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Interaction between circadian rhythms and stress

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A B S T R A C T

Life on earth has adapted to the day-night cycle by evolution of internal, so-called *circadian* clocks that adjust behavior and physiology to the recurring changes in environmental conditions. In mammals, a master pacemaker located in the suprachiasmatic nucleus (SCN) of the hypothalamus receives environmental light information and synchronizes peripheral tissues and central non-SCN clocks to geophysical time. Regulatory systems such as the hypothalamus-pituitary-adrenal (HPA) axis and the autonomic nervous system (ANS), both being important for the regulation of stress responses, receive strong circadian input. In this review, we summarize the interaction of circadian and stress systems and the resulting physiological and pathophysiological consequences. Finally, we critically discuss the relevance of rodent stress studies for humans, addressing complications of translational approaches and offering strategies to optimize animal studies from a chronobiological perspective.

Contents

1. The molecular clock

The ability to anticipate daily changes in the environment conveys an evolutionary advantage to most species on earth. Therefore, organisms ranging from plants to higher mammals have developed endogenous circadian clocks that allow them to estimate the time of day. In the absence of external time cues these clocks free-run with a period close to 24 h. In order to compensate

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discrepancies between this intrinsic period and the environmental cycle, circadian clocks entrain to external *Zeitgebers* (from German *time giver*), with light being the most potent one. Unlike most biochemical systems, the period of the circadian clock is temperature compensated, a feature that is especially important for poikilothermic species (Buhr and Takahashi, 2013).

In mammals, the circadian clock is based on a molecular oscillator present in virtually every cell of the body. It is built from transcriptional-translational feedback loops (TTLs) generating self-sustained oscillations even on the cellular level. The clock's core TTL is composed of the genes *brain and muscle arnt-like 1* (*Bmal1*), *circadian locomotor output cycles kaput* (*Clock*), *cryptochrome* (*Cry*) *1/2* and *period* (*Per*) *1–3*. BMAL1 and CLOCK proteins form the positive limb of this core TTL. They belong to the family of basic helix-loop-helix transcription factors and act as heterodimers binding to *E-box* regulatory elements within the promoters of *Cry* and *Per* genes (Fustin et al., 2009; Gekakis et al., 1998; Hogenesch et al., 1998; Yoo et al., 2004), activating transcription of *Per* and *Cry*. PER and CRY proteins constitute the negative feedback limb of the circadian core TTL. Over the course of the day they accumulate in the cytoplasm and form complexes that translocate back into the nucleus where they inhibit BMAL1/CLOCK-mediated transcription (Kume et al., 1999; Zheng et al., 2001). Before the next cycle can start, the BMAL1/CLOCK heterodimer has to be reactivated. This is achieved by proteasomal degradation of the PER and CRY repressor complex. PER1 and PER2 are subject to phosphorylation mediated by casein kinases 1 delta and epsilon. This phosphorylation mark leads to their ubiquitination and subsequent degradation by the ubiquitin proteasome system (Camacho et al., 2001; Eide et al., 2005). Similarly, adenosine monophosphate-activated protein kinase (AMPK) and glycogen synthase kinase 3 beta (GSK3β) phosphorylate CRY1 and CRY2, respectively (Harada et al., 2005; Lamia et al., 2009), so that they are ubiquitinated and degraded. Decreasing levels of CRY and PER terminate the repression of BMAL1/CLOCK-mediated transcriptional activation so that the clock can move to the next cycle.

Besides this core loop, there are accessory feedback loops and additional levels of regulation to stabilize the molecular oscillations and mediate additional fine-tuning. The most prominent accessory TTL consists of *reverse erythroblastoma* (*Rev-Erbα/β*) and *retinoic acid receptor-related orphan receptor* (*RORα-γ*) that also contain *E-boxes* within their promoter regions. The BMAL1/CLOCK heterodimer binds to these *E-boxes* and activates transcription of *Rev-Erbs* and *RORs* (Buhr and Takahashi, 2013). In turn, REV-ERB proteins exert a negative feedback, inhibiting *Bmal1* transcription (Liu et al., 2008; Triqueneaux et al., 2004). RORs, in contrast, are positive regulators of *Bmal1* transcription and compete with REV-ERBs for *retinoid orphan receptor response element* (*RORE*) binding sites within the *Bmal1* promoter (Akashi and Takumi, 2005). REV-ERBα and β are functionally redundant and are considered to be essential for *Bmal1* oscillation. RORs seem to have a modulatory function, but they are dispensable for rhythmic transcription of *Bmal1* per se (Liu et al., 2008).

This molecular clock machinery is present in all nucleus-containing cells of an organism. In order to synchronize single-cell oscillators with each other, the mammalian circadian system is organized in a hierarchical manner. A master clock resides in the suprachiasmatic nuclei (SCN) of the hypothalamus (Moore and Eichler, 1972; Ralph et al., 1990; Stephan and Zucker, 1972). The SCN receive light information from melanopsin-expressing cells in the retina via the retino-hypothalamic tract (Provencio et al., 2000). Time information is then passed on to subordinate peripheral tissues via humoral and neuronal signals (Buijs et al., 2003; Liu et al., 2007; Welsh et al., 2004; Yoo et al., 2004). In this way all peripheral and non-SCN tissue clocks are coordinated by the master clock.

2. Role of glucocorticoids in clock regulation

Among all peripheral oscillators, the adrenal gland holds a special role since the adrenal circadian clock can influence rhythms in other peripheral tissues via rhythmic release of hormones with clock-modulating properties. The adrenal gland is composed of an outer cortex and an inner medulla. The medulla releases catecholamines (epinephrine and norepinephrine), whereas the cortex secretes mineralocorticoids from the outer *zona glomerulosa*, glucocorticoids (GCs) from the intermediate *zona fasciculata*, and sex steroids from the inner *zona reticularis*. Cortisol and corticosterone, the main GCs in humans and rodents, respectively, display a very robust circadian oscillation with blood levels peaking shortly before the onset of the active phase (i.e. the early morning in humans and the early evening in nocturnal rodents). The circadian GC rhythm is overlaid by strong ultradian pulsatility with a period of around one hour and an amplitude that varies considerably during the day (Windle et al., 1998). GCs are secreted upon adrenocorticotropic hormone (ACTH) binding to melanocortin-2 receptors (MC2R) in the adrenal gland. ACTH itself is secreted from the anterior pituitary upon corticotrophin releasing hormone (CRH) signaling, which stems from the paraventricular nucleus (PVN) of the hypothalamus. Together, these tissues and factors constitute the hypothalamus-pituitary-adrenal (HPA) axis. Circadian oscillations are detectable for all components (CRH, ACTH, and GCs) (Chrousos, 1998; Girotti et al., 2009). However, it is not clear if rhythmic HPA axis activity is necessary for the circadian rhythm of GC secretion. On one hand, adrenal rhythms persist after hypophysectomy, when no ACTH is present (Fahrenkrug et al., 2008). On the other hand, ACTH is capable of phase-dependently resetting GC rhythms (Yoder et al., 2014). In addition, the HPA axis gets direct input from the SCN via the paraventricular nuclei of the hypothalamus (Dickmeis et al., 2013; Vrang et al., 1995) and the SCN controls GC rhythms as was shown in SCN-lesioned animals (Moore and Eichler, 1972). The circadian pattern of GC secretion can be abolished by specifically disrupting the circadian clock in the adrenal gland (Oster et al., 2006a; Son et al., 2008), indicating that this peripheral tissue clock finally governs GC secretory patterns.

Glucocorticoids act via mineralocorticoid (MR) and glucocorticoid receptors (GR), type-1 nuclear receptors with broad expression patterns throughout the body except for the SCN. GR signaling can mediate phase resetting of peripheral clocks, pointing at a special role of GC rhythms in the coordination of the organism's circadian network (Balsalobre et al., 2000). For instance, microarray analysis of murine liver revealed 100 rhythmic genes whose oscillation was directly dependent on adrenal signals, because rhythmicity of these genes is lost in adrenalectomized animals (Oishi et al., 2005). Finally, in a mouse model of *jet lag*, which is caused by an abrupt phase shift of light conditions, GC rhythms can modify the kinetics of entrainment to the new time zone (Kiessling et al., 2010).

On top of their phase shifting ability, GCs can stabilize peripheral rhythms against external perturbation. Timed food restriction can induce phase shifts in peripheral tissues so that peripheral clocks become uncoupled from the master clock in the SCN that stays tied to the light-dark cycle. The circadian system is more robust against such perturbations when GCs are high (Le Minh et al., 2001). In summary, rhythmic GC secretion is an important timing signal for the coordination of peripheral clocks.

3. Neurobiology of stress (HPA axis, glucocorticoids, catecholamines)

Besides their role in circadian coordination GCs are important vectors of the stress system. Stress refers to an external or internal