Differential cAMP Gating of Glutamatergic Signaling Regulates Long-Term State Changes in the Suprachiasmatic Circadian Clock

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We investigated a role for cAMP/protein kinase A (PKA) in light/glutamate (GLU)-stimulated state changes of the mammalian circadian clock in the suprachiasmatic nucleus (SCN). Nocturnal GLU treatment elevated cAMP; however, agonists of cAMP/PKA did not mimic the effects of light/GLU. Coincident activation of cAMP/PKA enhanced GLU-stimulated state changes in early night but blocked light/GLU-induced state changes in the late night, whereas inhibition of cAMP/PKA reversed these effects. These responses are distinct from those mediated by mitogen-activated protein kinase (MAPK). MAPK inhibitors attenuated both GLU-induced state changes. Although GLU induced mPer1 mRNA in both early and late night, inhibition of PKA blocked this event only in early night, suggesting that cellular mechanisms regulating mPer1 are gated by the suprachiasmatic circadian clock. These data support a diametric gating role for cAMP/PKA in light/GLU-induced SCN state changes: cAMP/PKA promotes the effects of light/GLU in early night, but opposes them in late night.

Key words: suprachiasmatic nucleus; glutamate; signal transduction; mPer1; protein kinase A; MAP kinase; rat

The circadian clock is a complex biological structure in which a dynamic set of cellular state progressions generates near 24 hr rhythms at behavioral, physiological, cellular, and molecular levels. The oscillatory mechanism is a self-sustained transcriptional–translational feedback loop (for review, see King and Takahashi, 1972; Pickard, 1982; Johnson et al., 1988). These afferent signals use specific signal transduction pathways to interface with core clock elements to induce long-term state changes, or phase shifts, in the clock. Clock sensitivity to state-altering signals and activation of signaling pathways are gated by the SCN (Gillette, 2000). For example, cAMP activation alters circadian timing only during subjective day, whereas sensitivity to cGMP-dependent state changes are restricted to subjective night (for review, see Gillette and Tischkau, 1999). Thus, the accessibility of specific signaling pathways is key to regulation of timing.

Light signaling pathways intersect with the molecular clockworks via the mammalian period1 (mPer1) gene. Translation of mPer1 mRNA is required for light-induced phase resetting in the early night (Akiyama et al., 1999). Light elevates mPer1 mRNA in both early and late night (Albrecht et al., 1997; Shearman et al., 1997; Shigeyoshi et al., 1997; Takumi et al., 1998; Zylka et al., 1998). Light responsiveness of other clock genes differs. Levels of mPer2 increase robustly after light in the early night, but the response in the late night is not as clear (Albrecht et al., 1997; Takumi et al., 1998; Zylka et al., 1998). mTim mRNA levels are also augmented in response to light in the early night [Tischkau et al., 1999], but see King and Takahashi (2000)]

Dissection of elements required for SCN processing of light information points to commonalities and disparities between early and late night. Glutamate (GLU) is the primary neurotransmitter transmitting light signals to the SCN (Pickard, 1982; Johnson et al., 1988; Castel et al., 1993; De Vries et al., 1993; Ding et al., 1994; Shirakawa and Moore, 1994; Hannibal et al., 2000). Throughout the night, a phase-resetting light stimulus evokes release of GLU from the retinohypothalamic tract (RHT) and activation of multiple GLU receptor types, of which NMDA receptors are critical (Colwell and Menaker, 1992; Ding et al., 1994; Shibata et al., 1994; Shirakawa and Moore, 1994; Mintz et al., 1999). The consequent influx of Ca2+ activates nitric oxide synthase (NOS) to produce nitric oxide (NO) (Ding et al., 1994; Amir et al., 1995). After liberation of NO, the light signaling pathways diverge (Gillette, 2000). In early night, light/GLU-induced state change, which delays the clock’s rhythm, requires activation of neuronal ryanodine receptors (RYRs) to release intracellular stores of Ca2+ (Ding et al., 1998). In late night, the light/GLU signal activates an RYR-independent, cGMP/protein kinase G (PKG)-dependent signal transduction cascade to initiate a phase advance (Weber et al., 1995; Mathur et al., 1996; Ding et al., 1998). Finally, light/GLU signaling in both early and late night induces phosphorylation of Ca2+/cAMP response element binding protein (CREB) and CRE-mediated transcriptional activation (Ginty et al., 1993; Ding et al., 1997; Obrietan et al., 1998, 1999).

Although activation of PKA can be downstream from NMDA receptor-triggered Ca2+ transients in other brain regions (Green-gard et al., 1991; Bito et al., 1997), a role for cAMP in nocturnal light/GLU signaling SCN state changes has yet to be determined. Recently, pituitary adenyl cyclase-activating polypeptide (PACAP) was demonstrated to modulate circadian state changes stimulated by light/GLU (Chen et al., 1999). These data suggest that activation of cAMP/PKA could contribute to light/GLU signal transduction. We hypothesized that a GLU-primed cAMP/PKA response system modulates light/GLU-induced state changes. We examined the effects of cAMP/PKA modulation of early and late night glutamatergic input to the SCN in terms of rodent behavioral and SCN electrical activity rhythms and on mPer1 mRNA.

MATERIALS AND METHODS

Animals and circadian time. Long-Evans rats (6–12 weeks old) were used for all in vitro experiments. This line has been imbred for >35 generations, surpassing the requirements for genetic homogeneity, which leads to low variation in physiological experiments. Rats were provided food and water ad libitum and entrained to a daily cycle of 12 hr light and 12 hr dark. Over the 2–3 d of experimentation in vitro, the SCN generates stable, near 24 hr oscillations in neuronal activity that do not deviate from the entrained 24 hr cycle (Prosser and Gillette, 1989). Therefore, in vitro clock time was determined from the lighting cycle in the donor colony. The time of lights-on was designated as circadian time (CT) 0; subjective day was CT
0–12. Subjective night (CT 12–24) corresponded to the dark portion of the donor’s cycle.

Preparation and treatment of brain slices. Brain slices were prepared according to the protocol of the day, obtaining a tissue slice of the desired thickness. The slice was then transferred to the brain slice chamber. Perifusion was stopped during treatment. GLU (10 mM, 10 min) was applied by microdrop (10 μl) to the slice at the onset of the dark phase of the light/dark cycle. A block of hypothermic tissue was cut with a mechanical chopper into 500 μm thick slices containing the SCN. Slices were studied for up to 3 in vitro with continuous perfusion of Earle’s Essential Balanced Salt Solution (EBSS, Sigma, St. Louis, MO), supplemented with 24.6 mM glucose, 26.2 mM NaHCO₃, and 2.5 mg/ml gentamicin, and saturated with 95% O₂/5% CO₂ at 37°C, pH 7.4. Neuronal activity, measured by single-unit recording, was low at night and peaks around midday (CT 7) (Gillette and Prosser, 1988). The time-of-peak, measurement of time-of-peak provides an accurate assessment of circadian phase (Gillette et al., 1995).

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Single-unit recordings of SCN neuronal activity. The technique used to record single units extracellularly from the ensemble of SCN neurons has been described in detail previously and validated thoroughly (Weber et al., 1988). Briefly, under visual guidance, a glass microelectrode filled with 5 M NaCl was lowered into the SCN using a hydraulic microdrive until the signal from a single cell was encountered. Electrical signals from single units were amplified, the background level was isolated, observed for stability, and counted for 4 min using LabView software. The electrode was advanced until a different cell was encountered and its activity was counted. After a complete pass through the brain slice, the electrode was repositioned within the SCN to sample throughout the entire nucleus over 15 min lags. The time-of-peak for each experiment was determined by counting. After a complete pass through the brain slice, the electrode was repositioned within the SCN to sample throughout the entire nucleus over 15 min lags. The time-of-peak for each experiment was determined by counting.

Glucose sensitivity and phase resetting. SCN slices exhibit a persistent, near 24 hr oscillation with a mean period of 24.6 hr before the onset of the dark phase of the light/dark cycle. A block of hypothermic tissue was cut with a mechanical chopper into 500 μm thick slices containing the SCN. Slices were studied for up to 3 in vitro with continuous perfusion of Earle’s Essential Balanced Salt Solution (EBSS, Sigma, St. Louis, MO), supplemented with 24.6 mM glucose, 26.2 mM NaHCO₃, and 2.5 mg/ml gentamicin, and saturated with 95% O₂/5% CO₂ at 37°C, pH 7.4. Neuronal activity, measured by single-unit recording, was low at night and peaks around midday (CT 7) (Gillette and Prosser, 1988). The time-of-peak, measurement of time-of-peak provides an accurate assessment of circadian phase (Gillette et al., 1995).

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Significance of phase resetting was measured as the distance between regression lines drawn through data from at least 5 d immediately before treatment and 5 d after reestablishment of a stable circadian rhythm after treatment (Ding et al., 1998).
PKA inhibition blocks GLU-induced phase delays but potentiates GLU-induced phase advances in vitro

A ubiquitous consequence of cAMP elevation is activation of PKA. To determine whether PKA mediates the effects of GLU that generate phase-shifting state changes in the SCN throughout subjective night, we used KT5720, an isoquinoline inhibitor of PKA. Application of KT5720 alone at either CT 14 (time-of-peak, CT 6.75 ± 0.18, n = 4) or CT 20 (time-of-peak 6.66 ± 0.25, n = 4) had no effect on the time-of-peak electrical activity from the ensemble of SCN neurons (Figs. 3, C, H and F, H, respectively). Coapplication of KT5720 completely blocked the GLU-induced phase delay of the SCN electrical activity rhythm at CT 14 (time-of-peak 6.98 ± 0.30, n = 3) (Fig. 3D,H). Conversely, at CT 20, KT5720 potentiated the phase-advancing effects of GLU. KT5720 + GLU caused a 6.25 ± 0.42 hr phase advance, which is significantly larger (p < 0.01) than the phase advance induced by GLU application alone at this time. Thus, although GLU stimulation increases cAMP levels throughout the night, the resultant PKA activation produces opposite consequences on phase resetting in response to glutamatergic stimuli in early versus late night.

Mitogen-activated protein kinase inhibition partially blocks both GLU-induced phase delays and advances

GLU-induced signal transduction can also activate the mitogen-activated protein kinase (MAPK) cascade to initiate neuronal state changes (Bading and Greenberg, 1991; Rosen et al., 1994). CAM-dependent activation of the MAPK pathway has been implicated in the phase-resetting effects of light/GLU in the SCN (Ginty et al., 1993; Obrietan et al., 1998). To determine whether the cAMP-dependent effects that we observed were caused by activation of MAPK, we inhibited the MAPK signal transduction cascade at two different sites in conjunction with GLU treatment at CT 14 and CT 20. Olomoucine (Abraham et al., 1995) and apigenin (Kuo and Yang, 1995) inhibit p44 MAPK. PD98059 inhibits MEK (MAP kinase kinase) (Kultz et al., 1998). Each inhibitor partially blocked the GLU-induced phase delay at CT 14 (Fig. 4) (n = 3 for each inhibitor). Likewise, but in contrast to the effects of KT5720 in tandem with GLU at CT 20, each MAPK inhibitor partially blocked the GLU-induced phase advance. Thus, our data support a role for MAPK as one of several elements essential to signal transduction events stimulated by GLU that initiate phase resetting at night. However, permissive effects of PKA on the MAPK pathway do not account for the effects we observe with KT5720. Importantly, inhibition of MAPK alone cannot explain the potentiation of the GLU-induced phase advance observed in the presence of KT5720.

PKA inhibition potentiates light-induced phase shifts in vivo

The importance of activation of cAMP/PKA signal transduction in response to light/GLU in the SCN was further evaluated in vivo. A 20 lux light pulse at the time of maximal sensitivity to phase resetting by light caused a 1.24 ± 0.37 hr (n = 8) phase advance in the onset of the wheel-running rhythm in the hamster (Fig. 5). Injection of KT5720 at 30 min before a light pulse significantly potentiated light-induced phase advances (1.92 ± 0.25 hr, n = 10; p < 0.05) (Fig. 5B,D). Injection of KT5720 alone had no effect on the phase of wheel-running activity (Fig. 5C) (n = 8).

Application of 8-Br-cAMP at CT 14 (time-of-peak, CT 6.75 ± 0.25, n = 3) or CT 20 (time-of-peak, CT 6.62 ± 0.15, n = 4) had no effect on the subsequent phase of the SCN neuronal activity rhythm (Fig. 2, B and E, respectively), confirming earlier observations (Prosser and Gillette, 1989). GLU application at CT 14 stimulated a characteristic (Ding et al., 1994) phase delay (time-of-peak, CT 9.9 ± 0.35, n = 5) of ~3 hr (Fig. 2C,H). Interestingly, coapplication of 8-Br-cAMP potentiated the phase delay stimulated by GLU. The 4.29 ± 0.15 hr phase delay that ensued after treatment with 8-Br-cAMP + GLU at CT 14 (time-of-peak, CT 11.09 ± 0.13; n = 4) (Fig. 2D,H) was significantly greater (p < 0.05) than the phase delay produced by GLU alone.

At CT 20, GLU induced a phase advance of ~3.5 hr (Fig. 2F,H) (time-of-peak CT 3.29 ± 0.32, n = 4), corroborating previous studies (Ding et al., 1994). When 8-Br-cAMP was applied in combination with GLU at CT 20 (Fig. 2G,H), the time-of-peak neuronal activity occurred, as in controls, at CT 6.55 ± 0.17 (n = 4). Thus, 8-Br-cAMP blocked the GLU-induced phase advance at CT 20.

Figure 2. Exogenous cAMP modulates GLU-induced phase resetting of the SCN electrical activity rhythm in vitro. A, A control recording demonstrated that the electrical activity rhythm persists for 2 d in vitro, with a peak near CT 7 on both days. B, The membrane-permeable cAMP analog 8-Br-cAMP (200 μM) had no effect on the time-of-peak electrical activity at CT 14. C, At CT 14, GLU (10 mM) induced a 3 hr phase delay in the SCN electrical activity rhythm. D, 8-Br-cAMP significantly increased (p < 0.05, Student’s t test) the magnitude of the GLU-induced phase delay from ~3 to ~4.5 hr. E, 8-Br-cAMP (200 μM) had no effect on the electrical activity rhythm when applied alone at CT 20. F, At CT 20, GLU (10 mM) advanced the electrical activity rhythm by ~3 hr. G, 8-Br-cAMP (200 μM) blocked the GLU-stimulated phase advance. H, Summary of the phase shifting effects of GLU ± 8-Br-cAMP at CT 14 and CT 20. Bars indicate the mean ± SEM of three to six experiments. a, b, and c indicate values significantly different from each other and from controls (p < 0.05; Student’s t test).
PKA inhibition differentially alters GLU-induced mPer1 mRNA accumulation in a state-dependent manner

To determine whether cAMP/PKA mediates the rapid, transient elevation of mPer1 mRNA required for both phase delays (Akiyama et al., 1999) and phase advances (S. A. Tischkau and M. U. Gillette, unpublished observations) in response to nocturnal light/GLU, in situ hybridizations were performed after treatment of SCN slices with GLU ± KT5720. Endogenous mPer1 mRNA was low at CT 14 and CT 20 (Fig. 6A, D). Consistent with the effects of light in vivo (Albrecht et al., 1997; Shearman et al., 1997; Shigeyoshi et al., 1997; Zylka et al., 1998), mPer1 mRNA was significantly elevated 60 min after GLU treatment at either CT 14 or CT 20 (Fig. 6B, E) \((n = 6)\). There was no significant difference in total content of GLU-stimulated mPer1 at either time (Student’s \(t\) test), but at CT 14, GLU-stimulated mPer1 was observed throughout the SCN, whereas GLU-stimulated mPer1 was restricted to the retinorecipient SCN at CT 20. KT5720 blocked the GLU-stimulated rise in mPer1 at CT 14 (Fig. 6C) \((n = 6)\) but did not diminish the accumulation of mPer1 after GLU treatment at CT 20. In contrast to samples treated with GLU alone at CT 20, mPer1 was observed throughout the SCN in these samples (Fig. 6F) \((n = 6)\). KT5720 had no effect on mPer1 levels when applied alone at either time (data not shown).

**DISCUSSION**

Overt SCN clock sensitivity to reagents that activate cAMP pathways (the neuromodulatory ligands PACAP and serotonin), stimulate cAMP accumulation (forskolin, cAMP phosphodiesterase inhibitors), or activate PKA (cAMP analogs) is restricted to subjective day; cAMP/PKA at night has no effect on clock phase (Prosser and Gillette, 1989; Medanic and Gillette, 1992; Shibata et al., 1992; Hannibal et al., 1997). Thus, daytime activation of cAMP signal transduction is typical of a primary signaling pathway. Direct activation of a downstream component, cAMP, evokes the same response as an extracellular signal, PACAP or serotonin, whereas inhibition of a downstream component, PKA, blocks the response to the extracellular signal.

This study reveals a different role for cAMP/PKA-dependent processes in modulation of nocturnal glutamatergic input to the SCN. PKA acts to gate long-term state changes invoked by GLU, because inhibition of cAMP/PKA alters the GLU effects, but cAMP/PKA has no effect independent of GLU. cAMP/PKA gating provides regulation by either blocking or enhancing signal flow through a primary pathway (Iyengar, 1996), as observed in other systems. Developmentally, PKA elevation blocks differentiation by the morphogenetic Hedgehog signal in Drosophila (Blair, 1995; Jiang and Struhl, 1995, Li et al., 1995; Perrimon, 1995) and mice (Fan et al., 1995). cAMP blocks H-Ras-induced transformation of NIH-3T3 cells (Chen and Iyengar, 1994) and EGF-induced Raf-1 phosphorylation in rat1 fibroblasts (Wu et al., 1993), whereas cAMP alone is ineffective. In contrast, cAMP/PKA activation promotes synaptic potentiation by BDNF in Xenopus neuromuscular synapses (Boulanger and Poo, 1999) and the early phase of postsynaptic long-term potentiation (LTP) (Blitzer et al., 1995, 1998), whereas exogenous cAMP has no effect.

Interestingly, both the positive and negative functions of the
cAMP/PKA gate occur in the SCN (Fig. 7A,B). Light/GLU activation of the cAMP/PKA gate in early night permits signal transduction through the primary pathway but blocks signal transmission through the same primary signaling pathway in late night. Thus, the clock itself determines the effects of cAMP/PKA activation, and as such, cAMP/PKA contributes contextual information regarding clock state at the time of light exposure.

State changes initiated by GLU in both early and late night are mediated by NMDA receptor-mediated Ca$^{2+}$ influx that activates NOS (Ding et al., 1994). Subsequent signaling activates multiple pathways specific to clock state but encompasses parallel, divergent, and convergent elements that induce phase-delays and state change in early night, or phase-advancing state change in late night. Convergent elements may include the MEK/MAPK pathway and CREB. MAPK (Obrietan et al., 1998), CREB phosphorylation (Ginty et al., 1993; Ding et al., 1997; Obrietan et al., 1998), and CRE-mediated gene transcription (Obrietan et al., 1999) have been implicated in light/GLU-induced state changes in the SCN. Activation of MAPK, but not PKA, can substitute for light/GLU to induce CRE-mediated gene transcription in the SCN (Obrietan et al., 1999).

This study provides functional evidence that MAPK signaling contributes to the effects of light/GLU on clock phase in both early and late night. Inhibition of MAPK attenuates both GLU-induced phase shifts by ~50% (Fig. 4). Although higher concentrations of the MAPK inhibitors were not tested, we predict that 50% is the maximal response. Like KT5720, each of the MAPK inhibitors was used at a concentration two times the IC$_{50}$. More importantly, these same concentrations of olomoucine and PD98059 block depolarization-induced CREB and Erk phosphorylation in hippocampal slices (Impey et al., 1998). Moreover, MAPK inhibition reduces light-induced phosphorylation of CREB on serine-133 in the SCN by 50% (Obrietan et al., 1998). PKA-dependent nuclear translocation of Erk/MAPK can lead to CREB phosphorylation (Impey et al., 1998).

These data predict that CREB functions as a convergence point, or coincidence detector, within the primary light/GLU-signaling pathway. An unidentified pathway(s) must contribute the other 50% of the light/GLU effect. Although PKA-dependent activation of MAPK can partially account for the signals that cause phase delay, the fact that PKA inhibition increases the magnitude of the phase advance suggests activation of an additional, alternative pathway. Although further studies are necessary to determine whether direct activation of MAPK can substitute for GLU to induce phase resetting at night, the aggregate data place MAPK on the primary pathway leading to long-term state changes in response to light/GLU.

Recent reports support a link between light signaling through GLU, NO, MAPK, and CREB phosphorylation to CRE elements in the mPer1 promoter (Obrietan, 2000; Tei et al., 2000). CREB is
Figure 7. A schematic summary of the effects of the cAMP/PKA gate on long-term changes in the SCN neuronal activity rhythm induced by light/GLU. 8-Br-cAMP is ineffective in altering circadian state when applied alone at night. However, raising cAMP enhances GLU-induced phase delays in early night but blocks GLU-induced phase advances in late night. In contrast, blocking PKA in the presence of GLU abolishes GLU-induced phase delays in early night but potentiates GLU-induced phase advances in late night. In contrast, inhibition of the MAPK kinase cascade attenuates both GLU-induced phase delays and phase advances by 50%. B, A model depicts interaction of the cAMP/PKA gating pathway with the primary GLU signaling pathway that confers state change in the SCN when activated at night. Nocturnal light activates an NMDA receptor-mediated pathway that leads to liberation of NO in both early and late night. After NO, the pathway leading to circadian phase delay in early night diverges from the pathway that evokes phase advance in late night. However, each pathway eventually leads to activation of mPer1 mRNA and ultimately alters circadian time. The cAMP/PKA gating pathway modulates activity of the main pathway in both early and late night. Activation of cAMP/PKA in the early night opens the gate, thereby permitting signal flow through the main pathway. In contrast, activation of cAMP/PKA in the late night hinders signal transfer through the primary pathway; the cAMP/PKA gate is closed. Thus, the phase of the circadian clock itself is the gatekeeper that determines the downstream consequences of cAMP/PKA activation. The mechanism for activation of cAMP/PKA remains speculative at this time as indicated by the question marks. NMDA receptor-mediated Ca^{2+} influx may both activate the primary pathway and the cAMP/PKA gate, as occurs during hippocampal LTP (Blitzer et al., 1995). Alternatively, NMDA receptor-mediated Ca^{2+} influx may prime the system for other extracellular signals, such as PACAP (Chen et al., 1999) or serotonin (Moriya et al., 1998), to activate adenylyl cyclase.

not likely a direct substrate for cAMP/PKA because nocturnal activation of cAMP/PKA alone in the SCN does not activate CRE-mediated gene transcription (Obrietan et al., 1999). This argues for placing CREB on the primary pathway, independent from the cAMP/PKA gating pathway. CRE elements in the promoter allow tentative placement of mPer1 on the primary pathway because the light/GLU stimulus that induces mPer1 (Albrecht et al., 1997; Shearman et al., 1997; Shigeyoshi et al., 1997; Zylka et al., 1998; present study) also generates phosphorylated CREB (Ginty et al., 1993; Ding et al., 1997; Obrietan et al., 1998) and stimulates CRE-mediated transcription. Furthermore, antisense oligonucleotides against mPer1 completely block both GLU-induced phase delays (Akiyama et al., 1999) and phase advances (Tischkau and Gillette, unpublished observations). Although we cannot exclude placement of mPer1 on the gating pathway in early night, the fact that inhibition of PKA does not inhibit mPer1 in late night eliminates it from a position on the cAMP/PKA gating pathway at that time. Thus, we favor placement of mPer1 on the primary pathway, downstream of CREB, with intersection of the cAMP/PKA gate upstream of these convergent elements (Fig. 7).

Our results demonstrate that activation of the cAMP/PKA gate, a signaling consequence of adding GLU to the slice, attenuates activity of the primary GLU pathway in the late night. PKA inhibition releases this brake, potentiating GLU-induced phase resetting. However, PKA inhibition did not potentiate the induction of mPer1 in the presence of GLU. These differences may reflect interpretative limitations imposed by experimental design. mPer1 induction was evaluated 60 min after GLU-stimulation of rat SCN slices. In the mouse, light-stimulated mPer1 in vivo is a rapid, transient event that is initiated by 15–30 min in the retinorecipient SCN, peaks at 60 min, and spreads throughout the nucleus by 120 min (Albrecht et al., 1997; Shearman et al., 1997; Shigeyoshi et al., 1997; Takumi et al., 1998; Zylka et al., 1998). Our data suggest that the kinetics of mPer1 induction may differ between species and/or may depend on the experimental paradigm. Sixty minutes after light in early night, expression of mPer1 in the rat is not limited to the retinorecipient SCN but has spread throughout the nucleus (Fig. 6B). In contrast, 60 min after light in late night, mPer1 is found only in the retinorecipient SCN (Fig. 6, compare B and E). However, consistent with the concept that PKA inhibition removes a brake on the GLU-signaling pathway, mPer1 was expressed throughout the entire SCN (Fig. 6, compare F and E) when slices were co-treated with a PKA inhibitor and GLU in late night. PKA inhibition may enhance flow through the signaling pathway, altering the kinetics of mPer1 induction in response to GLU. A thorough time course may reveal differences in total mPer1 that ultimately reflects the GLU-induced response.

Mechanisms for intersection of cAMP/PKA gating pathway with the primary light/GLU pathway are unknown. Because cAMP has opposite effects on the light/GLU-induced signal transduction in early versus late night, the intersection between the cAMP/PKA gating pathway and the primary pathway likely occurs at elements unique to each pathway. For example, it seems unlikely that cAMP/PKA would activate the NMDA receptor in early night and then inhibit this same molecule in late night. Therefore, it is not unreasonable to speculate that the intersection points between the cAMP/PKA gating pathway and the primary signaling pathways lie somewhere downstream of NO, the divergence point (Fig. 7B). In the early night, cAMP/PKA must regulate the primary pathway upstream of mPer1, because inhibition of PKA blocks mPer1 induction, i.e., the signal is blocked before it reaches mPer1.

The mechanism for raising cAMP in response to GLU remains unresolved. Gating pathways (Kobayashi and Iyengar, 1998) can be constitutively active, as in cAMP gating of the Hedgehog signal during development (Blair, 1995; Fan et al., 1995; Jiang and Struhl, 1995, Li et al., 1995; Perrimon, 1995), activated by intracellular signals, as in cAMP gating of early hippocampal LTP (Blitzer et al., 1995), or activated by a second extracellular signal, as in cAMP gating of neuropeptide-induced survival of retinal ganglion cells (Meyer-Franke et al., 1995). In the SCN, cAMP is not constitutively elevated. Peak levels occur near the end of subjective day and near the end of subjective night; low levels of cAMP are observed throughout the period of sensitivity to light/GLU (Prosser and Gillette, 1991). GLU could activate both the primary pathway and the cAMP/PKA gate.

More likely, GLU activates the primary pathway and then permits activation of the cAMP pathway via a second extracellular signal. Both PACAP (Chen et al., 1999), an extracellular messenger that colocalizes with GLU in the RHT (Hannibal et al., 2000), and the 5-HT1A-specific agonist MKC-242 (Moriya et al., 1998) have the same modulatory effects as cAMP/PKA on light/GLU-induced state changes. Thus, any afferent signal capable of accessing the
cAMP/PKA gate in the presence of light/GLU may play a modulatory role. The cAMP/PKA gating pathway may provide contextual information to allow integration of multiple signals that occur concurrently with light/GLU. Moreover, cAMP/PKA gating may explain discrepancies between the magnitudes of light-induced phase shifts in vivo (as in Fig. 5), where the response reflects integration of information from the RHT, as well as other afferents, and GLU-induced phase shifts in vitro (as in Figs. 2–4), where the response is isolated to the SCN. Elucidating the sites of intersection between the cAMP/PKA gating pathway and the primary signaling pathways stimulated by GLU will provide insights into mechanisms for integrating multiple signals to generate adaptive behavioral state changes.

References


