

Disruption of normal circadian rhythms and cardiovascular events

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Conflicts of interest: None.

Abstract

The intrinsic properties of the heart and the vascular tree exhibit marked oscillations over 24 h. Diurnal variations in the response of the cardiovascular system to environmental stimuli are mediated by the complex interplay of extracellular (ie, neurohumoral factors) and intracellular (ie, circadian clock) influences. The intracellular circadian clock comprises a series of transcriptional modulators that together allow the cell to “perceive” the time of day, thus enabling suitable responses to expected stimuli. These molecular timepieces have been identified and characterized within both vascular smooth muscle cells and cardiomyocytes, giving rise to a multitude of hypotheses regarding the potential role of the circadian clock as a modulator of physiological and pathophysiological cardiovascular events. This article summarizes the evidence available at present linking circadian rhythm disruption and cardiovascular disease.

■ *Heart Metab.* 2009;44:11–15.

Keywords: Circadian clock, diurnal variations, cardiovascular disease

Introduction

Circadian clocks have been identified in every mammalian cell investigated to date, including key components of the cardiovascular system, such as cardiomyocytes and vascular smooth muscle cells [1–4]. There is universal appreciation of the presence of diurnal variations in cardiovascular function, in both physiological and pathophysiological circumstances. The recent identification [5] of a molecular “machinery” within cells in the cardiovascular system, with the potential to modulate an array of cellular processes, has sparked increasing interest among researchers. Historically, diurnal variations in blood pressure, heart rate, and cardiac output, in addition to major cardiovascular events (ie, myocardial infarction and sudden death), have been attributed primarily to the occurrence of circadian changes in the autonomic

nervous system – that is, sudden increase in sympathetic activity [6,7]. However, it has become apparent that changes in the ability of the cardiovascular system to respond to neurohumoral stimuli in an appropriate and timely manner are likely to be of equal importance.

Intracellular circadian clocks provide a means by which the heart and the vasculature can “anticipate” diurnal variations in stimuli, such as autonomic nervous activity, ensuring an optimal response. The attenuation or malfunction of this molecular mechanism could therefore impair the ability of the heart, the vasculature, or both, to respond appropriately to environmental stimuli, which in turn may contribute to the development of cardiovascular disease. This article summarizes current knowledge regarding circadian clocks within the cardiovascular system, the biological processes they influence, and how a

disturbance of these circadian rhythms can lead to cardiovascular disease.

The central circadian clock

Almost all living organisms have developed biological rhythms linked to the day/night or light/dark cycles of the sun [8]. The impact that such rhythms that follow the time of day and season of the year exert on a variety of physiological functions in humans has been recognized for a long time [8]. The internal oscillator, or control station regulating the body's circadian clock, is the suprachiasmatic nucleus, a tiny structure (comprising approximately 70 000 neurons) located in the hypothalamus, above the optic chiasm [9]. The suprachiasmatic nucleus processes external signals such as ambient light and inputs from the brain to regulate a variety of cyclic functions, including body temperature, sleep/wake cycles, and secretion of hormones such as cortisol, melatonin, thyroxin, and vasopressin [8].

Evidence gathered over the past 15 years suggests that melatonin influences several functions of the cardiovascular system. Similar to other organs and systems, the cardiovascular system exhibits diurnal and seasonal rhythms in heart rate, cardiac output, and blood pressure [10], which are likely to be modulated by the suprachiasmatic nucleus and, possibly, the melatonergic system. The circadian pacemaker within the suprachiasmatic nucleus stimulates the pineal gland to produce melatonin at night. This process is set by the phase-shifting actions of light, such that normal physiological plasma concentrations of melatonin during the day are very low, but they begin to increase at night, from around 22.00 h onwards, with a peak at approximately 03.00 h, and a return to daytime values by 09.00 h. Endogenous production of melatonin is approximately 30 µg per day [11], associated with peak plasma concentrations of approximately 100 pg/mL, although these are highly variable among individuals, and decrease with age [12].

The enterochromaffin cells within the gastrointestinal tract also synthesize melatonin; this probably accounts for the normal low daytime plasma concentrations of this hormone, and is not under the circadian control of the suprachiasmatic nucleus [13].

Melatonin and cardiovascular disease

In previous studies, our group of investigators has reported a relationship between melatonin and light/dark variations in the production of inflammatory systemic markers such as interleukin-6, C-reactive protein, matrix metalloproteinase-9, and soluble vascular cell adhesion molecule-1 [14–17]. We have also

demonstrated, in a prospective study in patients with ST-segment elevation myocardial infarction, that melatonin may have a prognostic role. These findings, taken together, appear to suggest that patients who develop adverse events (heart failure or cardiac death) have significantly lower nocturnal concentrations of melatonin than patients who do not experience such events [18]. Moreover, in a recent study in patients with ST-segment elevation myocardial infarction undergoing primary percutaneous coronary intervention, we observed a relationship between melatonin concentrations and ischemia-modified albumin, a marker of myocardial ischemia. Our data suggest that melatonin acts as a potent antioxidant, reducing myocardial damage induced by ischemia-reperfusion [19].

Expression of circadian clock genes in the heart

Oscillations in gene expression in the heart have been examined extensively in mouse models, using both real-time polymerase chain reaction and expression array analysis [20,21]. Genes encoding both core clock components and clock-controlled genes, relevant to cardiac function, have demonstrated dramatic oscillations in heart tissue isolated at intervals throughout the circadian cycle [22]. Included in these oscillating transcripts are genes relevant to carbohydrate utilization, mitochondrial function, and fatty acid metabolism [23].

Although the circadian clock within the heart drives cardiac physiology, the function of this clock can be disrupted in pathological conditions. In a model of experimentally induced cardiac hypertrophy, the core molecular oscillator continues to cycle, but the amplitude of oscillations in transcription factors such as D-element binding protein are blunted [24], and the circadian cycle of expression of metabolic genes is lost. Hence, the tissue would be less prepared to respond to increases in physiological demand, predisposing it to metabolic abnormalities. Streptozotocin-induced diabetes in the rat is another model of contractile dysfunction that alters clock gene expression in the heart: clock component oscillations show normal amplitude, but their phase is advanced by approximately 3 h in this model [25]. Thus the impact of disease states on the cardiac circadian clock seems to be at the levels both of circadian clock genes and of clock-controlled output genes relevant to tissue-specific functions.

Circadian rhythms within the cardiovascular system

Intracellular circadian clocks exist within at least two major cells types in the cardiovascular system, namely

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cardiomyocytes and vascular smooth muscle cells. This molecular mechanism is present in all mammalian cell types, but it has not been fully characterized in, for example, endothelial cells [26]. Circadian clocks within individual cells of the cardiovascular system can influence physiological cardiovascular responses – for example, increasing sympathetic nervous activity before awakening – thereby ensuring an appropriately rapid response when required. In the in-vivo setting, a complex interplay between environmental influences and intrinsic mechanisms (ie, central and peripheral circadian clocks) exists that contributes to changes in cardiovascular function over the course of the day (Figure 1) [27].

Circadian rhythms in blood pressure that occur in humans [28–31] are lowest at night, reaching a trough at around 03.00 h and a peak at around 09.00 h. A second peak in blood pressure is often seen early in the evening (19.00 h) [11]. Day-to-night differences in physical and mental activity appear to be major determinants of the circadian rhythms in blood pressure [28,32]. In humans, shift workers show an essentially complete resynchronization of blood pressure rhythms within the first 24 h of the shift rotation [33,34]. Rhythms in heart rate appear to be driven largely by diurnal variations in autonomic nervous system activity [35,36]. Several lines of evidence suggest that the circadian rhythms of these two cardiovascular parameters might be differentially regulated. Hu et al [37] have reported that, in humans,

circadian rhythmicity in heart rate variability persisted, peaking in the early hours of the morning, even when their analyses controlled for sleep/wake and behavior cycles. Such studies expose an intrinsic component influencing normal cardiovascular function. It could be hypothesized that diurnal variations in autonomic stimulation (driven by the suprachiasmatic nucleus) and circadian-clock-driven diurnal variations in responsiveness of the heart to autonomic stimulation act together as major determinants of heart rate circadian rhythms. Environmental modulation of the synchronization between peripheral and central clocks may contribute to the development of cardiovascular disease; however, this remains speculative at present. A loss of synchronization of this type occurs in patients with diabetes mellitus, obesity, and sleep apnea, and in shift workers, all of whom are associated with increased risk for cardiovascular disease [27].

It has been shown that shift workers experience physical and psychological changes during the night. The exact mechanisms leading to these changes and their clinical impact are poorly understood at present. Stress-related biological variables, such as cortisol and body temperature, have a circadian pattern characterized by increased values during daytime, when the individual is active (awake), and lower values during the sleeping hours [38]. Some studies have shown a strong influence of physical activity levels on the circadian changes in heart rate and blood pressure [39–41].

Furlan et al [42] reported that continuous weekly changes in time of maximum and minimum output in the cardiac sympathetic and vagal autonomic control may play a part in the excessive rate of cardiovascular disease in shift workers. Circadian changes in autonomic activities have been postulated to be one of the reasons for the increased incidence of ischemic heart disease, stroke, and sudden death [6] in these individuals.

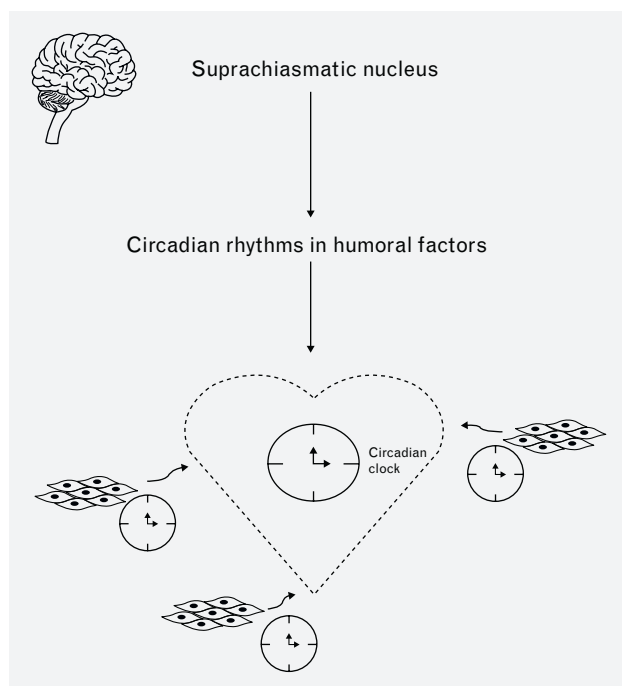


Figure 1. Circadian rhythms in the intrinsic properties of the heart are potentially mediated by intracellular (ie, circadian clock within the cardiomyocyte) or extracellular (ie, neurohumoral) influences, or both.

The circadian clock in physiological and pathophysiological states

Circadian clocks are altered in various animal models of cardiovascular disease. Young et al [23,24] found that the rhythmic expression of genes regulated by the circadian clock (eg, *dbp*, *hlf*, *tef*, *pdk4*, and *ucp3*) is significantly attenuated in the rat heart during left ventricular hypertrophy induced by pressure overload. Consistent with these observations, Mohri et al [43] reported that oscillations in circadian clock genes are severely attenuated in the hypertrophic hearts of Dahl rats fed a high-salt diet (an animal model of hypertension). In contrast, Naito et al [44] reported an augmentation of circadian clock gene oscillations in

the aortae and hearts from a different rat model of hypertension, the spontaneously hypertensive rat, which is associated with amplified rhythms in *pai-1*.

In humans, circadian rhythms in pathophysiological cardiovascular events are also well documented. We have known for a number of years that circadian fluctuations affect, and perhaps even orchestrate, a variety of pathophysiological states. The onset of myocardial infarction, sudden cardiac death, and stroke, are all increased between the hours of 06.00 h and 12.00 h. These responses may be related, at least in part, to increased sympathetic activity after an individual gets out of bed, the interaction between catecholamines and platelets thus affecting atherosclerotic plaque pathophysiology [6]. Circadian variations have also been observed in relation to hemodynamic responses, including blood pressure, myocardial blood flow, and heart rate, and cardiovascular events. In addition, circadian alterations have been documented in the response of platelets to aggregating stimuli, the concentration of plasma fibrinogen and coagulation factors, and the activity of the fibrinolytic system [6].

Implications for research

The existence of circadian clocks within components of the cardiovascular system has far-reaching implications, which extend beyond the clinical setting. Given the diversity of diurnal variations in the intrinsic properties of the cardiovascular system, which manifest at several levels, namely gene and protein expression and cellular and organ function, extreme caution is required when research studies are being designed. Both in-vivo and in-vitro studies may be affected by circadian variations, therefore considering time of day may be important in the design of research experiments. Performance of experiments at an inappropriate time of the day or the omission of suitable time controls may lead to erroneous conclusions or uninterpretable data. Such temporal considerations will undoubtedly help to reduce discrepancies between studies performed in different laboratories, and also discrepancies between gene and protein expression measurements and animal and human models.

Conclusions

Circadian rhythms are regulated by three components: (1) the circadian pacemaker or “clock”, (2) an “input” mechanism, which allows the clock to be reset by environmental stimuli, and (3) an “output” mechanism, which regulates physiological and behavioral processes. For many years, it has been accepted that neurons in the suprachiasmatic nucleus

were responsible for the control of circadian rhythms in peripheral tissues, acting via neural and humoral signals (eg, melatonin). It is currently believed that cells in other systems, including the cardiovascular system (ie, cardiomyocytes and vascular smooth muscle cells), are under the influence of circadian clocks similar to that in the suprachiasmatic nucleus.

Many aspects of cardiovascular physiology are subject to diurnal variations, and serious adverse cardiovascular events, including myocardial infarction and sudden cardiac death, appear to be conditioned by the time of day. It has therefore been suggested that biological responses, which are under the control of the “molecular clock”, may interact with environmental cues to influence the phenotype of human cardiovascular disease. Thus numerous mediators of cardiovascular disease display a circadian variation, and evidence for molecular clock regulation of these mediators is beginning to unfold. The mechanisms that regulate the circadian clock and the clinical implications of disturbances in circadian rhythm remain a fertile field of investigation. ■

REFERENCES

1. Davidson AJ, London B, Block GD, Menaker M. Cardiovascular tissues contain independent circadian clocks. *Clin Exp Hypertens*. 2005;27:307–311.
2. Durgan DJ, Hotze MA, Tomlin TM, et al. The intrinsic circadian clock within the cardiomyocyte. *Am J Physiol Heart Circ Physiol*. 2005;289:H1530–H1541.
3. Ederly I. Circadian rhythms in a nutshell. *Physiol Genomics*. 2000;3:59–74.
4. McNamara P, Seo SP, Rudic RD, Sehgal A, Chakravarti D, Fitzgerald GA. Regulation of CLOCK and MOP4 by nuclear hormone receptors in the vasculature: a humoral mechanism to reset a peripheral clock. *Cell*. 2001;105:877–899.
5. Quyyumi AA. Circadian rhythms in cardiovascular disease. *Am Heart J*. 1990;120:726–733.
6. Muller JE, Tofler GH, Stone PH. Circadian variation and triggers of onset of acute cardiovascular disease. *Circulation*. 1989;79:733–743.
7. Prinz PN, Halter J, Benedetti C, Raskind M. Circadian variation of plasma catecholamines in young and old men: relation to rapid eye movement and slow wave sleep. *J Clin Endocrinol Metab*. 1979;49:300–304.
8. Reiter RJ. The melatonin rhythm: both a clock and calendar. *Experientia*. 1993;49:654–664.
9. Damiola F, Le Minh N, Preitner N, Kornmann B, Fleury-Olela F, Schibler U. Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus. *Genes Dev*. 2000;14:2950–2961.
10. Sewerynek E. Melatonin and the cardiovascular system. *Neuro Endocrinol Lett*. 2002;23:79–83.
11. Lane EA, Moss HB. Pharmacokinetics of melatonin in man: first pass hepatic metabolism. *J Clin Endocrinol Metab*. 1985;61:1214–1216.
12. Sharma M, Palacios Bois J, Schwartz G, et al. Circadian rhythms of melatonin and cortisol in aging. *Biol Psychiatry*. 1989;25:305–319.
13. Bubenik GA. Gastrointestinal melatonin: localization, function, and clinical relevance. *Dig Dis Sci*. 2002;47:2336–2348.
14. Dominguez-Rodriguez A, Abreu-Gonzalez P, Garcia M, et al. Light/dark patterns of interleukin-6 in relation to the pineal hormone melatonin in patients with acute myocardial infarction. *Cytokine*. 2004;26:89–93.

Main clinical article

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15. Dominguez-Rodriguez A, Garcia-Gonzalez M, Abreu-Gonzalez P, Ferrer J, Kaski JC. Relation of nocturnal melatonin levels to C-reactive protein concentration in patients with ST-segment elevation myocardial infarction. *Am J Cardiol.* 2006;97:10–12.
16. Dominguez-Rodriguez A, Abreu-Gonzalez P, Garcia-Gonzalez MJ, Reiter RJ. Relation of nocturnal melatonin levels to serum matrix metalloproteinase-9 concentrations in patients with myocardial infarction. *Thromb Res.* 2007;120:361–366.
17. Dominguez-Rodriguez A, Abreu-Gonzalez P, Garcia-Gonzalez MJ, Samimi-Fard S, Kaski JC, Reiter RJ. Light/dark patterns of soluble vascular cell adhesion molecule-1 in relation to melatonin in patients with ST-segment elevation myocardial infarction. *J Pineal Res.* 2008;44:65–69.
18. Dominguez-Rodriguez A, Abreu-Gonzalez P, Garcia-Gonzalez M, Reiter RJ. Prognostic value of nocturnal melatonin levels as a novel marker in patients with ST-segment elevation myocardial infarction. *Am J Cardiol.* 2006;97:1162–1164.
19. Dominguez-Rodriguez A, Abreu-Gonzalez P, Garcia-Gonzalez MJ, Samimi-Fard S, Reiter RJ, Kaski JC. Association of ischemia-modified albumin and melatonin in patients with ST-elevation myocardial infarction. *Atherosclerosis.* 2007;199:73–78.
20. Storch KF, Lipan O, Leykin I, et al. Extensive and divergent circadian gene expression in liver and heart. *Nature.* 2002;417:78–83.
21. Curtis AM, Seo SB, Westgate EJ, et al. Histone acetyltransferase-dependent chromatin remodelling and the vascular clock. *J Biol Chem.* 2004;279:7091–7097.
22. Reilly DF, Westgate EF, FitzGerald GA. Peripheral circadian clocks in the vasculature. *Arterioscler Thromb Vasc Biol.* 2007;27:1694–1705.
23. Young ME, Razeghi P, Cedars AM, Guthrie PH, Taegtmeyer H. Intrinsic diurnal variations in cardiac metabolism and contractile function. *Circ Res.* 2001;89:1199–1208.
24. Young ME, Razeghi P, Taegtmeyer H. Clock genes in the heart: characterization and attenuation with hypertrophy. *Circ Res.* 2001;88:1142–1150.
25. Young ME, Wilson CR, Razeghi P, Guthrie PH, Taegtmeyer H. Alterations of the circadian clock in the heart by streptozotocin-induced diabetes. *J Mol Cell Cardiol.* 2002;34:223–231.
26. Nagoshi E, Brown SA, Dibner C, Kornmann B, Schibler U. Circadian gene expression in cultured cells. *Methods Enzymol.* 2005;393:543–557.
27. Young ME. The circadian clock within the heart: potential influence on myocardial gene expression, metabolism, and function. *Am J Physiol Hear Circ Physiol.* 2006;290:H1–H16.
28. Degaute JP, van de Borne P, Linkowski P, Van Cauter E. Quantitative analysis of the 24-hour blood pressure and heart rate patterns in young men. *Hypertension.* 1991;18:199–210.
29. Dennler S, Itoh S, Vivien D, ten Dijke P, Huet S, Gauthier JM. Direct binding of Smad3 and Smad4 to critical TGF beta-inducible elements in the promoter of human plasminogen activator inhibitor-type 1 gene. *EMBO J.* 1998;17:3091–3100.
30. Hill L. On rest, sleep and work and the concomitant changes in the circulation of the blood. *Lancet.* 1998;1:282–285.
31. Khatri IM, Freis ED. Hemodynamic changes during sleep. *J Appl Physiol.* 1967;22:867–873.
32. Clark La, Denby L, Pregibon D, et al. A quantitative analysis of the effects of activity and time of day on the diurnal variations of blood pressure. *J Chronic Dis.* 1987;40:671–681.
33. Chau NP, Mallion JM, de Gaudemaris R, et al. Twenty-four-hour ambulatory blood pressure in shift workers. *Circulation.* 1989;80:341–347.
34. Sundberg S, Kohvakka A, Gordin A. Rapid reversal of circadian blood pressure rhythm in shift workers. *J Hypertens.* 1988;6:393–396.
35. Krauchi K, Wirz-Justice A. Circadian rhythm of heat production, heart rate, and skin and core temperature under unmasking conditions in men. *Am J Physiol Regul Integr Com Physiol.* 1994;267:R819–R829.
36. Scheer FA, van Doornen LJ, Buijs RM. Light and diurnal cycle affect human heart rate: possible role for the circadian pacemaker. *J Biol Rhythms.* 1999;14:202–212.
37. Hu K, Ivanov PC, Hilton MF, et al. Endogenous circadian rhythm in an index of cardiac vulnerability independent of changes in behavior. *Proc Natl Acad Sci U S A.* 2004;101:18223–18227.
38. Moore-Ede MC, Richardson G. Medical implications of shift work. *Annu Rev Med.* 1985;36:607–617.
39. Munakata M, Ichii S, Nunokawa T, et al. Influence of night shift work on physiological state and cardiovascular and neuroendocrine response in healthy nurses. *Hypertens Res.* 2001;24:25–31.
40. Baumgart P, Walger P, Fuchs G, Dorst KG, Vetter H, Rahn KH. Twenty-four-hour blood pressure is not dependent on endogenous circadian rhythm. *J Hypertens.* 1989;7:331–334.
41. Sternberg H, Rosenthal T, Shamiss A, Green M. Altered circadian rhythm of blood pressure in shift workers. *J Hum Hypertens.* 1995;9:349–353.
42. Furlan R, Barbic F, Piazza S, Tirelli M, Seghizzi P, Malliani A. Modifications of cardiac autonomic profile associated with a shift schedule of work. *Circulation.* 2000;102:1912–1916.
43. Mohri T, Emoto N, Nonaka H, et al. Alterations of circadian expressions of clock genes in Dahl salt-sensitive rats fed a high-salt diet. *Hypertension.* 2003;42:189–194.
44. Naito Y, Tsujino T, Fujioka Y, Ohyanagi M, Iwasaki T. Augmented diurnal variations of the cardiac renin-angiotensin system in hypertensive rats. *Hypertension.* 2002;40:827–833.