In cerebrovascular circadian rhythms, EETs keep the beat. Focus on “Rhythmic expression of cytochrome P450 epoxygenases CYP4x1 and CYP2c11 in the rat brain and vasculature”

William J. Pearce
Loma Linda University School of Medicine, Loma Linda, California

CIRCADIAN RHYTHMS PERMEATE virtually all life. In mammals, these rhythms are evident in circulating glucose and hormone levels, immune cell abundance and activity, whole body metabolic rate and temperature, and blood pressure. Correspondingly, the function and activity of all major organ systems also exhibit strong circadian rhythms. From a cardiovascular perspective, many of these functional oscillations are driven by circadian changes in the activity of the sympathetic nervous system and concomitant changes in systemic vascular reactivity. In the cerebral circulation, however, the contractile efficacy of the sympathetic perivascular innervation is limited (14), and thus other mechanisms must mediate the long-established circadian patterns in cerebral blood volume, blood flow, hypercapnic reactivity, and cerebral oxygen consumption (7). Daily oscillations in circulating glucocorticoids (12) or melatonin (5) may contribute to these cerebral rhythms, but the exact mediators remain uncertain and controversial.

From a clinical perspective, knowledge of the mechanisms mediating cardiovascular circadian rhythms is highly relevant to the management of patients at high risk for stroke and myocardial infarction. As recognized long ago, most adverse cardiovascular events occur early in the morning for reasons that remain poorly understood despite intensive investigation (4). A pragmatic response to this pattern has been to adjust the timing of pharmacotherapy for optimum efficacy in the early morning hours (2). Despite the success of these largely empirical chronopharmacologic strategies, fundamental advances in therapeutic management of these patients await deeper understanding of the mechanisms driving circadian rhythms.

Fig. 1. Circadian rhythms in cerebral epoxyeicosatrienoic acid (EET) production. The superior chiasmatic nucleus (SCN) serves as a master internal clock whose endogenous periodicity is determined by multiple clock genes with cyclical rates of transcription that are regulated by posttranslational feedback. These rhythms are further synchronized with the environment through photoperiod cues conveyed via visual pathways. Outputs from the SCN through neural and neuroendocrine pathways synchronize the periodicity of peripheral molecular clocks that govern metabolic activity in many cell types. Carver et al. demonstrate that within astrocytes and cerebral endothelium, peripheral clock genes regulate transcription of genes coding for the P450 enzymes that catalyze synthesis of EETs. Cyclical oscillations of EETs, in turn, could contribute to circadian rhythms in cerebrovascular resistance and perfusion. How EETs integrate with the effects of other clock-driven vasoactive molecules remains unknown, but it seems likely that some of these molecules serve as feedback signals to fine-tune the rhythms generated by the central molecular clock within the SCN.

Address for reprint requests and other correspondence: W. J. Pearce, Loma Linda Univ. School of Medicine, Loma Linda, CA 92350 (e-mail: wpearce@llu.edu).
Early studies attributed circadian rhythms to variations in food intake, physical activity, and sleep. The ability of these rhythms to persist despite artificially imposed patterns of food intake and activity, however, motivated exploration of the hypothesis that circadian rhythms were generated endogenously. These investigations identified the hypothalamic superior chiasmatic nucleus (SCN) as a master internal clock that drives circadian rhythms (Fig. 1). Further investigation identified melatonin (5) and possibly also neuropeptide-Y (6) as neuroendocrine output signals from the SCN that mediate whole body circadian synchronicity. More recently, the search for a molecular clock within the SCN has identified multiple genes whose patterns of transcription and translation follow circadian rhythms, even when cultured in vitro (9). These exciting findings, in turn, have led to the discovery of oscillating “clock” genes (Bmal1, Clock, Cry1, Dhp, Npas2, Per1, Per2, etc.) in many different tissues. Such findings suggest that the “master clock” in the SCN is not primarily responsible for driving peripheral circadian rhythms, but instead serves to synchronize “peripheral clocks” that govern local rhythms in function and metabolism (11). In reciprocal fashion, these peripheral clocks may also have the potential to communicate with the SCN to effect two-way communication and establish integrated, feedback control of whole body circadian rhythms. The pathways and molecules involved in such communication remain under active investigation.

As in other tissues, the heart and vasculature abundantly express dynamically active peripheral clock genes, some of which may be intimately involved in circadian rhythms in vascular contractility (12). Studies of peripheral clocks in the vasculature have been greatly facilitated by the development of mouse strains in which key clock genes have been deleted, but interpretation of results from these mice is complicated by the inability to distinguish central (SCN) from peripheral effects of gene knockout (10). An important finding from studies of these knockout mice is that multiple clock genes appear to influence vascular structure and function independent of their time-keeping roles (1). This complexity, however, suggests that physiological studies in wild-type animals will be required to better distinguish central from peripheral regulation of circadian vascular rhythms.

The study by Carver et al. (3) in this issue of *Am Journal of Physiology-Cell Physiology* directly addresses this topic. Using samples of hippocampus, middle cerebral artery, and superior vena cava harvested from wild-type Wistar rats, together with cultures of hippocampal astrocytes and brain microvascular endothelial cells, the authors demonstrated dynamic oscillation in the peripheral clock genes *Per1* and *Per2*. These results also indicated oscillation in the clock-controlled genes *E4bp4*, *Reverba*, and *Dhp*, and more importantly revealed synchronized oscillations in the regulation of the cytochrome isofor x3 and Cyp2c11, and their products, the highly vasoactive epoxyeicosatrienoic acids (EETs). Together, these results offer new evidence of a pathway coupling peripheral clock activity to release of compounds capable of potently influencing local vascular resistance. Of particular importance was the finding that the rhythmic production of multiple types of EETs was observed in both endothelial cells and astrocytes. Previous studies have linked *Per2* mutation to impaired endothelial function, as indicated by depressed release of nitric oxide (NO) and prostaglandins (15). Carver et al. extend this observation to implicate endothelial EETs as major mediators of circadian periodicity in the cerebral circulation. The parallel finding that astrocytes can also rhythmically release EETs provides supporting evidence for previous suggestions that astrocytes are also involved in circadian oscillations in cerebral blood flow (8).

Many questions remain about circadian regulation of cerebral blood flow. For example, what fraction of circadian variation in cerebral blood flow is mediated by pulsations in EET production? How are the effects of locally produced EETs integrated with the effects of NO and vasodilator prostaglandins released from the endothelium (15)? Do other clock genes play a role in EET release? For example, the clock gene *Bmal1* helps regulate von Willebrand factor expression in endothelial cells (13); does *Bmal1* also influence EET production by cerebrovascular endothelium? How are the peripheral clock genes in hippocampal arteries and astrocytes synchronized with the SCN, and do EETs constitute a circulating feedback signal? The ability of the study by Carver et al. to stimulate so many questions is clear evidence that the results it offers represent an important step toward better understanding of circadian regulation of the cerebral circulation.

**GRANTS**

The work reported in this manuscript was supported by National Institute of Child Health and Human Development Grant HD31266, National Institute of Neurological Disorders and Stroke Grant NS076945, and the Loma Linda University School of Medicine.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author.

**AUTHOR CONTRIBUTIONS**

W.J.P. prepared figure, drafted manuscript; edited and revised manuscript; approved final version of manuscript.

**REFERENCES**


