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Circadian Rhythms of Glucocorticoid Hormone Actions in Target Tissues: Potential Clinical Implications

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Abstract

Organisms face unforeseen short- and long-term changes in the environment (stressors). To defend against these changes, organisms have developed a stress system that includes the hypothalamic-pituitary-adrenal (HPA) axis, which employs glucocorticoids and the glucocorticoid receptor (GR) for signal transduction. In addition, organisms live under the strong influence of day-night cycles and, hence, have also developed a highly conserved circadian clock system for adjusting their activities to recurring environmental changes. This regulatory system creates and maintains internal circadian rhythmicity by employing a self-oscillating molecular pacemaker composed of the Clock-Bmal1 heterodimer and other transcription factors. The circadian clock consists of a central master clock in the suprachiasmatic nucleus of the brain hypothalamus and peripheral slave clocks in virtually all organs and tissues. The HPA axis and the circadian clock system communicate with each other at multiple levels. The central clock controls the HPA axis, creating the diurnal oscillation of circulating adrenocorticotrophic hormone and cortisol, and the HPA axis adjusts the circadian rhythmicity of the peripheral clocks in response to various stressors through the GR. Further, Clock-Bmal1 regulates the response to glucocorticoids in peripheral tissues through acetylation of the GR, possibly antagonizing the biologic actions of diurnally fluctuating circulating cortisol. Importantly, dysregulation in the clock system and the HPA axis may cause similar pathologic manifestations—including obesity, metabolic syndrome, and cardiovascular disease—by uncoupling circulating cortisol concentrations from tissue sensitivity to glucocorticoids.

Presentation Notes

Slide 1: *Science Signaling* logo

The slideshow and notes for this presentation are provided by *Science Signaling* (www.sciencesignaling.org).

Slide 2: Title page

In this talk, I will discuss the latest understanding of the circadian regulation and dysregulation of glucocorticoid hormone actions in target tissues and their clinical implications.

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Editor's Note: This contribution is not intended to be equivalent to an original research paper. Note, in particular, that the text and associated slides have not been peer-reviewed.

Slide 3: Life on Earth

All organisms living on Earth, including humans, face short- and long-term environmental changes called “stressors,” which are unforeseen and may occur irregularly and unpredictably (1–3).

Slide 4: Examples of stressors in life

Examples of such stressors include physical challenges such as excessive heat or cold, food deprivation, trauma, and pathogen infection, as well as hurtful memories, social conflicts, aging-related organ dysfunction, and neoplasias (1, 4).

Slide 5: Human stress systems

To adjust the body’s activity to such external and internal stressors, organisms developed two adaptive systems: the hypothalamic-pituitary-adrenal (HPA) axis and the locus caeruleus–norepinephrine–autonomic nervous system, which includes the adrenal medulla (4, 5). Whereas the former employs glucocorticoids secreted from the adrenal glands as end-effector hormones, the latter uses catecholamines secreted from the adrenal medulla and the sympathetic nerve terminals (4, 5). Here, I will focus on the HPA axis.

Slide 6: HPA axis and the glucocorticoid signaling system

This slide shows the organization and components of the HPA axis (5). In the left panel, high brain centers, including the hippocampus, integrate input signals from multiple peripheral sensory organs and stimulate the corticotropin-releasing hormone (CRH) and arginine-vasopressin (AVP) neurons located in the paraventricular nucleus (PVN) of the hypothalamus to secrete CRH and AVP into the pituitary portal system (5). These two signaling peptides then stimulate production and secretion of the adrenocorticotrophic hormone (ACTH) from corticotrophs residing in the anterior pituitary gland (5). ACTH secreted into the systemic circulation reaches the adrenal cortex and stimulates secretion of cortisol, the major glucocorticoid produced in humans (5). As shown in the right panel, circulating cortisol enters peripheral tissues and cells and binds to its intracellular receptor protein, the glucocorticoid receptor (GR) (6). Glucocorticoid-bound GR translocates into the nucleus through the nuclear pore and regulates the transcription of glucocorticoid-responsive genes either by binding to glucocorticoid response elements (GREs) located in gene regulatory regions or by physically interacting with other transcription factors or co-regulators to modulate their effects on their own target genes (7). These actions of glucocorticoids through the GR are necessary for the maintenance of basal and stress-related homeostasis in virtually all organs and tissues (7). TF, transcription factor; TFREs, transcription factor response elements.

Slide 7: Human glucocorticoid receptor

This slide shows the structure of the human *GR* gene. It consists of nine exons, generates the *GR* (hereafter called *GR*) mRNA through alternative use of exon 9 , and subsequently produces the GR protein (8). The human GR consists of three major domains: the N-terminal domain (NTD, also known as the immunogenic domain), the central DNA binding domain (DBD), and the C-terminal ligand-binding domain (LBD) (9). The hinge region (HR), located between the DBD and LBD, plays an important role in the binding of dimerized GRs to GREs (10). ATG, translation start site; TGA, translation stop site.

Slide 8: The major and continuous environmental changes on Earth

In addition to coping with various unpredictable external and internal stressors, organisms must also adjust to the recurring environmental changes associated with the rotation and revolution of Earth. Rotation of Earth causes recurring day-night or light-dark changes in the

environment, and its revolution around the Sun generates seasonal alterations and variation in day-night length. In this presentation, I focus on the influence of day-night changes on living organisms.

Slide 9: Adaptation to day-night changes

Organisms adjust their body functions, such as physical activity versus rest and sleep, to day-night or light-dark cycles, so that they can increase their chances of survival and reproductive capacity. For example, diurnal animals, including humans, are generally more physically active during the day (light) period, whereas they rest and sleep during the night (dark) period. Thus, all body activities, including the energy-producing or -conserving systems, body temperature control, and brain functions, are synchronized to the day-night changes of the environment.

Slide 10: Circadian clock system

Living organisms, including humans, have developed and maintain the highly conserved circadian clock system to adjust the body's activity to the circadian changes of the environment (11, 12). The clock system creates internal circadian rhythmicity under the strong influence of light-dark information provided by the eyes (13, 14). There are two components in the circadian clock system. One is the central master clock, which is located in the suprachiasmatic nucleus (SCN) of the hypothalamus and generates the body's circadian oscillation rhythms (14). The other is the peripheral slave clock, which is ubiquitous in all organs and tissues (14). Importantly, circadian rhythms of the peripheral clock are synchronized to that of the central clock, possibly through neural and humoral connections, although details of such connections have not yet been completely elucidated (3, 15). The peripheral clock is organized by the master clock but continues to operate even when cells are uncoupled from the master clock, such as by explant and culture, though their periodicity will gradually wind down (16).

Slide 11: Circadian clock influences virtually all physiologic functions and organs

The central circadian clock system influences every aspect of body activities associated with day-night changes, such as wakefulness and sleep, feeding, thermoregulation, energy expenditure, and intermediary metabolism (3, 12, 15). The peripheral clocks located in all organs also influence organ-specific activities through the circadian information provided by the central clock (3).

Slide 12: Circadian clock transcriptional loop

At the molecular level, the clock is a transcriptional loop composed of the Clock-Bmal1 heterodimer and other transcription factors, such as members of the Period (Per) and Cryptochrome (Cry) families, that creates and maintains intrinsic, self-oscillating circadian rhythms in both the central and peripheral clock systems (11, 12). The Clock-Bmal1 heterodimer stimulates expression of genes encoding Pers and Crys, which, in turn, suppress the transcriptional activity of the Clock-Bmal1 heterodimer on their own promoters, creating the main circadian transcriptional loop that oscillates approximately every 24 hours (3, 12). In addition to this major loop, Clock-Bmal1 also stimulates expression of genes encoding other transcription factors, such as the retinoic acid receptor-related orphan receptor (ROR) and RevErbA, which together create an auxiliary loop for stabilizing the activity of the main loop (3). Importantly, these clock-related transcription factors influence the transcriptional activity of other clock-responsive genes to transduce circadian information to numerous cellular activities (17). RRE, ROR response element.

Slide 13: Interaction of the circadian clock system and the HPA axis

I hypothesized that the circadian clock system and the stress-responsive HPA axis might have strong interactions with each other, because both systems organize fundamental adaptive activities in response to environmental changes.

Slide 14: Serum cortisol circadian rhythms

For example, the SCN of the hypothalamus projects neurons to the PVN stress center and regulates clock-dependent cortisol secretion from the adrenal glands (3, 18). This results in the circadian rhythmicity of serum cortisol concentrations, which reaches its peak in the early morning and its low point in the late evening (19). I was interested in how glucocorticoid effects are regulated in peripheral target tissues. Therefore, I hypothesized that the Clock-Bmal1 circadian transcriptional system and the glucocorticoid-responsive GR signaling system may interact at the transcriptional level.

Slide 15: Clock-Bmal1—Circadian rhythm transcription factors

To test my hypothesis, I focused on the Clock and Bmal1 transcription factors. Clock and Bmal1 are both members of the bHLH-PAS [basic helix-loop-helix (bHLH)–Period (Per)–aryl hydrocarbon receptor nuclear translocator (ARNT)–single-minded (SIM) (PAS)] superfamily of transcription factors (11, 14, 19, 20). Clock functions as a histone acetyltransferase (HAT) (21) and has high structural similarity to one of the p160-type nuclear receptor coactivators with HAT activity, the activator of thyroid and retinoic acid receptors (ACTR) (21, 22). Both Clock and ACTR proteins have one bHLH and two PAS domains in the N-terminal portion and a serine-rich and nuclear receptor-interacting domain in the middle portion (20, 21). Both have a polyQ domain in the C-terminal portion, which has HAT activity (20, 21). Given that several nuclear receptor coactivators physically interact with and acetylate steroid hormone receptors, it is highly possible that Clock also binds and acetylates GR to regulate its transcriptional activity (10).

Slide 16: Clock-Bmal1 represses GR-induced transcriptional activity

I first studied a simple reporter assay, in which I transfected Clock- and Bmal1-encoding plasmids together with a glucocorticoid-responsive mouse mammary tumor virus (MMTV) promoter-driven luciferase construct. Clock-Bmal1 suppressed GR transcriptional activity on the MMTV promoter stimulated by the synthetic glucocorticoid dexamethasone (DEX) in both human cervical carcinoma HeLa cells and colon cancer HCT116 cells (23). RLU, relative luciferase unit; n.s., not significant. * $P < 0.01$ versus control with dexamethasone; error bars indicate SE calculated from three independent results.

Slide 17: Circadian rhythms of the GR transcriptional activity mirrors to those of the Clock-Bmal1 mRNA expression

I next examined the mRNA expression profiles of *Clock* and *Bmal1* and monitored the transcriptional activity of GR in HeLa cells whose circadian rhythms had been synchronized with serum shock, as observed in the diurnal fluctuation of the *Clock* and *Bmal1* mRNA abundance [(A) and (C)]. Treatment of the cells with pulses of DEX every 6 hours for 60 hours caused expression of the glucocorticoid-responsive *glucocorticoid-inducible leucine zipper* (*GILZ*) to oscillate in 24-hour cycles, but its expression fluctuated opposite that of *Clock* and *Bmal1* (E) (23). Knocking down Clock and Bmal1 by RNA interference greatly reduced the abundance of *Clock* and *Bmal1* transcripts, and neither gene exhibited cyclic expression [(B) and (D)]. When Clock and Bmal1 were knocked down, *GILZ* expression also lost the fluctuation (F). These results support the hypothesis that Clock and Bmal1 act as negative regulators of GR transcriptional activity and create its circadian rhythms.

siRNA, small interfering RNA. Error bars indicate SE calculated from three independent results.

Slide 18: GR interacts with Clock in GST pull-down assays

Given that Clock has a nuclear receptor-interacting domain (24), I tested its interaction with GR in glutathione *S*-transferase (GST) pull-down assays. As shown in the left panel, in vitro-translated and -radiolabeled GR interacted with the C-terminal half of bacterially produced and GST-fused Clock (the portion of Clock that contains a nuclear receptor-interacting domain) (23, 24). As shown in the right panel, in vitro translated and radiolabeled Clock interacted with GST-GR fusion proteins containing the GR C-terminal LBD (23). I also confirmed the interaction of endogenous Clock and GR by using an immunoprecipitation assay in human peripheral blood mononuclear cells (PBMCs) (25).

Slide 19: Clock represses GR-induced transcriptional activity in a HAT-dependent fashion

Because Clock has a HAT domain, I created an expression construct containing a HAT-defective Clock mutant and tested its effect on GR-induced transcriptional activity (23). As indicated in the left panel, wild-type (WT) Clock suppressed GR-induced transcriptional activity at the MMTV promoter, but the HAT-defective Clock mutant had no such effect (23), indicating that Clock represses GR-induced transcriptional activity in a HAT-dependent fashion. The right panel demonstrates that WT Clock and the HAT-defective mutant were produced at similar abundance throughout the experiment (23). * $P < 0.01$ versus control with dexamethasone; error bars denote SE calculated from three independent results.

Slide 20: Clock acetylates multiple lysines located in the GR hinge region

I next examined acetylation of the human GR in Western blots. I expressed constructs encoding WT or lysine-mutated human GR proteins together with Clock-Bmal1 in HCT116 cells, precipitated GR with an anti-GR antibody, and detected the acetylated GR with an antibody that recognizes acetylated lysine. WT GR was acetylated in a DEX-dependent fashion, and replacement of lysines (K) with alanines (A) in the amino acid positions indicated on the slide reduced acetylation of GR (23). Simultaneous replacement of lysines 480, 492, 494, and 495 almost completely abolished GR acetylation. I also performed reporter assays to test the effect of Clock-Bmal1 on the transcriptional activity of these GR lysine mutants and found that the lysine residues I identified as acetylated by Clock mediated the suppressive effect of Clock-Bmal1 on GR-induced transcriptional activity (23). Taken together, these results suggest that Clock-Bmal1 represses GR-induced transcriptional activity by acetylating human GR on lysines 480, 492, 494, and 495.

Slide 21: Clock-Bmal1 reduces the GR-GRE binding in ChIP assays

I then examined the effect of Clock-Bmal1 on the binding of GR to GREs of the *GILZ* and glucose-6-phosphatase (*G6P*) genes in chromatin immunoprecipitation (ChIP) assays. WT Clock suppressed binding of GR to the GREs of these genes' promoters in HeLa and HepG2 cells, and a HAT-defective Clock mutant lost this suppressive effect (23). Thus, Clock-mediated suppression of the binding of GR to GREs is HAT domain-dependent. * $P < 0.01$ versus control with dexamethasone; error bars denote SE calculated from three independent results.

Slide 22: Clock-Bmal1 represses GR-induced transcriptional activity through acetylation

These experiments led to the model of GR regulation by Clock-Bmal1 shown here. Glucocorticoid-bound GR stimulates transcription of its responsive genes by binding to GREs located in gene regulatory regions. Clock-Bmal1 reduces the binding of GR to GREs

by acetylating GR, thus repressing GR-induced transcriptional activity (3, 23). Clock finds GR through physical interaction with the GR LBD and acetylates lysines 480, 492, 494, and 495 located in the hinge region of the receptor (3, 23).

Slide 23: How do Clock-mediated GR acetylation and GR transcriptional activity fluctuate in humans?

I obtained the above results from experiments performed in cultured cells, so I next examined Clock-mediated regulation of GR-induced transcriptional activity in humans, in whom circulating cortisol exhibits marked diurnal fluctuation.

Slide 24: Study design

For this purpose, I recruited 10 healthy volunteers. I sampled their blood at 8 a.m. and 8 p.m. for determining serum cortisol and plasma ACTH concentrations. In PBMCs isolated from these same samples, I also examined GR acetylation and expression of known glucocorticoid-responsive genes and Clock-related genes. I tested glucocorticoid responsiveness of these genes by treating unsynchronized Epstein-Barr virus (EBV)-transformed human lymphocytes with hydrocortisone (HC) for 6 hours. In the participants enrolled in this study, both serum cortisol and plasma ACTH concentrations at 8 a.m. were about threefold higher than those at 8 p.m., indicating that circulating levels of these hormones fluctuated in a circadian fashion, as expected (25).

Slide 25: Circadian mRNA expression of CLOCK-related genes

Transcripts of *Clock*, *Bmal1*, and other Clock-related genes showed distinct expression patterns. Whereas the abundance of *Clock* and *Bmal1* mRNAs was greater in the morning (day) than in the evening (night), *Per1*, *Cry1*, and *ROR* mRNAs showed an opposite expression pattern, being higher in the evening and lower in the morning (25). * $P < 0.01$, comparing the two conditions indicated; error bars denote SE calculated from 20 independent results.

Slide 26: GR is acetylated in a circadian fashion in PBMCs

GR was highly acetylated in the morning, correlating with the abundance of *Clock* during this period (25). In contrast, such GR acetylation was attenuated in the evening. * $P < 0.01$, comparing the two conditions indicated; error bars denote SE calculated from 15 independent results.

Slide 27: Gene-specific circadian mRNA expression of glucocorticoid-responsive genes in PBMCs (transactivation)

I examined the expression of glucocorticoid-responsive genes that are stimulated by glucocorticoids. I chose (i) *GILZ*, (ii) *tristetraprolin*, (iii) *annexin A1*, and (iv) *dual-specificity phosphatase 1 (DUSP1)* as representative genes that are transactivated by glucocorticoids in cultured cells (19). These genes responded similarly positively to a 6-hour incubation with HC in unsynchronized EBV-transformed lymphocytes, as shown in the top panels (25). The bottom panels show that expression of *GILZ* and *tristetraprolin* was higher in the morning and lower in the evening (25), possibly responding to the diurnal fluctuation of circulating cortisol. In contrast, *annexin A1* and *DUSP1* did not show diurnal fluctuation (25), suggesting that the responsiveness of these genes to circulating cortisol is suppressed in vivo by some other factor(s). * $P < 0.01$, comparing the two conditions indicated; error bars denote SE calculated from 3 (top panels) and 20 (bottom panels) independent results, respectively.

Slide 28: Gene-specific circadian mRNA expression of glucocorticoid-responsive genes in PBMCs (transrepression)

I next examined expression of four glucocorticoid-responsive genes that are down-regulated by glucocorticoids: (i) *tumor necrosis factor- α* (*TNF α*), (ii) *interleukin-1 β* (*IL-1 β*), (iii) *IL-12 p40*, and (iv) *interferon- γ* (*IFN γ*) (19). Expression of these genes was reduced after a 6-hour incubation with HC in unsynchronized EBV-transformed lymphocytes (25). Also, expression of *IL-1 β* , *IL-12 p40*, and *IFN γ* was lower in the morning than in the evening (25), possibly responding to diurnally fluctuating circulating cortisol. In contrast, expression of *TNF α* did not show a diurnal fluctuation (25). Diurnal changes of *IFN γ* mRNA were also blunted as compared with its high responsiveness to HC in unsynchronized EBV-transformed lymphocytes (25). These results suggest that the effects of circulating cortisol on the transcription of some glucocorticoid-responsive genes repressed by this steroid are also influenced by additional factor(s) in vivo. * $P < 0.01$, comparing the two conditions indicated; error bars denote SE calculated from 3 (top panels) and 20 (bottom panels) independent results, respectively.

Slide 29: Clock effects on GR ex vivo

I then examined the effect of the peripheral (slave) clock on GR-induced transcriptional activity in ex vivo cultured PBMCs that are not under the influence of the central (master) clock-dependent diurnally fluctuating circulating cortisol. I therefore purified PBMCs at 6 a.m. and subsequently performed timed experiments in the absence of circulating cortisol.

Slide 30: Circadian rhythm of PBMCs cultured ex vivo

In PBMCs cultured ex vivo, expression of *Clock* and *Cry1* demonstrated circadian rhythmicity (25). Because PBMCs were sampled at 6 a.m., hour 0 in the x axis corresponds to early morning in vivo. * $P < 0.01$ versus control (time “0”); error bars denote SE calculated from 12 independent results.

Slide 31: GR acetylation fluctuates in PBMCs ex vivo

Acetylation of GR also demonstrated circadian rhythmicity in PMBCs cultured ex vivo, being higher at the 0-hour (early morning) time point and lower at the 12-hour (evening) time point; the same rhythms were observed in vivo (25). * $P < 0.01$ versus control (time “0”); error bars denote SE calculated from 12 independent results.

Slide 32: GR acetylation is dependent on the presence of Clock in PBMCs ex vivo

To examine the contribution of Clock to the acetylation of the GR in PBMCs ex vivo, I knocked down Clock by transfecting a construct encoding a siRNA targeting *Clock* into PBMCs. As shown in the right panel, circadian fluctuation of GR acetylation was dependent on the presence of Clock (25). The left panel indicates that *Clock* siRNA efficiently reduced *Clock* mRNA abundance (25). * $P < 0.05$ and ** $P < 0.01$, compared to the values obtained in the absence of HC at the same time point; error bars denote SE calculated from six independent results.

Slide 33: Gene-specific fluctuation of glucocorticoid-responsive gene expression in PBMCs ex vivo

In PBMCs cultured ex vivo, expression of *tristetraprolin* and *IL-1 β* , which showed circadian fluctuation in PBMCs in vivo, demonstrated steady expression (25). These results suggest that diurnal changes in the expression of these genes observed in vivo may be caused by the fluctuation of circulating cortisol. In contrast, expression of *DUSP1* and *TNF α* , which showed steady expression in vivo, demonstrated fluctuations opposite that of *Clock* expression and GR acetylation ex vivo (25). This result suggests that these genes are

sensitive to GR acetylation, demonstrating circadian rhythmicity under the influence of the local (peripheral) circadian clock system and subsequent acetylation of GR by Clock in the absence of circulating cortisol. $*P < 0.01$, compared to the values obtained in the absence of HC at the same time point; error bars denote SE calculated from 12 independent results.

Slide 34: Clock knockdown abolishes diurnal fluctuation of DUSP1 and TNF α mRNA expression in PBMCs ex vivo

To confirm this hypothesis, I knocked down Clock in PBMCs cultured ex vivo. As expected, Clock knockdown completely abolished fluctuation of *DUSP1* and *TNF* mRNA abundance (25), suggesting that the fluctuation of these genes' expression is dependent on the presence of Clock locally and possibly on GR acetylation in these cells. $*P < 0.01$ compared to the values obtained in the absence of HC at the same time point; error bars denote SE calculated from six independent results.

Slide 35: Clock-mediated gene-specific regulation of glucocorticoid action in peripheral tissues

Circulating cortisol concentrations demonstrate a circadian fluctuation with a peak in the early morning and the low point in the evening. Acetylation of GR is also in circadian rhythmicity, is synchronized with the fluctuation of serum cortisol concentrations, and causes reciprocal fluctuation of glucocorticoid sensitivity in target tissues in a gene-specific and possibly tissue-specific fashion.

Slide 36: Further interactions of circadian clock transcription factors and GR

In 2009, I first reported findings on Clock-mediated acetylation of GR and its regulation of tissue glucocorticoid actions (23), and two groups have subsequently reported regulation of GR activity by Per2 and Cry2 as well (26, 27). Per and Cry proteins form heterodimers and function as transcription factors that counteract the activities of Clock-Bmal1 (27). These reports indicate that the interaction between the molecular clock transcriptional loop and GR activity is multifactorial.

Slide 37: Multiple interactions between the circadian clock system and the HPA axis

Multilevel interactions exist between the circadian clock system and the HPA axis. Thus, the master clock strongly influences hypothalamic secretion of CRH and AVP by the PVN and creates diurnal fluctuation of circulating ACTH and cortisol (3, 18). I and others found that the peripheral clock negatively regulates GR transcriptional activity, functioning as a gene-specific counter-regulatory mechanism against the fluctuation of circulating glucocorticoids (23, 26, 27). A previous report indicated that glucocorticoids reset the peripheral clocks, but not the central clock, which may be important for adjusting the body's response to stress (28). In addition to these interactions, the central clock also sensitizes the adrenal glands to ACTH through neural connections (29). Peripheral clocks residing in the adrenal glands, and possibly in the pituitary gland, further influence the secretion of ACTH and cortisol from these organs locally (30, 31).

Slide 38: Pathologic implications of Clock-GR interaction in glucocorticoid target tissues

I would like to discuss pathologic implications of our findings on the interaction between the circadian clock system and the stress-responsive HPA axis and GR.

Slide 39: Loss of circadian rhythms and glucocorticoid excess cause similar metabolic disturbances

This slide shows that loss of circadian rhythms in animals and the human pathologic condition associated with glucocorticoid excess (Cushing syndrome) result in similar

metabolic disturbances, such as hyperglycemia, insulin resistance, central obesity, and hypertension (3). These pieces of evidence indicate that the circadian clock system and the HPA axis strongly cooperate with each other in physiology and pathophysiology.

Slide 40: Examples of pathologies due to aberrant coupling of the clock system and the HPA axis

Dysregulated communication between the circadian clock system and the stress-responsive HPA axis is also linked to development of human pathology. Conditions including chronic stress, working night shifts, and Cushing syndrome are associated with aberrant coupling of these systems. Indeed, they are all characterized by high risk for cardio-metabolic disease (3, 19).

Slide 41: Uncoupling between circadian rhythms of serum cortisol and tissue glucocorticoid sensitivity

I have demonstrated that glucocorticoid actions in target tissues fluctuate in a circadian fashion, being higher in the evening and lower in the morning through GR acetylation by Clock (23, 25). Participants under stress, such as those with melancholic depression and Cushing syndrome patients, demonstrate flattening of diurnal fluctuation of circulating cortisol and are highly susceptible to the development of metabolic syndrome and consequent cardiovascular complications (20, 32). It is likely that relatively high tissue sensitivity to glucocorticoids in the evening may potentiate the biologic actions of elevated evening cortisol seen in these patients, who then subsequently develop pathologic complications associated with glucocorticoid excess. Participants with decoupling of the central clock-HPA axis and the peripheral clock system, such as those who work night shifts or frequently travel between different time zones, are also susceptible to similar cardiovascular complications (33), possibly through exposure of the tissues with elevated sensitivity to glucocorticoids (3). CS, chronically stressed individuals; NS, nonstressed individuals.

Slide 42: Questions to be answered

Studying the pathologic impact of clock and HPA axis dysregulation and the prevention of disorders associated with such dysregulation is strongly warranted. The ultradian fluctuation in serum cortisol concentrations has not been studied extensively to date and is also an important research target (34). Finally, studies are lacking on the effect of seasonal rhythms on the clock and the HPA axis in health and disease.

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