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REVIEW

Circadian rhythm and cardiovascular disorders

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Abstract: Circadian rhythmicity affects all living organisms on earth. Central and peripheral cellular clocks have the ability to oscillate and be entrained to environmental cues, thus allowing organisms to anticipate and synchronize their physiologic processes and behavior to recurring daily environmental alterations. Disruption of the circadian rhythm in modern life, such as by shift work and jet travel, leads to dyssynchrony of the central and peripheral clocks, and is an independent risk factor for cardiovascular disease and the metabolic syndrome. Aging has also been associated with attenuated cellular rhythmicity. Here we summarize the clinical observations linking cardiovascular health to circadian rhythm. In addition, we discuss recent advances in experimental models for understanding the clock machinery in terms of a variety of physiologic processes within the cardiovascular system. Together, these studies build the foundation for applying our knowledge of circadian biology to the development of novel therapy for cardiovascular disorders.

Keywords: circadian rhythm, diurnal variation, cardiovascular

Introduction

Cardiovascular disease is the leading cause of morbidity and mortality in the USA, affecting more than one in three US adults and resulting in enormous health care expenditure, which was $444 billion in 2010.[1] Given the huge impact of cardiovascular disease, intensive effort is being made to understand the basic mechanisms governing cardiovascular function in health and disease. One area of investigation that has gained momentum in recent years is the influence of circadian rhythms on cardiovascular biology.

Circadian (from the Latin, circa diem, meaning "approximately daily") rhythm refers to any biological process that exhibits a 24-hour periodicity. Physiologic parameters of the cardiovascular system, such as heart rate, blood pressure (BP), vascular tone, and QT interval, show significant diurnal variation.[2–4] Pathologic states, such as arrhythmogenic sudden cardiac death (SCD), myocardial infarction (MI), aneurysmal rupture, and stroke, also show a daily rhythmic pattern, with peak susceptibility in the early morning hours.[5–8] In addition, disruption of the circadian rhythm either in the brain (central clock) or in the peripheral tissues (peripheral clock) leads to cardiovascular disease in both human and animal models.[9–19] In this review, we summarize our current understanding of the interplay between circadian regulation and cardiovascular disease, as well as future directions in development of therapy.

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Clinical observations linking circadian and cardiovascular biology

Blood pressure

BP is known to show diurnal variation, with a peak in the mid-morning and a trough at night, with a 10% variation in ambulating humans.[2] Diurnal BP variation results primarily from cyclic physical activity, including sleep–awake cycles, as demonstrated by studies in shift workers.[20] Endogenous factors, although influenced by activity, contribute to the diurnal variation of BP via circadian variation in the autonomic nervous system, arterial vascular tone, and humoral factors, including the renin angiotensin aldosterone axis, catecholamines, and cortisol. The heritability of circadian BP and heart rate variation may reflect the heritability of these endogenous factors.[21]

The association between this diurnal variation and predisposition to disease has been noted in both normotensive and hypertensive individuals. O'Brien et al were the first to observe the increased risk of stroke in nondippers.[22] Subsequent studies classified subjects into four groups according to their diurnal/nocturnal BP ratio (100× (mean diurnal BP − mean nocturnal BP)/mean diurnal BP): nondippers (ratio <10%), dippers (ratio 10%–20%), extreme dippers (ratio >20%), and inverse dippers or risers (ratio <0%, as mean nocturnal BP is higher than mean diurnal BP). Nondipping was associated with secondary hypertension and[23,24] endothelial dysfunction,[23,25] as well as a higher risk for cardiovascular events and adverse outcomes, including heart failure and MI,[26,27] stroke, left ventricular hypertrophy,[28] deterioration of kidney function,[29,30] and progression to end-stage renal disease.[31] Further, nondipping hypertensive individuals were shown to have a three-fold increase in adverse cardiovascular events than dipping hypertensives.[32] The Ohasama study of 1,542 Japanese people revealed a linear relationship between the nocturnal decline in BP and cardiovascular mortality. Each 5% decrease in the decline (nondipping) was associated with an approximately 20% increase in the risk for cardiovascular mortality.[33] The group with riser BP had the highest risk for both fatal and nonfatal stroke when compared with the other three groups.[34] Several mechanisms have been proposed to explain the higher night-time BP and associated worse outcome, ie, nocturnal autonomic dysfunction, disturbed baroreflex sensitivity, sleep apnea, abnormal sodium handling, and nocturnal volume overload.

The role of the morning surge in BP, however, is more controversial. A steeper surge of morning BP has been associated with an increase in intima media thickness,[35] increased inflammatory markers (such as a higher number of macrophages and T-lymphocytes, as well as more ubiquitin–proteasome, tumor necrosis factor-α, and nuclear factor-kappa B activity),[36] and higher stroke rates.[37] The largest study of relevance so far was reported by Li et al, who analyzed 5,645 individuals over a median follow-up of 11.4 years and concluded that people in the top 10% of the morning systolic surge are associated with increased all-cause mortality as well as adverse cardiovascular events.[38] However, given that the morning surge is defined as the difference between the lowest night-time BP or preawakening BP and the first morning BP, it is not surprising that a blunted "dipping" and a blunted morning surge (overall flat) may be closely linked. Indeed, Verdecchia and Hermida observed that a blunted morning surge was associated with an increased risk for cardiovascular events, but did not find an effect on total mortality.[39] More standardized definition and measurement is critical to reconcile these seemingly discrepant findings.

Arrhythmia/sudden cardiac death

Electrocardiographic measurements, such as P-wave duration and area, P-R interval, QRS duration, and corrected QT (QTc) interval all show circadian variation in healthy individuals.[3,4] Both atrial and ventricular tachyarrhythmias show a circadian clustering, with a peak in the early morning hours, as reviewed previously by Portaluppi et al.[40] The only exception seems to be vagally mediated atrial fibrillation, which tends to occur at night, with a second peak after lunch when vagal drive dominates.

The majority of SCDs are thought to result from coronary artery disease, whether fatal ventricular arrhythmia due to an acute plaque rupture or chronic ischemic cardiomyopathy.[41] Implantable cardiac defibrillators allow faithful extended recording of ventricular arrhythmias in the outpatient setting and have greatly improved our understanding of the circadian rhythmicity of cardiac arrhythmia and SCD. The primary early morning peak and secondary late afternoon peak has been confirmed by a number of investigators, including in a meta-analysis by Cohen et al, who reported a morning excess in the incidence of SCD based on analysis of 19,390 patients, with 30% of SCD occurring between 6 am and noon (relative risk 1.29, 95% confidence interval 1.26–1.32).[5] The early morning excess of SCD is also independent of whether ischemic heart disease is present,[42–44] suggesting mechanisms other than ischemia, eg, other endogenous circadian factors, such as metabolites, and intrinsic susceptibility of the myocardium.

Another interesting observation is patients with obstructive sleep apnea, who seem to have a marked nocturnal peak

for SCD, have the highest incidence between midnight and 6 am (relative risk 2.57, 95% confidence interval 1.87–3.52).[45] Obstructive sleep apnea is associated with increased nocturnal sympathetic tone, BP, autonomic dysfunction, and platelet aggregation, all of which increase the risk for adverse cardiac events. In addition, hypoxia can lead directly to cardiac ischemia and arrhythmia. Oxygenation can improve the electrical stability of the myocardium in animal models and alleviate arrhythmia in humans.[46,47] However, it is not known if the standard of care, ie, the nocturnal continuous positive airway pressure device, can restore normal sleep physiology and circadian rhythm in these individuals.

Patients with long QT (LQT) syndrome provide a unique opportunity to study the biology of the time-of-day dependency of arrhythmia. Stramba-Badiale et al were the first to demonstrate that QT prolongation at night is genotype-dependent, with only LQT3 patients showing significant lengthening of QTc at night, which is consistent with the clinical observation that night-time events are most frequent in LQT3 patients with mutations in *SCN5A*.[48] This was the first suggestion that the intrinsic circadian rhythm of a singular channel might contribute to the diurnal distribution of arrhythmic events.

Myocardial infarction

In 1985, Muller et al were the first to confirm the long suspected early morning clustering of MI, using creatine kinase to estimate the time of occlusion.[6] This finding was confirmed in subsequent studies, including a meta-analysis of 60,000 patients by Cohen et al[7] and a study of 45,218 patients from the National Cardiovascular Data Registry reported by Mogabgab et al.[49] Similar findings have been reported for unstable angina, stent thrombosis, and transient myocardial ischemia.[50,51] This circadian effect is abolished by a β-blocking agent or aspirin, suggesting involvement of both adrenergic activity and platelet aggregation.[6,52]

Despite the agreement regarding time of onset, circadian variation in the size of MI remains a focus of study and debate. Multiple groups have attempted to answer this challenging question in humans. Two retrospective studies and one prospective study have independently demonstrated that a larger infarct size, estimated by biomarker release, in patients with ST-elevation MI undergoing primary percutaneous coronary intervention is associated with onset of symptoms in the sleep-wake transition.[53–55] However, a recent prospective multicenter, multiethnic cohort study did not find a clear circadian dependence of infarct size either in the entire data set or in each of the three participating countries separately.[56] Unlike symptoms of ischemia, infarct size is influenced not only by the circadian biology of the patient, but also by the circadian biology of their health care providers, who are shift workers themselves and under different levels of stress in a time-of- day-dependent manner. This added complexity must be taken into consideration and may require larger cohorts and better designed controls, both of which are extremely challenging in human studies.

Stroke/thromboembolism/ pulmonary embolism

It has long been recognized that, like MI, arrhythmia, and SCD, ischemic cerebrovascular events have a peak incidence in the early morning with a secondary peak in the afternoon. A large multicenter observational study reported the most frequent time of onset of stroke to be between 8 am and 11 am.[7] This observation suggests a common mechanism that likely involves circadian rhythmicity of hemodynamic (BP, vascular tone, autonomic function), hemostatic (platelet aggregation), and fibrinolytic (thrombogenic versus thrombolytic) factors. Interestingly, although occurring via a different mechanism, hemorrhagic cerebrovascular events also follow the same trend, according to multiple studies.[57–61] Increased sympathetic tone and an arterial BP surge in the morning have been considered to be relevant driving forces.

Aneurysm rupture/dissection

Abdominal and thoracic aortic dissection and aneurysm rupture have been observed to have a time-of-day-dependent incidence, with a peak in the 6 am to noon window. It was first reported in two retrospective Italian studies[62,63] of acute rupture of thoracic aortic aneurysm and abdominal aortic aneurysm, respectively, which identified a primary peak at 10 am and a secondary peak at 8 pm. Subsequent studies worldwide have come to the same conclusion;[8,64–67] in particular, a large study by Mehta et al[8] evaluated 957 patients enrolled in the International Registry of Acute Aortic Dissection (IRAD) between 1996 and 2000 and found a significantly higher frequency of acute aortic dissection between 6 am and noon in the entire data set as well as in an analysis of subgroups according to age, sex, type of dissection, and BP. This interesting observation suggests that the underlying mechanism is independent of these factors, including BP, which was thought to be the driving factor for the diurnal variation of adverse cardiovascular events. The only subgroup that did not show a significant circadian variation was patients with diabetes, and this has been observed similarly in MI.

Diabetes/autonomic dysfunction

Diabetic patients are at very high risk for adverse cardiovascular events, but fail to show normal circadian fluctuations in the occurrence of MI[68–70] or acute aortic dissection.[8] The reduction in diurnal heart rate and variation in QT interval associated with increased autonomic dysfunction in patients with diabetes has been known for 30 years.[71,72] Blunted nocturnal BP "dipping" is also prevalent in diabetic patients.[73–75] Disruption of circadian rhythm may explain the change in peak incidence of cardiovascular events in these patients. In addition, loss of normal circadian rhythm may result in a continuous vulnerability to adverse events and explain the excess disease burden in these patients.

Shift work

Shift work is common in many occupations, including the health care professions. Recent epidemiologic studies in the US and in Europe show that 15%–20% of employees are shift workers. Shift work leads to dyssynchronization of the central (brain) and peripheral clocks (eg, liver and heart; please refer to the next section for a detailed discussion on these). It has also been associated with behavioral risk factors, including smoking and poor dietary habits. Further, increased stress leads to neurohormonal changes that may adversely affect BP, lipid profile, and systemic metabolism. It has long been known that there is an increased risk of coronary artery disease amongst shift workers, and more recently diabetes and metabolic syndrome, which are direct risk factors for coronary artery disease.[9,10,76,77] Other risk factors such as hypertension and dyslipidemia have also been associated with shift work. Knutsson et al reported a significantly increased risk of ischemic heart disease in 504 paper mill shift workers when compared with day workers and a dose response to the duration of shift work (relative risk 2.2 for 11–15 years; relative risk 2.8 for 16–20 years). It was generally acknowledged, including in several meta-analyses, that shift work increases the risk of cardiovascular disease by about 40%.[9,78,79] However, more recent studies have shown mixed results, including a meta-analysis by Frost et al which did not find an increased risk for ischemic heart disease in shift workers.[80] Overall, most studies point in the direction of an adverse effect of shift work.

Molecular clock mechanism, the central clock, and the peripheral clock

The molecular circadian clock mechanism, which synchronizes changes in gene expression with recurring patterns of daily life, such as eating and sleeping, is identified in all tissues and cell types. In mammals, the central clock exists in the suprachiasmatic nucleus in the hypothalamus, and peripheral clocks exist in all other tissues and cell types. The core molecular clock is composed of a transcriptional/translational feedback loop that synchronizes rhythmic gene expression downstream (reviewed by Takahashi et al).[81] CLOCK and BMAL1 constitute the positive limb and drive transcription of *Period (PER)* and *Cryptochrome (CRY)* in the negative limb. Once PER and CRY reach a threshold level, they enter the nucleus and inhibit the CLOCK:BMAL1 heterodimer on their own promoters. The nuclear hormone receptors, REV-ERBα and β and RAR-related orphan receptor α (ROR-α), are both transcription targets of the CLOCK:BMAL1 complex and represses and activates BMAL1 transcription, respectively, to facilitate the robustness and stability of the clock. In addition, casein kinase Iε and δ (CsK) also contribute to the regulation of clock proteins.

Recent landmark research by O'Neill et al demonstrated biochemical oscillations in the oxidation-reduction state of peroxiredoxin, an antioxidant protein, as a conserved timekeeping mechanism in all three kingdoms of life.[82] The oxidation-reduction cycle of peroxiredoxin is completely independent of transcription, as was observed in human mature red blood cells, which are anucleated as well as *Ostreococcus tauri* in the darkness, under which condition, transcription is completely shut off.[83,84] This work turned an exciting new page in the field of circadian rhythm and cellular reduction-oxidation state.

The central clock controls the entrainment of various biological activities to zeitgebers (from the German meaning "time givers"), mainly light. The central clock receives photic input from the retina, projects to different regions of the brain, and secretes circulating factors to mediate physiologic rhythms both within the brain and in the periphery, thus coordinating the physiology and behavior of the organism.[81] Lesions of the suprachiasmatic nucleus in rats abolish the circadian pattern of BP and heart rate without affecting minute-to-minute variation, thus providing evidence in support of central regulation of BP and heart rate through the clock in the suprachiasmatic nucleus. However, food intake also became arrhythmic, which suggested that activity is similarly affected.[11] The critical role of the central clock in the cardiovascular system is highlighted further by the aforementioned increased risk of cardiovascular events and the metabolic syndrome in shift workers.

The peripheral clock allows the multicellular organism to anticipate environmental changes, coordinate metabolic

processes, and maximize energy efficiency. The zeitgebers for the peripheral clocks are less clear, because they are not only coupled to the central clock but are also able to oscillate autonomously. Indeed, environmental cues, such as restricted feeding, were found to be able to override the central clock locally and cause dyssynchrony between the central and peripheral clocks, as seen in the shift work paradigm.[85] In the cardiovascular system, it is believed that neurohumoral factors play important roles in synchronizing the central and peripheral clocks, although the detailed mechanisms are as yet not well understood. High throughput studies confirm that approximately 10% of transcripts in each peripheral tissue type oscillate in a circadian fashion under the control of core clock genes; however, there is little overlap between the different tissues, and the total number of oscillating genes is estimated to be close to 50% of the transcriptome.[86–88] The role of the peripheral clock in the cardiovascular system is becoming a fast emerging field, and is elucidated further in the next section.

Mechanistic basis for circadian control of cardiovascular biology

Animal models of genetically engineered mutant core clock machinery have been instrumental in isolating and identifying the role of the clock component in the cardiovascular system. This section discusses the experimental evidence for circadian regulation of the intrinsic components (cardiac and vascular) of the cardiovascular system as well as extrinsic factors (neurohumoral and hematological) that act directly on the cardiovascular system and affect its function.

Intrinsic factors

Cardiac

The peripheral clock in cardiomyocytes has been shown to affect all major processes of the myocardium, from energy metabolism to contractile function and from response to injury to electrophysiologic properties.

Energy metabolism

The peripheral clock regulates many aspects of myocardial metabolism and allows the heart to anticipate and adapt to different sources of energy and demand efficiently in a time-of-day-dependent manner. Chatham and Young recently reviewed this topic in detail. Briefly, the clock machinery regulates myocardial glucose uptake, flux via the glycolysis and hexosamine biosynthetic pathway, and pyruvate oxidation, as well as glycogen, triglyceride, and protein turnover.[89] For example, the homozygous *Clock* mutant mouse model mimics the metabolic syndrome of hyperleptinemia, hyperlipidemia, hepatic steatosis, hyperglycemia, and hypoinsulinemia.[90] Cardiomyocyte-specific *Clock* mutant (CCM, dominant negative) mice show attenuated induction of myocardial fatty acid-responsive genes during fasting.[91] Moreover, in CCM hearts, myocardial oxygen consumption and fatty acid oxidation rates were increased and cardiac efficiency was decreased, without alterations in mitochondrial content or structure and only modest mitochondrial dysfunction.[14]

Contractile

Basal contraction and intracellular calcium levels were significantly greater in rat cardiomyocytes isolated during resting periods versus active periods. The increase in systolic intracellular calcium in response to isoproterenol was also significantly greater in resting periods than in active periods, reflecting a greater calcium load in the sarcoplasmic reticulum in the resting period.[92] Wild-type hearts but not CCM hearts showed a marked diurnal variation in responsiveness to an elevation in workload ex vivo, with a greater increase in cardiac power and efficiency during the dark (active) phase than in the light (inactive) phase.[93]

Qi and Boateng put forward an interesting hypothesis that CLOCK localizes to the sarcomeric z-disk and senses myofilament cross-bridge activity in neonatal cardiomyocytes.[94] However, this has been challenged recently by Wang et al because of a lack of specificity of the key antibody used in the first study.[95] In addition, Lefta et al showed subtle shifts in titin isoform composition, altered myosin heavy chain gene expression at the mRNA level, and disruption of sarcomere structure in BMAL1 null hearts, although passive tension in single cardiomyocytes was unaltered.[96] These studies suggest a possible role for the molecular clock in regulating the passive properties and structure of sarcomeres in addition to their active contractile properties, but more definitive studies are needed.

Hypertrophy

Wild-type mice were found to show a five-fold increase in cardiac hypertrophy when challenged with the hypertrophic agonist isoproterenol at the active-to-sleep phase transition compared with administration of isoproterenol at the sleep-to-active phase transition. This diurnal variation was not seen in CCM mice, which showed exaggerated hypertrophy at baseline.[14] Global BMAL1-deficient mice develop dilated cardiomyopathy with advancing age, and cardiomyocyte-specific knockout mice show increased biventricular weight and *Mcip1* expression.[14,96] Further, Martino et al have demonstrated that pressure overload (transverse aortic constriction)

in mice maintained in a disruptive 20-hour rhythm versus a normal 24-hour rhythm environment leads to worsened dilated cardiomyopathy.[97] Most interestingly, captopril, an angiotensin-converting enzyme inhibitor, benefited cardiovascular remodeling with improved cardiac function only when administered during sleep; wake-time captopril has an identical effect on cardiac remodeling and function to that of placebo, although achieved the same BP control as sleep time administration.[98]

Ischemia/reperfusion

In a rat coronary ischemia/reperfusion model, the amplitude of circadian clock gene oscillation, measured by messenger RNA level, was rapidly attenuated in the ischemia/reperfusion region when compared with the nonischemic region. This attenuation was not observed with hypoxia induced by a hypobaric chamber, suggesting that the clock machinery is involved in the response to ischemia/reperfusion, independent of hypoxia.[99] Durgan et al showed that wild-type mouse hearts subjected to ischemia/reperfusion at the sleep-to-wake transition (ZT12) had a 3.5-fold increase in infarct size compared with hearts subjected to ischemia/reperfusion at the wake-to-sleep transition (ZT0) and this variation was abolished in CCM mice. This study provides the first evidence that there is a time-of-day-dependent susceptibility to ischemia/reperfusion that is intrinsic to cardiomyocytes.[13] Studies by Virag et al showed that *mPer2* mutant (functional null) mice have reduced infarct by 43% after nonreperfused MI, by 69% after ischemia/reperfusion, and by 75% after preconditioned ischemia/reperfusion, respectively.[15,16] However, Eckle et al found that m*Per2* mutant mice had larger infarct sizes and loss of the cardioprotection conferred by ischemic preconditioning when compared with wild-type mice.[100] The same group also found a reduction in infarct size at ZT12 and ZT18 compared with ZT0, which is different from the first report by Durgan et al.[13] Although different surgical techniques and protocols (time-of-day) were used and may have contributed to the different results, more studies are needed to elucidate the reasons for this discrepancy.

Adenosine signaling has been implicated in the cardiac adaptation to limited availability of oxygen. Eckle et al identified PER2 as a target of adenosine receptor A2b, signaling of which leads to stabilization of PER2 during myocardial ischemia and subsequent stabilization of hypoxia-inducible factor-1α and induction of glycolysis. Most interestingly, stabilization of PER2 in the heart achieved by exposing mice to intense light resulted in transcriptional induction of glycolytic enzymes and PER2-dependent cardioprotection from ischemia.[100]

Excitability

Our group provided the first molecular evidence of circadian transcriptional regulation of channel activity as a mechanism for cardiac arrhythmogenesis. Specifically, we reported that cardiac ion channel expression and QT interval duration (an index of myocardial repolarization) show endogenous circadian rhythmicity under the control of a clock-dependent oscillator, Krüppel-like factor 15 (KLF15). KLF15 transcriptionally controls the rhythmic expression of Kv channel-interacting protein 2, a critical subunit required for generating the transient outward potassium current. Both a deficiency and an excess of KLF15 cause loss of rhythmic QT variation, abnormal repolarization, and enhanced susceptibility to ventricular arrhythmias.[101] Schroder et al subsequently demonstrated that cardiomyocyte-specific *Bmal1* knockout (iCSΔBmal1-/-) mice had a slowed heart rate, prolonged R-R and QRS intervals, and increased episodes of arrhythmia. Isolated iCSΔBmal1-/- hearts were more susceptible to arrhythmia during electromechanical stimulation. Further, the same group identified *Scn5a*, which encodes the principal cardiac voltage-gated sodium (+) channel (Na [V] 1.5) and mediates the circadian variation in susceptibility to arrhythmia in humans, as a potential target.[17] Interestingly, as mentioned, patients with LQT3 due to *SCN5a* mutations show a significant prolongation of QTc interval at night as well as increased adverse events during sleep.

Vascular

Circadian rhythmicity has been shown to affect the function of all major cell types in the vasculature. This section discusses vascular smooth muscle cells and endothelial cells.

Vascular smooth muscle cells

Under the dual regulation of neurohumoral input and the endothelial/nitric oxide system, vascular smooth muscle cells are critical in fine-tuning vascular resistance. Vascular smooth muscle cells also possess an intrinsic biological clock. Serum shock, angiotensin II, and retinoic acid have all been shown to synchronize the oscillation of clock gene expression.[102,103] Chalmers et al demonstrated synchronized rhythmic expression of core clock genes and clock-controlled genes, such as *tissue inhibitor of metalloproteinase 1 and 3* (*Timp1* and *Timp3*, respectively), *collagen 3a1* (*Col3a*), *transgelin 1* (*Sm22α*) and *calponin 1* (*Cnn1*) in a mouse smooth muscle cell line (Movas-1) using norepinephrine or forskolin as

zeitgebers.[104] Circadian expression of core clock genes (*PER2 and BMAL1*) is attenuated in senescent human smooth muscle cells. Ectopic expression of *TERT* (telomerase) in senescent cells or treatment with forskolin, a PKA activator, restores circadian rhythmicity and serum responsiveness via activation of cyclic adenosine monophosphate response element-binding (CREB) protein.[105] In addition, Saito et al showed that Rho-associated kinase 2 (ROCK2) plays a pivotal role in generating the intrinsic circadian rhythm of vascular contractility by receiving a cue from ROR-α.[106]

Vascular endothelial cells

Endothelial function measured by brachial artery flow-mediated endothelium-dependent vasodilation is reduced in the early morning (6 am) in healthy individuals.[107] In vitro studies have confirmed the function of the peripheral clock in vascular endothelial cells and identified important clock-controlled genes, including *thrombomodulin*, which codes for a membrane protein with anticoagulation activity.[108] Aortic rings from mice with the *Per2* mutation show impaired endothelium-dependent relaxation in response to acetylcholine, associated with decreased production of nitric oxide and vasodilatory prostaglandins and increased production of cyclooxygenase-1, which is independent of BP or dyslipidemia.[12] Similar findings in *Bmal1* knockout mice and *Clock* mutant mice have been reported, and thought to be due to attenuated AKT signaling and reduced production of nitric oxide, at least in *Bmal1* knockout arteries.[109]

In endothelial cells, the *tissue plasminogen activator inhibitor 1 (PAI-1)* promoter is under the direct regulation of cycle-like factor (CLIF)/CLOCK, BMAL1/CLOCK, and BMAL2/CLOCK heterodimers,[110,111] and is inhibited by CRY and PER proteins. Westgate et al observed a diurnal variation of thrombosis in response to photochemical injury.[19] In addition, this diurnal variation was abolished in *Clock* mutant mice, which showed a significantly longer time to thrombotic vascular occlusion, whereas global and endothelial deletion of *Bmal1* was associated with a loss of circadian oscillation and a shortened time to thrombotic vascular occlusion.[19]

Endothelial progenitor cells have an important function in endothelial repair and postnatal neovascularization.[112] A reduced number of circulating endothelial progenitor cells has been associated with increased cardiovascular risk.[113] Landmark studies by Méndez-Ferrer et al reported that circulating hematopoietic stem cells and their progenitors (including endothelial progenitor cells) show robust circadian fluctuations, peaking 5 hours after the initiation of light and reaching a nadir 5 hours after darkness. The cyclic release of hematopoietic stem cells is under the regulation of photic stimuli via core clock genes, the sympathetic nervous system, and stromal derivative factor-1 (CXCL12).[114] Normal individuals showed the highest number of circulating endothelial progenitor cells in the evening (10 pm).[115] On the other hand, studies in diabetic patients and in a diabetic rat model showed decreased release of endothelial progenitor cells, which was attributed to bone marrow neuropathy and decreased vascular reparative capacity.[93] Endothelial progenitor cells from *Per2* mutant mice showed reduced mobilization and response to vascular endothelial growth factor stimulation. Interestingly, endothelial progenitor cells from *Per2* mutant mice and *Bmal1* knockout mice showed an opposite effect on AKT activation, although both showed impaired endothelial function.[116] This may represent their opposing relationship in the core clock transcription regulatory loop and anti-phasic expression. It is conceivable that the Yin of increased injury due to increased shear stress and vascular tone and the Yang of concomitantly lowered repair mechanisms due to reduced endothelial progenitor cell number and function contribute to the excess adverse cardiovascular events in the early morning.

Extrinsic factors

Neurohumoral

A host of neurohumoral factors acting on the cardiovascular system demonstrate circadian rhythmicity, and have an important role in entraining the peripheral cardiovascular system to the central clock. Sympathetic activity dominates during the day and peaks in the morning, whereas parasympathetic activity peaks at night. The renin-angiotensin-aldosterone system,[117,118] vasoactive intestinal peptide,[119] and atrial natriuretic peptide[120] have all been shown to have diurnal variation in humans. Interestingly, secretion of cortisol, renin, and aldosterone show morning surges independent of activity;[121,122] however, other processes, such as surges of catecholamines, are attenuated if the individual remains in bed in the morning.[123,124]

Neurohumoral factors such as norepinephrine, epinephrine, and angiotensin II, when added to aortic smooth muscle cells in vitro, can serve as a zeitgeber as predicted and synchronize clock gene oscillation. However, oscillation of clock genes was preserved ex vivo in aortic, heart, and liver tissues harvested from *dopamine beta-hydroxylase* knockout mice (*Dbh*-/-), which could not synthesize either norepinephrine or epinephrine and was chronically treated with both propranolol and terazosin, thereby excluding compensation by

dopamine.[125] Thus, although sympathetic signaling affects the peripheral cardiovascular diurnal rhythm, circadian rhythmicity is preserved even without any adrenergic input.

Hematologic

Macrophages

Chronic systemic inflammation and macrophage infiltration is closely associated with an increased risk of cardiovascular events, and is a dynamic area of research. In mice, the spleen, lymph nodes, and peritoneal macrophages all contain a peripheral clock and more than 8% of the macrophage transcriptome oscillates in a circadian fashion, including many important regulators for recognition of pathogens and secretion of cytokines. Lipopolysaccharide is a robust zeitgeber for macrophages in culture. The peripheral clock regulates inflammatory innate immune function, with isolated spleen cells stimulated with lipopolysaccharide at different circadian times displaying circadian rhythms in secretion of tumor necrosis factor-alpha and interleukin-6.[126] Rev-erbα has been shown to regulate the expression of important genes involved in innate immunity, including *IL6*, *IL19*, *CXCL6*, *CXCL1*, and *CCL2*.[127] Sato et al recently demonstrated that Rev-erbα directly suppresses *CCL2* expression via a RORE element in the promoter and regulates infiltration of inflammatory macrophages.[128] An elegant study by Cheng et al, using an arterial isograft transplant mouse model, demonstrated that wild-type grafts when anastomosed to either wild-type mice or mice with disrupted circadian clocks exhibit no pathology, whilst aortic grafts from *Bmal1* knockout or *Per2*,*3* double-knockout mice transplanted into wild-type mice led to development of robust arteriosclerotic disease, with upregulation of T-cell receptors, macrophages, and infiltrating cells in the vascular grafts, that was independent of hemodynamics and B-cell-mediated or T-cell-mediated immunity.[18] This clearly demonstrates the crucial role of the peripheral clock in maintaining vascular health independent of the central clock.

Other hematologic factors

Both the number of circulating platelets and platelet aggregation show circadian variation.[129,130] The hypercoagulability noted in the early morning is also associated with a peak in tissue factor pathway inhibitor and activated factor VII levels.[131,132] Further, the number of vascular cell adhesion molecule-1-positive microparticles released from the endothelium in human plasma was reported to show a peak at 9 am, and was associated with increased coagulability through the tissue factor pathway.[133] Further, levels of both prothrombin fragment (a marker for intravascular thrombin generation) and the plasmin–plasmin inhibitor complex (representing the degree of intravascular plasmin generation) were found to peak at 8 am.[132] However, variations in platelet reactivity, fibrinogen, alpha 2-antiplasmin, and plasminogen were abolished in supine individuals.[124] Fibrinolytic activity, in contrast, peaks in the afternoon and troughs in the morning.[129,134] Tissue plasminogen activator inhibitor 1 peaks at 4 am[135] and this diurnal variation persists in supine individuals.[124] Interestingly, tissue-type plasminogen activator antigen also peaks in the morning; however, tissue-type plasminogen activator activity was found to be lower in the morning due to increased tissue plasminogen activator inhibitor 1 activity.[136] Indeed, a high level of tissue plasminogen activator inhibitor 1, associated with low fibrinolytic activity, was found to be an independent risk factor for first acute MI in both men and women.[137] The combination of increased platelet aggregability,[138] blood viscosity,[139] and thrombotic activity,[19] as well as decreased fibrinolytic activity,[135,140–142] would not only increase the size of an otherwise nonoccluding thrombus but would also increase its resistance to thrombolysis.[141]

An interesting study reported by Mou et al demonstrated the expression of tissue-type plasminogen activator and tissue plasminogen activator inhibitor 1 in the suprachiasmatic nucleus and their involvement in modulating photic phase shifts via activation of brain-derived neurotrophic factor[143] in brain slices. It will be exciting to see follow-up in vivo studies in this regard.

Therapeutic implications

The circadian clock is altered or dampened in multiple human cardiovascular disorders and animal models, including hypertension, MI, and diabetes. Disturbed circadian rhythm is a risk factor for cardiovascular disease, as evidenced by studies in shift workers. In this section, we discuss therapeutic strategies that take advantage of our understanding of circadian regulation in the cardiovascular system.

Chronotherapy

The effectiveness and toxicity of many drugs varies according to time of administration because of the circadian rhythmicity of a number of biochemical, physiological, and behavioral processes. Chronotherapy became widely used in clinical practice when the alternate morning oral corticosteroid regimen was introduced in the early 1960s.[144]

As mentioned earlier, nondippers tend to have worse cardiovascular outcomes. The possibility of achieving "dipping" by

pharmacologic intervention has been attempted in both animal and human studies with some success. Martino et al showed in mice that the benefit of captopril (a short-acting angiotensin-converting enzyme inhibitor) in cardiac remodeling after pressure overload is only observed when administered during sleep time but not during wake time despite the same level of BP control.[98] Similarly, human studies showed that ramipril 5 mg daily as monotherapy taken at bedtime was associated with significantly reduced BP during sleep (ie, improved "dipping") when compared with taking the drug on awakening. The proportion of patients with controlled ambulatory BP at 6 weeks increased from 43% to 65% (P=0.019) with treatment at bedtime.[145] Further, in the Heart Outcomes Prevention Evaluation (HOPE) study, ramipril was administered at night, although without a direct control group, and the benefits seen were three times greater than predicted from previous studies based on BP reduction alone.[146] The Ambulatory Blood Pressure Monitoring for Prediction of Cardiovascular Events (MAPEC) study directly compared morning versus nighttime doses and concluded that night dosing achieved overall better BP control in 2,156 individuals with a mean follow-up of 5.6 years, and this was associated with a lower risk of total cardiovascular disease events (relative risk 0.39, confidence interval 0.29–0.51, $P<0.001$).[147,148]

Chronotherapy as part of the personalized medicine that takes age, gender, and genetic background into consideration for an optimized regimen will be the goal of the foreseeable future. Drug delivery strategies incorporating knowledge of circadian rhythmicity will enable us to harness the benefit of chronotherapy without requiring meticulous compliance and adherence on the part of patients. Further, strategized therapies that aim to minimize or avoid the vulnerable window in the early morning for certain cardiovascular events, such as lethal MI and arrhythmia, will allow us to achieve chronoprevention.

Resynchronization

After a 12-hour light-dark shift, as experienced by human shift workers, central clock (suprachiasmatic nucleus)-mediated entrainment of BP and heart rate occurs in 1–2 days in humans.[149,150] However, the entrainment of cardiac peripheral clock and clock-controlled genes takes at least 5 days to occur,[91] thus creating a window of dyssynchrony. Therapies aimed at facilitating the transition of the peripheral clock will have implications for shift workers as well as frequent jet travelers.

Aging has been shown to attenuate circadian oscillation in vascular smooth muscle cells,[105] although the methodology used cannot distinguish whether this is due to overall dampened oscillation or an inability to synchronize within the cell population. Pharmacologic and behavior modifications that reinforce the "ticking of the clock" may ameliorate the cardiovascular risk factors associated with aging. In addition to changes in lighting conditions, nonphotic stimuli such as physical activity and feeding schedule have been shown to play key roles in entrainment of the peripheral clock.[85,151,152] A small pilot study by Scheer et al showed that daily oral melatonin for 3 weeks reduced BP during sleep, with improved "dipping". Although sleep was also improved, a direct correlation between improved sleep and decreased BP was not observed, suggesting that the beneficial effect on BP is independent of the improvement in sleep.[153] More human studies are required to fully evaluate the potential of resynchronizing therapy.

Resetting the "clock"

A recent advance in the field has been the ability to directly manipulate the clock machinery. Two high throughput screens identified small molecules that inhibit the function of CKIα/δ and CKIε/δ, thus preventing degradation of PER2 and significantly lengthening the circadian period in human and mouse cell lines as well as living zebra fish in one study.[154,155] Chen et al reported additional molecules that affect the period length and, interestingly, amplitude "damper" and "enhancer", which further expand our toolbox for manipulating the clock.[156] Of interest is a report by Solt et al that administration of a synthetic REV-ERB ligand in mice alters circadian behavior and the circadian pattern of gene expression in the hypothalamus as well as in peripheral tissues. Mice with diet-induced obesity treated with this ligand showed decreased obesity and a markedly improved metabolic profile with regard to dyslipidemia and hyperglycemia.[157] These results suggest that directly manipulating the core clock machinery may be a new therapeutic opportunity in the treatment of cardiometabolic disease.

Conclusion

Our lives are intimately linked to the solar cycle. The cardiovascular system has evolved an intricate circadian rhythm that oscillates intrinsically and can be entrained to the environment by zeitgebers, such as activity, temperature, and feeding. Our knowledge of how peripheral tissue clocks coordinate with the central suprachiasmatic nucleus clock to regulate cardiovascular physiology has greatly advanced in recent years, from the simple observation of diurnal fluctuation of physiologic parameters, to the molecular mechanisms of clock and their regulation on tissue-specific clock output

genes, to chemical tools for potential intervention in the clock itself. However, our insight into the molecular basis of circadian timing in a tissue-specific fashion is still limited and will continue to be a proliferating field of research in the immediate future. Discovering local physiologic and pharmacologic zeitgebers will not only shed light on circadian biology but also provide opportunities for specific intervention. Non-transcriptional control of the clock machinery is a brand new chapter in circadian biology, and on further exploration, may offer quicker and more local adaptation of the peripheral clock. Our ability to resynchronize or directly "reset" the clock is rapidly expanding, and holds promise for development of future therapeutic tools.

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Disclosure

The authors report no conflicts of interest in this work.

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