

Pathobiochemistry

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Syllabus Pathobiochemistry 2015/2016

1. Introduction, the importance of studying pathobiochemistry. The scope and requirements for successful completion of the course exam, recommended literature. Understanding the regulation of metabolism. Biochemical communication. Receptors.
2. The nucleic acid metabolism disorders of purine and pyrimidine. Hyperuricemia, orotacidurie, therapy. (MB)
3. Metabolism disorders, types and causes. Hereditary metabolic diseases. Enzymes, regulation of metabolism. Causes increased activity of cellular enzymes in the plasma. Clinically significant enzymes.
4. Amino acid metabolism and its disorders. Types of diseases and therapy. (MB)
5. Pathobiochemistry of carbohydrates, glucose metabolism and its disorders. Glycemic control disorders. Pathobiochemistry of diabetes mellitus, types of DM. Disorders of glycogen metabolism, glykogenesis.
6. Disorders of lipid metabolism. Cholesterol, lipoproteins. Lipidosy, dyslipoproteinaemia. (18.3 MB)
7. Blood, blood plasma proteins. Blood clotting, coagulopathy. Dysproteinaemias. Porphyrins. Biosynthesis, metabolism disorders. Porphyria, hemoglobinopathies.
8. Xenobiotics and their effects on the body. Detoxification mechanism. Biological oxidation. The effects of free radicals on the organism. Lipoperoxidation antioxidants.
9. Tumor, tumor markers. Basic characteristics of tumor cells. Strategy laboratory tests. Requirements ideal tumor marker. Used tumor markers.
10. Analysis of urea and the urinary sediment. Immunochemical methods. (.)
11. Mechanization and automation in clinical biochemistry. Analyzers, their distribution from different perspectives. Diagnostic kits. The organization of work in clinical-biochemical laboratory, laboratory and hospital information systems.
12. Pathobiochemistry of arteriosclerosis. Ischemic heart failure - cardiac markers.
13. Relation between Pathobiochemistry and Clinical Biochemistry. Clinical and biochemical analysis and its specific features. Terminology of Clinical Biochemistry. The analyzed material. Material removal. ()

Syllabus of practical exercises:

1. Practice: Analysis of tumor suppressor by immunodetection on membrane. (23.2. MB)
2. Practice: Basic biochemistry. Biochemical analyzer Dimension. (JJ)
3. Practice: Immunochemical methods. Immulite Immunoassay Analyser. (. JJ)
4. Practice: Hematologic methods. (.)
5. Final test. (MB)

Literature:

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- Murray et al. *Harper's Illustrated Biochemistry. 29th Edition.* Lange, 2012.
- KARLSON, P.; GEROK, W.; GROSS, W. *Pathobiochemie.* Academia, Praha, 1987.
- *Laboratorní diagnostika.* Edited by Tomáš Zima. 1. vyd. Praha: Galén, 2003. ISBN 80-7262-201-3.
- *Clinical biochemistry :metabolic and clinical aspects.* Edited by S. K. Bangert - William J. Marshall. New York: Churchill Livingstone, 1995. ISBN 0-443-04341-8. MASOPUST, Jaroslav. *Klinická biochemie. Požadování a hodnocení biochemických vyšetření.* 1. vyd. Praha: Karolinum, 1998. část I. a část II. ISBN 80-7184-649-3.
- *Clinical guide to laboratory tests.* Edited by Norbert W. Tietz. 3rd ed. Philadelphia: W.B. Saunders Company, 1995. ISBN 0-7216-5035-X.

The exam from Pathbiochemistry:

conditions for exam

credit from practical course
(100% presence, credit test 80%)

Exam: 2 parts

- test (60% limit)

.....

- oral examination

from A (90-95% of test)

B (90-80%), C(90-80%), D (80-70%), E (70-60%)

Regulation of the metabolism

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

ENZYMES -BIOCATALYSATORS

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

RECEPTORS (Type of membrane receptors and intracellular receptors)

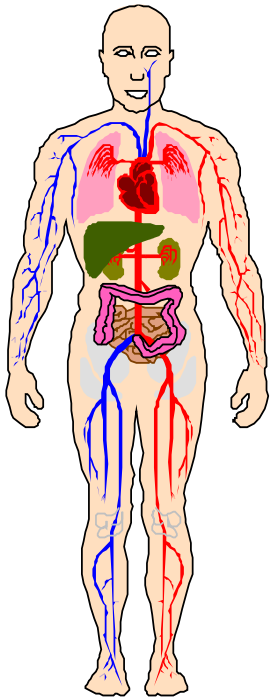
ENZYMES

VITAMINS

METABOLIC REGULATIONS

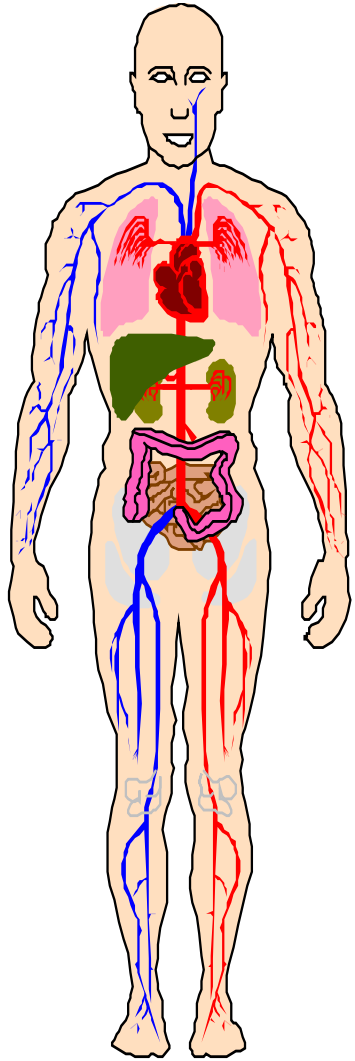
Hormones, Receptors, and Signal Transduction

General principles



- Higher organisms, from the fruit fly to humans, are comprised of **cells**.
- The **cells** often unite to form **tissue** which come together to form **organs** which together make up the **organism**.
- Cells of an **organism** do not live in isolation.
- The **communication** between cells ultimately controls growth, differentiation, and metabolic processes within the organism.
- **Communication** between cells is often by direct cell to cell contact.
- **Communication** frequently occurs between cells over **short and long distances**.

General principles cont...

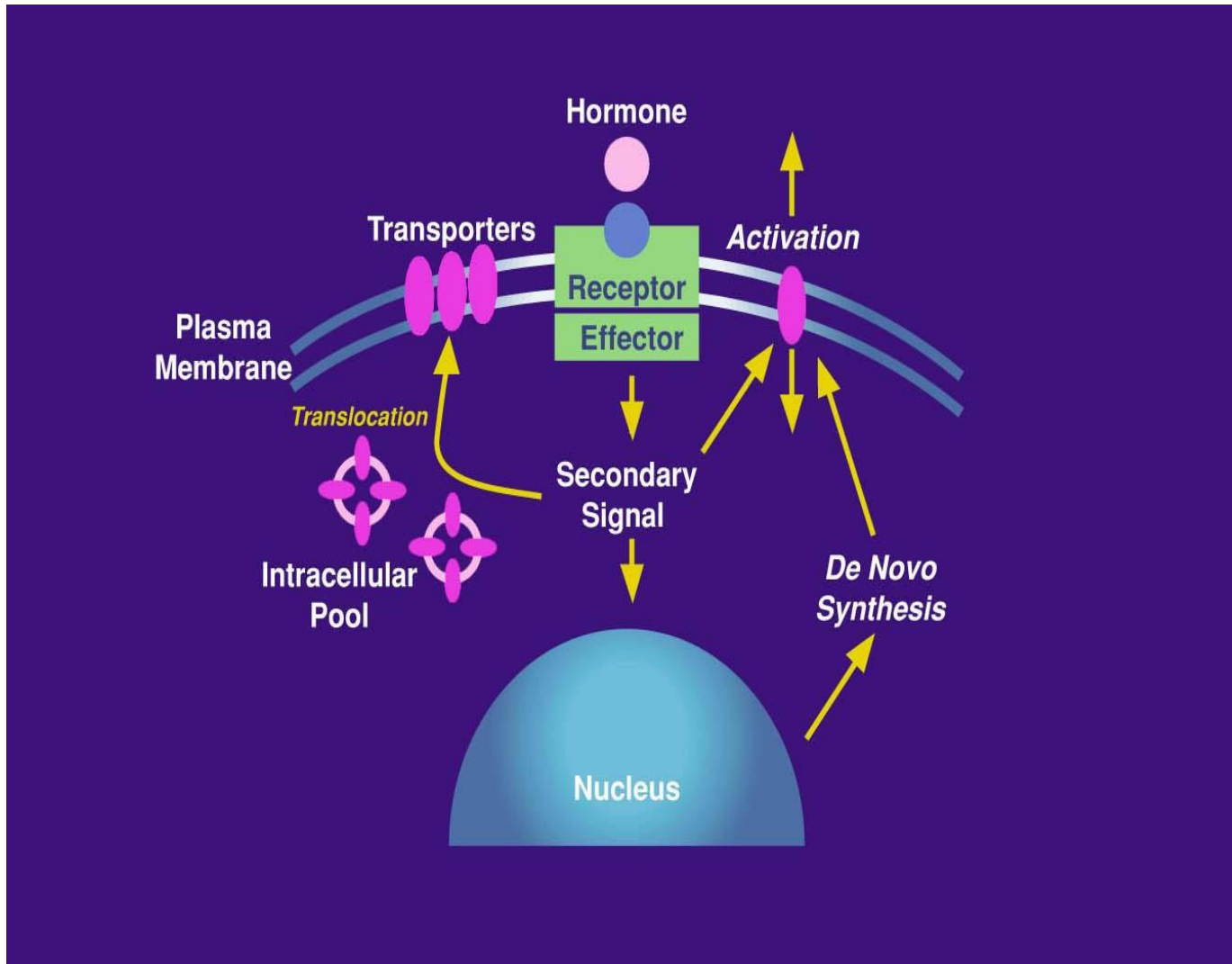


- In cases of short and long distance communication, a substance may be **released** by one cell and recognized by a different **target** cell.
- In the target cell, a **specific** response is induced.
- Cells use an amazing number of **signaling** chemicals.
- These signaling molecules are termed **"hormones."**
- The ability of a hormone to induce a response in a target cell is usually mediated by a hormone **receptor** on, or in, the target cell.

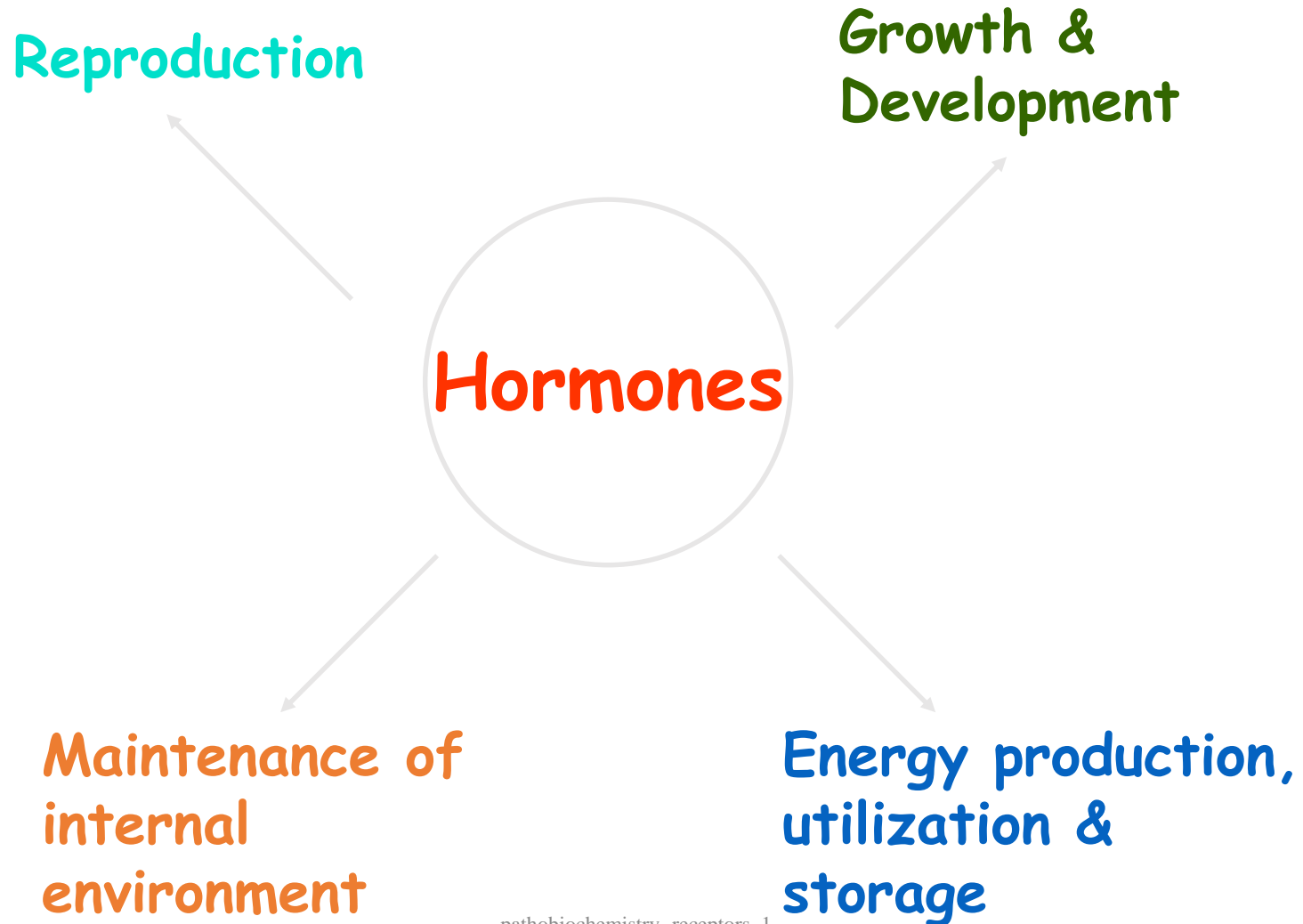
General characteristics of hormones

- Hormones are molecules synthesized by **specific** tissue. Classically these tissue were called **glands**.
- Hormones are secreted directly into the blood which **carries** them to their sites of action.
- Hormones are present at very **low** levels in the circulatory system.
- Hormones specifically affect or alter the activities of the responsive tissue (**target** tissue).
- Hormones act specifically via **receptors** located on, or in, **target** tissue.

Hormone/Receptor Interaction Secondary Signals



The four primary arenas of hormone action

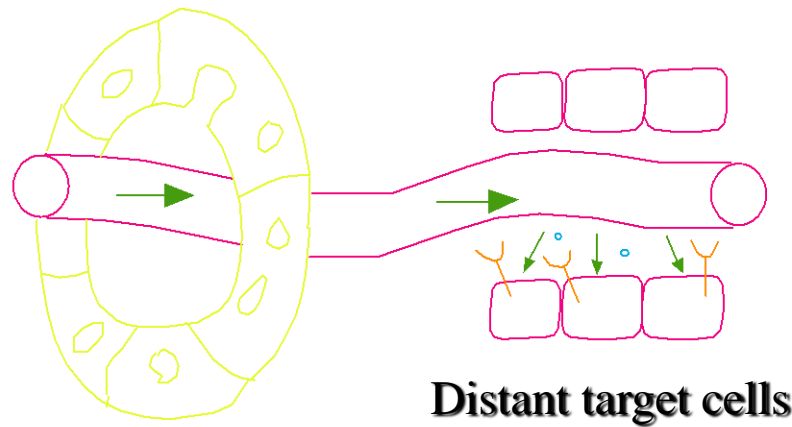


Definitions

- 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

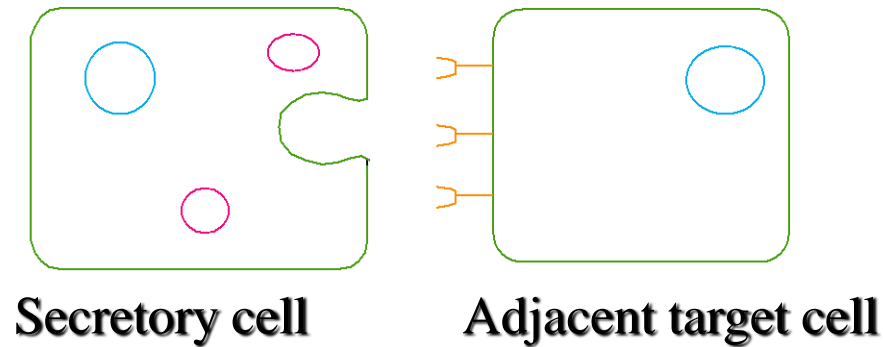
Hormone secretion into blood by endocrine gland



Blood vessel

Distant target cells

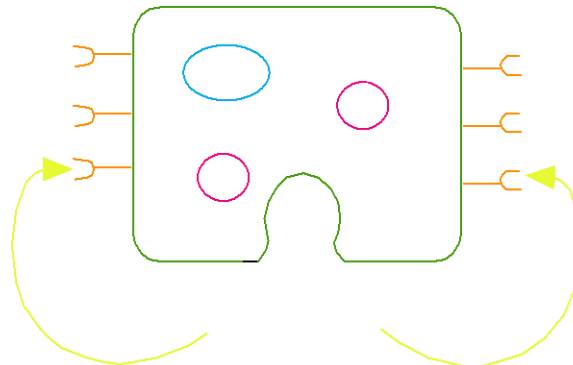
Paracrine



Secretory cell

Adjacent target cell

Autocrine



Receptor

Hormone or other extra cellular signal

Target sites on same cell

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 |
|---|--|
| HORMONES | secreted by endocrine glands, scattered glandular cells, eicosanoids a lot of other types of cells |
| NEUROHORMONES | secreted by neurons into the blood circulation |
| NEUROTRANSMITTERS | secreted in the synaptic ending |
| CYTOKINES, GROWTH FACTORS, EICOSANOIDS | 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)

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.

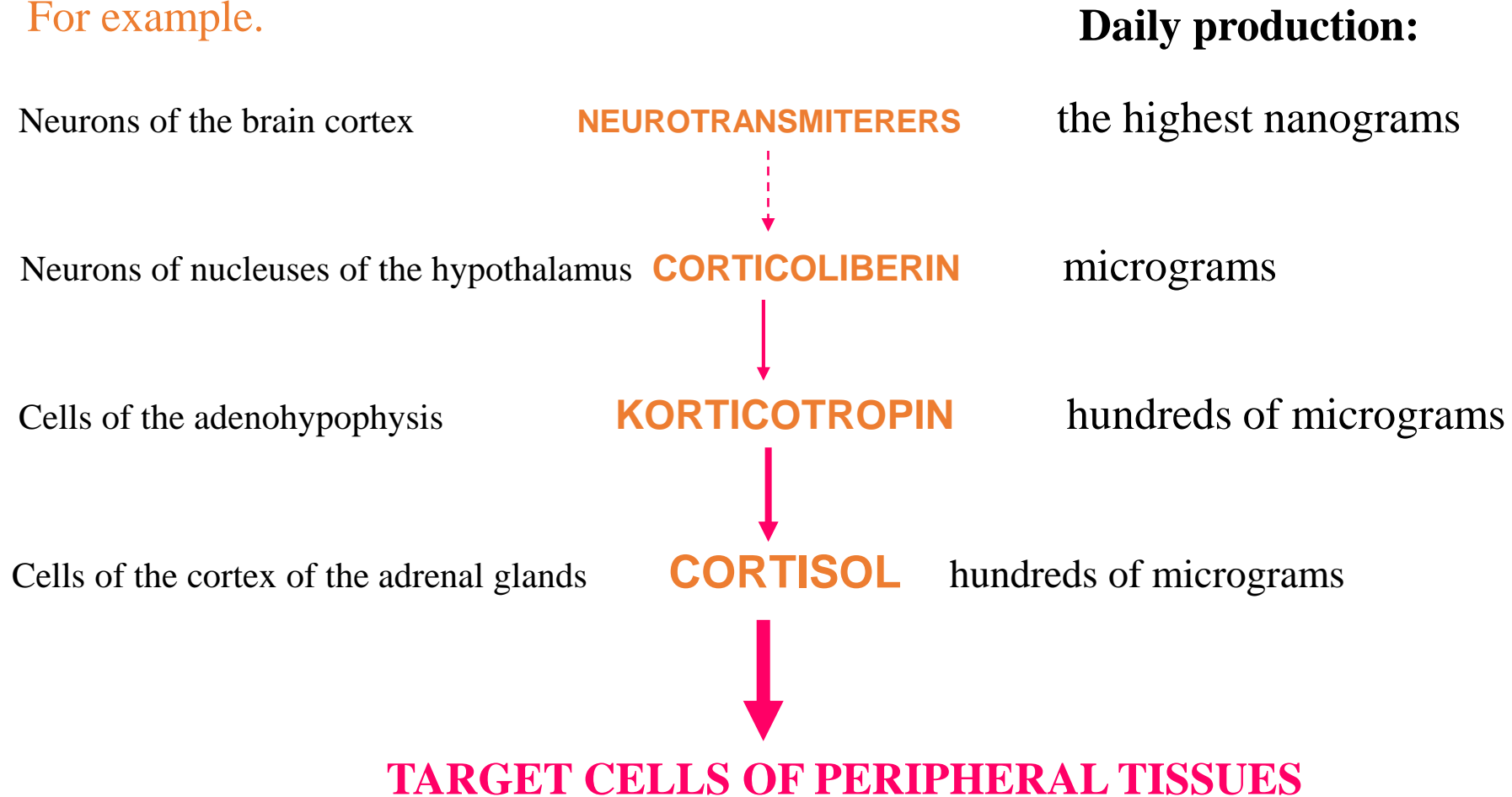
Activated receptor can enter into the reaction with other cellular components and realise the process of the signal transduction.

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 10⁴–10⁵ 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

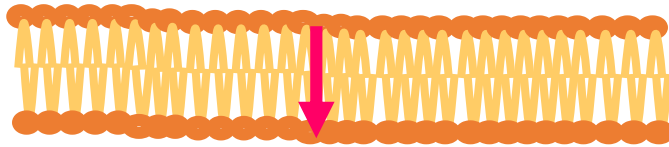
Nonpolar signal molecules
(steroids, thyroid hormones, retinoids)

TEST

Membrane and intracellular receptors

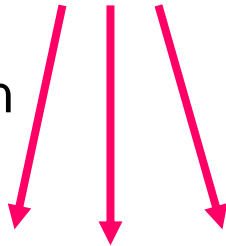
Polar signaling molecules

Membrane receptor



Transduction of signal

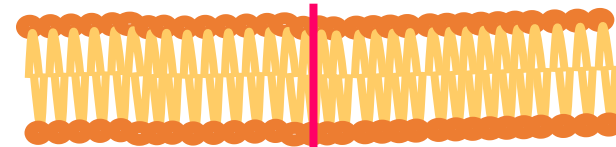
Amplification



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

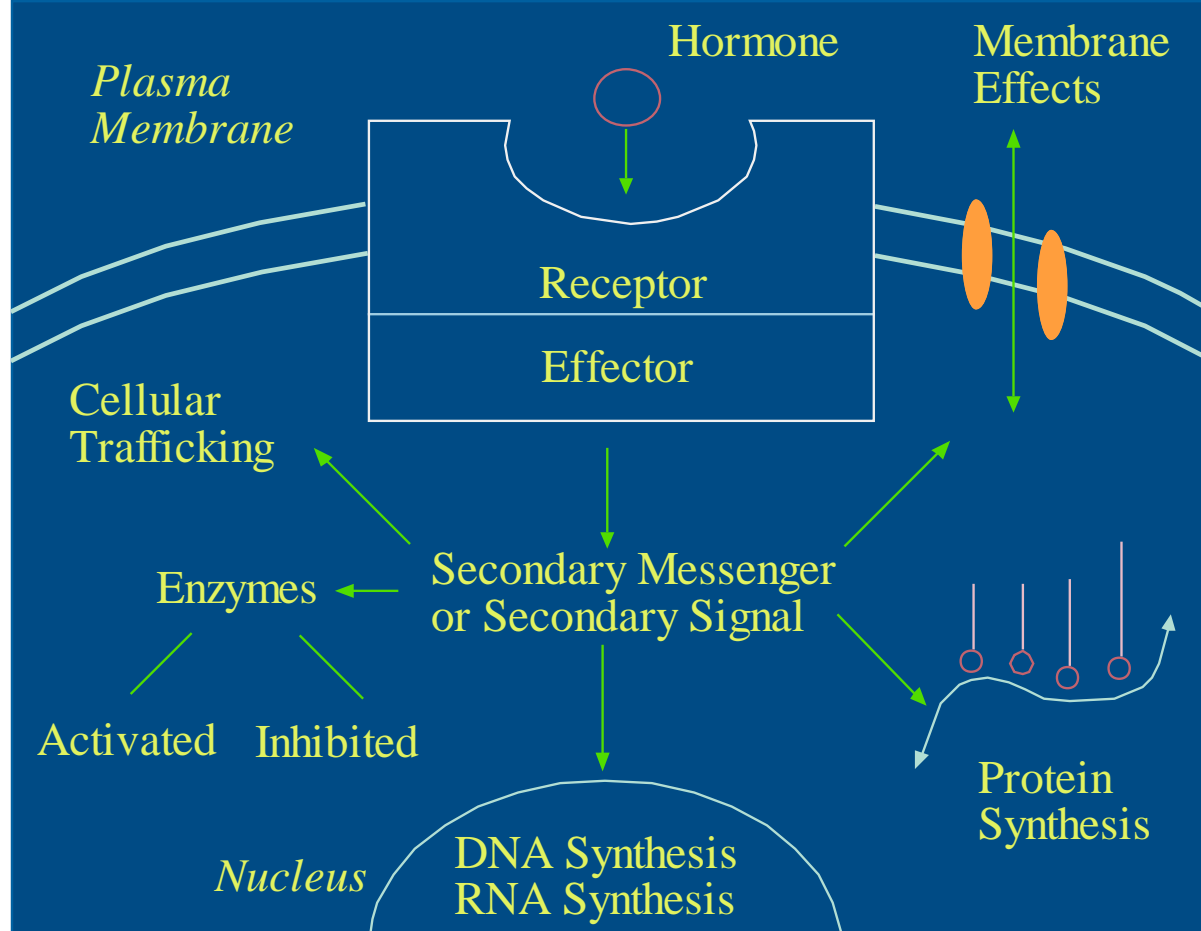
Biological effect
(slower effect)

Receptors

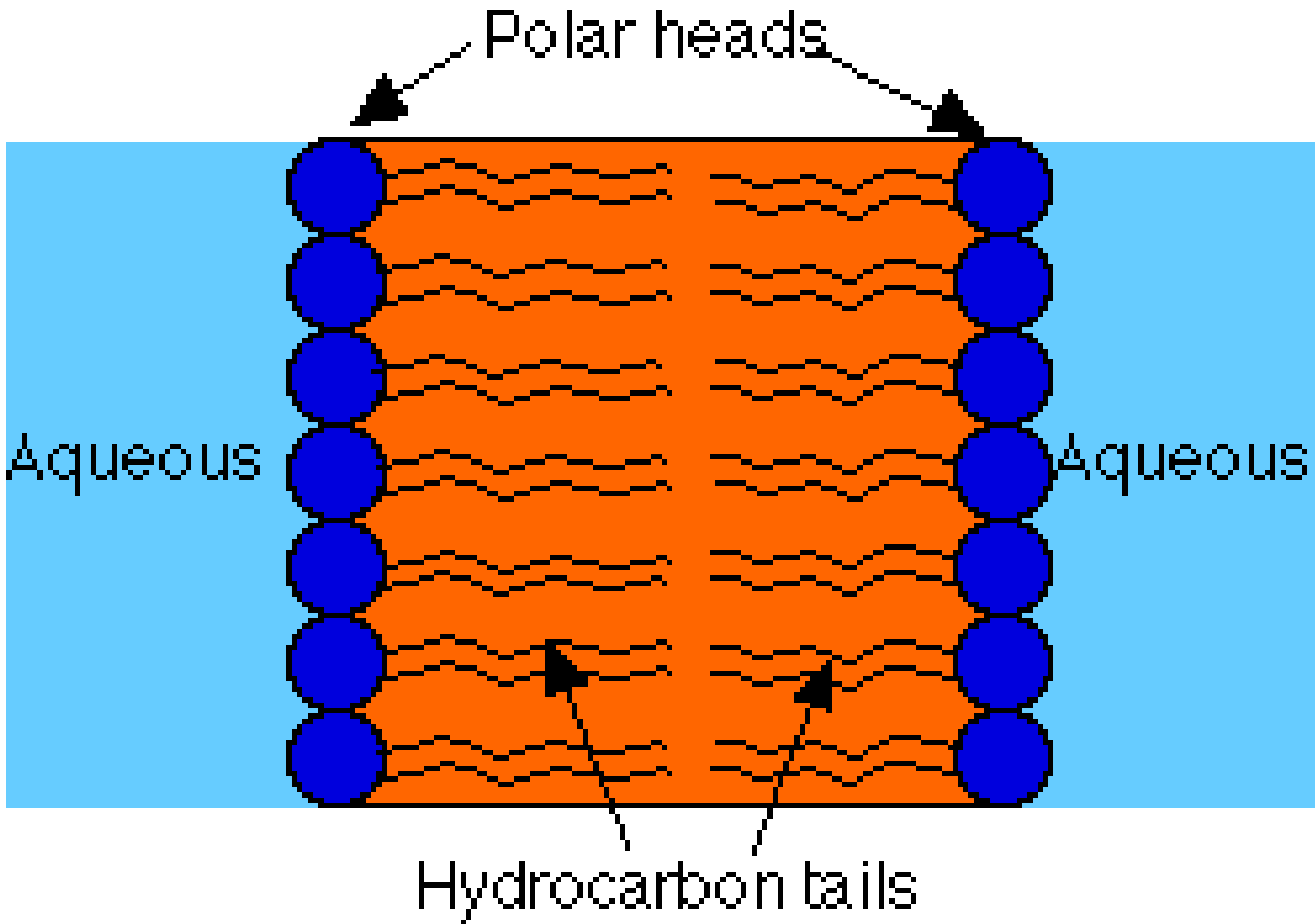
Nuclear receptors
estrogens

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 |

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**

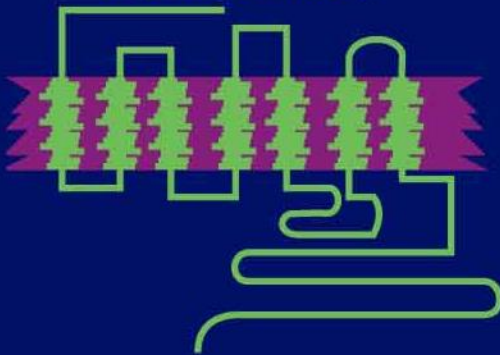
b) **proteinkinase**

IV. Receptors cooperating with the non-receptor tyrosine kinases

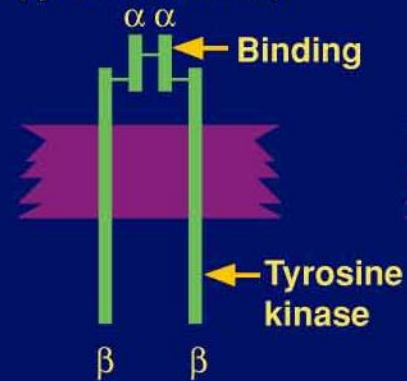
(eg. JAK) – receptors for somatotropin (GRH), prolactin, erythropoietin, interferons, interleukins and other cytokines.

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

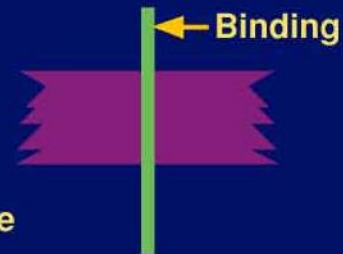
β -adrenergic receptor
(7-transmembrane domains;
G-protein linked)



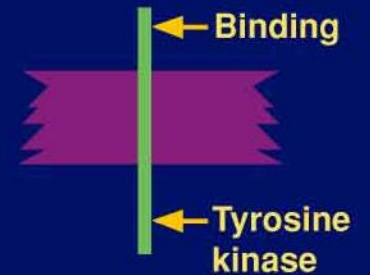
Insulin receptor
(tyrosine kinase)



GH receptor



EGF receptor
(tyrosine kinase)

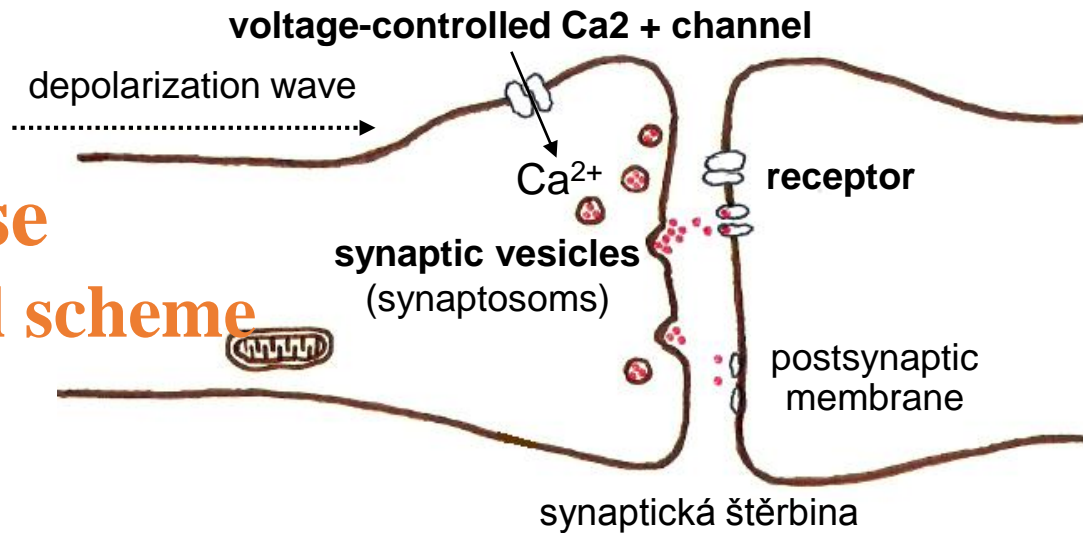


I. Receptors – ion channels

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 Na^+/K^+ ,

glutamate (CNS, some afferent sensory neurons)

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

inhibitory receptor **GABA A** (CNS) - channel for Cl^-

Metabotropic receptors activating G proteins, e.g.

protein **G_s** adrenergic β_1 a β_2 , receptor GABAB, dopamine D1,

protein **G_i** adrenergic α_2 , dopamine D3,

muscarinic acetylcholine M2 (also opens K^+ channel),

protein **G_q** muscarinic acetylcholine M1, adrenergic α_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.

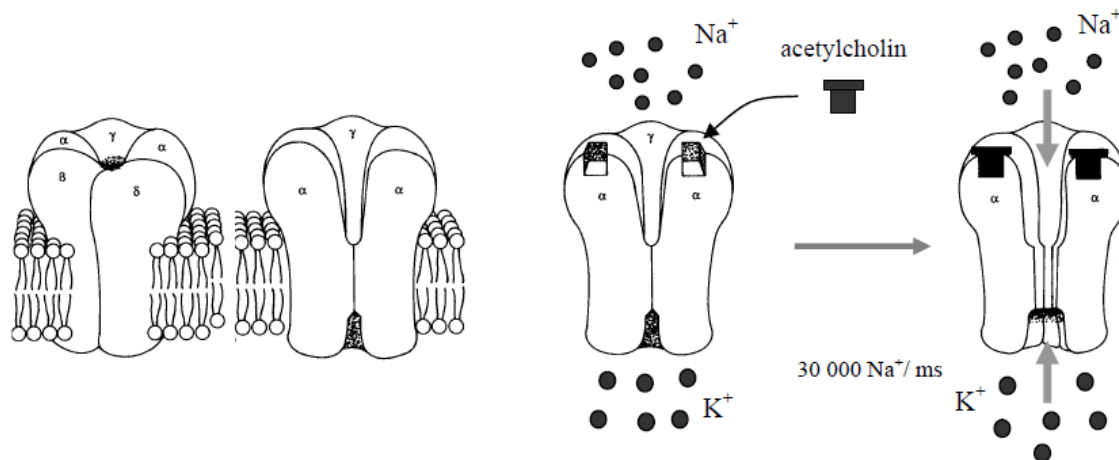
In peripheral nervous system

- efferent neurons
- excitatory **acetylcholine**
- noradrenaline**
- primary sensory afferent
- excitatory glutamate
- (A β fibers, tactile)
- substantion P (peptide)
- (C A δ 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 α , β , γ , δ , ϵ) 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 Ca²⁺ channels, which open when the action potential is expanded to the membrane. Increased levels of Ca²⁺ in the neuron endings activates the Ca²⁺-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⁺ 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.

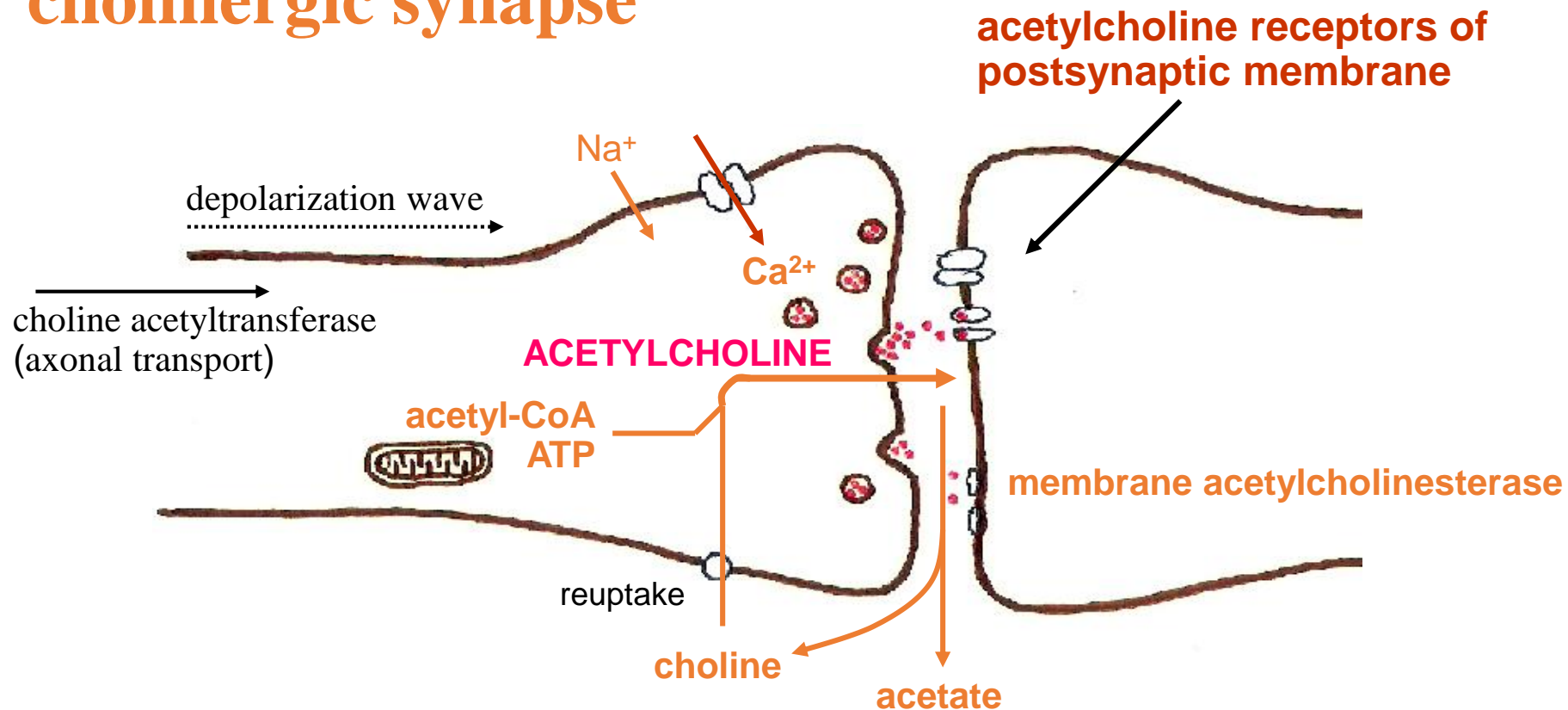


Major neurotransmitter receptors

Acetylcholine receptors

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

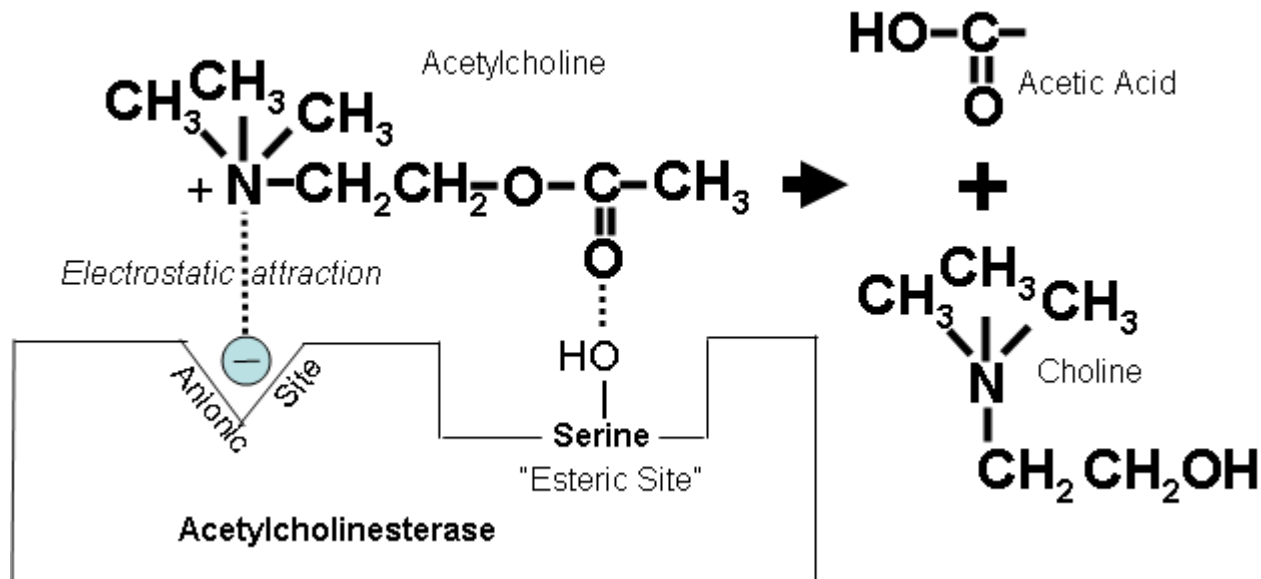
cholinergic synapse



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.

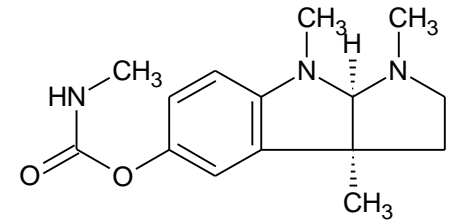
Acetylcholinesterase

- 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)



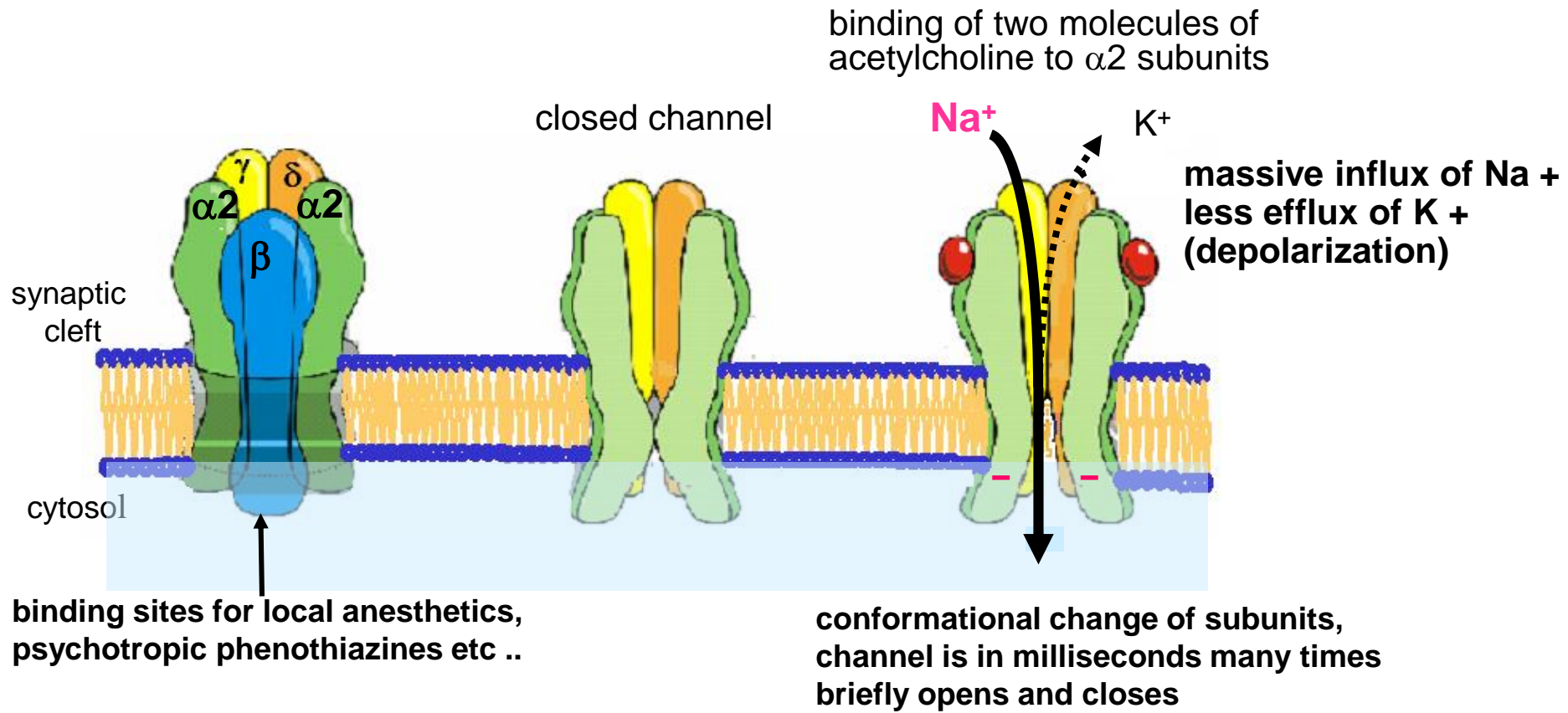
physostigmine

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

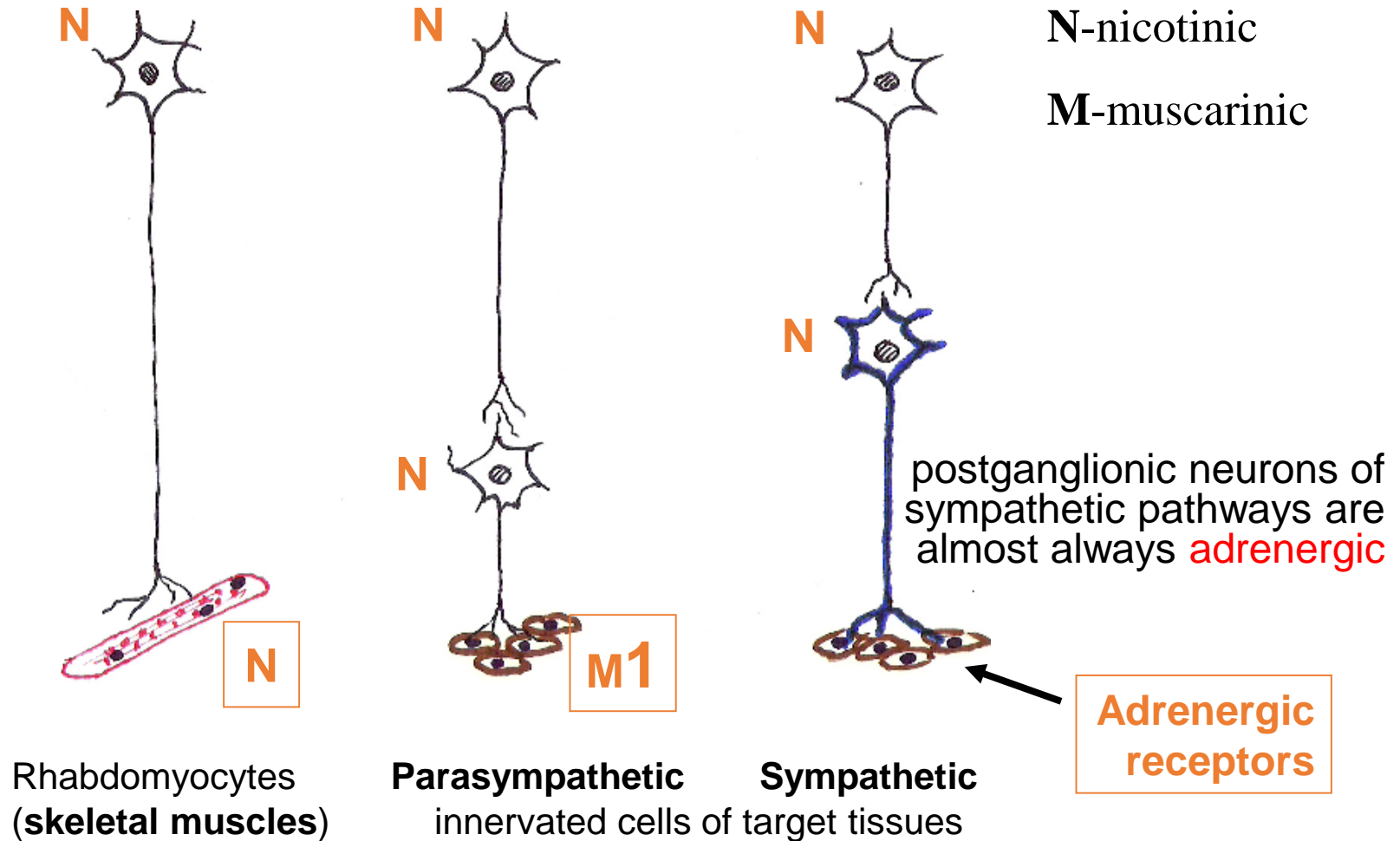
irreversible - formation of covalent bonds between enzyme and organophosphate

Nicotinic acetylcholine receptor of the nicotine type

e.g. at the neuromuscular junction - Na^+ / 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.

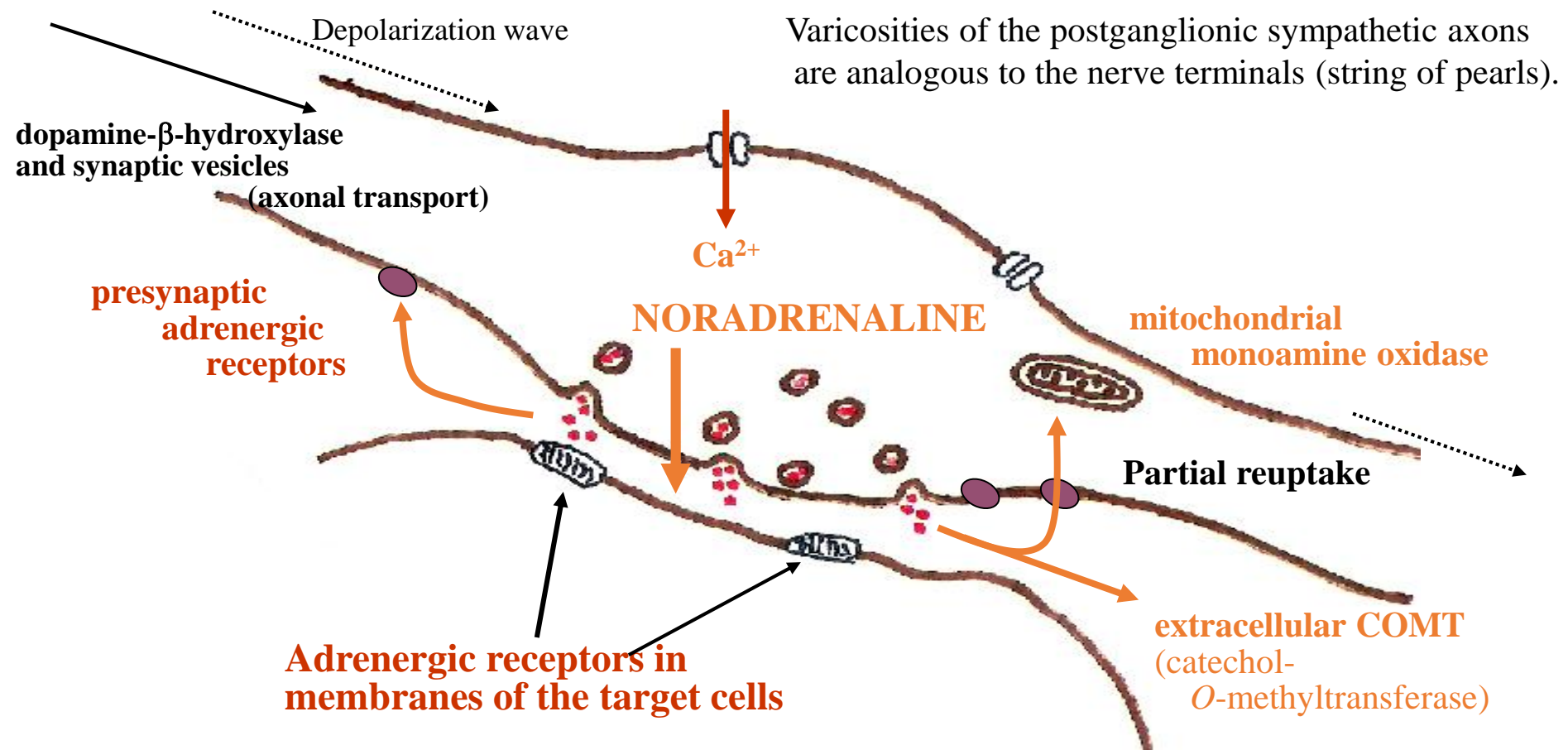
Muscarinic cholinergic receptors

| Type | Principle of action | Location |
|----------------|---------------------|--|
| M ₁ | G _q | Autonomic ganglia, CNS, exocrine gland cells |
| M ₂ | G _i | heart, K ⁺ channels opening |
| M ₃ | G _q | Smooth muscle |
| M ₄ | G _i | CNS |
| M ₅ | G _q | 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

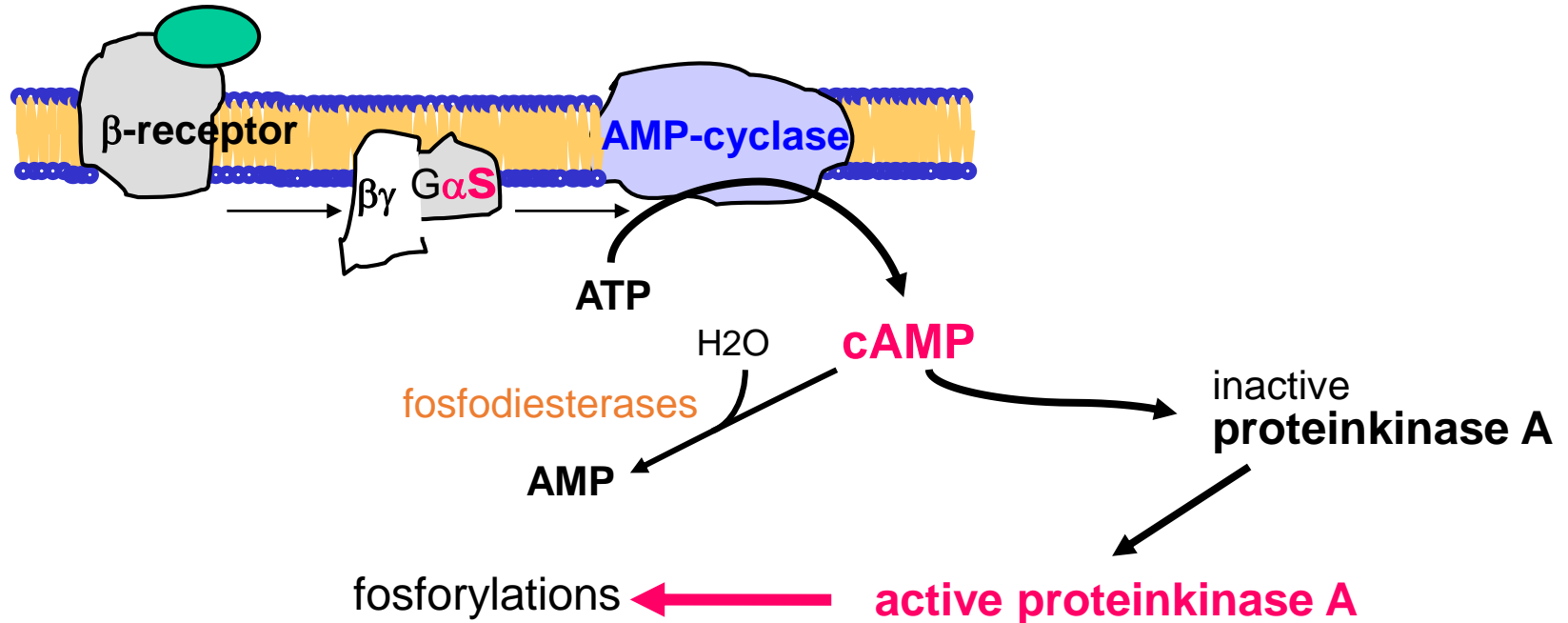
- Dopamine is synthesized in the cytoplasm
- Dopamine is transported into vesicles (ATP-dependent process, against concentration gradient).
- Final hydroxylation of dopamine to noradrenaline occurs in vesicles.

Adrenergic receptors

| Receptor | α_1 | α_2 | β_1 | β_2 |
|-----------------------------|---|---|---|--|
| G-protein | Gq | Gi | Gs | |
| Second messenger | DG + IP ₃ | cAMP ↓ | cAMP ↑ | |
| Examples of location | <ul style="list-style-type: none"> • GIT smooth muscle (sphincters) and skin blood vessels (contraction) | <ul style="list-style-type: none"> • adrenergic and cholinergic nervous terminals (transmitter releasing inhibited) • pancreas (glandular secretion inhibited) • thrombocytes (agregation) | <ul style="list-style-type: none"> • myocard (intensity and frequency of contractions increased) | <ul style="list-style-type: none"> • smooth muscle in the uterus, bronchi (relaxation) • GIT smooth muscle (peristalsis) • pancreas (glandular secretion inhibited activated) |

β -Adrenergic receptors

noradrenalin / adrenalin



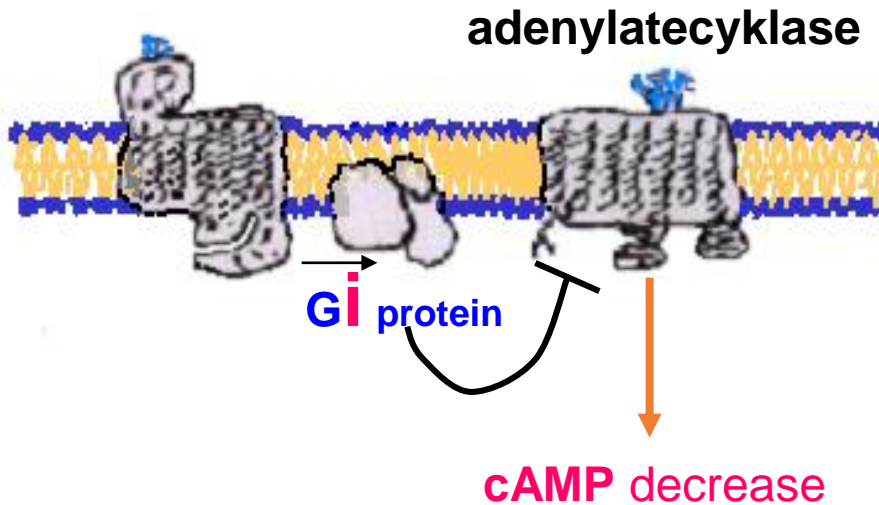
The typical effects of β -stimulation

- β_1 – tachycardia, inotropic effect in the myocard,
- β_2 – bronchodilation, vasodilation in the bronchial tree,
- β_3 – mobilization of fat stores, thermogenesis.

TEST

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

$\alpha 2$ -receptors

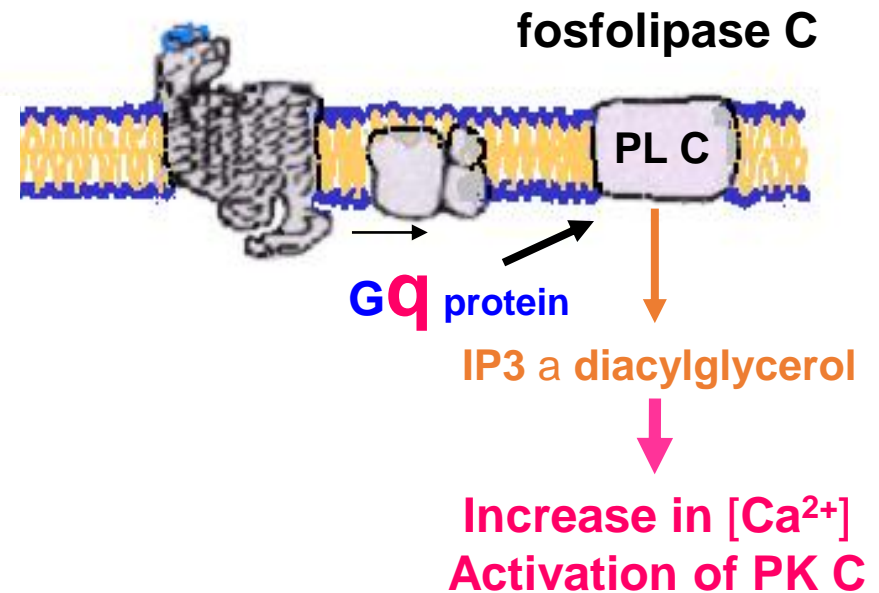


The typical effects of adrenergic

$\alpha 2$ -stimulation:

glandular secretion inhibited

$\alpha 1$ -receptors



$\alpha 1$ -stimulation:

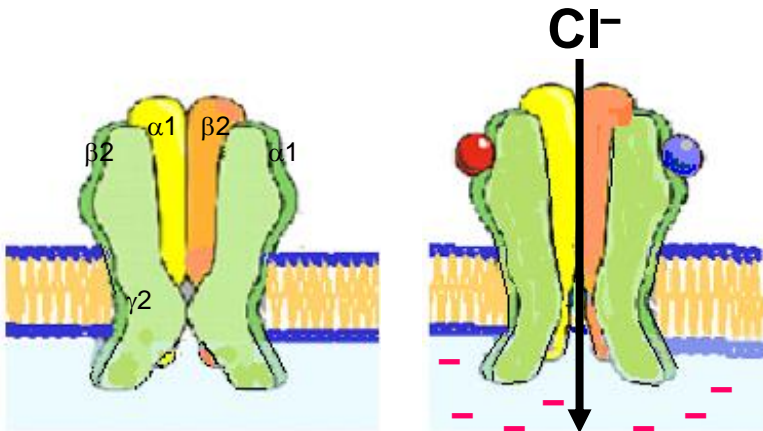
vasoconstriction

bronchoconstriction

motility of GIT inhibited

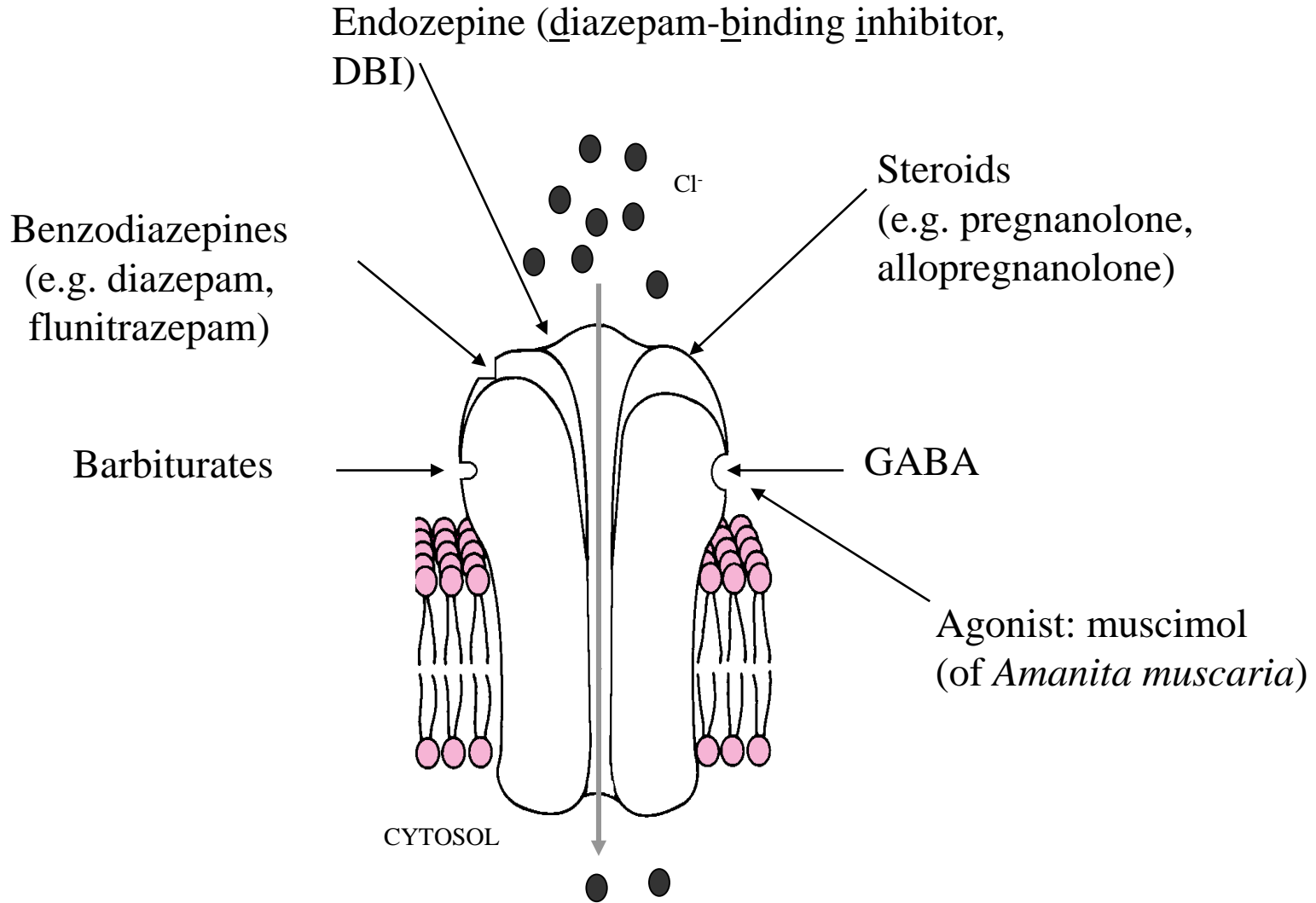
Inhibitory GABA_A receptor

Ligand-gated channel (ROC) for **chloride anions**. The interaction with **γ -aminomáselnou kyselinou (GABA)** opens the channel. The influx of 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

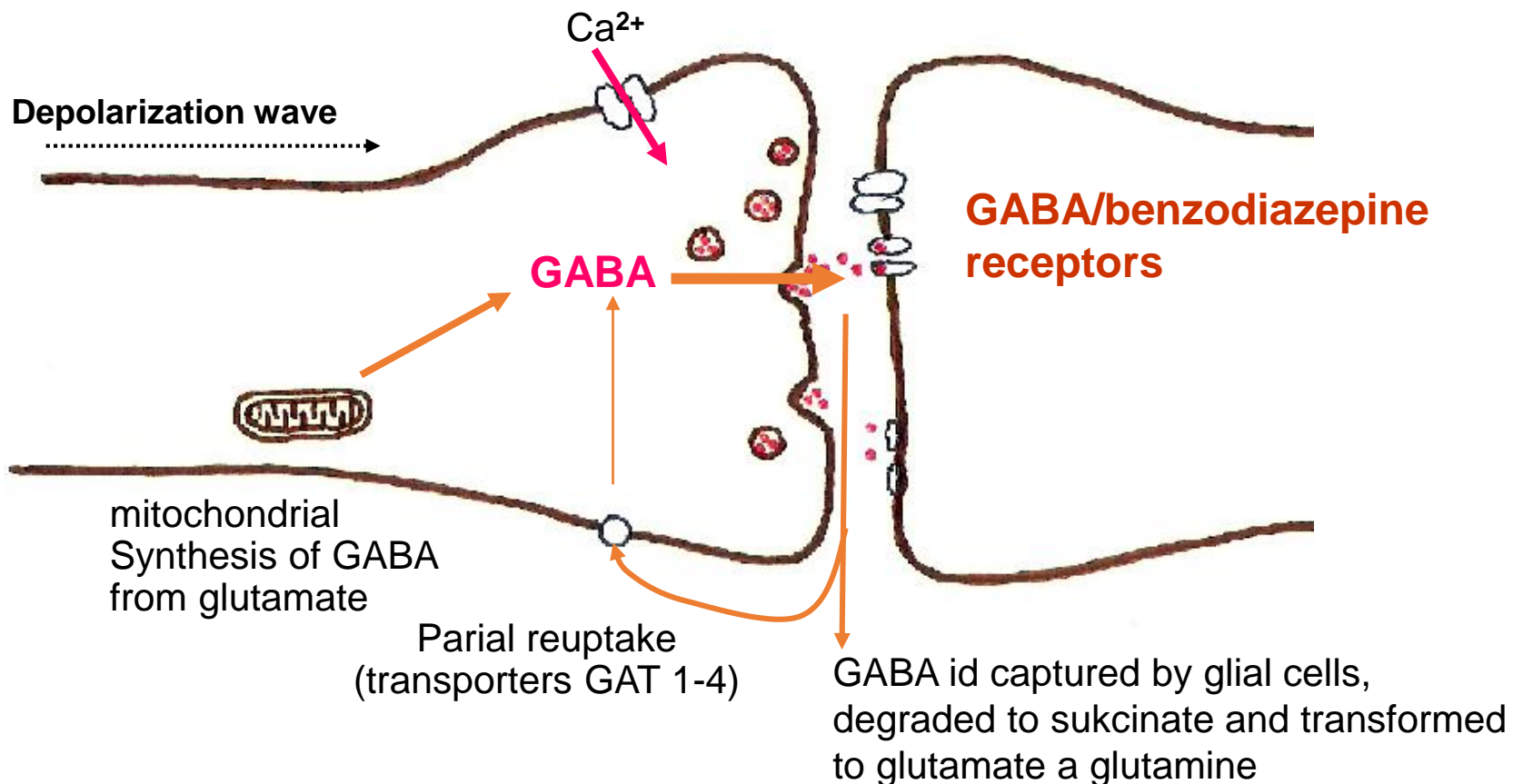
More than eleven **allosteric modulation sites** for substances increasing the effect of endogenous GABA (calming down, reduction of anxiety and myorelaxation): anesthetic, ethanol and numerous drugs such as benzodiazepines meprobamat and also various barbiturates.

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 brainstem 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

γ -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 _s (increasing cAMP) | G _i (decreasing cAMP) | G _q (increasing IP ₃ /DG) |
| <u>Na⁺/K⁺</u> – acetylcholine nicotinic | – | acetylcholine muscarinic M _{2,4} | acetylcholine muscarinic M _{1,3,5} |
| – | adrenergic β _{1,β2,β3} | adrenergic α ₂ | adrenergic α ₁ |
| <u>Na⁺/Ca²⁺/K⁺</u> – glutamate ionophores | – | glutamate mGluR skupiny II a III | glutamate mGluR class I |
| – | dopamine D _{1,5} | dopamine D _{3,4} | dopamine D ₂ |
| – serotonin 5-HT ₃ | serotonin 5-HT _{4,6} | serotonin 5-HT ₁ | serotonin 5-HT ₂ |
| – | histamine H ₂ | histamine H _{3,4} | histamine H ₁ |
| – | – | – | tachykinin NK ₁ for substance P |
| <u>Cl⁻</u> – GABA _A – glycine | GABA _B (metabotropic) | – | – |

II. Receptors interacting with heterotrimeric G-proteins

Common structural features:

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

Few minutes

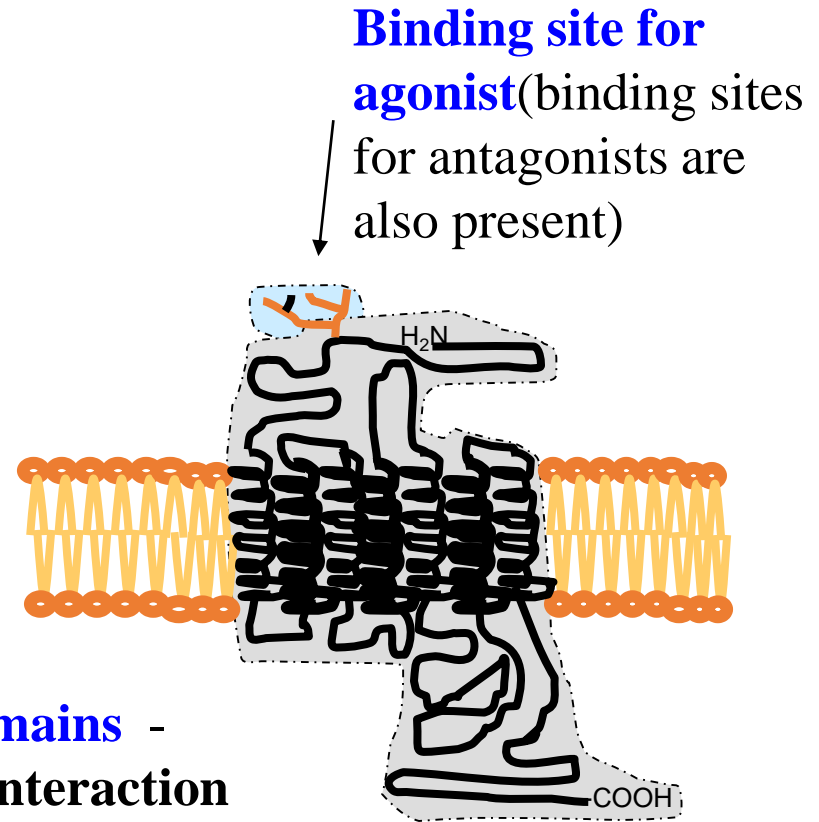
Neurotransmitters

Hormones

Agonist-ligand causes
signal transduction

Antagonist- prevents

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



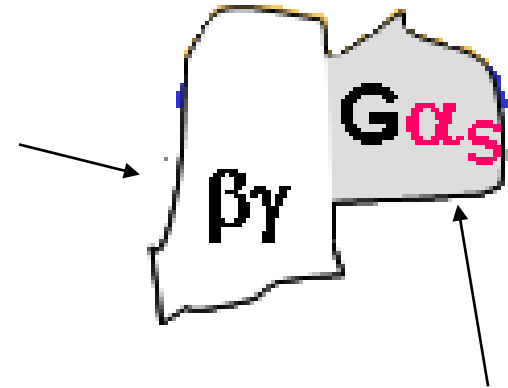
Heterotrimeric G-proteins

Proteins **binding GDP or GTP**

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

Subunits α , β a γ .

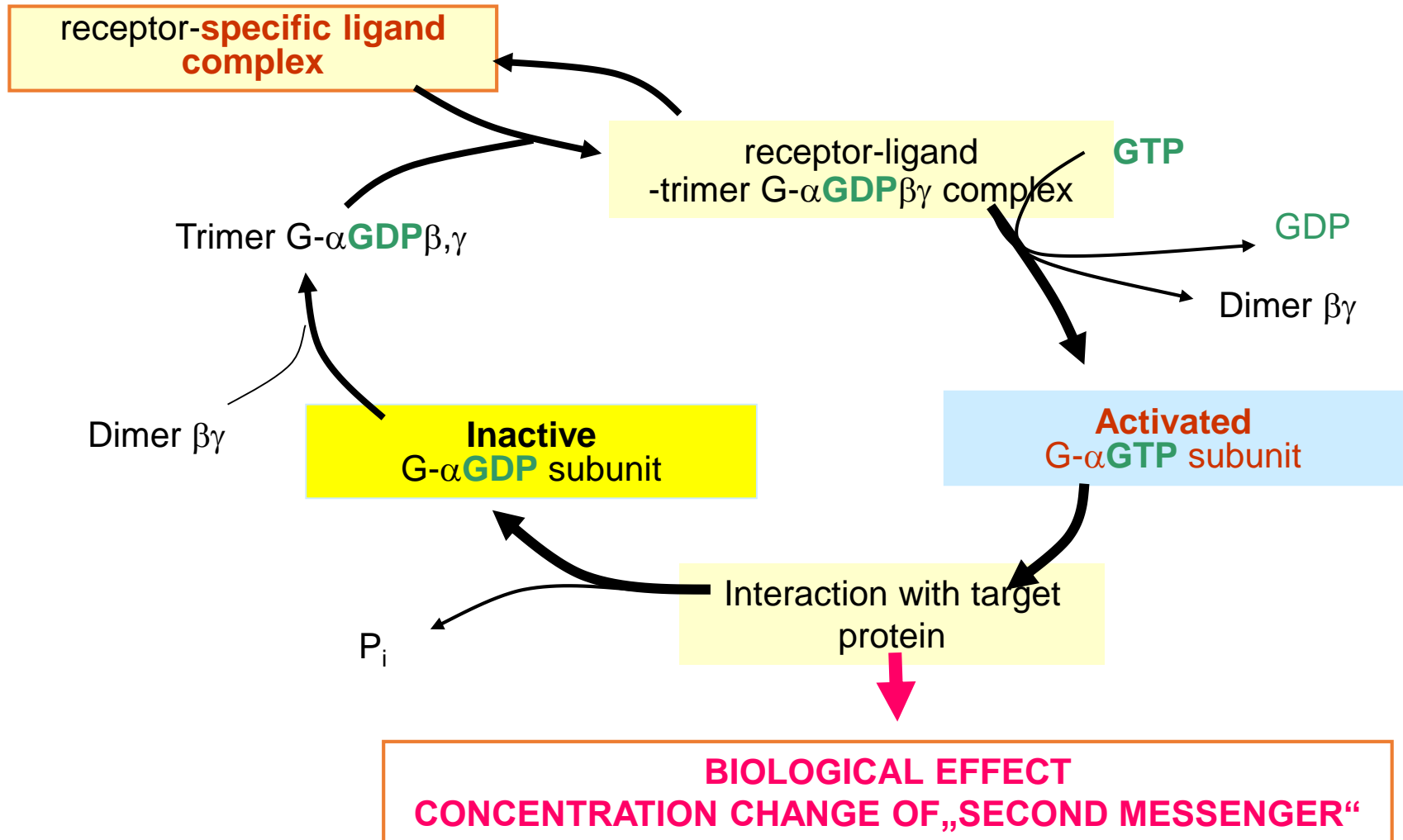
Subunits $G\beta$
and $G\gamma$ are
hydrophobic
and are not
specific.



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

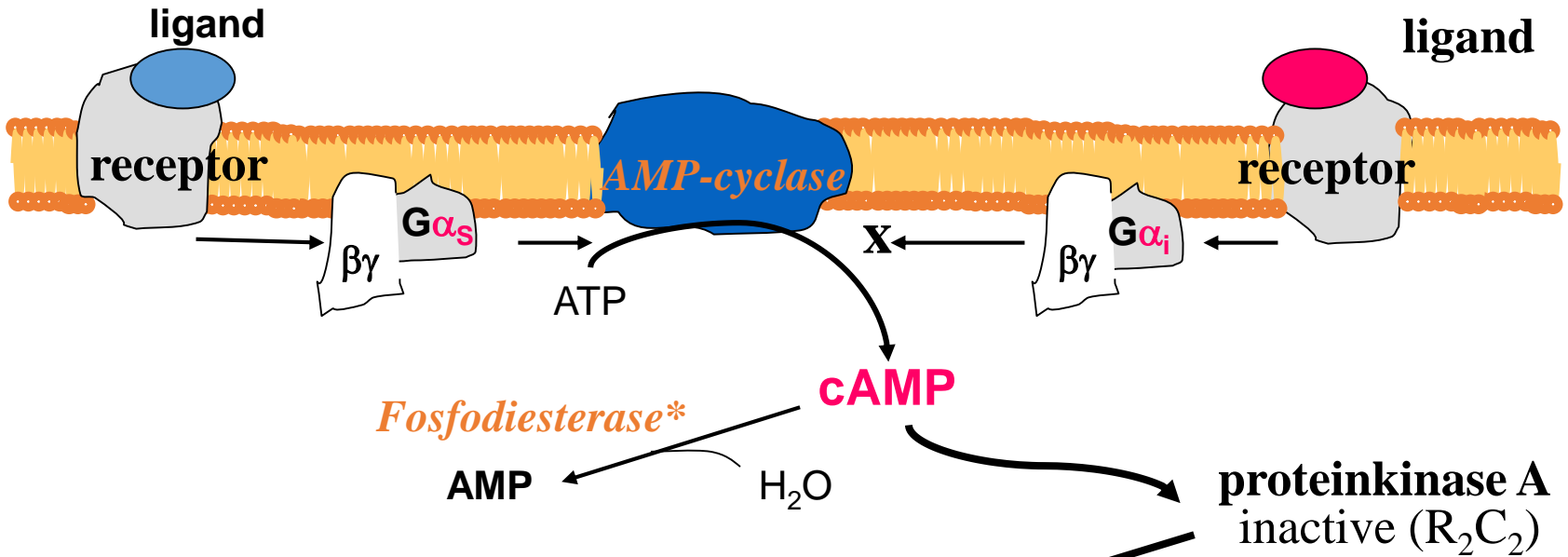
| Type of $G\alpha$ subunit | Examples of activating receptor | Effect of activated $G\alpha$ To target protein (intracellular signal) |
|--|--|--|
| $G\alpha_s$ (stimulating) | glucagon parathyrine β -adrenergic | Adenylatecyclase stimulation (cAMP, Ca ²⁺) |
| $G\alpha_i$ (inhibitory) | somatostatin α_2 -adrenergic | Adenylatecyclase inhibition (cAMP, K ⁺) |
| $G\alpha_q$ (activating PI cascade) | vasopressin V1 endothelin ETA,B acetylcholine M1 α_1 -adrenergic | phospholipase C stimulation (DG+IP ₃ , Ca ²⁺) |
| $G\alpha_t$ (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)

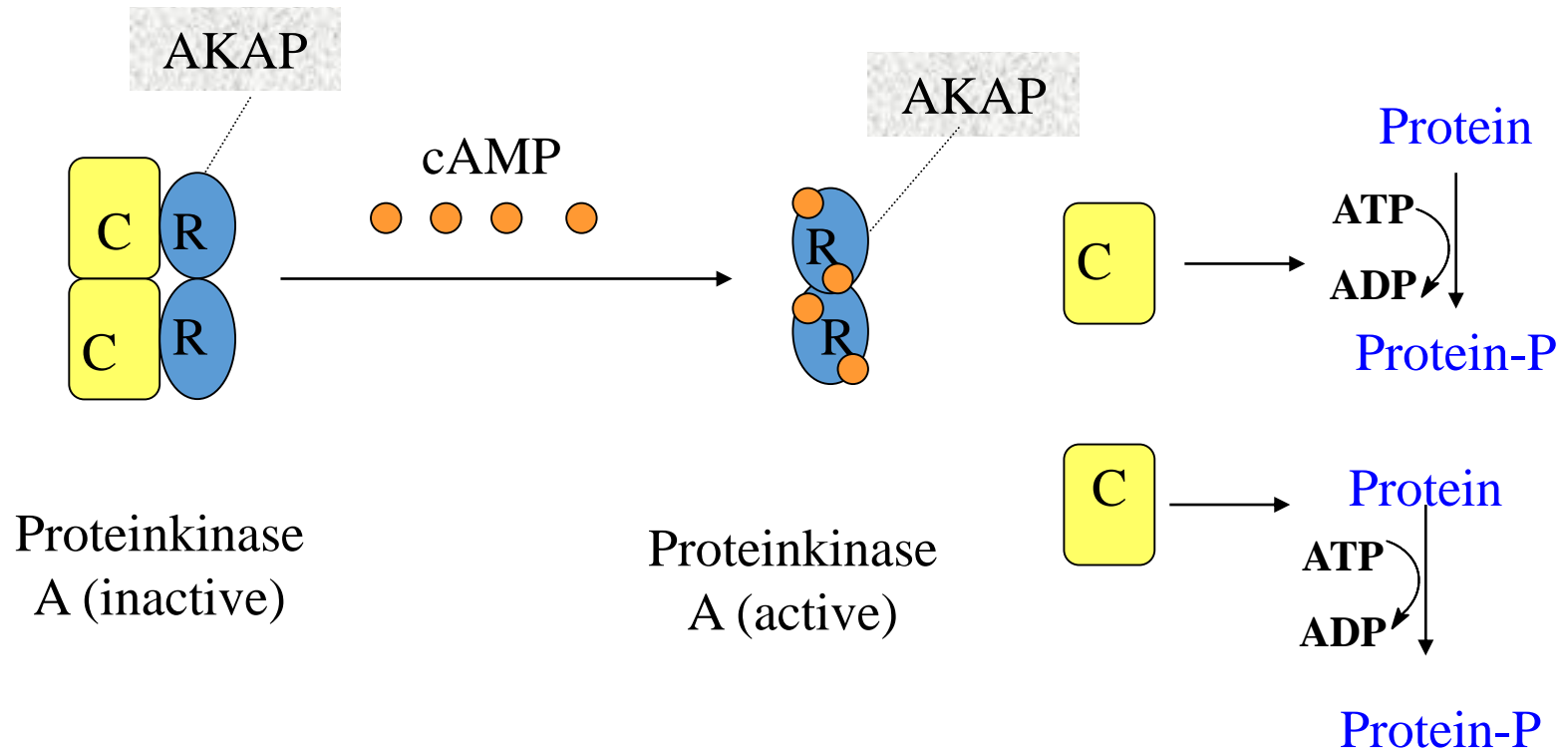


active protein kinase A



* 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.

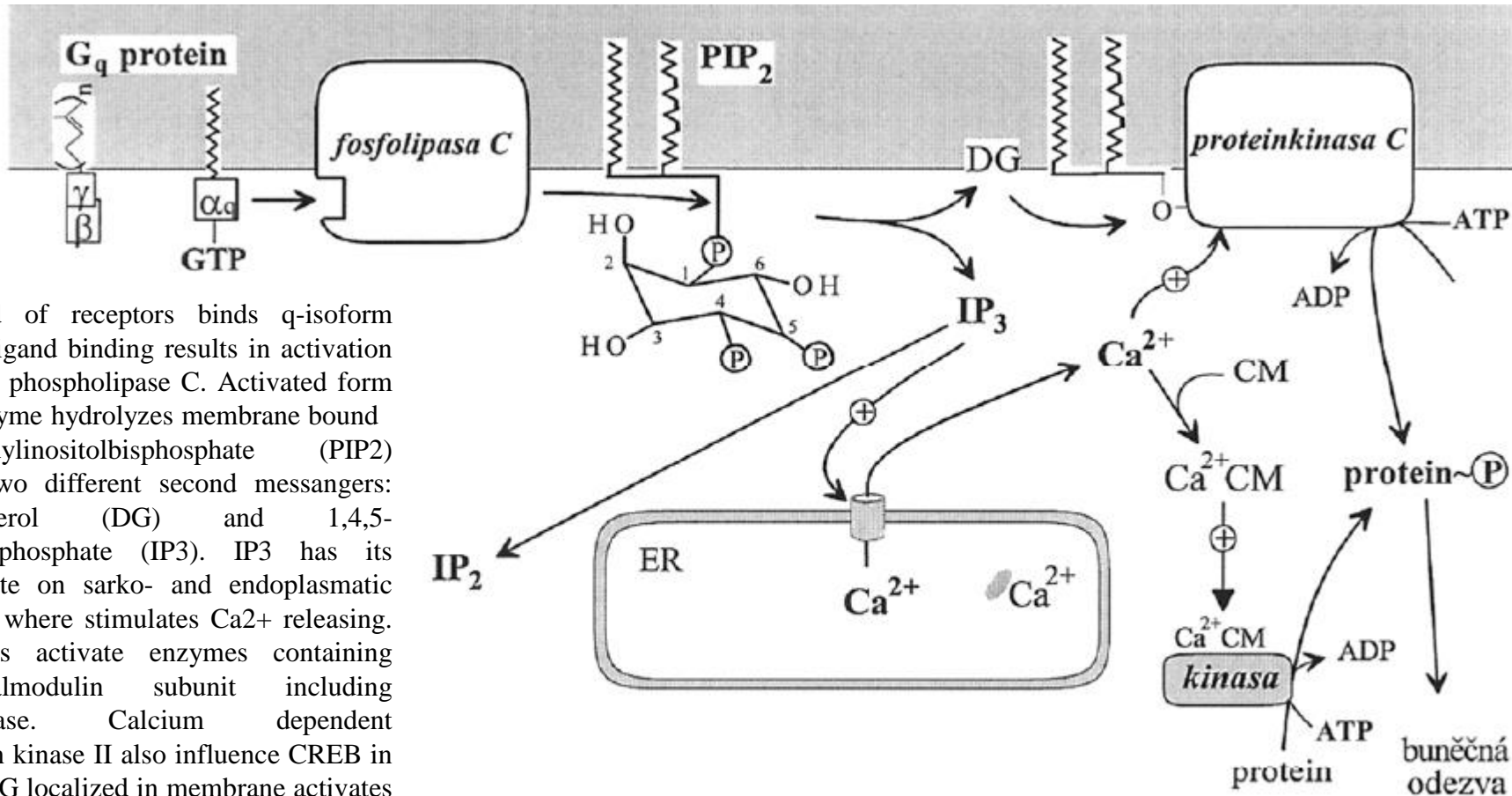
Proteinkinase A is heterotetrameric molecule, containing two regulatory and two catalytic subunits. In inactive state, subunits are bound to each other. cAMP binds regulatory subunits causing their separation from catalytic subunits, which become active and catalyze phosphate transmission from ATP to 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 α_s subunit is „frozen“ in active state continually producing cAMP. cAMP effect is active channel for Cl^- 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

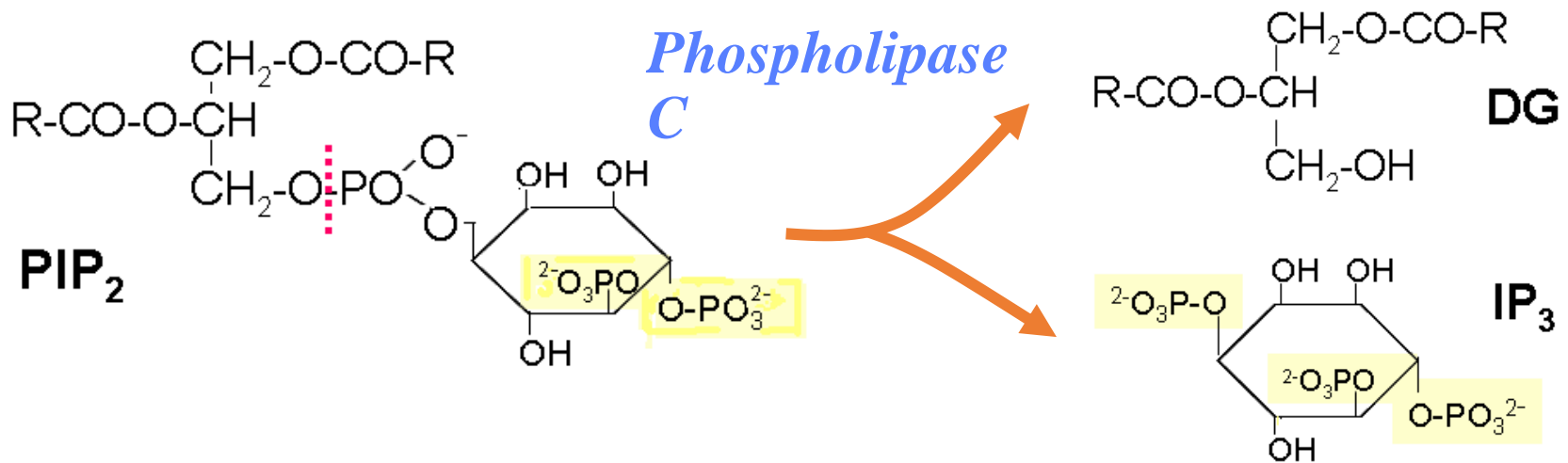
| Hormone | Localization of the effect |
|------------------------------|--|
| CRH | Adenohypophysis |
| TSH | Thyroid follicle |
| LH | Testicular Leydig cells, yellow body (corpus luteum) |
| FSH | Ovaria follicle cells, testicular Sertoli cells |
| ACTH | Adrenal cortex |
| ADH | Kidney distale tubule cells |
| PGI ₂ | Thrombocytes |
| Adrenaline, noradrenaline | β - 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.

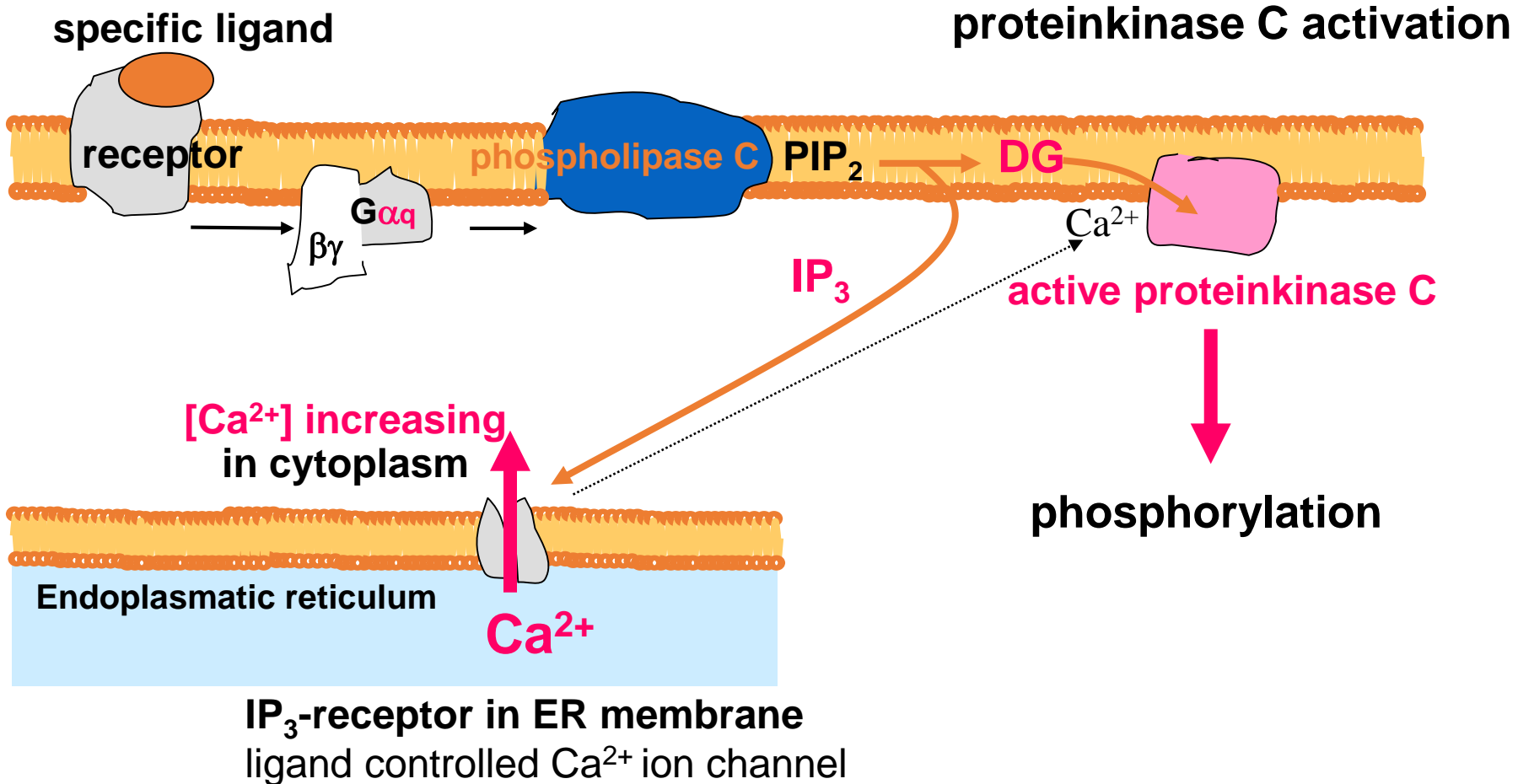
III. Receptors activating G_q protein stimulate phospholipase C triggering phosphatidylinositol cascade



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 Ca^{2+} concentration changes

- Basal 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
- Ca^{2+} increasing may be caused by

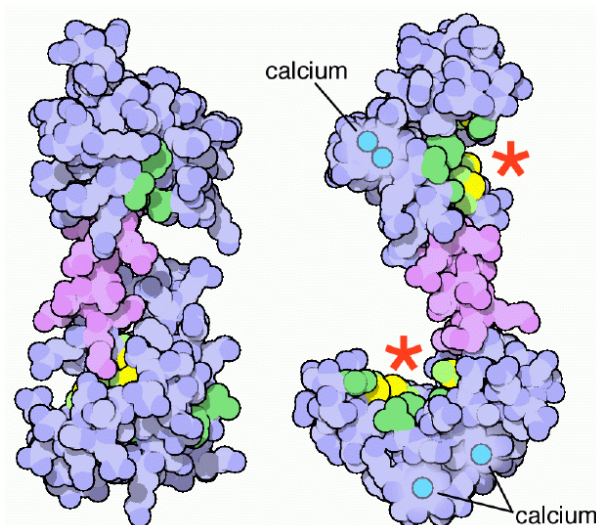
Ca^{2+} influx through cytoplasmatic membrane (e.g. Smooth muscle contraction)

releasing from intracellular supplies (ER, mitochondrie) e.g. IP_3 dependent Ca^{2+} channel in ER or ryanodine channels in skeletal and cardiac muscle

Regulatory protein calmodulin

Increasing Ca^{2+} level activates numerous [Ca²⁺-dependent](#) proteins forming family of small calcium dependent proteins.

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



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 | α_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:

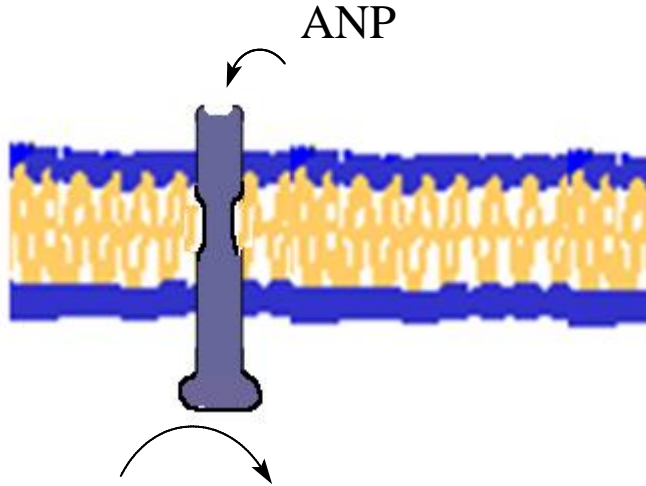
- membrane
- cytoplasmatic

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 quanylatecyclase 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 in the atria as a response to increasing of blood volume or pressure



phosphodiesterase

GMP

H_2O

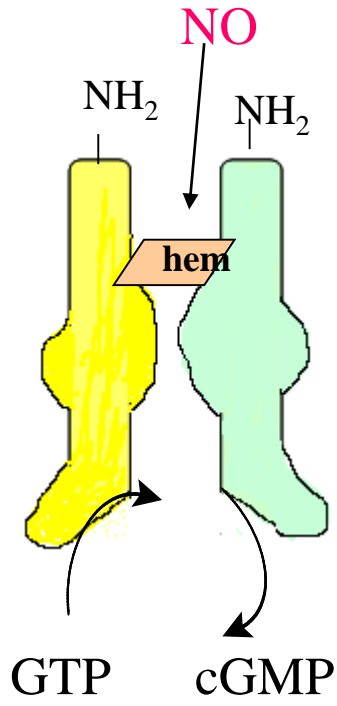
active proteinkinase G (PKG)

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



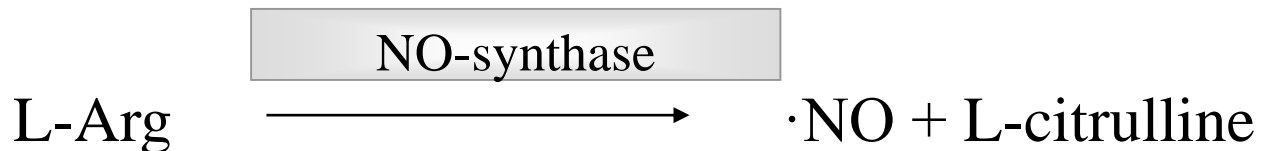
Relaxation of smooth muscles

Meaning of NO/cGMP signalization in smooth muscles of vessels

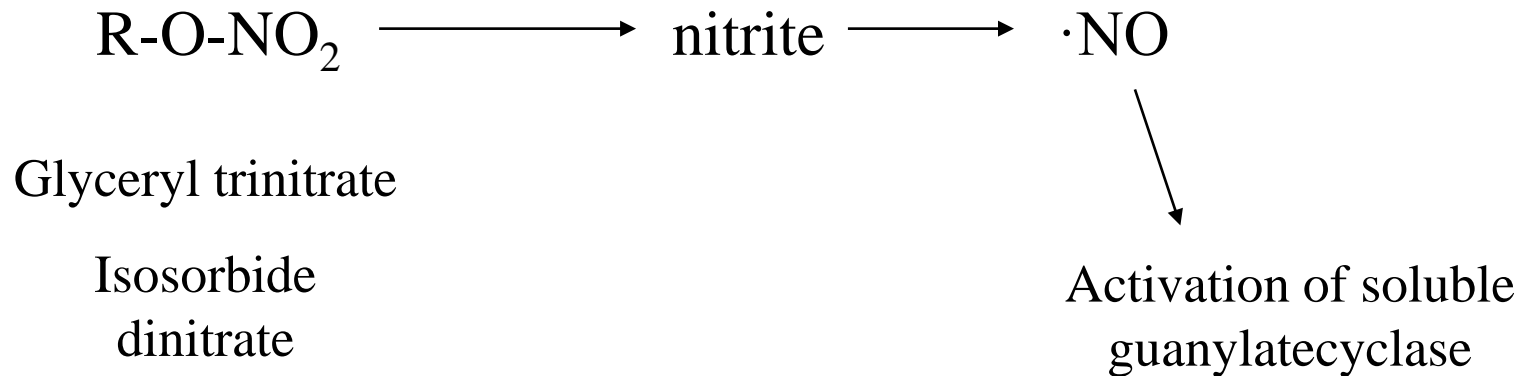
cGMP is a crucial second messenger for an induction of relaxation of smooth muscles in vessels

⇒ 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



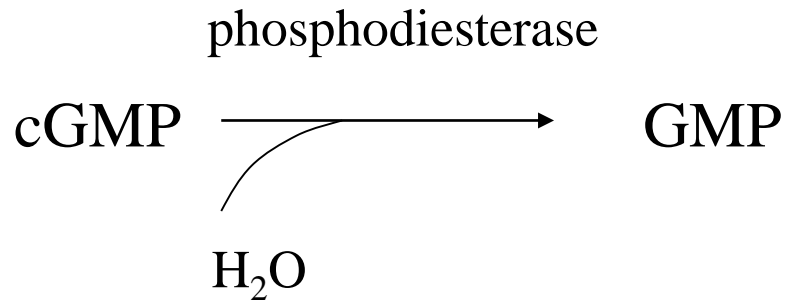
Drugs like organic nitrates are a source of exogenous NO



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.

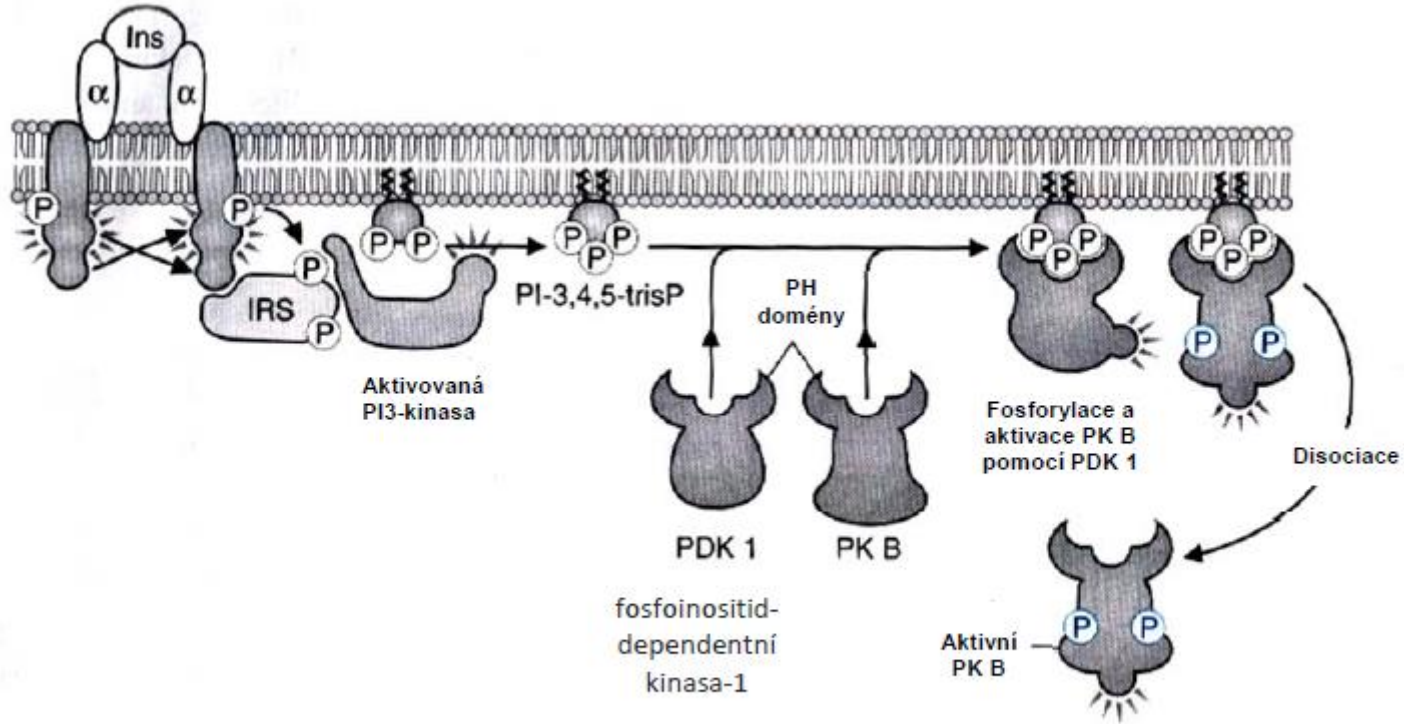
Drug sildenafil (Viagra) is selective inhibitor of phosphodiesterase 5 (PDE5) which is highly expressed in smooth muscles of vessels. Viagra promotes the effect of NO· which is released during sexual stimulation by inhibition of PDE5 and rises a concentration of cGMP in *corpora cavernosa*. The result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

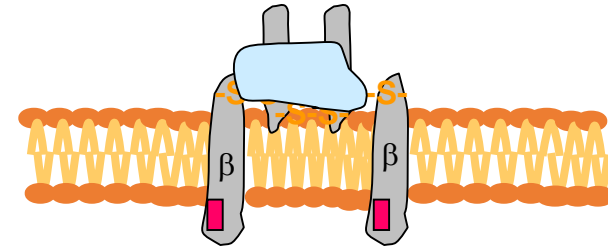
TEST

Insulin receptor

Dimeric structure

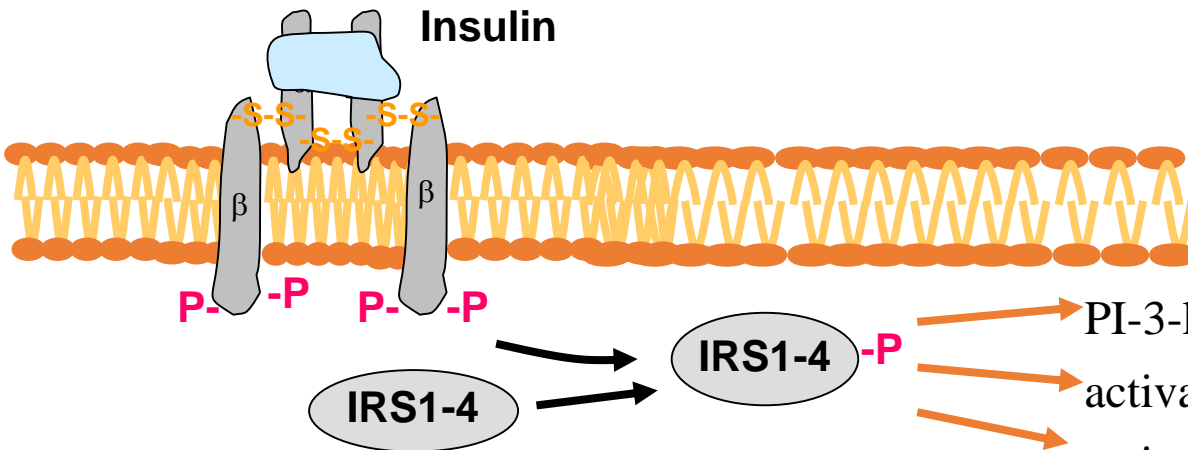
Binding site for insulin on α - subunits

Tyrosinkinase activity on β -subunits

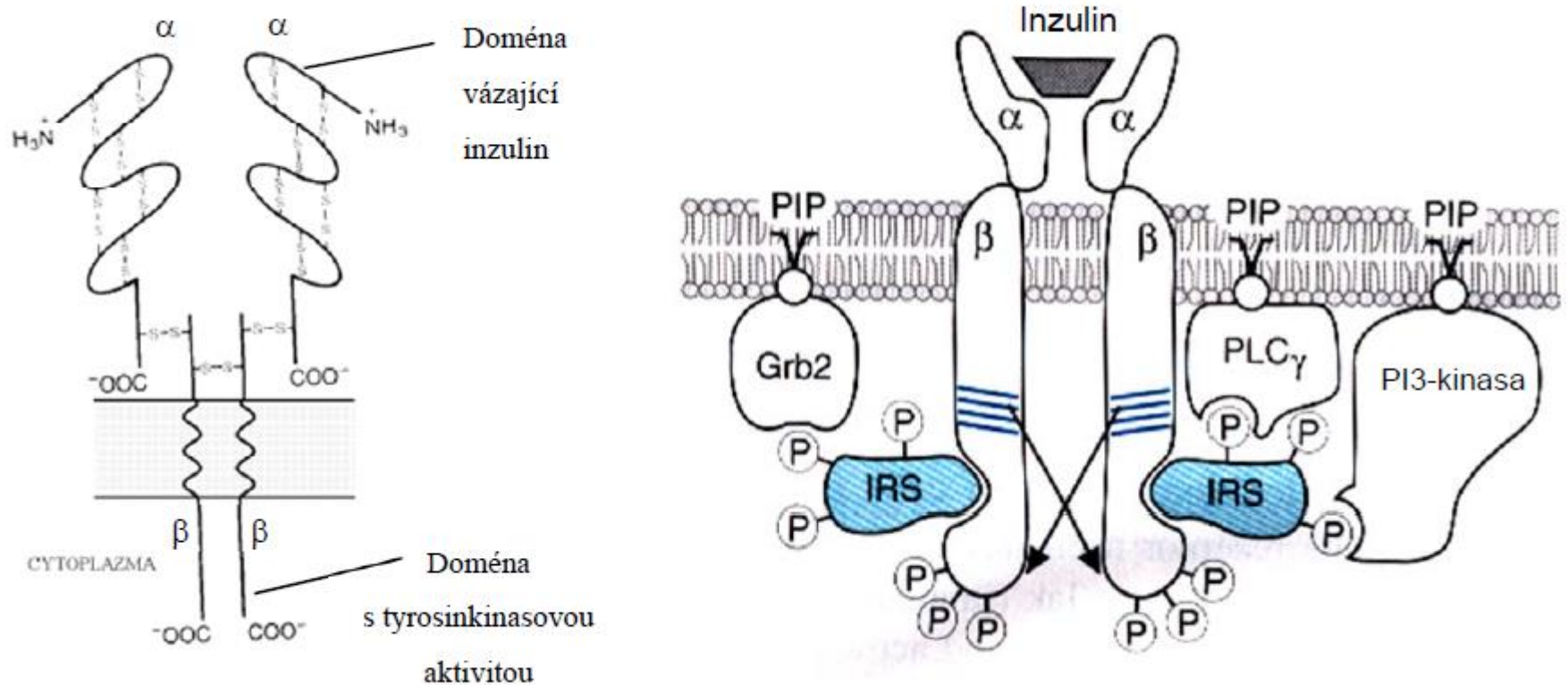


Binding insulin to the receptor causes internalization of complex hormone receptor, receptors are partially recycled

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



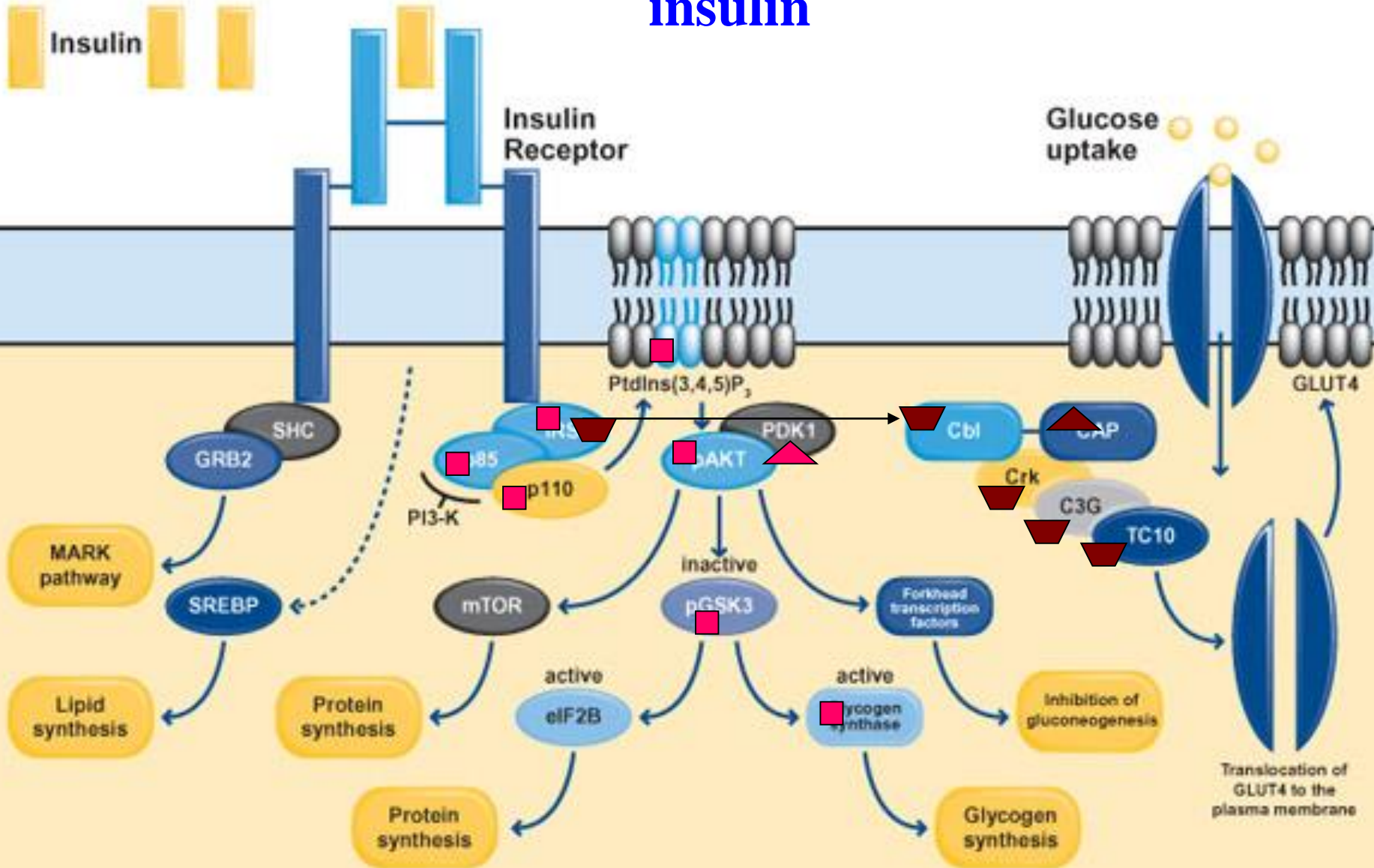
PI-3-kinase binding to the membrane
activation of phosphoprotein phosphatase-1
activation Ras – expression of genes



Insulin receptor

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

Some of the signaling pathways of insulin



The substrates of the insulin receptor IRS1-4 are **adapter proteins**.
If the insulin-receptor complex phosphorylates 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 (AKT) 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

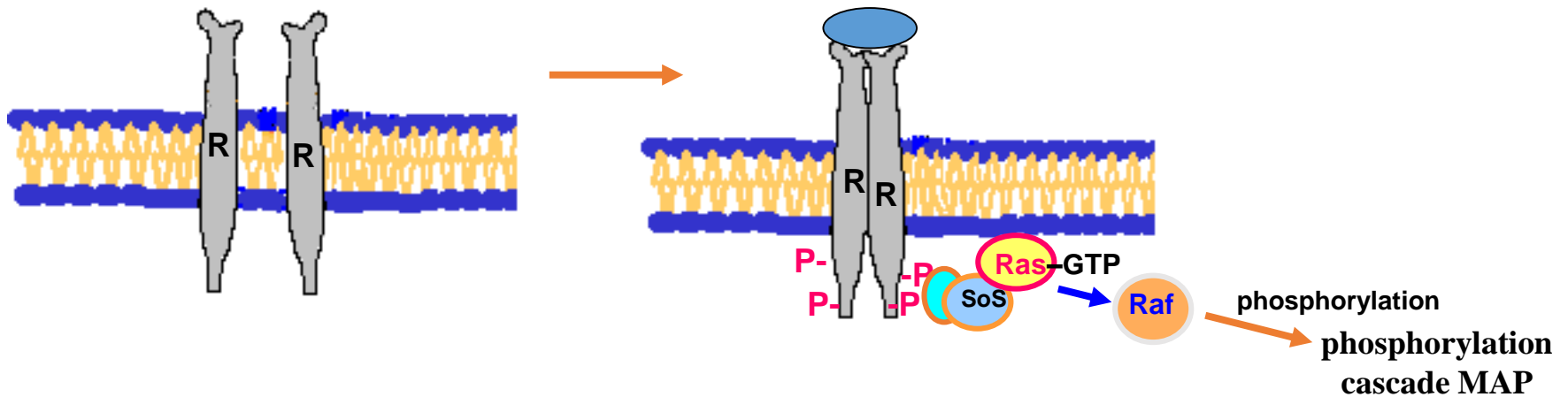
C3G activates TC10 (G-protein)

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.

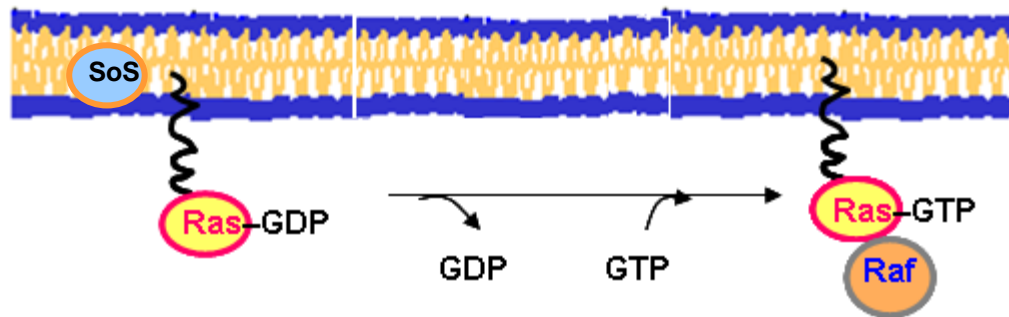
Receptor autophosphorylation

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 α -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)

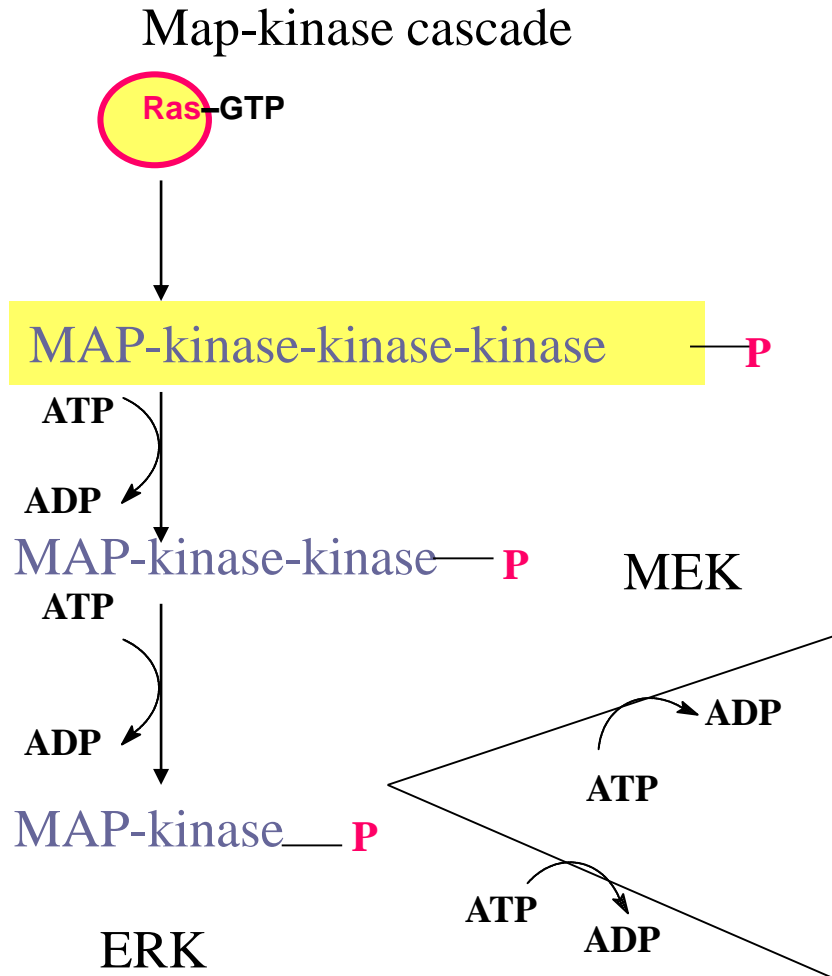
Monomer G-proteins, which play an important role in regulating growth, morphogenesis, cell motility, cytokinesis like.

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.



MAPK3, 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)

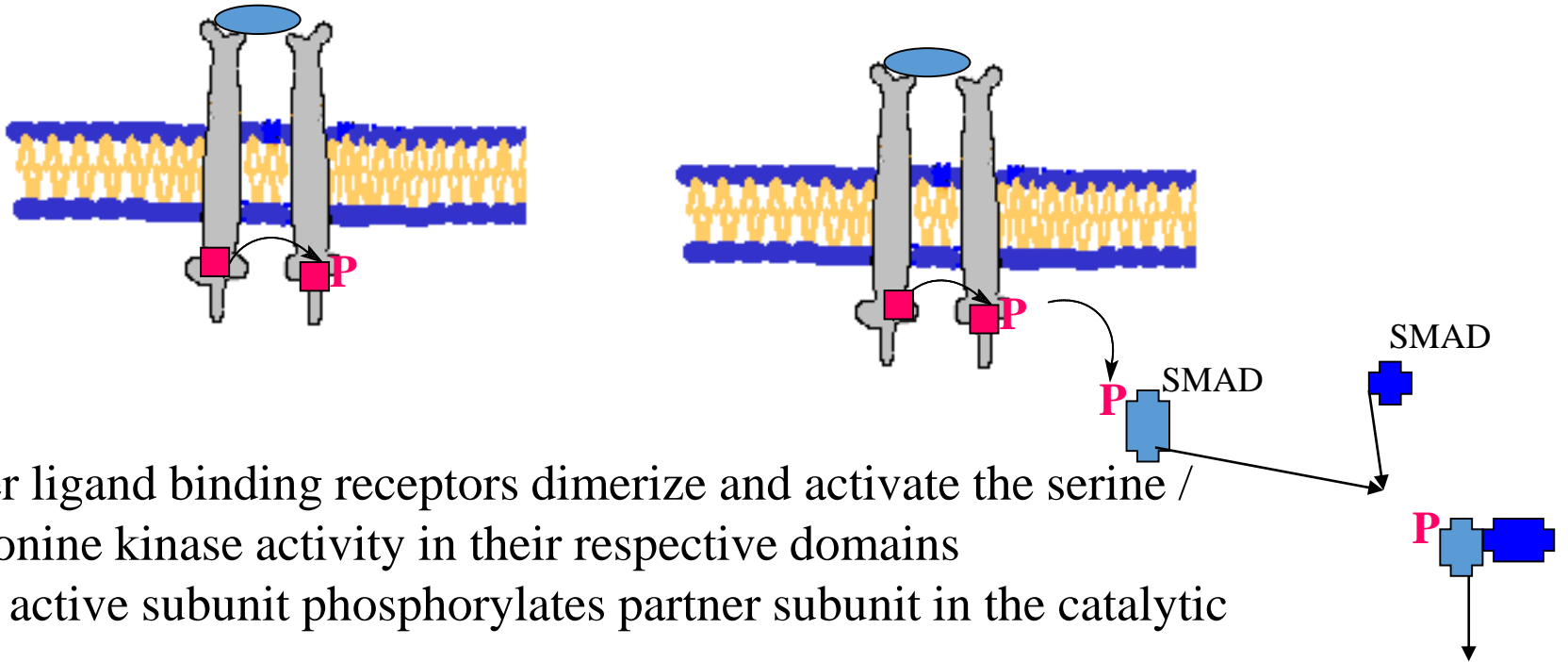
The mitogens - growth factors supporting the proliferation

Examples of mitogen:

| Abbreviation | Name | Function |
|---------------------|-----------------------------------|---|
| 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- β (TGF- β)



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

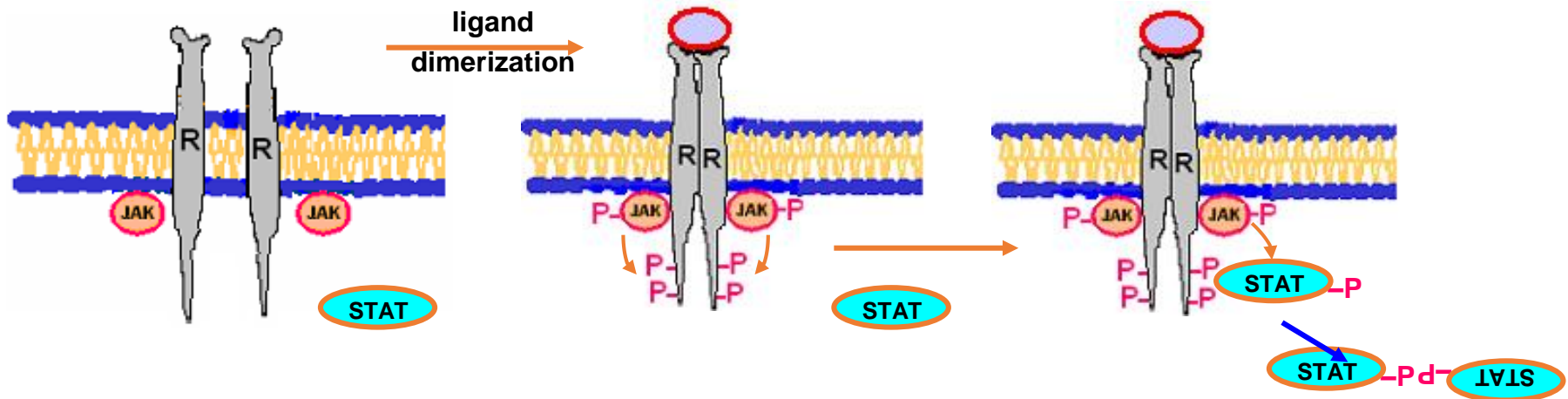
Smad proteins are activated by phosphorylation and forms dimers with other SMAD proteins

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 β -adrenergic receptor

Upon binding of the ligand to the receptor activates BARK (β -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)

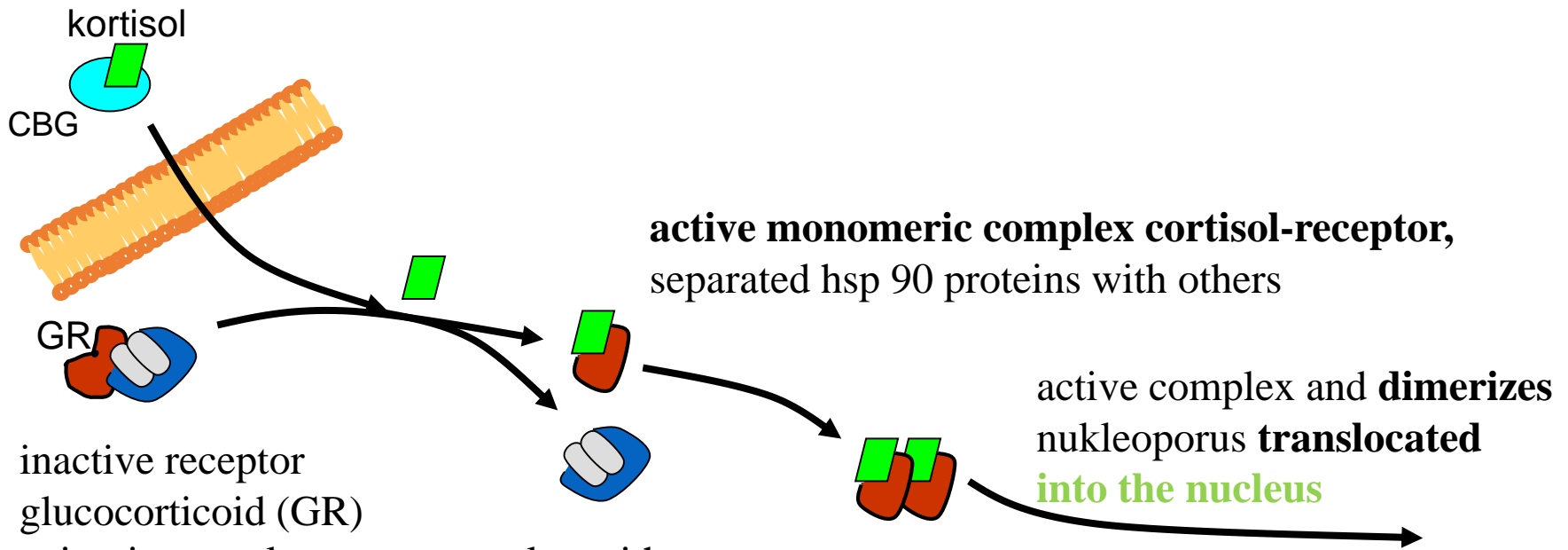
Superfamily of steroid and thyroid receptors - a family of structurally related proteins.

Activation of transcription is a slower process, a response delay

Example cortisol (glucocorticoid GK, their receptors GR)

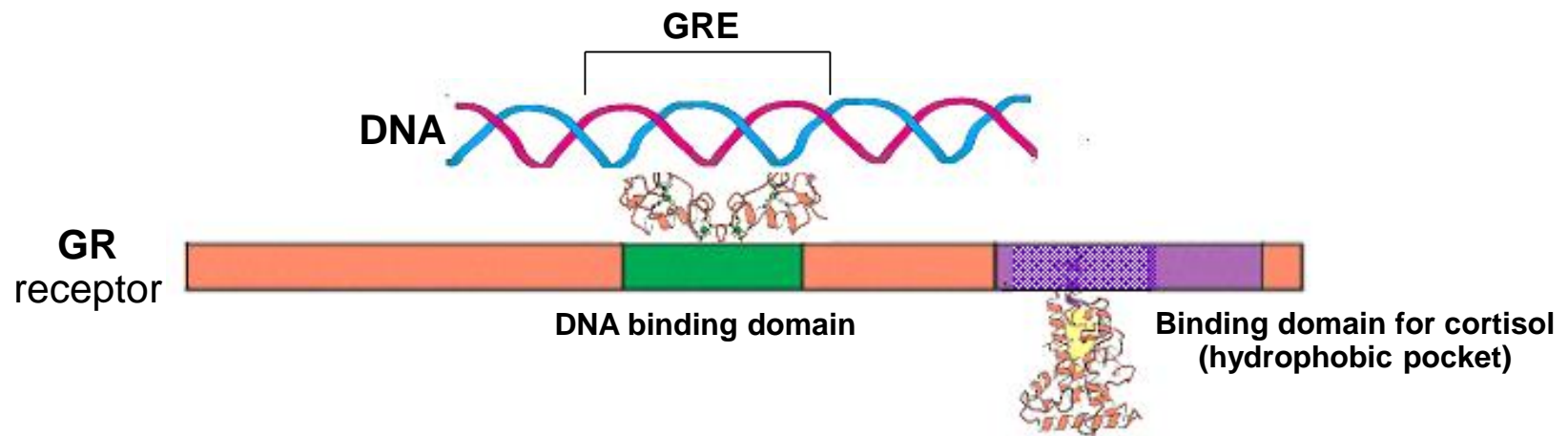
cortisol in the extracellular space transmitted CBG (corticosteroid-binding globulin)

hydrophobic molecule penetrates the cell hormone



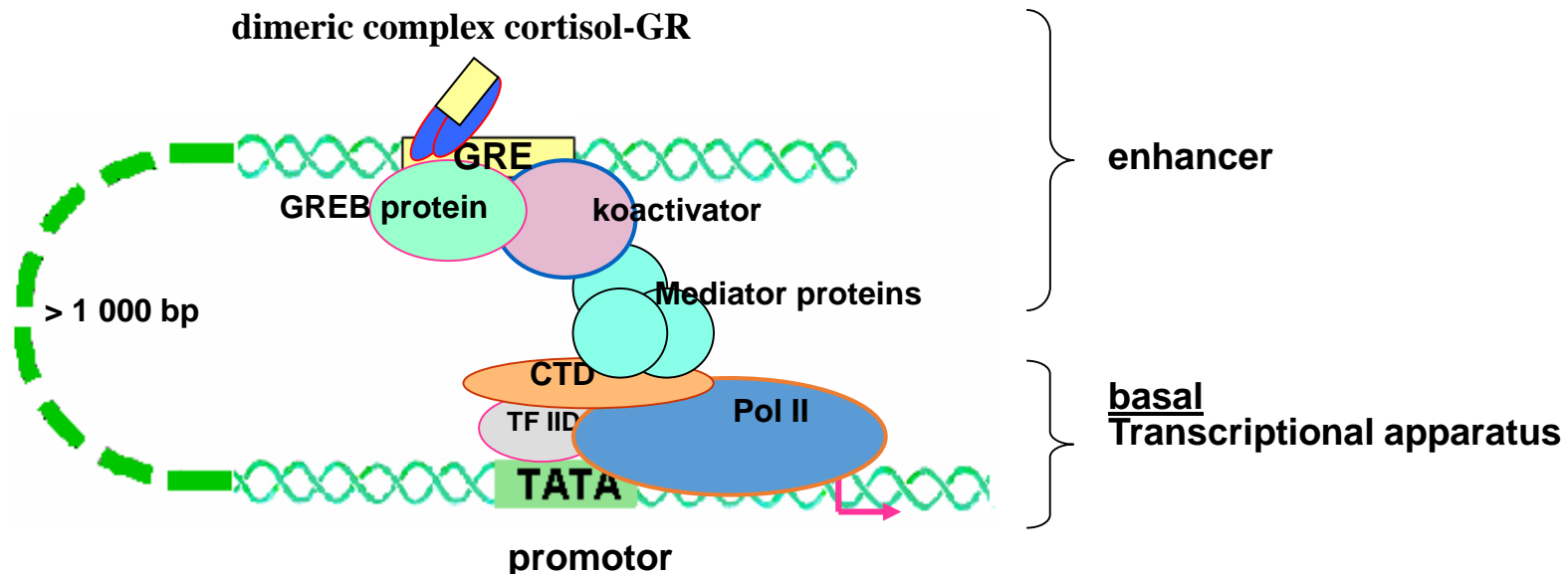
inactive receptor
glucocorticoid (GR)
exists in cytoplasm as a complex with
a protein dimer
hsp 90 (chaperone) and other proteins

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 ₁ , M ₃ , M ₅ G _q | M ₂ , M ₄ G _i |
| Second messenger | - | DG + IP ₃ | cAMP |
| Location | <ul style="list-style-type: none">•autonomic ganglia neurons•neuromuscular junction•chromaffin cells of the adrenal medulla | <ul style="list-style-type: none">•Brain•Smooth muscle•Glandular cells | <ul style="list-style-type: none">•Myocardium•Brain |
| Block receptor | Tubokurarine | Atropine | |

Adrenergic synapses

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| Receptor | α_1 | α_2 | β_1 | β_2 |
|----------------------|--|--|--|---|
| G-protein | G_q | G_i | G_s | |
| Second messenger | DG + IP ₃ | cAMP | cAMP | |
| Examples of location | <ul style="list-style-type: none"> •smooth muscles of the GIT (sphincters) and skin blood vessels (contraction) | <ul style="list-style-type: none"> •adrenergic and cholinergic nerve endings (inhibition of transmitter release) •pancreas (exocrine secretion inhibition) •platelets (aggregation) | <ul style="list-style-type: none"> •myocardium (increase in strength and frequency of contractions) | <ul style="list-style-type: none"> •smooth muscle of the uterus, bronchus (relaxation) •smooth muscle of GIT (peristalsis) •pancreas (exocrine secretion activation) |

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"> •dendrites ganglion neurons | <ul style="list-style-type: none"> •membranes of target cells | <ul style="list-style-type: none"> •chromafinní buňky dřeně nadledvin •dendrity neuronů ganglií | <ul style="list-style-type: none"> •sweat glands | <ul style="list-style-type: none"> •membranes of target cells |