TABULÆ ANATOMICÆ

TABULA II.
Novas exhibet corundem rennum, incumbentium glandularum, & vasorum figuras.
- 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.

- Glucocorticoids were regarded as compounds of both glucocorticoid and mineralocorticoid activities.
Aldosterone and cortisol synthesis

Cholesterol → Pregnenolone → Progesterone → 11-Deoxycorticosterone

CYP11A → CYP17 → 17OH-Pregnenolone → 17OH-Progesterone → Corticosterone

HSD3B2 → CYP17 → CYP21

CYP11B1 → 18OH-Corticosterone

CYP11B2 → Aldosterone

Capsule: Glomerulosa → Aldosterone

Fasciculata: Cortisol

Reticularis: Androgens

Medulla: Epinephrine, Norepinephrine

Glucocorticoid

Mineralocorticoid
Aldosterone is at 1000 fold lower concentrations than cortisol

<table>
<thead>
<tr>
<th></th>
<th>Plasma concentration (µg/dl)</th>
<th>Secretion rate (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortisol</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>Corticosterone</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>11-Deoxycortisol</td>
<td>0.16</td>
<td>0.40</td>
</tr>
<tr>
<td>Deoxycorticosterone</td>
<td>0.07</td>
<td>0.20</td>
</tr>
<tr>
<td><strong>Aldosterone</strong></td>
<td><strong>0.009</strong></td>
<td><strong>0.15</strong></td>
</tr>
<tr>
<td>18-OH Corticosterone</td>
<td>0.009</td>
<td>0.10</td>
</tr>
<tr>
<td>Dehydroepiandrosterone sulfate</td>
<td>115</td>
<td>15</td>
</tr>
</tbody>
</table>
Aldosterone in the blood

- Aldosterone was isolated in 1953 (21 mg aldosterone from 500 kg of bovine adrenals), a year later its structure 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 increases its water solubility and facilitates its removal with the urine; similarly in the case of cortisole)
Overproduction of aldosterone – Conn’s disease

(described in 1955 by J.W. Conn; it fact it was first described by M. Lityński in 1953, but he published it in the polish journal)

Cause:

- Mostly tumors developing from adrenal cortex cells (adrenal adenoma), usually at the age 30-50. It can be also caused by adrenal hyperplasia.

Symptoms:

• Strong hypertension,
• Hypokalemia,
• Alcalosis,
• Light hyernatremia,
• Polyuria,
• Tiredness,
• Weakness of muscles.
Variable proportions of aldosterone (MR) and glucocorticoid (GR) binding sites among human tissues

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>MR</th>
<th>GR</th>
<th>MR/GR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal collecting duct</td>
<td>10,000/cell</td>
<td>20,000/cell</td>
<td>1/2</td>
</tr>
<tr>
<td>Colonic epithelium</td>
<td>7,000/cell</td>
<td>21,000/cell</td>
<td>1/3</td>
</tr>
<tr>
<td>Brain hippocampus</td>
<td>100 fmol/mg protein</td>
<td>100 fmol/mg protein</td>
<td>1/1</td>
</tr>
<tr>
<td>Arterial smooth muscle cells</td>
<td>1,000/cell</td>
<td>30,000/cell</td>
<td>1/30</td>
</tr>
<tr>
<td>Cardiac myocytes</td>
<td>10 fmol/mg protein</td>
<td>300 fmol/mg protein</td>
<td>1/30</td>
</tr>
</tbody>
</table>
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.
Regulation of ligand selectivity for MR does not occur at the receptor level, but at the level of 11β-HSD2 activity (epithelium in kidney tubules, 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. However, 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)._
Sodium absorption by the renal tubular system

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

Coffman, 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 the 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.
Regulation of aldosterone action

- Concentration of aldosterone decreases in response to increase in the volume of extracellular fluid.

**I. Hypervolemia** is checked in atrium of the heart, which releases **atrial natriuretic peptide (ANP)** in response to atrial dystension. ANP binds to the receptors in zona glomerulosa and decreases the synthesis of aldosterone.

**II. Hypervolemia** is also checked in juxtaglomerular apparatus (JGA) in the kidney. In response to hypervolemia the production of **renin** decreases leading to reduced synthesis of **angiotensin-II (AngII)** and decreased synthesis of aldosterone. Even small changes in AngII lead to strong responses in the aldosterone level.
Hypertension

* Silent Killer – harmful complications,

* causes dizziness, headache, and visual difficulties,

* Leading risk factor in cardiovascular diseases

* Number one reason for drug prescription.

* 25% of population; among them: ~5%

Normal: 120/80 +/- 10/5
Mild + 20, Moderate +40, Severe +80; Malignant - > 210/120
Consequences of Hypertension:

Cardiovascular system:

Hypertensive cardiomyopathy

Atherosclerosis
Consequences of Hypertension:

Brain:

- Stroke (infarction)
- Haemorrhage
Consequences of Hypertension:

Eye:

Hypertensive retinopathy

- Flame haemorrhage
- Hard exudates
- Cotton wool spot
- Papilloedema
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 cardiomyocytes and activation of macrophages.

- Fibrosis is possible a secondary repair process.

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

Healthy myocardium


Rat heart

COX-2

MCP-1
Spironolactone competitively antagonizes aldosterone binding but may cause endocrine disturbances as a result of its nonselective binding affinity for progesterone and androgen receptors (used in male-to-female transsexual people).

Fig. 3. Survival curves among patients treated with placebo or spironolactone. Risk of death was 30% lower in the spironolactone-treated group in the RALES trial (P < 0.001). [Reproduced with permission from B. Pitt et al.: N Engl J Med 341:709, 1999 (76).]
- Corticosteroids are not stored, but are always synthetised de novo from cholesterol
- Level of circulating corticosteroids is the highest in the morning,
- Circulating corticosteroids are associated with transcortine (cortisol binding globulin, CBG, α2-globulin glycoprotein, 75-80%) and 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.
Hypophysial hypofunction – Addison disease

- Fatigue, no tolerance for even small stress,
- Fever,
- Insulin oversensitivity,
- Fasting hypoglycemia,
- No appetite,
- Nausea,
- Loss of weight,
- Anemia,
- Weakness,
- Low blood pressure,
- Consuming large amounts of salt,
- Increased number of lymphocytes and eosinophils, decreased neutrophils,
- Hair loss,
- Increased pigmentation of skin and mucosa (because of increased secretion of ACTH and activation of propio-melanocortine).
Hyperactivity of adrenal cortex – Cushing syndrome

Causes:
- treatment with pharmacological doses of corticosteroids
- overproduction of ACTH (e.g. pineal cancer, hyperplasia of pineal gland)

Symptoms:
- abdominal obesity with bull hump and round face,
- osteoporosis,
- thin skin with red striae,
- muscle weakness and atrophy,
- brushes after even weak trauma,
- hair loss,
- impaired wound healing,
- weak response to infections,
- hyperglycemia and increased neoglucogenesis,
- aggressiveness and depression
- high blood pressure
Cortisol activity

• ↑ glukoneogenesis, ↓ 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 the cells in number of 3,000 - 30,000 molecules per cell.

- GR without ligands are located in the cytoplasm, where are bound with Hsp90

- 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

Effect of GC (e.g., increased transcription)

Diminished Effect

(e.g. increased transcription)
Cortisol and immunological system

• in pharmacological doses is used as an antiinflammatory compound, which prevents also transplant rejection

• induces lipocortine, which inhibits phospholipase A2 producing arachidonic acid, a precursor of prostaglandins; thus it inhibits prostaglandin synthesis

• stabilizes lysosomal membranes, decreasing local release of proteolytic enzymes and hialuronidase in the site of inflammation

• decreases proliferation of mastocytes and thereby inhibits production of histamine in the inflammed tissue (but does not inhibits release of histamine from existing mastocytes)

• decreases leukocytic infiltrations, decreasing the synthesis of chemoattractants, and decreasing the permeability of endothelium

• cortisol inhibits the expression of e.g. IL-1, IL-6, IFNγ, TNFα (but may upregulates their receptors on the target cells)
Cellular effects of glucocorticosteroids
Corticosteroids and gene transcription

### Increased transcription
- Annexin-1 (lipocortin-1, phospholipase A₂ inhibitor)
- β₂-adrenergic receptor
- Secretory leukocyte inhibitory protein
- Clara cell protein (CC10, phospholipase A₂ 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 A₂
- Inflammatory receptors
  - Tachykinin NK₁-receptors, NK₂-receptors
  - Bradykinin B₂-receptors
- Peptides
  - Endothelin-1
Healthy synovial joint

- The synovial joint is composed of two adjacent bony ends each covered with a layer of cartilage, separated by a joint space and surrounded by the synovial membrane and joint capsule.

- The synovial membrane is normally <100 µm thick and the synovial lining consists of a thin (1–3 cells) layer of synoviocytes (macrophage derived and fibroblast derived);

- Only a few, if any, mononuclear cells are interspersed in the sublining connective tissue layer, which has considerable vascularity. The synovial membrane covers all intra-articular structures except for cartilage and small areas of exposed bone and inserts near the cartilage–bone junction.
Rheumatoid arthritis

- Rheumatoid arthritis (RA) is characterized by an inflammatory response of the synovial membrane conveyed by a transendothelial influx and/or local activation of T cells, B cells, plasma cells, dendritic cells, macrophages, mast cells, as well as by new vessel formation.

The lining layer becomes hyperplastic (a thickness of >20 cells) and the synovial membrane expands and forms villi.

- The hallmark of RA is bone destruction. The destructive cellular element is the osteoclast; destruction mostly starts at the cartilage–bone–synovial membrane junction. Bone repair by osteoblasts usually does not occur in active RA.

- The neutrophils' enzymes, together with enzymes secreted by synoviocytes and chondrocytes, lead to cartilage degradation.
Rheumatoid arthritis

healthy

arthritic
Rheumatoid Arthritis: Key Features

- **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)**
Rheumatoid Arthritis

- Affects approximately 1% of the adult U.S. population
- Incidence increases with age
- Occurs 2-4 times more often in women
- Shortens lifespan by 3-18 years
  (average of 10 years)
Role of Tumor Necrosis Factor in Rheumatoid Arthritis

TNF

- Bone resorption
- Joint inflammation
- Cartilage degradation
- Bone erosion
- Pain/joint inflammation
- Joint space narrowing
Joint destruction

Kirvan et al. Z Rheumatol, 2000
Asthma

- Inflammatory reaction and reversible constriction of muscles.

- Oversensitivity of bronchioles.

- Mild and moderate asthma:
  - infiltration of airways with lymphocytes and eosinophils
  - 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
Eosinophils – asthma- glucocorticoides

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

- They play a crucial role in pathogenesis of asthma and other allergic diseases. 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. Glucocorticosteroids increase phagocytosis of eosinophils by macrophages and epithelial cells.

*phagocytosis of eosinophils by epithelial cell*
- Glucocorticoids are the basic drugs in treatment of asthma.
- Currently the major way of corticosteroid application is inhalation.
SF1 (splicing factor-1)

- The human SF-1 gene encodes a 461-aminoacid orphan nuclear receptor.

- SF-1 binds to DNA as a monomer and recognizes variations of the DNA sequence motif, T/CCA AGGTCA. In most cases, SF-1 functions cooperatively with other transcription factors to modulate the timing and level of gene expression.

- It is possible that SF-1 is regulated independently of a specific ligand; its pattern and level of expression, interaction with other transcription factors, or posttranslational modifications represent plausible means to control its action.
In the mouse, SF-1 is first expressed in the urogenital ridge at embryonic d9. After gonadal determination (e13), SF-1 is expressed in a population of rapidly proliferating cells in the developing testis (20).

- In Sertoli cells, SF-1 regulates Mullerian inhibiting substance expression, leading to regression of Mullerian structures in males.

- In Leydig cells, SF-1 regulates the steroidogenic enzyme genes that control testosterone biosynthesis.

- SF-1 also plays an important role in the normal development and function of the hypothalamic-pituitary-gonadal axis. It is expressed in the hypothalamus and in pituitary gonadotropes.

- Thus, SF-1 regulates an many genes involved in sex determination and differentiation, reproduction, and steroidogenesis.
SF1

- SF-1 is a master regulator of reproduction, because its targets include genes at every level of the hypothalamic-pituitary-gonadal axis, as well as most genes involved in gonadal and adrenal steroidogenesis.

-SF-1 knockout mice have adrenal and gonadal agenesis. The XY mice exhibit male-to-female sex reversal, including persistent Mullerian structures, reflecting the absence of fetal androgens and Mullerian inhibiting substance.

![Mouse embryo](image)

**Fig. 2. Structures of the Testes and Internal Genitalia of Gonad-Specific SF-1 KO Mice**

Adult WT and gonad-specific SF-1 KO males were killed and their internal structures were displayed. **Left**, WT male. **Right**, SF-1 KO male. Testes in the gonad-specific SF-1 KO male are indicated by the **dotted ovals**. T, Testis; SV, seminal vesicle; E, epididymis; B, bladder; U, ureter; VD, vas deferens.

Jeuyasuria et al. 2004, Mol Endocrinol; Gut et al., Development 2005
Regulation of SF-1 activity

Fowkes and Burrin, J Endocrin 2003
DAX1 (dosage-sensitive sex reversal-adrenal hypoplasia congenita critical region on the X chromosome, gene 1)

-DAX-1 encodes an atypical nuclear hormone receptor that contains the ligand-binding domain but lacks DNA-binding motif.

- Alternative splicing may generate a second DAX-1 isoform.

- DAX-1 can inhibit the function SF-1 and other nuclear receptors adding.

-Like SF-1, DAX-1 is expressed not only in the adrenal primordium from its earliest stages of development but also in developing gonads, and pituitary gonadotropes.

- Expression of DAX is positively regulated by SF-1.
DAX1 (dosage-sensitive sex reversal-adrenal hypoplasia congenita critical region on the X chromosome, gene 1)

- Mutations in the DAX1 gene in humans cause the X-linked cytomegalic form of adrenal hypoplasia congenita, a rare disorder characterized by impaired development of the adrenal cortex and hypogonadism (HHG). Affected boys develop adrenal failure shortly after birth or during early childhood, whereas hypogonadism is visible during puberty.

- In Dax-1 KO mice, spermatogenesis was impaired, suggesting a distinct role for DAX1 in sperm development.

http://www.jamesonlab.northwestern.edu/
SF1 and DAX

-The coexpression of DAX-1 with SF-1 in the gonadal and adrenal axes and the adrenal failure seen in patients with mutations in these genes, suggest that DAX1 and SF-1 interact in a common genetic pathway.

- An SF-1 response element has been identified in the DAX1 promoter, and SF-1 activates Dax1 expression.

- In vitro studies show that DAX-1 represses SF-1–mediated transactivation. DAX-1 inhibits transcription of SF-1 target genes.

- These complex, reciprocal interactions may represent feedback loops between SF-1 and DAX1 that maintain the appropriate expression level of target genes in the adrenal cortex.
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 GRα and GRβ – implications in therapy
- DAX1 and SF-1: general characteristics

Slides can be found in the library and at the Heme Oxygenase Fan Club page:

https://biotka.mol.uj.edu.pl/~hemeoxygenase