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Review Article

Circadian redox rhythms in the regulation of neuronal excitability

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ABSTRACT

Oxidation-reduction reactions are essential to life as the core mechanisms of energy transfer. A large body of evidence in recent years presents an extensive and complex network of interactions between the circadian and cellular redox systems. Recent advances show that cellular redox state undergoes a ~24-h (circadian) oscillation in most tissues and is conserved across the domains of life. In nucleated cells, the metabolic oscillation is dependent upon the circadian transcription-translation machinery and, vice versa, redox-active proteins and cofactors feed back into the molecular oscillator. In the suprachiasmatic nucleus (SCN), a hypothalamic region of the brain specialized for circadian timekeeping, redox oscillation was found to modulate neuronal membrane excitability. The SCN redox environment is relatively reduced in daytime when neuronal activity is highest and relatively oxidized in nighttime when activity is at its lowest. There is evidence that the redox environment directly modulates SCN K^+ channels, tightly coupling metabolic rhythms to neuronal activity. Application of reducing or oxidizing agents produces rapid changes in membrane excitability in a time-of-day-dependent manner. We propose that this reciprocal interaction may not be unique to the SCN. In this review, we consider the evidence for circadian redox oscillation and its interdependencies with established circadian timekeeping mechanisms. Furthermore, we will investigate the effects of redox on ion-channel gating dynamics and membrane excitability. The susceptibility of many different ion channels to modulation by changes in the redox environment suggests that circadian redox rhythms may play a role in the regulation of all excitable cells.

1. Introduction

The suprachiasmatic nucleus (SCN) of the hypothalamus is the master regulator of the circadian rhythms in mammals. It generates the daily rhythms of behavior, metabolism, and other important physiological processes. The main driver of the circadian clock is a transcription-translation feedback loop of core circadian genes. However, emerging evidence suggest that metabolic oscillators also play a crucial role in the generation of circadian rhythms. Circadian rhythms in cycles of oxidation and reduction have been reported in a broad array of mammalian tissues and cell types and are conserved across the domains of life [1]. The discovery of a near-24-h oscillation of redox state in the SCN also revealed that cellular metabolic state could modulate neuronal excitability, an integral component of SCN timekeeping, via modification of redox-sensitive K^+ channels [2]. These studies demonstrate that redox homeostasis is dynamic, displays circadian characteristics, and may play a role in the regulation of daily rhythms of electrically excitable cells.

Since the pioneering work of Hodgkin and Huxley [3], the scientific community has developed deep insights into neuronal membrane dynamics. Neuronal excitability is linked directly to ion channel activity. A change in permeability of ions across the plasma membrane can lead to significant changes in resting membrane potential. The electrical properties of neurons and other excitable cells rely on many different

Abbreviations: AA, ascorbic acid; BK, large conductance Ca^{2+}- and voltage-activated K^+ channel; ChT, chloramine-T; CK1, casein kinase 1; CNS, central nervous system; CO, carbon monoxide; CRY, cryptochrome; DHA, dehydroascorbic acid; DRG, dorsal root ganglion; DTNB, 5,5-dithio-bis-(2-nitrobenzoic acid); DTT, dithiothreitol; ER, endoplasmic reticulum; FAD, flavin adenine dinucleotide; GPx, glutathione peroxidase; GSH, glutathione; GSSG, glutathione disulfide; Kv, voltage-gated potassium channel; HVA, high-voltage-activated; IP_3, inositol triphosphate; LVA, low-voltage-activated; MetO, methionine sulfoxide; MsrA, methionine sulfoxide reductase; NADH, nicotinamide adenine dinucleotide; NADPH, nicotinamide adenine dinucleotide phosphate; NAMPT, nicotinamide phosphoribosyltransferase; NO, nitric oxide; NOX, NADPH oxidase; NPAS2, neuronal PAS domain protein 2; PER, period; PPP, pentose phosphate pathway; Prx, peroxiredoxin; ROR, RAR-related orphan receptor; ROS, reactive oxygen species; RyR, ryanodine receptor; SCN, suprachiasmatic nucleus; SIRT1, sirtuin 1; SIRT3, sirtuin 3; Trx, thioredoxin; VIP, vasoactive intestinal peptide; V_m, membrane potential

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types of voltage-gated, ligand-gated, and leak channels that are permeable to ions such as Na^+, K^+, Cl^-, and Ca^{2+}. Membrane potential (V_m) is determined by the differential distribution of these ions. Ion channels are regulated by a vast number of ligands, post-translational modifications, and other mechanisms. They are susceptible to modulation by phosphorylation, second messengers, gaseous signaling molecules such as carbon monoxide (CO) and nitric oxide (NO), and by the redox environment [2,4–7].

Cellular redox encompasses the dynamic regulation of reactive oxygen species (ROS), antioxidants, and redox-sensitive metabolic cofactors. ROS have been viewed historically as toxic. There are many studies regarding their detrimental effects to the body and contributions to disease and aging. However, there is also increasing evidence that ROS signaling is integral to a multitude of normal cellular processes and signaling pathways [8–10]. ROS generation and redox homeostasis are no longer only relevant as sources of oxidative stress. Mounting evidence in the past few years shows circadian rhythms in redox state are an intrinsic and dynamic feature of all cell types and may contribute to daily regulatory processes.

Intrinsic circadian oscillations of signaling molecules exist in the SCN and hippocampus, opening windows of excitability and susceptibility. Cyclic changes that gate activity have been termed "iterative metaplasticity" to describe states permissive for plasticity mechanisms that are expressed as daily cycles [11]. In nucleated cells, the molecular circadian clock is reciprocally connected to the redox system. These interactions produce daily rhythms in redox state which can then modulate neuronal activity via regulation of ion channels [2]. Thus, day/night differences in redox state may play a role in the generation of daily changes in brain states that underlie the potential to establish long-lasting changes in brain function that we know as memory. Indeed, changes in the redox state have been found to modulate cognitive decline [12,13].

In this review, we examine fundamental features of circadian rhythms, the role of the SCN as master circadian clock, and explore the reciprocal connections between circadian timekeeping and the cellular redox oscillation, including the redox modulation of neuronal excitability. Then, we consider the many reports of redox modification of ion channel activity. We propose that circadian regulation of neuronal excitability via redox-sensitive ion channels may not be unique to the SCN.

2. Circadian rhythms

The 24-h cycle of day and night generated by the Earth's rotation has accompanied and driven the evolution of most organisms. As a result, myriad life forms, from some prokaryotes to all eukaryotes, have developed intrinsic daily rhythms in cellular processes, behavior, and metabolism. The self-sustained circadian oscillation provides an evolutionary advantage as it allows organisms to coordinate their internal states and anticipate changes in the timing and duration between night and day so that cellular, physiological, and behavioral events occur at appropriate times. Misalignment of the internal clock with the external environment can disrupt these functions and lead to disease [14–17].

3. The mammalian suprachiasmatic nucleus as master clock

The mammalian circadian system is organized hierarchically into a master oscillator and secondary oscillators in the brain and body. The suprachiasmatic nucleus (SCN) of the hypothalamus is the master circadian clock in mammals that synchronizes peripheral clocks in other brain regions and organ systems [18–20]. The SCN is a pair of small nuclei on either side of the third ventricle, directly above the optic chiasm in the anterior hypothalamus (Fig. 1) [21]. Each nucleus is composed of approximately 10,000 tightly compacted cells whose collective activity is considered to be the central “pacemaker” of circadian rhythm [22]. Destruction of the SCN results in the loss of daily rhythms in sleep-wake cycle, body temperature, locomotor activity, drinking, and endocrine release [23,24].

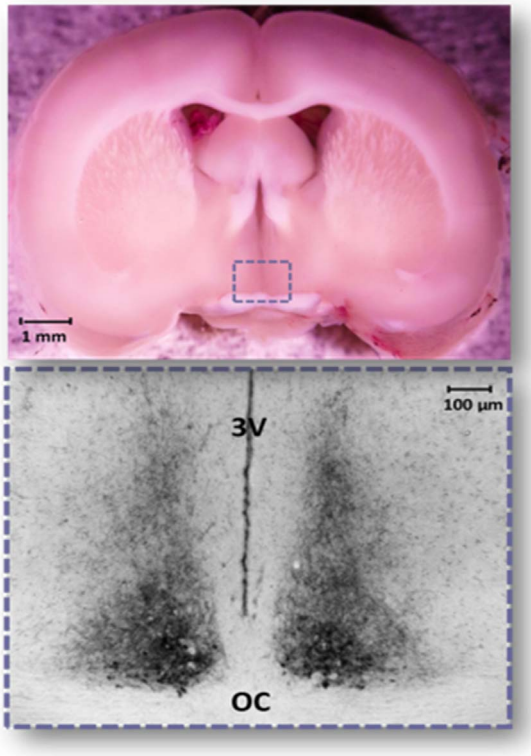

Fig. 1. The mammalian circadian clock is located in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus. *Upper:* In this fresh coronal section of the brain of a rat, the SCN lies near the base (within the dashed box). *Lower:* Vasoactive intestinal peptide (VIP) immunoreactive staining shows the pair of small hypothalamic nuclei is positioned medially, on either side of the third ventricle (3 V), and directly superior to the heavily myelinated optic chiasm (OC).

3.1. Oscillation of clock genes and proteins in the SCN

Circadian rhythms are generated by a transcription-translation feedback loop of clock genes and proteins that form an oscillatory molecular clock [25]. This core molecular clock consists of a heterodimeric complex of protein products of the genes *CLOCK* and *BMAL1,* which positively regulate the expression of *Period* (*Per1, 2, and 3*) and *Cryptochrome* (*Cry1 and 2*) genes. The accumulated protein products form their own heterodimeric transcriptional repressor complex of PER and CRY that represses the activity of CLOCK and BMAL1. The transcriptional-translational loop of these core clock genes repeats with a period of approximately 24 h and is the basis for the mammalian molecular clock.

An additional interlocking feedback loop involves the BMAL1/CLOCK-mediated transcription of nuclear receptor genes, *Rev-Erbα/β* and *RAR-related orphan receptor alpha (Rorα*). To complete the circadian loop, REV-ERB and ROR proteins then compete for binding sites within the promoters of *Bmal1* and *CLOCK,* where REV-ERB inhibits transcription and ROR initiates transcription. Additionally, casein kinase 1 (CK1)-mediated phosphorylation contributes to timekeeping through the destabilization of PER proteins [26]. Post-translational modifiers like protein kinases and small molecule messengers such as cAMP and Ca^{2+} play important roles to determine and modify the intrinsic circadian rhythm [27–32]. Together, these modifiers work to synchronize firing rate, gene expression, and secretion across the SCN.