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Circadian Clocks: Showtime for the Adrenal Cortex

THE LAST 10 yr have seen remarkable advances in understanding the control and interrelationships of circadian rhythms in the brain and peripheral organ systems of mammals and of their critical importance to human health. Disturbances in circadian timekeeping have been linked to cardiovascular disease, hypertension, metabolic syndrome, gastrointestinal disorders, affective disorders, and cancer (1). The adrenal gland, and the adrenal cortex in particular, has increasingly been seen as a critical source of signals to coordinate and temporally program metabolic activities throughout the body. For many years the prominent circadian oscillations in steroidogenesis and glucocorticoid secretion of the adrenal cortex were thought to be a consequence of similar circadian rhythms in ACTH secretion. However, evidence of functional neural innervation of the adrenal cortex (2, 3) and findings of nycthemeral rhythms in adrenal responsiveness to ACTH that could be altered extremely rapidly (4, 5) led to the recent demonstration of direct neural control of adrenal rhythms (6, 7). In this issue of *Endocrinology*, a study by Valenzuela and Torres-Farfan and colleagues (8) takes another step in advancing understanding of the regulation of adrenocortical rhythms by demonstrating the presence of an intrinsic circadian oscillator in the adrenal cortex of the capuchin monkey (*Cebus apella*) and by showing that expression of its clock genes are influenced by melatonin, another major role-player in the circadian rhythm story (9).

Circadian rhythms are generated and synchronized by endogenous oscillators that maintain a rhythm with an approximate 24-h period in the absence of any time cues. This rhythm can be entrained or kept "on schedule" by a time signal or Zeitgeber, the most potent of which is the day/night cycle. Over 35 yr ago, the first evidence was obtained for the existence of a "master clock" responsible for generating and synchronizing mammalian circadian rhythms residing in the paired suprachiasmatic nuclei (SCN) of the anterior hypothalamus, just above the optic chiasm (10, 11). However, it has only been recently that the mechanisms by which the SCN generates a circadian rhythm have been elucidated. Light is signaled to the SCN by direct input from the retina via the retinohypothalamic tract. The basis of the timekeeping mechanism is in interacting positive and negative feedback loops in gene expression that include both transcriptional and posttranslational regulatory mechanisms (1).

Members of the *Period* (*Per1*, *Per2*, and *Per3*) and *Cryptochrome* (*Cry1* and *Cry2*) gene families are major components of the clock. Transcription of these genes at the onset of daylight is activated by the positive arm of the system, heterodimeric protein products of *Clock* and *Bmal1* genes acting through the E-box regulatory sequences of the *Per* and *Cry* genes. CRY and PER proteins, which increase until reaching a peak at the end of daytime, inhibit *Clock* and *Bmal1* transcription, thus eventually inhibiting their own activation until their mRNA levels reach a nadir near daybreak. Redundancy of control is achieved by additional negative feedback loops operating through the *Rev-erb-α* protein to inhibit *Bmal1* transcription during the day and through inhibition of *Rev-erb-α* transcription by CRY and PER with a resultant nighttime increase in *Bmal1* transcription as daylight approaches again. Translational expression of *Bmal1* and *Clock* is in antiphase with the expression of *Cry* and *Per* genes.

In addition to the master clock in the SCN, independent circadian oscillators expressing the same clock genes have been found in a number of peripheral tissues in mammals, including liver, heart, lung, and skeletal muscle, and these clock genes have been found to maintain circadian rhythmicity *in vitro* (12, 13). The adrenal cortex would also appear to be a very likely candidate to function as a peripheral pacemaker. Adrenal glucocorticoids display a prominent circadian rhythm that is influenced by at least two mechanisms: ACTH secretion from the pituitary gland and a multisynaptic neural pathway between the SCN and the adrenal that has been identified by transneuronal virus tracing (6). Light rapidly stimulates glucocorticoid secretion and resets adrenal circadian function, and these responses are dependent on adrenocortical innervation (7). Glucocorticoid signaling from the adrenal has been shown to transiently change circadian gene expression in the liver, kidney, and heart (14), and to reset the daily rhythms of serotonin synthesis in the raphe nuclei (15).

Evidence of rhythmic expression of clock genes in the adrenal cortex has been found in mice (7, 16–18), rats (19), and monkeys (20, 21), but until the report by Valenzuela *et al.* (8), they have not been demonstrated to maintain transcriptional oscillations *in vitro*. The authors measured clock gene expression in the SCN and adrenal cortex. The expression of the clock gene mRNAs *Per2*, *Bmal1*, *Clock*, and *Cry2* were detected in both tissues, and in each case, *Per2* and *Bmal1* were found to oscillate in antiphase. The oscillation of the transcriptional expression of the adrenal clock genes was accompanied by rhythmic expression of the steroidogenic enzyme 3β-hydroxysteroid dehydrogenase. Cultured adrenal explants maintained rhythmic transcriptional expression of *Per2* and *Bmal1* for 36 h, demonstrating intrinsic oscillatory capacity (Fig. 1).

Melatonin secretion from the pineal gland exhibits a pronounced circadian rhythm and has also been shown to be a potent Zeitgeber for the entrainment of peripheral circadian oscillators (9). The phase relationships between glucocorticoid and melatonin secretion in nocturnal *vs.* diurnal mam-

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Abbreviation: SCN, Suprachiasmatic nuclei.

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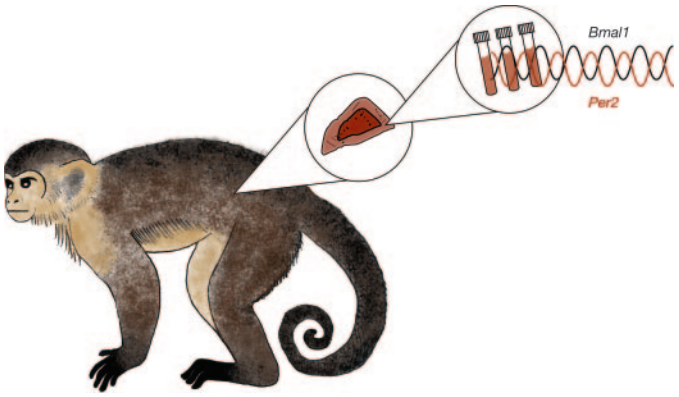

FIG. 1. Valenzuela *et al.* (8) demonstrated that adrenocortical explants from diurnal New World capuchin monkeys (*Cebus apella*) transcriptionally expressed the circadian clock genes *Bmal1* and *Per2* during 36 h in culture and that the expression of the two genes oscillated in antiphase.

mals reveal one of the major unanswered questions regarding regulation of circadian rhythms. Glucocorticoid secretion is linked to daily activity, with peak levels occurring coincident with the time of awakening: morning in diurnal mammals and evening in nocturnal animals. Melatonin secretion peaks in the middle of the night in both nocturnal and diurnal animals. Similarly, electrical and metabolic activities of the SCN are generally highest during the day regardless of an animal's activity patterns, but not all features of SCN rhythms are universal (22). The question arises as to the mechanism by which rhythms in sleep, locomotion, feeding, and metabolism are reversed in relation to SCN and pineal rhythms in diurnal relative to nocturnal mammals. The vast majority of studies of circadian rhythms in mammals have used nocturnally active species, and the coordinating mechanisms between the SCN and peripheral rhythms are necessarily different than those in humans or other diurnal primates. Further complicating the generalization of research on circadian integration to humans is that diurnality has evolved independently in different mammalian lineages (22), and even diurnal rodents may not provide suitable models for human circadian regulation. Thus, work in diurnal primates such as the capuchin monkey is especially valuable in attempting to understand mechanisms of human circadian coordination.

Earlier work by Serón-Ferré's group (23) has made progress in unraveling the interrelationships between melatonin and glucocorticoid rhythms in diurnal primates. They demonstrated the presence of the mt1 melatonin receptor in the capuchin adrenal, the first such finding in a primate, and showed that melatonin inhibits ACTH-stimulated cortisol production. The present study by Valenzuela *et al.* (8) built on this finding by showing that melatonin has a direct inhibitory effect on the expression of clock genes by adrenocortical explants. The adrenal cortex has been shown to be at a neuroendocrine crossroad in the integration of peripheral circadian rhythms in that: 1) it receives light-related neural input from the SCN and neuroendocrine signals via ACTH and melatonin, all of which regulate its circadian timekeeping as well as steroidogenesis; 2) it maintains intrinsic circadian oscillations; and 3) its glucocorticoid output has been shown to entrain, activate, and synchronize other peripheral pacemakers as well as serotonin rhythms in the central nervous system.

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