Rev-erbα: a potential target for the treatment of circadian disorders

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Abstract

Circadian variations are observed in many physiological processes, and alterations in these oscillations are closely linked to mood disorders, metabolic abnormalities and cardiovascular complications. The nuclear receptor Rev-erbα is a crucial component of the clock mechanism. It is an important metabolic regulator and a target for lithium, a drug used in the treatment of bipolar disorders. The recent identification of natural (heme) and synthetic ligands for Rev-erbα suggests that pharmacological modulation of Rev-erbα activity may be a potential therapeutic option in the treatment of circadian disorders including circadian-related metabolic and cardiovascular diseases.
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Many physiological processes follow daily variations, amongst which alternation between rest and activity, blood pressure, body temperature, feeding behavior, and carbohydrate and lipid metabolism are a few examples. These variations are orchestrated by an internal biological clock that relies on interlocked transcriptional regulation, starting, in mammals, by the transcriptional activation of the *per* and *cry* genes by the heterodimer, CLOCK–Bmal1. In turn, the Per and Cry proteins repress CLOCK–Bmal1-activated transactivation, thereby allowing a new cycle to start. This loop allows rhythmic expression of the core clock genes [1]. In addition, CLOCK–Bmal1 activates the transcription of the nuclear receptor Rev-erbα, resulting in daily fluctuations of its expression which, in turn, results in the repression of Bmal1. This additional loop is believed to improve the robustness of the clock. Additional post-transcriptional steps, protein phosphorylation, sumoylation, and acetylation, participate in the fine adjustment of the circadian period to 24 h.

Circadian rhythms are driven by a ''master'' hypothalamic clock residing in the suprachiasmatic nucleus, which aligns our circadian behavior to the day/night cycle and seasonal variations in light intensity. In addition, autonomous, self-sustained oscillators exist in all peripheral tissues and are synchronized by the master clock via neuroendocrine signals. This arrangement allows resonance between the different clocks throughout the body. Nevertheless, other time cues – such as the time of availability of food – can entrain peripheral pacemakers – such as the one in the liver – independently of the master clock [2]. These peripheral oscillators are important to the timely tuning of local circadian physiology. In the liver, for instance, up to 20% of the transcripts cycle, including those encoding enzymes and transporters involved in the pathways of synthesis of fatty acids, cholesterol, and bile acids, among many others [3]. Interestingly, nuclear receptors that are involved in the regulatory control of numerous key steps of biological processes, such as lipid and glucose

metabolism, display circadian variations in their expression, and thus are believed to entrain circadian variations in the expression of their target genes [4]. It is therefore not surprising that circadian misalignment between our internal timing system and the environment, for instance as a result of jet-lag or shift work, results in metabolic disorders. Shift work is associated with increased prevalence of obesity, dyslipidemia, altered glucose concentrations, and cardiovascular events [5]. Sleep cycles are equally important for the regulation of metabolism, and numerous data have revealed that sleep disorders are intricately linked to features of the metabolic syndrome [6].

Recent studies have revealed a direct link between the molecular clock and the regulation of metabolic pathways. Turek et al [7] have shown that *clock*$^{\Delta 19}$ mutant mice display an altered circadian feeding pattern, are hyperphagic, become obese, and develop hyperlipidemia and hyperglycemia in response to being fed a high-fat diet. Furthermore, *Bmal1*$^{-/-}$ mice display altered circadian oscillations in plasma glucose concentration, in addition to glucose intolerance [8], and Bmal1 seems to participate in the control of adipogenesis [9].

Emerging evidence obtained in humans demonstrates that several *clock* polymorphisms are associated with body weight and increased susceptibility to obesity [10,11]. Bmal1 has also been associated with type 2 (non insulin-dependent) diabetes and hypertension in humans [12]. These data demonstrate a direct link between clock genes and metabolic abnormalities recapitulating the metabolic syndrome.

Rev-erbα is a nuclear receptor that acts as a constitutive transcriptional repressor by recruiting the nuclear receptor co-repressor (NCoR) and histone deacetylase 3 (HDAC3) [13]. Rev-erbα is an important clock component, and Rev-erbα-deficient mice exhibit a 0.5 h shorter circadian period and are more sensitive to phase shifts induced by light pulses than are wildtype controls. In vitro, stability of the Rev-erbα protein is important for synchronizing and maintaining circadian rhythms after a serum shock [14]. Besides its role in the molecular clock system, Rev-erbα exerts an important role in metabolic functions: it regulates lipid metabolism, and Rev-erbα$^{-/-}$ mice display increased plasma concentrations of triglyceride and very-low-density lipoproteins, in addition to increased liver expression of apolipoprotein C-III [15]. In addition, Rev-erbα$^{-/-}$ mice exhibit altered bile acid synthesis, and diurnal variations in the expression of genes involved in bile acid synthesis and regulation are either phase-shifted or dampened, indicating that Rev-erbα links bile acid physiology to the circadian clock [16]. Rev-erbα plays also a modulatory role in the regulation of adipogenesis [17,18]. It may therefore act as a gatekeeper between the molecular clock and metabolic regulatory networks.

Although Rev-erbα has long been considered as an orphan receptor, recent reports have demonstrated that the Rev-erbα–NCoR interaction is increased after heme ligand binding by Rev-erbα [19,20]. Moreover, Meng et al [21] have recently reported a novel synthetic Rev-erbα ligand that enhances Rev-erbα repressive transcriptional activity. Interestingly, this ligand can influence circadian rhythms, inducing phase advance or delay in cultured fibroblasts. Rev-erbα is therefore becoming an attractive target for the manipulation of the biological clock, and it is tempting to speculate that modifying Rev-erbα activity to gate the physiological response to the correct circadian time is a promising strategy in the treatment of metabolic and cardiovascular abnormalities secondary to circadian disorders.

Accumulating evidence supports a role of the endogenous circadian clock in the pathogenesis of mood disorders. Indeed, shift workers are at risk of developing, not only features of the metabolic syndrome, but also anxiety and emotional problems, and are frequent users of tranquilizer drugs [22]. Bipolar disorder affects about 1–3% of the general population and is characterized by alternation between manic and depressive episodes; it is usually more prevalent during the winter season when the light period is reduced. During manic episodes, affected individuals show major disturbances in circadian processes such as sleep, activity, and hormonal secretions [23]. Very recently, disturbances of circadian rhythms have also come to be understood as being at the basis of depression. Difficulty in falling asleep, early awakenings, shifts in the cycle of body temperature and endocrine activities have been observed in depressed patients and thought to be clinical manifestations of a strong disruption of circadian rhythms [24]. Acting on MT1, MT2, and 5HT2C receptors in the suprachiasmatic nucleus, agomelatine, a drug licensed for the treatment of depression in Europe in 2009, resets the internal biological clock and restores circadian rhythms, thus leading to a strong clinical efficacy on depressive symptoms [25,26]. Treatments currently successful in preventing or attenuating these episodes rely on a stable pattern of sleep and activity along with pharmacological treatment, whereas disruption of these rhythms worsens the manic episodes [27].

Studies in human populations have identified several polymorphisms in clock genes that are associated with mood disorders. Although contrasting findings have been reported concerning a potential association between polymorphisms in the *Rev-erbα* gene and bipolar disorder [28,29], a polymorphism has been found in the 3′ flanking region of *clock* that associates with an increased number of manic episodes, insomnia, early waking time, and decreased need for sleep [30]. Interestingly, Roybal et al [31] have reported a

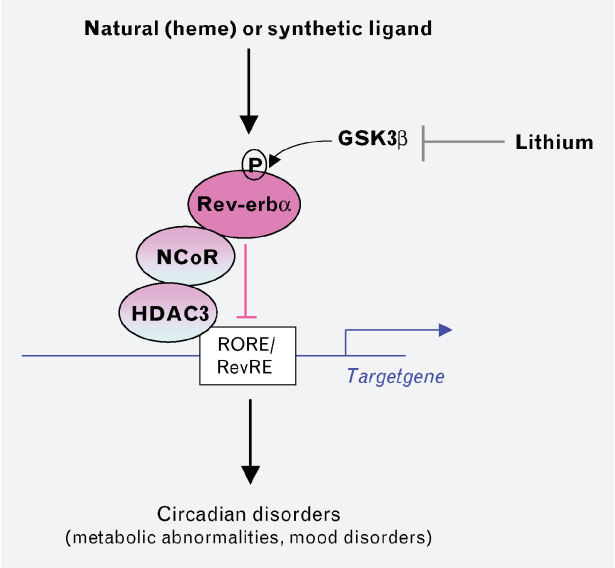

Figure 1. Involvement of the nuclear receptor, Rev-erbα, in various circadian disorders. GSK3β, glycogen synthase 3β; HDAC3, histone deacetylase 3; NCoR, nuclear receptor co-repressor; RORE/RevRE, Rev-erbα response element.

remarkably similar overall human mania-like behavior in *clock*$^{\Delta 19}$ mutant mice, (hyperactivity and depression-like behavior) [31], a phenotype normalized by chronic administration of therapeutic doses of lithium. Lithium is a commonly prescribed mood-stabilizing drug, lengthening the circadian period in rodents and humans, and this effect may be important for its therapeutic efficiency. Lithium targets glycogen synthase kinase 3β (GSK3β), a central regulator of the circadian clock. Interestingly, inhibition of GSK3β with lithium leads to rapid proteasomal degradation of Rev-erbα, and activation of Bmal1 [14]. This effect was abrogated in cells overexpressing a mutant form of Rev-erbα that is resistant to lithium-induced degradation. This indicates that lithium targets the clock molecular machinery through the stabilization of Rev-erbα.

The involvement of Rev-erbα in the various aspects of circadian disorders discussed above is displayed schematically in *Figure 1*

Plasminogen activator inhibitor type 1 (PAI-1)* plays a major regulatory role in the fibrinolytic cascade and promotes the development of atherothrombosis. Interestingly, PAI-1 plasma and tissue mRNA levels display circadian oscillations with a peak in the early hours, a time when the incidence of cardiovascular diseases such as myocardial infarction, and high blood pressure occur at higher frequency. Rev-erbα has been shown to repress PAI-1 gene expression, and this repressive effect is further enhanced by GSK3β-mediated phosphorylation and stabilization of Rev-erbα and blunted by lithium administration [32]. Altogether these data identify Rev-erbα as a major determinant of the circadian PAI-1 expression and a potential modulatory role of the morning susceptibility to myocardial infarction.

Conclusion

Rev-erbα is a gateway aligning the circadian system and physiology and, as such, it represents a potentially useful target for the treatment of aberrant circadian-related diseases.

*See glossary for definition of these terms. ■

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