VIP Activates and Couples Clock Cells. Focus on “Disrupted Neuronal Activity Rhythms in the Suprachiasmatic Nucleus of Vasoactive Intestinal Polypeptide-Deficient Mice”

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Neurons of the suprachiasmatic nucleus (SCN), located in the ventromedial hypothalamus, comprise the central mammalian circadian pacemaker (Reppert and Weaver 2002). In dispersed culture, these neurons can generate autonomous circadian (ca. 24 h) oscillations in spontaneous firing rate (Welsh et al. 1995). In vivo, they are normally synchronized to the 24 h light/dark cycle by input from the retina (Berson 2003). Even in constant darkness, SCN neurons remain coupled to one another to produce a coherent, rhythmic output signal, orchestrating robust circadian rhythms of physiology and behavior. Exactly how this coupling is achieved has proven to be a challenging question (Aton and Herzog 2005), but recent work has implicated VIP signaling through VPAC2 receptors as one important mechanism. In this issue of the Journal of Neurophysiology (p. 2553–2558), Brown et al. examine circadian firing rhythms of single SCN neurons in brain slices from VIP-deficient mice. Consistent with previous data on whole animal behavior and dissociated SCN cells, they find impairments in both amplitude and coordination of rhythms in VIP knockouts, but they also find even greater effects of a VPAC2 receptor antagonist, suggesting that other ligands at this receptor may partially substitute for VIP.

In the initial knockout studies, mice deficient in either the VPAC2 receptor (vipr2−/−) (Harmar et al. 2002) or VIP itself (vip−/−) (Colwell et al. 2003) were found to have weak locomotor activity rhythms in constant darkness, with short period and abnormal phase relationships to a prior light/dark cycle. It appeared that the vipr2−/− mice had weaker rhythms than vip−/− mice, but such a comparison is problematic because the VIP−/− mice were on a mixed genetic background, and a more recent study using congenic lines found minimal differences (Aton et al. 2005). From these initial studies, it was not clear whether the weaker behavioral rhythms were due to weaker rhythms in individual SCN neurons or to cellular desynchronization, although the presence of multiple periodic components in the behavioral data were suggestive of desynchronization (Aton et al. 2005).

So the next step was to look at single cells. Aton et al. (2005) found evidence for both weaker rhythms and desynchrony. They dissociated VIP−/− or VIPr2−/− SCN neurons, cultured them at high density on multielectrode arrays, and found that neurons of both genotypes showed similarly weak, desynchronized circadian firing rhythms. That is, compared with wild-type, fewer VIP−/− or VIPr2−/− SCN neurons were rhythmic, those rhythms were of lower amplitude, and there was a wider distribution of circadian phases and periods among cells. The desynchrony result was less than definitive, however, because even wild-type SCN neurons are mostly desynchronized in dissociated culture. Disruption of synchrony is better studied in an SCN slice preparation, where coupling and other cellular interactions are preserved. Using this preparation, Brown et al. (2005) found weaker circadian firing rhythms in vipr2−/− neurons than in wild type, consistent with Aton et al. (2005), but did not sample enough cells to reach a conclusion about desynchrony. Stronger evidence for a role of VPAC2 receptors in SCN synchrony came from Maywood et al. (2006), who were able to monitor circadian function of more cells in SCN slices using optical reporters of clock gene expression instead of neuronal firing. They demonstrated that vipr2-−/− neurons were clearly desynchronized as well as more weakly rhythmic than wild type. Thus, at the cellular level, VPAC2 receptors appear to be important for maintaining both circadian rhythm amplitude and synchrony.

Phenotypes of VIP−/− (as opposed to vipr2−/−) neurons in SCN slices were still unknown, however, so an important gap remained. In the present study, Brown et al. (2007) now find that VIP−/− cells adopt altered circadian periods and phases, conclusively demonstrating a breakdown in synchronization as a result of disrupted VIP signaling, consistent with the work on dissociated cells and VIPr2−/− slices. Single cells also have shorter periods in the absence of VIP signaling, which is consistent with behavioral data in both VIP−/− and VIPr2−/− mice (Aton et al. 2005; Brown et al. 2005; Colwell et al. 2003) and with data on firing rhythms in vipr2−/− SCN slices (Brown et al. 2005). Indeed, some recent mathematical models have predicted that global coupling lengthens periods of component oscillators in a multiscillator system (Garcia-Ojalvo et al. 2004; Gonze et al. 2005). Perhaps most interesting, Brown et al. (2007) find not only that rhythms of VIP−/− neurons in SCN slices are weak, consistent with previous studies, but also that they are not as weak as those of VIPr2−/− neurons until application of a VPAC2 receptor antagonist. This suggests that another ligand at the same receptor (e.g., PACAP) may partially substitute for VIP, at least in VIP−/− knockout mice. If the other ligand is PACAP, a transmitter found in SCN retinal afferents but not in SCN neurons themselves, this could explain why no differences were seen between VIPr2−/− and VIP−/− cells in dissociated SCN cultures (Aton et al. 2005), where retinal terminals would be absent. In summary, the present paper completes an impressive recent body of work indicating that VIP plays two roles in the SCN: to sustain circadian rhythms of single cells and to synchronize them to one another.

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How these two roles might be causally related, however, is not yet clear. Aton et al. (2005) suggested that the smaller number of rhythmic cells seen with loss of VIP signaling might result from decoupling of rhythmic pacemakers from damped follower cells. But the weaker rhythms could also be due to loss of a tonic VIP drive for per gene transcription that is required for sustained rhythmicity in most SCN cells. VIP depolarizes most SCN neurons (Pakhotin et al. 2006), and SCN neuronal firing rates (Cutler et al. 2003) and per expression (Harmar et al. 2002) are low in vipr2−/− mice. Furthermore, in the absence of VIP signaling, a tonic (nonrhythmic) excitatory drive provided by gastrin-releasing peptide (GRP) or membrane depolarization is sufficient to restore amplitude of single cell circadian rhythms to wild-type levels (Brown et al. 2005; Maywood et al. 2006). Thus, decoupling appears not to be the cause of weaker cellular rhythms in vipr2−/− mice. In fact, the reverse could be true, and it remains to be proven whether VIP has a coupling role independent of effects on cell amplitude. It would be interesting to extend the duration of tonic depolarizing treatments in vipr2−/− SCN slices, to test for a coupling defect that persists despite stable restoration of single-cell amplitude.

REFERENCES


