General principles of endocrine functions

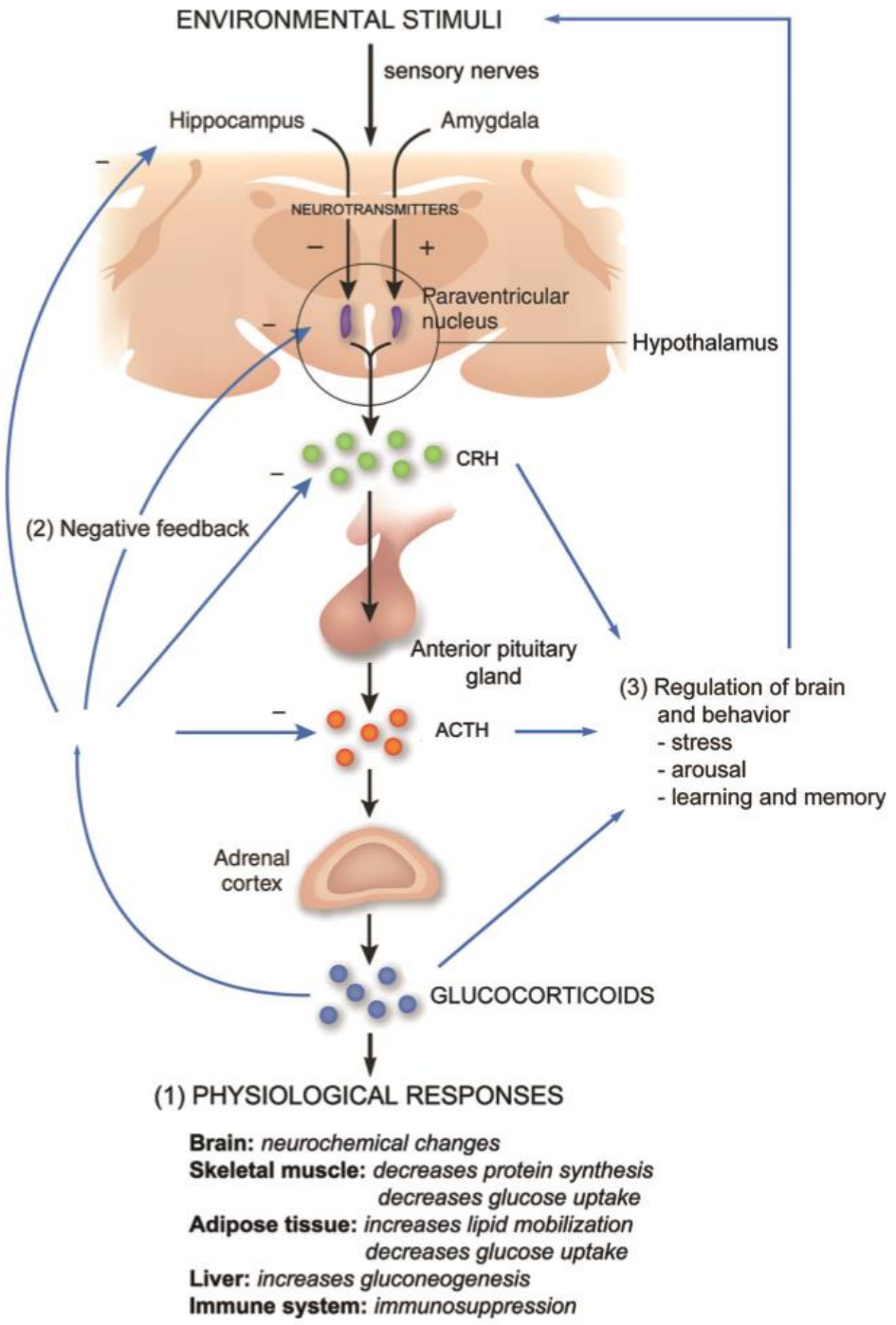
Integration systems of the organism

- Integration and coordination = maintaing the integrity and activity of the organism on all levels in the relation to the changing external and internal environments

- **Hormonal system**

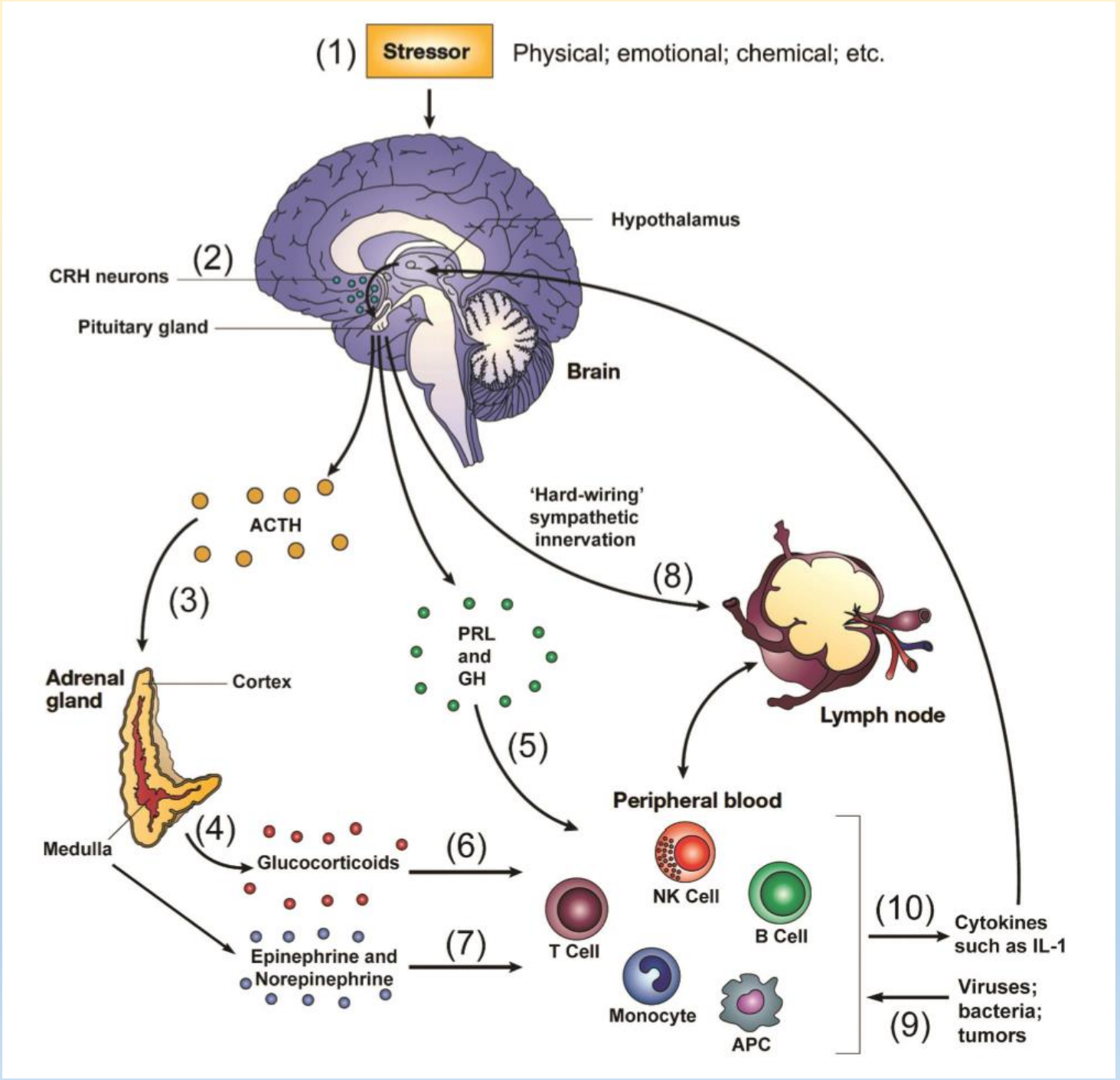
- **Nervous system**

- ***Immune system***



No system works independently = functional integration

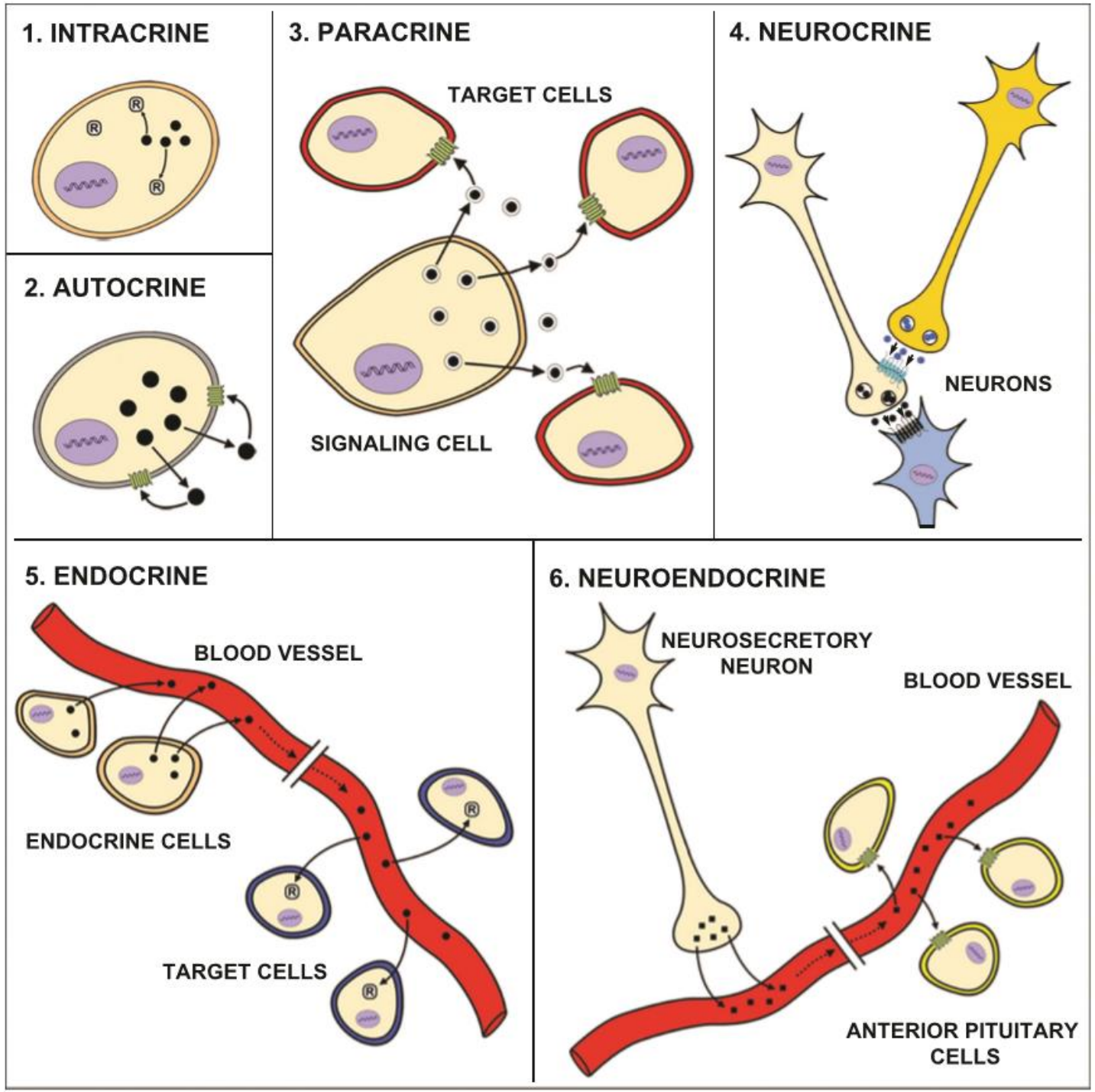
- **Hormones**
- **Neurohormones**
- **Neurotransmitters**
- **Paracrine (autocrine) effectors**



How do cells communicate?

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

source → environment → target cell



source

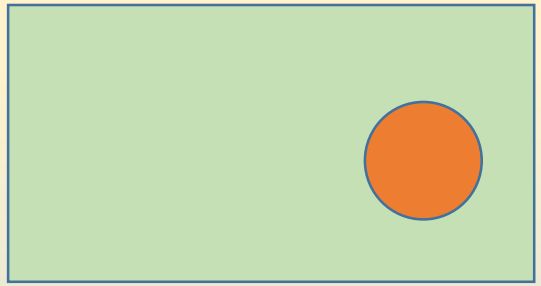
environment

target cell

endocrine

gland

blood



- synthesis/secretion
- no influence on specificity of effect

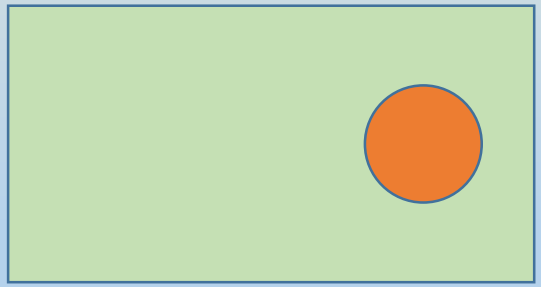
- universal environment
- dilution and interactions

- 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

paracrine

kidneys

Interstitial fluid

BMP4

proteases, chordin, noggin

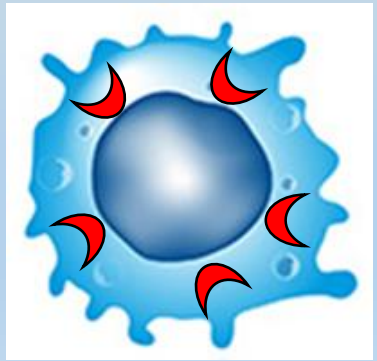
differentiation

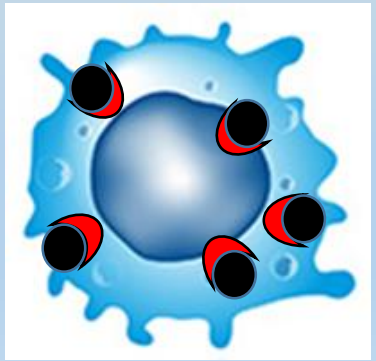
bone

bone formation

autocrine

Interstitial fluid/IVF



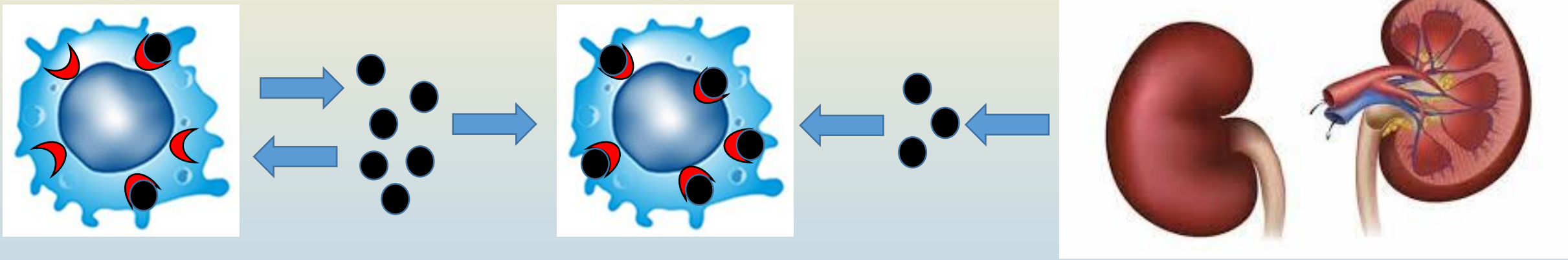


antimicrobial peptides

macrophage

1,25-$[OH]_2D_3$

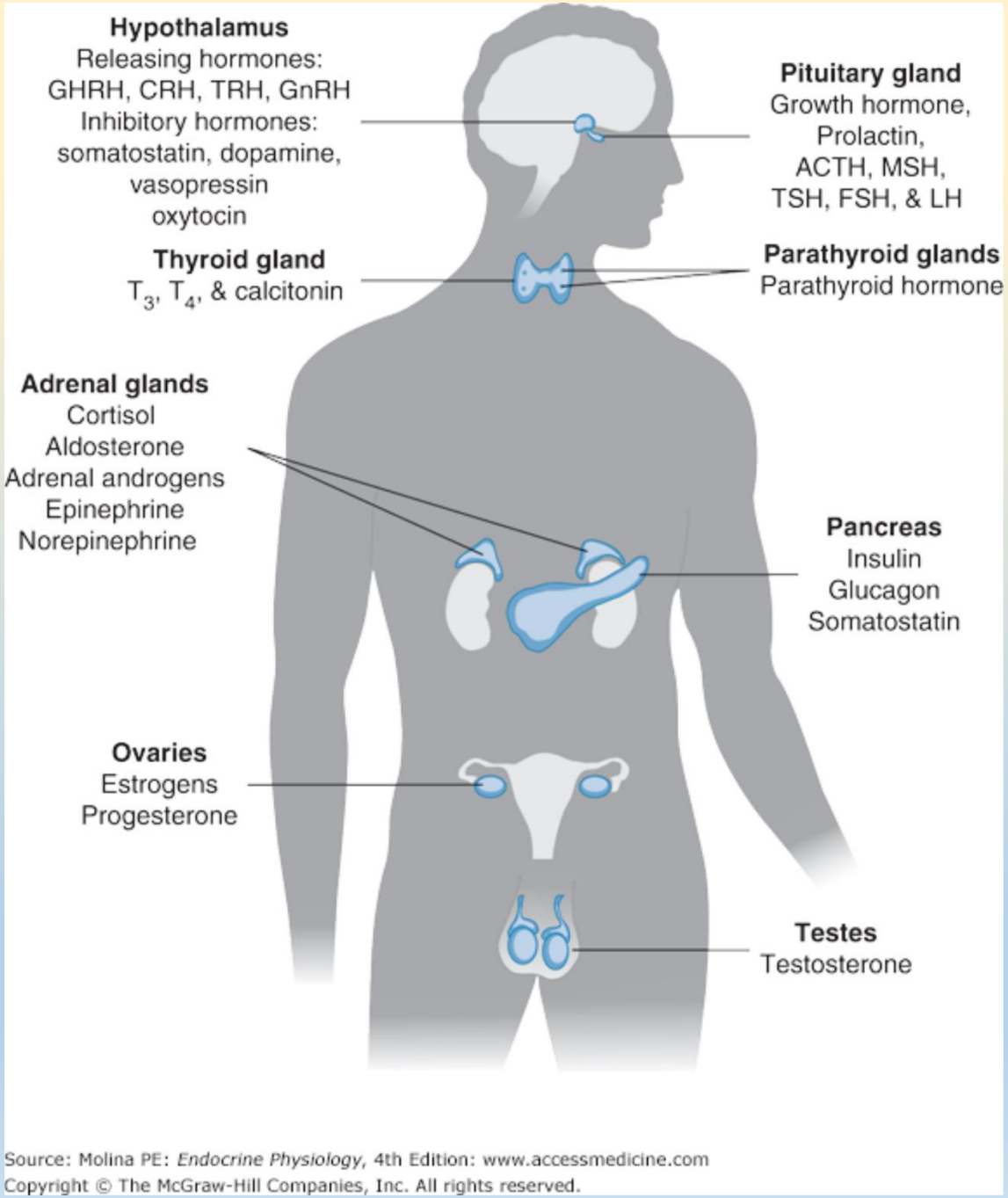
autocrine **paracrine** **endocrine**



macrophage $1,25\text{-}[OH]_2D_3$ $1,25\text{-}[OH]_2D_3$ kidney – proximal tubule

Hormones

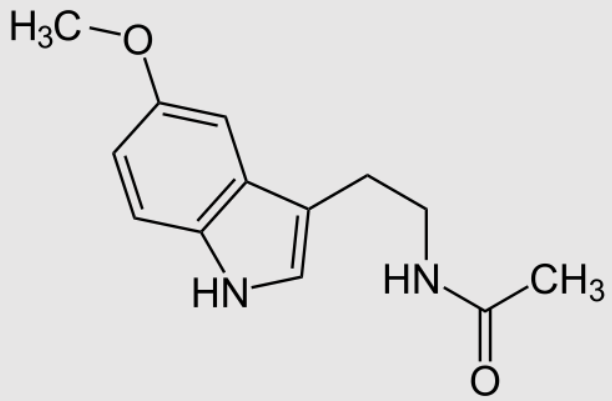
- **Starling 1905 - *secretin***
- Glandotropic hormones
- Aglandotropic hormones
- Target cells
- Limited time of effect



Chemical nature of hormones

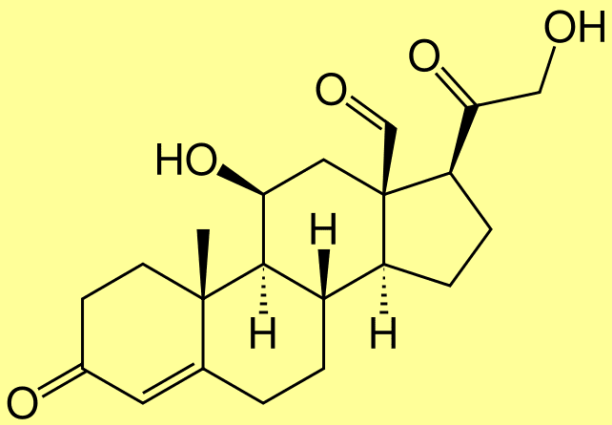
DERIVED FROM AMINOACIDS

-Adrenaline
-Noradrenaline
-Dopamine
-Melatonine
-T3/T4



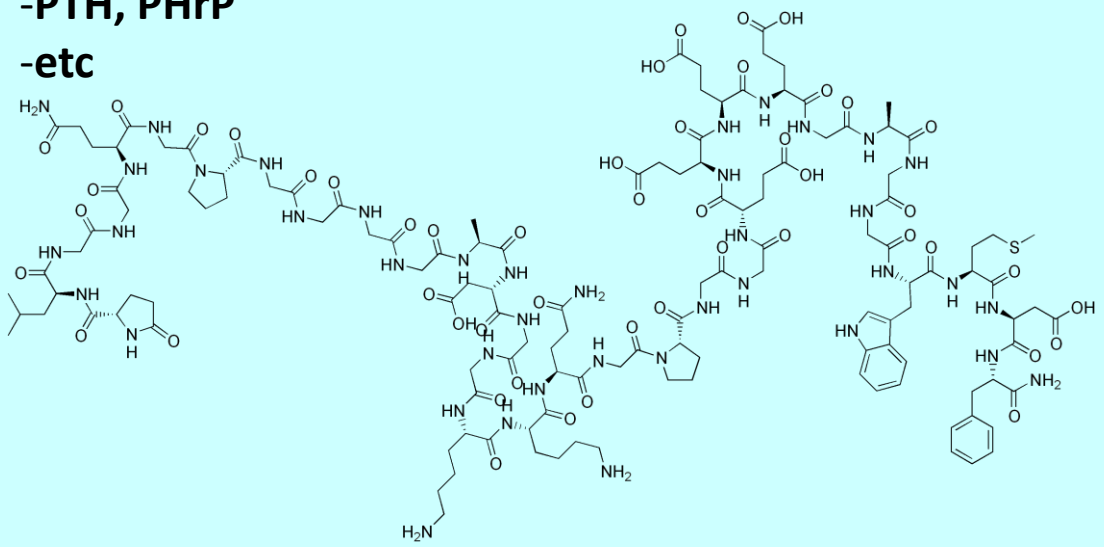
STEROID

-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



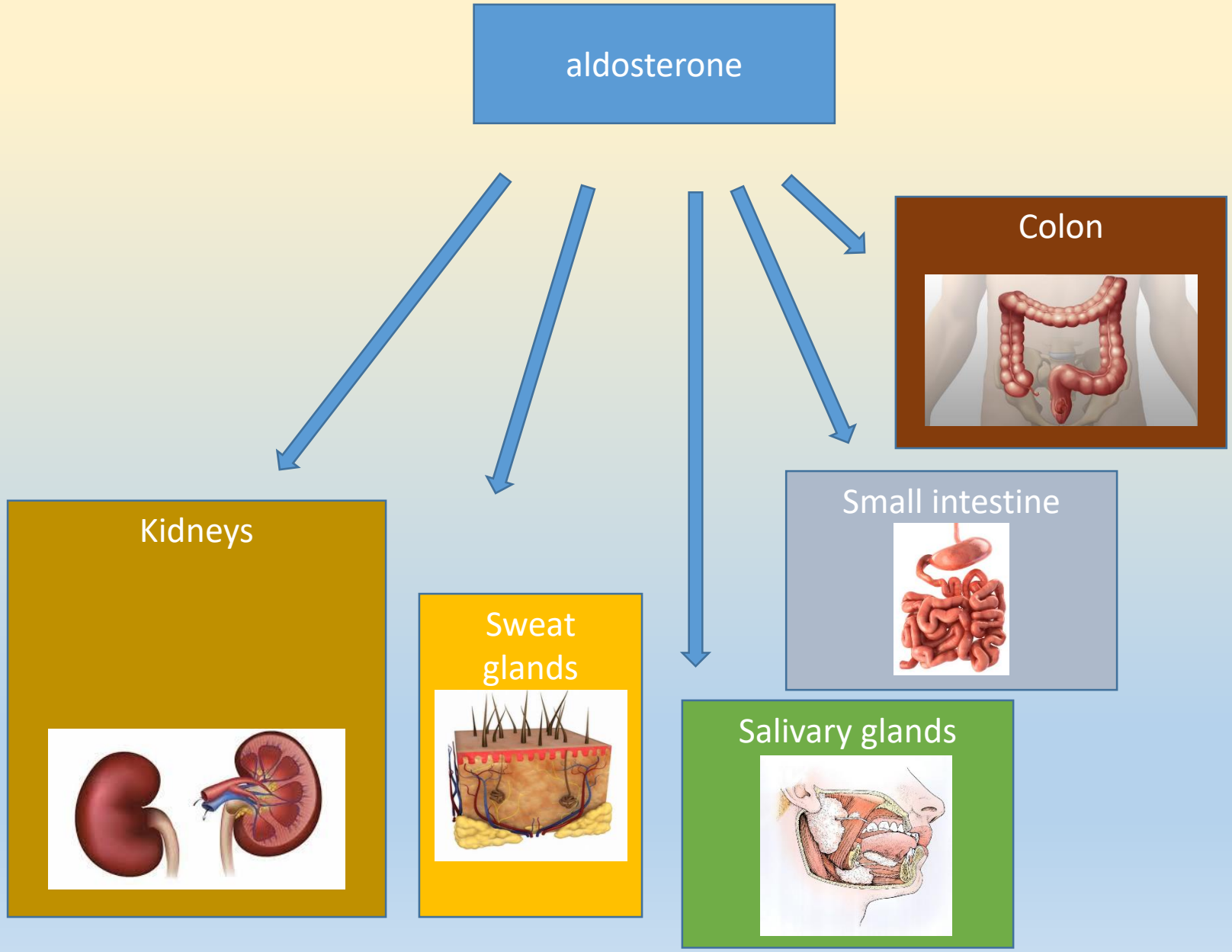
Chemical nature of hormones

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

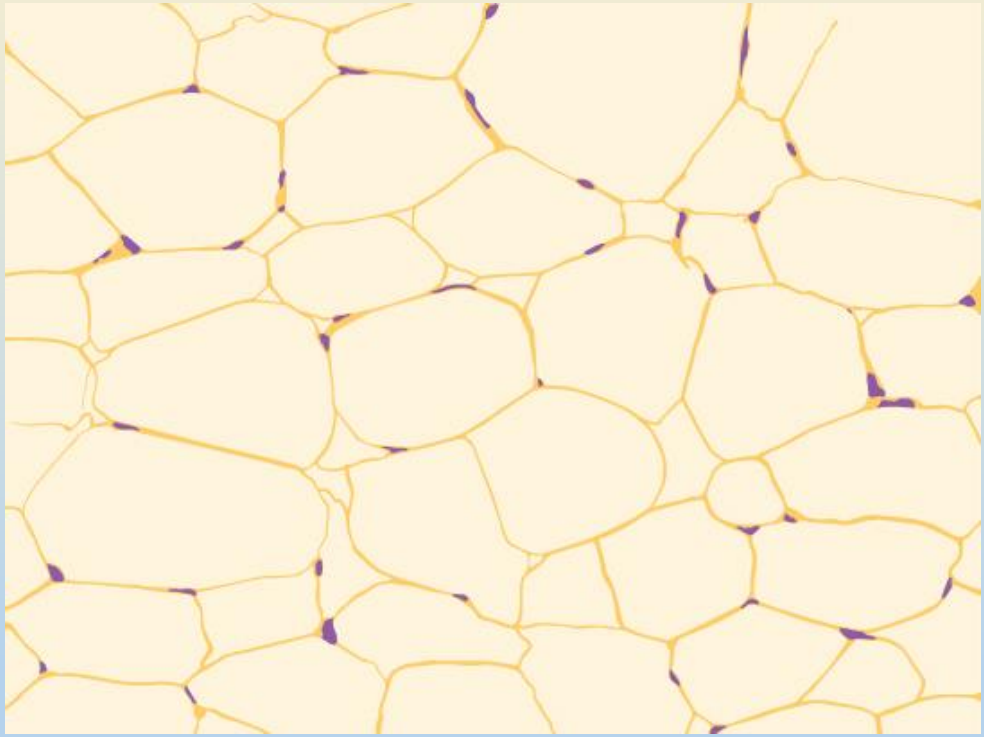


adrenaline

glucagon

noradrenaline

ghrelin

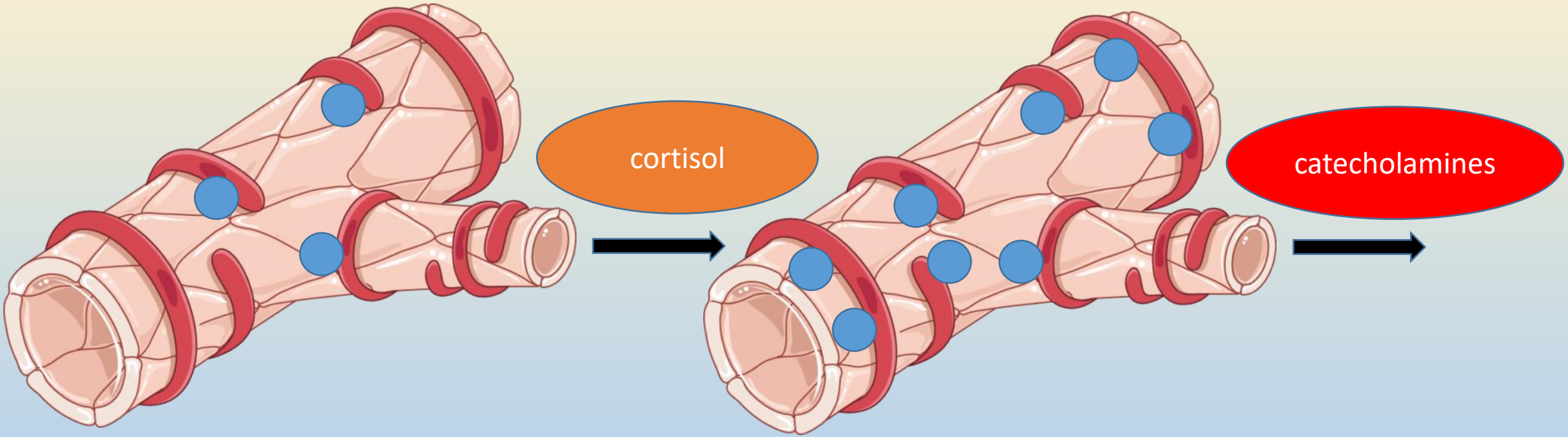


growth hormone

testosterone

adipose tissue

lipolysis

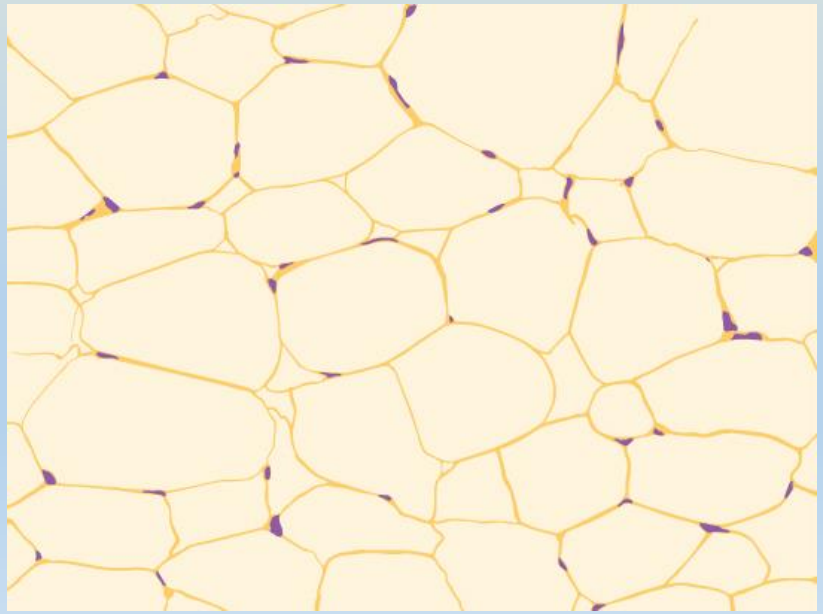


vasoconstriction

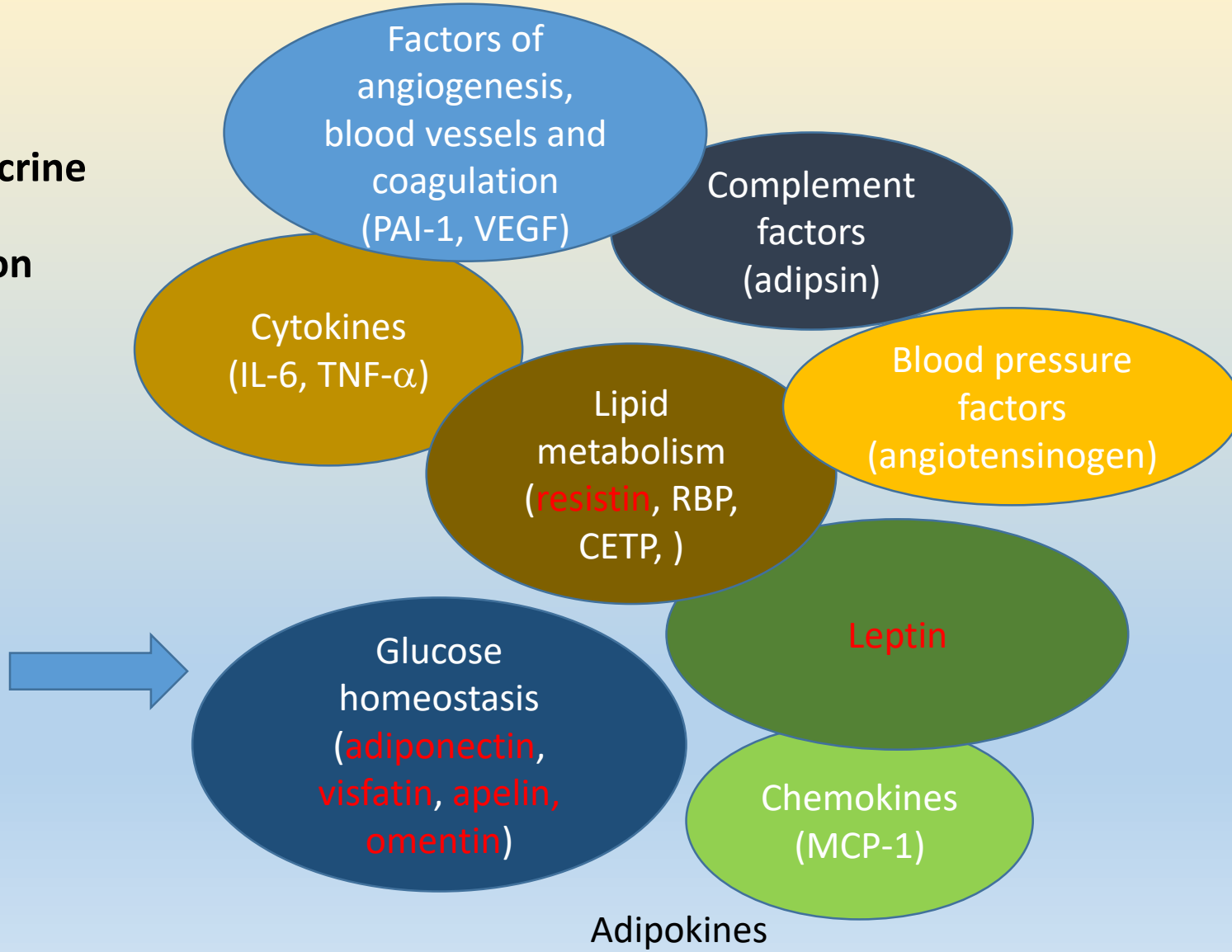
Arterioles – α2 receptors

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

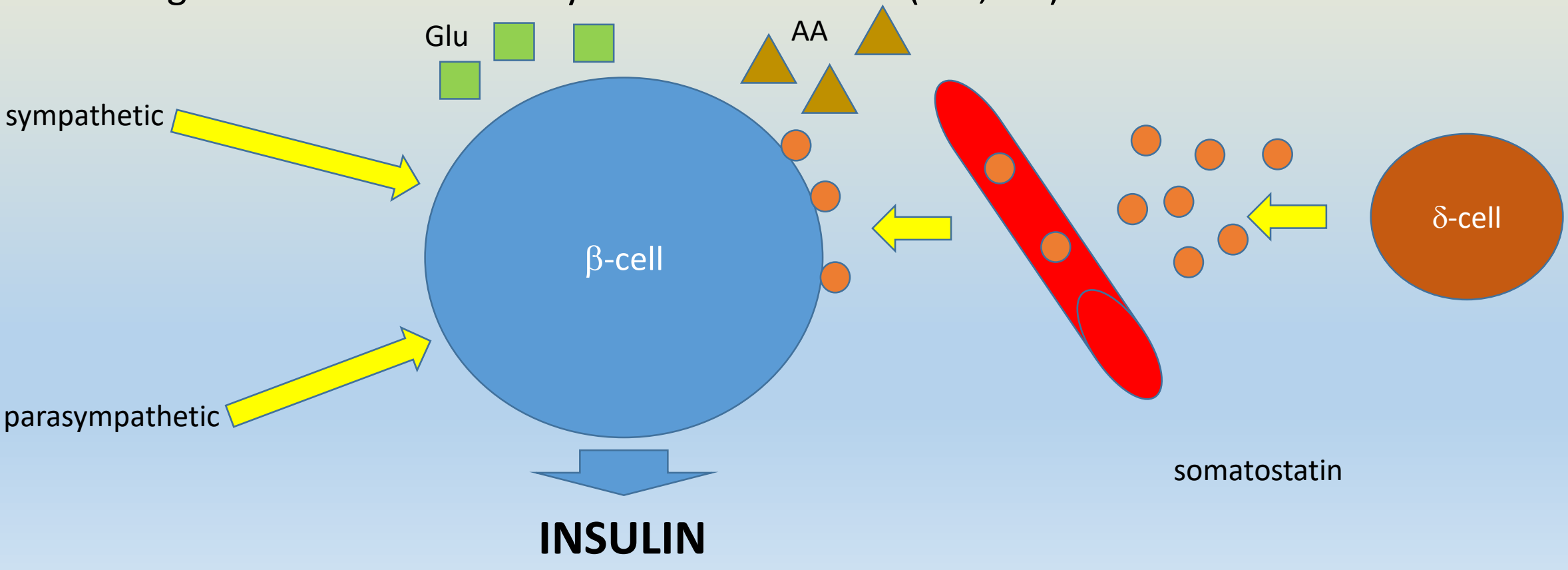
Lung tumors
– ADH (hyponatremia)
– ACTH (Cushing syndrome)
– PTHrP (hypercalcaemia)

Liver and kidney tumors
– erythropoietin (polycythemia)

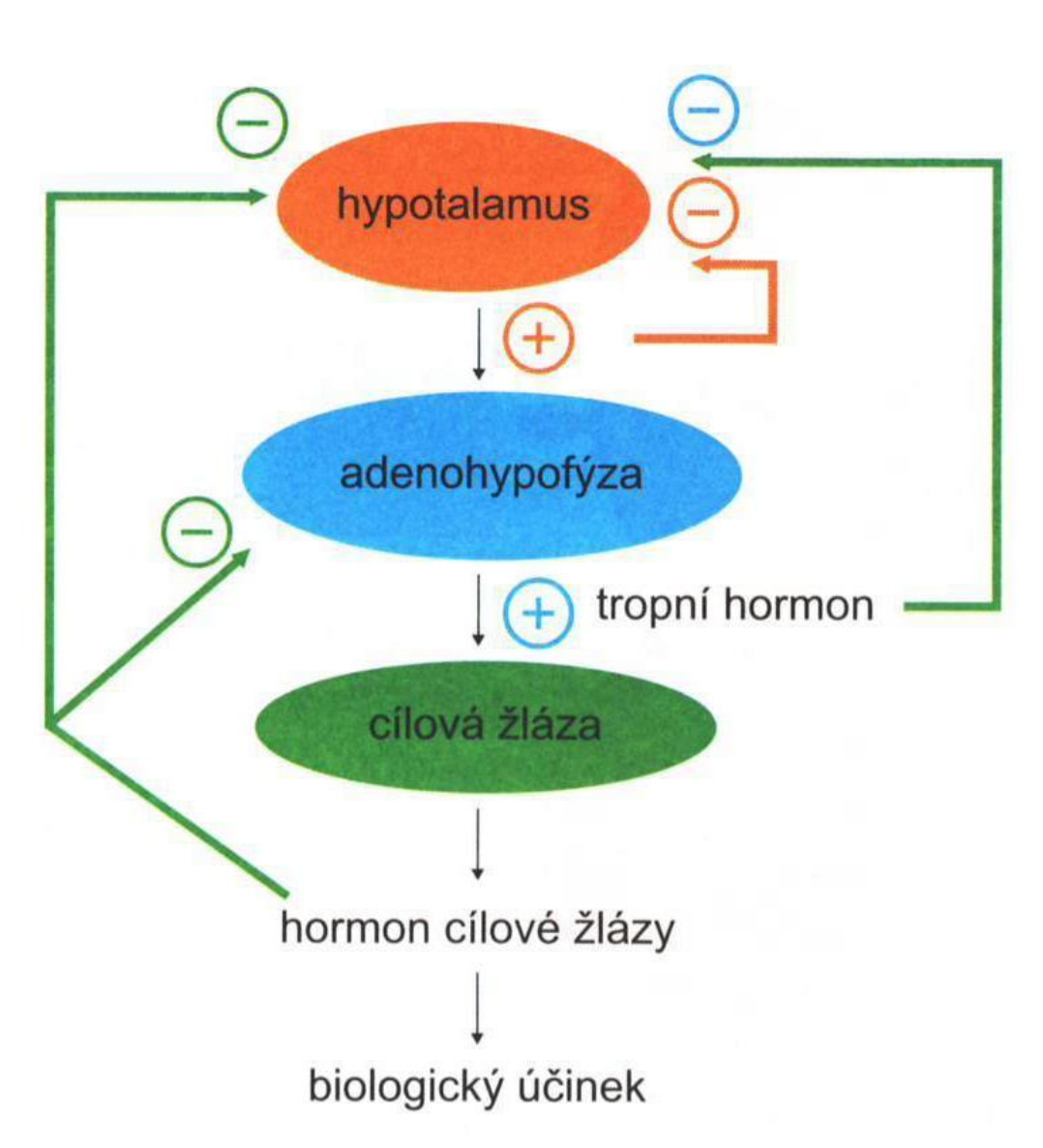
GIT tumors
– ACTH (Cushing syndrome)

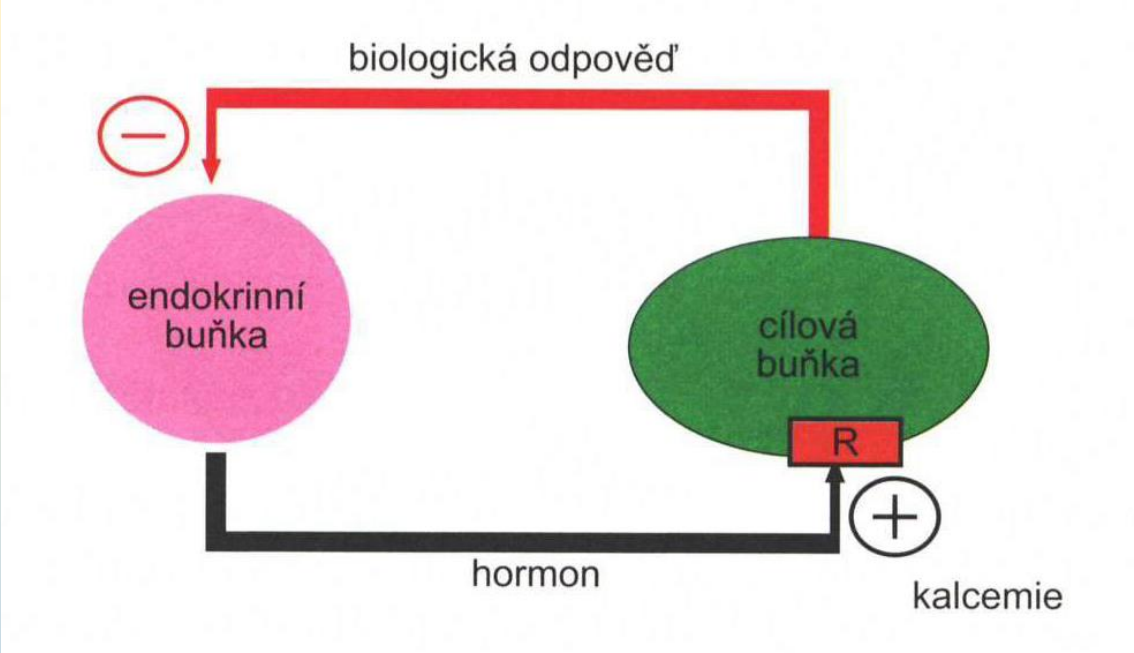
Secretion of hormones and its regulation

- 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

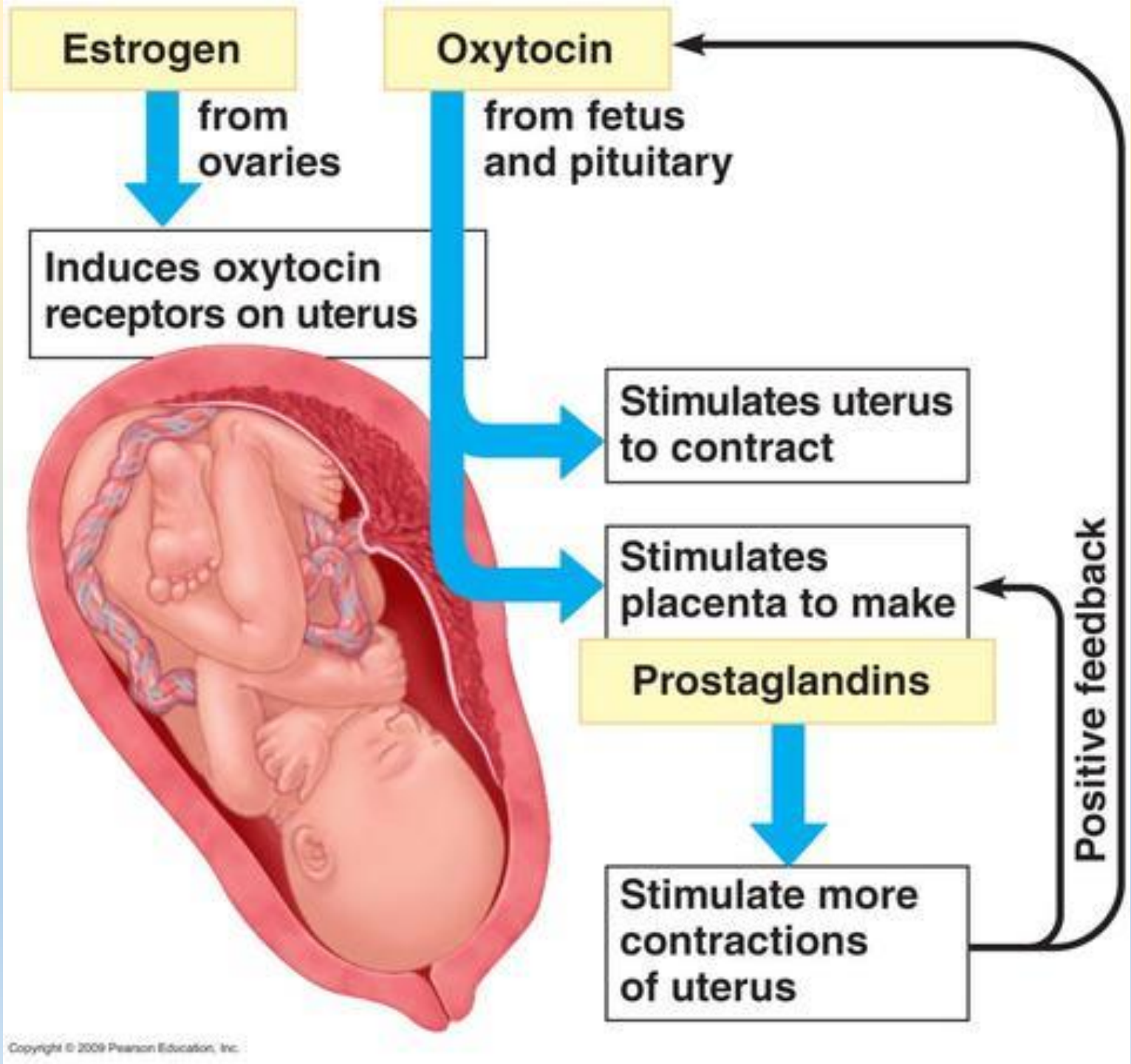


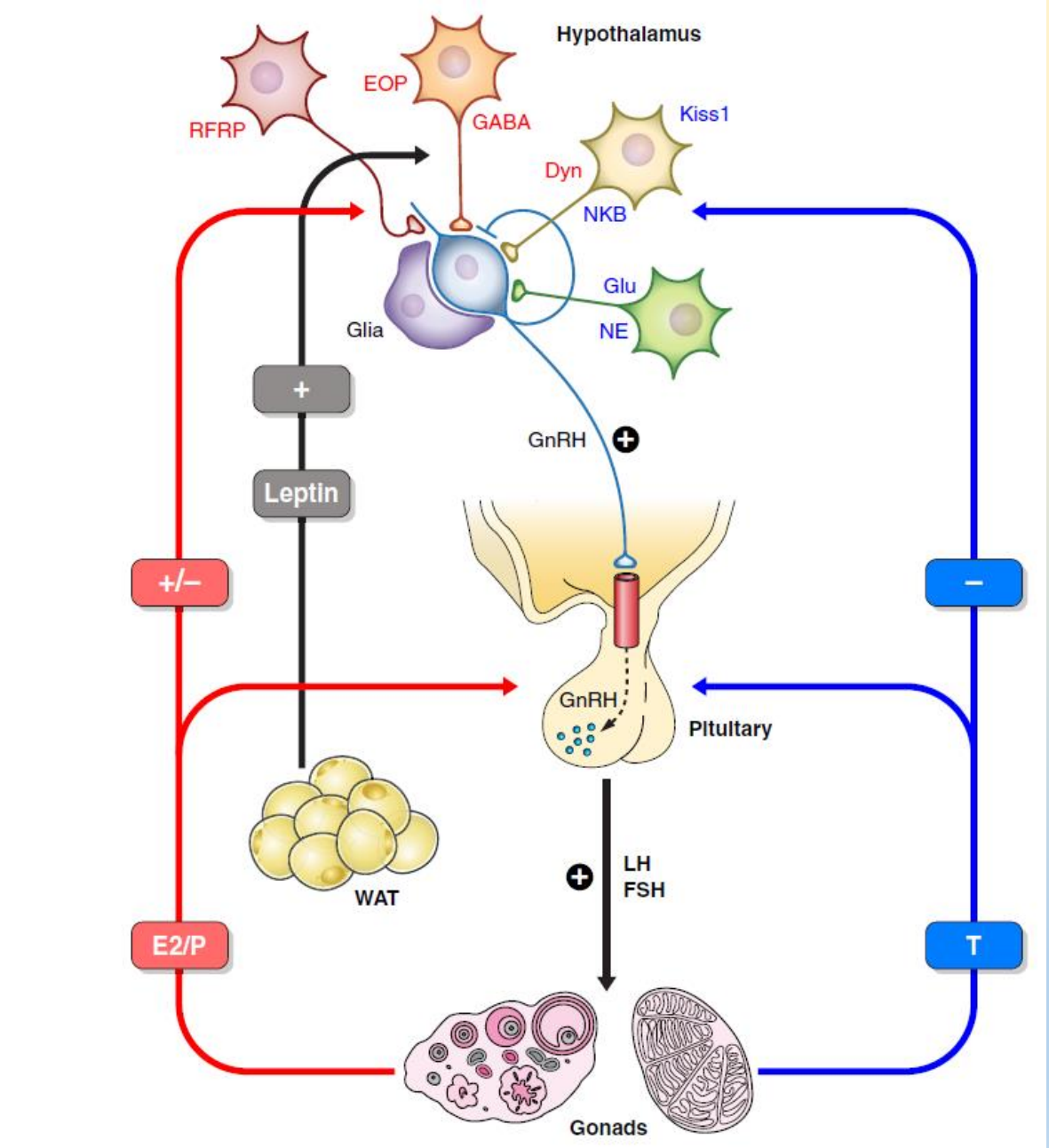


Feedback
- negative X positive
- simple X complex

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

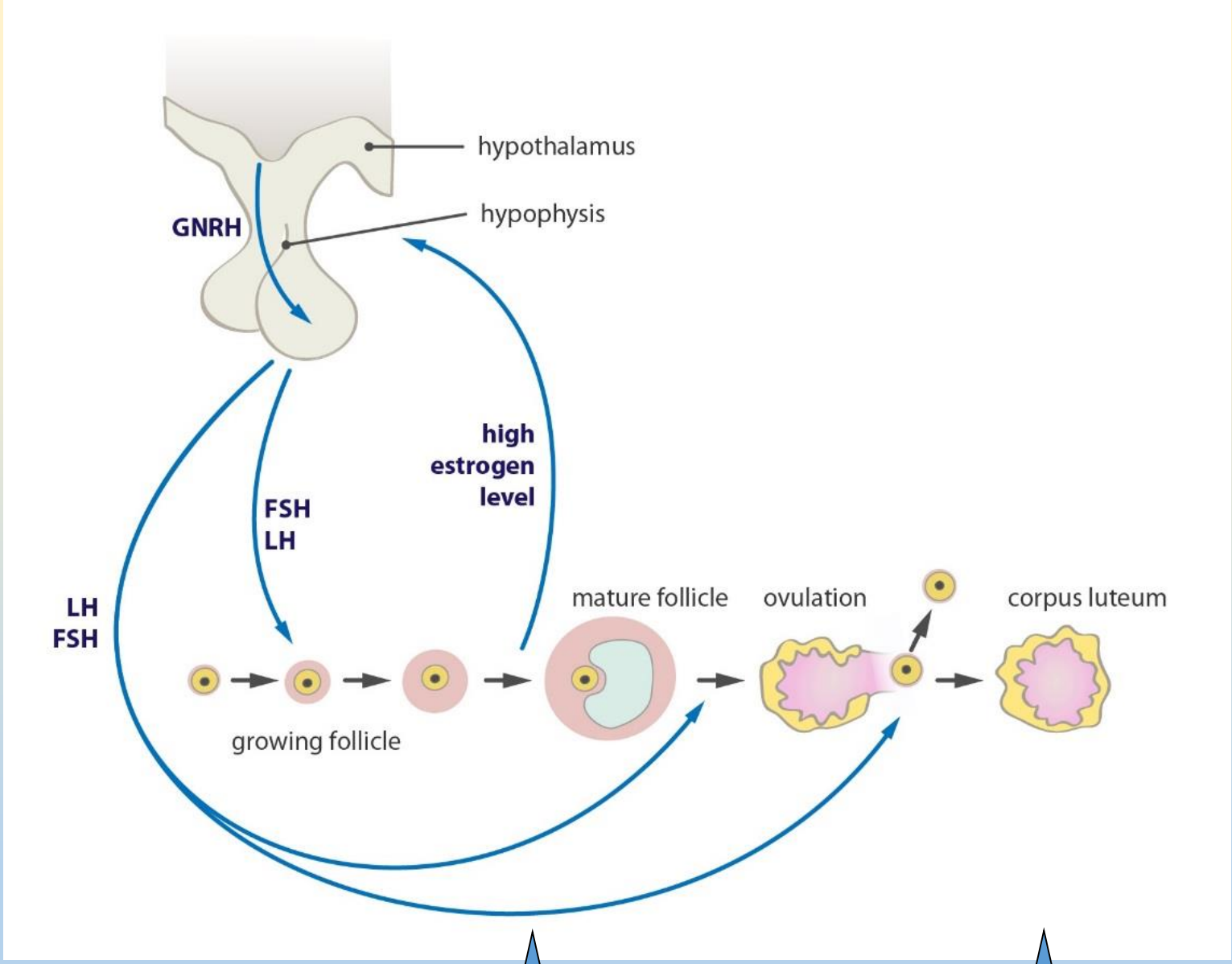
Positive feedback – why?

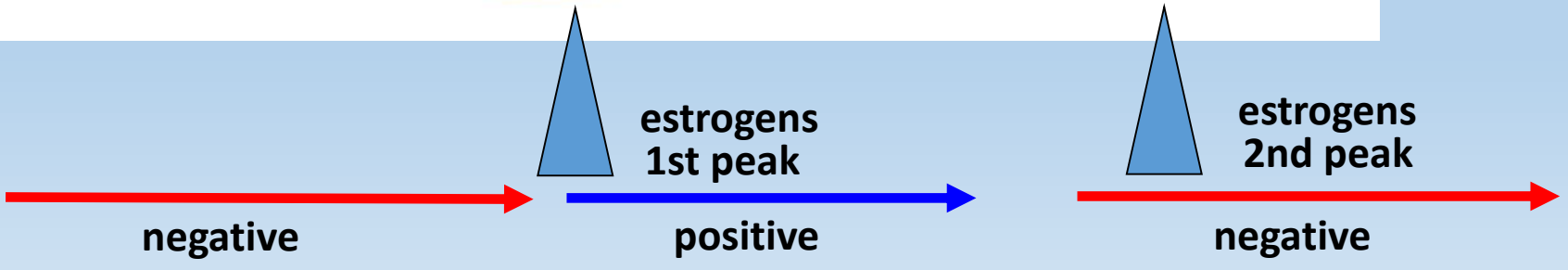




Pinilla, L., Aguilar, E., Dieguez, C., Millar, R. P., Tena-Sempere, M., 2012. KISSPEPTINS AND REPRODUCTION: PHYSIOLOGICAL ROLES AND REGULATORY MECHANISMS. Physiological Reviews. 92**, 1235-1316.**

FIGURE 1. Neurobiology of the hypothalamic-pituitary-gonadal (HPG) axis. Schematic presentation of the major elements of the neuroendocrine axis controlling reproduction: the HPG axis. Hypothalamic GnRH neurons, which receive trans-synaptic and glial inputs, release GnRH to the hypophysial portal blood system. In turn, GnRH dictates the pulsatile secretion of gonadotropins, LH and FSH, that stimulate the maturation and regulate the function of the gonads; note that in the scheme, both the ovary and testis are presented. These major hormonal elements are connected via feed-forward and feedback regulatory loops. The function of the HPG axis is under the regulation of several peripheral signals that include gonadal steroids, responsible for feedback control: testicular testosterone (T) conducts inhibitory actions on GnRH/gonadotropin secretion (negative feedback), whereas ovarian steroids, mainly estradiol (E2) and progesterone (P), can carry out both negative- and positive-feedback actions depending on the stage of the ovarian cycle. Other peripheral regulators of the HPG axis are metabolic hormones; among those, the prominent stimulatory/permissive roles of leptin, produced by the white adipose tissue (WAT), are depicted. Some of the central transmitters involved in the control of the HPG axis are also shown: predominant inhibitory transmitters are depicted in red, whereas excitatory factors are labeled in blue. Among the excitatory signals to GnRH neurons, Kiss1 neurons are highlighted. Please note that to concise presentation, discrimination between direct and indirect afferents to GnRH neurons is not made in the figure. Likewise, for sake of simplicity, some of the stimulatory and inhibitory signals to GnRH neurons are depicted in the same neurons; except for the Kiss1/NKB/Dyn neurons, this does not denote necessarily coexpression of these molecules in the same cells. Glu, glutamate; GABA, γ-aminobutyric acid; EOP, endogenous opioid peptides; NE, norepinephrine; NKB, neurokinin-B; Dyn, dynorphin; RFRP, RF-related peptides. [Adapted from Roa and Tena-Sempere (377).]

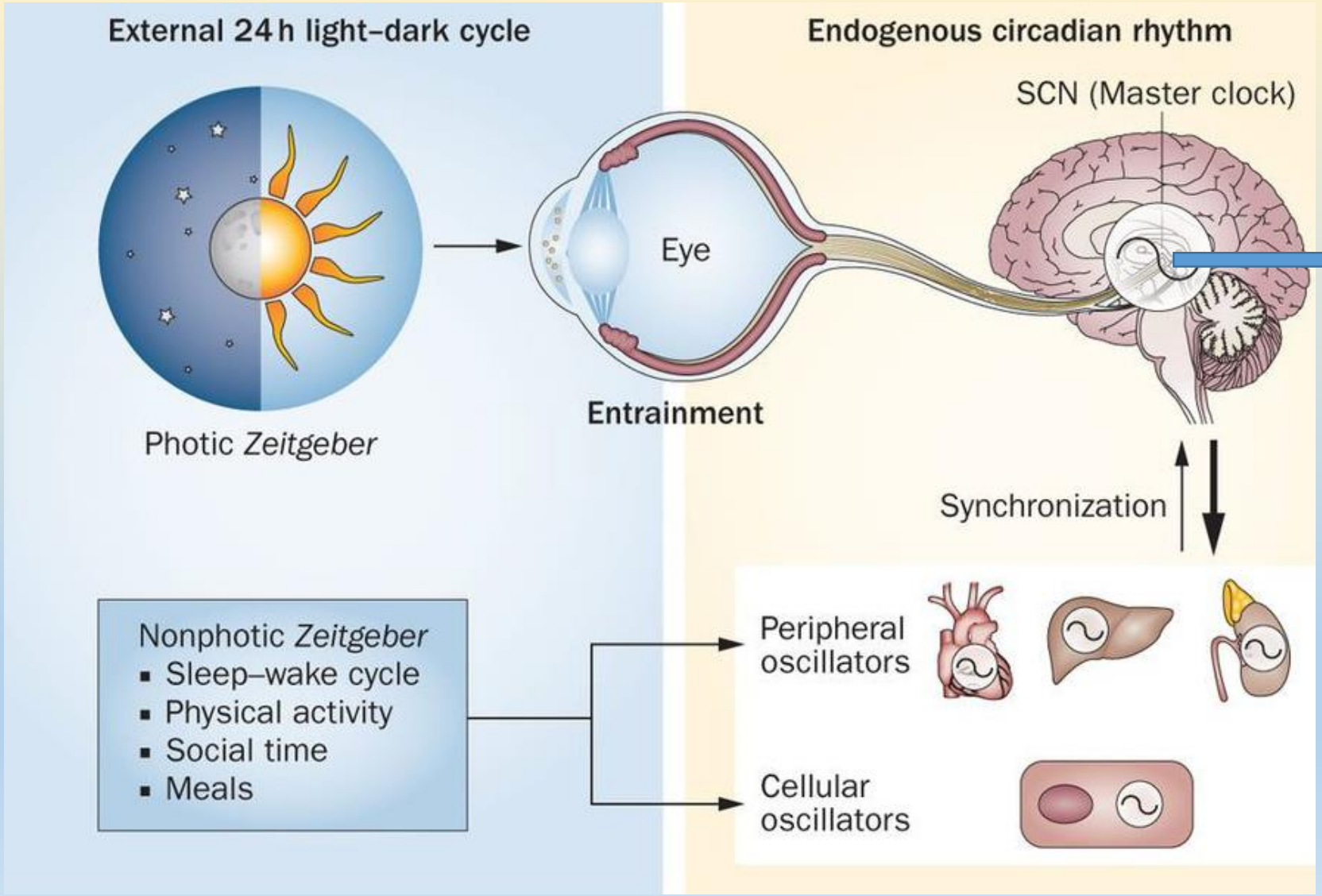

hypothalamus
hypophysis
GNRH
high
estrogen
level
FSH
LH
LH
FSH
mature follicle
ovulation
corpus luteum
growing follicle


estrogens
1st peak
estrogens
2nd peak
negative
positive
negative

Cyclic changes in hormone secretion

SCN:
- Afferent – retina
- Efferent – hypothalimic nucleus

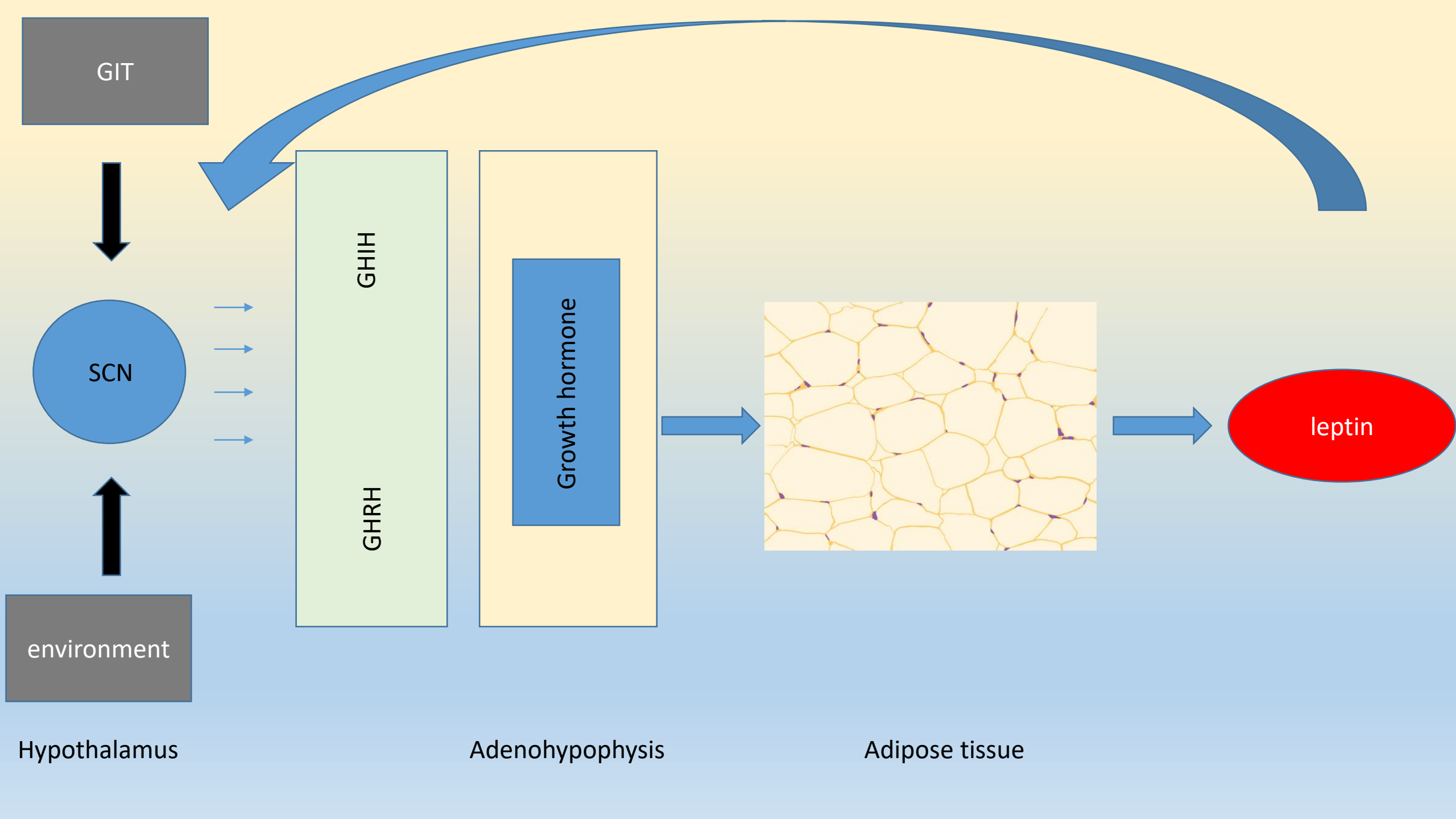
- Melatonin
- ADH
- ACTH – cortisol
- Insulin
- Ghrelin
- Adiponectin
- Leptin



Neuronal/hormonal = SNC-dependent

Satiety/fasting

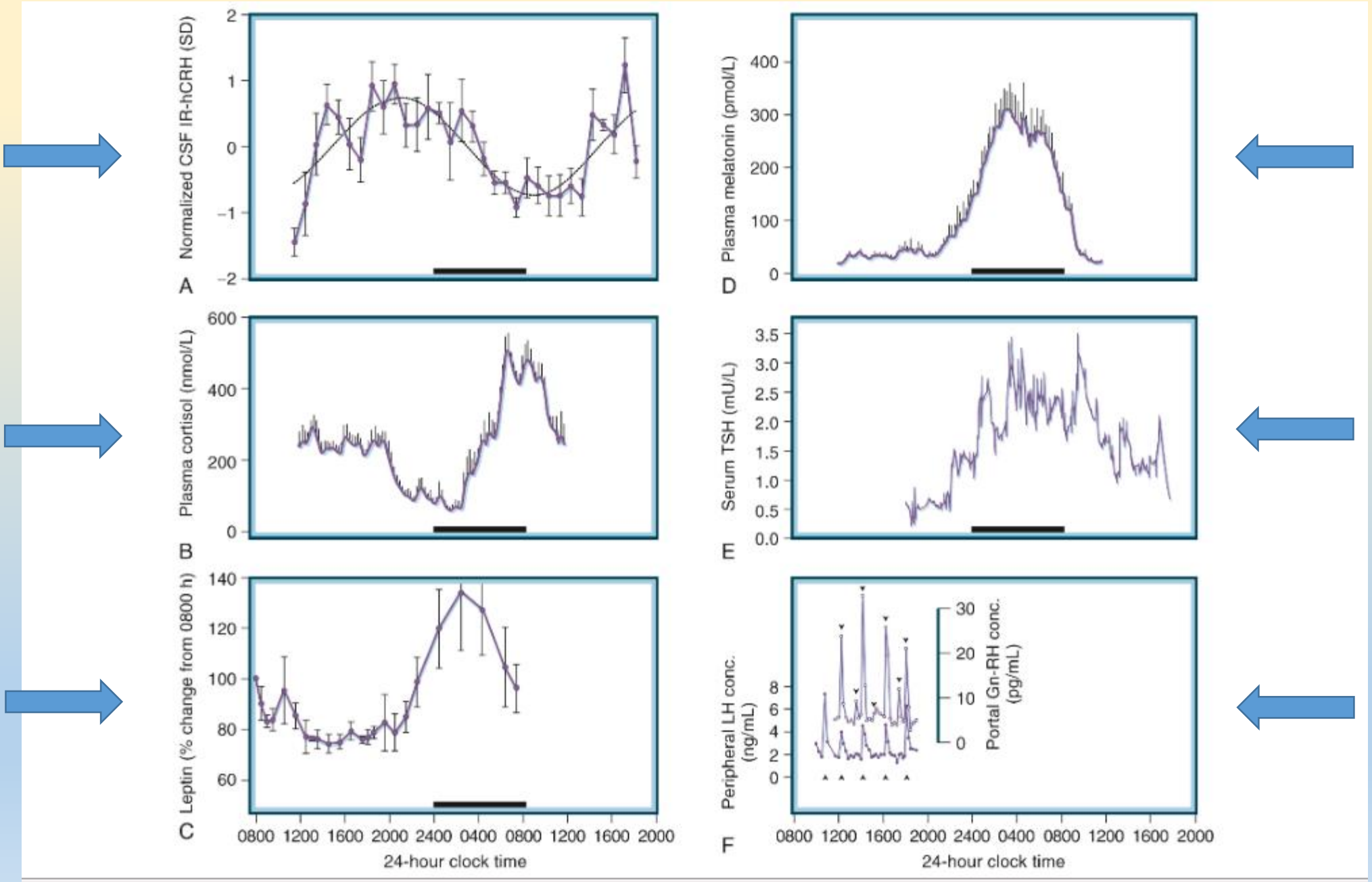
Body temperature


GIT
SCN
environment
GHIH
GHRH
Growth hormone
leptin
Hypothalamus
Adenohypophysis
Adipose tissue

CRH

cortisol

leptin



melatonin

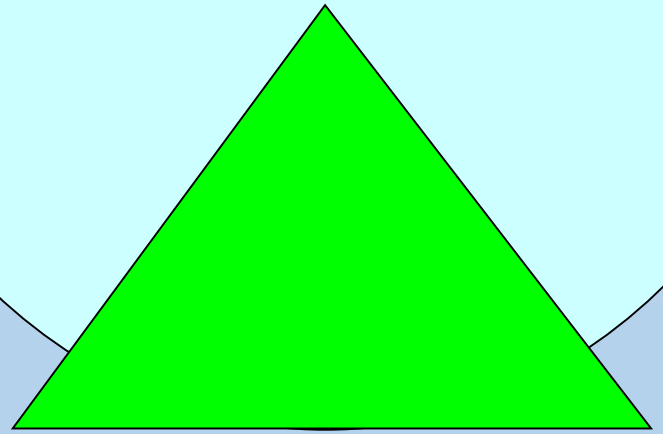
thyrotropin

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

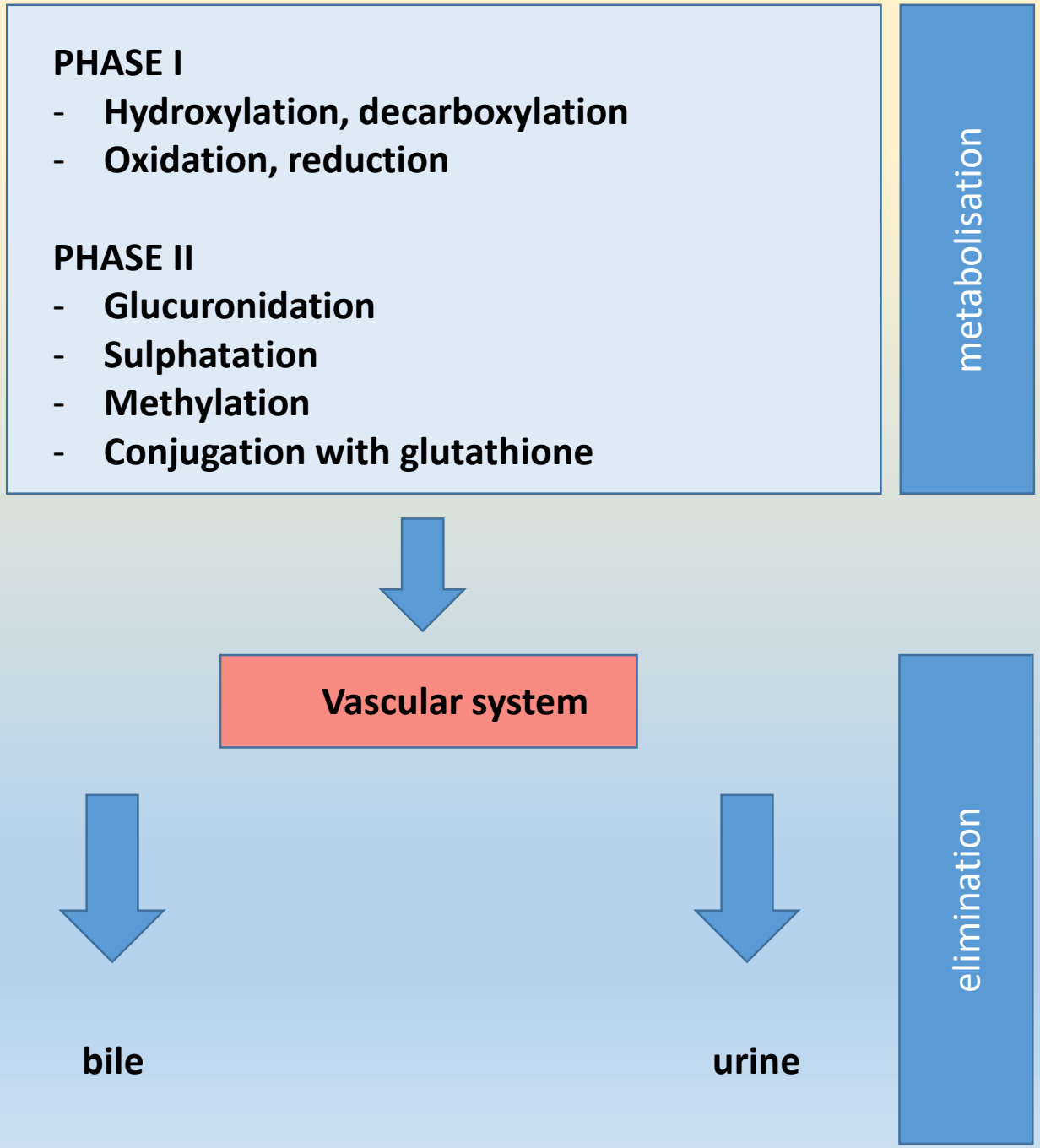
- Protection
- Reservoir
- Ubiquitous distribution
- Transport across plasmatic membrane (SHBG – megalin)



DYNAMIC BALANCE BETWEEN HORMONE AND TRANSPORT PROTEIN

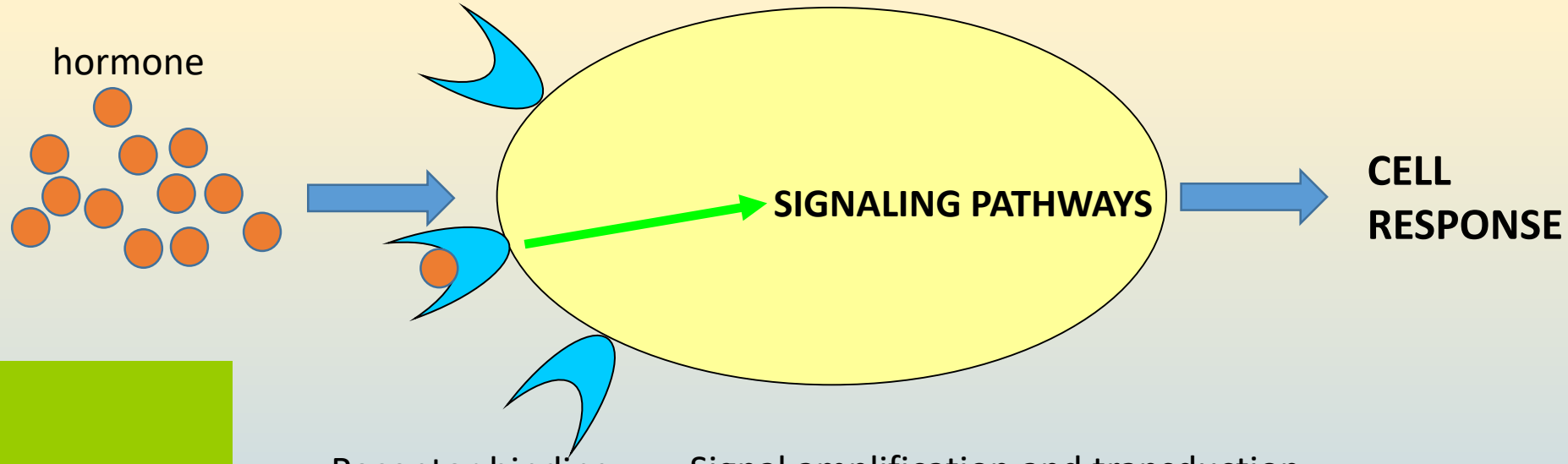
Hormone elimination

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



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

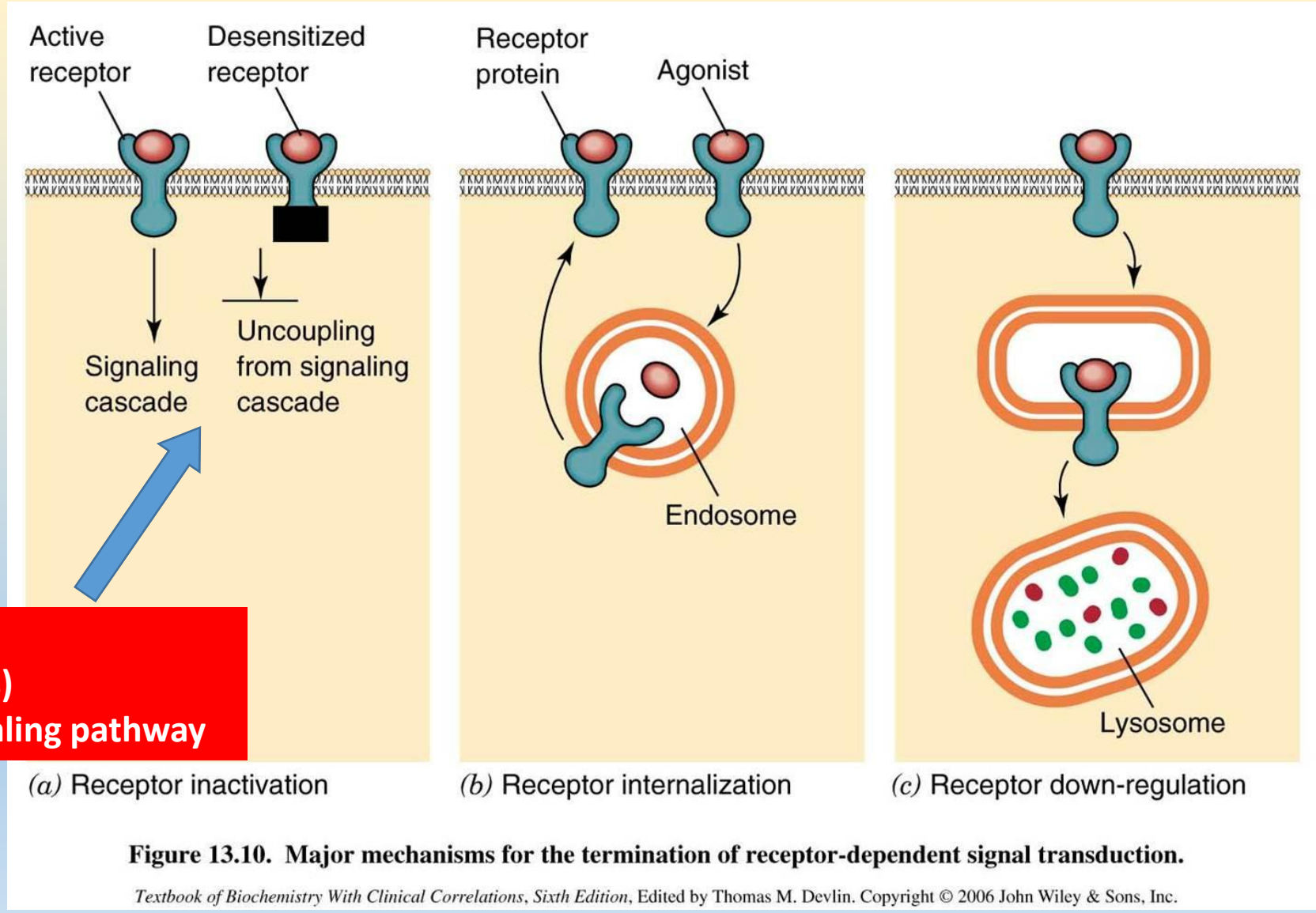
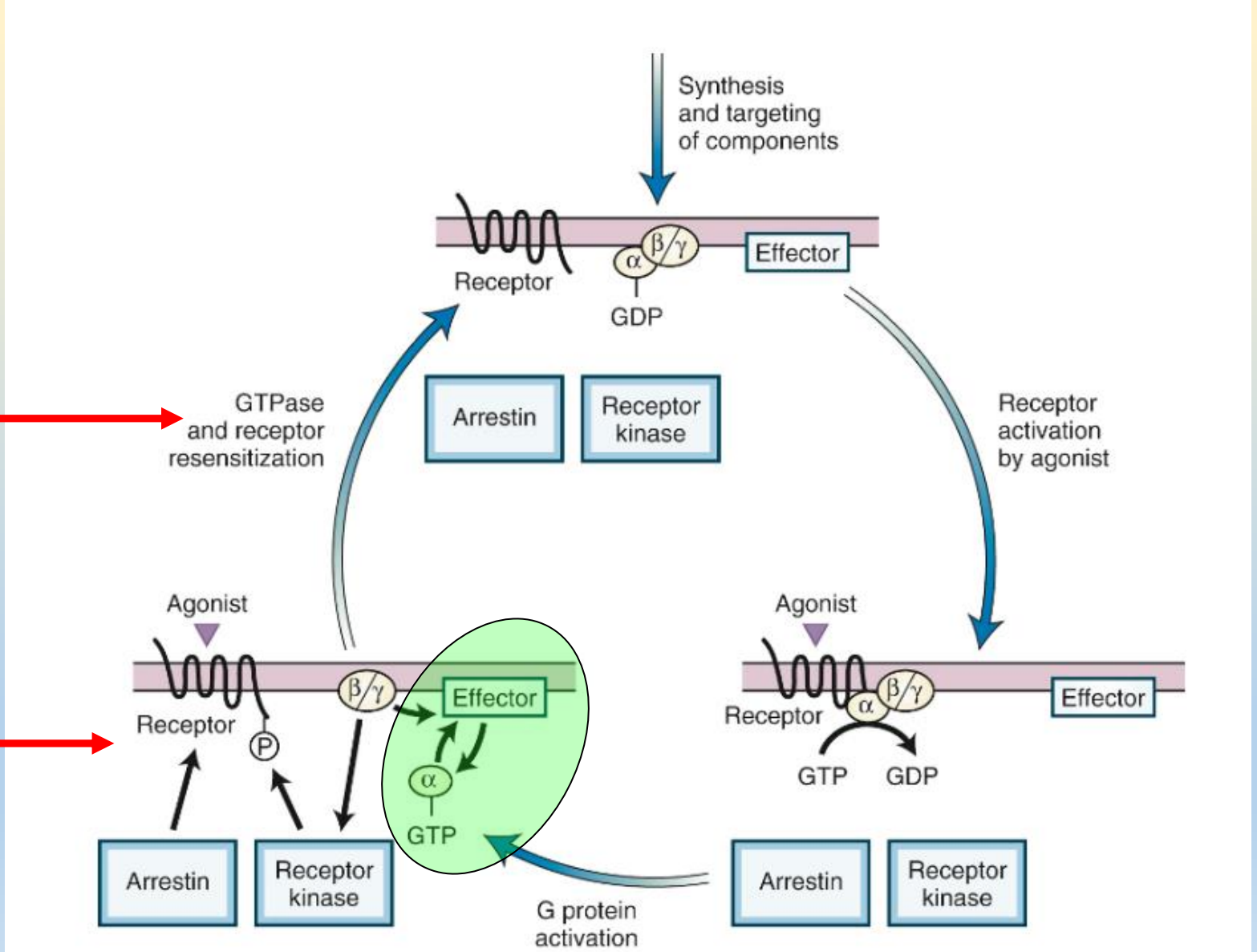


Figure 13.10. Major mechanisms for the termination of receptor-dependent signal transduction.

Sensitisation and desensitisation of G protein-coupled proteins

- α subunit with GTPase activity
  - resensitisation
  - desensitisation

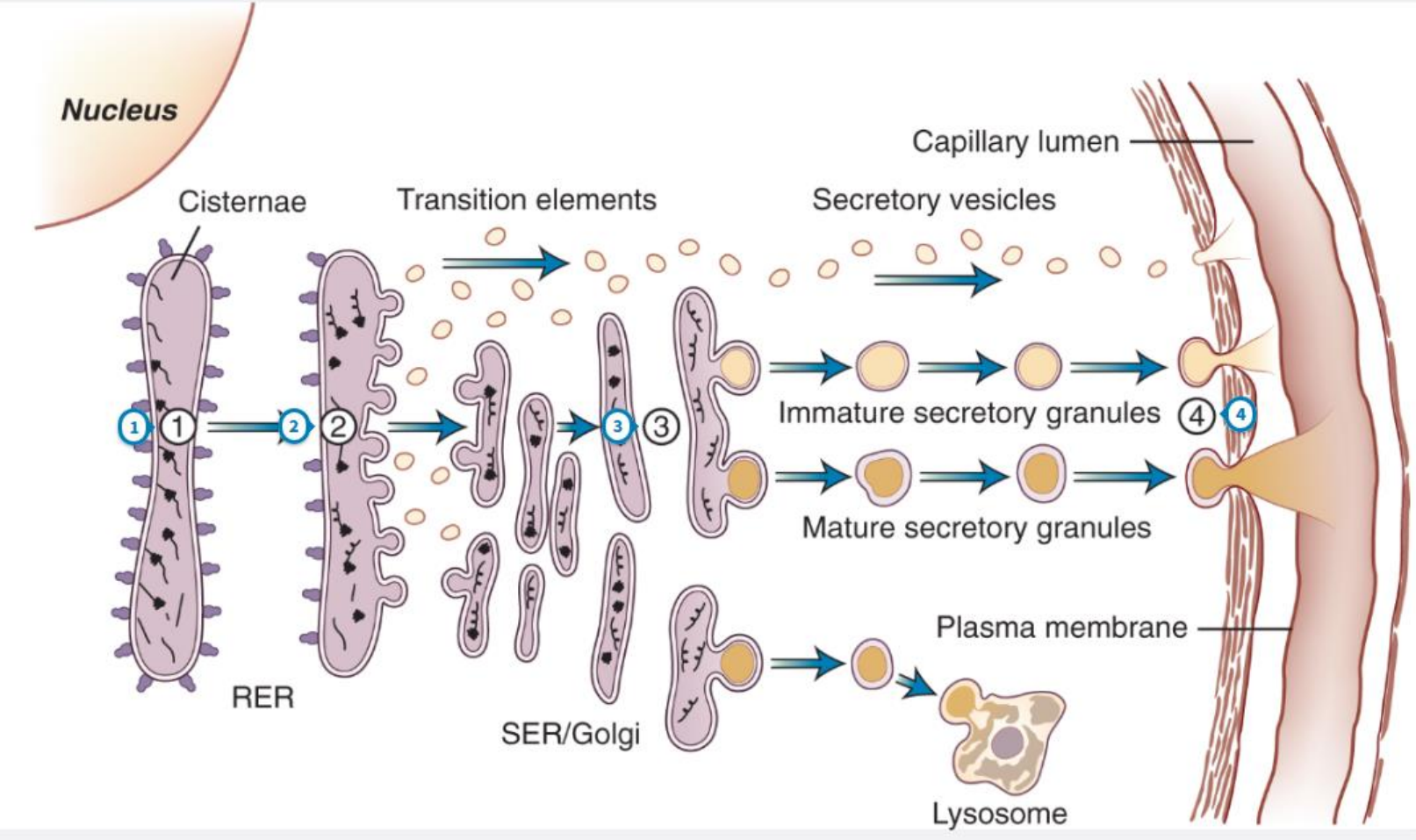


Hormones – proteins and peptids

„classic“ hormones

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

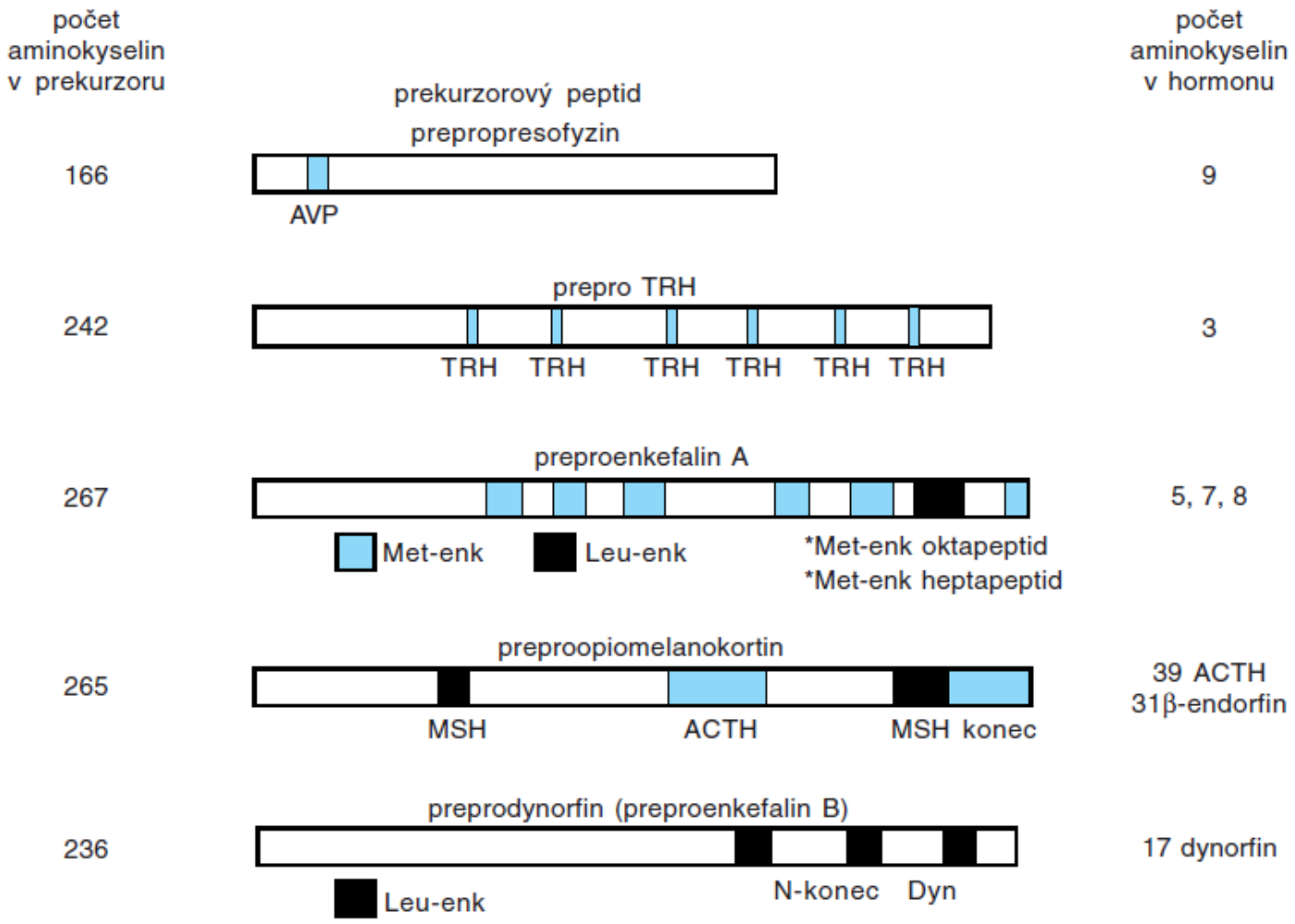
Paracrine/autocrine peptides



Receptors associated with plasmatic membrane

preprohormone – prohormone – hormone (+ fragments)

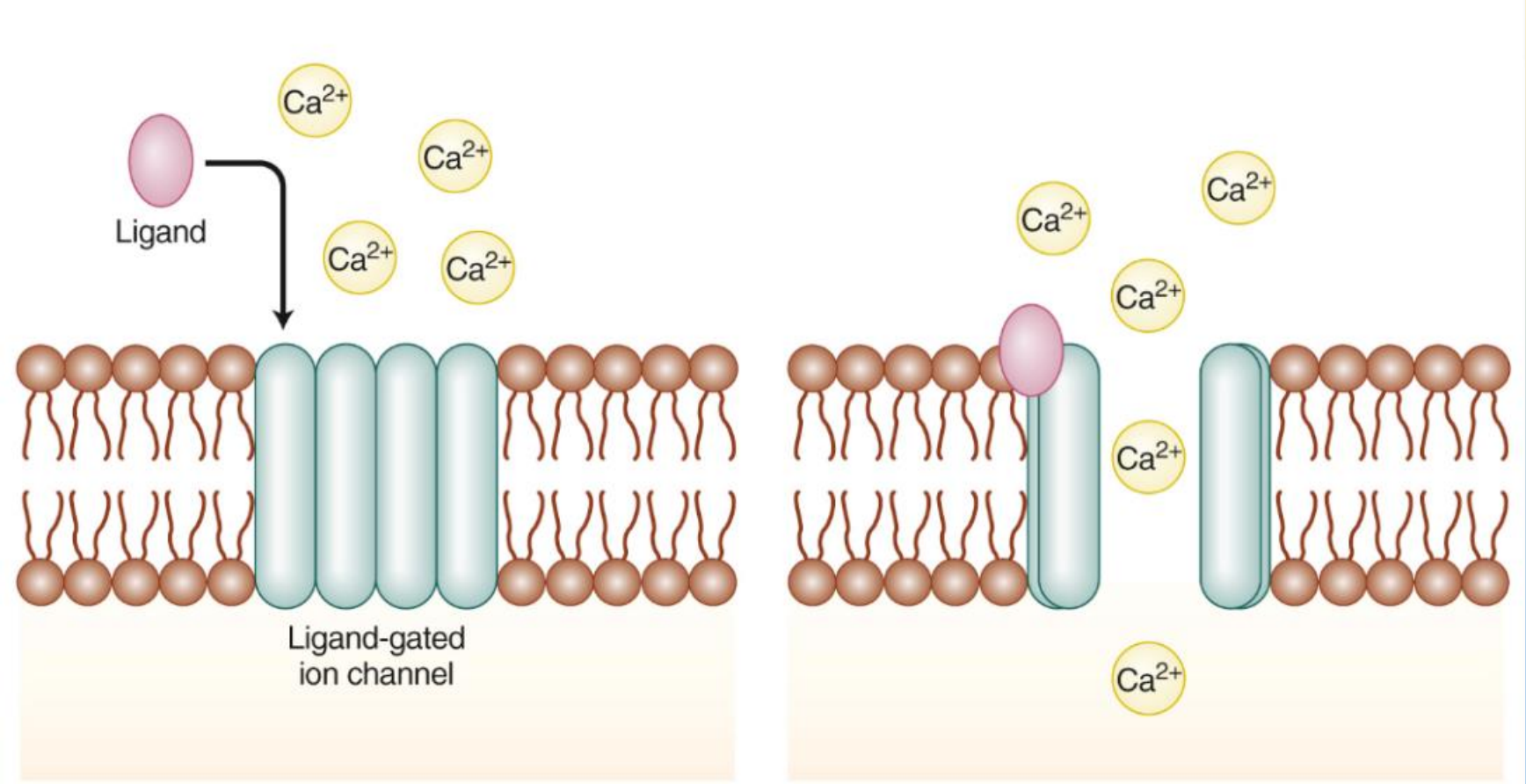
Peptide hormones as a part of preprohormones



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

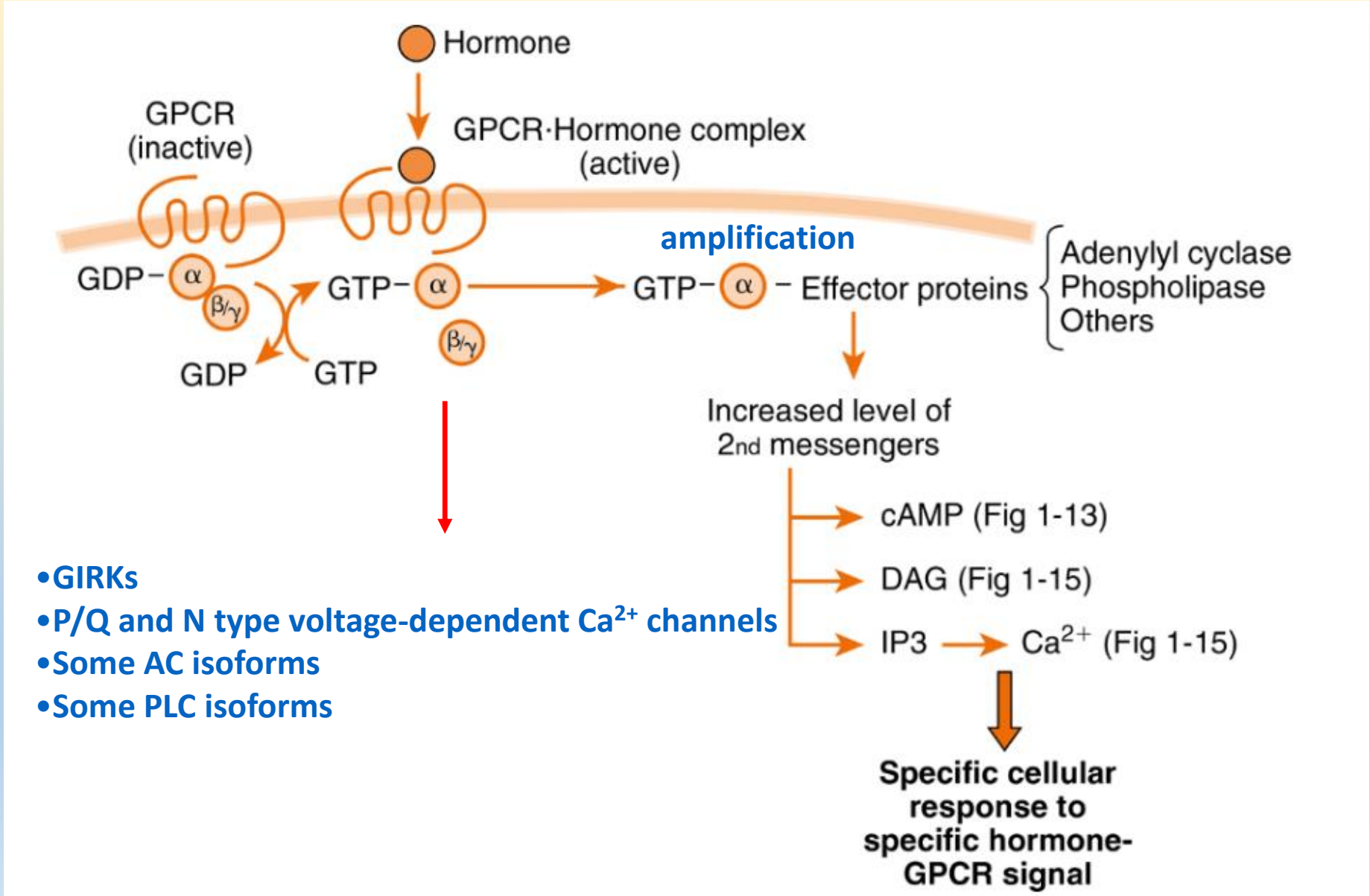
Taken from Ganong, W. F. Přehled lékařské fyziologie. 20th edition. Galén 2005.

Ligand-gated ion channels



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 Ca^{2+} channels**
- **Some AC isoforms**
- **Some PLC isoforms**

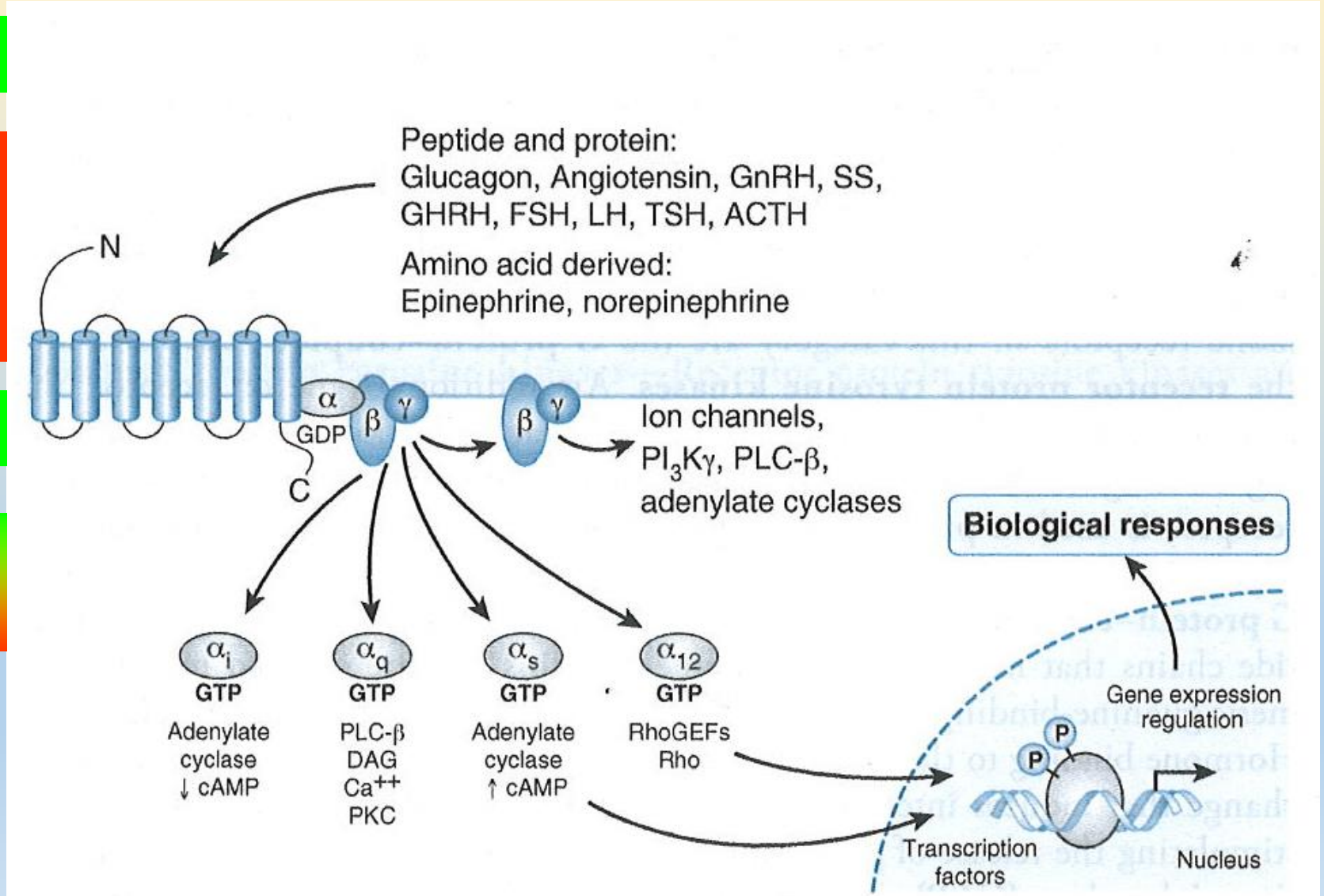
G protein-coupled receptors (GPCR)

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)

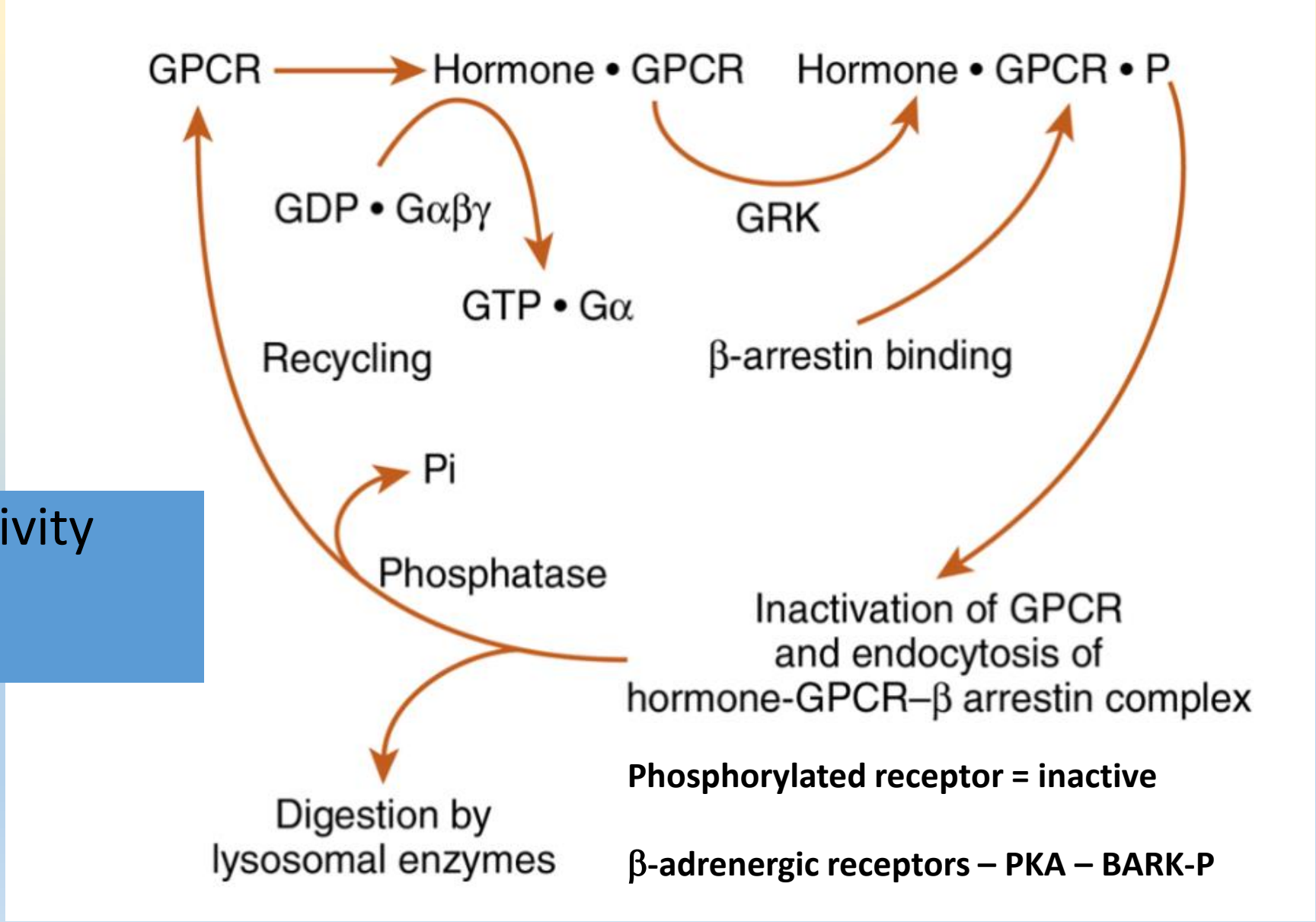
$G_{q/11}$ – activation of PLCβ

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

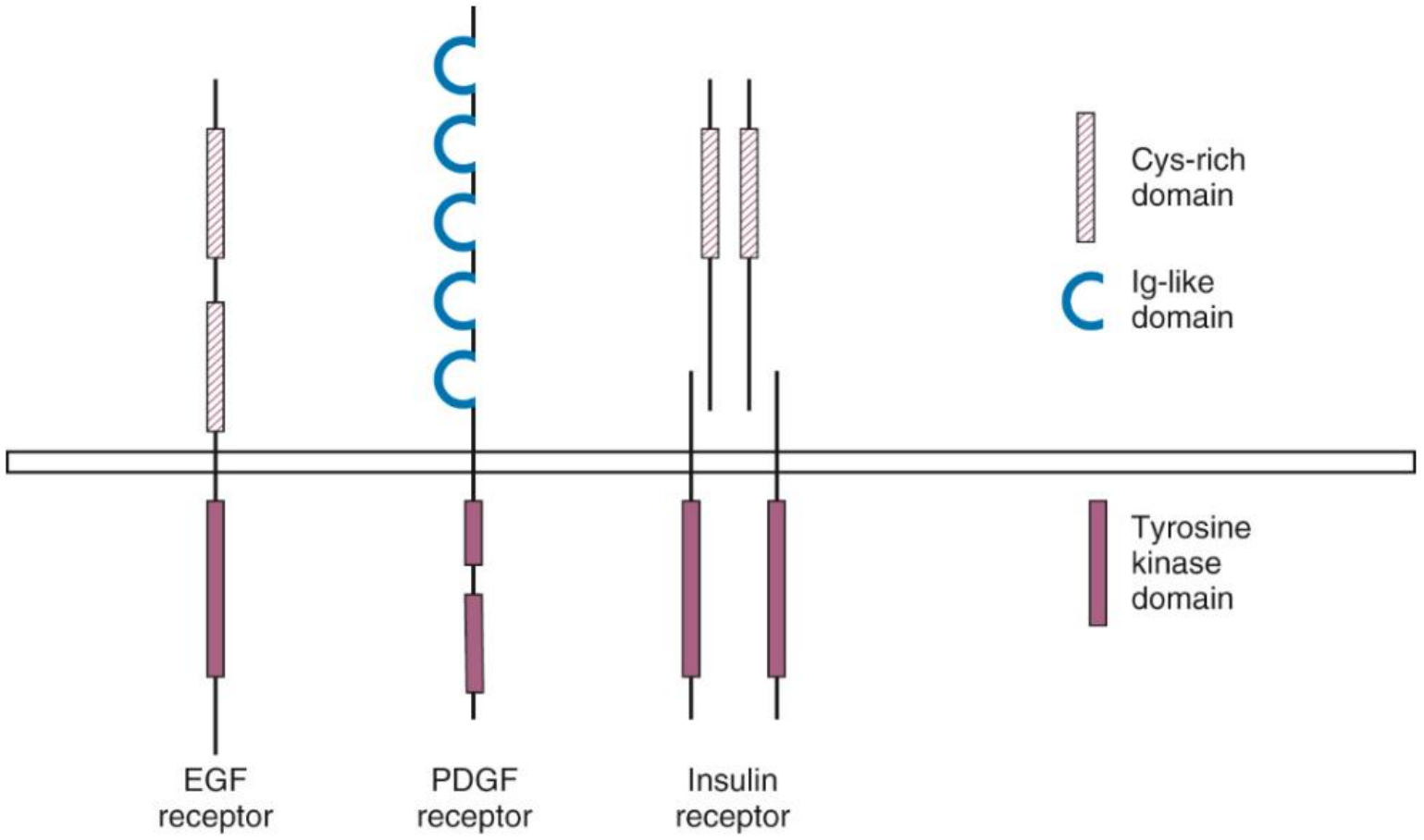


End of activation and limitation of cell response

- Intrinsic GTPase activity
- Endocytosis



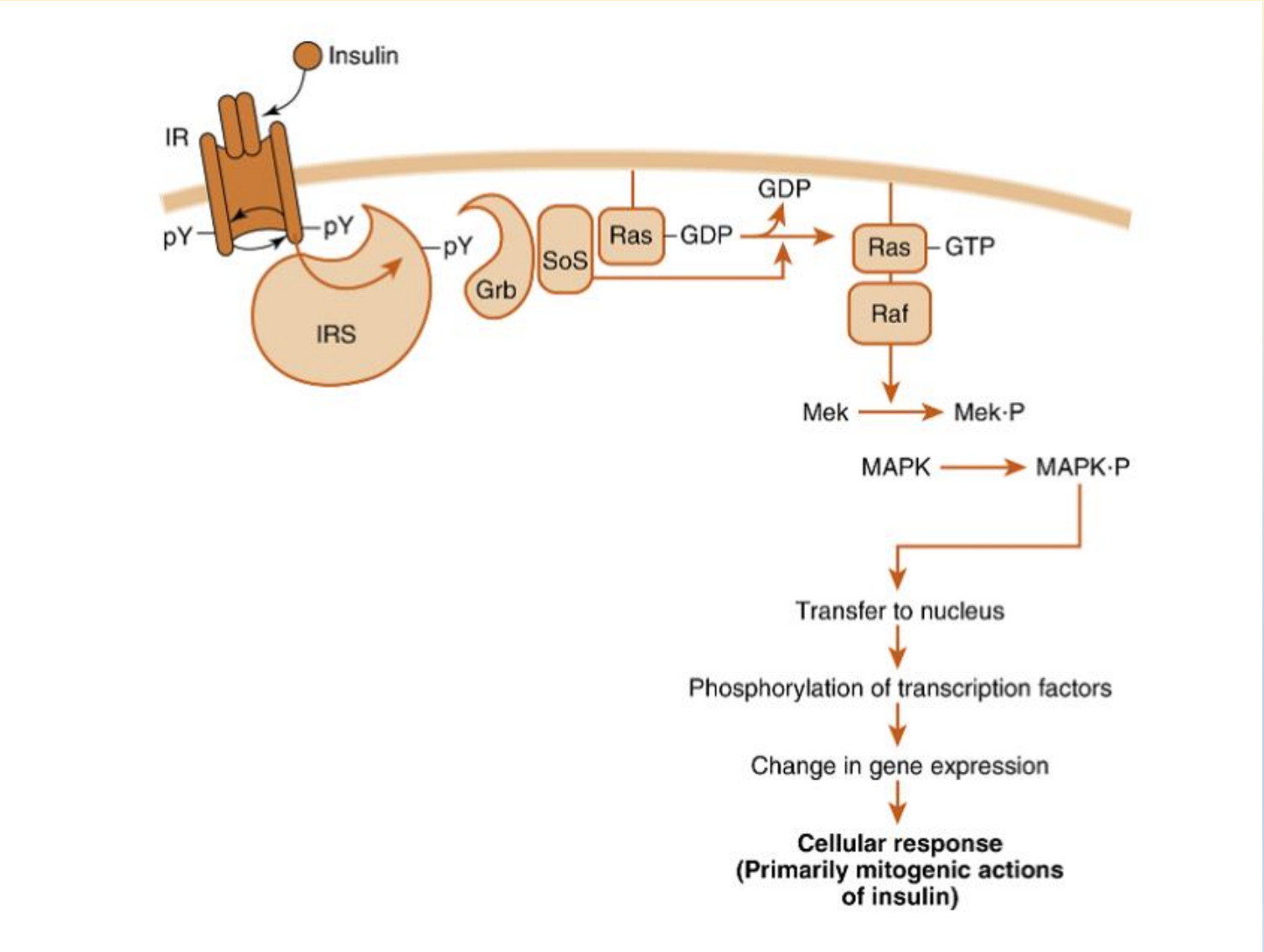
Receptor tyrosinkinases



- 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

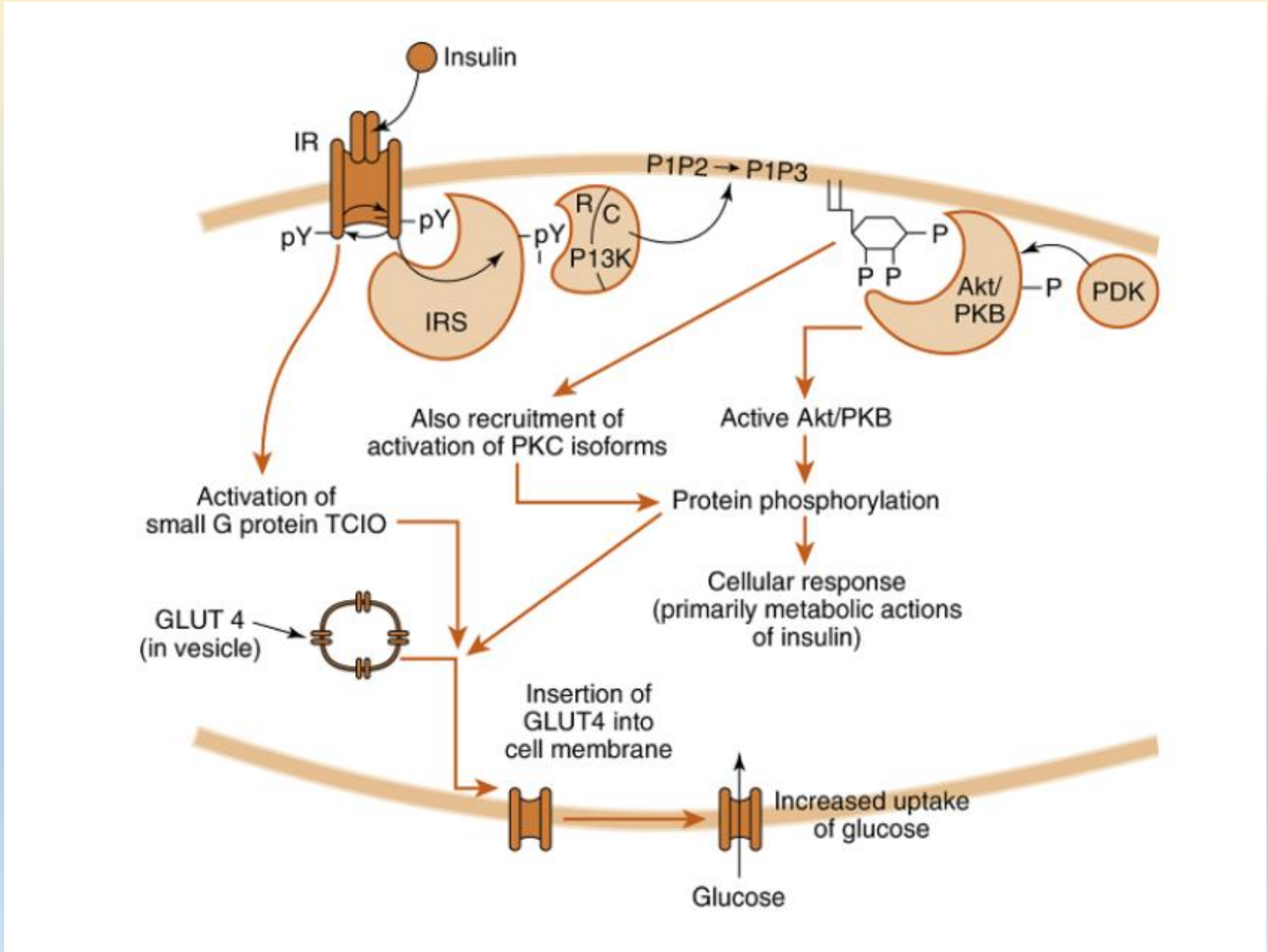
Insuline receptor – genomic effects

- 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



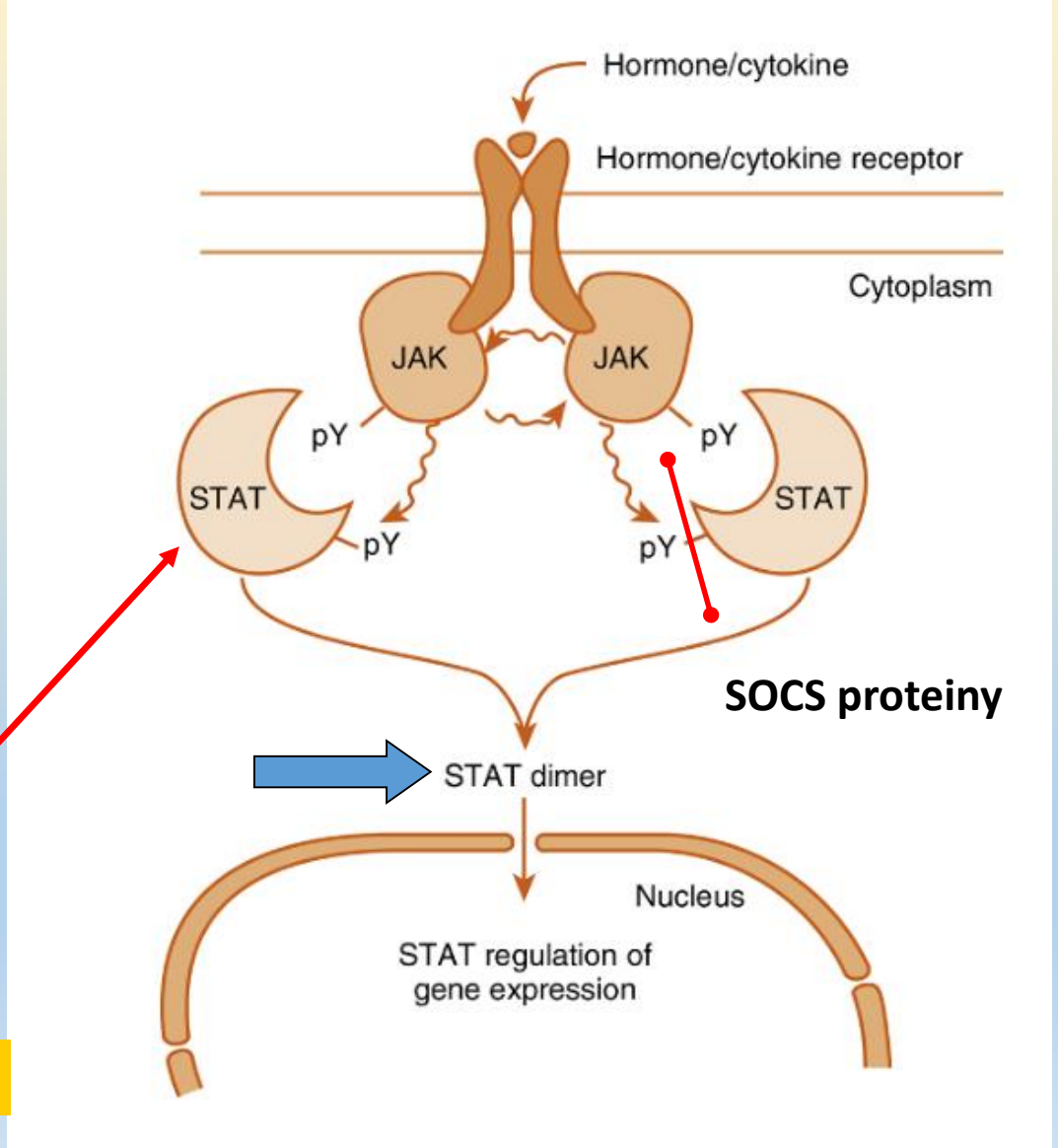
Insulin receptor – metabolic effects

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



Receptors associated with cytosolic TK

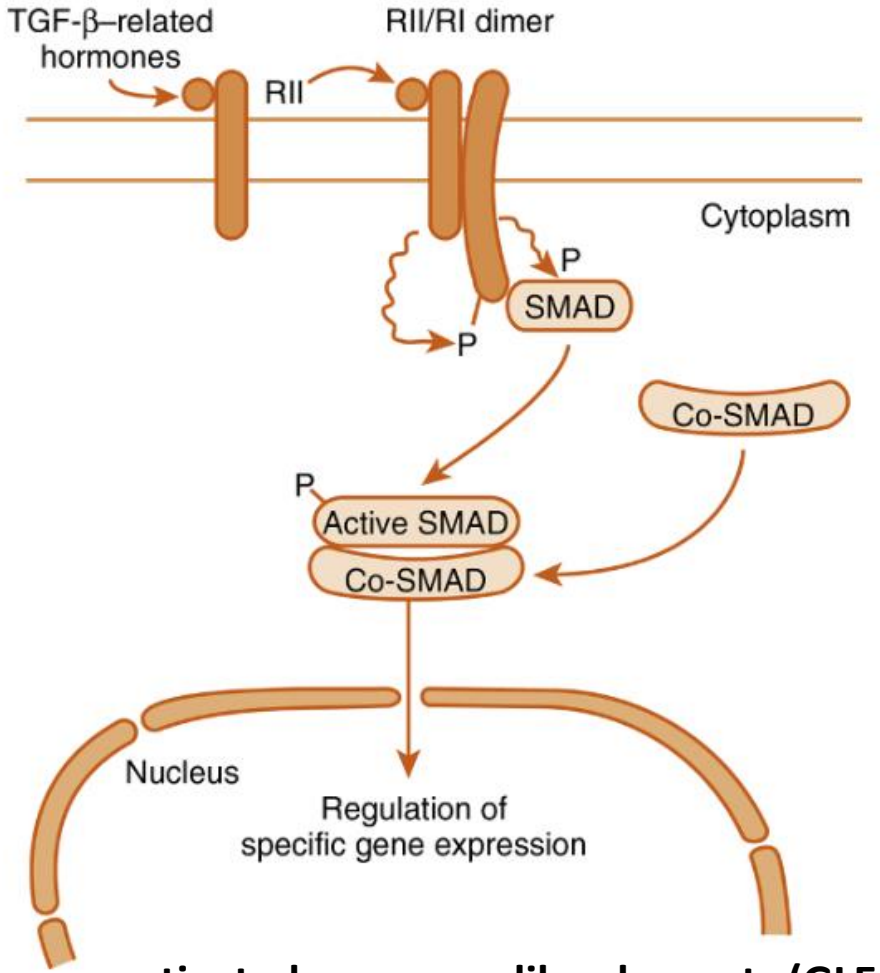
- GH, prolactin, leptin, erythropoietin
- Dimeric receptor **without** TK activity
- Association with JAK kinase
- After ligand binding – dimerisation, transphosphorylation, activation



signal transducers and activators of transcription

Receptor serin/threonin kinases

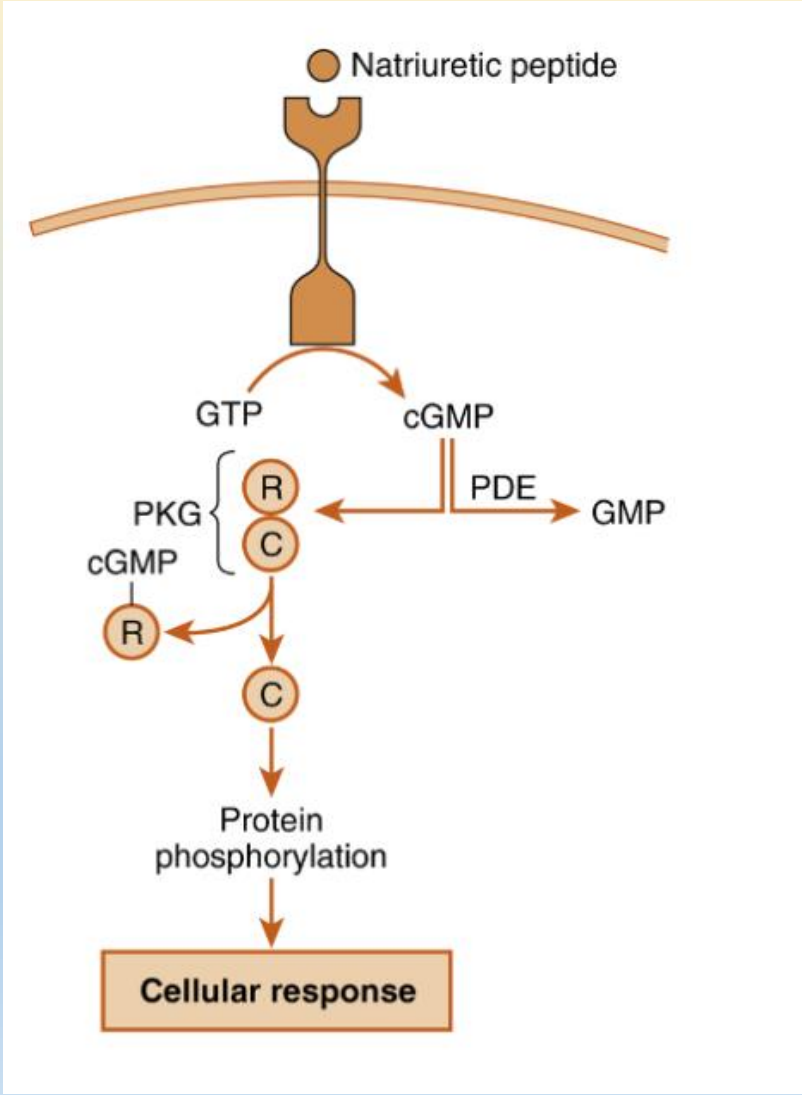
- Anti-Müllerian hormone, inhibitin
- Form of dissociated heterodimer
- SMAD = „latent transcription factors“



gamma-activated sequence-like elements (GLEs, 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

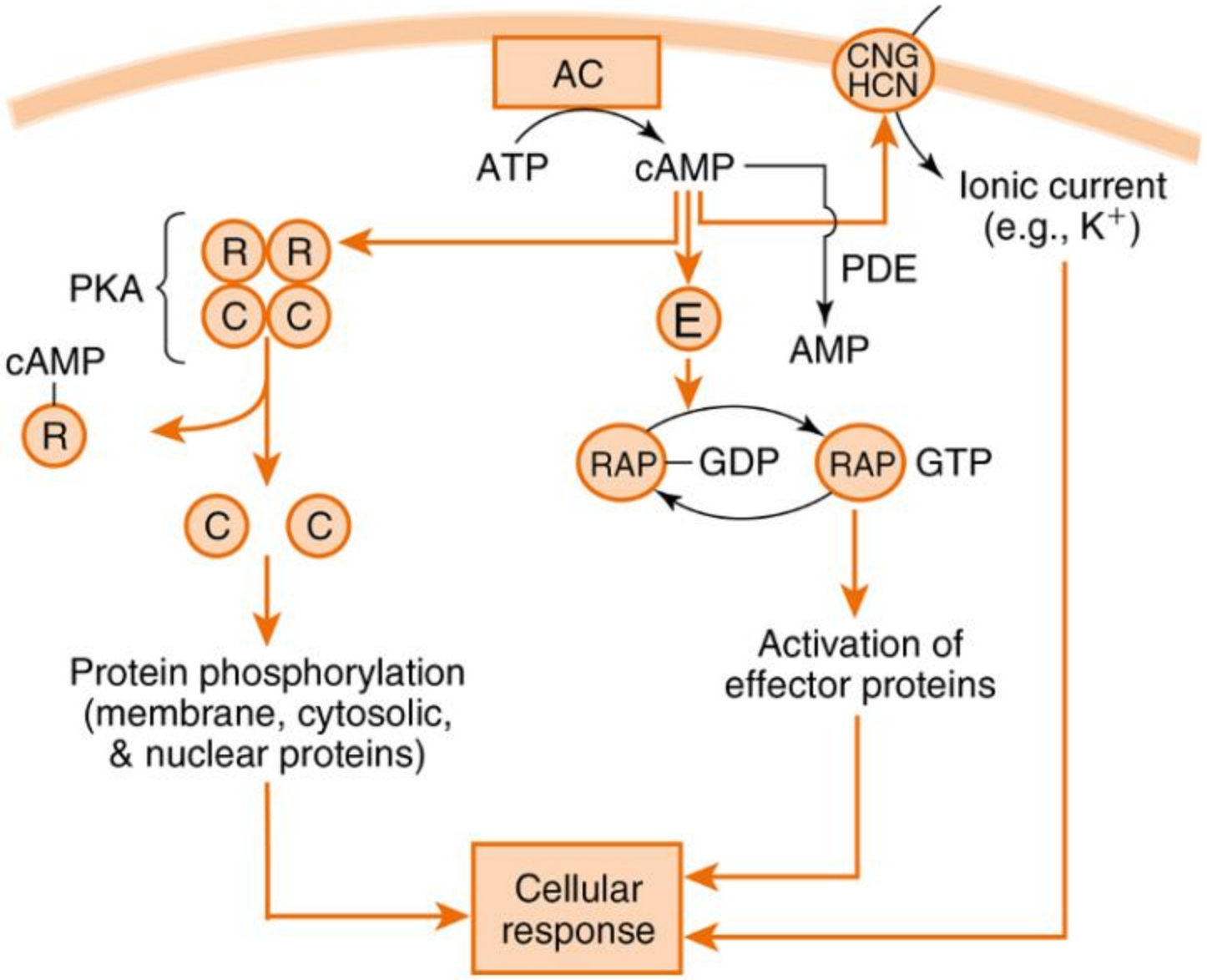
- DAG and IP_3
  - PIP_2 – phospholipase C system

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

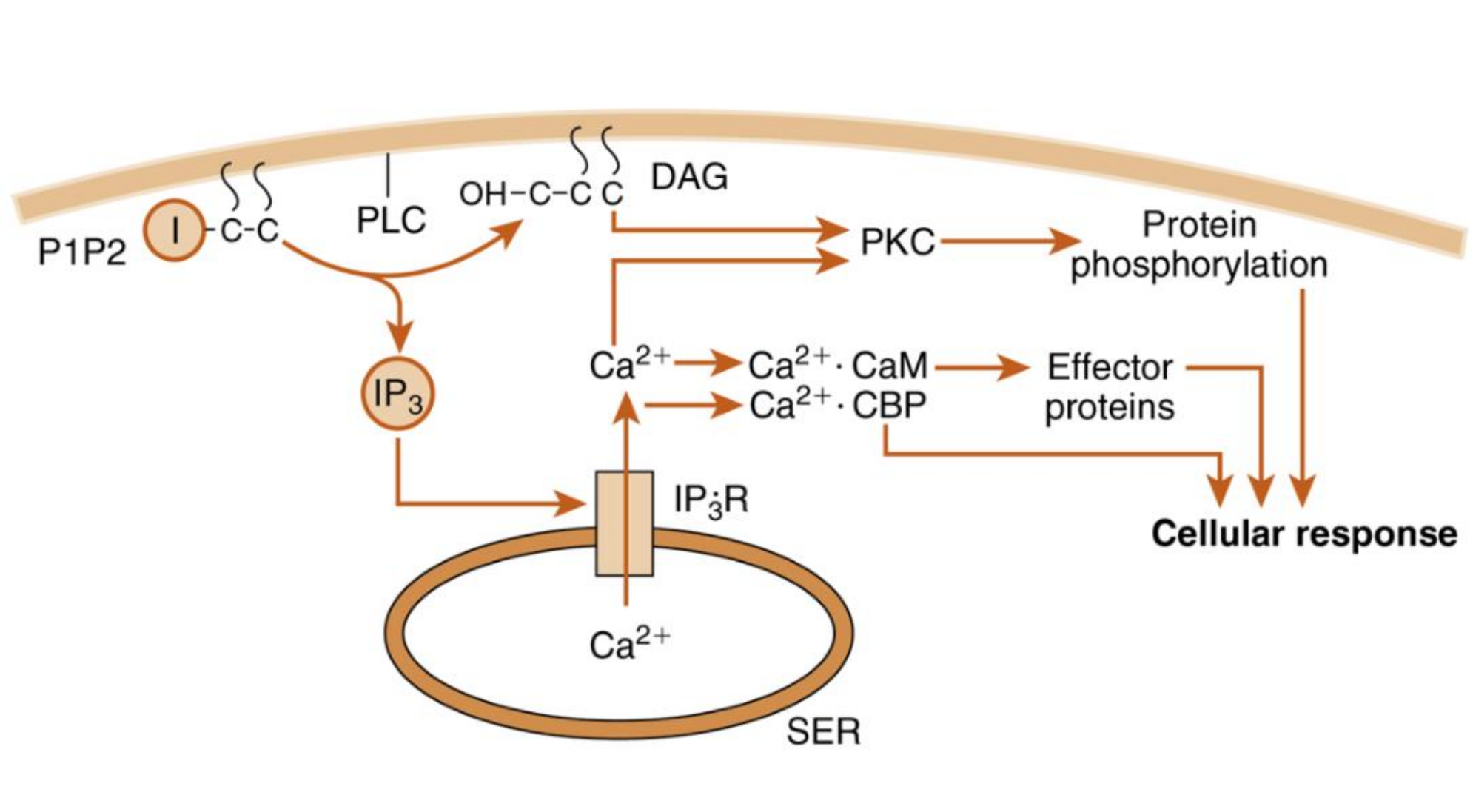
EXTRACELLULAR SIGNAL MUST BE CONVERTED TO INTRACELLULAR RESPONSE

AC – cAMP system

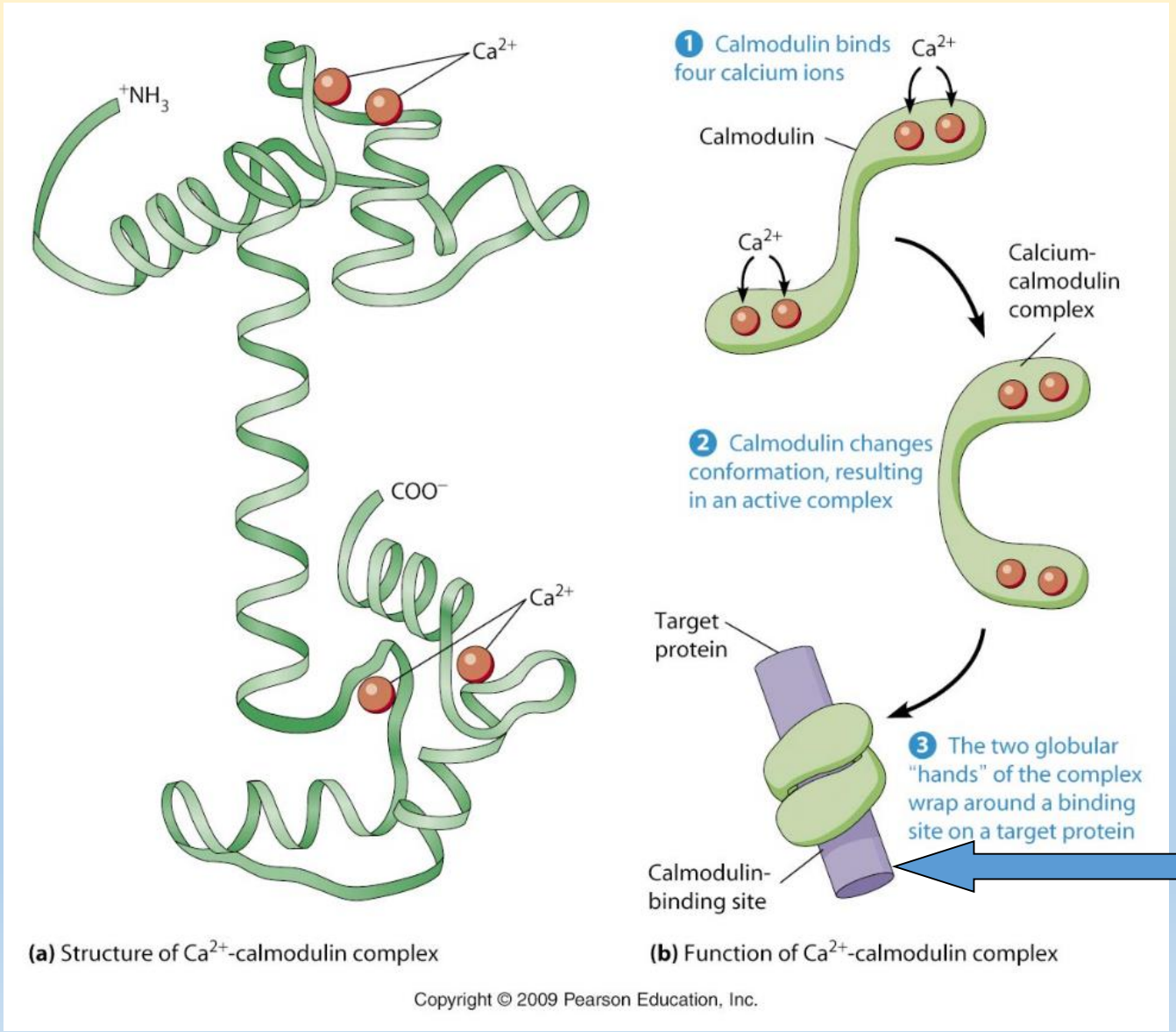
- PKA
- CREB (cAMP-responsive element–binding protein)
- Epac (E) as an another effector molecule (exchange protein activated by cAMP)
- cyclic nucleotide gated (CNG) channels
- hyperpolarization-activated cyclic nucleotide modulated (HCN) channels
- phosphodiesterases



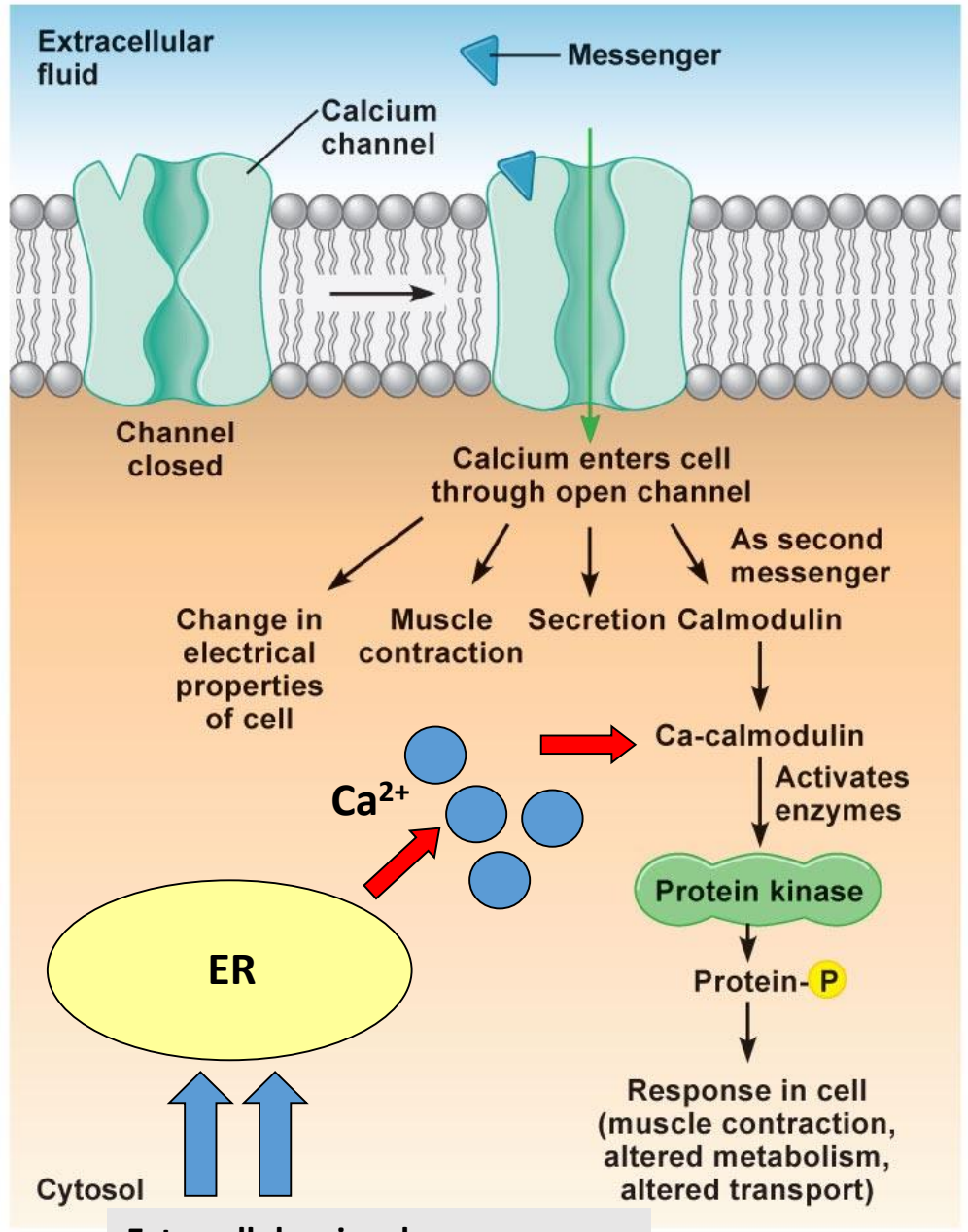
PLC - DAG and IP_3 system



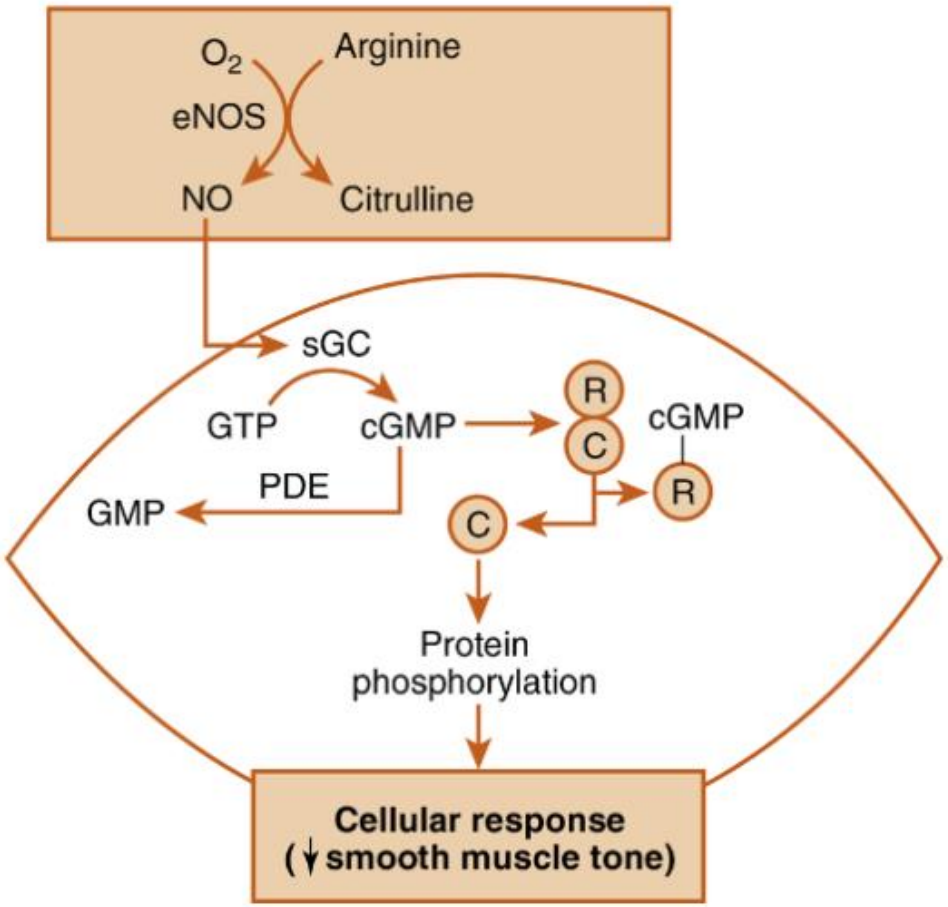
Ca^{2+} - calmodulin system

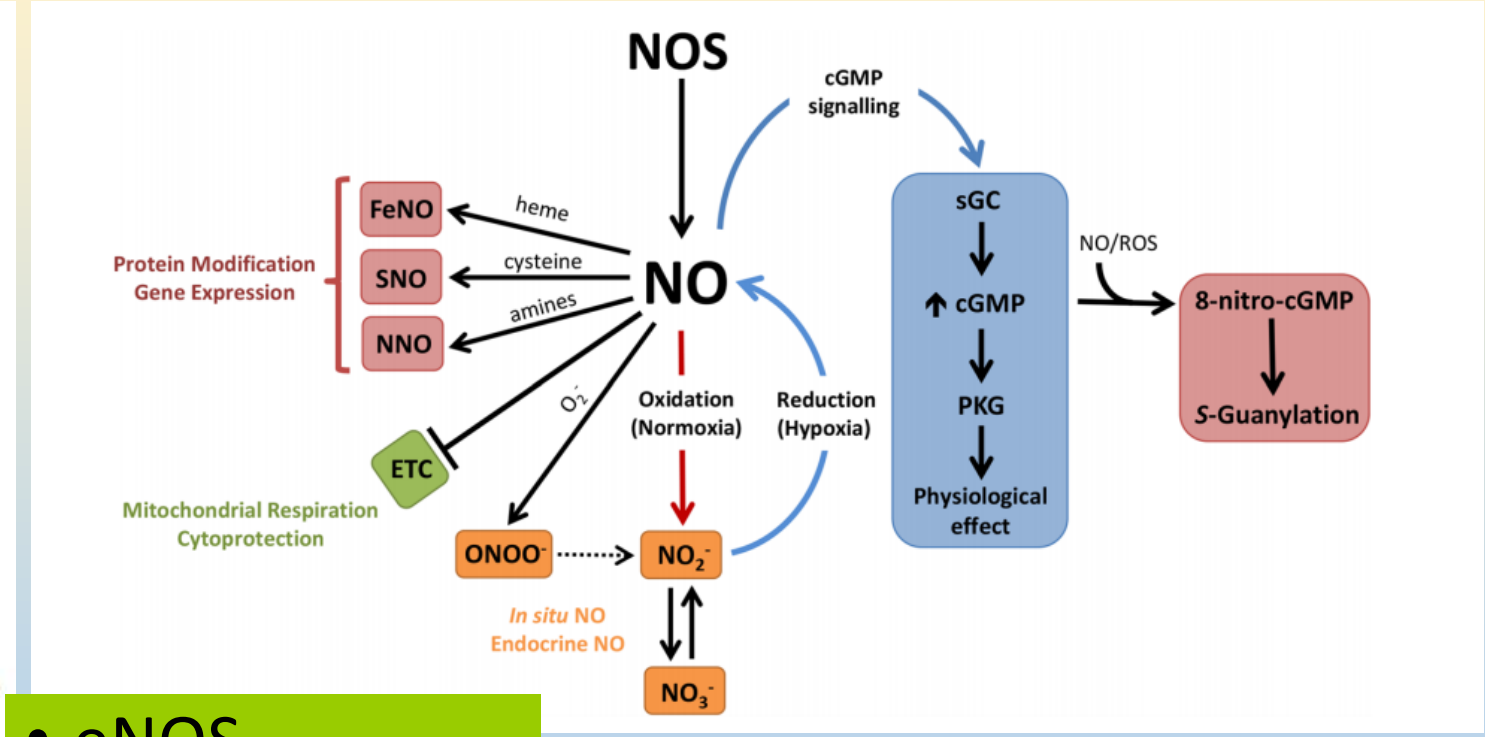


calmodulin-dependent kinases



NO as a signalling molecule - cGMP

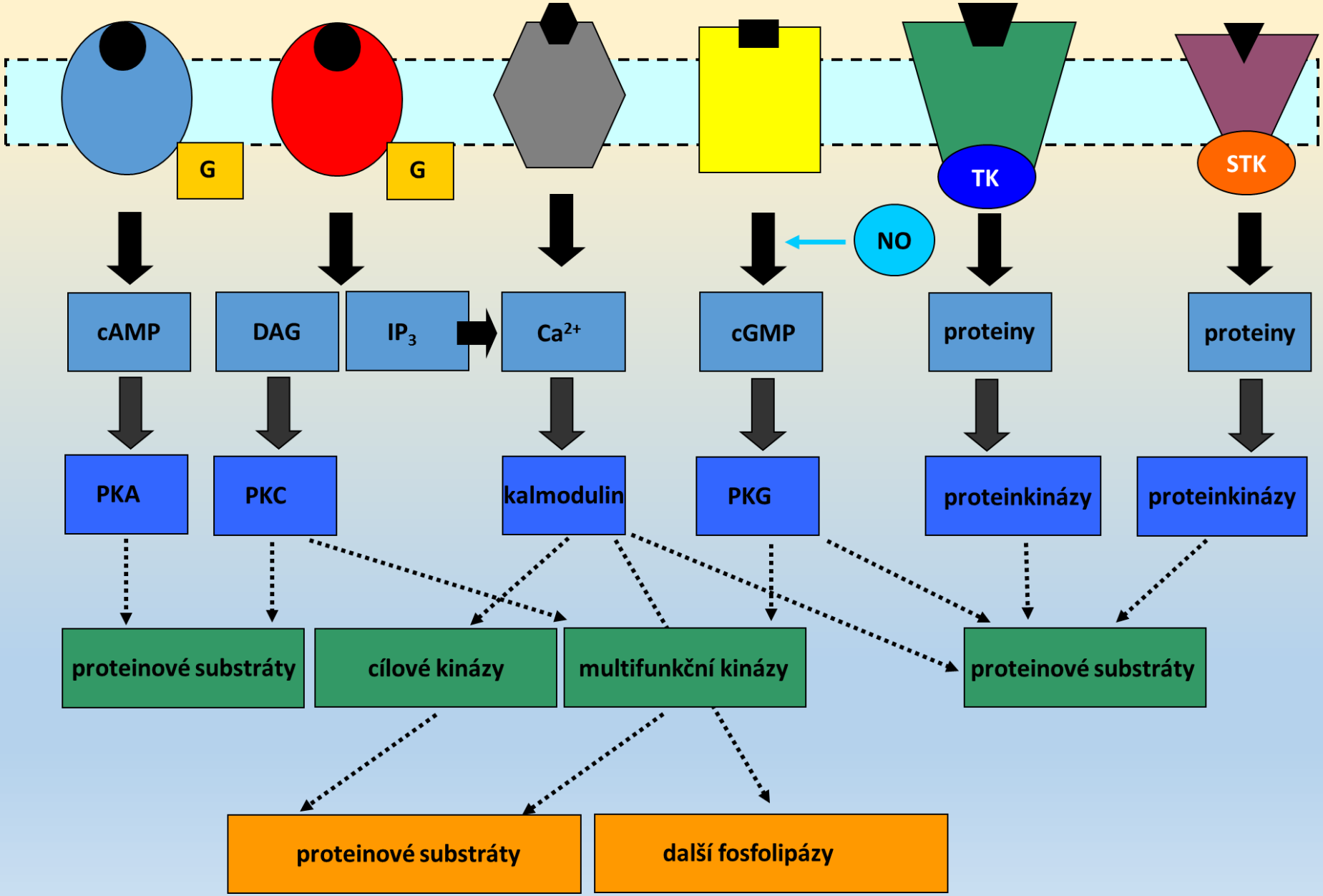




- eNOS
- nNOS
- iNOS

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.***

Summary – membrane receptors and associated systems



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 ← heterodimers

-Estrogens – ERα/β

-Testosterone - AR

-Progesterone - PR

-Aldosterone - MR

-Cortisol - GR

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

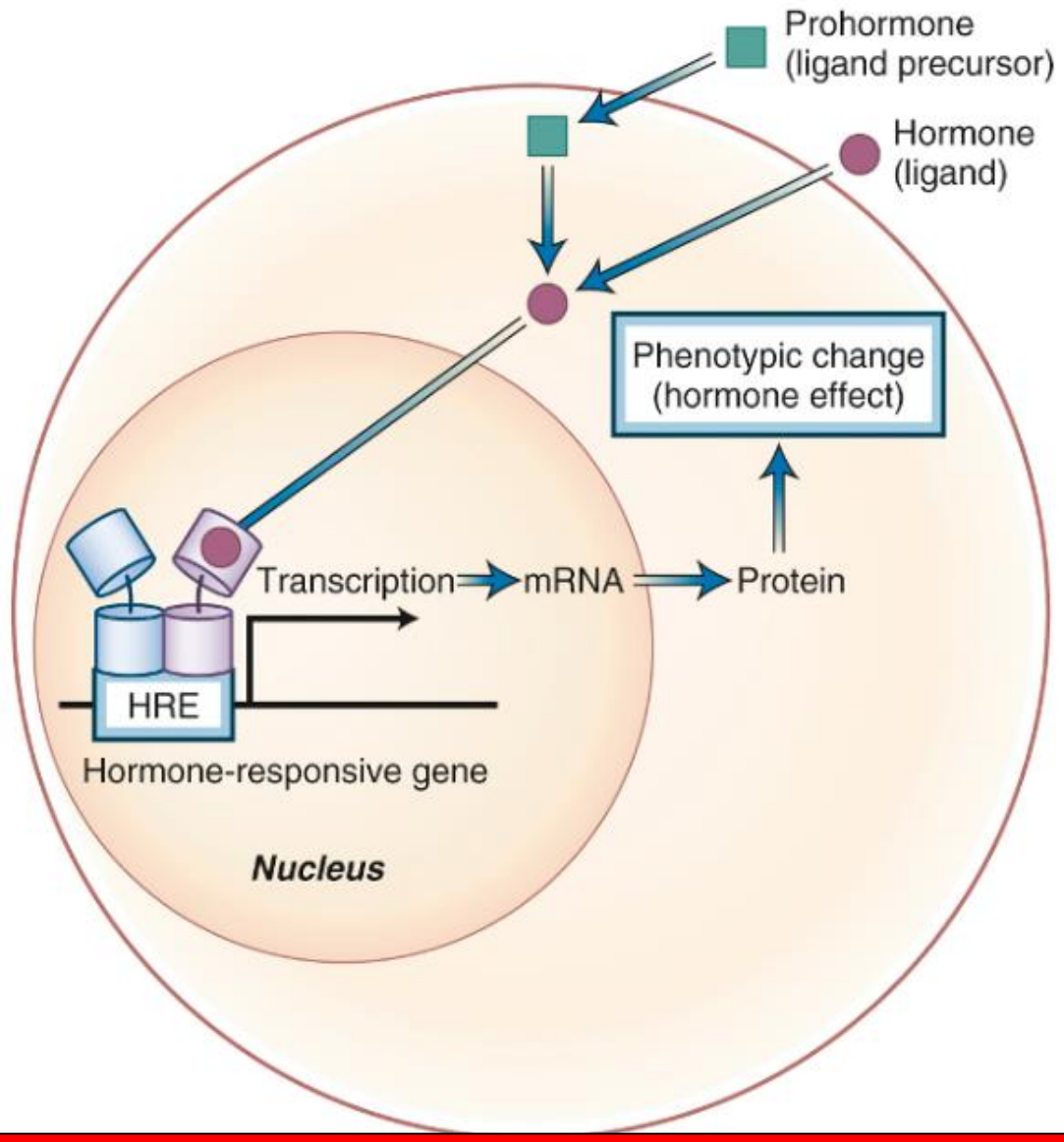
-Variable receptors

Explanation of some effects and pathologies

General mechanism of effect of hormones acting through nuclear receptors

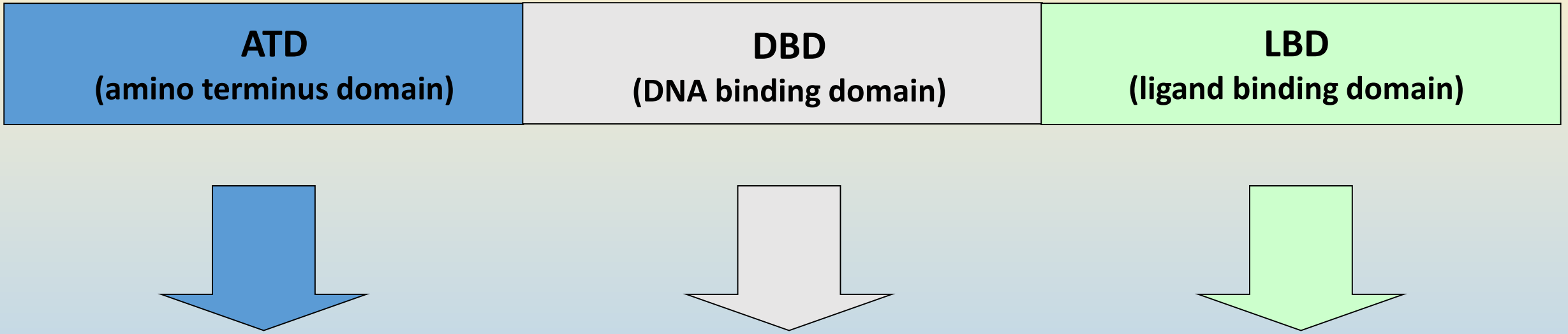
-High affinity of ligand bond = due to R structure
-Recognition of specific promotor region
-Dimerisation of receptors (homodimers, heterodimers)
-Remodelation of chromatin for gene expression (HDAC)
-Gene expression at the end decreased or increased

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

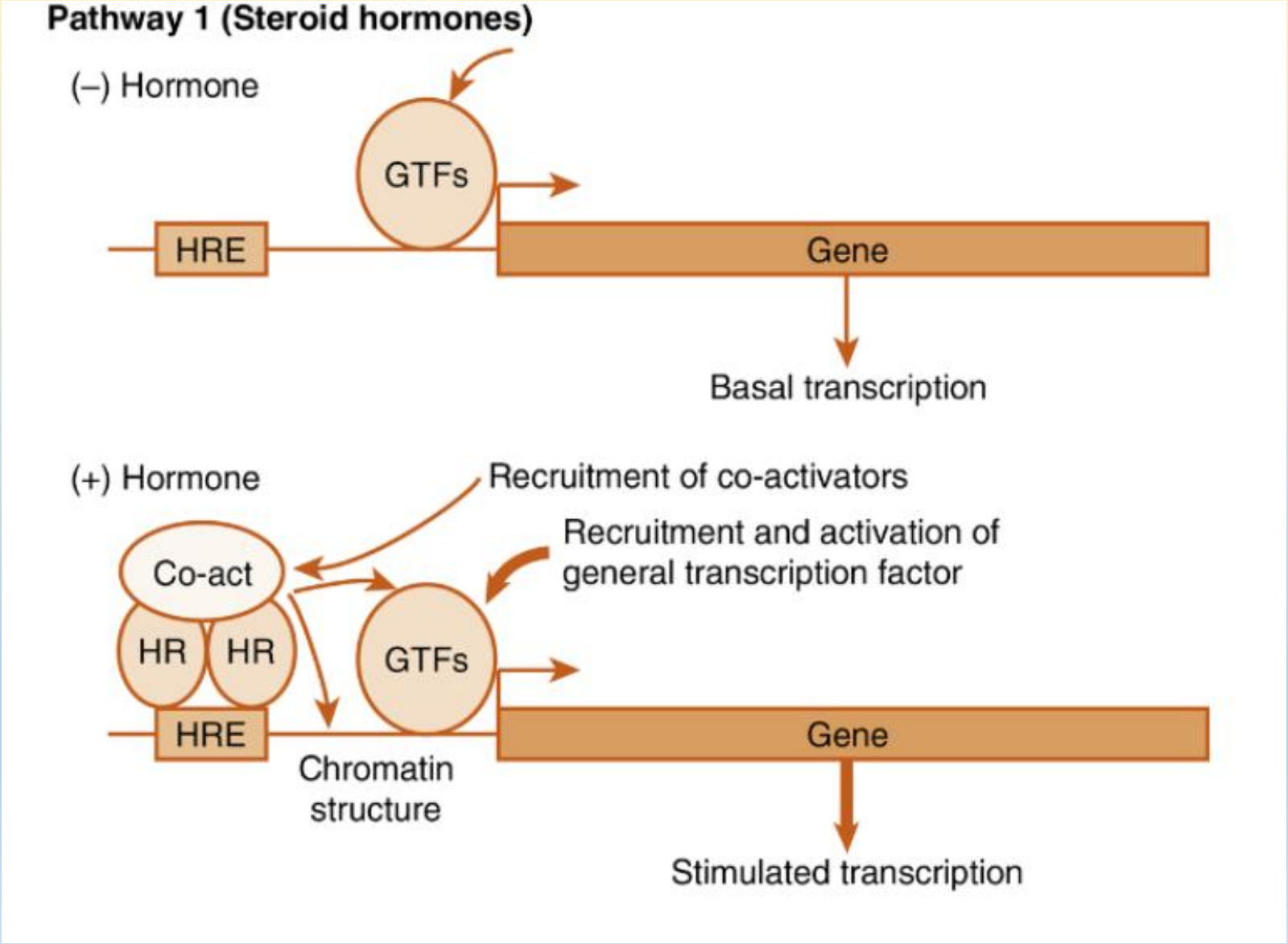
-DNA binding (zinc fingers)
-Dimerisation
-ERE, PRE, GRE, MRE, ARE

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

Example – steroid hormones

GTFs = general transcription factors (remodulators of chromatin)

HAT = histon acetyltransferase



Example – thyroid hormones

THRs, VDR, PPARs, RXRs

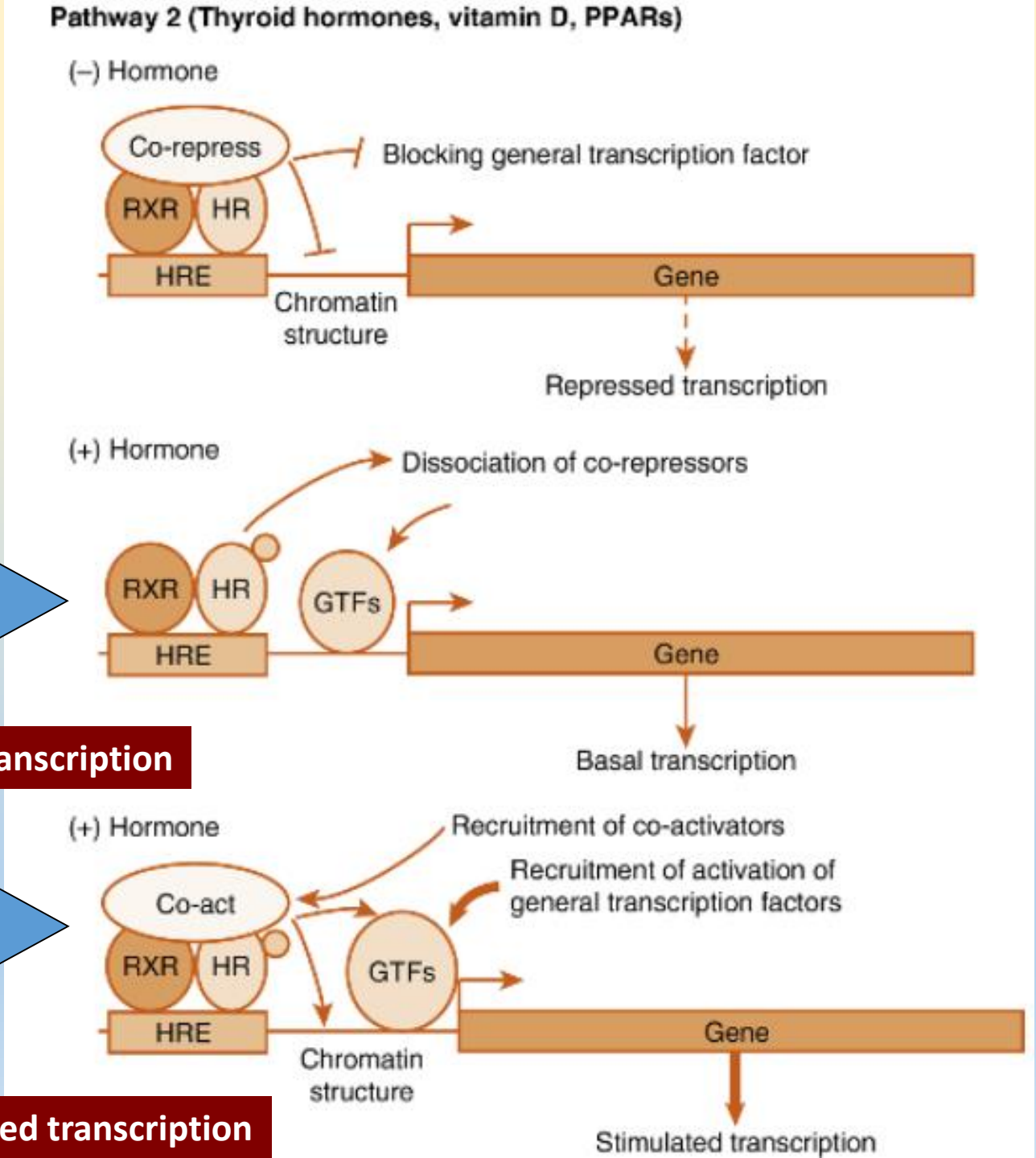
THR = heterodimer

hormone

basic transcription

hormone + RA

stimulated transcription



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)

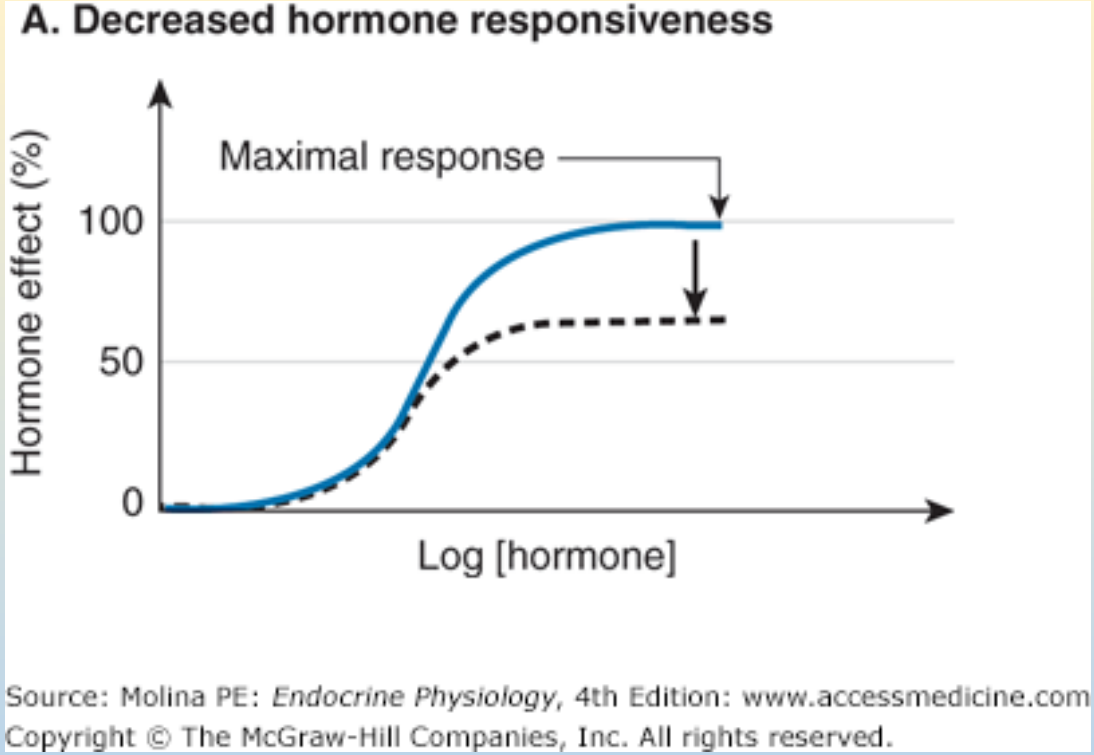
Inner enzymatic activity and its regulation

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

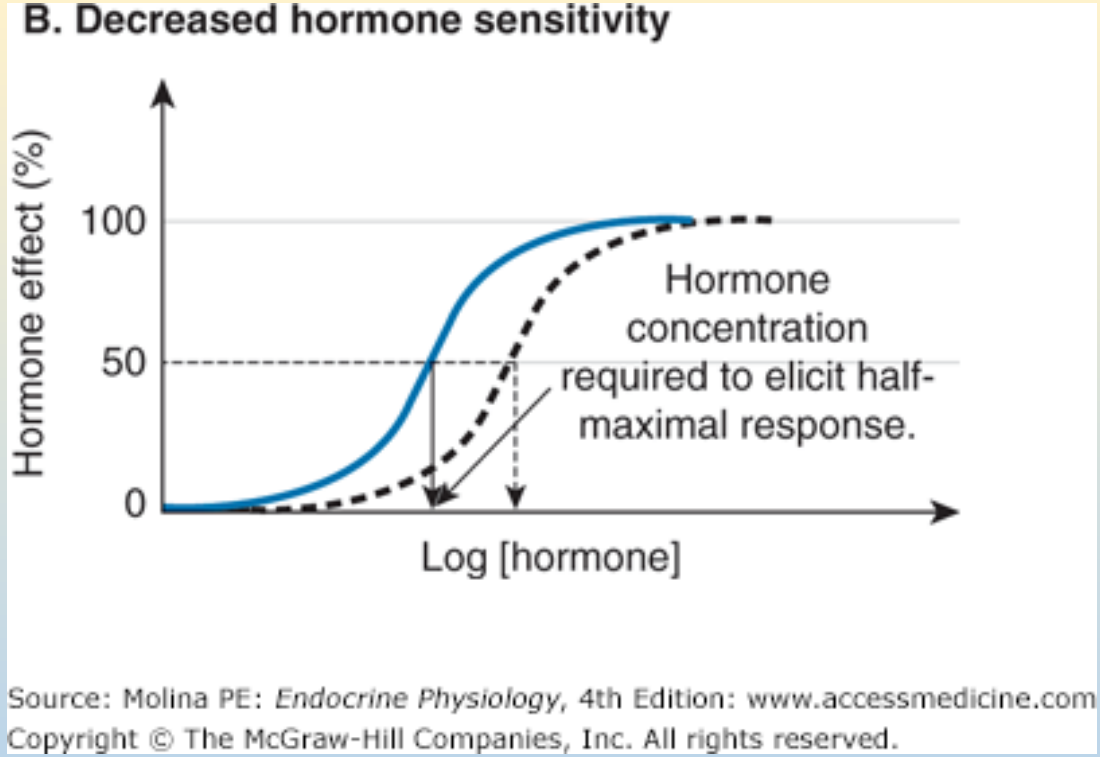
- Changes of transport hormones production during physiological conditions (pregnancy)

Clinical aspects



Source: Molina PE: *Endocrine Physiology*, 4th Edition: www.accessmedicine.com

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



Source: Molina PE: *Endocrine Physiology*, 4th Edition: www.accessmedicine.com

- **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

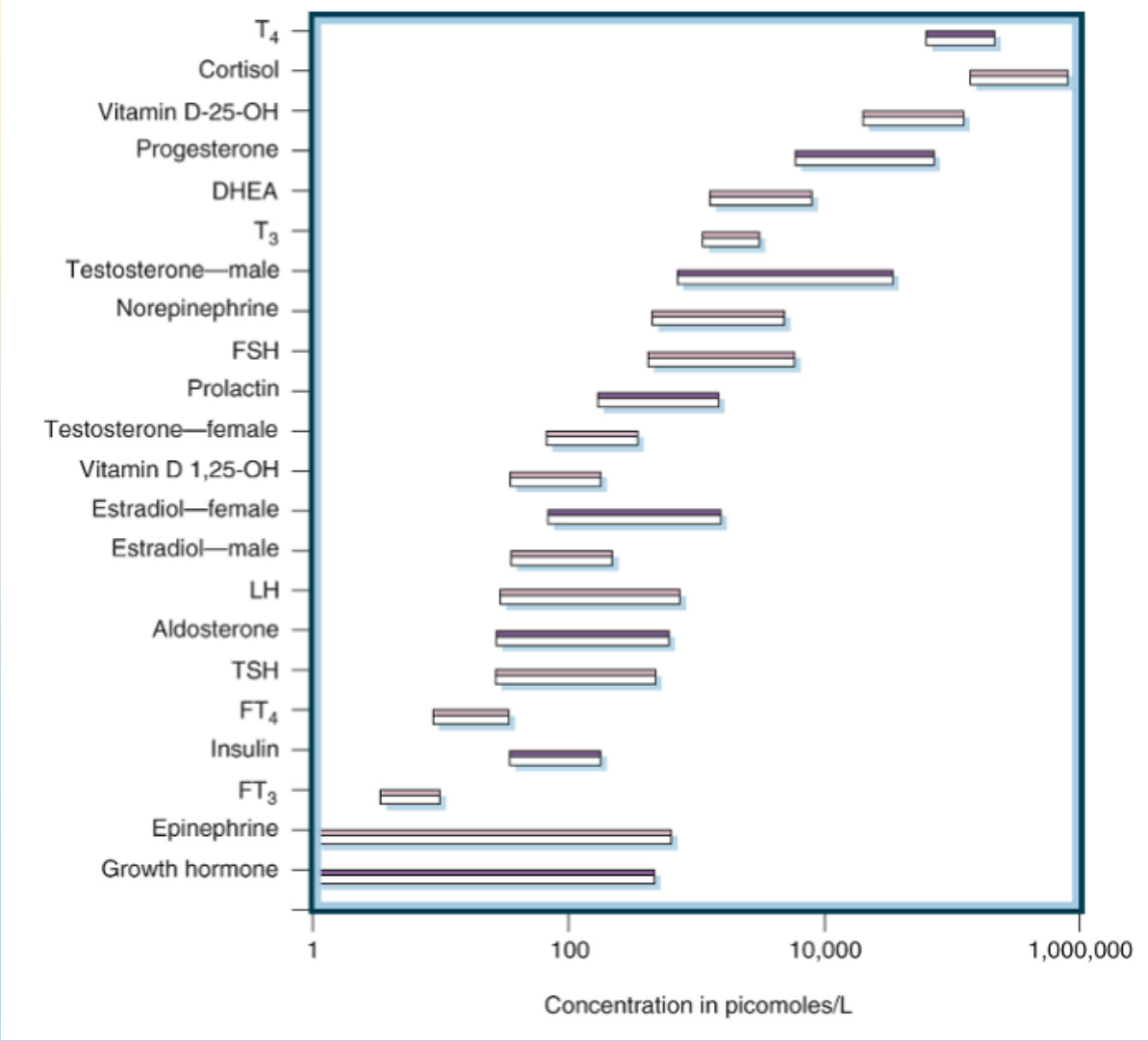
-HIGH SENSITIVITY DEMANDS
-WIDE CONCENTRATION RANGE

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

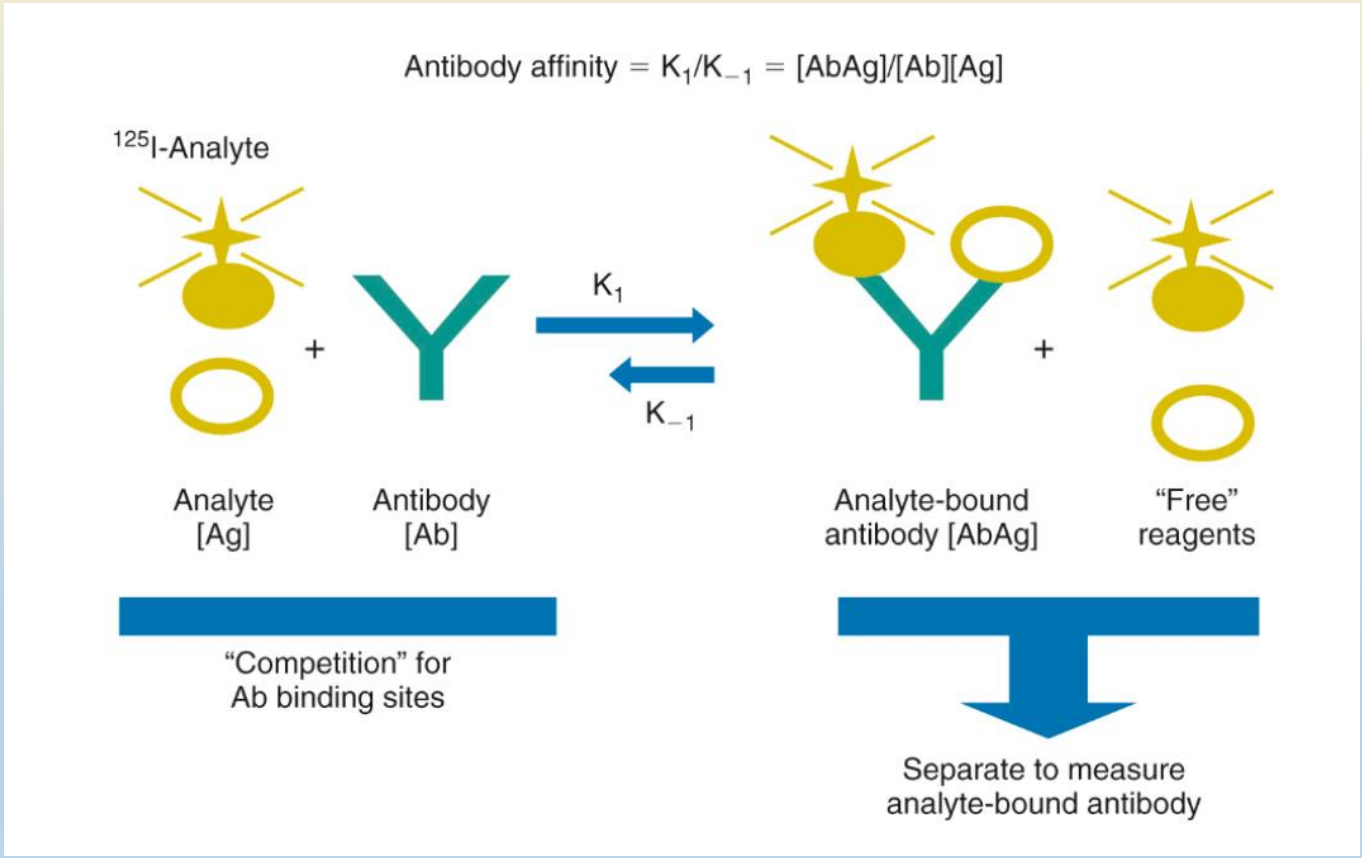
Nucleic acid-based methods
-hybridization techniques
-restriction fragmentation, electrophoresis, sequencing

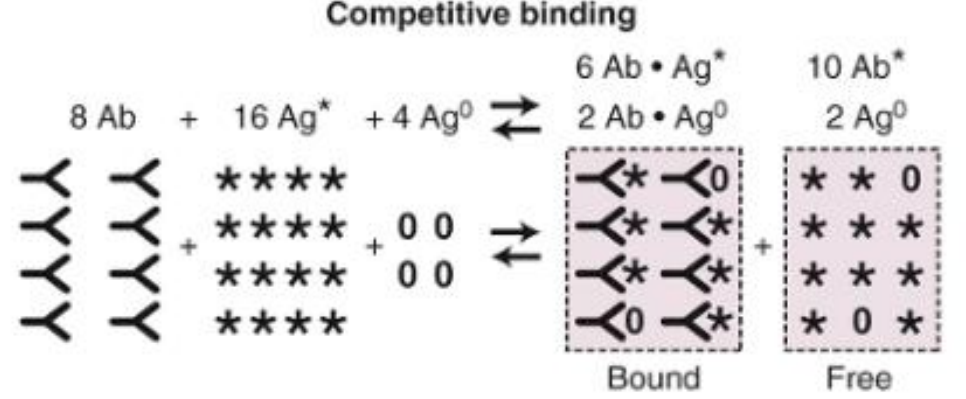
Separation techniques – free X bound hormones
- dialysis

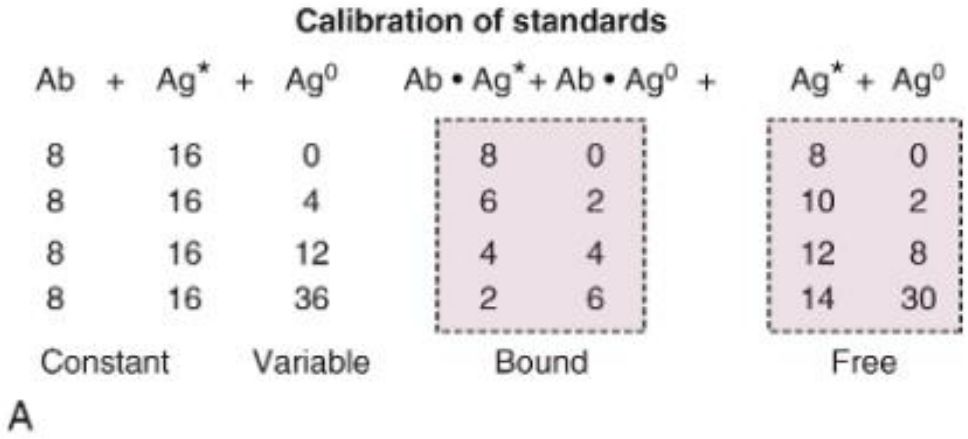


EXTREMELY LOW LEVELS OF HORMONES IN BLOOD

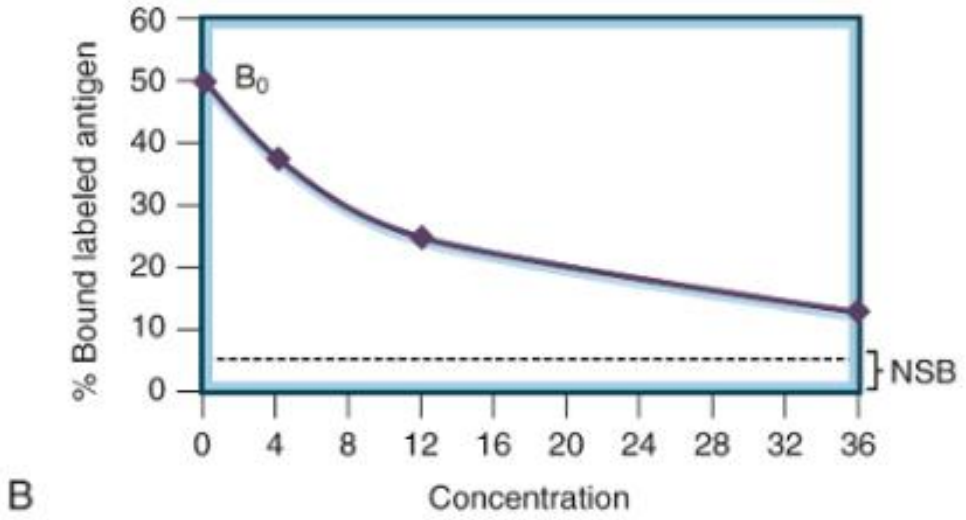
RIA = radioimmunoassay





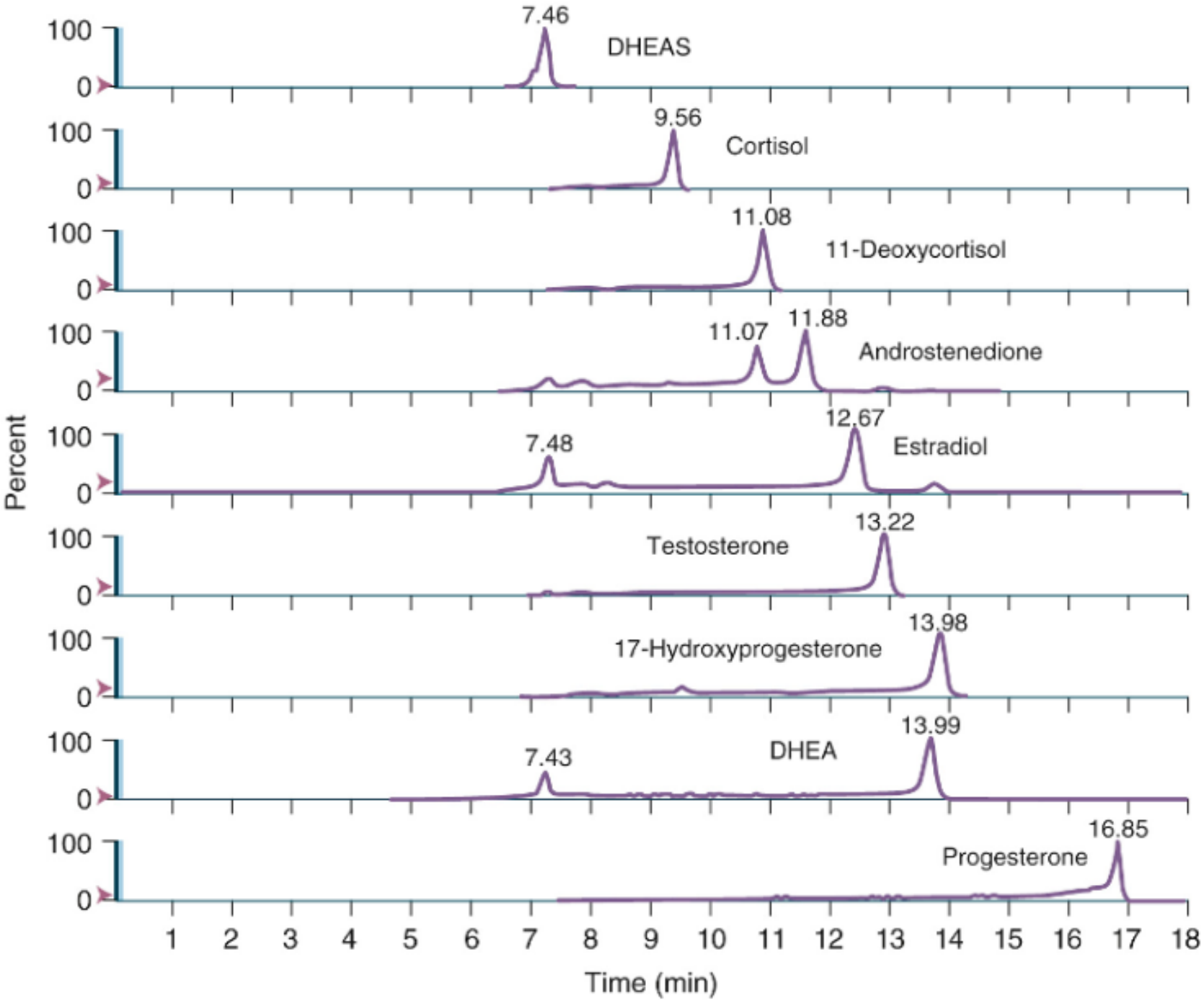


A



B

HPLC-MS


7.46
DHEAS
9.56
Cortisol
11.08
11-Deoxycortisol
11.07
11.88
Androstenedione
7.48
12.67
Estradiol
13.22
Testosterone
13.98
17-Hydroxyprogesterone
7.43
13.99
DHEA
16.85
Progesterone
100
0
Percent
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
Time (min)