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Diurnal Regulation of Renal Electrolyte Excretion: The Role of Paracrine Factors

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Abstract

Many physiological processes, including most kidney-related functions, follow specific rhythms tied to a 24-h cycle. This is largely because circadian genes operate in virtually every cell type in the body. In addition, many noncanonical genes have intrinsic circadian rhythms, especially within the liver and kidney. This new level of complexity applies to the control of renal electrolyte excretion. Furthermore, there is growing evidence that paracrine and autocrine factors, especially the endothelin system, are regulated by clock genes. We have known for decades that excretion of electrolytes is dependent on time of day, which could play an important role in fluid volume balance and blood pressure control. Here, we review what is known about the interplay between paracrine and circadian control of electrolyte excretion. The hope is that recognition of paracrine and circadian factors can be considered more deeply in the future when integrating with well-established neuroendocrine control of excretion.

INTRODUCTION

The control of renal fluid and electrolyte handling is an extremely complex effort involving a wide range of endocrine, paracrine, and autocrine factors that balance intake with output. In terms of endocrine factors, the amount of work dedicated to the renin-angiotensin-aldosterone system is immense and arguably the most studied aspect of kidney function. The physiological role of antidiuretic hormone is also very well established, as is the role of atrial natriuretic peptide, which is now approaching the fortieth anniversary of its discovery (1). Paracrine and autocrine control of renal excretory function, such as endothelin (ET) (2), ATP/purinergic receptors (3), kinins (4), arachidonic acid and P450 metabolites (5), and nitric oxide (NO) (6), has been studied and reviewed in isolation, but few studies have integrated the balance of actions. To make matters even more complicated, renal hemodynamics and neural inputs can directly and indirectly modulate the activity of these systems. Although most would agree that intrarenal hemodynamics can impact renal tubular function, the difficulty in measuring medullary blood flow has resulted in few laboratories considering this as impacting their system. The role of renal nerves has gained considerable new attention in the past 10+ years as the result of a series of clinical trials demonstrating promise for renal denervation for the treatment of resistant hypertension (7). Furthermore, only in recent years have we recognized that these balances are greatly impacted by sex and time of day.

While we have known for over 100 years that kidney excretory function follows a diurnal pattern, we have only begun to explore how this system works. A handful of labs have generated some very convincing evidence that the cell autonomous molecular clock functions to regulate renal tubular and vascular function in a circadian fashion (8). Given the current state of the field and the many reviews of individual factors, we focus here on a few of the major paracrine/autocrine factors known to impact renal excretory function, specifically endothelin-1 (ET-1) and purinergic receptors, with a specific focus on how the intrinsic kidney clock controls renal excretory function.

KIDNEY CLOCK AND CIRCADIAN PHYSIOLOGY

We have long known that several aspects of kidney physiology in humans follow specific rhythms within a single 24-h day (summarized in **Figure 1**). In 1950, Sirota et al. (9) recruited 18 healthy adults and conducted a thorough study examining diurnal variations of renal function. They showed that urine volume during the night was significantly lower compared with daytime. Tubular reabsorption was considerably higher at night (9). Shortly thereafter, Mills & Stanbury (10) carried out a study in which healthy adults were placed on a 12-h routine in place of the normal 24-h pattern. They found that diurnal urinary excretion of water and electrolytes, namely sodium and potassium, was not significantly affected by the 12-h cycle of eating, drinking, and sleeping patterns (**Figure 2**). These findings led to the original hypothesis for an intrinsic mechanism for regulating renal function. However, it was not until the past decade or so that we started to gain a further mechanistic understanding with regard to how this kidney rhythm was regulated.

The 2017 Nobel Prize in Physiology and Medicine was awarded to Jeffery Hall, Michael Rosbash, and Michael Young for their discoveries of molecular mechanisms controlling circadian rhythms. The readers are referred to excellent review articles for molecular circadian clock regulation (11, 12). Briefly, circadian proteins Clock and Bmal1 act as master transcription factors and activate *Period* (*Per*), *Cryptochrome* (*Cry*), and many other target genes. *Per* and *Cry* translocate to the nucleus and act as the repressive part in the feedback loop (**Figure 3**). As shown in **Figure 4**, several critically important genes related to sodium transport in the kidney are expressed in a circadian pattern such as the sodium/hydrogen exchanger 3 (*NHE3*), the sodium-glucose cotransporter 2 (*SGLT2*), and the epithelial sodium channel (*ENaC*) (13). The utilization of global and tissue-specific knockout animal models has facilitated a more thorough

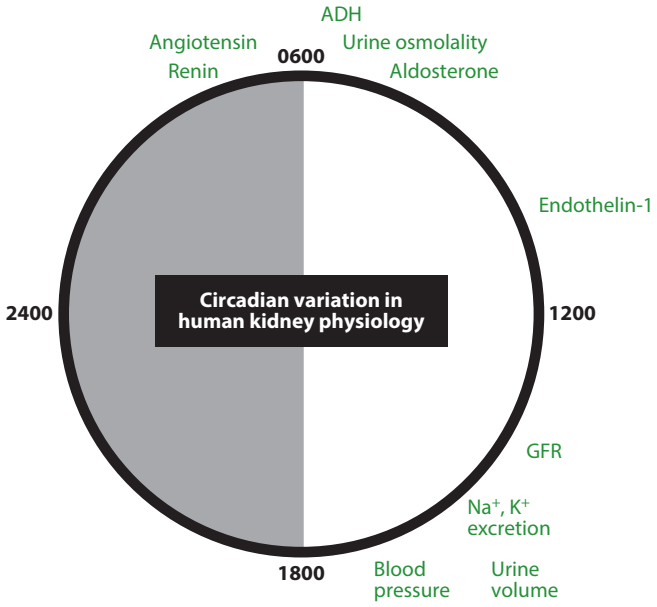

Figure 1

Approximate time of day when a variety of major renal excretory systems reach their maximum level. Minimums generally occur at the opposite side of this 24-h clock. Abbreviations: ADH, antidiuretic hormone; GFR, glomerular filtration rate.

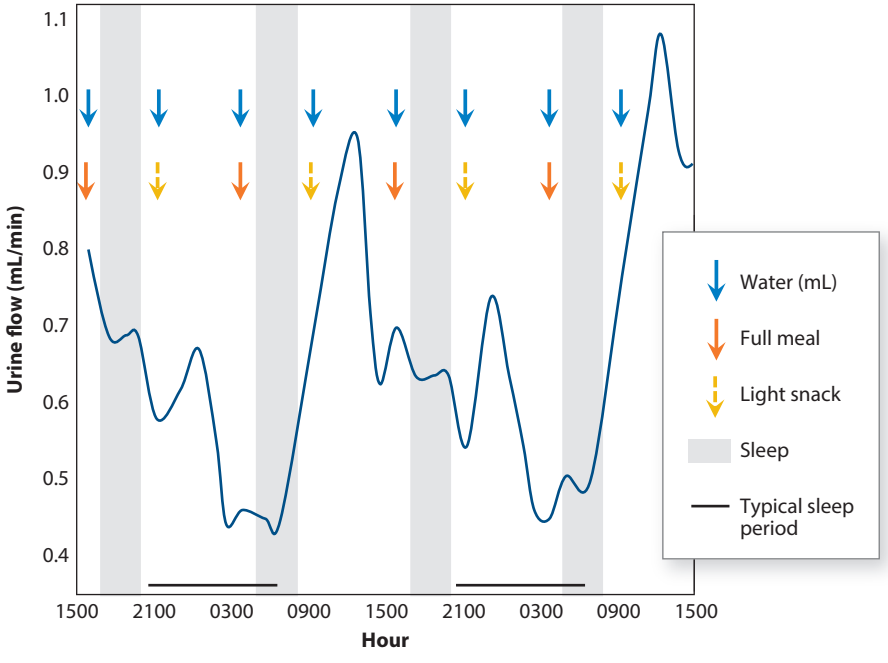

Figure 2

Persistence of circadian urine flow despite having a 12-h eating and sleeping pattern in healthy volunteers (10).

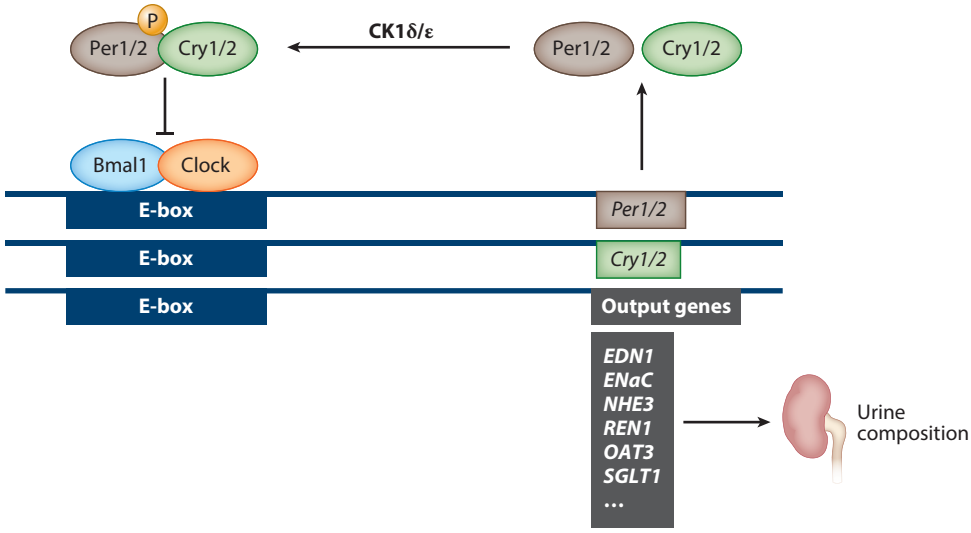

Figure 3

Simplified view of the core circadian clock. Bmal1 and Clock heterodimerize before binding to E-box elements on the promoters of a wide range of genes including *Per* and *Cry*. Per and Cry proteins then heterodimerize before then inhibiting Bmal1 and Clock binding to the E-box. However, this translocation is dependent upon phosphorylation by CK1δ/ε. Figure based on a variety of sources but primarily Reference 8. Abbreviations: *EDN1*, endothelin-1; *ENaC*, epithelial sodium channel or *SCNN1A*; *NHE3*, sodium/hydrogen exchanger 3 or *SLC9A3*; *OAT3*, organic anion transporter 3 or *SLC22A8*; *REN1*, renin 1; *SGLT1*, sodium-glucose cotransporter 1 or *SLC5A1*.

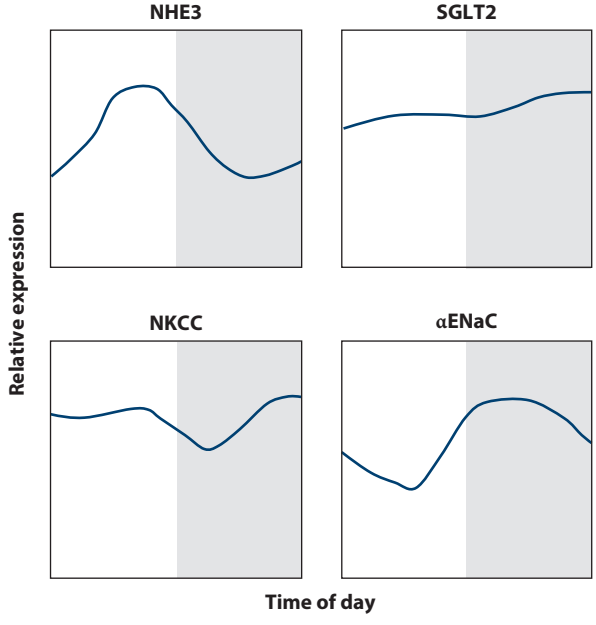

Figure 4

Approximate rhythm of mRNA expression for major Na^+ transporters in the mouse kidney. Figure based on the Circadian Expression Profiles Database (CircaDB) originally established by Panda et al. (13). Abbreviations: αENaC, epithelial sodium channel alpha subunit; NHE3, sodium/hydrogen exchanger 3; NKCC, potassium chloride channel; SGLT2, sodium-glucose cotransporter 2.

Table 1 Known mechanisms of molecular clock function in the nephron

Regulator	Target gene (mRNA)	Nephron segment	Reference(s)
Bmal1-Clock	*NHE3*	Proximal tubule	125
Per2, Cry1	*Bmal1-Clock*	Proximal tubule	125
Per1	*NHE3*	Proximal tubule	126
Per1	*SGLT1*	Proximal tubule	126
Bmal1	*OAT3*	Proximal tubule	22
Bmal1 (Arntl)	*Renin*	Thick ascending limb	23
Per1	*pNCC*	Distal tubule	28
Aldosterone	*Per1*	Collecting duct	24, 26
Per1	*αENaC* (*Scnn1A*)	Collecting duct	27
CK1δ/ε	*Per1*	Collecting duct	127

understanding of the specific roles of circadian components in different organs, including the kidney (**Table 1**). After the liver, the kidney has the second greatest number of genes that follow a circadian expression pattern (14), yet little is known about how these systems impact excretory function. Here, we list animal models deficient in clock components and summarize recent progress in the understanding of circadian regulation of renal physiology with a focus on blood pressure (summarized in **Figure 5**) and renal electrolyte excretion. Readers are directed to recent review articles in circadian renal function and its clinical implications (15, 16).

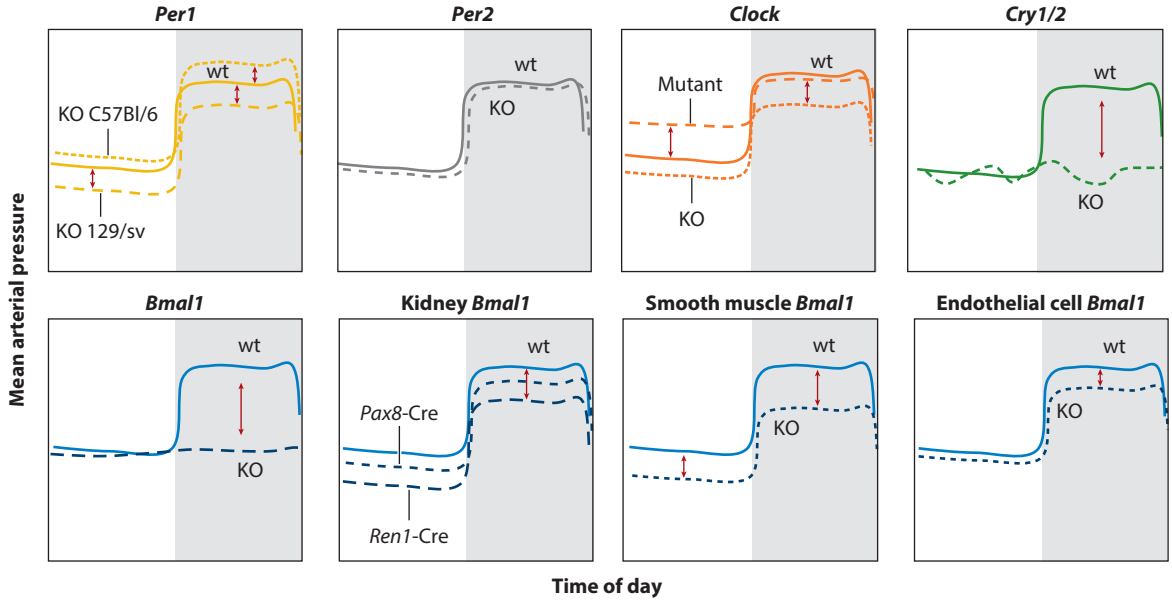

Figure 5

Approximation of blood pressures over the course of 24 h in mice with various clock gene knockouts (KO) compared to wild types (wt). All panels are based on 12:12 light:dark conditions while maintained on standard rodent diets. Red arrows depict significant differences in KO versus wt in light and dark conditions. *Per1*KO strains are shown for two different genetic backgrounds, C57Bl/6 and 129/sv. For *Clock*, there is a mutant strain that has a nonfunctional protein expressed and a traditional KO strain. Kidney *Bmal1*–specific KO include the *Pax8*-Cre that is specific for the entire nephron and the *Ren1*-Cre that is specific for cells expressing renin, which includes juxtaglomerular cells as well as renal epithelium.

Bmal1

Bmal1 has been one of the most intensively studied circadian genes and is often referred to as a component of the core clock, not to be confused with the central clock that refers to the molecular clock within the central nervous system, specifically in the suprachiasmatic nucleus. Various mouse models with specific depletions of *Bmal1* exhibited disrupted blood pressure phenotypes with or without impaired renal electrolyte excretion. The global *Bmal1* knockout (*Bmal1*KO) mouse was first generated in 2000 by Bunger et al. (17) and was known to have a complete loss of circadian rhythmicity in activity. Subsequently, Curtis et al. (18) showed that *Bmal1*KO mice did not have a circadian blood pressure rhythm such that blood pressure did not rise during the active phase. Further efforts have been made to uncover the mechanisms underlying *Bmal1* control of circadian blood pressure and renal electrolyte excretion using a variety of tissue-specific knockout mice. Wang et al. (19) showed that loss of peroxisome proliferator-activated receptor gamma, or *PPARγ*, in smooth muscle (*SM22*-Cre) led to decreased day/night variation of blood pressure in mice, which was further shown to be caused by impaired rhythmicity of *Bmal1*. Xie et al. (20) utilized the same *SM22*-Cre and observed that depletion of *Bmal1* in smooth muscle reduced the night-day amplitude of circadian blood pressure rhythms in mice without affecting their locomotor activity. In addition, Chang et al. (21) generated perivascular adipose tissue (PVAT)-specific *Bmal1*KO mouse and showed that loss of *Bmal1* in PVAT led to a superdipper phenotype, where the inactive phase of blood pressure was low compared with control mice. Collectively, these studies were of great importance because they provided solid evidence that peripheral circadian components participate in circadian blood pressure regulation.

Regarding the kidney clock, Firsov and colleagues (22) have generated a renal tubular *Bmal1*-deficient mouse model using *Pax8*-Cre. These mice lack *Bmal1* expression in the entire nephron. They observed a slight decrease in systolic but not diastolic blood pressure and an intact circadian blood pressure rhythm. In addition, the rhythms of sodium and potassium excretion were preserved, although we do not know how the mice would respond to a challenge of low or high dietary electrolytes. In another kidney-specific *Bmal1*KO model generated by the same group, depletion of *Bmal1* in renin-secreting cells (*Ren1*-Cre) increased glomerular filtration rate (GFR), attenuated diurnal rhythms of sodium and water excretion, and reduced plasma aldosterone concentrations (23). However, circadian blood pressure rhythms remained intact even though both systolic and diastolic blood pressure were significantly decreased compared to control mice. These studies suggested that kidney-specific Bmal1 may be important in maintaining blood pressure and/or electrolyte rhythms; however, further studies are needed to determine whether Bmal1 in the kidney controls the rhythmicity of blood pressure.

Period

The Gumz lab (24) has published a line of work illustrating an important role for Per1 in the regulation of blood pressure and renal electrolyte excretion as well as the renal effects of aldosterone. This lab generated mice where the clock gene *Per1* was deleted in two strains of mice (the salt-sensitive 129/sv and salt-resistant C57Bl/6). Blood pressure phenotypes were different between these two strains of mice. Deletion of *Per1* in 129/sv mice produced a significantly lower blood pressure compared with control mice; however, loss of *Per1* in C57Bl/6 mice resulted in an increase in blood pressure observed only during the active phase (25). Following high salt plus mineralocorticoid treatment, male knockout mice developed a nondipping blood pressure pattern; that is, blood pressure was not reduced during the inactive (lights on) phase (25). However, females did not display this nondipping phenotype (26). Consistent with the blood pressure changes, Per1 also positively regulates sodium transporters in the kidney (27, 28). Male *Per1*KO mice have a loss

of diurnal sodium excretion and elevated ET-1 mRNA in the kidney; changes in ET-1 were not observed in female *Per1*KO mice. Given the sex differences in ET-1-dependent natriuresis in the kidney (29), these findings suggest that there could be a sex difference in how the clock regulates ET-1-dependent sodium homeostasis. Importantly, a recent study by Alli and colleagues (30) reported that knockdown of *Per1* with siRNA in *Xenopus* 2F3 distal nephron cells reduced ENaC activity as assessed by patch clamp techniques.

Mice lacking another isoform of the *Per* gene, *Per2*, were shown to have aortic endothelial dysfunction and significantly lower diastolic blood pressure during the active phase; however, systolic and mean arterial pressure were not different compared to control mice (31). Pati et al. (32) treated a series of Period isoform knockout (*Per2*KO, *Per2*,*3*KO, and *Per1*,*2*,*3*KO) mice with a low-salt diet and were able to show a loss of circadian blood pressure due to high inactive phase blood pressure, which was restored by losartan. These findings suggest that angiotensin contributes to Period-mediated regulation of blood pressure, but whether this response has effects on kidney electrolyte handling is unknown.

Cryptochrome, *Clock*, and *Dec*

Doi et al. (33) generated double *Cryptochrome* (*Cry1* and *Cry2*) null mice and showed that they exhibited a loss of circadian blood pressure rhythm, although the 24-h average blood pressures were similar to wild-type mice. Interestingly, these mice developed significant hypertension relative to wild-type controls when placed on a high salt diet due to extremely high levels of circulating aldosterone; this effect was mitigated by administration of a mineralocorticoid receptor antagonist, eplerenone.

In contrast to the *Cry1/2* knockouts, *Clock* mutant mice that generate a nonfunctional protein have intact circadian blood pressure rhythms; however, the blood pressure dipping was reduced such that the night-day amplitude was significantly attenuated compared to wild-type controls (34). This abnormality in circadian blood pressure and heart rate rhythms was entirely abolished by adrenalectomy (34). Furthermore, Zuber et al. (35) generated *Clock*-deficient mice that have a lower expression of ENaC at the beginning of the inactive phase that is consistent with an observed increase in urinary sodium excretion. In addition, *Clock* mutant mice had an arrhythmic plasma aldosterone concentration and disrupted plasma potassium level compared to wild-type controls. It is important to note that these two *Clock* mutant/-deficient mouse strains (34, 36) were generated on different genetic backgrounds, which we know can lead to distinct differences in phenotype, as reported by the Gumz lab (24) for *Per1* where mice on the 129/sv background had normal blood pressure rhythms, but at a lower level than wild-types, but *Per1*KO on the C57Bl/6 background were hypertensive but only during the active period (25).

Dec1 has been considered a fifth member of the clock gene family and functions to repress the transcription of *Per1* induced by the Clock/Bmal1 heterodimer (37). Nakashima et al. (38) showed that mice lacking the *Dec1* gene had significantly lower blood pressure but an intact circadian rhythm. Evidence that Dec1 directly regulates *Atp1b1*, a gene that encodes the beta subunit of sodium/potassium ATPase, was also reported in this study.

PARACRINE CONTROL AND CIRCADIAN EXCRETION

Endothelin-1

In this section, we summarize our current understanding of the connections between the circadian system and ET-1 system and their synergistic impact on renal electrolyte excretion. ET-1 is a 21-amino-acid peptide with very potent effects on many cell types throughout the body (39). Over the past several decades, considerable progress has been made characterizing the effects of

ET-1 in the kidney and the renal microcirculation that serves, in large measure, to facilitate the excretion of high dietary salt (40). The primary sources of ET-1 production in the kidney are the collecting duct and particularly the inner medullary collecting duct (IMCD), but considerable ET-1 is also produced by the vascular endothelium and most likely a few other sources. Production of ET-1 is largely stimulated by high dietary salt. This occurs in both the collecting duct and vascular endothelium. In an autocrine fashion, ET-1 in the collecting duct primarily stimulates the ET_B receptor, which, in turn, activates nitric oxide synthase 1β (NOS1β) to produce NO, cGMP, and subsequent inhibition of ENaC activity to facilitate natriuresis. However, the role of ET-1 in response to high-salt diet is evident in the renal microcirculation as well. Fellner et al. (41) showed that impaired autoregulation by the renal afferent arteriole resulting from a chronic high-salt diet is restored following ET_B receptor blockade, suggesting that ET-1 can contribute to increased GFR during high-salt conditions. In recent years, considerable attention has been paid to circadian aspects of the ET-1 system following the important discovery of the Gumz lab (42) showing that the largest changes in gene expression produced by aldosterone in collecting duct cells were *Per1* and the ET-1 gene (*EDN1*).

In 1998, Hwang et al. (43) recruited 11 normotensive subjects and 23 hypertensive patients and conducted an elegant study to determine the relationship between hypertension and the diurnal excretion rate of ET-1. Subjects with hypertension, but not end-organ damage, exhibited regular circadian blood pressure rhythm. However, there was a one-hour phase delay in the hypertensive group compared with normotensive controls; that is, the peak in blood pressure consistently occurred at a later time. In general, the asynchrony of functional rhythms is thought to increase disease risk. Urine samples from these patients were collected 4 times per day for 3 consecutive days. Under a control diet (7g NaCl every 24 h), urinary sodium and ET-1 excretion showed a similar diurnal rhythm between normotensive and hypertensive subjects. In addition, the diurnal pattern of ET-1 excretion was correlated with sodium excretion. Following saline infusion, nearly half of the hypertensive group showed a delayed natriuretic response where urinary sodium excretion was not increased compared with baseline during the first 4 h. Interestingly, less urinary ET-1 excretion was also observed in these patients. This delayed response in sodium and ET-1 excretion happened in the morning hours (8 AM–12 PM), which is roughly the beginning of the active period for humans. In contrast, the other half of hypertensive subjects showed an exaggerated natriuretic response as well as increased ET-1 excretion following saline infusion. This early study in humans demonstrated that in a specific population of hypertensive subjects, the timing of urinary sodium excretion can be delayed, which could be related to circadian excretion of ET-1. Consistent with these results, Johnston et al. (44) showed that rats with dysfunctional ET_B receptors (ET_B-def) failed to excrete sodium in a timely manner. When challenged with an acute salt load at the beginning of the inactive period, both male and female ET_B-def rats had a delayed natriuretic response compared with control rats. However, when the salt load was given at the beginning of the active period, female ET_B-def rats were able to more promptly excrete the sodium, whereas the natriuretic response in males was significantly delayed. This delay could be due, at least in part, to increased ET_A receptor activity when ET_B activity is reduced because administration of an ET_A antagonist, atrasentan, was able to improve the delayed natriuretic response. These two studies from humans and rats suggest that diurnal/circadian excretion of sodium requires the ET-1 system in which the natriuretic effect mediated by the ET_B receptor may be opposed by the ET_A receptor. Furthermore, males and females have differential regulatory mechanisms of the ET-1 system that are time dependent and may explain why females have less salt-dependent hypertension.

Dhaun et al. (45) conducted a clinical trial examining nocturnal blood pressure dipping and arterial stiffness in nondiabetic chronic kidney disease (CKD) patients and healthy controls. They found that elevated plasma ET-1 levels were associated not only with hypertension but also

nondipping blood pressure that is characteristic in CKD patients. In addition, 6-week treatment with an ET_A receptor antagonist, sitaxentan, significantly improved the nocturnal dipping, suggesting the involvement of the ET system in the diurnal variation of blood pressure in the setting of CKD. The authors were not able to detect circadian variations in plasma ET-1 or urinary ET-1, which could be due to the relatively small sample size. It is also possible that the lack of observation for circadian activity of the ET-1 system may be related to difficulties in measuring paracrine activity in vivo. In rats, plasma ET-1 exhibits a discernable rhythm within 24 h (46). Urinary excretion of ET-1 is higher in the active period compared with the inactive period in normal rats but not in ET_B-def rats, suggesting an impaired diurnal renal production of ET-1 when ET_B receptors are not functional, which occurs in many forms of salt-sensitive animals.

High salt intake causes a highly significant phase shift (a 5.5-h phase delay) of *Bmal1* mRNA expression in the inner medulla but not the cortex of control rats (46). However, this high salt–induced alteration in *Bmal1* expression was absent in ET_B-def rats. *Bmal1* mRNA expression in IMCD3 cells decreased following exogenous ET-1 treatment (47). In addition, *Bmal1* knockdown reduced the expression of ENaC. These results provide strong evidence for involvement of Bmal1 in ET-1 system-mediated regulation of renal sodium excretion. Of note, high-salt diet causes an increase in skin sodium content during the active phase compared with the inactive phase in ET_B-def rats, which may contribute to the increased blood pressure amplitude observed in these rats following high-salt diet (48).

As noted earlier, Per1 also appears to be heavily involved in regulating the ET-1 system in the collecting duct. Gumz et al. (27) first discovered in 2009 that Per1 positively regulates ENaC. Subsequently, the same lab showed that knockdown of *Per1* in collecting duct cells leads to an induction of ET-1 and several other negative regulators of ENaC (24). In addition, mice lacking *Per1* on a 129/sv background have lower blood pressures overall despite maintaining a normal night-day amplitude (24). This was associated with increased renal production of ET-1 from both the inner medulla and cortex. In another study, this lab also reported that ET_A and ET_B receptor expression in the kidney is time dependent but not regulated by Per1 (49); mRNA levels of ET_A and ET_B receptors were assessed in wild-type and *Per1* heterozygous mice at the middle of the inactive and active phases. In both wild-type and *Per1* heterozygous mice, ET_A expression was lower at the active phase compared to the inactive phase in both the renal cortex and inner medulla. In both wild-type and Per1 het strains, ET_B expression was higher at the active phase compared to the inactive phase in the inner medulla, but cortical expression was lower at the active phase compared to the inactive phase. In contrast to Bmal1, Per1 acts as an upstream regulator of ET-1 production, which likely serves as a mediator for ET-1 regulation of sodium excretion.

Nitric Oxide

NO signaling is also key to promoting natriuresis and diuresis. NO inhibits sodium reabsorption in different segments along the nephron, primarily the thick ascending limb and the collecting duct. Work primarily from the Garvin lab (50–52) has established that NO decreases chloride absorption in the thick ascending limb of the loop of Henle and reduces the activity of the sodium, potassium 2 chloride cotransporter (NKCC2). In the collecting duct, direct evidence has established that NO inhibits the amiloride-sensitive ENaC (53). Additionally, NO can independently inhibit water reabsorption in the collecting duct (54). All three isoforms of NO synthase (NOS1, 2, and 3) are constitutively expressed along the nephron, but the distribution of these isoforms is quite specific. Most of the effects of NO on sodium and water handling have been attributed to NOS3 in the thick ascending limb and NOS1 in the collecting duct. However, actual NOS activity has been shown to be at least tenfold higher in the collecting duct than any other segment.

Advances in understanding NO function in salt-dependent hypertension were once hindered largely due to the normotensive blood pressure phenotype in global *NOS1*KO mice (55). It was later found that the original global *NOS1*KO was in fact a strain that only lacks the alpha subunit of NOS1, while the splice variant, NOS1β, remains active in the so-called *NOS1*KO mouse. Hyndman et al. (56) demonstrated that depletion of NOS1β in principal cells of the collecting duct in mice leads to impaired sodium excretion and salt-sensitive hypertension, which alters the salt-resistant pressure-natriuresis relationship observed in C57Bl/6 mice. The synergistic effect of the ET-1 and NO systems in regulating sodium excretion has been confirmed by several labs. ET-1 stimulates *NOS3* expression in the thick ascending limb and thereby increases the production of NO through activating the ET_B receptor (57). The signaling mechanism for the rapid (seconds to minutes) paracrine/autocrine effects of ET-1 on NO production is not clear. In primary cultures of rat IMCD cells, Ye et al. (58) provided evidence that endogenous ET-1 stimulates *NOS3* gene expression, although they did not measure NOS1 or its splice variants. Jennifer Pollock's lab (59) has shown that NOS1 and NOS3 activities are significantly lower in the inner medulla of ET_B-def rats compared with those of normal rats. In addition, both ET_A and ET_B receptors were shown to regulate NOS activity. Gao et al. (60) showed that mice lacking *NOS3* in the nephron (*PAX8*-Cre) displayed delayed sodium excretion and salt-sensitive hypertension. In addition to the thick ascending limb and collecting duct, NOS activation in the macula densa has been shown to be important in regulating blood pressure and sodium excretion. Lu et al. (61) showed that loss of NOS1β in mice leads to salt-sensitive hypertension and impaired sodium and potassium excretion following acute volume expansion.

Although recent evidence suggests a strong capacity for the NO regulation of renal sodium and water excretion, whether these NO-mediated effects depend on time of day remains largely unknown. An early study conducted by Tunctan et al. (62) measured NOS activity over 24 h in the kidney, brain, testis, lung, and aorta from male BALB/C mice. NOS activity measured in kidney homogenates exhibited circadian rhythmicity when mice were placed in a light/dark condition (14 h light, 10 h dark). However, under constant lighting conditions, circadian NOS activity in the kidney was absent, which was different from the brain and lung where NOS activities displayed circadian rhythms under both light/dark and constant lighting conditions. This suggests that NOS activity in the kidney could be entrained by light.

Functional evidence that NO signaling is heavily regulated by the circadian clock comes from studies in the vasculature. Anea and colleagues (63) showed that expression of phosphorylated NOS3 (or eNOS), an indirect measure of activity, was decreased in the carotid arteries of *Bmal1*KO mice, which was consistent with the reduced endothelial-dependent vascular function observed in both *Bmal1*KO and *Clock* mutant mice. Subsequently, this group showed that loss of Bmal1 was associated with increased production of endothelial superoxide by uncoupling NOS3 in the aorta (64). Westgate et al. (65) found that plasma nitrate and nitrite, indirect measures of NO production, displayed diurnal variations in control mice as well as endothelial-specific *Bmal1*KO mice. In mesenteric resistance arteries from male rats, maximal contraction to phenylephrine was greater early in the inactive period compared with responses in the active period, while the acetylcholine relaxation was the opposite being greater in the active period (66). NOS3 protein expression also exhibited a time-of-day variation with higher levels being observed during the active period corresponding to the greater acetylcholine response. Chronic blockade of NOS with L-nitroarginine methyl ester (L-NAME) resulted in hypertension with a maintained circadian rhythm, although at high doses, the amplitude of the blood pressure rhythm was exaggerated (67). *NOS3*KO mice are hypertensive compared to genetic controls, but the amplitude of blood pressure variation is greater due to the increase in blood pressure during the active period (68). Although this clearly indicates that NOS3 is not required for a blood pressure rhythm, it does indicate that NOS3

functions in a circadian pattern to impact blood pressure. Unfortunately, none of these studies examined the kidney or kidney NOS in circadian blood pressure control, but given the critical role of the kidney in blood pressure control, studies are clearly justified to explore the mechanism responsible for diurnal NOS activity and whether this occurs in the kidney.

In aged mice, Kunieda and coworkers (69) provided evidence that NO increases Per1 promoter activity and also helps to maintain circadian expression of Per2 in aortic smooth muscle cells. They also found that protein levels of phosphorylated NOS3 in aged mice exhibited an impaired circadian rhythm with lower levels during the inactive period compared with young mice. Once again, work in this area is underdeveloped and has not been addressed as to whether these mechanisms occur in the kidney. However, it has been reported that urinary nitrate excretion and cyclic GMP excretion, which are indicators for NO production, exhibited circadian rhythms in healthy subjects but not hypertensive patients (70).

Purinergic Receptors

ATP-binding purinoceptors, P2 receptors, are expressed throughout renal tubular segments and renal vasculature and have been shown to be highly involved in paracrine regulation of renal tubular transport. Using microperfusion techniques, Bailey (71) demonstrated that administration of a P2Y1 receptor agonist, 2meSADP, inhibited NHE3 activity and led to decreased sodium transport; this decrease could be abolished by co-administration of an P2Y1 antagonist, MRS2179. Pochynyuk et al. (72) provided direct evidence that purinergic signaling regulates ENaC in the collecting duct. This group showed that ATP could decrease ENaC activity through P2Y2 receptors on the apical side of the principal cells. Moreover, ENaC activity measured directly using patch-clamp methods was increased in collecting ducts isolated from *P2Y2*KO mice compared to genetic controls. Inconsistent with these findings, protein expression of αENaC was reported to be reduced in *P2Y2*KO mice while exhibiting salt-resistant hypertension along with lower plasma aldosterone and renin levels, increased expression of NKCC2, and increased water reabsorption (73). Additionally, these mice exhibit salt-resistant hypertension. Wildman et al. (74) showed that P2X4 may also be involved in local regulation of ENaC in the collecting duct, which is consistent with the hypertensive phenotype of the *P2X4*KO mice (75). Of note, *P2X4*KO mice have impaired endothelial-dependent relaxation. Knockdown of *P2X4* receptors in cultured endothelial cells inhibited NO production response to increases in shear stress. These studies demonstrate that P2 signaling is important in regulating sodium transport and most likely blood flow that may impact renal excretion of salt and water.

More recent evidence has shown that renal purinergic signaling and the ET-1 system may have some interaction within the collecting duct. Pandit et al. (76) showed that flow-induced ET-1 expression in cultured IMCD3 cells could be prevented by inhibiting the P2Y2 and P2X7 receptors. Gohar and colleagues (77, 78) observed that infusion of the P2 receptor agonist, UTP, into the renal medulla produced ET-1-dependent natriuresis in male rats but not females. Further studies are needed to determine the specific mechanisms, including the more specific subtypes of P2 receptors that are responsible for the purinoceptor-dependent ET-1 production.

Although direct evidence for circadian involvement in renal purinergic signaling has yet to be investigated, it is reasonable to speculate that the paracrine regulation of renal electrolyte excretion mediated by purinoceptors is, to some extent, time of day dependent, largely due to the intrinsic circadian characteristics of most, if not all, sodium transporters and the mounting evidence for circadian control of the ET-1 system. Of note, Palomino-Doza et al. (79) conducted a study that involved more than 1,400 subjects from nearly 250 families and showed that nighttime diastolic blood pressure was strongly associated with P2X receptor polymorphism.

However, research in this area has yet to determine whether renal purinergic signaling works in a circadian-dependent manner.

The Intrarenal Renin-Angiotensin System

In 1990, Ingelfinger et al. (80) utilized in situ hybridization to detect the existence of angiotensinogen in the kidney proximal tubule. This led to a tremendous amount of work in this area, as recently reviewed in detail elsewhere (81, 82). In general, research in this area has provided evidence that the intrarenal renin-angiotensin system (RAS) may also play a pivotal role in regulating blood pressure and electrolyte homeostasis in addition to the classic hormonal RAS. Gonzalez-Villalobos et al. (83) showed that angiotensin II (Ang II) generated by the angiotensin-converting enzyme (ACE) paradoxically led to increases in intrarenal Ang II. Moreover, in the presence of ACE inhibitors, the hypertensive response to chronic Ang II infusion was suppressed (84). Subsequently, this group conducted another study where they generated mice lacking kidney *ACE* and determined the role of renal ACE in hypertension induced by either Ang II infusion or NOS inhibition (85). Mice lacking renal *ACE* were hypertensive, and following Ang II infusion, the increases in intrarenal Ang II accumulation and activation of sodium transporters were inhibited.

In regard to humans, Isobe et al. (86) recruited 36 CKD patients and 14 non-CKD subjects and conducted a study to determine the relationship between circadian blood pressure and circadian rhythms of the intrarenal RAS. As expected, they observed that in non-CKD subjects, daytime blood pressure is significantly higher compared with nighttime. In addition, levels of urinary angiotensinogen excretion, an indicator of intrarenal RAS activation, are similar between daytime and nighttime. Based on nighttime blood pressure data, CKD subjects were divided into riser (nighttime systolic blood pressure higher than the 24-h average) and nonriser categories (nighttime systolic blood pressure lower than the 24-h average). In nonriser CKD patients, urinary angiotensinogen excretion significantly decreased at night, which was similar to the blood pressure pattern. In risers, the diurnal pattern of urinary angiotensinogen excretion was not evident. Importantly, the nighttime/daytime ratio of urinary angiotensinogen positively correlated with the nighttime/daytime ratio of urinary sodium excretion. Of note, nighttime activation of intrarenal RAS in CKD patients was associated with impaired melatonin secretion, which is often observed in CKD subjects (87). In addition, in a rat model of nephritis, an increase in the amplitude for circadian fluctuation of intrarenal RAS components was found (88). Given the role of melatonin in regulating a variety of circadian behaviors, these findings suggest a possible association between melatonin and intrarenal RAS activity.

Figure 6 summarizes some of the known factors that are linked to clock genes and/or circadian control of renal tubular function. Several other elements such as 20-hydroxyeicosatetraenoic acid (20-HETE), bradykinin, and prostaglandin E2 (PGE2), have also been shown to participate in tubular function (89–92). However, evidence for their interactions with circadian control of excretory function is relatively lacking, although Nikolaeva et al. (36) observed a circadian oscillation of 20-HETE in the kidney. It is important to note that this list is relatively sparse compared to the many cellular mechanisms that control what appears in the final urine. In that sense, this field is only in its infancy. Given the link between endocrine and paracrine factors such as aldosterone and ET-1 and the molecular clock, it is clear that this is an area that will be of great interest to hypertension investigators in the near future.

POTASSIUM HOMEOSTASIS AND CIRCADIAN RHYTHMS

The role of circadian regulation in potassium balance was reviewed recently (93). Thus, we summarize recent progress with a focus on clinical implications of circadian misalignment of

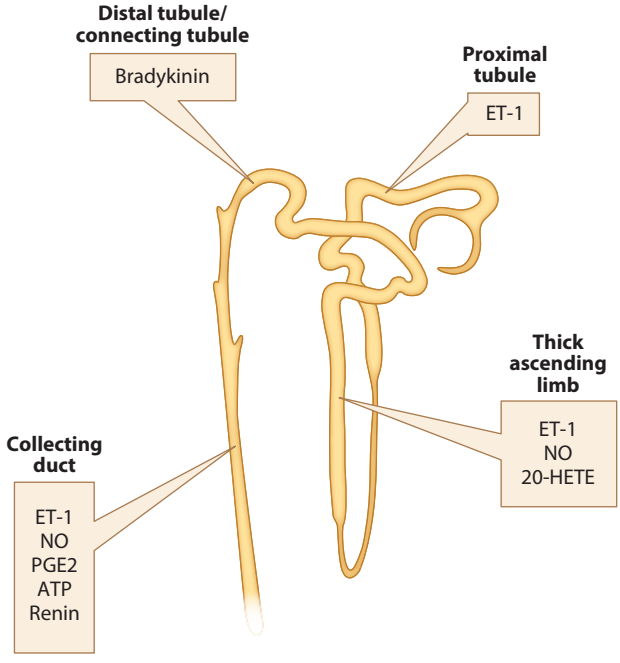

Figure 6

Primary site of action of major paracrine/autocrine factors in the nephron. Although these and other paracrine factors may have other sites of action, these have been established in vivo. Abbreviations: ET-1, endothelin 1; NO, nitric oxide; PGE2, prostaglandin E2.

potassium homeostasis along with some novel insights from rodent studies. In 1999, Wilson et al. (94) determined the effects of dietary potassium supplements on nocturnal blood pressure in African American adolescents. Participants that presented with normal blood pressures at baseline, but who were salt sensitive, were given potassium supplements following a high-sodium diet. High-sodium diet caused nondipping blood pressure in nearly half of the participants but was reversed to dipper status after potassium intervention. Similar results were achieved in salt-sensitive subjects in China (95). In CKD patients, Agarwal (96) showed that urinary excretion of potassium was significantly higher at night compared with daytime, whereas the majority of potassium is normally excreted during daytime in healthy individuals (97). Bankir et al. (98) also reported a similar relation between diurnal potassium excretion and circadian blood pressure in a larger population. It is possible that the beneficial effect of potassium supplementation on blood pressure may be due to a resetting of the diurnal pattern of potassium excretion and possibly aldosterone. Microarray analyses on mouse kidneys taken every 4 h over 24 h found that the majority of known potassium-regulatory genes were expressed in a circadian-dependent manner, including genes that encode Na/K ATPase, H/K ATPase, Kir1.3, ROMK, and the BK channel (35). In two distinct tissue-specific *Bmal1*KO models using *Ren1(d)* Cre and *PAX8* Cre, the diurnal variation in potassium excretion was not consistently different compared to control mice (22, 23). However, the *Clock* knockout mouse has an increased inactive period potassium excretion under both normal light/dark and constant dark conditions (36). Future efforts should be directed to determine the mechanisms underlying circadian potassium regulation, and in particular, its relationship with circadian regulation of sodium balance.

IMPLICATIONS FOR HYPERTENSION

In 2017, the American Heart Association and American College of Cardiology (99) released an updated guideline for the prevention, diagnosis, and treatment of high blood pressure in adults, in which the definition for stage 1 hypertension is systolic blood pressure ≥130 mm Hg or diastolic blood pressure ≥80 mm Hg. This new guideline places an additional 14% of the US population into the category of having hypertension. With nearly half of the US population considered hypertensive, it is imperative that more accurate and reliable diagnostic methods are established, including the use of ambulatory blood pressure monitoring (ABPM). ABPM provides enhanced abilities for diagnosing complicated yet not uncommon types of hypertension, including masked hypertension, white-coat hypertension, and nocturnal hypertension. Specifically, neither office blood pressure measurement or regular home blood pressure monitoring is able to accurately determine the existence of nocturnal hypertension, which has been shown to be prevalent in hypertensive patients (100).

The common definition for nocturnal high blood pressure is that the nighttime blood pressure decreases less than 10% of daytime level. Based on the nighttime dipping level, blood pressure can be recognized in several categories: extreme dipper (larger than 20% dipping), dipper (10–20% dipping), nondipper (less than 10% dipping), and riser (higher than daytime blood pressure) (100, 101). The prevalence of nocturnal hypertension varies depending on ethnicity, gender, age, body mass index (BMI), sleeping disorder, and many other factors. The Ambulatory Blood Pressure Collaboration in Patients with Hypertension (ABC-H) meta-analysis contains multiple studies from 10 cohorts with 17,312 hypertensive patients (102). The proportion of combined nondippers and risers ranges from 37% to 65%. In another study by Harshfield et al. (103), African American youth showed significantly higher nighttime blood pressure compared to European Americans despite comparable daytime blood pressure. It was also noted in this study that sex was an important predictor of nocturnal blood pressure; males were more prone to exhibit less frequent nighttime blood pressure dipping. Li and colleagues (104, 105) showed a higher prevalence of isolated nocturnal hypertension in East Asians compared to Africans and Europeans, which was associated with higher sodium consumption and lower potassium intake. BMI has been shown to be related to nocturnal blood pressure dipping. Cuspidi et al. (106) showed that overweight (BMI >25 kg/m^2) hypertensive patients had reduced nocturnal blood pressure dipping compared to lean patients. Obstructive sleep apnea has been shown to increase nighttime blood pressure, and the severity of hypertension is correlated with apnea hypopnea index (107, 108). However, the extent of renal involvement in the daytime versus nighttime blood pressures has not been established.

The nighttime dipping of blood pressure is often attributed to endogenous circadian control of autonomic systems as well as exogenous factors such as activity and stress (109, 110). Nighttime blood pressure may also serve as an independent target for blood pressure control (111). The absence of appropriate nocturnal dipping of blood pressure is associated with various cardiovascular events and end organ complications. In the ABC-H study, the nocturnal blood pressure decline was an independent predictor of various adverse outcomes, including myocardial infarction and stroke (102). In addition, among the various categories of blood pressure dipping, risers were shown to have the worst prognoses for events mentioned above.

Although nocturnal hypertension is often seen in patients with essential hypertension, it is not uncommon in several other pathological conditions. In fact, patients with CKD often exhibit an attenuated nocturnal blood pressure decline (112, 113). In a large cohort of CKD patients of Chinese ethnicity, isolated nocturnal hypertension was observed in more than 20% of participants. In addition, it was shown that CKD patients with isolated nocturnal hypertension had worse renal

function and more prominent left ventricular hypertrophy (114). Moreover, nondipping blood pressure is considered a risk factor for the progression of CKD. Davidson et al. (115) conducted a retrospective study among hypertensive patients with normal baseline renal function and showed that nondipping blood pressure preceded the decline of GFR and associated with an increase in serum creatinine level during a follow-up of 3.6 years. Garcia-Ortiz et al. (116) showed an inverse relationship between nocturnal blood pressure dipping and albuminuria and that nondipping was a predictor of renal damage in hypertensive patients. In a prospective study conducted by Agarwal & Andersen (117), nighttime blood pressure was associated with end-stage renal disease and was a stronger predictor of total mortality compared with pressures during the day. In addition to CKD, nocturnal hypertension is a common comorbidity of both type 1 and type 2 diabetes and a major cardiovascular risk factor in diabetic patients (118). The mechanisms underlying this nondipping phenomenon in diabetic patients remain to be elucidated although renal dysfunction, autonomic neuropathy, and poor glycemic control have all been suggested (119, 120).

Theoretically, impaired sodium handling by the kidney may play an important role in elevated nocturnal blood pressure. Many have hypothesized that the loss of dipping in nighttime blood pressure is a pressure-natriuresis mechanism meant to compensate for an impaired ability to excrete sodium during the day (98, 121, 122). Bankir et al. (98) recruited 325 individuals of African descent and investigated the relationship between nighttime blood pressure and daytime urinary sodium excretion. They found that daytime urinary sodium concentration was inversely related to nocturnal systolic blood pressure and suggested that daytime sodium excretion was a determinant factor for nocturnal blood pressure. Similarly, another study carried out by Uzu et al. (123) showed that, in Japanese patients who have metabolic syndrome, nondipping blood pressure was associated with higher sodium sensitivity. Furthermore, it has been shown that patients with CKD have a decreased day-versus-night ratio of natriuresis and require longer time at night until their blood pressure starts to fall, which may very well be the mechanism underlying the nondipping blood pressure phenotype observed in subjects with impaired renal function (124).

FUTURE ISSUES

1. The gap in knowledge regarding mechanisms that control renal electrolyte handling is enormous, and only a handful of labs have focused on this significant area of research. Furthermore, in vivo validation of the impact of specific circadian genes on electrolyte excretion and blood pressure is needed. Such work related to time-of-day physiological variability will facilitate our understanding of renal and cardiovascular disease risk.
2. When performing clinical, whole animal physiology, and even cell culture studies, the time-of-day fluctuation of potential targets, caused by either their intrinsic circadian rhythms or the result of the central clock, must be taken into consideration. This has been an overlooked aspect related to scientific rigor and reproducibility.
3. More studies are needed to determine the effect of diurnal potassium homeostasis on circadian blood pressure rhythm and its interaction with sodium transport, as many investigators often overlook the role of the renin-angiotensin-aldosterone system in control of potassium homeostasis and instead focus solely on sodium balance. This is important not only for different types of hypertension but also for kidney diseases, especially IgA nephropathy, diabetic nephropathy, and CKD.

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