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Cirkadiánní regulace sekrece glukokortikoidů

Circadian regulation of glucocortioid secretion

Bakalářská práce

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Prohlášení:

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Podpis: ________________

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Abstrakt

Glukokortikoidy jsou skupina steroidních hormonů, které jsou syntetizovány v nadledvinách. Vykonávají četné funkce související s metabolismem, imunitní odpovědí a ontogenezí. Glukokortikoidy účinkují jako výkonné molekuly hypothalamo-hypofýzo-nadledvinové (HPA) osy a jako takové je jejich hladina v krvi zvýšena po vystavení stresu. Bazální hladina glukokortikoidů také vykazuje nápadný denní rytmus svědčící o vlivu cirkadiánních hodin na regulaci HPA osy. Studie ukázaly, že na regulaci denní sekrece glukokortikoidů se kromě HPA osy podílí i další regulační mechanismy. Poruchy těchto regulačních mechanismů mohou vést k vážných patologiím. Tato práce popisuje podstatu rytmické povahy výlevu glukokortikoidů a mechanismy, kterými cirkadiánní hodiny uplatňují svůj vliv na zmíněný rytmus. Dále se práce stručně zaměřuje na vysvětlení povahy zpětné vazby glukokortikoidů na hodinový systém. Závěrem jsou shrnuty některé příklady úlohy abnormální sekrece glukokortikoidů v několika vybraných chorobách.

Klíčová slova

Glukokortikoidy, Cirkadiánní hodiny, HPA osa, Nadledviny, Autonomní nervový systém

Abstract

Glucocorticoids belong to a family of steroid hormones synthesized in the adrenal gland. They fulfill a variety of functions related to metabolism, immune response and ontogenesis. Glucocorticoids function as the end-effector of hypothalamic-pituitary-adrenal (HPA) axis and as such, their levels in blood are elevated after exposure to stressors. The basal levels of glucocorticoid also show a pronounced diurnal rhythm, suggesting involvement of the circadian clock in the regulation of HPA axis. Studies have shown that other regulatory mechanisms apart from the HPA axis are involved in regulation of diurnal glucocortioid secretion. The disturbances of the regulatory mechanisms may lead to serious pathological conditions. This thesis describes the rhythmic nature of glucocorticoid release and mechanisms by which the circadian clock exerts its influence over the rhythm. Thereafter, the feedback of glucocorticoids onto the clock system is briefly explained. Finally, some examples of a role of abnormal glucocorticoid secretion in selected pathologies are provided.

Keywords

Glucocorticoids, Circadian clock, HPA axis, Adrenal gland, Autonomic nervous system

Contents

1 Introduction

Due to the presence of day-night cycles on Earth, organisms began to integrate mechanisms that allowed them to optimize their behavioral activity and internal functions. These mechanisms are now present at many different levels and act through both humoral and neural mechanisms. By doing so, organisms could anticipate periodic changes in their surroundings and make necessary precautions, hence gaining an evolutionary advantage over their peers and dominating the planetary ecology. We call these mechanisms "biological clocks". The clocks that oversee daily rhythms are called "circadian clocks". (in latin "circa diem" means "approximately a day")

The molecular makeup of circadian clocks is composed of self-sustaining transcriptional-translational feedback loops, a principle well conserved among organisms. Its complexity varies from the simple clock in cyanobacteria to rather complicated mammalian clock. In order to achieve such a higher level of coordination needed to keep several systems in synchrony, mammals harbor a central, or "master" circadian clock, which directly receives information from the outside world and then relays it to other clocks in peripheral tissues.

In mammals, some parts of the endocrine system are under tight circadian regulation, including an important endocrine organ, the adrenal gland. One of the hormone families secreted by the adrenal gland are glucocorticoids. Glucocorticoid secretion follows diurnal pattern under normal circumstances and if the organism loses its inherent rhythmicity, the rhythm of secretion generally disappears as well.

The rhythm of glucocorticoids and its persistence in an organism are very important. If not present, serious pathological consequences may develop. It is therefore important to understand the mechanisms that govern over its rhythmicity. The aim of this work is thus i) to reiterate the characteristics of glucocorticoids, ii) to underline the basic mechanisms and outlook of mammalian circadian clockwork, iii) to summarize the mechanisms by which the clock influences adrenal gland function and glucocorticoid secretion and iv) to provide examples of several disorders which are linked with abnormal glucocorticoid secretion.

2 Glucocorticoids and their synthesis

Glucocorticoids are adrenocortical hormones of steroid nature synthesized in adrenal gland (Payne 2004), whose action is tightly linked with intermediate metabolism and the stress response. The member of glucocorticoid family found in human body in largest volumes is cortisol, while many other mammals, such as rodents, utilize corticosterone in its place. Glucocorticoids show a wide array of physiological effects throughout organisms, including regulation of inflammatory reaction and general immunosuppression, stimulation of gluconeogenesis, lipid breakdown and other functions tied with metabolism, ontogenesis or immune system. Due to these effects, glucocorticoids are widely used in modern medicine, for example, for treatment of rheumatoid arthritis (Hoes et al. 2010).

The adrenal gland is divided into two distinct tissues – cortex and medulla. Additionally, the gland itself is surrounded by a capsule of connective tissue in order to maintain its structure and shape. Adrenal cortex is made up by three distinct zones. The outer zona glomerulosa (ZG), central zona fasciculata (ZF) and inner zona reticularis (ZR). ZG synthesizes mineralocorticoids, which affect salt and water homeostasis and ZR produces adrenal androgenes. ZF is the primary site of glucocorticoid production and is also responsible for small amount of androgen production (Simpson 1988).

Biosynthesis in ZF begins with the transport of cholesterol into mitochondria, a process regulated by steroidogenic acute regulatory protein (StAR), where it is converted to pregnenolone by cholesterol side chain cleavage enzyme P450scc (Miller 1998). This is the first and rate-limiting step in glucocorticoid synthesis and, therefore, it is the subject to physiological regulation (Miller 1998). Pregnenolone may be hydroxylated to create 17α-OH-pregnenolone and then is converted to (17α-OH-)progesterone, which undergoes another reaction to form deoxycorticosterone (or deoxycortisol if 17α-OH-progesterone was used) and in the final step, deoxycorticosterone or deoxycortisol is converted to corticosterone or cortisol (Fig.1). In ZG, corticosterone undergoes one more reaction, forming aldosterone.

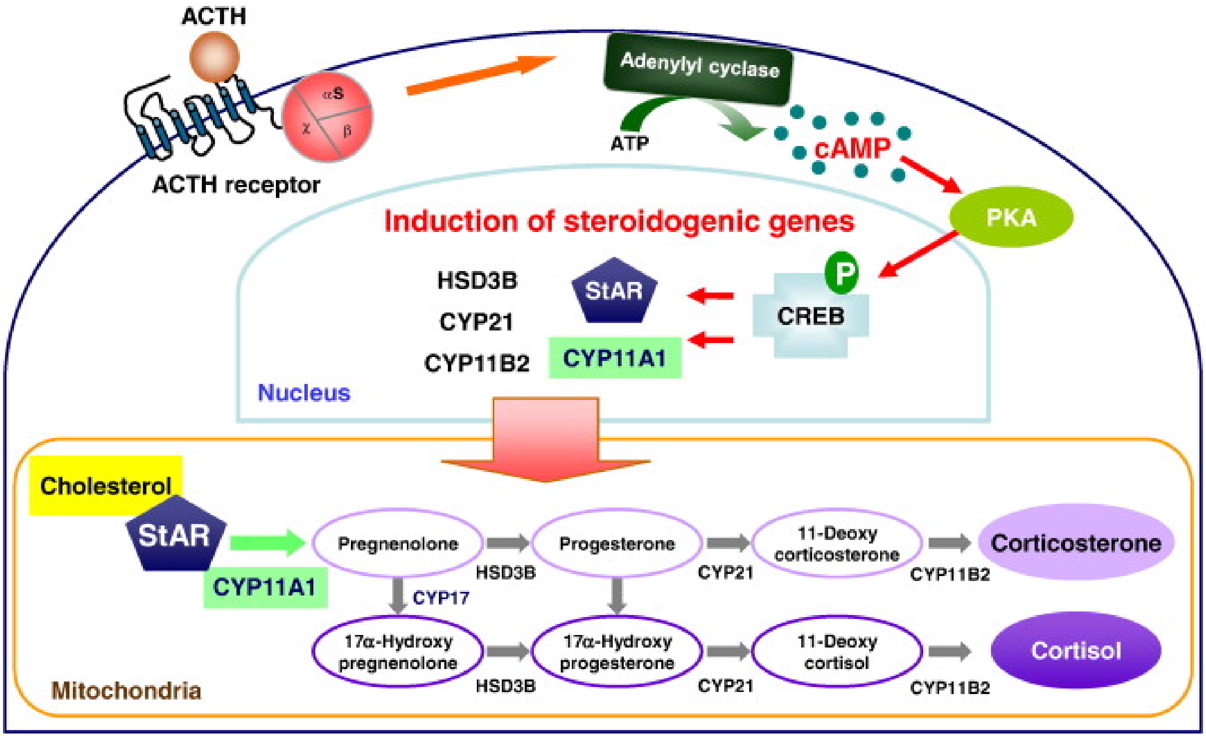

Fig.1. Overview of important proteins, enzymes and reactions in biosynthetic pathway of glucocorticoids. Binding of ACTH onto its receptor induces a signaling cascade promoting gene expression of steroidogenic proteins. Abbreviations as following: ACTH – Adrenocorticotropic hormone; PKA - Protein kinase A; CREB – cAMP response element-binding protein; StAR – Steroidogenic acute regulatory protein; CYP11A1 – P450ssc cholesterol side-chain cleavage enzyme; CYP17 – steroid 17α-hydroxylase; HSD3B - 3-β-hydroxysteroid dehydrogenase; CYP21 - steroid 21-hydroxylase; CYP11B2 steroid 18-hydroxylase (Chung 2011)

Glucocorticoids act either by binding to the cytoplasmic glucocorticoid receptors, forming complexes with chaperone proteins, such as HSP90 (Kumar 2005), or to the mineralocorticoid receptors, which bind both glucocorticoids and mineralocorticoids (De Kloet et al. 1998). Following ligand binding, the hormone-receptor complex dissociates from HSP molecules and is actively transported to nucleus, where it interacts with their respective response elements or with other transcription factors (Ray 1993).

One of the key characteristics of glucocorticoid secretory profile that can be detected in blood and/or saliva samples, is its pulsatility (Fig.2). Moreover, the highest systemic levels are present around the onset of the activity period (i.e. early morning in diurnal, or in the evening in nocturnal, species), while the nadir occurs just before the beginning of the rest period.

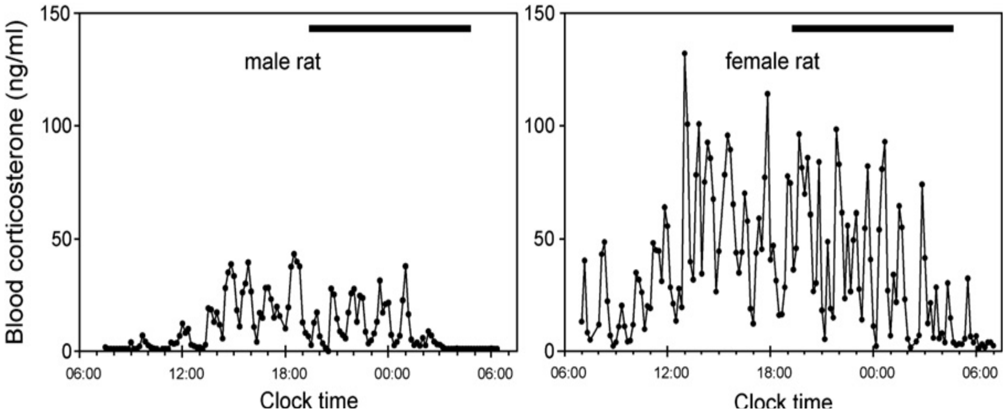

Fig.2. Representation of a rhythmic corticosterone secretion in male and female Sprague-Dawley rat. Horizontal bars represent the dark portion of day. (Lightman et al. 2008)

3 The Circadian Clocks

The nature of the circadian clocks and their role in organism has been recognized relatively recently in modern biology. The internal timekeeping system allows organism not only to anticipate and prepare for regular changes in external environment, but also to separate their own biochemical processes (Ishikawa 1976). The simplest circadian clock known so far is present in cyanobacteria and although only consisting of three proteins, it is able to produce quite accurate 24-hour oscillations. This clock has been reproduced in vitro (Nakajima et al. 2005), which may bring new perspectives to clock research. In mammals, circadian clocks are hierarchically organized to form a coherent circadian system. The presence of a master clock, which is the only clock synchronized by photic input, is necessary to drive output behavioral rhythms and keep them synchronized with external light/dark cycle (Stephan 1972; Moore 1972). Apart from the central clock, the mammalian body houses multiple other oscillators with similar makeup in peripheral tissues (Yagita et al. 2001), which are continually entrained by the central clock (Yamazaki et al. 2000).

3.1 The central circadian clock

The central circadian oscillator in mammals is the suprachiasmatic nucleus (SCN), which is located in the anterior hypothalamus above the chiasma opticum on both sides of the third ventricle (Ralph 1990). The SCN is comprised of two major subdivisions - ventral core and dorsal shell region (Moore 1983). The core region is adjacent to chiasma opticum, receives direct input from retina and projects to shell region.

Each cell of the SCN is capable of generating its own circadian rhythm by spontaneous firing of action potentials (Welsh et al. 1995). Importantly, the cells of SCN are coupled to form cohesive circadian oscillation. The main tools of this arrangement are action potentials within the SCN (Yamaguchi et al. 2003). Oscillations in the dorsal shell cells exhibit shorter period than those of the entire SCN, while the oscillatory periods of cells in ventral core region correspond to it. It has been proposed that the dorsal oscillators are entrained by the ventral ones (Noguchi et al. 2004). This also correlates with numerous projections from the ventral to the dorsal SCN, whereas those in opposite direction are quite sporadic (Leak 1999). Core neurons produce mostly vasoactive intestinal polypeptide (VIP) or gastrin-releasing peptide (GRP) and project to the shell, which is made up largely from neurons that produce arginine vasopressin (AVP). Both the core and shell neurons are colocalized with GABA. The shell region projects to different areas than the core, which are localized in other parts of the brain (Moore 2002).

Intact SCN determines the presence of circadian period and of the coherent output signals necessary to maintain circadian rhythmicity. Transplantation of the SCN tissue to hamsters with SCN lesions restored circadian rhythms of locomotor activity (Lehman et al. 1987). The length of the restored period depends on donor's genotype and the recipient adopts it (Ralph et al. 1990). However, transplantation of the SCN does not restore all of the recipient's rhythmicity (Meyer-Bernstein et al. 1999), implying that neuronal projections are not the only mechanism taking part in the SCN output signals.

Mammalian circadian clockwork is made up from interconnected transcriptional-translational feedback loops of a set of genes and their protein products. All cells in mammalian species exhibiting circadian rhythms depend basically on the same basic clock mechanism. During the day, proteins of *Clock* (circadian locomoter output cycles kaput) and *Bmal1* (brain and muscle aryl hydrocarbon receptor nuclear translocator-like) genes (Gekakis et al. 1998) heterodimerise in the cytoplasm and translocate into nucleus, where they bind to E-box sequences on promoters of various genes, including *Per* (period) and *Cry* (cryptochrome) (Kume et al. 1999). PER and CRY proteins heterodimerise and inhibit their own transcription by interacting with CLOCK/BMAL1 dimers (Shearman et al. 2000). The PER/CRY dimers decay during the night, and a new cycle begins at onset of a day. The auxiliary loop regulates Bmal1 transcription by expression of *Rev-Erbα* and *RORα*, whose promoters both contain E-boxes. Their corresponding proteins then compete for binding at RORE (retinoic orphan nuclear receptor element) on the Bmal1 promoter. Whereas REV-ERBα represses Bmal1 transcription, RORα activates it (Guillaumond et al. 2005). Crosstalk between these two genes and their proteins thus rhythmically controls Bmal1 expression in anti-phase with expression of Per and Cry genes (Fig.3).

The precision of these mechanisms and period of these oscillations are modulated by additional post-translational modifications. A role of casein kinase 1ε (CK1ε) and FBXL3 protein is to be noted. CKIε phosphorylates BMAL1, PER and CRY proteins and its mutation leads to a shortened period (Lowrey et al. 2000). BMAL1 is also post-translationally sumoylated, which supports its rhythmicity (Cardone 2005). FBXL3 is a protein of the E3 ubiquitin ligase complex, which degrades CRY1 protein. Mutation of *FBXL3* gene has been identified in a long period (~26 hours) circadian mutant, called Overtime or Afterhours. Higher stability of CRY protein in the mutant leads to prolonged CRY-dependent repression of the circadian cycle (Siepka et al. 2007; Godinho et al. 2007).

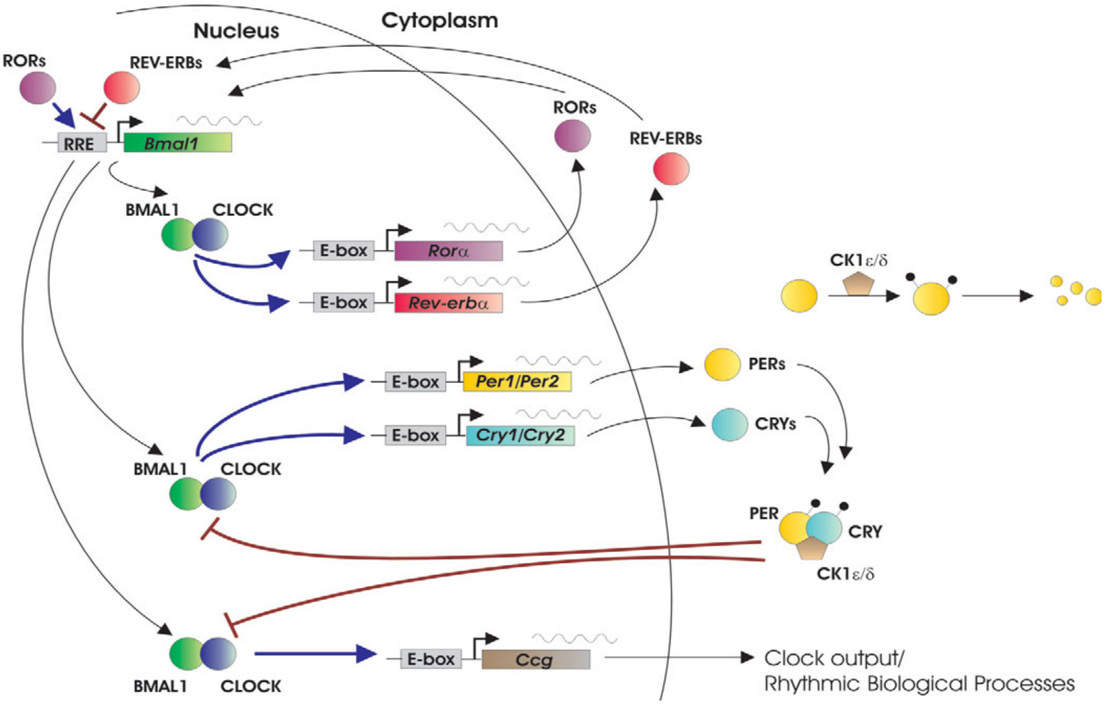

Fig.3. A model representing the feedback loops of mammalian circadian clockwork. Ccg stands for clock-controlled genes. (Ko 2006). For abbreviations see text.

When isolated from external time cues, the endogenous period (tau) differs from 24 hour cycle (T), therefore, organisms need to receive information about the outside conditions and entrain their clock accordingly. The most important Zeitgeber (time giver) in organisms is light (Roenneberg 1997). Photic signals from the environment are transferred to the SCN via intrinsically photoreceptive retinal ganglion cells (ipRGCs), which utilize a pigment melanopsin to record external light intensity (Berson 2003). These cells are monosynaptically connected with neurons of the core SCN and thus form a major pathway to SCN - the retinohypothalamic tract (RHT). The RHT uses glutamate as neurotransmitter (Ebling 1996). Light activates a signaling pathway involving phosphorylation of CREB (cAMP response element-binding) protein, which is able to induce *Per* transcription during subjective night (Travnickova-Bendova et al. 2002). The SCN also receives input from intergeniculate leaflet (IGL) located in thalamus. The IGL receives projections from the retina and projects to the SCN via the geniculohypothalamic tract (GHT) which utilizes neuropeptide Y as its main neurotransmitter. The GHT has been attributed to play a role in photoperiodic and non-photic entrainment of the SCN (Freeman 2004; Maywood 1997). Another non-photic entrainment pathway to the SCN is conveyed by raphe nuclei of the midbrain. Serotonin projected by dorsal (DRN) and median (MRN) nuclei modulates SCN response to light (Rea 1994). SCN projects diverse output signals to many regions of the brain, as evidenced by experimental application of tracers (Watts 1987). SCN also utilizes autonomic nervous system to indirectly control several tissues (see below). The overview of output signals and signaling molecules exceeds the scope of this work, therefore, only those involved in regulation of glucocorticoid secretion will be mentioned in the text below.

3.2 Peripheral circadian clocks

Apart from the SCN, the circadian clocks are present in other brain areas and also in non-neural peripheral tissues of the organism, including the adrenal gland. Peripheral oscillators in bodily tissues are self-sustained (Yoo et al. 2004). These cellular clocks are not coupled in the same way as those in the central clock. The paracrine signaling has been suggested as a possible alternative mechanism of their coupling (Nagoshi et al. 2004), however, intact SCN is required to keep phases of individual oscillators synchronised with each other (Guo et al. 2006). Since mammalian peripheral clocks lack the means to perceive photic cues, their main Zeitgeber are various non-photic cues, for example the food intake because feeding activity usually occurs during the activity period of the animal. Feeding restricted to improper time of a day can alter the phase of the peripheral clocks significantly while leaving the phase of the SCN unaffected and, therefore, uncouple the central clock with the peripheral ones (Damiola et al. 2000).

3.3 Influence of timekeeping on transcriptome

The circadian clock also shows a significant influence on the organism's own transcriptome (Schaffer et al. 2001). It has been discovered that a significant part of mRNAs cycle over the 24-hour period (Storch, 2002). These cycling mRNAs not only represent genes involved in the circadian clock mechanism itself, but also genes whose proteins are involved in various cellular tasks (Akhtar et al. 2002). These genes are called "clock-controlled genes". The circadian signal is transduced via activation of response elements in the gene promoter regions, such as E-box motifs binding sites for CLOCK/BMAL1, RORE motifs binding RORE and REV-ERBα (Ueda et al. 2005) and D-elements binding DBP (D-site binding protein) (Lavery 1993).

It should be noted that levels of rhythmic transcripts overlap only slightly between tissues (Panda 2002). The clock-controlled genes therefore seem to be not only subjected to circadian control of gene expression, but also to tissue-specific control. The central SCN clock plays an important role in entrainment of the transcript cycling and its ablation attenuates or abolishes the rhythms at the tissue or organ level (Sakamoto et al. 1998).

4 Circadian regulatory mechanisms of glucocorticoid secretion

The circadian rhythmicity of glucocorticoid secretion has been well known but the mechanisms that govern over its regulation are still being researched. These mechanisms can be divided into those originating from the central clock in the SCN, and those which are tied with the peripheral clock of the adrenal gland.

4.1 HPA axis

The hypothalamic-pituitary-adrenal (HPA) axis is comprised of several elements which form a complex system whose main function is to maintain and adjust homeostasis in accordance to stress stimuli by regulating organism's behavior and metabolism. The three basic elements of the HPA axis are paraventricular nucleus (PVN) of the hypothalamus, anterior lobe of the pituitary gland and adrenal cortex. (Chrousos 1992).

HPA axis action begins with activation of neurons in the paraventricular nucleus of the hypothalamus, which results in release of AVP and corticotropin-releasing hormone (CRH) to the pituitary. These hormones stimulate median eminence, which controls the synthesis and secretion of adrenocorticotropic hormone (ACTH) from the anterior pituitary (Watts 2004). ACTH stimulates glucocorticoid secretion in the ZF of the adrenal gland. Secreted glucocorticoids in turn show negative feedback onto the whole system, rapidly inhibiting it by nongenomic mechanisms after reaching a threshold (Evanson et al. 2010).

ACTH is transported by blood flow to the adrenal gland, where it binds to adrenocorticotropic hormone receptor (ACTHR) in the ZF. The receptor then activates heterotrimeric G proteins, thus stimulating adenylyl cyclase (Côté et al. 2001). The rising levels of intracellular cAMP then activate protein kinase A (PKA) nuclear transcription factors, like cAMP response element binding protein (CREB). The transcription factors then modulate expression of genes involved in the adrenal glucocorticoid biosynthesis (Mayr 2001).

The SCN has a significant role in the regulation of HPA axis and thus the glucocorticoid secretion. Studies clearly show that ablation of the SCN in rats abolished circadian fluctuation of glucocorticoid levels in blood (Abe et al. 1979). SCN-lesioned hamsters provided with SCN grafts did not show restored glucocorticoid rhythms, which shows the importance of axonal connections in their circadian regulation (Meyer-Bernstein et al. 1999)

Projection from the SCN to the medioparvocelullar (mpPVN) zone of the hypothalamus, which contains a subset of neuroendocrine neurons, which produce CRH and AVP (Sawchenko 1984), constitutes a direct regulatory link (Vrang 1995), but indirect projections to the median eminence of the hypothalamus are also important. The SCN contacts the subparaventricular (subPVZ) and the dorsomedial (DMH) zone of the hypothalamus, which are directly connected to the mpPVN, which then projects directly onto median eminence (Fig.5) (Buijs et al. 2003).

The levels of glucocorticoids are known to increase after SCN ablation in rats (Buijs 1993), suggesting an inhibitory role of the central clock in regulation of the HPA axis and secretion of glucocorticoids. The inhibitory effect of the SCN on the HPA axis is generally attributed to AVP, which is an important neurotransmitter in the SCN projections (Buijs et al. 1993) towards several areas in the hypothalamus. Studies have shown that infusion of AVP into the PVN/DMH zones inhibited corticosterone release in rats with SCN lesions whereas administration of AVP antagonist in the same area increased its secretion (Kalsbeek et al. 1992).

The role of AVP in the regulation of HPA axis is thus of dualistic nature. Its release into the median eminence from mpPVN stimulates, but its release into PVN and DMH inhibits the secretion of glucocorticoids.

These mechanisms, however, are all tailored for the nocturnal rat. The circadian variations in mRNA levels of AVP have been reported to be similarly phased in the rat and the diurnal mice *Arvicanthis ansorgei* (Dardente et al. 2004). In accordance with results of in vitro experiments demonstrating the role of AVP in hypothalamic GABAergic inhibition in rats (Hermes et al. 2000), the proposed model now distinguishes the nocturnal and diurnal mammals (Fig.4). Their circadian regulatory mechanisms of the HPA axis exhibit different innervation. This model explains the inverse effect of AVP action and also explains how the excitatory AVP can be part of inhibitory response.

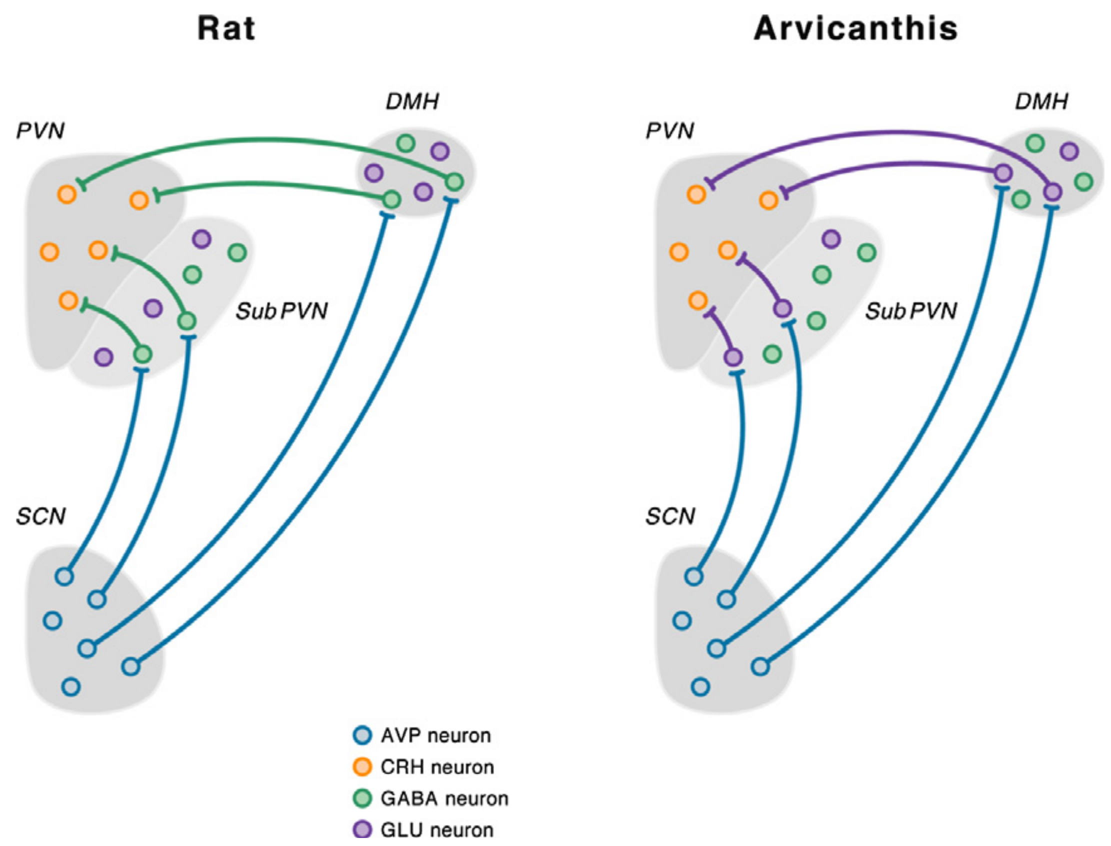

*Fig.4. Opposite effects of AVP on the HPA axis in nocturnal (rat) and diurnal (*Arvicanthis*) animals. AVP is released during the light period in both species. In rats, released AVP inhibits the CRH-containing neurons in the hypothalamic PVN by contacting GABAergic neurons in the subPVN and DMH. In* Arvicanthis ansorgei *mice, however, the released AVP contacts glutamatergic excitatory neurons in the subPVN and DMH, which in turn activates the CRH-containing neurons in the PVN. (Kalsbeek et al. 2012)*

However, the described regulation of HPA axis is not by itself sufficient to establish a robust rhythm of glucocorticoid levels. The rhythm of ACTH has relatively lower amplitude compared with the amplitude of the rhythm in glucocorticoid levels (Watts 2004), suggesting a necessity of an additional regulatory mechanisms. It also appears that the intact rhythm of glucocorticoids does not solely depend on the rhythmic release of ACTH, since the rhythm persisted in hypophysectomized rats receiving ACTH via pellets (Meier 1976).

4.2 Autonomic nervous system

The discrepancies mentioned above lead to a belief that another mechanisms independent of ACTH secretion must exist in order to produce the observed rhythm. Experiments have shown that the rhythm in plasma glucocorticoids may persist in hypophysectomized rats, but disappears if their adrenal gland is denervated (Ottenweller 1982), which further supports a theory of another, neural regulatory mechanism.

Evidence for a significance of the autonomic nervous system in the regulation of adrenal gland and its secretion of glucocorticoids has been uncovered in a series of experiments (Edwards 1993), which clashed with the role of ACTH as the sole control mechanism of the secretion. The anatomy of the connection of autonomic nervous system and SCN was revealed by a transneuronal retrograde virus tracing from adrenal gland (Buijs et al. 1999). The labeling showed that the adrenal gland is connected to sympathetic neurons in the intermediolateral column of the spinal cord (IML), which receives input from neurons in the autonomic division of the PVN of the hypothalamus. These neurons receive an input from SCN efferents through AVP or VIP (Fig. 5).

The neural pathway enables regulation of glucocorticoid secretion by relaying information about external light from the SCN. Light exposure alters adrenal gland gene expression via the SCN and the sympathetic nervous system without affecting ACTH secretion (Ishida et al. 2005). This change in gene expression is followed by change of glucocorticoid levels. The light pulse is being followed by a rise in adrenaline in adrenal gland, which is a candidate molecule for the signal transmission. This observation is also confirmed by the fact that animals with SCN lesion do not exhibit increased adrenal nerve activity after light stimulation (Niijima et al. 1993).

The other part of evidence about the regulation via autonomic nervous system is based on the finding that the adrenal innervation alters the gland's sensitivity to ACTH in a diurnal manner. The adrenal gland's sensitivity peaks alongside circulating ACTH levels (Dallman et al. 1978). This is also dependent on an intact SCN, as in ablated rats, the ACTH-induced corticosterone secretion is shown independent of the time of day (Sage 2002). Also, adrenal denervation led to a decrease of glucocorticoid secretion in the evening in rats (Ulrich-Lai

2006). Thus, circadian system also exerts its influence over the glucocorticoid levels by altering the activity of autonomic nervous system.

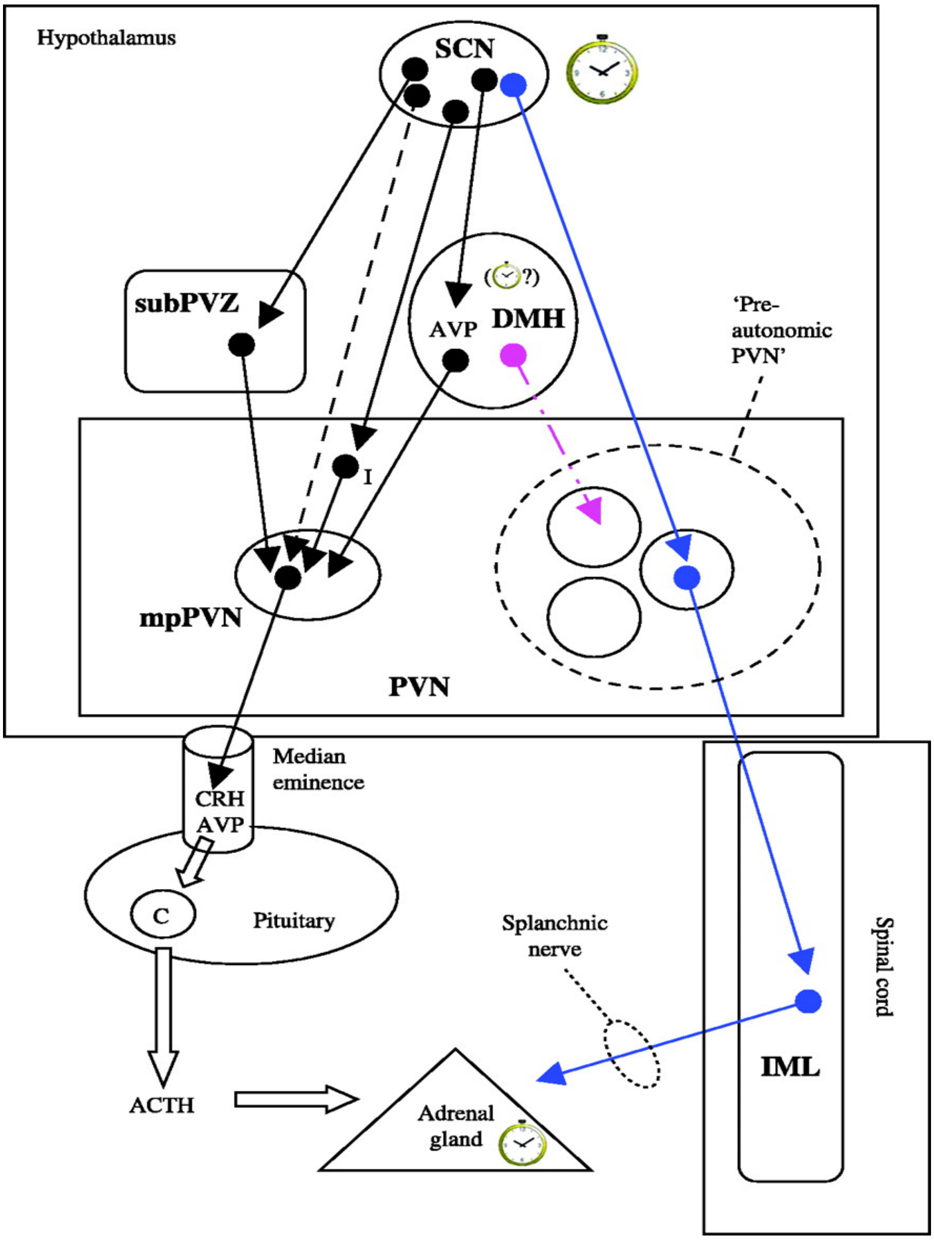

Fig.5. Overview of circadian regulatory pathways of glucocorticoid secretion. Black arrows represent projections targeting the HPA axis, blue arrows symbolize the ANS pathway. The purple arrow shows projection from the DMH to PVN distinct from the SCN-adrenal gland pathway. Clock show presence of autonomous circadian pacemakers. (Dickmeis 2009) For abbreviations see text.

4.3 Adrenal-intrinsic mechanisms

The adrenal gland shows circadian rhythms in metabolic activity and glucocorticoid release in vitro without any input from the central clock (Andrews 1964). In later experiments, the clock gene expression has been uncovered (Lemos 2006), revealing that adrenal gland hosts its own peripheral clock. This observation became the basis for research aimed to uncover the role of the adrenal clock in regulation of glucocorticoid secretion.

As mentioned above, significant portion of the mouse genome is under the influence of circadian clock (Oster et al. 2006). Among these genes, there are several components of ACTH signaling pathway and a few genes involved in transport and biosynthesis of cholesterol. Thus, Oster and colleagues proposed a theory of so-called "gating mechanism" in regulation of the glucocorticoid levels. According to their hypothesis, the peripheral clock of the adrenal gland regulates the diurnal rhythm of glucocorticoid production by altering the expression of clock-controlled genes in accordance to daytime and thus regulates, i.e., gates, its sensitivity to ACTH (Oster et al. 2006). They performed several experiments with adrenal cultures from wild-type and *Per2/Cry1* mutant mice. The wild-type gland slices exhibited a pronounced response to the evening ACTH stimulation, whereas clock-deficient mutant slices failed to do so. This finding provides a strong evidence supporting the "gating" mechanism (Oster et al. 2006)

One of the mechanisms of how the adrenal clock regulates glucocorticoid secretion is by rhythmic expression of other genes, which are directly involved in biosynthesis of glucocorticoids. Among the genes, a Steroidogenic acute regulatory protein (StAR) acts as a rate-limiting step in the biosynthetic pathway by transporting cholesterol into the mitochondria for the P450scc (Clark et al. 1994). The StAR protein has been shown to have a pronounced daily rhythm in expression, which was absent in arrhythmic mutant mice. The promoter of *StAR* gene houses an E-box, so its expression falls under control of CLOCK/BMAL1 heterodimer. The levels of StAR protein correspond with the daily glucocorticoid levels. These findings implicate that the StAR protein is another possible link between the adrenal clock and the steroidogenic pathway (Son et al. 2008).

5 The influence of glucocorticoids on biological clocks

It is evident that the circadian clocks exert a major influence over the systemic levels of glucocorticoids. However, glucocorticoids themselves regulate many aspects of the circadian clockwork. This regulation will be reviewed in this chapter.

First, glucocorticoids are believed to be involved in entrainment of the peripheral clocks. Treatment of rat fibroblast with dexamethosane, a glucocorticoid receptor agonist, has led to induction of circadian gene expression and it also phase shifted clocks in the liver, kidney and heart. In mutant mice without glucocorticoid receptor, dexamethasone did not phase-shift the clocks, revealing that glucocorticoid receptor expression is required for dexamethasone-induced phase shifting (Balsalobre et al. 2000). The role of glucocorticoids as a timing cue for peripheral clocks is further reinforced by the discovery that from a set of 169 genes cycling in the mouse liver, 100 of these have lost the circadian rhythmicity of their expression in adrenalectomised animals (Oishi et al. 2005). Glucocorticoids do not only regulate circadian gene expression in the peripheral tissues, but also in the brain. In adrenalectomised animals, the PER2 expression rhythm was abolished in oscillator in the oval nucleus of the bed nucleus of the stria terminalis (BNST-OV) (Amir et al. 2004). This may represent an additional regulation next to projections from the SCN (Leak 2001) because the BNST-OV is involved in regulation of behavioral and endocrine responses linked to stress and other emotional stimuli. Adrenalectomy did not affect the rhythm in PER2 in other regions of the amygdala, (Lamont et al. 2005). The rhythm of PER2 expression has been successfully restored in rats after introducing a rhythm in corticosterone via adding it into drinking water. Replacing corticosterone by constant-releasing pellets had no effect (Seagall et al. 2006).

Another regulatory role of glucocorticoids is related with the mechanism of entrainment of peripheral clocks by the restricted feeding regime. The food-entrainable oscillator works independently of the central clock and is able to decouple the phases of central and peripheral oscillators in a few days. Temporal limitation of the food availability also increases animal's behavioral activity in anticipation to food presentation. The food anticipatory activity is linked with an increase of adrenal glucocorticoid secretion (Stokkan et al. 2001). Glucocorticoids seem to fulfill a regulatory role in decoupling of the central and peripheral clocks, because adrenalectomised animals or animals with mutated glucocorticoid receptor gene exhibited faster phase shifting due to exposure to restricted feeding (Le Minh et al. 2001).

Cell cycle is another process that is known to exhibit variation dependent on the time of day (Bjarnason 1999). Studies on zebrafish larvae have shown that light is an important factor able to induce S phase in different cell types across the organism (Dekens et al. 2003). Recent research have shown that cell cycle rhythms are less prominent in zebrafish mutants with lesser corticotrope pituitary cells. High-amplitude cell cycle rhythms have been rescued by introduction of larvae to a tonic concentration of dexamethasone, showing that circadian input from central and peripheral clocks is not by itself enough to govern over cell proliferation across the organism and needs another signal, specifically glucocorticoids, to properly exert its influence (Dickmeis et al. 2007).

6 Clinical aspects of abnormal glucocorticoid secretion

Glucocorticoids are essential molecules, whose biosynthesis and secretion are necessary for physiological functions of an organism. Abnormal secretion and systemic levels of glucocorticoids are associated with many pathological conditions and therefore, a better understanding of regulatory mechanisms of glucocorticoid secretion might be beneficial to improve treatment of these diseases.

Cushing's syndrome is a term describing various conditions associated with the pathological elevation of systemic glucocorticoid levels. The diurnal rhythm of plasma cortisol is also abolished, implying abnormalities in circadian regulatory mechanisms (Raff 1998). The symptoms of Cushing's syndrome include central weight gain, thickening of the facial fat, glucose intolerance, hypertension, general weakness and others. These factors contribute to the increased mortality of patients with Cushing's syndrome (Steffensen et al. 2010). The high levels of glucocorticoids can be achieved either by high levels of plasma ACTH, which stimulate the adrenal gland (ACTH-dependent Cushing's syndrome, also called "Cushing's disease") or by excessive production of glucocorticoids by abnormal cells in the adrenal gland (ACTH-independent Cushing's syndrome) (Orth 1995). Treatment of Cushing's disease is usually transsphenoidal microadenomectomy, which may be followed by additional procedures (such as pituitary irradiation) should the first surgery fail. Patients with ACTH independent primary adrenal disease require adrenalectomy or treatment with adrenal enzyme inhibitors (Orth 1995).

Another disease associated with dysregulations in glucocorticoid levels is chronic fatigue syndrome (CFS). Its symptoms are sleep disturbance, impaired concentration, muscle and/or joint pain, headaches, exacerbated fatigue after physical exertion, sore throat and tender lymph nodes (Fukuda et al. 1994). The cause of CFS is not fully understood, but patients diagnosed with CFS exhibit reinforced negative feedback of glucocorticoids and hypocortisolism (Cleare 2003). The disturbances in HPA axis, however, develop after the onset of CFS. Low cortisol seems to act as one of the maintaining factors of the CFS and low-dose replacement cortisol therapy has reported an improvement of the symptoms (Cleare 2004).

Increased levels of glucocorticoids and hyperactivity of HPA axis are also well recognized in patients suffering from depression (Juruena et al. 2006). This has been accredited to impaired function of glucocorticoid receptor, which plays a role in negative feedback of glucocorticoids on the HPA axis (Anacker et al. 2011). Elevated glucocorticoid levels result in decreased hippocampal neurogenesis, which correlates with increased anxiety and depressed behavior (Murray 2008). Discrepancies in the circadian system may be responsible for malfunctions in the circadian control of HPA axis and the glucocorticoid secretion, inducing depression.

In modern life, many professions require subjects to change their daily routine significantly. Night shifts, sleep deprivation, jet lag and irregular eating can lead to chronic disruption of timekeeping system and development of metabolic syndromes (Buijs 2006). This is defined as a group of conditions that put humans at risk for heart disease, diabetes and obesity. Patients with metabolic syndrome show increased activity of the HPA axis and thus are victims of hypercorticolism. Repairing the dysregulated cortisol secretion by identifying the regulatory abnormalities may thus relieve many people of symptoms of metabolic syndrome (Walker 2006).

7 Conclusion

Regulation of such a complex process as secretion of adrenal hormones glucocorticoids is deceptively complicated. Several regulatory systems cooperate and impinge on the gland to produce a pronounced rhythm in the hormone levels that are capable of acting in the whole organisms at different levels. This rhythm may even be interpreted differently in various tissues, possibly also dependent on the time of day.

The variation of glucocorticoid levels throughout the day is also an important factor needed for negative feedback onto HPA axis, which additionally regulates the secretion of glucocorticoids. Keeping the glucocorticoid secretory profile in its normal state is very important since higher or lower concentrations may lead to development of various pathologies. Maintaining the proper levels of glucocorticoids is also important in mounting a response to stress.

Since a large part of the present knowledge available on regulatory mechanism of glucocorticoids has been published relatively recently, not everything is yet clear. A link between these health conditions and the rhythm in systemic levels of glucocorticoids has not yet been extensively studied. The chronobiological aspects of this research may shed light onto pathophysiological mechanisms of those diseases and help in their precautions and therapies.

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