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A low salt diet and circadian dysfunction synergize to induce angiotensin II-dependent hypertension in mice

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Abstract

Blood pressure exhibits a robust circadian rhythm in health. In hypertension, sleep apnea, and even shift work, this balanced rhythm is perturbed via elevations in nighttime blood pressure, inflicting silent damage to the vasculature and body organs. Herein, we examined the influence of circadian dysfunction during experimental hypertension in mice. Using radiotelemetry to measure ambulatory blood pressure and activity, the effects of angiotensin II administration were studied in wild-type (WT) and Period isoform knockout mice (Per2-KO, Per2,3-KO and Per1,2,3-KO/PerTKO mice). On a normal diet, administration of Ang II caused caused non-dipping blood pressure and exacerbated vascular hypertrophy in the Period isoform knockout mice. To study the endogenous effects of Ang II stimulation, we then administered a low salt diet to the mice, which does stimulate endogenous Ang II in addition to lowering blood pressure. A low salt diet decreased blood pressure in WT mice. In contrast, Period isoform knockout mice lost their circadian rhythm in blood pressure on a low salt diet, due to an increase in resting blood pressure, which was restorable to rhythmicity by the angiotensin receptor blocker losartan. Chronic low salt caused vascular hypertrophy in Period isoform knockout mice which also exhibited increased renin levels and altered AT1 receptor expression. These data suggest that circadian clock genes may act to inhibit or control renin/angiotensin signaling. Moreover, circadian disorders such as sleep apnea and shift work may alter the homeostatic responses to sodium restriction to potentially influence nocturnal hypertension.

Keywords

Circadian; hypertension; angiotensin; sodium restriction; renin; clock; vascular

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Disclosures

None.

Hypertension remains a major risk factor for cardiovascular disease and death. Reducing sodium intake in the diet to control blood pressure is important in blood pressure management.[1, 2] However, recent observations have emerged that challenge the absolute benefits of salt control in cardiovascular health.[3–15] To date, the mechanisms and physiologic interactions that underlie the potentially complex effects of sodium restriction and cardiovascular disease are unclear.

A significant characteristic of blood pressure is its circadian rhythm. In humans[16] and animals,[17] blood pressure rises and falls over 24 hours. The molecular mechanism responsible for circadian rhythms, the circadian clock, is important in the regulation of basal blood pressure.[18] The circadian clock is driven by a heterodimeric interaction between the bHLH transcription factors Bmal1 and Clock or Npas2/MOP4. Bmal1/Clock or Bmal1/Npas2 heterodimers bind promoter elements of genes within the circadian clock loop (to control circadian rhythm) and outside of the clock loop to control function and physiology (output genes). The Period isoform genes (Per1, Per2, Per3) and Cryptochrome genes (Cry1 and Cry2) comprise the negative feedback limb of the circadian clock loop. Single, double and triple gene targeted mutations of the Per isoforms, revealed that while Per1 and Per2 are critical for central locomotor behavioral rhythms.[19] The Per isoform knockout mice (as well as the Clock mutant mice) exhibit a relatively normal locomotor rhythm in normal light cycle conditions, but lose locomotor rhythm during free running conditions in which 24 hours of constant dark/DD conditions are maintained,[20, 21] analgous to the Clock mutant mouse.[22]

Recent evidence has demonstrated the importance of the circadian clock in key organs that are also involved in blood pressure regulation, including blood vessels,[23–26] heart,[27, 28] and kidneys.[29, 30] Moreover, the significance of blood pressure rhythms in human disease is highlighted in human conditions where rhythms are absent, as in non-dipper hypertensive patients[31] that exhibit a raised incidence of cardiovascular morbidity.[32] Moreover, the prevalence of non-dipping blood pressure is not trivial, but may be up to 45% in humans[33] and may even further impact individuals with HIV[34] while targeting non-dipping blood pressure with night-time administration of antihypertensives may even be beneficial in diabetes.[35]

Herein, we undertake studies to examine the interactions between angiotensin II, a key peptide in blood pressure regulation, and hypertension during circadian dysfunction, leading us to unexpected findings with regard to salt restriction.

Material and Methods

Animals

Care of the mice was in accordance to institutional guidelines. Mice were kept on 12h light-12h dark cycles prior to and during portions of the time subjected to experimentation. Studies were performed on 4- to 6- month-old male littermate control (WT), Per2-KO, and Per1, 2, 3 triple knockout (Per-TKO) mice as indicated. Period isoform triple knockout mice (Per-TKO) were raised to a colony (provided to us by Dr. David Weaver) in our laboratory. Studies were validated in the 3 different Per isoform knockout models of the mice. In these

studies we demonstrated that deletion of Per2-KO, Per2,3-KO, and Per-TKO mice exhibited comparable phenotypes with regard to blood pressure and activity rhythms. The strategy Per-TKO mice were implemented in studies as a more comprehensive These mice were originally generated by gene targeting in 129sv embryonic stem cells followed by chimeric males bred to isogenic 129/sv females. Thus, the genetic background of the Per-TKO mice is 129S1/SvImJ. Detailed methods are provided in the online-only Data Supplement.

Statistical analysis

Comparisons were made with 2-way ANOVA and 1-way ANOVA with a Bonferroni post-test or with unpaired Student t tests. For studies, N=4–7, as indicated. SEM was used for error bars. Differences were considered statistically significant when $P<0.05$. Graph pad Prism (GraphPad Software Inc., La Jolla, CA) and cosinor analysis (Refinetti R, Lissen GC, Halberg F (2007) were used for analysis of data.

Results

Blood Pressure Rhythm Is Sustained in Per2-KO Mice Despite Loss of Locomotor Rhythm in Free Running Conditions

The circadian clock plays an important role in control of baseline blood pressure rhythm in standard light cycle conditions/LD.[18, 26, 36–38] Given the significant impact of free running conditions to worsen locomotor rhythm in circadian clock mutant mice, we sought to determine blood pressure rhythms in Per2-KO mice in conditions of constant darkness/DD. In LD, (12 hours light and 12 hours dark), blood pressure did not differ between WT and Per2-KO mice (Figure 1A). Similarly, locomotor activity in LD also exhibited a circadian rhythm in both WT and the Per2-KO mice (Figure 1B). DD conditions, however, effectively impaired the circadian rhythm in activity in the Per2-KO mice but not WT mice (Figure 1B). Despite the loss of activity rhythm in DD in Per2-KO mice, blood pressure rhythm remained intact in both WT and Per2-KO mice (Figure 1C). In 6 days of DD, resting (daytime) blood pressure of Per2-KO mice did modestly increase averaging 119±2.34 mmHg versus 114±2.99 mm Hg in WT mice (Figure 1C, inset). Also, while WT mice appeared to have a phase delay in BP rhythm in response to DD while Per2-KO mice were absent this response.

Angiotensin II infusion causes non-dipping hypertension in Per2-KO mice

We next sought to determine if the response to experimentally-induced hypertension was conditioned by circadian clock gene dysfunction. Thus, WT and Per2-KO mice were infused with Angiotensin II (Ang II). As observed in Figure 1A, baseline blood pressure and activity rhythms were not different between WT and Per2-KO mice (Figure 2A), (Figure S1A). Exogenous administration of Ang II in LD caused a dramatic increase in blood pressure in the mice, but the elevation and rhythm of blood pressure was similar in WT and Per2-KO mice (Figure 2B) although activity was decreased in Per2-KO mice (Figure S1B). In contrast, in DD, Ang II caused a robust impairment in the blood pressure rhythm of Per2-KO mice (Figure 2C; WT=85.1% and Per-2 KO=61.6%, robustness analyzed by Cosinor). The mean blood pressure over 24 hours was significantly higher in AngII-infused Per2-KO mice (WT= 140.8±3.88 mm Hg and Per2-KO=150.4±1.46 mm Hg, Figure 2D). The overall elevation in blood pressure in Per2-KO mice was due to non-dipping blood pressure during

the rest (day) period. (subjective day MAP; WT 134.3±2.58 mm Hg ; Per2KO 150.3±1.70 mm (Figure 2E). In DD, Locomotor activity was decreased, and arrhythmic as expected, in Per2-KO mice (Figure S1C). To determine if the day time elevation in blood pressure caused an increase in blood vessel medial thickness, an index of vascular disease, aorta were harvested from vehicle treated and Ang II treated mice. WT and Per2-KO mice exhibited no differences in medial thickness under baseline conditions (Figure 2F). However, after the Ang II treatment, medial thickening was observed in WT mice but thickening was worsened in Per2-KO mice (Figure 2G). We expanded our studies to include more comprehensive Per isoform disrupted mice using both Per2,3-KO mice (Figure S2) and Per1,2,3-KO/Per-TKO mice (Figure S3). Importantly, we were able to demonstrate a congruence of medial thickening phenotype among the isoform knockout of Per (Per2-KO, Per2,3-KO and Per-TKO).

A Low Salt Diet Causes Non-Dipping Hypertension during Circadian Dysfunction

A low salt diet is a potent stimulus to stimulate the endogenous renin-angiotensin-aldosterone system in humans[39] and animals.[40] To determine the impact of the endogenous angiotensin II pathway in the context of circadian dysfunction, we next undertook studies to stimulate the endogenous renin-angiotensin system by administration of a low salt diet. In these studies, we implemented studies in mice absent all Per isoforms, Per-TKO mice, as these mice should be absent any potential compensatory function of alternate Per isoforms, although we did find congruence of phenotype among the single double and triple mutants. On a normal salt diet, Per-TKO mice had similar blood pressure to WT mice in LD conditions (Figure 3A), and Per2-KO mice (Figure 1A, 2A). After mice were placed on a low salt diet for 3 days, the WT mice exhibited a reduction in blood pressure, falling from ~130 mmHg peak blood pressure (Figure 3A), to ~122 mmHg (Figure 3B), with a comparable drop in blood pressure troughs (105 mmHg to 98 mmHg), thus exhibiting the expected blood pressure drop in response to low salt conditions. However, in Per-TKO mice the response to low salt diet was completely different. The rhythm in blood pressure was abolished by the low salt diet, resulting in non-dipping blood pressure (Figure 3B) in Per-TKO, analogous to the effects of Ang II administration In Per2-KO mice (Figure 2). With low salt, daytime blood pressure was significantly elevated in Per-TKO mice (WT= 104.5± 2.86 mm Hg and Per-TKO=118.7±4.60 mm Hg, Figure 3B). To determine if non-dipping blood pressure in the Per-TKO mice caused by low salt was angiotensin II-dependent, we administered the mice the AT_1 receptor antagonist losartan. While Losartan decreased blood pressure in both Per-TKO and WT mice as expected, losartan also restored the blood pressure rhythm in Per-TKO mice (Figure 3C). Blood pressure rhythm in Per-TKO mice was abolished with removal of losartan from the drinking water after 4 days (Figure S6A). In contrast to losartan, administration of the aldosterone antagonist spironolactone lowered blood pressure but was ineffective in restoring blood pressure rhythm (Figure S6B).

While circadian clock mutant mice are crucial in elucidating our understanding of the role of the circadian clock in the cardiovascular system, light-cycle derangement in WT mice may also mimic perturbed states such as shift work and sleep disruption. Thus, we next subjected WT mice that were administered a low salt diet to a dramatically shortened light-dark cycle (4:4 hr LD conditions). After 18 days of the shortened light-cycle, rhythmic blood pressure

was intact (Figure S7A), but as time progressed to 31 days (Figure S7B) and even longer to 43 days, rhythmic blood pressure was abolished in WT mice (Figure 3D) while the arrhythmic blood pressure also persisted in the long-term in Per-TKO with the chronic low salt (Figure 3E). The effect of low salt in the long-term was detrimental to the vasculature, as aortas from Per-TKO mice (Figure 3F) showed significantly increased medial thickening. Per-TKO mice on a chronic low salt diet also exhibited cardiac hypertrophy (Figure S8A) and decreased body weight (Figure S8B).

Low salt diet and circadian dysfunction impair regulation of the renin-angiotensin pathway

To determine if a low salt diet caused misregulation of the renin-angiotensin pathway in the context of circadian dysfunction, we measured renin levels in cardiovascular tissues in WT and Per-TKO. WT and Per-TKO mice on a normal salt diet displayed a similar pattern of renin mRNA expression in kidney (Figure S5B). On a low salt diet, renin mRNA expression in kidney was increased approximately 2-fold in Per-TKO compared to WT (Figure 4A). Plasma renin concentration was significantly elevated as well as measured by two distinct assays (Figure 4B, Figure S5A). Within the vasculature, renin protein expression in aorta also showed a significant increase in Per-TKO mice (Figure 4C). Thus, circadian clock dysfunction increased renin, which is a key enzyme that ultimately leads to the pro-proliferative and hypertensive peptide Ang II. Also, Per-TKO mice showed an approximately 12 hour phase advance in expression of atrial natriuretic peptide (ANP) in heart, a regulator of renin, compared to WT mice (Figure S9). We also sought to determine whether Period disruption affects AT1 receptors. Interestingly, we found that the temporal pattern of basal AT1 expression differs in Per-TKO mice compared to WT mice. Both WT and Per-TKO mice showed circadian oscillation, yet AT1 expression in Per-TKO mice displayed a phase delay in aorta (Figure 4D and kidney (Figure 4E) with higher daytime expression. To assess the direct effect of Per2 on AT1, we used an siRNA strategy to knockdown Per2 in human aortic smooth muscle cells (Figure 4F). Per2 knockdown in HASMCs decreased AT1 expression, which was further exacerbated with AngII treatment (Figure 4G). We also examined the effect of AngII on Per2 using isolated peritoneal macrophages from circadian clock reporter mice (Per2-luciferase). These studies revealed Per2 promoter activity was decreased in Ang II treated cells compared to control cells (Figure S10), suggesting there as a reciprocal relationship between angiotensin signaling and the circadian clock.

Discussion

Despite the numerous and effective therapies available, fewer than half of hypertensives under treatment have their blood pressure controlled to target levels.[41] Among the confounding issues could be the prevalence of masked, resistant,[42] and non-dipping hypertension. The prevalence of non-dipping hypertension has recently been reported to be as high as 45% in individuals,[33] and even higher in patients being treated with antihypertensives (53%).[43] Indeed, it is established that non-dipping or nocturnal hypertension has a substantially negative influence on patient morbidity.[32]

Studies in mice with genetically mutated circadian clocks have revealed the importance of the Bmal1 clock component in the control of basal blood pressure rhythm[18] and the Cryptochrome clock component in high salt sensitive hypertension.[44] In addition, other studies showed that the circadian period of mean arterial pressure (MAP), heart rate (HR), and locomotor activity is shortened in Per2 mutant mice in constant darkness under basal conditions.[45] More recently, Bmal1 in vascular smooth muscle has been shown to play a key role in circadian blood pressure regulation.[46] These studies have established that the circadian clock genes are fundamentally important in blood pressure regulation. In the current study, with regard to basal blood pressure regulation, we find that DD may phase advance blood pressure in WT mice, comparable to the phase advance that occurs in WT mice in locomotor activity in DD, an effect absent in the Per2-KO mice. Moreover, we find that experimental hypertension induced by angiotensin II infusion caused non-dipping hypertension in Period knockout mice that worsened Ang II-induced pathological vascular remodeling in the aorta. Surprisingly, a low salt diet, which is a known stimulus of the endogenous renin-angiotensin system,[39] caused non-dipping blood pressure and medial thickening in circadian clock knockout mice but also in WT mice that were induced to circadian derangement by a shortened light cycle. In mice with intact circadian rhythm and function, low salt did as expected, reduce blood pressure. While the absolute levels of sodium reduction induced experimentally in these mouse studies may not be achievable in humans, it maybe the relative change in sodium levels that is critical; the sodium restriction model is at very least a valuable approach to examine the endogenous stimulation of angiotensin II.

While low salt is clearly of benefit in controlling volume-expanded hypertension, our data suggests that in conditions of circadian dysfunction, low salt hyperactivates the renin angiotensin system. Indeed, recent human data hints that sodium restriction may potentially exhibit condition-dependent detrimental effects[4, 8, 13, 42] albeit the raised study limitations.[47] Aside from the effect on volume depletion, reduction of sodium in diet can also induce an array of changes as demonstrated in the human urinary metabolome, affecting pathways related to cardiovascular risk, nitric oxide production, oxidative stress, osmotic regulation, and metabolism.[48] Studies exploring the effect of sodium restriction and its effect on *rhythmic* blood pressure are limited [49–51] and little is known with regard to how low salt diets influence blood pressure during human circadian dysfunction and circadian decline, as in sleep disorders,[2, 52–54] shift work,[55] and aging,[1] given the prevalence of hypertension in these conditions.

In conclusion, the circadian clock may exert a significant restraint on the renin-angiotensin pathway. Infused angiotensin II or low salt-induced renin-angiotenisin signaling synergize with a dysfunctional circadian clock to produce night-time hypertension and vascular hypertrophy. Ultimately such studies may provide additional insight into the etiology of hypertension, the complex effects of sodium restriction in blood pressure regulation, and the underlying contribution of the circadian clock.

Perspectives

Blood pressure control remains a major challenge in preventing cardiovascular disease despite the numerous and effective antihypertensives available and the general knowledge regarding the benefits of lifestyle modifications. Aside from the difficulties in managing blood pressure, there is even still complexity identifying a uniformly indicated target blood pressure. For example, the 2014 JNC8 report suggested that blood pressure may not need be as aggressively lowered to standard normotensive ranges in elderly patients[56] however, more recent results from the SPRINT trial suggest that aggressive blood pressure reduction to below 120 mmHg diastolic does offer increased health benefit in nondiabetic patients at increased cardiovascular risk.[57] While current standards are based on day-time blood pressure, standards for appropriate night-time blood pressure are lacking. Indeed, the current data, albeit in mice, underscores the significance of time of day variation in blood pressure, with even modest changes in dipping blood pressure profile causing pathological vascular remodeling. Greater implementation of ambulatory blood pressure readings in humans could help establish more defined standards of 'healthy' blood pressure according to time of day, and even may prove to identify more effective chronotherapeutic antihypertensive strategies.[58, 59] In addition, the current experimental data suggests that circadian dysfunction may interact with sodium restriction to cause non-dipping blood pressure and pathological remodeling. Whether this unexpected effect of reduced dietary salt observed in mice translates into human circadian disorders such as sleep dysfunction, shift work, or even circadian decline in aging, remains to be seen. Mechanistically, these data do suggest that the circadian clock comprises a significant influence to restrain renin-angiotensin signaling and thereby impact blood pressure and vascular remodeling, which ultimately may shed new information into the molecular influences in hypertension.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Ando H, Ushijima K, Kumazaki M, Takamura T, Yokota N, Saito T, Irie S, Kaneko S, Fujimura A. Influence of age on clock gene expression in peripheral blood cells of healthy women. J Gerontol A Biol Sci Med Sci. 2010; 65:9–13. [PubMed: 19861640]
2. Archer SN, Laing EE, Moller-Levet CS, van der Veen DR, Bucca G, Lazar AS, Santhi N, Slak A, Kabiljo R, von Schantz M, Smith CP, Dijk DJ. Mistimed sleep disrupts circadian regulation of the human transcriptome. Proc Natl Acad Sci U S A. 2014; 111:E682–691. [PubMed: 24449876]
3. Kupferschmidt K. Report Reignites Battle Over Low-Salt Diets. Science. 2013; 340:908. [PubMed: 23704540]
4. Alderman MH. Reducing Dietary Sodium The Case for Caution. JAMA-Journal of the American Medical Association. 2010; 303:448–449.

5. Cohen HW, Hailpern SM, Alderman MH. Sodium intake and mortality follow-up in the Third National Health and Nutrition Examination Survey (NHANES III). Journal of General Internal Medicine. 2008; 23:1297–1302. [PubMed: 18465175]

6. Jessup M, Abraham WT, Casey DE, Feldman AM, Francis GS, Ganiats TG, Konstam MA, Mancini DM, Rahko PS, Silver MA, Stevenson LW, Yancy CW, Hunt SA, Chin MH. Heart Failure Writing C, Writing Comm M. 2009 Focused Update: ACCF/AHA Guidelines for the Diagnosis and Management of Heart Failure in Adults A Report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. Circulation. 2009; 119:1977–2016. [PubMed: 19324967]

7. Paterna S, Parrinello G, Cannizzaro S, Fasullo S, Torres D, Sarullo FM, Di Pasquale P. Medium Term Effects of Different Dosage of Diuretic, Sodium, and Fluid Administration on Neurohormonal and Clinical Outcome in Patients With Recently Compensated Heart Failure. American Journal of Cardiology. 2009; 103:93–102. [PubMed: 19101237]

8. Thomas MC, Moran J, Forsblom C, Harjutsalo V, Thorn L, Ahola A, Waden J, Tolonen N, Saraheimo M, Gordin D, Groop PH. FinnDiane Study G. The Association Between Dietary Sodium Intake, ESRD, and All-Cause Mortality in Patients With Type 1 Diabetes. Diabetes Care. 34:861–866. [PubMed: 21307382]

9. Garg R, Sun B, Williams J. Effect of low salt diet on insulin resistance in salt-sensitive versus salt-resistant hypertension. Hypertension. 2014; 64:1384–1387. [PubMed: 25185125]

10. Garg R, Williams GH, Hurwitz S, Brown NJ, Hopkins PN, Adler GK. Low-salt diet increases insulin resistance in healthy subjects. Metabolism. 2011; 60:965–968. [PubMed: 21036373]

11. Petrie JR, Morris AD, Minamisawa K, Hilditch TE, Elliott HL, Small M, Connell JM. Dietary Sodium Restriction Impairs Insulin Sensitivity in Noninsulin-Dependent Diabetes Mellitus. The Journal of Clinical Endocrinology & Metabolism. 1998; 83:1552–1557. [PubMed: 9589654]

12. O'Donnell MJ, Yusuf S, Mente A, Gao P, Mann JF, Teo K, McQueen M, Sleight P, Sharma AM, Dans A, Probstfield J, Schmieder RE. Urinary Sodium and Potassium Excretion and Risk of Cardiovascular Events. Jama-Journal of the American Medical Association. 306:2229–2238.

13. O'Donnell M, Mente A, Rangarajan S, McQueen MJ, Wang X, Liu L, Yan H, Lee SF, Mony P, Devanath A, Rosengren A, Lopez-Jaramillo P, Diaz R, Avezum A, Lanas F, Yusoff K, Iqbal R, Ilow R, Mohammadifard N, Gulec S, Yusufali AH, Kruger L, Yusuf R, Chifamba J, Kabali C, Dagenais G, Lear SA, Teo K, Yusuf S, Investigators P. Urinary sodium and potassium excretion, mortality, and cardiovascular events. N Engl J Med. 2014; 371:612–623. [PubMed: 25119607]

14. Ivanovski O, Szumilak D, Nguyen-Khoa T, Dechaux M, Massy ZA, Phan O, Mothu N, Lacour B, Drueke TB, Muntzel M. Dietary salt restriction accelerates atherosclerosis in apolipoprotein E-deficient mice. Atherosclerosis. 2005; 180:271–276. [PubMed: 15910852]

15. Lu H, Wu C, Howatt DA, Balakrishnan A, Charnigo RJ Jr, Cassis LA, Daugherty A. Differential effects of dietary sodium intake on blood pressure and atherosclerosis in hypercholesterolemic mice. J Nutr Biochem. 2013; 24:49–53. [PubMed: 22705323]

16. Millar-Craig MW, Bishop CN, Raftery EB. Circadian variation of blood-pressure. Lancet. 1978; 1:795–797. [PubMed: 85815]

17. Rudic RD, Fulton DJ. Pressed for time: the circadian clock and hypertension. J Appl Physiol. 2009; 107:1328–1338. [PubMed: 19679741]

18. Curtis AM, Cheng Y, Kapoor S, Reilly D, Price TS, Fitzgerald GA. Circadian variation of blood pressure and the vascular response to asynchronous stress. Proc Natl Acad Sci U S A. 2007; 104:3450–3455. [PubMed: 17360665]

19. Bae K, Jin X, Maywood ES, Hastings MH, Reppert SM, Weaver DR. Differential functions of mPer1, mPer2, and mPer3 in the SCN circadian clock. Neuron. 2001; 30:525–536. [PubMed: 11395012]

20. Albrecht U, Sun ZS, Eichele G, Lee CC. A differential response of two putative mammalian circadian regulators, mper1 and mper2, to light. Cell. 1997; 91:1055–1064. [PubMed: 9428527]

21. Zheng B, Larkin DW, Albrecht U, Sun ZS, Sage M, Eichele G, Lee CC, Bradley A. The mPer2 gene encodes a functional component of the mammalian circadian clock. Nature. 1999; 400:169–173. [PubMed: 10408444]

22. Vitaterna MH, King DP, Chang AM, Kornhauser JM, Lowrey PL, McDonald JD, Dove WF, Pinto LH, Turek FW, Takahashi JS. Mutagenesis and mapping of a mouse gene, Clock, essential for circadian behavior. Science. 1994; 264:719–725. [PubMed: 8171325]

23. Anea CB, Ali MI, Osmond JM, Sullivan JC, Stepp DW, Merloiu AM, Rudic RD. Matrix Metalloproteinase 2 and 9 Dysfunction Underlie Vascular Stiffness in Circadian Clock Mutant Mice. Arterioscler Thromb Vasc Biol. 2010; 30:2535–U2387. [PubMed: 20829506]

24. Anea CB, Zhang M, Stepp DW, Simkins GB, Reed G, Fulton DJ, Rudic RD. Vascular disease in mice with a dysfunctional circadian clock. Circulation. 2009; 119:1510–1517. [PubMed: 19273720]

25. Cheng B, Anea CB, Yao L, Chen F, Patel V, Merloiu A, Pati P, Caldwell RW, Fulton DJ, Rudic RD. Tissue-intrinsic dysfunction of circadian clock confers transplant arteriosclerosis. Proc Natl Acad Sci U S A. 2011; 108:17147–17152. [PubMed: 21969583]

26. Viswambharan H, Carvas JM, Antic V, Marecic A, Jud C, Zaugg CE, Ming XF, Montani JP, Albrecht U, Yang Z. Mutation of the circadian clock gene Per2 alters vascular endothelial function. Circulation. 2007; 115:2188–2195. [PubMed: 17404161]

27. Ingle KA, Kain V, Goel M, Prabhu SD, Young ME, Halade GV. Cardiomyocyte specific Bmal1 deletion in mice triggers diastolic dysfunction, extracellular matrix response and impaired resolution of inflammation. American Journal of Physiology Heart and Circulatory Physiology. 2015; 309:H1827–H1836. [PubMed: 26432841]

28. Young ME, Brewer RA, Peliciari-Garcia RA, Collins HE, He L, Birky TL, Peden BW, Thompson EG, Ammons BJ, Bray MS, Chatham JC, Wende AR, Yang Q, Chow CW, Martino TA, Gamble KL. Cardiomyocyte-specific BMAL1 plays critical roles in metabolism, signaling, and maintenance of contractile function of the heart. J Biol Rhythms. 2014; 29:257–276. [PubMed: 25238855]

29. Gumz ML, Stow LR, Lynch IJ, Greenlee MM, Rudin A, Cain BD, Weaver DR, Wingo CS. The circadian clock protein Period 1 regulates expression of the renal epithelial sodium channel in mice. J Clin Invest. 2009; 119:2423–2434. [PubMed: 19587447]

30. Solocinski K, Richards J, All SC, Cheng KY, Khundmiri SJ, Gumz ML. Transcriptional Regulation of NHE3 and SGLT1 by the Circadian Clock Protein Per1 in Proximal Tubule Cells. American Journal of Physiology Renal Physiology. 2015; 309:F933–942. [PubMed: 26377793]

31. O'Brien E, Sheridan J, O'Malley K. Dippers and non-dippers. Lancet. 1988; 2:397. [PubMed: 2899801]

32. Verdecchia P, Porcellati C, Schillaci G, et al. Ambulatory blood pressure. An independent predictor of prognosis in essential hypertension. Hypertension. 1994; 24:793–801. [PubMed: 7995639]

33. Viera AJ, Zhu S, Hinderliter AL, Shimbo D, Person SD, Jacobs DR Jr. Diurnal blood pressure pattern and development of prehypertension or hypertension in young adults: the CARDIA study. J Am Soc Hypertens. 2011; 5:48–55. [PubMed: 21269909]

34. Kent S, Burkholder G, Tajeu G, Overton ET, Muntner P. Mechanisms Influencing Circadian Blood Pressure Patterns Among Individuals with HIV. Curr Hypertens Rep. 2015; 17:1–9.

35. Rossen NB, Knudsen ST, Fleischer J, Hvas A-M, Ebbehøj E, Poulsen PL, Hansen KW. Targeting Nocturnal Hypertension in Type 2 Diabetes Mellitus. Hypertension. 2014; 64:1080–1087. [PubMed: 25259747]

36. Masuki S, Todo T, Nakano Y, Okamura H, Nose H. Reduced alpha-adrenoceptor responsiveness and enhanced baroreflex sensitivity in Cry-deficient mice lacking a biological clock. J Physiol. 2005; 566:213–224. [PubMed: 15860530]

37. Sei H, Oishi K, Chikahisa S, Kitaoka K, Takeda E, Ishida N. Diurnal amplitudes of arterial pressure and heart rate are dampened in Clock mutant mice and adrenalectomized mice. Endocrinology. 2008; 149:3576–3580. [PubMed: 18403480]

38. Stow LR, Richards J, Cheng K-Y, Lynch IJ, Jeffers LA, Greenlee MM, Cain BD, Wingo CS, Gumz ML. The Circadian Protein Period 1 Contributes to Blood Pressure Control and Coordinately Regulates Renal Sodium Transport Genes. Hypertension. 2012; 59:1151–1156. [PubMed: 22526258]

39. Oliver WJ, Cohen EL, Neel JV. Blood pressure, sodium intake, and sodium related hormones in the Yanomamo Indians, a “no-salt” culture. Circulation. 1975; 52:146–151. [PubMed: 1132118]
40. Ingelfinger JR, Pratt RE, Ellison K, Dzau VJ. SODIUM-REGULATION OF ANGIOTENSINOGEN MESSENGER-RNA EXPRESSION IN RAT-KIDNEY CORTEX AND MEDULLA. Journal of Clinical Investigation. 1986; 78:1311–1315. [PubMed: 3533999]
41. Go AS, Mozaffarian D, Roger VL, Benjamin EJ, Berry JD, Borden WB, Bravata DM, Dai S, Ford ES, Fox CS, Franco S, Fullerton HJ, Gillespie C, Hailpern SM, Heit JA, Howard VJ, Huffman MD, Kissela BM, Kittner SJ, Lackland DT, Lichtman JH, Lisabeth LD, Magid D, Marcus GM, Marelli A, Matchar DB, McGuire DK, Mohler ER, Moy CS, Mussolino ME, Nichol G, Paynter NP, Schreiner PJ, Sorlie PD, Stein J, Turan TN, Virani SS, Wong ND, Woo D, Turner MB. Heart Disease and Stroke Statistics—2013 Update: A Report From the American Heart Association. Circulation. 2013; 127:e6–e245. [PubMed: 23239837]
42. Calhoun DA, Jones D, Textor S, Goff DC, Murphy TP, Toto RD, White A, Cushman WC, White W, Sica D, Ferdinand K, Giles TD, Falkner B, Carey RM. Resistant Hypertension: Diagnosis, Evaluation, and Treatment: A Scientific Statement From the American Heart Association Professional Education Committee of the Council for High Blood Pressure Research. Circulation. 2008; 117:e510–e526. [PubMed: 18574054]
43. de la Sierra A, Redon J, Banegas JR, Segura J, Parati G, Gorostidi M, de la Cruz JJ, Sobrino J, Llisterri JL, Alonso J, Vinyoles E, Pallarés V, Sarría A, Aranda P, Ruilope LM. Investigators obotSSoHABPMR. Prevalence and Factors Associated With Circadian Blood Pressure Patterns in Hypertensive Patients. Hypertension. 2009; 53:466–472. [PubMed: 19171788]
44. Doi M, Takahashi Y, Komatsu R, Yamazaki F, Yamada H, Haraguchi S, Emoto N, Okuno Y, Tsujimoto G, Kanematsu A, Ogawa O, Todo T, Tsutsui K, van der Horst GT, Okamura H. Salt-sensitive hypertension in circadian clock-deficient Cry-null mice involves dysregulated adrenal Hsd3b6. Nat Med. 16:67–74. [PubMed: 20023637]
45. Vukolic A, Antic V, Van Vliet BN, Yang Z, Albrecht U, Montani JP. Role of mutation of the circadian clock gene Per2 in cardiovascular circadian rhythms. American journal of physiology Regulatory, integrative and comparative physiology. 2010; 298:R627–634.
46. Xie Z, Su W, Liu S, Zhao G, Esser K, Schroder EA, Lefta M, Stauss HM, Guo Z, Gong MC. Smooth-muscle BMAL1 participates in blood pressure circadian rhythm regulation. J Clin Invest. 2015; 125:324–336. [PubMed: 25485682]
47. Oparil S. Low Sodium Intake — Cardiovascular Health Benefit or Risk? New England Journal of Medicine. 2014; 371:677–679. [PubMed: 25119614]
48. Jablonski KL, Klawitter J, Chonchol M, Bassett CJ, Racine ML, Seals DR. Effect of Dietary Sodium Restriction on Human Urinary Metabolomic Profiles. Clinical Journal of the American Society of Nephrology. 2015; 10:1227–1234. [PubMed: 25901092]
49. Uzu T, Ishikawa K, Fujii T, Nakamura S, Inenaga T, Kimura G. Sodium Restriction Shifts Circadian Rhythm of Blood Pressure From Nondipper to Dipper in Essential Hypertension. Circulation. 1997; 96:1859–1862. [PubMed: 9323073]
50. Uzu T, Nishimura M, Fujii T, Takeji M, Kuroda S, Nakamura S, Inenaga T, Kimura G. Changes in the circadian rhythm of blood pressure in primary aldosteronism in response to dietary sodium restriction and adrenalectomy. Journal of Hypertension. 1998; 16:1745–1748. [PubMed: 9869007]
51. Simonetti GD, Farese S, Aregger F, Uehlinger D, Frey FJ, Mohaupt MG. Nocturnal dipping behaviour in normotensive white children and young adults in response to changes in salt intake. Journal of Hypertension. 2010; 28:1027–1033. doi:1010.1097/HJH.1020b1013e328337854d. [PubMed: 20173652]
52. Ackermann K, Plomp R, Lao O, Middleton B, Revell VL, Skene DJ, Kayser M. Effect of sleep deprivation on rhythms of clock gene expression and melatonin in humans. Chronobiol Int. 2013; 30:901–909. [PubMed: 23738906]
53. Evans DS, Parimi N, Nievergelt CM, Blackwell T, Redline S, Ancoli-Israel S, Orwoll ES, Cummings SR, Stone KL, Tranah GJ. Study of Osteoporotic F, Osteoporotic Fractures in Men Study G. Common genetic variants in ARNTL and NPAS2 and at chromosome 12p13 are associated with objectively measured sleep traits in the elderly. Sleep. 2013; 36:431–446. [PubMed: 23449886]

54. Lippert J, Halfter H, Heidbreder A, Rohr D, Gess B, Boentert M, Osada N, Young P. Altered dynamics in the circadian oscillation of clock genes in dermal fibroblasts of patients suffering from idiopathic hypersomnia. PLoS One. 2014; 9:e85255. [PubMed: 24454829]

55. Bracci M, Manzella N, Copertaro A, Staffolani S, Strafella E, Barbaresi M, Copertaro B, Rapisarda V, Valentino M, Santarelli L. Rotating-shift nurses after a day off: peripheral clock gene expression, urinary melatonin, and serum 17-beta-estradiol levels. Scand J Work Environ Health. 2014; 40:295–304. [PubMed: 24402410]

56. James PA, Oparil S, Carter BL, Cushman WC, Dennison-Himmelfarb C, Handler J, Lackland DT, LeFevre ML, MacKenzie TD, Ogedegbe O, Smith SC Jr, Svetkey LP, Taler SJ, Townsend RR, Wright JT Jr, Narva AS, Ortiz E. 2014 evidence-based guideline for the management of high blood pressure in adults: report from the panel members appointed to the Eighth Joint National Committee (JNC 8). JAMA. 2014; 311:507–520. [PubMed: 24352797]

57. A Randomized Trial of Intensive versus Standard Blood-Pressure Control. New England Journal of Medicine. 2015; 373:2103–2116. [PubMed: 26551272]

58. Hermida RC, Ayala DE, Mojon A, Fernandez JR. INFLUENCE OF CIRCADIAN TIME OF HYPERTENSION TREATMENT ON CARDIOVASCULAR RISK: RESULTS OF THE MAPEC STUDY. Chronobiology International. 2010; 27:1629–1651. [PubMed: 20854139]

59. Smolensky MH, Hermida RC, Ayala DE, Tiseo R, Portaluppi F. Administration-time-dependent effects of blood pressure-lowering medications: basis for the chronotherapy of hypertension. Blood Pressure Monitoring. 2010; 15:173–180. [PubMed: 20571367]

Novelty and Significance

What Is New?

- Circadian clock dysfunction can exacerbate angiotensin induced hypertension.
- In conditions of low salt diet, circadian arrhythmicity may also cause paradoxical non-dipping blood pressure.

What Is Relevant?

This work is relevant to our understanding of high blood pressure in that it demonstrates an interaction of the circadian clock and circadian rhythm in renin-angiotensin system regulation, hypertension development, and resultant vascular remodeling.

Summary

- Angiotensin II infusion causes non-dipping hypertension in Period isoform knockout mice
- Endogenous stimulation of the angiotensin II system by low salt also causes a non-dipping blood pressure profile during circadian dysfunction
- The renin-angiotensin signaling pathway is hyperactivated in Per-TKO mice

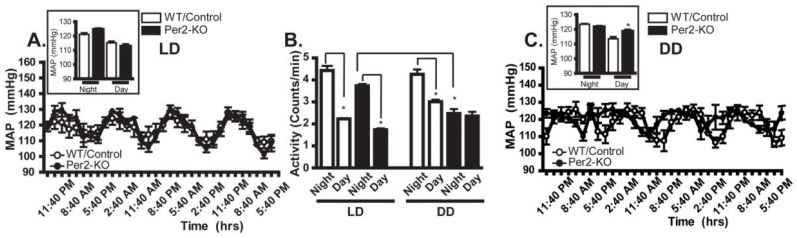

Figure 1. Basal blood pressure rhythm is maintained in Per2-KO mice in LD and DD conditions

Radiotelemetric transmitters were placed in WT and Per-2 KO mice. (**A**) In LD conditions, blood pressure exhibited a circadian rhythm in Per-2 KO mice that was indistinguishable from WT mice in LD conditions, shown as blood pressure trace (left panel) and day/night quantification (inset). (**B**) Locomotor activity was derived from radiotelemetry and quantified by, averaging values during peaks (night) and troughs (day) of activity traces in conditions of LD and DD. (n=6, *p<0.05, 1-way ANOVA). (**C**) Placement of mice in DD conditions caused a phase change in blood pressure rhythm (peaks and troughs were temporally shifted), however, rhythm persisted (n=6, *p<0.05). 1-way ANOVA was used to compare differences in blood pressure values.

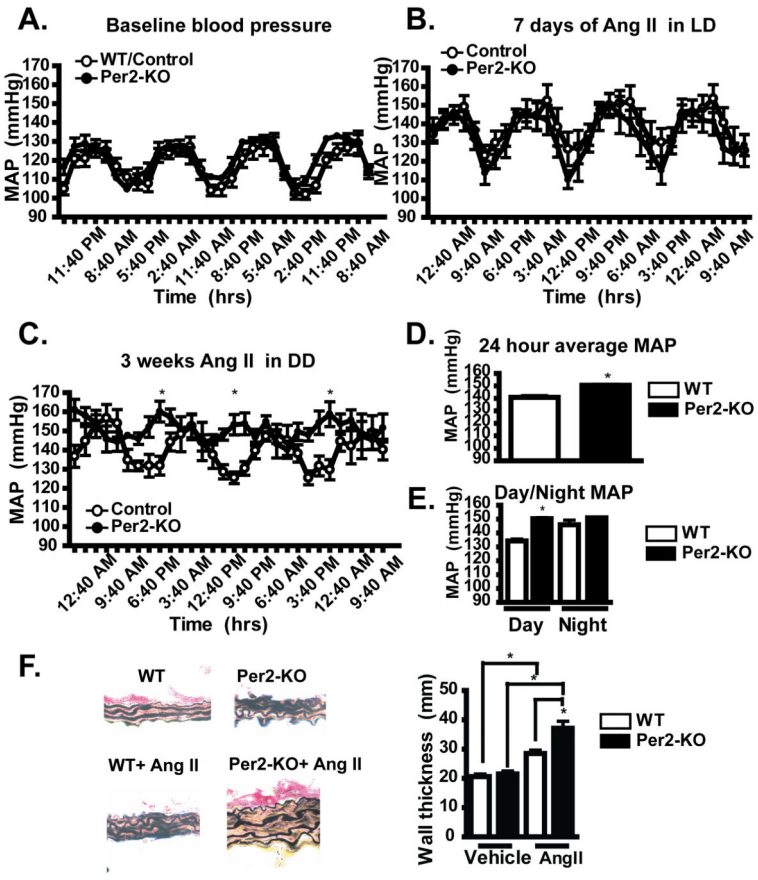

Figure 2. Per2-KO mice display non-dipping hypertension when administered Ang II in DD conditions

(**A**) Baseline blood pressure in LD is similar between WT and Per2-KO mice. (**B**) Angiotensin II was administered by osmotic mini-pumps (Alzet 2004) to mice at a dose of 1000 ng/kg/min which caused hypertension in WT and Per2-KO. After 1 week, blood pressure was elevated after in LD conditions but there was no difference between WT and Per-2 KO mice. (**C**) Mice were placed in DD for 2 weeks while administered Ang II. Under these conditions, a robust impairment in the blood pressure rhythm occurred in Per2-KO (n=4, *p<0.05, 2-way ANOVA followed by a Bonferroni post-test used to compare the difference between groups.) (**D**) Mean blood pressure over 24 hours is significantly higher in Per2-KO (WT= 140.8±3.88 mm Hg and Per2-KO=150.4±1.46 mm Hg. (**E**) Blood pressure values were then analyzed by segregating values to nighttime and daytime. Daytime blood pressure was significantly higher in Per2-KO mice, while nighttime blood pressure did not differ from WT mice (WT daytime blood pressure=134.3±2.58 mm Hg and Per2-KO=150.3±1.70 mm Hg. Nighttime WT=145.9±5.31 mm Hg and nighttime Per2-KO= 150.9±3.17 mm Hg.) (n=4, *p<0.05, 1-way ANOVA was used to find the difference in means.) (**F**) Van Giesen staining of aortic cross sections showed similar medial thickness in untreated WT and Per2-KO mice quantified by morphometry. (**G**) After AngII was infused chronically by osmotic minipump, aortic medial thickness was dramatically increased in Per2-KO mice relative to WT mice. (n=5, *p<0.05, Unpaired Student *t* test)

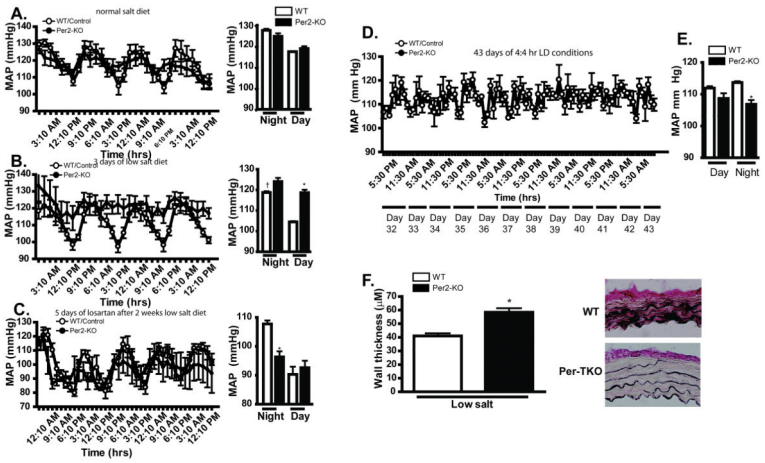

Figure 3. A low salt diet causes non-dipping hypertension during circadian dysfunction

(**A**) Blood pressure measured on a 0.3% salt diet (TD8604) diet (Teklad) which is the standard mouse diet, shown as blood pressure trace (left panel) and night/day quantification (right panel). (n=5) (**B**) Mice were then placed on a 0.01–0.02% low salt diet (TD90228) which caused non-dipping hypertension in Per-TKO mice, but did cause an expected reduction in blood pressure in WT mice. Daytime blood pressure was significantly elevated in Per-TKO mice compared to WT mice (n=4, *p=0.0002) while in WT mice nighttime blood pressure was significantly higher than daytime pressure (n=5, †p=0.0002). (**C**) WT and Per-TKO mice that were on low-salt diet were then administered the angiotensin 1 receptor blocker (AT1) losartan in drinking water (0.6 g/L). Losartan lowered blood pressure, in both WT and Per-TKO mice, but also partially restored the circadian variation in blood pressure in Per-TKO mice. (**D**) The LD cycle was shortened from 12hr:12hr L:D to 4hr:4hr L:D while mice were maintained on a low salt diet. By ~5 weeks (32 days) blood pressure rhythm was abolished in WT mice. (**E**) Blood pressure analysis of telemetric data during conditions of shortened light cycle revealed that blood pressure rhythm was abolished in WT mice while the ablation of rhythm persisted in Per-TKO mice on a chronic low salt under a 4hr:4hr L:D cycle. (**F**) Quantitative morphometry of the aorta and Van Giesen staining of aortic cross sections isolated from WT and Per-TKO mice under conditions of chronic low salt, showed exacerbated medial thickening in Per-TKO mice compared to WT mice (n=5, *p<0.05, Unpaired Student *t* test),

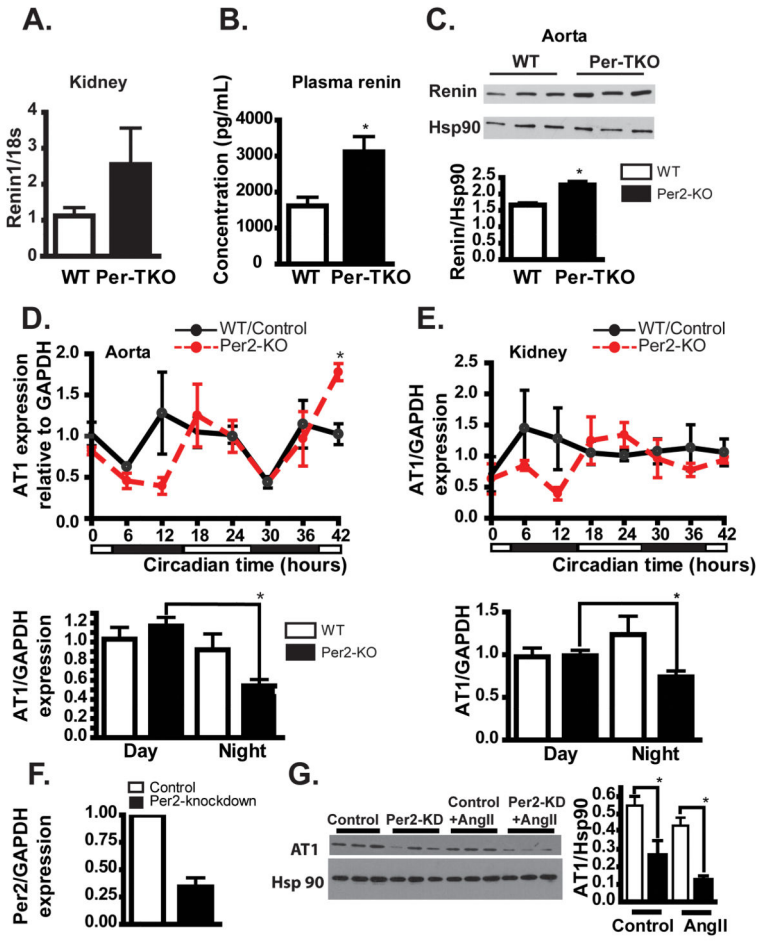

Figure 4. Low salt alters the renin-angiotensin pathway in Per-TKO mice

(**A**) The level of renin expression in kidney was measured by qRT-PCR. Kidney renin expression in Per-TKO was increased over 2-fold compared to WT in conditions of low salt. (**B**) Plasma renin concentration was determined with an ELISA kit (Mouse Renin 1 DuoSet, R&D Systems). Per-TKO mice display a significant elevation in plasma renin (*p<0.05, Unpaired Student *t* test). (**C**) In aorta, renin expression was determined by Western blotting which also showed a significant increase in renin in low salt-treated Per-TKO mice. Shown as western blot of three representative WT and three representative Per-TKO mice and densitometry (WT n=5, KO n=3, *p<0.05, Unpaired Student *t* test). Aorta (**D**) and Kidneys (**E**) were harvested from WT and Per-TKO mice in LD at 6 hour intervals for 42 hours under normal diet conditions. Aorta and kidney AT1 expression show a phase shift with higher daytime expression in Per-TKO versus WT mice. (F) Relative mRNA expression by Q-PCR of control and Per2 with siRNA transfection (50 nmol). (**G**) Western blotting shows Per2 knockdown decreases AT1 receptor expression in HASMCs and is further decreased with 24 hours of angiotensin II treatment (100 nmol/L), quantified by densitometry (n=3 per group, *p<0.05, 1-way ANOVA).