Time Is of the Essence
Vascular Implications of the Circadian Clock

R. Daniel Rudic, PhD

The circadian clock is a molecular network of genes and proteins that control biological timing. In the suprachiasmatic nucleus of the brain, this unique signaling pathway operates to control the 24-hour sleep-wake cycle. In the periphery, the circadian clock acts to regulate local functions that follow circadian rhythms. Indeed, the cardiovascular system of mammals follows a circadian rhythm, exhibiting a functional oscillation of a 24-hour period. Blood pressure follows a 24-hour profile, rising and falling during the circadian day. Heart rate, endothelial function, circulating levels of humoral signals, and myogenic tone also follow a circadian rhythm. Recent data have demonstrated that the circadian clock resides in blood vessels; further evidence is defining the significance of the circadian clock in vascular cell signaling, blood pressure control, vascular function, and disease.

The Circadian Clock

Circadian rhythms are generated through an interlocking set of transcriptional and posttranslational feedback loops to yield molecular and functional cycles that approximate 24 hours. The molecular essence of this loop—the circadian clock—resides in the precise control of transcription by negative feedback, the oscillating components of which then impinge on target genes and proteins to facilitate circadian physiology. At the core of this oscillating signaling network are the positive limb (Bmal1, Clock, and Npas2) and negative limb (Period and Cryptochrome) circadian clock components that interact to form a negative feedback loop. Bmal1, Clock, and Npas2 are transcription factors containing basic helix-loop-helix and PER-ARNT-SIM (PAS) domains, which confer the ability to bind a promoter-enhancing element called the E-box (CACGTG) and the ability to form heterodimeric interactions, respectively. The circadian cycle begins when Bmal1 binds either Clock or Npas2 to form a heterodimeric protein interaction that can then transactivate the E-box sequence of the Period (Per1, Per2, and Per3) and Cryptochrome (Cry1, Cry2) genes (Figure 1).[1–3] Translated Per and Cry proteins then form heterodimers and translocate back into the nucleus to inhibit Bmal1-Clock/Npas2,[4] an inhibition that occurs through a direct physical interaction between Cry and Bmal1 or Clock.[5] The result is that the Per and Cry proteins act to suppress their own transcription. Additional interlocking/accessory loops control Bmal1 transcription and oscillation via the nuclear receptors Rev-Erbα[6] and ROREα,[7] which repress and stimulate Bmal1, respectively.

The transcriptional-translational feedback loops alone are not sufficient to generate rhythms of 24 hours; thus, posttranslational mechanisms, including phosphorylation and ubiquitination, control trafficking and protein abundance to fine-tune timing of the circadian clock.[8] The serine-threonine kinase casein kinase 1 epsilon (CKIϵ) phosphorylates Bmal1 to induce transcription[9] and phosphorylates Per proteins at discrete residues either to promote nuclear translocation by way of a heteromeric CKIϵ-Per-Cry complex[10,11] or to direct Per proteins to undergo proteosomal degradation.[12] Little is known about CKIϵ in blood vessels, although CKIϵ has been reported to bind and phosphorylate the tight junction protein occludin in human umbilical vein endothelial cells,[13] which may confer a mechanism for the circadian clock to control the endothelial barrier and permeability because previous studies have also shown rhythmicity in junctional proteins.[14]

Additional posttranslational regulation of the circadian clock occurs through GSK3β, which phosphorylates and stabilizes Rev-erbα to influence Bmal1 transcription.[15] Regulation of protein turnover is also central to regulating timing of the circadian clock. On phosphorylation, Cry binds FbxL3,[16] and Per can bind bTrCP,[17] both of which are ubiquitin ligases that target Cry and Per proteins for proteosomal degradation. Although recent work has emerged to implicate the core loop components (Bmal1, Clock, Per, and Cry) in multiple aspects of vascular biology, little is known about the operation and function of the accessory regulatory loops and posttranslational control mechanisms in the vasculature.

The Circadian Clock: Redundancy and Robustness

In the mammalian system, groundbreaking discoveries in gene-modified mice established the identity of the circadian clock and its role in the control of rhythmic behavior. These primary studies assessed effects on locomotor rhythm, the difference in pattern of movement/activity that occurs between night and day. From these observations, important insight is garnered into the complexity and robustness of the circadian clock and its influence outside the central nervous system, which may provide additional clues as to how the circadian clock may operate in the vascular system and how central clock function may impinge on the periphery.

From the Department of Pharmacology and Toxicology, Medical College of Georgia, Augusta.
Correspondence to R. Daniel Rudic, PhD, Department of Pharmacology and Toxicology, 1120 15th St, Medical College of Georgia, Augusta, GA 30912. E-mail rrudic@mcg.edu

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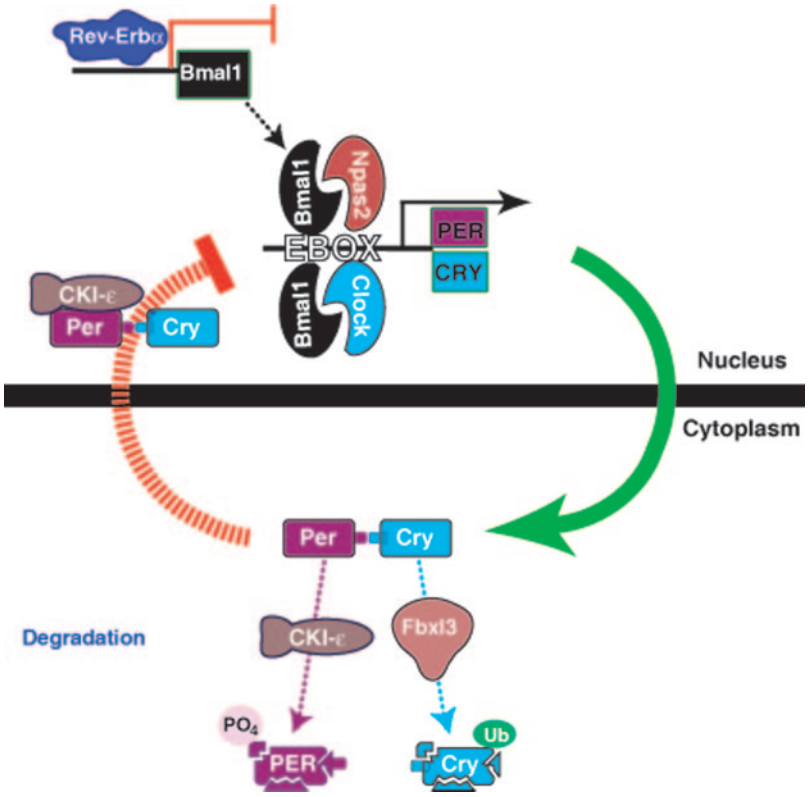

Figure 1. The circadian clock signaling pathway. Bmal1-Clock or Bmal1-Npas2 protein heterodimers transactivate the Cry and Per genes to induce transcription. Translated Per and Cry proteins form heterodimers and translocate into the nucleus to repress Bmal1. Timing is tuned to approximate 24 hours through posttranslational modifications, including CKI-dependent phosphorylation and FbxL3-dependent ubiquitination, which lead to proteosomal degradation of the Per and Cry proteins, respectively.

Characteristic of circadian clock components is that disruption or mutation of the genes either abolishes the amplitude of the rhythm, causing complete arrhythmicity, or shortens (fast clock) or increases (slow clock) the period of the 24-hour circadian rhythm in locomotor function (Table 1). Indeed, the disruption/mutation of distinct core clock components produces variable phenotypes in circadian dysfunction as it pertains to these locomotor/behavioral rhythms. Under standard light cycle (LD) conditions, 12-hour light/12-hour dark, mice with targeted disruption of the Bmal1 gene (Bmal1-KO) exhibit the most profound impairment among components of the circadian clock, exhibiting a reduction in total activity and delay in the onset of activity that can manifest as arrhythmicity that is independent independent of light cycle.[18] This may stem in part from the fact that Bmal1 appears to have no functionally redundant paralogs in the regulation of centrally regulated circadian rhythms. Although Bmal2/MOP9/CLIF is an apparent ortholog of Bmal1,[19] subserving a role comparable to Bmal1 in its ability to bind Clock,[20,21] the complete impairment in locomotor rhythm caused by Bmal1 disruption alone in mice suggests that Bmal2 is not sufficient to sustain circadian rhythms in centrally regulated activity. The other components of the circadian clock do have paralogs/homologs that are capable of functional redundancy—Clock/Npas2, Per1/Per2 (in cultured fibroblasts, Per1 and Per2 are not functionally redundant, both appearing necessary for circadian oscillation[22]), and Cry 1/Cry 2—so that single mutants of these genes exhibit only subtle changes in period length of circadian rhythm under standard LD conditions as opposed to complete arrhythmicity.[23–33] However, during free-running conditions (constant darkness [DD]), these phenotypes can worsen to complete arrhythmicity when both paralogs/homologs are mutated, which may reflect a light-driving component input into the circadian clock, as was suggested in studies assessing Cry1-Cry2 double-knockout (KO) mice and their light cycle–dependent impairment in Per2 and locomotor rhythm.[34]

Table 1. Circadian Clock–Mutant Mice Exhibit Impaired Rhythm in Locomotor Circadian Rhythm

Core Clock Mice	LD	DD
Wild type	Normal rhythm	Normal rhythm
Bmal1 KO[18]	Arrhythmic*	Arrhythmic
Clock mut[23]	Slow Clock	Arrhythmic
Clock KO[24]	Normal rhythm	Fast clock
Npas2 KO[25]	Normal	Fast clock
Clock.Npas2 dKO[26]	Subtle	Immediately arrhythmic
Per1 mutant[27]	Fast clock	Progress to arrhythmicity over time
Per2 mutant[28,29]	Fast clock	Progress to arrhythmicity over time
Per3 mutant[30]	Normal rhythm	Slightly shortened period
Per1.Per2 dKO[31]	Slow clock	Immediately arrhythmic
Per1.Per2.Per3 tKO[32]	Slow clock	Immediately arrhythmic
Cry1 KO[33]	Normal	Fast clock
Cry2 KO[33]	Normal	Slow clock
Cry1.Cry2 dKO[33]	Normal	Arrhythmic

dKO indicates double KO; tKO, triple KO. Gene disruption or mutation of different core clock components affects circadian rhythm in locomotor function, albeit differently. Because circadian defects can be masked by normal light cycles (LD), DD conditions are the standard to identify circadian rhythms and their defects. *In LD, the timing of activity onset is delayed and the amount of activity is significantly lower in Bmal1-KO mice than wild-type mice.[18]

Despite genetic disruption of the circadian clock, rhythms persist for the most part in LD conditions (except Bmal1-KO mice), demonstrating the "robustness" of the circadian system; ie, rhythmicity persists despite dysfunction in the circadian clock signaling mechanism. Recent evidence has demonstrated that robustness of the circadian network involves redundancy, compensatory responses by other circadian clock components,[35] and with particular relevance to the central clock/suprachiasmatic nucleus, intercellular coupling. Fibroblasts and liver cells lack the capacity to implement intercellular coupling as a mechanism to maintain rhythm,[22] although it is not known whether vascular cells exhibit this feature, which may or may not be unique from established mechanisms of vascular cell communication such as gap junctions, paracrine signals, and electric impulses. The inability to use intercellular coupling as a mechanism to preserve rhythm may make vasculature and other peripheral tissues more prone to the detrimental impacts of cell-localized circadian dysfunction. Clearly, more studies are necessary to determine the importance of vascular cell communication in vascular rhythms.

The complexity of circadian clock signaling is underscored by the range of phenotypes in the mutant mice. For example, disruption of either the positive limb component Bmal1[36] or negative limb component Per2[37] exhibits the same impairment with respect to endothelial function. Conversely, the effect on vascular cell signaling can be opposed between these components because the phosphorylating kinase phosphoinositide-dependent kinase-1 is decreased in Bmal1-KO mice[36,38] but increased in Per2-mutant mice.[38] Phenotypic congruence among circadian clock mutants may be evidence for the importance of "rhythm" per se, whereas disparity may reflect consequences in discrete clock targets. Alternatively, the variability of phenotypes may reflect nuances in the buffering of vascular circadian rhythm, which may be vascular cell specific or even vascular function specific. To date, the nature and/or presence of functional redundancy and/or robustness in the vascular system among circadian clock components are largely unstudied, with very little known about the particularities of circadian clock dynamics in the vasculature. Is the circadian clock differentially expressed and oscillating in endothelial cells compared with smooth muscle cells? Are there vascular, endothelial, or smooth muscle cell–specific partners for the circadian clock that modulate circadian clock signaling in blood vessels? Indeed, there may be such basic helix-loop-helix/PAS proteins unique to the vasculature such as endothelial PAS1 (endothelial PAS1/hypoxia-inducible factor-2α), a protein important in vascular remodeling[39] and angiogenesis. In fact, Bmal2, which does not appear to be critical for locomotor rhythm, exerts an influence in the control of endothelial cell signaling. Thrombomodulin, a glycoprotein important in coagulation, KLF-5, and vascular endothelial growth factor are substantially upregulated in human umbilical vein endothelial cells cotransfected with Bmal2 and Clock.[40] In addition, Bmal2, when partnered with Clock, can transactivate the plasminogen activator inhibitor type 1 promoter in endothelial cells.[21,41] Dissection of this complex circadian network in the vasculature will be of utmost importance in understanding the basic vascular biology with ramifications for the development of targeted therapeutic strategies for the future.

The Circadian Clock Oscillates in Blood Vessels: A Vascular Clock

In the suprachiasmatic nucleus of the brain, rhythmic oscillation of clock genes is responsible for rhythms in sleep and locomotor activity, as shown recently in brain-specific rescue studies.[42] The circadian clock is also functional in nonneural tissues in the periphery,[43] including the vasculature. Indeed, Bmal1, Clock, Npas2, Per, and Cry—the core circadian clock—are expressed and oscillating in vascular tissue.[14,44] In the aorta, the circadian clock component Per2 exhibits robust oscillation but is out of phase with Per2 oscillation in the suprachiasmatic nucleus of the brain. Thus, timing in vascular tissue may be controlled in a manner to facilitate vascular-specific functions. Coordination of vascular contractility, endothelial signaling, and cell turnover may be precisely timed to optimize blood flow and tissue perfusion in accordance with tissue demands. Moreover, not all blood vessels are created equally. Arteries follow a different timing than veins, exhibiting circadian oscillations (Period gene oscillation) of varying phases that persist for as many as 12 circadian cycles ex vivo.[45] Vascular bed–specific rhythms may distinctly regulate signaling in accordance with the vascular bed–specific functions of the circadian clock. An arterial clock may uniquely control mechanics, structure/remodeling, and signaling, which may influence the progression to disease, whereas its dysfunction may influence atherosclerosis and remodeling. In fact, dysfunction of the circadian clock was recently shown to influence vascular remodeling and the response to injury in conduit arteries.[36] In arterioles, the circadian clock may control vasoactive signals that regulate peripheral vascular resistance to influence blood pressure,[46] and in veins, it may further influence hemostasis and thrombosis. Microvessels may similarly be regulated by the circadian clock to regulate tissue perfusion and even angiogenesis, as has been recently demonstrated in Per2-mutant mice,[38] although to date no studies have directly explored clock function in microvessels. Because there are differences in circadian clock oscillation across the vascular tree,[45] it may be that hemodynamic forces such as shear stress and wall stress, which vary throughout the vascular tree, may act as an external cue to uniquely entrain the arterial, venous, and microvessel clocks in a manner analogous to the way that the light-dark cycle resets the central clock[47] and food entrains the liver clock.[48,49]

Evidence is emerging that numerous vasoactive signals also modulate circadian clock signaling in vascular cells. Indeed, circadian rhythm can be recapitulated in vitro. Although single cells in culture have an oscillating circadian clock, cell populations en masse are arrhythmic because of asynchronous circadian clock oscillation among cells.[50] Thus, a phase-aligning stimulus must be applied to cultured cells to elicit a uniform circadian rhythm.[51] In cultured fibroblasts, a short duration of high concentrations of horse serum[52] or even glucose[53] phase aligns or synchronizes the circadian clock among cells to evoke a uniform oscillatory clock signal (ie, Bmal1, Per2 oscillation), whereas other signals alter the

Table 2. Circadian Clock–Modifying Signals in Vascular Cells

Cue	Cell Type	Effect on the Circadian Clock
Horse serum	F,[52] VSMC[44]	Synchronize
Glucose	F[53]	Synchronize
Glucocorticoids	F,[54] VSMC[44]	Synchronize
Retinoic acid	VSMC, aorta[44]	Phase shift
Angiotensin II	VSMC[55]	Phase shift
NE, E	VSMC[56]	Phase shift
endothelin	F[57]	Synchronize
PGE_2	F, heart[58]	Synchronize (F); phase shift (heart)
PGJ_2	Glioma cells[59]	Synchronize
PPARγ agonist	EC[60]	Decrease Bmal1 expression
NO (GSNO, L-NAME)	EC, VSMC[61]	Phase shift

F indicates fibroblasts; VSMC, vascular smooth muscle cells; NE, norepinephrine; E, epinephrine; PGE_2, prostaglandin E_2; PGJ_2, prostaglandin J_2; PPARγ, peroxisome proliferator–activated receptorγ; GSNO, S-nitrosoglutathione; L-NAME, *N*-nitro-L -arginine methyl ester; and EC, endothelial cells. A number of signals with established roles in the control of vascular function have a demonstrable role in the control of circadian clock expression and oscillation. Because a homogeneous population of cultured cells exhibit circadian oscillation that is of varying phase, cell to cell, an emerging number of mediators phase align or synchronize cellular clocks. In addition, other signals act to phase shift the circadian clocks in cells and in tissue.

timing of the clock to phase advance or delay the rhythm. Recent studies have begun to identify these "vascular clock"–modifying signals.[44,53–61] Surprisingly, signals with established roles in the control of vascular contractility, tone, and remodeling also impart a significant influence on the circadian clock. These include endothelin, prostanoids, angiotensin, and even nitric oxide (NO) (Table 2).

The Circadian Clock and Endothelial NO Synthase Signaling

A novel twist is emerging in NO biology, with evidence mounting that the circadian clock regulates endothelial NO synthase (eNOS) signaling and the upstream mechanisms that control its phosphorylation, an axis integral to the short- and long-term responses of blood vessels.[62] The phosphorylated form of eNOS[61] and its activating kinase, Akt, exhibit circadian oscillation,[14,63] an important pathway that enhances NO production,[64,65] protects cells from apoptosis,[66,67] and modulates vascular function.[68] More direct evidence to implicate the circadian clock in the control of the Akt-eNOS pathway has been demonstrated in mice with a dysfunctional circadian clock because phosphorylated eNOS expression is decreased in arteries of Bmal1-KO mice. Further upstream, multiple lines of evidence demonstrate concomitant dysregulation of phosphoinositide-dependent kinase-1 in Bmal1-KO[36] and Per2-mutant mice,[38] a kinase that phosphorylates Akt-1. It remains unclear how the circadian clock regulates this pathway, whether occurring through mechanisms of E-box activation or other methods of circadian regulation, and whether PI-3 kinase is involved.

In addition to the circadian clock controlling NO, NO may regulate the circadian clock. NO donors activate Per1 transcriptional activity and conversely increase Bmal1 expression in endothelial cells. Because NO donors may release amounts of NO that exceed endogenous production, it is of further biological relevance that arteries of eNOS-KO mice exhibit a phase shift of the circadian clock (Per), whereas *N*-nitro-L-arginine methyl ester similarly phase advances Per2 expression in vascular smooth muscle cells, suggesting that both endogenous eNOS and neuronal NOS play a modulatory role in control of the circadian clock.[61] In contrast to the effect of NO in the vasculature, neither eNOS-KO nor neuronal NOS–KO mice have any aberration in behavioral circadian function,[69,70] suggesting that this reciprocal relationship between NO and the circadian clock may be a vascular-specific phenomenon.

The Circadian Clock in Vascular Function and Disease

Circadian Clock and Blood Pressure

In humans, blood pressure rises steadily during the day and falls during the night, exhibiting a circadian rhythm.[71] Conversely, nocturnal animals such as mice follow a rhythm that peaks at night and troughs in the daytime. Moreover, many hypertensive patients exhibit circadian disturbances in their blood pressure rhythm; nondipping hypertensives are absent the nighttime fall in blood pressure.[72] There is now compelling evidence that the circadian clock plays an important role in the regulation of the circadian variability in blood pressure.[37,46,73–76]

Targeted deletion of Bmal1 in mice (Bmal1-KO) completely abolishes the circadian variation in blood pressure as a result of a hypotensive phenotype during the activity period that may in part be due to a defect in catecholamine production.[46] Similarly, blood pressure rhythm is also abolished in Cry1-Cry2 double-KO mice acclimated to constant darkness, although the impairment is due to a hypertensive phenotype during the rest period.[73] Per2-mutant mice exhibit lower blood pressures,[37] a phenotype more similar to Bmal1-KO mice. Thus, both positive and negative limb mutants exhibit impairments in blood pressure and its rhythm, evidence that the circadian clock genes/proteins play a significant role in the regulation of blood pressure.

Recent data also suggest that peroxisome proliferator–activated receptor-γ interacts with the circadian clock to influence blood pressure.[60] Endothelial cell–specific KO mice of peroxisome proliferator–activated receptor-γ, which have reduced Bmal1 expression and oscillation, exhibit a blunted blood pressure rhythm resulting from hypotension during the activity period, albeit to a lesser extent than Bmal1-KO, a phenotype remarkably similar to endothelial cell–specific KO mice of Bmal1,[76] although less marked than the impairments in Bmal1-KO blood pressure rhythms. In addition, urine levels of catecholamines are attenuated, consistent with the studies in Bmal1-KO mice. In addition to normal blood pressure control, there is evidence to implicate a role for the circadian clock in hypertension because there are known variants of the Bmal1 gene in rat models of hypertension and human hypertension.[77] Future studies to assess blood pressure regulation will prove informative in understanding the nature of the interaction between the circadian clock and hypertension.

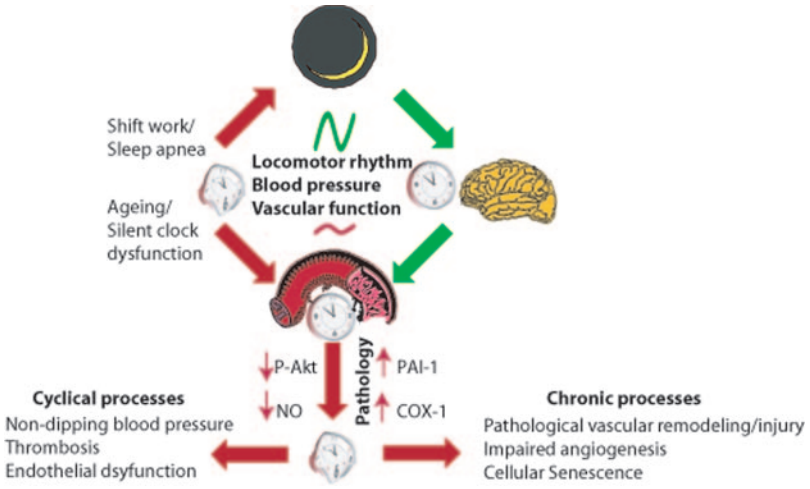

Figure 2. Central, vascular, and broken clocks. In the central nervous system, sunlight is the dominant external cue that resets timing of the circadian clock, which is localized in the suprachiasmatic nucleus of the brain and mediate rhythms in locomotor/behavior. In blood vessels, hormones and hemodynamics may be auxiliary cues that set timing in vascular function. One manner of circadian dysfunction occurs during aberrations in behavior (shift work, sleep apnea) that disrupt the rhythmic interaction with the environment. The effect is to impair circadian clock gene oscillation, which may impinge on the vasculature to induce pathology. In addition, there are silent circadian dysfunctions (nondipping hypertension) that may induce or even originate from local circadian clock dysfunction and subsequently impinge on signaling pathways to induce vascular pathology.

The Circadian Clock in Endothelial Function

There is growing evidence that the genetic components of the circadian clock exert a significant influence on the function of blood vessels. Multiple independent observations have revealed that circadian clock dysfunction impairs endothelial function. The relaxant response to acetylcholine in aortas from Per2-mutant mice,[37] Bmal1-KO mice, and Clockmut mice[36] is attenuated in the face of normal vascular smooth muscle cell function. Not only does the endothelial dysfunction manifest in discrete models of genetic clock disruption, but further evidence suggests that these impairments are directly dependent on deterioration of circadian rhythms. Clockmut mice have normal endothelial function in LD conditions but develop dysfunction only when acclimated to DD conditions,[36,78] which are known to impair circadian rhythm in this particular genetic mutant model (Table 1). It remains unclear whether these impairments in circadian rhythms are due to central clock dysfunction or to vascular clock dysfunction; no studies have characterized the effects of LD versus DD in the periphery or in the vasculature. However, indirect evidence from studies in Per2-mutant mice may indicate that vascular clock impairment in the face of intact central clock function may be sufficient to induce dysfunction because Per2-mutant mice in standard LD retain central rhythm.

Key signals that regulate endothelial function are also dysregulated in circadian clock–deficient mice. Vasculoprotective signals, including the phosphorylated, activated forms of Akt and eNOS, are reduced in arteries of circadian clock–mutant mice,[36,38] whereas plasminogen activator inhibitor type 1, which is prothrombotic and antifibrinolytic, is upregulated in Bmal1-KO mice.[36] Additional evidence suggests that elevation in pressor mechanisms, particularly those mediated by cyclooxygenase-1, may also contribute to the endothelial dysfunction observed in mice with dysfunctional clocks. Cyclooxygenase-1 protein expression is increased in the aortas of Per2-mutant mice, and the contractile response to indomethacin is exacerbated.[37] As for the particular prostaglandins responsible, whether it is prostaglandin $F_{2\alpha}$ or E_2 or whether in fact there are also differences in vasodilatory prostaglandins (prostaglandin I_2) remains unknown. But clearly, the circadian clock exerts a tonic influence on an array of regulatory signals that control vascular function and further condition the long-term response to vascular injury.

Thrombosis and the Circadian Clock

The mechanisms that govern endothelial function are important to counterbalance pathological processes; in fact, circadian clock–deficient mice exhibit gross abnormalities in their response to vascular injury. Acute experimental thrombosis induced by photochemical injury exhibits a circadian rhythm, a rhythm that is absent in Clockmut mice.[76] Clockmut mice actually exhibit a delay in occlusion time, suggesting that mutation of the Clock gene may actually protect against thrombosis. In mice with endothelial cell–specific disruption of Bmal1, rhythm in occlusion time is also abolished, but this is due to an accelerated time to occlusion, suggesting that these mice are, in contrast, more prone to injury. Thus, the circadian clock conditions acute thrombotic events in a manner that may be uniquely controlled by individual circadian clock components.

The Circadian Clock in the Long Term: Vascular Remodeling, Injury, and Angiogenesis

In addition to controlling the short-term daily oscillations that occur in hemostasis, the genetic components of the circadian clock exert a significant influence on the long-term response to vascular injury and remodeling. Arterial ligation of the common carotid artery or vascular injury of the femoral artery results in pathological remodeling and worsened intimal hyperplasia in mice with a dysfunctional clock.[36] Moreover, because these impairments occur only when Clockmut mice are acclimated in DD but not LD (Table 1), the light cycle–dependent worsening of vascular injury in Clockmut mice suggests that the circadian clock and rhythm per se exert an important regulatory role in vascular remodeling and the response to injury. Other lines of evidence also suggest a role for the circadian clock in the remodeling of the microvasculature. The angiogenic response to hind-limb ischemia is blunted and causes limb loss in Per2-mutant mice.[38] Thus, the circadian clock may play a significant role in the progression of large-artery and peripheral vascular disease.

Intuitively, vascular remodeling and angiogenesis are not processes that follow daily cycles. Although circadian rhythms are most appreciated for their importance in the

navigation of daily cycles, the latter evidence demonstrates that the circadian clock may also play an important role in the control of long-term adaptation in the vasculature such that long-term dysfunction in circadian rhythm may exacerbate the disease process (Figure 2).

The Aging Clock

Indeed, time is of the essence as it pertains to the circadian clock, and its impact reaches beyond 24-hour intervals. Aside from the circadian clock acting to influence long-term processes like remodeling and angiogenesis, the passage of time in the long term also conditions the circadian clock with important ramifications in the vasculature. Bmal1-KO mice exhibit a phenotype of premature aging manifesting in hindlimb arthropathy,[79] increased end-organ disease,[80] and increased mortality.[81] In the vasculature, aging has been shown to have a reciprocal interaction with the circadian clock whereby aging stunts the circadian clock and circadian clock dysfunction accelerates senescence.[82] Bmal1 and Per2 oscillation and expression are blunted in high-passage senescent human aortic smooth muscle cells and aortas from old wild-type mice,[82] whereas endothelial cells and aortic tissue of Per2-mutant mice have elevated markers of senescence.[38] Moreover, the circadian rhythm in phosphorylated eNOS is attenuated in aged mice, and Per2 oscillation is attenuated in aortas and vascular cells of aged wild-type mice and eNOS-KO mice,[61] suggesting that there is an age-conditioned relationship between NO and the circadian clock that exhibits reciprocity. Because locomotor rhythm, ie, central clock function, is normal in neuronal NOS–KO[69] and eNOS-KO mice,[70] endogenous NO may exert a vascular-specific role in the control of the circadian clock and thus may present as a "silent" dysfunction. Indeed, there is evidence for other circadian disorders that are not necessarily linked with behavioral abnormalities. Of particular relevance, nondipping hypertensive patients lack the nighttime fall in blood pressure but exhibit no abnormal patterns of locomotor rhythm.[83] Future consideration needs to be given to the assessment of silent circadian dysfunction in the vascular system and other peripheral tissues, which may play an underappreciated role in pathogenic processes.

Conclusions

Circadian rhythms are a primitive survival mechanism, empowering organisms with the ability to anticipate light to harvest its energy or alternatively to avoid its damaging ultraviolet radiation. The circadian clock, the molecular basis of circadian rhythms, permits single-cell organisms to receive temporal environmental input but has further evolved in its capacity and complexity to anticipate even in cells without direct photic input, as occurs in the vasculature of the mammalian system. Blood vessels contain a circadian clock that is identical to that in the central nervous system, but it has unique features and signaling components that govern function. Emerging data now demonstrate that the circadian clock has an elaborate role in the control of vascular cell signaling and function. In addition to affecting the daily cycles of endothelial function, blood pressure, and hemostasis, the circadian clock conditions the long-term adaptation of blood vessels during angiogenesis and remodeling. Behavioral modifications that directly affect central clock function such as shift work and sleep disorders may impinge on vascular homeostasis through the release of humoral or endocrine signals that are relayed to the periphery. However, circadian dysfunction may extend beyond these locomotor anomalies. Silent worsening of circadian clock function occurs locally in blood vessels at least in part as a result of advancing age as circadian clock protein levels and subsequent rhythm diminish. One direct impact is on the vasculature, impairing vascular cell signaling and function, but worsening clock function may also affect blood vessels by impairing extravascular tissues. Glucose[84] and lipid homeostasis[85] and adiposity[86] are under clock control, and mice with aberrant clock function exhibit features of the metabolic syndrome.[87] Thus, circadian clock dysfunction is rapidly emerging as an important molecular link in the progression of vascular disease, and its pervasive impact may underlie mechanisms that contribute to multisystemic disorders such as the metabolic syndrome.

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Disclosures

None.

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R. Daniel Rudic

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