General adaptation syndrome (GAS)

Definition of stress and GAS

Phases of stress reaction

Consequences of GAS

GAS was introduced by Hans Selye

Stages of stress & their purpose
(1) Alarm reaction (AR)

- adaptive, enabling surveillance by alteration of metabolism, cardiovascular & respiratory functions, decreasing pain perception (analgesia) and, at the same time, inhibition of processes decreasing surveillance chance such as reproductive functions and food intake and processing
  - metabolic alterations → increase of glycemia using catecholamines (CAT) and glucocorticoids (GC)
    - ↑ insulin-stimulated glucose uptake
    - ↓ protein, fatty acid and glycogen synthesis
    - ↑ lipolysis and proteolysis (incl. part of the immune systems which is “sacrificed” in order to gain AA)
    - ↑ glycogenolysis by CAT (short-term effects on glycemia)
    - ↑ gluconeogenesis by GC (long-term effects on glycemia)
  - cardiovascular and respiratory alterations → glucose and oxygen traffic to muscles, heart and brain using CAT, GC and ADH
    - apart from CAT effects on cardiovascular and respiratory functions and effect of GC on Na retention in the kidney there is also release of ADH to help ↑ circ. volume
  - stress-induced analgesia (SIA) → decrease of pain perception by 2 ways
    - opiates-dependent SIA: enkephalins and β-endorphins
    - opiates independent SIA: glutamate
  - cognitive and emotional alterations
    - ↑ motivation, arousal, vigilance, anxiety by ↑ delivery of NE to CNS structures

Analysis of the situation by CNS

- (1) analysis of the potentially threatening situation
  - higher cognitive areas → prefrontal cortex
    - major connections to amygdala and LC, however, gives us a voluntarily option to modify automatic responses (conscious control over anxiety)
      - process of extinction = stimulus that triggers a conditioned fear gradually loses this effect
      - involved in the final phase of confronting a danger, after the initial automatic, emotional reaction, the action that is best for us is chosen (people whose frontal cortex is damaged - “frontal syndrome” can’t plan the simplest task)
  - respective autonomic and neuroendocrine reactions
    - limbic system
      - hippocampus
        - memory (storage and retrieving), connections to amygdala – origin of strong emotions triggered by particular memories
        - processing of the context of a situation
        - connections to hypothalamus
      - amygdala
        - essential for decoding emotions, and in particular stimuli that are threatening to the organism
      - thalamus
        - sensory information to amygdala
      - hypothalamus
        - activated by the structures of the limbic system
        - controls activity of pituitary and ANS (LC)
    - brain stem
      - pons
        - locus coeruleus (LC) – afferentation from hypothalamus, controls activity of the SNS and other CNS parts by NE release → increase of cognitive functions (prefrontal cortex), motivation, HPA activity (↑ CRH), SNS activation
      - medulla oblongata – n. tractus solitarii

Limbic system

Various nature of the stressors vs. uniform reaction

- stress response can be elicited by various stressors:
  - real
    - external (sensed)
      - from sensory cortex via prefrontal cortex
    - internal (detected/quantified)
      - from various hypothalamic centers, somatic and autonomous afferent nerves perceived
  - emotional (amygdala)
    - memory (hippocampus)

- no matter what was the initial stressor, reaction is carried by uniform pathways involving limbic system
  - LC - SNS - adrenal medulla - CAT
  - hypothalamus - HPA - cortisol
  - higher CNS functions (motoric, cognitive, behavioral reactions)
(1a) AR – nervous response - ANS

SNS → adrenal medulla → CAT

- adrenal medulla = stress-responsive CAT reservoir whose activity is modulated by limbic system, LC and hypothalamus (CRH), pituitary (ACTH) and cortisol
- produces
  - 80% of E (thus majority of response during F&F reaction is carried predominately by E from adrenal medulla)
  - 20% of NE
- CAT circulate bound to albumin, rapid degradation in liver → quite short-acting and thus flexible regulators
- CAT synthesis and metabolism (see Fig.)
  - rate-limiting enzyme: tyrosin hydroxylase
  - inherited defects of synthesis (enzyme defects)
  - overproduction in pheochromocytoma cells
- effects
  - ↓ GIT secretion, motility, digestion
  - ↓ salivation
  - ↑ heart rate, conduction & contractility
  - ↑ respiration & bronchodilation
  - blood redistribution from splanchnic circulation and skin to muscles, brain and heart
  - ↑ activity and vigilance
  - metabolic effects (↑ Glc and FFA)
- effects mediated by post-synaptic adrenergic receptors and respective signaling cascades
  - G-protein coupled receptor superfamily
    - α (1 and 2) – PLC/IP3/DAG pathway
    - β (1 to 3) – cAMP/PKA pathway

Metabolic effects of E - glycemia

Overall metabolic effects of E
**(1b) AR – humoral response – HPA**

- CRH produced in the paraventricularis (PVN) reaches the pituitary via the hypothalamo-hypophyseal portal system, stimulating release of ACTH.
  - From the precursor proopiomelanocortin (POMC).
  - ACTH binds to G-protein receptor → cAMP → effects of CRH.
- HPA-mediated metabolic action of cortisol.
- Non-HPA-mediated action in the immune system.
- Cortisol half-life in circulation ~90 min.
  - Majority bound to cortisol-binding globulin (CBG, ~75%) and albumin (~15%).
- Peripheral tissue-specific modulation of cortisol availability by enzymes:
  - 11β hydroxysteroid dehydrogenase type 1 (11βHSD1).
  - 11β hydroxysteroid dehydrogenase type 2 (11βHSD2).
- Pathological stress responses:
  - Hypo-cortisolism (Addison disease).
  - Pan-hypopituitarism (Sheehan syndrome).

**Regulation of cortisol production**

- Several factors influence cortisol production:
  - (1) Diurnal rhythm.
  - (2) Negative feedback:
    - Cortisol → ACTH and CRH.
    - Substrates (Glc) → insulin → HPA.
  - (3) Stress.
- GC participate in all 3 stages of GAS:
  - Alarm reaction is associated with short-term activation of HPA.
  - Sustained activation represents the stage of resistance.
  - Upon further prolongation of stress GC overproduction induces stage of exhaustion.

**ACTH action**

- Various steroid biosynthetic pathways:
  - p450 enzymes in mitochondria, each catalyses several reaction steps.
  - 3βHSD (3β-hydroxysteroid dehydrogenase) in cytoplasm, bound to endoplasmic reticulum.
  - 17βHSD and p450aro are found mainly in gonads.
**Physiologic effects**

- **GCs have receptor (GR) existing in two isoforms**
  - cytoplasmic (cGR)
  - membrane bound (mGR)

- Therefore, GCs have several modes of action
  - genomic – mediated by cGR (action of GR) upon binding to DNA, regulates transcription
  - non-genomic – mediated by mGR and non-specific effects by interaction with other proteins and cell membranes

- **(A) genomic effects** – via cGR
  - majority of metabolic effects are achieved by genomic effects
    - GC responsive genes represent ~20% of all coding genes, indispensable for life
    - GCs have receptor (GR) existing in two isoforms
    - GR act as hormone dependent
      - membrane bound (mGR)
    - GR knock-out animals are not viable!!

- **Non-genomic effects**
  - mediated by cGR, mGR and non-specific effects by interaction with other proteins and cell membranes
  - effects:
    - (1) transactivation binding to GRs
      - (a) transactivation binding to GRs
        - due to specific sequences of DNA located in promoters
        - (2) transactivation binding to negative GREs (GR/GRE) or interaction with other TF (GR)(GR)
        - repression of transcription or inactivating action of other TF
        - on gene regulation
      - the whole sequence of events following binding of GCs to cGRs takes at least ~100 min
        - increase affinity of GRs for GCs (~100 fold)

- **(B) non-genomic effects**
  - many of anti-inflammatory and immunosuppressive effects
  - cGR has 3 domains: N-terminal transactivation domain / DNA-binding domain / ligand-binding domain
  - following synthesis GRs are located in the cytoplasm in complexes with molecular chaperones
  - the receptor dimerizes
  - increase affinity of GRs for GCs
  - translocation to nucleus and homodimerisation
  - effects:
    - (1) transactivation by certain cellular signalling pathways
    - (2) non-genomic effects
      - membrane-bound receptors

**Steroid hormone receptor signalling**

- GR act as hormone dependent nuclear transcription factor
- upon entering the cell by passive diffusion, the hormone (H) binds the receptor
- which is subsequently released from heat shock protein
- to nuclear translocates to the nucleus
- there, the receptor dimerizes
- binds specific sequences in the DNA
- acts upon the cell
- this latter step can be modulated by certain cellular signalling pathways
- glucocorticoids are hormone specific receptors in nuclear or cytoplasmic compartment
- (1) transactivation = binding to GREs
  - (2) non-genomic effects

**Peripheral modulation of GC availability**

- peripheral tissue-specific modulation of cortisol availability by enzymes catalysing
- interconversions of active and inactive forms of GCs
  - (a) 11β hydroxysteroid dehydrogenase type 1 (11βHSD1)
    - act as a reductase regenerating cortisol from cortisone → in intracellular corticosteroid concentration
    - expression of 11βHSD1 is higher in visceral than subcutaneous fat – visceral fat is therefore more flexible pool of energy substrates
    - often co-localises with GR (e.g. in liver and adipose tissue) and thus locally amplifies the GC action
    - 11βHSD1 overexpressing mice develop obesity, while mice lacking 11βHSD1 are protected from overeating-induced obesity
    - liver and fat-tissue specific inhibitors of 11βHSD1 could be used for treatment of metabolic syndrome and obesity
    - pathology associated with 11βHSD1
      - Cushing syndrome – higher expression of 11βHSD1 in visceral fat – normally first source of substrate, but higher suppression with GC, while enhanced GC action leads to lipolysis in adipose tissue, the fat accumulates in visceral
      - congenital deficiency of 11βHSD1 (apparent cortison reductase deficiency) = compensatory over-activation of HPA axis → adrenal androgen excess, oligomenorrhea, hirsutism in women
      - oversuppression of 11βHSD1 in subcutaneous tissue (congenital or acquired) leads to lipodystrophy
      - 11βHSD1 plays a role in the pathogenesis of polycystic ovary syndrome
      - regulation: starvation, cortisol, other hormones

- (b) 11β hydroxysteroid dehydrogenase type 2 (11βHSD2)
  - act as a dehydrogenase degrading cortisol to cortisone → a intracellular corticosteroid concentration
  - mainly in kidney
  - by degrading cortisol 11βHSD2 enables tissue-specific preferential action of aldosterone on MR, even though concentration of plasma cortisol
  - pathology associated with 11βHSD2
    - congenital deficiency of 11βHSD2
    - (apparent mineralocorticoid excess) → mineralocorticoid form of hypertension
  - (c) 11β hydroxysteroid dehydrogenase type 3 (11βHSD3)
    - (apparent mineralocorticoid excess) → mineralocorticoid form of hypertension
    - (d) 11β hydroxysteroid dehydrogenase type 4 (11βHSD4)
    - (apparent mineralocorticoid excess) → mineralocorticoid form of hypertension
    - (e) 11β hydroxysteroid dehydrogenase type 5 (11βHSD5)
      - (apparent mineralocorticoid excess) → mineralocorticoid form of hypertension

**Metabolic effects of GC – increased turnover of free and stored substrates**

<table>
<thead>
<tr>
<th>Tissue/organ</th>
<th>Physiologic effects</th>
<th>Effects of overproduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>Hepatic gluconeogenesis (↑ Glic) (stimulation of key enzymes – pyruvate carboxylase, PEPCK, G6Pase)</td>
<td>Impaired glucose tolerance/diabetes mellitus</td>
</tr>
<tr>
<td></td>
<td>Lipogenesis (↑ FA and VLDDL) (stimulation of key enzymes acetyl-CoA carboxylase and FA synthase)</td>
<td>Steatosis/steatohepatitis</td>
</tr>
<tr>
<td>Adipose tissue</td>
<td>Lipolysis in subcutaneous fat (↑ FFA) (activation of HSL and inhibition of LPL)</td>
<td>Insulin resistance in the muscle (competition of FFA with Glic for oxidation)</td>
</tr>
<tr>
<td></td>
<td>↑ Glic uptake (down-regulation of IRS, inhibition of P38K, Glut4 translocation)</td>
<td>Insulin resistance by interference with insulin post-receptor signalling</td>
</tr>
<tr>
<td></td>
<td>Adipocyte differentiation in visceral fat (expression of GR and 11βHSD1 different in adipose and visceral fat)</td>
<td>Truncal (abdominal) obesity, metabolic syndrome</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>↑ Glic uptake (down-regulation of IRS, inhibition of P38K, Glut4 translocation)</td>
<td>Insulin resistance by interference with insulin post-receptor signalling</td>
</tr>
<tr>
<td></td>
<td>Proteolysis → proteosynthesis (↑ AA) (counteracting effect of IGFs, activation of ubiquitin-mediated degradation, induction of phosystatin and glutamine synthetase)</td>
<td>Muscle atrophy, weakness, steroid myopathy</td>
</tr>
<tr>
<td>Pancreas (β cells)</td>
<td>Insulin secretion (suppression of GLUT2 and K+ channel, apoptosis)</td>
<td>Impaired glucose tolerance/diabetes mellitus</td>
</tr>
</tbody>
</table>
Summary – availability of GCs

- if the stress does not cease shortly (after completing F&E reaction) increased energetic demands must be met by metabolism increasing availability of Glc and FFA – mainly via regulation of hepatic gluconeogenesis and adipose tissue lipolysis
- also, optimisation of the survival following trauma or infection is achieved by alterations of immune function – suppression of adaptive immunity – activation of certain parts of innate immunity, while suppressing the others
- there are many non-metabolic, non-immune effects of GC (see Tab.) which are desirable in short-term prospect but become adverse in long-term

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<tr>
<th>Tissue/organ</th>
<th>Physiologic effects</th>
<th>Effects of overproduction</th>
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<tbody>
<tr>
<td>Bone and connective</td>
<td>osteoblast action and bone formation vs. osteoclast action and bone resorption</td>
<td>osteoporosis</td>
</tr>
<tr>
<td>tissue</td>
<td>collagensynthesis and proteolysis</td>
<td>osteoporosis, poor wound healing, easy bruising, thin skin</td>
</tr>
<tr>
<td>GIT</td>
<td>gastric juice secretion, + mucus secretion</td>
<td>stress-induced peptic ulcers</td>
</tr>
<tr>
<td>Kidney</td>
<td>Na retention, glucocorticoid-activated kinase, increased Na channel and angiotensigen expression (liver)</td>
<td>hypertension, hypokalemia</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>erythropoietin and PMN maturation</td>
<td>poliglobulia, granulocytosis</td>
</tr>
<tr>
<td>Reproductive system</td>
<td>suppression of production of estradiol and testosterone</td>
<td>oligomenorhea, infertility</td>
</tr>
<tr>
<td>Behaviour</td>
<td>expression of GR in hippocampus</td>
<td>post-traumatic stress disorder, “burn-out”, depression, anxiety</td>
</tr>
<tr>
<td>Fetal and neonatal development</td>
<td>surfactant and fetal lung maturity, fetal hepatic and gastrointestinal enzyme systems</td>
<td>fetal lung immaturity</td>
</tr>
</tbody>
</table>

GC action on immunity

- suggested to be mediated via:
  - genomic effects (I)
    - transactivation and transpression of many immunoproteins
  - non-genomic effects (II)
    - GR by sequestering proteins
      - e.g. kinases (MAPK) → blockade of action
    - mGR (III) - multi-protein complexes with other membrane receptors → blockade of action
      - e.g. growth factors
        - alternatively, induction of apoptosis
    - direct interactions of GC with cellular membranes (IV) → intercalation into membrane → stabilisation
      - inhibition of Na/Ca exchange
      - increase of proton leak in mitochondria → less ATP
      - ATP-dependent cellular membrane system (cytokinesis, migration, phagocytosis, antigen processing and presentation, Ig synthesis, cytotoxicity, ...)

GCs and immune system

Glucocorticoid effects on primary and secondary immune cells

<table>
<thead>
<tr>
<th>Monocytes / macrophages</th>
<th>Number of circulating cells (↑ myelopoiesis, ↓ release)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Expression of MHC class II molecules and Fc receptors</td>
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<tr>
<td></td>
<td>Synthesis of pro-inflammatory cytokines (e.g. IL-1, -2, -6, TNFα) and prostaglandins</td>
</tr>
<tr>
<td>T cells</td>
<td>Number of circulating cells (redistribution effects)</td>
</tr>
<tr>
<td></td>
<td>Production and action of IL-2 (most important)</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>Number of circulating neutrophils</td>
</tr>
<tr>
<td></td>
<td>Number of eosinophile and basophile granulocytes</td>
</tr>
<tr>
<td>Endothelial cells</td>
<td>Vessel permeability</td>
</tr>
<tr>
<td></td>
<td>Expression of adhesion molecules</td>
</tr>
<tr>
<td></td>
<td>Production of IL-1 and prostaglandins</td>
</tr>
<tr>
<td>Fibroblasts</td>
<td>Proliferation</td>
</tr>
<tr>
<td></td>
<td>Production of fibronectin and prostaglandins</td>
</tr>
</tbody>
</table>
Examples of multiple action of GCs on immunity

Balance of Th1/Th2 immune responses - Th2 shift as a consequence of stress

Summary – effects of GC on immunity

(3) Stage of exhaustion (wear and tear)

- examples of clinically significant consequences:
  - one of the non-heritable components of metabolic syndrome (→ cardiovascular events)
  - high incidence of death in the first years of retirement for those not prepared for it (sudden end of years in a stressful, demanding job without much relaxation)
  - weekend headache
  - high incidence of illness during holidays
  - anxiety or depressive disorders
Subjective stress responsibility

- flourishes or perish
- there are enormous interindividual differences in the response to stress due to
  - prenatal stress
  - early-life experiences
    - postnatal handling and mother care, mother separation, ...
    - e.g., animal (rodent) models – maternal care (licking and grooming) produces exploratory, open-to-novelty and less emotionally active animals = neophilic with↓ HPA activity
      - interestingly, apart of stressoric effect, abuse of the young (rough handling by the mother) leads to attachment rather than avoidance → increases the chance of survival by continuing to obtain food until weaning (much longer and complex in humans though!)
  - adult-life experiences
    - school, work, family, relationships, sleep deprivation, ...
      - e.g., someone who has been treated badly in the job and/or has been fired will approach a new job quite differently than someone with altogether positive employment history
  - sex differences
  - genetics - sensitivity to stress/reward mediators
    - many genes/common alleles! e.g. serotonin transporter, brain-derived neurotropic factor, glucocorticoid receptor, ...

Common indications for therapeutic use of GCs and major adverse effects

- respiratory diseases
  - allergies, asthma, sarcoidosis, prevention/treatment of ARDS
  - renal diseases
    - some types of nephrotic syndromes and glomerulonephritides
  - gastrointestinal disease
    - ulcerative colitis, Crohn’s disease, autoimmune hepatitis
  - rheumatological diseases
    - systemic lupus erythematosus, polymyalgia rheumatica, temporal arthritis, juvenile idiopathic arthritis, vasculitides, rheumatoid arthritis
  - emergency situations
    - cerebral oedema
  - skin diseases
    - allergies, pemphigus, eczema
  - tumours
    - Hodgkin’s lymphoma, other lymphomas
  - transplantation immunosuppression

side-effects

- physiological
  - adrenal and/or pituitary suppression
- pathological
  - increased blood pressure, peptic ulceration (or exacerbation), pancreatitis, polyuria, nocturia, depression, asthma, psychosis, insomnna, weight gain, impaired glucose tolerance/diabetes, impaired growth, amenorrhea, osteoporosis, proximal myopathy and wasting, asptic necrosis of the hip, pathological fractures, skin thinning, easy bruising, cataracts (including inhaled drug), increased susceptibility to infection, sepsis, reactivation of TB, fungal infections

Phylogenetic differences in stress response

- stress response plays different roles in different species according to the characteristic patterns of social behaviors, environment etc.
  - example: dominant and subdominant primates (males)
    - in stable conditions (no territorial emergency), dominant males have lower GC levels than subdominant ones
    - in unstable conditions, however, GC levels in dominant males increase and they are the same or higher than in subdominant males
  - i.e., “personal power” of dominant male correlates with low GCs levels during rest conditions
  - human beings compared to other species are used and ready to adapt to the long-term stress situations by elevated activity of certain systems (survival advantage)
    - nowadays the outcome of such selection contributes to the common pathologic conditions such as metabolic syndrome