Diurnal physiology: core principles with application to the pathogenesis, diagnosis, prevention, and treatment of myocardial hypertrophy and failure

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Sole MJ, Martino TA. Diurnal physiology: core principles with application to the pathogenesis, diagnosis, prevention, and treatment of myocardial hypertrophy and failure. J Appl Physiol 107: 1318–1327, 2009. First published June 25, 2009; doi:10.1152/japplphysiol.00426.2009.—The circadian system has been shown to be fundamentally important in human health and disease. Recently, there have been major advances in our understanding of daily rhythmicity, and its relevance to human physiology, and to the pathogenesis and treatment of cardiac hypertrophy and heart failure. Cardiovascular tissues, such as heart and blood vessels, show remarkable daily variation in gene expression, metabolism, growth, and remodeling. Moreover, synchrony of daily molecular and physiological rhythms is integral to healthy organ growth and renewal. Disruption of these rhythms adversely affects normal growth, also the remodeling mechanisms in disease, leading to gross abnormalities in heart and vessels. These observations provide new insights into the pathogenesis, diagnosis, treatment, and prevention of heart disease. In this review, we focus on the recent advances in circadian biology and cardiovascular function, with particular emphasis on how this applies to human myocardial hypertrophy and heart failure, and the implications and importance for translational medicine.

circadian; clock; chronobiology; cardiomyopathy; cardiac remodeling; cardiovascular

Life on Earth has evolved under the evolutionary pressure of Earth’s 24-h day-night cycle. Before the 20th century and the absence of substantive artificial lighting, human activity was synchronized to natural light-dark (L/D) rhythms, and adequate sleep was a cornerstone of the therapy of disease. Two giants, Claude Bernard in France and Walter Cannon in America primarily influenced the tenets of modern physiology; both championed the importance of homeostasis in modern medicine. This concept of maintenance of biological steady state so dominated medical thinking that the importance of biological rhythms, the apparent antithesis of steady state fell into the shadows. Both homeostasis and biological rhythms are keystones of normal physiology, yet contemporary society appears to have lost interest in these physiological foundations of good health, as it focuses on 24/7 schedules and the therapies of modern medicine.

Heart failure has emerged as a major health problem during the past 3 decades (48, 60). In spite of our advances, presently available therapeutic interventions have had only limited success in improving the long-term survival of patients suffering from this disease. The modern pharmacological therapy of heart failure has been focused at three levels: 1) improving symptoms by the amelioration of fluid overload, and hemodynamic abnormalities; 2) reducing the ongoing loss of cardiomyocytes by decreasing myocardial tension and inappropriate neurohormonal or cytokine stimulation; and 3) preventing the primary causes of myocyte loss or decompensation, such as myocardial infarction or hypertension, by treating risk factors, such as high blood pressure, hyperlipidemia, glucose intolerance, obesity, and smoking.

In recent years, the measurement of neurohormonal rhythms and the subsequent discovery of actual molecular clocks that endow cells with the ability to anticipate physiological demands have renewed scientific interest in the importance of circadian or diurnal rhythms. However, circadian physiology appears to have had little impact on clinical cardiology or indeed medicine; the exception, perhaps, is the growing recognition that obstructive sleep apnea (OSA) is a risk factor for diabetes, obesity, and cardiovascular disease (83). Other papers in this series of reviews will focus on the molecular details of central and peripheral clocks, with a particular emphasis on metabolism, including diabetes. In this review, we will assume a basic understanding of the molecular events, which underlie circadian rhythms (23, 80, 86, 95). We will take a translational...
approach to the physiology of biological rhythms to emphasize their role in the maintenance of cardiovascular structure and especially their relevance to the pathogenesis, prevention, and therapy of myocardial hypertrophy and failure.

Common practice reserves the term diurnal to relate to the lighted part of the day, and circadian to mean any 24-h cycle. Cellular molecular circadian clocks are endogenously generated and have a fixed periodicity; however, they are susceptible to modulation (e.g., time of onset) by external environmental zeitgebers (timekeepers). The key zeitgeber for the central or “master” clock in the suprachiasmatic nucleus (SCN) of the hypothalamus is the 24-h day-night rhythm of light and darkness. Feeding and activity are important zeitgebers for peripheral tissues, such as heart and liver (61, 62). Neurohormones particularly relevant to the cardiovascular system, such as melatonin, glucocorticoids, catecholamines, growth hormone, atrial natriuretic factor, angiotensin II, aldosterone, and renin, exhibit diurnal variation (21, 37, 67, 73, 96) and possibly synchronize peripheral tissue molecular circadian clocks to the SCN signal (1, 4, 5, 19, 28, 69, 88, 96).

Humans are diurnal, thus they are active and eat in the light of day and sleep at night when it is dark; this is in contrast to most rodents used in basic rhythms research, which are nocturnal. This human/rodent difference is reflected not only by behavior, but also by rhythmic gene expression (9). This distinction is important, because this review article is ultimately focused on humans, under normal diurnal 24-h L/D cycling (not dim or dark, “pure” circadian) conditions.

DIURNAL RHYTHMS IN NORMAL CARDIOVASCULAR PHYSIOLOGY AND HEART DISEASE

Circadian clocks allow us to entrain to environmental cues and hence anticipate the differing physiological and behavioral demands of daily events (23, 80, 86, 95). We observe the output of these entrained clocks as daily rhythms, such as sleep-wake cycles, body temperature cycles, and cyclic variations in heart rate (HR) and blood pressure (BP).

The temporal profile of HR and BP follows the diurnal variation of our autonomic nervous system and is lowest during the period of vagal dominance at nighttime, during sleep, and in the early morning hours (35, 46). HR and BP increase just before and at wake time, to help sustain cardiac output, and to meet the demands of the working day; they decrease again toward the evening and late at night. Although much of this variation is neurohormonal, reflecting day-night changes in the neuroendocrine and autonomic milieu, some of this variation is intrinsic to molecular rhythms within the cardiomyocyte. Cardiomyocyte-specific clock mutant mice exhibit an attenuation of diurnal HR variability in the absence of any overt conduction system abnormalities (14). Using this model, Bray and colleagues (14) have also shown that the intrinsic myocardial clock contributes to the selection of glucose vs. fatty acid substrate by the cardiomyocyte and plays a role in the regulation of cardiac contractile function, including the response of the heart to an increase in workload. The occurrence of adverse cardiovascular events, such as sudden cardiac death, and myocardial infarction exhibit diurnal variation, with a peak incidence in the morning hours, just before or after awakening (22, 35, 43, 67, 93); there is evidence that this timing is directly linked to the intrinsic circadian clock, as opposed to “the stress of awakening” (22, 43). Diurnal rhythms have also been documented for vasomotor tone, platelet aggregability, and factors involved in thrombosis or thrombolysis (2, 27, 55, 72).

CARDIOVASCULAR GENE EXPRESSION EXHIBITS SIGNIFICANT VARIATION OVER THE DAY-NIGHT CYCLE

Cardiomyocytes do not replicate after development, although recent evidence supports replacement by progenitor cells over a period of many years (6). In contrast, cardiomyocytes turn over their protein contents and lipid membranes every few weeks, in effect, renewing cell structure (16); for example, the half-life of the contractile protein myosin in the heart is considered to be ~15 days (74). Contemporary clinical medicine presumes (incorrectly) that this biology is a continual housekeeping activity, constant over the 24-h day.

Circadian clock gene expression is a property of virtually all tissues, except perhaps the testes (9, 23, 47, 54, 56, 81, 85, 95, 98). The core genetic constituents of the cellular circadian clock, clock, casein kinase 1ε, period (per1, per2), ARNTL (bmal1), Rev/erb-a, and cryptochromes, comprise an autoregulatory feedback loop that cycles approximately every 24 h. These important data had been acquired under pure circadian [i.e., constant (dim) lighting] conditions, controlling external variables using a methodical yet reductionist approach. As most life is exposed to an environment of varying light and dark conditions, we examined global cardiac gene expression in the mouse by microarray and bioinformatics under normal conditions of 24-h L/D cycling (56); the animals also exhibited their normal patterns of activity and feeding behavior. We demonstrated that ~13% of the global cardiac transcriptome cycled under 24-h rhythmic L/D conditions (56). Although we used, for the first time, a global array approach under “natural world” L/D conditions for analyzing the cardiac transcriptome, we note that expression of the core cardiac clock genes per and bmal1 was similar to that reported earlier on by Young and colleagues using semiquantitative polymerase chain reaction (98), which helped to validate our approach (56). Indeed, all of the core components of the cellular circadian clock were expressed in the heart appropriately; radar diagrams illustrated two principal gene expression peaks: one in the light phase, and a second in the dark (Fig. 1A). Interestingly, a third subset of genes showed remarkably abrupt change in expression only at L/D transition times. Gene expression profiles were classified using the Gene Ontology Consortium and were found to map to key biological processes, particularly those linked to cardiac metabolism, growth and remodeling, transcription, translation, and molecular signal pathways (56). Bray et al. (14) have recently shown that many genes expressed in the heart are under direct control of the cardiomyocyte circadian clock.

We similarly examined gene expression in aorta under normal diurnal conditions of L/D cycling, using a microarray and bioinformatics approach (58). This also revealed two major peaks in rhythmic gene expression (one in the light and one in dark), although notably those peaks occurred at slightly different times than the ones in the heart. A third minor peak in the aorta occurred in the dark (Fig. 1B). Rudic et al. (81) have also reported circadian rhythms in the expression of genes in the mouse aorta. They consolidated their data in functional cassettes and demonstrated these rhythms were particularly
relevant to genes associated with vascular structural integrity and metabolism. Thus, taken together, these studies clearly demonstrate that gene expression in the heart and vasculature is remarkably different day vs. night.

We then examined diurnal gene expression in compensatory cardiac remodeling. For this study (58), we used a model of pressure-overload myocardial hypertrophy produced by transverse aortic constriction (TAC) in the mouse. Although the amplitude of expression for some genes was augmented or decreased, rhythmic expression of core cycling genes in the TAC mice under L/D conditions was virtually superimposable in time (Fig. 1, C and D), that is, the core cycling transcriptome retained period and phase in TAC heart compared with normal or sham-operated heart. Similarly, for TAC aorta subject to high pressure (above the ligature) or low pressure (below the ligature), the core cycling transcriptome maintained the same rhythmic profile as in normal (or sham-operated) vasculature. In addition, we noted the expression of many genes, not exhibiting a cosinor rhythmic pattern in either sham or TAC heart, that not only differed between sham and TAC, but also exhibited abrupt, reproducible transitions, with some active only during the day and some only at night; that is, the difference in expression between sham and TAC was evident only either at night or during the day.

Fig. 1. Rhythmic gene expression in heart and aorta is examined by microarray and bioinformatics (COSOPT) analyses and plotted on radar diagrams. A: global gene expression in the heart shows a biphasic pattern with two major peaks: one in the light phase, and one in the dark phase. B: the aorta similarly reveals two major peaks in gene cycling, one in the light and one in the dark, although, notably, these peaks occur at slightly different times than those for the heart. There is also a third minor peak in the aorta that occurs in the dark. C and D: microarray data from further studies demonstrate conserved global gene rhythms in heart/vessel disease [transverse aortic constriction (TAC) heart]. Temporal profiles of TAC heart (C) and TAC aorta (D) are shown. Phase histograms show only the genes with robust cycling profiles, as analyzed using the COSOPT bioinformatics program. Rhythms help coordinate thousands of biochemical and biophysical pathways and responses daily. Presumably, the specificity of peak and phase helps ensure that processes occur during a biologically optimal time of day for each tissue/organ as needed. Images are adapted from our papers; for further details, see Refs. 56, 58.

Young et al. (98) in the TAC rat and Mohri et al. (63) in the salt-fed Dahl salt-sensitive hypertensive rat have also reported conservation of phase for clock gene expression. Naito et al. (68) studied the circadian variation of gene expression for various components of the renin-angiotensin system in spontaneously hypertensive rats (SHR) and control Wistar-Kyoto (WKY) rats. The amplitude of mRNA expression of renin, angiotensinogen, angiotensin-converting enzyme (ACE), and angiotensin type 1a and type 2 receptors was greater in the SHR compared with WKY, particularly during the dark phase. The phase relationships of the expression of these genes between WKY and SHR were complex. It was reported that ACE activity did not track the rhythm of ACE mRNA gene expression; however, only two points of time were assessed. If one looks at their ACE gene expression profiles and then accounts for an ~6 h delay between gene and protein expression, this observation could also be explained as an artifact of their chosen measurement times. Diabetes may result in phase alterations of the circadian clock; this has been reported for the expression of circadian clock genes in the streptozotocin-induced diabetic rat (99) and for BP circadian rhythm in the diabetic db/db mouse (87).

Although direct links between the circadian clock and myocardial growth and remodeling have not yet been defined, several promising avenues of investigation have opened recently. The protein of the clock gene has been found recently within the myofilament Z-disk colocalizing with α-actinin; also, myocyte contractility can directly alter the subcellular distribution of Clock protein (11, 77). Clock protein is also implicated in chromatin remodeling, histone acetylation (29), and possibly the modification of other nuclear and cellular proteins (34). Indeed, there is a growing belief that many hundreds of genes may be under the direct regulation of the local molecular clockwork. For example, ornithine decarboxylase, an enzyme associated with muscle cell growth and protein synthesis, exhibits significant circadian variation within the myocardium (91).

Another link between cardiac hypertrophy or remodeling and the circadian clockwork may be through glycogen synthase kinase-3β (GSK-3β). GSK-3β has recently been discovered to be an integral component of the mammalian circadian clock, perhaps promoting the nuclear translocation of PER2 advancing (GSK-3β increased) or delaying (GSK-3β decreased) clock phase (45). GSK-3β has been demonstrated to stabilize Rev-erb-α protein and, through it, clock gene regulation (94). It is of particular interest for the present discussion that GSK-3β also negatively regulates cardiac hypertrophy; activation of GSK-3β by phosphorylation at the serine 9 residue antagonizes the cardiac hypertrophic response to stimuli, such as pressure overload or catecholamine stimulation (36).

THE HEART RENEWS AND REMODELS DURING SLEEPING HOURS

These data suggest that cardiovascular growth and remodeling is dynamic and does not occur uniformly over the day-night cycle; the patterns seen also suggest that myocardial growth and renewal is most active during the period normally allocated to sleep (the subjective night). Also, differential incorporation of labeled leucine into rat myocardial protein over 24 h indicates that myocardial protein...
may be synthesized at the greatest rate late in the light period (rats asleep) with the least synthesis occurring 12 h later (rats active) (79). Although this paper was published in 1975, the authors perspicaciously concluded, “These preliminary studies are not conclusive but they support the hypothesis that there is a circadian rhythm of protein synthesis in the heart.” Neurohormones with anabolic activity relevant to the cardiovascular system, such as growth hormone, atrial natriuretic peptide, aldosterone, angiotensin II, renin, and proopiomelanocortin, exhibit diurnal variation (18, 21, 37, 67), with gene and/or protein expression peaking during sleeping hours. Rat hearts isolated during the subjective day (dark phase) and perfused ex vivo exhibit greater cardiac power than those isolated during the subjective night (97); the capacity for carbohydrate oxidation and oxygen consumption was also increased during the subjective day.

Assessment of all of the molecular data described above are consistent with our hypothesis that myocardial renewal and growth is diurnal, with significant activity occurring during the subjective night when HR and BP are at their lowest and physiological stress is at minimum. Cell energy and resources then can be turned from coping with external physiological demands toward cellular repair and growth.

There is also considerable support at the clinical level for the concept that myocyte maintenance or growth predominantly takes place during sleep time. Normal human BP, when taken across the diurnal cycle, exhibits a 10% decrease at night, with a pressure surge in the morning, just before and on awakening (25, 39, 46). Patients with hypertension fall into two primary groups of BP profile (25, 39). One group parallels the cyclic variation in pressure exhibited by normotensive subjects, including the nocturnal “dip” in BP, but at an overall elevated level. A second group, known as “nondippers,” shows a failure to decrease BP by 10%, with a few even exhibiting a nocturnal increase. The nondipper (nocturnal hypertension) group exhibits an increased risk of target organ damage, with greater left ventricular hypertrophy and an increased risk of cardiovascular and renal disease (25, 39, 90). Moreover, although infarcts are more common just before or in the morning after awakening, myocardial infarcts occurring during in the middle of nighttime sleep are larger, exhibiting a greater risk of heart failure than those experienced during the day (66). Furthermore, there is evidence documenting the adverse effects of OSA on myocardial structure (12, 13, 83). Continuous positive airway pressure, even though applied only during sleep, yields permanent long-term benefits, including reverse (beneficial) cardiac remodeling (12, 13). Finally, conversion from conventional to nocturnal hemodialysis results in significant regression of left ventricular hypertrophy in patients with end-stage renal disease (20). All of these data suggest that the heart is most susceptible to remodeling, or renews, significantly during the sleeping hours.

**DRUG THERAPIES BENEFITTING CARDIOVASCULAR STRUCTURE MAY BEST BE ADMINISTERED DURING THE SUBJECTIVE NIGHT**

Chronotherapeutic strategies in clinical medicine have been employed on a largely empirical basis or on drug clearance and metabolism data. Low-dose evening aspirin has been shown to have a mild antihypertensive effect and less gastric irritability than the same dose taken in the morning (40). The importance of administration time-dependent effects of treatment on BP profile has been well reviewed (41). Discovery of tissue clocks has paved the way for more basic mechanistic studies. For example, a recent study has demonstrated a link between tissue sensitivity to cyclophosphamide chemotherapy and the molecular state of the tissue circadian clock (33). In the clinical realm, oncology studies have demonstrated diurnal variation in tumor tissue growth and sensitivity to chemotherapy; high-dose radiotherapy causes less high-grade oral mucositis and less weight loss when administered in the morning vs. the late afternoon in patients with head and neck cancer (7–10). The outcome of coronary artery angioplasty at night is less successful than that during the day, even when accounting for shift variables, such as operator fatigue (26, 32, 38).

The hypothesis that the heart and perhaps blood vessels remodel at night provides a molecular rationale for the temporal targeting of remodeling. Our analysis of gene expression cycling in the normal murine heart and aorta, and also these same tissues in murine TAC (58), showed phase conservation of the core clockwork genes in heart and vasculature, but with increased expression of the “output” genes involved in cardiac responses, such as BP homeostasis, myocyte hypertrophy, and tissue remodeling, including ACE. ACE inhibitors (ACEi) are central to abating or reversing adverse myocardial remodeling in patients with hypertension, heart failure, or postmyocardial infarction. These benefits of ACEi were originally demonstrated in cognate animal models, including the ascending aorta clipped/constriction model of hypertensive myocardial hypertrophy (15, 92). Thus we investigated the diurnal efficacy of the short-acting ACEi, captopril, given by intraperitoneal injection, on cardiac remodeling in TAC mouse (89). Captopril, given when the mice normally slept, significantly improved cardiovascular function and reduced adverse remodeling. Remarkably, captopril administered during the murine waking/active hours did not have this effect; indeed, cardiac outcome was as poor as in TAC mice given vehicle alone. Morgan et al. (65) reported similar data in the one-clipping renal hypertensive rat. Captopril, 75 mg/kg per day, consumed in the drinking water, effectively lowered BP over most of the 24 h; captopril given intraperitoneally (15 mg/kg per day) at the onset of the subjective night produced an identical decrease in BP, but only for a few hours. Both doses produced an identical regression of cardiac hypertrophy, despite the markedly shorter duration of the sleep time drug. The same laboratory (64) also reported the converse experiment. BP in rats was elevated briefly during sleep time by intraperitoneal injections of angiotensin II during the early subjective night or increased continuously by 24-h infusion; achieved BP peaks were identical. Despite the great difference in exposure, both groups exhibited the same cardiac enlargement. These data further support the hypothesis that cardiovascular growth and remodeling are rhythmic and occur predominantly during sleeping hours. They also underscore the importance of diurnal considerations to maximize the benefit of ACEi in the treatment of cardiovascular disease; moreover, these chronotherapeutic benefits should be particularly apparent for ACEi (or angiotensin receptor antagonists) with shorter half lives.
DISTURBED DIURNAL RHYTHMS EXACERBATE CARDIOVASCULAR DYSFUNCTION

The 20th century saw the development of ubiquitous artificial lighting, rapid long-distance transmeridian air flight, multinational communications, the internet, and e-mail. Social and vocational activity was 24/7, no longer dependent on our diurnal environment. Recently, new central stimulants, such as Mondafanil, promise “the conquest of sleep” (53). Society and contemporary medicine regard adequate and regular sleep time as primarily for neuropsychological health. Yet regular day-night cycles, adequate sleep time, and synchrony between external and internal environment also seem to be critical for cardiovascular health. This would be supported by the increased prevalence of adverse cardiovascular events found in shift workers, transmeridian flight crews, patients with sleep apnea, and other sleep disturbances (49, 51, 52). Disrupted sleep-wake cycles in these conditions have been reported to lead to resistant hypertension, obesity, abnormal lipid profiles, glucose intolerance, and increases in oxidative stress and circulating inflammatory factors, an ideal biological milieu for atherosclerotic heart disease and stroke (31, 82, 84). Animal models also exhibited increased mortality, if not allowed to adapt to the external L/D environment. For example, mice exposed to phase advances of the L/D cycle that mimicked chronic jet-lag exhibited higher mortality than unshifted control mice or even those exposed to phase delays (24). Repeated phase shifts in the L/D cycle also reduced longevity in cardiomyopathic Syrian hamsters (76).

OSA has been the subject of intensive study and has been clearly linked as a culprit in the pathogenesis of cardiac arrhythmias, high BP, coronary artery disease, myocardial dysfunction, obesity, and diabetes (12, 13, 83). The pathology resulting from OSA has been associated with increases in inflammatory factors, nocturnal hypertension, increased ventricular transmural pressure from the negative intrathoracic pressure during apneic periods, and abnormalities in lipid and glucose metabolism (12, 13, 83). OSA also significantly disrupts sleep, and continuous positive airway pressure helps to restore it. Disruption in normal diurnal cycling, itself, may be a major contributor to the adverse effects of OSA in patients with and without preexisting cardiac disease.

Using our murine TAC model of pressure overload cardiac hypertrophy, we examined the effects of a rhythm-disturbed environment on cardiovascular pathology (Fig. 2, A–F) (58). In these animals, transmural pressure is increased in the left ventricle and thoracic aorta in the presence of sleep disruption; thus this model displays some of the physiological characteristics of OSA. The “rhythm-disturbed” TAC mice were housed

Fig. 2. Diurnal rhythms in cardiac remodeling genes. A–F: the TAC heart disease model. A: normal heart gross morphology; B: normal cardiac myocytes (hematoxylin and eosin stain); C: normal myocardium (Masson’s); D: TAC heart is greatly increased in size due to pressure overload; E: myocyte growth (hypertrophy) is evident; also F: cardiac fibrosis (blue) throughout the myocardium. G: diurnal rhythms in remodeling genes atrial natriuretic factor (ANF), brain natriuretic peptide (BNP), angiotensin-converting enzyme (ACE), and Col3a1 are altered in a 20-h rhythm-disrupted environment, compared with a normal 24-h environment, and this exacerbates (worsens) the cardiac remodeling and heart disease. Return to a normal 24-h environment [reverse (Rev)] restores gene expression and normal remodeling processes. *P < 0.05. Images are modified from our papers; for further details, see Refs. 56, 58.
in an altered L/D environment (20 h vs. the normal 24-h diurnal cycle). Rhythm-disturbed TAC mice exhibited a complete disruption of sleep/wake, feeding, and activity behavior, unable to consolidate any. These animals exhibited gross increases in left ventricular end-systolic and end-diastolic diameters and reduced contractility of the heart, with an increase in BP, compared with “nonrhythm-disrupted” TAC mice. Also, histology was strikingly abnormal. Despite the increased pressure load, myocyte hypertrophy in both blood vessels and heart was markedly constrained, while fibrous tissue accretion in both vessels (perivascular) and heart was significantly increased. Effectively, both heart and blood vessel walls were inappropriately remodeled relative to the BP burden. The inability to harmonize internal rhythms with the external environment, as demonstrated by their nonconsolidated behavior patterns, was also manifested at the level of the molecular clock. The 20-h TAC mice exhibited abnormal cycling of bmal1 and per2 in the heart; also, there was decreased expression of key genes in hypertrophic pathways, including atrial natriuretic factor, brain natriuretic peptide, ACE, and Col3a1 (a structural collagen), compared with TAC mice in the normal 24-h conditions. However, once the external rhythm was allowed to correspond to the animals’ innate 24-h internal rhythm, the clock normalized, and BP fell to hypertensive levels seen in control TAC mice. Unexpectedly, there was a dramatic and paradoxical increase in myocyte hypertrophy, along with increased expression of hypertrophic genes (Fig. 2G). That is, normal compensatory expression of hypertrophic genes during the period was exhibited. These data demonstrated that desynchronization between external and internal rhythms prevented the appropriate tissue histological and genetic response to a rise in BP. Failure to harmonize internal and external rhythms augmented cardiovascular target organ damage.

Despite all of the epidemiological, physiological, and molecular data above, circadian disorganization alone had never been shown to be a direct and sufficient cause of cardiovascular disease or, indeed, pathology in any organ. We explored the direct long-term effects of rhythm desynchronization on normal organ physiology, such as might occur in humans with recurrent jet lag, chronic sleep disturbance, or shift work (57). We used a prototypic model of circadian rhythm disruption that had been linked with reduced longevity, hamsters carrying a mutation in casein kinase-1ε (τ mutants) (44, 71, 78). The mutant allele reduces the free-running circadian period from 24 h in the wild type to 22 h in τ+/− heterozygotes. When τ+/− (22 h) hamsters are entrained to a 24-h day, there was early onset and significant fragmentation of sleep, activity, and feeding; life expectancy was shortened (44, 71, 78). We demonstrated that these animals, although normal when young, developed profound cardiac and renal pathology over the long term (57) (Fig. 3). Ultimately, they died prematurely with severe, dilated cardiomyopathy and renal failure. In contrast, hamsters on 22-h L/D cycles appropriate for their genotype, behavioral patterns, life expectancy, and heart and renal structure and function were normal. Also, pathology did not develop in homozygous τ/τ hamsters, presumably because their extremely short intrinsic (20-h) circadian period is able to dominate the external environment with little conflict. Similarly, abnormal cardiorenal pathology was not seen in τ+/− raised in darkness or in those with their SCN removed. In these latter models, no conflict developed between internal and external rhythms; in the former, rhythm was dictated internally, and, in the latter, by the external environment. Thus in animals bearing the heterozygote +/τ mutation, organ pathology only appeared when normal internal circadian rhythmicity was disrupted or conflicted. In this case, with the τ hamsters (57), and in the case of the TAC mice (58) described above, cardiac, vascular, and renal pathology developed when there was a conflict between the endogenous tissue clock and diurnal signals coming from the SCN (Fig. 3).

Recently, Anea et al. (3) have supported these conclusions, linking disrupted rhythmic gene expression to vascular disease. The investigators studied Bmal1 knockout and Clock mutant mice. Bmal1 knockouts exhibited a loss of normal behavioral and cellular rhythm (17, 50), whereas clock mutants exhibited their circadian defect only under constant dark conditions and were rescued in the normal L/D environment (50). Both models, with rhythm disruption, exhibited acute endothelial dysfunction and, in the long term, pathological vascular remodeling in response to pressure stress and an abnormal response to intraluminal injury; these abnormal responses appeared to be largely due to disruption of normal signaling through phosphoinositide-dependent kinase 1, Akt, and endothelial nitric oxide synthase and were exacerbated by age (3).

Thus circadian dysregulation can be profoundly important in the etiology or exacerbation of cardiac, vascular, and renal disease; undoubtedly, this will extend to other tissues, including the central nervous system. Indeed, genes that integrate clock and light signaling and their coordinated regulation (i.e., harmony between internal and external cues) are critical to the normal growth and development of plants (70). The relationship between clocks in different organs or in different tissues in the same organ and the consequences if this relationship or harmony is disrupted are important areas for future exploration.
SUMMARY AND IMPLICATIONS FOR PHYSIOLOGY AND MEDICINE

These studies suggest a much broader range of issues from day-night schedule disruption than just impaired cognitive function or degraded job performance due to fatigue, the foci of contemporary thought to date. These studies highlight three important physiological principles.

1) The expression of genes relevant to cardiovascular metabolism, growth, and remodeling is dynamic and does not occur uniformly over the day-night cycle. Gene expression in the heart and blood vessels is dramatically different in the day compared with the night; growth and renewal appears to occur predominantly during the subjective night.

2) Synchrony between intrinsic and extrinsic diurnal/circadian rhythms is integral to healthy organ growth and renewal. Disruption of this synchrony has a devastating effect on the heart, kidney, and possibly other organs.

3) The risk-to-benefit ratio of some therapeutic interventions, for example ACEi, is not uniform across the 24-h cycle, but occurs in a diurnal fashion. These principles draw attention to several issues of importance for bench and bedside (Fig. 4).

4) Diurnal variation in gene expression and the differences between nocturnal animals and diurnal humans must be considered in studies dissecting the molecular events underlying disease or studies focused on the search for biomarkers. For example, in the heart failure research laboratory, when assessing murine gene expression by microarray, comparison between normal and disease must control for the time of day when tissues are harvested; as noted in the TAC experiments, there were differences in gene expression between sham and TAC that were evident only either at night or during the day. Diurnal variation in gene expression and proteins has great potential for discovering and characterizing diurnal markers in the blood or plasma and relating this to the pathophysiology, progression, or prognosis of human disease (59).

2) Our results also suggest that disregard for diurnal rhythms may occasionally account for differences in responses to therapy sometimes seen between nocturnal animal models vs. human patients. Drugs are usually initially tested on rodents during the day, rodent sleep time, to assess their possible effects on patients when administered during the human day. Clinical trials may take no account of changing safety and efficacy profiles across the diurnal cycle, as trials are largely conducted during the day for convenience. Chronotherapy offers novel approaches to the treatment of cardiovascular disease, as well as disease in other organ systems, potentially leading to greater benefit, fewer side effects, appropriate dosing, and perhaps reduced costs.

3) Environment may be a determinant of whether genes that underlie a disorder may actually result in a disease phenotype. Above, we showed an interaction between environment and a casein kinase-1e (τ) gene (57). Sleep or synchronized diurnal rhythm should be considered as an important environmental variable required for phenotypic expression or the extent of target organ damage. For example, our TAC studies (58) suggest chronic diurnal rhythm disruption in patients with hypertension should exacerbate both hypertension and target organ damage. The clinical observations that an abnormal sleep profile is a recognized cause of resistant hypertension, heart attack, heart failure, and stroke, are consistent with this approach. One would also anticipate that chronic diurnal rhythm disruption may exacerbate the phenotype of familial hypertrophic or dilated cardiomyopathy or impair the quality of tissue repair following myocardial infarction.

4) Daily rhythms, such as sleep, are viewed mainly from a neuroscience perspective; an article in Nature as recently as 2005 (42) was entitled “Sleep is of the brain, by the brain and for the brain”. Sleep may be “of” the brain, but biological rhythms are found in all organs (“by” all organs), and the above studies show that the integrity of biological rhythms including sleep are likely “for” all organs, certainly for the health and integrity of the cardiovascular and renal systems. Modern hospitals, particularly in intensive and cardiac care units, still use multibeded rooms and seem to ignore the importance of undisturbed diurnal rhythms for the healing process, even in our critically ill (30,75). Finally, save for possible inquiry regarding OSA, clinicians and society largely disregard regular day-night schedules or sleep as a risk factor for disease. The importance of regular and sufficient sleep and of a consistent pattern of the zeitgebers, light and dark, eating and activity, maintaining harmony between these external cues and internal rhythms, for the management of chronic diseases, such as cardiomyopathy and heart failure, is an area worthy of formal investigation.

New drugs that promise to grant a longstanding human wish, reduce the need for sleep, have been described as “a new wave
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of lifestyle drugs that promise to do for sleep what the con-31650-31651 receptive pill did for sex—unshackle it from nature” (53). Be careful what you wish for!

CONCLUSION

Investigating the response of cellular molecular rhythms to varying diurnal conditions is important, if we are to understand how we adapt to our real-world environment. Synchrony between external and internal diurnal rhythms and harmony among the molecular rhythms within the cell appears to be essential for normal organ biology. The substrate and enzymatic components of a given pathway must be present, not only in the right compartment within the cell, but also at the right time. We believe that a consistent L/D, eating, and activity schedule with adequate sleep is a lifestyle key to good health.

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REFERENCES


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