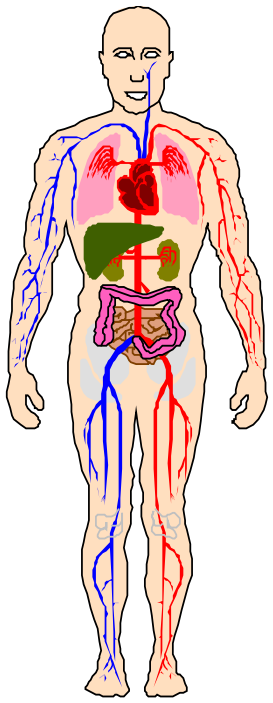
Regulation of the metabolism

PRINCIPLES OF THE REGULATION OF THE METABOLISM:
THEORETIC BASES

ENZYMES -BIOCATALYSATORS
HORMONES (Common mechanisms of the effect of hormones and neurotransmiters)
RECEPTORS (Type of membrane receptors and intracelular receptors)
ENZYMES
VITAMINS
METABOLIC REGULATIONS

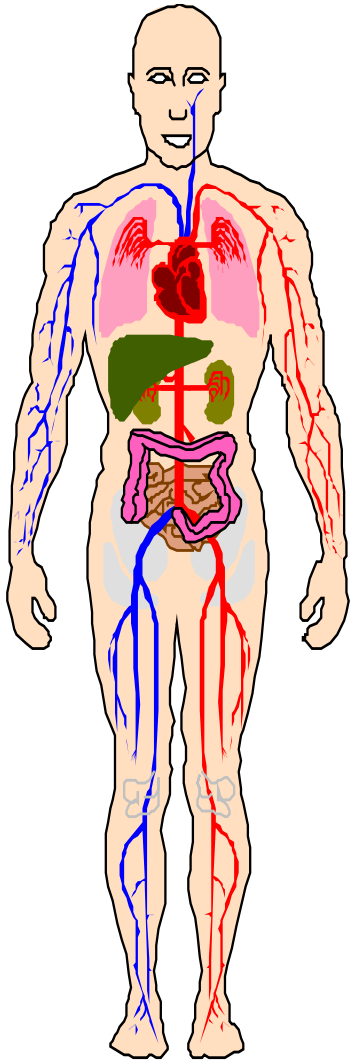
Hormones, Receptors, and Signal Transduction

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...

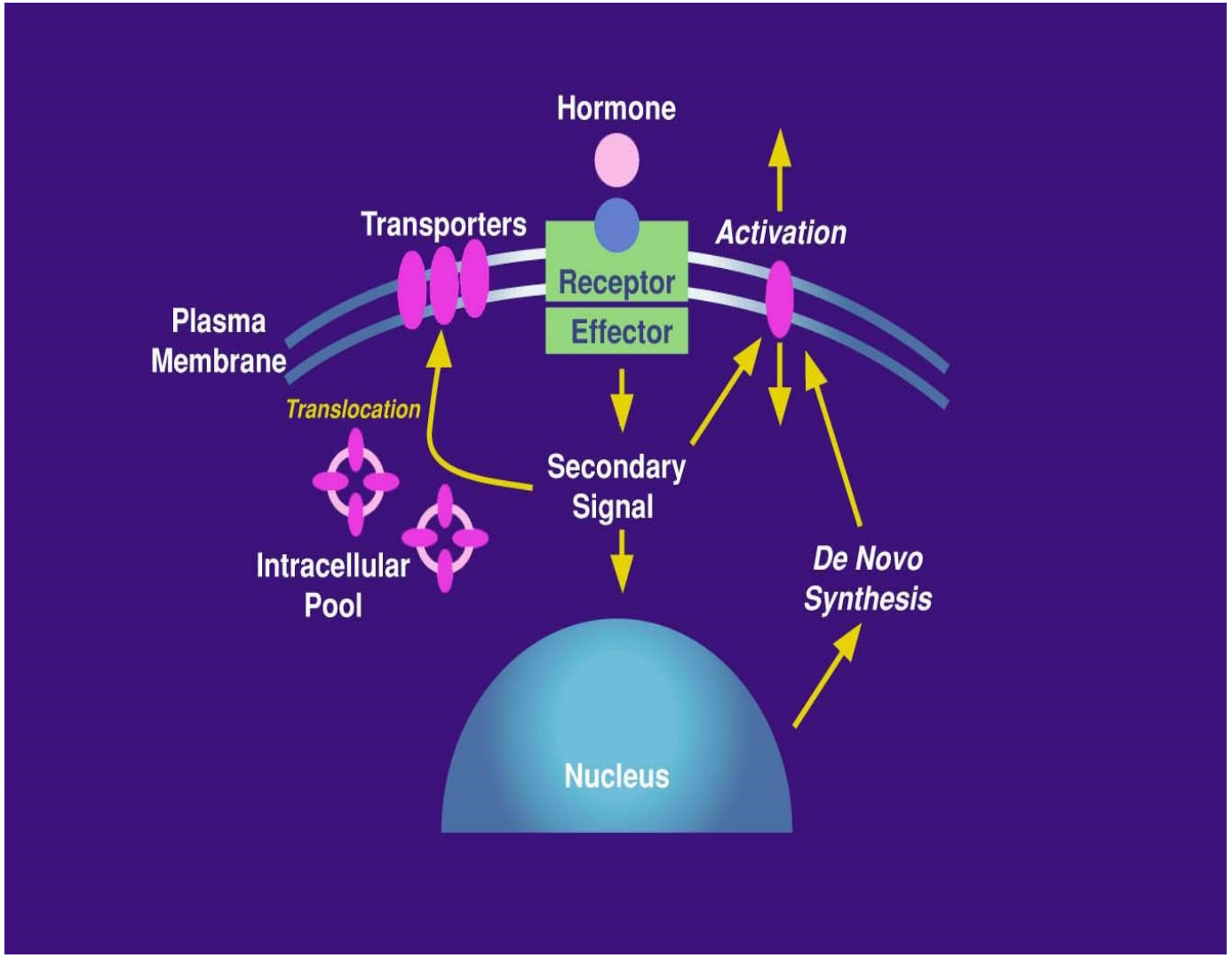


- In cases of short and long distance communication, a substance may be **released** by one cell and recognized by a different **target** cell.
- In the target cell, a **specific** response is induced.
- Cells use an amazing number of **signaling** chemicals.
- These signaling molecules are termed "**hormones.**"
- 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.

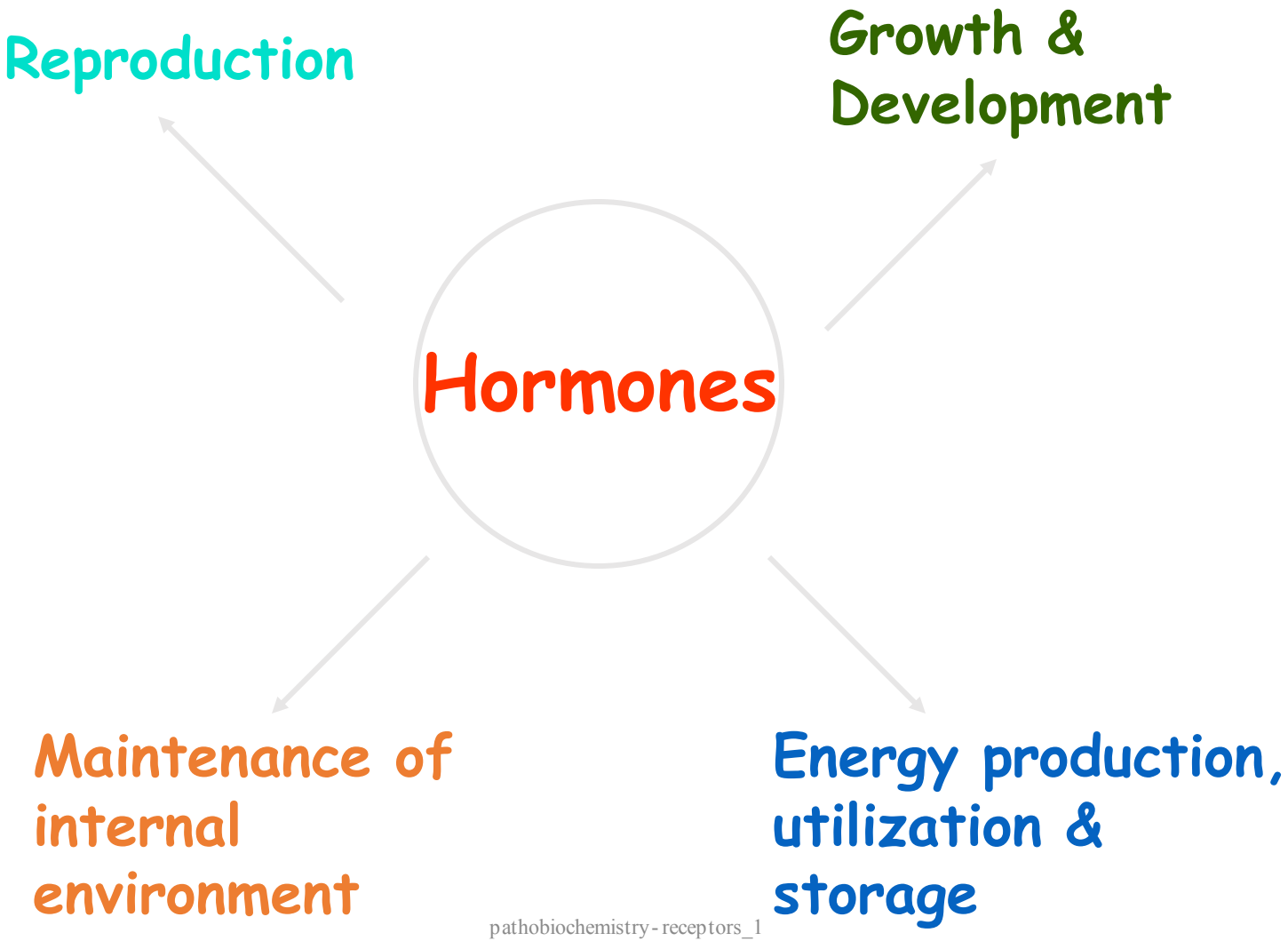
General characteristics of hormones

- 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.

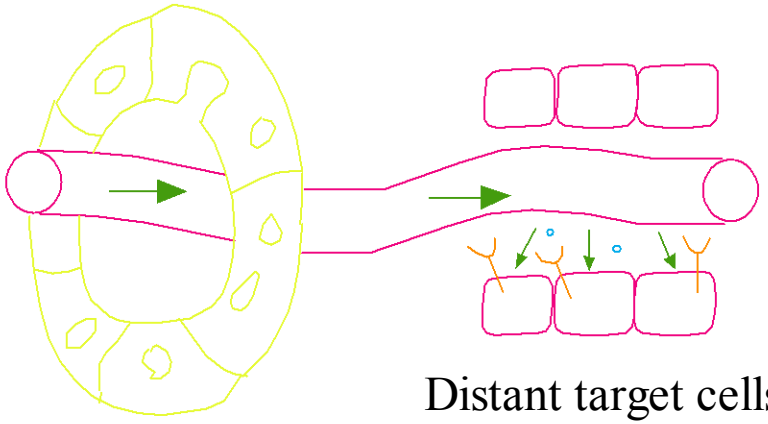
Hormone - Substances released by an endocrine gland and transported through the bloodstream to another tissue where it acts to regulate functions in the target tissue (classic definition).

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.

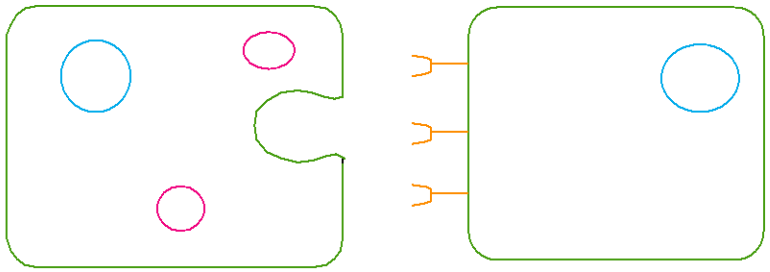
Endocrine



Blood vessel

Hormone secretion into blood by endocrine gland

Distant target cells

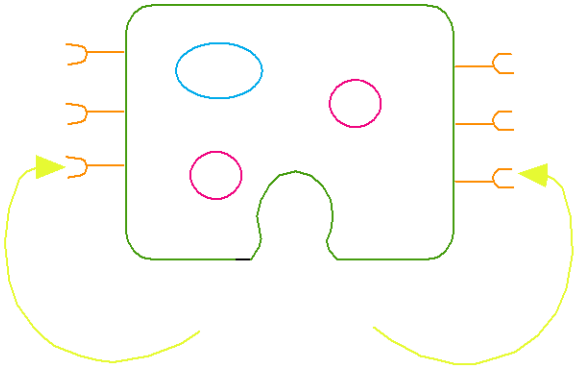


Paracrine

Secretory cell

Adjacent target cell

Autocrine



Target sites on same cell

Receptor

Hormone or other extra cellular signal

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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 ale similer 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 take definite time that the concentration of the hormone in blood will rise and the concentration of the homone in blood stays for definite time (a few minute or hour) rised.

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 concenration 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 concentratrion in the surroundings of the cell, the signal molecule is separated. Paracrine signaling is determined for fast and localised communication between cells.

Autocrine –The cell secretes the signal molecule and it is also a target. Features ale similer like paracrine effect.

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

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Common mechanisms of the effect of hormones and neurotransmiters.

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
NEUROTRANSMITERS	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, izoenzymes, 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)

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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 bindind 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 cytoplazmatic membrane or intracellulary**. In their structure there are two main components: (1) domain binding the ligand, which ensures the specifity of the binding with the relevant ligand; (2) efector domain, wich start a genesis of the biological answer after the binding of the ligand.

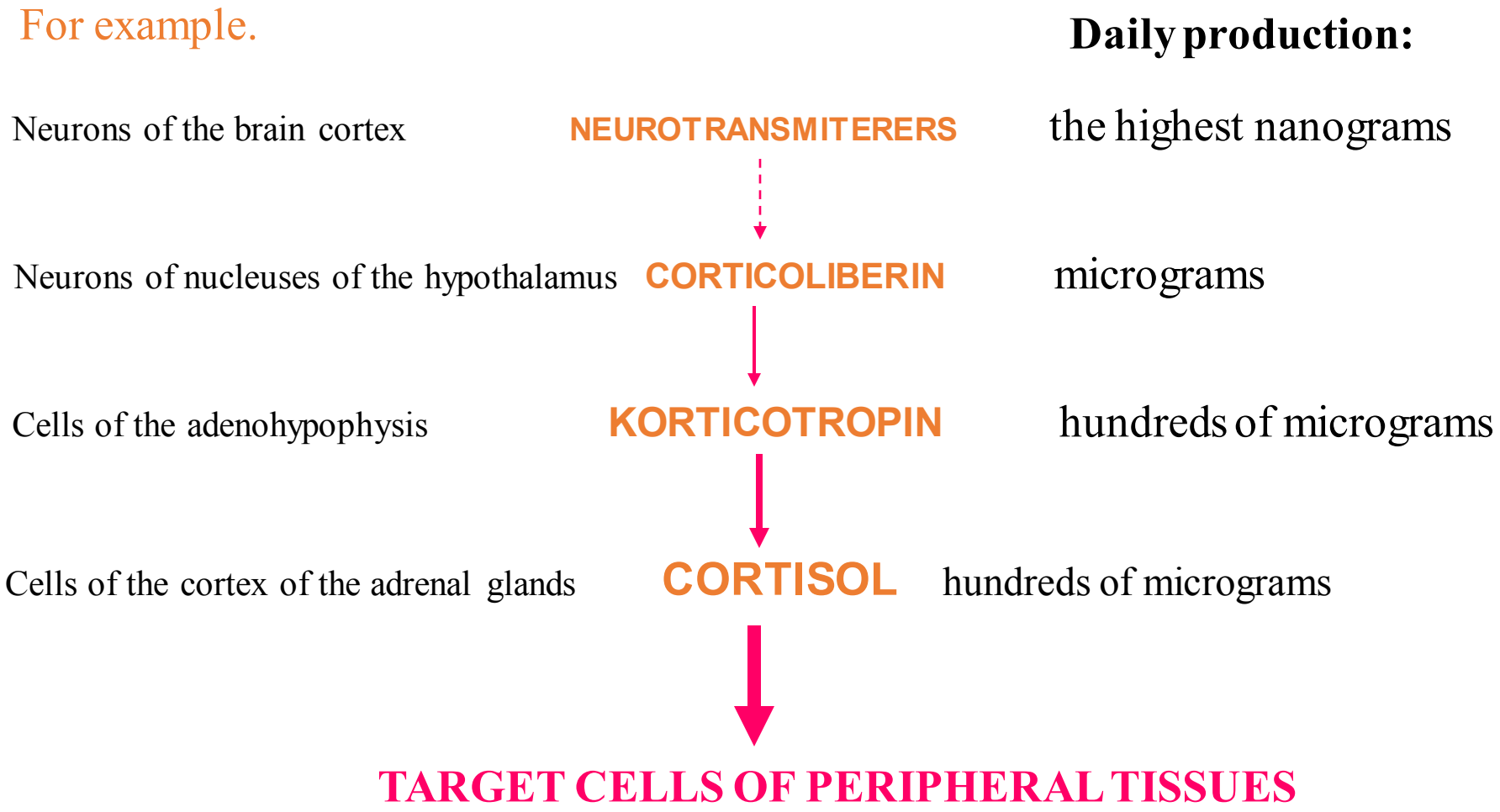
Activated receptor can enter into the reaction with other cellular components and realise the process of the signal transduction.

Tissues, whose cells have no molecules of the specific receptor, can´t react to the relevant hormone.

Charakteristic feature of a transport of the signal by receptors is its **amplification**, when the only one molecule of the hormone is able to cause celullar answer with 104–105 times higher intensity.

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

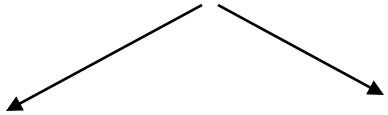
For example.



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 amins, eicosanoids)

Intracelular receptors

Nepolar signal molecules (steroids, jodthyronins, retinoats)

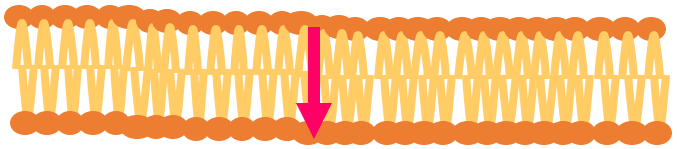
TEST

Membrane and intracelular receptors

Polar signaling molecules

↓

Membrane receptor



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Transduction of signal

100

Amplification

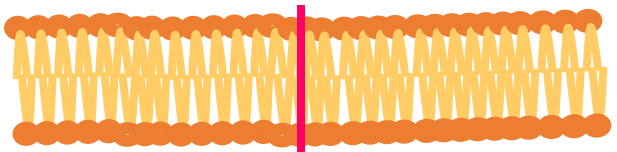
10 000

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 hormon-sensitive element of DNA

↓

Biological effect
(slower effect)

Receptors

Nuclear receptors

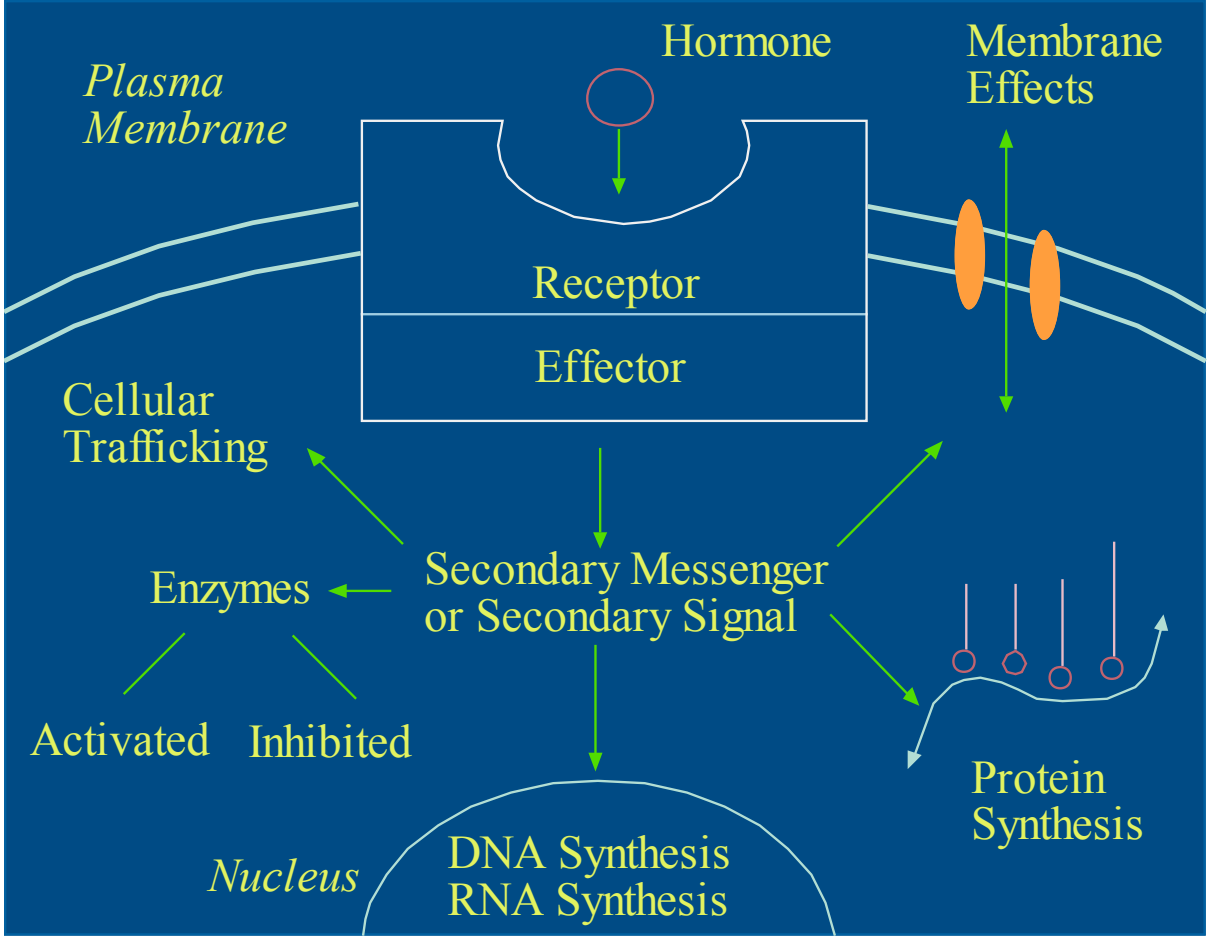
estrogens

Cytoplasmic receptors

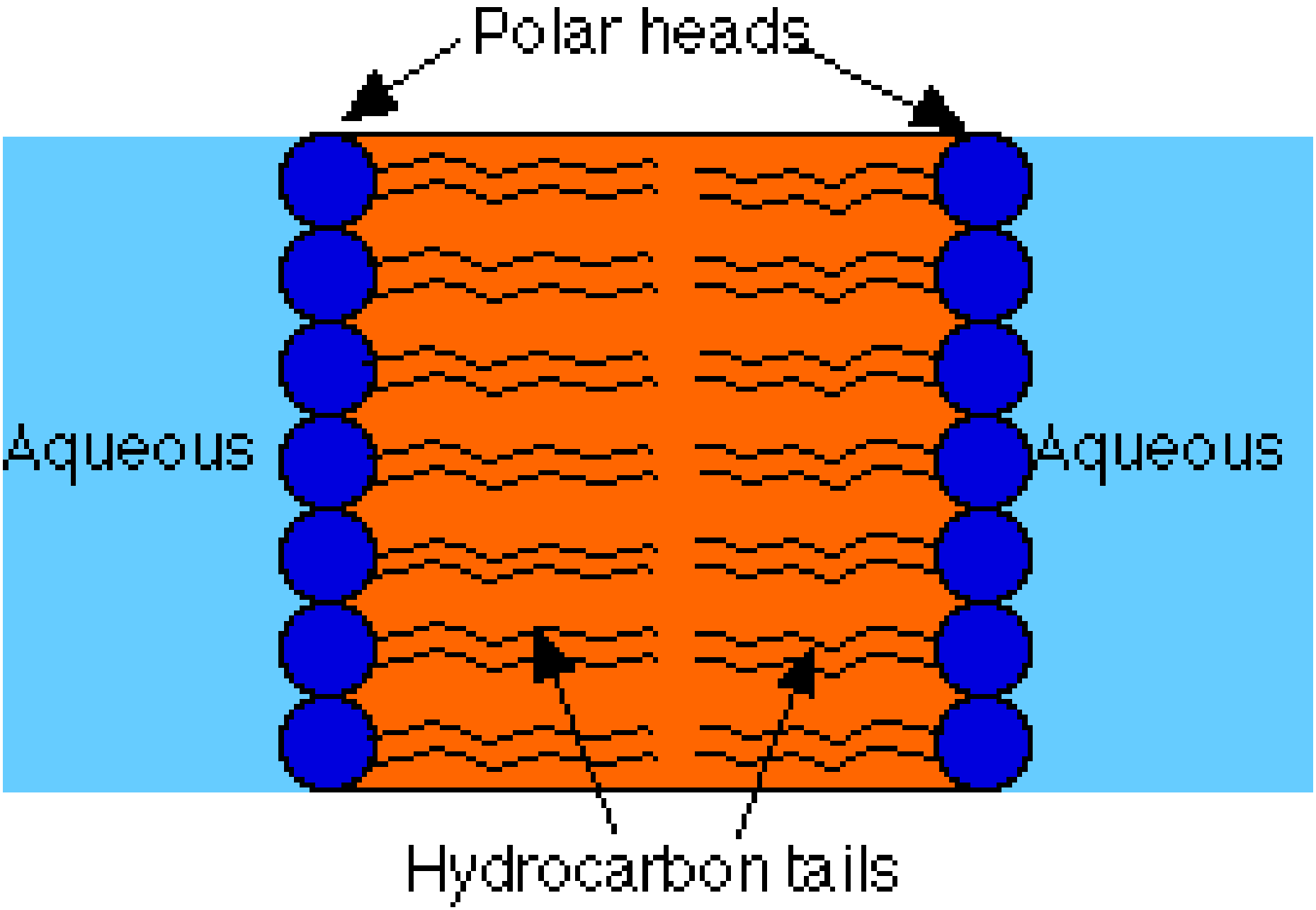
Most steroid and thyroid hormones

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.


Polar heads
Aqueous
Aqueous
Hydrocarbon tails

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

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Main types od 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

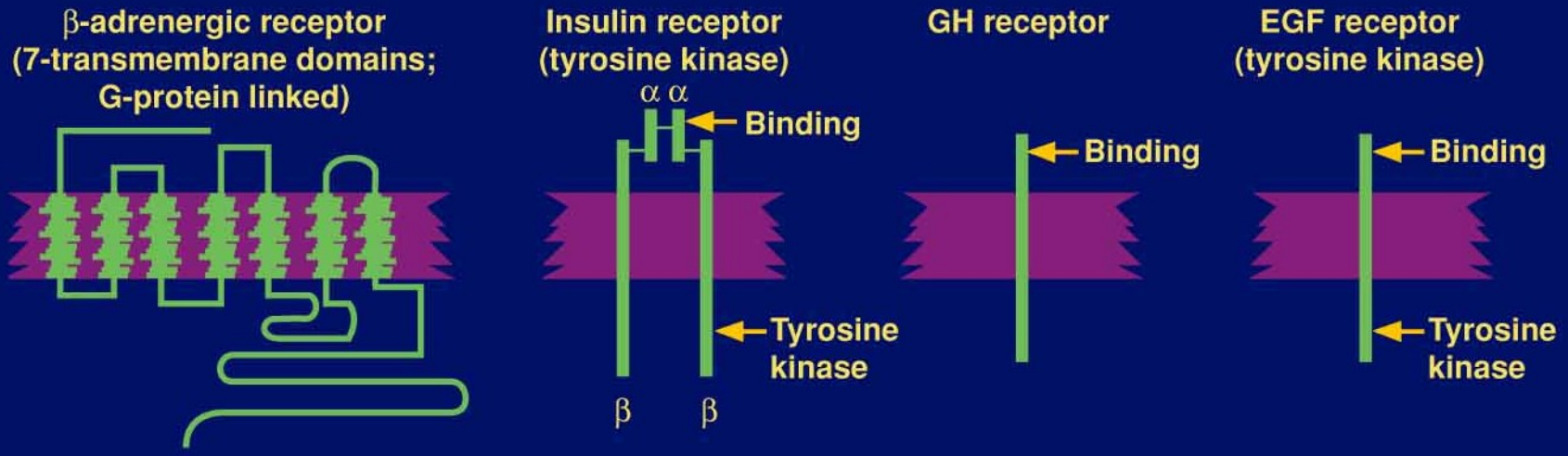
a) **guanylate-cyclase**

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.

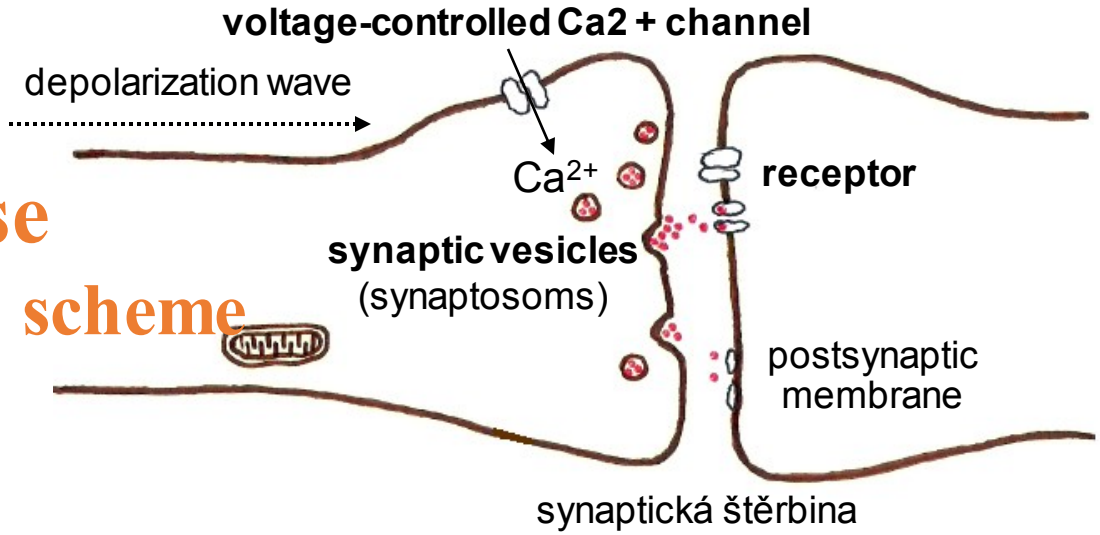


I. Receptors – ion channels

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)

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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 $Na^+/K^+/Ca^{2+}$,

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

Metabotropic receptors activating G proteins, e.g.

protein G**s** **adrenergic β1** a **β2**, receptor GABAB, dopamine D1,

protein G**i** **adrenergic α2**, dopamine D3,

muscarinic acetylcholine M2 (also opens K + channel),

protein G**q** **muscarinic acetylcholine M1**, **adrenergic α1**.

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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 than10 %)

acetylcholine (about 10 %)

dopamine(about 1 %, in striatum 15 %)

serotonine

histamine

aspartate

noradrenalin

(less than1 %, in hypotalamus 5 %)

adenosine

Neuromodulation endorphins and enkephalins, endozepines, delta-sleep-inducing peptide etc.

In peripheral nervous system

– <u>efferent neurons</u>

excitatory **acetylcholine**

noradrenaline

– <u>primary sensory afferent</u>

excitatory glutamate

(Aβ fibers, tactile)

substantion P (peptide)

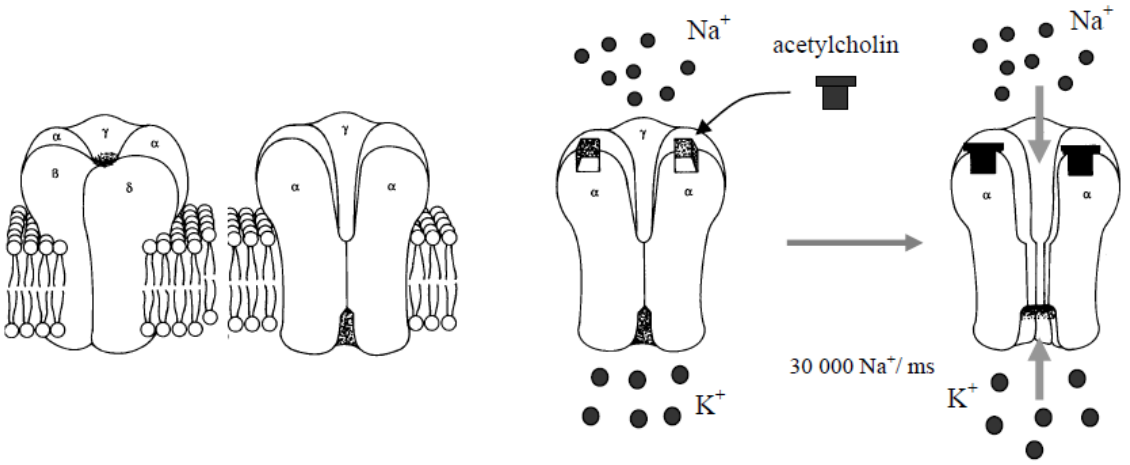
(C Aδ fiber nociceptive)

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 (2a, b, g, 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 Ca + -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.



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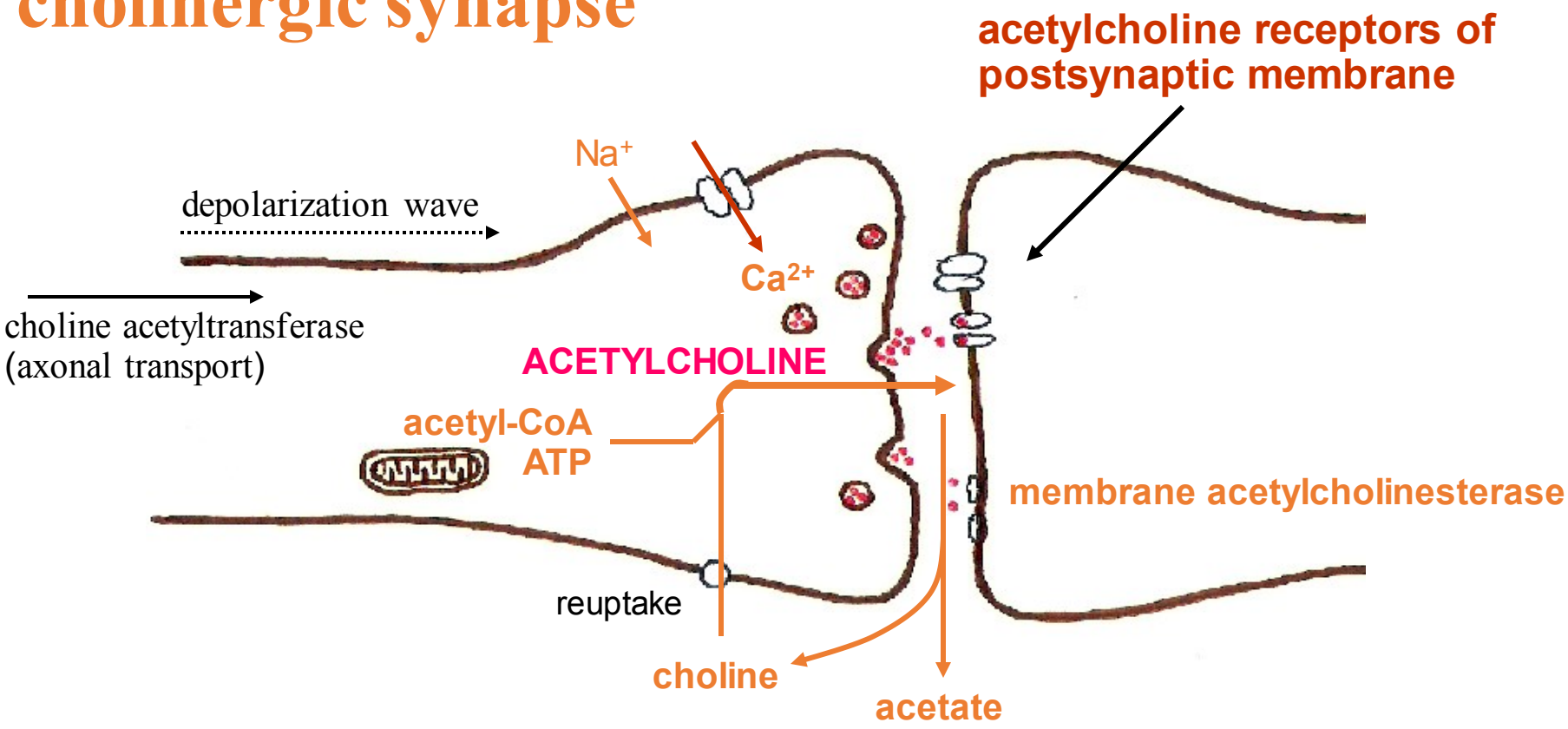
Major neurotransmitter receptors

Acetylcholine receptors

Receptor	Nicotine	Muscarinic	
	ion channel	M_1, M_3	M_2
Mechanism of action		G_q	G_i
The second messenger		$DG + IP_3$	cAMP
occurrence	• autonomic ganglia neurons, • neuromuscular junction, • chromaffin cells of the adrenal medulla	• brain, • smooth muscle, •glandular cells	• myocardium, • brain
	tubocurarine	atropin	

TEST

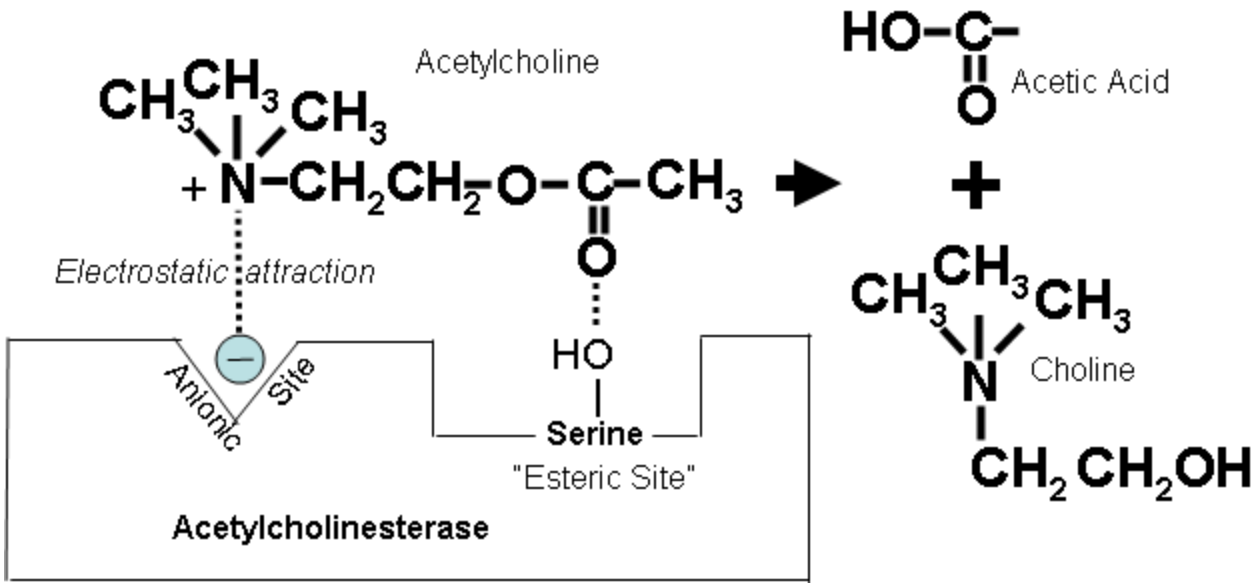
cholinergic synapse



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.

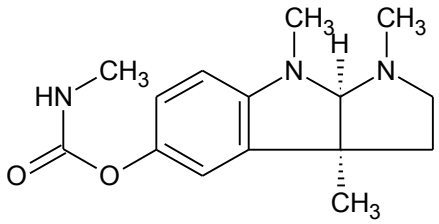
Acetylcholinestherase

- 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)



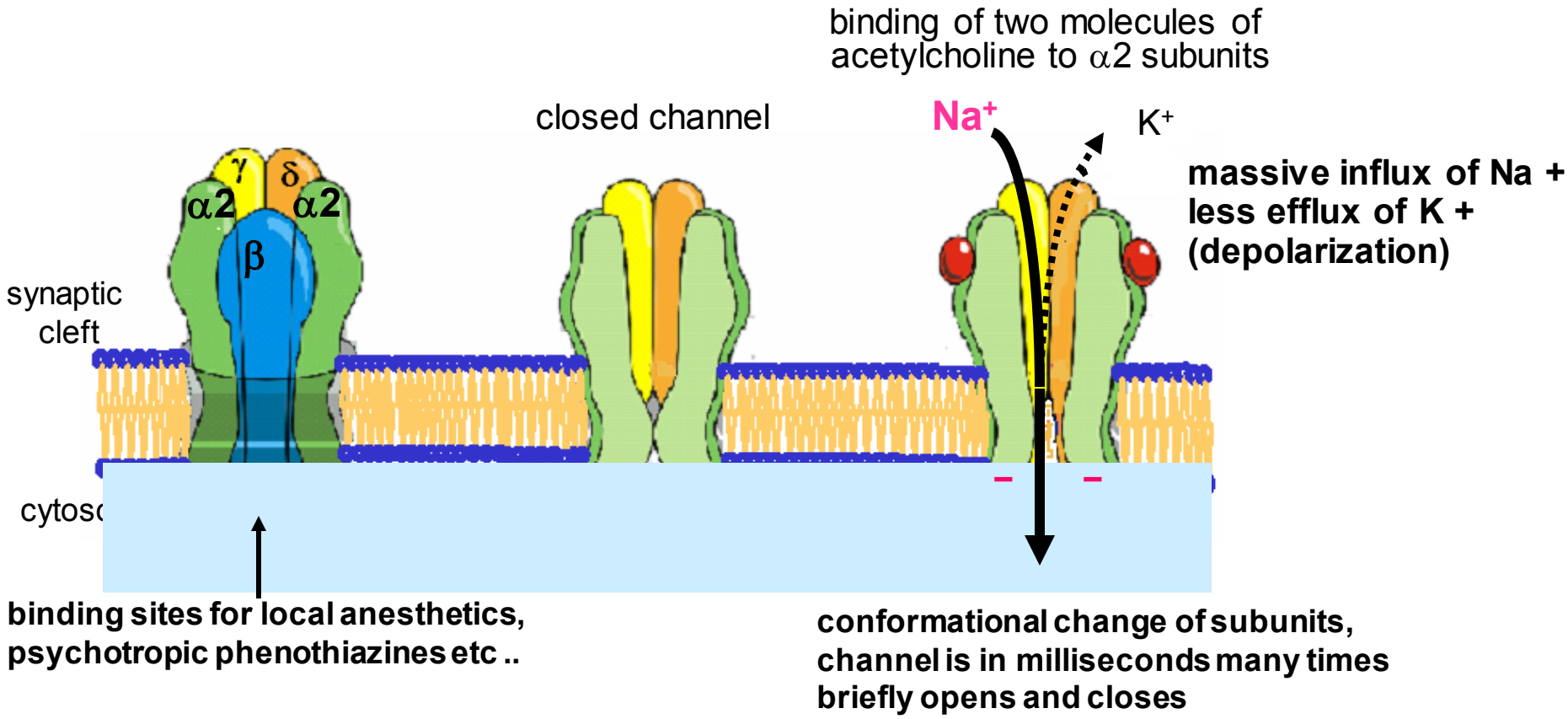
fyzostigmin

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

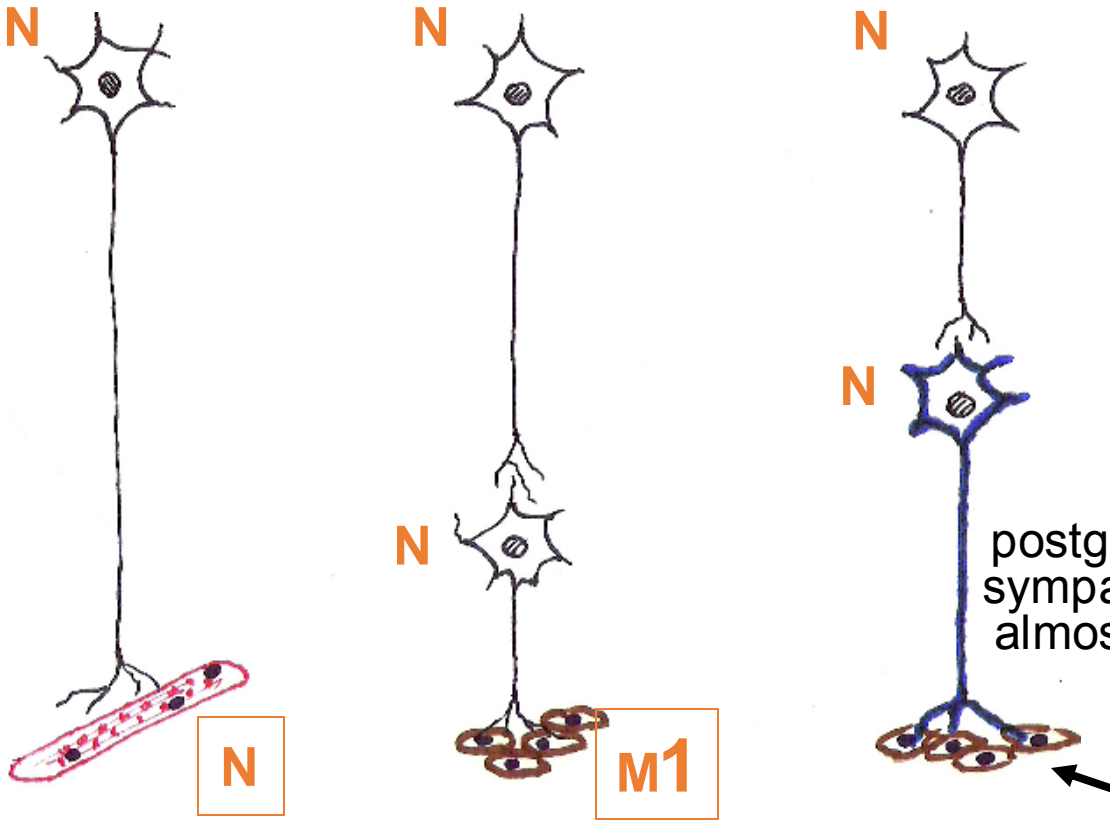
irreversible - formation of covalent bonds between enzyme and organophosphate

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



N-nicotinic

M-muscarinic

postganglionic neurons of sympathetic pathways are almost always adrenergic

Adrenergic receptors

Rhabdomyocytes (**skeletal muscles**)

Parasympathetic innervated cells of target tissues

Sympathetic

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.

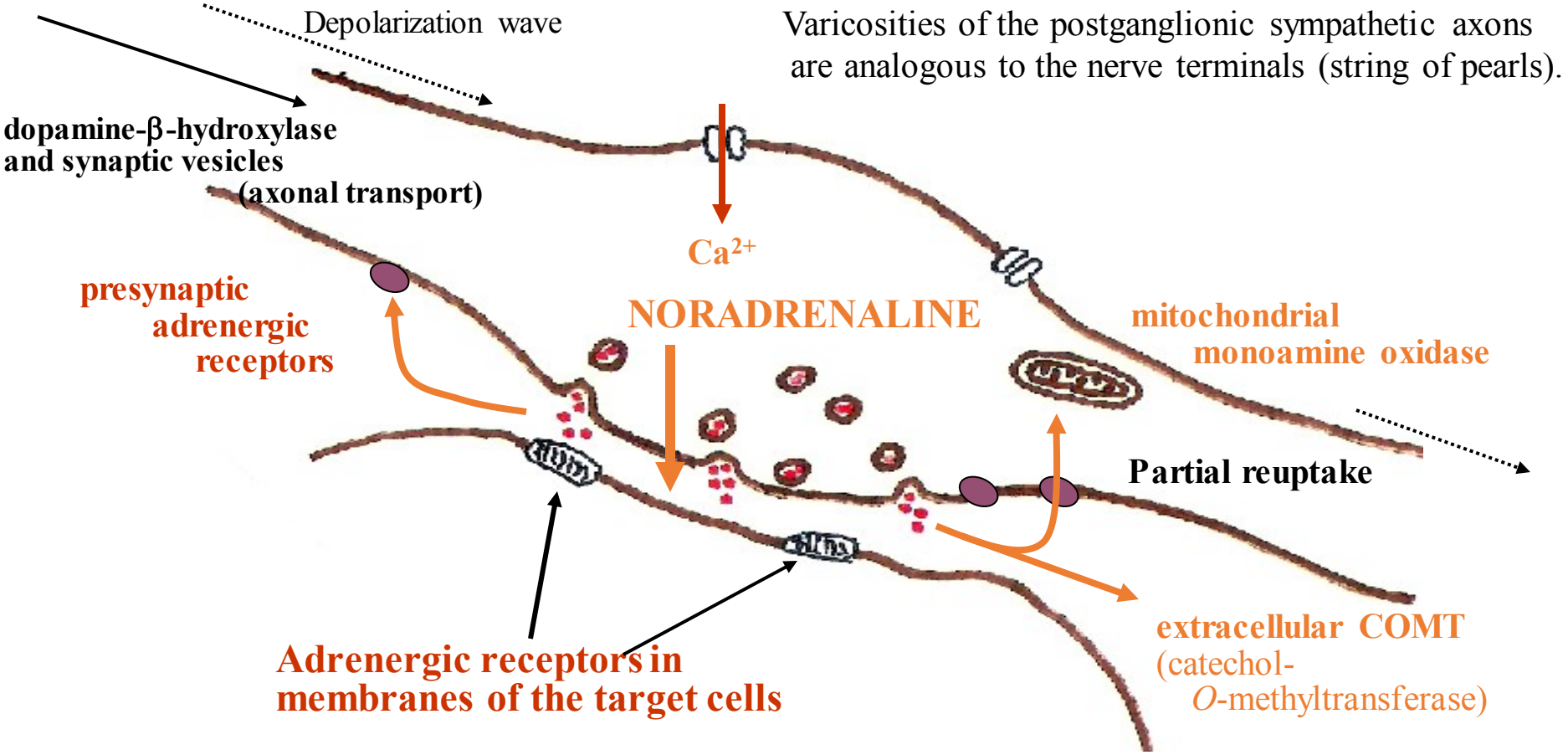
Muscarinic cholinergic receptors

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.



TEST

Synthesis and storage of catecholamines

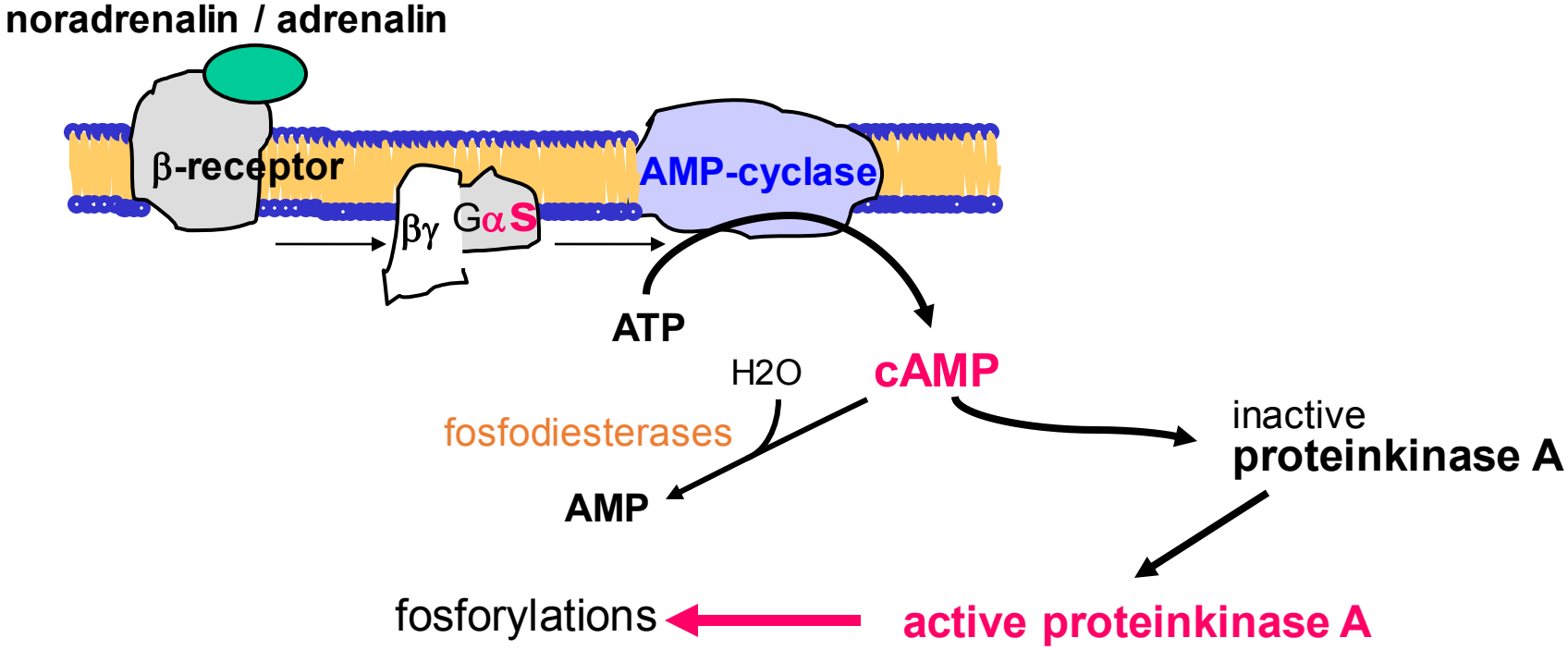
- Dopamine is synthesized in the cytoplasm
- Dopamine is transported into vesicles (ATP-dependent process, against concentration gradient).
- Final hydroxylation of dopamine to noradrenaline occurs in vesicles.

TEST

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	• GIT smooth muscle (sphincters) and skin blood vessels (contraction)	• adrenergic and cholinergic nervous terminals (transmitter releasing inhibited) • pancreas (glandular secretion inhibited) • thrombocytes (agregation)	• myocard (intensity and frequency of contractions increased)	• smooth muscle in the uterus, bronchi (relaxation) • GIT smooth muscle (peristalsis) • pancreas (glandular secretion inhibited activated)

β-Adrenergic receptors



The typical effects of β-stimulation

β1 – tachycardia, inotropic effect in the myocard,

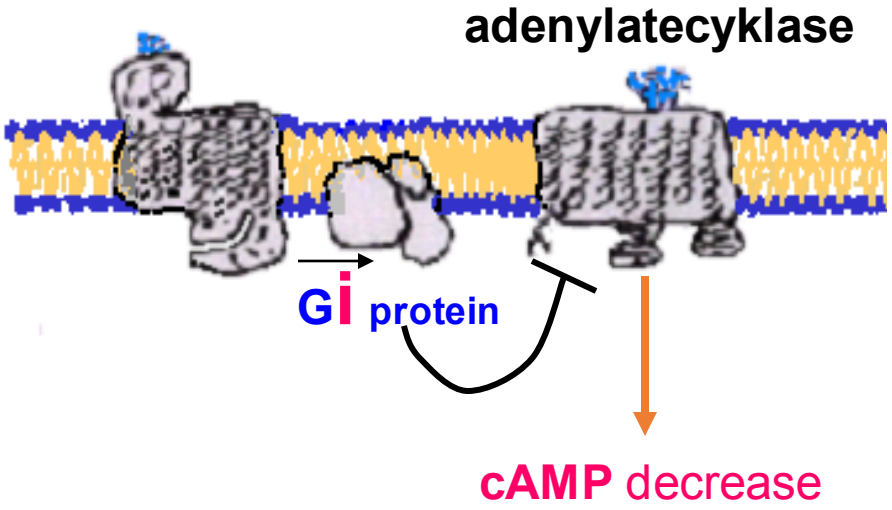
β2 – bronchodilation, vasodilation in the bronchial tree,

β3 – mobilization of fat stores, thermogenesis.

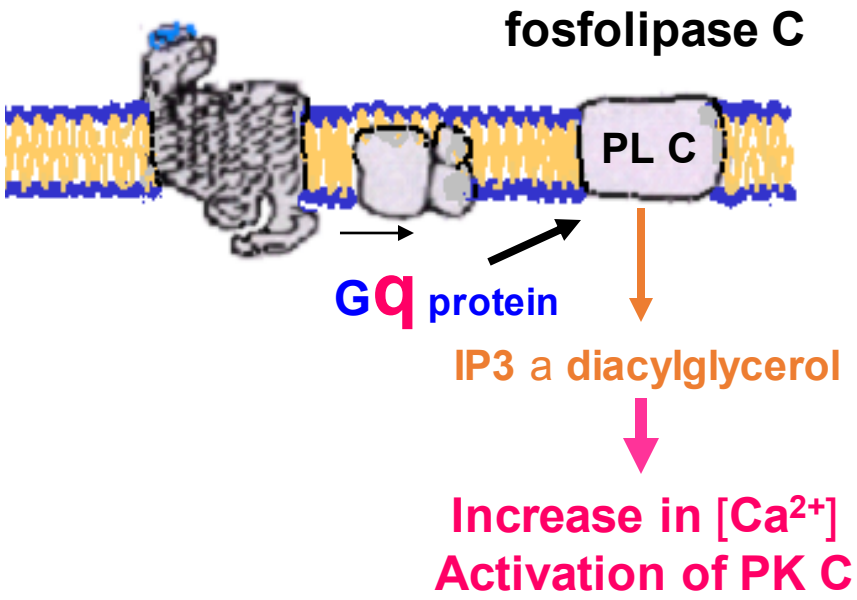
TEST

Adrenergic receptors α2 a α1

α2-receptors



α1-receptors



The typical effects of adrenergic

α2-stimulation:

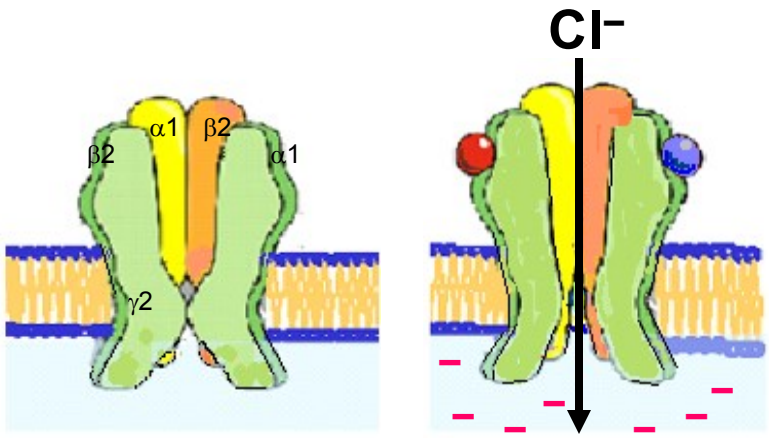
glandular secretion inhibited

α1-stimulation:

vasoconstriction
bronchoconstriction
motility of GIT inhibited

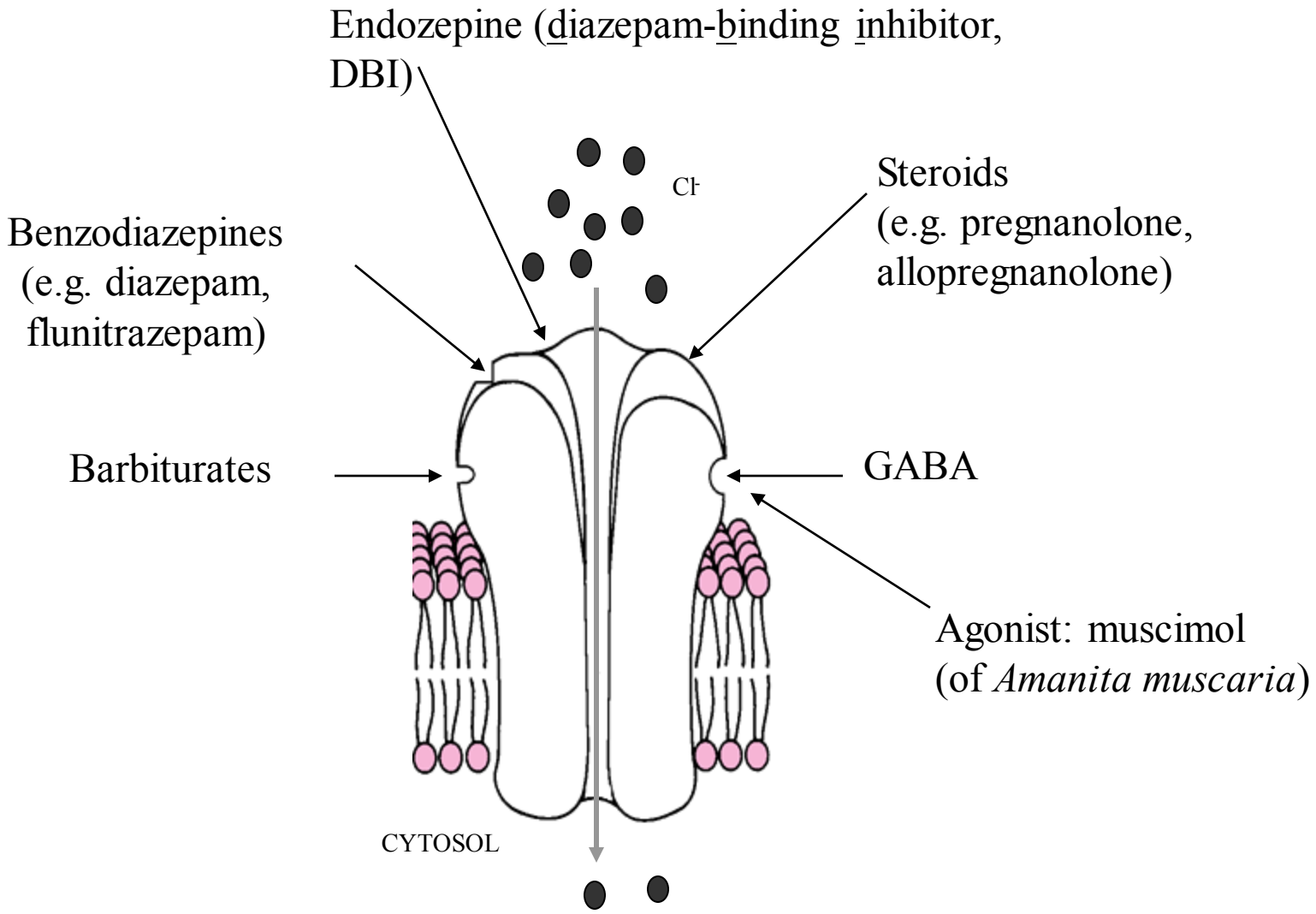
Inhibitory $GABA_A$ receptor

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

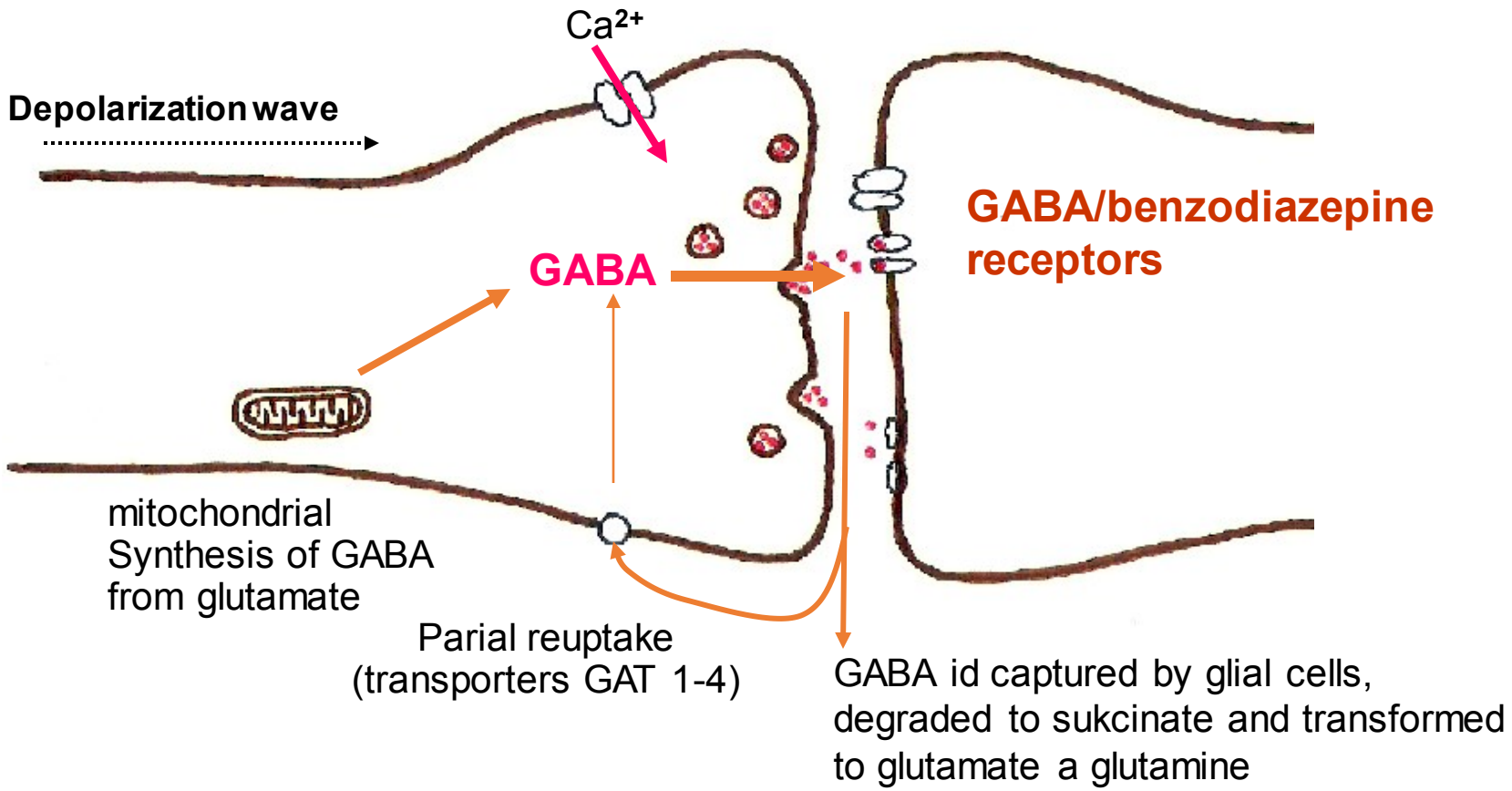
More than eleven **allosteric modulation sites** for substances increasing the effect of endogenous GABA (calming down, reduction of anxiety and myorelaxation): anesthetic, ethanol and numerous drugs such as benzodiazepines meprobamat and also various barbiturates.

On the contrary, another ligands compete for benzodiazepine binding site or act also as the **antagonists** of GABA (inverse agonists), ⇒ 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

γ-Aminobutyric acid(GABA) is the main inhibitory neurotransmitter in CNS. Gabaergic synapses represent about 60 % of all brain synapses.



TEST

Receptors of the most important neurotransmitters

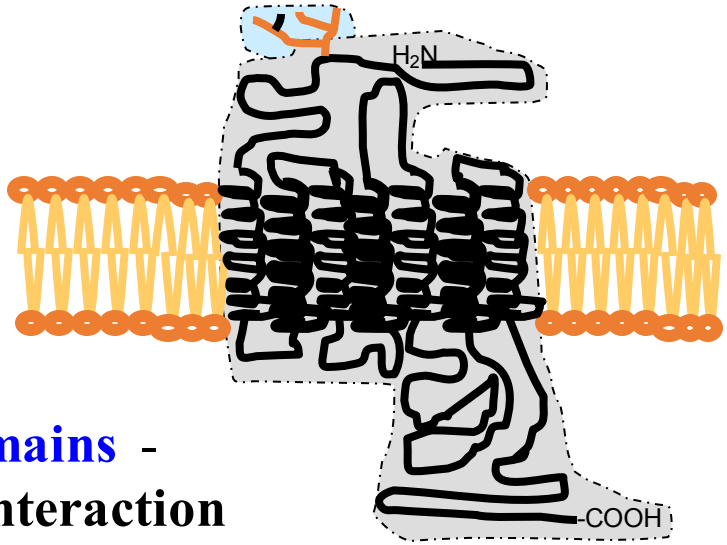
Ion channels (ROC)	Receptors associated with G-proteins		
	Gs (increasing cAMP)	**Gi (decreasing cAMP)**	**Gq (increasing IP3 /DG)**
$\underline{Na^+/K^+}$ – **acetylcholine nicotinic**	–	acetylcholine muscarinic M2,4	acetylcholine **muscarinic M1**,3,5
–	**adrenergic β1,β2**,β3	**adrenergic α2**	**adrenergic α1**
$\underline{Na^+/Ca^{2+}/K^+}$ – glutamate ionophores	–	glutamate mGluR skupiny II a III	glutamate mGluR class I
–	dopamine D1,5	dopamine D3,4	dopamine D2
– serotonin 5-HT3	serotonin 5-HT4,6	serotonin 5-HT1	serotonin 5-HT2
–	histamine H2	histamine H3,4	histamine H1
–	–	–	tachykinin NK1 for substance P
$\underline{Cl^-}$ – GABAA – glycine	GABAB (metabotropic)	–	–

II. Receptors interacting with heterotrimeric G-proteins

Common structural features:

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

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



Few minutes

Neurotransmitters

Hormones

Agonist-ligand causes signale transduction

Antagonist- prevents

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

Heterotrimeric G-proteins

Proteins **binding GDP or GTP**

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

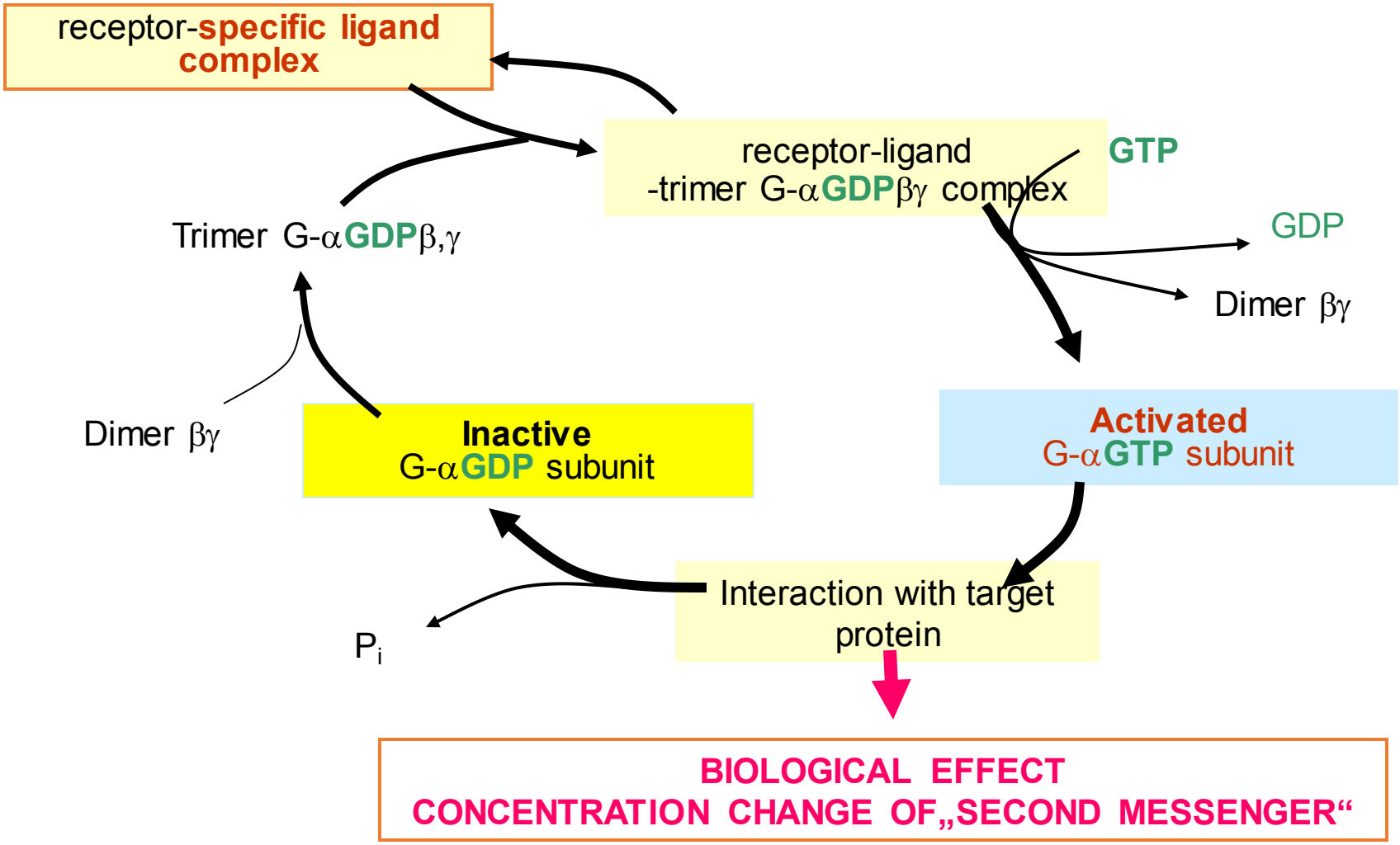
Subunits α, β a γ.

Subunits Gβ and Gγ are hydrophobic and are not specific.

Subunits Gα are the biggest, bind GDP or GTP and are **specific** for every type of transduction mechanism.

Identified more than 20 types of different Gα subunites.

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



TEST

Chosen types of G-proteins

Type of Gα subunit	Examples of activating receptor	Effect of activated Gα To target protein (intracellular signal)
$G\alpha_s$ (stimulating)	glucagon parathyrine β-adrenergic	Adenylatecyclase stimulation (cAMP, Ca2+)
$G\alpha_i$ (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+IP3, Ca2+)
$G\alpha_t$ (inhibitory) (transducin)	rhodopsin	phosphodiesterase cleaving cGMP stimulation

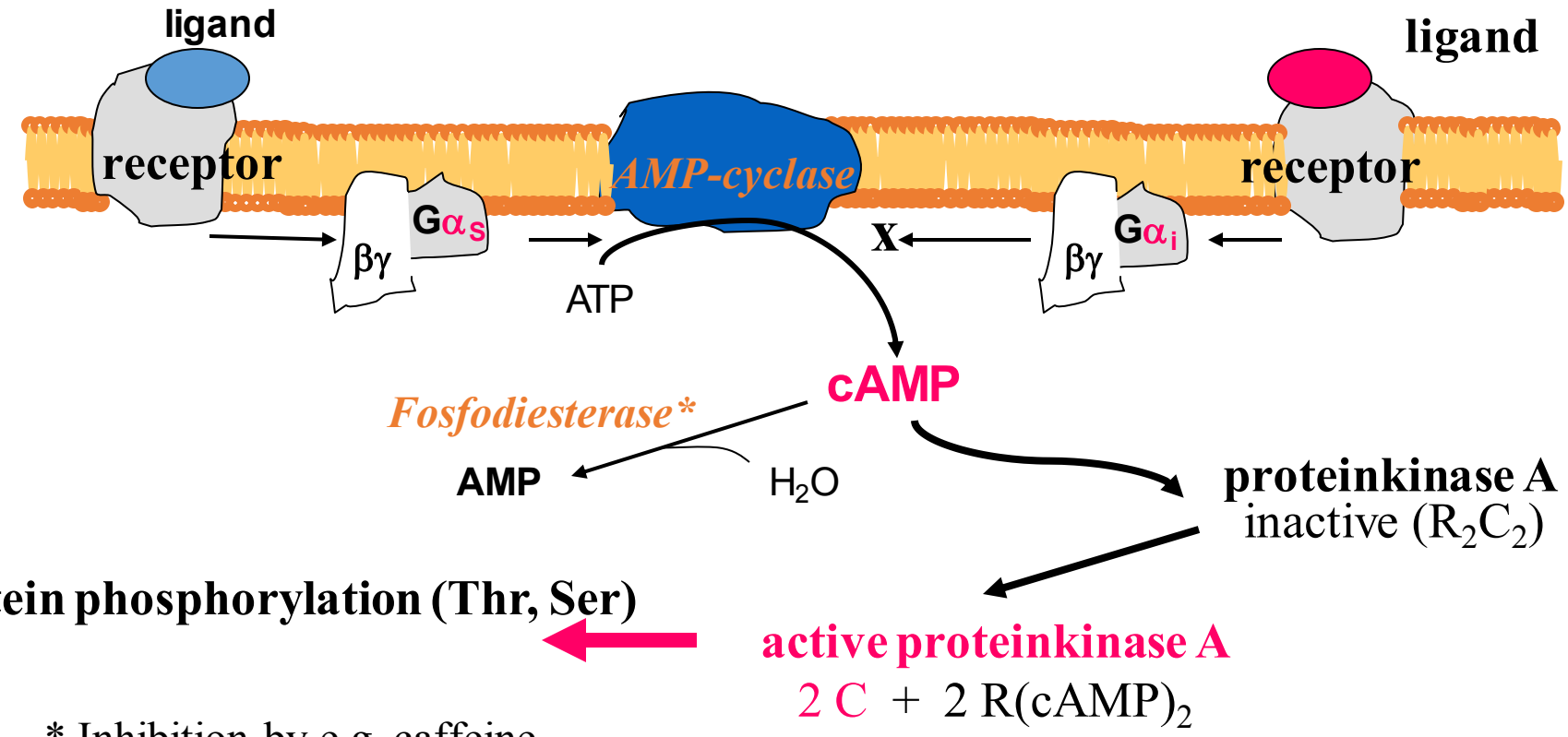
Receptors activating G_s and G_i stimulate or inhibit adenylatecyclase

TEST

Adenylatecyclase - membrane enzyme catalysing reaction

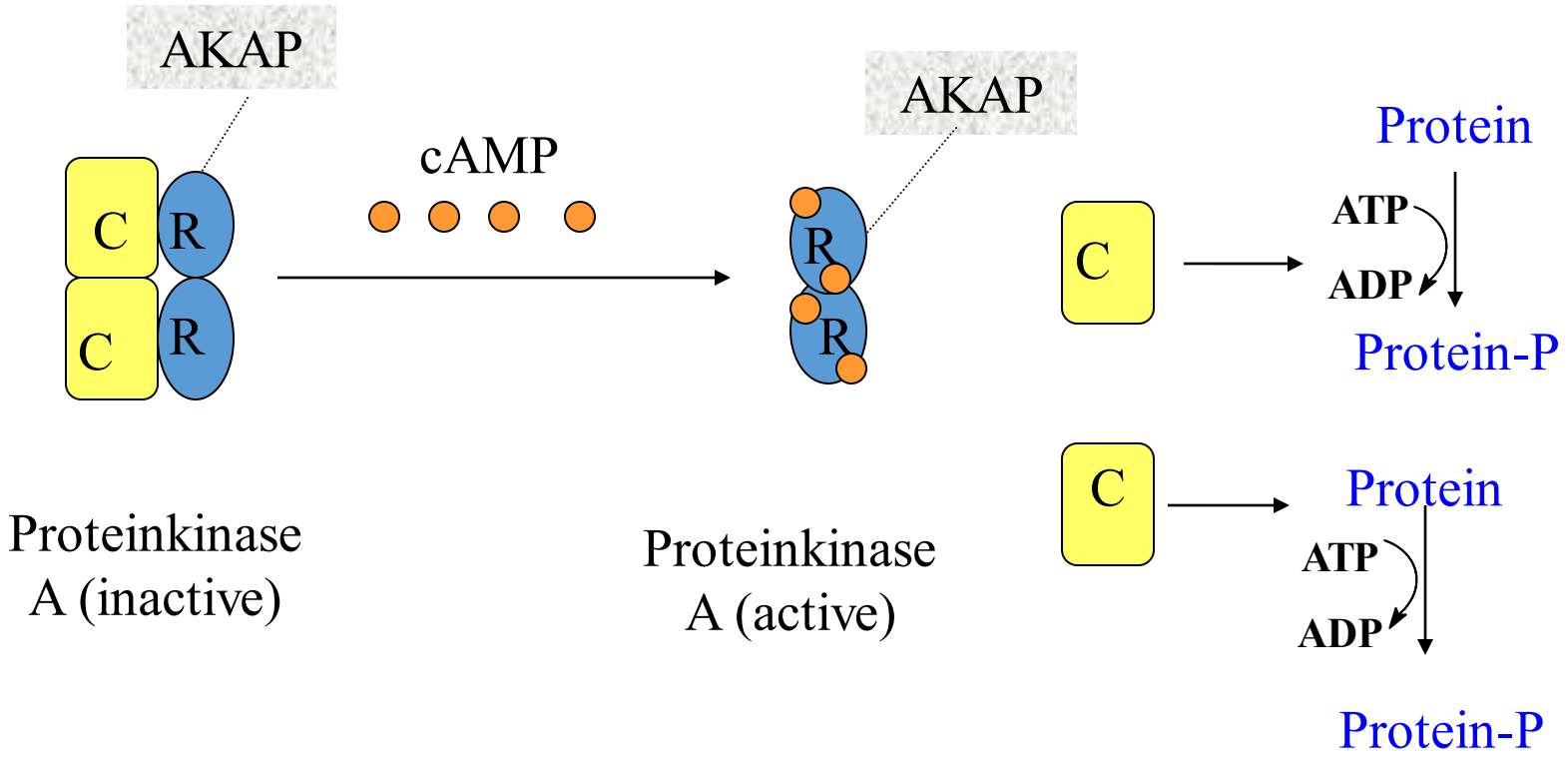
$ATP \rightarrow cAMP + PP_i$;

cAMP is second messanger.



* 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)

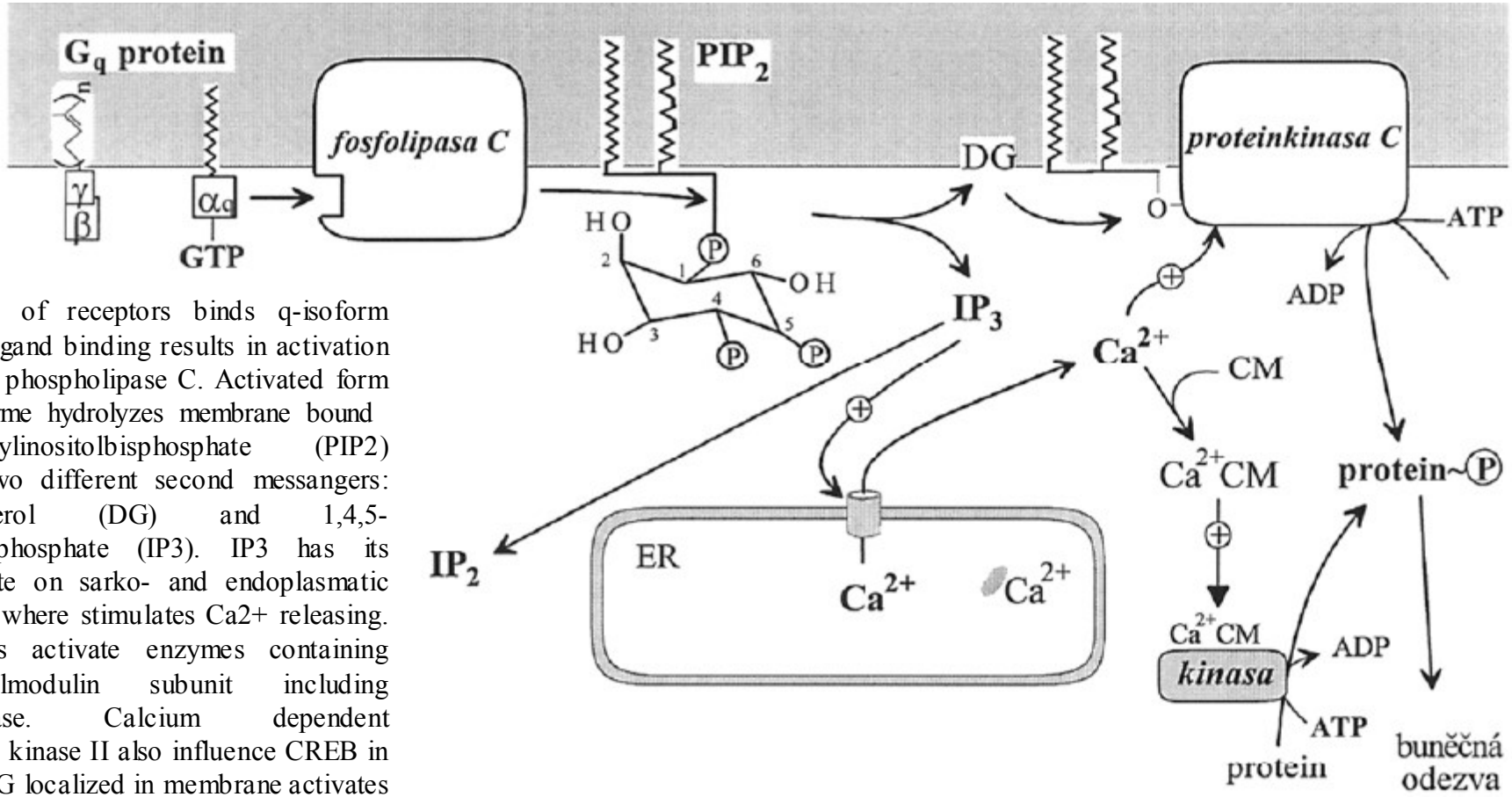
cAMP provides many different effects in the cell.
One of the most important effect is **proteinkinase A** activation, which phosphorylates many others metabolic enzymes. The effects of kinases can by aimed for certain proteins phosphorylation. That is maintained by specific proteins binding kinases. 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 aktivity 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 phoshate transmission from ATP to serine or threonine residues of target proteins. Proteinkinase A catalytic subunit also enters the nucleus where prosphorylate gen 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*. Choleratoxin is protein causing by its effect inhibition of GTPasa activity of Gs protein subunit. Modificated □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 secernation to intestinal lumen. Inhibitory G-protein is the target of pertusis 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

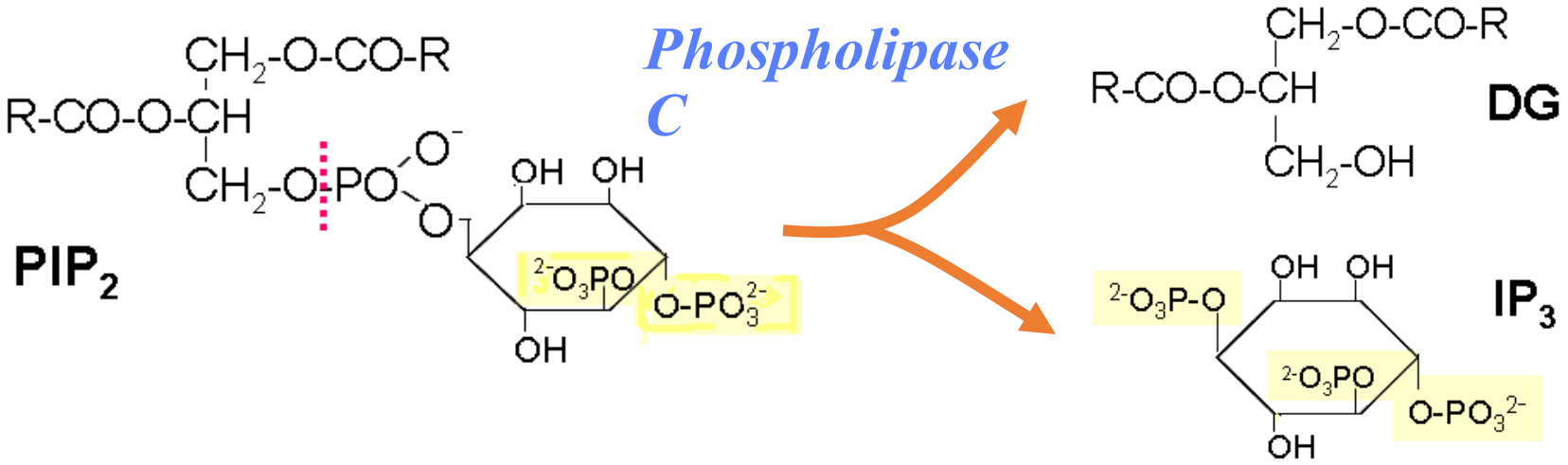
Hormone	Localization of the effect
CRH	Adenohypophysis
TSH	Thyroid follicle
LH	Testicular Leydig cells, yellow body (corpus luteum)
FSH	Ovaria follicle cells, testicular Sertoli cells
ACTH	Adrenal cortex
ADH	Kidney distale tubule cells
PGI_2	Thrombocytes
Adrenaline, noradrenaline	β - receptors in many cells
glukagon	Livers

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



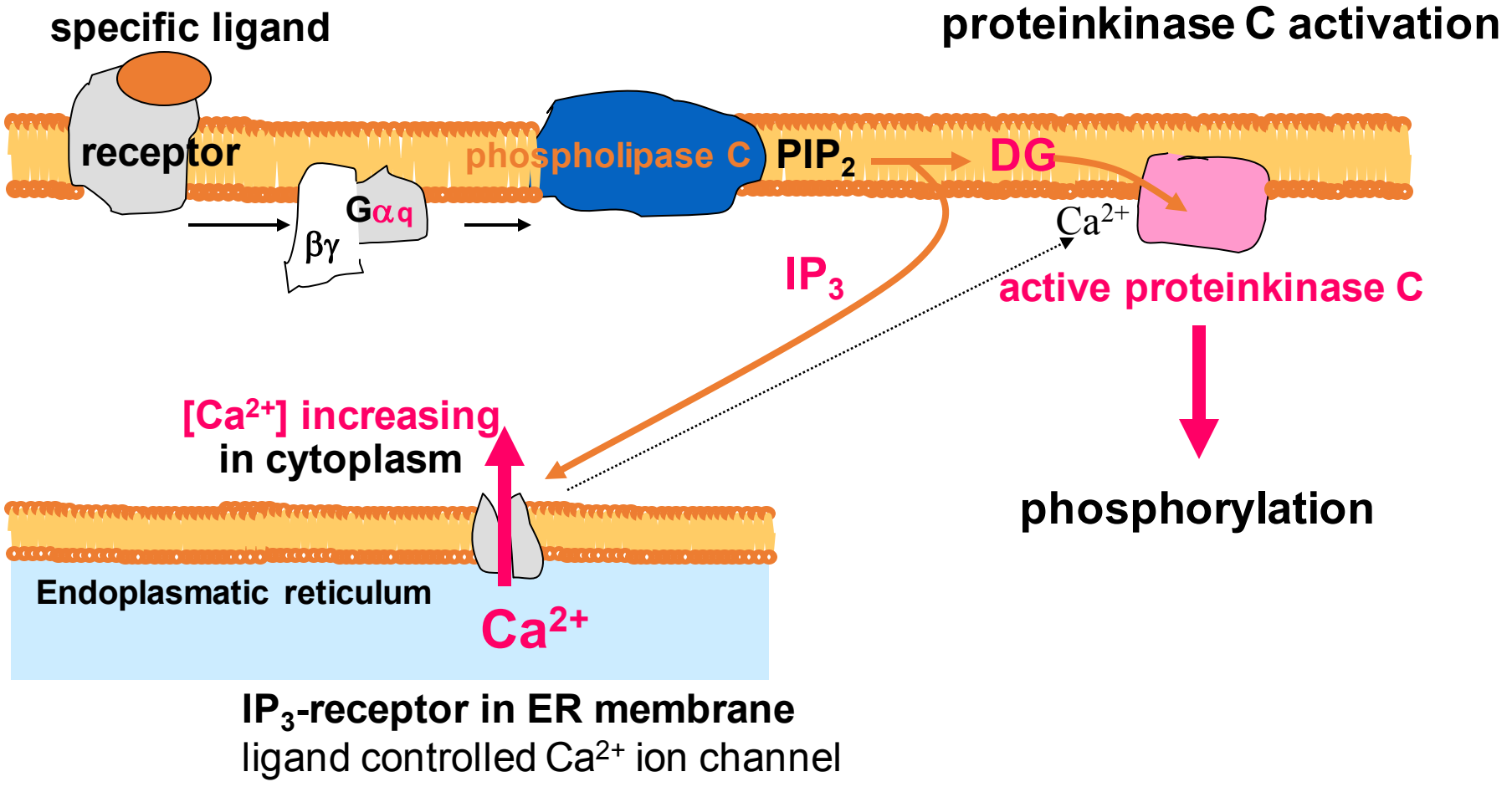
That kind of receptors binds q-isoform subunit. Ligand binding results in activation of enzyme phospholipase C. Activated form of this enzyme hydrolyzes membrane bound phosphatidylinositolbisphosphate (PIP2) forming two different second messangers: diacylglycerol (DG) and 1,4,5-inositoltrisphosphate (IP3). IP3 has its binding site on sarko- and endoplasmatic reticulum, where stimulates Ca2+ releasing. Ca2+ ions activate enzymes containing calcium-calmodulin subunit including proteinkinase. Calcium dependent calmodulin kinase II also influence CREB in nucleus. DG localized in membrane activates proteinkinase 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.

Phosphatidylinositol cascade



IP_3-receptor in ER membrane
ligand controlled Ca^{2+} ion channel

Regulation of metabolism by cytoplasmatic Ca^{2+} concentration changes

- Basal Ca^{2+} concentration in cytoplasm ~ 1.10^{-7} mol/l
- Concentration increasing to ~ 1.10^{-6} fast and effectively activates different cellular process
- Ca^{2+} increasing may be caused by

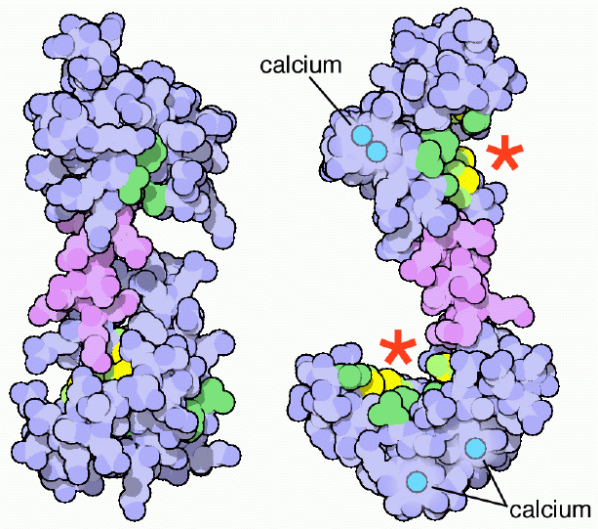
  Ca^{2+} influx through cytoplasmatic membrane (e.g. Smooth muscle contraction)

  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 Ca^{2+}-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

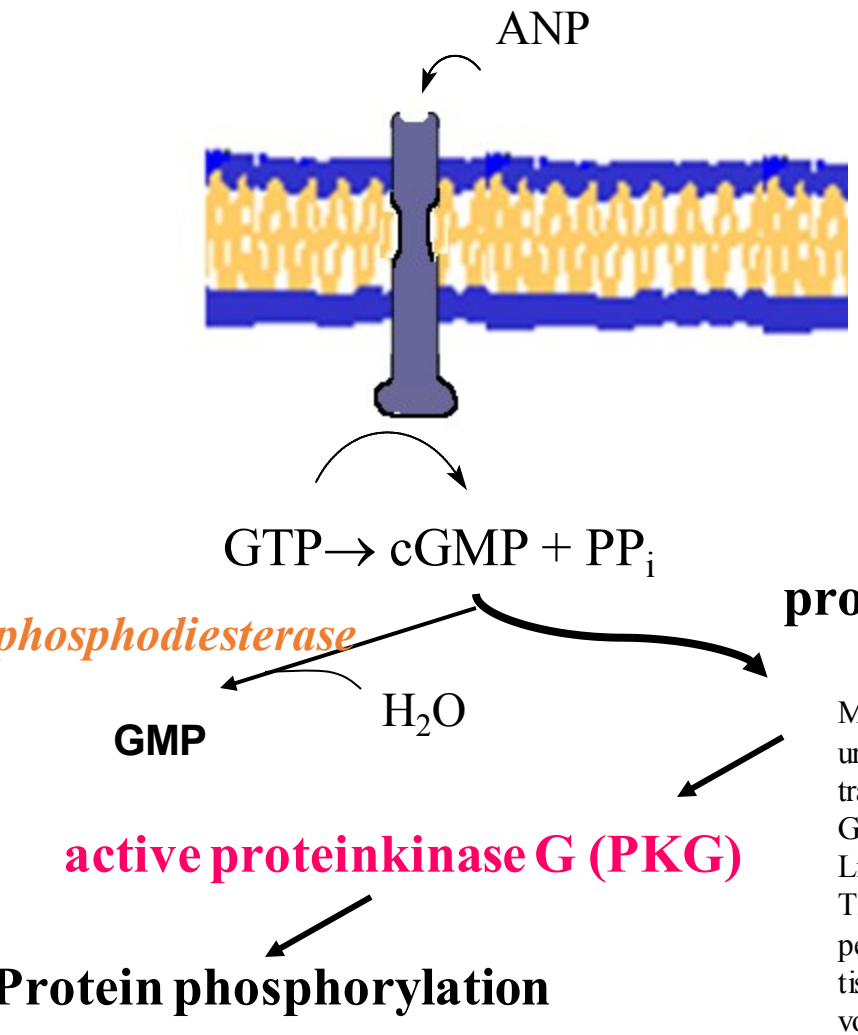
Two kinds of receptors:

- 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 dirrectly 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



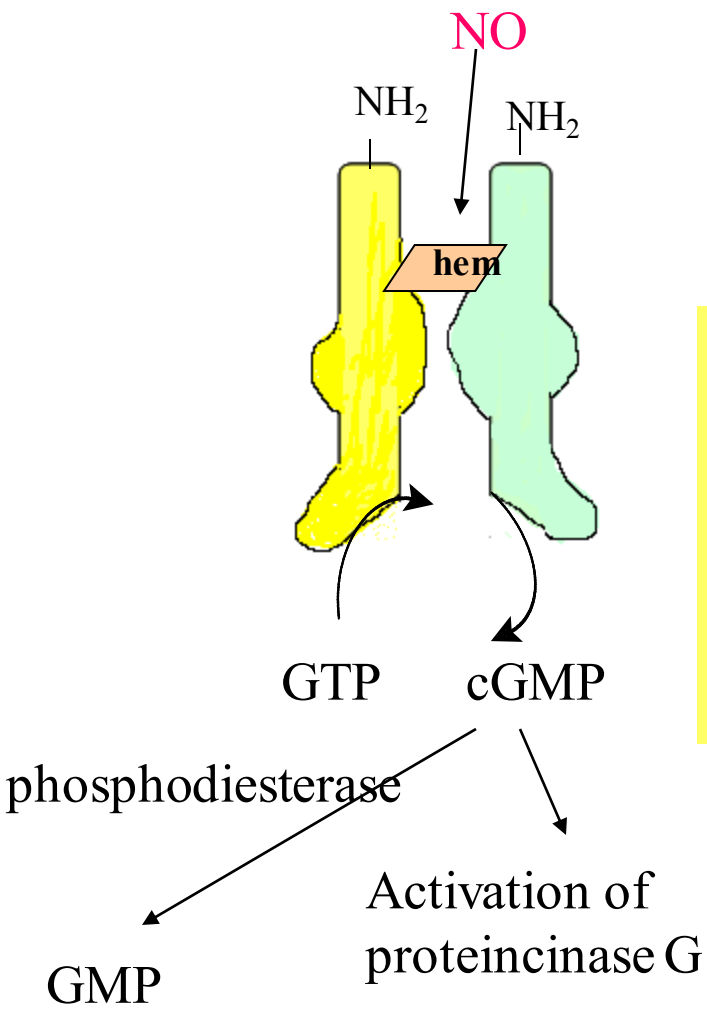
Receptors for ANP

Present especially in vascular smooth muscle and in kidneys

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

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 secernated by myocytes atria as a response to increasing blood volume or pressure in right atrium causing vasculature relaxation. That leads to decrasing 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 a 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 synthetised 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 degradeted 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.

Proteinkinase G

cGMP sensitive proteinkinase G

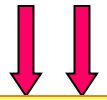
It is spread in a lot of tissues

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 $\Rightarrow \downarrow$ 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



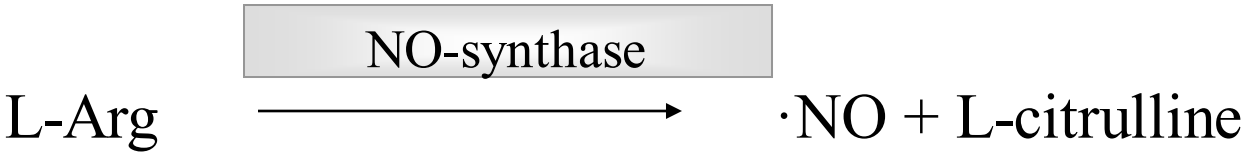
Relaxation of smooth muscles

Meaning of NO/cGMP signalization in smooth muscles of vessels

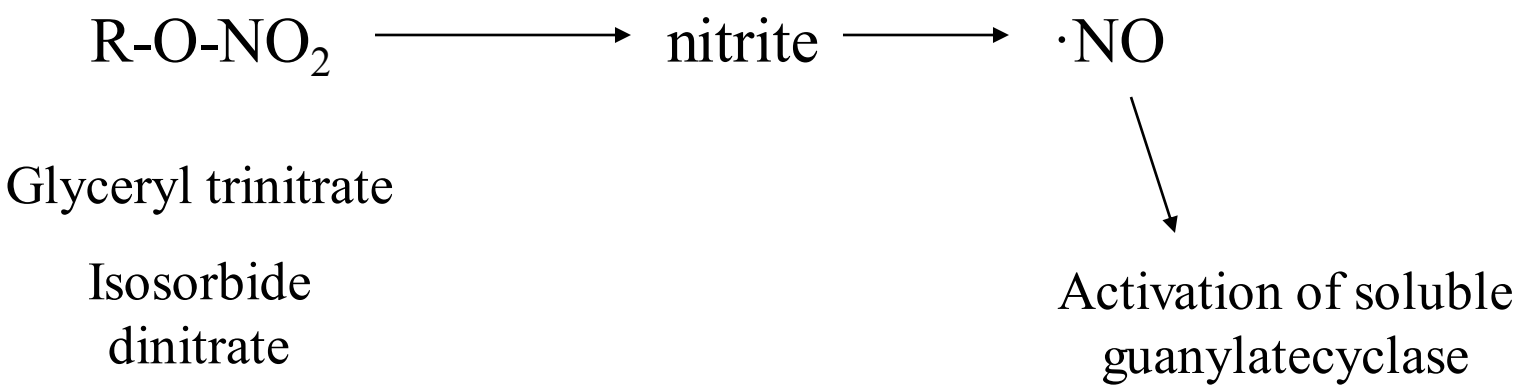
cGMP is a crucial second messenger for an induction of relaxation of smooth muscles in vessels

$\Rightarrow$ 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



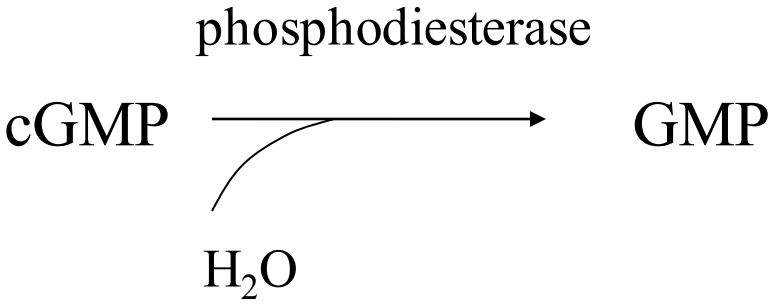
Drugs like organic nitrates are a source of exogenous NO



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

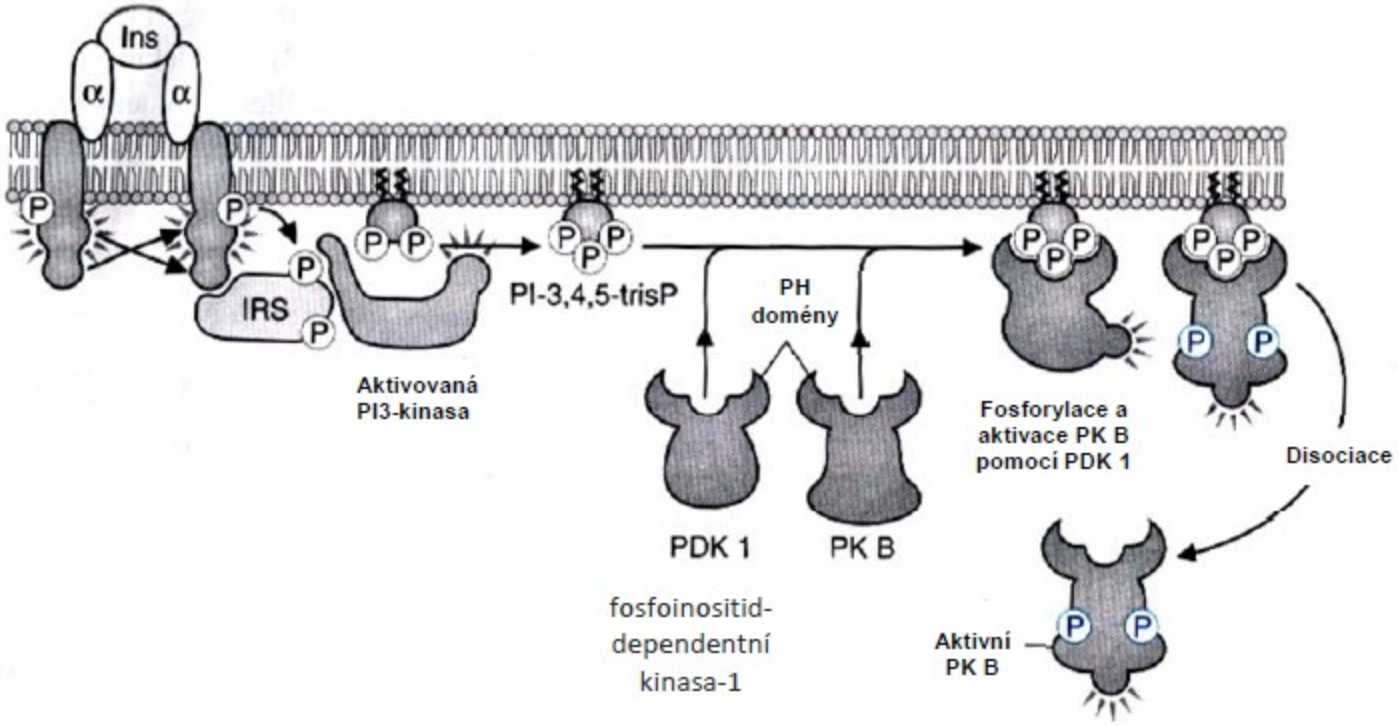
• it caused autophosphorylation of tyrosine of receptor alternatively other proteins (IRS)

•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 stimulate 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.

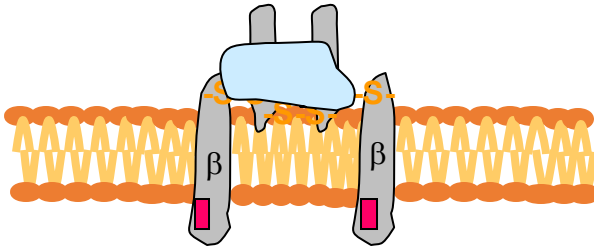
TEST

Insulin receptor

Dimeric structure

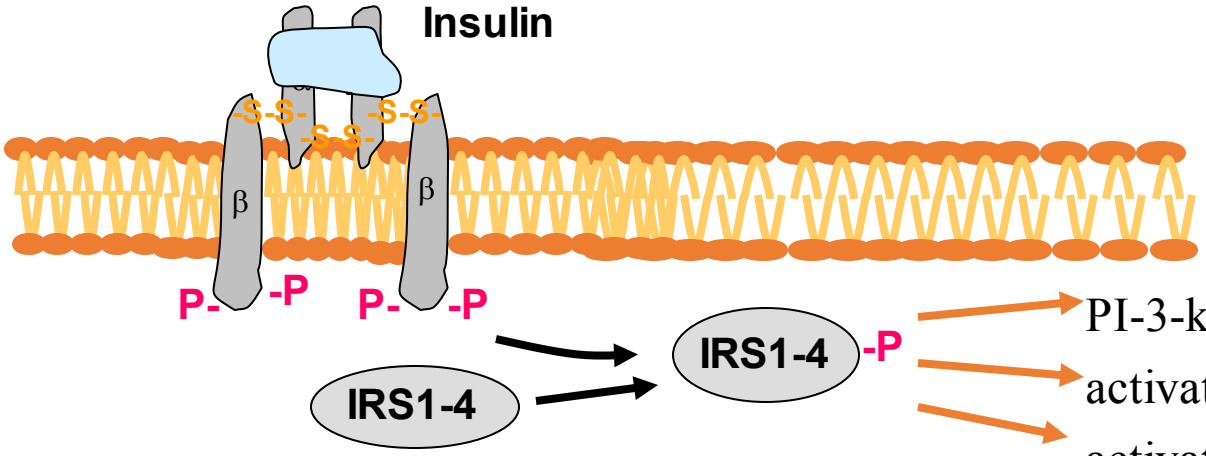
Binding site for insulin on α- subunits

Tyrosinkinase activity on β-subunits



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

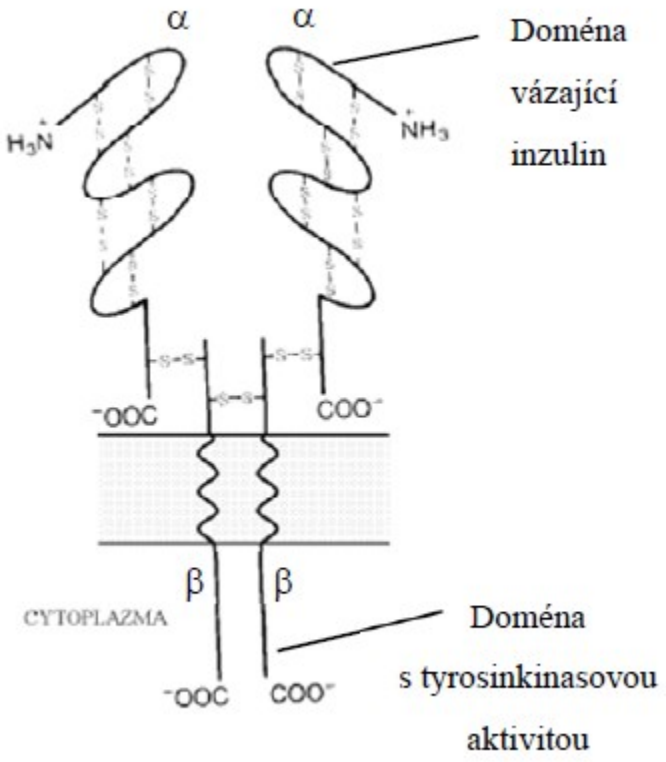
Binding insulin to the receptor causes internalization of complex hormon receptor, receptors are partially recyclated

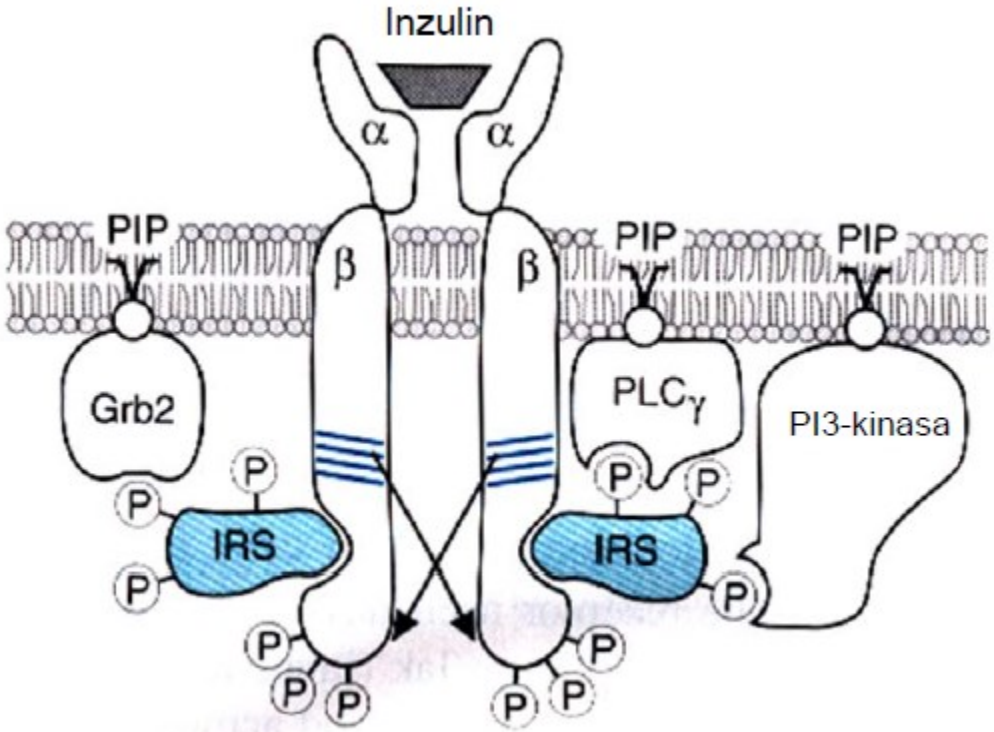


PI-3-kinase binding to the membrane

activation of phosphoproteinphosphatase-1

activation Ras – expression of genes

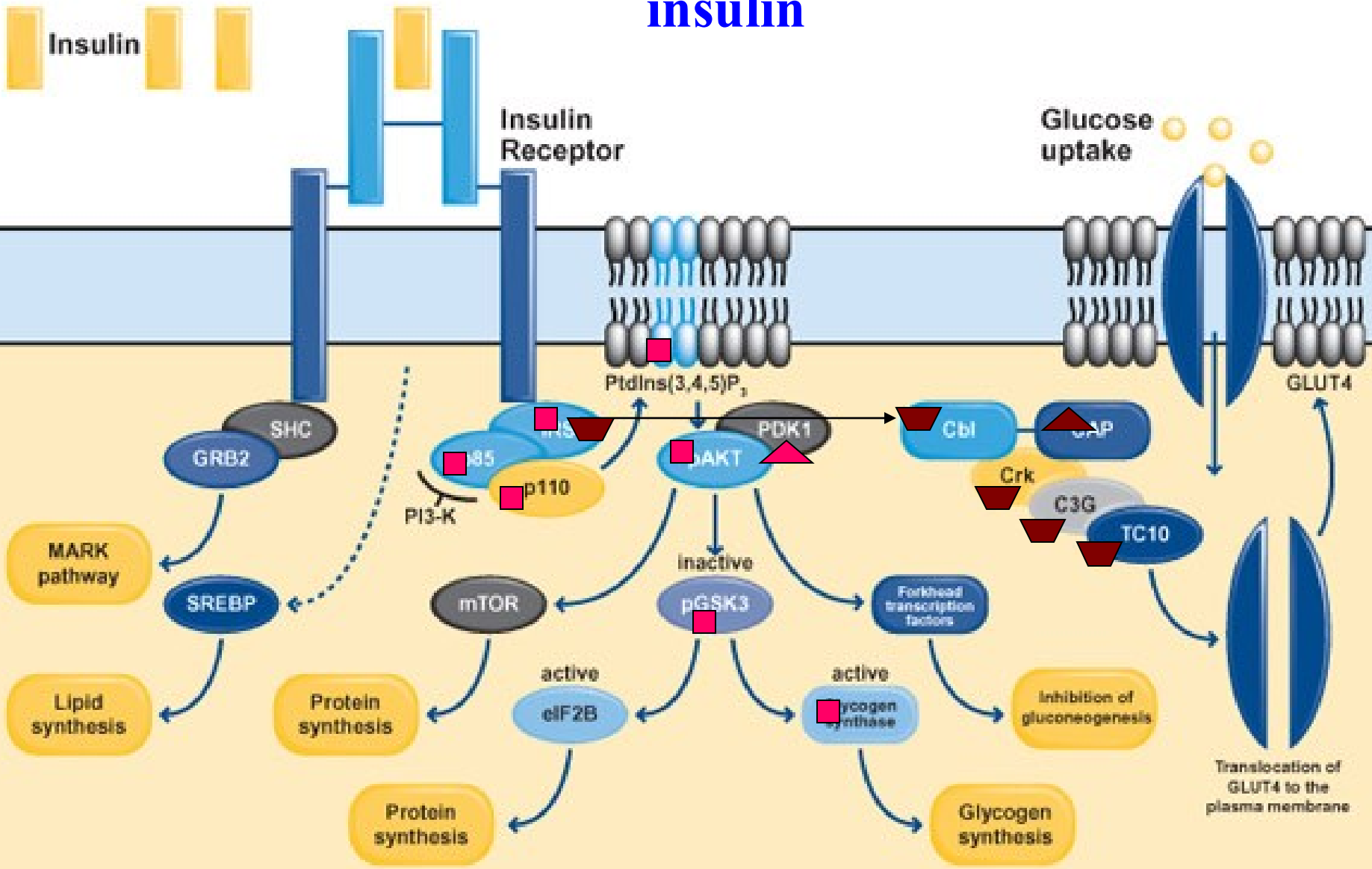




Insulin receptor

Insulin receptor is in membranes like a dimmer. 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



http://www.abcam.com/index.html?pageconfig=resource&rid=10602&pid=7

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 foforylates (inactivates) glykogensynthasa 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 proteinCrk
Crk associated with C3G

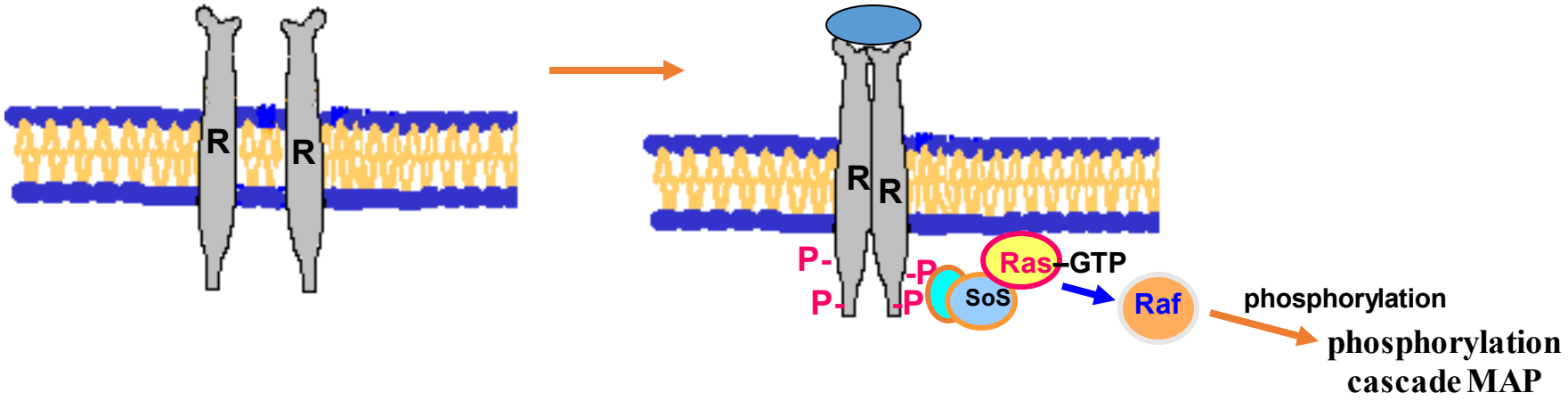
C3G activates TC10 (G-protein)

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.

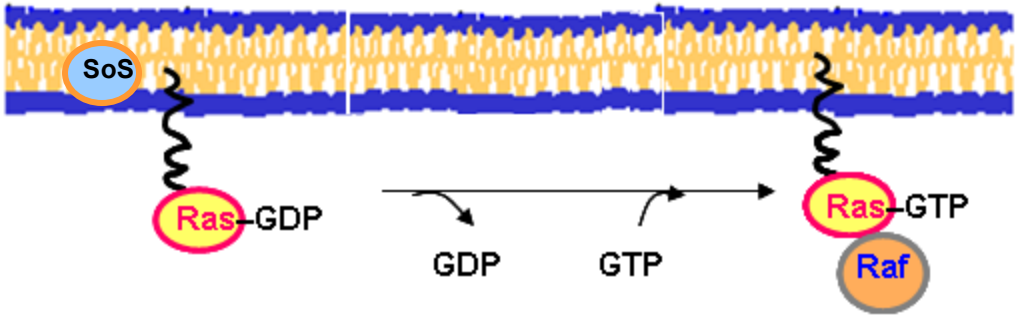
Receptor autophosphorylation

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

Through SOS protein is activated by G-protein Ras → aktivace 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

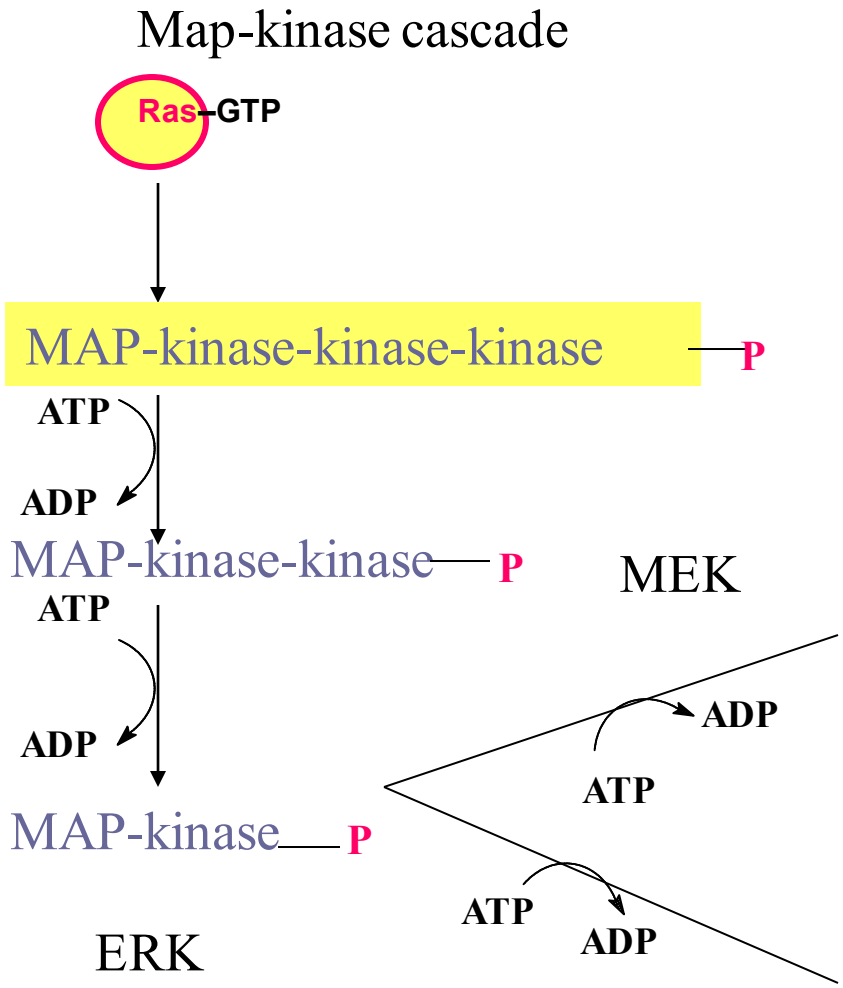
Anchored to the lipid membrane by lipid anchors (myristoyl, farnesyl)

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.

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)

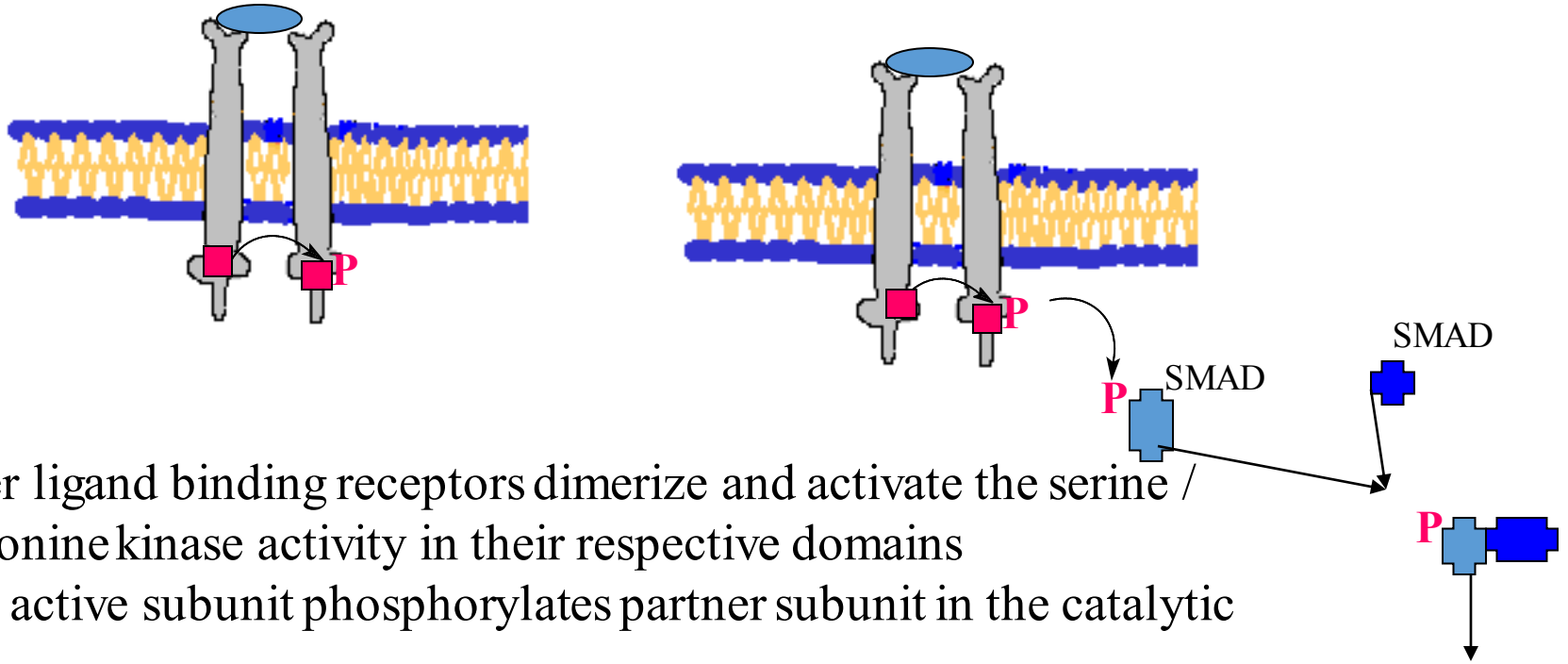
The mitogens - growth factors supporting the proliferation

Examples of mitogen:

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

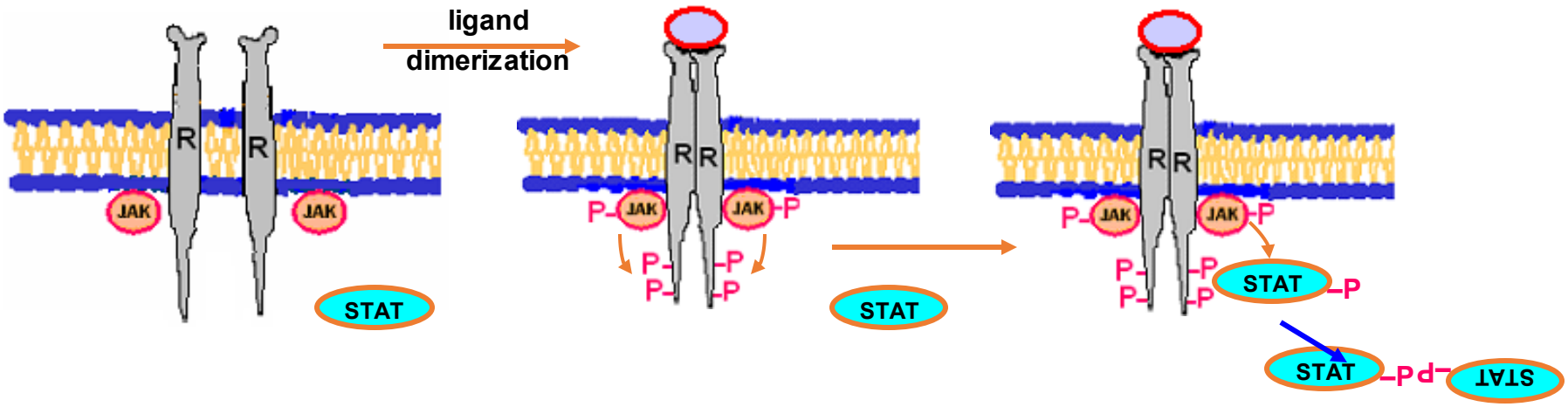
Smad proteins are activated by phosphorylation and forms dimers with other SMAD proteins

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

TEST

IV. Receptors activating non-receptor tyrosine kinase

JAK-STAT receptors (**J**anus **K**inase – **S**ignal **T**ransducer and **A**ctivator of **T**ranscription)



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

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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)

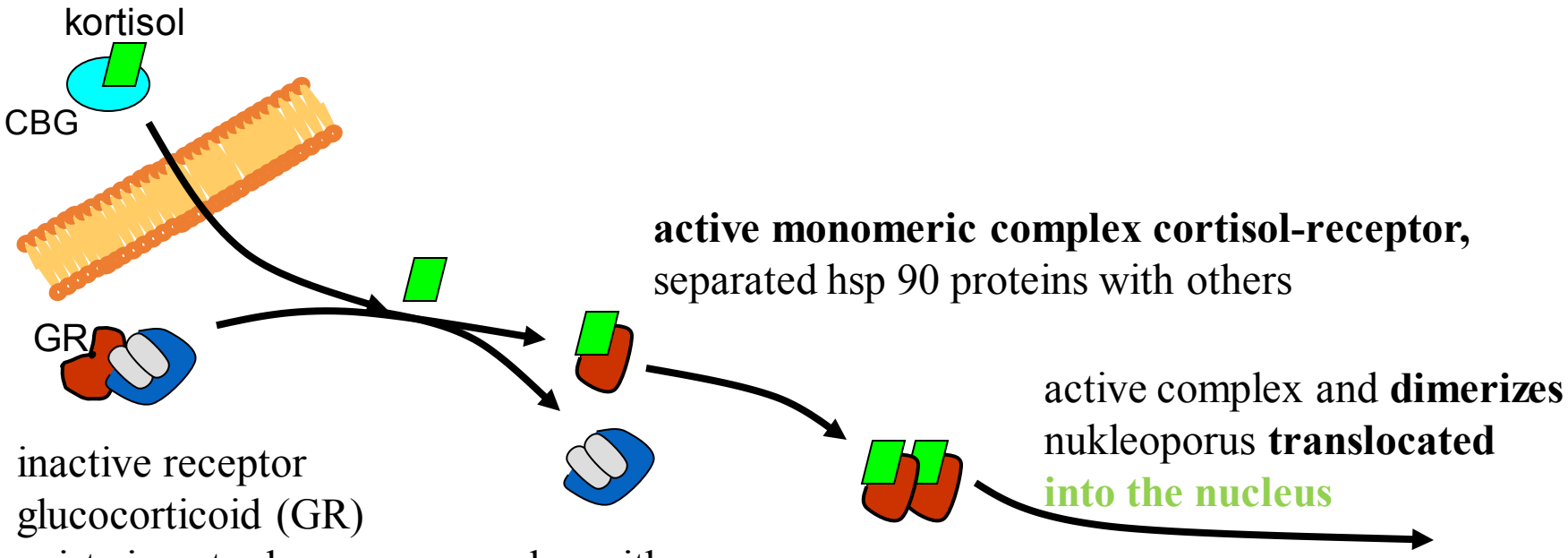
Superfamily of steroid and thyroid receptors - a family of structurally related proteins.

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

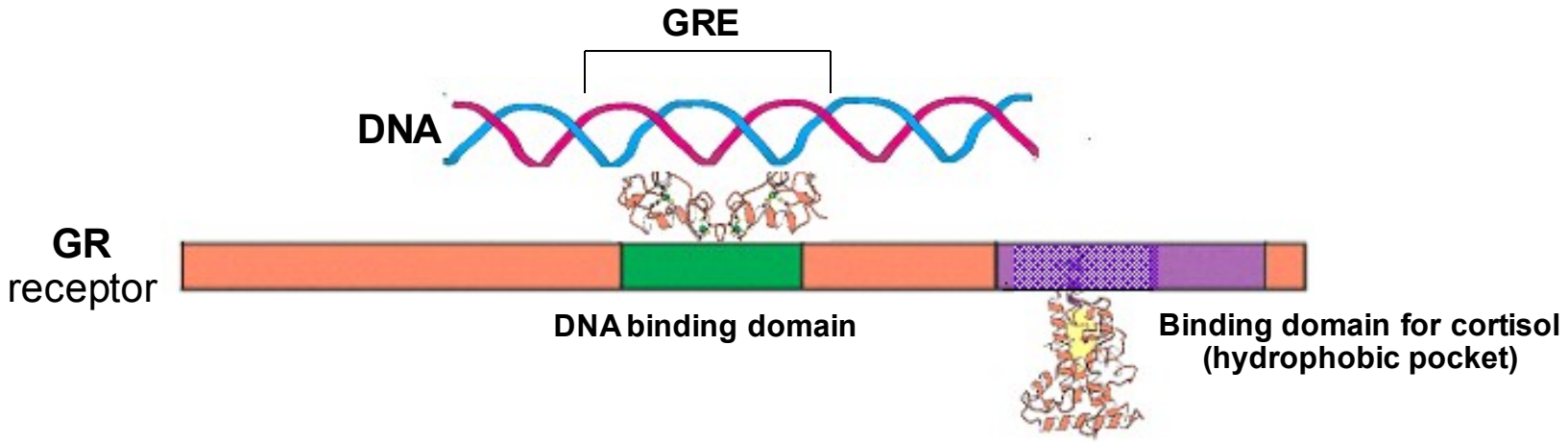


active monomeric complex cortisol-receptor,
separated hsp 90 proteins with others

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

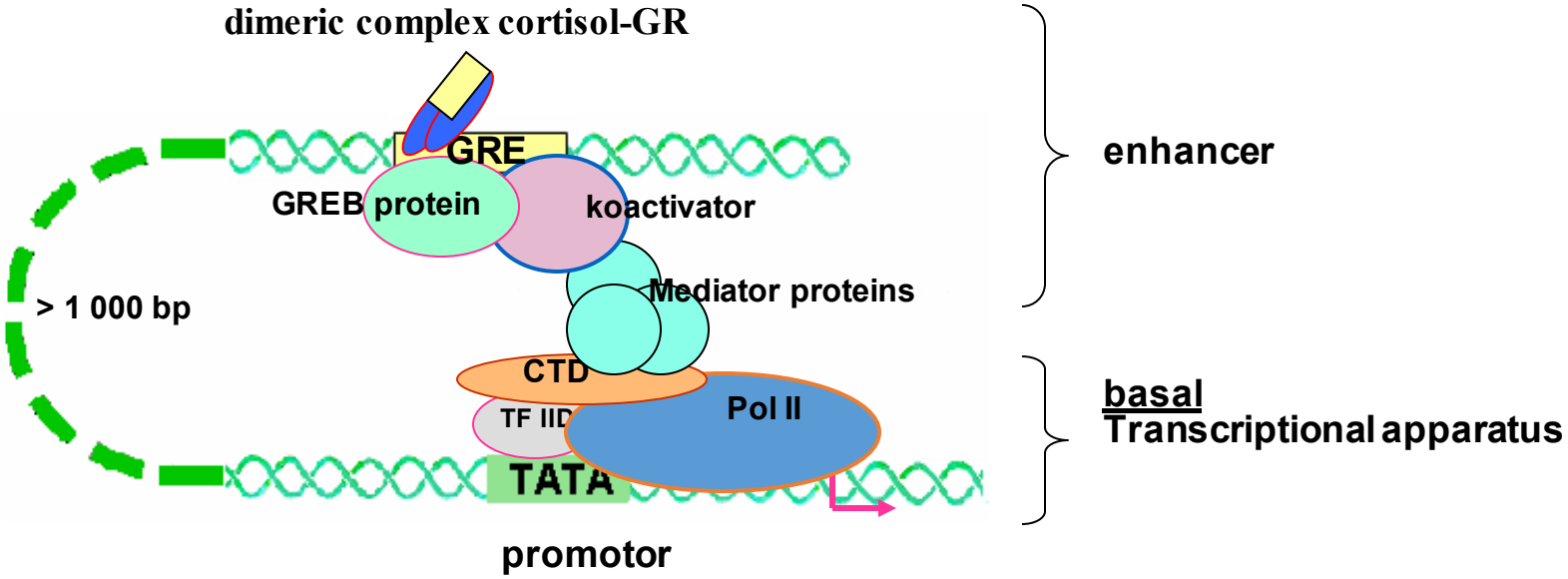
inactive receptor
glucocorticoid (GR)
exists in cytoplasm as a complex with
a protein dimer
hsp 90 (chaperone) and other proteins

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)

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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	•autonomic ganglia neurons •neuromuscular junction •chromaffin cells of the adrenal medulla	•Brain •Smooth muscle •Glandular cells	•Myocardium •Brain
Block receptor	Tubokurarine	Atropine	

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Adrenergic synapses

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	•smooth muscles of the GIT (sphincters) and skin blood vessels (contraction)	•adrenergic and cholinergic nerve endings (inhibition of transmitter release) •pancreas (exocrine secretion inhibition) •platelets (aggregation)	•myocardium (increase in strength and frequency of contractions)	•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	Parasympaticus		Sympaticus		
Mediator	Acetylcholine		Acetylcholine		Noradrenaline
Receptor	nicotine	muscarine	nicotine	muscarine	$\beta_1, \beta_2, \alpha_2, \alpha_1$
Location	•dendrites ganglion neurons	•membranes of target cells	•chromafinní buňky dřeně nadledvin •dendrity neuronů ganglií	•sweat glands	•membranes of target cells