Between a ROCK and a Hard Place
How to Align Our Circadian Rhythms?

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Many, if not all, aspects of biology are temporally controlled to align our temperaments and behavior with a 24-hour world. Such rhythms are driven by the dynamic interplay of a master molecular clock, housed in the suprachiasmatic nucleus, and peripheral tissue clocks, all conditioned by environmental inputs, most of which we poorly understand. Expression of 20% of genes in most tissues oscillates in a circadian fashion, and the tightly regulated transcriptional regulation of the clock is complemented by additional controls—by microRNAs and posttranslational and epigenetic modifications. Further underscoring the importance of this system, the molecular clock is highly conserved, exhibits marked elemental redundancy, and is central among the networks that link biological networks that are prominent in distinct tissues.

Longstanding clinical interest in circadian rhythms has been fostered by temporal variability in the incidence of symptoms of many diseases—asthma, endogenous depression, myocardial infarction, and stroke among them—as well as in the efficacy and metabolism of commonly used drugs. Coincident with the diurnal pattern of cardiovascular events, diurnal variations have been reported in the pressor response to infused vasoconstrictors, sympathetic nerve activity, the renin–angiotensin system, QT interval, plasma fibrinolytic activity, platelet aggregability, and vascular contractility

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In this issue of Circulation, Saito et al turn their attention to the mechanism by which vascular contractility is subject to clock control, focusing on the phosphorylation state of the myosin light chain protein (MLC). A circadian rhythm of thrombin or endothelin-1–induced MLC phosphorylation is apparent in cultured vascular smooth muscle cells with a peak at 36 hours after synchronization. Inhibition of Rho-associated kinase 2 (ROCK), alone of 4 enzymes capable of calcium-induced MLC phosphorylation, abolishes this rhythm. The phosphorylation state of MLC is also controlled by MLC phosphatase. Both MLC and a noncatalytic subunit of MLC phosphatase, MYPT1, can be phosphorylated on different residues by ROCK2, Zipper-interacting kinase (ZIPK) or integrin-like kinase (ILK), with opposing effects on their activities. The ROCK2 promoter contains 2 retinoic acid–related orphan receptor response elements that are responsive to RAR-related orphan receptor α, and its suppression removes the rhythm of ROCK2 expression. Expression of ROCK2 protein and phosphorylation of MLC both peak at 36 hours after synchronization in vascular smooth muscle cells—the transition to the light phase in mice, strengthening the case of ROCK2 controlling a pattern in vascular contractility.

Phosphorylation of the MLC phosphatase subunit MYPT1 at Thr853, attributable mainly to ROCK2, is rhythmic and in phase with the peak of ROCK2 protein and MLC phosphorylation. Therefore, the rhythm in MLC phosphorylation state can be controlled by ROCK2 in 2 ways: by directly phosphorylating MLC at Thr18 and Ser19, or by phosphorylating MYPT1 on Thr853 and thus inhibiting the inhibitor, MLC phosphatase. As the substrate specificity of ROCK2 is greater for MYPT1 than MLC, the authors propose that the mechanism of generating a circadian rhythm in MLC activity is likely attributable to the removal of inhibition of MLC through inactivation of MLC phosphatase by ROCK2 phosphorylation.

Diurnal variation in aortic myofilament Ca2+ sensitivity correlates with the increase in MLC phosphorylation and ROCK2 expression ex vivo, and lack of a functional RAR-related orphan receptor α protein abolishes rhythms in both in staggerer mice, already known to have altered blood pressure and vasomotor tone.

So how does this relate to the human condition? Not easily. Unlike many other aspects of cardiovascular function in the mouse, including blood pressure and endothelial function, MLC phosphorylation and myofilament Ca2+ sensitivity do not bear a simple antiphase relationship to the temporal incidence of cardiovascular events in humans (Figure). Although the diurnal variation of blood pressure in staggerer mice has not been reported here or previously, it seems odd that this
biomarker, which mirrors the temporal incidence of clinical events, should be such a trailing indicator of a ROCK2-dependent rhythm in vascular contractility. Extrapolation of circadian studies from mice to humans is complicated by their dependent rhythm in vascular contractility. Extrapolation of events, should be such a trailing indicator of a ROCK2-biomarker, which mirrors the temporal incidence of clinical events.

Saito et al reveal an important role for RAR-related orphan receptor α-driven ROCK2 in the temporal control of vascular reactivity. On the other hand, their work reveals how much more we have yet to learn about how the environment and our molecular clocks interact to create the timing that drives our lives.

“It’s always about timing. If it’s too soon, no one understands. If it’s too late, everyone’s forgotten.”

Anna Wintour

References


Disclosures

None.

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