Chronic stimulation of the hypothalamic vasoactive intestinal peptide receptor lengthens circadian period in mice and hamsters

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Pantazopoulos H, Dolatshad H, Davis FC. Chronic stimulation of the hypothalamic vasoactive intestinal peptide receptor lengthens circadian period in mice and hamsters. Am J Physiol Regul Integr Comp Physiol 299: R379–R385, 2010. First published May 12, 2010; doi:10.1152/ajpregu.00176.2010.—Evidence suggests that circadian rhythms are regulated through diffusible signals generated by the suprachiasmatic nucleus (SCN). Vasoactive intestinal peptide (VIP) is a neurotransmitter expressed in many neuronal processes in the retinohypothalamic tract and transmit information to other SCN cells and adjacent hypothalamic areas. Studies using knockout mice indicate that VIP is essential for synchrony among SCN cells and for the expression of normal circadian rhythms. To test the hypothesis that VIP is also an SCN output signal, we recorded wheel-running activity rhythms in hamsters and continuously infused the VIP receptor agonist BAY 55-9837 in the third ventricle for 28 days. Unlike other candidate output signals, infusion of BAY 55-9837 did not affect activity levels. Instead, BAY 55-9837 lengthened the circadian period by 0.69 ± 0.04 h (P < 0.0002 compared with controls). Period returned to baseline after infusions. We analyzed the effect of BAY 55-9837 on cultured SCN from PER2::LUC mice to determine if lengthening of the period by BAY 55-9837 is a direct effect on the SCN. Application of 10 μM BAY 55-9837 to SCN in culture lengthened the period of PER2 luciferase expression (24.73 ± 0.24 h) compared with control SCN (23.57 ± 0.26, P = 0.01). In addition, rhythm amplitude was significantly increased, consistent with increased synchronization of SCN neurons. The effect of BAY 55-9837 in vivo on period is similar to the effect of constant light. The present results suggest that VIP-VPAC2 signaling in the SCN may play two roles, synchronizing SCN neurons and setting the period of the SCN as a whole.

suprachiasmatic nucleus; vasoactive intestinal peptide; per2; BAY 55-9837; bioluminescence

IN MAMMALS, circadian rhythms are regulated by the suprachiasmatic nucleus (SCN) of the hypothalamus. Transplantation of an SCN in arrhythmic animals restores circadian rhythmicity, suggesting that the SCN regulates rhythms through diffusible signals (34, 40). Studies have identified three candidate output signals, TGF-α, cardiotrophin-like cytokine, and prokineticin 2, all of which inhibit locomotor activity (5, 22, 23). Vasoactive intestinal peptide (VIP) is a neurotransmitter expressed in many neuronal soma of the ventromedial SCN, which receives photic input from the retinohypothalamic tract (20). Processes from these neurons extend throughout the SCN as well as into the adjacent subparaventricular zone (SPZ). VIP in the SCN is rhythmically expressed in a light-dark cycle (LD) (1, 38), and exposure to constant light decreases VIP peptide content (39). Knockout mice for VIP (vip−/−) or its receptor (vpac2−/−) lack normal circadian activity, probably because of loss of synchronization among SCN neurons and a loss of rhythmicity in individual cells (2, 3, 6, 8, 17, 29). Moreover, SCN transplants that are successful in restoring rhythms express VIP (24, 34), suggesting that VIP is a possible SCN output signal.

In addition to possible roles in coupling and output, VIP-VPAC2 receptor (VPAC2) signaling may be involved in transduction of photic input to SCN cells. Studies have shown that VIP pulses result in phase delays or phase advances of locomotor activity, depending on circadian time of administration (32). These effects are similar to the phase-shifting effects of light pulses. Electrophysiological investigations have shown that application of VIP, or a VPAC2 receptor agonist, have similar phase-shifting effects on the firing rates of SCN neurons (36). VIP pulses have also been shown to induce expression of the clock genes per1 and per2 (30), similar to the effect of light pulses on per gene expression.

Taken together, these findings indicate that VIP mediates several important aspects of circadian rhythm regulation by the SCN. Whereas mice lacking VPAC2 or VIP show either shorter periods or complete loss of rhythmic activity (2, 7), the effect of chronic VPAC2 receptor activation has not been examined. Based on the evidence of a role for VIP-VPAC2 signaling in integrating light information to SCN neurons, together with disruptions in wheel-running activity and synchrony of SCN neurons in vip−/− mice, we hypothesized that chronic stimulation of the VPAC2 receptor may result in lengthening of the period, similar to the effect of constant light (9). In addition, chronic stimulation of the VPAC2 receptor could result in increased or decreased synchrony among SCN cells. If synchrony is decreased, activity rhythms could be disrupted similar to knockout mice. If synchrony is increased, it is expected that the amplitude of a rhythm expressed by a population of rhythmic cells will increase. For example, this might be seen as higher peak expression of a key circadian regulatory gene, per2. Because VIP processes extend outside the SCN, VIP might also act as an output signal from the SCN. If so, chronic stimulation of the VPAC2 receptor in regions adjacent to the SCN through intraventricular infusion should affect activity levels. To test these hypotheses, we chronically infused a potent VPAC2 receptor agonist [BAY 55-9837 (15, 44)] in the third ventricle of Syrian hamsters while monitoring wheel-running activity. In addition, we examined the effect of chronic application of BAY 55-9837 on period and amplitude of PER2 expression measured by bioluminescence in cultured SCN explants.

METHODS

Research was performed in accordance with the “Guiding Principles in the Care and Use of Animals” (American Physiological Society), and protocols were reviewed and approved by the Institutional Animal Care and Use Committee of Northeastern University, an

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**Chronic infusion of VPAC2 agonist.** Seventeen male Syrian hamsters (*Mesocricetus auratus*, LVG; Charles River Laboratories, Kingston, NY), ~3 mo old, were housed in cages with running wheels in a 14:10-h LD cycle for 3 wk. Hamsters were assigned to two groups [9 control artificial cerebral spinal fluid (acsf)- and 8 BAY 59-9837-infused animals]. Animals were then transferred to cages with running wheels in constant darkness (DD) for 3 wk to record baseline activity. Activity was monitored using ClockLab (ActiMetrics, Evanston, IL). After 3 wk, stereotaxic surgery was performed to implant an ALZET mini-osmotic pump (model no. 2004; 0.25 µl/h; Durect, Cupertino, CA) containing either acsf (in mM: 144 NaCl, 2.7 KCl, 1 MgCl₂, 1.2 CaCl₂, and 2 NaPO₄, pH 7.4) or acsf with 200 µM of the VPAC2 agonist BAY 59-9837 (Tocris Biosciences, Ellisville, MO), connected to a 30-gauge infusion cannula (model no. 3300P; Plasticons, Inc. Roanoke, VA) with polyethylene tubing. Animals were anesthetized with intraperitoneal injection of ketamine/xylazine (130/26 mg/kg body wt) and placed in a stereotaxic device (David Kopf Instruments, Tujunga, CA). A bur hole was drilled at bregma, and a cannula was lowered in the third ventricle. The plastic cap holding the cannula was cemented to the skull using Denmat dental cement (Denmat, Santa Maria, CA). Forces were used to place the osmotic pump connected to the cannula under the skin between the shoulder blades. Following surgery, animals were returned to clean running-wheel cages in DD, and their activity was recorded for a minimum of 7 wk (4 wk of pump infusion and 3 wk after completion of pump infusion). Animals that developed infection from the surgery (1 control and 2 BAY 59-9837-infused animals) were killed and removed from the experiment, resulting in eight acsf (control)- and six BAY 59-9837-infused animals.

Application of BAY 59-9837 to cultured SCN expressing PER2::LUC. PER2::LUC knockin mice (43) were obtained from Mary Harrington, with the permission of J. Takahashi, and bred in-house. Fifteen male PER2::LUC heterozygous mice were entrained to a 12:12-h LD cycle. Animals were killed by cervical dislocation, the brain was removed, and the SCN was dissected in cold (4°C) Hanks’ buffered saline solution under a dissecting microscope. Sections involved cutting a small block of tissue from the base of the hypothalamus and dividing it at the midline using irredectomy scissors. Individual SCN nuclei, ~1 mm², were placed on millicell-CM support membranes (Millipore) in 35-mm culture dishes with 1.2 ml of Luciferase Culture Medium (11, 42) consisting of 1.2 ml of Dulbecco’s modified Eagles medium (Sigma) with 10 mM HEPES (pH 7.2), 2% B27 (GIBCO), 25 units penicillin, 25 mg streptomycin, and 0.1 mM beetle luciferin (Molecular Imaging Products). Dishes were covered with a cover slip, sealed with silicone grease, and placed in a 36°C incubator. Bioluminescence was measured using photomultiplier tubes (ActiMetrics Lumicycle System; ActiMetrics), with a sample (counts/2 min) taken every 10 min. After 7 days, cover slips were removed, and the media was changed with either 1.2 ml of control media (6 SCN) or media containing BAY 59-9837 (Tocris Biosciences) at concentrations of 100 nM (6 SCN), 1 µM (6 SCN), and 10 µM (6 SCN), recovered with a cover slip, and returned to the Lumicycle System for recording. Following 7 days of recording after the first media change, the media was changed again to fresh control media to analyze washout of the agonist. Samples were recorded for 7 days following the second media change.

**Data analysis.** Free-running periods were calculated from wheel-running activity measurements by chi-squared periodograms, and the amount of activity was calculated using ClockLab (ActiMetrics). Statistical analyses of period and amount of activity during infusion and after infusion were performed on the data after subtracting the pretreatment baseline period or amount of activity, respectively, for each animal.

Bioluminescence was analyzed using Lumicycle Analysis software (ActiMetrics). The 3 days of data beginning after the first full day following media change were analyzed for each 7-day period of recording (pretreatment: days 2–5; drug application: days 8–11; and washout: days 15–18). Baseline drift was subtracted using a polynomial curve equal to the number of days analyzed (three). Baseline-subtracted data were used for all bioluminescence analyses. Period of PER2 expression measured by bioluminescence was calculated using the Levenberg-Marquardt algorithm to fit a sine wave. Amplitude of bioluminescence was calculated using baseline drift-subtracted data. Amplitude for each sample was averaged over 3 days following the first full day of recording for each condition (pretreatment, VPAC2 agonist, and washout). Average amplitude for each sample for each time period (baseline, drug application, or washout) was used for repeated-measures ANOVA comparisons. For subsequent group comparisons, average amplitude for each sample during drug application or washout was divided by the average amplitude for each sample during pretreatment and multiplied by 100 to obtain a percent change from baseline recordings. Wheel-running activity data were analyzed by ANOVA using JMP version 5.0.1a (SAS Institute, Cary, NC). PER2-driven bioluminescence data were analyzed by repeated-measures ANOVA using PASW Statistics 18.0 (SPSS, Chicago, IL) to test the effect of time (baseline, drug application, washout), drug (100 nM, 1 µM, or 10 µM BAY 59-9837 and acsf), and the interaction of time × drug. ANOVA with JMP was also used to compare treatment groups during baseline, drug application, and washout.

**RESULTS**

**Chronic infusion of the vpac2 receptor agonist in vivo lengthened the circadian period.** Wheel-running activity was recorded for 3 wk in DD before surgery. The average free-running period during this time was 24.13 ± 0.04 h for the control group that received acsf and 24.07 ± 0.06 h for the BAY 59-9837 group (P = 0.40; Fig. 1). During 28 days of infusion, the average period of the control group was unchanged. In contrast, 200 µM of BAY 59-9837 lengthened the period by an average of 0.69 h (P < 0.0002; Fig. 1E). Upon completion of the 28-day infusions, the average periods of the experimental and control groups were again similar (24.17 and 24.08 h, respectively) as were the average changes relative to the free-running period before surgery (−0.06 and 0.10 h, P = 0.41). A small aftereffect on period following completion of BAY 59-9837 infusion was observed in two animals (for example, see Fig. 1B) but did not result in a significant long-lasting effect on period when periods of all animals were averaged (Fig. 1D).

**Chronic infusion of BAY 59-9837 did not affect the amount of wheel-running activity.** The amount of wheel-running activity expressed as average counts per 6 min was compared between groups during 21 days before surgery, 28 days of infusion, and for 21 days following completion of infusion. A general decrease in the amount of wheel-running activity was observed following surgery in both groups (Fig. 2). No significant difference in the amount of wheel-running activity was observed between acsf- and BAY 59-9837-infused animals before, during, or after infusion (Fig. 2).

**BAY 59-9837 lengthened the period and increased amplitude of PER2 expression in SCN explants in vitro.** Four groups of SCN explants (6 explants each) from adult male PER2::LUC mice were placed in media (see METHODS) in individual petri dishes, and bioluminescence was recorded for 7 days to obtain pretreatment (baseline) recording of SCN PER2 expression rhythms. The groups did not differ in the average periods of PER2 bioluminescence rhythms before treatment (Fig. 3A). On

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Fig. 1. Infusion of vasoactive intestinal peptide receptor (VPAC2) agonist in vivo lengthens the period of wheel-running activity. Shown are double-plotted actograms of hamsters infused with control artificial cerebral spinal fluid (acsf) (A) and 200 μM BAY 55-9837 (B and C). All hamsters displayed similar circadian periods before surgery. During 28 days of infusion (gray area), animals in B and C displayed significant lengthening of the circadian period. In comparison, the animal in A did not show a change in the period during pump infusion. Hamsters in B and C returned to a shorter period following completion of pump infusion. The bar graph (D) shows the average period for each group before surgery (baseline), during infusion (infusion), and during 3 wk following completion of pump infusion (recovery). Bars represent means for each group, horizontal lines represent 95% confidence intervals, and black circles represent individual values. E: bar graph showing change in period during infusion (period during infusion minus baseline) in control acsf- and BAY 55-9837-infused animals.

Fig. 2. BAY 55-9837 does not affect the amount of wheel-running activity. Bar graph showing the amount of wheel-running activity during baseline, drug infusion, and after pump infusion. There was no significant difference in activity level between animals infused with BAY 55-9837 and control acsf-infused animals. Activity level decreased following surgery for both the acsf (**P = 0.04) and BAY 55-9837 (+ + P = 0.001) group compared with activity levels before surgery. Bars represent means of each group, horizontal lines represent 95% confidence intervals, and black circles represent individual values.

of treatment was observed in any group (F = 0.79, P = 0.39 for the 100 nM group, F = 1.42, P = 0.26 for the 1 μM group, and F = 1.64, P = 0.23 for the 10 μM group). In contrast, a significant interaction of time × treatment was observed in the 10 μM group (F = 4.60, P = 0.02) but not in the other dosage groups (F = 0.35, P = 0.71 for the 100 nM group and F = 2.94, P = 0.76 for the 1 μM group). Comparisons of each drug group with acsf controls during the drug application time period using ANOVA also indicated that low concentrations of BAY 55-9837 did not have a significant effect on the average period of PER2 bioluminescence rhythms (Fig. 3A). The highest concentration (10 μM) of BAY 55-9837 significantly lengthened the average period of PER2 bioluminescence rhythms compared with the control group during the drug application period (P < 0.01; Fig. 3, A and B).

A significant effect of time on amplitude of PER2 bioluminescence was observed using repeated-measures ANOVA for all groups (F = 26.43, P < 0.001 for the 100 nM group, F = 64.93, P < 0.001 for the 1 μM group, and F = 52.64, P < 0.001 for the 10 μM group). No significant effect of treatment was observed in any group (F = 0.13, P = 0.72 for the 100 nM group, F = 0.26, P = 0.62 for the 1 μM group, and F = 0.02, P = 0.86 for the 10 μM group). A significant interaction of time × treatment was detected only in the 10 μM group (F = 0.001, P = 0.99 for the 100 nM group, F = 2.08, P = 0.15 for the 1 μM group, and F = 3.91, P = 0.03 for the 10 μM group).
Comparisons of drug groups with acsf controls during the drug application time period using ANOVA further indicated that 10 μM BAY 55-9837 increased the amplitude of the PER2 bioluminescence rhythm compared with control samples during the drug application period (Fig. 4, A and B). Following 7 days in media containing BAY 55-9837, all samples were switched to fresh media, and PER2 bioluminescence was recorded for seven additional days. During washout, no significant differences in period or amplitude of PER2 bioluminescence were observed between the 10 μM BAY 55-9837 group and the control group (Figs. 3A and 4C).

**DISCUSSION**

Chronic in vivo stimulation of the VPAC2 receptor by BAY 55-9837 lengthened the free-running period of hamster wheel-running activity rhythms (Fig. 1). This effect was reversed upon completion of pump infusion. Chronic application of BAY 55-9837 to cultured SCN explants also lengthened the period of the PER2 bioluminescence rhythm (Fig. 3). The observed lengthening of period by chronic VPAC2 receptor stimulation, together with the reported shortening of period in vip−/− and vpac2−/− mice (2, 4, 7, 12, 18), suggests that

Fig. 3. VPAC2 agonist in vitro lengthens the period of PER2 expression rhythm in the suprachiasmatic nucleus (SCN). A: there was no difference in the period between groups during baseline recording and washout, but there was a significant lengthening of the period in SCN given 10 μM BAY 55-9837 compared with standard media. B: bar graph showing the change in period of the PER2 expression rhythm in control samples and in samples treated with 10 μM BAY 55-9837. Bars represent means for each group, horizontal lines represent 95% confidence intervals, and black circles represent individual values.

Fig. 4. BAY 55-9837 (10 μM) increases the amplitude of bioluminescence. A: representative recordings of bioluminescence from samples given 10 μM BAY 55-9837 (gray) or standard control media (black). Peak bioluminescence is increased in samples treated with 10 μM BAY 55-9837. B and C: change in amplitude of bioluminescence (relative to pretreatment amplitude) during drug treatment and after washout. Bars represent means for each group, horizontal lines represent 95% confidence intervals, and black circles represent individual values.
VIP-VPAC2 signaling plays a role in the determination of circadian period. Period is a functionally important property of circadian clocks affecting the entrained phase relationship between an organism’s rhythms and environmental cycles (33). The period of the mammalian central clock, the SCN, is determined by both the intrinsic period of oscillations generated within individual cells and by cellular interactions that integrate them to produce a population value (25, 26, 41). When cells are coupled, they necessarily express similar periods, and coupling therefore involves adjustments in period. Because VIP-VPAC2 signaling is already thought to be important for coupling (2, 6, 17), the regulation of period and coupling mechanisms may be closely linked.

Previous studies have suggested several functions for VIP-VPAC2 signaling in the SCN as follows: mediation of synchronization among SCN neurons (2, 6, 17), modulation of responses to light (7, 12, 14, 16, 18, 30, 32, 36), and maintenance of cellular oscillations by a contribution to intracellular cAMP rhythms (see below). The present study is the first to report effects of chronic VPAC2 activation. Although chronic activation and the resulting effect on period are likely related to the other effects associated with VIP-VPAC2 signaling, exactly how they are related is unclear. For example, VIP-VPAC2 signaling has been shown to increase cAMP in cells (13, 19, 27, 35), and cAMP appears to have a critical role in sustaining the circadian transcription/translation feedback loop in SCN cells (31). In addition, inhibition of adenylyl cyclase caused a lengthening of the period in vitro (PER2::LUC rhythms) and in the activity rhythms of mice when an inhibitor was infused in the brain (31). It is unclear why adenylyl cyclase inhibition (reducing cAMP) and VPAC2 activation (the present study) should both cause a lengthening of period. Similarly, overexpression of the VPAC2 receptor in mice caused a shortening of the period (37). In this case, activation of VPAC2 signaling might still occur only at times when an endogenous ligand is released in contrast to the present study where an exogenous ligand was provided at constantly high levels.

The observed lengthening of the period by chronic VPAC2 receptor activation is similar to the effect of constant light on the circadian period in nocturnal rodents (9). This, together with previous reports showing that pulses of VIP-VPAC2 agonists can phase shift locomotor activity and the firing rate rhythm of SCN neurons, and can induce SCN per expression (30, 32, 36), suggests that VIP-VPAC2 signaling has a role in transmitting photic input across SCN cells. In addition, although previous studies showed that the activity of vpac2−/− mice is mostly restricted to the dark phase of a LD cycle, this is primarily the result of the negative masking effects of light rather than entrainment of a coherent activity rhythm (14, 16). The activity onsets of both vpac2−/− and vip−/− mice that do express activity rhythms when released in DD show that entrainment was abnormal, specifically that the phase of the activity rhythm was advanced relative to that of wild-type animals. An advanced phase relationship is consistent with a shorter than normal free-running period and/or reduced phase delay responses to light. Both vpac2−/− and vip−/− mice show reduced phase shift responses to light and abnormal cellular responses in the SCN, including reduced responses during subjective night (especially delay shifts) and greater responses during subjective day. Taken together, these findings indicate that VIP-VPAC2 signaling contributes to responses of the circadian clock to light and could therefore mediate the period-lengthening effect of constant light. In vitro effects of VPAC2 stimulation reported here and by others (36) indicate that activation of VIP-VPAC2 signaling resembles effects of light in vivo by acting within the SCN and not through effects on the retina or presynaptically through the retinohypothalamic tract.

If VIP-VPAC2 signaling mediates the effects of constant light on the period, then constant light would be expected to increase VIP-VPAC2 signaling. In rats, however, light exposure decreased the level of VIP peptide and mRNA in the SCN (38, 39). In rats, VIP is also lower during the light of a LD cycle, but this has not been reported in mice or hamsters (10, 12). Although the evidence is strong that VIP is involved in the integration of light information in SCN cells, the differences in VIP SCN levels in response to light across animal models and types of exposures remain unexplained.

In addition to an effect on period, chronic application of BAY 55-9837 to cultured SCN explants increased the amplitude of PER2 bioluminescence (Fig. 4). Although, to our knowledge, the stability of BAY 55-9837 has not been thoroughly characterized, the results of our chronic infusion of this drug in vivo indicate that it was active for at least the 28 days of infusion. Therefore, under similar conditions in vitro, it is reasonable to assume that BAY 55-9837 was active for at least 7 days. It is possible that VPAC2 stimulation increases the amplitude of each individual cell, resulting in increased amplitude of PER2 bioluminescence from SCN explants as a whole. Alternatively, or in addition, chronic application of BAY 55-9837 may generate greater synchrony among cells, such that greater numbers of cells reach peak and trough PER2 expressions at the same times, resulting in increased amplitude of the population rhythm. This is consistent with previous results indicating that VIP-VPAC2 is required for synchrony among SCN cells. In particular, vpac2−/− or vip−/− mice showed decreased synchrony among SCN cells in per1 expression (6, 17, 29) and firing rates (2, 3). When cells lose synchrony, their individual amplitudes also fall (2, 17, 29). Pulses of VPAC2 agonist are sufficient to restore synchrony among the firing rhythms of dispersed SCN neurons (2) and increase the amplitude of PER2 bioluminescence rhythms expressed by cultured cortical astrocytes (28). Moreover, depolarization of cells in SCN slices from vpac2−/− mice by application of K+ increased amplitude of per1-driven bioluminescence and synchrony among cells, indicating that depolarization of cells by VIP-VPAC2 signaling could lead to synchronization of SCN cells (29). This effect may be occurring postsynaptically through cAMP signaling. Decreased cAMP, as well as blockade of the hyperpolarization-activated and cyclic nucleotide-gated ion channels (HCN), leads to decreased amplitude of PER2 bioluminescence (31). VIP-VPAC2 signaling may activate cAMP and increase per expression and synchrony among cells by activating HCN channels. The depolarization of membrane potential might also make cells more receptive to other signals.

Although we did not see an effect of chronic VPAC2 receptor stimulation on the level of wheel-running activity in hamsters, we are unable to rule out a role for VIP as an SCN output signal. Neurons in a principal efferent target area of the SCN, the SPZ, express VPAC2 receptors (21). Recently, SPZ...
neurons that receive monosynaptic GABAergic input from the SCN were found to be either depolarized by VIP or show increased inhibitory postsynaptic currents (IPSCs), indicating presynaptic modulation of SCN input. In both cases, the same VPAC2 agonist used here (BAY 55-9837) had effects similar to VIP (15). In the SCN, application of VIP has been reported to increase the frequency of γ-aminobutyric acid IPSCs in SCN neurons through the VPAC2 receptor and a cAMP/protein kinase A-dependent pathway (19). Electrophysiological work in *vpaC2*−/− mice reported hyperpolarized resting membrane potential of SCN cells in these animals (2), consistent with a depolarizing effect of VIP-VPAC2 signaling. Together, these studies indicate that VIP-VPAC2 signaling potentially has both inhibitory and excitatory postsynaptic effects in both the SCN and target areas such as the SPZ. Chronic infusion of a VPAC2 agonist may have conflicting effects and a small net effect on downstream targets. Further studies are needed to elucidate the role that VIP-VPAC2 signaling may play in transmitting circadian clock information to adjacent brain regions.

**Perspectives and Significance**

In humans as in other mammals, the SCN is specialized for the generation and coordination of circadian rhythms. It receives direct retinal input and is essential for the expression and entrainment of circadian rhythms in physiology. The SCN consists of thousands of cells, and its functional properties emerge at least in part from their interactions. VIP is a functional component of the circadian clock, its endogenous period. Ultimately, pharmaco-stimulation reversibly changed a fundamental property of the SCN.

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**DISCLOSURES**

No conflicts of interest are declared by the authors.

**REFERENCES**


