Ruled by the Clock

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Circadian Rhythms Govern Cardiac Repolarization and Arrhythmogenesis
Jeyaraj et al

The ability of organisms to respond to time allows for anticipation of cyclic changes in the environment that provide a survival advantage. The time variance of environmental influences on an organism may occur over years, months, or days. Biological clocks are ubiquitous and hierarchical in mammals and provide cell autonomous, transcriptionally mediated mechanism(s) to regulate function over several time scales.1 The master clock in mammals is in the suprachiasmatic nucleus (SCN) of the hypothalamus, which is regulated by external cues (zeitgebers), prominently light. Circadian rhythmicity is maintained by endogenous 24-hour cycle clocks that can be regulated by external signals to produce diurnal behaviors. Circadian clocks are characteristic of most mammalian tissues and are coupled to produce complex diurnal behaviors.2

The main molecular components of mammalian biological clocks include a series of transcription factors that regulate the expression of clock-controlled genes (CCG) in a negative feedback fashion. CLOCK and BMAL1 heterodimerize and bind to an E-box motif, activating transcription of the period (PER1, 2, 3) and cryptochrome (CRY1, 2) proteins. As period proteins accumulate, they complex with cryptochromes and translocate to the nucleus, suppressing CLOCK/BMAL1 mediated transcription. Casein kinase 1 (CK1ε) phosphorylates period proteins targeting them for proteasomal degradation, derepressing CLOCK/BMAL1-mediated transcription. These core components of the clock are regulated by a number of other CCGs including arginine vasoressin and other transcription factors such as D-element–binding proteins, hepatic leukemia factor, and thyrotrophic embryonic factor. The central clock in the SCN regulates autonomic nervous system function, levels of humoral factors, and peripheral clocks to produce circadian variation in physiological function.1 Peripheral clocks exist in virtually every tissue in the body and are believed to have similar molecular mechanisms.

Cardiovascular physiology and the clinical presentation of heart and vascular disease exhibit diurnal variation. Heart rate, blood pressure, vascular reactivity, inflammation, metabolism, and thrombogenicity change in a diurnal fashion. Neurohumoral regulators of the cardiovascular system that change over the course of a day may be particularly relevant to complex phenotypes such as myocardial infarction and sudden cardiac death (SCD). In rodents, genes that encode repolarizing K currents, specifically the transient outward (I_out, Kv4.2) and ultrarapid (I.ur, Kv1.5) currents exhibit circadian variation in transcript levels.9

Figure. Peripheral clocks in the heart are under the control of circadian oscillators in the suprachiasmatic nucleus (SCN) of the hypothalamus. Circadian variation of expression of Klf15 drives the diurnal variation of expression of the potassium channel subunit Kcnip2, which alters the expression of the major repolarizing current in the rodent ventricle, Ito. At the extremes of expression, the action potential duration (APD) and QTc are either increased or decrease resulting in heightened susceptibility to lethal ventricular arrhythmias and sudden cardiac death (SCD). (Illustration: Cosmocyte/Ben Smith).
In the report by Jeyaraj and coworkers, a link between endogenous myocyte circadian rhythms and arrhythmias is described. These investigators serendipitously discovered that the Krüppel-like factor 15 (Klf15) exhibits circadian variation in expression in the mouse heart. Microarray expression data in Klf15+/− hearts suggested that the Ito,s subunit KChIP2 (encoded by Kcnd2) was a transcriptional target of Klf15. KLF15 is a member of the family of Krüppel-like factors, which are zinc-finger–containing transcription factors. KLF15 is highly expressed in liver, kidney, heart, and skeletal muscle and is an important component of stress induced remodeling in the heart, inhibiting cardiac fibrosis and myocyte hypertrophy.

The promoter region of Klf15 contains 4 E-box regions that are consensual binding sites for the essential transcription factors (CLOCK and BMAL1 also known as ARNTL) in the circadian clock. Chromatin immunoprecipitation assays demonstrated circadian variation in BMAL1 binding to the Klf15 promoter in wild-type mice. Interestingly, mice that are null for expression of any one of a number of circadian genes such as Bmal1-Per2- and Cry1-null hearts have disrupted expression of Klf15. To explore the endogenous cyclic behavior of the basis of cardiac repolarization, QTc intervals were tracked in mice placed in the dark and K current transcript levels were measured. Endogenous circadian variation was noted in the QTc, Kv4.2 (Kcnd2) and KChIP2 but not Kv1.5 or Kir2.1. Klf15-null mice exhibited no rhythmic variation in KChIP2; moreover, Kv4.2 expression was suppressed and shifted in time. Transgenic expression of Klf15 (Klf15-Tg) driven by an attenuated α-MHC promoter restored KChIP2 expression. The KChIP2 promoter was noted to have numerous consensus Krüppel-binding sites [C(A/T)CCC] and the activity of KChIP2-luc reporter was increased by full length KLF15 but not by a mutant KLF15 with an absent Zn-finger DNA binding domain. Chromatin immunoprecipitation of flag-KLF15 from Klf15/Tg hearts revealed increased KLF15 binding to the endogenous KChIP2 promoter both findings consistent with KChIP2 being the target of Klf15.

Interestingly, increased or decreased expression of KLF15 did not alter the oscillatory expression of several components of the core clock machinery (eg, Bmal1, Per2, and Cry1); the endogenous clock is using KLF15 to regulate KChIP2 expression. In contrast, a change in expression of Klf15 did alter ventricular repolarization and the diurnal variation of this electrophysiological metric (Figure). In Klf15-null mice, the QTc was prolonged and its diurnal variation was attenuated. Overexpression of Klf15 shortened the QTc and was associated with J-point elevation on the ECG and eliminated diurnal variation in repolarization. Consistent with the changes in the QTc, Klf15-null myocytes exhibited a marked reduction in fast component (Kv4.2 and KChIP2 driven) (Ito,s) but not slow component (Ito,o) or steady-state components (Ito) of the Ito current and increase in the action potential duration (APD). The Klf15-Tg ventricular myocytes had an augmented Ito,s, Ito,o, and Ito and a shorter APD. Based on the change in the AP morphology Ito,s appears to be central to APD shortening. Extremes of repolarization may lead to electric instability and indeed excessive shortening of repolarization may be more lethal than prolongation. The arrhythmic phenotypes of the variant mouse strains are consistent with this speculation. There were no spontaneous arrhythmias in Klf15-null or wild-type, but polymorphic ventricular arrhythmias were more easily induced in the null mice. In comparison, Klf15-Tg mice had a more malignant phenotype with spontaneous arrhythmias and early mortality by 4 months of age. The findings do not appear to be the result of structural changes in the heart induced by Klf15 underexpression or overexpression; Klf15-null mice exhibit no reduction in left ventricular function, fibrosis or apoptosis in the basal state. These data emphasize the importance of divergent molecular mechanisms that control the expression of proteins that exhibit circadian cycling. The changes in expression are driven hierarchically by the core clock components, but specific downstream factors such as Klf15 have distinct targets, allowing for diversification of circadian and diurnal signaling.

The circadian changes in cardiac electrophysiology add to the growing knowledge base about diurnal variation in heart and vascular function that underlie the variation in cardiovascular physiology and help to explain the peaks in major cardiac events such as myocardial infarction and sudden death. Moreover, uncoupling of physiological functions that occur over the course of the day may be a primary driver of time dependent changes in susceptibility to adverse cardiac events. Specifically in the heart, circadian rhythmicity influences excitability, metabolism, response to stress, and in this study ventricular repolarization. Underlying structural heart disease may influence one or more of the circadian cycles, perhaps exaggerating the uncoupling and in the context of basal changes in function create a highly vulnerable, time-varying substrate for acute events. Although caution about extrapolation of these data to larger mammals is warranted, it is likely that circadian clock driven mechanisms underlie the diurnal susceptibility to major adverse cardiac events such as SCD in humans. The question of whether a link between variations ion channel expression, alterations in ventricular repolarization and susceptibility to lethal ventricular arrhythmias underlie the diurnal variation in the occurrence of SCD remains open. Although changes Ito is not apt to produce much change in ventricular APD in humans, it is quite possible that diurnal changes in a single or few ion channel genes could alter spatial gradients in repolarization that could contribute to the production of potentially lethal arrhythmias. It is interesting to speculate whether regional alterations in the circadian clock exist in the heart with the potential to exaggerated the heterogeneity of the electric property such as repolarization rendering the heart highly susceptible. The underlying susceptibility may be increased by the presence of structural heart disease, but in many instances a diurnal variation of events is retained. The contributions of other time varying properties of the heart and vasculature are likely to contribute to the heightened susceptibility to SCD in the early morning hours. The work by Jeyaraj and coworkers highlight that regardless of the drivers, circadian variation in mediators or modulators of cardiac electric properties account for at least part of the diurnal variation in SCD.
Disclosures
None.

References
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