Circadian regulation of renal function and potential role in hypertension.

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The authors have declared that no conflict of interest exists.

Key points: 1. The circadian clock controls a large number of specific renal functions. 2. The circadian clock is involved in renal sodium handling through a variety of recently elucidated mechanisms. 3. Alteration of circadian rhythms in humans is associated with several conditions, including decreased glucose sensitivity, hypertension and cancer. 4. Chronopharmacotherapy is an emerging field that may help treating conditions like hypertension and cancer.

Purpose of review: Previous studies have shown that a variety of specific renal functions exhibit circadian oscillations. This review aims (i) to provide an update on the molecular mechanisms underlying circadian rhythms in the kidney, and (ii) to discuss how dysregulation of circadian rhythms can interfere with kidney function.

Recent findings: The molecular mechanism responsible for generating and maintaining circadian rhythms has been unraveled in great detail. This mechanism, known as circadian clock, drives circadian oscillation in expression levels of a large number of renal mRNA transcripts. Several proteins critically involved in renal homeostatic functions have been shown to exhibit significant circadian oscillation in their expression levels or in their posttranslational modifications. In transgenic mouse models, disruption of circadian clock activity results in dramatic changes in the circadian pattern of urinary sodium and potassium excretion and causes significant changes in arterial blood pressure. A growing number of evidence suggests that dysregulation of circadian rhythms is associated with the development of hypertension and accelerated progression of chronic kidney disease (CKD) and cardiovascular disease in humans. Chronotherapy studies have shown that the efficacy of antihypertensive medication is greatly dependent on the circadian time of drug administration.

Summary: Recent research points to the major role of circadian rhythms in renal function and in control of blood pressure.

Keywords: kidney, circadian clock, sodium, blood pressure, chronopharmacology.

Introduction

Circadian rhythmicity of renal function is a long-studied phenomenon which was first documented in the middle of the 19th century when Edward Smith and other pioneers in chronobiology noted that urinary water and urea excretion exhibit significant circadian oscillations [1]. The existence of circadian rhythms was subsequently revealed in a number of specific renal functions. Despite the apparent correlation of renal circadian rhythms with rhythms of activity and feeding, physiological studies demonstrated that several important rhythmic processes in the kidney (e.g. circadian rhythm of urinary potassium excretion) are largely self-sustained and intrinsic in origin (reviewed in [2,3]). On the molecular level, it has been shown that circadian rhythms are primarily driven by the circadian clock, a complex mechanism that tightly coordinates all major cellular functions with the time of the 24-hour geophysical cycle (circadian time). This coordination provides an important functional advantage which consists in time-of-day-dependent preconditioning of cells and organs to recurrent environmental circadian changes.

Long-term misalignment of intrinsic circadian rhythms with environmental time has been shown as an independent risk factor for the progression of a variety of diseases, including diabetes, cardiovascular disease, chronic kidney disease and cancer (reviewed in [4,5]). Genome Wide Association Studies (GWAS) have suggested a genetic link between single nucleotide polymorphisms (SNPs) in genes involved in the circadian clock and type 2 diabetes [6], hypertension [7] and mood disorders [8]. A growing number of mouse models in which the activity of the circadian clock was modified have demonstrated that this mechanism is indispensable for many vital processes, including renal homeostatic function and blood pressure control. In this review we summarize advances achieved within the last 12-18 months in the understanding of the role of the circadian timing system in kidney health and disease.

The circadian clock Recent research has revealed that circadian rhythms are driven by a highly complex mechanism operating at different molecular levels. The first discovered and most studied circadian clock mechanism involves the transcriptional factors BMAL1, CLOCK and NPAS2 and their repressors PER and CRY (reviewed in [**9]). These proteins constitute the core of a transcriptional/translational feedback loop that generates transcriptional rhythms with a periodicity of ~24 hours. The BMAL1/CLOCK or BMAL1/NPAS2 heterodimers activate transcription and translation of PER and CRY during the daytime. Accumulation of PER and CRY proteins in the cytoplasm results in their heterodimerization and translocation into the nucleus where PER/CRY complexes bind to and inhibit the transcriptional activity of BMAL1/CLOCK and BMAL1/NPAS2. In the absence of *de novo* synthesis, PER and CRY are progressively depleted during the night by the ubiquitination/proteosome degradation pathway, thus resulting in the relief of the transcriptional repression (so completing the 24-hour cycle). Activity of this circadian clock in peripheral tissues is largely self-sustained and cell-autonomous, but its synchronization with the environmental time is dependent on a central pacemaker located in the suprachiasmatic nucleus of hypothalamus. In addition to the regulation of PER and CRY transcription, the BMAL1/CLOCK and BMAL1/NPAS2 heterodimers are thought to generate circadian oscillations of 2-10% of cellular transcriptome. For instance, Zuber et al. have shown that hundreds of mRNA transcripts in the microdissected distal convoluted tubule (DCT), connecting tubule (CNT) and cortical collecting duct (CCD) exhibit significant circadian oscillations, and Nikolaeva et al. have demonstrated that circadian rhythmicity of many of these transcripts is abolished in mice devoid of CLOCK [10,**11]. Many among the oscillating transcripts are crucially involved in different homeostatic renal functions e.g.,

aquaporin 2- and 4- water channels, the V2 vasopressin receptor, the NHE3 sodium proton exchanger, serum- and glucocorticoid- induced kinase 1 (Sgk1), the H,K-ATPase type 2 and the regulator of the Na,K-ATPase Fxyd5 [10,12,*13,14].

This classical model of the circadian clock based on the transcriptional oscillations has been seriously challenged by several recent studies. Menet et al. and Koike et al. have shown that oscillations of only about 20% of mature circadian mRNAs result from the circadian transcription [15,**16]. These studies pointed to the very important role of posttranscriptional events in the generation of circadian transcriptome. Another level of complexity in the circadian clock was revealed by the discovery of so-called "metaclocks" involving anti-oxidant proteins peroxiredoxins (PRX). PRXs exhibit transcription-independent oscillations in their oxidation states that parallel circadian production of reactive oxygen species (ROS) in mitochondria. Importantly, Edgar et al. have shown that the PRX oxidation state can influence the circadian transcriptional component, thereby demonstrating the interconnectivity between different circadian mechanisms [*17]. Finally, Jouffe et al. have recently demonstrated that the circadian clock controls ribosome biogenesis, a mechanism which is thought to be involved in the dynamic regulation of energy homeostasis in the cell [18].

Circadian rhythms in the kidney Accumulating evidence suggests that most if not all specific renal functions exhibit circadian oscillations. For instance, many renal rhythms are imposed by the circadian rhythmicity in the glomerular filtration rate (GFR), the renal blood flow (RBF) and the plasma concentration of filtered solutes. As an example, both the GFR and the plasma concentration of amino acids rise by ~50% during the activity phase of the circadian cycle, thereby generating significant circadian oscillations in the filtered load of amino acids (cumulative amplitude of ~100%) [19]. This, in turn, imposes a requirement for circadian adaptation in the reabsorption capacity for amino acids in the proximal tubule. How does this adaptation occurs at the molecular level has not been investigated, but our analyses

of renal circadian transcriptomes has shown that several major apical and basolateral transporters of amino acids exhibit significant circadian oscillations in their mRNA expression levels (Figure 1). A similar rationale can be applied to a wide variety of filtered substances since Dallmann et al. and Eckel-Mahan et al. have recently shown that at least 15% of blood metabolites cycle with a 24-hour rhythmicity (this rationale is depicted in Figure 2) [*20,*21].

Previous functional studies have suggested that the circadian clock is involved in blood pressure control. For instance, Bankir et al. demonstrated that the circadian dynamic of urinary sodium excretion is an important determinant of dipping in nocturnal blood pressure [22]. The discovery of the clock genes has allowed a direct assessment of the role of the circadian clock in renal function and in blood pressure control. Woon et al. have identified several polymorphisms in the promoter region of the human *BMAL1* gene that are associated with hypertension [7]. Mice with targeted disruption of any of the positive or negative elements of the circadian clock based on the transcriptional feedback loop display either decreased or increased blood pressure (reviewed in [2,23]). Recent studies have begun to shed light on the possible role of the kidney in these dysfunctions. As mentioned above, a great number of renal mRNA transcripts exhibit circadian expression pattern. However, only a few proteins have been experimentally shown to undergo circadian oscillations. For instance, Saifur Rohman et al. have shown that mRNA and protein expression of sodium-proton exchanger 3 (NHE3) displays a circadian expression pattern which was blunted in CRY1/CRY2 double knockout mice [24]. Recently, Susa et al. have demonstrated that proteins involved in renal sodium homeostasis and blood pressure control can be modified in a circadian manner on the posttranslational level [**25]. By analyzing WNK-OSR1/SPAK-NCC signaling cascade they were able to show that the phosphorylation level of OSR1 and SPAK kinases as well as the sodium-chloride co-transporter (NCC) display significant

circadian oscillation with the peak of phosphorylation at the time of transition between the inactive and active phases of the circadian cycle. The authors also demonstrate that this rhythmicity in phosphorylation state is driven by aldosterone and they interpreted the circadian phosphorylation kinetic of the cascade as a requirement to decrease sodium reabsorption during the active phase and to increase during the rest phase.

Aldosterone is the major regulator of sodium balance and blood pressure. Doi et al. have shown that secretion of this mineralocorticoid hormone by the adrenal glands is controlled by the circadian clock [26]. The authors demonstrated that beta-hydroxyl-steroid dehydrogenase (Hsd3b6), a steroidogenic enzyme involved in aldosterone biosynthesis, is transcriptionally regulated by the molecular clock. In Cry1/Cry2 double knockout mice that exhibit constantly elevated activity of the circadian clock, the plasma aldosterone levels are markedly increased causing salt-sensitive hypertension. Gumz and colleagues have shown that aldosterone can influence sodium reabsorption in the distal nephron and the collecting duct by interfering with the circadian clock activity. They demonstrated that aldosterone upregulates the expression level of the alpha subunit of the epithelial sodium channel (αENaC) in part through the stimulation of Per1 expression [27,28]. This finding was recently supported and extended by Richards et al. who demonstrated that αENaC expression and ENaC activity are significantly reduced upon inhibition of casein kinases 1δ and 1ε (CK1δ/ε) which are required for the phosphorylation and nuclear localization of PER/CRY heterodimers [*29]. The same group has recently reported that Per1 knockout mice exhibit low blood pressure [12].

Recently, we have shown that mice devoid of CLOCK exhibit dramatic changes in the circadian dynamic of urinary sodium, potassium and water excretion [**11]. Further functional analysis revealed that suppression of CLOCK abolishes the normal circadian rhythmicity of plasma aldosterone levels and results in a significant reduction in blood

pressure. Importantly, all these changes occurred despite normal pattern of physical activity and water intake in the knockout mice. To identify molecular mechanism underlying these dysfunctions we analyzed transcriptional changes in kidneys of these mice. Interestingly, the most significant difference was observed in the expression levels of cytochrome-P450 enzymes (Cyp4a12 and Cyp4a14) involved in the oxidation of arachidonic acid to 20-Hydroxyeicosatetraenoic acid (20-HETE). Analysis of temporal changes in 20-HETE levels in the kidney revealed a circadian-like pattern that was significantly modified in the knockout mice. 20-HETE has been shown to have a complex influence on blood pressure by inducing vasoconstriction and by inhibiting sodium reabsorption in the renal tubule. Collectively, this study suggested that the Cyp4a/20-HETE pathway could be one of the regulatory mechanisms by which the circadian clock participates in blood pressure control.

The circadian clock and human diseases. Given the pivotal role of the circadian clock in a variety of cellular functions, numerous studies have addressed the role of the circadian clock genes in pathogenesis of human diseases. Different animal models have been created and/or used to evaluate this issue. One of the most striking finding was obtained in the model of golden hamster heterozygous for a loos-of-function mutation in the circadian regulatory gene casein kinase-1epsilon [30]. These animals exhibit extensive fibrosis leading to cardiomyopathy, renal insufficiency and premature death when exposed to the light-dark cycles longer than the period of their intrinsic circadian clock, while the same animals kept in the light-dark cycle synchronized with their intrinsic circadian clock do perfectly well [31]. Another example is given by mice devoid of the three clock output genes DBP-HLF-TEF which display lower blood pressure, lower aldosterone levels and cardiac hypertrophy [32].

In humans, evidence showing a link between the circadian clock and diseases is weaker and more indirect. Unbiased genome-wide association studies (GWAS) have associated single nucleotide polymorphisms in loci close to genes involved in the molecular

clock with several traits. When searched in the catalog of published GWAS (http://www.genome.gov/gwastudies/), we found that rs900145, close to *BMAL1 (ARNTL)*, was associated with age at menarche [33] and plasminogen activator inhibitor-1 (PAI-1) plasma levels [34], and that the *CRY2* variant rs11605924 was associated with fasting glucose levels and beta cell function [6,35]. Moreover, a *CLOCK* variant was associated with agreeableness in one study [8] and *NPAS2* with a variant form of Creutzfeldt-Jakob disease in another study [36]. The *PER1, PER2, CRY1, DBP, TEF* and *HLF* or other genes coding for important components of the molecular clock have not display any significant association in GWAS conducted so far. In order to establish causality, one would need to study human beings with proven and unique deletion or mutation leading to significant disruption of the circadian clock. The only Mendelian disease known to be associated with the circadian clock is the autosomal dominant familial advanced sleep-phase syndrome (OMIM 604348) in which the genes *PER2* and *CSNK1D* are mutated [37-39]. However, these families are extremely rare, poorly characterized and no data on their long-term health status is available. Another rare syndrome recently associated with the circadian clock function is the Smith-Magenis syndrome in which haploinsufficiency of *RAI1* gene leads to a complex clinical phenotype characterized by low intellectual capabilities, sleep-disorders disturbances, obesity and various neurobehavioral and congenital anomalies. One of the hallmarks of this syndrome is an inverted melatonin rhythm that leads, as a consequence, to diurnal somnolence and nocturnal sleep difficulties. RAI1 was shown to be an important regulator of the expression of *CLOCK* and other components of the clock system [40].

One of the most prominent circadian traits in human is blood pressure. Nocturnal dip of blood pressure is a well-known phenomenon, but the underlying physiological mechanism(s) still remains largely elusive. Numerous studies have attempted to address the role of the circadian clock in this phenomenon in shiftworkers [41-44]. These studies,

however, have yielded in conflicting results. In total, evidence establishing causality between shiftwork and hypertension are scarce and do not allow definitive conclusion.

Chronotherapy for hypertension? Accumulating evidence suggests that the antihypertensive medication should be preferentially taken at vesper time. Using HYGIA cross-sectional data and MAPEC prospective data, Hermida and colleagues showed that prescription of at least one anti-hypertensive drug at bedtime controlled hypertension significantly better than when taking the treatment exclusively in the morning [45]. These and other data were recently reviewed in a meta-analysis of 21 studies that included ~2000 patients suffering from primary hypertension [46]. Evening taking of antihypertensive drugs was associated with a significantly better control of 24 hour blood pressure than morning dosing (SBP: -1.71 mm Hg, 95% CI: -2.78 to -0.65; DBP: -1.38 mm Hg, 95% CI: -2.13 to -0.62), but the clinical relevance and the effect on harder endpoints such as cardiovascular events remains unexplored. The mechanisms by which this effect would take place are still speculative. Pharmacokinetics and/or pharmacodynamics profiles for a given drug might be more optimal in the evening, even though almost any kind of antihypertensive drug tested so far has shown better effect when taken in the evening. This suggests a more general, drug-independent mechanism.

Conclusions

Identification of the molecular components of the clock system has allowed significant advances in the comprehension of circadian rhythms at the transcriptional level, and has brought forward new mechanisms of regulation e.g. at translational level or by redox reaction. The renal and hypertension fields start to benefit from these progresses. In particular, circadian rhythms should be taken into account in daily interpretation of data collected at

different times of the day. Research should be oriented at deciphering circadian rhythms of complex traits i.e. traits in which several levels of circadian-dependent regulation are taking place. Chronotherapeutics may help ameliorating the control of blood pressure in humans and open new perspective in other fields.

Figure legend.

Figure 1. Circadian expression patterns of mRNAs encoding different amino acid (AA) transporters in the mouse proximal tubule. Y-axis is the expression level in arbitrary units. ZT - Zeitgeber time units: ZT0 is the time of light on and ZT12 is the time of light off.

Figure 2. Different origin of circadian rhythms in the kidney. The circadian rhythmicity in filtered loads results from the circadian rhythms in the GFR, the RBF and the circadian oscillations in blood levels of filtered compounds. The circadian rhythms in urinary excretion is the sum of circadian rhythms in filtered loads and tubular reabsorption/secretion. Of note, the period length of these functional rhythms is approximately the same (~ 24 hours), but the difference between their maximum and minimum values (amplitude) as well as the time of the day when the given process function at a maximal rate (acrophase) can vary considerably.

ACKNOWLEDGMENTS This work was supported by the Swiss National Science Foundation research grants 31003A-132496 (to D.F.) and PP00P3-133648/1 (to O.B.).

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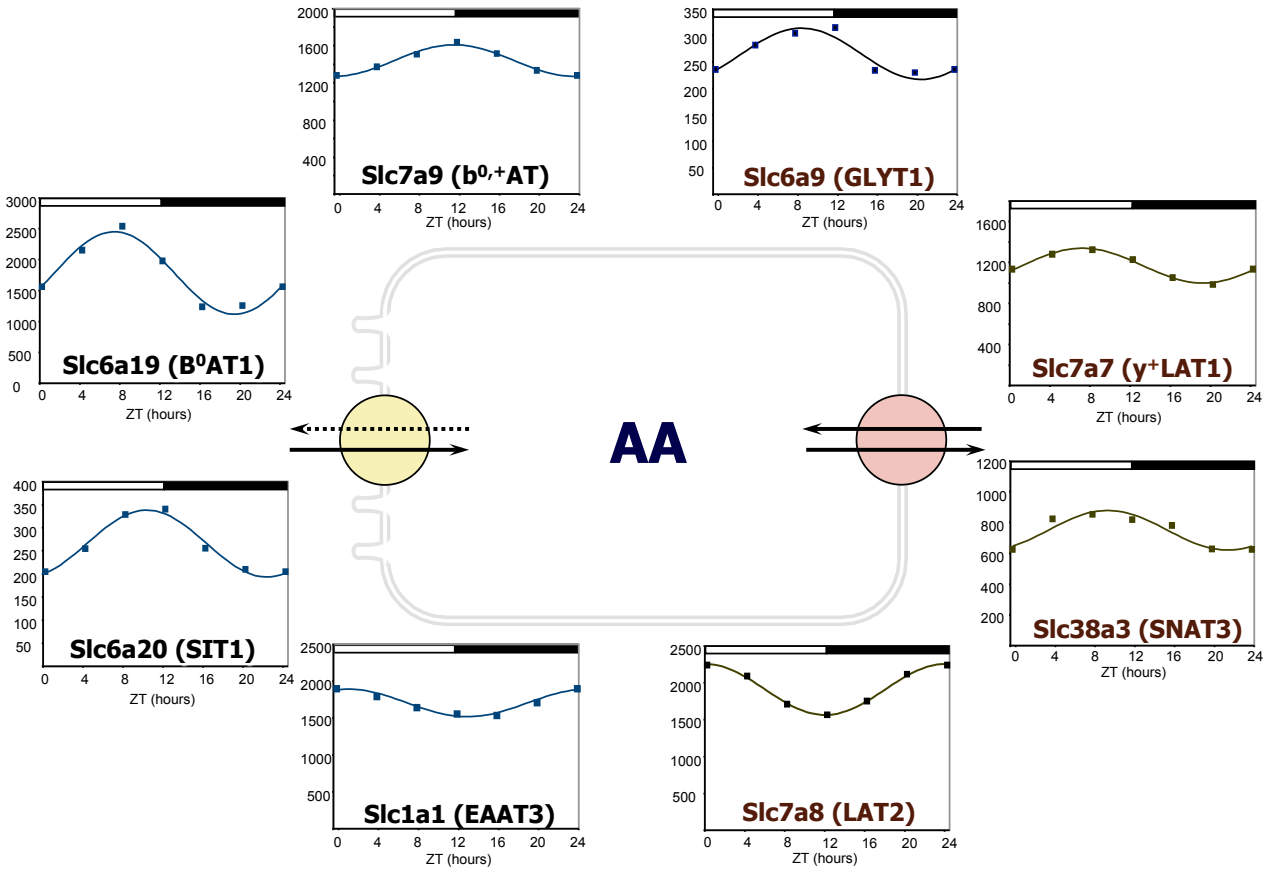

Figure 1. Bonny & Firsov

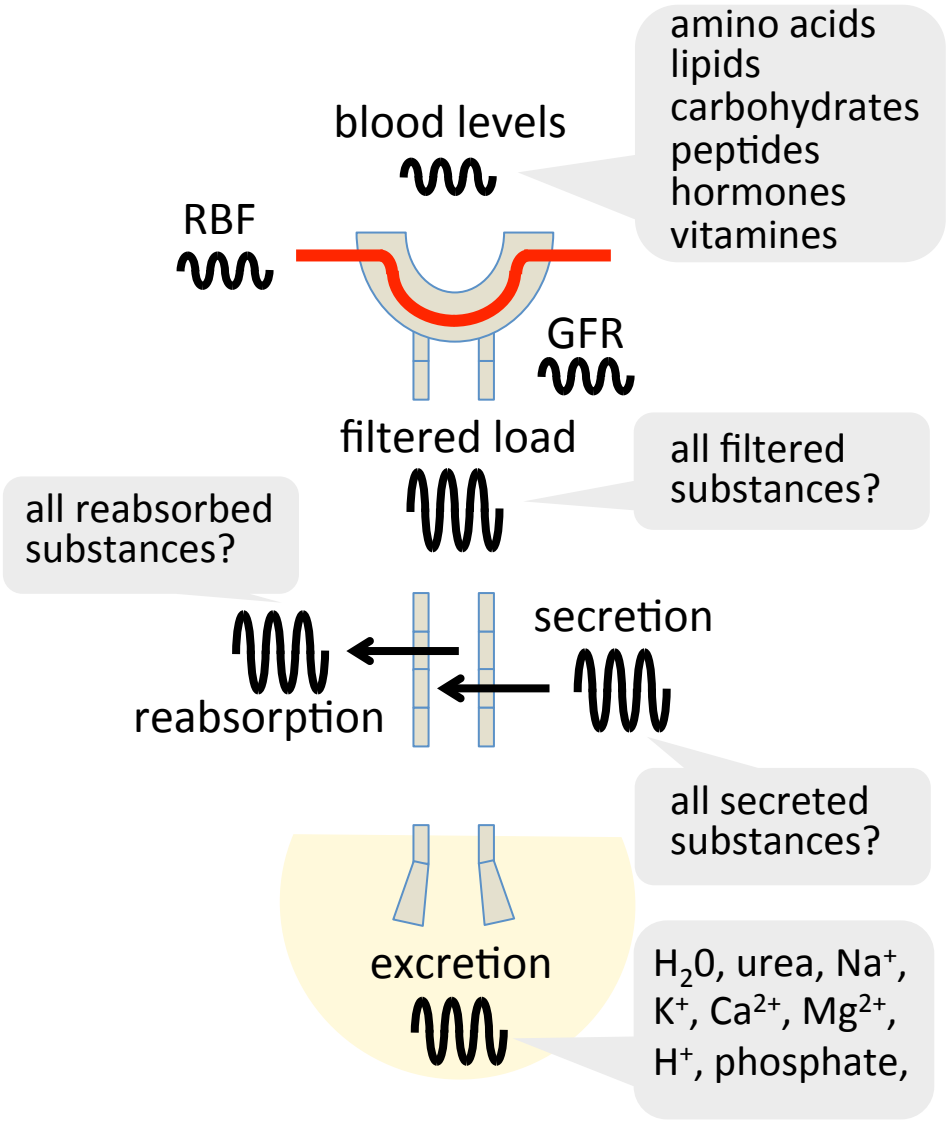

Figure 2. Bonny & Firsov