General principles of endocrine functions

Integration systems of the organism

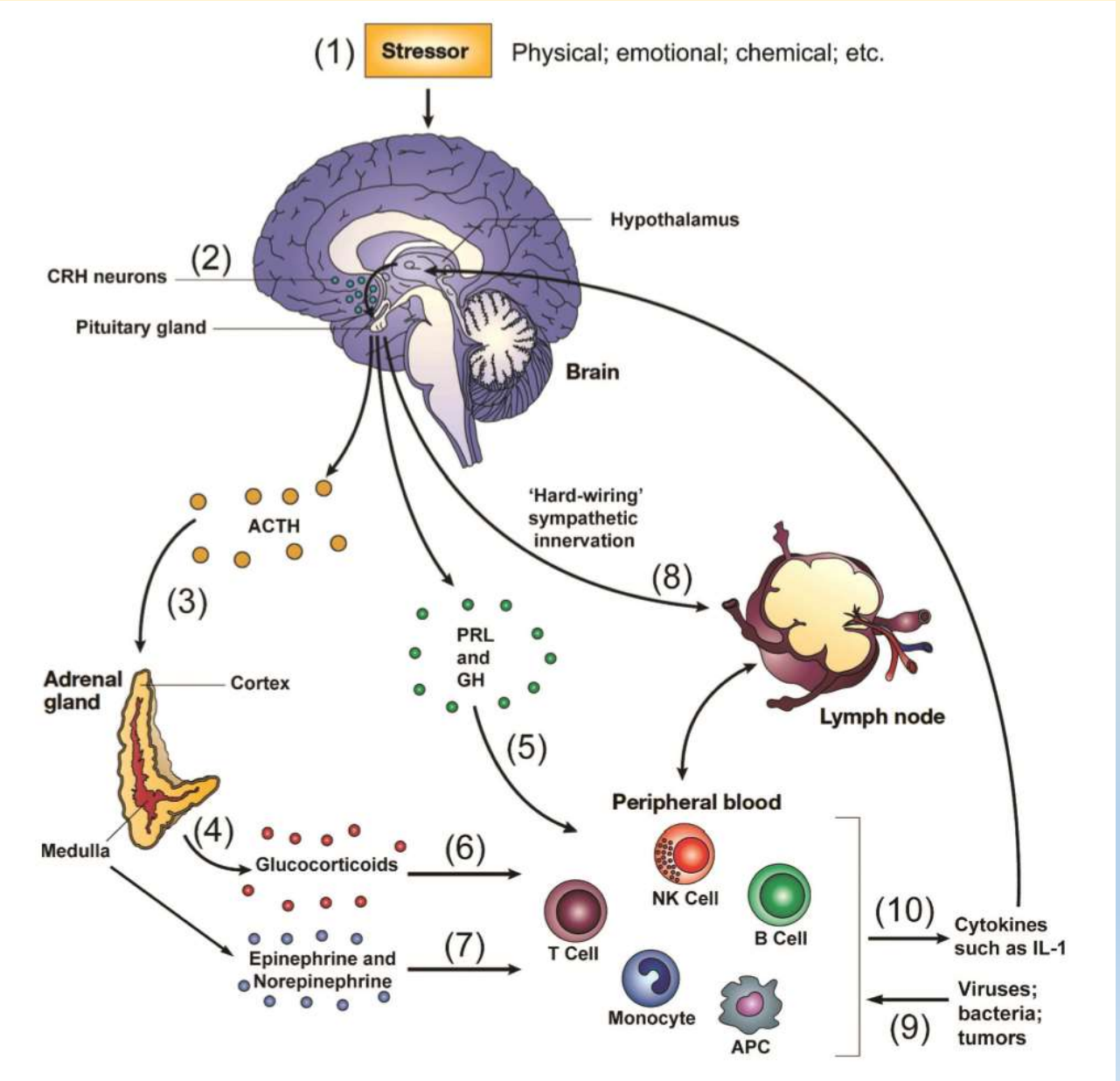
- **Hormonal system**
- **Nervous system**
- ***Immune system***

Hormones

Neurohormones

Neurotransmitters

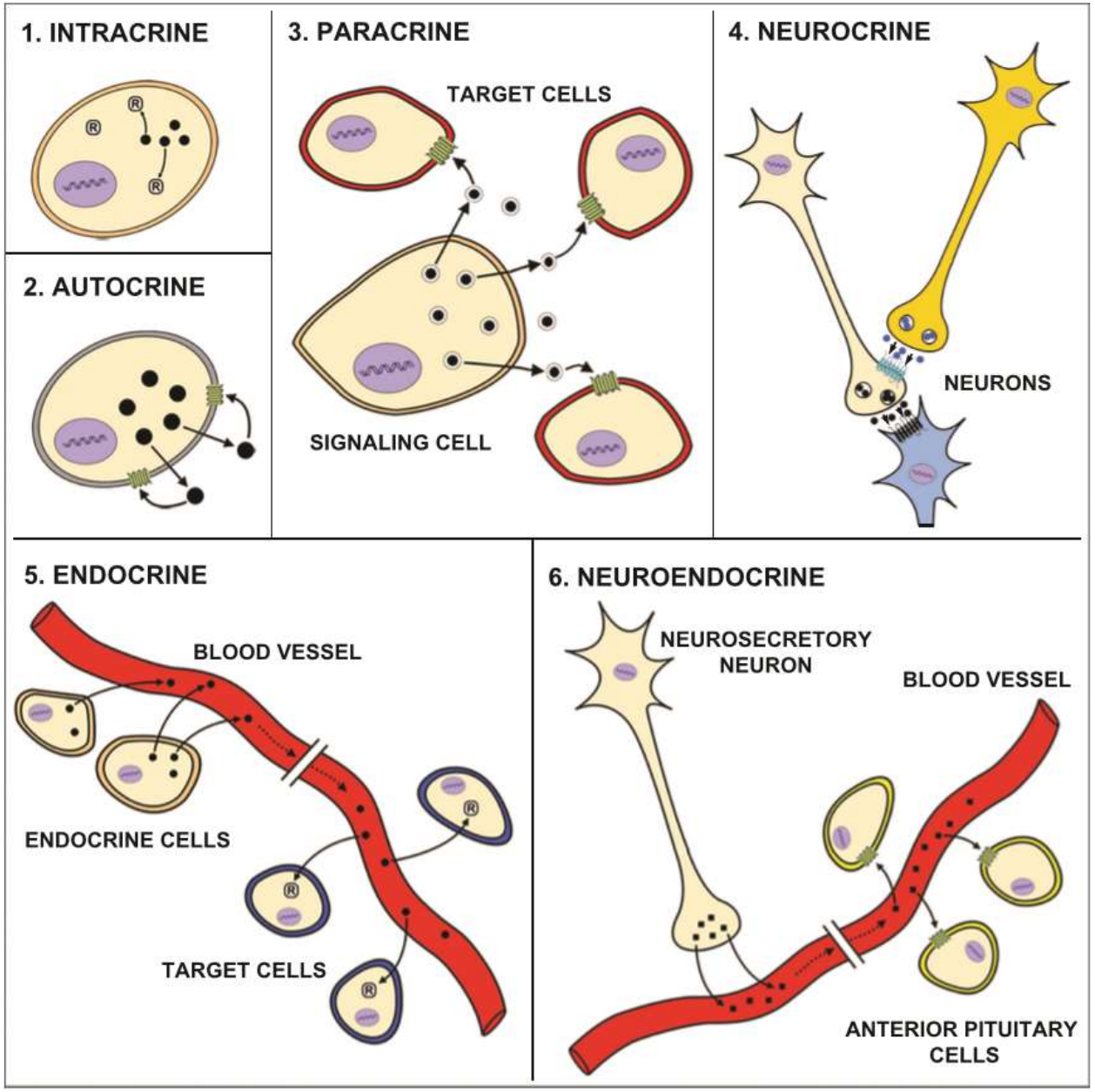
Paracrine (autocrine) effectors

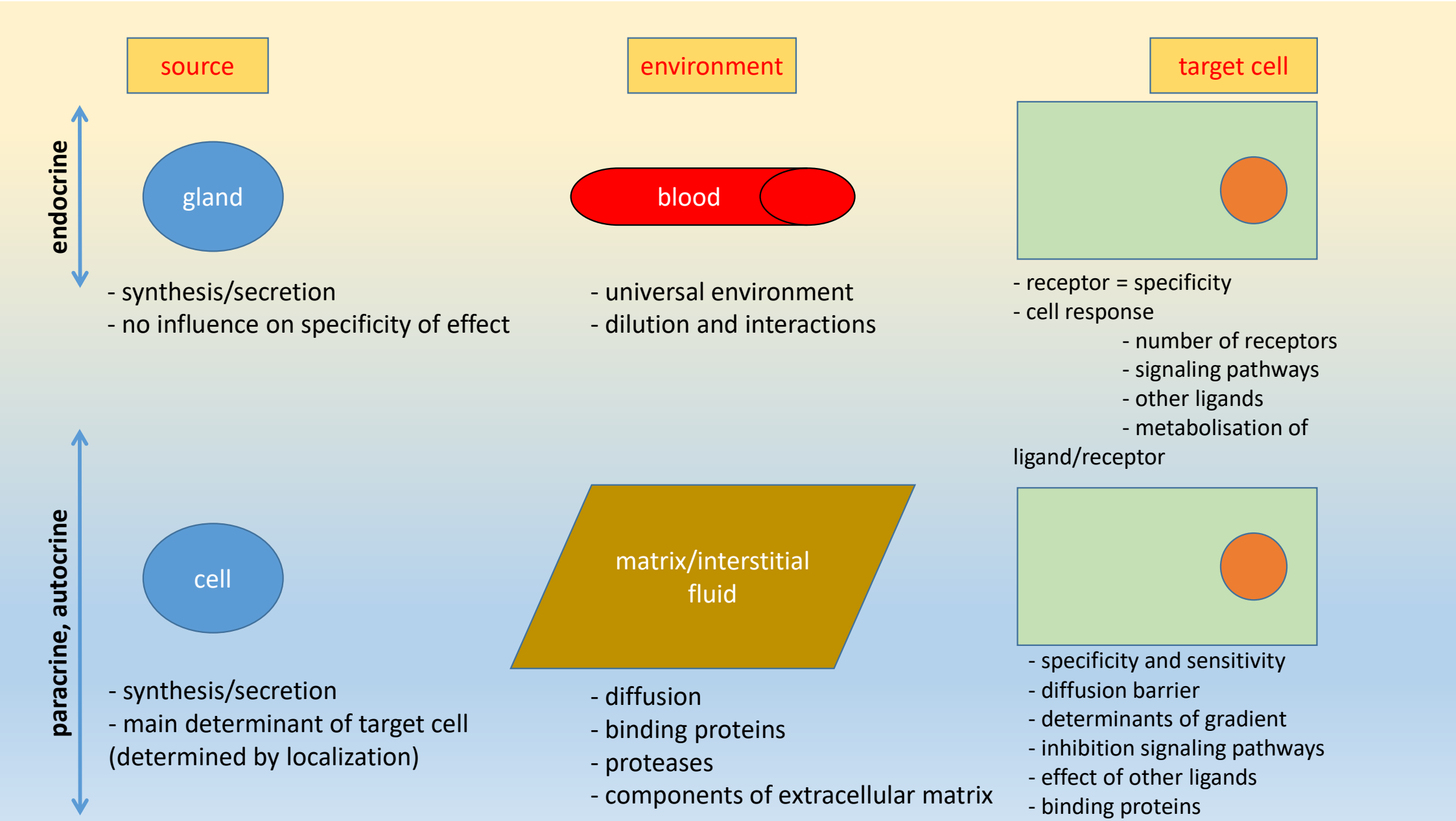


How do cells communicate?

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

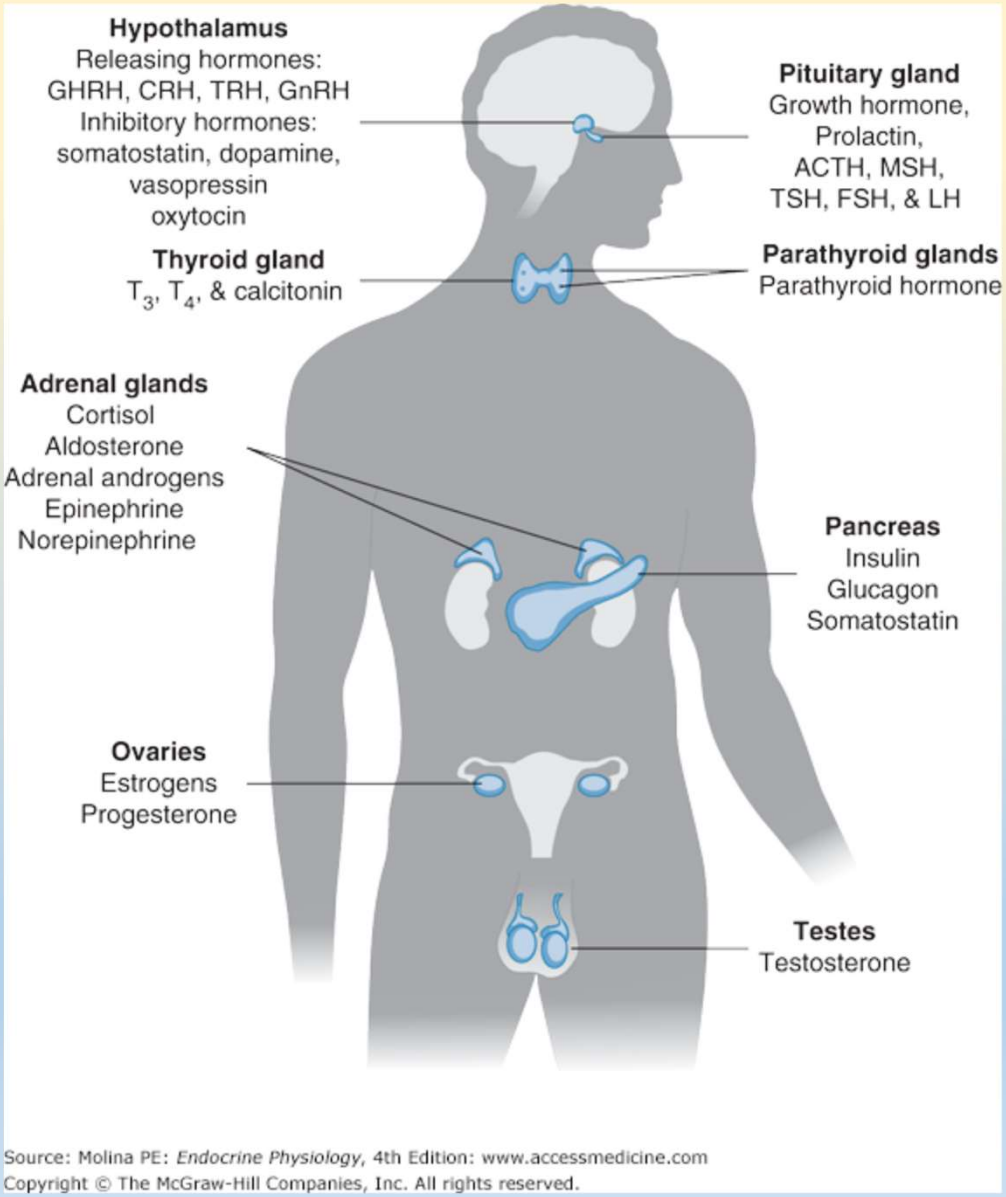
source → environment → target cell




source
environment
target cell
endocrine
gland
blood
- synthesis/secretion
- no influence on specificity of effect
- universal environment
- dilution and interactions
- receptor = specificity
- cell response
- number of receptors
- signaling pathways
- other ligands
- metabolisation of ligand/receptor
paracrine, autocrine
cell
matrix/interstitial fluid
- 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

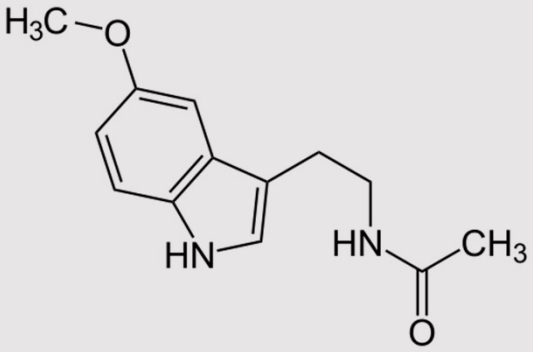
Hormones

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

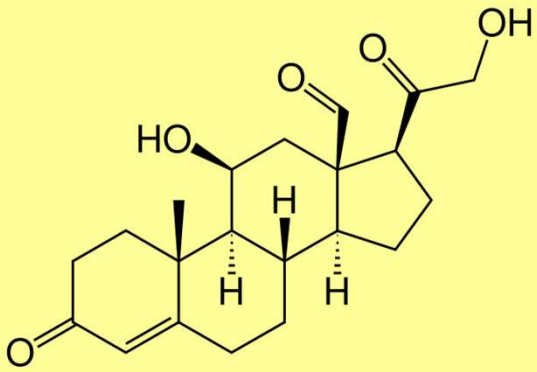


Chemical nature of hormones

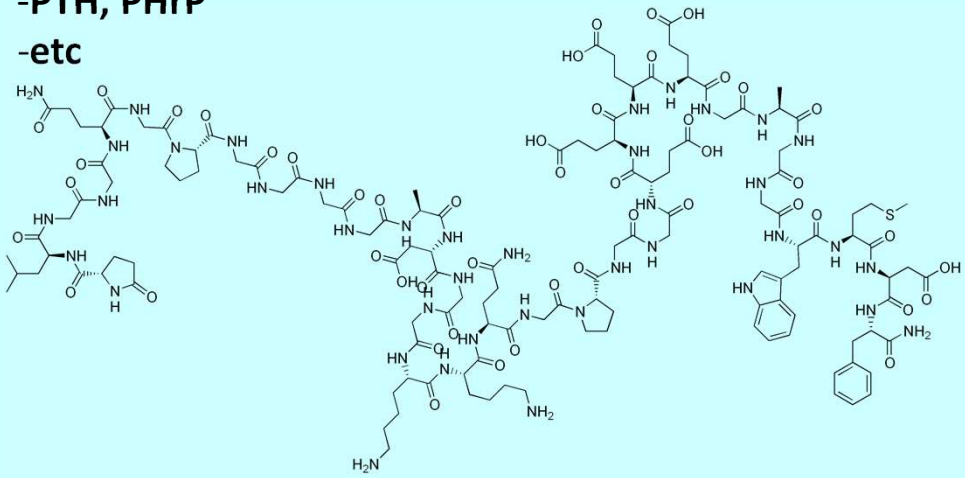
DERIVED FROM AMINOACIDS
- -Adrenaline
- -Noradrenaline
- -Dopamine
- -Melatonine
- -T3/T4



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



PEPTIDES AND PROTEINS
- -Hypothalamic hormones
- -Adenohypophyseal hormones
- -Insulin, glucagon, somatostatin
- -Gastrin, cholecystokinin, secretin
- -Natriuretic peptides
- -Erythropoietin, thrombopoietin
- -PTH, PHrP
- -etc



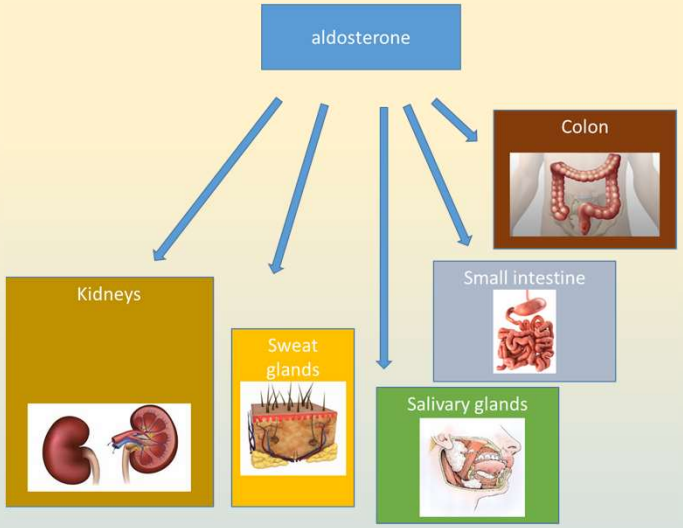
Chemical nature of hormones

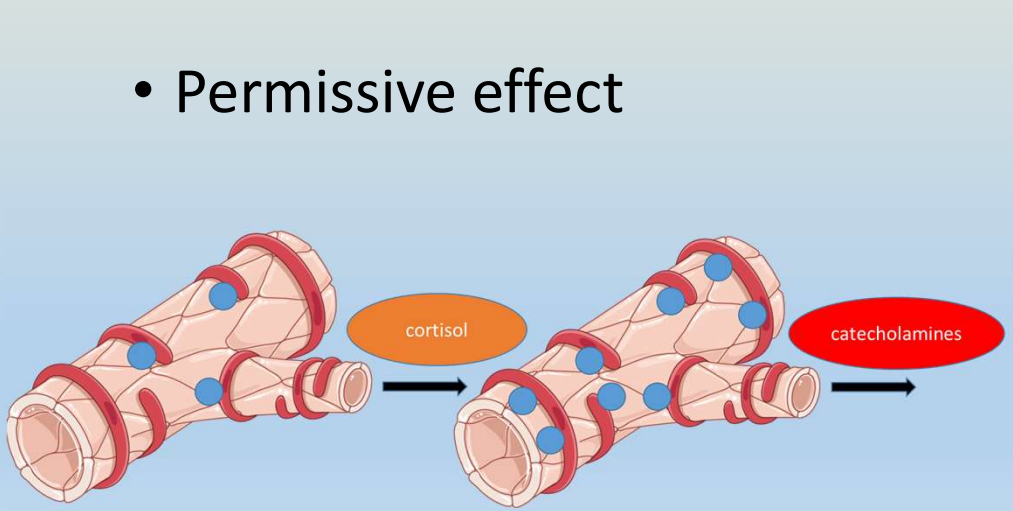
Hormone – characteristics	Peptides – proteins	Catecholamines	Steroid hormones	Thyroid hormones
Ph-CH properties	hydrophilic	hydrophilic	lipophilic	lipophilic
synthesis	proteosynthesis	Tyr modification	CH precursors	Tyr modifications
storage	secretory granules	secretory granules	not present	colloid
secretion	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

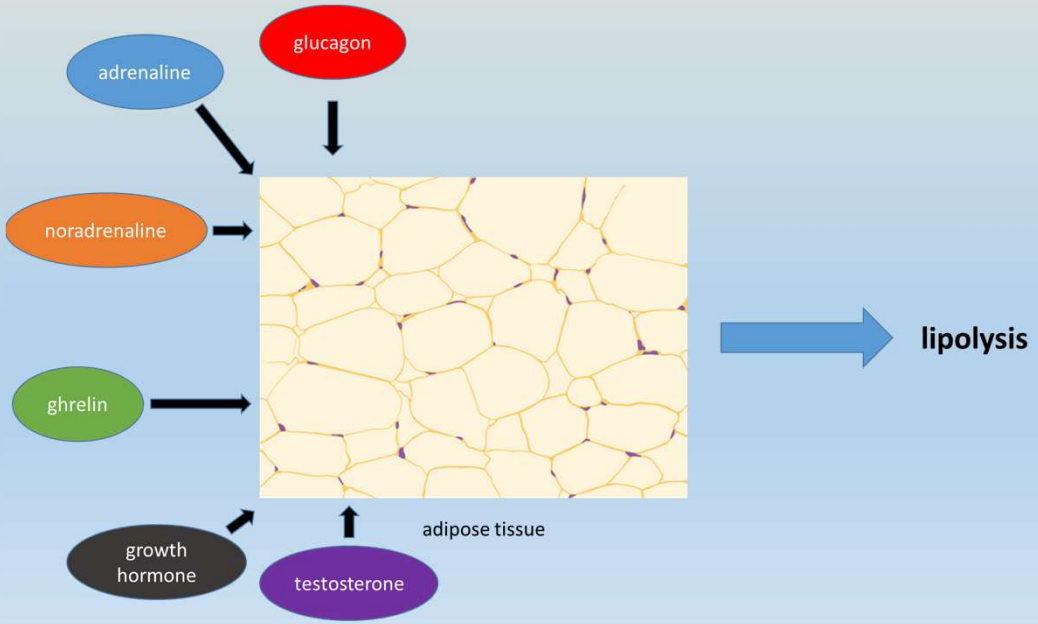
CHEMICAL STRUCTURE OF HORMONES DETERMINES THEIR BIOSYNTHESIS, STORAGE, RELEASE, TRANSPORTATION, ELIMINATION HALF-LIFE, WAY OF ELIMINATION AND THE MECHANISM OF EFFECT ON TARGET CELLS

Hormones

- Pleiotropic effects
- Multiplicity
- Permissive effect

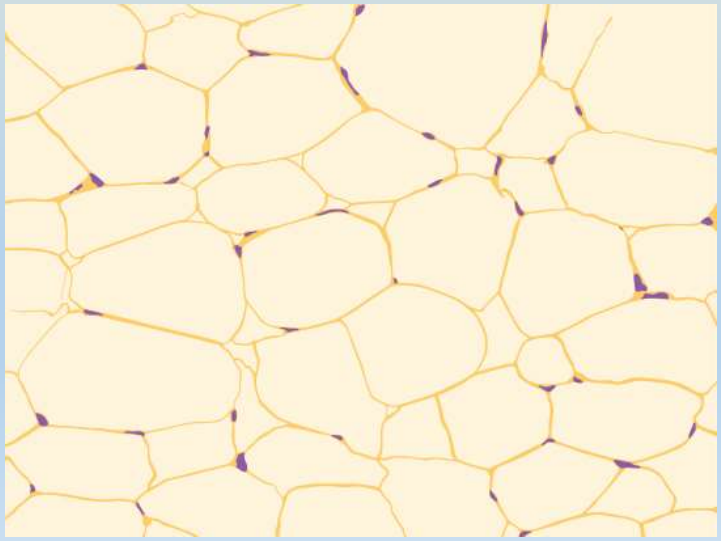




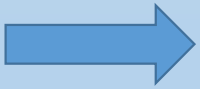


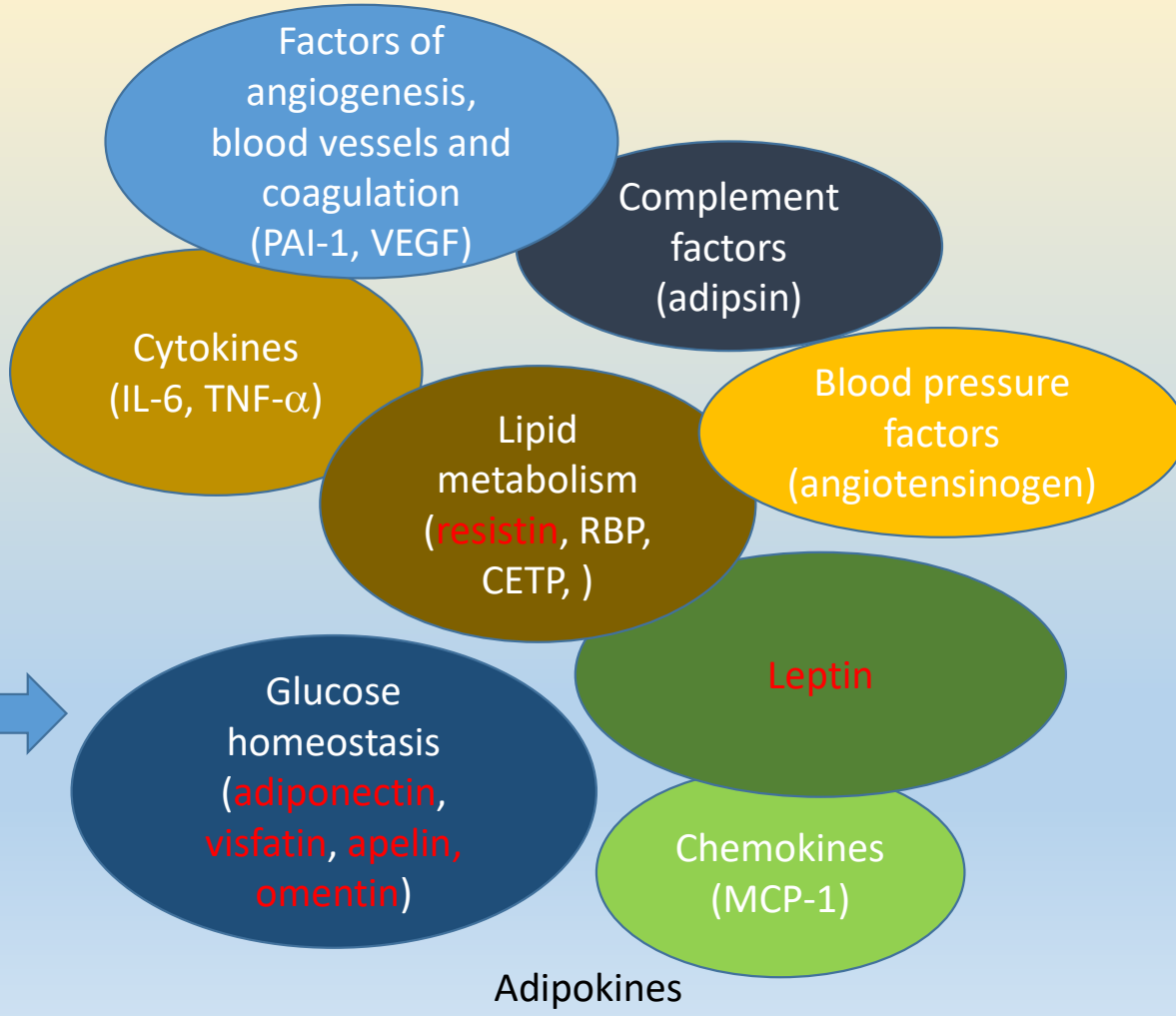
Endocrine organs

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



adipose tissue





Adipokines

Clinical aspects

- Production of hormones by tumors – PARANEOPLASTIC SYNDROMES

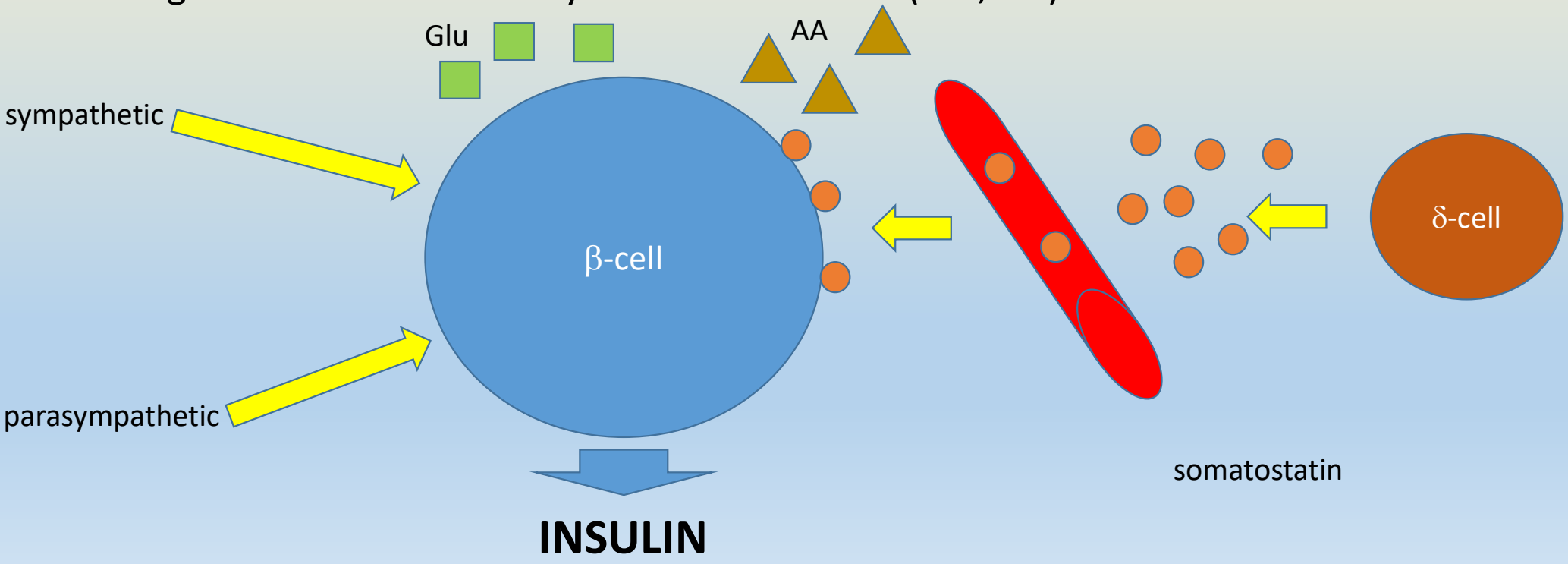
Lung tumors
– ADH (hyponatremia)
– ACTH (Cushing syndrome)
– PTHrP (hypercalcaemia)

Liver and kidney tumors
– erythropoietin (polycythemia)

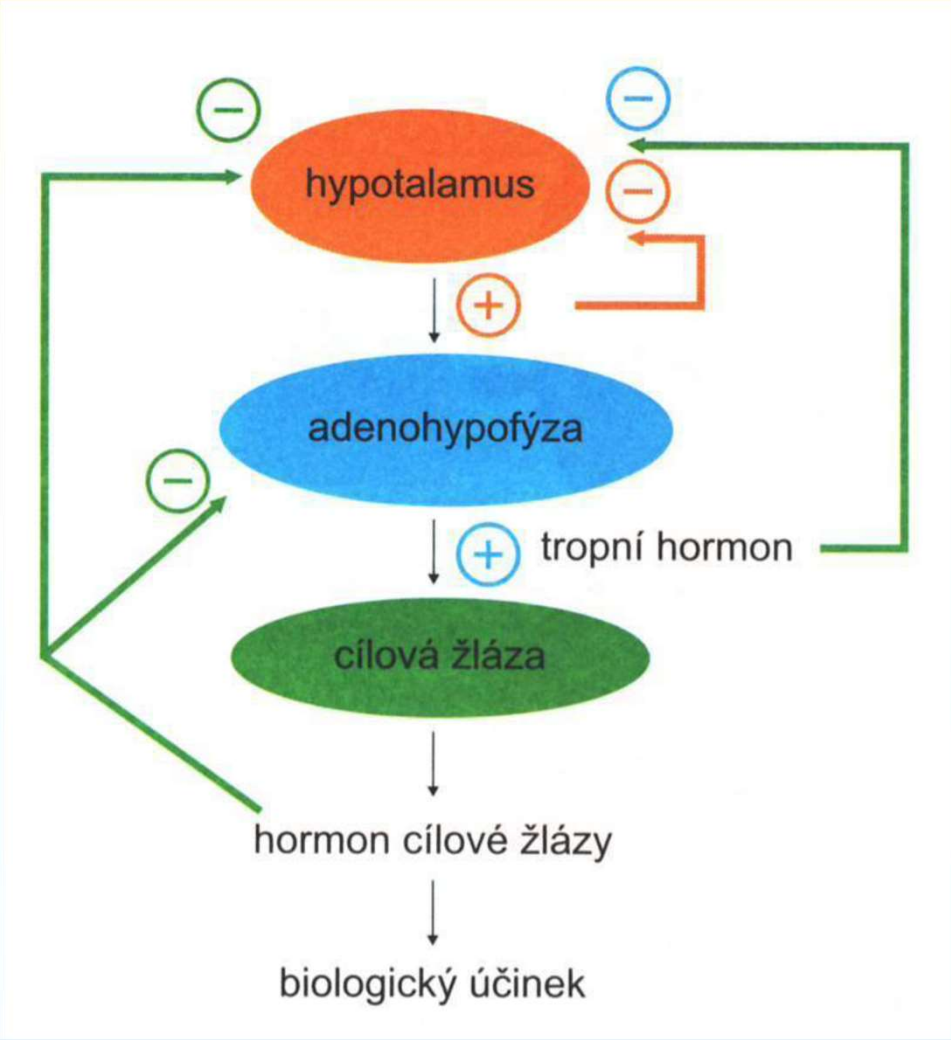
GIT tumors
– ACTH (Cushing syndrome)

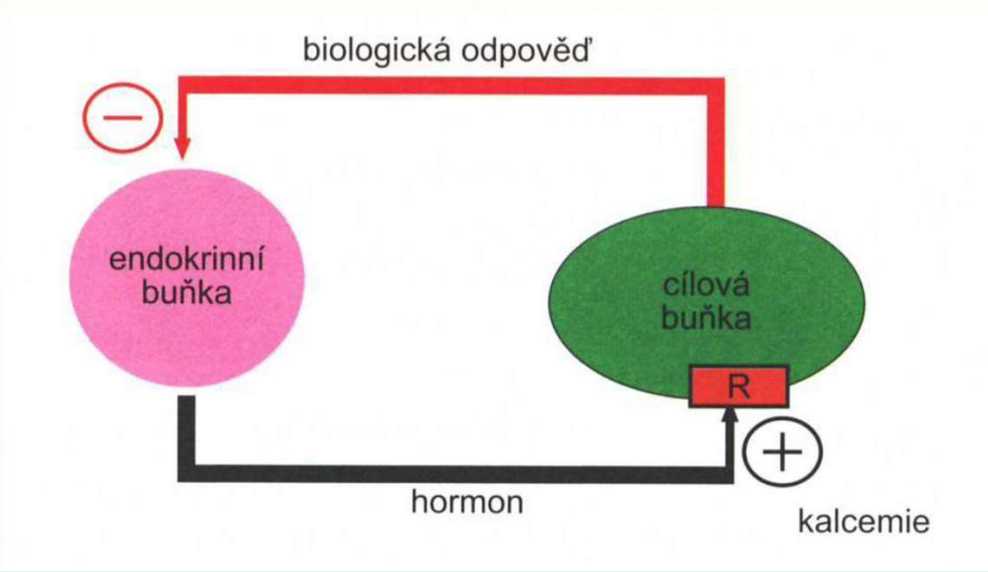
Secretion of hormones and its regulation

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



Hormone secretion is controlled by feedback system





Feedback

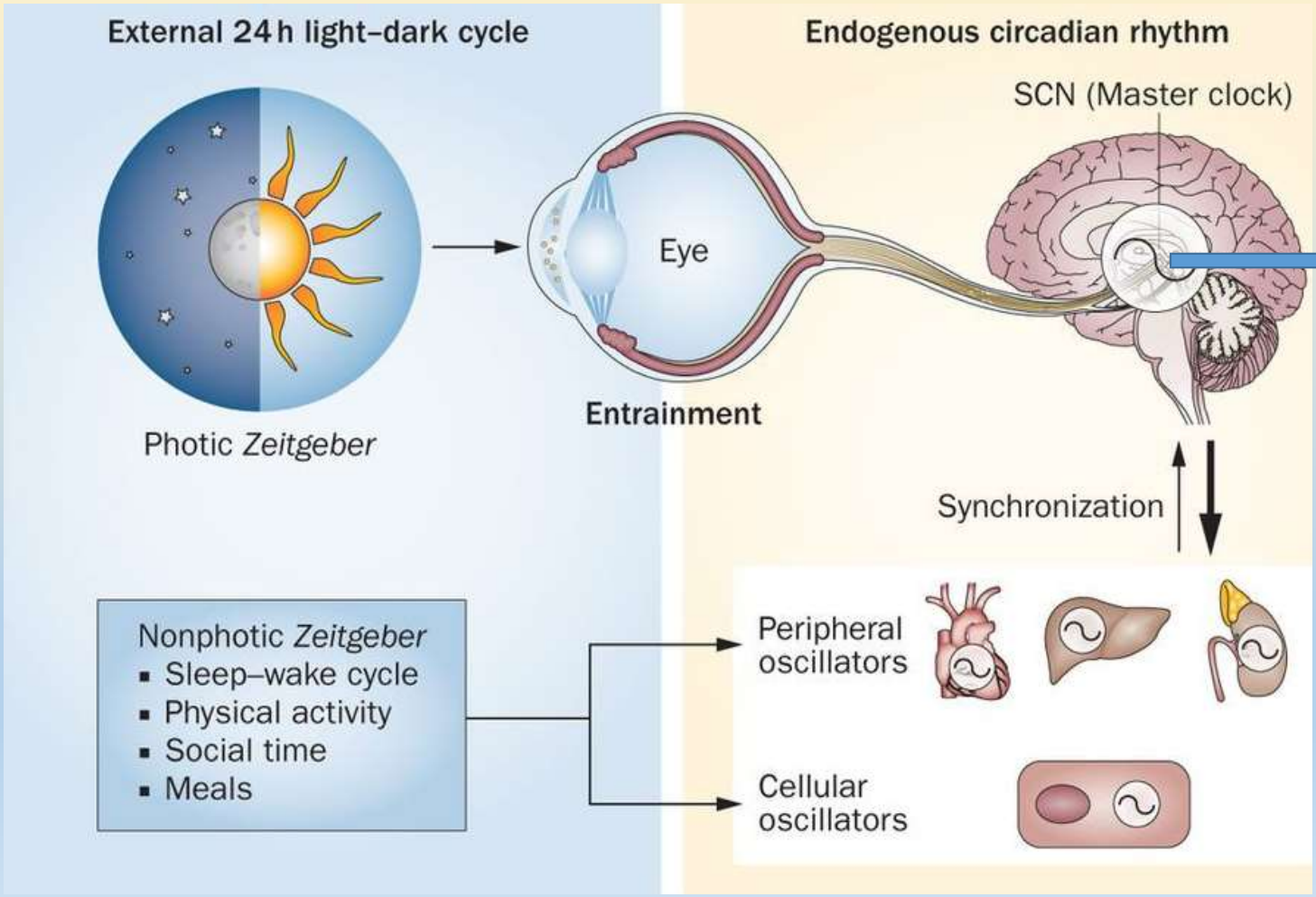
negative X positive

simple X complex

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

Cyclic changes in hormone secretion

SCN:
- Afferent – retina
- Efferent – hypothalamic nucleus



- Melatonin
- GHRH/GHIH
- ADH
- ACTH – cortisol
- Insulin
- Ghrelin
- Adiponectin
- Leptin

Neuronal/hormonal = SNC-dependent

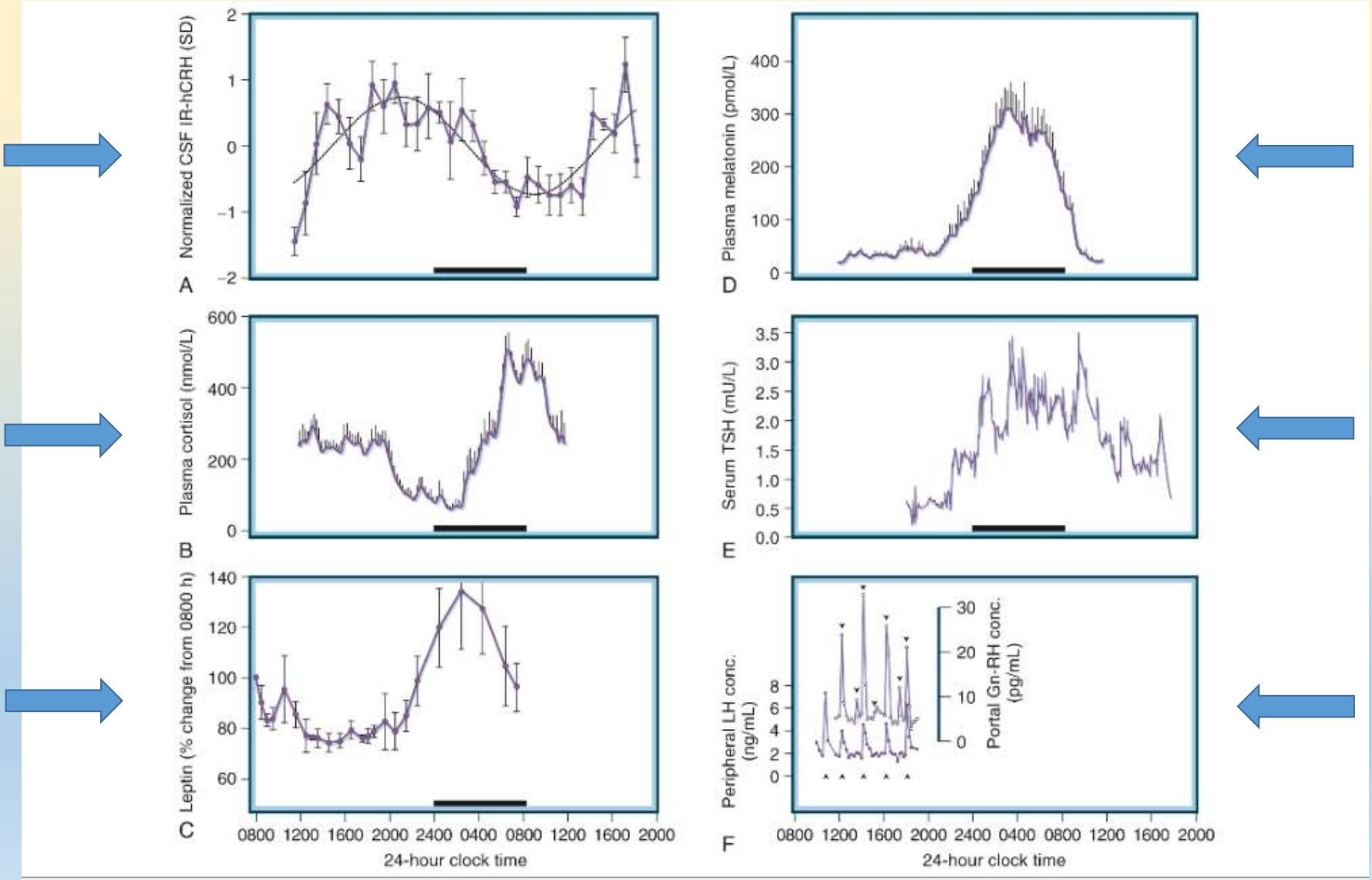
Satiety/fasting

Body temperature

CRH

cortisol

leptin



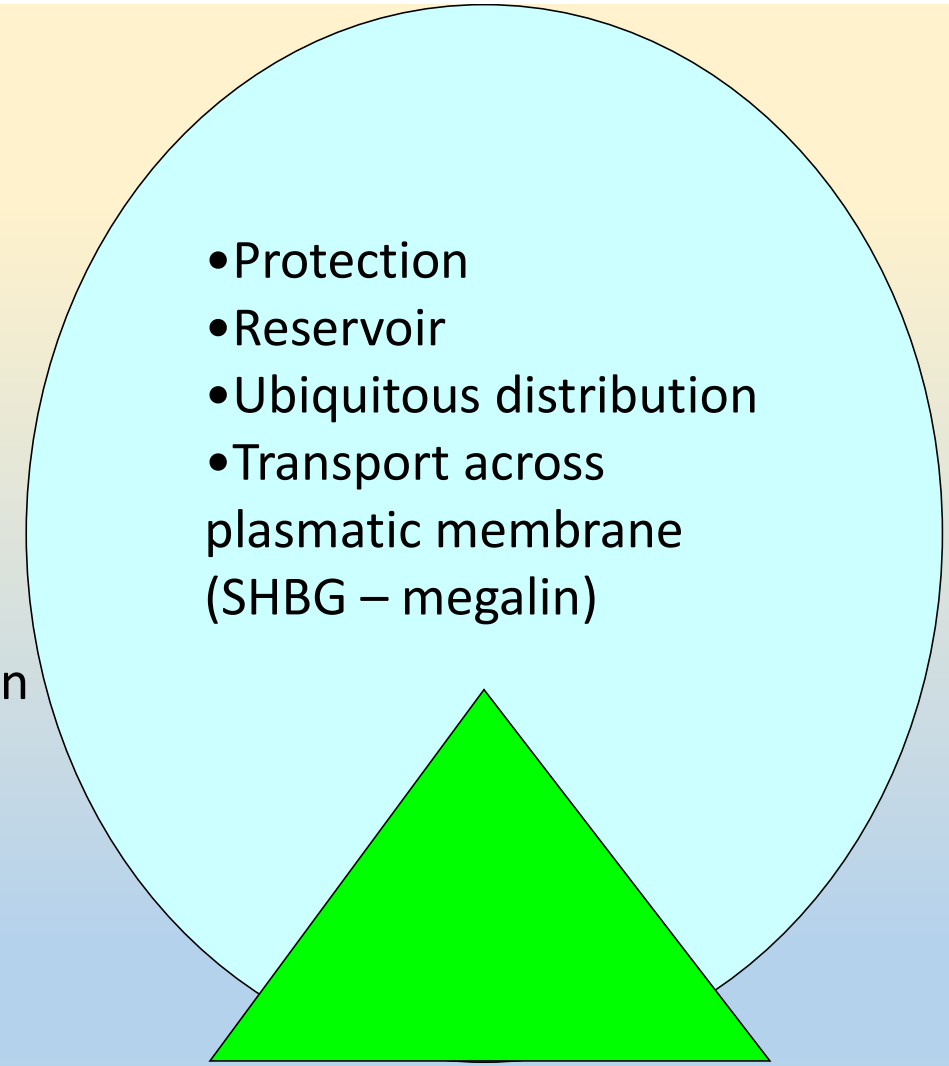
melatonin

thyrotropin

LH

Hormone transport

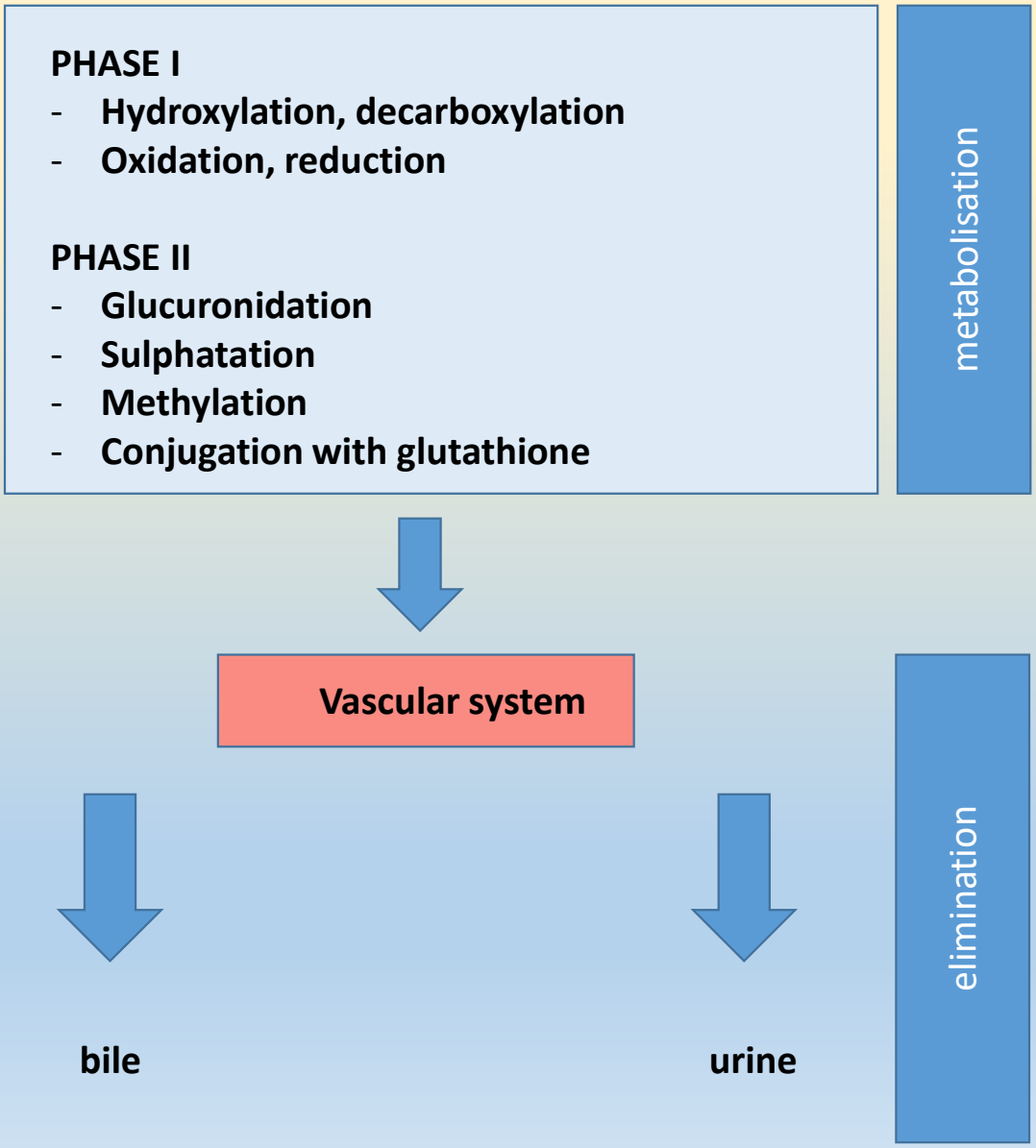
- Chemical properties of hormone
- Transport protein(s) bond and its significance
  - Albumin
  - Globulins
  - Specific proteins – TBG, SHBG, CBG
- Bond strength
- „Alternative“ binding – TBG versus transthyretin



DYNAMIC BALANCE BETWEEN HORMONE AND TRANSPORT PROTEIN

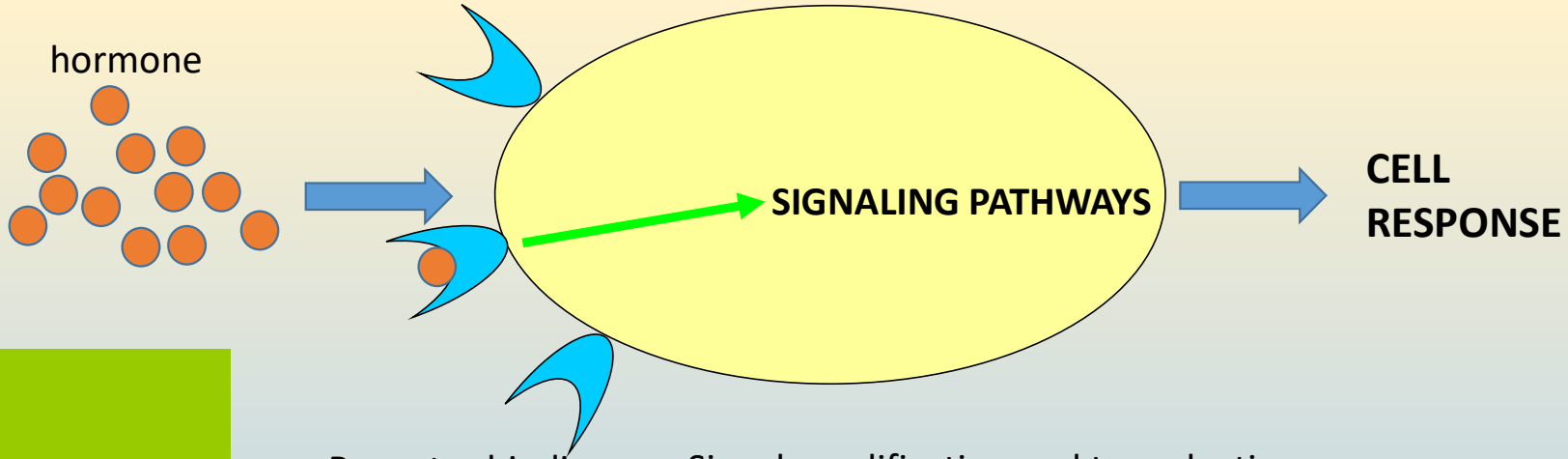
Hormone elimination

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



Hormones and cell response

- Target cells
- Specificity
- High affinity
- Selectivity



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

Receptor binding

% of occupied receptors
conformation change

Signal amplification and transduction efector molecules

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

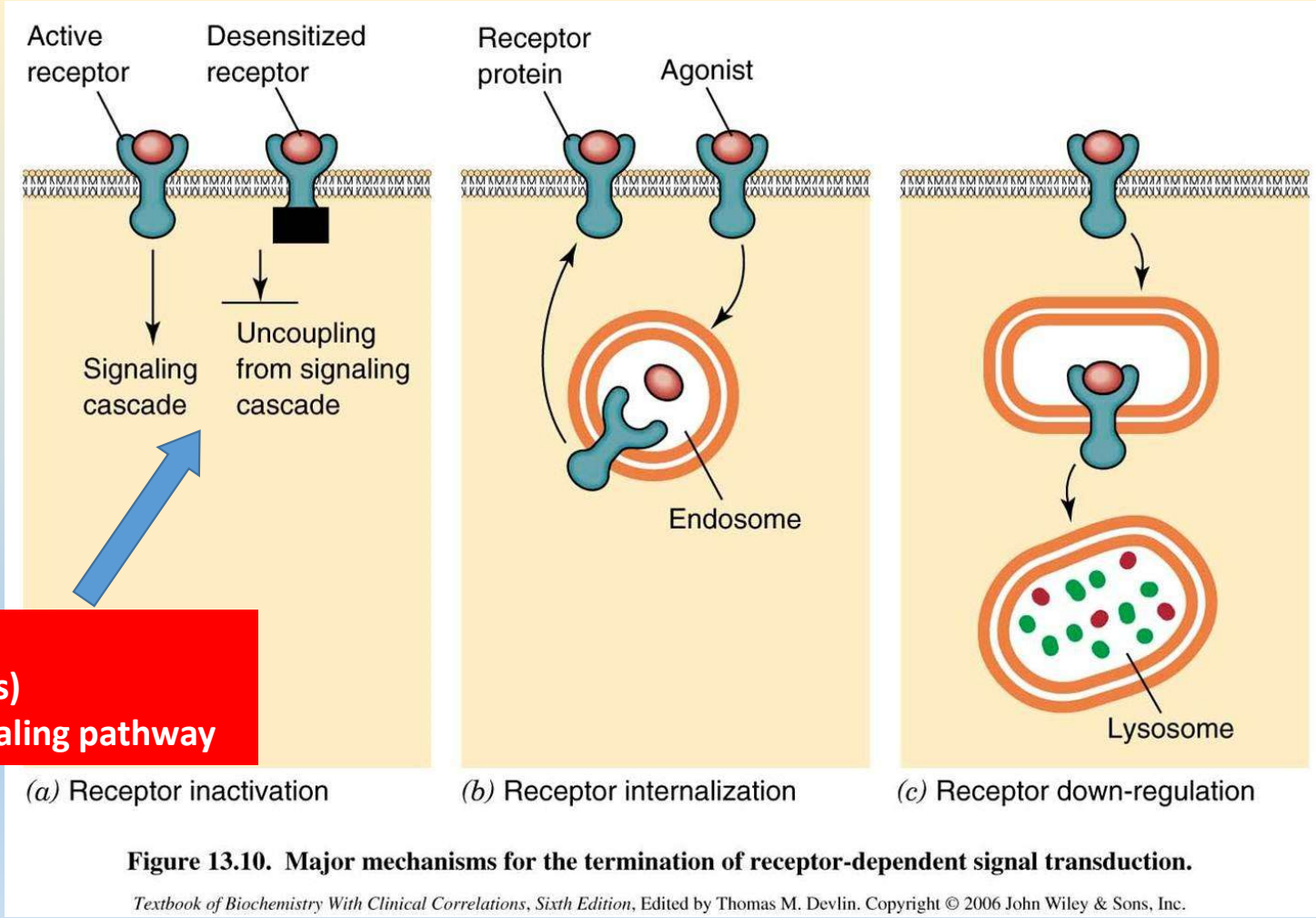
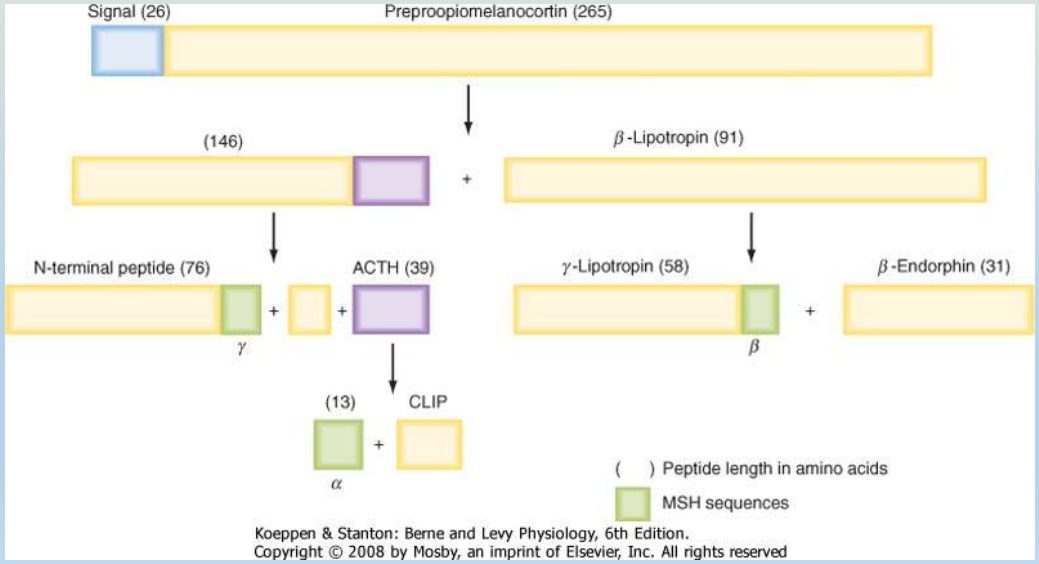


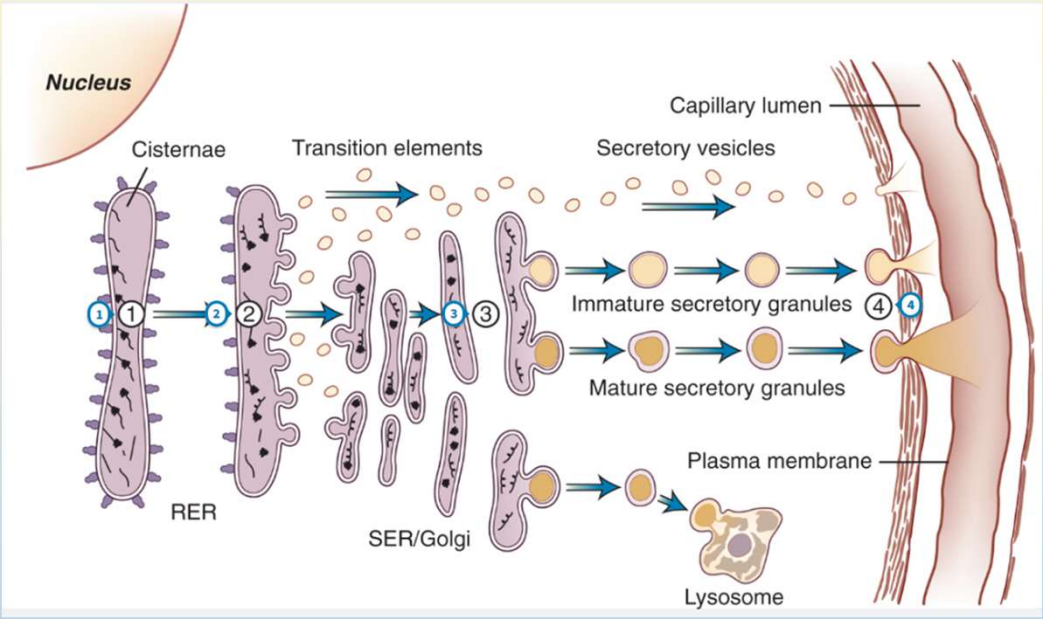
Figure 13.10. Major mechanisms for the termination of receptor-dependent signal transduction.

Hormones – proteins and peptids

Hormones

Paracrine/autocrine peptides





preprohormone – prohormone – hormone (+ fragments)

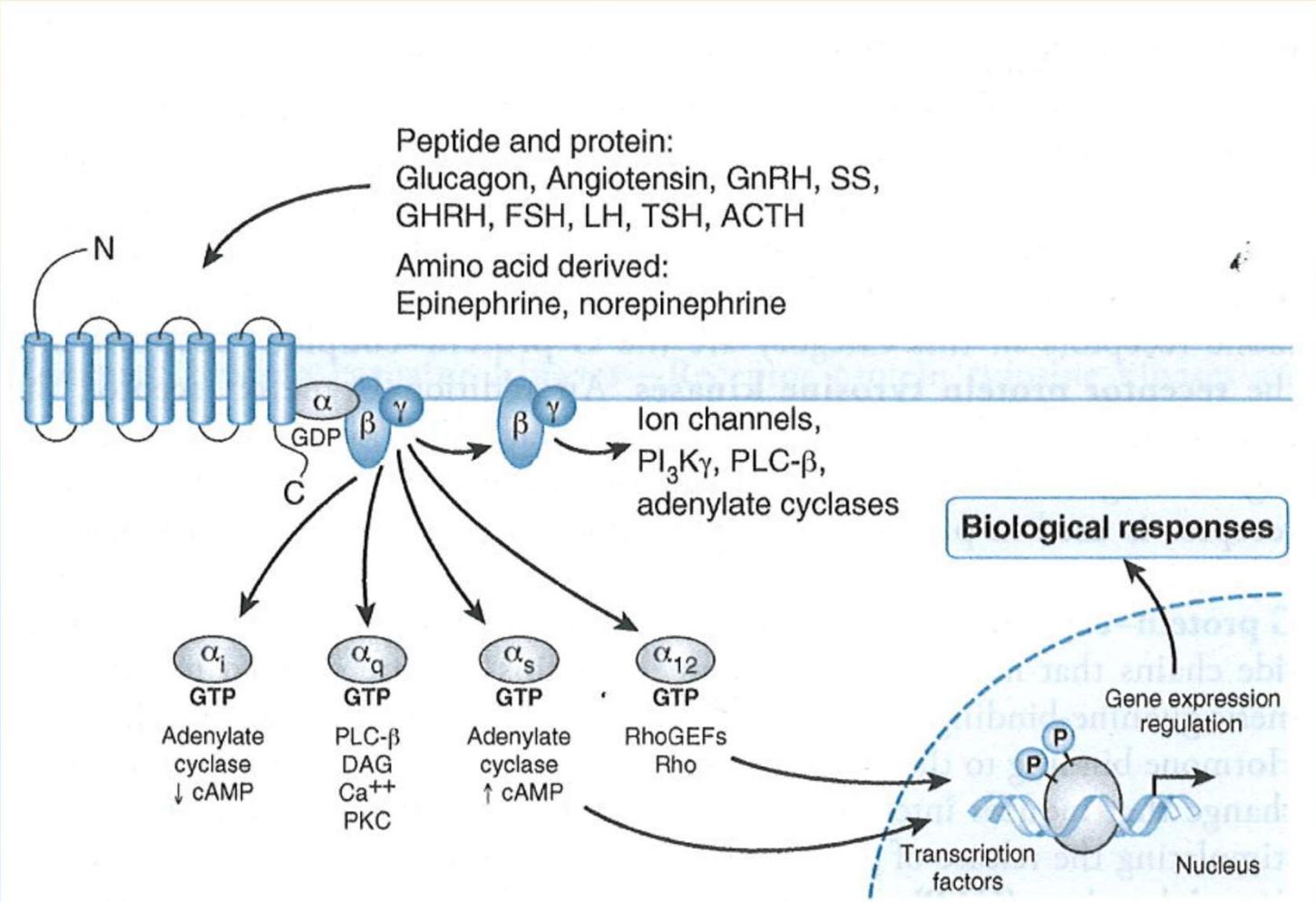
G protein-coupled receptors (GPCR)

G_s – G_s, G_{olf} – activation of AC

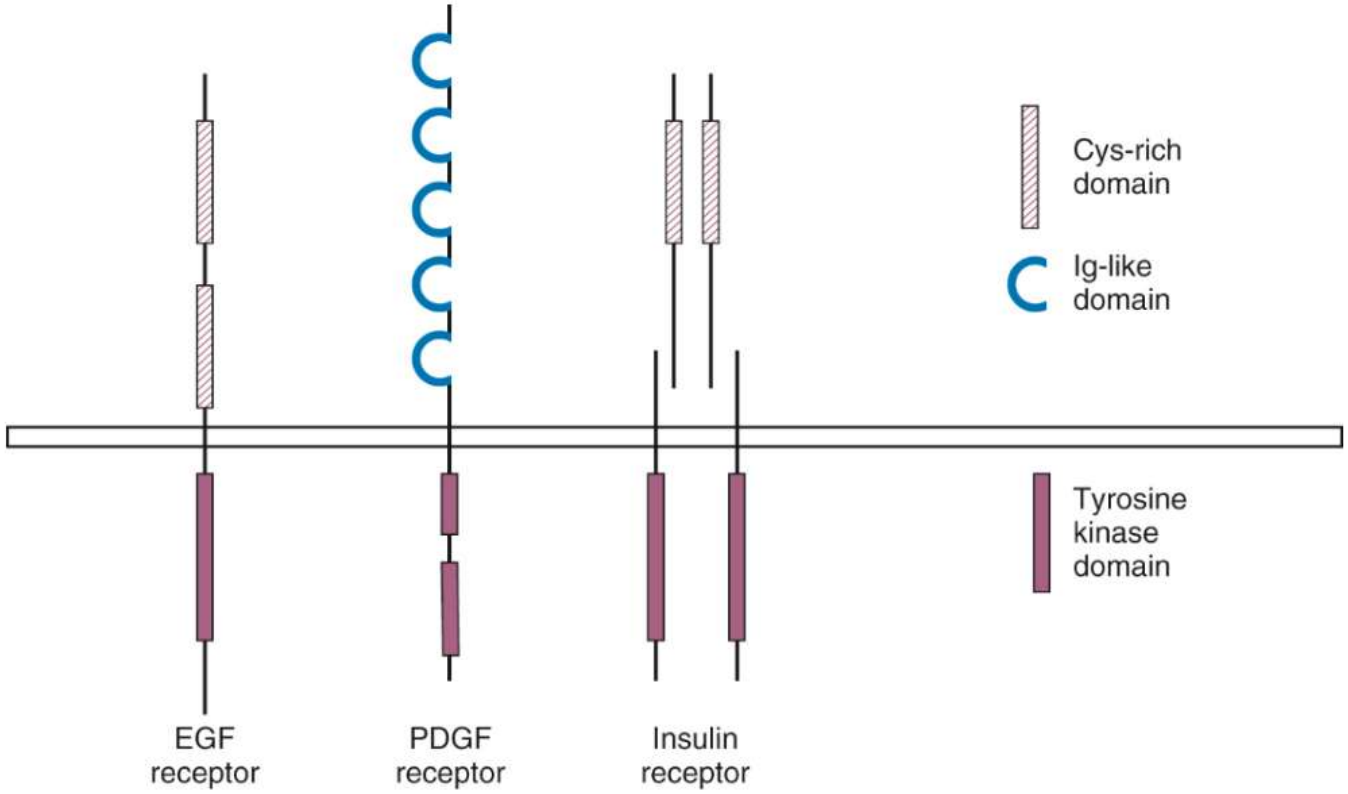
G_i – inhibition of AC
- G_0 (2, brain)
- G_t (2, photorec. – cAMP-PDE)
- G_z (inhibition of K^+ channels)

$G_{q/11}$ – activation of PLCβ

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



Receptor tyrosinkinases

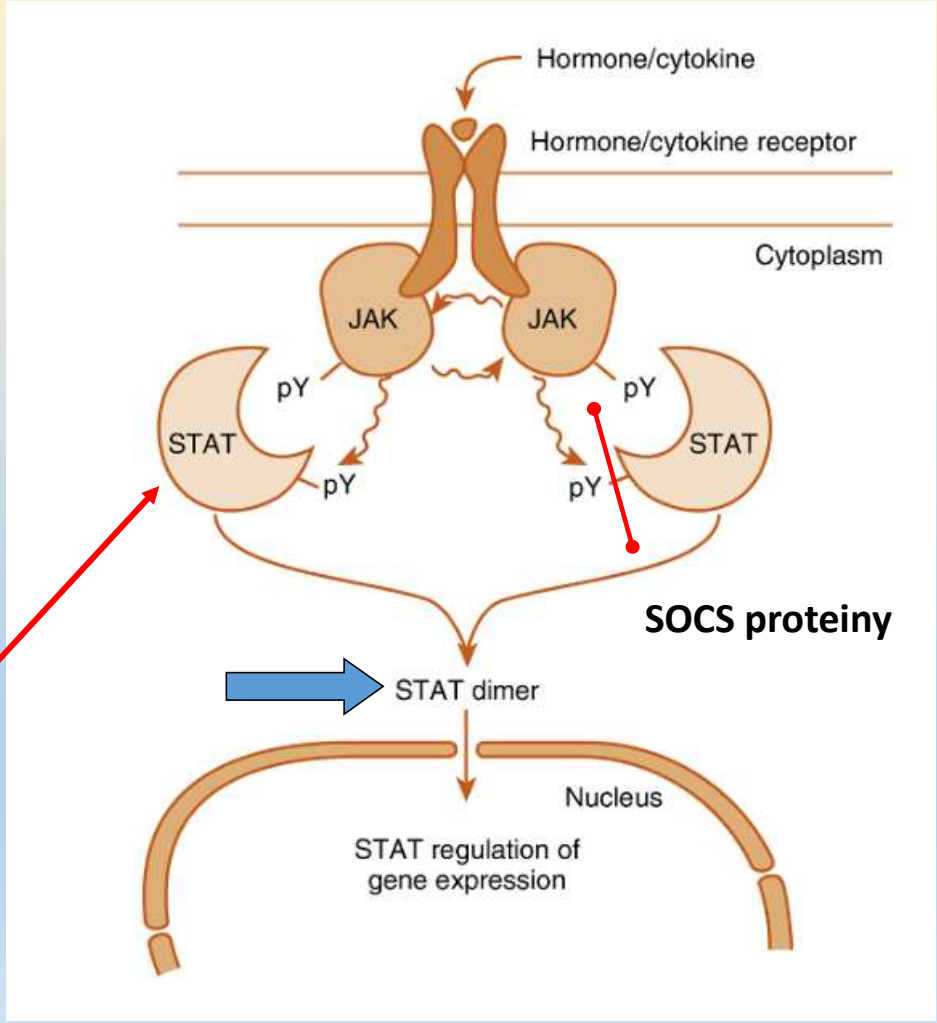


- 58 RTKs/20 subfamilies
- Usually dimerisation after ligand binding
- ATP as a source of P for phosphorylation of intracellular domains/associated proteins
- Insulin
- IGF-1/2

Receptors associated with cytosolic TK

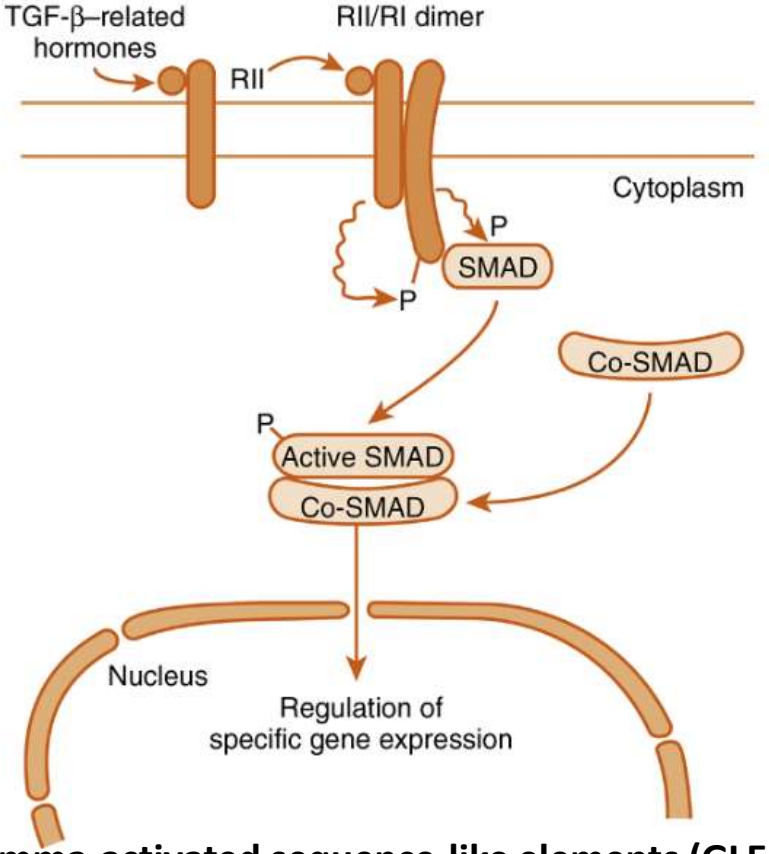
- GH, prolactin, leptin, erythropoietin
- Dimeric receptor **without** TK activity
- Association with JAK kinase
- After ligand binding – dimerisation, transphosphorylation, activation

signal transducers and activators of transcription



Receptor serin/threonin kinases

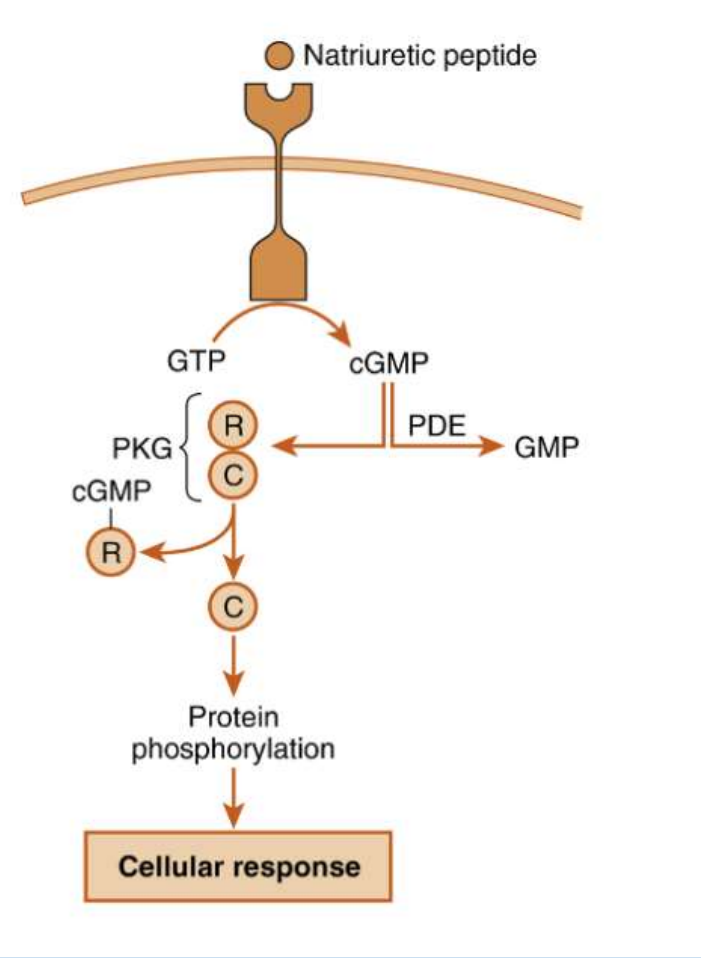
- Anti-Müllerian hormone, inhibitin
- Form of dissociated heterodimer
- SMAD = „latent transcription factors“



gamma-activated sequence-like elements (GLEs, promotor 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

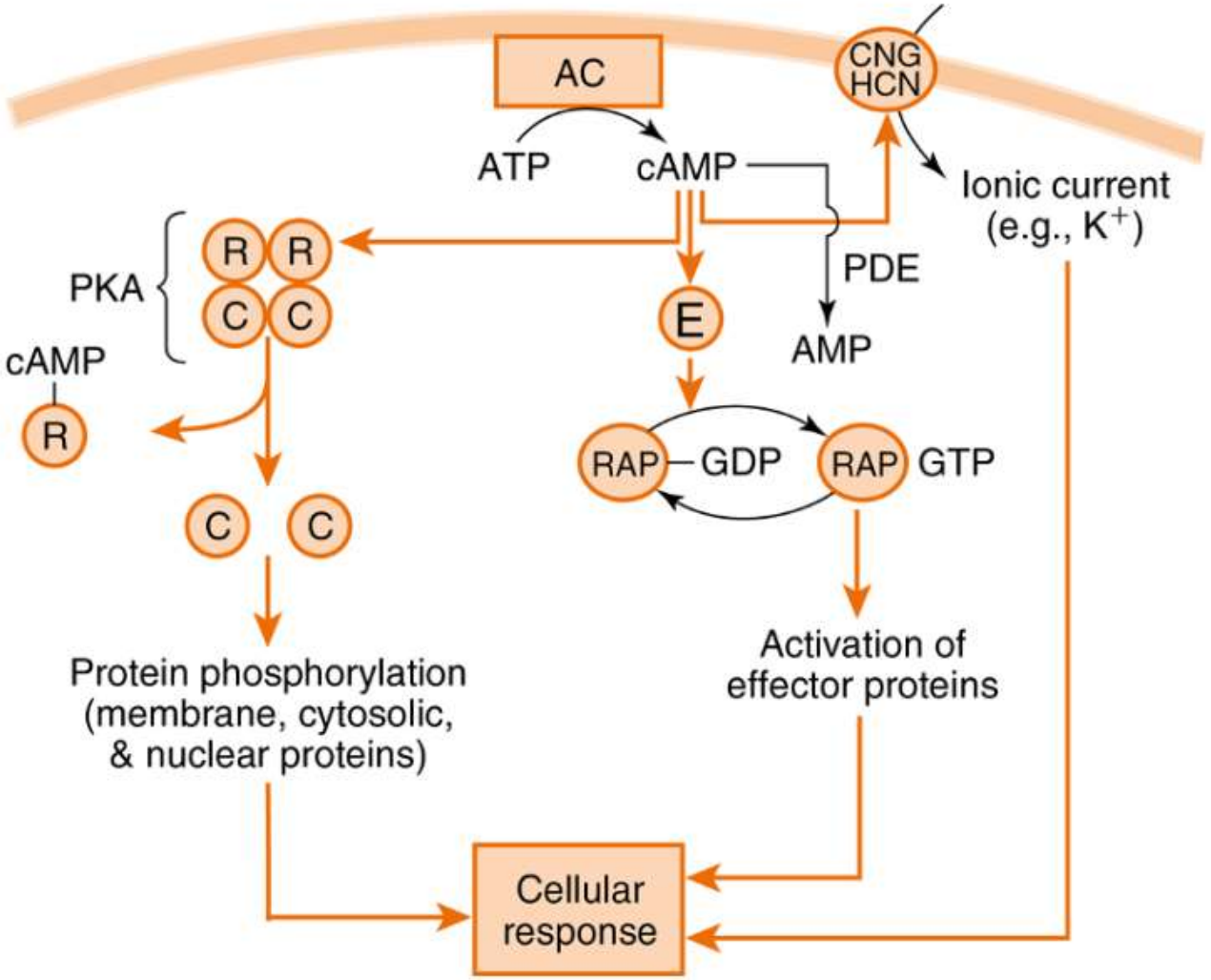
- DAG and IP_3
  - PIP_2 – phospholipase C system

- Ca^{2+}
  - Ca^{2+}/Ca^{2+}- calmodulin

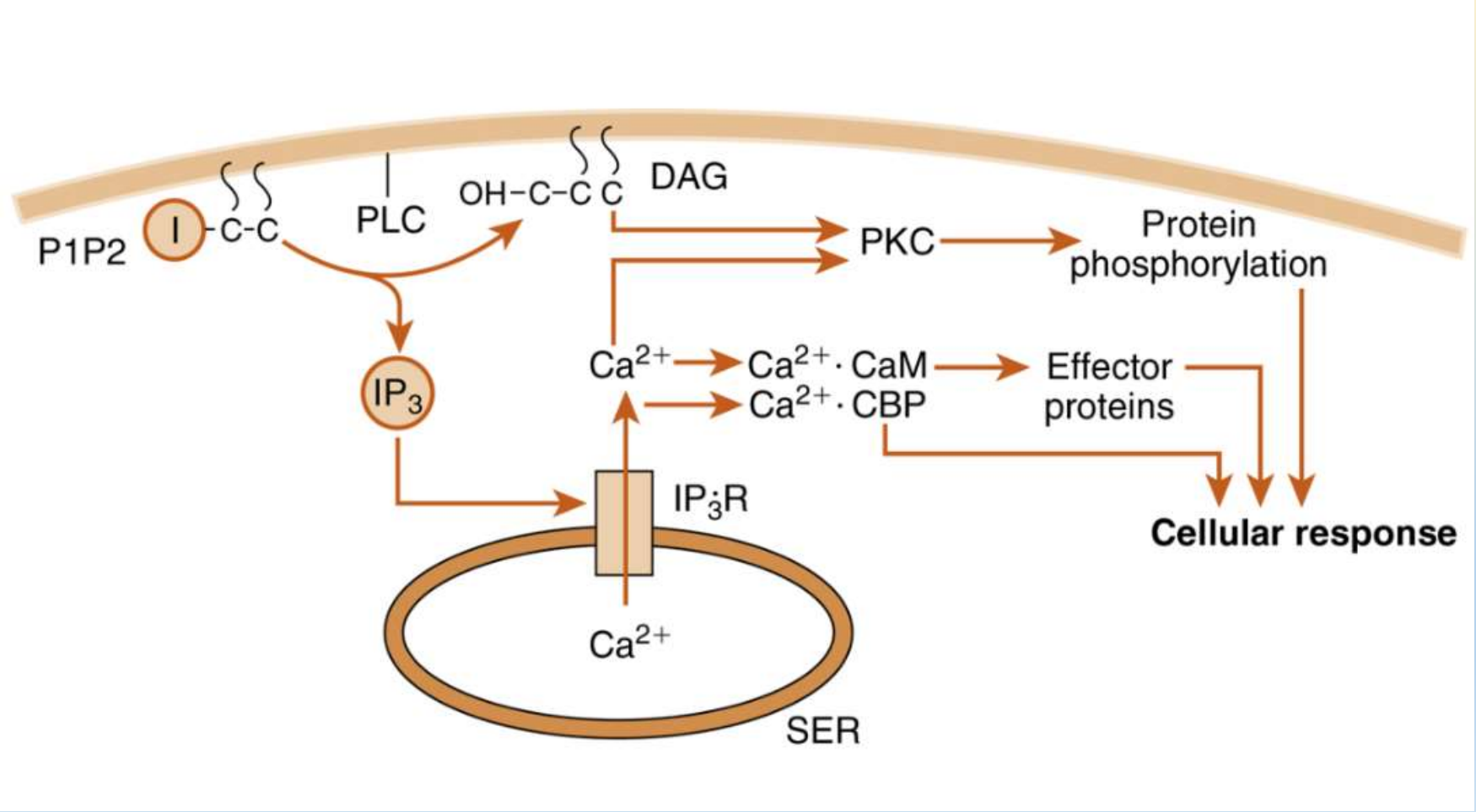
EXTRACELLULAR SIGNAL MUST BE CONVERTED TO INTRACELLULAR RESPONSE

AC – cAMP system

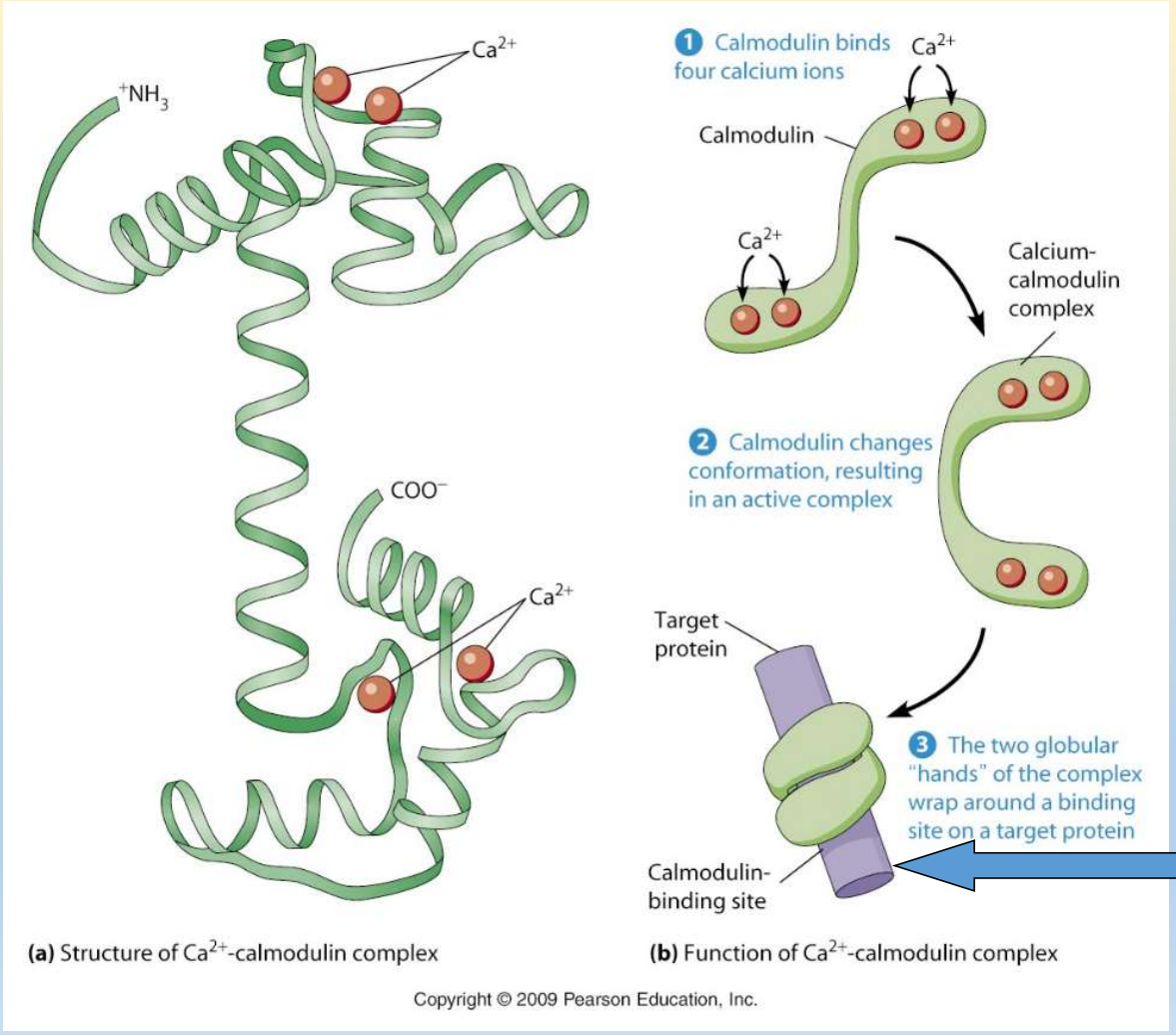
- PKA
- CREB (cAMP-responsive element–binding protein)
- Epac (E) as an another effector molecule (exchange protein activated by cAMP)
- cyclic nucleotide gated (CNG) channels
- hyperpolarization-activated cyclic nucleotide modulated (HCN) channels
- phosphodiesterases



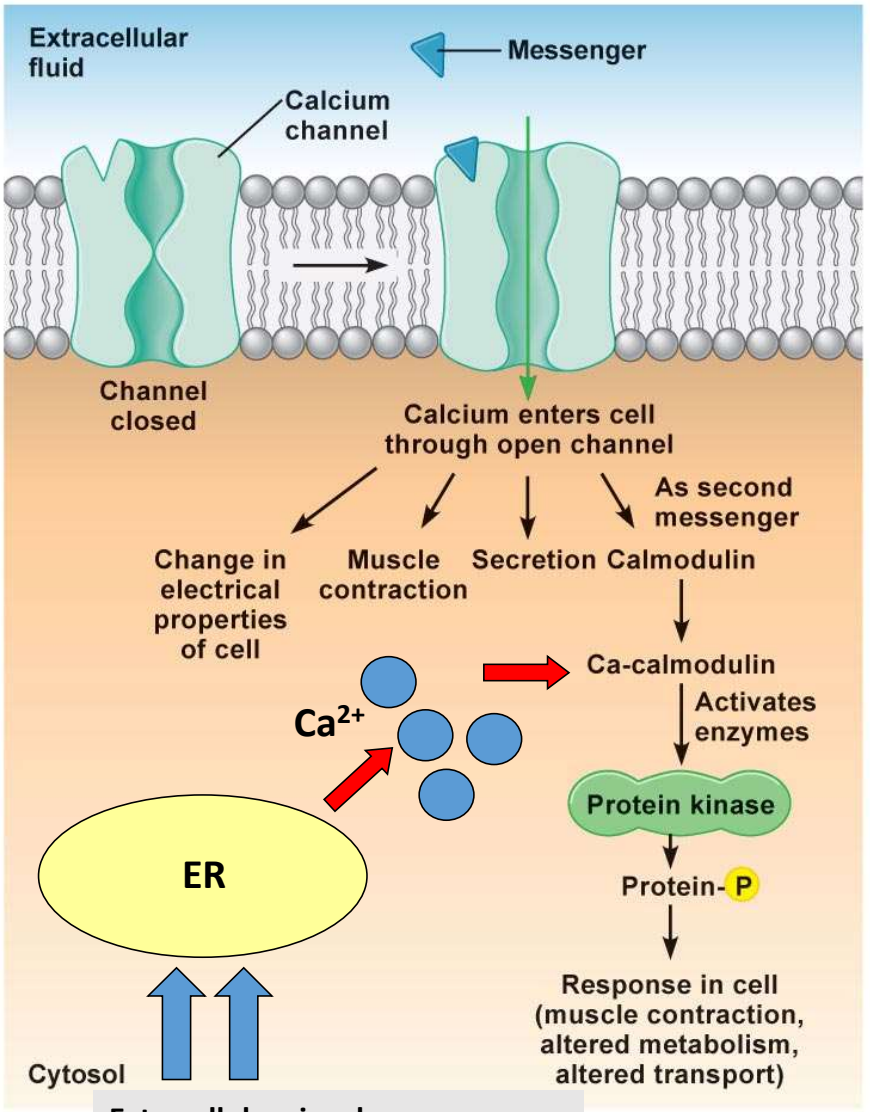
PLC - DAG and IP_3 system



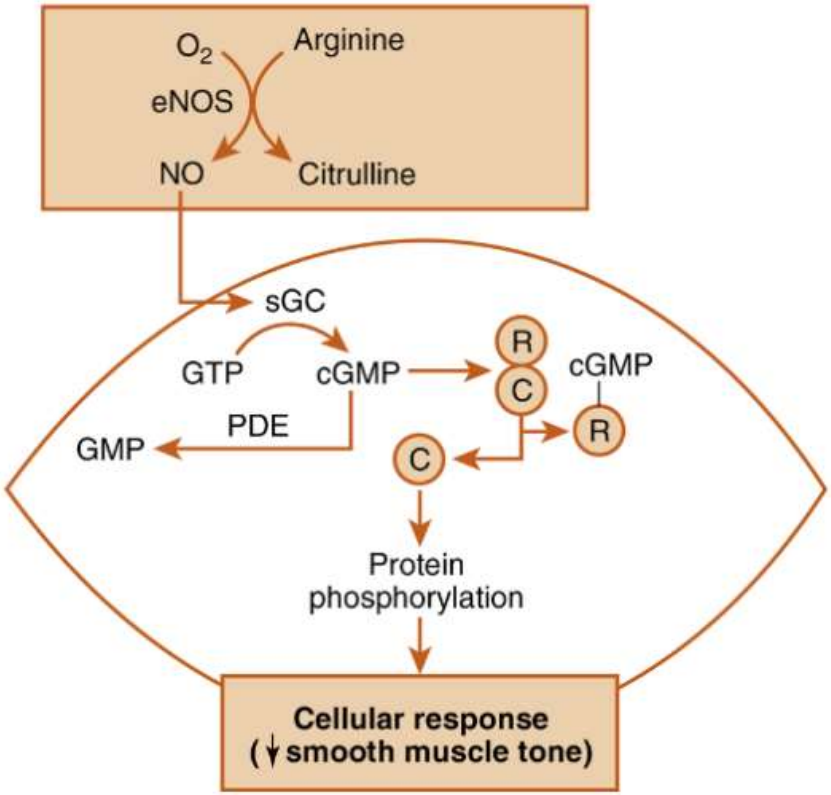
Ca^{2+} - calmodulin system

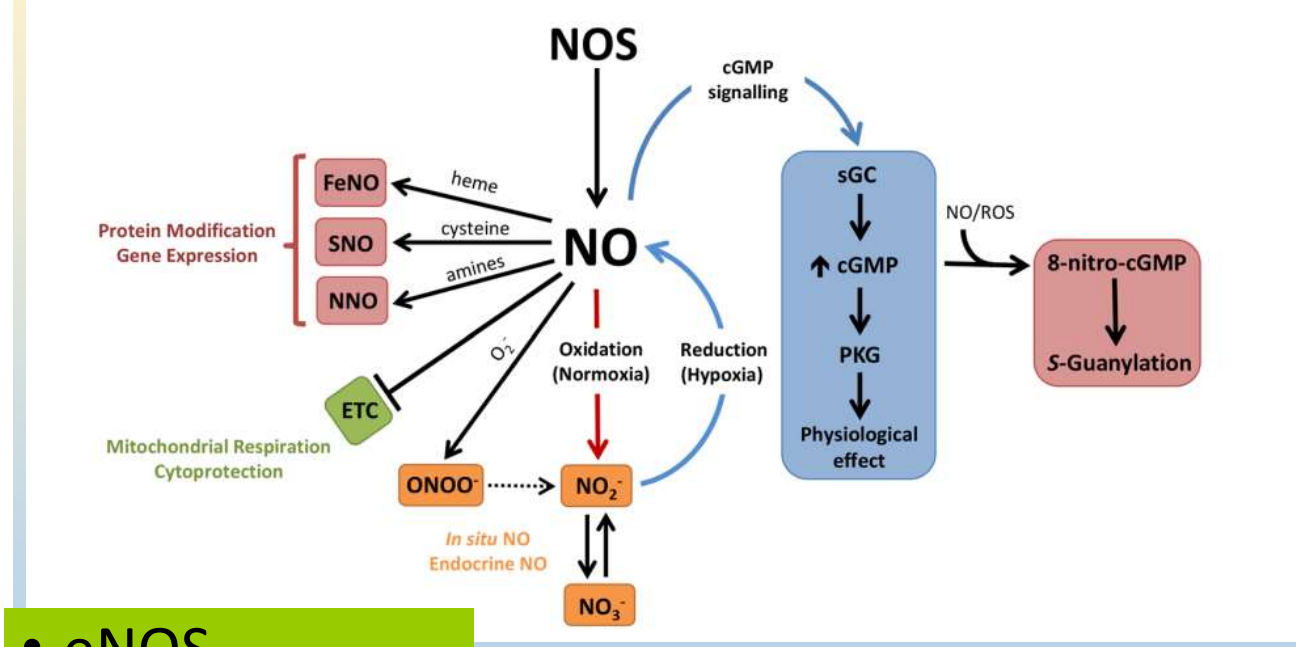


calmodulin-dependent kinases



NO as a signalling molecule - cGMP





- eNOS
- nNOS
- iNOS

Donald JA, Forgan LG, Cameron MS: **The evolution of nitric oxide signalling in vertebrate blood vessels.** ***J Comp Physiol B-Biochem Syst Environ Physiol 2015, 185(2):153-171.***

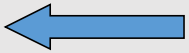
Clinical aspects

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

Hormones acting through nuclear receptors

HORMONES

- Thyroid hormones – TRα/β
- Estrogens – ERα/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

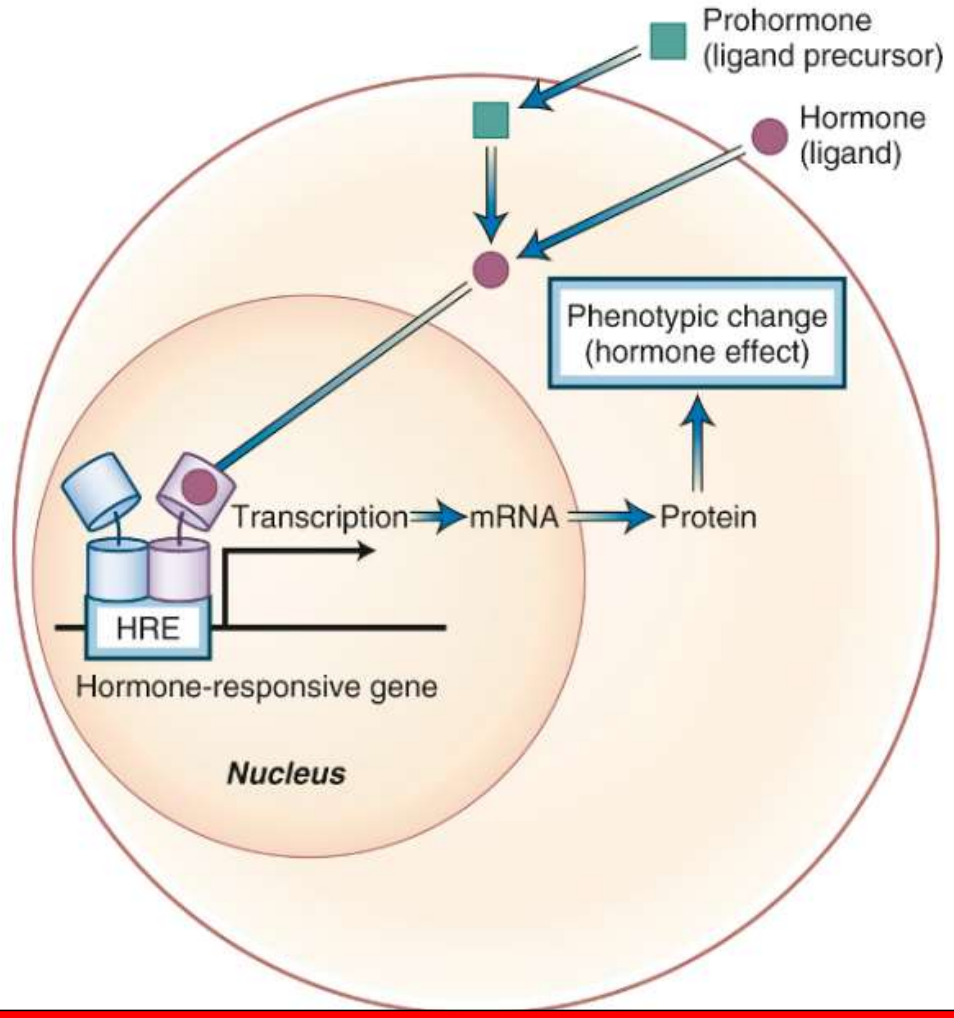
- 1,25-[OH]2D3 - VDR
- All-*trans*-retinoic acid – RA receptors α, β, γ
- 9-*cis*-retinoic acid – retinoid X receptor RXR α, β, γ

- Orphan receptors
- Variable receptors

Explanation of some effects and pathologies

General mechanism of effect of hormones acting through nuclear receptors

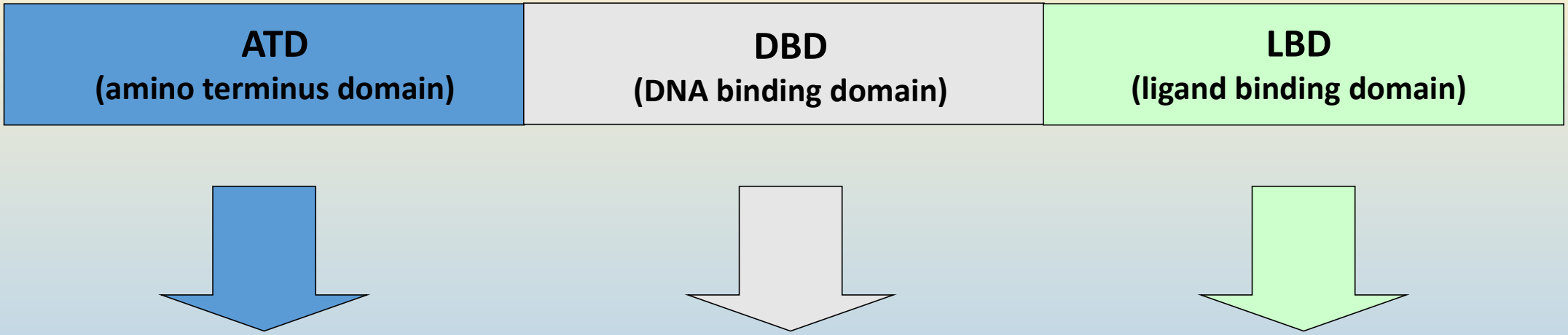
-High affinity of ligand bond = due to R structure
-Recognition of specific promotor region
-Dimerisation of receptors (homodimers, heterodimers)
-Remodelation of chromatin for gene expression (HDAC)
-Gene expression at the end decreased or increased



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

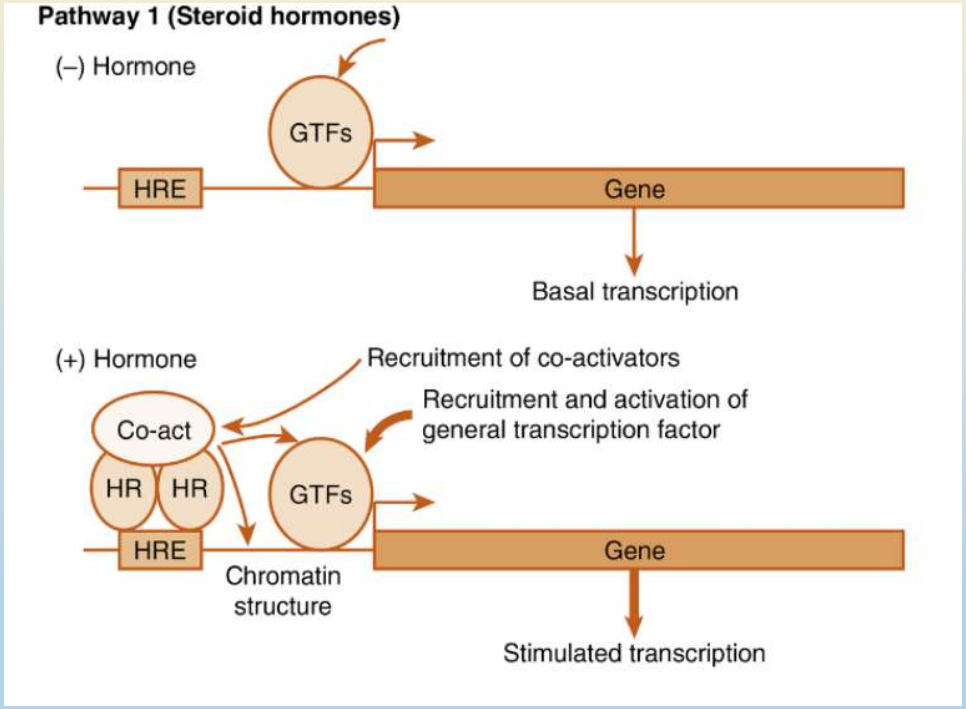


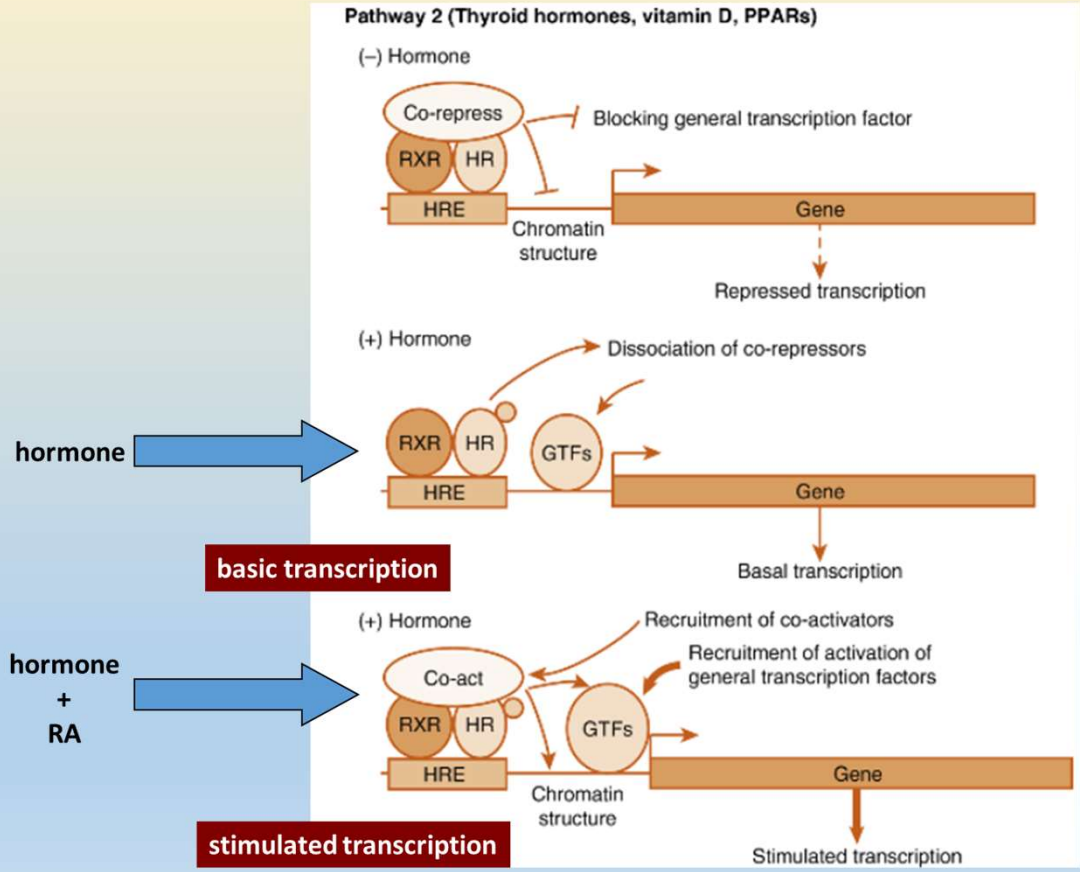
-Coregulatory proteins binding (independent on ligand)
- Phosphorylation sites

-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 X thyroid hormones





Termination of hormone action

Receptor-mediated endocytosis and subsequent lysosome degradation

Phosphorylation/ dephosphorylation of receptor or proteins of signaling pathway

Ubiquitination and proteosomal degradation

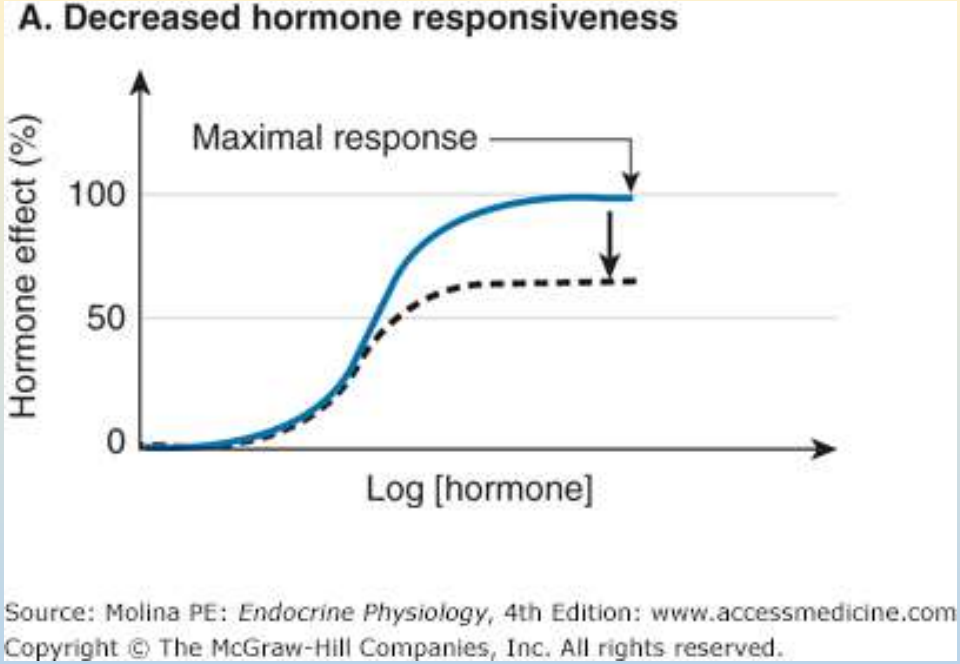
Binding of regulatory factor on corresponding protein (enzyme)

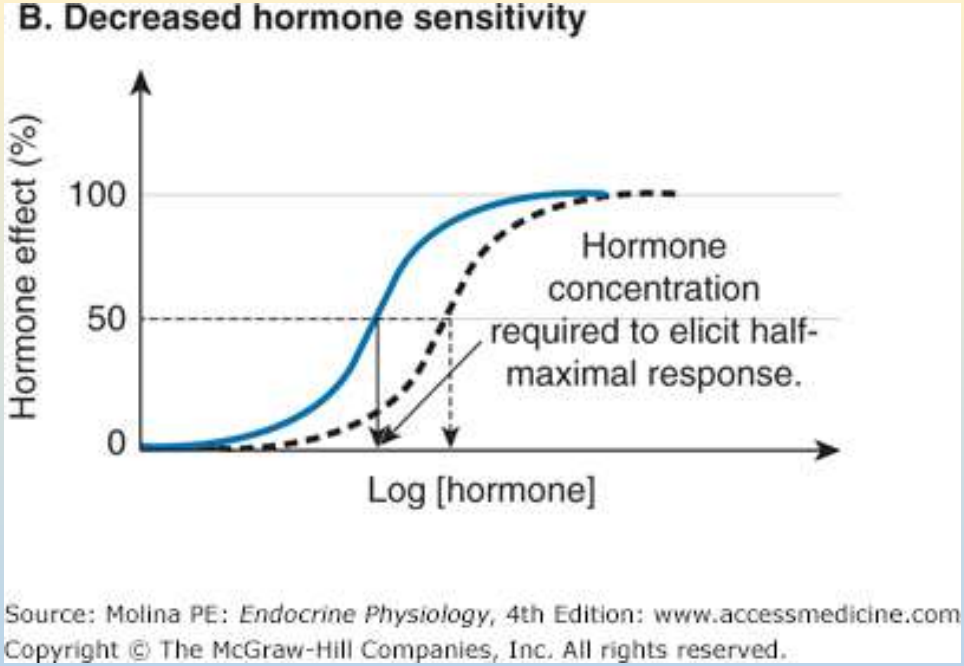
Inner enzymatic activity and its regulation

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





- **Decreased number of receptors**
- **Decreased concentration of hormone-activating enzyme(s)**
- **Increased concentration of non-competitive inhibitor**
- **Decreased number of target cells**

- **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

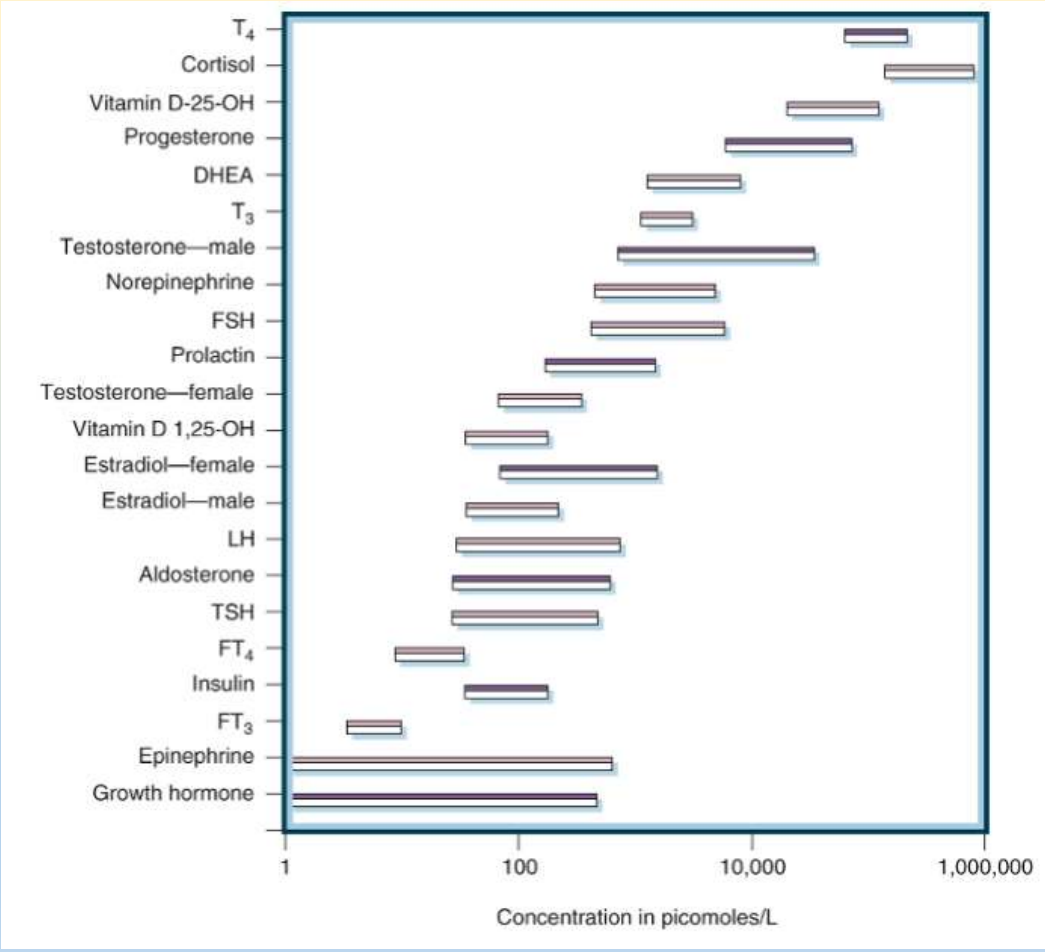
-HIGH SENSITIVITY DEMANDS
-WIDE CONCENTRATION RANGE

Antigen-antibody interaction-based methods
-Anibody 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