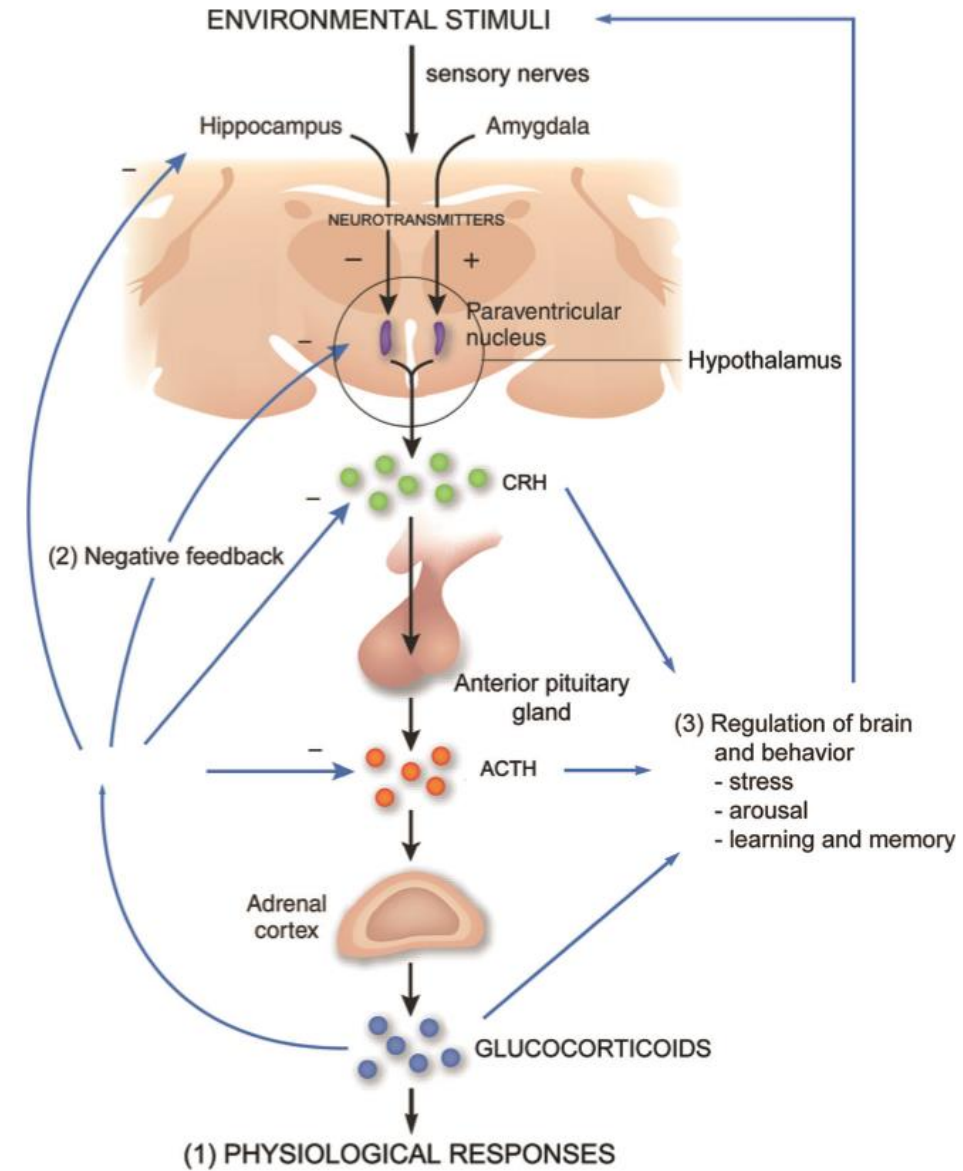


General principles of endocrine functions

Integration systems of the organism

- Integration and coordination = maintaining the integrity and activity of the organism on all levels in the relation to the changing external and internal environments
- **Hormonal system**
- **Nervous system**
- *Immune system*



(1) PHYSIOLOGICAL RESPONSES

Brain: *neurochemical changes*

Skeletal muscle: *decreases protein synthesis
decreases glucose uptake*

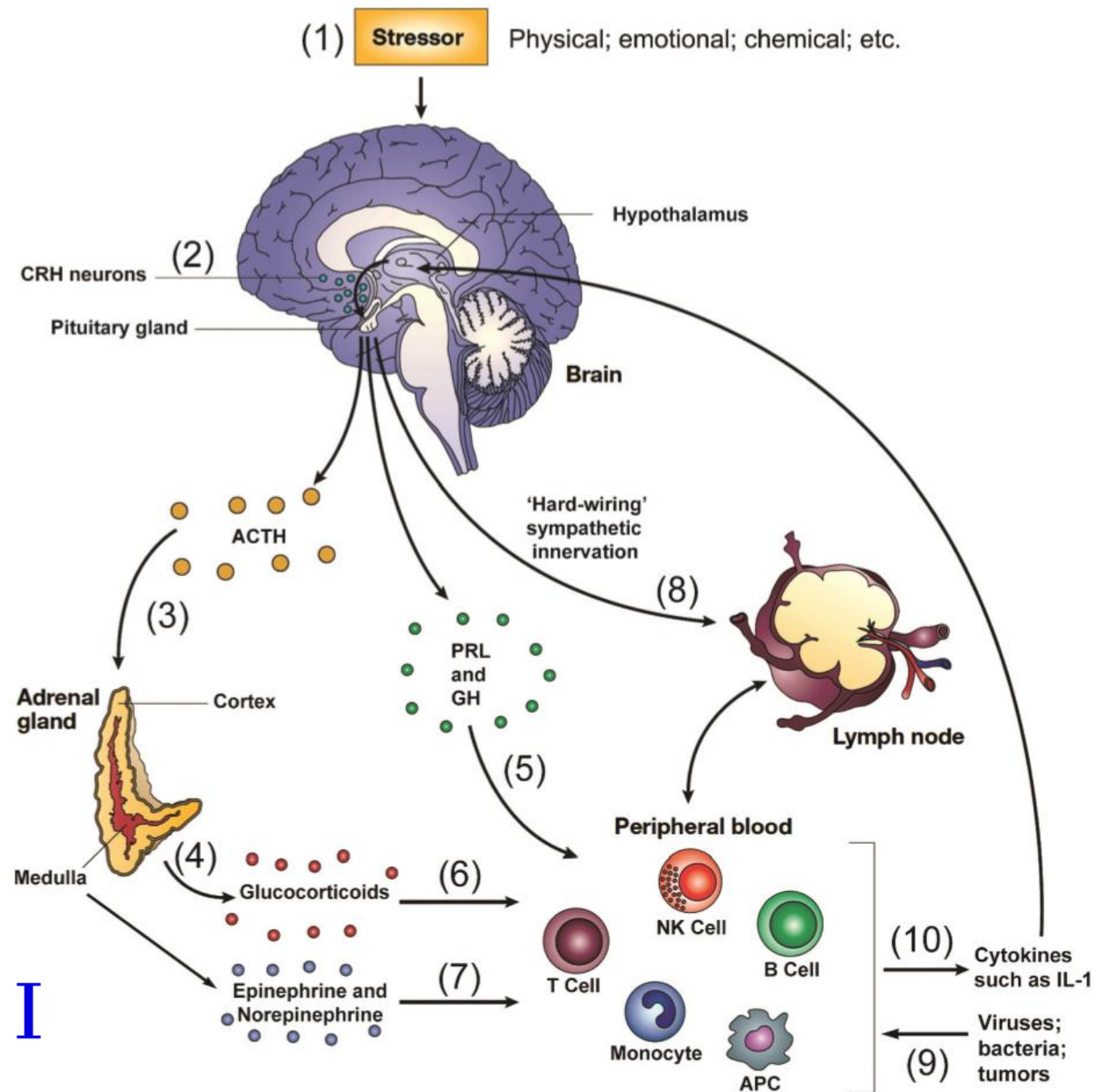
Adipose tissue: *increases lipid mobilization
decreases glucose uptake*

Liver: *increases gluconeogenesis*

Immune system: *immunosuppression*

No system works independently
= functional integration

- Hormones
- Neurohormones
- Neurotransmitters
- Paracrine (autocrine) effectors



How do cells communicate?

- Intracrine
- Autocrine
- Paracrine
- Neurocrine
- Endocrine
- Neuroendocrine

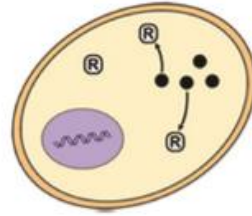
M U N I
M E D

source

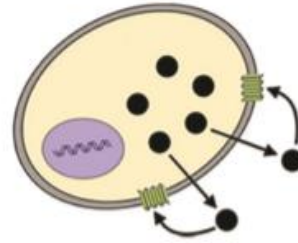
environment

target cell

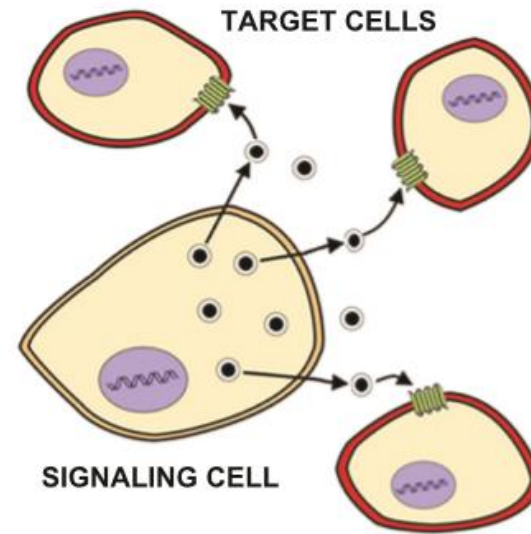
1. INTRACRINE



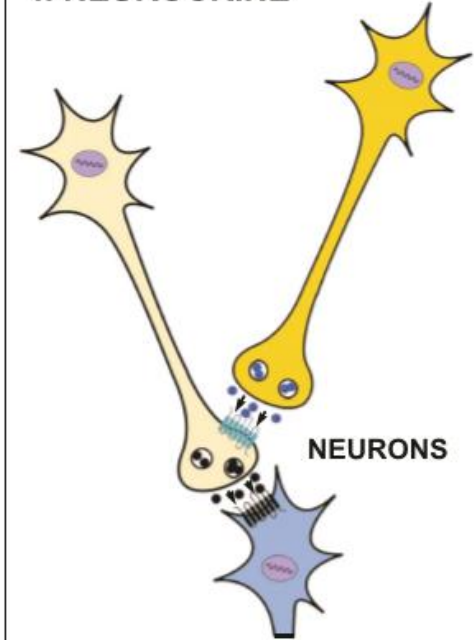
2. AUTOCRINE



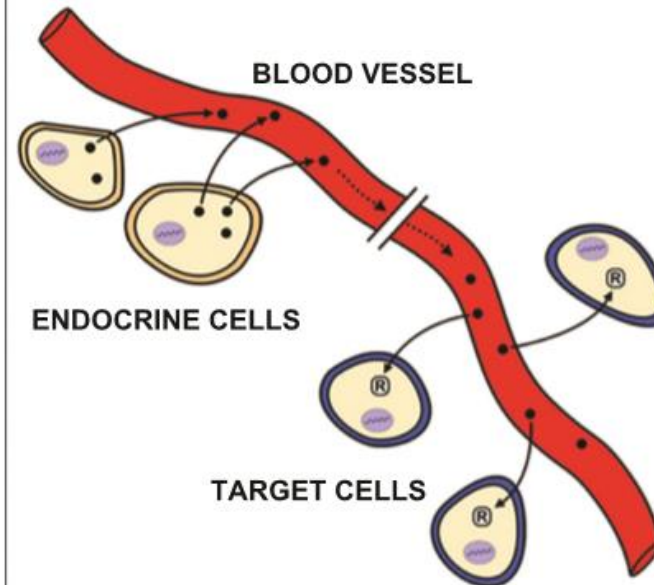
3. PARACRINE



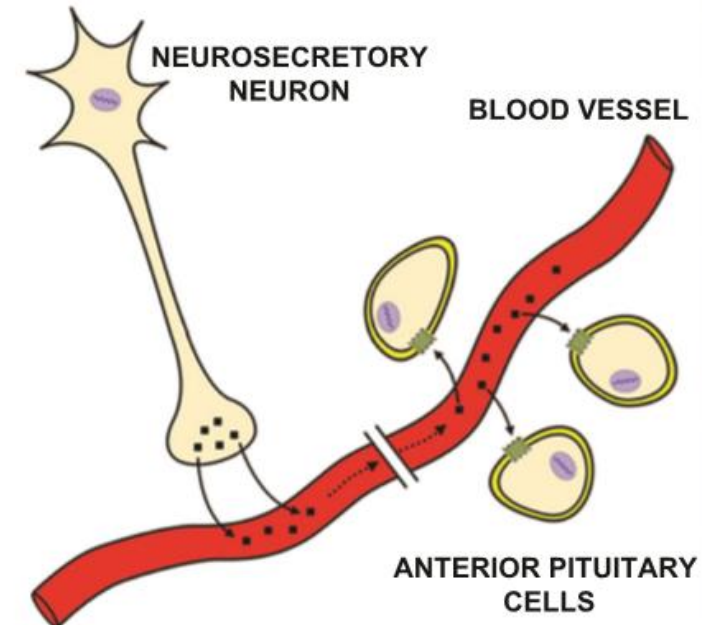
4. NEUROCRINE



5. ENDOCRINE



6. NEUROENDOCRINE



endocrine

source



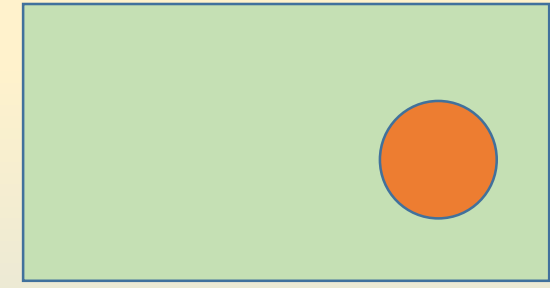
- synthesis/secretion
- no influence on specificity of effect

environment



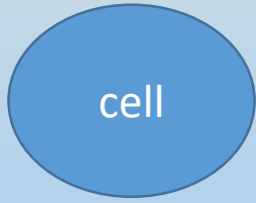
- universal environment
- dilution and interactions

target cell

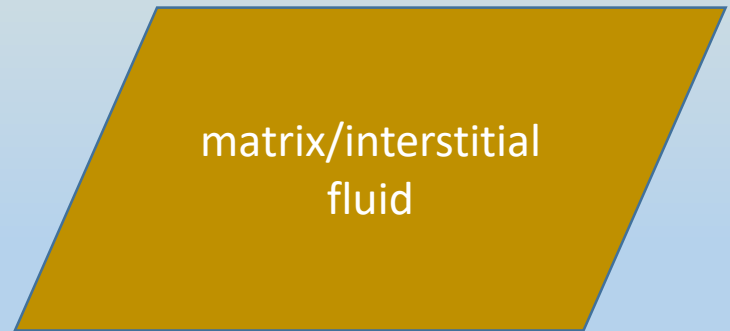


- receptor = specificity
- cell response
 - number of receptors
 - signaling pathways
 - other ligands
 - metabolism of ligand/receptor

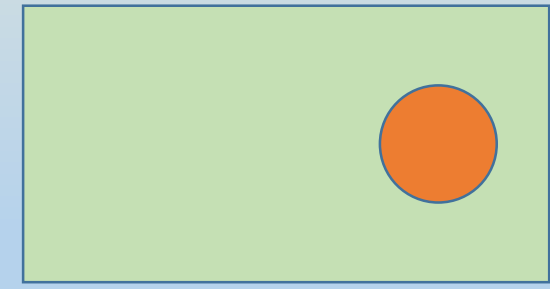
paracrine, autocrine



- synthesis/secretion
- main determinant of target cell (determined by localization)

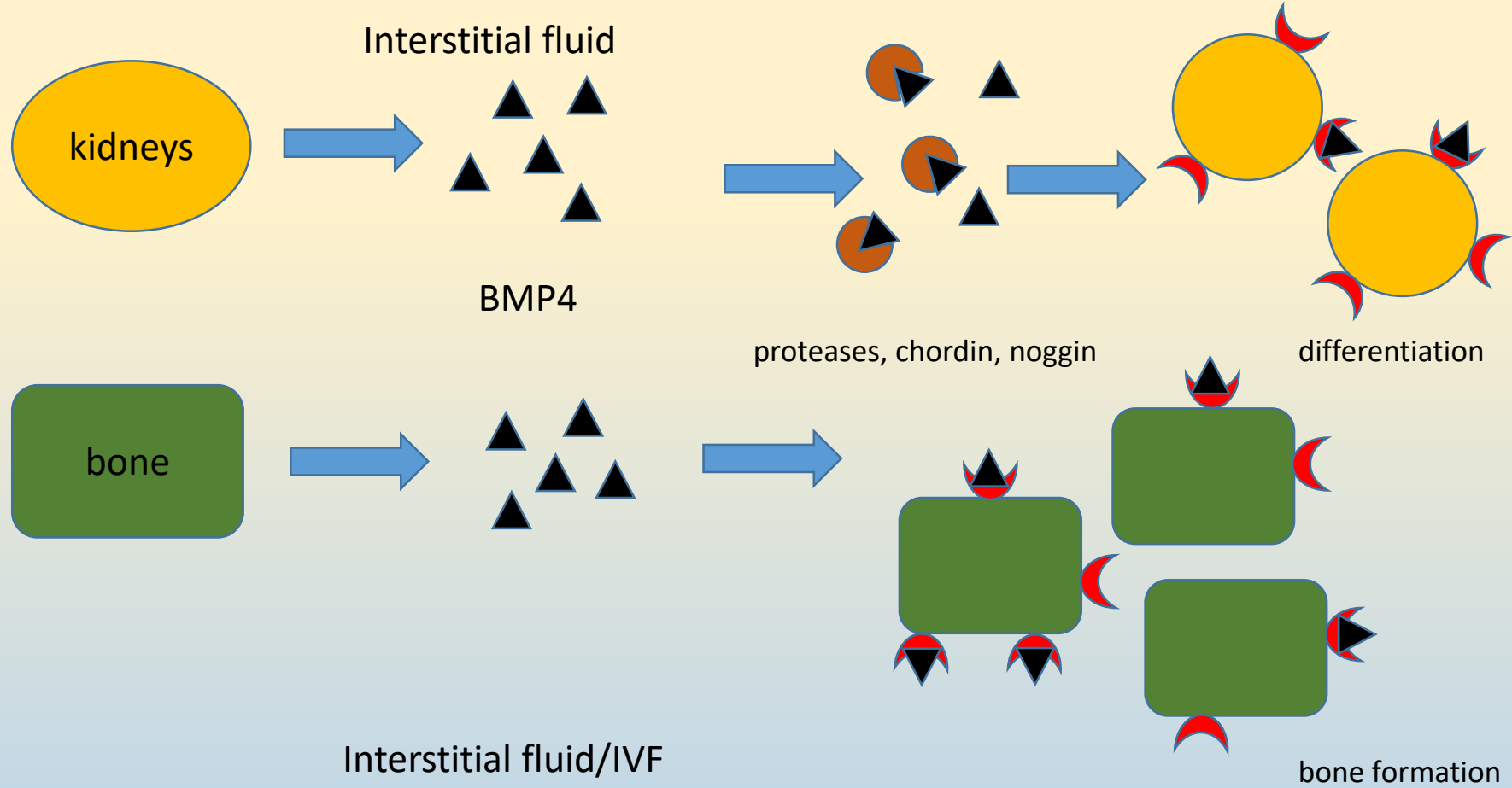


- diffusion
- binding proteins
- proteases
- components of extracellular matrix

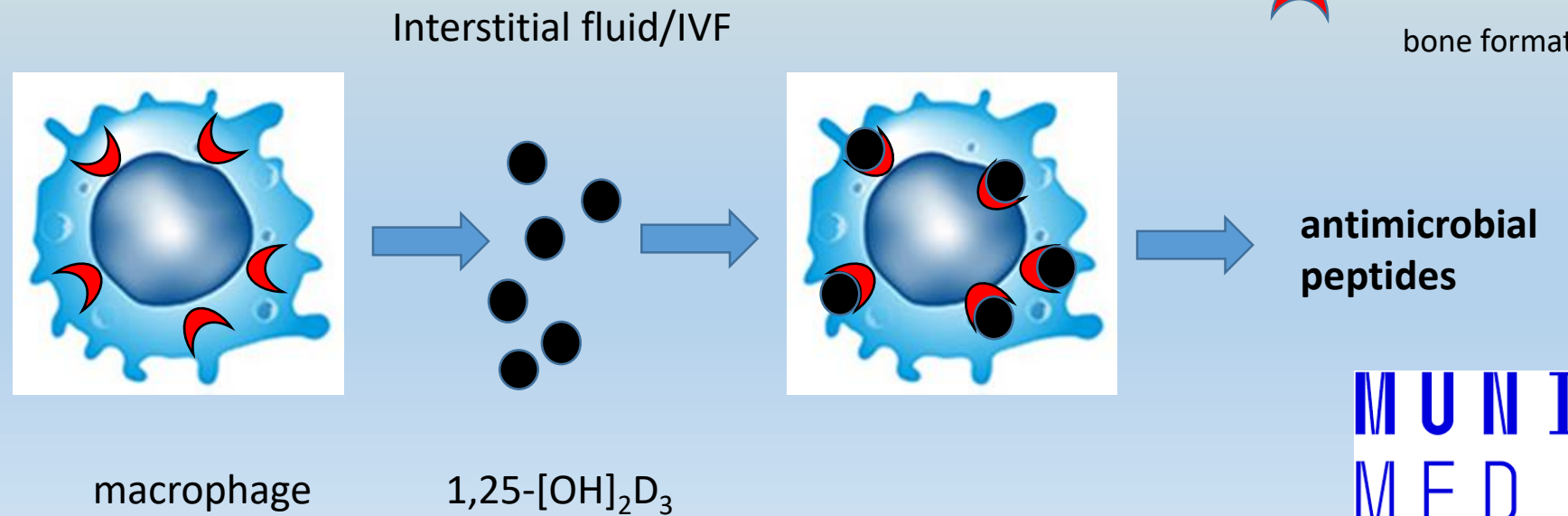


- specificity and sensitivity
- diffusion barrier
- determinants of gradient
- inhibition signaling pathways
- effect of other ligands
- binding proteins

paracrine



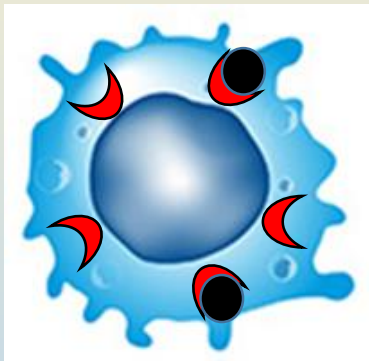
autocrine



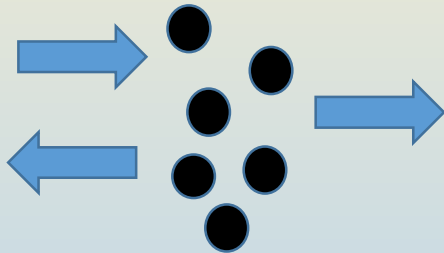
autocrine

paracrine

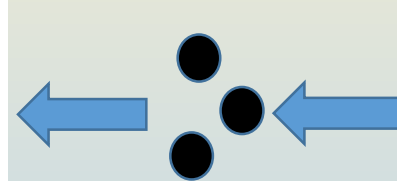
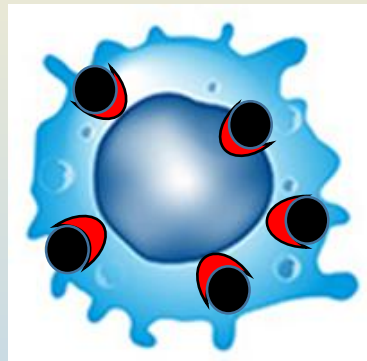
endocrine



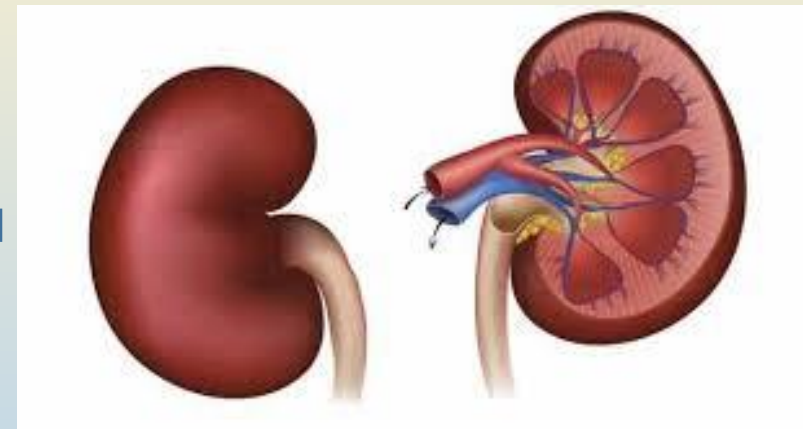
macrophage



1,25-[OH]₂D₃



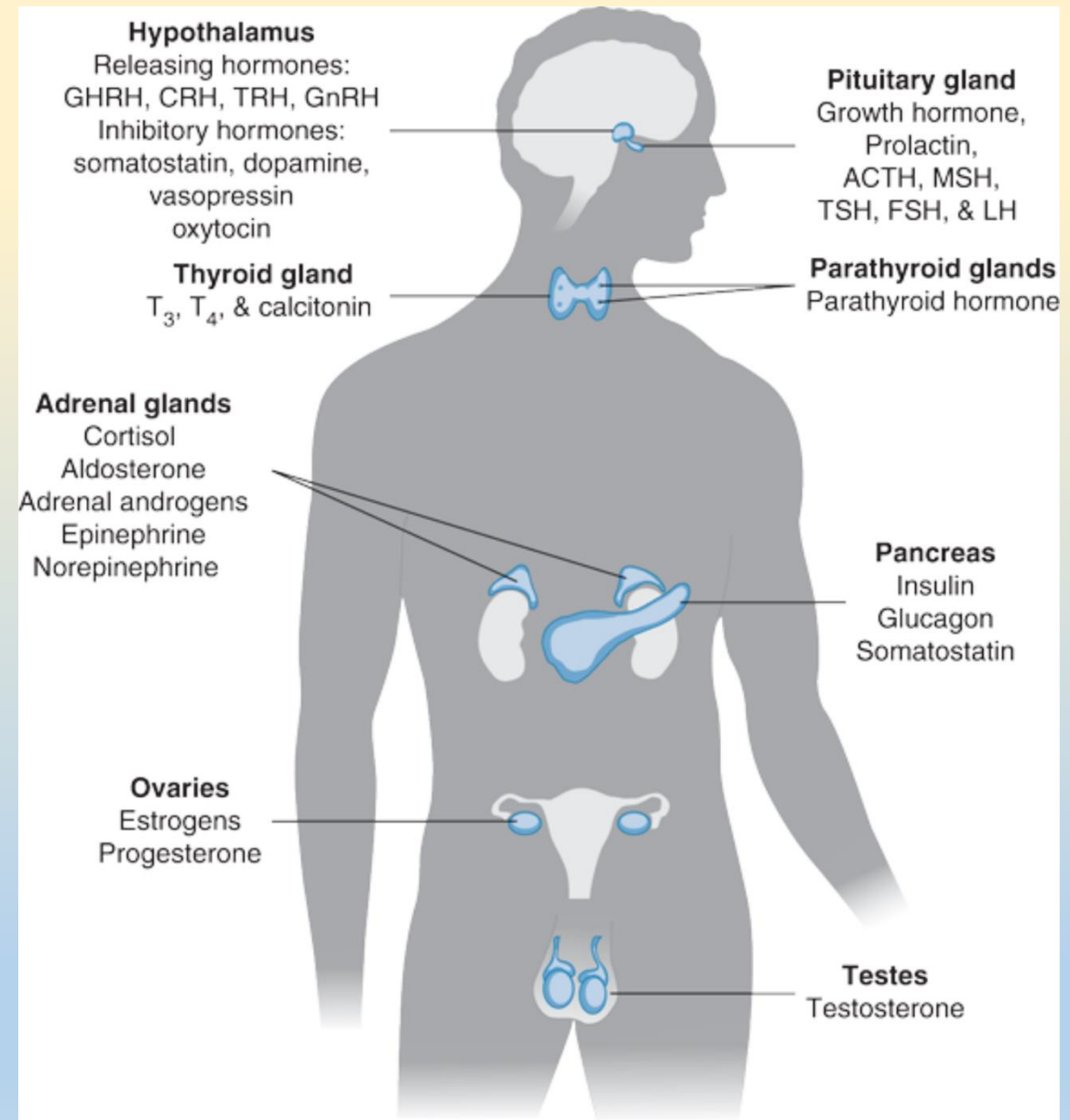
1,25-[OH]₂D₃



kidney – proximal tubule

Hormones

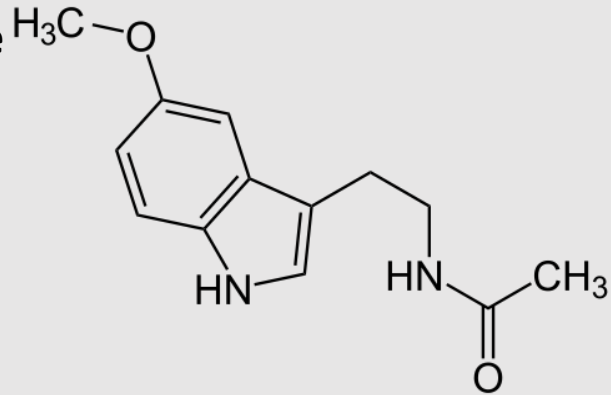
- Starling 1905 - *secretin*
- Glandotropic hormones
- Aglandotropic hormones
- Target cells
- Limited time of effect



Chemical nature of hormones

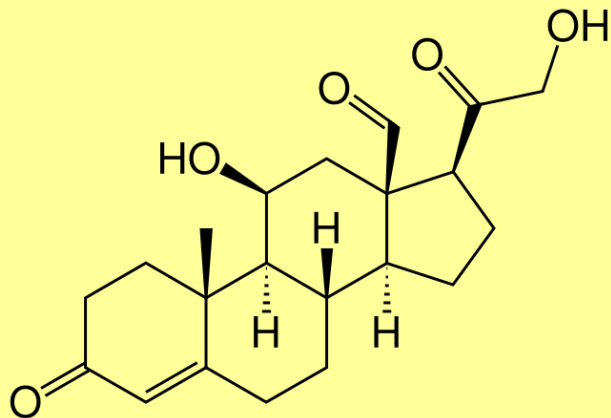
DERIVED FROM AMINOACIDS

- Adrenaline
- Noradrenaline
- Dopamine
- Melatonin
- T3/T4



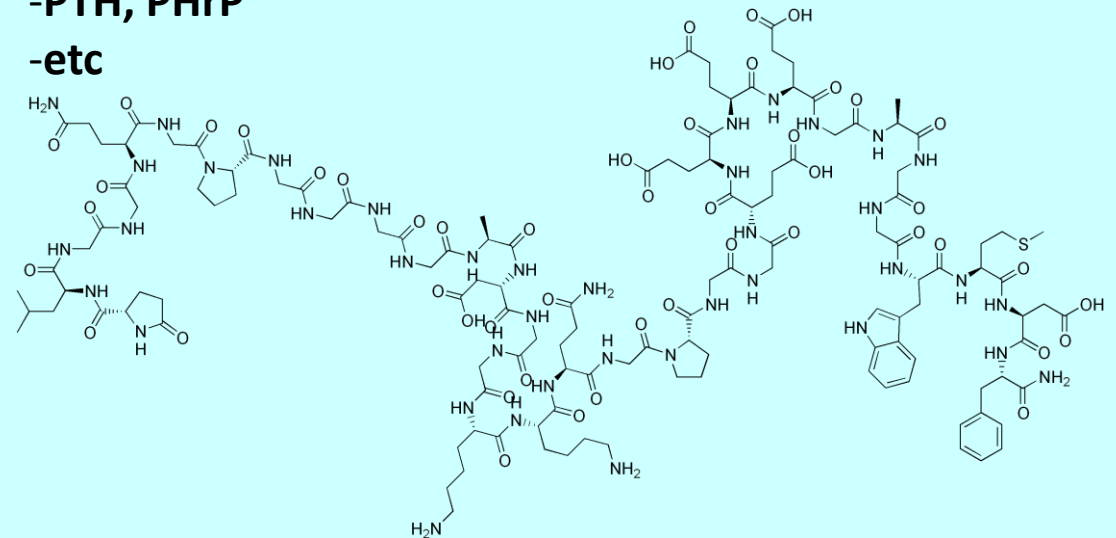
STEROID

- Cortisol
- Aldosterone
- Testosterone
- Progesterone
- Estradiol
- Calcitriol



PEPTIDES AND PROTEINS

- Hypothalamic hormones
- Adenohypophyseal hormones
- Insulin, glucagon, somatostatin
- Gastrin, cholecystikin, secretin
- Natriuretic peptides
- Erythropoietin, thrombopoietin
- PTH, PThrP
- etc



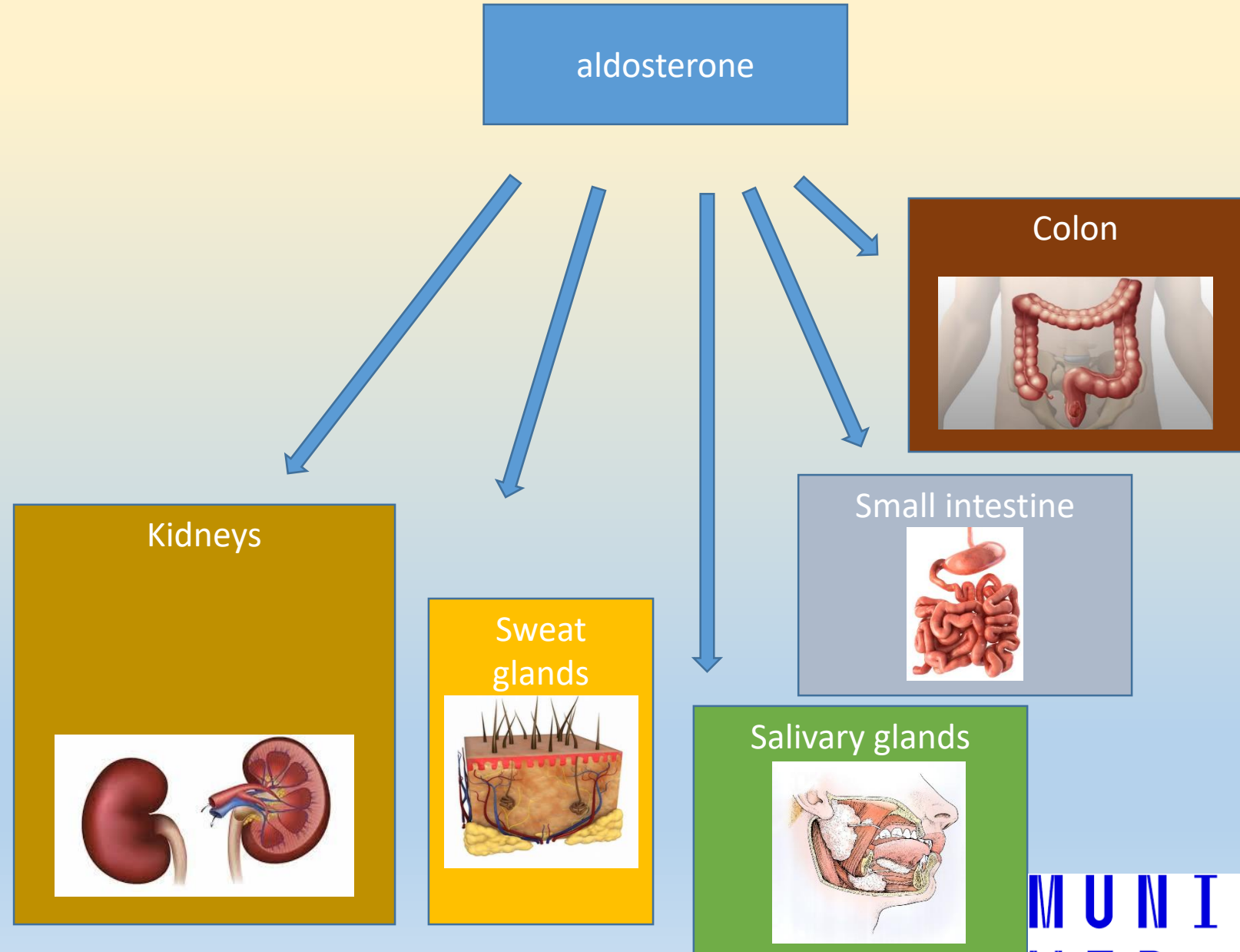
Chemical nature of hormones

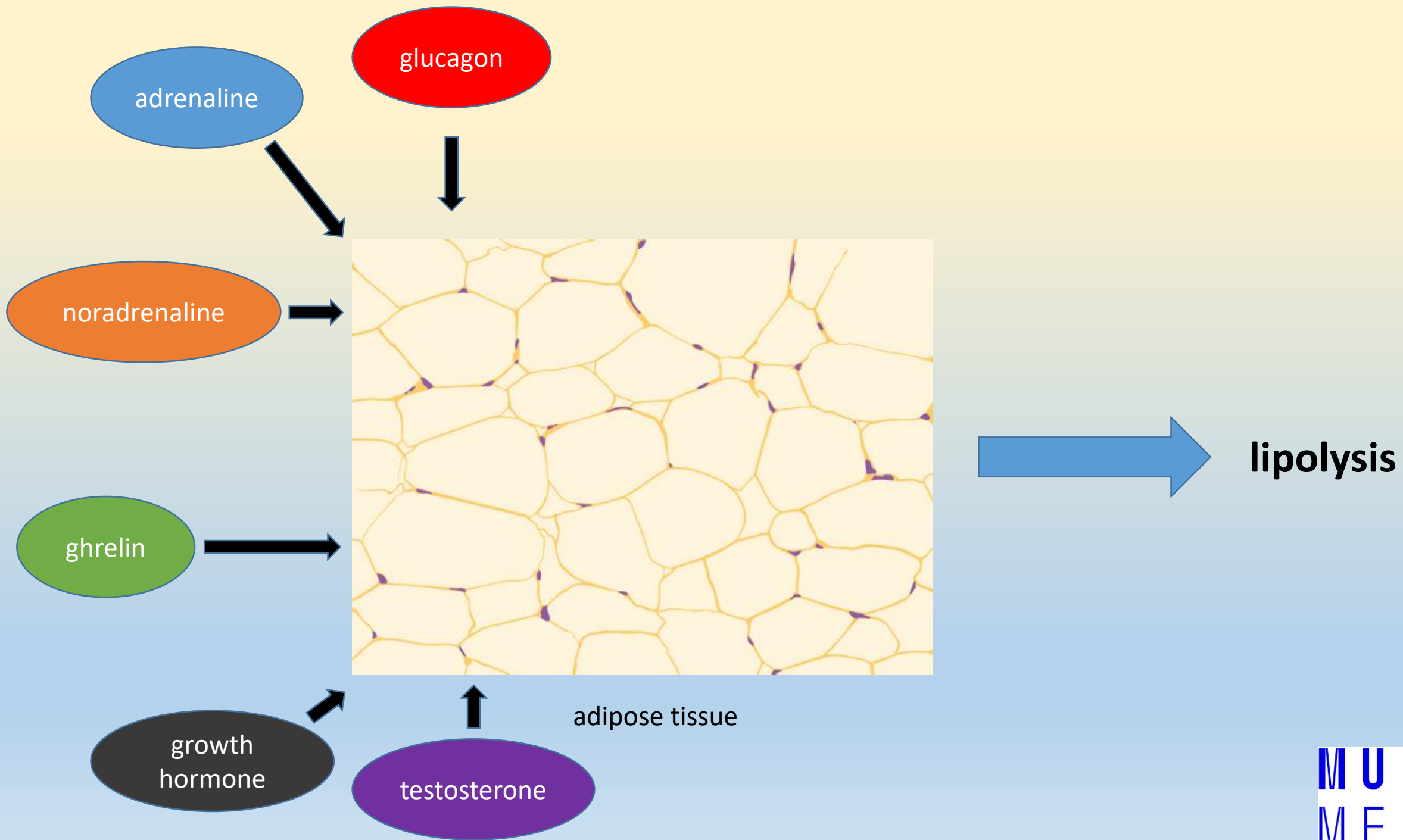
Hormone – characteristics	Peptides – proteins	Catecholamines	Steroid hormones	Thyroid hormones
Ph-CH properties	hydrophilic	hydrophilic	lipophilic	lipophilic
synthesis	proteosynthesis	Tyr modification	CH precursors	Tyr modifications
storage	secretory granules	secretory granules	not present	colloid
secretion	controlled exocytosis	controlled exocytosis	diffusion	diffusion
transport	free	free/weakly bound	bound	bound
elimination half-life	short (4 – 40 – 170 min)	very short (2 – 3 min)	moderate (up to 180 min)	long (20 hours – 7 days)
receptors	membrane	membrane	cytosol	nuclear
effect	short-term	very short-term	long-term	long-term
cell response	quick	very quick	slow	slow

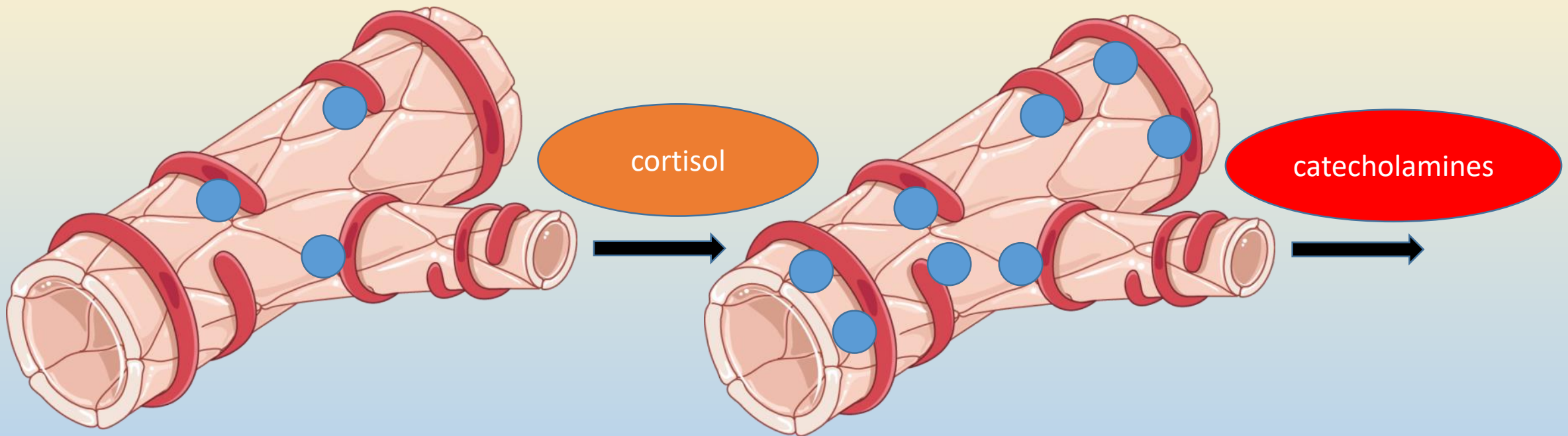
CHEMICAL STRUCTURE OF HORMONES DETERMINES THEIR BIOSYNTHESIS, STORAGE, RELEASE, TRANSPORTATION, ELIMINATION HALF-LIFE, WAY OF ELIMINATION AND THE MECHANISM OF EFFECT ON TARGET CELLS

Hormones

- Pleiotropic effects
- Multiplicity
- Permissive effect





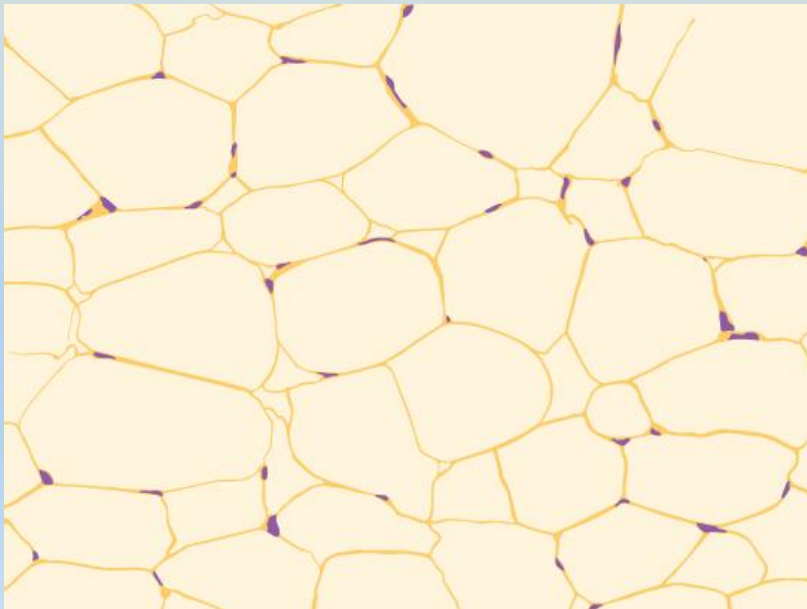


Arterioles – α_2 receptors

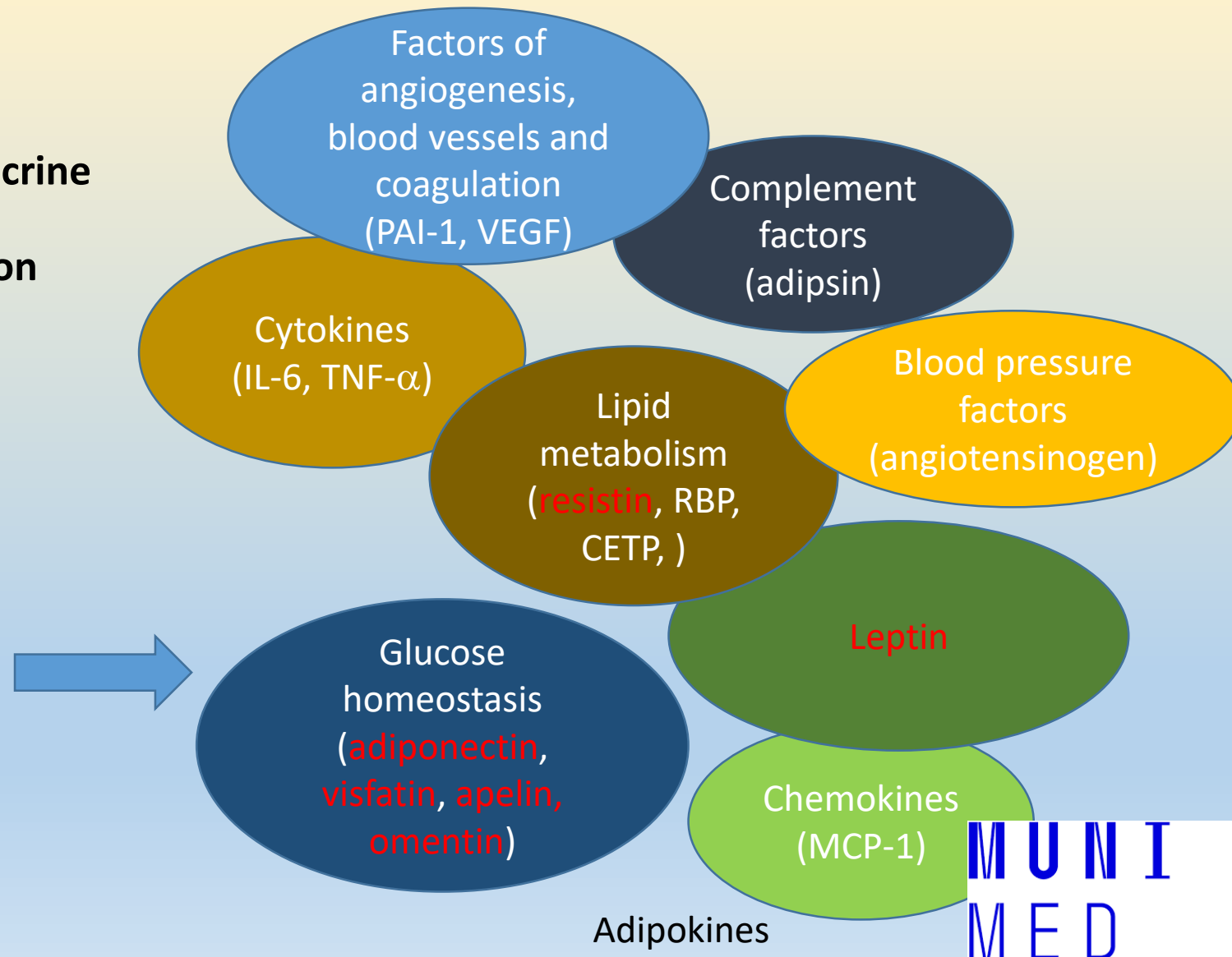
vasoconstriction

Endocrine organs

- specialised cells – specialised organs („endocrine“)
- „secretory“ cells – organs with endocrine function
- cells without specialised secretory function
- cells converting hormone precursors



adipose tissue



Clinical aspects

- Production of hormones by tumors – PARANEOPLASTIC SYNDROMES

Lung tumors

- ADH (hyponatremia)
- ACTH (Cushing syndrome)
- PTHrP (hypercalcaemia)

Liver and kidney tumors

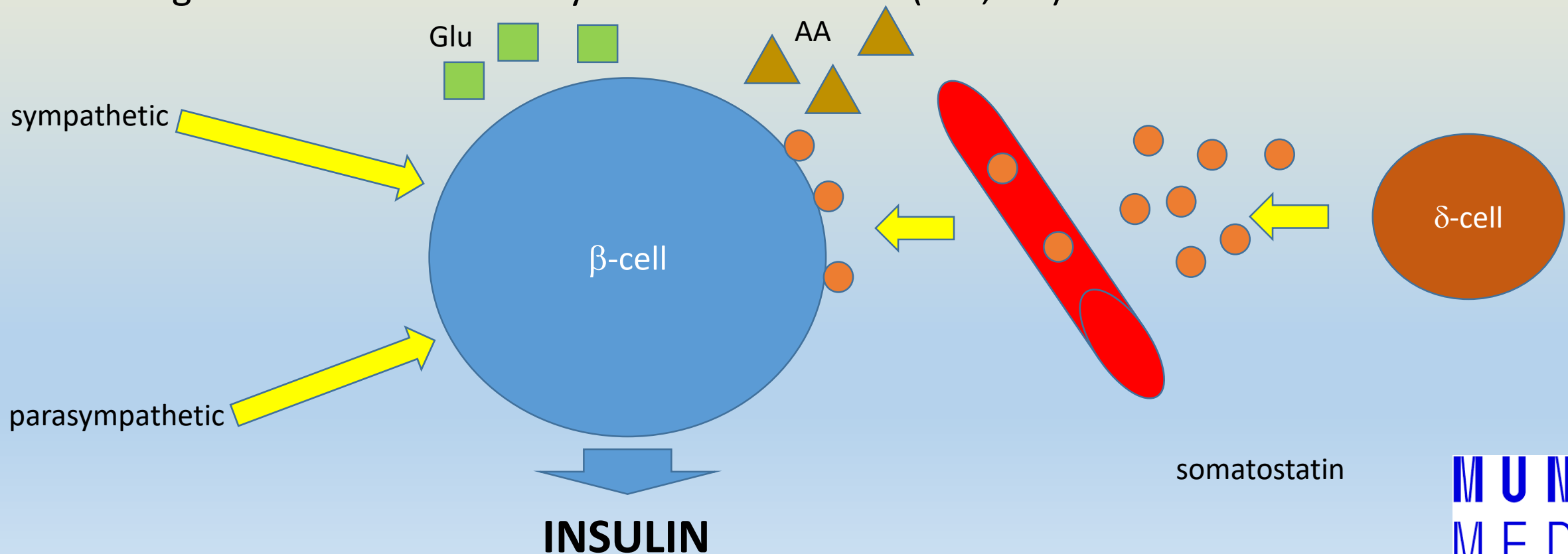
- erythropoietin
(polycythemia)

GIT tumors

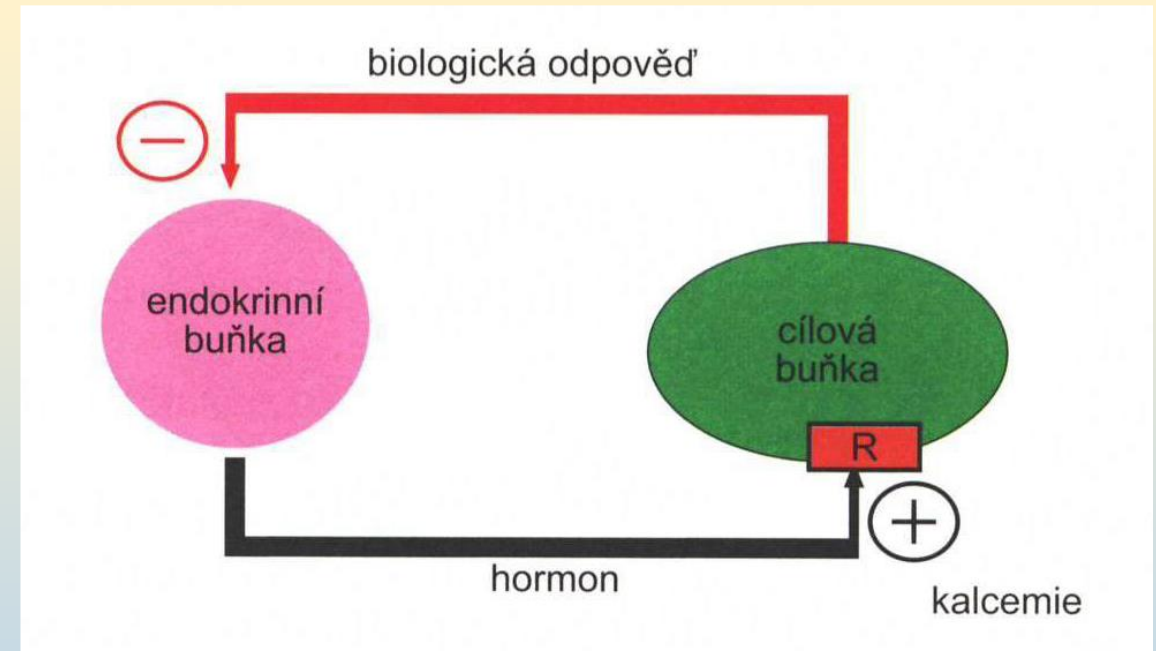
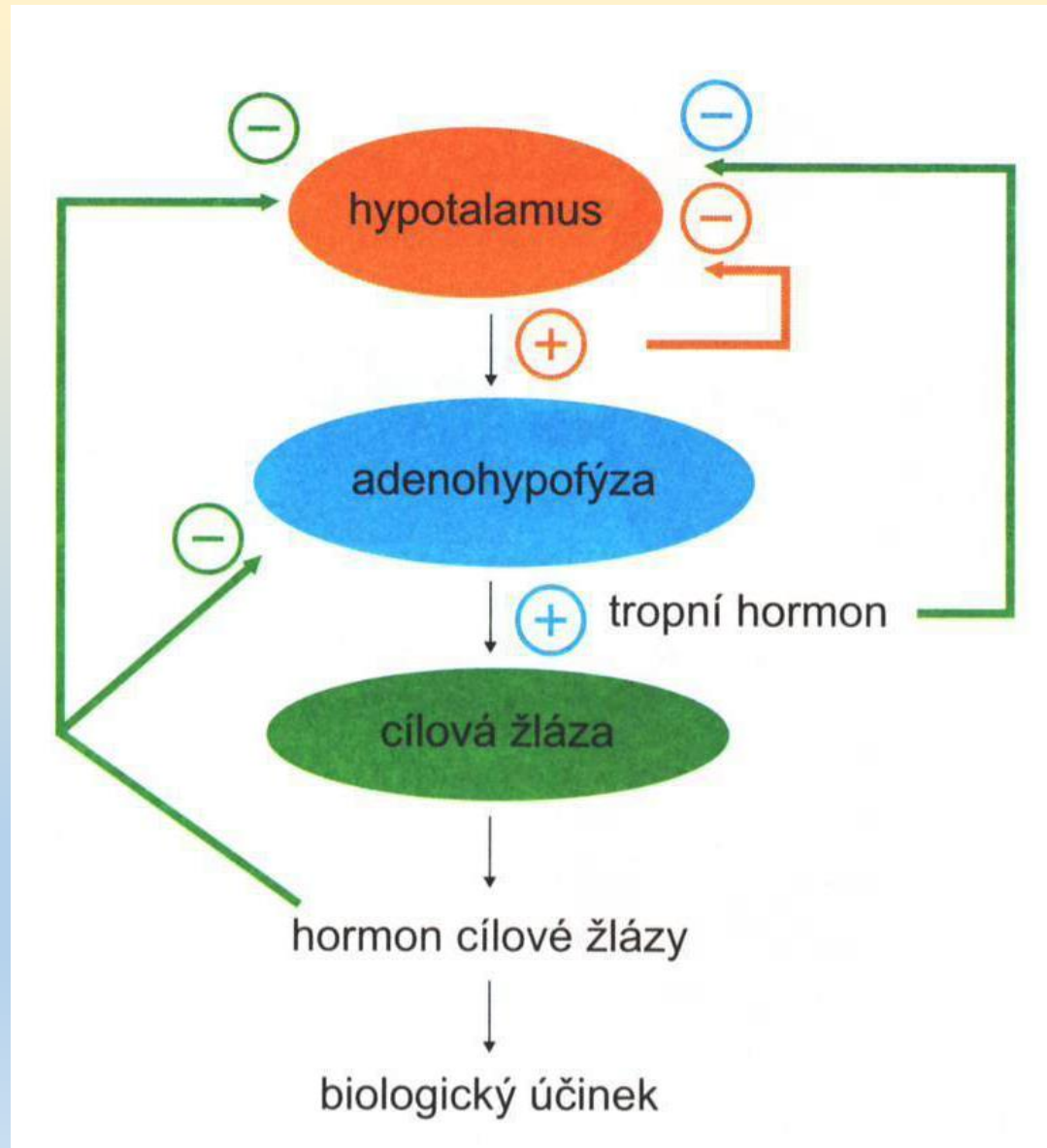
- ACTH (Cushing syndrome)

Secretion of hormones and its regulation

- Neuronal control
 - hypothalamus
 - sympathetic/parasympathetic nervous system
- Hormonal control
- Regulation of secretion by ions or substrates (Glu, AA)



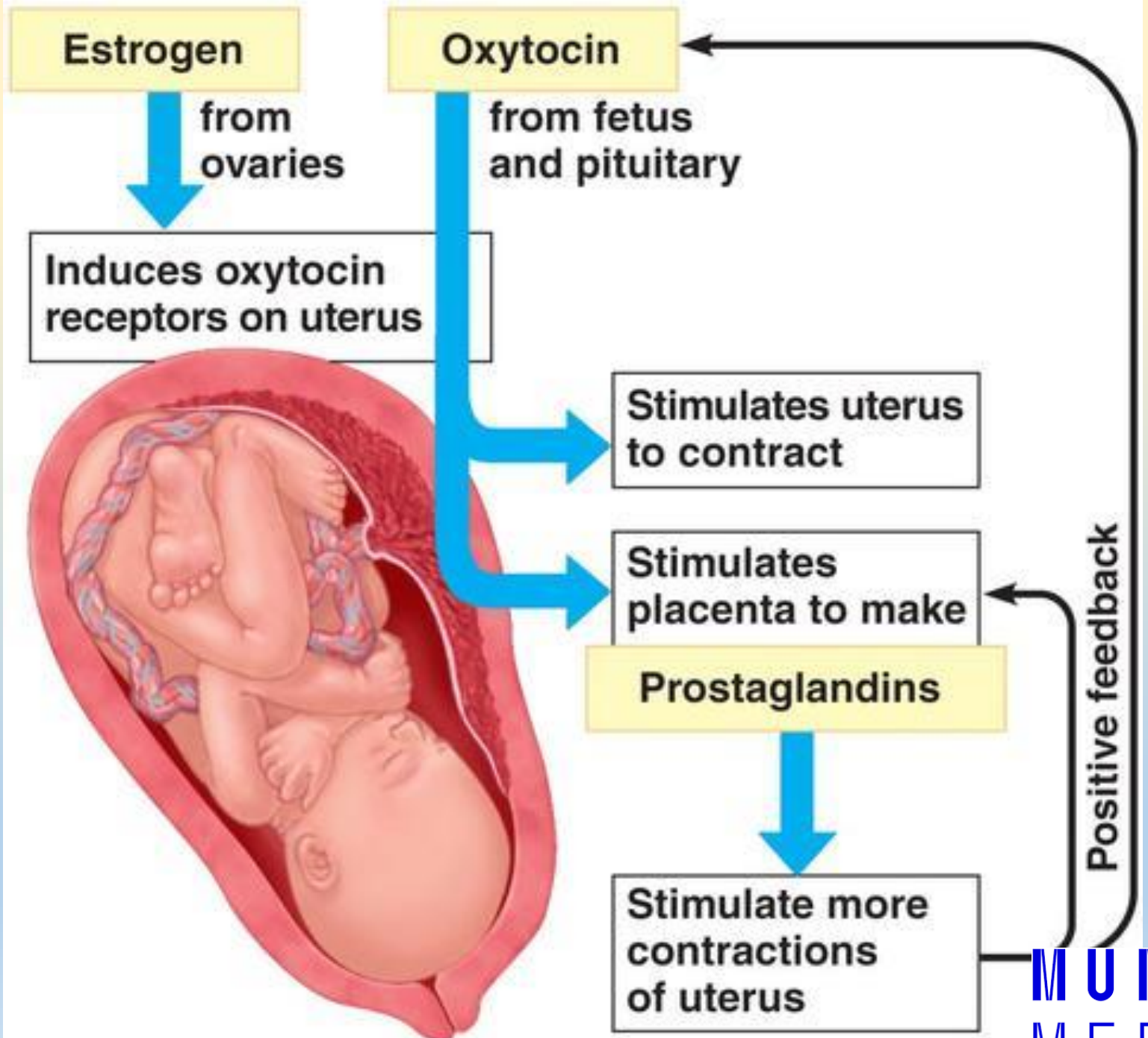
Hormone secretion is controlled by feedback system



Feedback
negative X positive
simple X complex

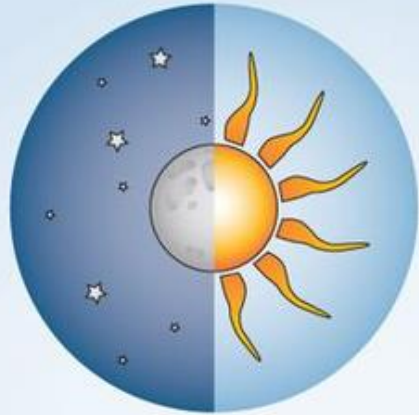
Taken from Kittnar et al. Lékařská
fyziologie. 1st edition. Grada 2011.

Positive feedback – why?



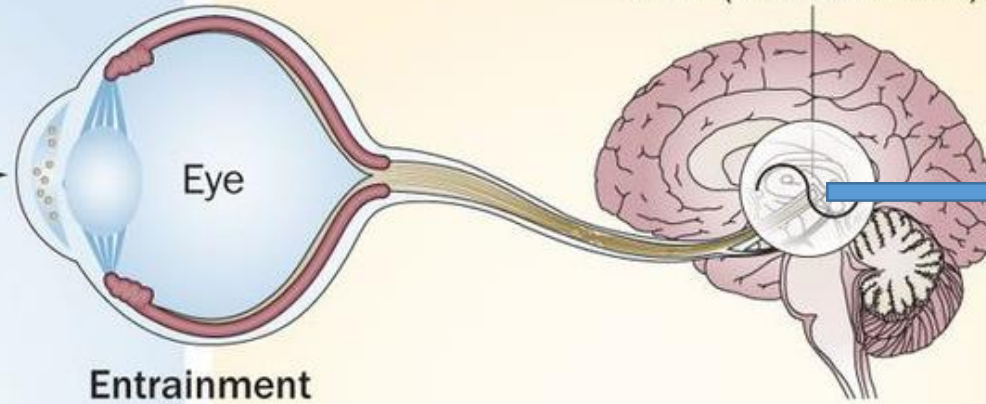
Cyclic changes in hormone secretion

External 24h light-dark cycle



Photic Zeitgeber

Endogenous circadian rhythm



Entrainment

Synchronization

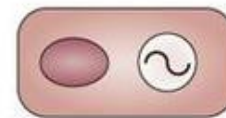
Nonphotic Zeitgeber

- Sleep-wake cycle
- Physical activity
- Social time
- Meals

Peripheral oscillators



Cellular oscillators



SCN:

- Afferent – retina
- Efferent – hypothalamic nucleus

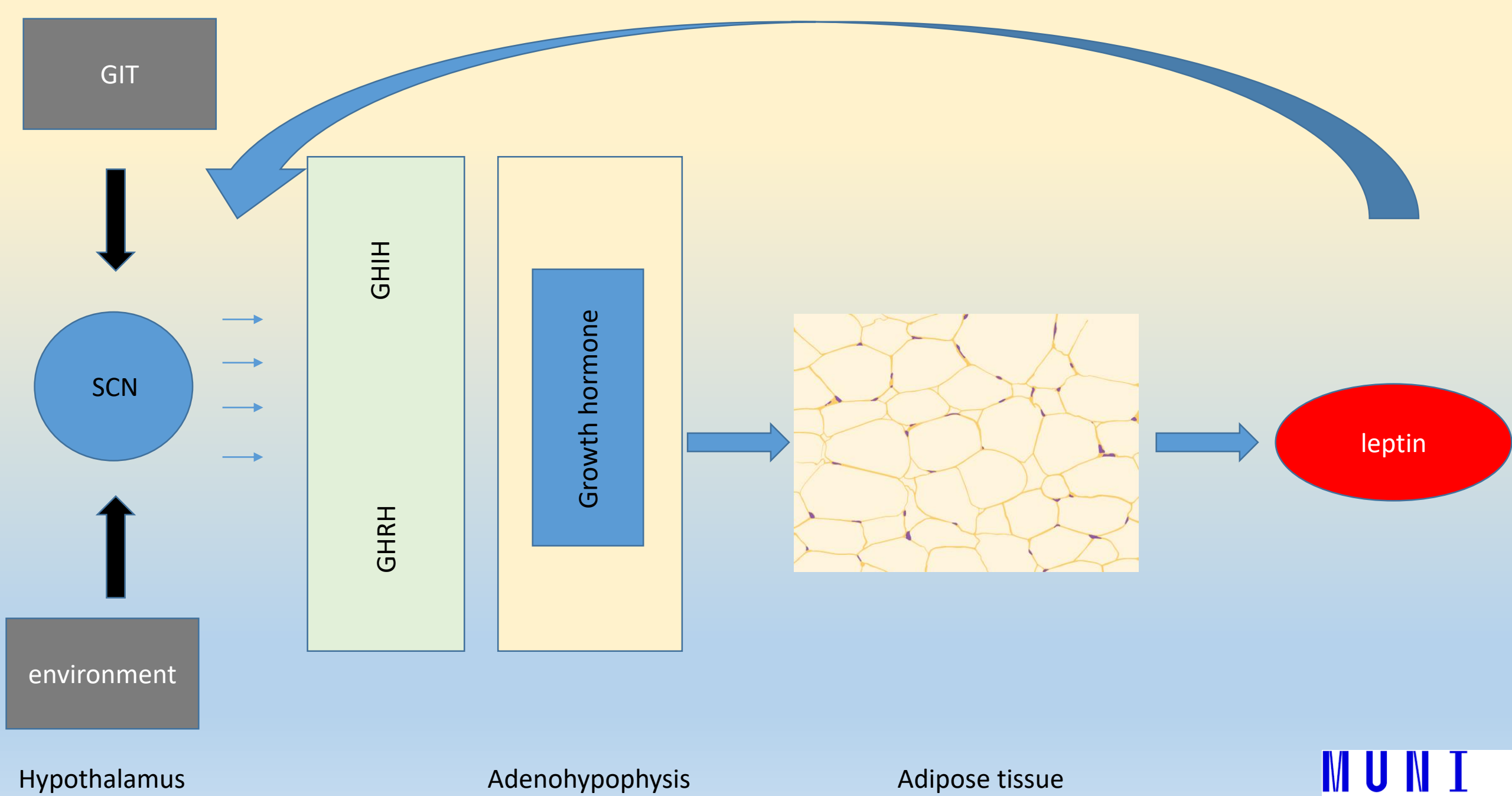
- Melatonin
- ADH
- ACTH – cortisol
- Insulin
- Ghrelin
- Adiponectin
- Leptin

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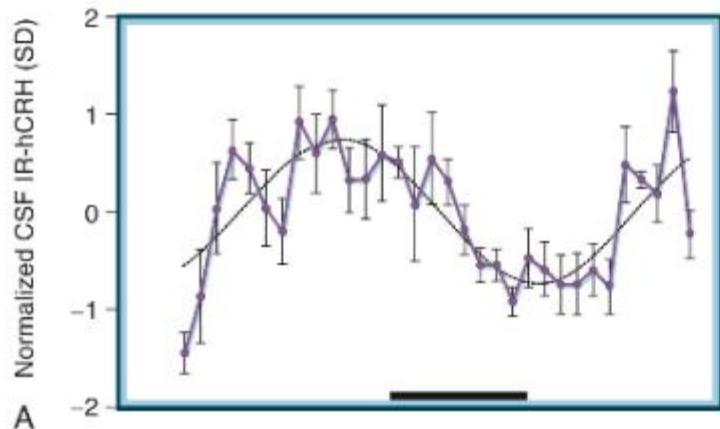
Neuronal/hormonal
= SNC-dependent

Satiety/fasting

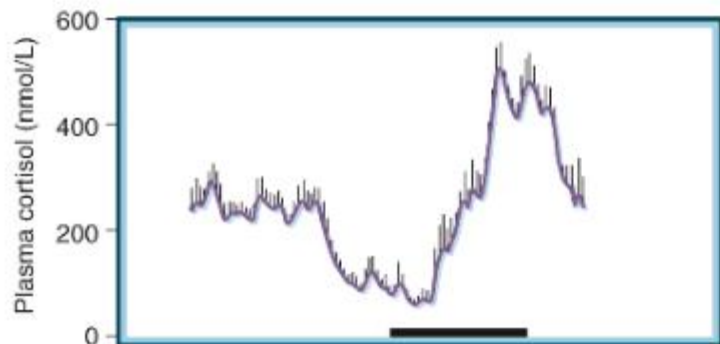
Body temperature



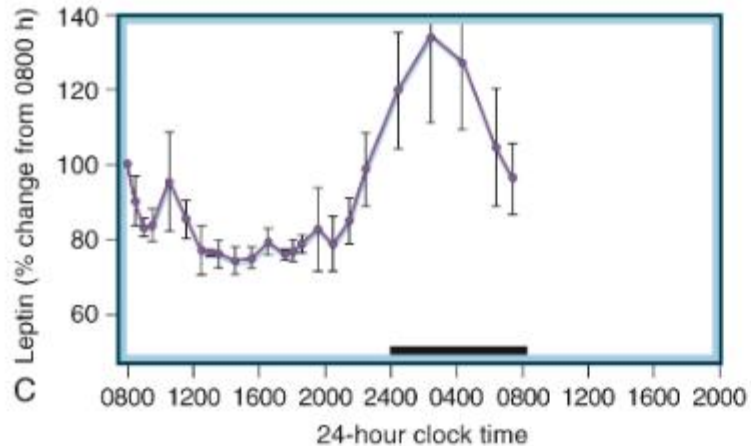
CRH



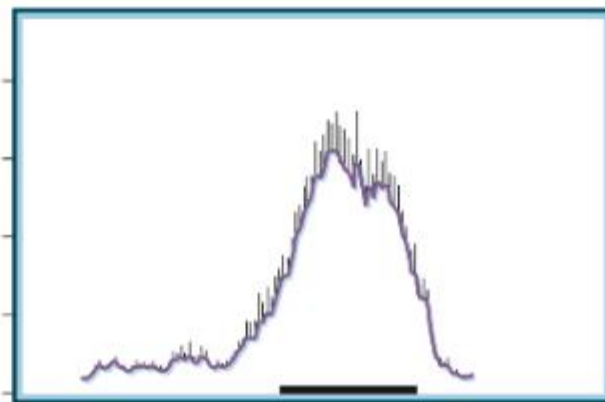
cortisol



leptin



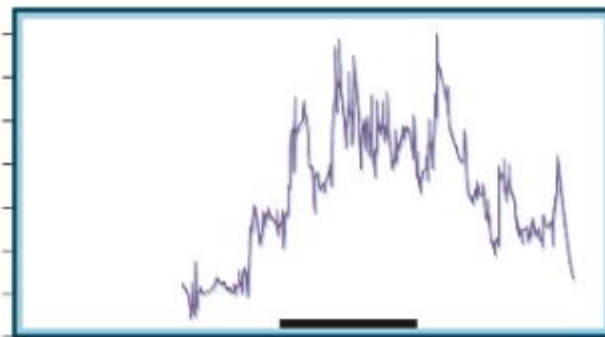
D Plasma melatonin (pmol/L)



melatonin



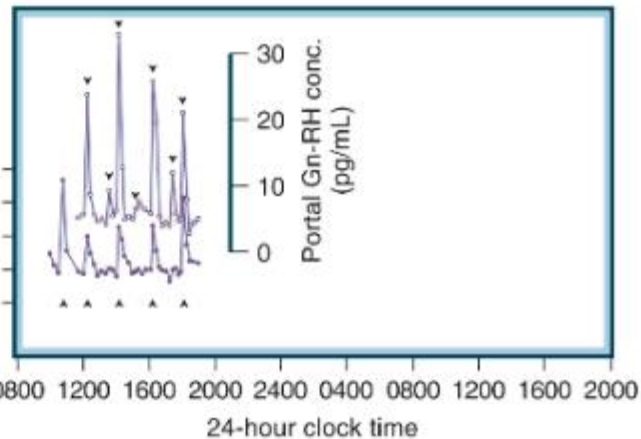
E Serum TSH (mU/L)



thyrotropin



F Peripheral LH conc. (ng/mL)



LH



Hormone transport

- Chemical properties of hormone
- Transport protein(s) bond and its significance
 - Albumin
 - Globulins
 - Specific proteins – TBG, SHBG, CBG
- Bond strength
- „Alternative“ binding – TBG versus transthyretin

- Protection
- Reservoir
- Ubiquitous distribution
- Transport across plasmatic membrane (SHBG – megalin)

DYNAMIC BALANCE BETWEEN HORMONE AND TRANSPORT PROTEIN

Hormone elimination

- Different length of time in circulation
- Metabolisation by
 - Target cells
 - Enzymatic systems in blood
 - Organs – mainly liver
- Elimination
 - Liver
 - Kidneys

PHASE I

- Hydroxylation, decarboxylation
- Oxidation, reduction

PHASE II

- Glucuronidation
- Sulphatation
- Methylation
- Conjugation with glutathione

metabolisation



Vascular system



bile



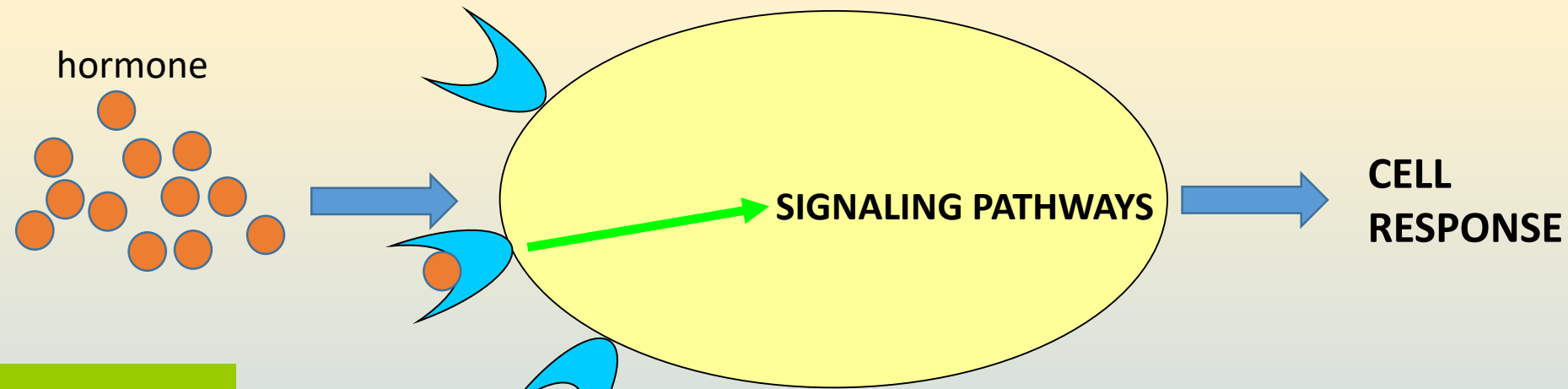
urine

elimination

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Hormones and cell response

- Target cells
- Specificity
- High affinity
- Selectivity



MECHANISMS

Conformation changes
Phosphorylation/dephosphorylation + protein recruitment
GTP binding (G proteins)
cAMP binding (effector proteins)
Precursor molecule generation in PM
Non-covalent Ca^{2+} bond

Receptor binding

Signal amplification and transduction
effector molecules

% of occupied receptors
conformation change

synergy
antagonism
possible loss of sensitivity
feedback-loop regulation

CELL RESPONSE IS MEDIATED BY RELEVANT RECEPTORS

Receptor level of cell response regulation

- Downregulation
- Upregulation
- Homologous desensitization
- Heterologous desensitization

Phosphorylation (specific kinases)
 Dephosphorylation (specific phosphatases)
 Modification by proteins of inhibited signaling pathway

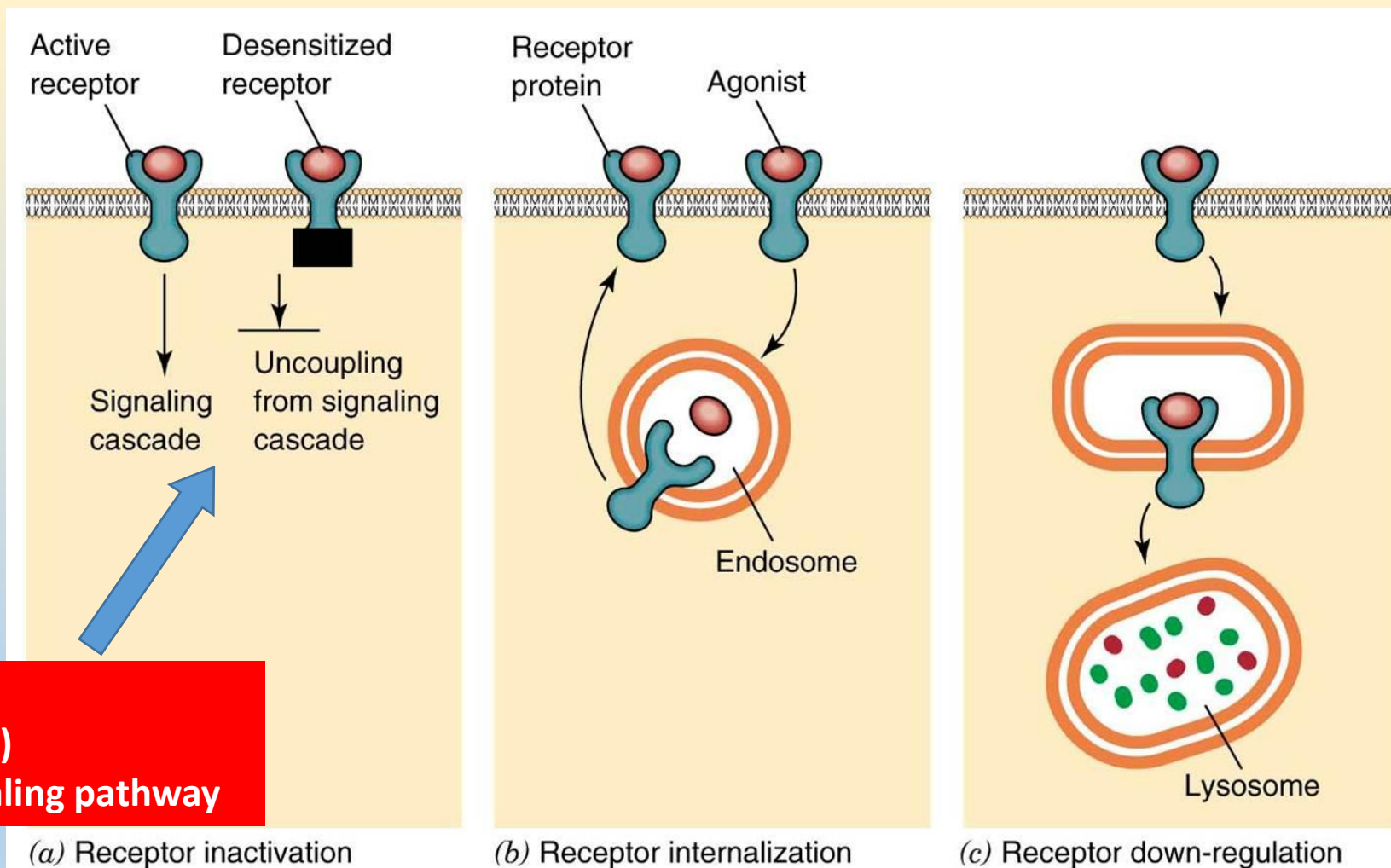


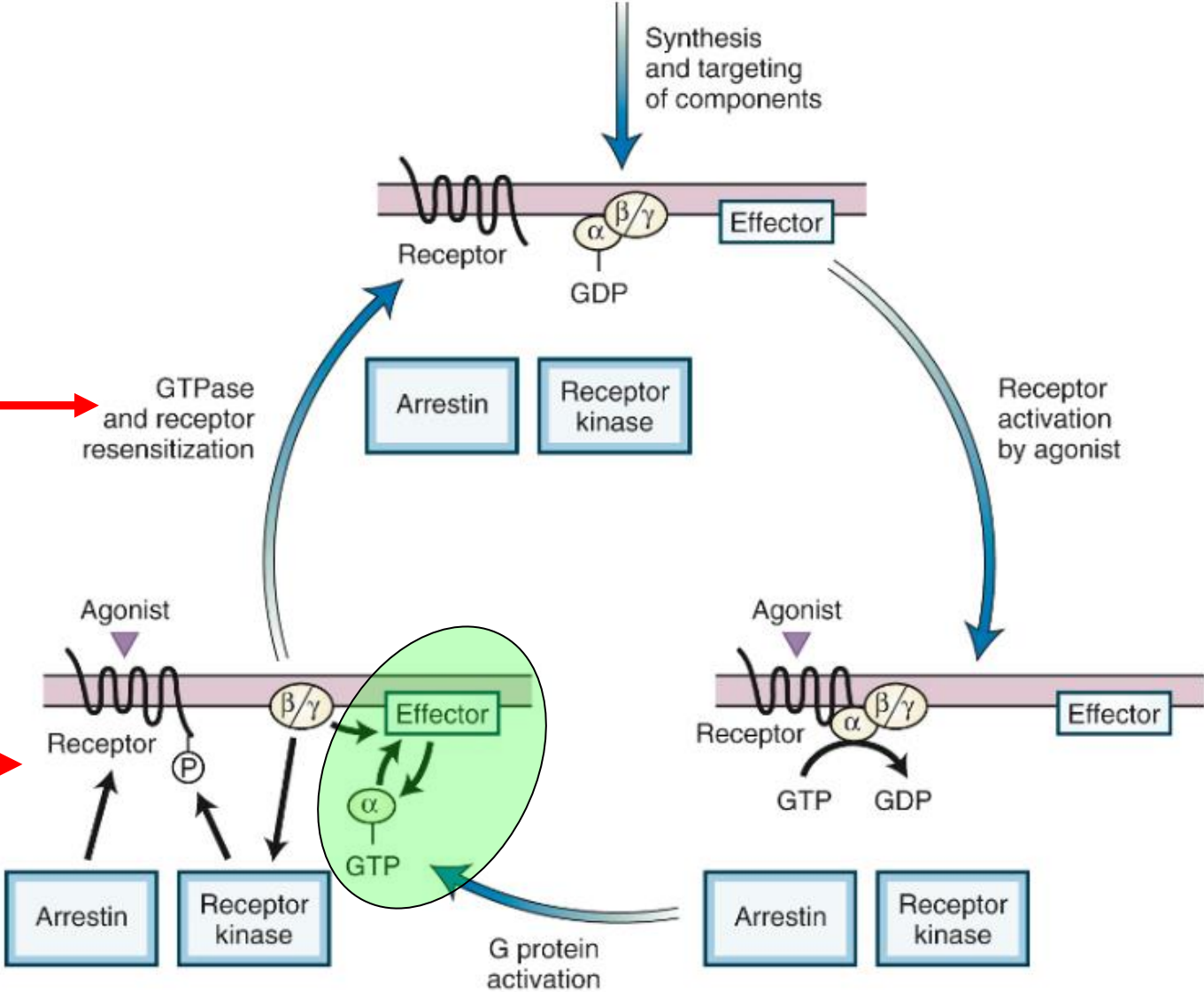
Figure 13.10. Major mechanisms for the termination of receptor-dependent signal transduction.

Sensitisation and desensitisation of G protein-coupled proteins

- α subunit with GTPase activity

- resensitisation

- desensitisation



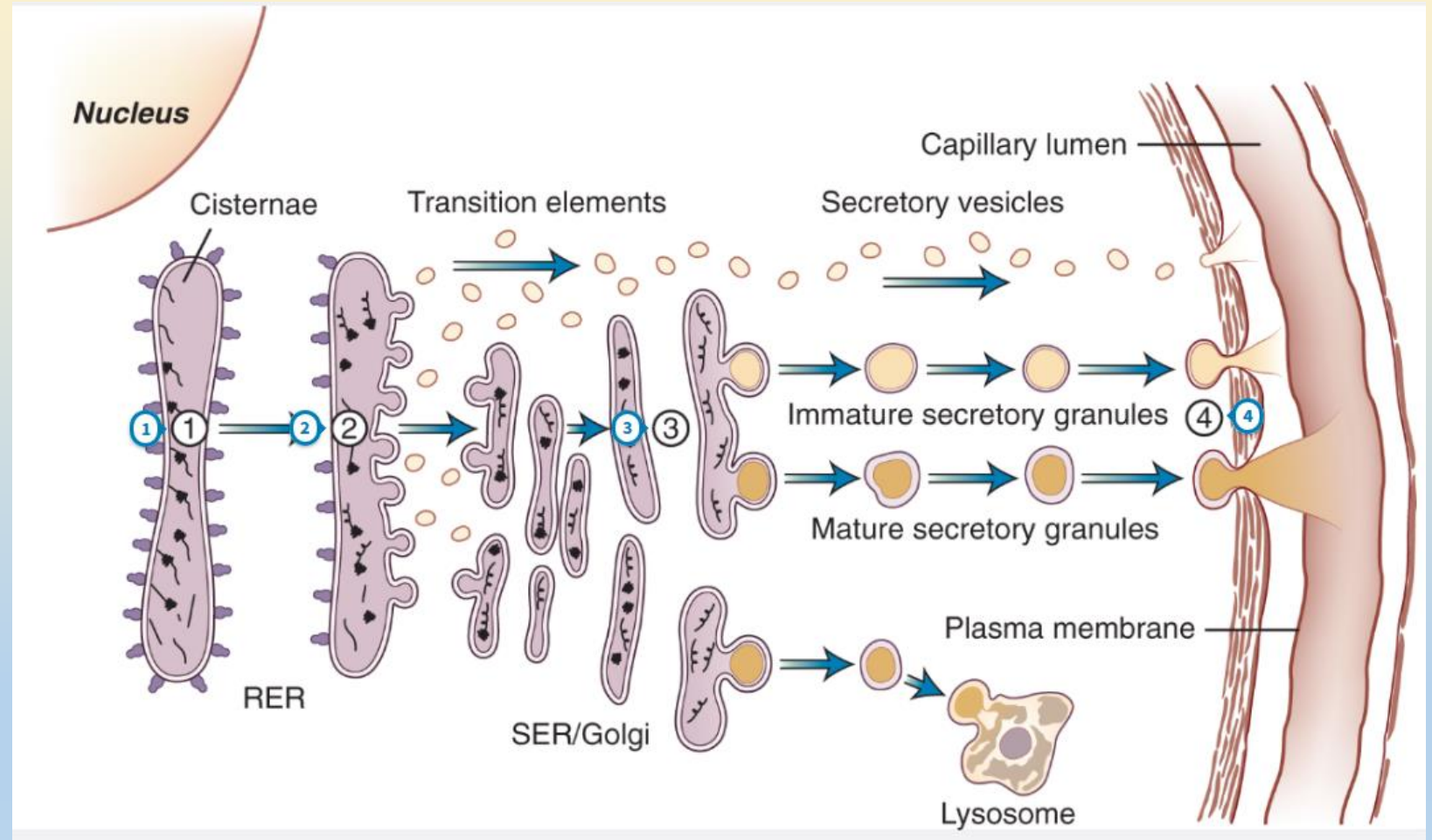
Hormones – proteins and peptides

„classic“ hormones

Hormones produced by non-specialised cells (e.g. *adipokines*)

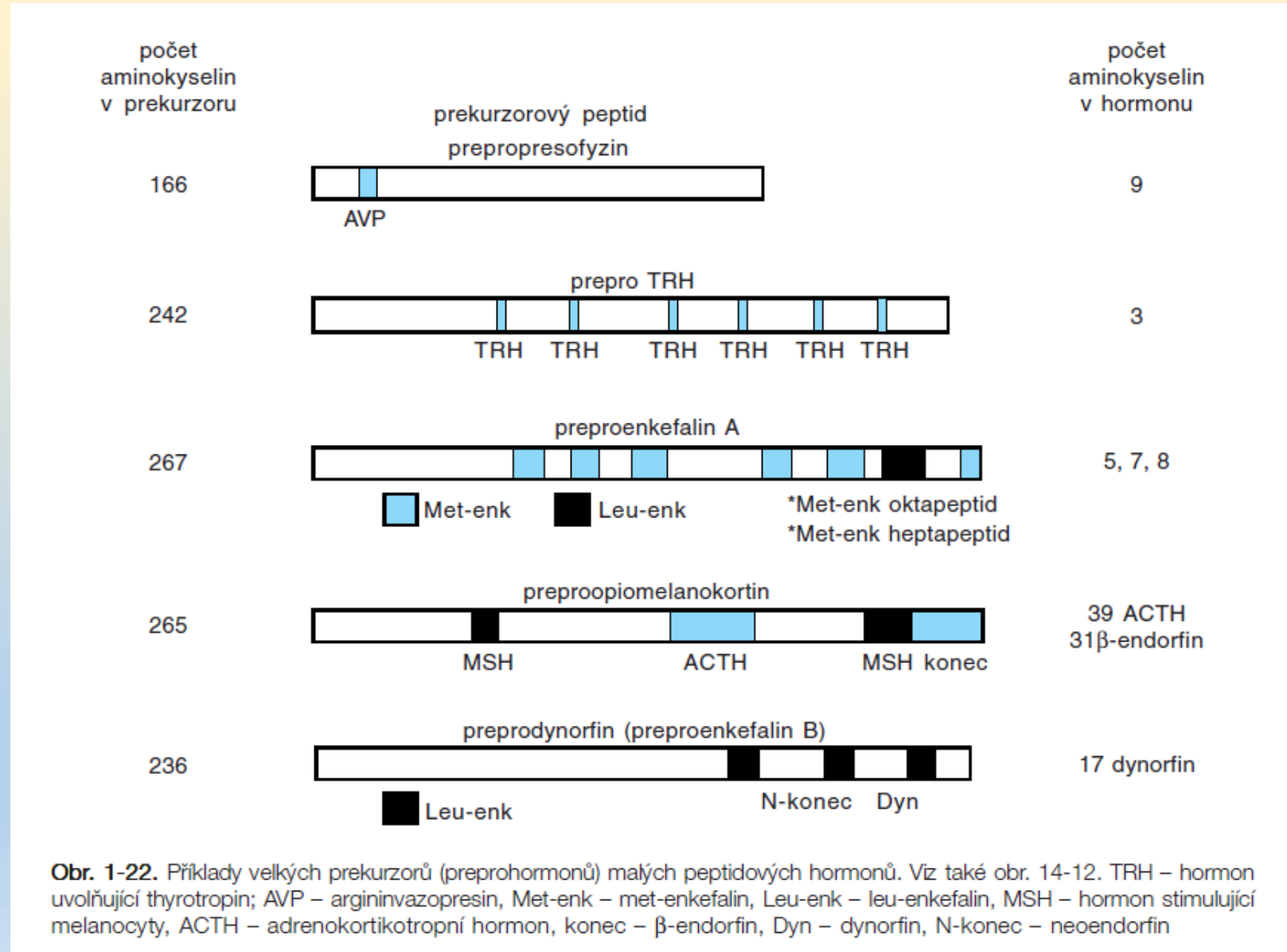
Paracrine/autocrine peptides

Receptors associated with plasmatic membrane



preprohormone – prohormone – hormone (+ fragments)

Peptide hormones as a part of preprohormones

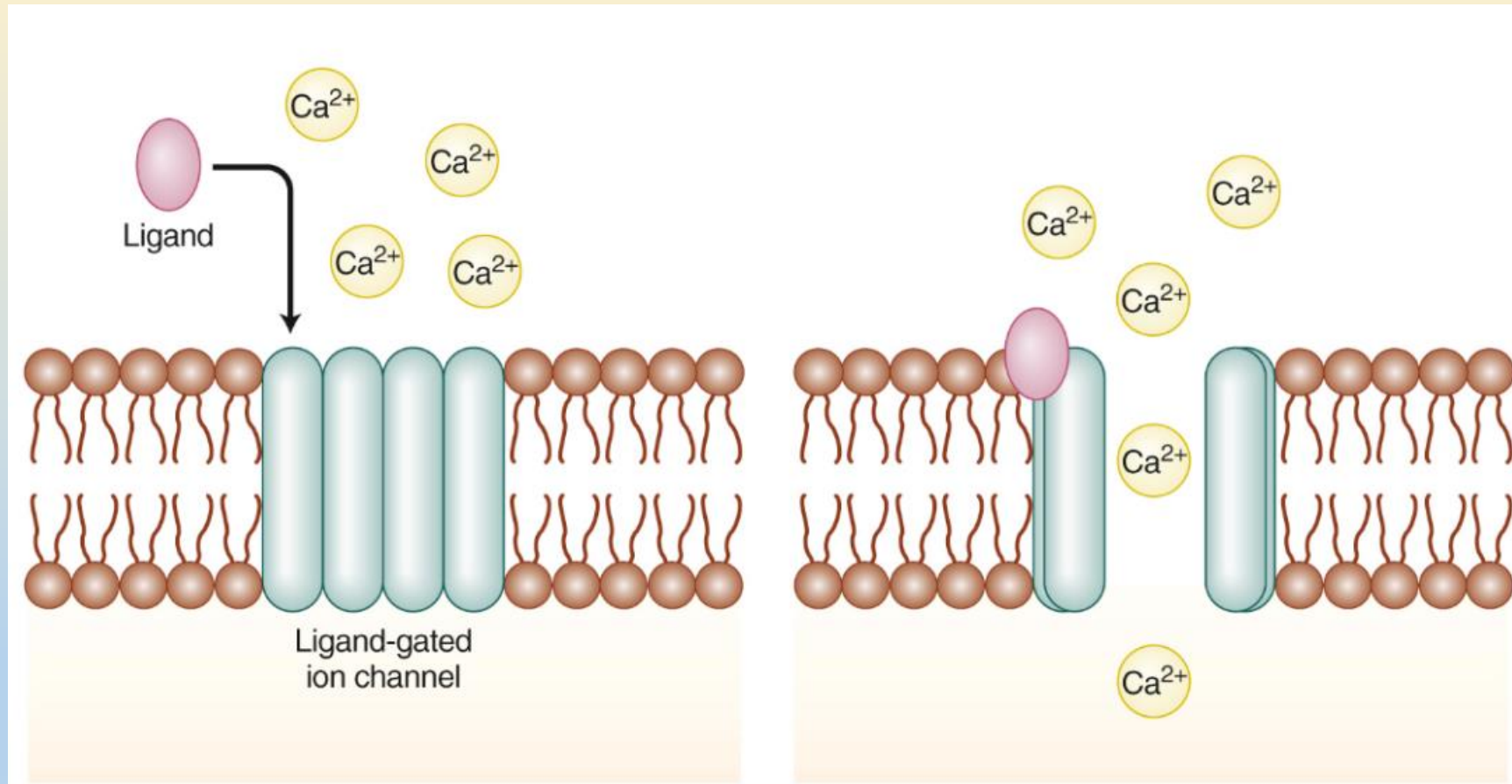


Obr. 1-22. Příklady velkých prekurzorů (preprohormonů) malých peptidových hormonů. Viz také obr. 14-12. TRH – hormon uvolňující thyrotropin; AVP – arginin vazopresin, Met-enk – met-enkefalin, Leu-enk – leu-enkefalin, MSH – hormon stimulujiící melanocyty, ACTH – adrenokortikotropní hormon, konec – β-endorfin, Dyn – dynorfin, N-konec – neoendorfin



Taken from Ganong, W. F. Přehled lékařské fyziologie. 20th edition. Galén 2005.

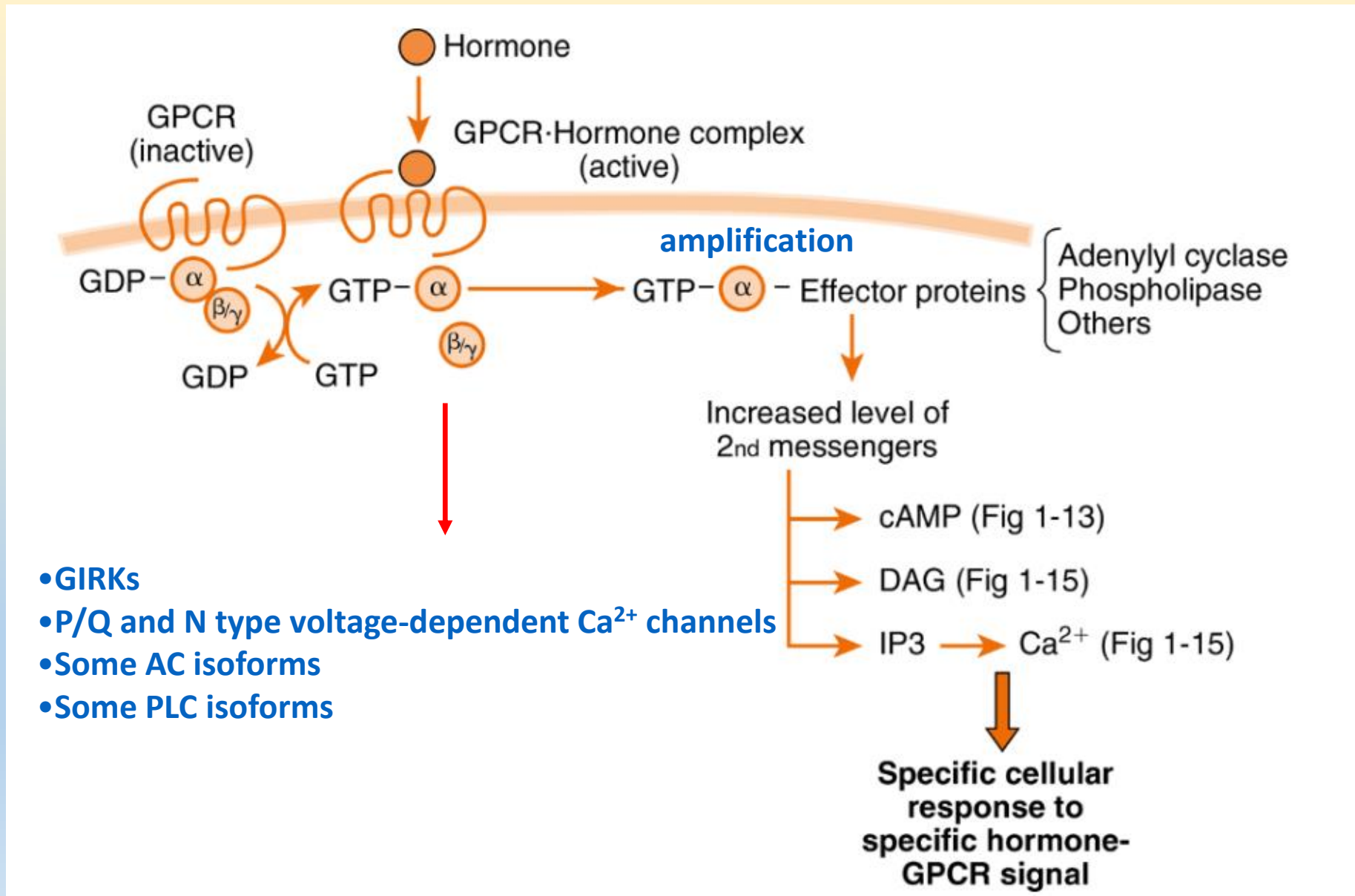
Ligand-gated ion channels



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SECRETION OF HYPOTHALAMIC HORMONES AFTER BINDING OF CORRESPONDING TYPE OF LIGAND (NEUROTRANSMITTER)

G protein-coupled receptors (GPCR)



- GIRKs
- P/Q and N type voltage-dependent Ca²⁺ channels
- Some AC isoforms
- Some PLC isoforms

G protein-coupled receptors (GPCR)

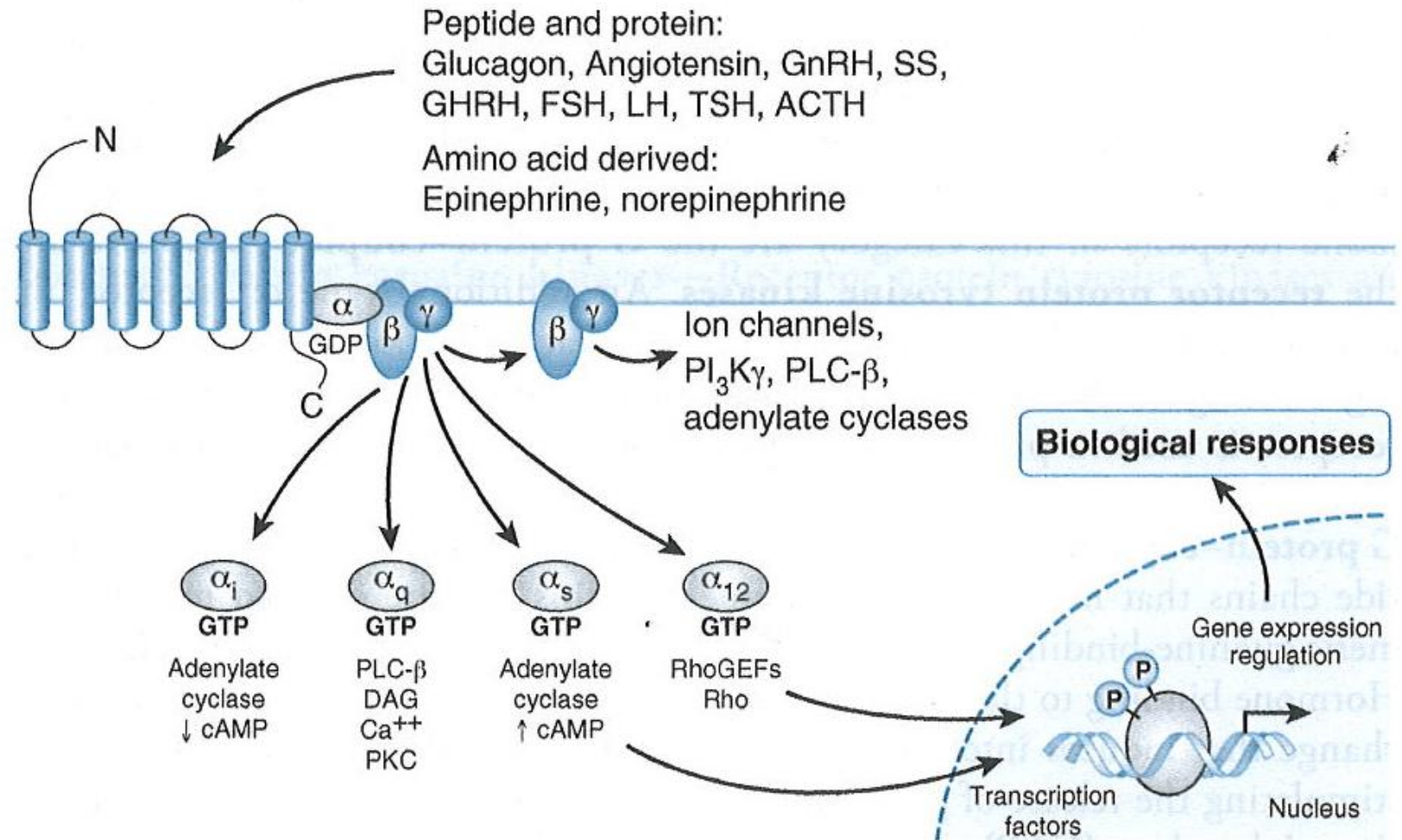
$G_s - G_{s'}$, G_{olf} – activation of AC

G_i – inhibition of AC

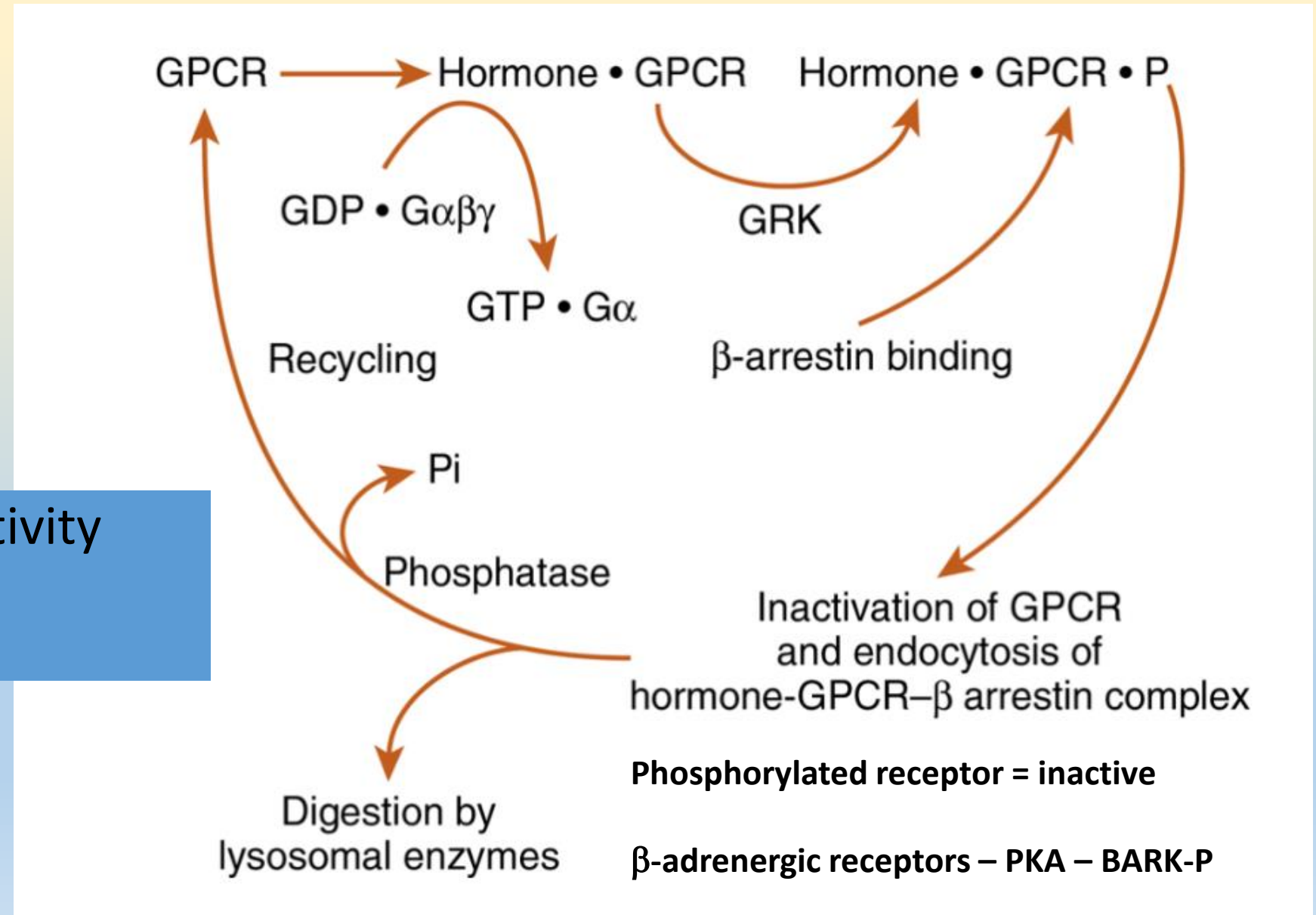
- G_0 (2, brain)
- G_t (2, photorec. – cAMP-PDE)
- G_z (inhibition of K^+ channels)

$G_{q/11}$ – activation of PLC β

$G_{12/13}$ – inhibition and activation of RhoGEF

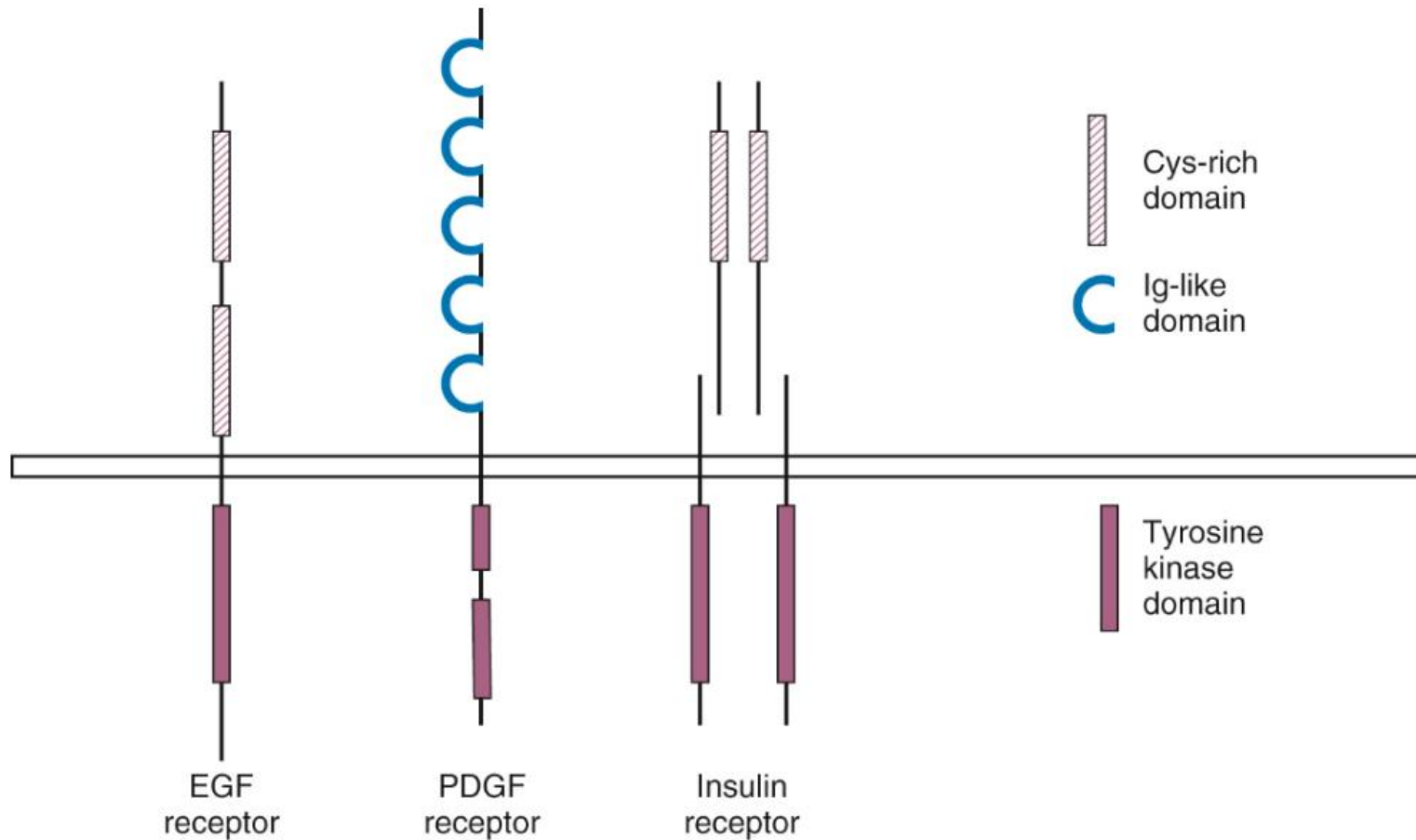


End of activation and limitation of cell response



- Intrinsic GTPase activity
- Endocytosis

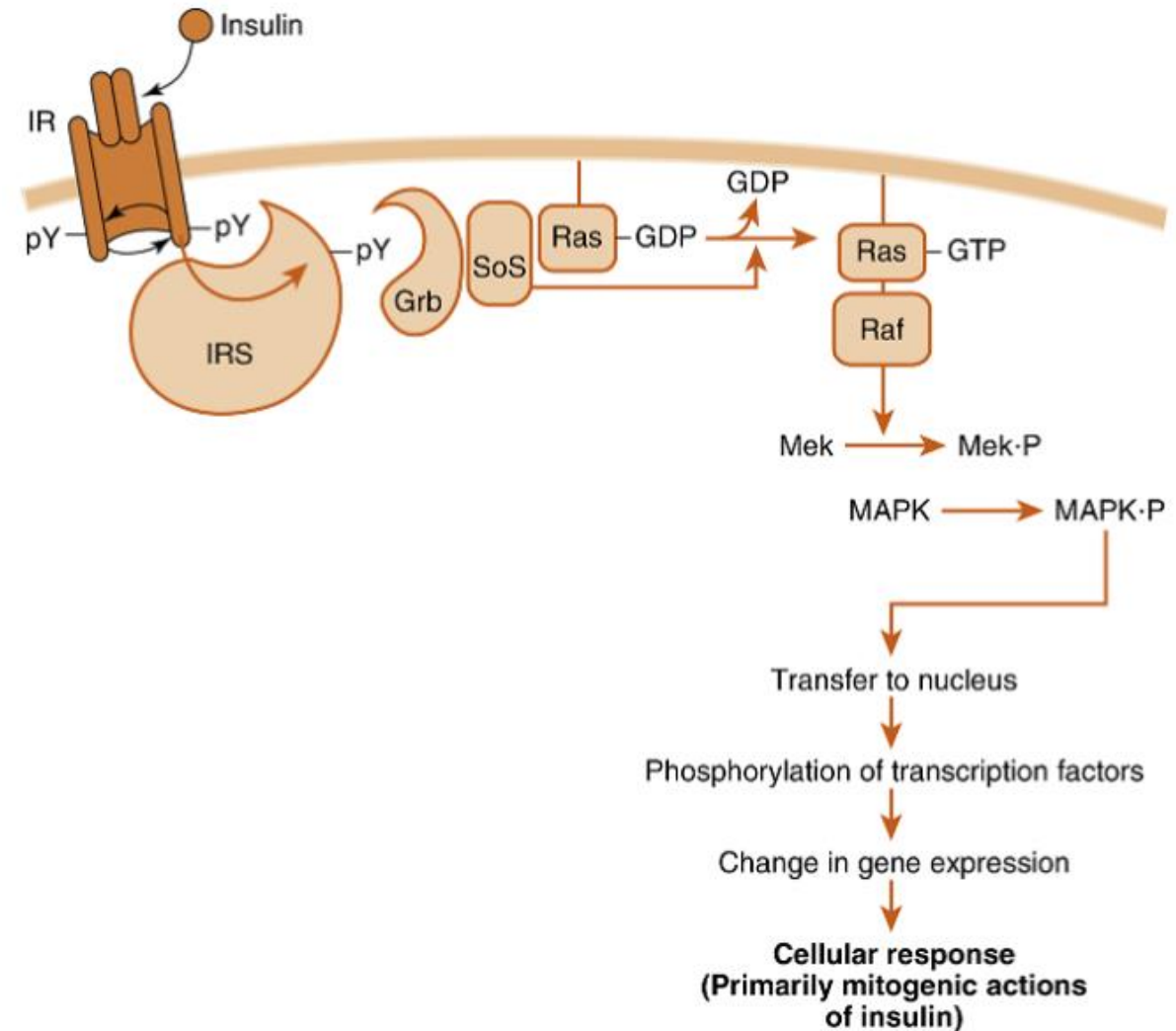
Receptor tyrosinases



- 58 RTKs/20 subfamilies
- Usually dimerisation after ligand binding
- ATP as a source of P for phosphorylation of intracellular domains/associated proteins
- Insulin
- IGF-1/2

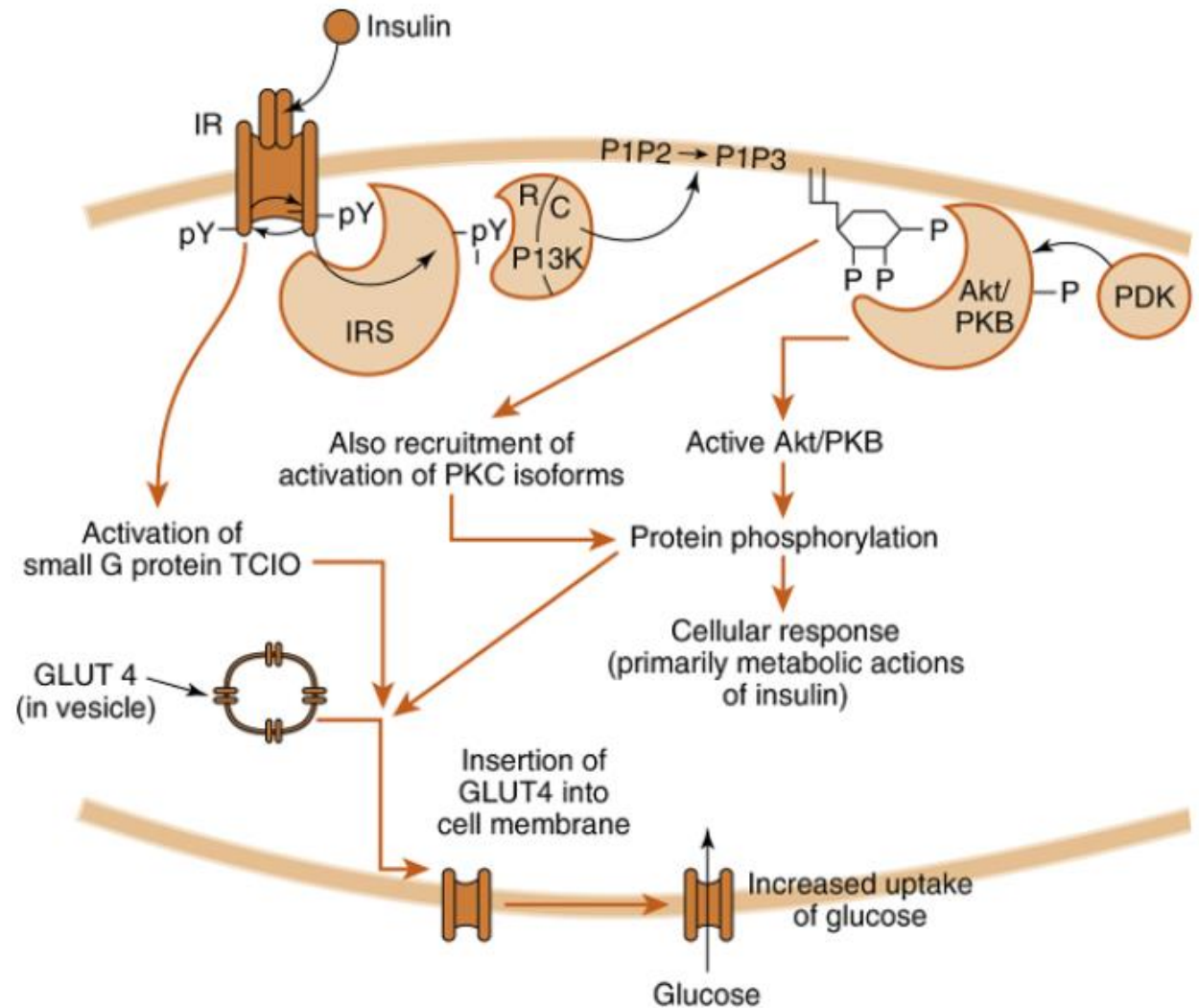
Insuline receptor – genomic effects

- IRS = insulin receptor substrate
- Grb = adaptor protein (growth factor receptor-bound protein)
- SoS = Son of sevenless homologue
- Ras = small GTPase-like proteins (ability to bind GTP)
- Raf = serin/threonin-specific proteinkinases



Insulin receptor – metabolic effects

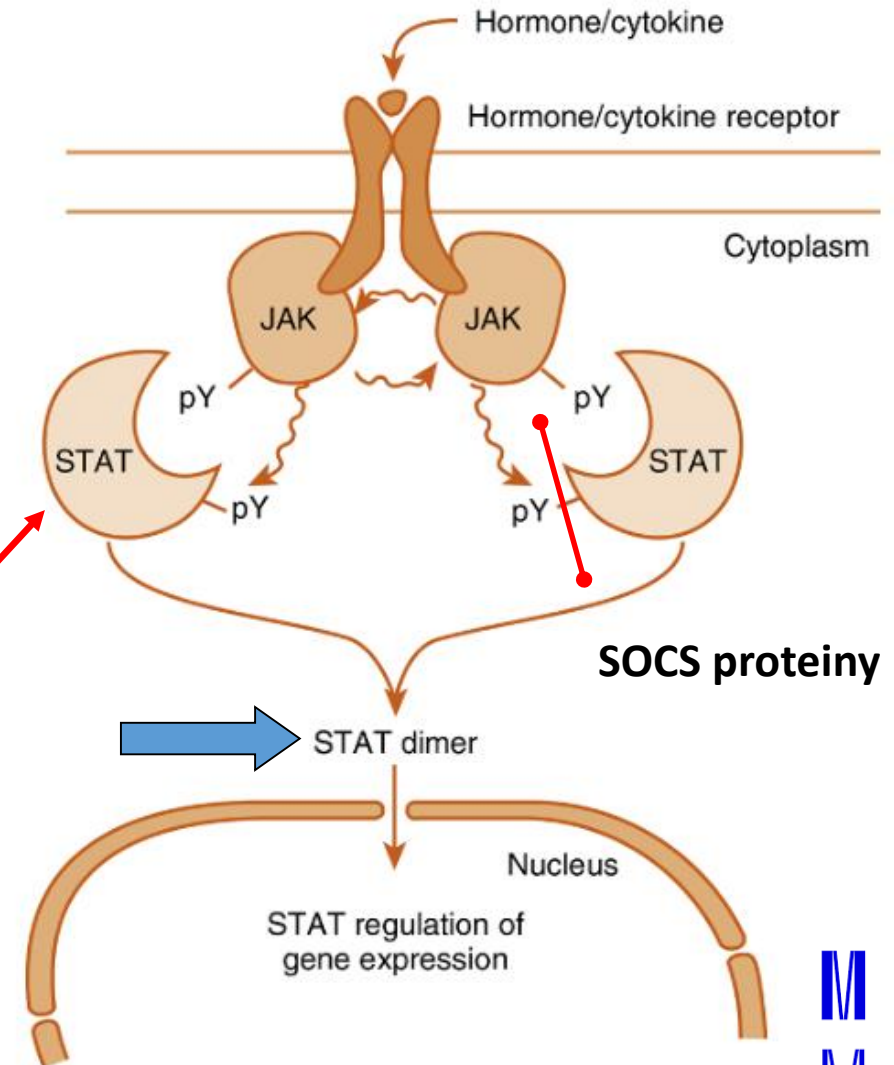
- P13K = phosphatidylinositol-3-kinase
- Akt = proteinkinase B



Receptors associated with cytosolic TK

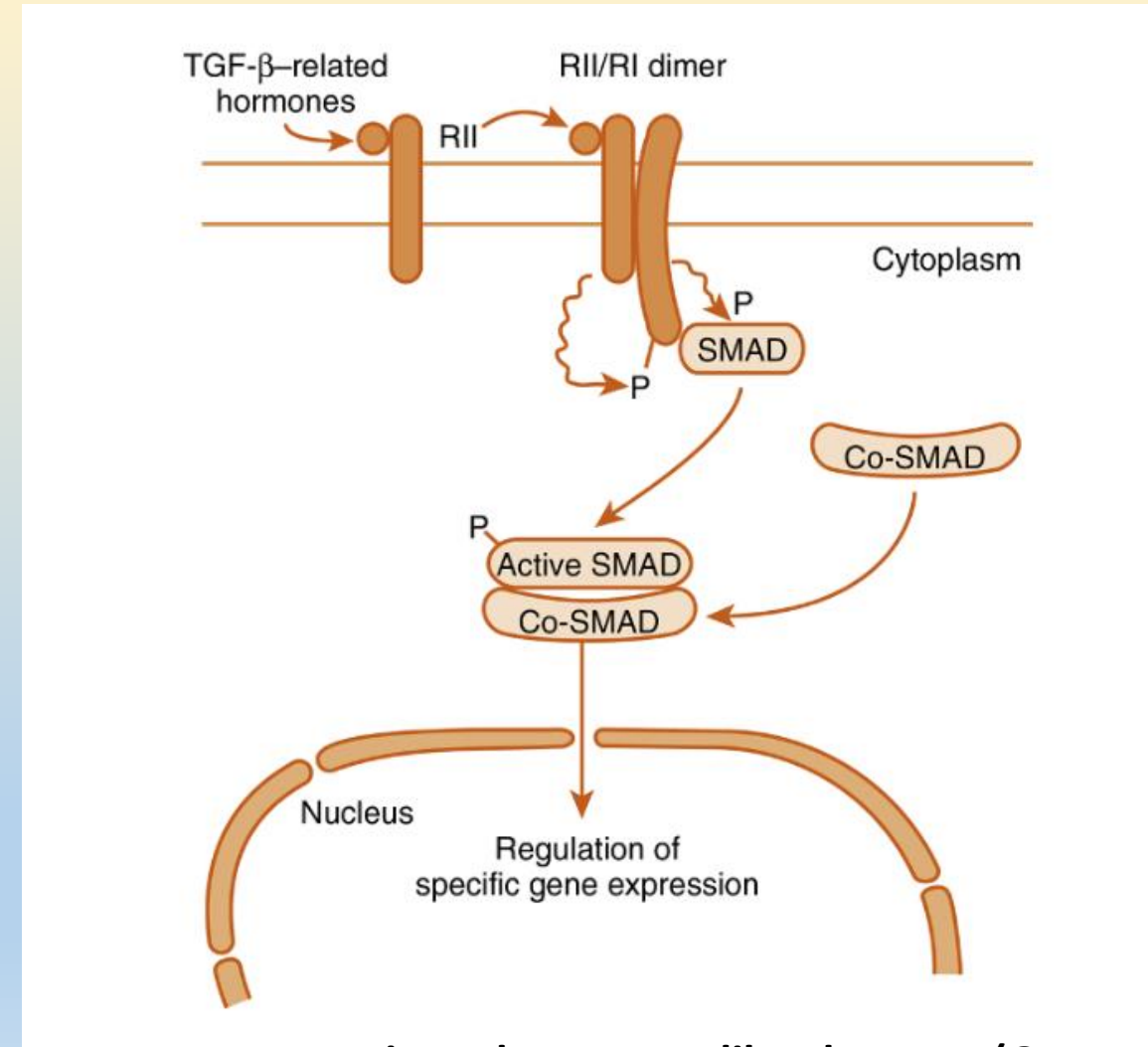
- GH, prolactin, leptin, erythropoietin
- Dimeric receptor **without** TK activity
- Association with JAK kinase
- After ligand binding – dimerisation, transphosphorylation, activation

signal transducers and activators of transcription



Receptor serin/threonin kinases

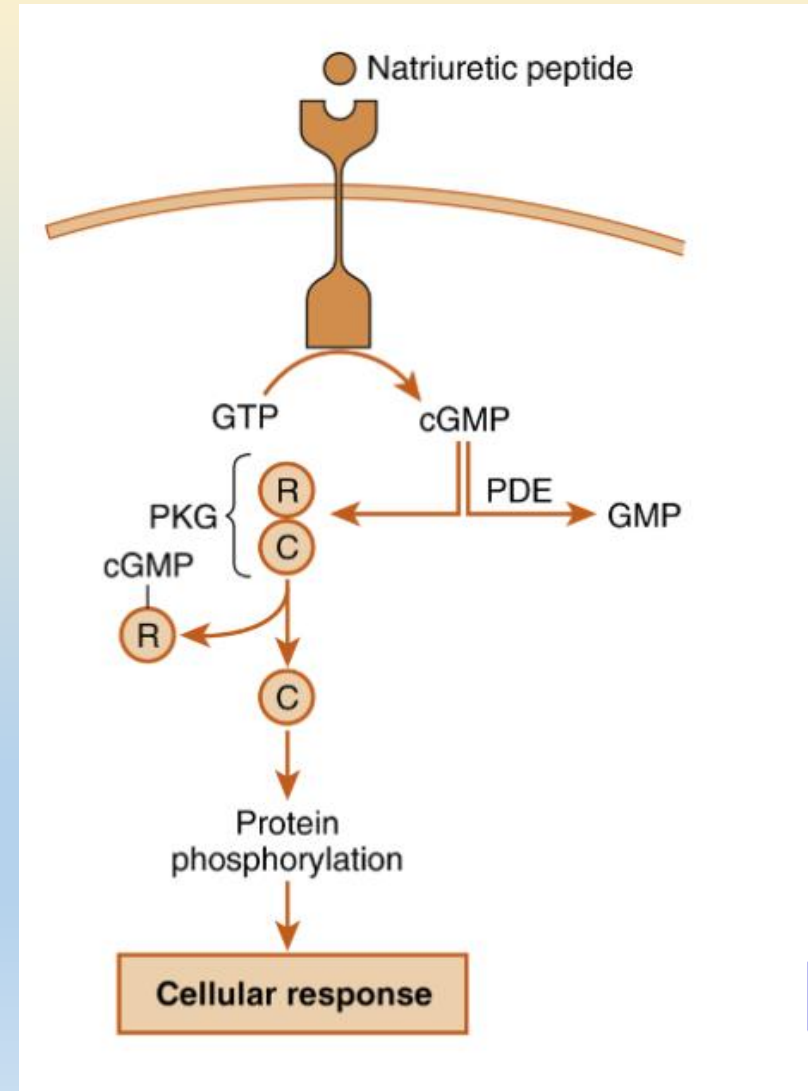
- Anti-Müllerian hormone, inhibitin
- Form of dissociated heterodimer
- SMAD = „latent transcription factors“



gamma-activated sequence-like elements (GLEs, promoter region of some genes)

Receptor guanylate cyklases

- Natriuretic peptides
- ANP, BNP, CNP



Signal transduction – system of second messengers

HORMONE = FIRST MESSENGER

INTRACELLULAR SIGNALING MOLECULE GENERATED AFTER HORMONE-RECEPTOR BONDING = SECOND MESSENGER

• cAMP

- TSH, glucagon, ACTH, hypothalamic hormones, ADH etc.
- Proteinkinase A
- Modulation of signaling pathways by compartmentalization (A-kinase anchoring proteins (AKAPs))

• cGMP

- ANP, BNP, CNP
- NO as a signaling molecule
 - Proteinkinase G

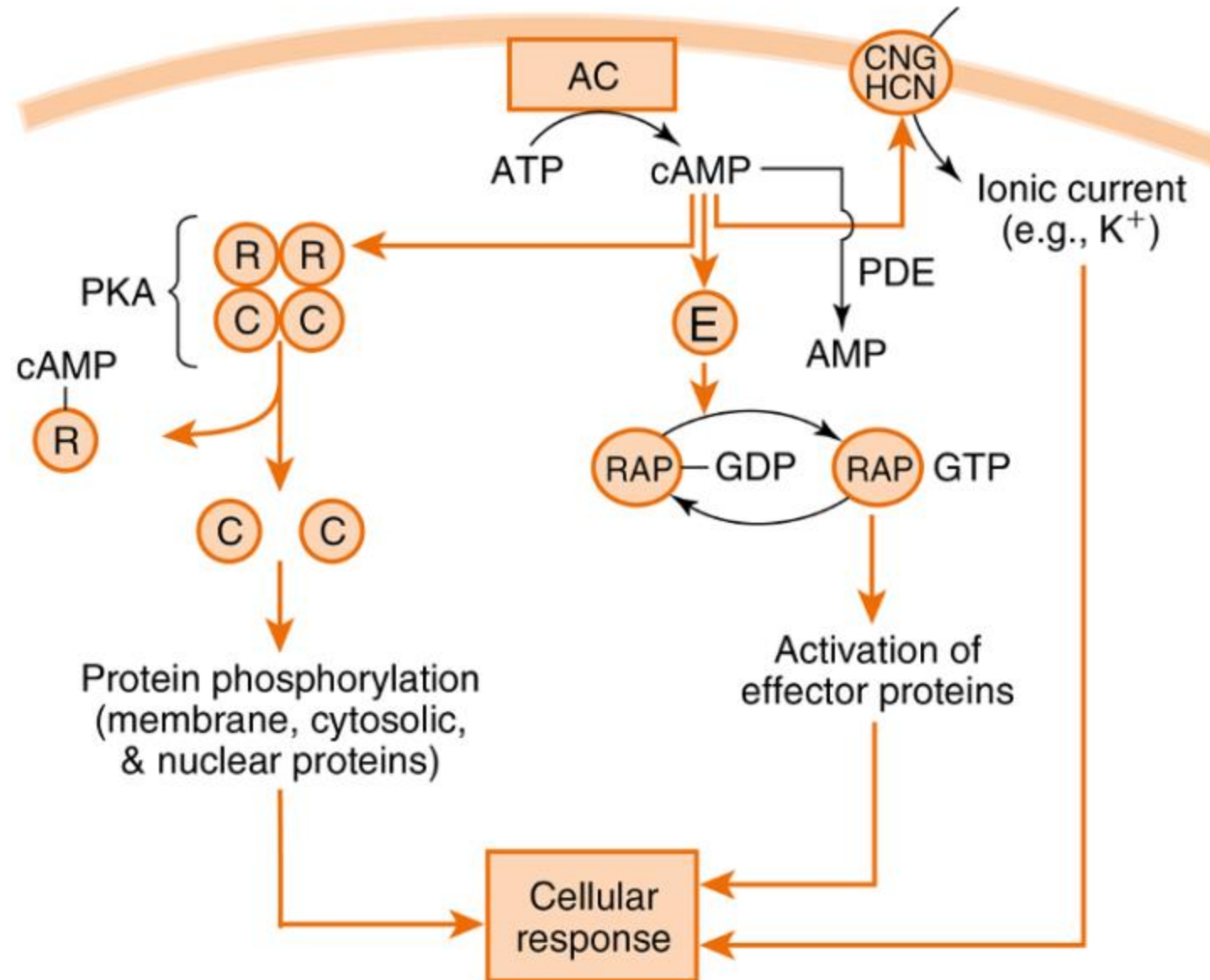
• DAG and IP₃

- PIP₂ – phospholipase C system
- Ca²⁺
 - Ca²⁺/Ca²⁺- calmodulin

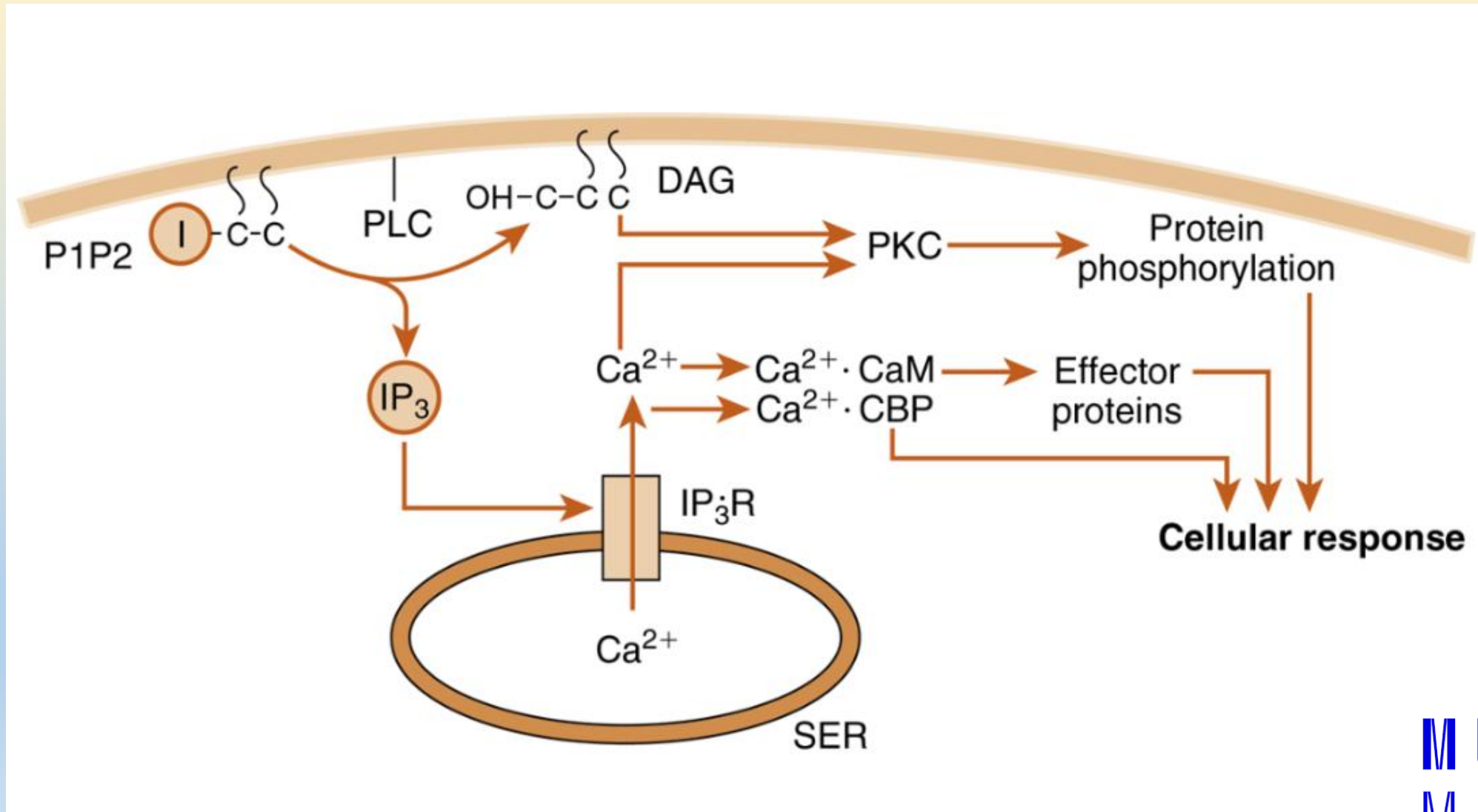
EXTRACELLULAR SIGNAL MUST BE CONVERTED TO INTRACELLULAR RESPONSE

AC – cAMP system

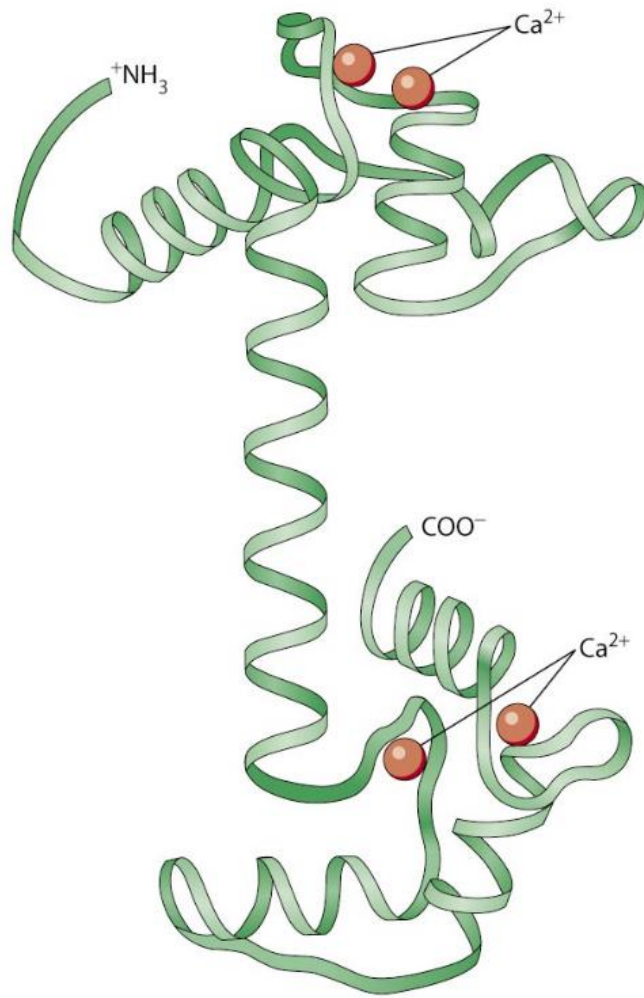
- PKA
- CREB (cAMP-responsive element-binding protein)
- Epac (E) as an another effector molecule (exchange protein activated by cAMP)
- cyclic nucleotide gated (CNG) channels
- hyperpolarization-activated cyclic nucleotide modulated (HCN) channels
- phosphodiesterases



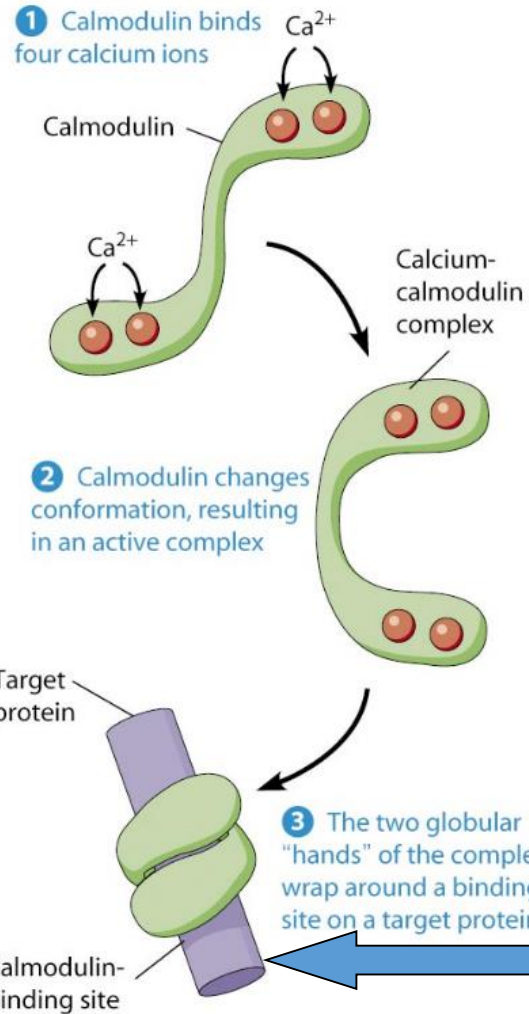
PLC - DAG and IP₃ system



Ca²⁺ - calmodulin system

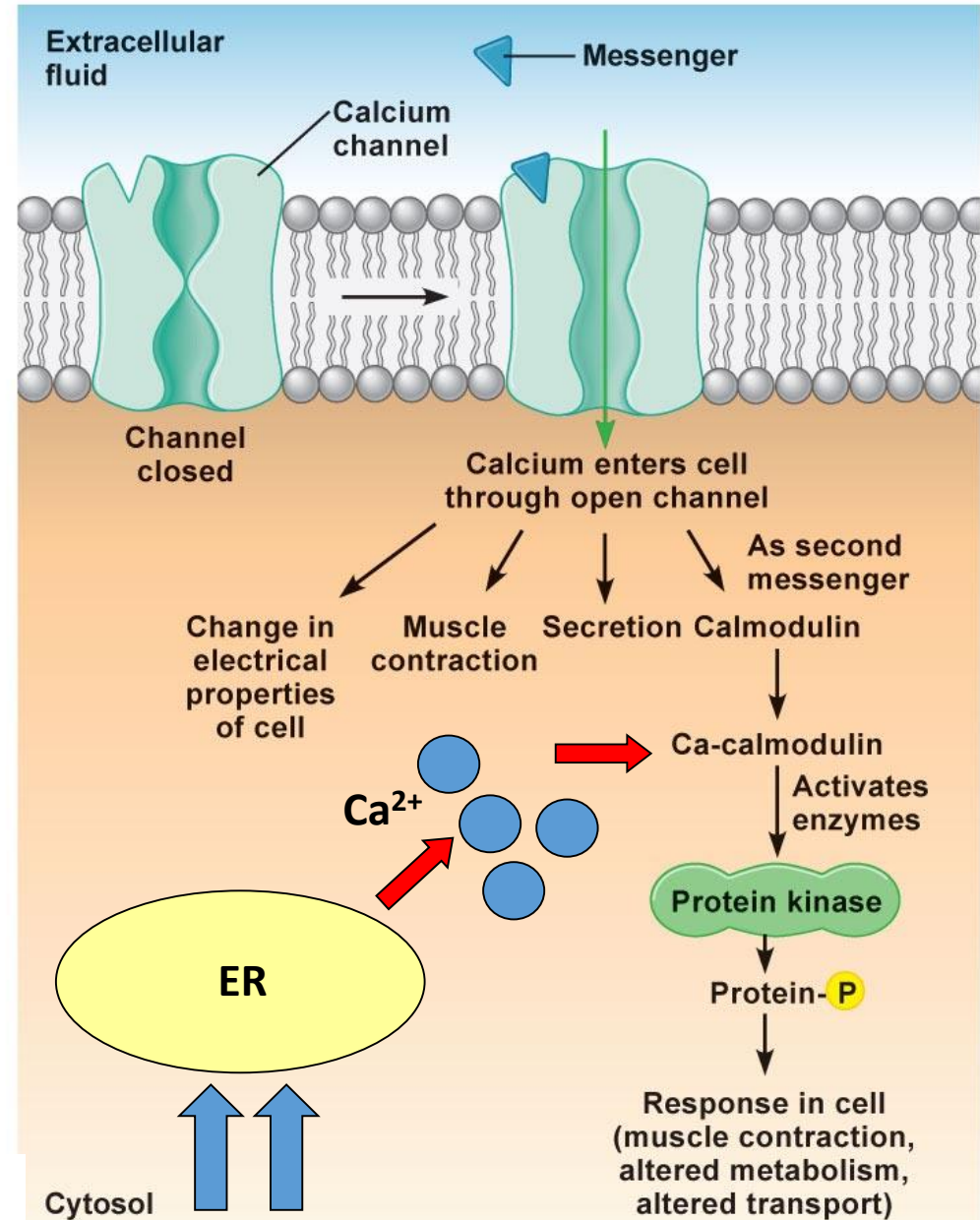


(a) Structure of Ca²⁺-calmodulin complex



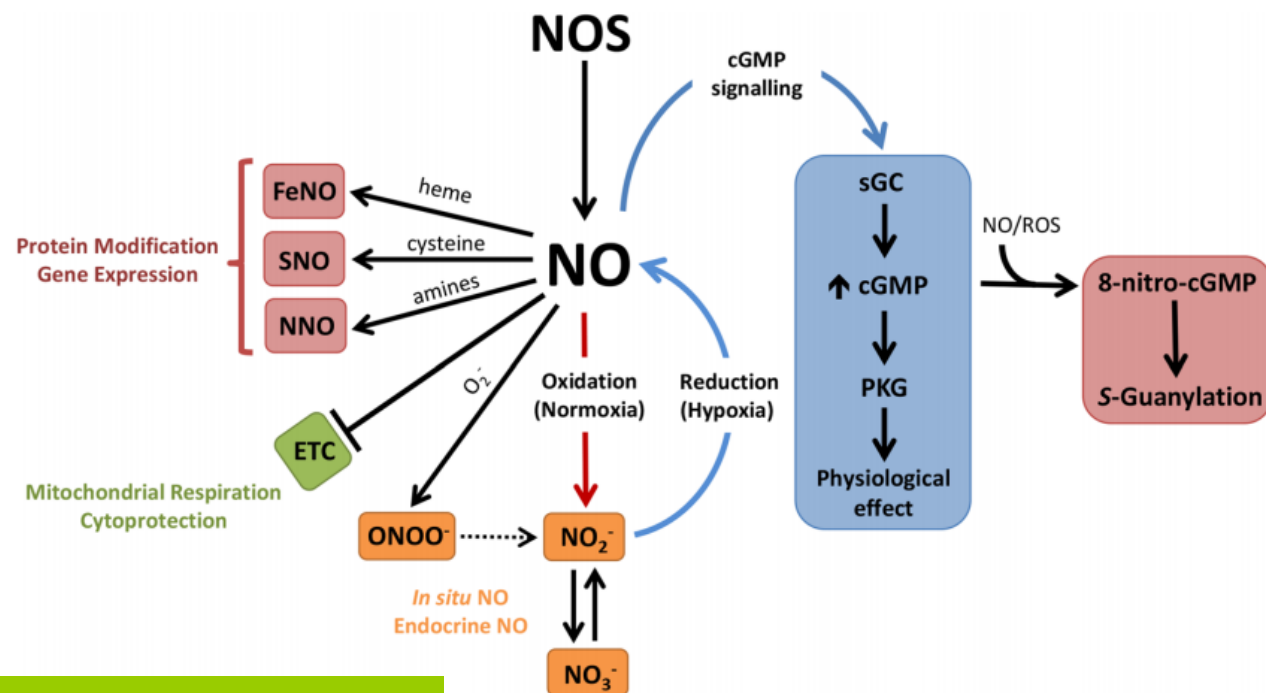
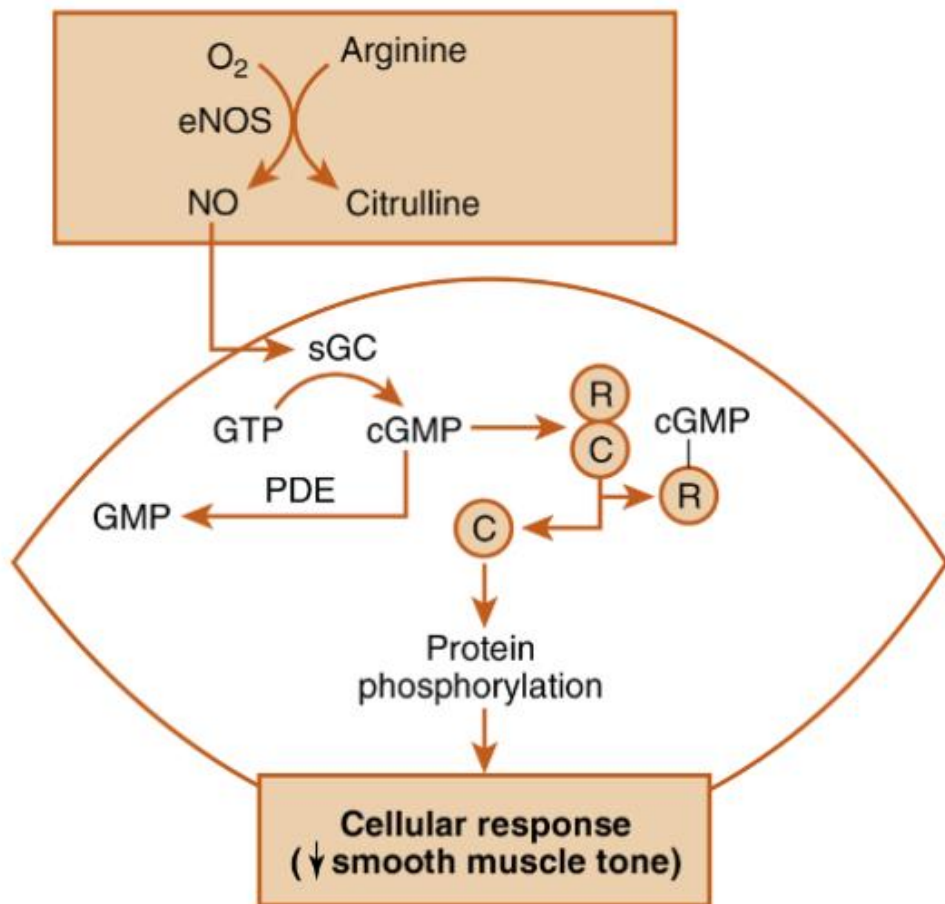
(b) Function of Ca²⁺-calmodulin complex

calmodulin-dependent kinases



Extracellular signals (hormones, neurotransmitters)

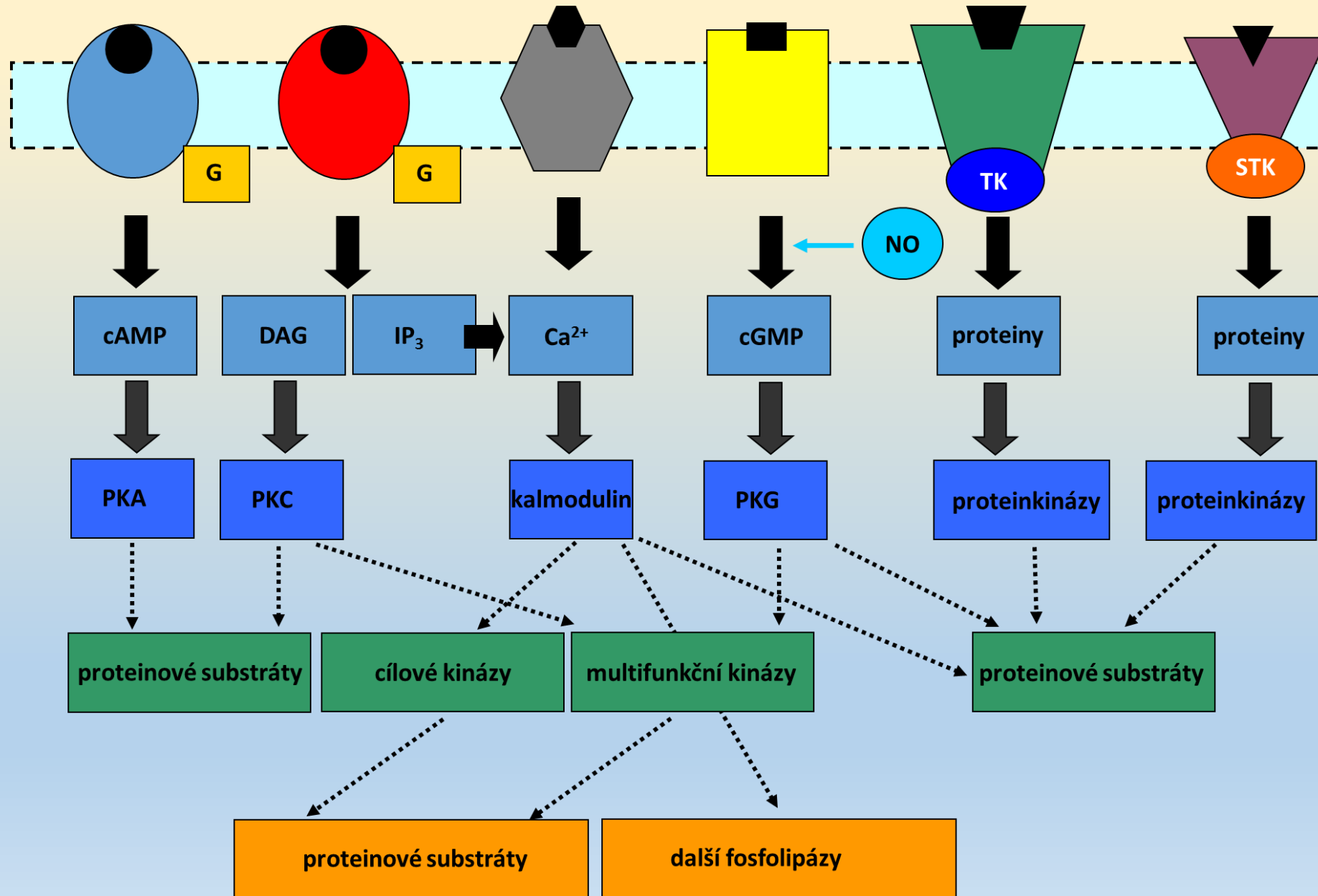
NO as a signalling molecule - cGMP



- eNOS
- nNOS
- iNOS

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Summary – membrane receptors and associated systems



Clinical aspects

- Syndromes of resistance to hormones (i.e. IR, IGF-1, TR β)
- Syndromes caused by CPCR and G proteins mutations
 - ADH – nephrogenic diabetes insipidus
 - ACTH – familiar ACTH resistance
 - GnRH – hypogonadotrophic hypogonadism
 - FSH – hypergonadotrophic ovarian dysgenesis
 - LH – male pseudohermaphroditism
 - Melanocortin 4 – obesity
 - PTH/PTHrP – Blomstrand lethal chondrodysplasia

Hormones acting through nuclear receptors

HORMONES

- Thyroid hormones – TR α/β
 - Estrogens – ER α/β
 - Testosterone - AR
 - Progesterone - PR
 - Aldosterone - MR
 - Cortisol - GR
- ← heterodimers
- homodimers
-

PRODUCTS OF METABOLISM AND XENOBIOTICS

- Fatty acids– PPAR α, β, γ
- Oxysterols – liver X receptor LXR α, β
- Bile acids - BAR
- Hem – RevErb α, β
- Phospholipids – homologue of liver receptor LRH-1, SF-1
- Xenobiotics – pregnane X receptor PXR
 - constitutive androstane receptor CAR

VITAMINS

- 1,25-[OH]2D3 - VDR
- All-*trans*-retinoic acid – RA receptors α, β, γ
- 9-*cis*-retinoic acid – retinoid X receptor RXR α, β, γ

- Orphan receptors
 - Variable receptors
-

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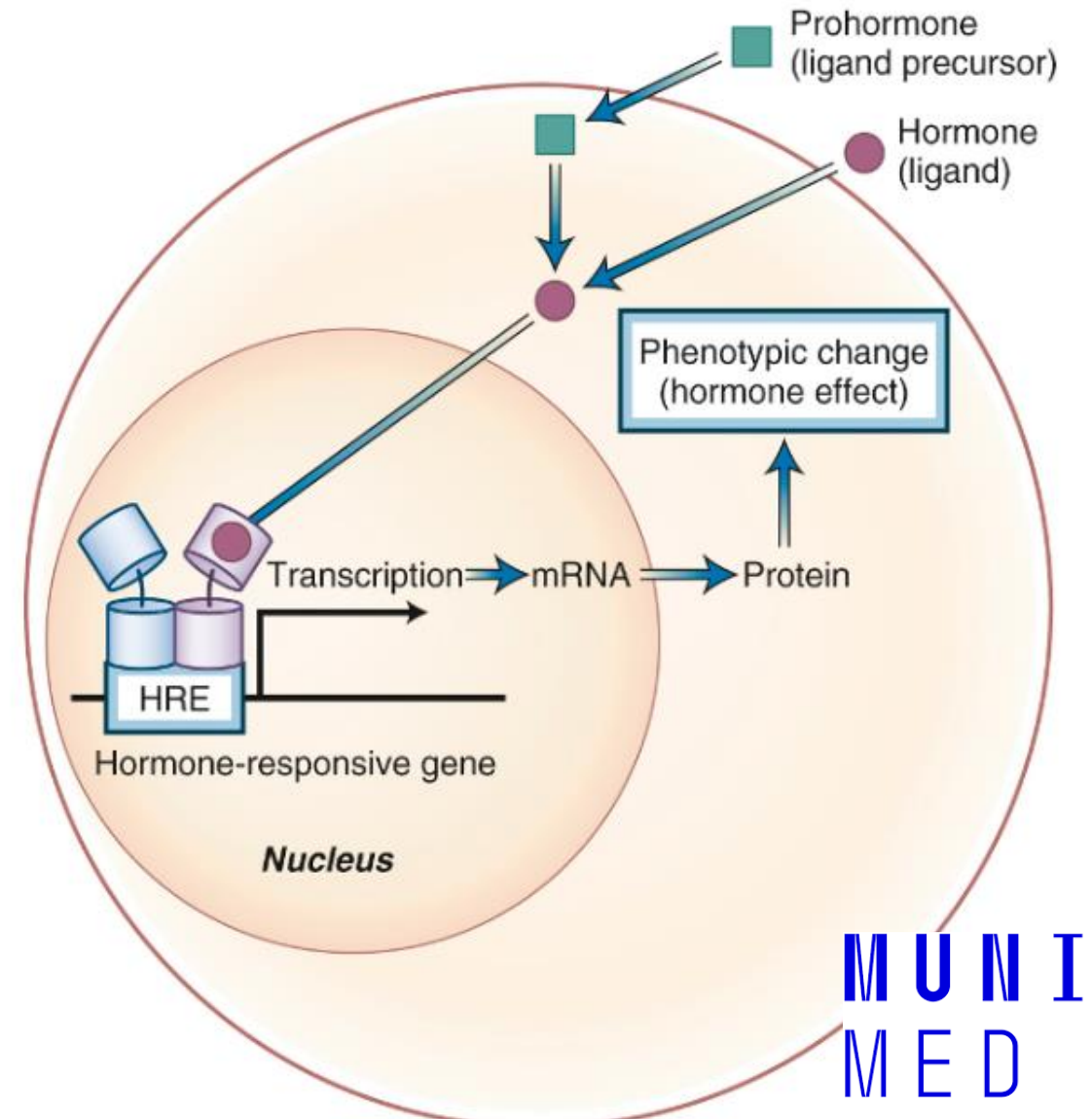
Explanation of some effects and pathologies

General mechanism of effect of hormones acting through nuclear receptors

- High affinity of ligand bond = due to R structure
- Recognition of specific promotor region
- Dimerisation of receptors (homodimers, heterodimers)
- Remodelation of chromatin for gene expression (HDAC)
- Gene expression at the end decreased or increased

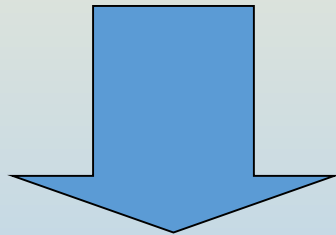
WHY ONLY NUCLEAR RECEPTORS?

- Synthesis in cytoplasm
- Stay until ligand binding or until transport to nucleus

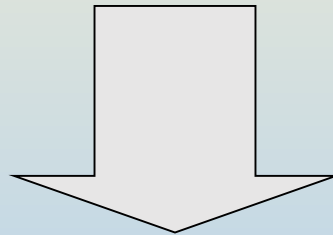


- Regulation mechanism – modification, count of receptors
- Important parameter – selectivity of target cells
- Tissue-specific factors, coactivators and corepressors

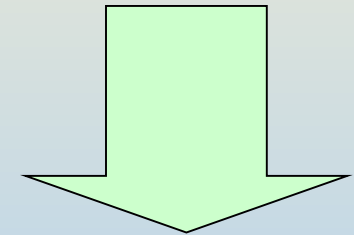
Nuclear receptors



- Coregulatory proteins binding (independent on ligand)
- Phosphorylation sites



- DNA binding (zinc fingers)
- Dimerisation
- ERE, PRE, GRE, MRE, ARE

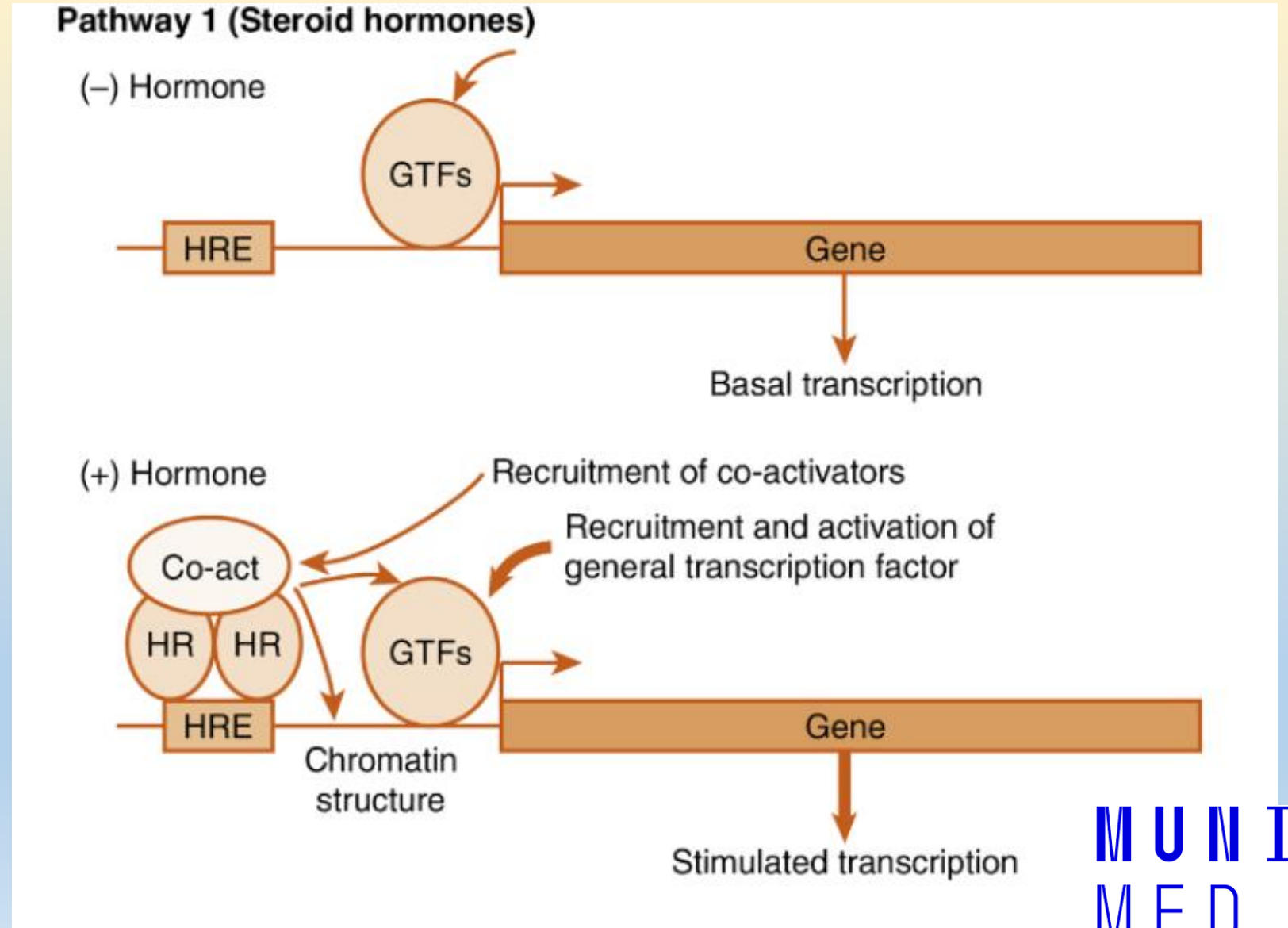


- Ligand binding (agonist, antagonist)
- Coregulatory proteins binding (dependent on ligand)
- Dimerisation
- Nuclear translocation
- Chaperone association (HSP)

Example – steroid hormones

GTFs = general transcription factors
(remodulators of chromatin)

HAT = histon acetyltransferase



Example – thyroid hormones

THRs, VDR, PPARs, RXRs

THR = heterodimer

hormone

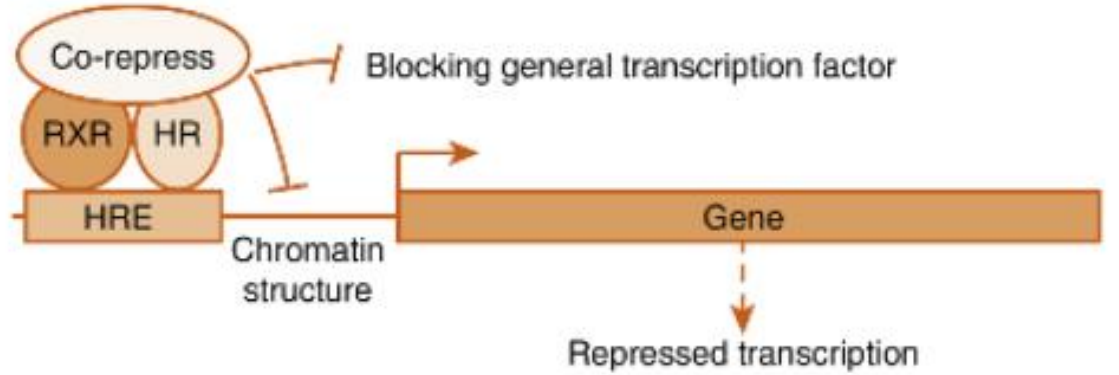
basic transcription

hormone + RA

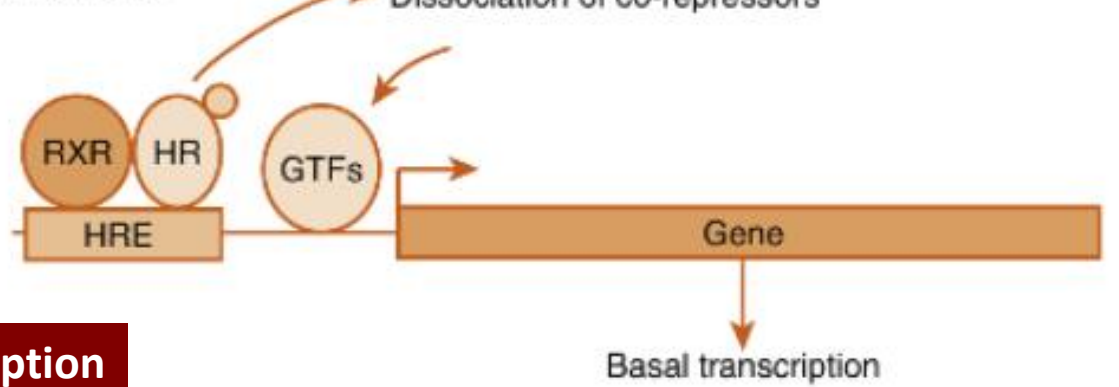
stimulated transcription

Pathway 2 (Thyroid hormones, vitamin D, PPARs)

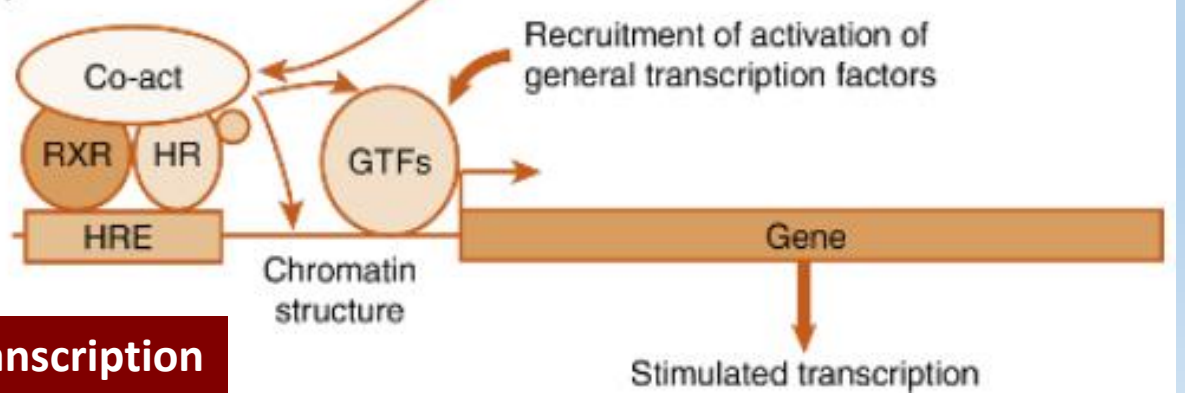
(-) Hormone



(+) Hormone



(+) Hormone



Termination of hormone action

Receptor-mediated endocytosis and subsequent lysosome degradation

Phosphorylation/ dephosphorylation of receptor or proteins of signaling pathway

Ubiquitination and proteosomal degradation

Binding of regulatory factor on corresponding protein (enzyme)

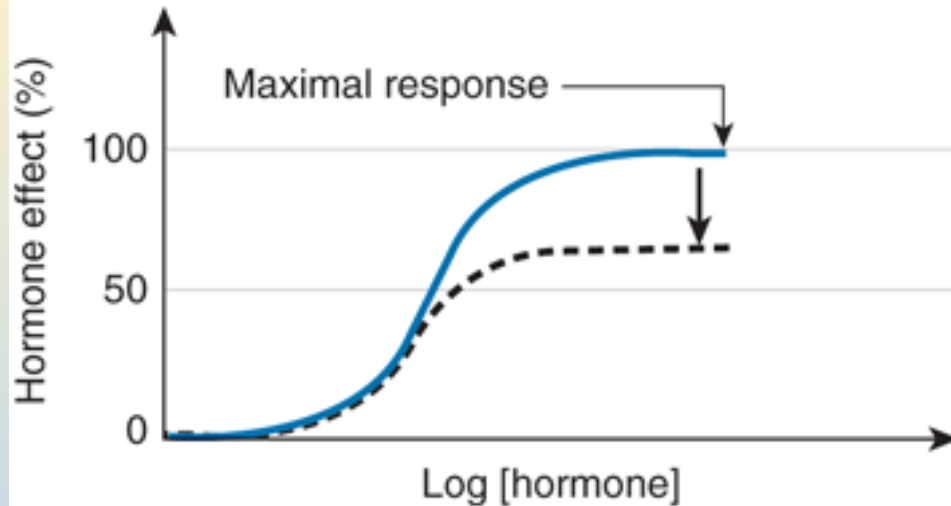
Inner enzymatic activity and its regulation

Clinical aspects

- Hormone overproduction
- Hormone underproduction
- Changes in sensitivity of target tissues and/or change in cell response
- Higher rate of inactivation or degradation of hormones
- Insufficient production or higher degradation of transport proteins
- Changes of transport hormones production during physiological conditions (pregnancy)

Clinical aspects

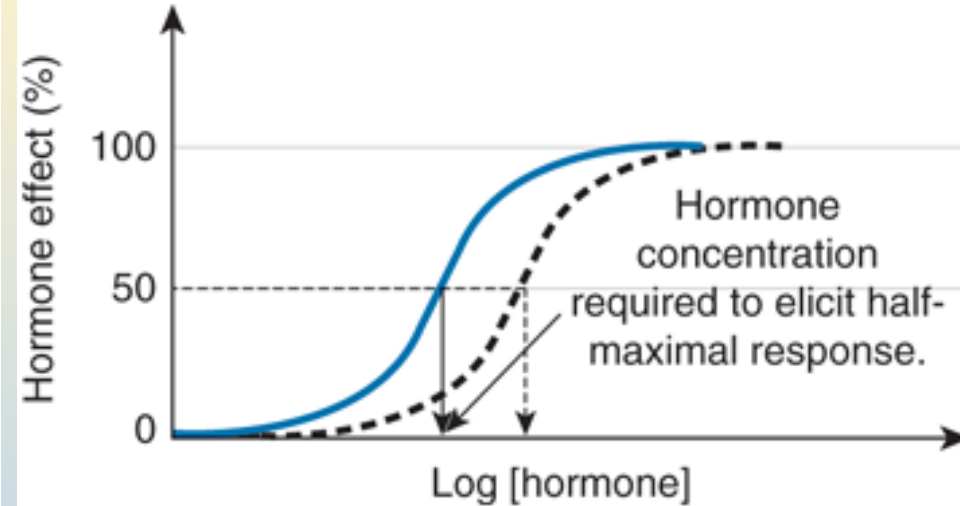
A. Decreased hormone responsiveness



Source: Molina PE: *Endocrine Physiology*, 4th Edition: www.accessmedicine.com
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- Decreased number of receptors
- Decreased concentration of hormone-activating enzyme(s)
- Increased concentration of non-competitive inhibitor
- Decreased number of target cells

B. Decreased hormone sensitivity



Source: Molina PE: *Endocrine Physiology*, 4th Edition: www.accessmedicine.com
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- Decreased affinity of hormone to receptor
- Decreased number of receptors
- Increased rate of hormone degradation
- Increased concentration of antagonists/competitive inhibitors

Determination of hormone levels in blood

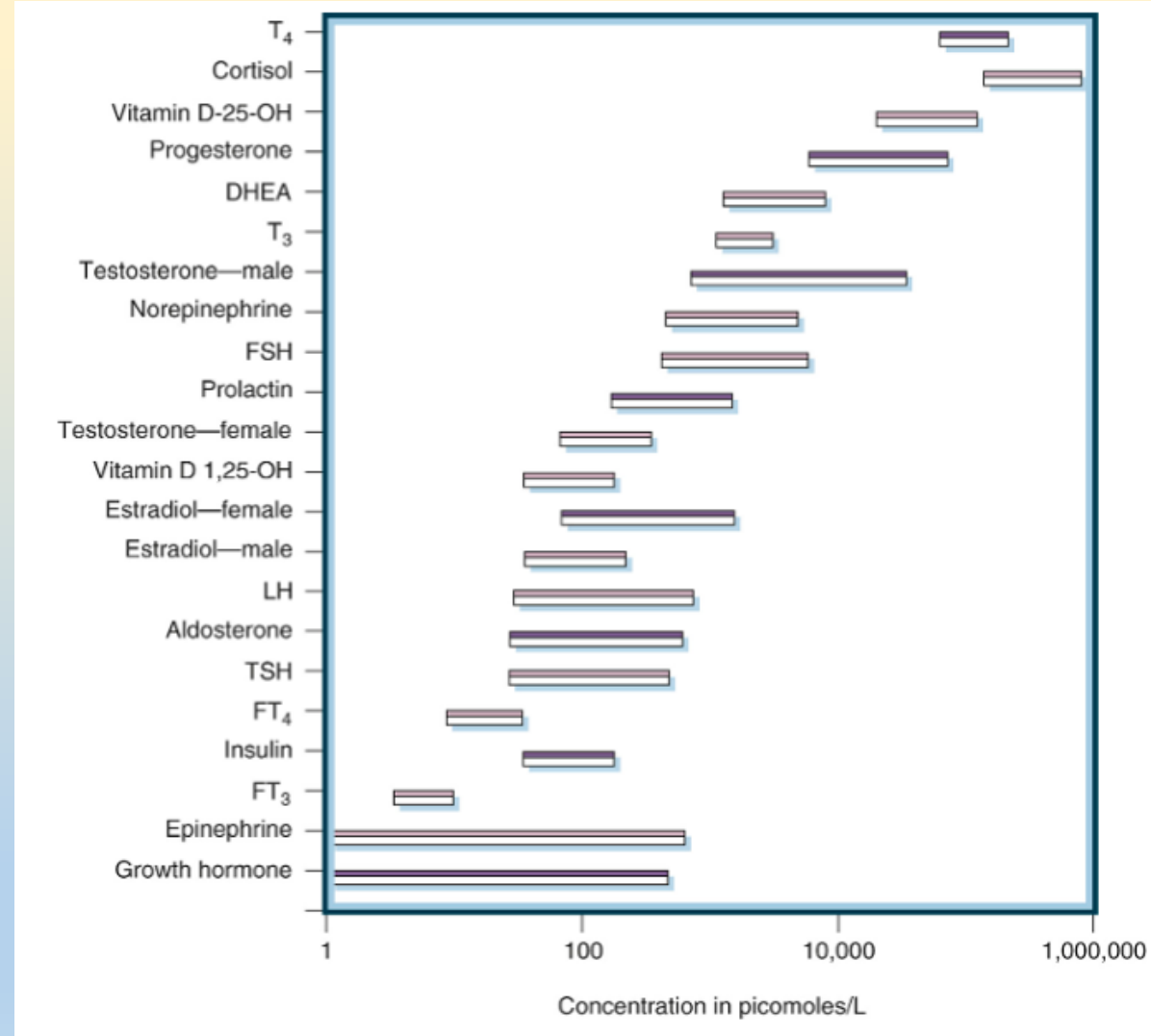
- HIGH SENSITIVITY DEMANDS
- WIDE CONCENTRATION RANGE

- Antigen-antibody interaction-based methods
- Antibody requirements (poly- X monoclonal)
- Monoclonal antibodies = specific epitopes
- Radioactive labeled antibodies
- Necessity of quantification!
- RIA, ELISA

Methods based on HPLC-MS

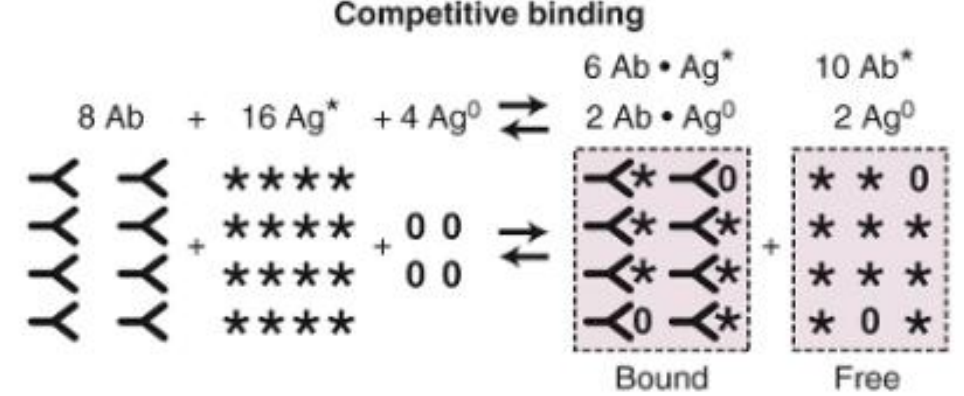
- Nucleic acid-based methods
- hybridization techniques
- restriction fragmentation, electrophoresis, sequencing

- Separation techniques – free X bound hormones
- dialysis



EXTREMELY LOW LEVELS OF HORMONES IN BLOOD

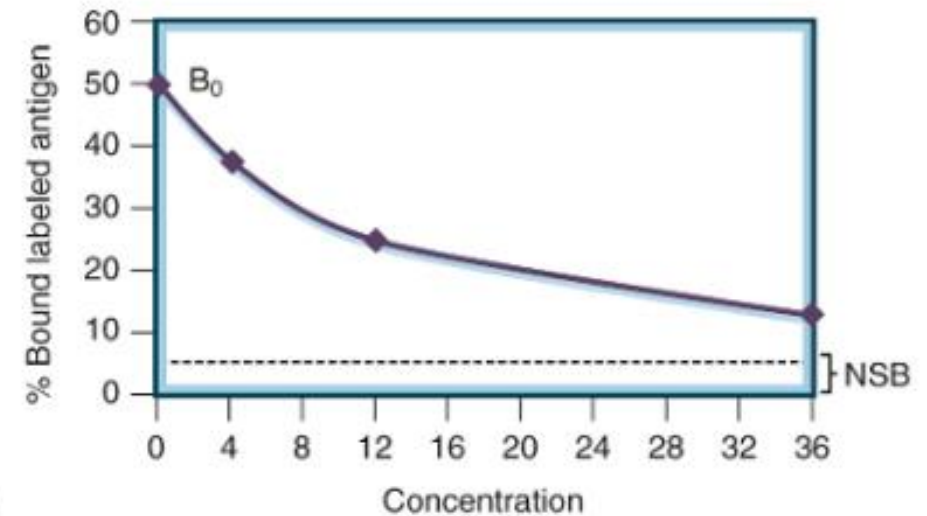
RIA = radioimmunoassay



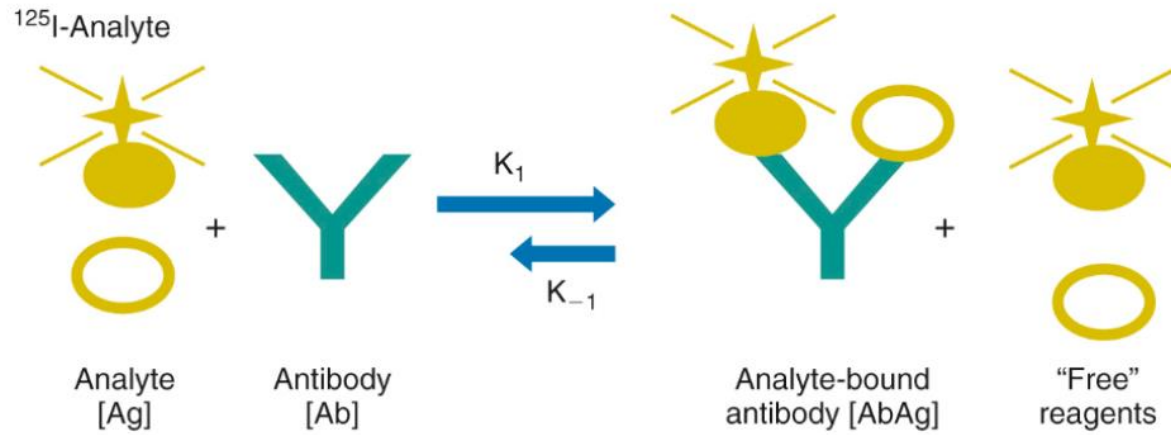
Calibration of standards

Ab	Ag*	Ag ⁰	Ab · Ag*	Ab · Ag ⁰	Ag* + Ag ⁰
8	16	0	8	0	8 0
8	16	4	6	2	10 2
8	16	12	4	4	12 8
8	16	36	2	6	14 30
Constant	Variable		Bound		Free

A



$$\text{Antibody affinity} = K_1/K_{-1} = [\text{AbAg}]/[\text{Ab}][\text{Ag}]$$



"Competition" for
Ab binding sites

Separate to measure
analyte-bound antibody

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HPLC-MS

