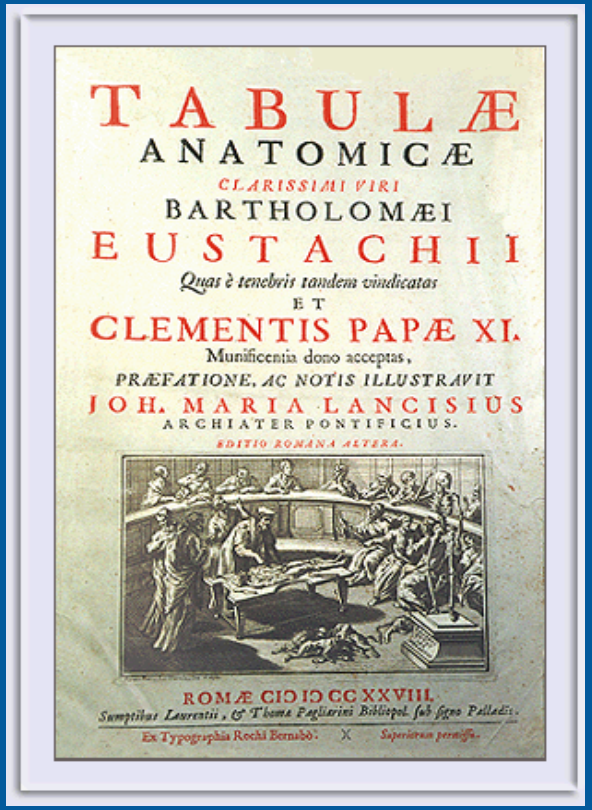
JAGIELLONIAN UNIVERSITY
IN KRAKOW

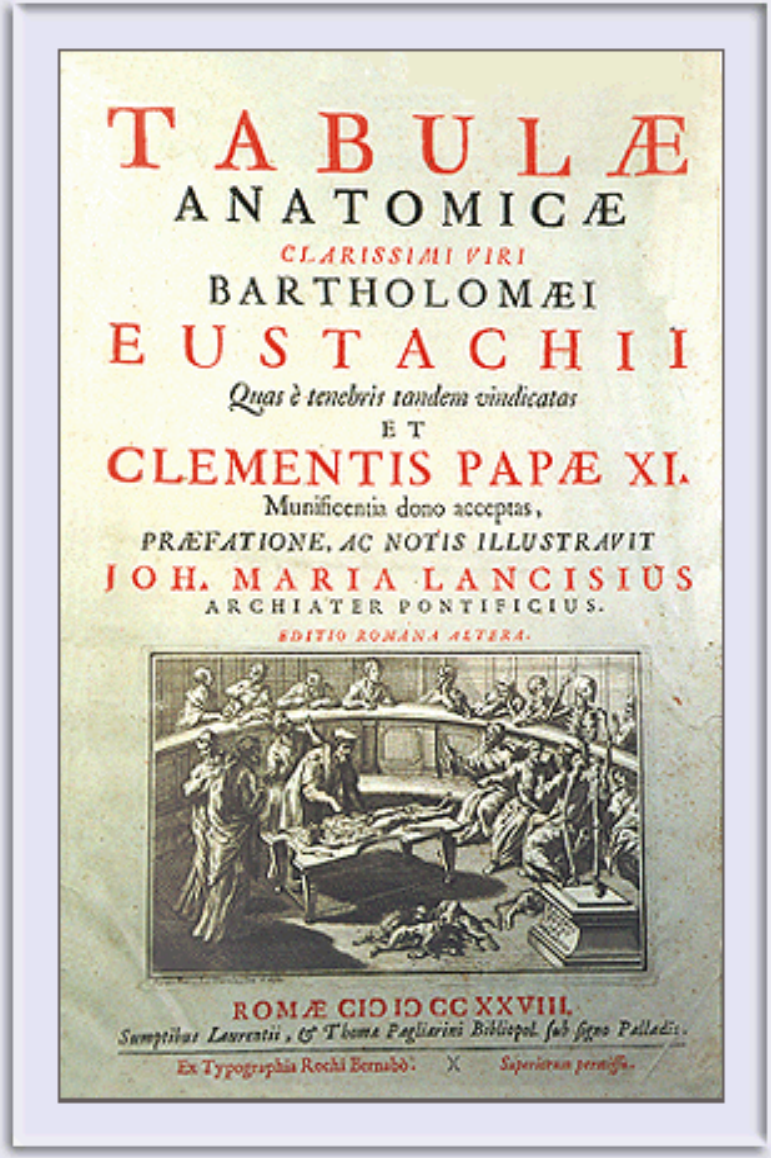


Nuclear receptors

Lecture 7

Adrenals

Adrenals


TABULÆ
ANATOMICÆ
CLARISSIMI VIRI
BARTHOLOMÆI
EUSTACHII
Quas è tenebris tandem vindicatas
ET
CLEMENTIS PAPÆ XI.
Munificentia dono acceptas,
PRÆFATIONE, AC NOTIS ILLUSTRAVIT
JOH. MARIA LANCISIUS
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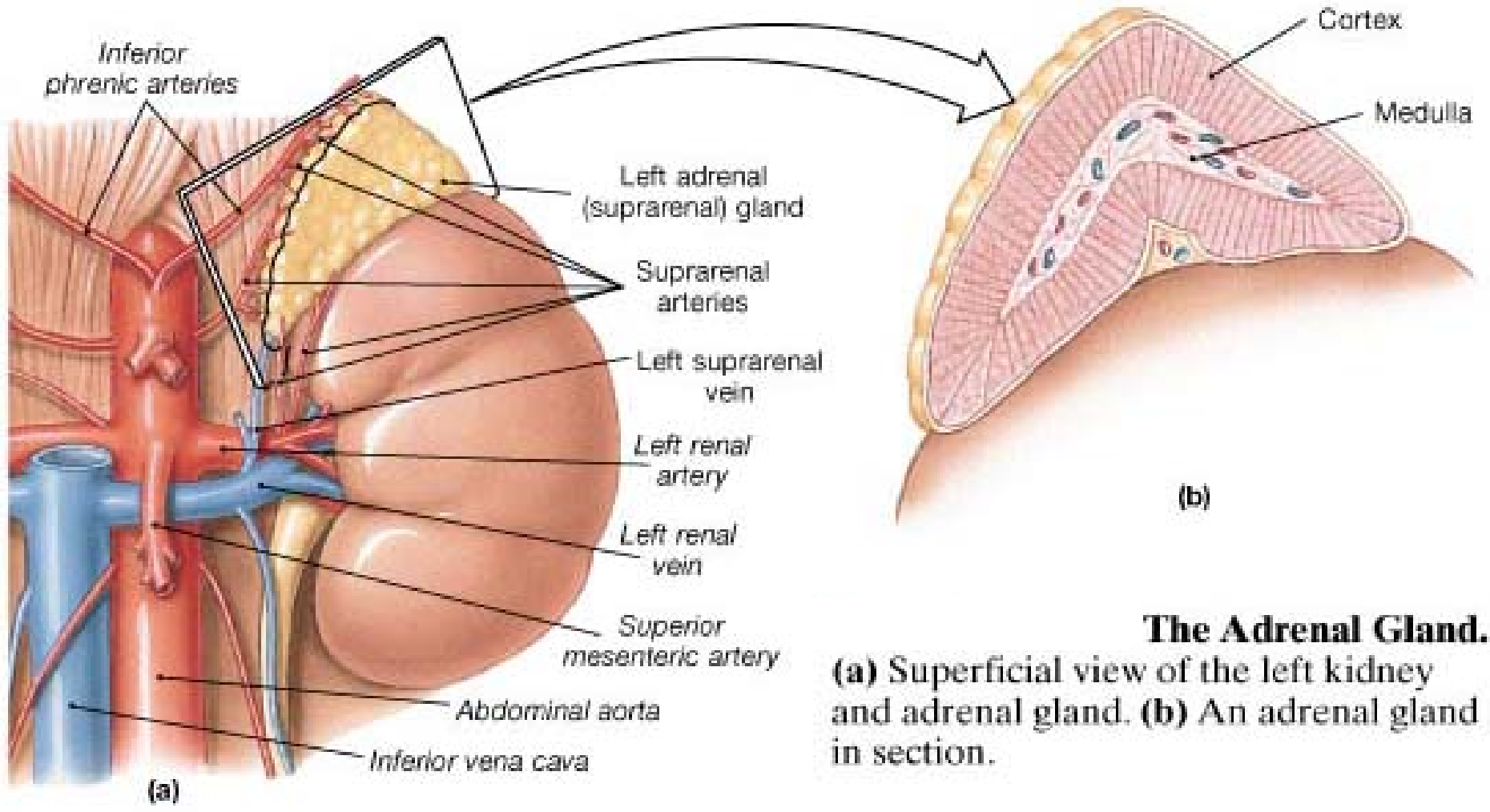
- The first description of adrenals origins from the year 1563. It is an illustration done by Bartolomeo Eustachio "Glandulae Renibus Incumentes" (published in 1714) .

- In 1849 Thomas Addison published the description of lethal effects of adrenal failure, which began the modern research of adrenal cortex physiology.

- Till the half of XX century most experiments on adrenal cortex focused on carbohydrates and glucocorticoids.

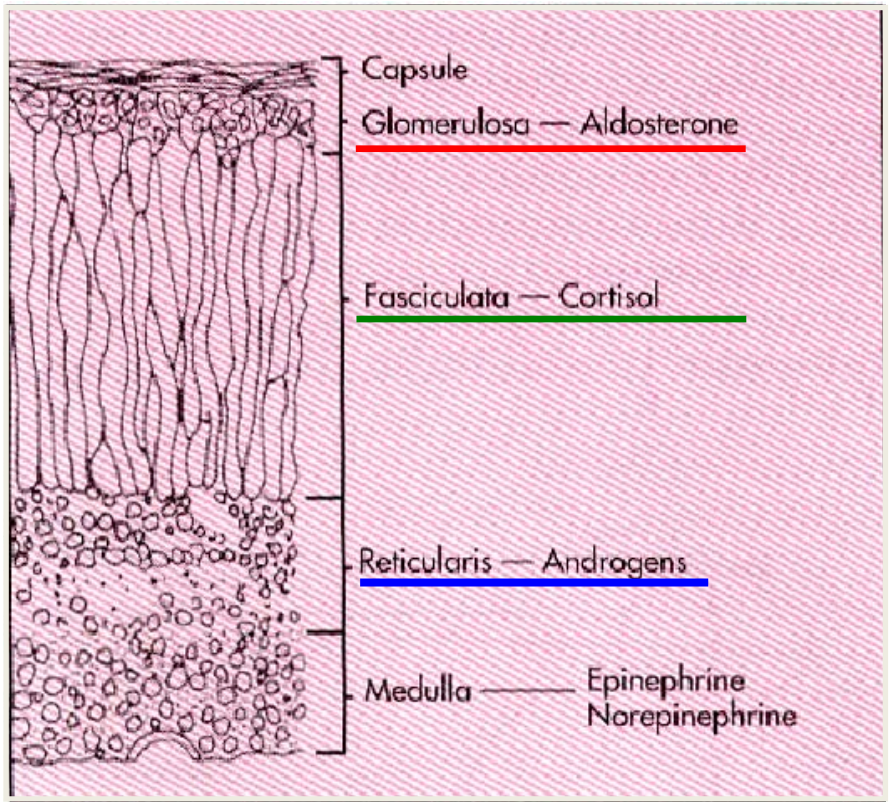
- Glucocrticoids were regarded as compounds of both glucocorticoid and mineralocorticoid activities.

Adrenal glands



The Adrenal Gland.
(a) Superficial view of the left kidney and adrenal gland. **(b)** An adrenal gland in section.

Aldosterone and cortisol synthesis



Cholesterol
CYP11A
Pregnenolone
HSD3B2
Progesterone
CYP21
11-Deoxycorticosterone
CYP17
17OH-Pregnenolone
HSD3B2
17OH-Progesterone
CYP17
11-Deoxycortisol
CYP21
Cortisol
CYP11B1
Glucocorticoid
Corticosterone
CYP11B1
18OH-Corticosterone
CYP11B2
Aldosterone
CYP11B2
Mineralocorticoid

Concentration of aldosterone and cortisol

Average 8 AM plasma concentration and secretion rates of adrenocortical steroids in adult humans

	Plasma concentration (μg/dl)	*Secretion rate (mg/dl)*
Cortisol	13	15
Corticosterone	1	3
11-Deoxycortisol	0.16	0.40
Deoxycorticosterone	0.07	0.20
Aldosterone	0.009	0.15
18-OH Corticosterone	0.009	0.10
Dehydroepiandrosterone sulfate	115	15

Aldosterone in the blood

- Aldosterone was isolated in 1953 (21 mg aldosterone from 500 kg of bovine adrenals…), a year later its stucture was characterized.

- Most aldosterone is synthetized in adrenal cortex, in zona glomerulosa.

- Aldosterone is also produced in other tissues, e.g. in the heart, blood vessels and brain.

- In the blood only ~50% aldosterone is bound to transporting proteins (mostly albumins) (cortisol: 90-95% is bound to proteins).

- Half-life time in the blood for aldosterone is ~20 minutes (cortisol: ~70 minutes).

- 90% aldosterone is removed after single passing through the liver (here aldosterone is bound to glucoronide acid, which facilitates its removal with the urine; similarly in the case of cortisole).

Overproduction of aldosterone – Conn's disease

Described in 1955 by Jerome W. Conn; it fact it was first described by Michał Lityński in 1953, but he published it in the Polish journal.

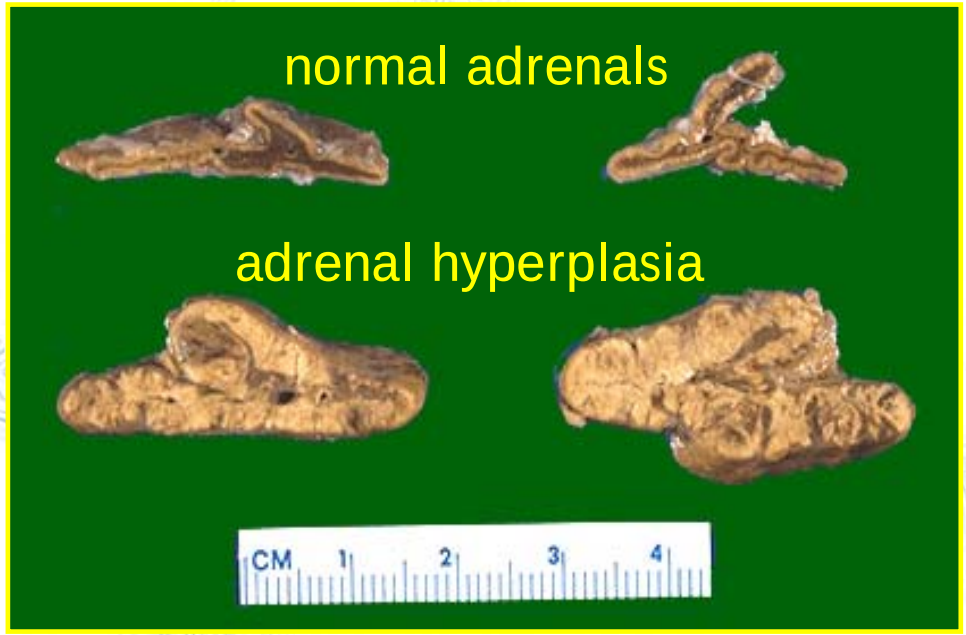
Michal Litynski

Cause:

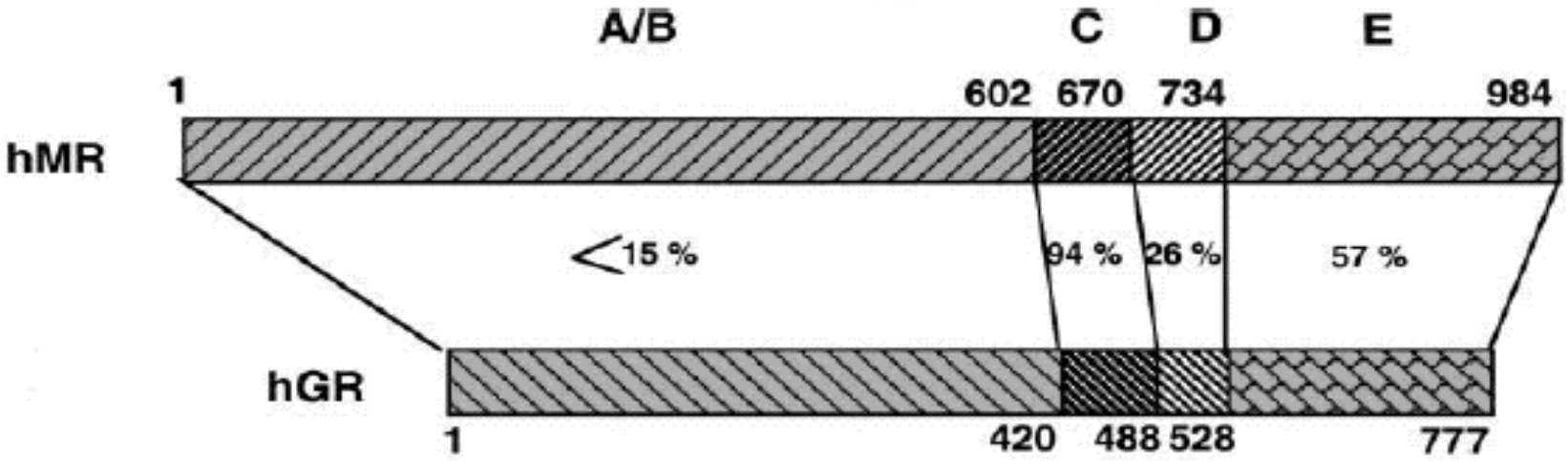
- Mostly tumors developing from adrenal cortex cells (adrenal adenoma), usually at the age 30-50.
- Adrenal hyperplasia.

Symptoms:

- Strong hypertension,
- Hypokalemia,
- Light hypernatremia,
- Polyuria,
- Tiredness,
- Weakness of muscles.



Schematic structure of MR and GR



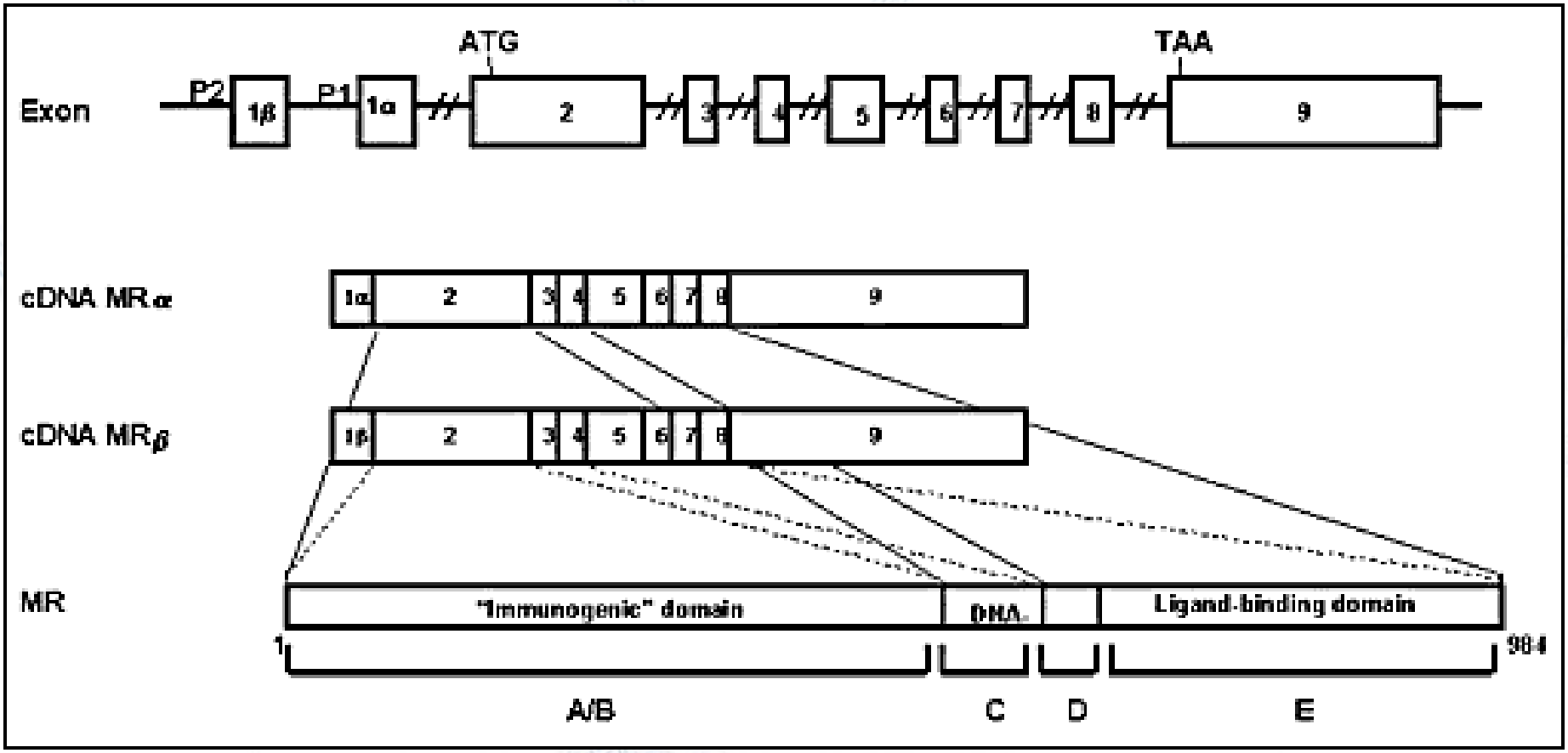
Variable proportions of aldosterone (MR) and glucocorticoid (GR) binding sites among human tissues

Cell Type	MR	GR	MR/GR
Renal collecting duct	10,000/cell	20,000/cell	1/2
Colonic epithelium	7,000/cell	21,000/cell	1/3
Brain hippocampus	100 fmol/mg protein	100 fmol/mg protein	1/1
Arterial smooth muscle cells	1,000/cell	30,000/cell	1/30
Cardiac myocytes	10 fmol/mg protein	300 fmol/mg protein	1/30

Mineralocorticosteroid receptor (MR)

- MR was cloned in 1987.

- The MR gene consists of 9 exons. It has two exons 1 (exon 1α and exon 1β), each with an alternative promoter. However, the finally translated MR protein is the same.



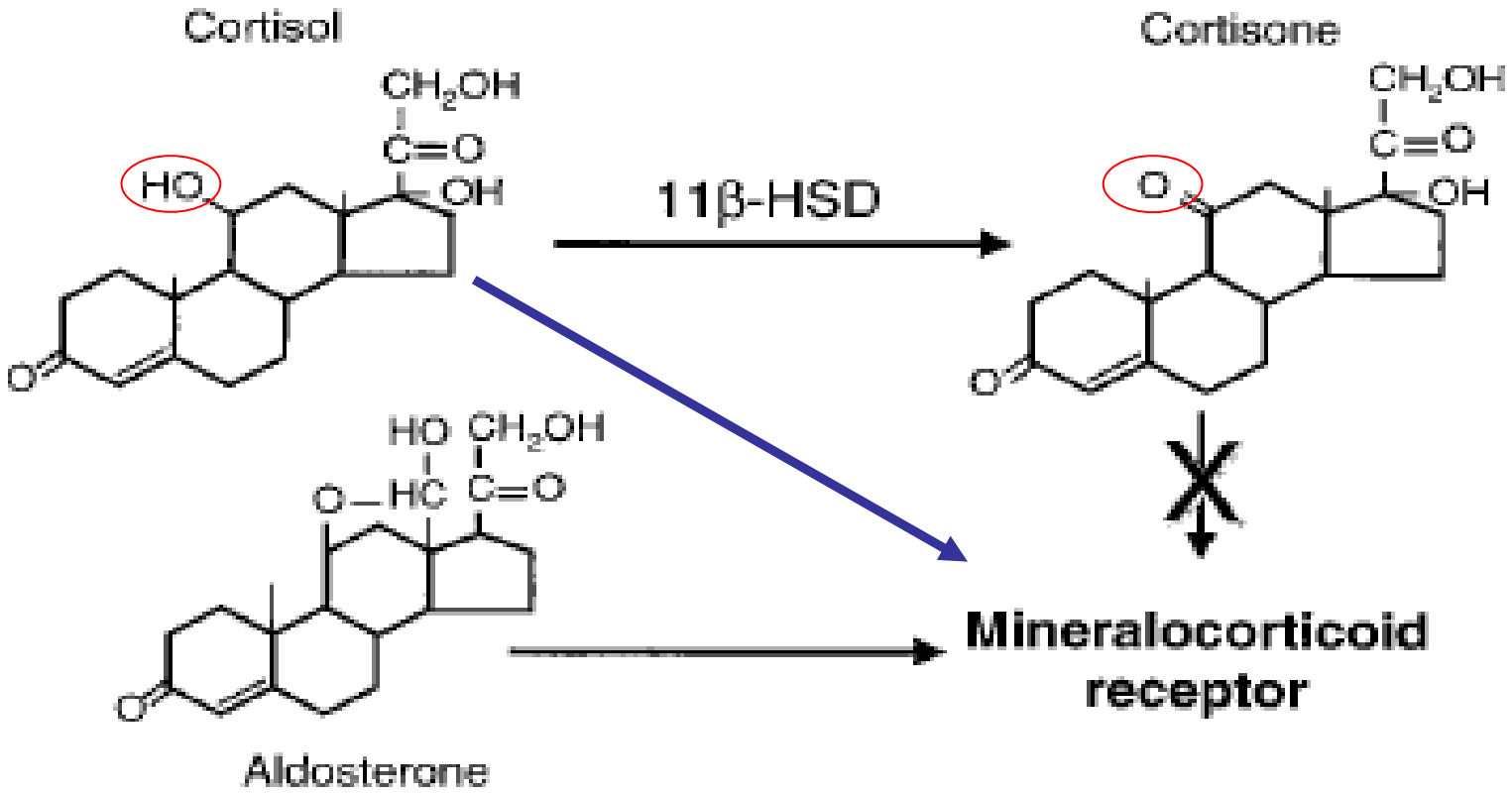
Mineralocorticosteroid receptor (MR)

Major ligands of MR:

* **aldosterone** – major MR ligand exerting physiological effects.

* **cortisol** – has higher affinity to MR than aldosterone, but in major target tissues for aldosterone (e.g. in kidneys) enzyme **11β-hydroxysteroid dehydrogenase (11β-HSD2)** metabolizes cortisol to cortisone, which does not bind to MR. In the case of defect or deficiency of this enzyme cortisol starts to act as a mineralocorticoid.

11β-hydroxysteroid dehydrogenase



- Regulation of ligand selectivity for MR does not occur at the receptor level, but at the level of 11β-HSD2 activity. In kidney epithelium, bladder, gastrointestinal tract, saliva glands, sweat glands, vascular smooth muscle cells and endothelium only aldosterone may activate MR. In the brain and miocytes, which do not express 11β-HSD2 – the major MR activator is cortisol.

Activity of aldosterone

- Major task for aldosterone is to safe water and sodium as well as maintain the appropriate volume of extracellular fluids (volume of primary urine reaches ~170 L/day and ~1.5 kg of salt…).

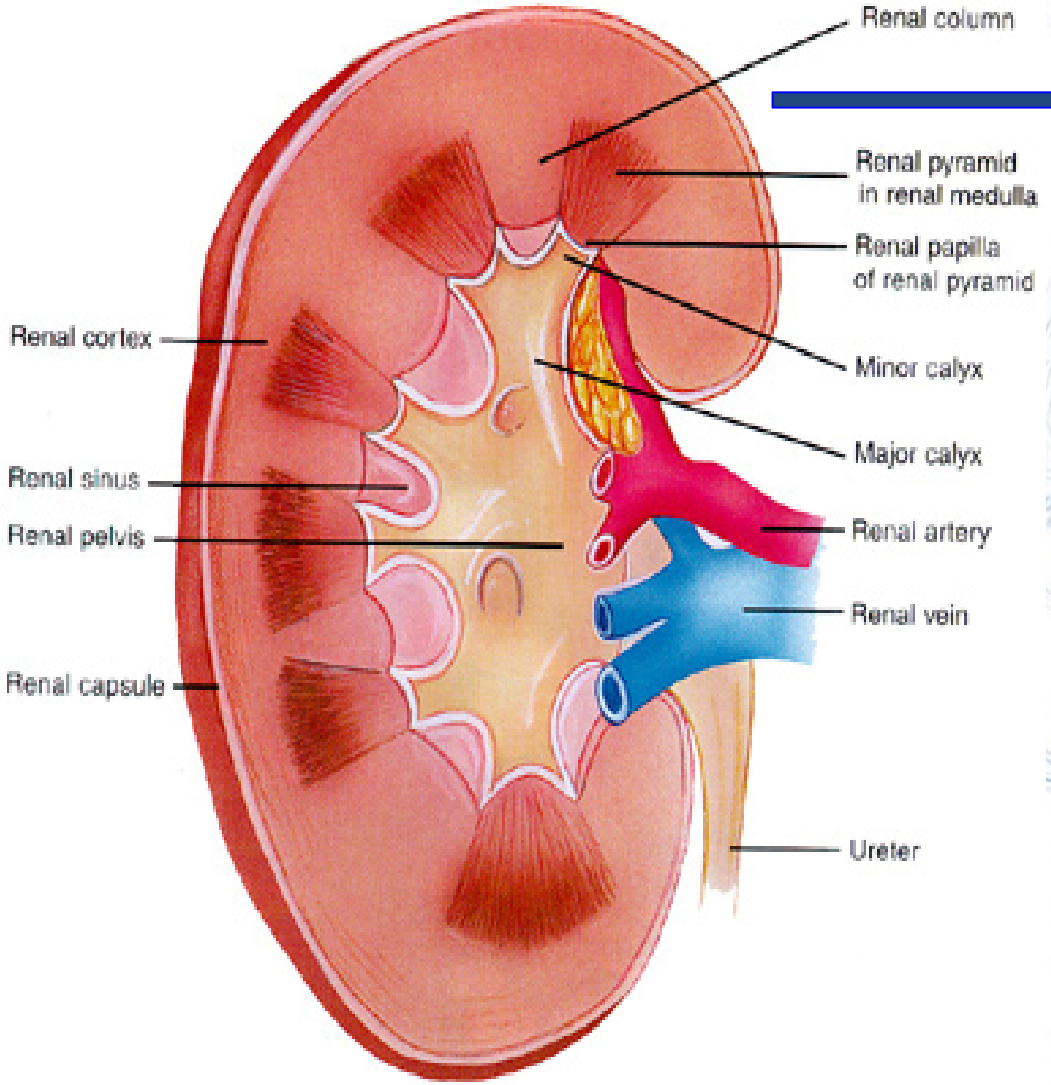
- Major target site for aldosterone are kidneys and their distal and collecting tubules, where aldosterone increases the resorption of Na+, decreasing removal of Na+ with urine. On the other hand, it increases removal of K+ and H+, because Na+ ions are exchanged to K+ and H+.

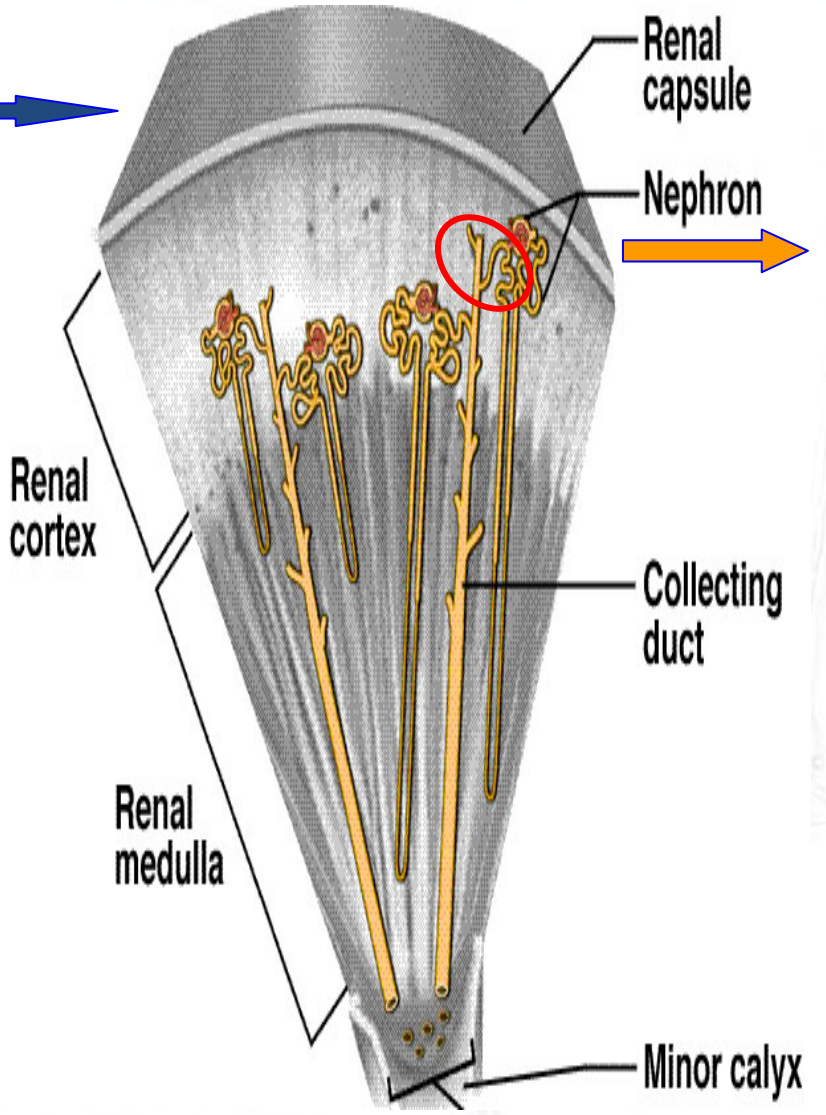
- Aldosterone increases the volume of extracellular fluids and increases blood pressure.

- Aldosterone decreases the loss of sodium with sweat and saliva.

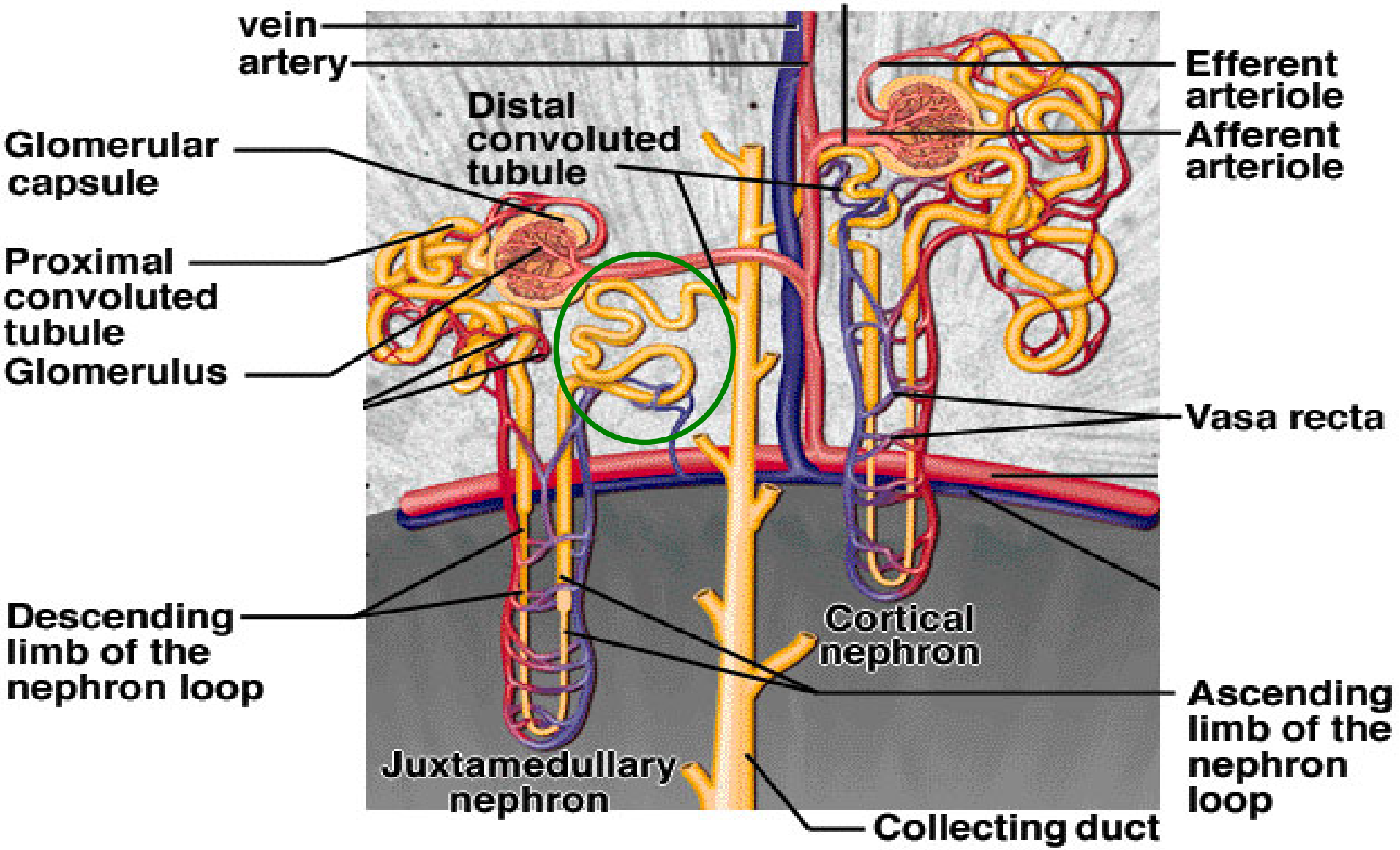
E.g. if in response to training someone starts to sweat, the first perspirate contains a lot of sodium. Decrease in volume of extracellular fluid leads to increased synthesis of aldosterone and decreased loss of sodium. The sweat becomes in practice sodium-free (thus, drinking the ”balanced” or ”isotonic drinks” is usually useless).

Activity of aldosterone

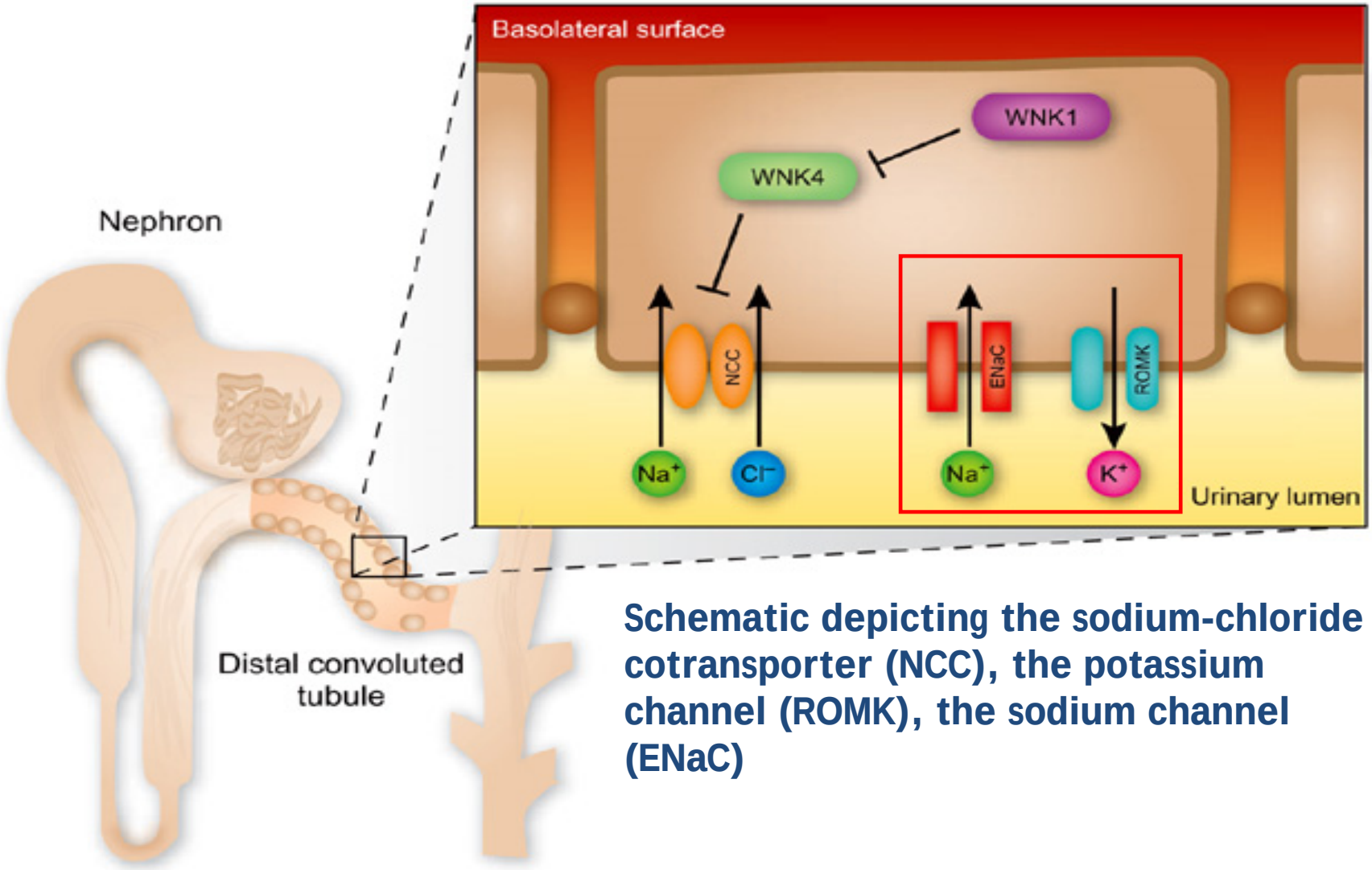




Activity of aldosterone



Sodium absorption by renal tubular system

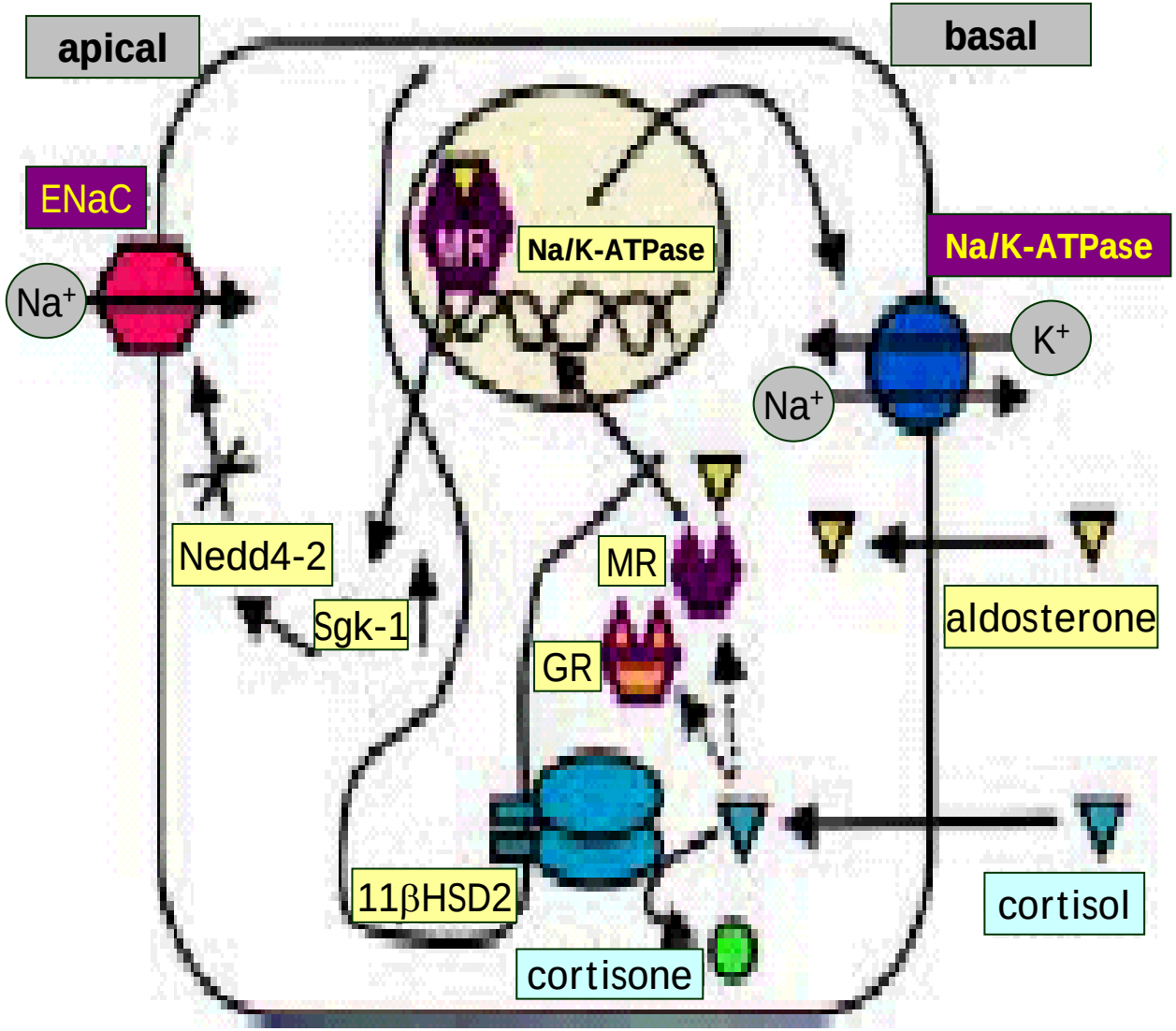


Schematic depicting the sodium-chloride cotransporter (NCC), the potassium channel (ROMK), the sodium channel (ENaC)

Kim Caesar

Coffman et al. Nat Genetics 2006

Regulation of sodium absorption



- Aldosterone binds to the MR;

- Activation of MR leads to increased expression of Sgk-1 which phosphorylates Nedd4-2.

- Phosphorylated Nedd4-2 no longer interacts with internalised ENaC, leading to increased expression of ENaC at the apical membrane.

- Activation of MR also leads to increased expression of Na+/K+-ATPase, thus causing a net increase in sodium uptake from the renal filtrate.

Aldosterone in the heart

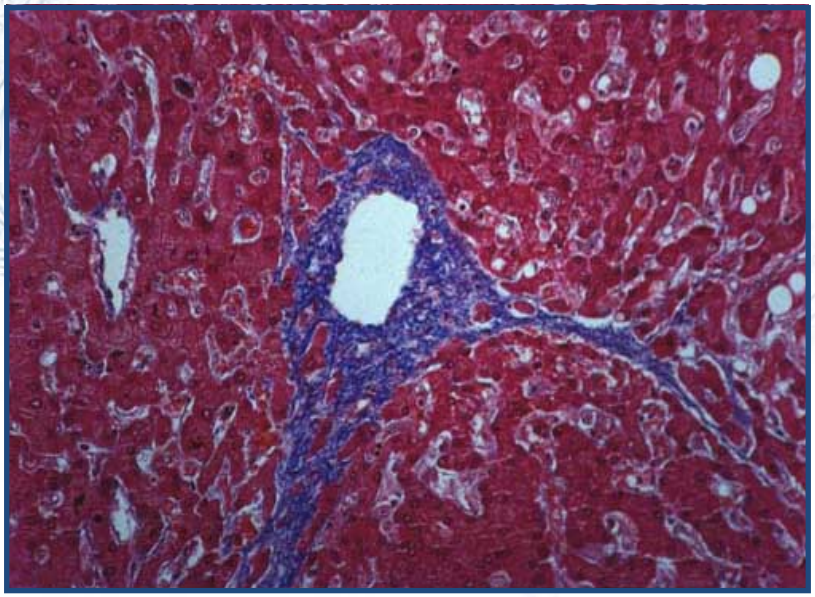
- High concentrations of aldosterone, especially combined with a high-salt diet leads to **cardiac fibrosis**.

- This effect is inhibited by **spironolactone** lub **eplerenone** – MR antagonists.

- Aldosterone in the heart may lead to necrosis of cadiomyocytes and activation of macrophages.

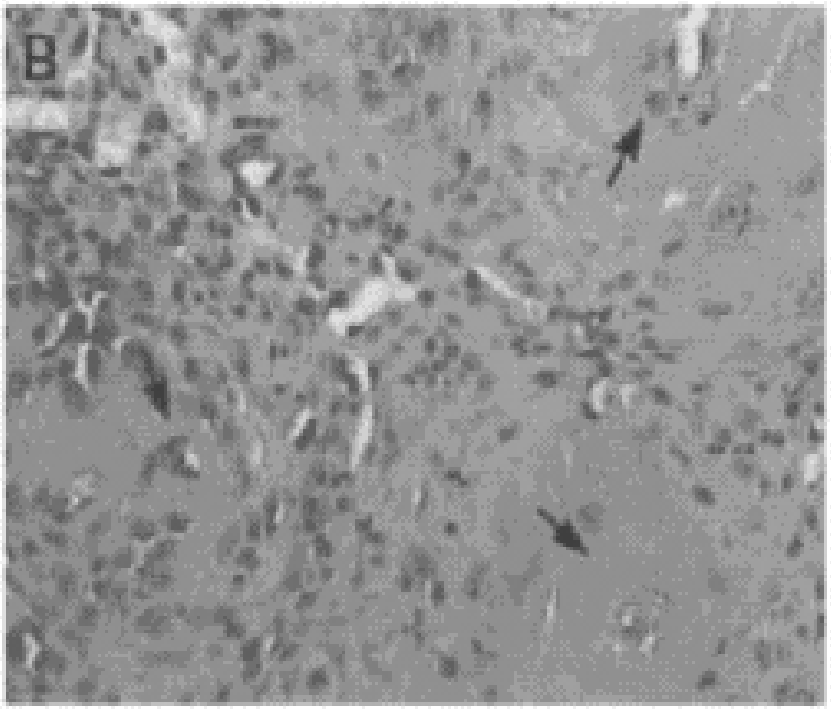
- Fibrosis is possible a secondary repair process.

- The primary cause of injury is inflammation and necrosis of cardiomyocytes.



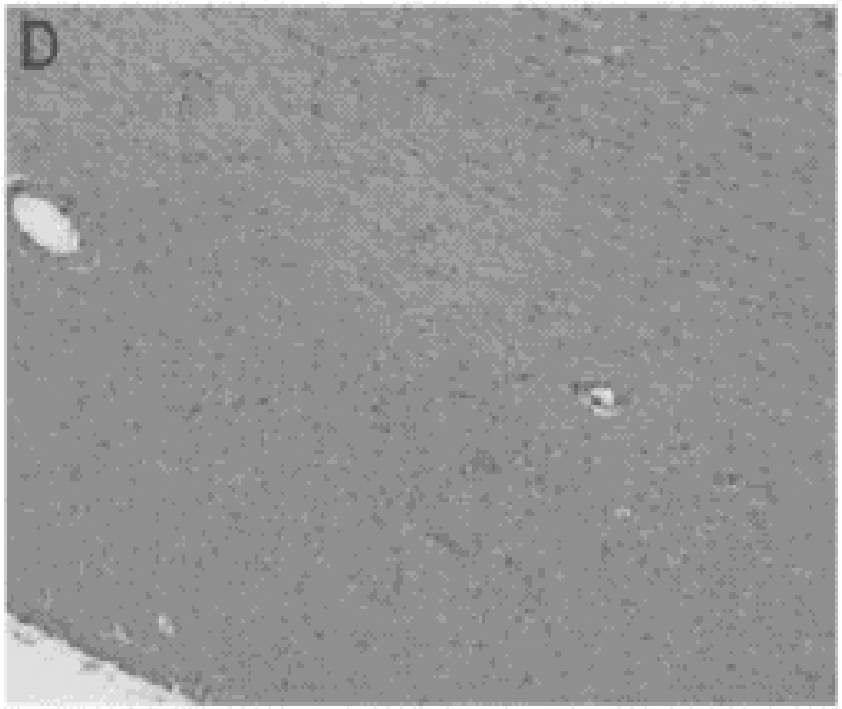
Aldosterone in the heart

Myocardial Histopathology in Aldosterone/NaCl-treated Hypertensive Rats



Aldo/NaCl

Inflammatory infiltrate

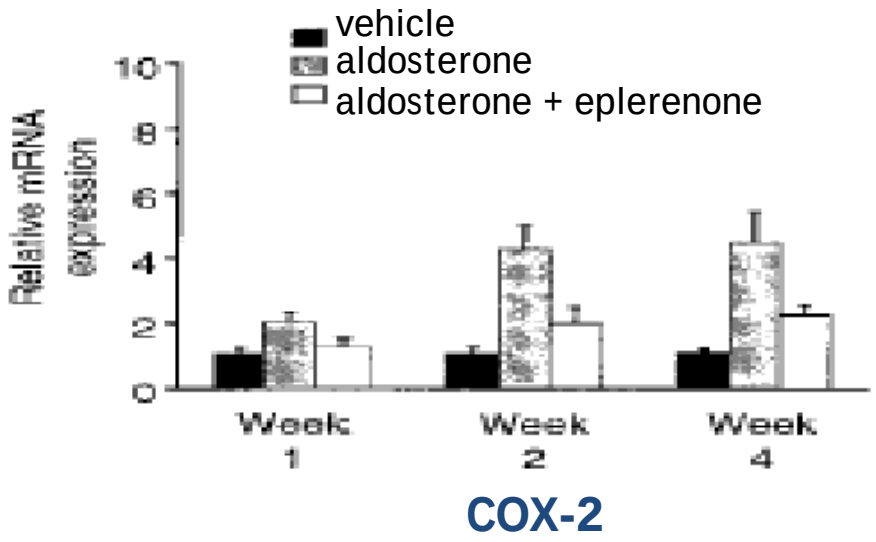


Aldo/NaCl + **eplerenone**

Healthy myocardium

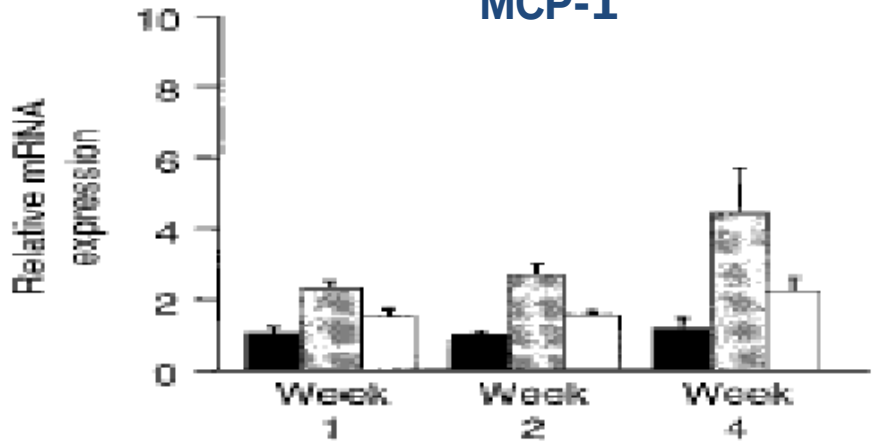
Rocha et al. Am J Physiol Heart Circ Physiol, 2002

Aldosterone in the heart



COX-2

MCP-1



Rat heart

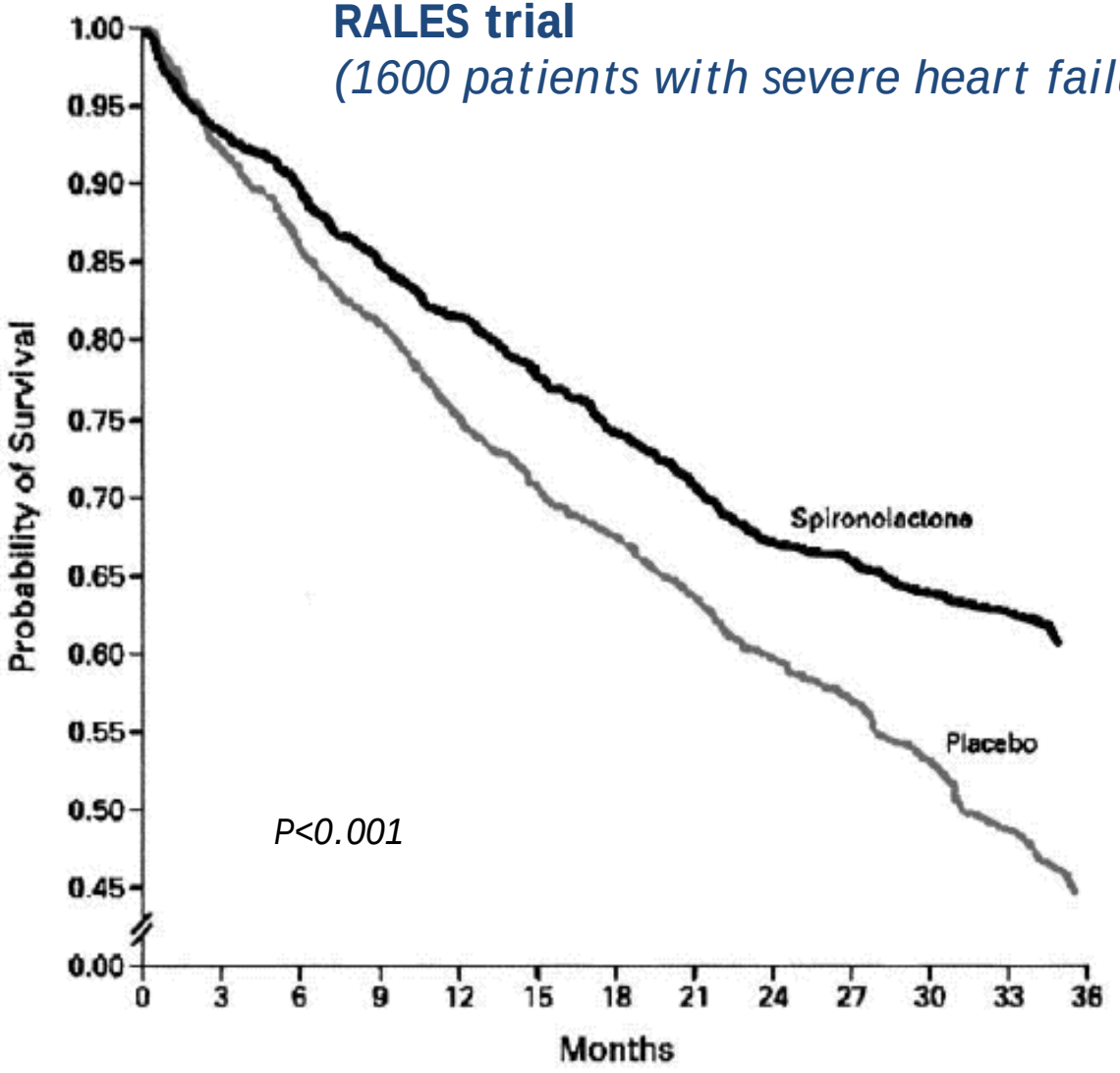
Myocardial Histopathology in Aldosterone/NaCl-treated Hypertensive Rats

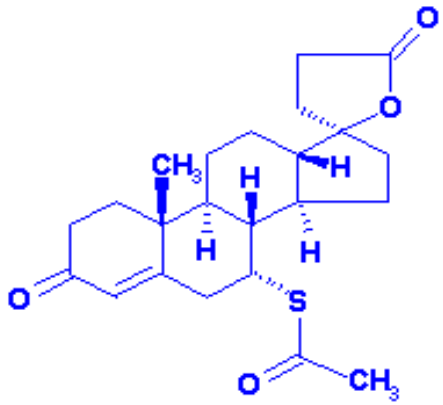
Rocha et al. Am J Physiol Heart Circ Physiol, 2002

Aldosterone in the heart

RALES trial

(1600 patients with severe heart failure)





Spironolactone

- competitively antagonizes aldosterone binding

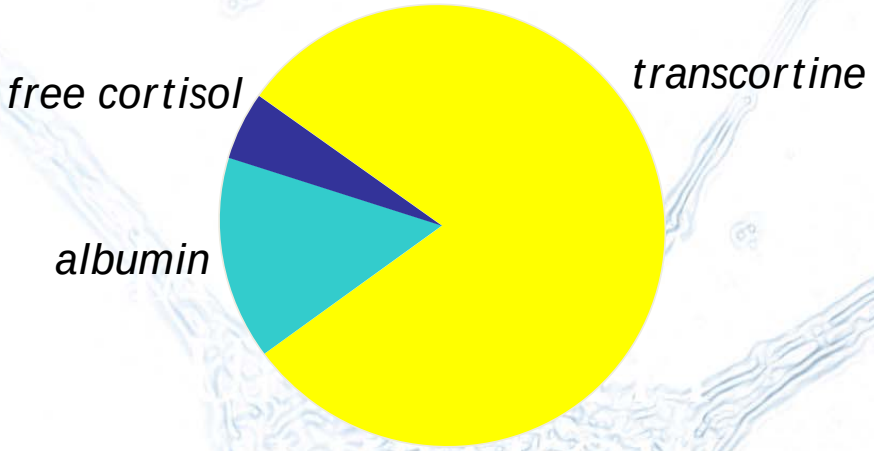
- may cause endocrine disturbances because of non-selective binding to:
  * progesterone receptors
  * androgen receptors

(used in male-to-female transsexual people).

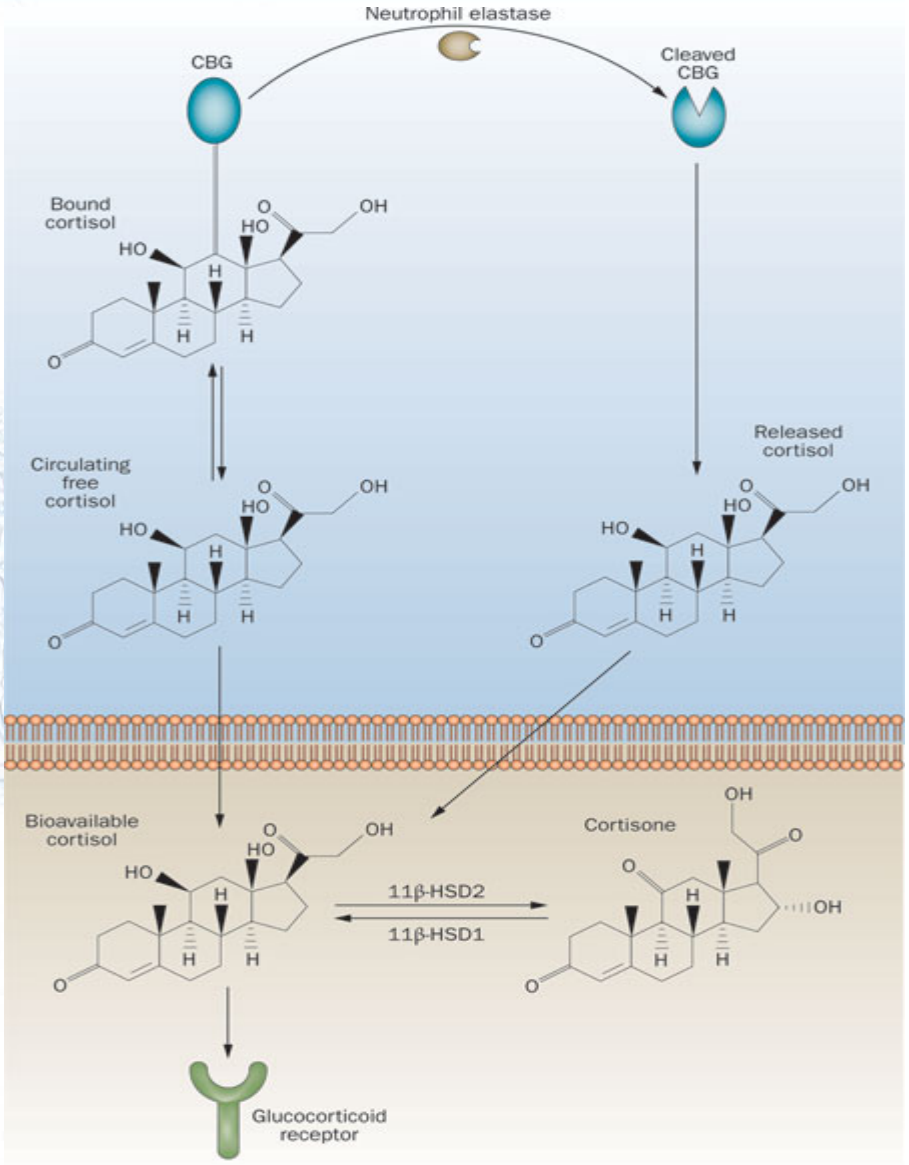
Cortisol

- Corticosterids are not stored, but are always synthetised de novo from cholesterol; level of circulating corticosterids is highest in the morning.

- Circulating corticosterids are associated with transcortine (cortisol binding globulin, CBG, α2-globulin glycoprotein, 75-80%) i albumins (15%). 5-10% is free.



- On cells there are membrane receptors for transcortine. Binding the ligands (complex transcortine-cortisol) leads to **elevation of cAMP** and mediates **non-genomic effects of cortisol**.



Perogamvros et al. Nat Rev Endocrinol. 2012

Cortisol activity

- ↑ gluconeogenesis, ↓ insulin sensitivity; results in **hyperglycemia**

- ↑ lipolysis (mostly in the extremities), ↓ lipogenesis, fat redistribution – **abdominal obesity** (belly, corpus, face)

- ↓ production of collagen type I, ↓ maturition of osteoblast progenitors, ↓ calcium absorbtion in intestine (too high level of cortisol leads to **osteoporosis**).

- in cardiovascular system it contributes to regulation of normal blood pressure: ↑ heart beating, ↑ response of arterioles to catechloamines which **increases blood pressure**, ↓ production of vasodilating prostaglandin, ↓endothelium permeability, which protects against edema in inflammed tissues.

- in the kidneys it acts in a **opposite way to aldosterone**: ↑ removal of water from organism, ↓ secretion of vasopresine (an antidiuretic hormone) from hypothalamus.

Glucocorticoid receptors

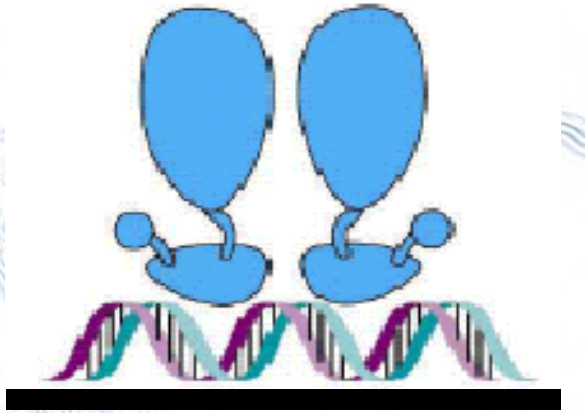
- GR is commonly expressed in many cell types.

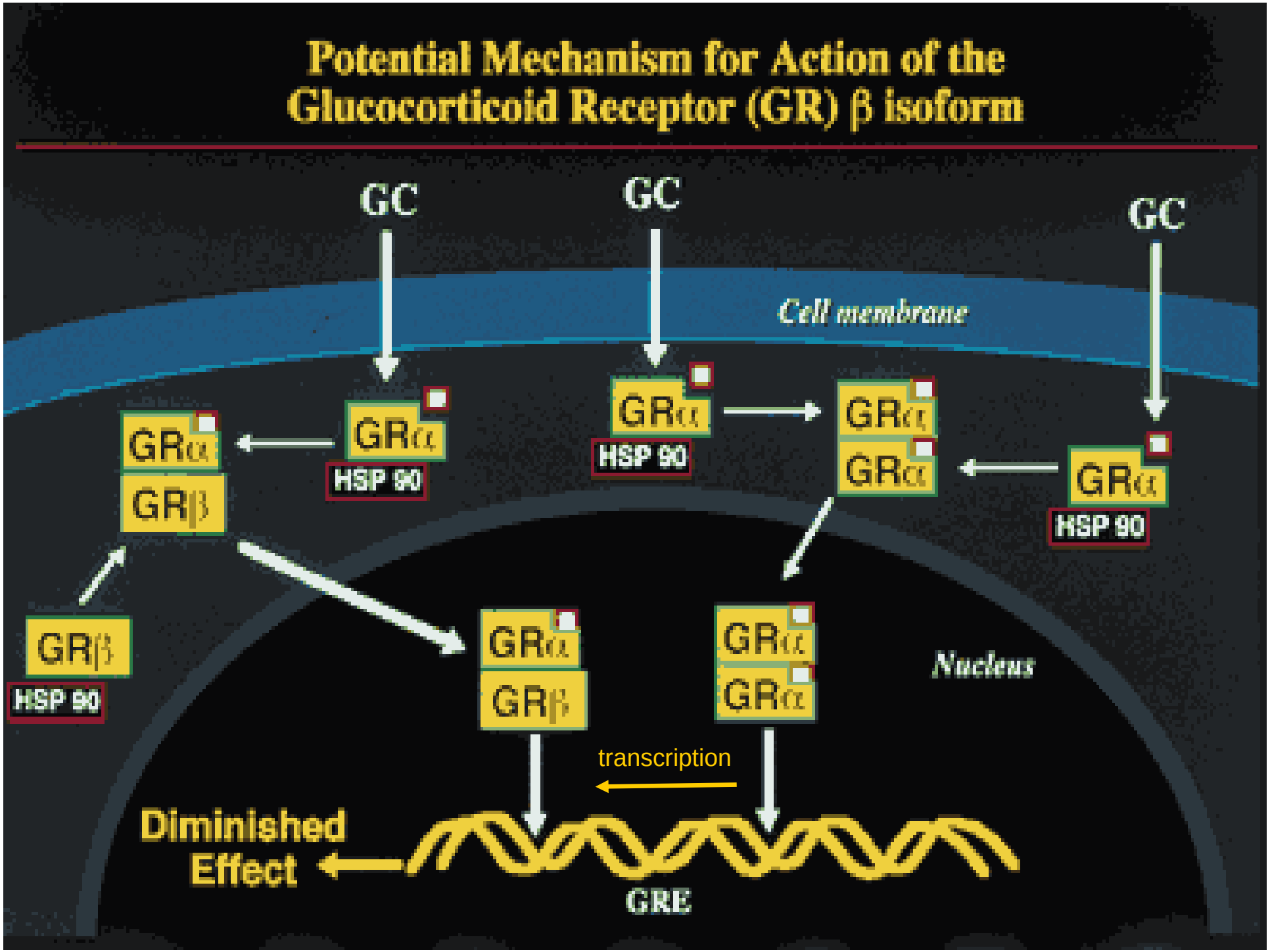
- GR without ligands are located in the cytoplasm, where are bound with HSP90, HSP70, immunophilin FKB56

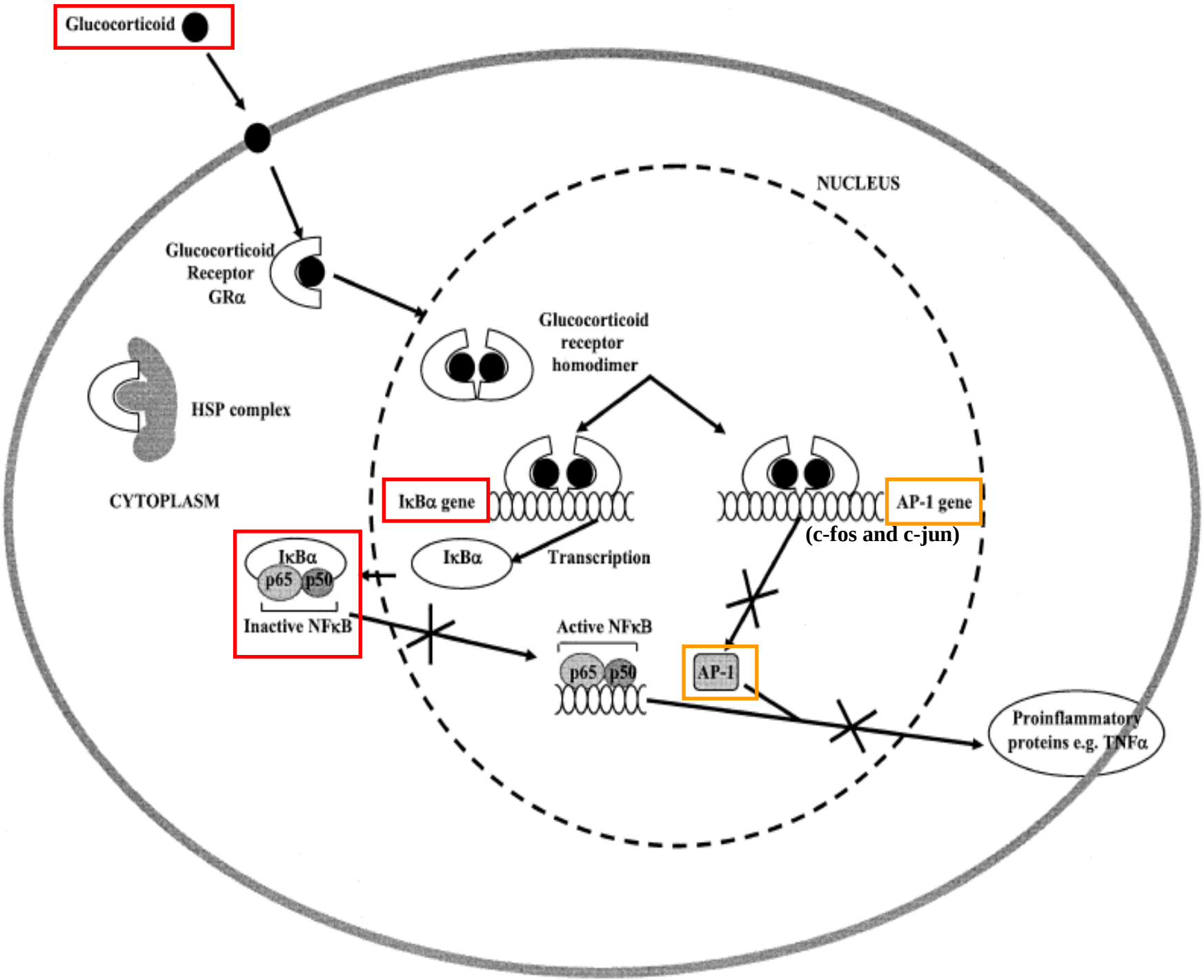
- GR is active as a homodimer, which recognize the palindromic sequence TGTTCT

- GR exists in two splicing forms:
  * α (777 aminoacids)
  * β (742 aminoacids, lack of C-terminal fragment)

- Isoform β cannot bind ligands, although it may bind to DNA. Possibly it may inhibit activity of glucocorticoids.




Potential Mechanism for Action of the Glucocorticoid Receptor (GR) β isoform
GC
GC
GC
Cell membrane
GRα
GRβ
HSP 90
Nucleus
transcription
Diminished Effect
GRE


Glucocorticoid
NUCLEUS
Glucocorticoid Receptor GRα
Glucocorticoid receptor homodimer
HSP complex
CYTOPLASM
IκBα gene
AP-1 gene
(c-fos and c-jun)
IκBα
p65
p50
IκBα
Transcription
Inactive NFκB
Active NFκB
p65
p50
AP-1
Proinflammatory proteins e.g. TNFα

Corticosteroids and gene transcription

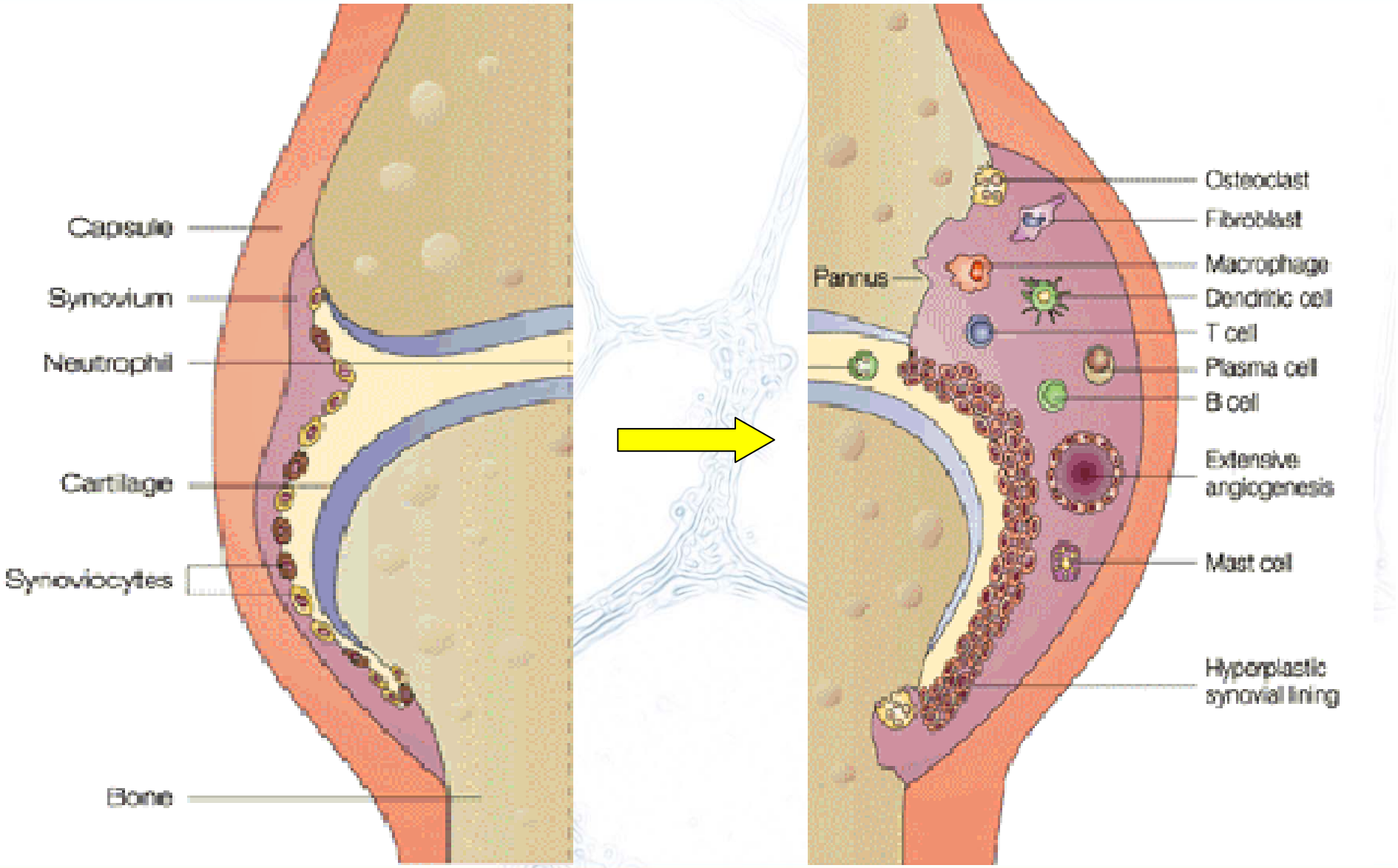
Increased transcription

- Annexin-1 (lipocortin-1, phospholipase A_2 inhibitor)
- β_2-adrenergic receptor
- Secretory leukocyte inhibitory protein
- Clara cell protein (CC10, phospholipase A_2 inhibitor)
- IL-1 receptor antagonist
- IL-1R2 (decoy receptor)
- IκBα (inhibitor of NF-κB)
- IL-10 (indirectly)

Decreased transcription

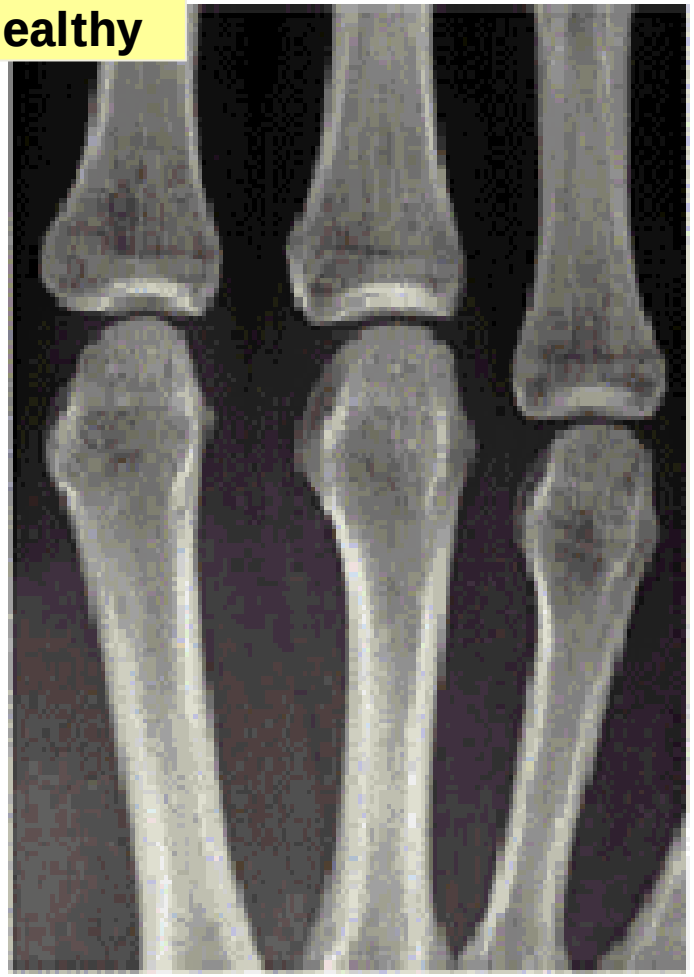
- Cytokines
  - IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-11, IL-12, IL-13, IL-16, IL-17, IL-18, TNF-α, GM-CSF, SCF
- Chemokines
  - IL-8, RANTES, MIP-1α, MCP-1, MCP-3, MCP-4, eotaxin
- Adhesion molecules
  - ICAM-1, VCAM-1, E-selectin
- Inflammatory enzymes
  - Inducible nitric oxide synthase
  - Inducible cyclooxygenase
  - Cytoplasmic phospholipase A_2
- Inflammatory receptors
  - Tachykinin NK_1-receptors, NK_2-receptors
  - Bradykinin B_2-receptors
- Peptides
  - Endothelin-1

Rheumatoid arthritis

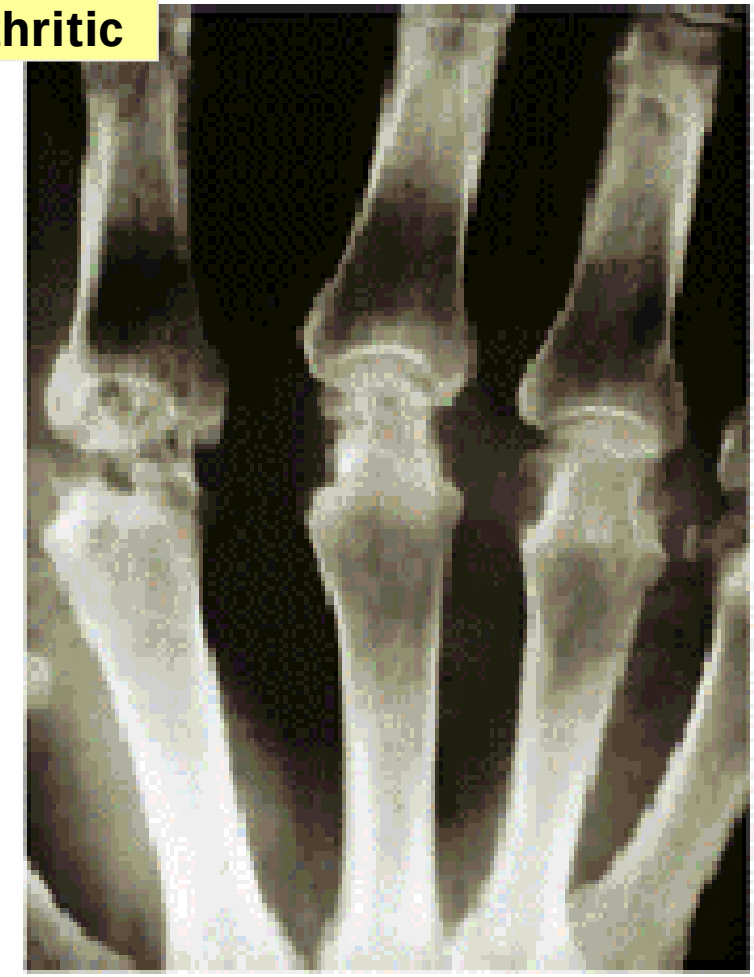


Rheumatoid arthritis

healthy



arthritic



Rheumatoid arthritis

Symptoms >6 weeks' duration

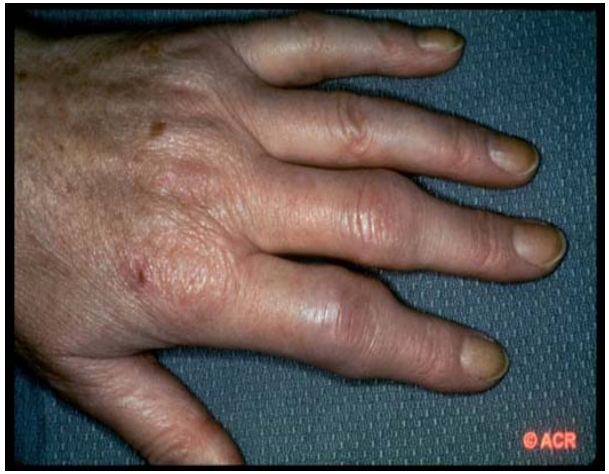
Often lasts the remainder of the patient's life

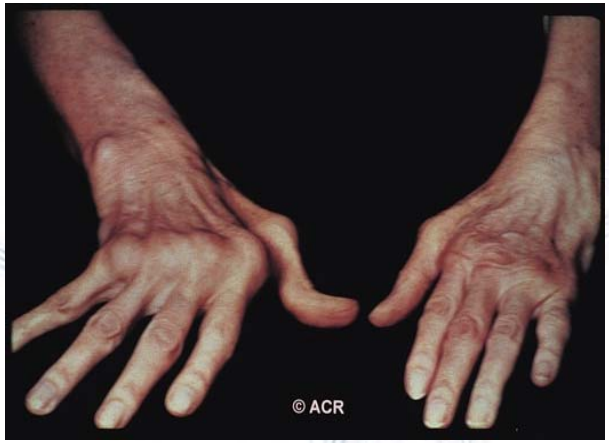
Inflammatory synovitis

Palpable synovial swelling

Morning stiffness >1 hour, fatigue

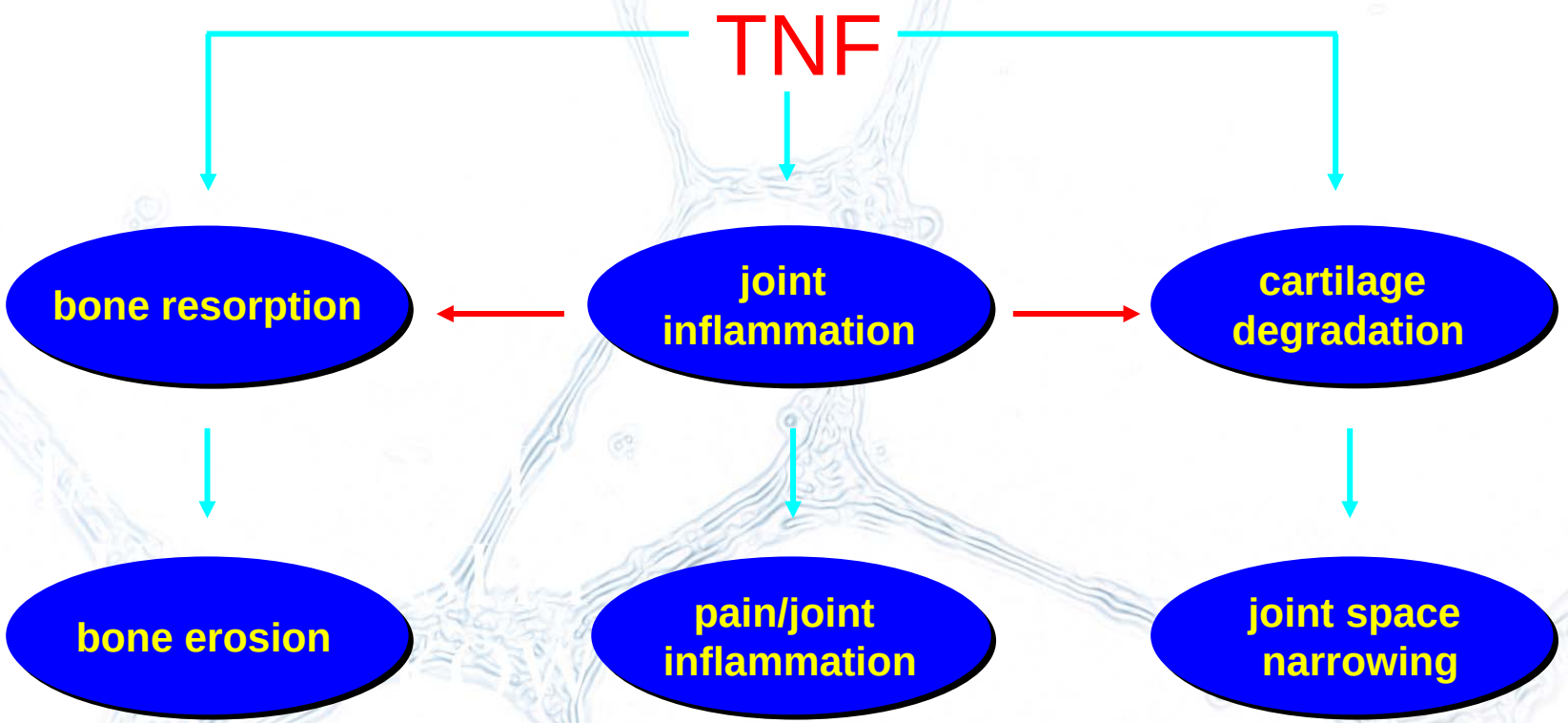
Symmetrical and polyarticular (>3 joints)



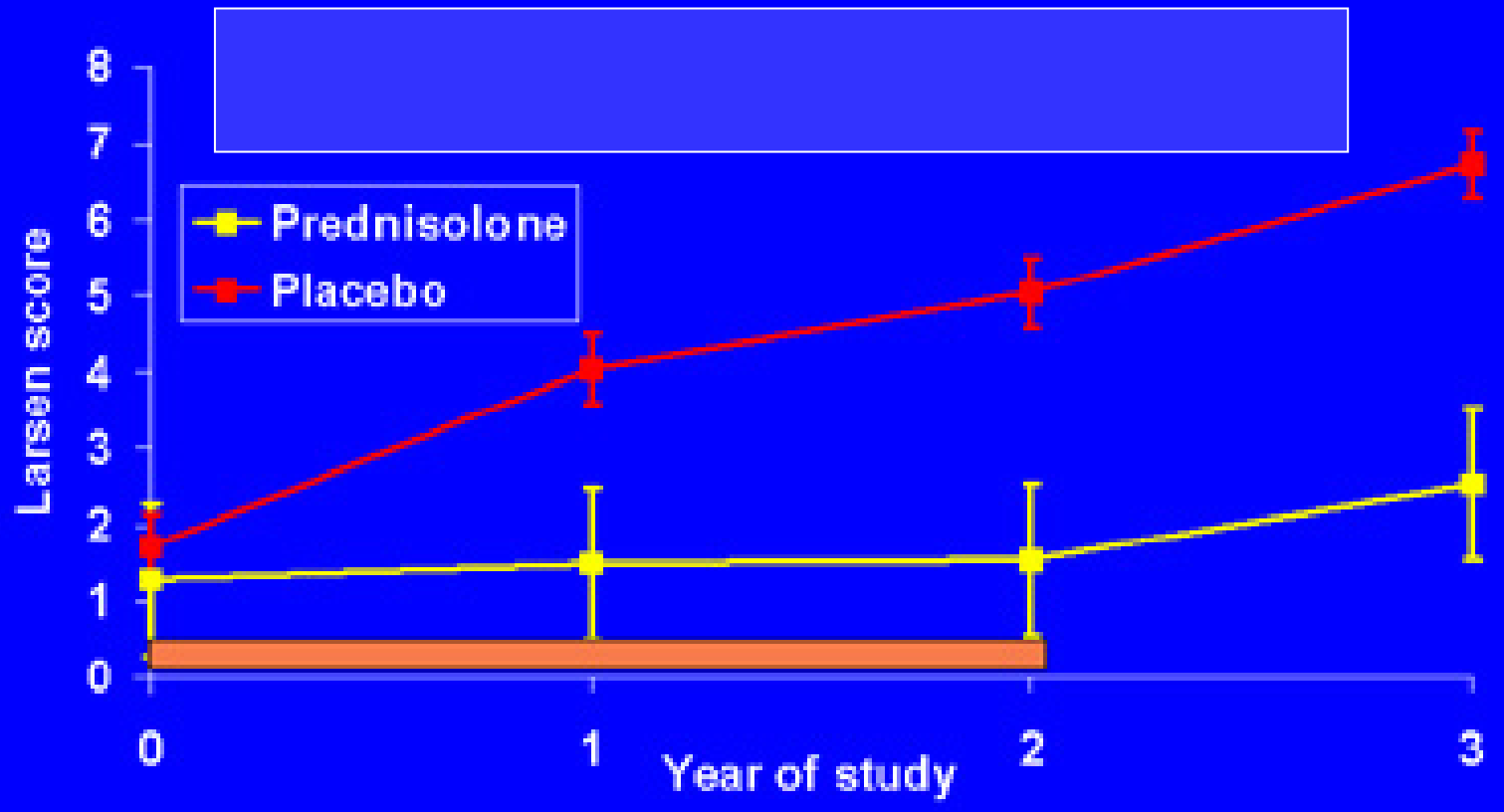


- Affects approximately 1% of the adult population
- Incidence increases with age
- Occurs 2-4 times more often in women
- Shortens lifespan by average of 10 years

TNFα in rheumatoid arthritis

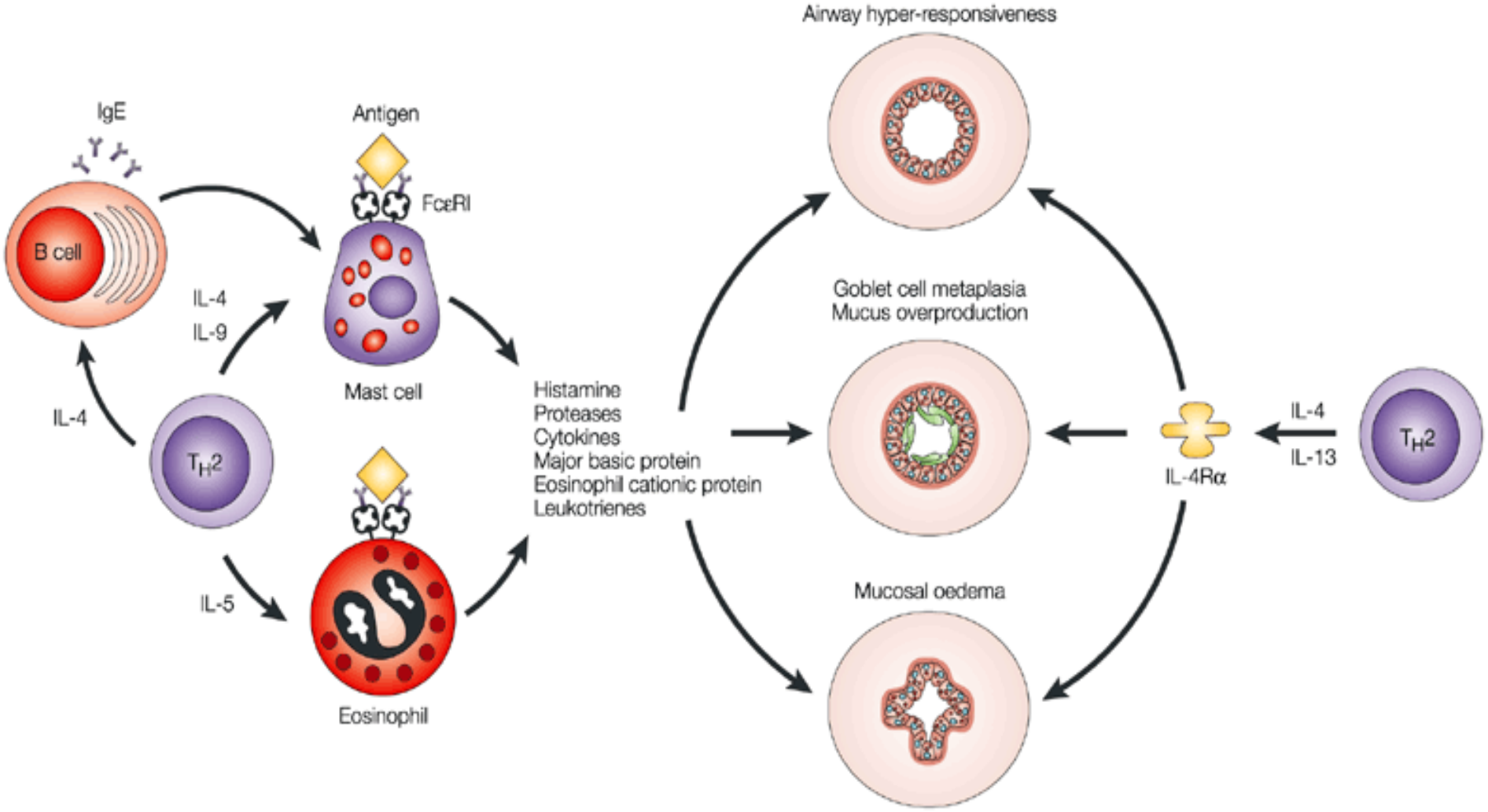


Joint destruction



Kirvan et al. Z Rheumatol, 2000

Asthma



Nature Reviews | Drug Discovery

Asthma

- Inflammatory reaction and reversible const-ruction of muscles.

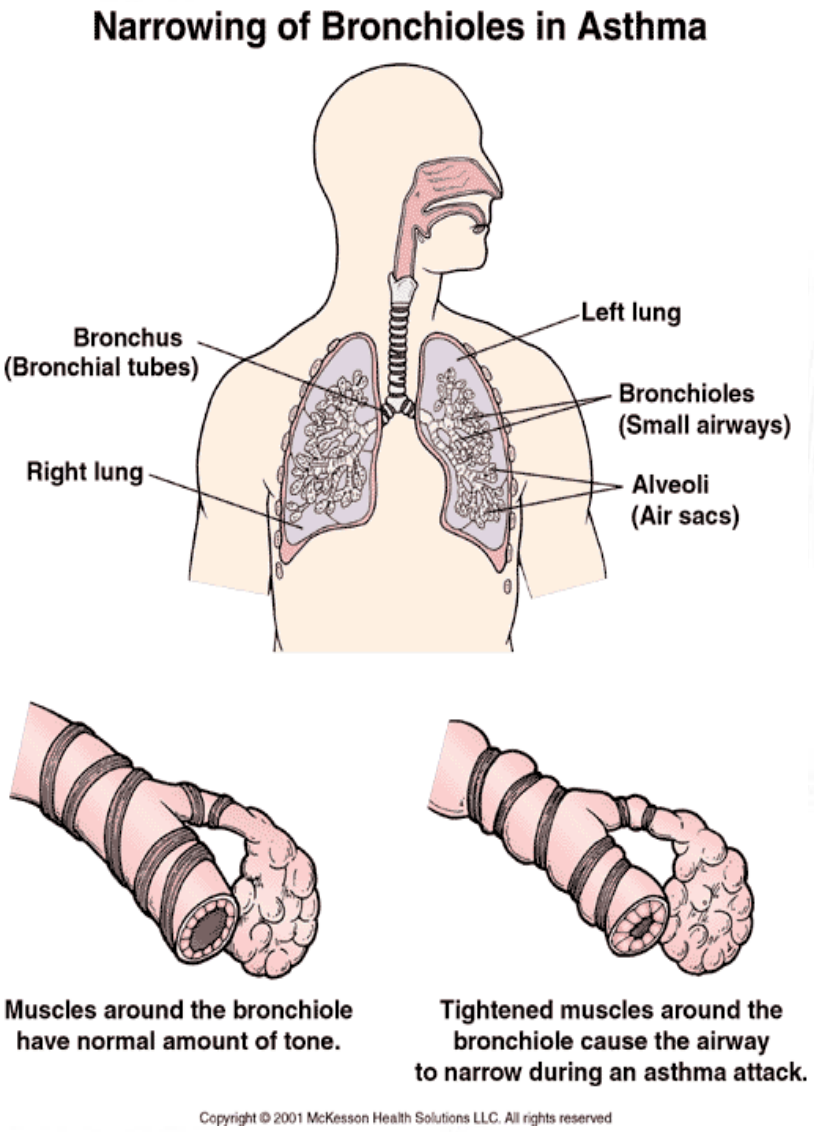
- Oversensitivity of bronchioles.

- **Mild and moderate asthma:**

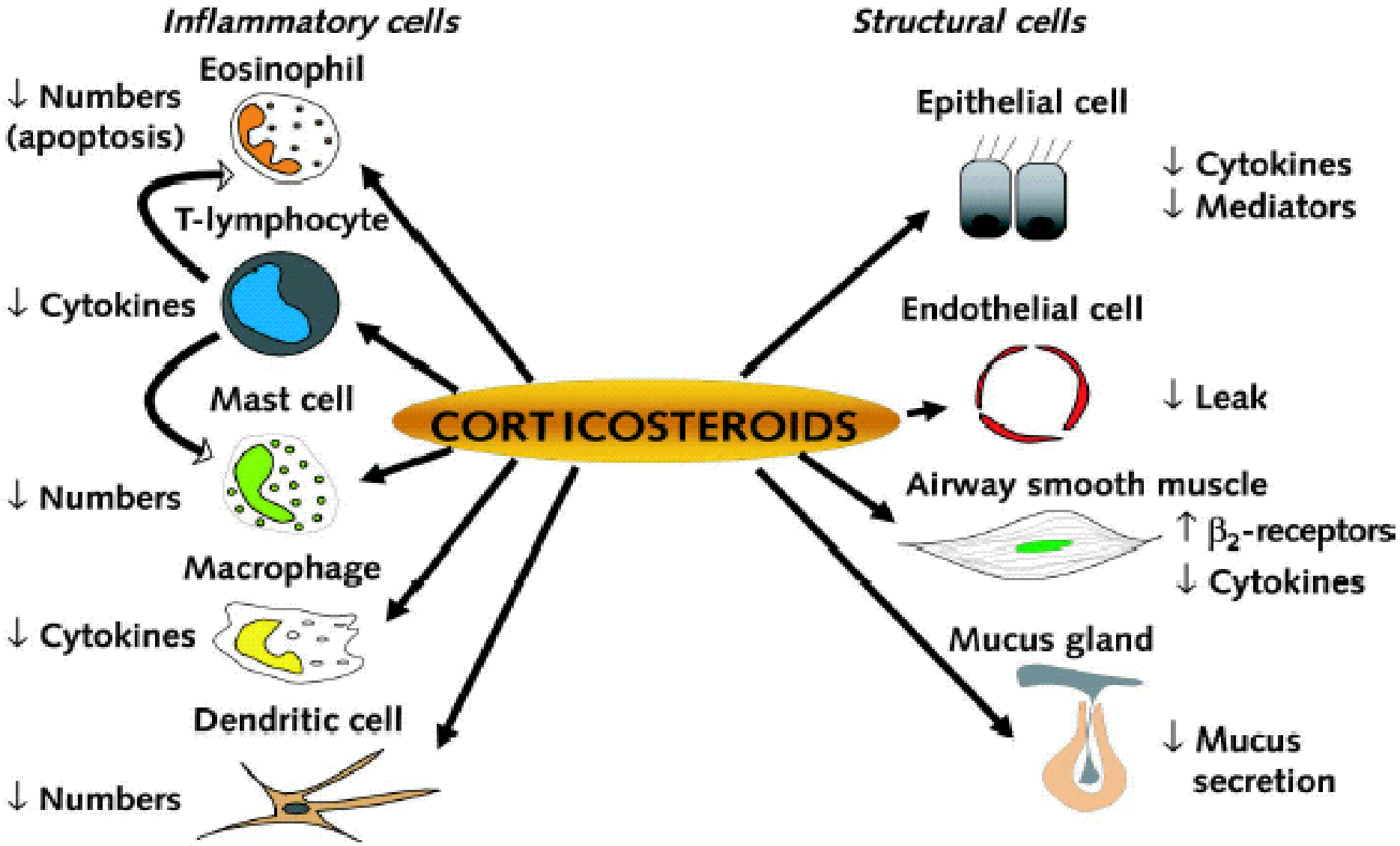
  * lymphocytic and eosinophilic infiltrations in airways
  * injury and lost of respiratory epithelium
  * degranulation of mastocytes
  * accumulation of collagen under basal membranes

- **In advanced asthma:**

  * occlusion of airways by mucus
  * hyperplasia/hypertrophy of smooth muscle cells
  * hyperplasia of epithelial cells



Cellular effects of cortisol



Eosinophils – asthma- glucocorticoids

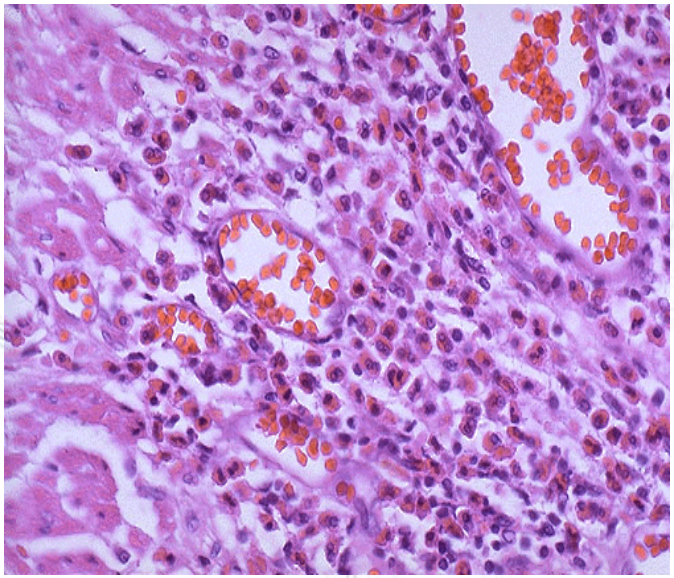
- Eosinophils are the major cells in response to parasites of respiratory system

- In patients with asthma there are massive eosinophil infiltration in the airways.

- Treatment with corticosteroids patients with asthma decreases inflammation in airways, mostly through induction of eosinophil apoptosis, then eosinophiles are phagocyted by macrophages and epithelial cells.

Some patients do not respond for treatment with corticosteroids. It can be associated with the presence of β splicing form of GR.

- Eosinophils isolated from patients with asthma resistant to corticosteroid are also resistant to corticosterone-induced apoptosis.

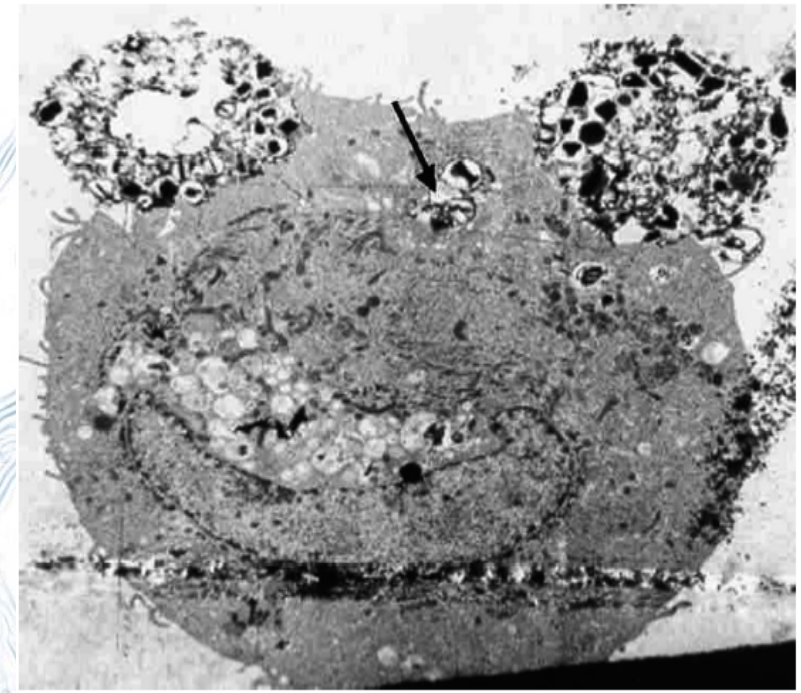


Eosinophils – asthma- glucocorticoids

- In the case of massive apoptosis important is a fast phagocytosis of the dead cells. If not – the secondary necrosis can occur. The content of cells is released and induces inflammation.

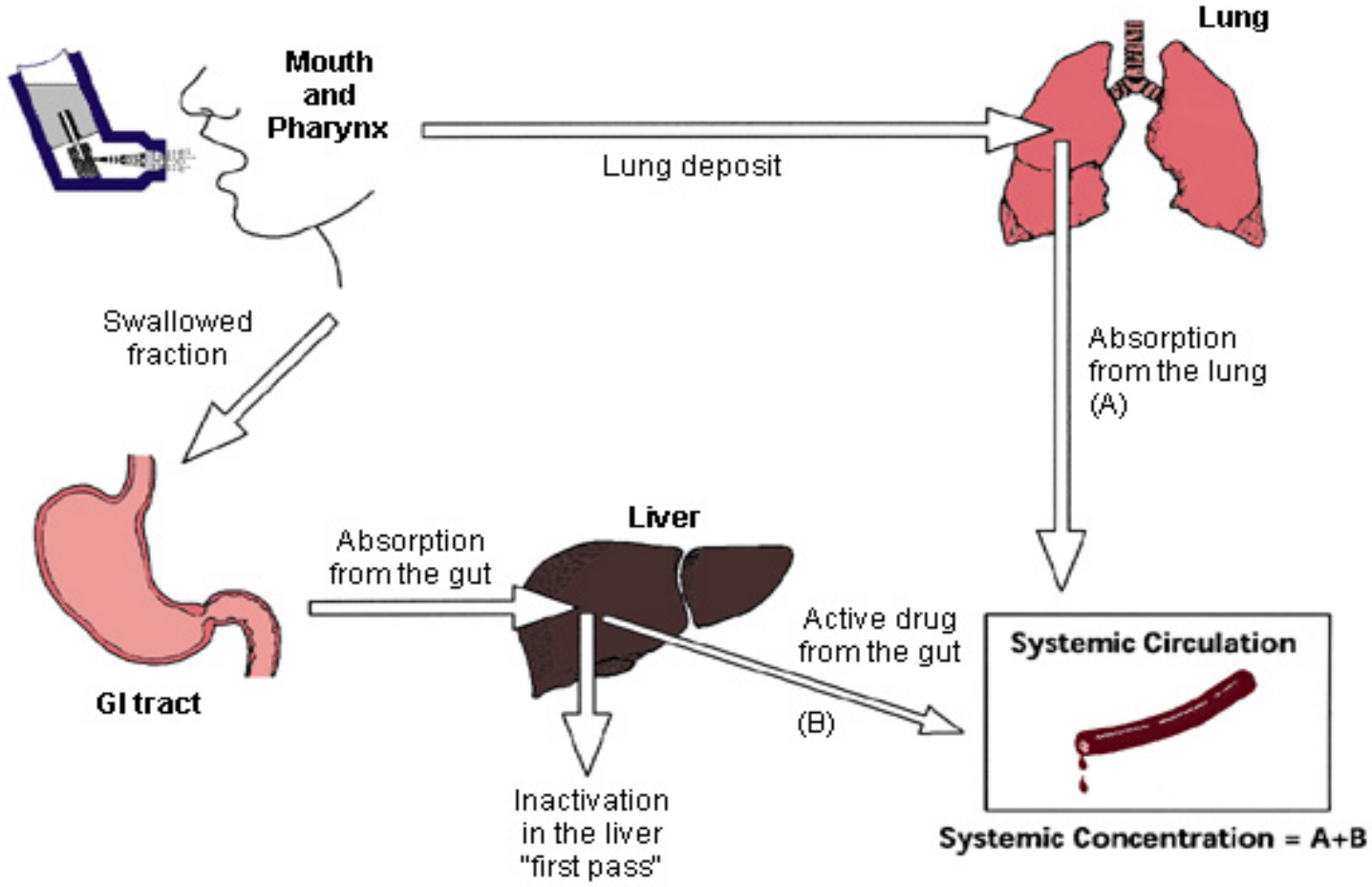
- Major cells responsible for removal of apoptotic eosinophils are macrophages. Glucocrticosteroids increase phagocytosis of eosinophils by macrophages and epithelial cells.

phagocytosis of eosinophils by epithelial cell



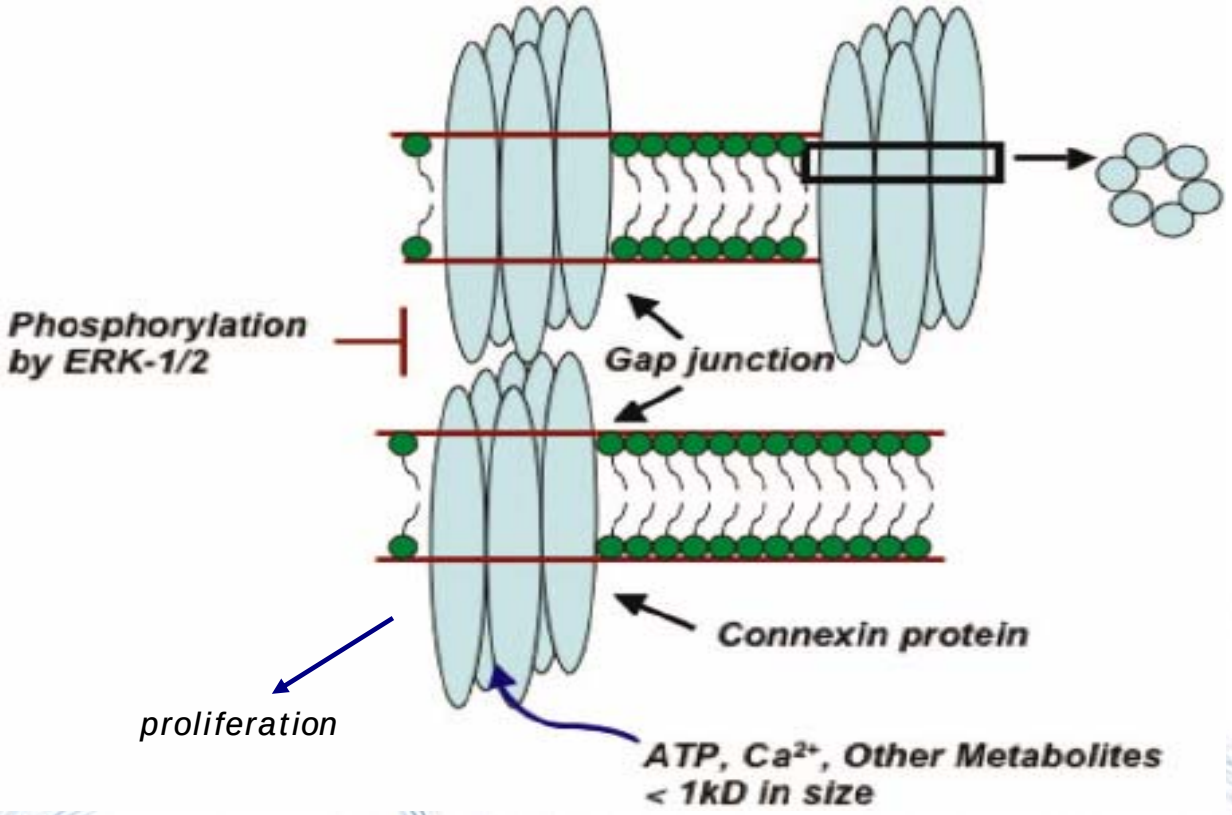
Asthma and glucocorticoids

Currently the major way of corticosteroid application is inhalation. Corticosteroids remain still the basic drug in tretatment of asthma.



Non-classical signaling by glucocorticoids

Six connexin proteins combine to form a gap junction hemi-channel. Two hemi-channels from adjacent cells form a gap junction through which metabolites and small molecules (less than 1 kD) can pass.



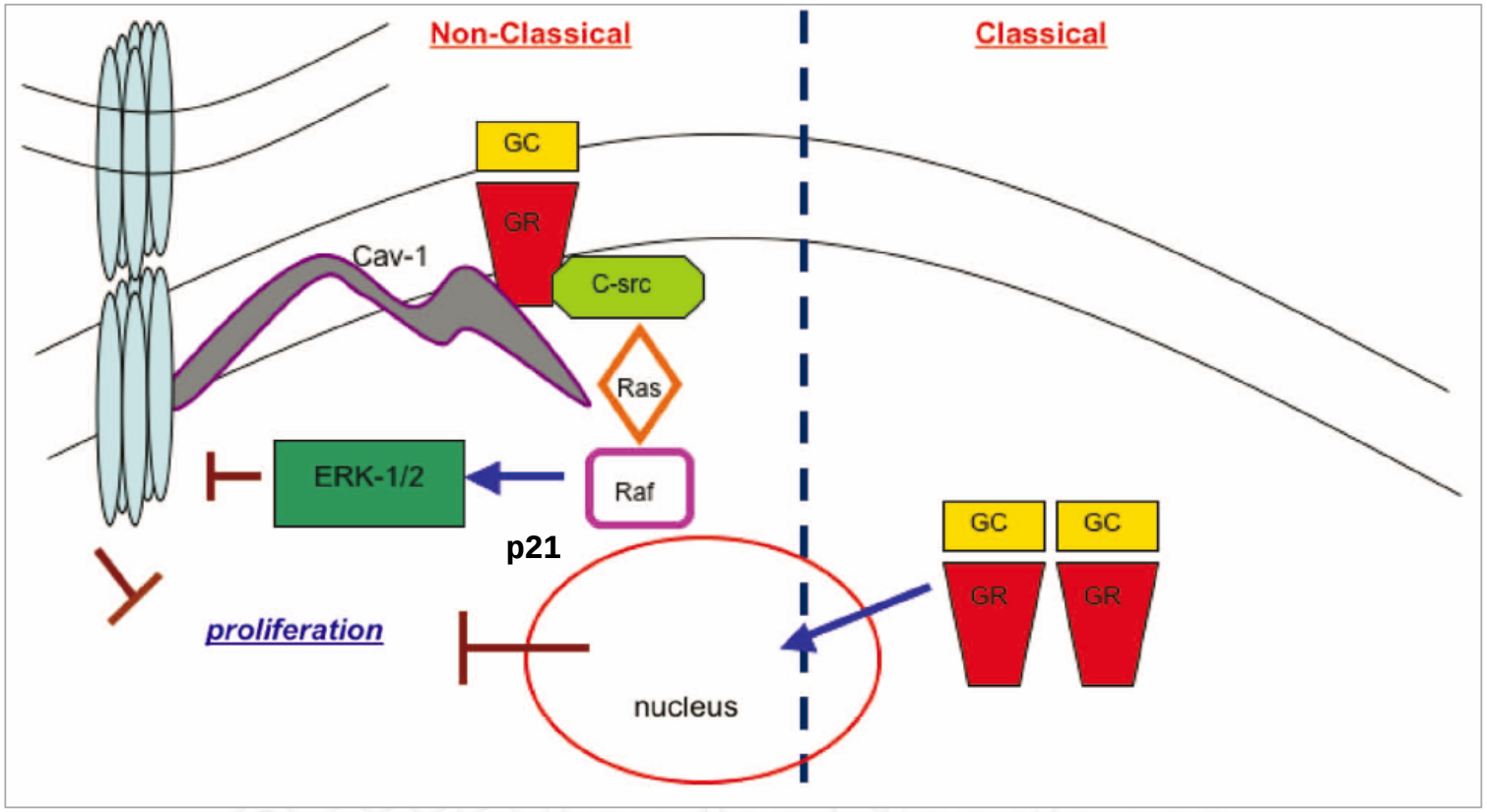
Connexin 43 gap junctions can be phosphorylated by ERK-1/2, and this phosphorylation leads to inhibition of gap junction intercellular communication

Non-classical signaling by glucocorticoids

Activation of non-classical and classical pathways by GCs alters cell proliferation

signaling by membrane GR associated with Cav-1, activation of c-src, activation of ERK1/2, phosphorylation of Cx43, and reduction of gap junction proproliferative signaling

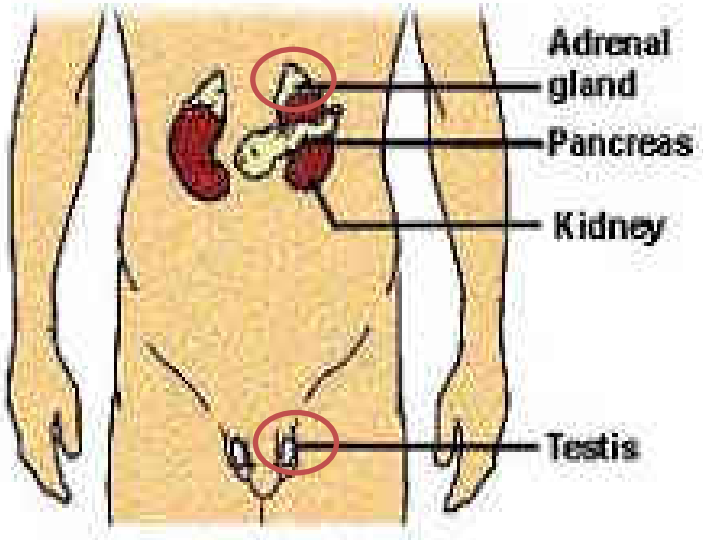
upregulation of p21 transcription through classical way

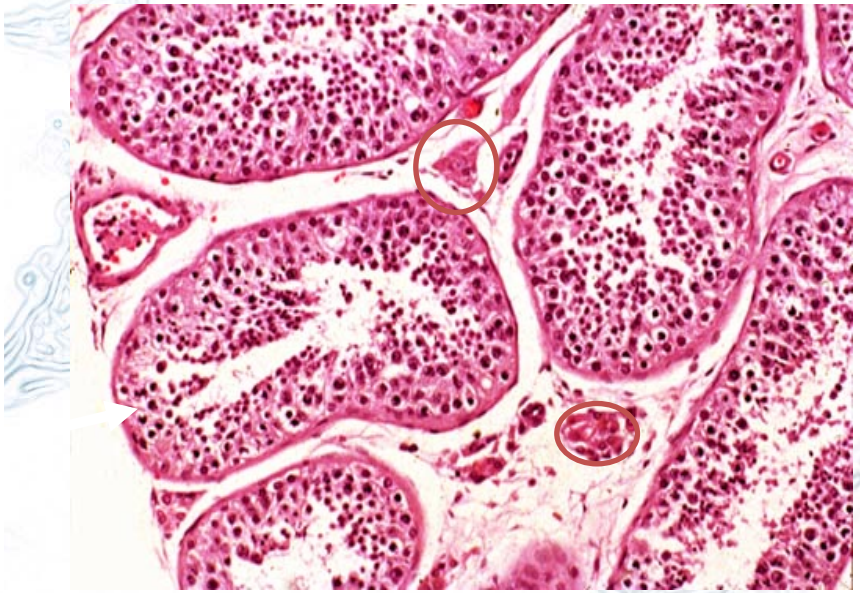


Samarasinghe et al. Cell Cycle 2012

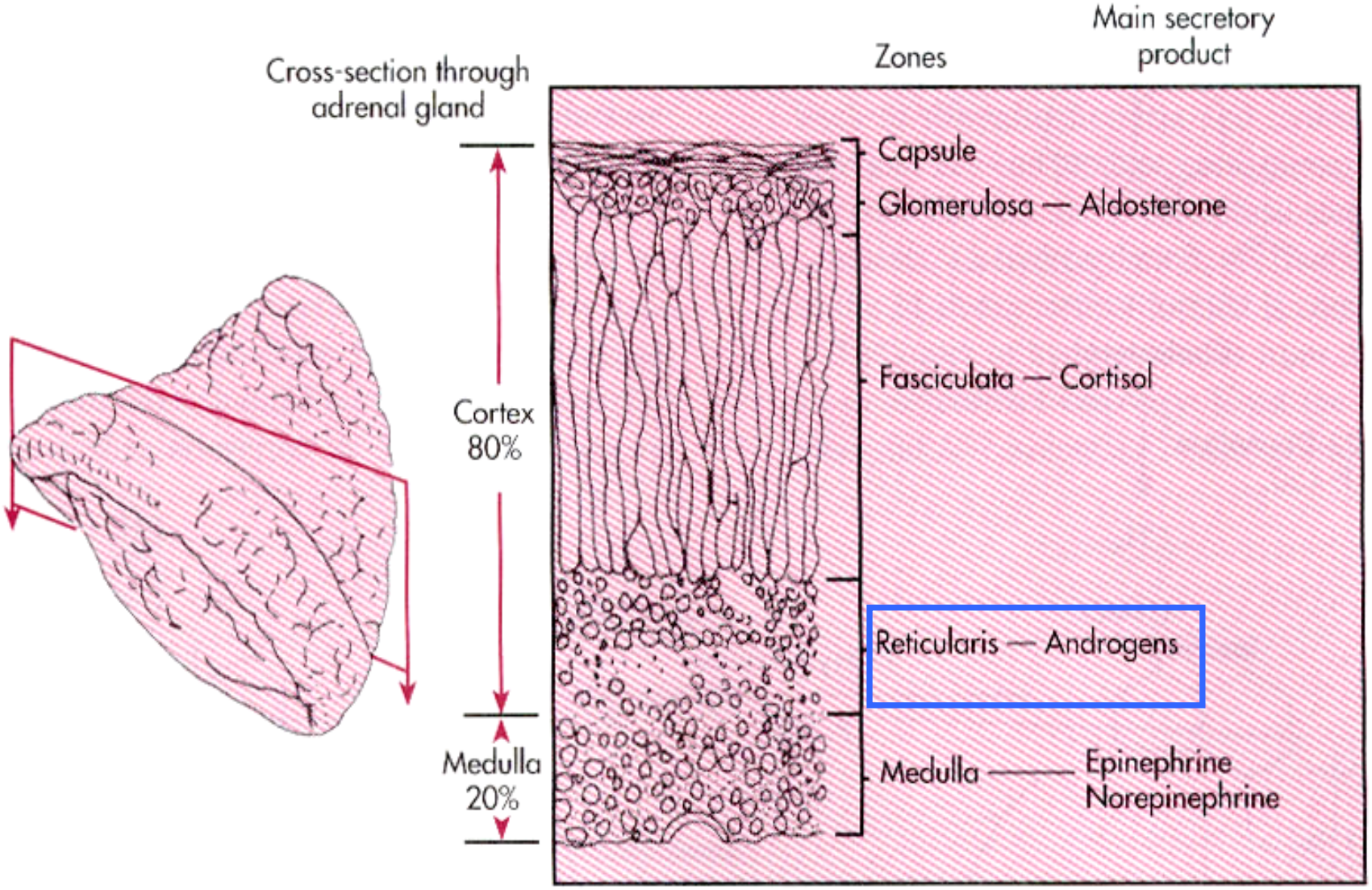
Androgens – general characteristics

- The most abundantly synthetized ligand of androgen receptors (AR) is testosterone, produced by the **Leydig cells** in response to luteinizing hormone produced by the pituitary gland.



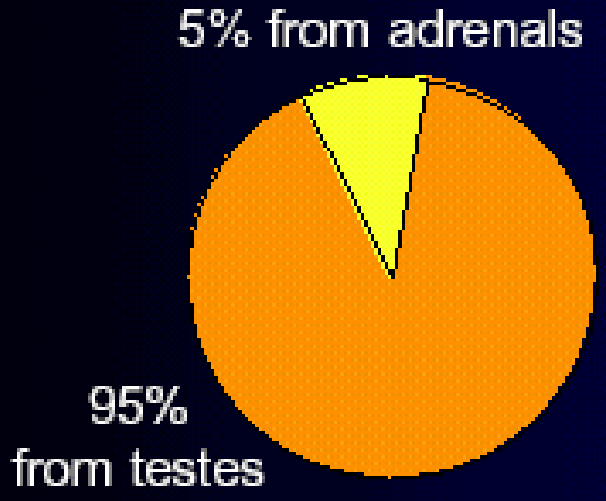


Adrenal glands

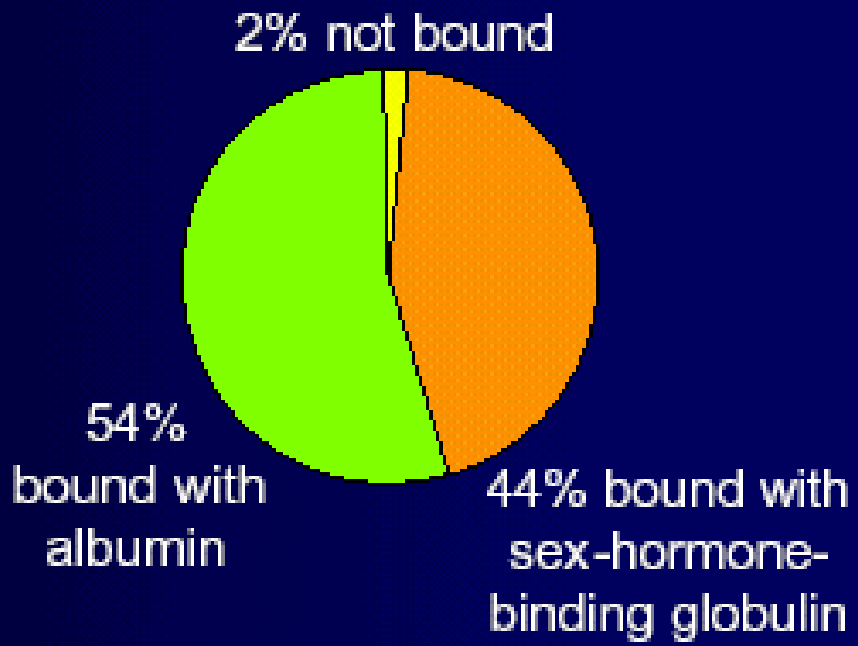


Testosterone

Origin



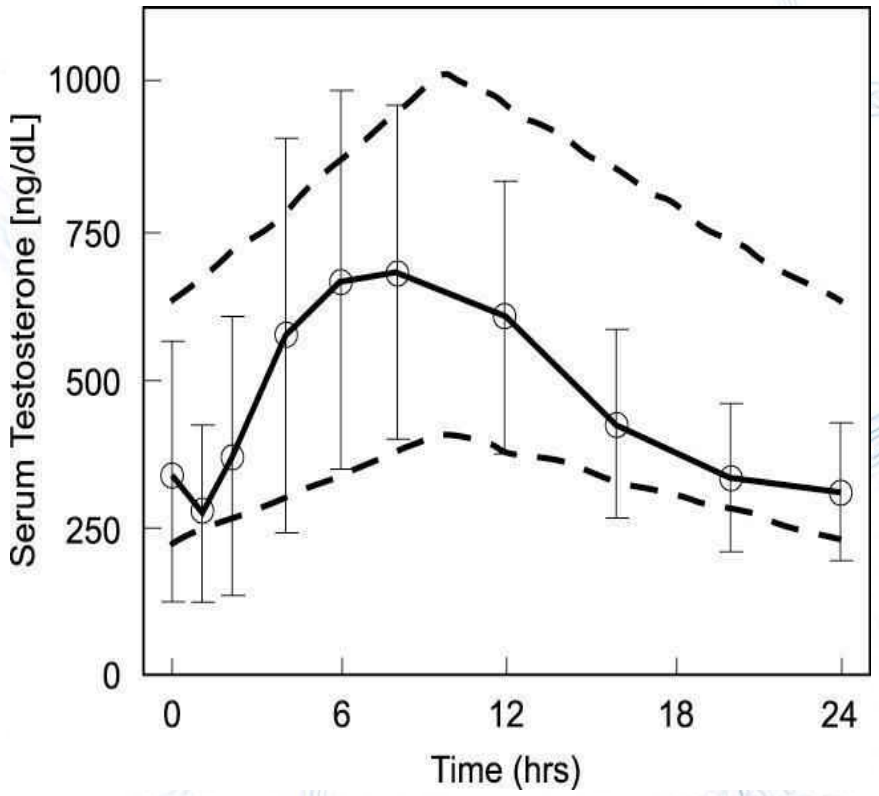
Distribution

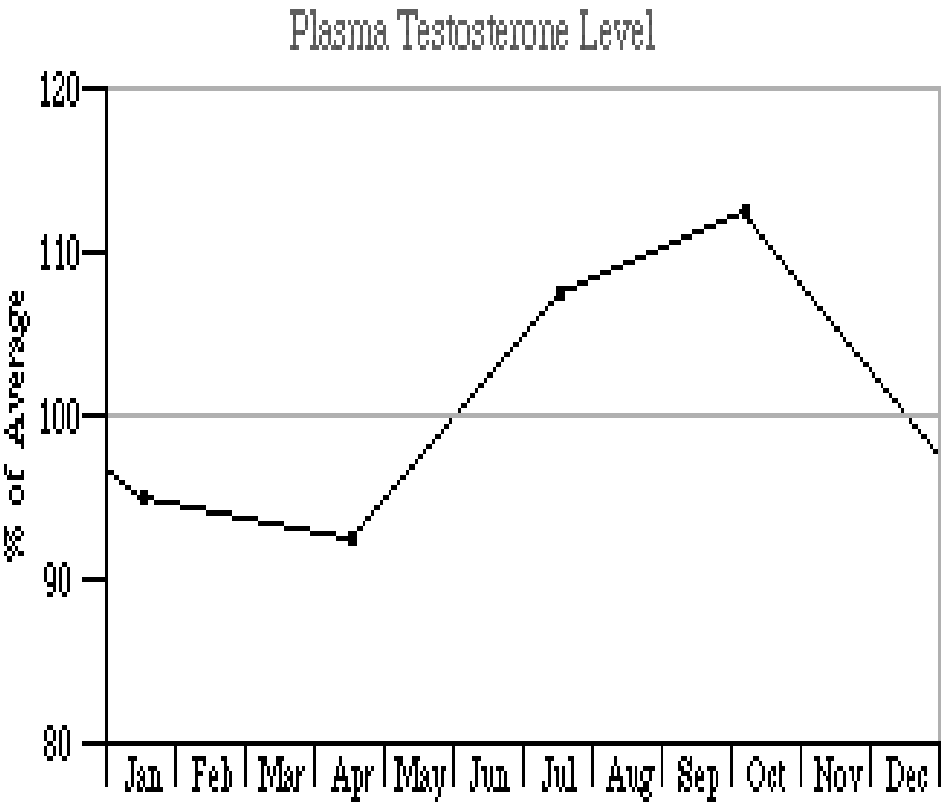


Source: Adapted from Coffey DS. In: Walsh PC, et al, eds. *Campbell's Urology*. 6th ed. 1992:221-266.

Androgens – general characteristics

- Production of testosterone changes periodically with circadian and seasonal peaks.





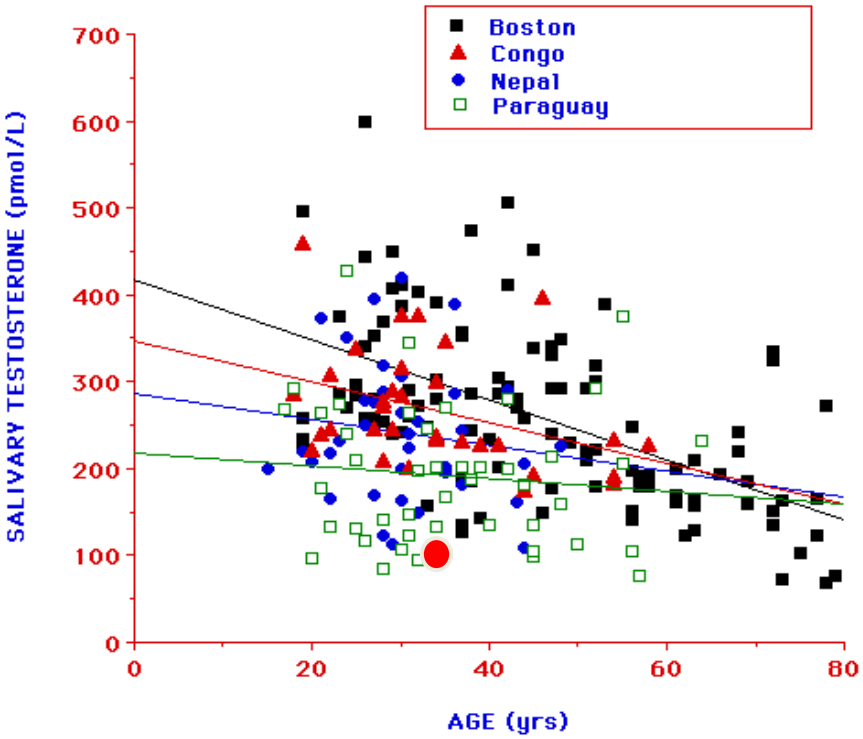
Testosterone – changes with age

- Level of testosterone gradually decreases with age (starting from the age 20-30).

- No data indicates the correlation between the level of testosterone and sexual behavior, unless the changes are within the physiological range.

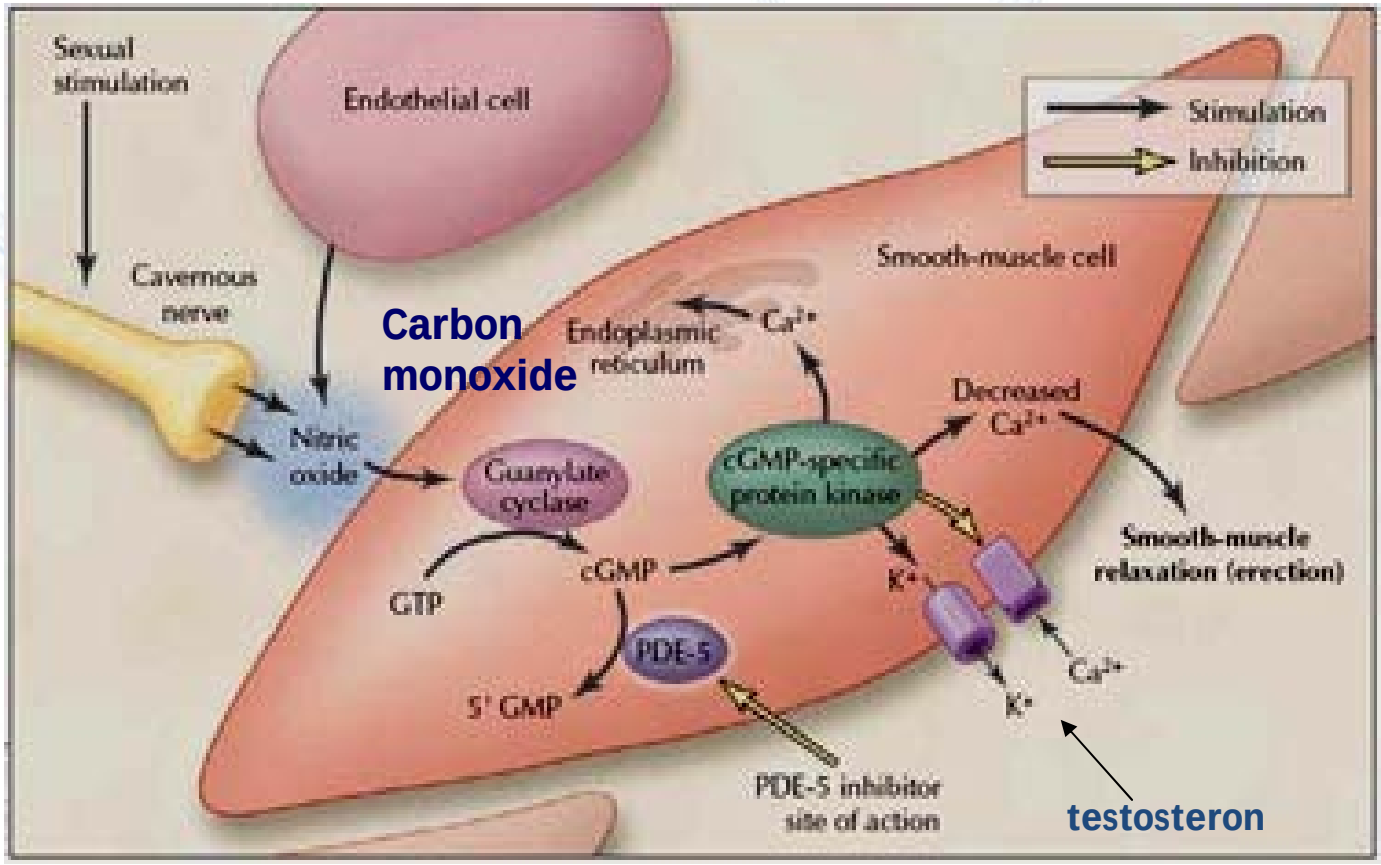
- In men with healthy gonads, but with erectile dysfuctions, supplementation with testosterone does not give any benefits.

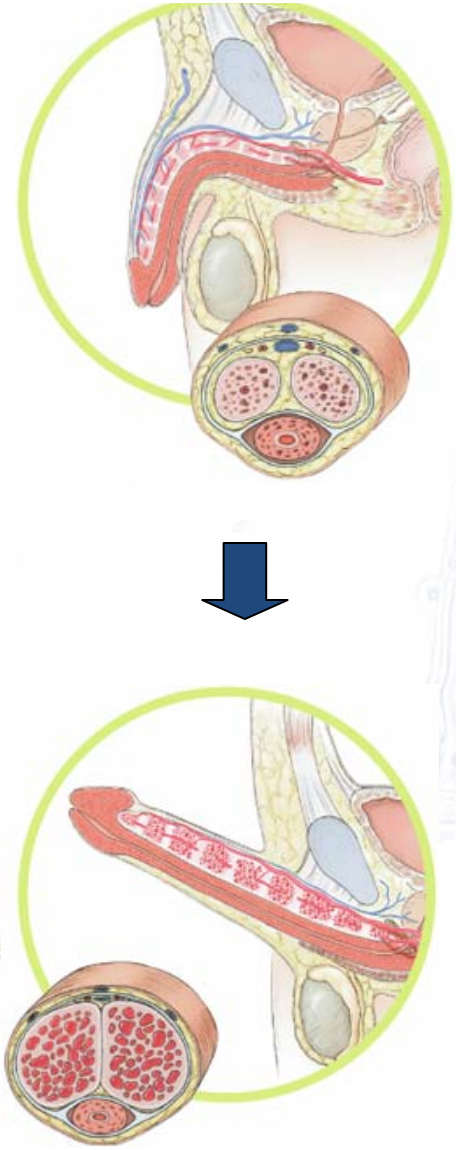
- In hypogonadal men it can give the increase in ejaculation frequency, but does not improves erection itself.



• **NO is released from nerve endings or from endothelial cells, and stimulates cGMP production. This induces smooth-muscle relaxation by reducing the calcium ion concentration.**

• **PDE-5 reverses this cascade by converting cGMP to GMP. PDE-5 inhibitors (e.g. sildenafil), work to inhibit this enzyme, thereby continuing smooth-muscle relaxation and prolonging an erection.**





Erectile dysfunction

- Decline in sexual function with age
- 1290 subjects (40-70 yrs)
  - 9.6% complete ED (5.1% at 40 to 15% at age 70)
  - 25.2% moderate ED
  - 17.2% minimal ED

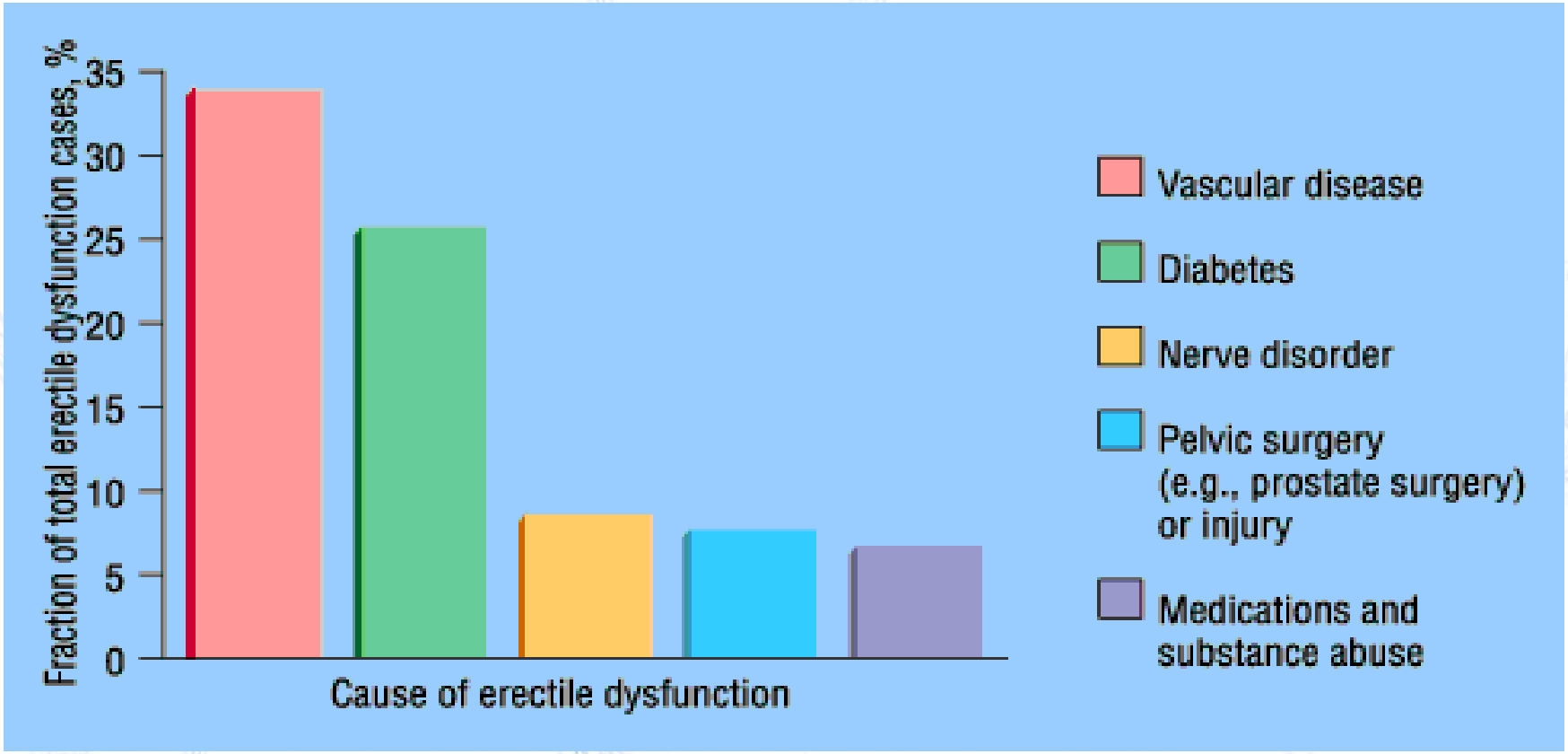
52%

Results: ED Prevalence by Country

ED prevalence for the entire sample was 19%

Erectile Dysfunction (ED)

- ED, once thought to be psychogenic
- Later, considered androgenic
- Now, found to be predominately vasculogenic



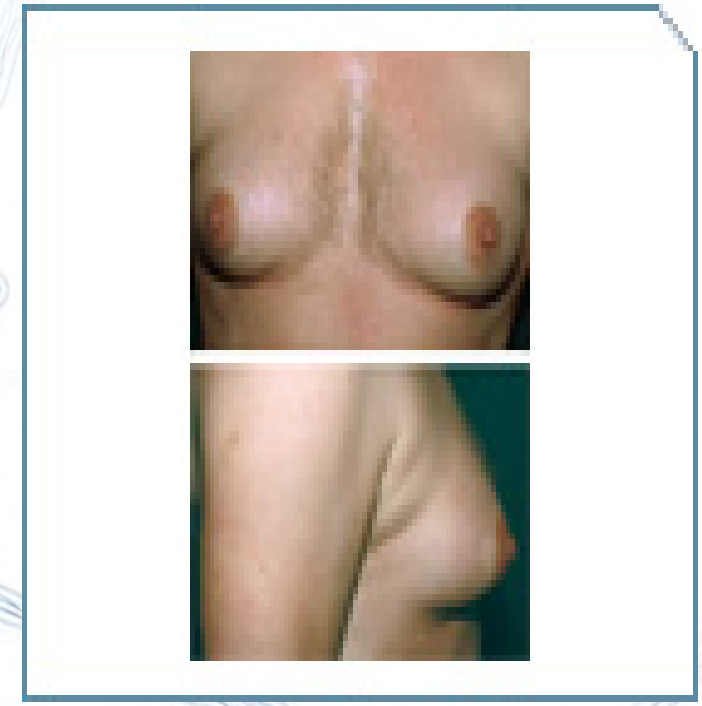
AR – androgen receptors

- Point mutations in AR:

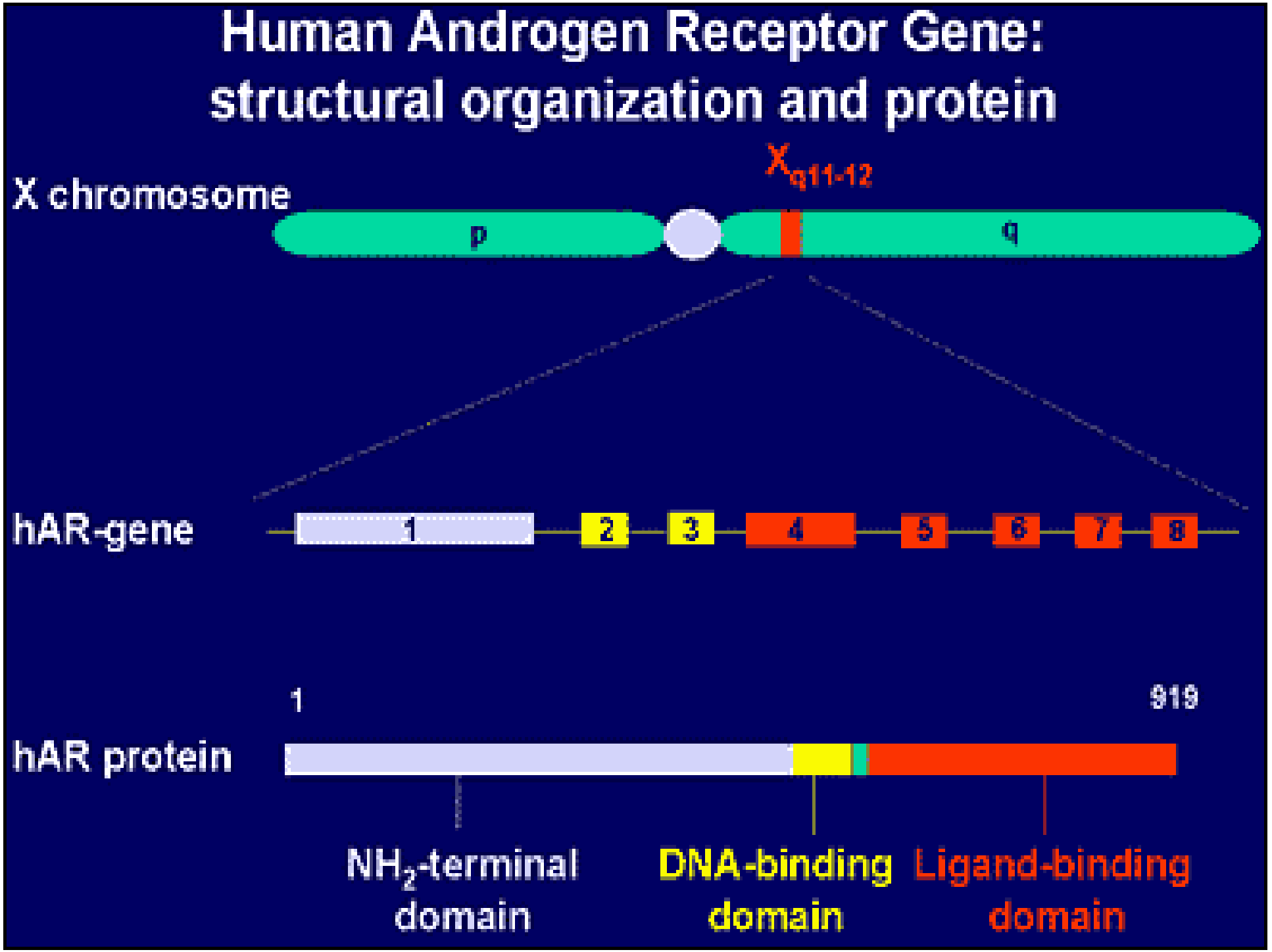
* May result in acquiring the sensitivity of AR protein to the other ligands (including anti-androgens).

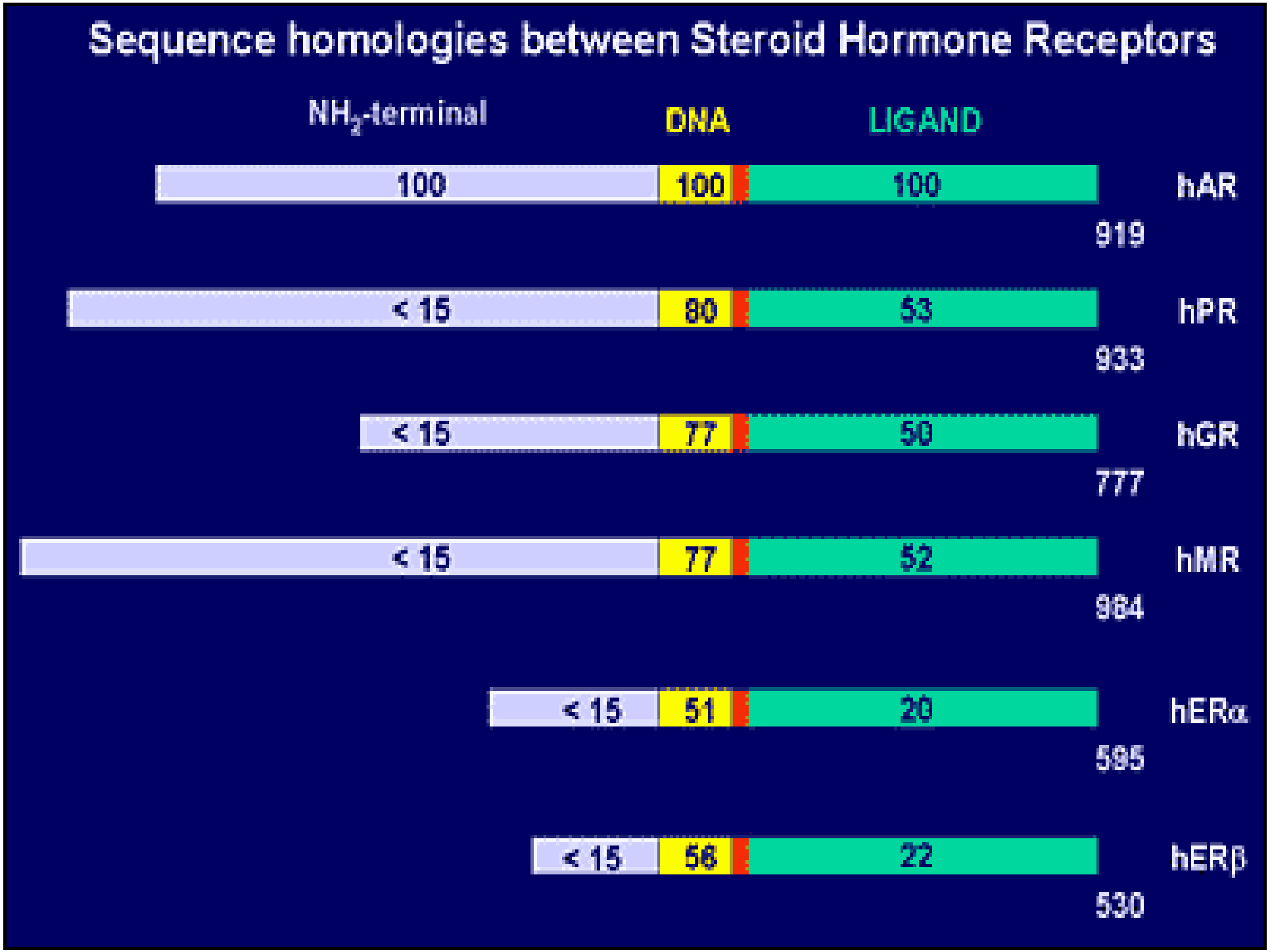
* May result in decreased sensitivity to androgens, leading to **Reifenstein's syndrome:**

- gynecomastia,
- atrophy of testes,
- oligosperm or azoosperm,
- increased level of gonadotropin,
- absence of sense of smell.



Gynecomastia in a man with Reifenstein's syndrome


Human Androgen Receptor Gene:
structural organization and protein
X chromosome
X_{q11-12}
p
q
hAR-gene
1
2
3
4
5
6
7
8
1
919
hAR protein
NH_2-terminal domain
DNA-binding domain
Ligand-binding domain


Sequence homologies between Steroid Hormone Receptors
NH2-terminal
DNA
LIGAND
100
100
100
hAR
919
< 15
80
53
hPR
933
< 15
77
50
hGR
777
< 15
77
52
hMR
984
< 15
51
20
hERα
595
< 15
56
22
hERβ
530

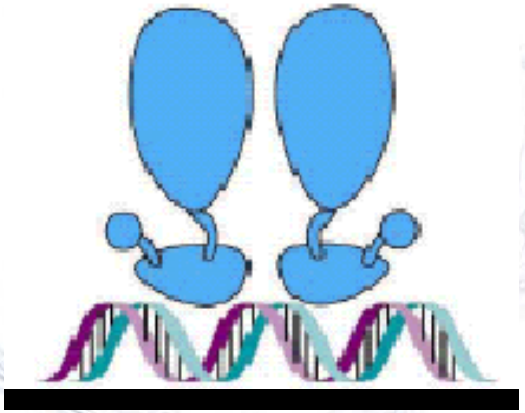
AR – androgen receptors

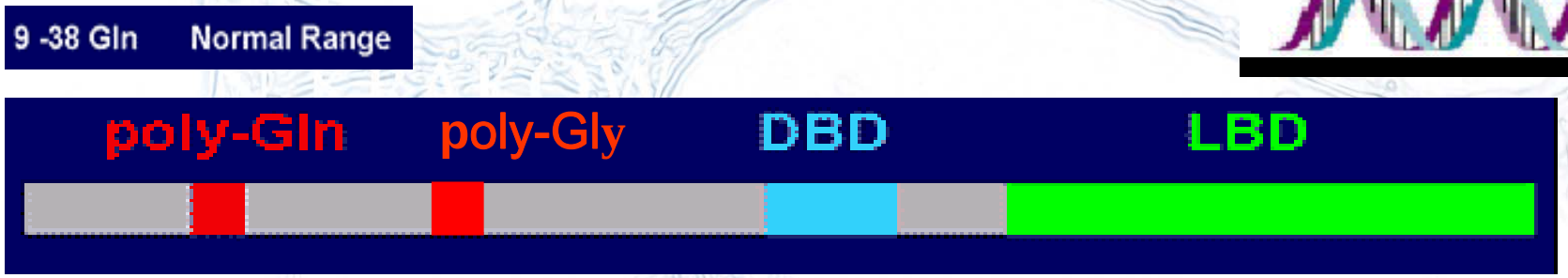
- AR was cloned in 1988. There are several isoforms of AR (98.4-100 kDa).

- Different sizes of AR proteins result from the polymorphism of glycine-reach sequence (GGC) or glutamine-reach sequence (GAC) at the N-terminus.

- Elongated GAC fragment **decreases** transcriptional activity of AR protein.

- N-terminal repeats of GAC are shorter in the primates phylogenetically more distant from human.





Kennedy's Syndrome

- Neurogenerative disease (described in 1911 by Dr. Foster Kennedy) manifested with:

  * decreasing sensitivity to androgens in adult men
  * contineous weakness and atrophy of muscle (e.g. facial).

- Symptoms result from lost of motoric neurons. The most pronounced weakness is observed in muscles of face and tongue (in the third to fifth decade):

  * weakness of facial and arm muscles,
  * tremor of hands,
  * increased level of kreatinin kinase.

- **Longer polyglutamine CAG** fragment in AR is associated with **earlier onset** of the disease.

- Women with long CAG fragment in AR have only some subtle neurological changes which can be detected during detailed examination.

Prostate cancer

- Mean number of **CAG** (glutamine) **repetitions** (Africans<Europeans<Asians) **correlates with risk of prostate cancer**.

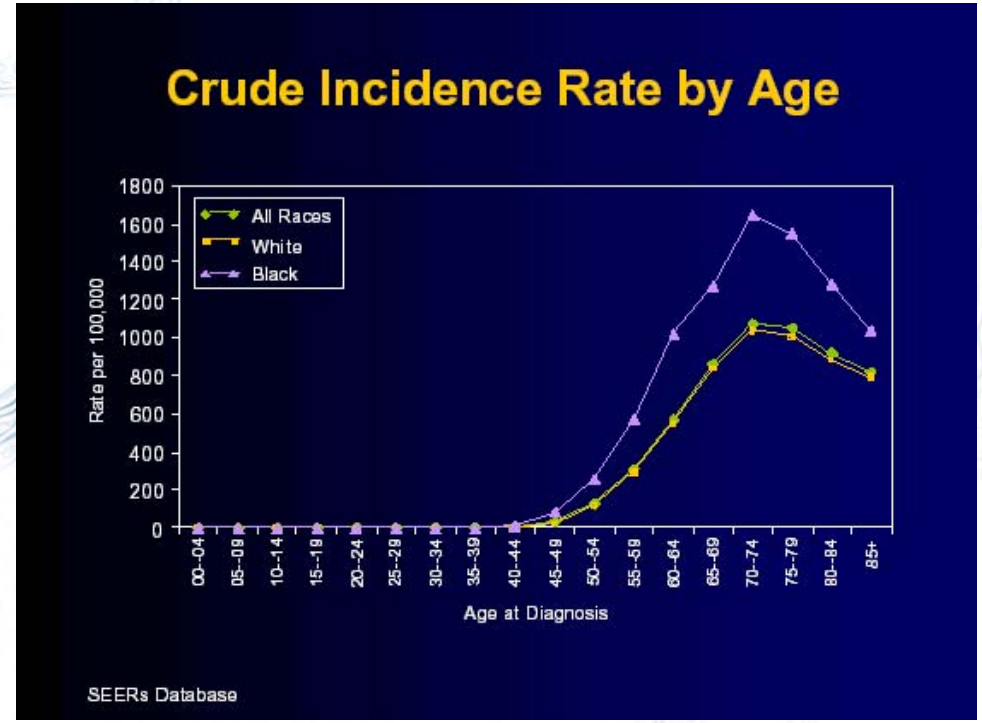
- Case report: in the healthy tissues AR had CAG=24, while in tumor CAG=18 (both lengths were within the normal values).

- Ethic differences may be associated with:

* higher level of testosterone in Africans

* lower activity of 5α reductase in Asians.

- In Japan less clinical cases of prostate cancer is noticed than in USA but in postmortem investigations the numbers of pre-clinical or latent tumors in both countries are similar.



Thank you and see you next week…

What would be profitable to remember in June:

- Ligands for MR – why cortisol acts as mineralocorticoid only in some tissues
- Regulation of aldosterone synthesis – effect on hypertension
- Antiinflammatory activities of corticosteroids
- Differences between activity of GRa and GRb
- Classical and nonclassical action of GS
- Effect of AR polymorphism on risk of diseases