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REVIEW

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Signaling in the suprachiasmatic nucleus: selectively responsive and integrative

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Abstract The suprachiasmatic nucleus (SCN) contains a biological clock that generates timing signals that drive daily rhythms in behaviors and homeostatic functions. In addition to this pacemaker function, the SCN gates its own sensitivity to incoming signals, which permits appropriate temporal adjustment to achieve synchrony with environmental and organismic states. A series of time-domains, in which the SCN restricts its own sensitivity to a limited set of stimuli that adjust clock phase, can be distinguished. Pituitary adenylyl cyclase-activating peptide (PACAP) and cAMP directly reset clock phase during the daytime domain; both cause phase advances only during the clock's day-time domain, but are without effect at night. In contrast, acetylcholine and cGMP analogs phase advance the clock only when applied during the night. Sensitivity to light and glutamate arises concomitant with sensitivity to acetylcholine and cGMP. Light and glutamate cause phase delays in the early night, by elevating intracellular Ca^{2+} via neuronal ryanodine receptors. In late night, light and glutamate utilize a cGMP-mediated mechanism to induce phase advances. Nocturnal responses of SCN primed by light or glutamate can be modulated by effectors of phase-resetting in daytime, namely, PACAP and cAMP. Finally, the dusk and dawn domains are characterized by sensitivity to the pineal hormone, melatonin, acting through protein kinase C. These changing patterns of sensitivities demonstrate that the circadian clock controls multiple intracellular gates, which ensures that they can be opened selectively only at specific points in the circadian cycle. Discerning the molecular bases of these changes is fundamental to understanding integrative and regulatory mechanisms in the circadian system.

Keywords PACAP · Glutamate · cAMP · Phase shift · Melatonin · cGMP · Acetylcholine · SCN

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As the central component of the mammalian circadian system, the suprachiasmatic nucleus (SCN) receives projections from numerous brain regions as well as a direct connection from the light-sensing retina via the retinohypothalamic tract (RHT). These inputs relay the organismic and environmental states to the SCN. Incoming signals are integrated by SCN neurons, which are amongst the smallest in the brain. The high relative surface to volume ratio of SCN neurons predicts their role as chemosensors. Signals impinging upon the SCN can either alter the state of the circadian clock directly, or modify responsiveness in an SCN primed by a primary regulator. However, the action of individual regulators or modulators is limited to specific segments of the circadian cycle, when the clock is receptive to them. Altering the phase in behavioral rhythms under clock control would be advantageous only if the phase-adjusting stimulus were an 'error' signal. In this context, the error signal would convey mismatch between the organismic or environmental states and clock time. For example, light occurring in the day is appropriate; however, light encountered at night would represent inappropriate phasing between the clock cycle and the environmental light/dark cycle. The signal(s) reset(s) clock time appropriately.

The SCN undergoes a programmed, near 24-h sequence of sensitivity to stimuli that can adjust its phase (Gillette et al. 1995). Receptive periods correlate with discrete stages in the clock's entrained cycle: some are restricted to subjective day, others to subjective night, and yet others to transitions in the day/night cycle (Fig. 1). The discrete regions of responsiveness significantly restrict the ability of specific signals to alter clock timing, and effectively determine whether the clock recognizes a stimulus as an error signal. In this way, the

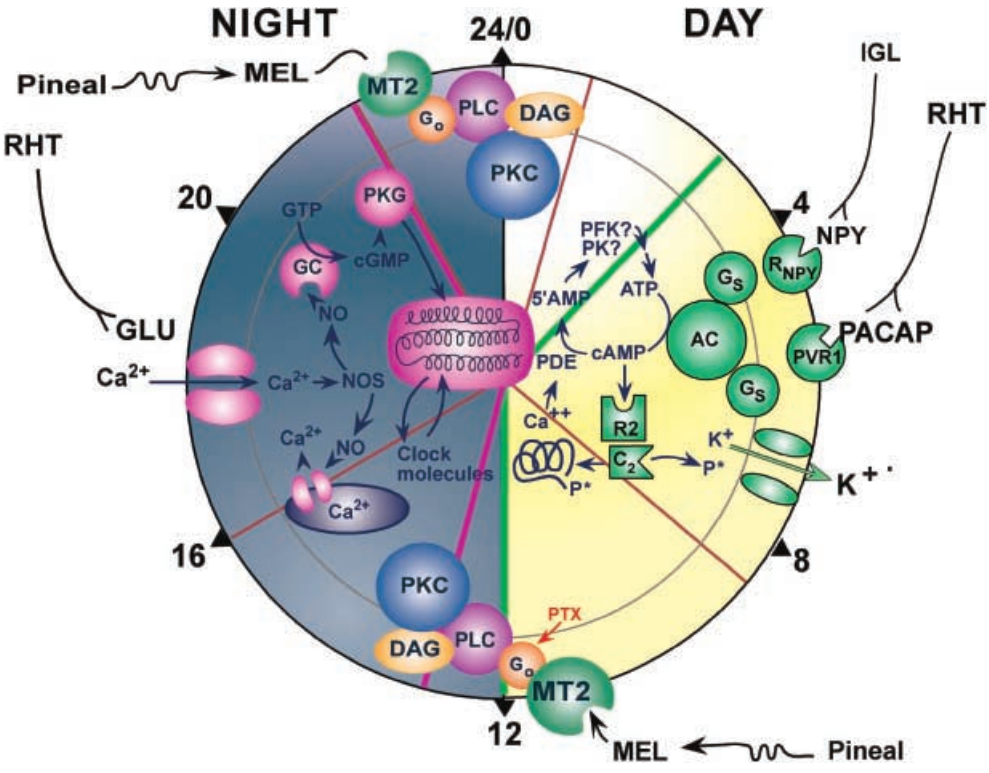

Fig. 1 Schematic representation of the 24-h circadian cycle, comprising four major time domains with respect to temporal sensitivities of the SCN to various signaling pathways (*MEL* melatonin, *MT2* melatonin receptor type 2, G_o G protein o, *PLC* phospholipase C, *DAG* diacylglyceride, *PKC* protein kinase C, *PKG* protein kinase G, *PK* phosphorylase kinase, *PFK* phosphofructokinase, *IGL* intergeniculate leaflet, *NPY* neuropeptide Y, *RHT* retinohypothalamic tract, *PVR1* PACAP type 1 receptor, G_s G protein stimulating, *AC* adenylyl cyclase, *R2* PKA regulatory subunit, C_2 PKA catalytic subunit, *PTX* pertussin toxin, *NO* nitric oxide, *NOS* nitric oxide synthase, *GC* guanylyl cyclase, *GLU* glutamate)

clock temporally filters, or gates, the information that can access its timekeeping mechanism across the circadian cycle.

Studying the temporal organization of the SCN of the rat in a hypothalamic brain slice preparation (Gillette 1991) enables the direct evaluation of properties endogenous to the SCN. Perhaps surprisingly, the SCN generates near 24-h oscillations in ensemble neuronal firing rate in vitro (Prosser and Gillette 1989), like those observed in vivo (Inouye and Kawamura 1979), when sectioned in a 500-μm coronal slice. This occurs despite surgical deafferentation, separation of the rostral from caudal components, and lack of endocrine and trophic factors. The pattern of neuronal activity of the collective SCN slice is sinusoidal (Gillette 1986), with mean activity peaking near midday, at CT 7.0±0.1 (Prosser and Gillette 1989) (CT = circadian time, where CT 0 in vitro corresponds to 'lights on' in the colony, and continues for 24 h; 24 h is the period (τ) of this rhythm over 3 days ex vivo; Prosser and Gillette 1989). The time of peak activity of the neuronal population is stable across several cycles and predictable in these inbred rats (Prosser and Gillette 1989). Therefore, the circadian rhythm in the firing rate of the collective SCN neurons can be used as a bioassay to probe for gating mechanisms.

Only at specific phases, or time domains, within the 24-h cycle, are animals susceptible to phase resetting. Likewise, the circadian clock in vitro is receptive to only a subset of the various resetting stimuli, and, therefore, the circadian clock regulates its own sensitivity to afferent signals. Thus, the states of specific molecular gates regulated by the clock change over the 24-h cycle and lie

Table 1 Activation of pathways is temporally restricted to distinct time domains and has characteristic effects on the clock phase. The effects on phase were determined by the shift in peak neuronal activity of SCN neurons to occur earlier (advance) or later (delay) than during the previous day (*n.d.* not determined, *PACAP* pituitary adenylyl cyclase-activating peptide, *NPY* neuropeptide Y, *NMDA N*-methyl-D-aspartate, *NOS* nitric oxide synthase, *RyR* ryanodine receptor, *HO-2* heme oxygenase 2, *PKA* protein kinase A, *PKC* protein kinase C)

Stimulus	Day	Early night	Late night	Dawn/ dusk
cAMP/PKA agonist	Advance	No effect	No effect	No effect
C/PKG agonist	No effect	Advance	Advance	n.d.
PKC agonist	No effect	n.d.	n.d.	Advance
PACAP	Advance	No effect	No effect	No effect
Serotonin	Advance	No effect	No effect	No effect
NPY	Advance	No effect	Advance	No effect
Light/GLU pathway				
Light/GLU	No effect	Delay	Advance	n.d.
NMDA	No effect	Delay	Advance	n.d.
Ca^{2+} influx	No effect	Delay	Advance	n.d.
NOS activation	No effect	Delay	Advance	n.d.
RyR activation	No effect	Delay	No effect	n.d.
C/PKG activation	n.d.	Not activated	Advance	n.d.
Cholinergic pathway				
Cholinergic agonist	No effect	Advance	Advance	n.d.
HO-2 activation	No effect	Advance	Advance	n.d.
C/PKG activation	No effect	Advance	Advance	n.d.
Melatonin	No effect	No effect	No effect	Advance

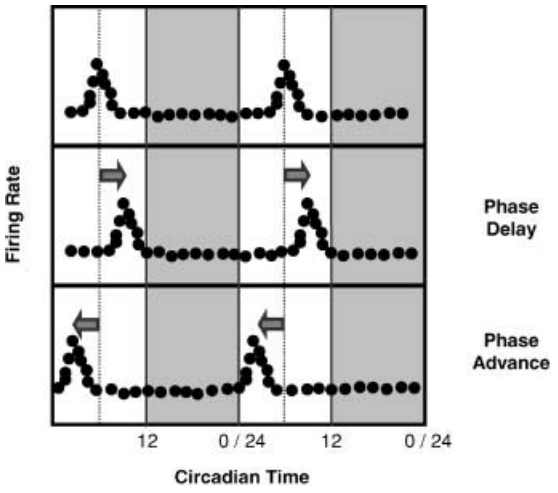

Fig. 2 Schematic representation of phase resetting of the spontaneous electrical activity of the collective SCN neurons of a hypothalamic brain slice. The electrical activity peaks at mid-subjective day (CT 7), which is marked with a *dashed line*. A phase delay results from a stimulus that causes the peak of neuronal activity to occur later than in controls. A phase advance occurs when the peak in neuronal activity appears earlier than in controls (*shaded areas* subjective night)

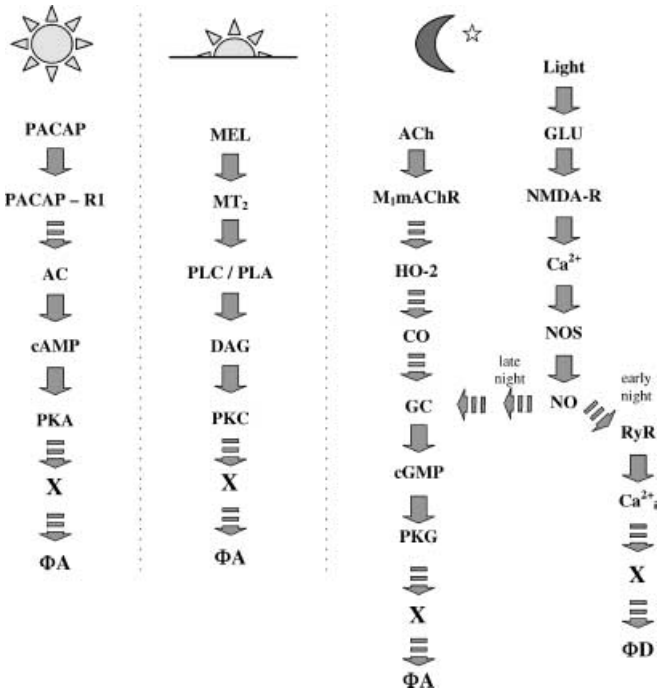

Fig. 3 Summary of the putative elements in signaling pathways that can access the clock in daytime, dusk/dawn and nighttime. *Broken arrows* indicate signaling processes with an unknown number of steps. Relative position of a critical gating site in each pathway is designated by an *X*. (*PACAP* pituitary adenylyl cyclase-activating peptide, *PACAP-R1* PACAP receptor 1, *AC* adenylyl cylase, *cAMP* cyclic AMP, *PKA* protein kinase A, *MEL* melatonin, MT_2 melatonin type 2 receptor, *PLC* phospholipase C, *PLA* phospholipase A, *DAG* diacyl glycerol, *PKC* protein kinase C, *ACh* acetylcholine, *M1 mAChR* M1 muscarinic acetylcholine receptor, *HO-2* heme oxygenase 2, *CO* carbon monoxide, *GC* guanylyl cyclase, *PKG* protein kinase G, *GLU* glutamate, *NMDA-R* *N*-methyl-D-aspartate receptor, *NOS* nitric oxide synthase, *NO* nitric oxide, *RyR* ryanodine receptor, Φ*A* phase advance, Φ*D* phase delay)

within the SCN. These sensitivities can be effectively probed by briefly applying stimuli to receptive regions of the SCN and evaluating effects of timing of the peak in the spontaneous oscillation of neuronal activity (see Table 1). Effects on clock timing are measured against the time of peak activity in controls, or in modulating a primary response over the next 1–2 days in vitro. If the peak appears earlier than in controls during the cycles after treatment, the phase of the rhythm has been advanced (Fig. 2). If it appears later than in controls, the phase has been delayed by the treatment (Fig. 2). The changing relationship between the circadian time of treatment and effect on phase can be visualized using a phase-response curve (PRC). This relationship graphically presents the temporal pattern of SCN sensitivity to activation of specific signaling pathways.

Temporal domains of clock phase resetting via specific signal transduction pathways comprise discrete portions of the circadian cycle with distinct sensitivity and response characteristics (Fig. 3). These temporal domains define periods of sensitivity to the various neurotransmitters systems that impinge upon this hypothalamic site. Clock-controlled gating of SCN responsiveness can be divided into four primary domains of the circadian clock: day, night, dawn and dusk. Day and night represent distinct domains, while dawn and dusk domains are more similar to each other. Characteristics of each domain as well as the nature of modulatory interactions will be considered in turn.

Daytime domain

The daytime domain is characterized by its selective sensitivity to cAMP and the cAMP-dependent protein kinase (PKA). Reagents that stimulate acute activation of PKA induce robust advance in the phase of the neural activity of the SCN (Gillette and Prosser 1988; Prosser and Gillette 1989). Effective reagents include: (1) membrane soluble cAMP analogs, which directly activate PKA and resist degradation; (2) forskolin, which results in enhanced synthesis of native cAMP through stimulation of adenylyl cyclase (AC); and (3) RO 20–1724, which inhibits the phosphodiesterase that degrades endogenous cAMP (see Table 1).

The cAMP/PKA-mediated response of the daytime domain is highly selective. A number of reagents, which have been shown to alter the circadian clock, are not functional during the daytime domain. Thus, at mid-subjective day, the SCN responds neither to activation of the protein kinase G (PKG) pathway by membrane soluble analogs of cGMP (8-bromo-cGMP, N^2O^2-dibutyryl-cGMP) (Prosser et al. 1989), nor to activation of the protein kinase C pathway through the addition of tetraphorbol acetate (TPA) (McArthur et al. 1997). Stimuli that mediate Ca^{2+} influx and NO production are also ineffec-

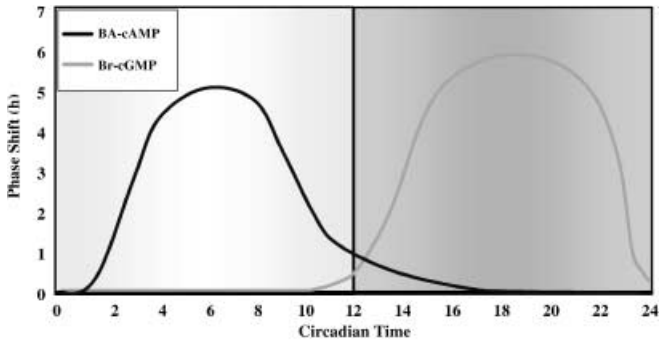

Fig. 4 Phase-response curves showing the sensitivity of the SCN clock to resetting by cAMP and cGMP analogs applied at different points in the circadian cycle. The data represent the shift in phase of the SCN rhythm (in hours) to a 1-h exposure to the analog initiated at the circadian time denoted. The domain of the clock sensitivity to BA-cAMP is during subjective daytime, while sensitivity to Br-cGMP occurs in antiphase, during the subjective nighttime. (Data replotted from Prosser et al. 1989)

tive in regulating the clock at this time (Ding et al. 1994, 1998).

Temporal gating of clock sensitivity in the rat SCN to activation of the cAMP/PKA pathway is clear in the phase response curve for membrane soluble analogs of cAMP (Fig. 4) (Prosser and Gillette 1989). Responsiveness of the SCN clock to bromo-cAMP (Br-cAMP) is limited to the daytime domain. Sensitivity to cAMP first appears early in the subjective daytime between CT 2 and 3, representing 2–3 h after the initiation of the light portion of the entrained 12-h light: 12-h dark cycle. The response rapidly peaks between CT 4 and 7, when Br-cAMP induces phase advances of 4–6 h. Then, it slowly wanes until phase is altered by less than 1 h when the cAMP analog treatment is administered at CT 11 or later into subjective night (Fig. 4). Other reagents that elevate endogenous cAMP are also ineffective at mid-subjective night, CT 18. This demonstrates that the gate permitting direct access of the cAMP/PKA signaling to the circadian clock is open during the daytime domain and closes at night.

The basis of the daytime domain's sensitivity and temporal selectivity to activation of the cAMP/PKA pathway is presently unknown. The obvious first level of control would be the cell membrane. There gating could occur through: the activation state of (1) membrane receptors, (2) G proteins available to couple ligand-bound receptors to intracellular effectors, or (3) effectors such as adenylyl cyclase or phosphodiesterase. While a range of evidence suggests that daytime behavioral arousal can alter clock phase via serotonin (Reebs and Mrosovsky 1989), possibly through a cAMP pathway (Medanic and Gillette 1992), recent evidence argues strongly that pituitary adenylyl cyclase-activating peptide (PACAP) may be the first messenger for cAMP (see Hannibal 2002: this volume). PACAP is localized in retinal ganglion cells that project to the retinorecipient region of the rat SCN and the PACAP type 1 receptor, which couples positively to adenylyl cyclase, is localized to SCN neurons in the retinorecipient area (Hannibal et al. 1997). When applied via microdrop to the SCN in vitro, PACAP induces a robust phase advance during the subjective day but not during the subjective night. The stimulus-response pattern is overlapping with that observed after direct stimulation of the cAMP/PKA pathway (Kopp et al. 2001). PACAP requires stimulation of cAMP for its effect. It will be important to determine what signaling agents can modulate the PACAP/cAMP response, to identify the next steps in the signaling pathway and to understand the mechanism that restricts a direct response to PACAP in the day and to glutamate at night, even though both are localized to retinal ganglion cells that project to the SCN (see Hannibal 2002: this volume).

While the clock may regulate the constituents of the cell membrane so that they vary and thus could restrict activation via receptor-mediated processes, clock-gated sensitivity to cAMP analogs demonstrates unequivocally that the clock controls the open state of intracellular gates at some point within the cell, downstream from cAMP production. These gates may include the state of PKA activation and localization as well as differing PKA targets, including transcriptional regulators. Considering the redundancy in biological control systems, multiple control points within a single pathway are likely. The fact that membrane permeable cAMP analogs that directly activate cAMP-dependent processes induce phase resetting only in daytime, reveals the existence of a critical gate positioned downstream from cAMP production. The identification of these gating sites and their molecular targets is presently a subject of intense research.

Nighttime domain

With the waning of the day and onset of night, the clock completely remodels the open state of gates to phase-altering stimuli. Whereas the daytime domain can be characterized by its sensitivity to direct activation of a PACAP/cAMP/PKA pathway, two distinct signaling pathways emerge with the initiation of night. The first pathway signals through elevation of cGMP level and subsequent activation of protein kinase G (PKG, the cGMP-dependent protein kinase). This pathway is initiated by muscarinic cholinergic stimulation. Although the behavioral context for cholinergic input is unknown, the cholinergic innervation of the SCN most likely originates from the basal forebrain and the pontine tegmental nuclei of the brainstem (Bina et al. 1993), both of which contribute to the control of a well-defined circadian behavior, sleep/arousal. The second pathway responds to environmental light. Photic stimulation is transmitted from the retina to the SCN via the retinohypothalamic tract (RHT), where it induces glutamate (GLU) release, *N*-methyl-D-aspartate (NMDA) receptor activation, Ca^{2+} influx and nitric oxide (NO) production. The SCN is sensitive to both pathways during the night, the signs of the two responses are opposite during early night, whereas

in late night they are the same (Fig. 5) (Ding et al. 1994; Liu and Gillette 1996). This suggests that the two pathways may activate different signaling pathways in the early night; however, the signaling pathways may converge during the late night. The data discussed below support this notion.

Muscarinic cholinergic response

Throughout the night, cholinergic stimulation of the SCN and activation of its muscarinic receptors induces significant phase advances in the SCN neuronal activity rhythm in a brain slice preparation from rat (Liu and Gillette 1996). The phase advance peaks (+6 h) in the middle of the night at CT 18 (Liu and Gillette 1996; Gillette et al. 2001). The SCN is insensitive to this treatment after CT 22 and throughout the subjective daytime. Whereas injections of cholinergic agonists at peripheral sites or centrally outside the SCN produce light-like responses (Zatz and Herkenham 1981; Earnest and Turek 1985), emerging evidence indicates that direct intra-SCN injection of the cholinergic agonist, carbachol (CARB), at night also causes phase advance of mouse behavioral rhythms (Buchanan and Gillette 2001). The pharmacological profile for SCN responsiveness to CARB is consistent with activation of M_1 muscarinic acetylcholine receptors (M_1 mACh-R) (Liu and Gillette 1996).

Interestingly, the window of sensitivity to phase shifting by the acetylcholine agonist CARB completely overlaps the cGMP-sensitive period in amplitude and duration (Fig. 4). Analogs of cGMP, which activate PKG, when applied throughout most of subjective night, whether early or late, induce robust phase advances in clock phase; a maximal phase advance of 6.5 h appears at CT 18 in the rat. The M_1mAChR has been shown to couple to cGMP activation (Hu and el-Fakahany 1993); thus, binding of the cholinergic stimulus to an M_1mAChR could activate the cGMP pathway in the SCN. In fact, at CT 18, CARB upregulates cGMP and activates PKG in the SCN (Liu et al. 1997a). The phase advance stimulated by the addition of CARB can be blocked by the addition of the KT5823, an inhibitor of PKG. Thus, acetylcholine localized in afferents to the SCN from brainstem and basal forebrain sleep structures (Bina et al. 1993) is a strong candidate for the first messenger of cGMP.

Recently, it has been determined that stimulation of heme oxygenase 2 (HO-2), with the resultant production of carbon monoxide (CO), one of the major activators of GC (Snyder et al. 1998), mimics the cholinergic effects on the SCN in vitro (Artinian et al. 2001). HO-2 is expressed in the rat SCN and inhibitors of HO-2 block the cholinergic phase advance and elevation in cGMP synthesis (Artinian et al. 2001). Therefore, the signal transduction pathway of cholinergic phase advance would seem to depend on HO-2 stimulation of cGMP production (see Fig. 3). It is noteworthy that gating of the cholinergic response matches that of cGMP analogs, which like cAMP analogs, act intracellularly. It follows that the SCN clock restricts access via this cholinergic pathway to the nighttime domain at a gating point within the cell downstream from cGMP.

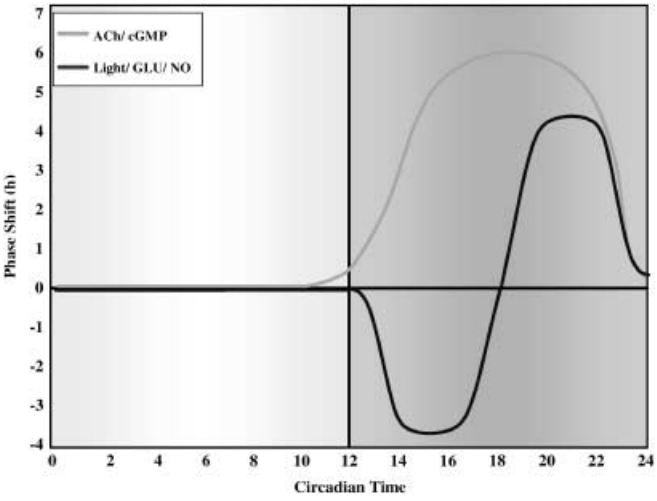

Fig. 5 Within the nighttime domain, the SCN exhibits distinct responses to different stimuli. Light/GLU can activate NMDA receptors with the resultant release of NO, which acts on different targets in early and late night, resulting in phase delay and phase advance, respectively. Throughout the night, activation of cholinergic muscarinic receptors, release of CO and elevation of cGMP cause phase advance

Light/glutamatergic response

The response of the SCN to light at night is the best-understood, yet most paradoxical domain of the clock. In animals maintained in constant darkness, stimulation by a pulse of light causes phase resetting of behavioral rhythms throughout the subjective night but not in subjective day (Summer et al. 1984). Unlike most stimulus-response relationships, the phase response curve for light is biphasic throughout the night, a pattern observed in all species examined (see Fig. 5). During early night, a light stimulus results in a delay in behavioral activity. About midway through the night, the response to light reverses so that late at night the result is an advance in behavioral activity.

The same bimodal response is elicited by brief application of GLU in vitro to brain slices, supporting the idea that GLU is the neurotransmitter of light to the SCN via the RHT. The SCN neurons express NMDA (NMDAR1, NMDAR2C), non-NMDA (GluR1, GluR2, GluR4) and metabotropic (mGluR1) glutamate receptors (Ebling 1996). The phase response generated by light/GLU can be replicated using NMDA, an agonist of the ionotropic GLU receptor or NO donors (Ding et al. 1994). Specific antagonists of the NMDA receptor or of nitric oxide synthase (NOS) block both GLU-stimulated phase delays and advances (Ding et al. 1994). The elements of this pathway have been largely corroborated in vivo through the light-like responses to NMDA injection

near the SCN (Mintz and Albers 1997) and blockage of the light response by intracerebroventricular injection of antagonists of the NMDA receptor (Ebling et al. 1991; Rea et al. 1993) or of NOS (Ding et al. 1994; Weber et al. 1995a). Concordance of both the timing of sensitivities and patterns of bimodal responses supports the idea that this complex response to light is mediated through a common signaling pathway involving GLU, activation of NMDA receptors, stimulation of NOS and production of NO. Therefore, the pathway inducing either a phase advance or a phase delay likely diverges downstream of NO.

Both the pattern and amplitude of the light response induced by GLU, NMDA, and NO differ from the response to acetylcholine and cGMP analogs (Fig. 5). In many systems, NO binds to an intracellular receptor in the form of a heme moiety of GC, stimulating the production of cGMP (Lincoln and Cornwell 1993). In fact, the phase advance induced by light in vivo (Weber et al. 1995b; Mathur et al. 1996) and by GLU in vitro (Ding et al. 1998) is blocked by a specific PKG inhibitor, KT5823. We hypothesize, therefore, that during the late night the pathway stimulated by light, GLU, NMDA-R and NO would activate GC/cGMP/PKG (see Fig. 3). Since the amplitude of the phase shift induced by the light/GLU pathway is smaller than that induced by stimulation by cGMP/PKG alone, it is likely that additional pathways may be activated by light.

On the other hand, early in the night inhibition of the cGMP/PKG pathway had no effect on the GLU-induced phase delay (Ding et al. 1998), suggesting that there is a bifurcation in the signaling pathway downstream from NO between early and late night (see Fig. 3). Whereas in late night NO is likely to interact with GC, the target of NO differs in early night. GLU-induced phase delays have been shown to require Ca^{2+}-induced Ca^{2+} release (CICR) (Ding et al. 1998). Specifically, activation of neuronal ryanodine receptors (RyR) by caffeine or immunosuppressive agents (FK506 or rapamycin) that stabilize the RyR in the open position mimic the effects of light/GLU in the early night. These reagents have no effect on phase in daytime or late night (Ding et al. 1998). Furthermore, the light/GLU-induced phase delay can be blocked by the RyR antagonist, dantrolene (Ding et al. 1998).

Despite the bifurcation in the signaling pathway between early and late night, stimulation by light, GLU or NO produces rapid and transient phosphorylation of the gene transcription factor CREB (P-CREB) (Ginty et al. 1993; Ding et al. 1997; von Gall et al. 1998) and activation of Ca^{2+}/cAMP response element (CRE)-mediated transcription (Obrietan et al. 1999). Therefore, it would seem that a gate upstream of CREB is an important gate to nocturnal phase shifting.

Modulation of the light response

The response to environmental light is not stereotypic. Rather, it is highly regulated by other signals impinging coincidentally upon the SCN. The emerging pattern is that primary effectors of phase-shifting in daytime play an alternative role as modulators at night, when the system is activated concomitantly with light/GLU. This modulatory role is well studied for PACAP/AC/cAMP/PKA signaling when the SCN has been primed by light/GLU. Nocturnal activation of the cAMP/PKA pathway or addition of PACAP in conjunction with stimulation of the light response by GLU causes a downward bias of the phase response curve (Fig. 6) (Chen et al. 1999; Tischkau et al. 2000). This translates differentially as the direction of the phase shift changes. In early night the phase delay is amplified, whereas in late night the phase advance is blocked (Chen et al. 1999; Tischkau et al. 2000). When the cAMP/PKA pathway is blocked by the isoquinoline inhibitor of PKA or inhibition of the PAC1 PACAP receptor, the light/GLU-induced phase response curve is shifted in the opposite direction (Fig. 6) (Chen et al. 1999; Tischkau et al. 2000). In this case, the phase delay is decreased and the phase advance is potentiated.

Co-stimulation with established daytime regulators such as neuropeptide Y (NPY) (Weber and Rea 1997; Yannielli and Harrington 2000) and serotonin (Weber et al. 1998; Quintero and McMahon 1999), or with melatonin (Benloucif and Dubocovich 1996), possibly acting through inhibition of PACAP pathway (von Gall et al. 2000), as well as concomitant activation of other signaling pathways, including the MAP kinase pathway (Obrietan et al. 1998; Tischkau et al. 2000), can modulate the effect of the light response. This type of interac-

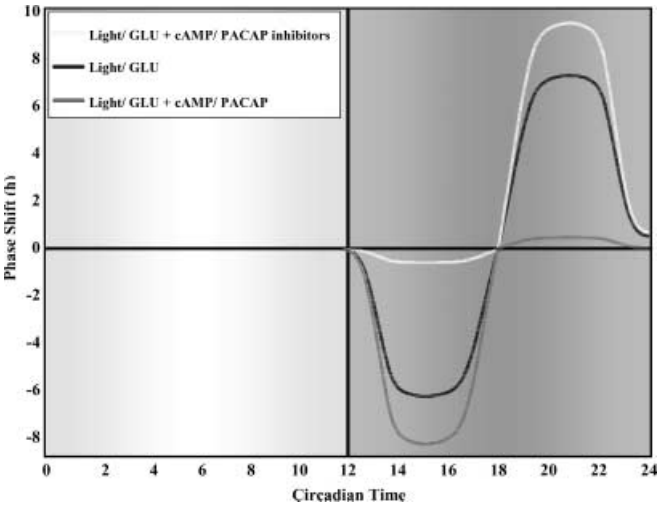

Fig. 6 A schematic summary of the modulatory effects of PACAP/cAMP on long-term changes in the SCN neuronal activity rhythm induced by light/GLU. PACAP and cAMP analogs are ineffective in altering circadian state when applied alone at night. However, they can modulate the response of the clock primed by exposure to light/GLU. Both PACAP and cAMP analogs enhance GLU-induced phase delays in early night, but block GLU-induced phase advances in late night. On the other hand, inhibitors of the PACAP or cAMP pathway block the GLU-induced phase delays in early night, and potentiate the GLU-induced phase advance in late night. (Redrawn from Tischkau et al. 2000)

tion among signaling pathways is not limited to the nighttime domain. There is also evidence for an interaction between GLU (Prosser 2001) and NPY (Prosser 1998), and for a modulation of the serotonin pathway by melatonin (Prosser 1999). Furthermore, interaction between melatonin and PACAP has also been reported in late subjective daytime (Kopp et al. 1997; von Gall et al. 1998). Together, these data reveal that phase-shifting stimuli have the potential to be influenced by signals from other events, with the result that the stimulation that actually accesses the clockworks is likely to be highly processed, integrating information from multiple relevant sources.

Dusk/dawn domains

In addition to the day and night domains, there are dusk/dawn domains that exhibit specific gating of select signaling pathways. Dusk and dawn, the periods surrounding the light-to-dark and dark-to-light transitions in the entraining lighting cycle, are relatively insensitive to simulation by cAMP, cGMP, Ca^{2+} influx, or NO. The presence of melatonin receptors in the SCN (Vanecek et al. 1987; Liu et al. 1997b; Hunt et al. 2001) suggests that melatonin feeds back to regulate SCN function. The duration of melatonin secretion changes in parallel with alterations in the relative durations of light and dark in the 24-h cycle. This predicts that the circadian clock should exhibit sensitivity to phase resetting by melatonin during these crepuscular domains, times where information concerning altered daylength is most altered by the seasons.

Indeed, the SCN clock is sensitive to melatonin at dusk and dawn (Stehle et al. 1989; McArthur et al. 1991, 1997; Starkey et al. 1995). Melatonin administered to the SCN in vitro advances the phase of the neuronal activity rhythm at both subjective dusk and subjective dawn, but is without effect on clock phase in day or night (McArthur et al. 1991, 1997). The melatonin-induced shift of clock phase is sensitive to pertussis toxin, invoking a G-protein-coupled receptor pathway (non-G_S) (McArthur et al. 1997). The SCN expresses MT_1 and MT_2, the only G-protein-linked melatonin receptors encoded by the rodent genome. The SCN from mice lacking the MT_1 receptor fail to show acute, modulatory effects of melatonin on spontaneous firing rate; however, they do exhibit normal phase-resetting of the neuronal activity rhythm (Liu et al. 1997b). These findings implicate residual MT_2 receptors as mediators of the effects on clock phase.

Additional evidence supports the hypothesis that melatonin may access the circadian clock via activation of the MT_2 receptor. SCN phase advance is blocked by the MT_2-selective antagonist, 4P-PDOT, at both dusk and dawn (Hunt et al. 2001). Melatonin activates protein kinase C (PKC) specifically at dusk and dawn, but not during daytime (McArthur et al. 1997). The effect of 4P-PDOT in SCN clock-resetting requires PKC activation at dusk and dawn (Hunt et al. 2001). Furthermore, pharmacological inhibitors of the PKC pathway block the melatonin-induced phase advance and phorbol esters, activators of the PKC pathway, induce melatonin-like effects. Thus at present, data obtained from studies on SCN tissue are consistent with a modulatory role for MT_1 receptors and a primary regulatory role for MT_2 receptors via a PKC-mediated pathway.

While compelling evidence indicates that PKC activation is an essential element in melatonin signal transduction, the molecular site gating melatonin's effectiveness in phase shifting has not been established. Based on our findings that gating of sensitivities to daytime and nighttime resetting stimuli occurs downstream from second messengers, we hypothesize that a critical gate for melatonin lies at a parallel level in its signaling cascade.

Conclusions

Together, a rapidly growing body of data reveals that the generation of both a 24-h timebase and a gated sensitivity to phase adjusting stimuli are fundamental properties of the circadian clock. On the basis of the temporal selectivity of the SCN to phase altering stimuli, which is present in the behaviors of animals under constant conditions and persists in the SCN in vitro, the circadian cycle can be divided into four primary time domains: day, dusk, night and dawn (Gillette 1996). The clock proceeds dynamically through these states of sensitivity, repeating them every 24 h.

At any given time, the SCN is sensitive to phase resetting by only a subset of signaling pathways. Direct phase-shifting effects of PACAP and cAMP occur only during the subjective daytime. Conversely, muscarinic cholinergic agonists and cGMP have access to the clock mechanism only during subjective night. Gates open to NMDA receptor-mediated glutamate stimulation also during the nighttime domain; however, the signal transduction pathway and phase response process for GLU differ remarkably between early and late night. Unique to the early night is the Ca^{2+}-mediated intracellular Ca^{2+} release by a neuronal ryanodine receptor resulting in phase delays. On the other hand, late night stimulation of the glutamate-mediated NMDA receptor and activation of the muscarinic cholinergic pathway converge at GC/cGMP/PKG; both induce phase advances. Finally, the sensitivity of the SCN to melatonin acting through an MT_2–like receptor-mediated activation of PKC is selective for the dusk/dawn domains.

Each of these gated responses is defined in the context of the signaling pathway whose activation is required in the SCN for the adjustment of circadian phase (see Fig. 3). The results are consistent in that gating of signaling pathways by the circadian clock occurs at multiple levels, including those beyond membrane receptors. We predict that molecular keys to gating-specific responses within the SCN are downstream from second messenger signaling. The changing pattern of sensitivities of the isolated SCN in vitro demonstrates that the

circadian clock controls multiple molecular gates in a way that ensures that they can be selectively opened in a permissive fashion only at specific points in the clock cycle. Integrative modulation of activating signals may take place at multiple levels as well. This ensures that the circadian cycle is adjusted appropriately according to the strengths of the various signals for change.

Understanding the molecular mechanisms that generate gating and compute the strengths of interacting signals is fundamental to understanding the SCN's role in integration and regulation of circadian rhythms. Gating allows the clock to prime itself to receive exogenous signals, investing them with temporal relevance. Accordingly, the endogenous clock anticipates significant exogenous cues that provide the clock with information critical for maintaining internal synchrony with the changes in darkness and light inherent to seasonal changes in the natural world. Signal integration fine-tunes resetting to the acute, day-to-day variations in the physiological, metabolic and behavioral milieu within the body. Together, clock-controlled temporal gating and integration of signaling pathways endow the individual with the plasticity necessary to function adaptively in a complex, ever-changing world.

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