Tick Tock: Time to Recognize the Kidney Clock

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A variety of physiologic functions related to the kidney, including BP, GFR, renal blood flow, and urinary sodium excretion, exhibit a circadian pattern of variation (reviewed by Stow and Gumz1). Whereas these clinical observations are well established, the underlying molecular mechanisms are not completely characterized. On the molecular level, the circadian clock consists of a complex series of transcriptional, translational, and post-translational feedback loops.2 More simply, the four core circadian proteins—Bmal1, CLOCK, Period (Per1, Per2, Per3) and Cryptochrome (Cry1, Cry2)—regulate expression of thousands of target genes via a transcriptional mechanism to perpetuate rhythms in physiologic function.3

There has long been a debate in the circadian field regarding the relationship between the central clock, located in the suprachiasmatic nucleus (SCN) of the brain, and the peripheral clocks, located in nearly every cell type and tissue of the body. Zeitgebers, or “time-givers,” act as inputs to the circadian clock and these cues include light and food (reviewed by Richards and Gumz4). The prevailing model at the present time is that the central clock in the SCN, entrained by light, acts as a “conductor” to coordinate the physiologic functions of the “orchestra” made up of peripheral clocks located throughout the body (reviewed by Richards and Gumz4). Neuronal and humoral signaling is involved in the function of the conductor to synchronize the peripheral clocks in the orchestra.

Generation of cell type–specific knockouts (KO) has recently begun to shed light on the extent to which the peripheral clocks may independently contribute to physiologic function. For example, studies by Young and colleagues in the cardiomyocyte-specific clock mutant mouse have demonstrated a clear role for the CLOCK protein in the metabolic function of the heart (reviewed by Richards and Gumz4). Specific deletion of Bmal1 in pancreatic β cells demonstrated a role for the circadian clock in glucose-stimulated insulin secretion and oxidative stress–induced β-cell failure.5 Until now, studies like these have been lacking for the kidney. In a groundbreaking report presented in this issue of JASN, Tokonami et al. demonstrate a role for a kidney-specific peripheral clock in the regulation of renal function and BP.6

The first report of a circadian clock–controlled gene in the kidney came from Saifur Rohman et al., with the demonstration that the Na/H exchanger NHE3 was directly regulated by Bmal1 and CLOCK via a transcriptional mechanism.7 Our subsequent reports showed that Per1 regulates the expression of the α subunit of the epithelial sodium channel and the activity of epithelial sodium channel.8,9 Consistent with these mechanistic molecular findings in renal models, studies in circadian KO mice have consistently demonstrated a role for each of the core clock proteins in BP control.10–13 An important role for the kidney in these BP phenotypes has often been proposed, but the lack of renal cell type–specific KO models of circadian genes has prevented the use of a genetic model to directly test this hypothesis.

In the report by Tokonami et al., floxed Bmal1 mice were crossed with Ren1ΔCre mice to generate mice lacking Bmal1 expression in renin-producing cells of the kidney. Specifically, Bmal1 expression was significantly reduced in the renin-secreting granular cells of the juxtaglomerular apparatus and in principal cells of the cortical collecting duct and the outer medullary collecting duct. Less dramatic decreases in Bmal1 expression were also observed in the medullary thick ascending limb. Reduction of Bmal1 expression in these specific
vascular and tubular kidney cell types was associated with significant changes in several parameters, including increases in GFR and urine volume and decreases in plasma aldosterone and both systolic and diastolic BP. These landmark findings clearly demonstrate the importance of the kidney clock in the control of BP and renal function. Moreover, because this study was performed in mice with an intact clock mechanism in the SCN, these results suggest that the function of the kidney clock may be somewhat independent of the central clock.

Interestingly, the overall phenotype of the kidney-specific Bmal1 KO is very similar to that of the global KO of CLOCK12,14 and that of Per1.8,13,15 The features of these phenotypes include reduced plasma aldosterone, mild polyuria, altered urinary sodium excretion, and reduced BP. These shared features between global loss of CLOCK or Per1 and a kidney-specific Bmal1 KO are consistent with the notion that the kidney significantly contributes to the phenotypes observed in the global KO mice.

An intriguing feature of the study by Tokonami et al. is the finding that although loss of Bmal1 from specific cell types in the kidney was associated with lower BP, the circadian rhythm of BP did not appear to be altered. This is in contrast with the phenotype of the global Bmal1 KO, which included disruption of the normal dipping pattern of BP.10 Indeed, time-independent effects of circadian rhythm proteins were previously observed and may be attributable to the general function of these proteins as transcription factors.16 Future studies combining cell type conditional KO of circadian proteins together with alteration of entraining signals such as food and light may help dissect time-dependent and independent effects of circadian proteins in a manner that can be correlated with tissue-specific function. Another explanation for the maintenance of the dipping pattern in the kidney-specific Bmal1 KO could be related to the function of nitric oxide in the vasculature; it has been proposed that vascular nitric oxide signaling may be a key determinant of the dipping pattern of BP.17 Nitric oxide action may explain the other provocative finding in this study, that GFR is increased in the kidney-specific Bmal1 KO in the absence of increased protein or glucose in the urine. The authors speculate that increased nitric oxide produced by tubular cells could explain the altered GFR. Future studies using additional cell type–specific Cre mice in combination with floxed circadian gene models will certainly help elucidate the role of clock proteins in nephron segments beyond those investigated by Tokonami et al.

The work of Tokonami et al. represents a significant milestone in our understanding and perception of the kidney clock. Knockout of Bmal1 expression in a subset of specific renal cell types resulted in alteration of urinary Na excretion, GFR, and BP. These findings clearly and specifically demonstrate a role for circadian clock proteins, localized in the kidney, in the regulation of renal function and BP control.

DISCLOSURES
None.

REFERENCES
Kidney transplant recipients have a markedly increased risk of premature cardiovascular disease (CVD) and death.\(^1,2\) Hypertension is an established risk factor for CVD in this population and is associated with reduced graft survival in registry analyses.\(^3-5\) Most renal transplant recipients routinely receive antihypertensive agents.\(^6,7\) Against this background, it is remarkable to those outside the transplant community that we lack robust information on selection of antihypertensive agents or BP targets. The only trial of antihypertensive therapy in kidney transplant recipients (Study on Evaluation of Candesartan Cilexetil after Renal Transplantation), which examined the benefits of the angiotensin-receptor blocker candesartan,\(^8\) was closed early because of a low endpoint rate; there seems to be little appetite or funding for the necessary trials of antihypertensive therapy in transplant recipients.

The paper by Carpenter and colleagues in this issue of JASN reinforces the power of post hoc analyses of large-scale clinical trials to inform on issues outside the primary aim of the study.\(^9\) The Folic Acid for Vascular Outcomes Reduction in Transplantation trial failed to show a benefit of folic acid therapy on CV events in 4110 kidney transplant recipients. However, it generated a large dataset of carefully phenotyped transplant recipients, with follow-up and validated endpoints, which the investigators have used to study the role of BP on CVD. The results are clear but challenging with respect to the underlying mechanism and implications for treatment. First, higher systolic BP is associated with increased cardiovascular risk; the risk for CVD is increased 43% with each 20-mmHg increase in systolic BP. This is intuitive and confirms both registry and prior post hoc trial analyses in kidney transplant recipients.\(^10,11\) Second, lower diastolic BP (at least <70 mmHg) is also associated with increased CV risk; the hazard ratio is 31% higher for each 10 mmHg below 70 mmHg. This latter observation is more difficult to reconcile, although it confirms previous findings from the Assessment of LEscol in Renal Transplantation study on the divergent relationships between systolic and diastolic BP and CV outcomes.\(^11\) In fact, the data from these two large-scale trials in transplantation come to near-identical conclusions—in different populations, including whites and nonwhites, patients with diabetes and those without diabetes—suggesting that these relationships are likely to have universal relevance in transplant populations.

We should not be surprised by these findings. Similar relationships between systolic BP and a J-shaped relationship for diastolic BP have been demonstrated in patients receiving maintenance hemodialysis, as well as in patients with less advanced CKD who do not require dialysis.\(^12,13\) In the absence of aortic valve insufficiency, the pattern of high systolic BP, low diastolic BP, and increased pulse pressure are a marker of vascular stiffness. This has been extensively studied in CKD and ESRD, and it reflects accelerated arteriosclerosis and vascular calcification in progressive renal disease.\(^14\) Such extensive, established peripheral vascular disease is the norm in incident transplant recipients, and although it does not progress as rapidly after transplantation, it does not regress. Moreover, it is strongly associated with the development of left ventricular hypertrophy, which, in turn, is linked to cardiovascular morbidity and mortality in kidney transplant recipients—specifically to sudden cardiac death.\(^15,16\) The increase in sudden death is believed to be due to increased myocardial mass, cardiac fibrosis, increased arrhythmogenicity, and reduced diastolic filling of the coronary circulation. Prevention of vascular calcification, uremic cardiomyopathy, and sudden cardiac death are the leading challenges in the management of patients with CKD.

Carpenter and colleagues identify BP as a risk factor; however, given that we cannot undo the underlying vascular disease, the question is how to treat it and which targets or agents to use. In practice, only systolic BP offers a manageable target; agents that decrease systolic BP will also reduce pulse pressure and, to a lesser extent, diastolic BP. For patients with a diastolic BP >70 mmHg, it is reasonable to use established targets for systolic BP until diastolic BP falls to <70 mmHg; for patients with a high systolic BP and diastolic BP <70 mmHg, one would need to balance the benefits of lowering systolic BP with the additional hazard of reduced diastolic BP and its consequences. The 2012 Kidney Disease Improving Global Outcomes clinical practice guideline for management of BP in CKD recommend a target BP of 130/80 mmHg in kidney transplant recipients, regardless of other risk factors.\(^17\) These guidelines are based on research in other

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**BP Targets in Renal Transplant Recipients: Too High or Too Low?**

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