecule is secreted into the ambient surroundings of the cell (local mediators). The signal molecule influences only the cells of the nearest surroundings. The concentration of the signal molecule in the surroundings of the cells is higher ( $10^{-9}$ – $10^{-6}$  mol/l). Affinity of the receptors to the signal molecule is lower – after decrease of the concentration in the surroundings of the cell, the signal molecule is separated. Paracrine signaling is determined for fast and localized communication between cells.

**Autocrine** – The cell secretes the signal molecule and it is also a target. Features are similar like paracrine effect.

**Juxtacrine** – signalling between cells or cell and extracellular matrix requires tight contact.

TEST

# Common mechanisms of the effect of hormones and neurotransmitters.

## Types signal molecules in the neurohumoral regulations:

Signal molecule	Source
<b>HORMONES</b>	secreted by endocrine glands, scattered glandular cells, eicosanoids a lot of other types of cells
<b>NEUROHORMONES</b>	secreted by neurons into the blood circulation
<b>NEUROTRANSMITTERS</b>	secreted in the synaptic ending
<b>CYTOKINES, GROWTH FACTORS, EICOSANOIDS</b>	secreted by a lot of types of cells, usually not from endocrine glands

## Regulation of the metabolism is in the different levels, but always on the molecular base

**The Regulation of enzymatic reactions is a central instrument of the regulation of the metabolism.**

Regulation **in the definite cellular compartment**

Regulation **in the complete cell**  
(proteome, specific receptors, isoenzymes, transporters, energetic state of the cell)

Regulations followed from the **communication between cells**

**Levels of the regulation overlap.**

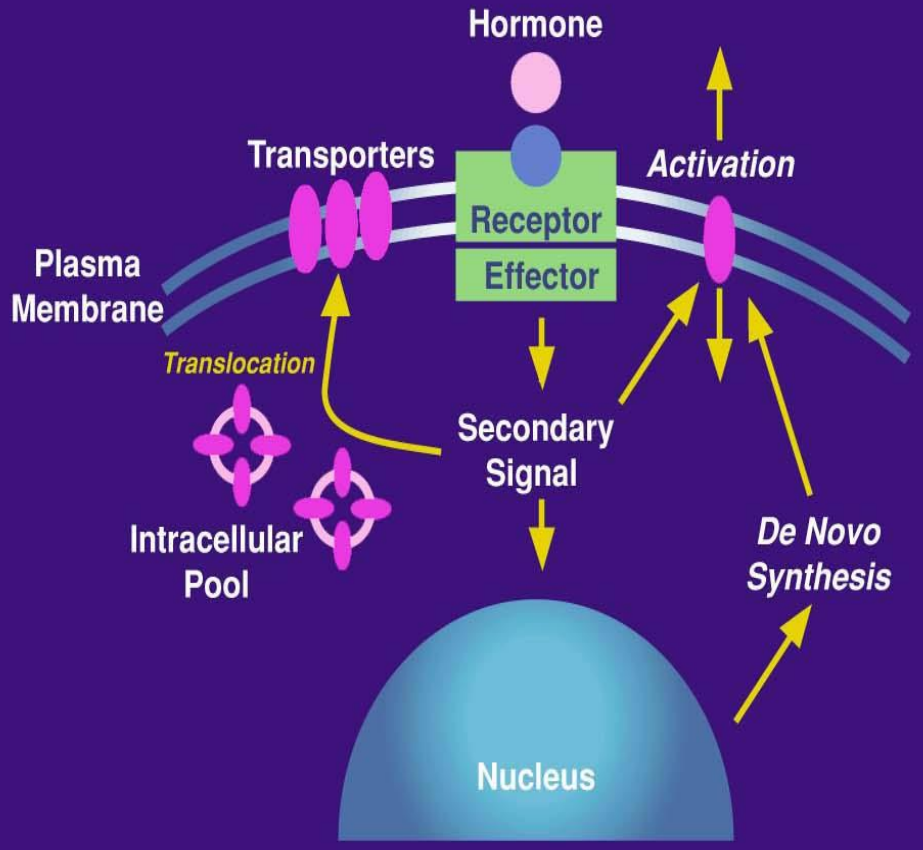
# Regulation of the enzymatic activity

- regulation of the **amount of enzyme** (synthesis and degradation)
- regulation of the **activity of the enzyme** (modification of the enzyme by proteolysis, covalent modification, allosteric regulation, interaction with the regulatory proteins)
- **availability and concentration** of the substrate (regulation of the transport)



# SIGNAL TRANSDUCTION

## Hormone/Receptor Interaction-Secondary Signals



# TEST

The collective feature of all **substances with modulating effects** to the cells is their effect by receptors.

**Receptors** are allosteric proteins, which change their conformation after the binding of a ligand.

**Ligands** are signal molecules.

**Agonists** are ligands, where after the binding on the receptor cause the transduction of signal, **antagonists** after the binding on the receptor defend the signal transduction.

Receptors are localised on the **outer surface of the cytoplasmic membrane or intracellularly**.

In their structure there are two main components: (1) domain binding the ligand, which ensures the specificity of the binding with the relevant ligand; (2) effector domain, which starts a genesis of the biological answer after the binding of the ligand.

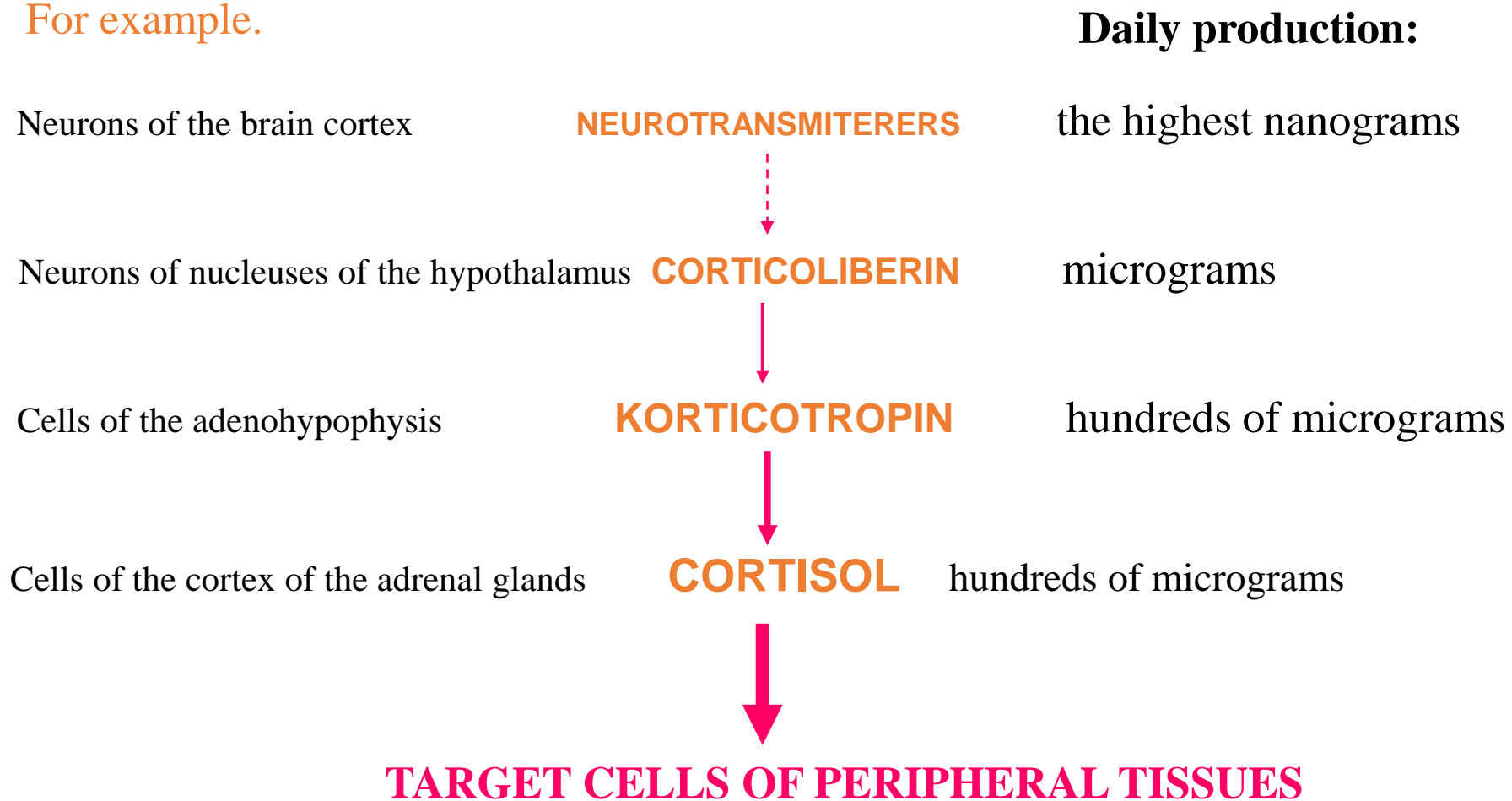
Activated receptor can enter into the reaction with other cellular components and realise the process of the signal transduction.

Tissues, whose cells have no molecules of the specific receptor, can't react to the relevant hormone.

Characteristic feature of a transport of the signal by receptors is its **amplification**, when the only one molecule of the hormone is able to cause cellular answer with  $10^4$ – $10^5$  times higher intensity.

# Principle of hierarchy in some hormonal regulations and amplification of the flow of information by signal molecules

For example.



# Signal transduction

**How does the cell take over the information carried by the chemical signal?**

↓  
Reaction of the signal molecule with the receptor

## Membrane receptors

Proteins and smaller signal molecules (peptides, amino acids, biogenic amines, eicosanoids)

## Intracellular receptors

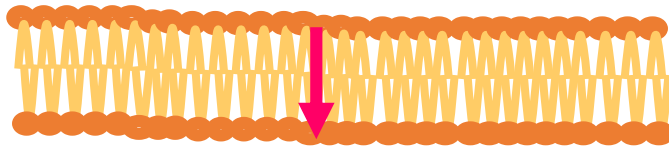
**Nonpolar signal molecules**  
(steroids, thyroid hormones, retinoids)

# TEST

## Membrane and intracellular receptors

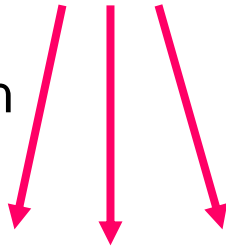
**Polar** signaling molecules

**Membrane receptor**



Transduction of signal

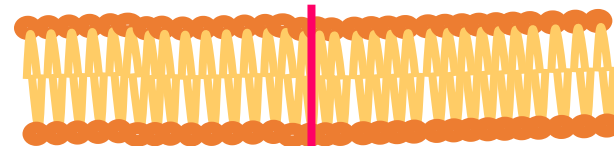
Amplification



**Biological effect**  
(fast effect, may be followed by belated action)

**Nonpolar** signaling molecules bound to plasma transport protein

Transport of signal molecule



**Intracellular receptor**

Interaction of the complex hormone- receptor with hormone-sensitive element of DNA

**Biological effect**  
(slower effect)

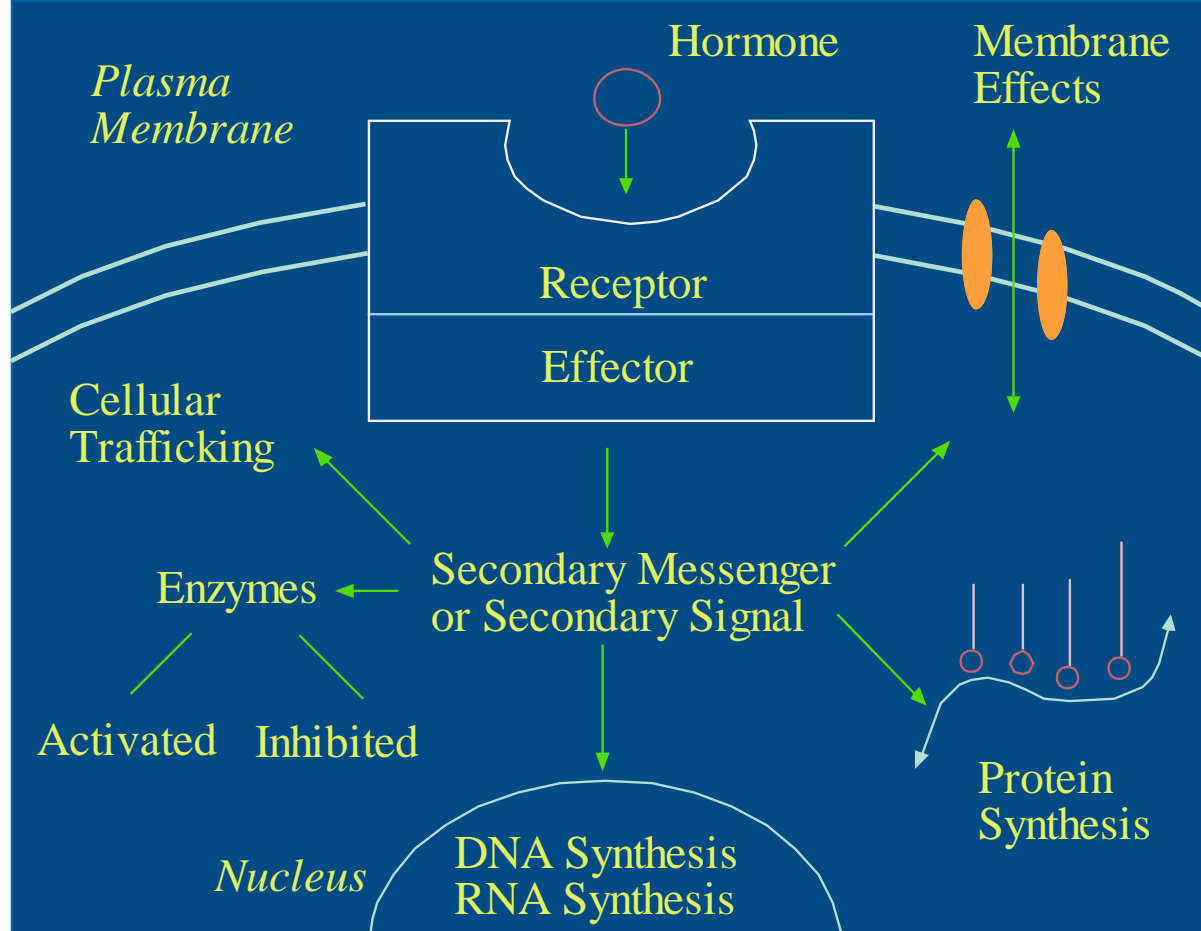
# Receptors

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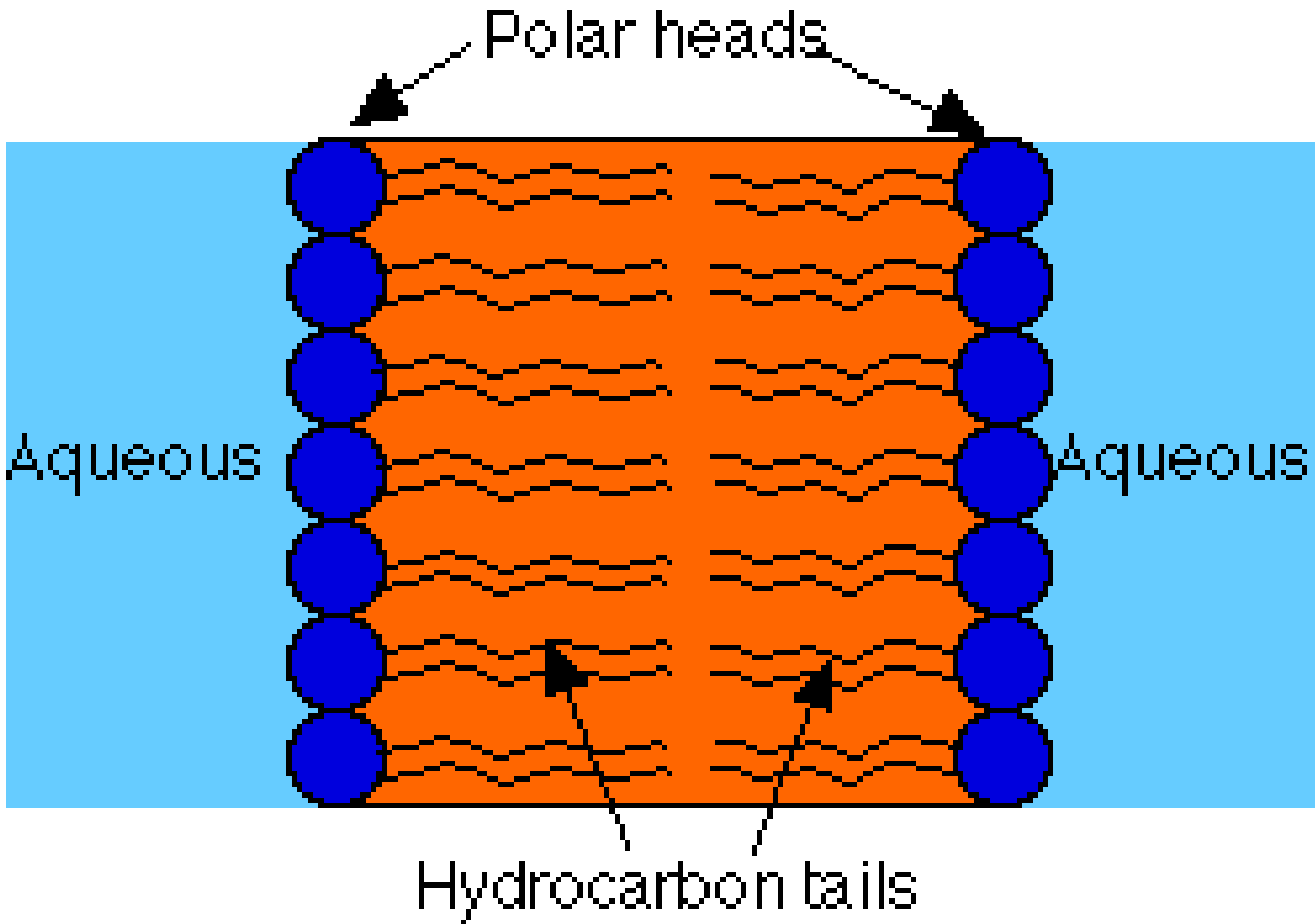
**Nuclear** receptors  
estrogens

**Cytoplasmic** receptors  
Most steroid and thyroid hormones

Cell surface **membrane** receptors  
Polypeptide hormones and catecholamines



A general model for the action of peptide hormones, catecholamines, and other membrane-active hormones. The hormone in the extra cellular fluid **binds** to the receptor and **activates** associated **effector(s)** systems, that may or may not be in the same molecule. This activation results in generation of an intracellular signal or **second messenger** that, through a variety of common and branched pathways, produces the final **effects** of the hormone on metabolic enzyme activity, protein synthesis, or cellular growth and differentiation.





# Types of receptors

Type of receptor	Ligand characteristics	Receptor characteristics
Membrane	Big signal molecules (peptides and proteins) Small, strongly hydrophilic molecules (amonoacides and their derivates)	Intergral membrane proteins
Intracellular	Small hydrophobic molecules (steroids, vitamin D, retinoids, thyroidal hormones)	Proteins in cytoplasm or in the nucleus

# Main types of membrane receptors

**I. Receptors - ion channels** (ROC, ligand-gated channels) only receptors for some neurotransmitters (ion channels controlled by neurotransmitters)

**II. Receptors** interacting with G-proteins (heterotrimeric)

**III. Receptors** with its own catalytic activity

a) **guanylate-cyclase**

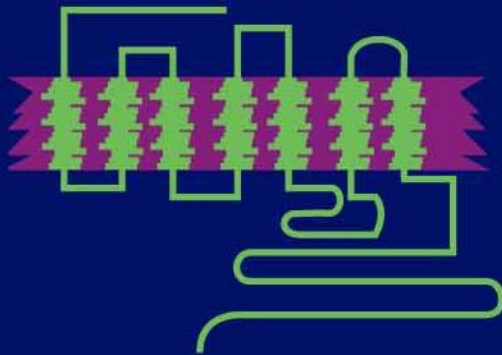
b) **proteinkinase**

**IV. Receptors cooperating with the non-receptor tyrosine kinases**

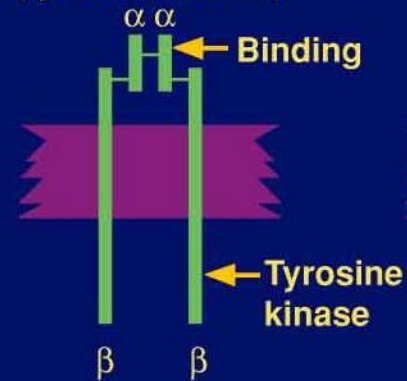
(eg. JAK) – receptors for somatotropin (GRH), prolactin, erythropoietin, interferons, interleukins and other cytokines.

## Representation of various types of membrane receptors with examples of each type.

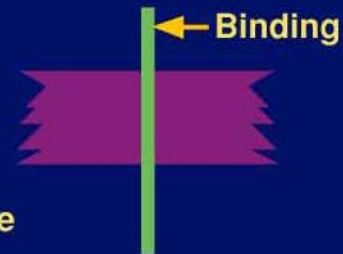
$\beta$ -adrenergic receptor  
(7-transmembrane domains;  
G-protein linked)



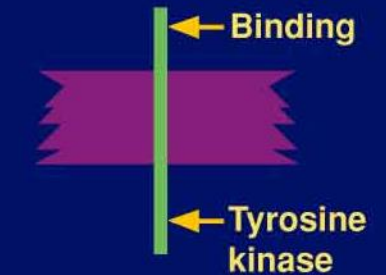
Insulin receptor  
(tyrosine kinase)



GH receptor



EGF receptor  
(tyrosine kinase)

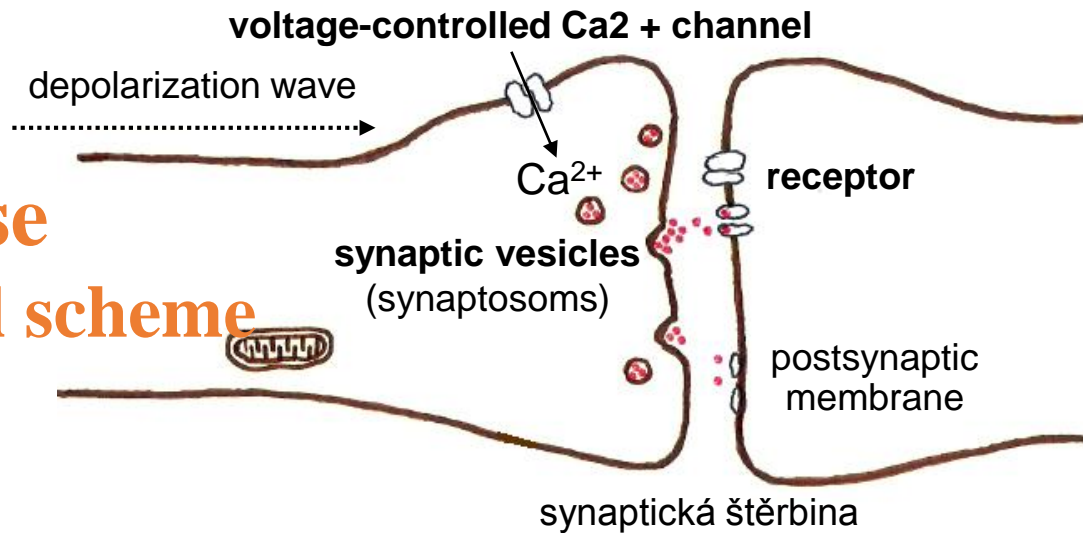


# I. Receptors – ion channels

Receptors of the type of ion channels are present in the synapses, their ligands are neurotransmitters.

Neurotransmitters - **chemical signals**, enable the transfer of of nerve impulses between neurons or between a neuron and the target cell

## Synapse General scheme



- Neurotransmitter binds directly to **the ion channel (ionotropic receptors)** → electrical signal (neuron -neuron)
- The neurotransmitter binds to a receptor that generates second messenger (metabotropic receptors) → chemical signal (eg. smooth muscle)

# Membrane receptors for the neurotransmitters

**Ionotropic receptors - ligand-controlled ion channels (ROC), e.g.**

excitatory **nicotinic acetylcholine** - channel for  $\text{Na}^+/\text{K}^+$ ,

glutamate (CNS, some afferent sensory neurons)

- channel for  $\text{Na}^+/\text{K}^+/\text{Ca}^{2+}$ ,

inhibitory **receptor GABA A (CNS)** - channel for  $\text{Cl}^-$

**Metabotropic receptors activating G proteins, e.g.**

protein **G<sub>s</sub>** adrenergic  $\beta_1$  a  $\beta_2$ , receptor GABAB, dopamine D1,

protein **G<sub>i</sub>** adrenergic  $\alpha_2$ , dopamine D3,

muscarinic acetylcholine M2 (also opens  $\text{K}^+$  channel),

protein **G<sub>q</sub>** muscarinic acetylcholine M1, adrenergic  $\alpha_1$ .

# Neurotransmitters

There are more than 30 different neurotransmitters (amino acids, biogenic amines caused their transformation, or very large peptides).

Examples:

## In central nervous system

- inhibitory **GABA** (minim. 50 % all synapses)
  - glycine** (prevails in the spinal cord)
  - excitatory **glutamate** (more than 10 %)
  - acetylcholine** (about 10 %)
  - dopamine** (about 1 %, in striatum 15 %)
  - serotonine**
  - histamine
  - aspartate
  - noradrenalin
  - (less than 1 %, in hypothalamus 5 %)
  - adenosine
- Neuromodulation endorphins and enkephalins,  
endozepines, delta-sleep-inducing peptide  
etc.

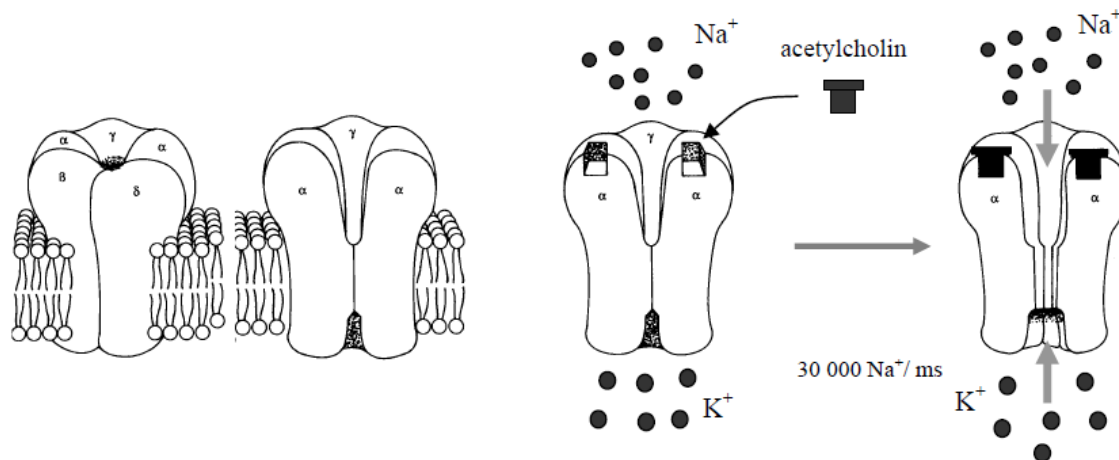
## In peripheral nervous system

- efferent neurons
- excitatory **acetylcholine**
- noradrenaline**
- primary sensory afferent
- excitatory glutamate
- (A $\beta$  fibers, tactile)
- substantion P (peptide)
- (C A $\delta$  fiber nociceptive)

# TEST Major neurotransmitter receptors

## Acetylcholine receptors

Occurs at neuromuscular junctions of skeletal muscle and in almost all peripheral dendrites of efferent neurons. It consists of five subunits (2 $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ,  $\epsilon$ ) penetrating the membrane. Acetylcholine is synthesized by the presynaptic neuron region of acetyl-CoA to choline and before release is stored in vesicles stored close to the active zone of the presynaptic membrane. Membrane also has the voltage-gated Ca<sup>2+</sup> channels, which open when the action potential is expanded to the membrane. Increased levels of Ca<sup>2+</sup> in the neuron endings activates the Ca<sup>2+</sup>-dependent protein kinase, which phosphorylates synapsin and other proteins, thereby effecting fusion of the vesicles with the presynaptic plasma membrane and release of acetylcholine into the synaptic cleft. Acetylcholine binds the two subunits and his binding causes a conformational change and short influx of sodium ions into the cell and potassium ions out of the cell. This causes depolarization of postsynaptic membrane, and if the threshold is reached, potential-dependent channels are opened for Na<sup>+</sup> and the action potential arises. Once acetylcholine secretion ceases, its concentration in the cleft decreases and acetylcholine stops to bind to receptors. Acetylcholine is decomposed by acetylcholinesterase, which is bound to the surface of the postsynaptic membrane.



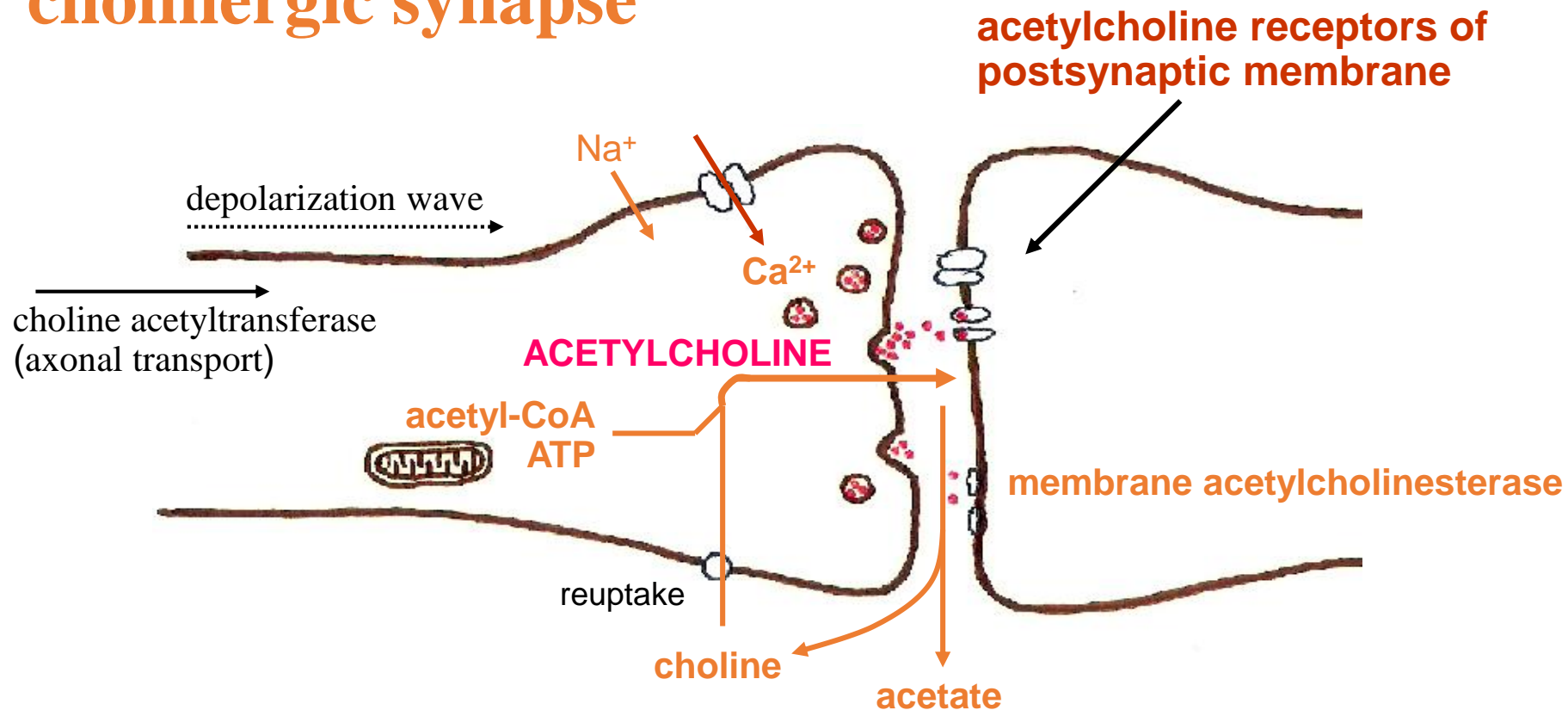
# Major neurotransmitter receptors

## Acetylcholine receptors

Receptor	Nicotine	Muscarinic	
		M <sub>1</sub> , M <sub>3</sub>	M <sub>2</sub>
Mechanism of action	ion channel	G <sub>q</sub>	G <sub>i</sub>
The second messenger		DG + IP <sub>3</sub>	cAMP
occurrence	<ul style="list-style-type: none"> <li>• autonomic ganglia neurons,</li> <li>• neuromuscular junction,</li> <li>• chromaffin cells of the adrenal medulla</li> </ul>	<ul style="list-style-type: none"> <li>• brain,</li> <li>• smooth muscle,</li> <li>• glandular cells</li> </ul>	<ul style="list-style-type: none"> <li>• myocardium,</li> <li>• brain</li> </ul>
	tubocurarine	atropin	



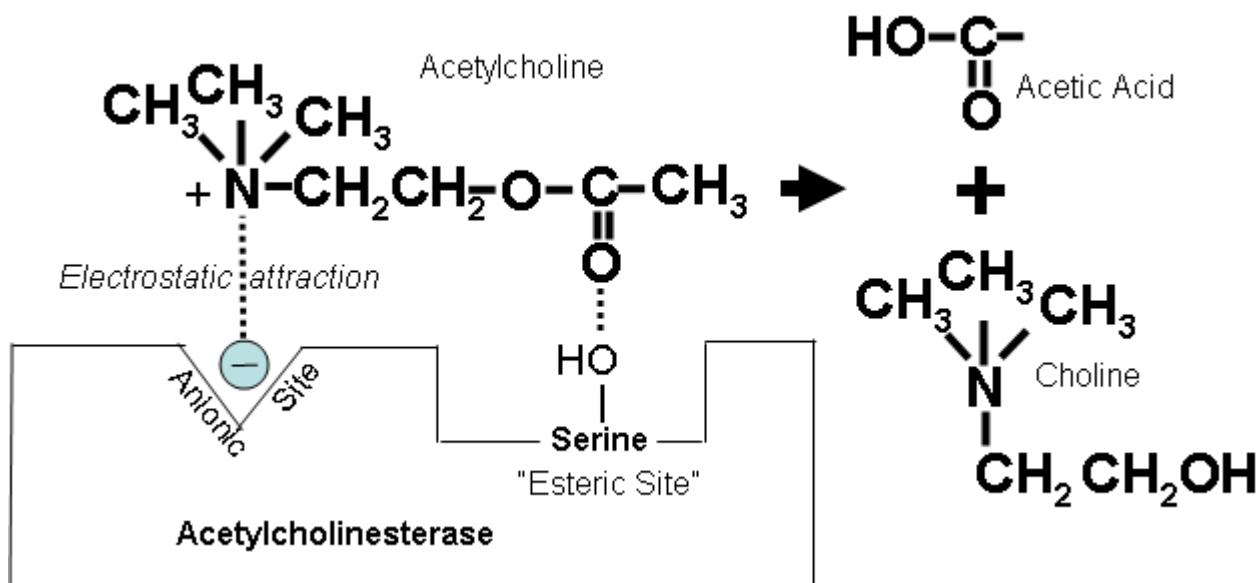
# cholinergic synapse



One nerve impulse in the neuromuscular junction releases approx. 300 vesicles, one contains about 40,000 molecules of acetylcholine; concentration of acetylcholine in the synaptic cleft rises to 10 000 times. The mediator is rapidly hydrolyzed by acetylcholinesterase.

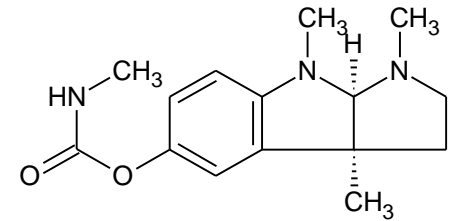
# Acetylcholinesterase

- The hydrolysis of acetylcholine to acetate and choline
- It is a serine hydrolase



# Acetylcholinesterase inhibitors

- a) reversible: carbamates (physostigmine, rivastigmine, neostigmine)
- b) irreversible: organophosphates (DFP, soman, sarin)



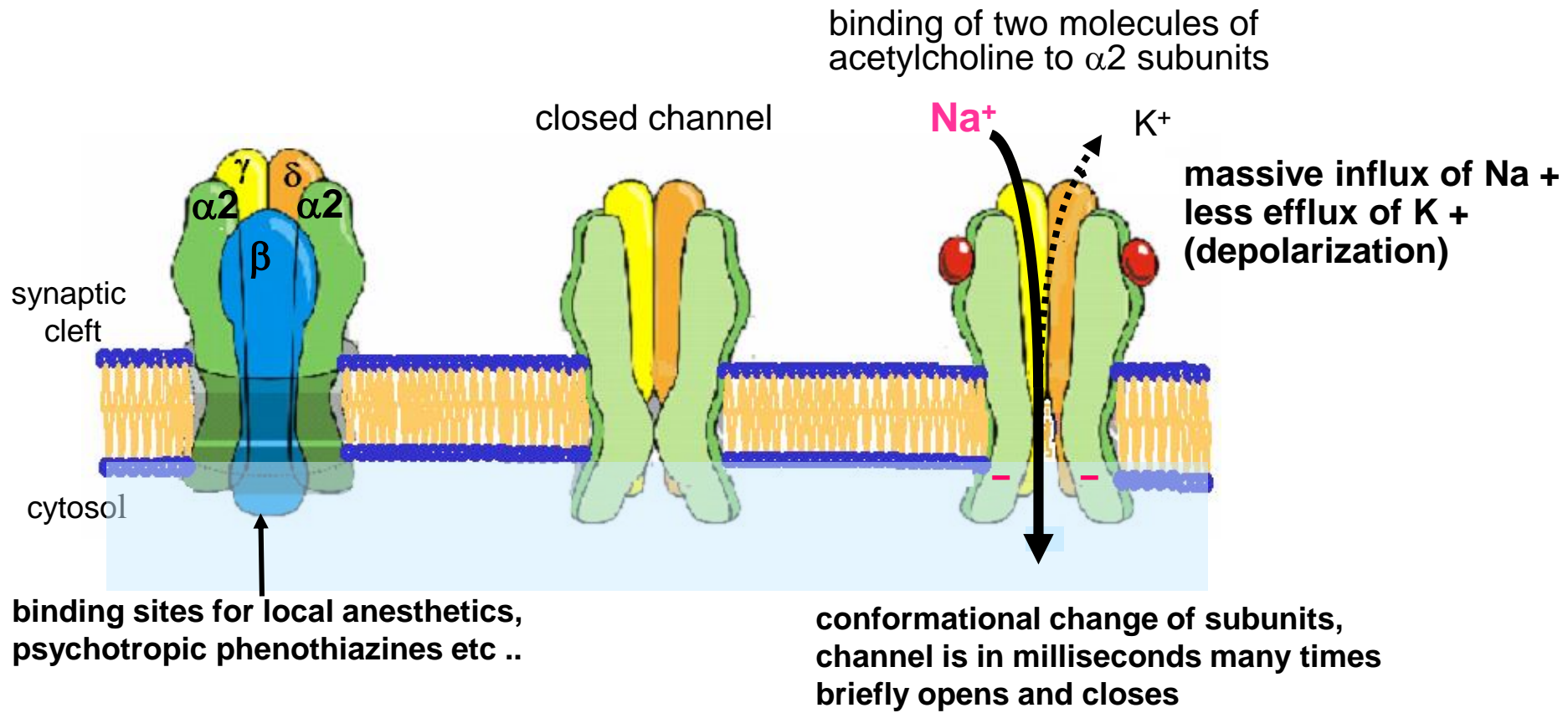
physostigmine

Binding of toxic organophosphates to cholinesterase is done in two stages:  
reversible can be affected by reactivators)

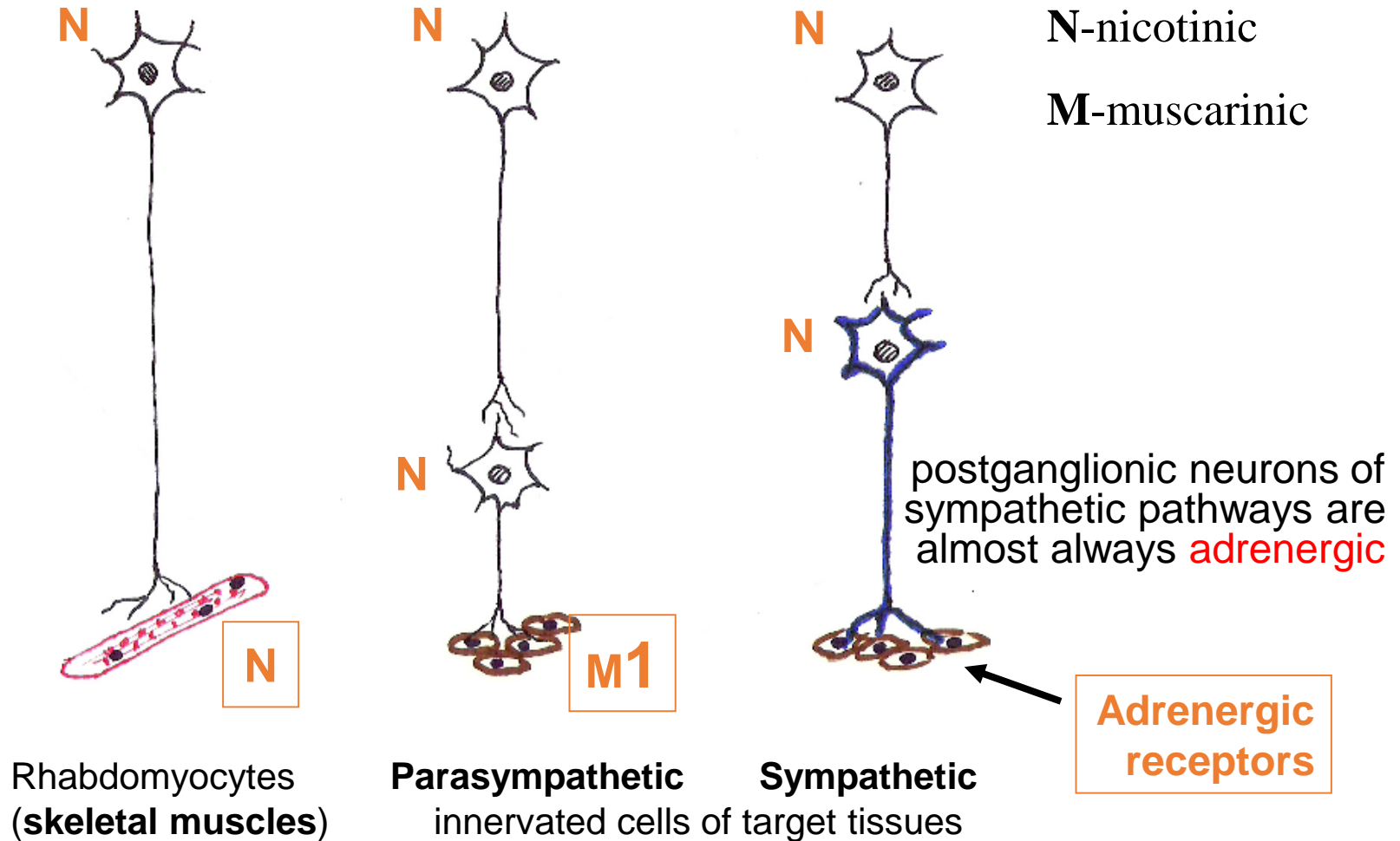
irreversible - formation of covalent bonds between enzyme and organophosphate

# Nicotinic acetylcholine receptor of the nicotine type

e.g. at the neuromuscular junction -  $\text{Na}^+ / \text{K}^+$  ionophore: asymmetrical pentamer of four types of homologous subunits penetrating the membrane.



# Acetylcholine (cholinergic) receptors in peripheral efferent neurons



# Ligands interacting with acetylcholine receptors of nicotine types

**D-tubocurarine** - **competitive antagonist** of acetylcholine, prevents the opening of ionophore (depolarization does not occur) paralysis of skeletal muscles  
pancuronium, vecuronium ad. - muscle relaxants during prolonged operations

**Succinylcholine** - **agonist** binds more efficiently than acetylcholine and depolarizes. The persistent depolarization leads to loss of electrical excitability of membrane. Short term myrelaxans.

**Botulotoxine** – protein complex from Clostridium botulinum. Inhibits the release of acetylcholine from the nerve endings.

**Nicotine** - binds to receptors in the peripheral and vegetative nervous system which controls the internal organs. Here causes increased activity of the digestive tract: increase of production of saliva and digestive juices and the increase in activity of smooth muscles. Also increases the production of sweat and may cause the contraction of pupil.

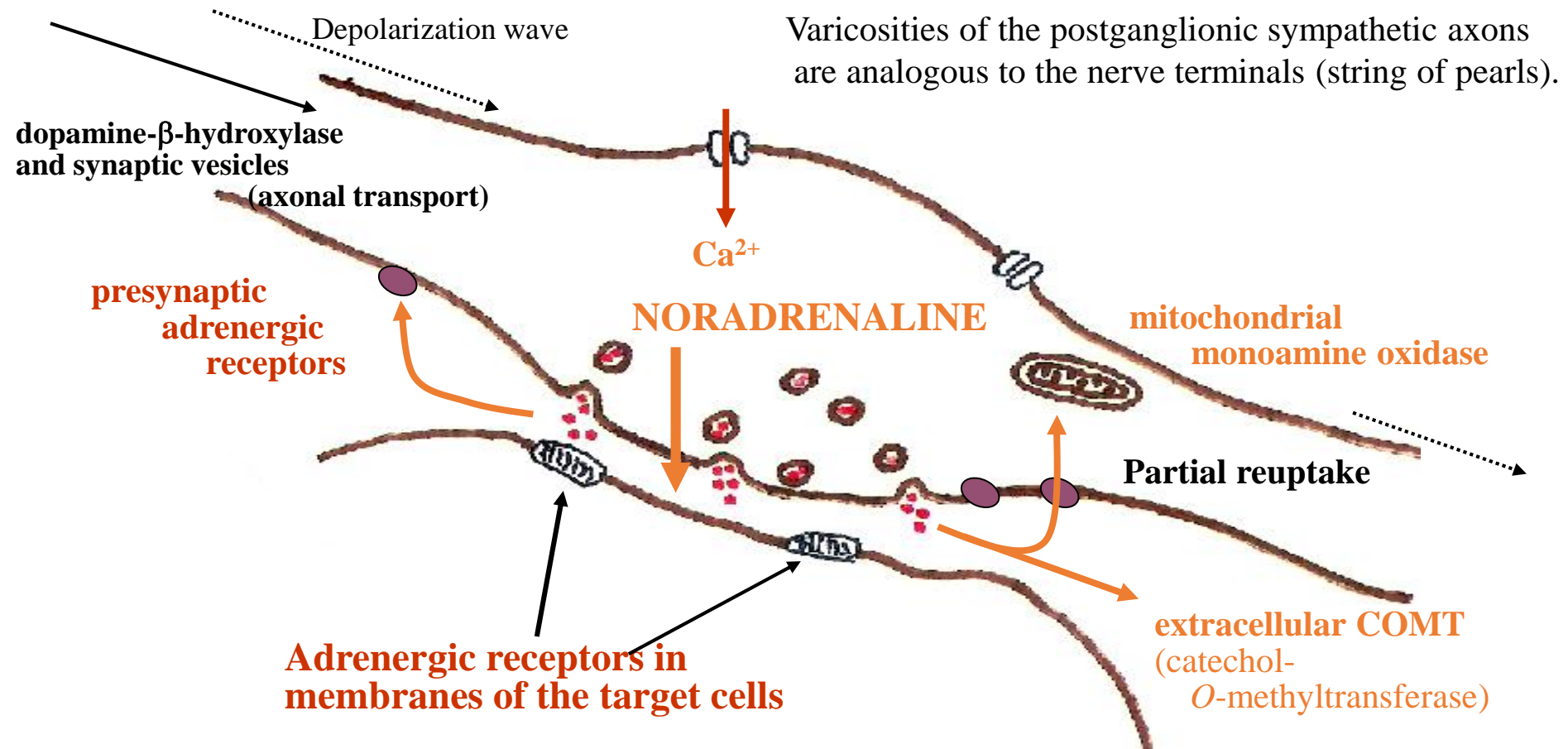
# Muscarinic cholinergic receptors

Type	Principle of action	Location
M <sub>1</sub>	G <sub>q</sub>	Autonomic ganglia, CNS, exocrine gland cells
M <sub>2</sub>	G <sub>i</sub>	heart, K <sup>+</sup> channels opening
M <sub>3</sub>	G <sub>q</sub>	Smooth muscle
M <sub>4</sub>	G <sub>i</sub>	CNS
M <sub>5</sub>	G <sub>q</sub>	CNS

The alkaloid **atropin** is antagonist of muscarinic receptors preventing acetylcholine binding.

# Adrenergic synapse

Neurotransmitter of most postganglionic sympathetic neurons is **noradrenaline**.  
Some nerves can be also influenced by adrenaline.





# Synthesis and storage of catecholamines

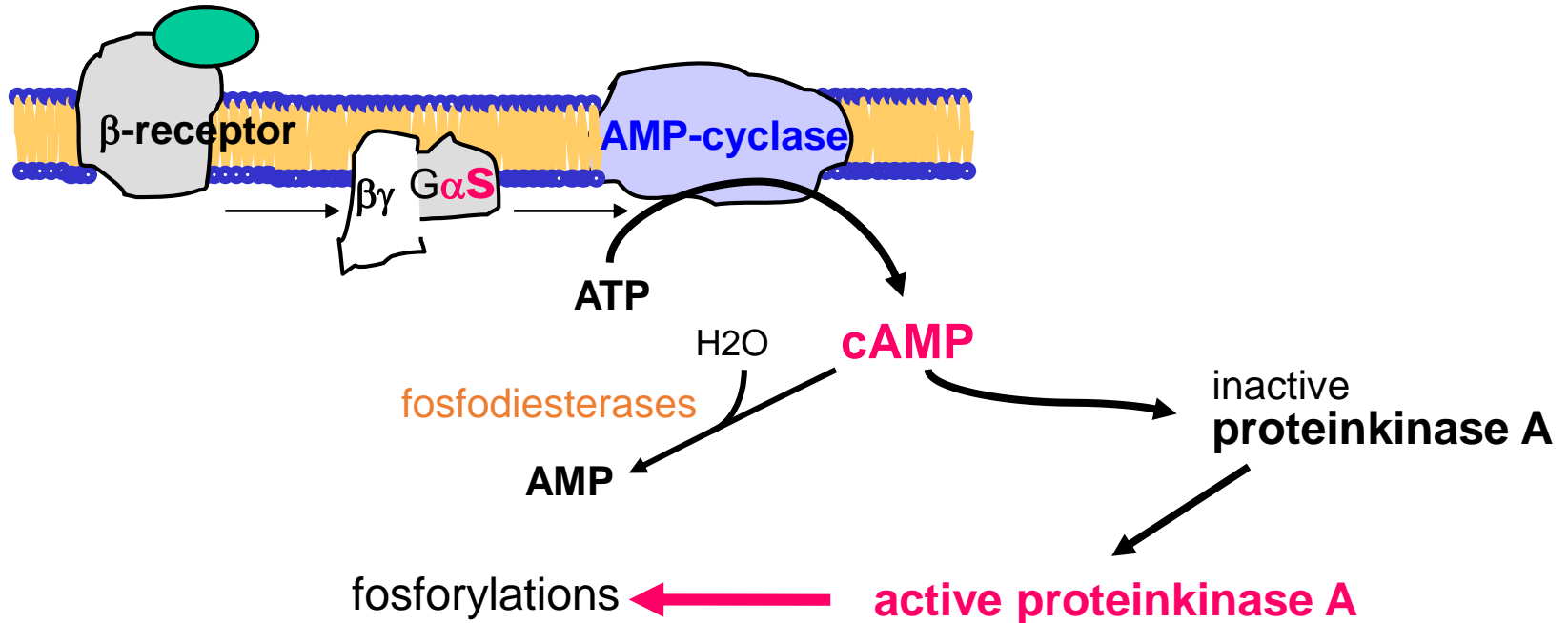
- Dopamine is synthesized in the cytoplasm
- Dopamine is transported into vesicles (ATP-dependent process, against concentration gradient).
- Final hydroxylation of dopamine to noradrenaline occurs in vesicles.

# Adrenergic receptors

Receptor	$\alpha_1$	$\alpha_2$	$\beta_1$	$\beta_2$
<b>G-protein</b>	Gq	Gi	Gs	
<b>Second messenger</b>	DG + IP <sub>3</sub>	cAMP ↓	cAMP ↑	
<b>Examples of location</b>	<ul style="list-style-type: none"> <li>• GIT smooth muscle (sphincters) and skin blood vessels (contraction)</li> </ul>	<ul style="list-style-type: none"> <li>• adrenergic and cholinergic nervous terminals (transmitter releasing inhibited)</li> <li>• pancreas (glandular secretion inhibited)</li> <li>• thrombocytes (agregation)</li> </ul>	<ul style="list-style-type: none"> <li>• myocard (intensity and frequency of contractions increased)</li> </ul>	<ul style="list-style-type: none"> <li>• smooth muscle in the uterus, bronchi (relaxation)</li> <li>• GIT smooth muscle (peristalsis)</li> <li>• pancreas (glandular secretion inhibited activated)</li> </ul>

# $\beta$ -Adrenergic receptors

noradrenalin / adrenalin



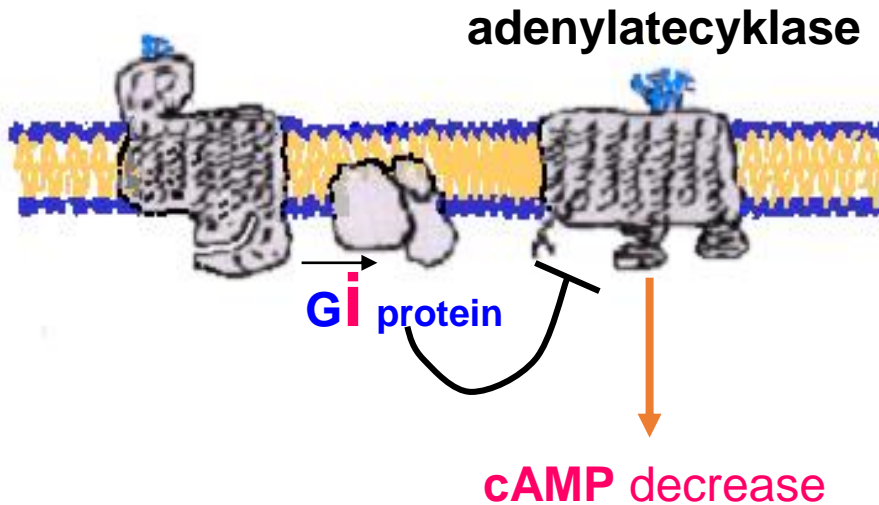
## The typical effects of $\beta$ -stimulation

- $\beta_1$  – tachycardia, inotropic effect in the myocard,
- $\beta_2$  – bronchodilation, vasodilation in the bronchial tree,
- $\beta_3$  – mobilization of fat stores, thermogenesis.

# TEST

## Adrenergic receptors $\alpha 2$ a $\alpha 1$

### $\alpha 2$ -receptors

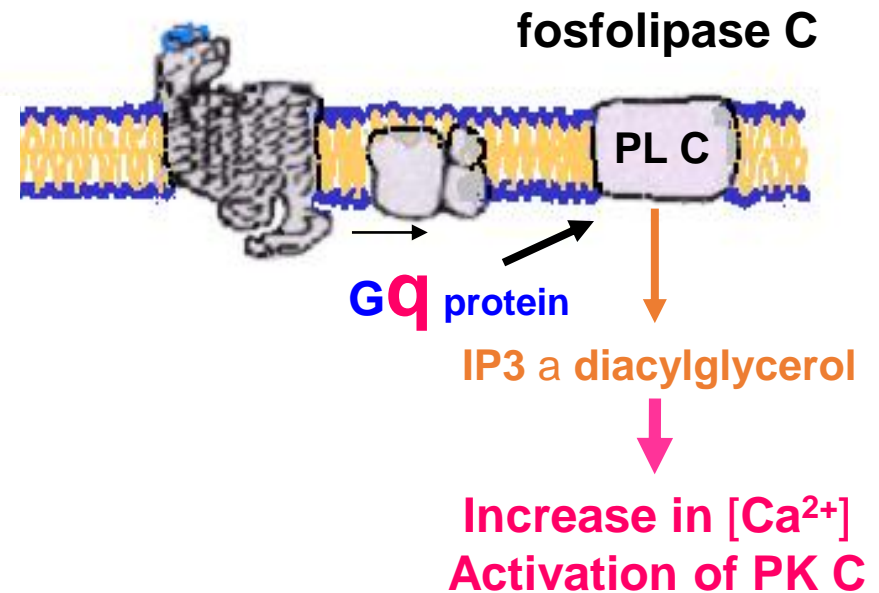


The typical effects of adrenergic

$\alpha 2$ -stimulation:

glandular secretion inhibited

### $\alpha 1$ -receptors



$\alpha 1$ -stimulation:

vasoconstriction

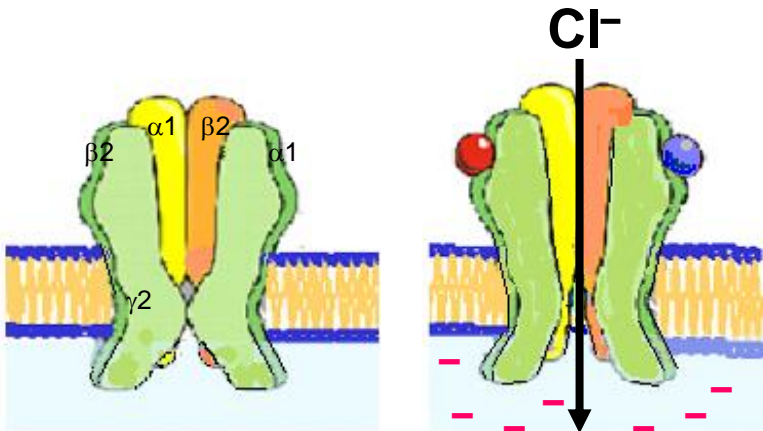
bronchoconstriction

motility of GIT inhibited

# Inhibitory GABA<sub>A</sub> receptor

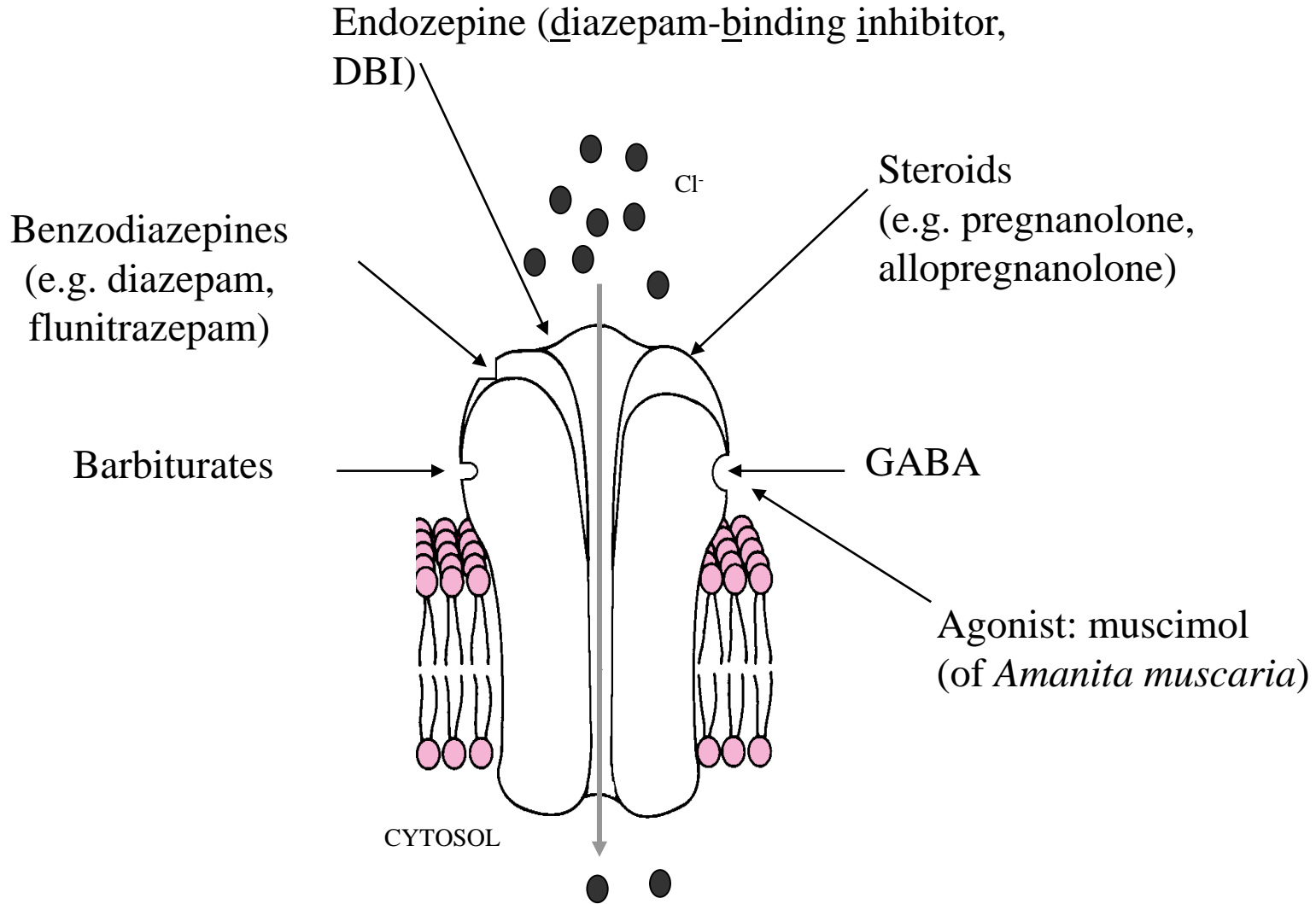
Ligand-gated channel (ROC) for **chloride anions**. The interaction with  **$\gamma$ -aminomáselnou kyselinou (GABA)** opens the channel.

The influx of  $\text{Cl}^-$  is the cause of **hyperpolarization** of the postsynaptic membrane and thus it's depolarization (formation of an action potential) disabled.



heteropentamer containig 3 subunits

# Another binding sites of GABA receptor



# Another binding sites of GABA receptor

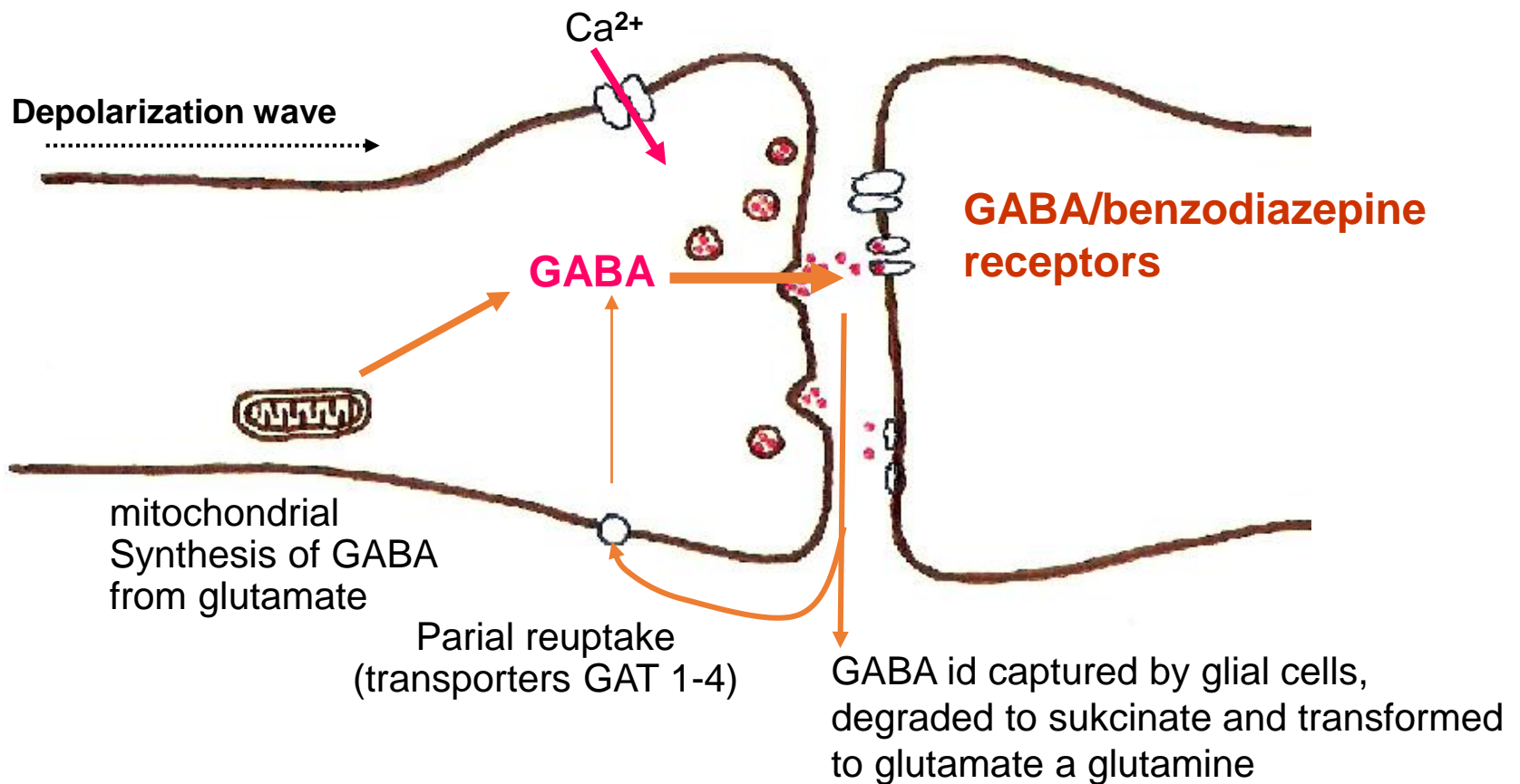
More than eleven **allosteric modulation sites** for substances increasing the effect of endogenous GABA (calming down, reduction of anxiety and myorelaxation): anesthetic, ethanol and numerous drugs such as benzodiazepines meprobamat and also various barbiturates.

On the contrary, another ligands compete for benzodiazepine binding site or act also as the **antagonists** of GABA (inverse agonists),  $\Rightarrow$  causing unease and anxiety (e.g. endogenous peptides called endozepines).

In brainstern and spinal cord, **glycin** has a similar function to GABA in brain. The inhibitory effect of glycinergic synapses is blocked by strychnine alkaloid, known seizure poison.

# Inhibitory GABAergic synapse

**$\gamma$ -Aminobutyric acid (GABA)** is the main inhibitory neurotransmitter in CNS. GABAergic synapses represent about 60 % of all brain synapses.





## Receptors of the most important neurotransmitters

Ion channels (ROC)	Receptors associated with G-proteins		
	G <sub>s</sub> (increasing cAMP)	G <sub>i</sub> (decreasing cAMP)	G <sub>q</sub> (increasing IP <sub>3</sub> /DG)
<u>Na<sup>+</sup>/K<sup>+</sup></u> – acetylcholine nicotinic	–	acetylcholine muscarinic M <sub>2,4</sub>	acetylcholine muscarinic M <sub>1,3,5</sub>
–	adrenergic β <sub>1,β2,β3</sub>	adrenergic α <sub>2</sub>	adrenergic α <sub>1</sub>
<u>Na<sup>+</sup>/Ca<sup>2+</sup>/K<sup>+</sup></u> – glutamate ionophores	–	glutamate mGluR skupiny II a III	glutamate mGluR class I
–	dopamine D <sub>1,5</sub>	dopamine D <sub>3,4</sub>	dopamine D <sub>2</sub>
– serotonin 5-HT <sub>3</sub>	serotonin 5-HT <sub>4,6</sub>	serotonin 5-HT <sub>1</sub>	serotonin 5-HT <sub>2</sub>
–	histamine H <sub>2</sub>	histamine H <sub>3,4</sub>	histamine H <sub>1</sub>
–	–	–	tachykinin NK <sub>1</sub> for substance P
<u>Cl<sup>-</sup></u> – GABA <sub>A</sub> – glycine	GABA <sub>B</sub> (metabotropic)	–	–

## II. Receptors interacting with heterotrimeric G-proteins

### Common structural features:

All of them have **seven hydrophobic  $\alpha$ -helical domains**, penetrate the membrane and connect extra- and intracellular loops.

Few minutes

Neurotransmitters

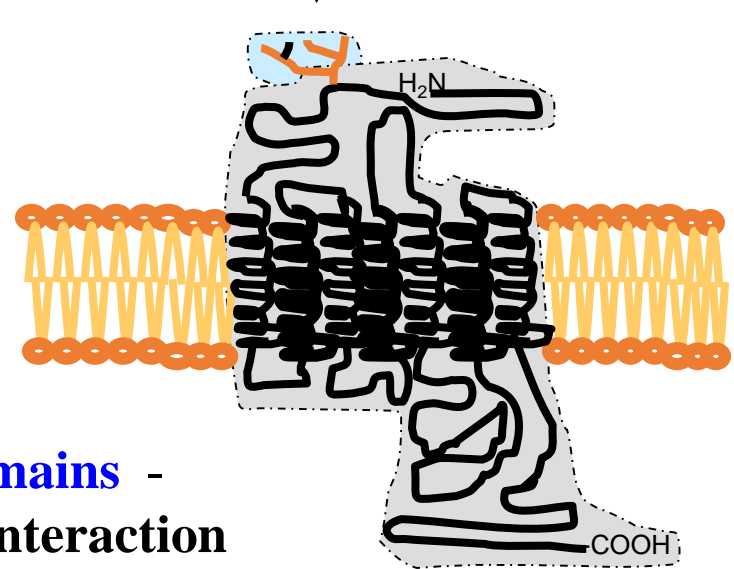
Hormones

Agonist-ligand causes  
signal transduction

Antagonist- prevents

**Intracellular domains** -  
binding site for **interaction**  
with G-protein of **single**  
**specific type.**

**Binding site for  
agonist**(binding sites  
for antagonists are  
also present)



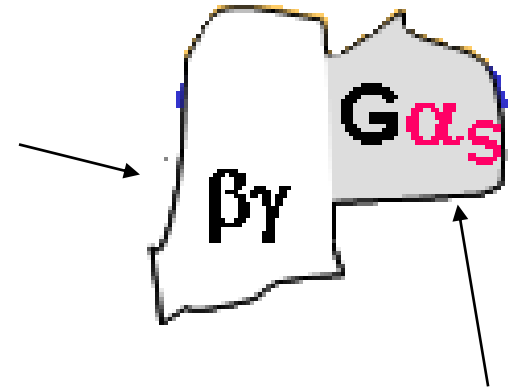
# Heterotrimeric G-proteins

## Proteins **binding GDP or GTP**

Mostly freely bound to cytoplasmatic membrane – they can move along its inner surface.

Subunits  $\alpha$ ,  $\beta$  a  $\gamma$ .

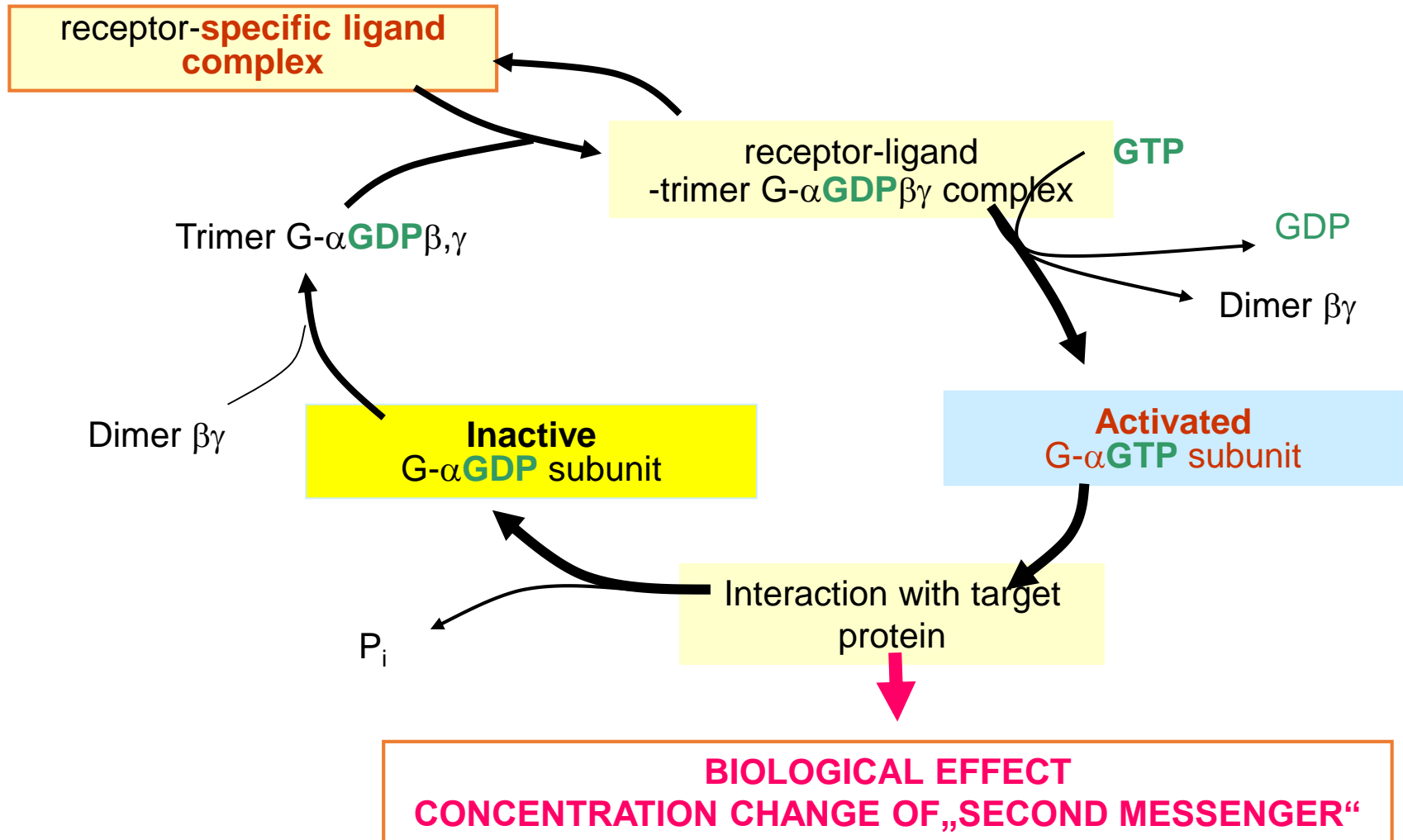
Subunits  $G\beta$   
and  $G\gamma$  are  
hydrophobic  
and are not  
specific.



**Subunits  $G\alpha$**  are the biggest, bind GDP or GTP and are specific for every type of transduction mechanism.

Identified more than 20 types of different  $G\alpha$  subunits.

# Heterotrimeric G-proteins activation cycle by interaction with receptor-specific ligand complex



## Chosen types of G-proteins

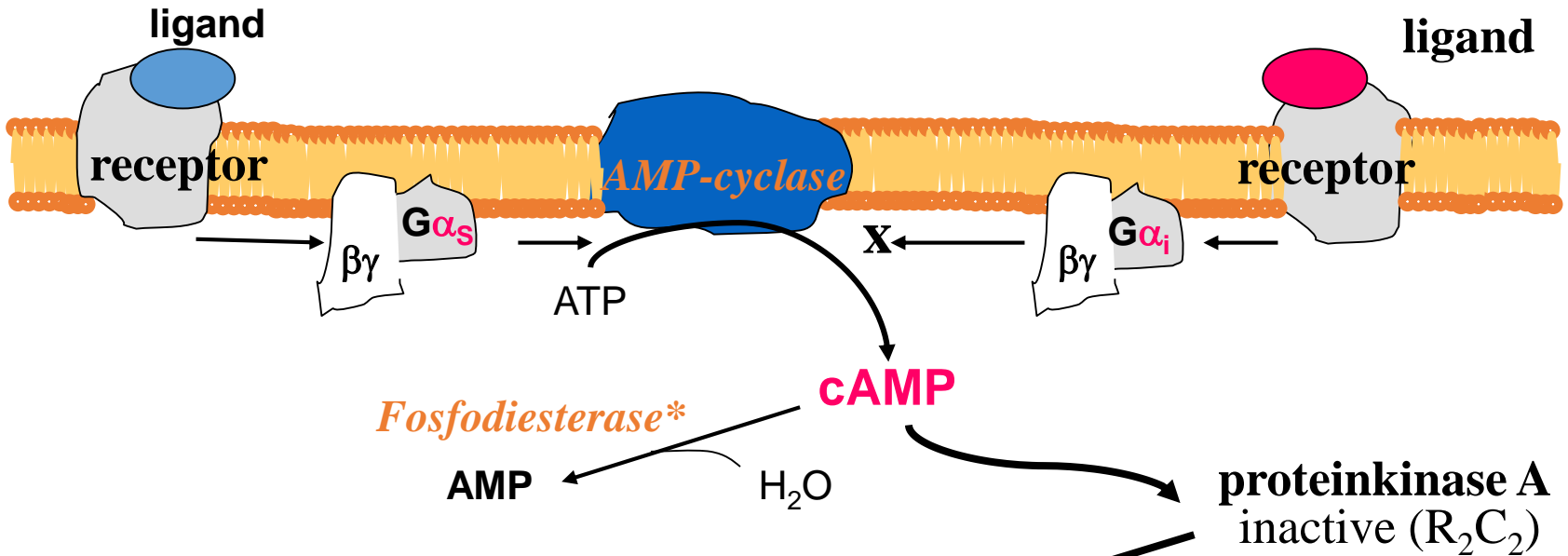
Type of G $\alpha$ subunit	Examples of activating receptor	Effect of activated G $\alpha$ To target protein (intracellular signal)
G $\alpha_s$ (stimulating)	glucagon parathyrine $\beta$ -adrenergic	Adenylatecyclase stimulation (cAMP, Ca <sup>2+</sup> )
G $\alpha_i$ (inhibitory)	somatostatin $\alpha_2$ -adrenergic	Adenylatecyclase inhibition (cAMP, K <sup>+</sup> )
G $\alpha_q$ (activating PI cascade)	vasopressin V1 endothelin ETA,B acetylcholine M1 $\alpha_1$ -adrenergic	phospholipase C stimulation (DG+IP <sub>3</sub> , Ca <sup>2+</sup> )
G $\alpha_t$ (inhibitory) (transducin)	rhodopsin <small>pathobiochemistry- receptors_1</small>	phosphodiesterase cleaving cGMP stimulation

# Receptors activating $G_s$ and $G_i$ stimulate or inhibit adenylatecyclase

*Adenylatecyclase* - membrane enzyme catalysing reaction



cAMP is second messenger.



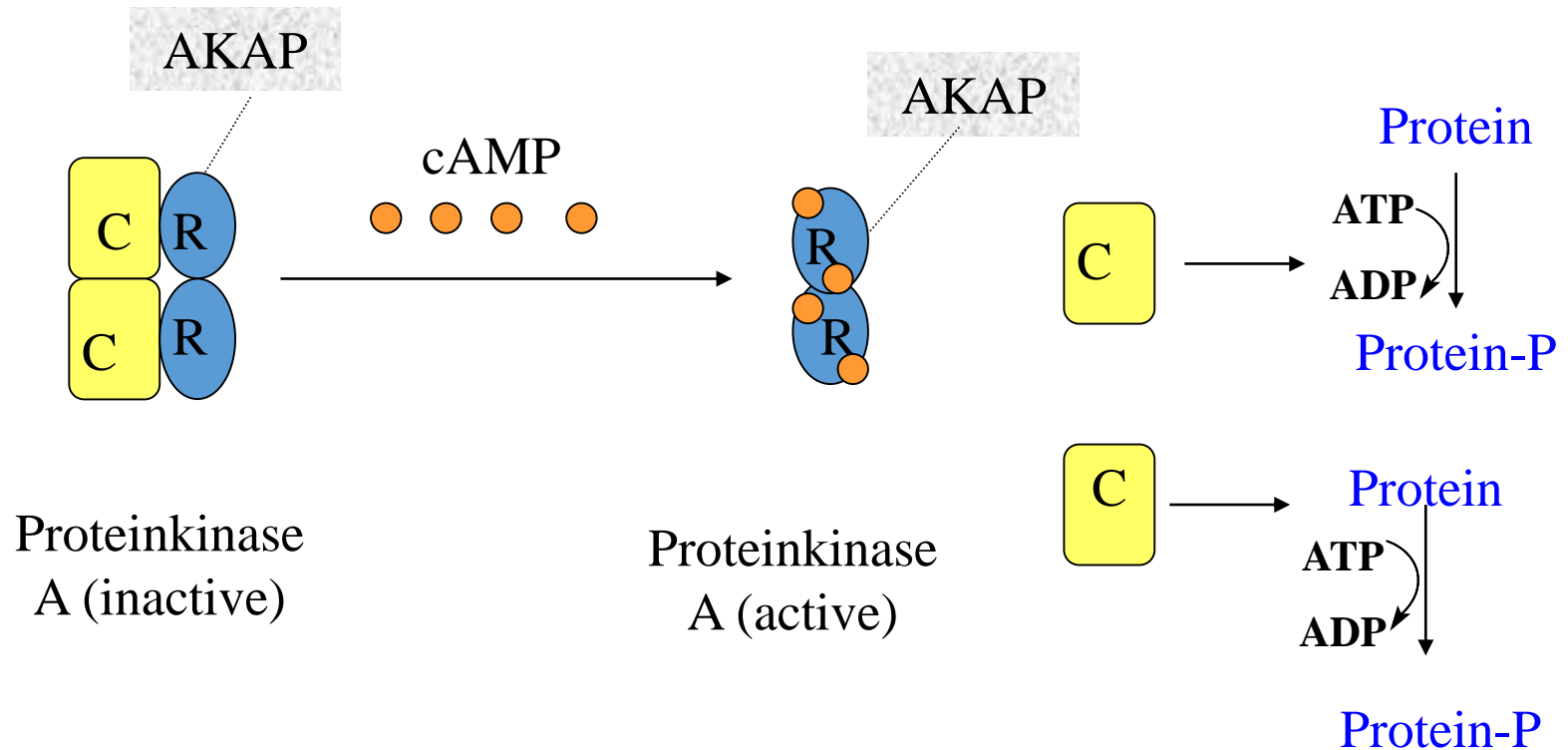
Protein phosphorylation (Thr, Ser)

**active proteinkinase A**



\* Inhibition by e.g. caffeine, theophylline (METHYLXANTHINES)

# Effects of cAMP in cells



## Protein phosphorylation.

In cytoplasm- most often metabolic enzymes (fast response)

In nucleus—gene specific transcription factor CREB (cAMP response element-binding protein) phosphorylation (pomalejší odpověď)

**cAMP** provides many different effects in the cell.

One of the most important effect is **proteinkinase A** activation, which phosphorylates many others metabolic enzymes. The effects of kinases can be aimed for certain proteins phosphorylation. That is maintained by specific proteins binding kinases. In the case of proteinkinase A, it is about so called AKAPs (A kinase anchoring proteins), which serve as supporting structure and localize proteinkinase A position near by certain substrate, which is supposed to be phosphorylated and at the same time, their spontaneous activity is reduced.

**Proteinkinase A** is heterotetrameric molecule, containing two regulatory and two catalytic subunits. In inactive state, subunits are bound to each other. cAMP binds regulatory subunits causing their separation from catalytic subunits, which become active and catalyze phosphate transmission from ATP to serine or threonine residues of target proteins. Proteinkinase A catalytic subunit also enters the nucleus where phosphorylate gene specific transcription factors so called CREB (cyclic AMP response element-binding protein). CREB binds cAMP-responsive element in unphosphorylated state and is poor transcription activator. After phosphorylation by proteinkinase A, CREB bind coactivator CBP (CREB-binding protein) causing transcription amplification. Some **bacterial toxins** modify G-proteins effect. Cholera is an infectious intestinal disease causing severe life threatening diarrheas. Diarrhea is caused by enterotoxin produced by bacteria *Vibrio cholera*. Cholera toxin is protein causing by its effect inhibition of GTPase activity of Gs protein subunit. Modified  $\alpha$ s subunit is „frozen“ in active state continually producing cAMP. cAMP effect is active channel for Cl<sup>-</sup> in intestinal cell membrane and its effect causes chloride ions and water secretion to intestinal lumen. Inhibitory G-protein is the target of pertussis toxin effect, which is produced with whooping cough by bacteria *Bordetella pertusis*. The result is Gi protein inactivation and cAMP overproduction.

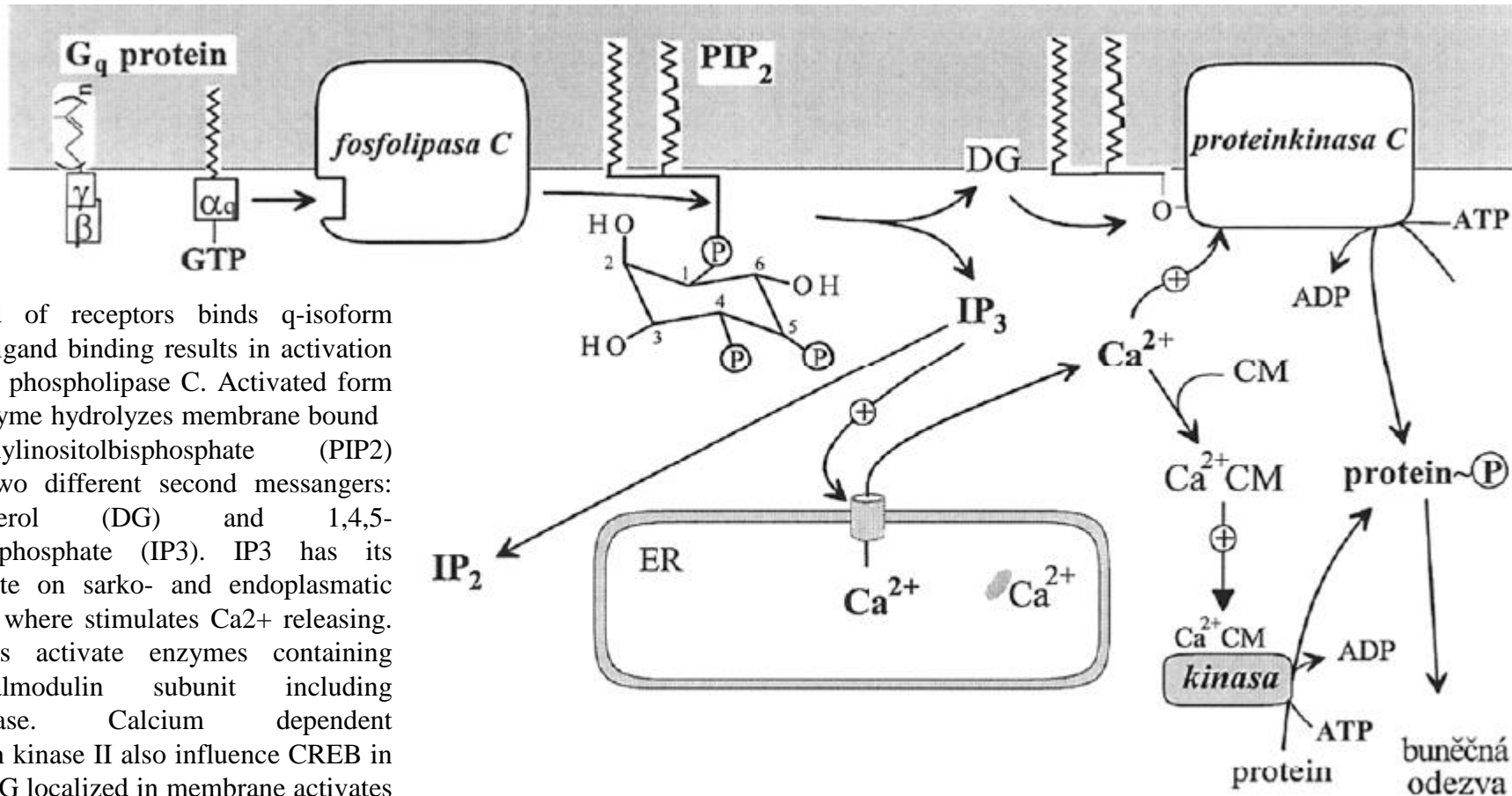
Besides proteinkinase A activation and proteins phosphorylation, cAMP or cGMP can bind also ion channels influencing their permeability. Those mechanisms find its use especially in activation of olfactory and visual perceptions.



# Examples of hormones effecting through PAK activation

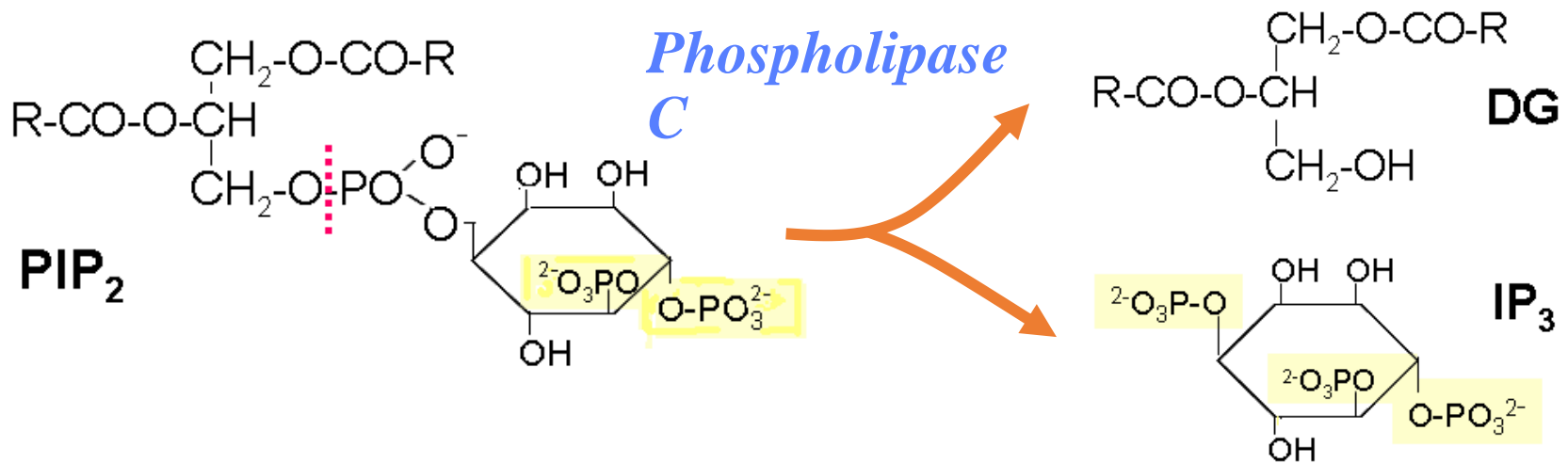
Hormone	Localization of the effect
CRH	Adenohypophysis
TSH	Thyroid follicle
LH	Testicular Leydig cells, yellow body (corpus luteum)
FSH	Ovaria follicle cells, testicular Sertoli cells
ACTH	Adrenal cortex
ADH	Kidney distale tubule cells
PGI <sub>2</sub>	Thrombocytes
Adrenaline, noradrenaline	$\beta$ - receptors in many cells
glukagon	Livers

# III. Receptors activating $G_q$ protein stimulate phospholipase C triggering phosphatidylinositol cascade



That kind of receptors binds q-isoform subunit. Ligand binding results in activation of enzyme phospholipase C. Activated form of this enzyme hydrolyzes membrane bound phosphatidylinositolbisphosphate ( $PIP_2$ ) forming two different second messengers: diacylglycerol (DG) and 1,4,5-inositoltrisphosphate ( $IP_3$ ).  $IP_3$  has its binding site on sarko- and endoplasmatic reticulum, where stimulates  $Ca^{2+}$  releasing.  $Ca^{2+}$  ions activate enzymes containing calcium-calmodulin subunit including protenskinase. Calcium dependent calmodulin kinase II also influence CREB in nucleus. DG localized in membrane activates protein kinase C amplyfying response by target proteins phosphorylation.

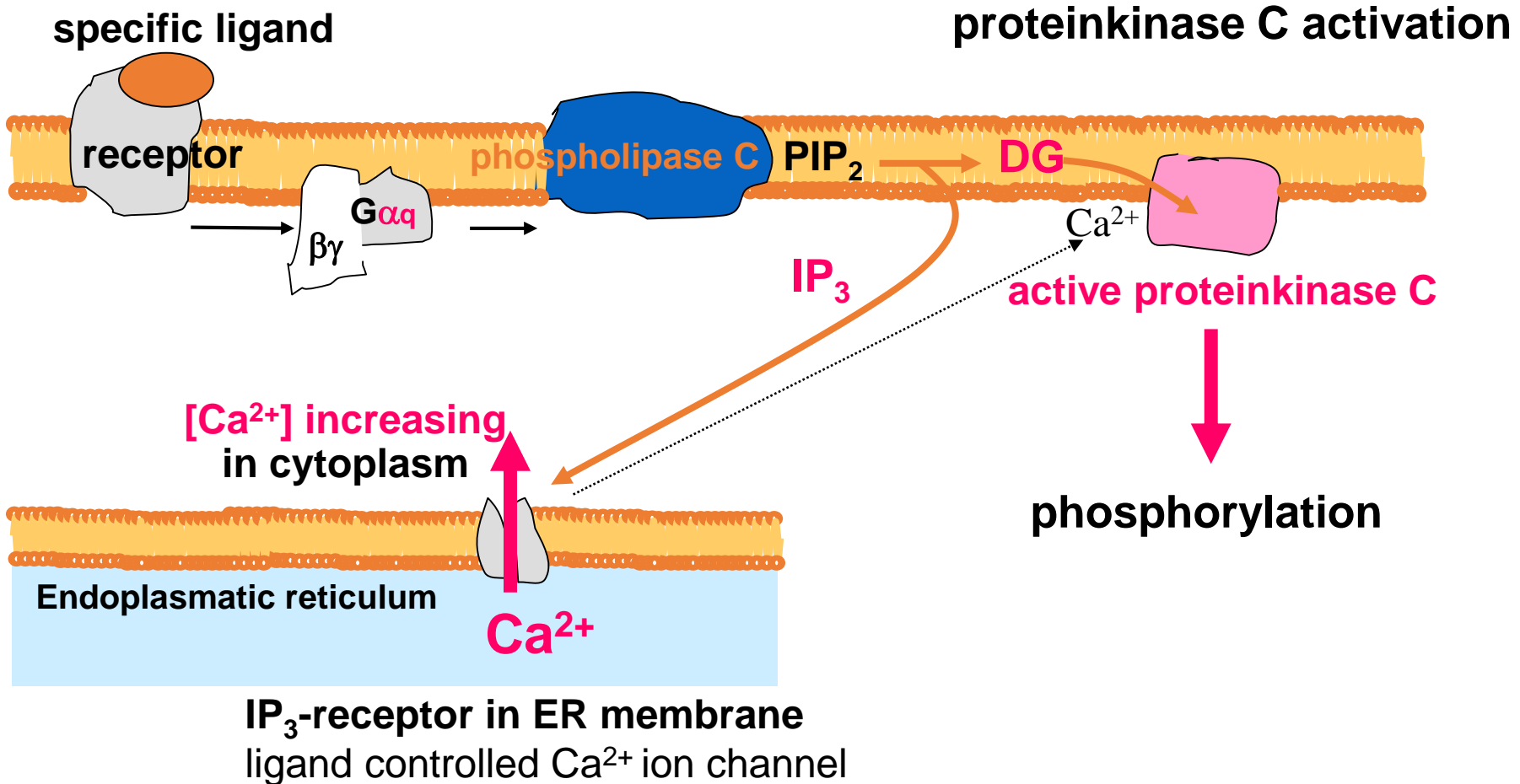
### III. Receptors activating $G_q$ protein stimulate phospholipase C triggering phosphatidylinositol cascade



**Both products are second „messengers“:**  
Inositol-1,4,5-trisphosphate opens  $Ca^{2+}$  channel in ER membrane,  
diacylglycerol activate membrane proteinkinase C.

# TEST

## Phosphatidylinositol cascade



# Regulation of metabolism by cytoplasmic $\text{Ca}^{2+}$ concentration changes

- Basal  $\text{Ca}^{2+}$  concentration in cytoplasm  $\sim 1 \cdot 10^{-7}$  mol/l
- Concentration increasing to  $\sim 1 \cdot 10^{-6}$  fast and effectively activates different cellular process
- $\text{Ca}^{2+}$  increasing may be caused by

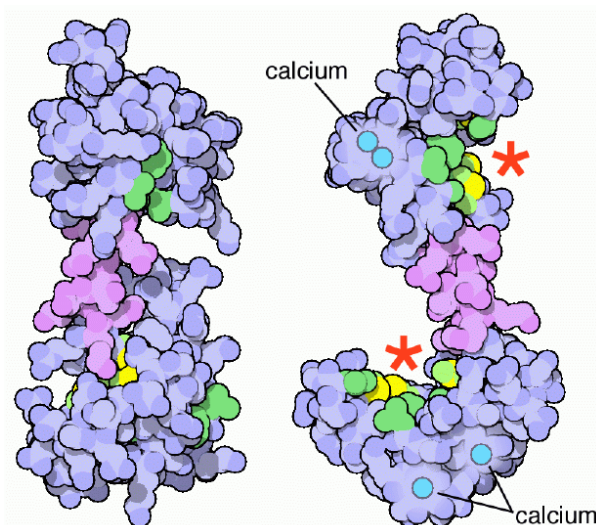
$\text{Ca}^{2+}$  influx through cytoplasmatic membrane (e.g. Smooth muscle contraction)

releasing from intracellular supplies (ER, mitochondrie) e.g.  $\text{IP}_3$  dependent  $\text{Ca}^{2+}$  channel in ER or ryanodine channels in skeletal and cardiac muscle

# Regulatory protein calmodulin

Increasing  $\text{Ca}^{2+}$  level activates numerous [Ca<sup>2+</sup>-dependent](#) proteins forming family of small calcium dependent proteins.

The most important is **calmodulin** which is present in almost all cells.



$\text{Ca}^{2+}$  binding to calmodulin (4 binding sites) changes its conformation and activates its interaction with another proteins, e.g. kinases, phosphatases and others.

Some of Ca-calmodulin-dependent kinases are highly specific, others have wide substrate specificity.

# Examples of hormones effecting through phosphatidylinositol system activation and PKC

Hormone	Localization of the effect
TRH	Adenohypophysis
GnRH	Adenohypophysis
TSH	Thyroid follicle
Angiotensin II/III	Adrenal cortex (zona glomerulosa)
Adrenaline	$\alpha_1$ - receptors

# III. Receptors with enzymatic activity

## III. A) Receptors with guanylatecyclase activity

After ligand binding transform GTP to cGMP

cGMP is second messenger

Activates proteinkinase G

Two kinds of receptors:

- membrane
- cytoplasmatic

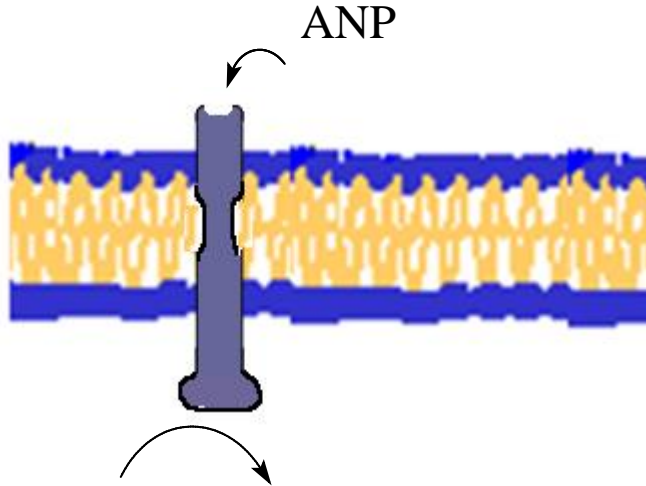
cGMP can be also second messenger. Unlike adenylatecyclase, guanylatecyclase isn't activated by G-proteins.

There are two different types of guanylatecyclase: membrane bound enzymes activated directly by extracellular ligands and soluble enzymes in cytoplasm, reacting to small diffusible molecules. Both types of guanylatecyclase are located in vascular smooth muscle cells.



# TEST

## Membrane receptors with guanylatecyclase activity



Receptors for ANP

Present especially in vascular smooth muscle and in kidneys

ANP is secreted by myocytes of the atria as a response to increasing of blood volume or pressure



*phosphodiesterase*

**GMP**

$\text{H}_2\text{O}$

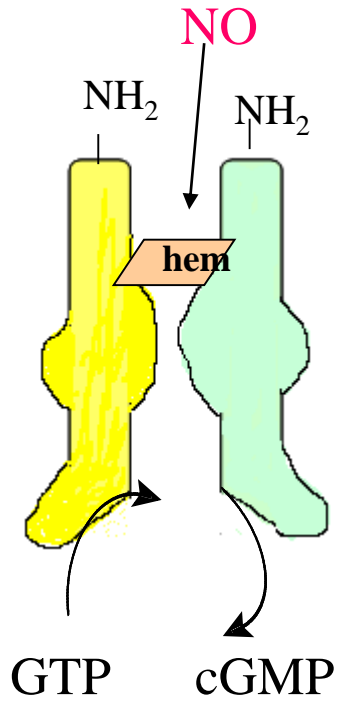
**active proteinkinase G (PKG)**

**Protein phosphorylation**

**proteinkinase G  
inactive**

Membrane bound enzyme is receptor for natriuretic peptides (ANP, BNP, urodilatin). Receptor contains extracellular domain for ligand binding, simple transmembrane helix and intracellular guanylatecyclase domain. Guanylatecyclase activity is initiated by ANP binding to extracellular domain. Likewise cAMP and cGMP has effect through proteinkinase activation. This kinase is called proteinkinase G according the convention. Natriuretic peptides receptors are localized in vascular smooth muscle, kidneys and other tissues. ANP is secreted by myocytes of the atria as a response to increasing blood volume or pressure in right atrium causing vasculature relaxation. That leads to decreasing of total peripheral resistance and improving of local blood flow. In kidneys, causes dilatation of afferent and narrowing of efferent glomerular arteriole and relaxation of mesangial cells. Glomerular capillary pressure and glomerular filtration are increased and that leads to increased sodium and water excretion.

# Cytoplasmic receptors with a guanylatecyclase activity



The receptor is dimeric and binds hem

NO is bound to the hem, its bond rises a catalytic activity of guanylatecyclase

NO is generated by nitroxide synthase (NOS)

NO goes through by membranes easily, it can be generated also by other cells and to the target cell penetrates by diffusion.

Soluble guanylatecyclase

Soluble guanylatecyclase is in the cytoplasm of many cells. It is a dimeric molecule with a hem. It binds NO which causes in its structure conformation changes and rises its enzymatic activity. NO is synthesised by nitroxide synthase (NOS) from arginine. It can be also generated in organism from some exogenous compounds (NO donors), for example nitroglycerine, nitropruside. cGMP is degraded by a few types soluble or in membrane bound cGMP phosphodiesterases. Inhibitors of cGMP phosphodiesterases cause also an increase of cGMP and prolong relaxation of smooth muscles.

phosphodiesterase

GMP

Activation of  
proteincinase G

# Proteinkinase G

cGMP sensitive proteinkinase G

It is spread in a lot of tissues

It phosphorylates different proteins (enzymes, transport proteins and so on)

## Effect of PKG in smooth muscles

Phosphorylation of proteins:

- inactivation of proteins which promote releasing of  $\text{Ca}^{2+}$  from ER  $\Rightarrow \downarrow \text{Ca}^{2+}$
- activation MLC phosphatase  $\Rightarrow$  inhibition of an actin-myosin interaction
- Decrease of an activity of  $\text{K}^{+}$ -channels which promote hyperpolarization  $\Rightarrow$  decrease of an influx  $\text{Ca}^{2+}$  into the cell



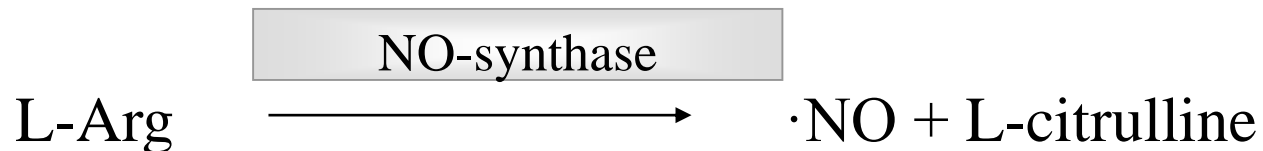
Relaxation of smooth muscles

# Meaning of NO/cGMP signalization in smooth muscles of vessels

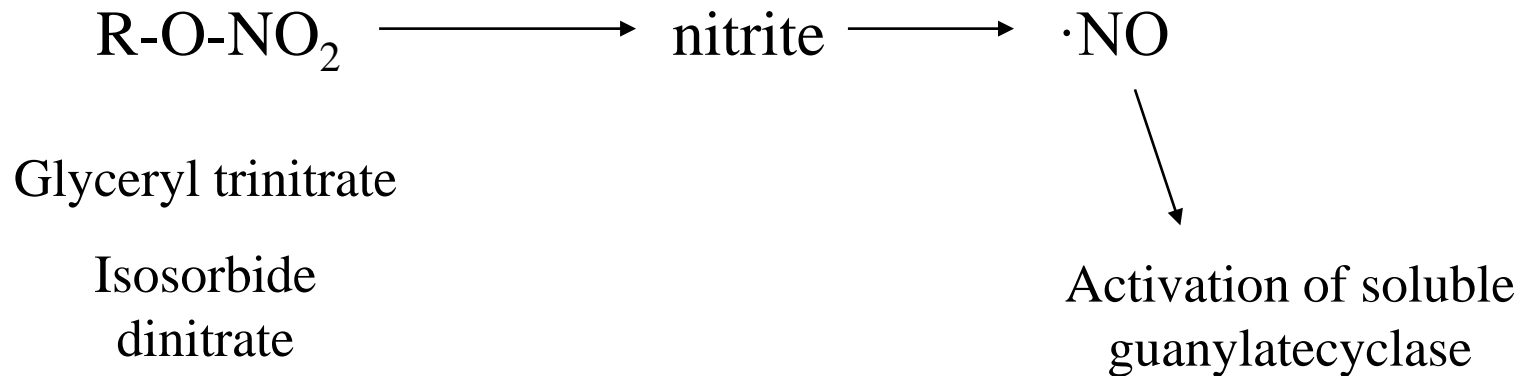
cGMP is a crucial second messenger for an induction of relaxation of smooth muscles in vessels

⇒ vasodilatation and increase flow of blood

NO is produced in endotel cells by nitroxide synthase from arginine (activation for example by acetylcholine) and diffuses into adjacent cells of smooth muscle



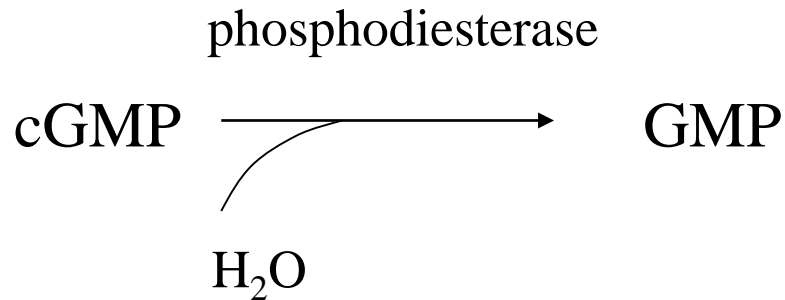
# Drugs like organic nitrates are a source of exogenous NO



## Therapy of angina pectoris

Vasodilatation effect to arteries releases coronary spasm and normalise a perfusion

# Inhibition of phosphodiesterase potentiates the effect of NO



There are more types of phosphodiesterases, depending in a type of cells.

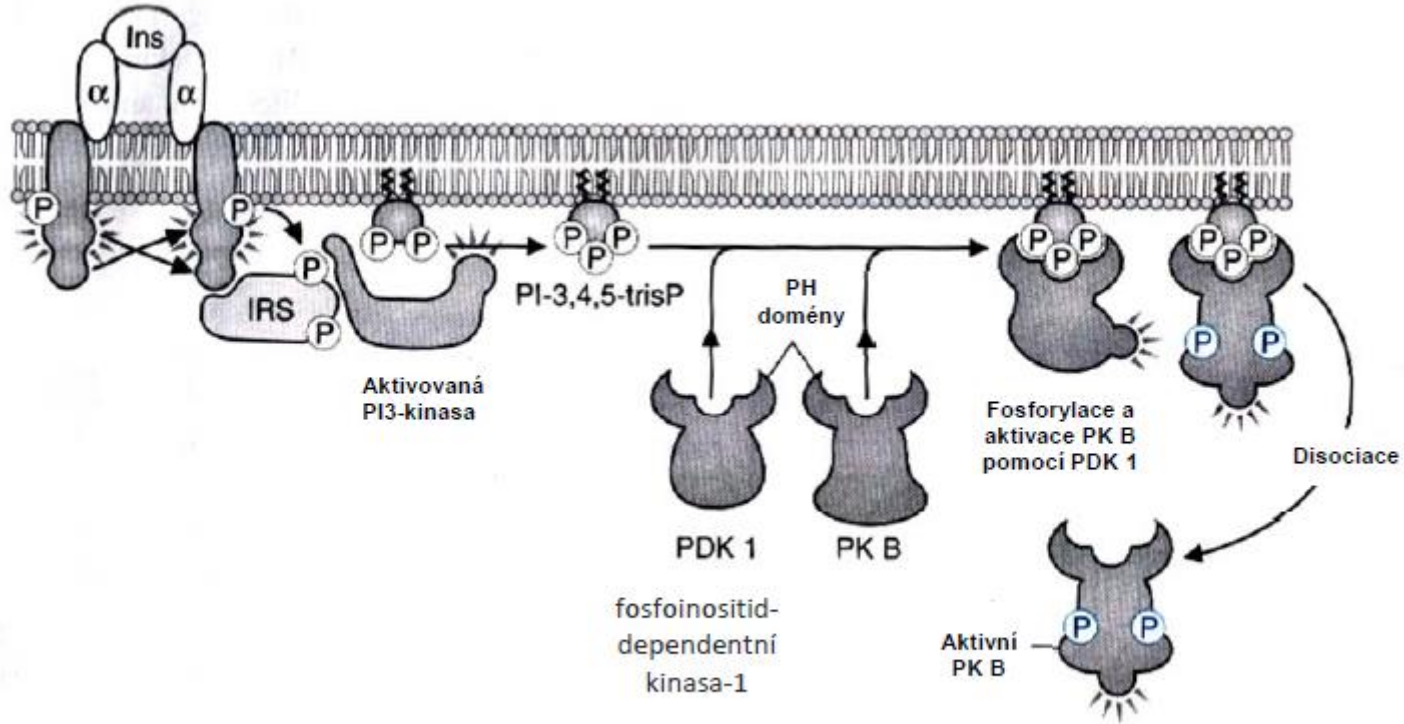
Drug sildenafil (Viagra) is selective inhibitor of phosphodiesterase 5 (PDE5) which is highly expressed in smooth muscles of vessels. Viagra promotes the effect of NO· which is released during sexual stimulation by inhibition of PDE5 and rises a concentration of cGMP in *corpora cavernosa*. The result is a relaxation of smooth muscle in vessels and perfusion of *corpora cavernosa*.

# III. B) Receptors with tyrosinkinase activity

## Collective features

- binding of the signal molecule to the receptor causes conformation changes
- tyrosinkinase activity of receptor is activated
- it caused autophosphorylation of tyrosine of receptor alternatively other proteins (IRS)
- other proteins (adapter molecules) bind to the phosphorylated receptor and substrates phosphorylated by the receptor
- adapter proteins bind to phosphotyrosine residue by SH2 domains (Src homologs of 2 domains).
- adapter proteins react with other molecules and a signal is carried by a cascade of phosphorylation/dephosphorylation reactions by changing of guanine nucleotides, conformation changes and so on.

Membrane receptor family with a tyrosinkinase activity is formed by receptors **for growth factors and insulin**. Growth factors stimulate mitosis, cell differentiation, migration of cells and apoptosis. Insulin stimulates an utilization of nutrients. Collective feature of receptors is intracellular tyrosinkinase domain.



For example IGF-1 (insulin-like growth factor-1) receptor; EGF (epidermal growth factor) receptor; PDGF (platelet-derived growth factor) receptor belong into the subfamily with a tyrosinkinase activity.



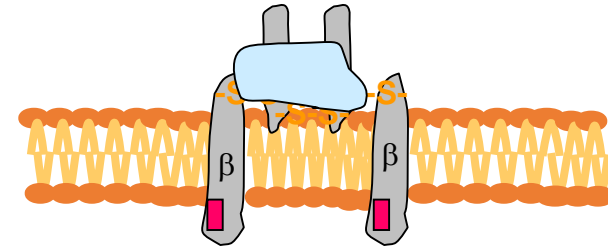
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## Insulin receptor

### Dimeric structure

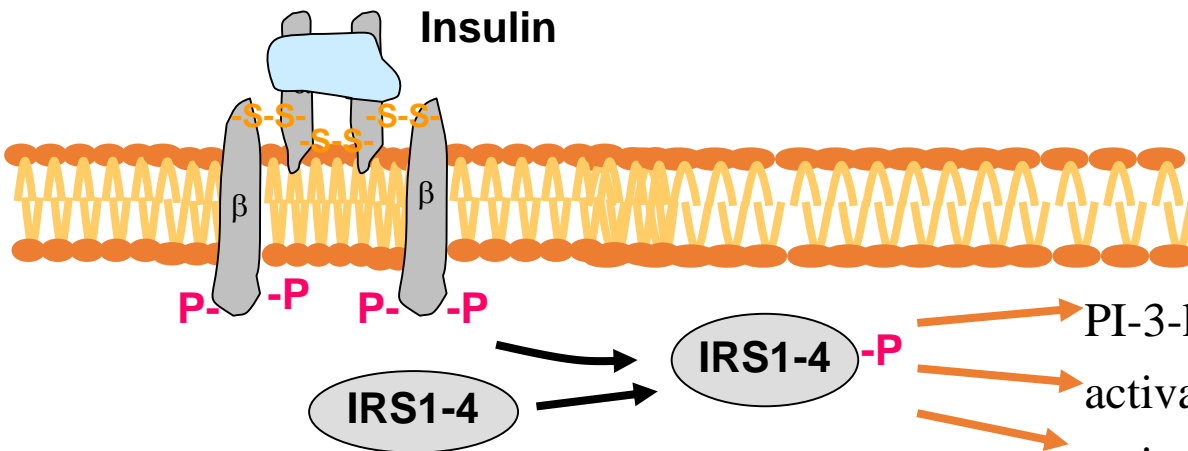
Binding site for insulin on  $\alpha$ - subunits

Tyrosinkinase activity on  $\beta$ -subunits

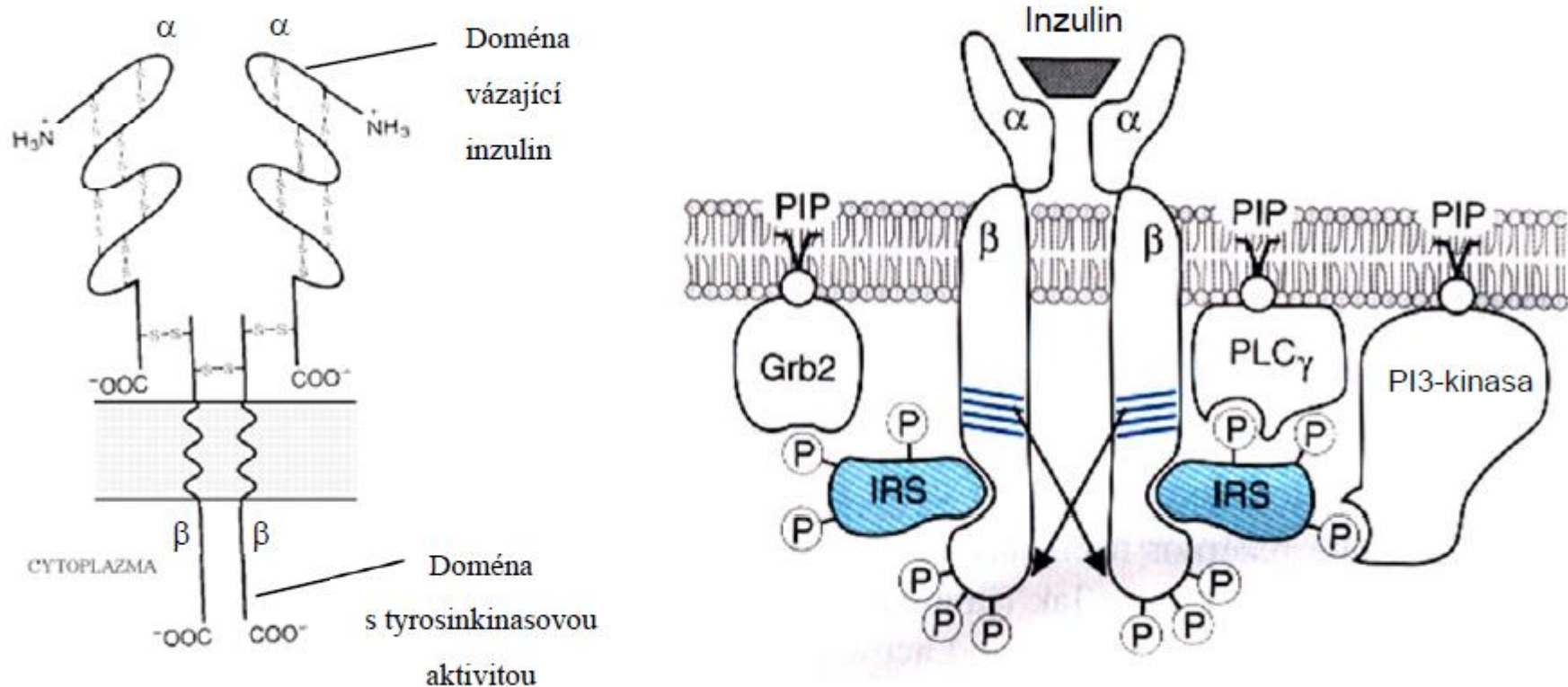


Binding insulin to the receptor causes internalization of complex hormone receptor, receptors are partially recycled

Insulin binding to the receptor  $\rightarrow$  tyrosinkinase activity  
autophosphorylation of  $\beta$ -subunits and **phosphorylation of proteins IRS 1-4**  
(insulin receptor substrates 1-4)



PI-3-kinase binding to the membrane  
activation of phosphoprotein phosphatase-1  
activation Ras – expression of genes



## Insulin receptor

Insulin receptor is in membranes like a dimer. Each monomer consist of extracellular subunit  $\alpha$  and integral membrane subunit  $\beta$ . Subunits  $\alpha$  a  $\beta$  are connected by disulfide bond and disulfide bond is also between monomers. Binding sites for insulin are on  $\alpha$  subunits. Subunits  $\beta$  include domains with own tyrosinkinase activity. After bindind insulin  $\beta$  subunits phosphorylate themselves.



The substrates of the insulin receptor IRS1-4 are **adapter proteins**.  
If the insulin-receptor complex phosphorylated these proteins, they follow more proteins and activate them so.

## **Examples mechanism of action of insulin receptor**

### **Glycogen synthesis**

**Phosphorylation of IRS activates the regulatory subunit of PI 3-kinase**

**The catalytic subunit of PI 3-kinase phosphorylates PIP2 to PIP3**

**PIP3 activates protein kinase B (AKT) activation of PKB (ACP) helps PDK**

**Activated Akt diffuses into the cytoplasm and phosphorylates (inactivates) glycogen synthase kinase**

**Glycogen synthesis is activated (the active form of glycogen synthase is dephosphorylated)**

### **Translocation of glucose transporters**

**Insulin receptor phosphorylates CBI (IRS)**

**CBI-CAP complex translocates into lipid raft membrane**

**CBI is reacted with an adapter protein Crk**

**Crk associated with C3G**

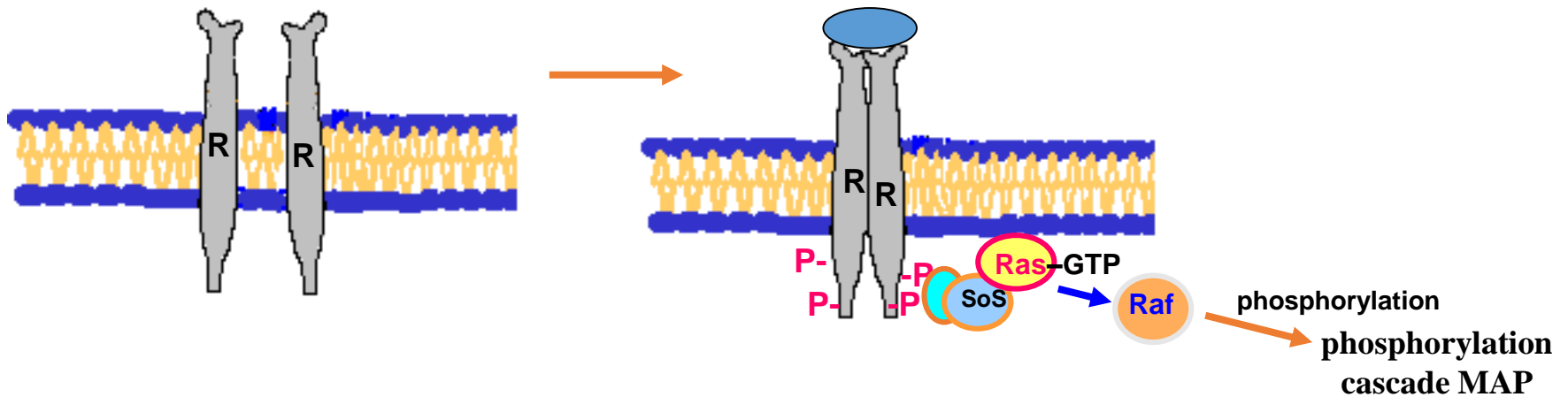
**C3G activates TC10 (G-protein)**

**Activates the translocation of transporters into the plasma membrane**

# The receptor for epidermal growth factor EGF

Receptor with tyrosine kinase activity

Once ligand binding occurs receptor dimerization



This activates the tyrosine kinase activity in the cytoplasmatic domain.

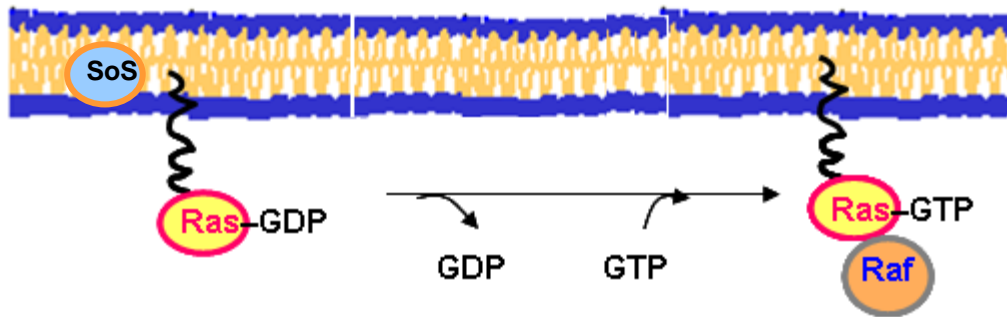
Receptor autophosphorylation

The phosphorylated sites bind Grb2 adapter protein (SH-2 domains).

Through SOS protein is activated by G-protein Ras → activate MAP-kinase cascade (Ras / MAP-cascade)

# Ras is a monomeric G-protein (structural analogue $\alpha$ -subunits)

Monomer G-protein - binds GTP and has simultaneously GTPase activity. Activated GTP binding site GDP



Activation of Ras - a key step in signal transduction. Inactive Ras-GDP switches to active Ras -GTP, which activates another member of the pathway. Inactivation of Ras - subsequent hydrolysis of GTP by using activating protein GTPase activity of G-protein

# Ras superfamily proteins

5 families: Ras, Rho, Arf, Rab, Ran

Anchored to the lipid membrane by lipid anchors (myristoyl, farnesyl)

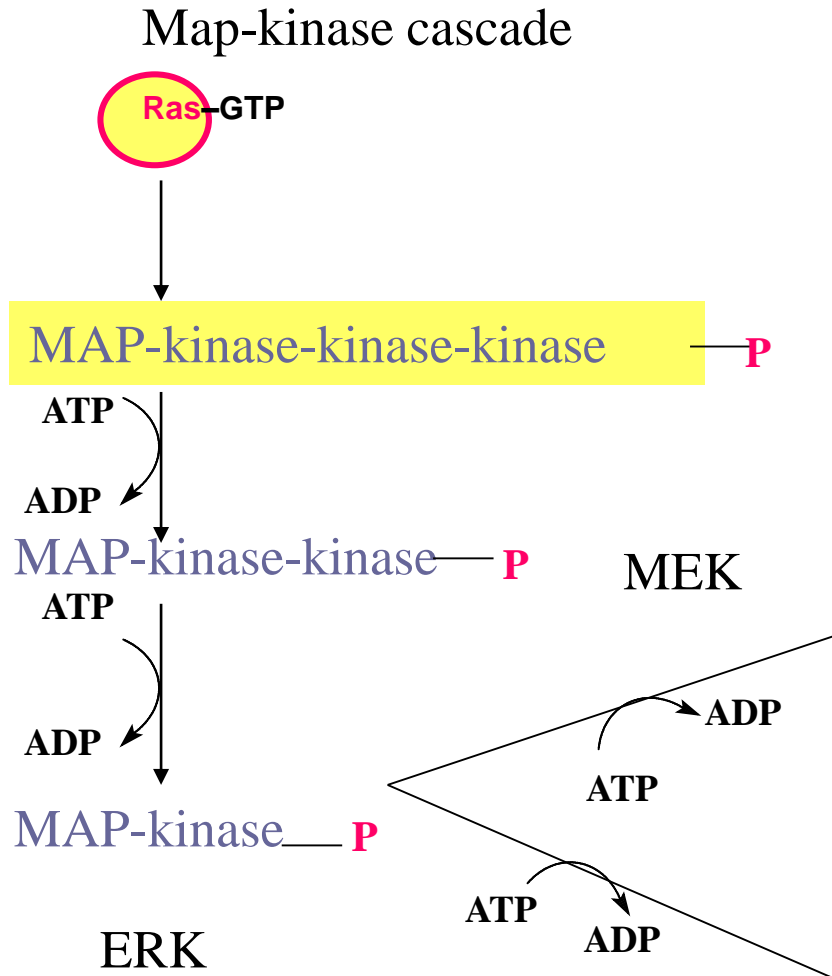
Monomer G-proteins, which play an important role in regulating growth, morphogenesis, cell motility, cytokinesis like.

Mutations in the Ras genes induce proliferation and pathologic antiapoptosis. Ras Mutations occur in about 30% of all human cancers.

# MAP-kinase signaling pathway (Mitogen activated protein kinase)

Described 3 systems, the most famous ERK.

Especially regulates cell growth and differentiation.



MAPKKK, Raf

Phosphorylation of membrane proteins or cytosolic

Phosphorylation of regulatory proteins in the nucleus, promotion of proliferation (e.g. activation of transcription factors Jun, Fos)



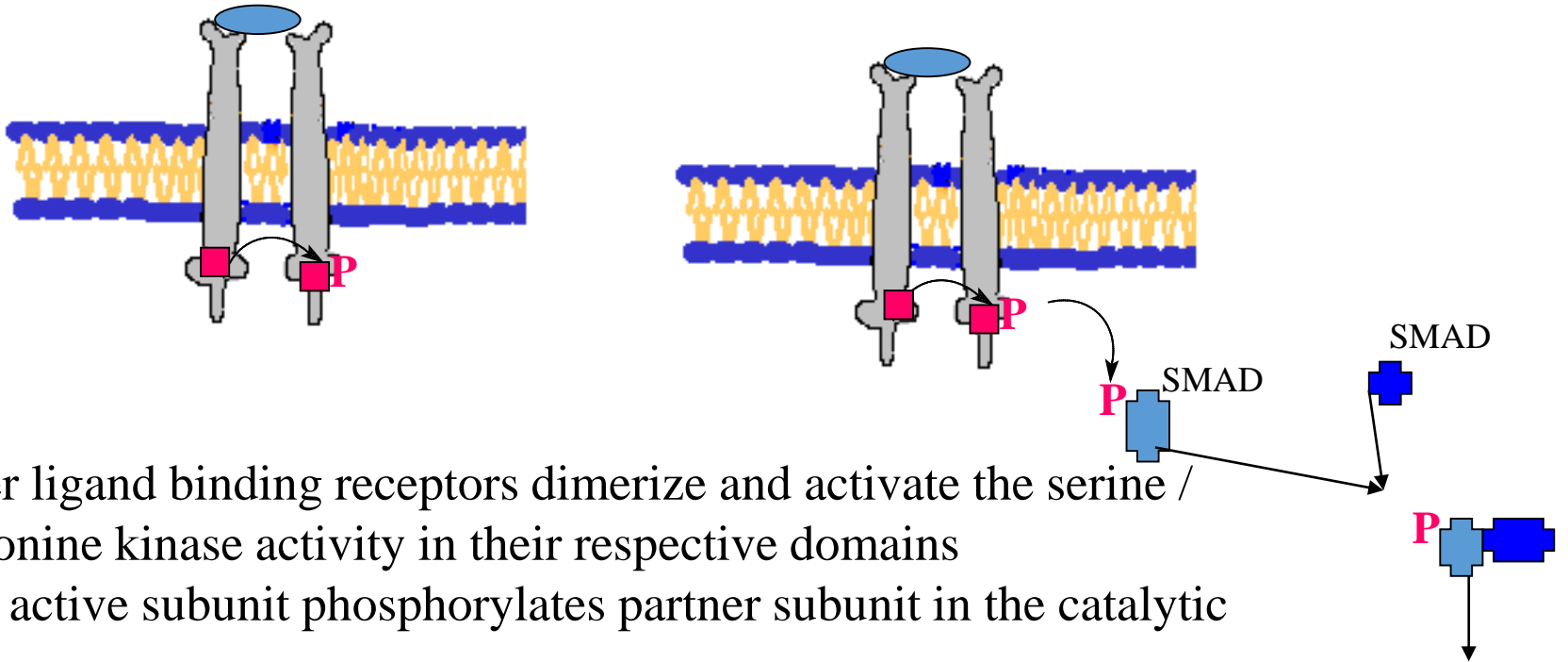
# The mitogens - growth factors supporting the proliferation

Examples of mitogen:

<b>Abbreviation</b>	<b>Name</b>	<b>Function</b>
PDGF	Derived Growth Factor platelet	mitogen for connective tissue cells and undifferentiated neuroglia
EGF	Epidermal growth factor	mitogen series of cells of mesodermal origin and ectodermal
FGF-2	Fibroblast growth factor 2	mitogen for a variety of cells such as fibroblasts, endothelial cells, myoblasts; induces embryonic mesoderm
IL-2	Interleukin 2	mitogen for T-lymphocytes

# Receptors with a serine / threonine kinase activity

The ligand is e.g. transforming growth factor-  $\beta$  (TGF-  $\beta$ )



After ligand binding receptors dimerize and activate the serine / threonine kinase activity in their respective domains

One active subunit phosphorylates partner subunit in the catalytic site

Phosphorylated partner subunit phosphorylates the cytoplasmic proteins SMAD

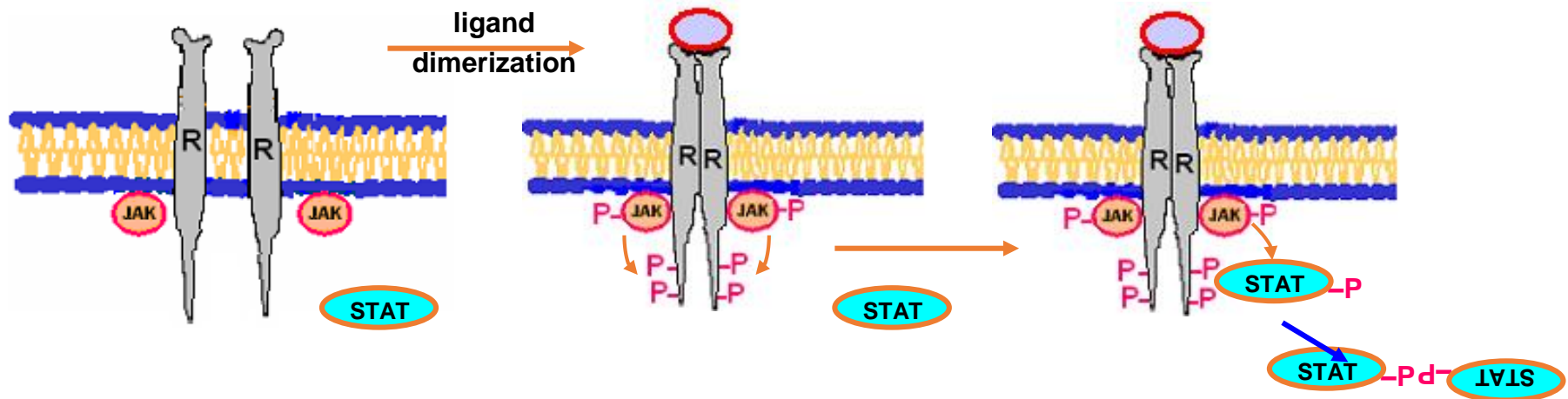
Smad proteins are activated by phosphorylation and forms dimers with other SMAD proteins

The translocation to the nucleus, where they interact with other regulation proteins

# TEST

## IV. Receptors activating non-receptor tyrosine kinase

JAK-STAT receptors (Janus Kinase – Signal Transducer and Activator of Transcription)



Receptor has kinase activity, but is associated with the tyrosine kinase JAK.  
After ligand binding receptors dimerize (homodimers or heterodimers)  
Activated somehow phosphorylate tyrosine residues on the receptor.  
The sites phosphorylated adapter proteins bind STAT (using SH2 domains)  
STAT are phosphorylated and dimerize.  
STAT dimers translocate to the nucleus, where they act as transcription factors

# JAK-STAT receptors - a family of receptors for cytokines \* (e.g.interferons, interleukins)

Diverse cytokine effects are caused by the existence of large amounts of STAT proteins

Receptors for various cytokines bind the various states which produces heterodimers in different combinations

Thus, it is possible that different cytokines affect different genes

Receptors cooperating with the JAK-STAT also have prolactin, erythropoietin ad.

\* Cytokines - small signaling proteins involved in the immune response significantly. They are produced by immune cells (macrophages, T-cells etc.) and are capable of inducing such as rapid division and differentiation of certain cell types involved in the fight against pathogens and other features of the immune defense

# Control Membrane Receptors

Regulation by changing the number of receptors (down regulation, up-regulation)

Control properties receptor (desensitization)

## For example: desensitization of $\beta$ -adrenergic receptor

Upon binding of the ligand to the receptor activates BARK ( $\beta$ -adrenergic receptor kinase)

The cytoplasmic portion of the receptor molecule, the phosphorylation  
The phosphorylated site binding arrestin protein that inhibits the ability to activate G-protein

## TEST

# Intracellular receptors

## Steroid hormones, calcitriol, and iodo thyronine Retinome

Receptors are located in the cytoplasm or in the nucleus

The hormone-receptor complexes bind to specific DNA sites and serve as **transcription factors**

Hormone-receptor complex to the DNA binding site HRE (hormone response element)

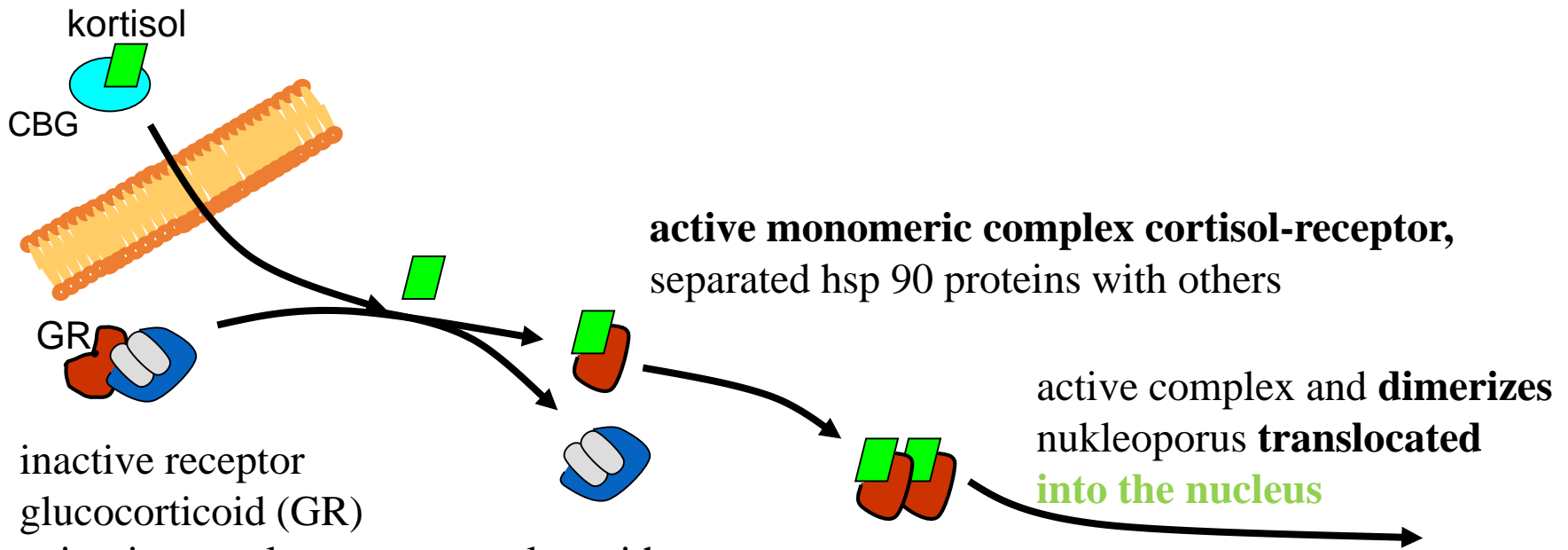
Superfamily of steroid and thyroid receptors - a family of structurally related proteins.

Activation of transcription is a slower process, a response delay

# Example cortisol (glucocorticoid GK, their receptors GR)

cortisol in the extracellular space transmitted CBG (corticosteroid-binding globulin)

hydrophobic molecule penetrates the cell hormone

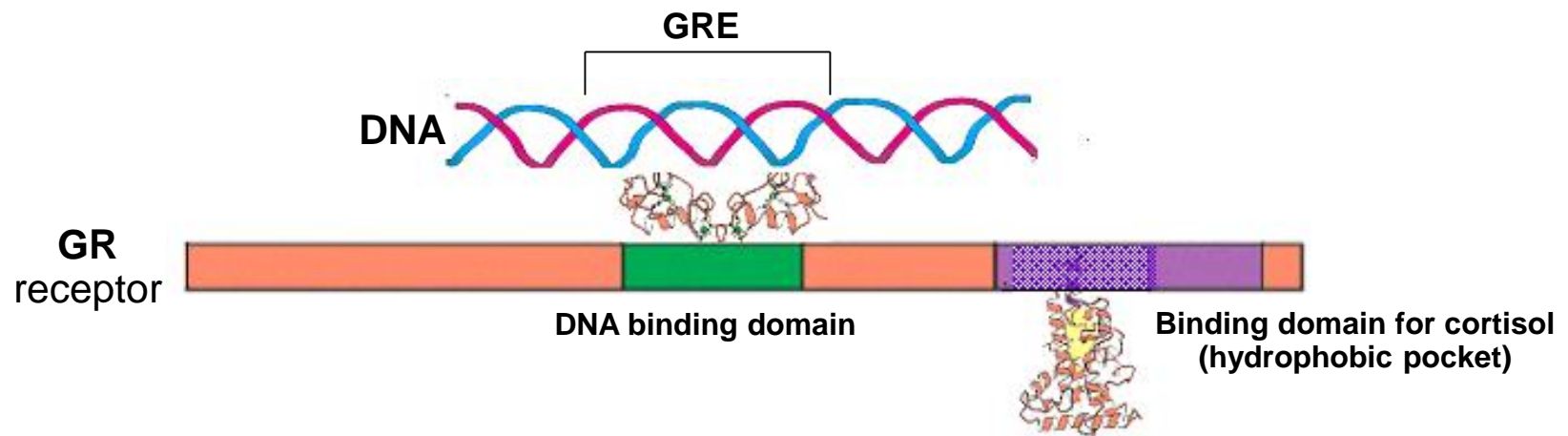


active monomeric complex cortisol-receptor,  
separated hsp 90 proteins with others

active complex and **dimerizes**  
nukleoporus **translocated**  
**into the nucleus**

inactive receptor  
glucocorticoid (GR)  
exists in cytoplasm as a complex with  
a protein dimer  
hsp 90 (chaperone) and other proteins

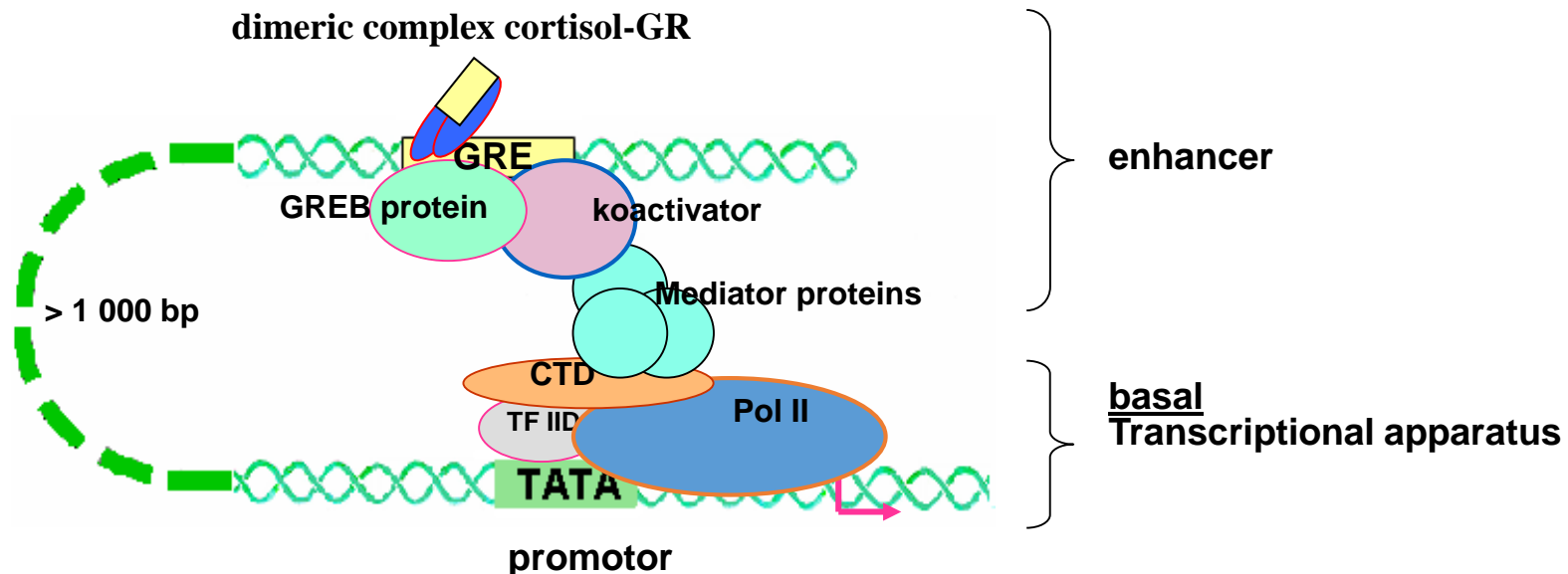
**Dimeric complex cortisol receptor in the nucleus continues to site-specific dsDNA sequences of GRE** (glucocorticoid response element), i.e. on the HRE (hormone response element) specific to glucocorticoids.





## Initiation of transcription cortisol

Active complex cortisol receptor **binds to DNA** in a sequence-specific site **GRE** (glucocorticoid response element, one of the many HRE - hormone response elements). Complex itself, however, hormone-receptor **binding to DNA** and affect transcription is not capable. Connects to a specific coactivator proteins **GREB**(glucocorticoid response element-binding proteins). This complex via **mediator proteins** builds upon the basal transcriptional apparatus to the promoter and initiates transcription.



**GR dimer** – intracellular glucocorticoid receptor (dimer)

**GRE** – glucocorticoid response element

**GREB protein** – GRE binding protein (specific transcription factor)

# TEST

## Overview of the most important neurotransmitter receptors

### Cholinergic synapsis

Receptor	Nicotine	Muscarinic	
Mechanism of action	Ion channel	M <sub>1</sub> , M <sub>3</sub> , M <sub>5</sub> G <sub>q</sub>	M <sub>2</sub> , M <sub>4</sub> G <sub>i</sub>
Second messenger	-	DG + IP <sub>3</sub>	cAMP
Location	<ul style="list-style-type: none"><li>•autonomic ganglia neurons</li><li>•neuromuscular junction</li><li>•chromaffin cells of the adrenal medulla</li></ul>	<ul style="list-style-type: none"><li>•Brain</li><li>•Smooth muscle</li><li>•Glandular cells</li></ul>	<ul style="list-style-type: none"><li>•Myocardium</li><li>•Brain</li></ul>
Block receptor	Tubokurarine	Atropine	

## Adrenergic synapses

# TEST

Receptor	$\alpha_1$	$\alpha_2$	$\beta_1$	$\beta_2$
G-protein	$G_q$	$G_i$	$G_s$	
Second messenger	DG + IP <sub>3</sub>	cAMP	cAMP	
Examples of location	<ul style="list-style-type: none"> <li>•smooth muscles of the GIT (sphincters) and skin blood vessels (contraction)</li> </ul>	<ul style="list-style-type: none"> <li>•adrenergic and cholinergic nerve endings (inhibition of transmitter release)</li> <li>•pancreas (exocrine secretion inhibition)</li> <li>•platelets (aggregation)</li> </ul>	<ul style="list-style-type: none"> <li>•myocardium (increase in strength and frequency of contractions)</li> </ul>	<ul style="list-style-type: none"> <li>•smooth muscle of the uterus, bronchus (relaxation)</li> <li>•smooth muscle of GIT (peristalsis)</li> <li>•pancreas (exocrine secretion activation)</li> </ul>

## The action of the neurotransmitters in the autonomic nervous system

System	Parasympathicus		Sympathicus		
Mediator	Acetylcholine		Acetylcholine		Noradrenaline
Receptor	nicotine	muscarine	nicotine	muscarine	$\beta_1, \beta_2, \alpha_2, \alpha_1$
Location	<ul style="list-style-type: none"> <li>•dendrites ganglion neurons</li> </ul>	<ul style="list-style-type: none"> <li>•membranes of target cells</li> </ul>	<ul style="list-style-type: none"> <li>•chromafinní buňky dřeně nadledvin</li> <li>•dendrity neuronů ganglií</li> </ul>	<ul style="list-style-type: none"> <li>•sweat glands</li> </ul>	<ul style="list-style-type: none"> <li>•membranes of target cells</li> </ul>