PACAP enlightenment of mouse circadian clock

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LOCOMOTOR ACTIVITY, BODY TEMPERATURE, and many biological functions are regulated by the circadian timing system in mammals. This system efficiently orchestrates physiology so that living organisms adapt to environmental cycles through partly elucidated complex and interacting dynamic mechanisms (15). The study reported by Colwell et al. (6) in this issue of the American Journal of Physiology-Regulatory, Integrative and Comparative Physiology highlights the role of pituitary adenylate cyclase activating peptide (PACAP) in the response of the circadian system to light.

The recognition that 24-h rhythms in behavioral or hormonal functions were not the mere reflection of the alternation of light and darkness but persisted in the absence of exogenous time cues led to the foundation of chronobiology in the 1950s. In addition to the essential characterization of the biological and mathematical properties of the bodily rhythms, the search striving for anatomic components of the circadian timing system has identified specific brain nuclei and structures and several neuroanatomic afferent and efferent pathways throughout the body (4, 15, 17).

The discovery of the suprachiasmatic nuclei (SCN) had led to an abrupt shift in the paradigm of the circadian timing system from a network composed of multiple oscillators to a single hypothalamic pacemaker responsible for the generation of all circadian rhythms within the organism. Indeed, the selective destruction of these nuclei in several rodent species suppressed the rhythmic alternation of rest and activity and other behavioral functions over 24 h (17). Locomotor activity was hence considered as a marker of circadian system function. This was convenient because this variable was easily amenable to simple and noninvasive monitoring in mammals. Interestingly, spontaneous or chemically induced alterations of the circadian phenotype in wheel running activity led to the discovery of the molecular bases of circadian rhythms, in particular clock genes *Clock* and *Casein Kinase*, whereas homologs and orthologs of the *Drosophila* clock genes such as *Per's* were found in the mammalian genome, and their properties in mammalian molecular clock function were characterized (15, 22, 23). Contrary to prior assumptions, however, circadian rhythms were demonstrated in cultured cells for clock gene expression and cellular function (1). Furthermore, rhythms in clock gene expression persisted in the tissues of mice despite SCN ablation (24), a finding we replicated for several cellular functions (9). These and other data revealed that mammalian tissues could maintain some level of circadian coordination in the absence of SCN, especially in animals under light-dark synchronization.

Light has long been known to reset the molecular clock and various rhythmic functions in the SCN as well as locomotor activity rhythms. Recently, melanopsin-containing ganglion cells in the retina were shown to be responsible for the conveyance of light messages to the circadian pacemaker (3). These cells contained PACAP, and this peptide was colocalized with glutamate in the retinohypothalamic tract from the retina to the SCN. PACAP further modulated glutamate effects on the SCN (5).

PACAP is a neuropeptide of the vasoactive intestinal peptide (VIP)-secretin-glucagon superfamily, which was initially isolated from the ovine hypothalamus and potently activates adenylate cyclase to produce cAMP in pituitary cells (25). PACAP-27 and PACAP-38, respectively, contain 27- and 38-amino acid residues and derive from the same precursor. The localization of both molecular forms can differ according to tissue and species, being mostly found in the hypothalamus and other brain areas, in the gut and in lymphoid tissues (25). PACAP exerts pleiotropic effects through G protein-coupled receptors with high PACAP affinity and different tissue distribution as well: PAC-1, which is specific for PACAP and VPAC (1), and VPAC-2, which is common for PACAP and VIP (25).

Colwell et al. (6) hypothesized that the response of the circadian system to light would be affected by PACAP deficiency. The ability of PACAP to alter circadian entrainment had been shown by the following: 1) an injection of the molecule in the vicinity or within the SCN phase shifted the wheel running activity rhythm in mice or hamsters (2, 5, 19, 21) and 2) exposure of SCN slice to PACAP altered both SCN neuronal firing and the expression patterns of early response gene c-fos and clock genes mPer1 and mPer2, with circadian time-dependent modulations of glutamate or light effects (20). However, opposite PACAP effects were reported on clock response depending on experimental conditions. Intriguingly, the phase delay of the wheel running activity rhythm to a light pulse at early subjective night was increased in PAC-1 receptor mutants (13), but decreased in PACAP–/– mice (16). In addition, light at late subjective night attenuated the usual phase advance of the running wheel activity rhythm in PACAP–/– mice, but it induced a phase delay in PAC-1–/– mice (16). As an attempt to resolve these apparent inconsistencies, Colwell et al. generated a new line of PACAP-deficient mice on C57BL/6 background and analyzed the response of the wheel running activity rhythm to a change in photoperiodic synchronization and to brief pulses of light at different stages of the circadian cycle [circadian times (CT)].

PACAP–/– mice had an overall level of running wheel activity that was greater than that of wild-type animals in constant darkness. In these conditions, as well as in constant light, the period was significantly shorter in the homozygous mutants than that in the wild-type animals, suggesting a role of PACAP in the duration of the endogenous circadian period. However, no difference was found in the rate of adjustment to a single 8-h phase advance or delay of a 12:12-h light/dark
The thalamus, the olivary pretectal nucleus, and the anterior and lateral hypothalamic areas other than the SCN. Thus a recent study showed that PACAP is required for the normal light-induced synchronization of the circadian system and modulates both light-induced advances and delays.

However, PACAP could also play a role in the response of the circadian system to light and possibly entrainment via structures other than the SCN. Thus a recent study showed that PACAP-containing ganglion cells in the retina projected not only to the SCN but also to the intergeniculate leaflet of the thalamus, the olivary pretectal nucleus, and the anterior and lateral hypothalamic areas among others (12), suggesting that PACAP fibers could transmit the light-dark information to structures other than the SCN.

Among its pleiotropic actions, PACAP displayed anti-inflammatory and antiapoptotic effects, whereas it promoted neuronal cell survival and neuronal stem cell proliferation and controlled smooth muscle contractility, especially in the intestine. PACAP further inhibited platelet aggregation, emphasizing its potential role in coagulation processes (7, 8, 10, 11, 18). One of the current challenges in the circadian field is to understand how the circadian clocks in the central nervous system and the peripheral organs adjust in a coordinated fashion to the photoperiodic environment. Such issues may be critical for understanding better the rhythmic mechanisms in immunologic, coagulation, cell proliferation, and gastrointestinal functions and effectively act on them.

Colwell et al.’s PACAP−/− mouse, together with other models of mutated PACAP and/or VIP receptors (14), constitutes the required tools for such exploration, which is just beginning at the SCN level.

REFERENCES