Endocrine regulation of circadian physiology

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Abstract

Endogenous circadian clocks regulate 24-h rhythms of behavior and physiology to align with external time. The endocrine system serves as a major clock output to regulate various biological processes. Recent findings suggest that some of the rhythmic hormones can also provide feedback to the circadian system at various levels, thus contributing to maintaining the robustness of endogenous rhythmicity. This delicate balance of clock–hormone interaction is vulnerable to modern lifestyle factors such as shiftwork or high-calorie diets, altering physiological set points. In this review, we summarize the current knowledge on the communication between the circadian timing and endocrine systems, with a focus on adrenal glucocorticoids and metabolic peptide hormones. We explore the potential role of hormones as systemic feedback signals to adjust clock function and their relevance for the maintenance of physiological and metabolic circadian homeostasis.

Introduction

Endogenous circadian clocks regulate a broad spectrum of biological functions via multiple routes. A major output pathway of the circadian clock is the endocrine system which allows for a systemic coordination of various physiological target systems according to the time of day. It has long been appreciated that many hormones show circadian rhythms in the circulation (Pincus et al. 1954, Moore & Eichler 1972). Both central and peripheral tissue clocks impinge on such endocrine rhythms. On the other hand, hormonal signals have been shown to feedback on circadian clock regulation. Modern life styles compromise the integrity of this feedback balance, and targeting the interaction between the two systems may provide novel solutions to improve general well-being. Various previous reviews have discussed clock regulation of specific hormonal systems (Haus 2007, Kalsbeek & Fliers 2013) as well as the impact of circadian misalignment in metabolic disorders (Marcheva et al. 2013). Here, we describe the clock–endocrine crosstalk with a focus on the hypothalamic–pituitary–adrenal (HPA) stress axis and peptide hormones involved in energy homeostasis (Fig. 1).

Molecular and anatomical makeup of the mammalian circadian system

The mammalian cellular clockwork consists of a transcriptional–translational feedback loop (TTL) of a set of core clock genes, with a positive and a negative arm active during subjective night and day time, respectively. The positive arm comprised circadian locomotor output cycles kaput (CLOCK) (in neural and vascular tissues, CLOCK can be functionally replaced by neuronal PAS domain-containing protein 2 (NPAS2)) and brain and muscle aryl hydrocarbon receptor nuclear translocator-like 1 (BMAL1). These transcription factors form
heterodimers which bind to E-box promoter elements to activate the negative arm genes, period (Per1-3) and cryptochrome (Cry1/2), as well as several other clock-controlled genes (CCGs). During the night, CRY and PER proteins accumulate in the cytoplasm and translocate into the nucleus where they inhibit CLOCK:BMAL1 activity and, thus, shut down their own transcription. Subsequent degradation of PER and CRY relieves the inhibition on CLOCK:BMAL1 toward the morning, followed by resumed transcription of Per/Cry and other CCGs. The cycle is further stabilized by accessory TTLs such as the one composed of the nuclear receptors REV-ERBa/β and RORα/β, repressing and activating Bmal1 transcription, respectively (Zhang & Kay 2010).

The mammalian circadian system is organized in a hierarchical manner. The molecular clockwork described above is present in essentially all cells, but the nature of CCGs differs between tissues (Zhang et al. 2014). In order to generate physiologically meaningful rhythms, these tissue clocks have to be properly aligned with each other and external time. In mammals, light is the major Zeitgeber (German for ‘time giver’). A master pacemaker located in the hypothalamic suprachiasmatic nucleus (SCN) acts as the major interface between endogenous rhythms and environmental time cues such as the light–darkness cycle (Husse et al. 2015). The SCN receives photic input from intrinsically photoreceptive retinal ganglion cells (ipRGCs) expressing the photopigment melanopsin to entrain SCN clock gene expression and neuronal activity (Hankins et al. 2008). The majority of efferent projections from the SCN target hypothalamic structures such as the subparaventricular zone (sPVZ), the arcuate nucleus (ARC), the paraventricular nucleus (PVN), and the dorsomedial hypothalamic nucleus (DMH). Through these, the SCN regulates a plethora of physiological systems (Saeb-Parsy et al. 2000). Mice with focal SCN lesions lose circadian...
behavioral and physiological rhythms and no longer entrain to light–darkness cycles (Moore & Eichler 1972, Stephan & Zucker 1972). It is still a matter of debate as to which of these functions require rhythmic input from the SCN and which are primarily controlled by clocks in downstream central or peripheral tissues. Environmental factors other than light can serve as Zeitgebers of the circadian system. For example, temporally restricted food availability is a potent synchronizer of peripheral tissue and some central nervous system (CNS) clocks, but has little effect on the SCN itself (Damiola et al. 2000, Stephan 2002).

**Circadian regulation of the HPA axis**

Many hormones and most of the major endocrine axes show robust circadian rhythms. Of these, circadian regulation of the HPA axis is particularly well studied, since blood levels of glucocorticoids (GCs) are among the most rhythmic functions observed in the mammalian system and because both GCs and catecholamines have been implicated in the synchronization of circadian clocks and rhythms in other tissues (Balsalobre et al. 2000, Terazono et al. 2003). HPA axis activity coordinates different physiological systems and behavior in response to stress. GCs are the end products of the activation of the HPA axis. Acute stress activates hypothalamic release of corticotropin-releasing hormone (CRH) to the anterior pituitary, where it stimulates the release of adrenocorticotropic hormone (ACTH) into the bloodstream. ACTH activates adrenocortical steroidogenesis through the melanocortin 2 receptor (MC2R), via a cyclic adenosine monophosphate/protein kinase A (cAMP/PKA)-dependent pathway (Miller & Buijs et al. 2008). Under nonstressed conditions, blood GC levels oscillate in both circadian and ultradian manners. Ultradian (aka. pulsatile) GC rhythms are controlled by a negative feedback loop in which GCs suppress ACTH production via the hypothalamus and the pituitary (Spiga et al. 2014). The circadian GC rhythm, in contrast, involves HPA activity, SCN-controlled autonomic innervation, and adrenocortical clocks. The peak of the circadian GC rhythm is phase-locked to the beginning of the active phase in order to organize various biological processes to prepare for the active behavior during the wake time (Moore & Eichler 1972). Circadian GC rhythms have long been established as a function of endogenous circadian clocks as they persist even under constant conditions. At the same time, surgical ablation of the SCN completely abolishes the circadian release of GCs into the blood (Moore & Eichler 1972). Retrograde neuronal tracing experiments revealed that the adrenal is connected to the SCN via the intermediolateral (IML) column of the spinal cord and the PVN (Buijs et al. 1999). Sympathetic nerve stimulation potentiates GC responses, suggesting a role of adrenal sympathetic innervation in the regulation of GC rhythms (Edwards & Jones 1993). The SCN projects inhibitory efferents to pre-autonomic and CRH-producing neurons in the PVN which ultimately lead to a suppression of adrenal GC secretion (Buijs et al. 1997). On the other hand, HPA axis activity upstream of the adrenal is also rhythmic, so circadian GC release may be an indirect response to SCN-controlled CRH expression. The latter view has been challenged by the discovery that the timing of expression of CRH in the hypothalamus and pro-opiomelanocortin (POMC) in the anterior pituitary and the plasma surge of GC is not organized in the expected sequential manner (Girotti et al. 2009). Moreover, in hypophysectomized rats, the implantation of constant-release ACTH pellets is sufficient to restore GC rhythmicity (Ottenweller & Meier 1982). Along this line, light signals have been found to be capable of modulating adrenal GC secretion via the SCN directly and independent of ACTH (Buijs et al. 1999, Ishida et al. 2005).

Experiments from the early 1960s suggested that the responsiveness of the adrenal steroidogenic machinery to ACTH stimulation shows circadian variations (Ungar & Halberg 1962). More than 40 years later, our group described an SCN-independent, adrenal clock-controlled gating mechanism underlying this observation (Oster et al. 2006). As a consequence, surgical or genetic ablation of adrenal clock function in mice results in dampened rhythms of GC secretion compared with sham-treated or wild-type controls (Oster et al. 2006, Son et al. 2008). Son and coworkers further showed that steroidogenic acute regulatory protein (STAR), one of the pacemakers of GC biosynthesis, is a direct target of CLOCK:BMAL1, suggesting a mechanistic function of adrenocortical clocks in the regulation of GC production (Son et al. 2008). The light pulse data discussed above suggest that adrenal clocks are entrained by neuronal mechanisms (Buijs et al. 1999, Ishida et al. 2005). However, Yoder and coworkers recently found that ACTH itself can also shift the phase of the adrenal Per2 rhythm (Yoder et al. 2014). One might speculate that, in this way, stress-induced HPA axis activation can reset adrenal clocks, thus affecting circadian GC release. Also, under acute stress conditions, the circadian clock plays a role in HPA axis regulation. SCN-lesioned animals show...
Review

A H TSANG and others

Endocrine rhythms

230:1

R4

Circadian regulation of metabolic hormones

Recent studies highlight the importance of the circadian clock in metabolic regulation. Behaviorally arrhythmic mice under constant light conditions (LL) or SCN-lesioned animals show increased body-weight gain together with impaired glucose tolerance and insulin sensitivity (Fonken et al. 2010, Coomans et al. 2013). Likewise, various metabolic phenotypes have been reported for different clock gene mutations (Bechtold & Loudon 2013). For instance, Bmal1-knockout (KO) mice display impaired glucose homeostasis with reduced gluconeogenesis and glucose intolerance (Rudic et al. 2004, Lamia et al. 2008, Marcheva et al. 2010, Kennaway et al. 2013), while ClockΔ19-knockout mice are obese and show hyperglycemia and hyperlipidemia (Turek et al. 2005). These and other metabolic disturbances are accompanied by altered endocrine functions. Both the master SCN clock and local clocks in peripheral tissues are involved in regulating the production and function of several metabolic hormones. Below, we summarize recent findings on the circadian regulation of insulin and its function in glucose homeostasis and on the ghrelin–leptin system controlling feeding behavior.

Circadian gating of insulin function in glucose homeostasis

Circulating plasma glucose levels, insulin concentrations, and whole-body insulin sensitivity display SCN-dependent circadian variations (La Fleur et al. 1999, Rudic et al. 2004, Lamia et al. 2008, Shi et al. 2013). Local clocks in pancreatic beta cells provide a gating mechanism for insulin secretion. Disruption of the positive arm of this clock (i.e. tissue-specifically deleting Clock or Bmal1) results in hypoinsulinemia (Marcheva et al. 2010, Sadacca et al. 2011), whereas deletion of the clock’s negative regulators (i.e. the Pers or Crys) is associated with hyperinsulinemia (Barclay et al. 2012, Zhao et al. 2012). In rodent and human pancreatic beta cells, the main functional targets of the clock machinery are a number of CCGs involved in peptide secretory pathways (Perelis et al. 2015, Saini et al. 2016). This mechanism further appears to be sensitive to the cellular redox status. Normalizing reactive oxygen species (ROS) levels can partially rescue the impairment of insulin secretion in Bmal1-deficient pancreatic beta cells (Sadacca et al. 2011). In line with these observations, mice with behaviorally disrupted circadian rhythms also exhibit hypoinsulinemia (Sadacca et al. 2011). Intriguingly, SCN-lesioned mice show hyperinsulinemia, but this may rather be an effect of elevated body weight and systemic insulin resistance (Coomans et al. 2013). Further studies will be needed to elucidate how SCN and local pancreatic clocks interact in regulating insulin secretion.

Circadian clocks play a further role in regulating insulin sensitivity at target tissues. Insulin resistance, a hallmark of the prediabetic state, has been associated with extended shift work in humans (Esquirol et al. 2012). Liver, skeletal muscle, and adipose tissues are the major effector organs of insulin’s hypoglycemic effects. Cell-type-specific Bmal1-mutant mice have been used to assess the role of the molecular clock in regulating insulin sensitivity in these tissues. Bmal1-knockout mice show impaired systemic insulin sensitivity accompanied by hyperglycemia over the course of a day (Rudic et al. 2004, Lamia et al. 2008, Shi et al. 2013). In contrast, mice with liver-specific deletion of Bmal1 show improved glucose tolerance, lowered blood glucose, but normal systemic

exaggerated GC secretion under acute stress, in line with an inhibitory role of the SCN on HPA axis activity (Buijs et al. 1997, 1999). Interestingly, we recently reported that Bmal1-deficient mice show a blunted stress-induced GC release along with reduced behavioral responses (Lelievski et al. 2014). Together, these data suggest that repetitive or chronic stress may alter the timing of GC-regulated metabolic processes. Not only GC production but also GC action has been found to be gated by the molecular clock. GC receptors (GRs) are rhythmically expressed in various tissues and subject to acetylation (and subsequent inactivation) by CLOCK (Charmandari et al. 2011). Recently, CRY1/2 proteins have been shown to directly repress the transactivational function of GR in a ligand-dependent manner (Lamia et al. 2011), providing another layer of complexity to the circadian regulation of GR signaling.

The pineal hormone melatonin may serve as an additional mean for the SCN to further modulate HPA axis activity. Melatonin secretion is controlled via multisynaptic innervation by the SCN pacemaker and plays a role in synchronizing peripheral tissue clocks (Hardeland et al. 2012). Melatonin administration suppresses HPA axis activity (Konakchieva et al. 1997, Torres-Farfan et al. 2003), and timed melatonin administration can entrain adrenal gland rhythms (Torres-Farfan et al. 2011). Together, melatonin and GC rhythms appear to act in concert to stabilize circadian phase and precision of different physiological systems.

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insulin sensitivity (Lamia et al. 2008). Hypoglycemia in these mice may be explained by blunted expression of GLUT2, which mediates the export of glucose from liver to the blood stream (Lamia et al. 2008). Alternatively, CRY1 has been shown to modulate gluconeogenesis by its interaction with the G-protein involved in glucagon signaling pathway (Sun et al. 2015) and PER2 binds to regulatory regions of the glucose-6-phosphatase gene, a key player in maintaining glucose levels during fasting periods (Schmutz et al. 2010). Skeletal muscle clocks have been suggested to play a role in preparing the transition from the rest/fasting to the active/feeding phase of the day when glucose becomes the predominant fuel. Skeletal muscle-specific Bmal1-knockout mice show impaired insulin-stimulated glucose uptake due to downregulated GLUT4 expression. However, the canonical insulin signaling pathway appears unaltered in these animals, as do systemic insulin sensitivity and blood glucose concentrations (Dyar et al. 2014). Skeletal muscle itself is an endocrine organ which secretes a number of peptide hormones known as myokines. Using human primary skeletal myotubes as a model, a recent study has unveiled that the clock machinery plays a pivotal role in regulating basal and circadian secretion of several myokines (Perrin et al. 2015). Given that many myokines are established regulators of energy metabolism, disruption of muscle clocks may link circadian misalignment to metabolic diseases. Adipose physiology shows strong variations over the course of the day. Adipocyte-specific Bmal1-knockout mice develop obesity. Surprisingly, though, these mice display normal systemic insulin sensitivity and glucose homeostasis (Paschos et al. 2012). Instead, the obesity phenotype appears to stem from mistimed feeding induced by altered adipose production of polyunsaturated fatty acids (Paschos et al. 2012).

Circadian regulation of leptin and ghrelin

The timing of food intake has recently been found to play an unexpected key role in body energy homeostasis (Zarrinpar et al. 2016). For example, scheduled feeding of a high-fat diet (HFD) during the inactive phase in mice leads to significant increase in body weight compared with feeding during the normal active phase, despite comparable caloric intake (Kohsaka et al. 2007). In line with that, scheduled feeding during the active phase (i.e. the night in nocturnal rodents) is protective against the metabolic dysregulation induced by HFD compared with chow fed controls ad libitum (Chaix et al. 2014). In a recent study in humans, restricting food intake to 12h during the day had beneficial effects on body-weight control (Gill & Panda 2015). The relative abundance of the antagonistic hormone duo, ghrelin and leptin, plays a major role in determining appetite regulation via signaling to the mediobasal hypothalamus (MBH) (Begg & Woods 2013). Blood levels of both hormones display circadian rhythms and are subject to acute regulation by food intake (see below).

Leptin is a cytokine-like peptide secreted from adipocytes to suppress food intake. In most obese individuals, leptin fails to suppress appetite despite increased blood leptin concentrations; a condition that has, in reference to insulin resistance in type 2 diabetes, been termed leptin resistance, though the mechanism of this lack of leptin action has still to be determined (Begg & Woods 2013). Baseline plasma leptin levels show SCN-dependent circadian variations (Kalsbeek et al. 2001) which are blunted and elevated in Clockδ19, Bmal1-knockout, as well as adipose-specific Bmal1-deficient mice (Turek et al. 2005, Paschos et al. 2012, Kennaway et al. 2013). Owing to increased fat mass in these genetic models, it has not yet been conclusively shown to which extent in vivo leptin secretion rhythms are directly regulated by adipocyte clocks or rather follow systemic cues such as food intake. Nevertheless, in vitro leptin secretion rhythms persist in cultured adipocytes (Otway et al. 2009). A recent study has identified an unexpected dual role of BMAL1:CLOCK in Leptin (Lep) transcription via an interaction with CCAAT/enhancer-binding protein (C/EBPα) at the proximal Lep promoter. When blood leptin levels are at their trough (corresponding to the early inactive phase in mice), CLOCK:BMAL1 modestly enhances the C/EBPα-mediated upregulation of Lep transcription, while acting in a suppressive way at high leptin blood levels (i.e. during the late inactive phase) due to direct competition with C/EBPα for Lep promoter binding (Kettner et al. 2015). In the same study, the authors also demonstrated that central leptin sensitivity is impaired in mice with genetically or behaviorally disrupted circadian rhythms (Kettner et al. 2015). How the molecular clock influences hypothalamic leptin signaling, however, remains unclear.

Ghrelin, on the other hand, is an orexigenic hormone secreted from the gastrointestinal (GI) tract in response to fasting. Both circulating ghrelin and gastric prepro-Ghrelin (Ghrl) mRNA levels display circadian rhythms which are lost in Bmal1-mutant mice (LeSauter et al. 2009, Laermans et al. 2015). Intriguingly, local GI clocks appear to be dispensable for regulating ghrelin secretion, as
siRNA-mediated downregulation of Bmal1 failed to affect ghrelin secretion from ghrelinoma cells (Laermans et al. 2015). Despite its role as a peripheral feedback signal to the central circadian system (see below), very little is still known about how the circadian system affects ghrelin secretion and sensitivity.

**Endocrine feedback on the circadian system**

Recent data suggest that endocrine functions are not merely an output of the circadian clock system, but also serve as feedback signals to regulate the circadian system at various levels. Alterations of such endocrine feedback have been implicated in the circadian disruption associated with conditions such as sleep disorders or shift work. In the following paragraphs, we will discuss the physiological relevance of hormones on the regulation of circadian rhythms with a focus on GCs and metabolic peptide hormones.

**GC circadian clock feedback**

As outlined above, the rhythmic secretion of GCs is involved in entraining clocks of peripheral and CNS tissues. GR is ubiquitously expressed throughout the body, with the notable exception of the SCN (Okamura 2007). It exerts a plethora of physiological effects by binding to GC-responsive elements (GREs) in the promoter of target genes and upregulating their expression (Yamazaki et al. 2009). The mechanism of GC-induced resetting of local clocks has been described. The proximal promoters of Per genes contain GREs and, thus, are activated by GCs to reset the phase of the TTL, very comparable to light-induced resetting of SCN clocks (Balsalobre et al. 2000). Very recently, a novel consensus promoter GR regulatory sequence (nGRE) has been discovered, binding of which results in repression of target gene transcription (Surjit et al. 2011). An auxiliary clock gene (Dec1) contains an nGRE in its promoter (Surjit et al. 2011). The two DEC proteins (DEC1/2; aka. BHLHE40/1) have been shown to modify the molecular clock in a tissue-specific manner (Rossner et al. 2008; Tsang et al. 2012). How GC-induced Dec suppression may affect tissue clock function, however, has not yet been studied.

Given the stimulatory role of GCs in arousal and alertness, it is not surprising that due to disruption of GC rhythms (such as in Cushing's (hypercortisolemia), Addison's (hypocortisolemia), and chronic stress diseases) patients often suffer from sleep and circadian rhythm disorders (Lelivskii et al. 2015). For Addison's patients, the standard therapeutic approach involves lifelong supplementation of GCs which often fails to replicate the normal circadian GC rhythm, thus compromising the patients' quality of life (Arlt & Allolio 2003). Attempts to modify the release profiles of GC replacement medications aimed to mimic the natural GC circadian rhythm have shown promising improvements in clinical studies (Chan & Debono 2010), highlighting the physiological importance of the circadian GC rhythm.

Transcontinental flight travel across multiple time zones results in jetlag which manifests in transient physiological and behavioral disturbances such as decreased alertness during the day and sleep problems during the night. It has been shown that GC rhythms are involved in mediating the adaptation of internal rhythms under jetlag conditions. In mice, by manipulating GC secretion phase during jetlag, behavioral adaptation after a shift of the light–darkness cycle can be accelerated (Kiessling et al. 2010). The potential clinical use of this treatment in humans warrants further pursuit.

**Metabolic hormone circadian clock feedback**

As outlined above, the timing of food intake is a major Zeitgeber to clocks residing in, both, the periphery and the CNS. When food access is restricted to the inactive phase of mice, it uncouples peripheral clocks from the SCN pacemaker (Damiola et al. 2000). In consequence, mistimed feeding alters circadian rhythms of locomotor activity and of various endocrine functions (Zarrinpar et al. 2016). Interestingly, already a mere change in nutrient composition can affect circadian rhythm regulation. Mice fed a high-fat diet ad libitum show blunted behavioral and molecular circadian rhythms (Kohsaka et al. 2007). These effects are correlated with altered regulation of metabolic hormone secretion.

All three metabolic hormones discussed above can provide feedback to the circadian clock system. Insulin has been shown to regulate food-induced resetting of liver clocks in vitro and in vivo, though the underlying mechanism has not yet been described (Tahara et al. 2011, Yamajuku et al. 2012). Our group has recently identified the incretin hormone oxyntomodulin (OXM) as an additional effector of food entrainment of peripheral tissues clocks (Landgraf et al. 2015). OXM is a 37-amino acid peptide of the glucagon family secreted from GI cells after meal intake. It promotes gastric acid secretion...
and gut motility, but suppresses appetite. Both glucagon (GCGR) and glucagon-like peptide 1 receptors (GLP-1R) have been shown to mediate a part of OXM signaling, but an, as yet uncharacterized, additional pathway has been suggested (Pocai 2014). In our study, we found that, in the inactive phase, postprandial increases of blood OXM levels are sufficient to reset the molecular clock and clock output gene rhythms in liver via a glucagon receptor-dependent signaling mechanism and induction of hepatic Per expression (Landgraf et al. 2015). Paradoxically, at a different time of day, glucagon itself can enhance fasting-induced liver Bmal1 expression (Sun et al. 2015). Further studies are needed to evaluate the temporal order and relative contributions of these and other metabolic hormones in mediating feeding-associated resetting of circadian transcriptional programs in peripheral tissues. Along this line, using a novel screening approach, Gerber and coworkers identified serum response factor (SRF) as an immediate early response transcription factor in the liver relaying signals from as yet unidentified oscillating blood-borne factors to control liver clock gene expression (Gerber et al. 2013).

Fibroblast growth factor 21 (FGF21) is a metabolic hormone that has recently been found to directly regulate circadian behavior and energy homeostasis via the SCN (Bookout et al. 2013). FGF21 is mainly produced by the liver, but is also secreted from muscle and white adipose tissues. Its signals are mediated via its cognate receptors (FGF receptors FGFR1c, FGFR2c, or FGFR3c) in complex with β-Klotho abundantly expressed in metabolic tissues (Degirolamo et al. 2016). FGF21 plays a major role in adaptive responses under starvation. The secretion of FGF21 is stimulated by fasting to enhance energy mobilization through gluconeogenesis, fatty acid oxidation, and ketogenesis (Degirolamo et al. 2016). FGF21 was also found to enhance insulin sensitivity after overfeeding (Xu et al. 2009). Together, much attention has been drawn to the therapeutic potential of FGF21 in treating metabolic syndromes. A recent study showed that the expression of the β-Klotho-encoding Klb gene is highly enriched in the SCN. Overexpression of FGF21 in mice was found to blunt circadian locomotor activity rhythms and suppress insulin, but to enhance GC secretion and reduce body weight. However, mice with conditional genetic ablation of Klb in the SCN lack these phenotypes, suggesting that the metabolic FGF21 effects are dependent of SCN clock function (Stephan 2002). Additionally, the FGF21–SCN axis has been found to play a key role in regulating female fertility via vasopressin–kisspeptin signaling controlling the proestrus surge of luteinizing hormone (Owen et al. 2013).

Both leptin and ghrelin can also directly regulate SCN function. In explant slices, leptin stimulation can reset the phase of SCN neuron firing rhythms (Prosser & Bergeron 2003). In vivo, systemic leptin injections result in modest induction of Per expression in the SCN and potentiate the phase-shifting effects of light in female, but not in male mice (Mendoza et al. 2011). Ob/ob mice (carrying a loss-of-function mutation of the Lep gene) show altered photic responses of the circadian clock at both behavioral and molecular levels, which can be normalized by timed injections of leptin. In the same study, however, the authors found that systemic leptin injections failed to stimulate canonical leptin signaling cascades in the SCN, suggesting an indirect mechanism for leptin’s circadian resetting effects (Grosbellet et al. 2015b). Db/ib mice (expressing a nonfunctional version of the long isoform of leptin receptor gene, Obrb) show severely disrupted locomotor activity and feeding rhythms together with exaggerated molecular photic responses in the SCN (Grosbellet et al. 2015a). Similar to leptin, ghrelin has been shown to directly impact on the SCN. In vitro, treatment of ghrelin phase resets the circadian neuronal firing and Per expression rhythms in organotypic cultured SCN-containing slices (Yannielli et al. 2007). Likewise, application of a synthetic ghrelin analog in in vivo phase resets circadian locomotor activity rhythms (Yannielli et al. 2007), but attenuates light-induced phase-shifts, which may involve inhibitory innervations from ghrelin-sensitive neurons in the ARC to the SCN (Yi et al. 2008). A recent study has discovered an unexpected role of ghrelin as a circadian regulator of the sleep/wake cycle. Ghrelin-positive neurons, receiving projections from the SCN and the visual system via the intergeniculate leaflet (IGL), suppress the activity of arousal-promoting orexigenic neurons in the lateral hypothalamus (LH) (Horvath et al. 2012).

Anticipatory behavioral activity before scheduled feeding (food anticipatory activity, FAA) is seen in animals with time-restricted access to food (RF) and is characterized by increases in locomotor activity, body temperature, and GC secretion just before feeding time (Krieger et al. 1977). It is believed to reflect foraging in anticipation of food intake. FAA rhythms represent a form of food entrainment of the circadian system as they develop in the absence of other time cues (such as the light–darkness cycle) and persist even when the scheduled feeding condition is lifted (i.e. they free-run) (Milstiber 2009). Although the anatomical and molecular makeup of this food-entrainable...
oscillator (FEO) underlying FAA rhythms is still largely unknown, both leptin and ghrelin have been shown to play a role in regulating FAA rhythms. ob/ob mice and Obrb-mutant Zucker rats show increased FAA under RF, which can be suppressed by the administration of recombinant leptin (Mistlberger & Marchant 1999, Ribeiro et al. 2011). In contrast, ghrelin receptor (Ghsr)-deficient mice show impaired FAA (Blum et al. 2009, LeSauter et al. 2009, Lamont et al. 2014); though intriguingly, ghrelin-deficient mice are normal under these conditions (Szentirmai et al. 2010). The mechanisms that mediate leptin and ghrelin effects on FAA remain unknown. Two neural substrates have been identified to modify FAA rhythms, which are also established targets of leptin and ghrelin: the dopaminergic reward circuitry Gallardo et al. 2014 and the mediobasal hypothalamic appetite-regulating circuitries centers (Bechtold & Loudon 2013). The SCN itself has been recognized as a negative regulator of FAA rhythms (Storch & Weitz 2009, Acosta-Galvan et al. 2011). Given that both of these two hormones can directly act on the SCN clock, it is plausible that the circadian pacemaker may mediate part of their FAA-regulating effects. Alternatively, it has been shown that the DMH is a positive regulator of FAA through suppression of SCN neuronal activity (Acosta-Galvan et al. 2011). The DMH receives input from several metabolic state-sensing brain nuclei such as the ARC (Bouret et al. 2004), thus acting as a relay mediating the FAA effects of metabolic hormones.

Concluding remarks

The endocrine system serves as a major output of the circadian clock. Circadian disruption is a frequent consequence of modern urban lifestyles. Occupations requiring shift work or irregular working hours, chronic stress, mistimed light exposure, e.g. through artificial lighting or the use of light-emitting electronic devices (Chang et al. 2015), and the frequent intake of arousal stimulating beverages such as coffee at night (Burke et al. 2015) are all established disruptors of the circadian system. Altered circadian hormonal regulation compromises one’s health such as by promoting the development of metabolic disorders. At the same time, many hormones have been shown to feed back on the circadian clock system at various levels, thus supporting the maintenance of robust physiological and behavioral rhythms. This central role of endocrine signals within the circadian system makes them attractive targets to manipulate physiological functions via the circadian clock, e.g. to affect circadian stabilization during jetlag (Kriessling et al. 2010) or shift work. The recent discovery of drugs directly impinging on molecular clock function (Hirota et al. 2010, Solt et al. 2012), on the other hand, offers the possibility to ameliorate disrupted endocrine regulation under desynchrony conditions through the circadian clock. Finally, reinstating behavioral and physiological rhythms, e.g. by restricting food intake to the active phase, may protect against metabolic dysregulation (Chaix et al. 2014, Gill & Panda 2015), providing a highly accessible mean for improving the general well-being.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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