HIGHLIGHTED TOPIC | The Role of Clock Genes in Cardiometabolic Disease

Pressed for time: the circadian clock and hypertension

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Hypertension is a major risk factor for cardiovascular disease and death. The chronic elevation of blood pressure is a silent disorder in that its progression occurs largely asymptotically. However, its impact is deafening—causing cardiovascular disease, end-organ damage, and death (44). This seemingly simple relationship between high blood pressure and cardiovascular disease that is heavily influenced by our behavior and what we eat (4, 9, 112) is also conditioned by the time of day. Indeed, circadian rhythm is a significant input into the regulation of blood pressure.

With each day, the human body experiences a reproducible rhythm in behavior, waking in the morning (or thereabouts) and sleeping in the evening—a circadian rhythm. This is a consequence of the brain “resting” and “waking” as evidenced by changes in electrical activity (26, 116). In the cardiovascular system, blood pressure exhibits a rhythm as well, as blood pressure rises in the daytime and falls at nighttime. Moreover, in human hypertension, there are significant deviations to this rhythm in blood pressure. Recent evidence suggests that the genetic components of the circadian clock exert a key and fundamental role in the regulation of blood pressure.

The circadian clock, the molecular basis of circadian rhythms, is an interlocking set of autoregulatory loops, regulated through transcriptional, translational, and posttranslational processes, to generate a 24-h period of oscillation (133). The core of this signaling pathway is a negative-feedback loop comprised of a positive limb of transcription factors (Bmal1, Clock, and Npas2) and a negative limb of regulatory proteins (Per1, Per2, Per3, Cry1, Cry2). Heterodimers between either Bmal1-Clock or Bmal1-Npas2 drive transcription of Period (Per) and Cryptochrome (Cry), which then feed back in a manner further regulated posttranslationally to inhibit the positive limb, resulting in rhythmic oscillation of all components [except for Clock which may oscillate in a tissue-specific manner (111, 130)]. In addition to these core clock genes, it is estimated that 5–10% of the transcriptome is under the control of the circadian clock (94) and rhythmic changes in the expression of these genes help to shape the function of organ systems. Such pervasive control is at least in part possible because the hands of the clock touch specialized promoters within all tissues, organs, and cells. Indeed, the same genetic components that comprise the central clock in the suprachiasmatic nucleus (SCN) are also found in peripheral clocks, including blood vessels (21, 111), liver (155), skeletal muscle (91, 94, 155), heart (127, 151), and kidneys (114). The present article addresses recent observations from human and animal studies that shed insight as to how the circadian clock regulates blood pressure, contributes to hypertension, and ultimately evolves to vascular disease.

Rhythms in Blood Pressure and Central Acting Signals

It is well established that blood pressure exhibits a circadian variation in mammals, including humans (93) and mice (83). In the absence of disease, there is a nighttime dip in blood pressure, a rise in the morning hours, followed again by a subsequent drop, in a cycle that occurs every 24 h. These cycles are inverted in nocturnal animals such as mice. With regard to the central clock, locomotor activity follows a circadian rhythm that originates in the SCN of the brain. The SCN is considered to be the master clock to all peripheral rhythms, and as such is also an important influence in the regulation of the blood pressure rhythm. Indeed, ablation of the SCN results in the loss of circadian variation of blood pressure (148).
Behavioral aberrations can also significantly affect the circadian variation in blood pressure as has been recently shown in β1/β2 adrenergic receptor knockout (KO) mice (68), and also in humans with particular relevance to the morning surge (66, 78). As such, there are numerous candidate signals that exhibit a dual role in modulation of activity and blood pressure rhythms. At the core of blood pressure regulation is the brain medulla, which contains the vasomotor center that is the source of sympathetic and parasympathetic drive to the heart, kidneys, and vasculature. Recent studies have shown that in models where the circadian rhythm in blood pressure and activity is disrupted, catecholamine levels are perturbed (17, 87, 145). In hypertensive rats, sympatholytic drugs are also more effective at suppressing circadian variations in blood pressure than nonsympatholytic vasodilators (57). Similarly, central administration of cholinergic agonists can induce phase shifts in circadian activity (29), while a high-fat diet-induced ablation of rhythmic blood pressure in canines coincides with a reduction in type 2 muscarinic receptors in the heart (105). Although M2-receptor-deficient mice have no significant difference in single-time point assessments of blood pressure (76), studies that continuously assess blood pressure and its circadian variation are lacking.

Additional central clock-modifying peptides have been identified that may also transmit circadian cues to the vasculature. Vasoreactive intestinal peptide (VIP) is known to condition central circadian clock function (1) and modulate vascular function (53), and its expression is reduced in hypertensive patients (37). Dysregulation of VIP even at the level of the central clock could impact blood pressure through effects on organs/tissues that are intrinsically important in blood pressure regulation, such as the vasculature (Fig. 1). Indeed, the receptor for VIP, VPAC2, which is also important in circadian function (5, 46), is located in the vascular wall (39). Another potential peptide mediator that is important in circadian rhythm is prokineticin (14, 82). Overexpression of the receptor for prokineticin-2 in the heart induces hypertrophy and capillary vessel leakage (138); however, whether it has a role in blood pressure regulation is unknown. Aside from peptides, a hormone shown to entrain the SCN is melatonin (85, 140). In patients with essential hypertension and impaired circadian variation in blood pressure, melatonin supplementation can improve the lowering of nocturnal blood pressure (117). However, the mechanism and targets of circadian blood pressure regulation, i.e., central or peripheral, remain obscure. Indeed melatonin receptors are found not just in the brain, but are also expressed in the cardiovascular system (27, 31, 143). Clearly, more studies are necessary to understand the contribution of central regulation of circadian variations in blood pressure.

**BLOOD PRESSURE RHYTHMS: MORE THAN RUNNING AROUND THE CENTRAL CLOCK**

The impetus underlying rhythmic changes in blood pressure is more than a consequence of changes in activity/locomotor/behavioral rhythms. In fact, there is considerable and growing evidence that rhythmic blood pressure may also be guided by circadian actions in the periphery. When studied over a full 24-h period, the relationship between locomotor and blood pressure rhythm varies during the course of the day, and this variation is not circadian (58). Moreover, in humans submitted to experimental conditions that force behavioral asynchrony (i.e., no locomotor rhythm), circadian rhythm in cardiovascular function persists (54). Thus there is significant evidence in humans that blood pressure rhythms can be uncoupled (be independent) from endogenous circadian rhythms of activity. These findings are analogous to the ability of food restriction in rodents to uncouple the peripheral (liver) clock from the central (SCN) (20, 125). Further evidence of uncoupling between blood pressure and activity is evident in transgenic, hypertensive rats that possess an extra copy of the renin gene [TGR(mREN2)27]. TGR(mREN2)27 rats develop an inverse blood pressure rhythm (inverse/reverse dipper), 6 wk after birth, transitioning from a blood pressure that peaks at night to a blood pressure that peaks in the day. Yet motor activity and rhythm in the TGR(mREN2)27 rats remain unperturbed (147). Thus regulation of rhythmic blood pressure is more than a surrogate of rhythmic locomotor activity driven by the SCN and may also be influenced by circadian clocks peripheral to the central nervous system. One emerging theme is that the genetic components that control circadian rhythm—the circadian clock—are critical in the control of circadian rhythm in blood pressure.
THE CIRCADIAN CLOCK IN BLOOD PRESSURE REGULATION

In mice, a role for core components of the circadian clock (Bmal1, Clock, Npas2, Per, and Cry) has now been established in the regulation of blood pressure (Table 1). In conditions of constant darkness, Cry1/Cry2 deficient mice (double knockout/DKO) are hypertensive in the daytime (a time when blood pressure falls in rodents) relative to wild-type mice (WT). This lack of a daytime or rest period dip abolishes the circadian variation in blood pressure, which rises in WT mice at night during the activity period but remains unchanged in the Cry deficient mice (90). Thus Cry deficient mice are hypertensive during the rest period and on average normotensive in the activity period. Targeted deletion of Bmal1 in mice (Bmal1-KO) also abolishes the circadian variation in blood pressure. However, this is due to a hypotensive phenotype during the activity period (17) that is distinct from the hypertensive period rest period phenotype observed in the Cry deficient mice. In contrast to global Bmal1 disruption (i.e., Bmal1-KO mice), the overall blood pressure rhythm remains intact in mice with endothelial cell (EC)-specific deletion of Bmal1 (EC-Bmal1-KO) (146). EC-Bmal1-KO mice do exhibit a reduction in blood pressure at discrete times within the activity phase, albeit the increment of hypotenstion is more modest than that observed in the global KO and not present in the resting phase. Future studies are needed to address the role of Bmal1 within the more predominant vascular smooth muscle cell layer in the regulation of blood pressure rhythm. In addition, as disruption of endothelial cell clock function appears to leave smooth muscle cell clock function intact (146) (although if the converse is true it is not known), more definitive answers as to the role of the vascular clock may emanate from approaches such as vascular cell-specific rescue in Bmal1-KO mice, comparable to studies by McDearmon et al. (92) where brain or peripheral tissue-specific rescue was implemented in mice with global Bmal1 disruption.

Mice with mutation of Npas2 retain blood pressure rhythm, albeit the mice are also hypotensive. Clock mutant mice exhibit only a subtle dampening of blood pressure when conditioned in light-dark (LD) on either the C57Bl/6(17) or Jcl/Icr background (120), which may reflect the influence of the light cycle conditioning in these mice and the functional redundancy of Bmal1 to bind either Clock or NPAS2 (22, 23). Thus Bmal1-KO, EC-Bmal1-KO, Npas2 mutant, Clock mutant, and Per2 mutant mice exhibit lower blood pressures than corresponding WT mice, while Cry deficient and Clockmut (Jcl/Icr) mice exhibit higher blood pressures than their WT counterparts (Table 1). Aside from genetic strain, which may condition phenotype as has been observed with metabolic phenotypes in circadian mutant mouse (102), another nuance to be considered is that most of the aforementioned blood pressure studies [aside from those in Cry deficient mice and Npas2 mutant mice] were conducted under standard LD conditions. Indeed, the locomotor impairment in circadian clock is variable depending on light cycle conditions, except for Bmal1-KO mice which are completely arrhythmic in either standard LD conditions or free-running conditions of constant darkness (DD). Mice with mutation in the other core circadian clock components only exhibit a difference in period, with the locomotor clock running faster or slower in LD, but the locomotor phenotype worsens in DD to arrhythmicity. Thus additional studies to examine blood pressure rhythm in LD versus DD may prove informative to reveal if blood pressure rhythm worsens in a manner comparable to locomotor function in free-running conditions or if in fact the blood pressure rhythm may be perturbed independent of light cycle-induced deterioration of behavioral rhythms. These studies also highlight the difficulty of identifying clock-dependent phenotypes and emphasize the need for multiple genetic models to counter the many hands of circadian clock.

In addition to these core circadian clock components, there are other mechanisms that may modulate the circadian clock to influence the circadian variation in blood pressure. Peroxisome proliferator-activated receptor-gamma (PPARY) is one such mechanism. Mice with targeted deletion of PPARγ in the endothelium (EC-PPARγ-KO) exhibit a striking phenotypic resemblance to EC-Bmal1-KO mice (145, 146). EC-PPARγ-KO mice have lower blood pressures at night, like EC-Bmal1-KO mice, and normal blood pressures during the day, with rhythm remaining largely intact. In contrast, smooth muscle cell disrupted PPARγ-KO mice (SMC-PPARγ-KO mice) actually have higher blood pressure in the daytime and only trend to a lower blood pressure at night. Importantly, locomotor rhythm remains intact in EC and SMC-PPARγ mice (145), further evidence that circadian inputs into blood pressure regulation are not exclusively emanating from the central clock or activity rhythms. The ability of PPARγ to modulate blood pressure may arise from its ability to transactivate Bmal1, and as such corresponding loss of PPARγ in the aorta of both EC and

<table>
<thead>
<tr>
<th>Mouse Mutant</th>
<th>Light Cycle</th>
<th>Mutant</th>
<th>WT</th>
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<tbody>
<tr>
<td>Cry1-Cry2-DKO</td>
<td>DD</td>
<td>Night</td>
<td>90</td>
</tr>
<tr>
<td>Bmal1-KO (17)</td>
<td>LD</td>
<td>Day</td>
<td>92</td>
</tr>
<tr>
<td>Npas2-mut (17)</td>
<td>LD (DD)</td>
<td>Night</td>
<td>104</td>
</tr>
<tr>
<td>Clock mut (17)</td>
<td>LD</td>
<td>Day</td>
<td>102</td>
</tr>
<tr>
<td>Clock mut (Jcl/Icr) (120)</td>
<td>LD</td>
<td>Night</td>
<td>130</td>
</tr>
<tr>
<td>Per2 mut (142)</td>
<td>LD</td>
<td>Day</td>
<td>110</td>
</tr>
</tbody>
</table>

Table 1. The circadian rhythm in blood pressure in circadian clock mutant mice

Mean Arterial Blood Pressure, mmHg

<table>
<thead>
<tr>
<th>Mouse Mutant</th>
<th>Light Cycle</th>
<th>Mutant</th>
<th>WT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Per2 mut (142)</td>
<td>DD</td>
<td>Night</td>
<td>105.9 ± 1.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Day</td>
<td>116.5 ± 2.1</td>
</tr>
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Four distinct studies have examined blood pressure in mice with mutated circadian clocks (citations shown in parentheses). Shown are the average mean arterial pressures at night and day. Studies in Per2 mutant (mut) mice are single time point studies, but the hypotensive phenotype that was observed in all circadian clock mutant mice except for the Cry1-Cry2 double knockout (DKO) mice was still observed. Values for Clock mut mice were approximated from bar graphs in accompanying studies. WT, wild type; DD, constant darkness condition; LD, light-dark condition; KO, knockout.
SMC-PPARγ-KO mice leads to reduced expression of Bmal1, Npas2, Cry1, Cry2, and Per2.

That EC-Bmal1-KO and EC-PPARγ-KO mice retain blood pressure rhythm, albeit blunted, does not necessarily diminish a role for the endothelial clock in the regulation of blood pressure rhythm. The apparent disparity (i.e., rhythmic blood pressure in face of aberrant circadian clock expression) may, in part, stem from the known robustness of circadian rhythm to perturbation to individual clock components, which may in part occur through redundancy, networking (6), and also intercellular coupling (84) of the circadian clock mechanism. It should be added that evidence for intercellular coupling as a mechanism to mediate robustness of the circadian clock appears to be a phenomenon unique to SCN neurons and not apparent in fibroelastic cell populations (84). Indeed, vascular cells are “coupled” and communicate through gap junctions, paracrine mediators, and electrical/voltage impulses. However, whether these communications intimate robustness of the vascular circadian clock remains unknown. Moreover, additional studies remain necessary to assess circadian gene expression in the more abundant resistance vessels (as opposed to conduit vessels such as the aorta) that better reflect the basis of hypertension. The divergent blood pressure rhythms in EC vs. SMC PPARγ-disrupted mice further highlight the complexity involved in blood pressure regulation and respective involvement of the circadian clock.

**METABOLIC CONTROL AND BLOOD PRESSURE**

There is an intimate relationship between the circadian clock, metabolism, and obesity, one that is now known to also influence blood pressure. Indeed, obesity and metabolic dysfunction are major risk factors for the impaired control of blood pressure (24, 109). In rodent models of type II diabetes, mean blood pressure is mildly elevated (12, 103). While this in itself is a risk factor for cardiovascular diseases, the elevation in blood pressure is accompanied by changes in the circadian variation of blood pressure as demonstrated in experimental models of type II diabetes (db/db mice). Indeed, several independent observations have now shown that the daytime fall in blood pressure in mice is significantly blunted in db/db mice, suggesting that the mechanisms orchestrating the circadian timing of blood pressure are impaired (36, 121, 129). In humans, type II diabetes also dramatically increases the frequency of deficits in circadian blood pressure regulation and blunted dipping is observed in up to 75% of patients (18). This loss or reversal of the nocturnal dip in blood pressure is of great import and is associated with increased cardiovascular mortality (51, 55, 128). While type II diabetes comprises the vast majority of cases of diabetes, individuals with type I diabetes also exhibit blunted circadian regulation of blood pressure, and this correlates with declining renal function (86). Interestingly, experimental induction of type I diabetes in rats by streptozotocin alters the phase of oscillation of Bmal1, Clock, Cry1, Cry2, Per1, Per2, and Per3 in heart tissue (152). Clearly, more studies are necessary to understand the target organs and mechanisms by which diabetes compromises the circadian regulation of blood pressure.

Studies in animal models document that in addition to modifying blood pressure rhythms, type II diabetes also affects the circadian regulation of heart rate (36, 129) and locomotor activity (129). Other aspects of blood pressure control are also altered in animals with type II diabetes, including renal function (16), circulating hormones (33, 124), autonomic reflexes (119), and vascular (7, 25, 42, 59, 61) and sympathetic tone (36). However, the links between these variables and the impaired circadian regulation of blood pressure are also poorly understood. Another unknown is the relative contribution of the central clock versus local or peripheral clocks. Locomotor activity is frequently used as a surrogate for the activity of the central clock, and lesion of the SCN disrupts the rhythms of feeding, activity, and blood pressure (148). Indeed, mice with type II diabetes exhibit a gross reduction in locomotor activity that accompanies the impaired daily variations in blood pressure. However, the circadian rhythm of locomotor activity remains intact, suggesting that it is the morbidly obese phenotype that is the primary constraint on activity rather than an alteration of the central clock. These findings are supported by molecular evidence that expression of circadian clock genes is unaltered in the SCN of mice with type II diabetes (74). In contrast, the oscillation of circadian genes in the periphery, in isolated blood vessels, and the liver is impaired (74, 129). These results support the concept that type II diabetes promotes asynchrony of the peripheral and central circadian clocks and also that this asynchrony may contribute significantly to cardiovascular disease.

The aspects of the metabolic dysfunction that contribute to the asynchrony of circadian genes are poorly understood. Food intake rhythms are impaired in db/db mice (43), and the restricted timing of food intake has been shown to entrain peripheral clocks (20, 126) and normalize circadian clock expression in the liver (74). However, it is not yet known whether restricted feeding can improve the circadian timing of blood pressure in type II diabetes. Other approaches such as PPARγ agonists (2) and ACE inhibitors (19) have shown efficacy.

The link between type II diabetes and altered circadian timing is further compounded by the ability of the circadian clock itself to influence metabolism and ultimately obesity and diabetes. Clock mutant mice exhibit altered feeding patterns leading to obesity and insulin resistance (137). Furthermore, in both humans and rodents, polymorphisms of the Bmal1 gene are associated with increased incidence of type II diabetes and hypertension (150). While it is clear that the circadian clock participates in these processes, it should also be emphasized that these effects are subtle and require the complex interplay of other genes that can be modified by genetic background (30) and also that the individual clock genes may have different effects on blood pressure.

**THE RHYTHM IN HUMAN HYPERTENSION AND CHRONOTHERAPY**

The influence of circadian rhythm that occurs during normotension also presides during conditions of hypertension. In hypertension, there is a rise in blood pressure that is silent and chronic, inducing pathological processes of vascular remodeling, inflammation, and/or atherosclerosis to ultimately compromise end-organ perfusion. The rhythm and pattern of blood pressure oscillation can frequently be abnormal during hypertension, with patients presenting as nondippers (101), extreme dippers (62), and reverse dippers (63). Importantly perturbation...
to the amplitude and rhythm of blood pressure effectuates worsened cardiovascular and clinical outcome (134), a point whose importance is also highlighted in the 2003 JNC7 report (15). Moreover, the time at which the peak blood pressure occurs (acrophase), although less well characterized, may also have a significant impact on the secondary endpoints of disease. These variations in blood pressure are much more difficult to detect clinically and add stealth to an already silent disease.

The clinical relevance of the blood pressure rhythm was underscored in the Heart Outcomes Prevention Evaluation Study Investigators (HOPE trial), where the angiotensin-converting enzyme (ACE) inhibitor ramipril had significant effects on the reduction in the rates of death, myocardial infarction, and stroke (153). Surprisingly, the effect on lowering blood pressure during office visits (daytime) was not significant, misleadingly suggesting that the ACE inhibitor impacted cardiovascular outcomes independent of blood pressure. A subsequent substudy revealed ramipril did in fact lower blood pressure, but the effect was most prominent at night (131). Three important messages emerge from these studies. First, restoring the circadian variation and nighttime dip via antihypertensive therapy is important to cardiovascular outcome. Second, in addition to the silence of hypertension itself, there is the compounding factor of silence in circadian dysfunction with regard to blood pressure, especially if occurring at night. Third, hypertension may be more effectively treated by reducing blood pressure at a particular time of day.

The time at which antihypertensives are actually administered, chronotherapy, also impacts blood pressure control (79, 80). For example, in hypertensive patients with chronic renal failure, the calcium channel blocker (CCB) isradipine exhibits a greater blood pressure-lowering effect on nighttime blood pressure when given at 8 PM when compared with an 8 AM administration and under these conditions effectively restores the circadian blood pressure rhythm (107). Similarly, the administration of an α-adrenergic antagonist to patients with primary hypertension exhibits more pronounced effects on blood pressure control when administered before sleeping (47). Indeed, the restoration of circadian variability in blood pressure appears to be an important factor in the control of cardiovascular disease. Future studies are needed to determine if these aberrations in blood pressure rhythm stem from tissue-specific circadian clock dysfunctions, where experimental animal models of hypertension will be of utmost utility.

**RAS IN EXPERIMENTAL HYPERTENSION**

Circadian influences have also been explored in animal models of renin-angiotensin system (RAS)-induced hypertension that mimic an important manifestation of human hypertension. In addition to the TGR(mREN2)27 rat, which exhibits profound impairments in blood pressure rhythm as discussed earlier, the spontaneously hypertensive rat (SHR) [which has a mutation in a genetic locus in close proximity to the ACE gene (50, 56)] exhibits a blood pressure rhythm whose acrophase is shifted further toward the resting period (123). With regard to locomotor rhythm, SHRs also exhibit aberrant circadian function. Activity period in SHRs begins 1.5 h earlier than in WKY control rats, and their response to light cycle shifts is altered, perhaps stemming from a defect in vasoactive intestinal peptide (106) whose receptor VPAC is important in circadian locomotor function (46). Perhaps the most striking evidence in the SHR to implicate the circadian clock in the pathogenesis of hypertension is the identification of a single nucleotide polymorphism in the essential circadian clock component Bmal1 (150). Indeed, SHRs exhibit enhanced expression of Clock, Bmal1, and Per2 in heart tissue, albeit their rhythms are retained (98). Furthermore, SHRs exhibit enhanced amplitudes in the oscillation of renin, angiotensinogen, ACE, and angiotensin type 1a (AT1a) and type 2 (AT2) receptors in the heart (97). However, there are no data to examine either circadian clock or RAS oscillation in the vasculature or kidneys of SHRs, which are also relevant tissues in the control of blood pressure. Indeed, ANG II does have significant effects on the circadian clock in vascular cells. In cultured vascular smooth muscle cells, ANG II has potent effects on the circadian clock to induce synchronous circadian oscillation of Per2 and Bmal1 expression (99).

In contrast to the more subtle effects observed in the RAS-defective SHRs, chronic ANG II infusion by osmotic minipump in rats has more robust effects on circadian blood pressure (115). ANG II abolishes the circadian rhythm of arterial pressure in a sex-independent manner and further causes a modest reverse dipper phenotype in female rats, reminiscent of the response of TGR(mREN2)27 rats. With regard to effects on locomotor rhythm, although exogenous administration of ANG II directly to SCN brain slices does stimulate neuronal depolarization (10), behavioral rhythm remains intact in ANG II-infused mice in the face of the profound effects on blood pressure rhythm (115). These results again reinforce the concept that blood pressure regulation is more complex and cannot be explained solely by changes in activity.

Mice mutated in discrete components of the RAS signaling pathway largely retain the circadian variation in blood pressure, but impairments emerge during conditions of experimental hypertension. ACE-KO mice exhibit an intact blood pressure rhythm, but in response to a high salt diet, the amplitude of the rhythm is enhanced in ACE heterozygotes and blunted in ACE-KOs relative to WT mice (11). In AT1a receptor-KO mice, which also exhibit intact rhythmic blood pressure, fructose feeding also increased the difference in blood pressure between night- and daytime (32). Moreover, 5 days of high-salt diet abolishes the normal light-dark blood pressure rhythm in AT1a-KO mice (13). There is additional evidence to suggest that brain AT2 receptor may play a role in regulation of the circadian rhythm in blood pressure, whereby adenoviral transduction of the AT2 receptor in the rostral ventral lateral medulla of rats abolishes the circadian variation in blood pressure by blunting the nighttime spike (35). Thus an important concept is that the variation in blood pressure while appearing normal in the basal state may be disturbed by diet and other peripheral challenges aside from light cycle shifts. Additionally, hypertensive disease may worsen clock function, which may feed forward to further impair the pressor response.

**THE CIRCADIAN CLOCK AND FLUID BALANCE**

The kidney is the major organ for long-term blood pressure regulation and is also under circadian regulation. In nondipping individuals, the normal circadian pattern of sodium excretion is
blunted in concert with blood pressure (28). Sodium restriction can convert nondippers into dippers, and alternatively sodium loading attenuates dipping, suggesting a vital role for the kidney and fluid volume in this process. (34, 49, 139). Thiazide diuretics which promote natriuresis can also restore the nighttime reduction in blood pressure (34). Further evidence for a role of the kidney comes from studies showing that loss of renal function following nephrectomy correlates with impaired circadian variation in blood pressure (38). Recent studies have begun to uncover putative molecular targets of the circadian clock in the kidney that account for the rhythmic changes in blood pressure. The Na\(^+\)/H\(^+\) exchanger (NHE3) appears to be a bona fide target of the circadian clock as its expression in the kidney oscillates with a circadian rhythm and its promoter contains functional E-boxes that participate in the transactivation by Bmal1 and Clock (113). However, the loss of NHE3 in mice does not appear to affect the circadian regulation of blood pressure (100). Similarly, loss of the Na-2Cl-K cotransporter does not affect blood pressure rhythm (67). Interestingly, deficiency of carbonic anhydrase II in mice, which is important in sodium and bicarbonate reabsorption in the kidney, alters the circadian period of locomotor rhythm (64).

Perhaps most compelling are recent observations that describe a reciprocal relationship between the circadian clock component Per1 and sodium balance (41). In these studies, aldosterone, which has been shown to oscillate in plasma (120), stimulated Per1 expression in the kidney medulla in vivo and in vitro, further corroborated by reporter assay studies of transcriptional regulation of the Per1 promoter. In these studies, the renal epithelial sodium channel αENaC has emerged as a novel circadian target. Per1 regulated the expression of αENaC while its expression was altered in mice lacking functional Period genes and by Per1 knockdown. These studies further demonstrated that urinary sodium excretion was increased in Per-deficient mice. Interestingly, these observations are consistent with observations in Clock mutant mice, which have decreased water intake (120). These results suggest that the circadian clock may be important in control of sodium balance, while also acting as a sensor to sodium levels via aldosterone.

In addition, circadian changes in renal function may also be dependent on genes extrinsic to the kidney (i.e., liver), and indeed the expression of a large number of genes that affect renal function are known to oscillate in a circadian pattern including renin/angiotensin, kinins, AVP, and uroguanylin to name but a few (40, 52, 73, 118, 135). All of these factors are further influenced by age and salt load (69).

THE CIRCADIAN CLOCK AND PERIPHERAL VASCULAR RESISTANCE

Increased peripheral vascular resistance is a trademark and a significant target of therapy in hypertension. Indeed, vascular tone is known to exhibit a circadian variation (104). Catecholamines have largely been thought to in fact be responsible for the circadian variation in blood pressure, in part through effects to increase vascular resistance and cardiac output. Recent data in mice with disrupted or disturbed circadian clocks further support the role of catecholamines in blood pressure rhythm. Circadian clock deficient mice (Bmal1-KO and Npas2 mutant) (17) and EC-PPARγ-KO mice (which have reduced Bmal1) (145) have been demonstrated to have substantially reduced levels of norepinephrine and epinephrine in plasma at both night- and daytime, consistent with the hypertensive phenotype of the mice. Mice with disruption of chomogranin A, which produces the catecholamine inhibitory fragment catestatin (88), have complete impairment in circadian blood pressure rhythm and reduction in adrenal catecholamine levels (87). Independent observations suggest that these effects may not stem from the β-adrenergic receptor per se, as β1/β2-adrenergic-KO mice exhibit a normal blood pressure rhythm (68). That said, mice deficient in dopamine β-hydroxylase, the rate-limiting enzyme in catecholamine production, do have an impaired blood pressure rhythm (132), further evidence for epinephrine and norepinephrine in the circadian variation in blood pressure. Thus it may be that catecholamines mediate circadian changes in blood pressure through alpha-1 adrenergic receptors to control vascular resistance as opposed to control of cardiac contractility through the β-adrenergic receptors. This is supported by observations demonstrating that the pressor response to an α1-adrenergic receptor agonist is suppressed in Cry-deficient mice (90), alterations in the expression of α-adrenergic receptors, and our own observations in isolated aortic rings of Bmal1-KO mice (Anea CB and Rudic RD, unpublished observations). What is lacking, however, are studies that examine circadian clock function and signaling in resistance arterioles of the microvasculature, which are more relevant to blood pressure regulation.

Nitric oxide (NO) has also been implicated as a control mechanism for the circadian variation in blood pressure. NO and the ability of the nitric oxide synthase (NOS) inhibitor \(\text{N}^\text{G}\)-nitro-L-arginine methyl ester (L-NAME) to increase blood pressure vary significantly with time. L-NAME also blunted the diurnal variation in blood pressure (149) and along with other NOS inhibitors was shown to modulate the function of the SCN (72). Additionally, the concentration of NO metabolites in the plasma also exhibits circadian variation (89). There are three isoforms of NOS, neuronal (nNOS), inducible (iNOS) and endothelial (eNOS), that are responsible for the generation of NO in higher mammals. Indeed, recent data suggest that eNOS signaling and endothelial function is impaired in mice with dysfunctional circadian clocks (3, 110, 144). eNOS expression is not modified in these mice, but there is evidence that posttranslational mechanisms regulating eNOS activity are compromised, consistent with observations demonstrating that eNOS activity exhibits a circadian variation (136), which may be a consequence of its phosphorylation state (3, 75, 109a, 144). Moreover, vascular disease is worsened in circadian clock mutant mice when challenged by arterial ligation or vascular injury (3). However, the absence of an effect on blood pressure rhythm per se in eNOS-KO mice (81, 141) suggests that circadian rhythms in blood pressure are either not mediated by this isoform or compensated for through other mechanisms, i.e., robustness of blood pressure rhythm. Similarly, the function of the SCN is unmodified in mice lacking nNOS, although it is not known whether nNOS contributes to circadian regulation of blood pressure (72). Although an unlikely contributor, there are no studies yet addressing whether iNOS can influence SCN or circadian blood pressure regulation.

Another mechanism that might account for a reduction in nocturnal pressure is a loss of endothelial function. That a circadian rhythm exists in the function of human blood vessels
is long known (60). Indeed, endothelial function varies according to time of day (65), and this variation is altered in mice with mutated circadian clocks (3, 142), while the downstream effector response to NO, through guanylyl cyclase, remains intact (3, 142), consistent with observations in humans which demonstrate that the response to sodium nitroprusside does not vary according to time of day (104). In individuals with compromised endothelial function, this diurnal variation in blood vessel function is blunted (122). Forearm blood flow is also compromised in nondipping vs. dipping hypertensives (48). These findings raise important questions as to whether a loss of circadian rhythms causes endothelial dysfunction or if loss of endothelial function results in the disruption of circadian regulation. Indeed, hypertension is known to modify the circadian clock in the heart and vasculature (95, 98, 151). Recent studies support the former, as endothelial function is attenuated in mice with genetic disruption of the circadian clock (3, 142).

The increased production of superoxide is a powerful antagonist to the vasculoprotective actions of NO and a frequent cause of endothelial dysfunction in cardiovascular disease states. Blood vessels from hypertensive animals overproduce superoxide and accompanying ROS, which alters vascular tone and induces endothelial dysfunction (108). Increased production of ROS occurs in kidneys and spleen from Bmal1-KO mice (71). Moreover, the levels of superoxide production in brain and neutrophils have been shown to exhibit circadian variation (8, 96), but the source of the increased ROS is poorly characterized and thus it is not yet possible to determine if disruption of circadian rhythms promotes increased ROS production (Noxes, mitochondria) or reduced antioxidant defenses such as superoxide dismutase, glutathione peroxidase, catalase, or even heme oxygenase. Increased production of ROS can also disrupt the timing of the circadian clock (45, 154), suggesting a potential mechanism for stimuli that induce ROS production, such as high blood pressure or diabetes, to disrupt the local circadian clock and promote vascular disease.

It is evident that the regulation of the circadian clock impinges on multiple mechanisms involved in blood pressure regulation. The hands of the clock may directly touch on the genes and proteins involved in blood pressure regulation, i.e., through direct transcriptional control and posttranslational regulation. Perturbations to the circadian clock may occur in a silent manner perhaps influencing only peripheral clocks, i.e., nondipping hypertension or through central behavioral aberrations, i.e., sleep apnea, sleep duration, and shift work (Fig. 1). One possibility is that the genetic components of the circadian clock may act to directly activate or repress such mechanisms in tissues intrinsically important in blood pressure regulation or in tissues extrinsic to the pressor mechanism by release of mediators such as hormones or circulating peptides. Transcriptional activation by the positive limb of peripheral circadian clocks may transactivate genes important in vascular (111), cardiac (127), or renal function (70), and these functions can be antagonized by proteins of the negative limb. Future studies are needed to examine if there is an impact of ROS production in vascular function and blood pressure regulation in mice with a disrupted circadian clock as ROSs are established negative modulators (77). Moreover, it will be important to identify the putative signals that act as intermediaries to blood pressure and circadian control.

**CONCLUSION**

Circadian rhythms have long been known to be a characteristic feature of blood pressure regulation and hypertension. Recent evidence demonstrates that the genetic components of the circadian clock have a direct influence on the rhythmic oscillation of blood pressure. This may occur through the regulation of vasoactive agents, but also via the sensitivity of the response to these mediators. As the circadian clock impinges on the multiple mechanisms orchestrating blood pressure control including metabolic function, vascular function, and fluid balance, future studies are needed to examine the influence of the circadian clock on these downstream molecular mechanisms involved in hypertension, which may ultimately provide breakthroughs in our understanding and treatment of this silent killer.

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