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Adenosine A_1 receptors regulate the response of the hamster circadian clock to light

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Abstract

Circadian rhythms are synchronized to the environmental light–dark cycle by daily, light-induced adjustments in the phase of a biological clock located in the suprachiasmatic nucleus. Ambient light alters the phase of the clock via a direct, glutamatergic projection from retinal ganglion cells. We investigated the hypothesis that adenosine A_1 receptors modulate the phase adjusting effect of light on the circadian clock. Systemic administration of the selective adenosine A_1 receptor agonist, N^6-cyclohexyladenosine (CHA), significantly ($p < 0.05$) attenuated light-induced phase delays and advances of the circadian activity rhythm. Selective agonists for the adenosine A_{2A} and adenosine A_3 receptors were without effect. The inhibitory effect of CHA on light-induced phase advances was dose-dependent (0.025–1.0 mg/kg, ED_{50} = 0.3 mg/kg), and this effect was blocked in a dose-dependent (0.005–1.0 mg/kg) manner by the adenosine A_1 receptor antagonist, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX). Injection of CHA (10 μM) into the region of the suprachiasmatic nucleus significantly attenuated light-induced phase advances, and this effect was also blocked by DPCPX (100 μM). The results suggest that adenosine A_1 receptors located in the region of the suprachiasmatic nucleus regulate the response of the circadian clock to the phase-adjusting effects of light. © 2001 Published by Elsevier Science B.V.

Keywords: Suprachiasmatic nucleus; Circadian rhythm; Adenosine; Circadian clock; Light

1. Introduction

Circadian rhythms in physiology and behavior, including rhythms in sleep and wakefulness, are driven by an endogenous biological clock located in the suprachiasmatic nucleus in the ventral hypothalamus (for review, see Klein et al., 1991). Abundant evidence has shown that circadian rhythmicity is an intrinsic property of the suprachiasmatic nucleus (Green and Gillette, 1982; Welsh et al., 1995), and that the circadian signal is transmitted to the rest of the brain through both synaptic and humoral processes (Stephan and Nunez, 1977; Silver et al., 1996). In addition, the phase of the circadian clock is adjusted on a daily basis by ambient light to maintain synchrony with relevant environmental rhythms (DeCoursey, 1964; Daan and Pittendrigh, 1976). In general, light exposure during the period of dusk twilight delays the phase of the clock, while light exposure around the time of dawn twilight causes phase advances of circadian rhythms (DeCoursey, 1964; Pittendrigh and Daan, 1976). Light exposure during the subjective daytime does not alter circadian phase. The process by which daily exposure to light synchronizes circadian rhythms with the cycle of light and darkness is termed “photic entrainment”.

Photic information is conveyed to the suprachiasmatic nucleus by at least three distinct pathways (Moore and Lenn, 1972; Card and Moore, 1982; Mikkelsen and Vrang, 1994). However, the retinohypothalamic tract, a monosynaptic projection from retinal ganglion cells to the suprachiasmatic nucleus, appears to be uniquely responsible for photic entrainment of the circadian clock (Johnson et al., 1988). Evidence from electrophysiological and pharmacological analyses of retinohypothalamic tract neuro-

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transmission indicates that glutamate serves as the principal excitatory neurotransmitter at the retinohypothalamic tract synapse, and is responsible for light-induced alterations in circadian phase (reviewed in Rea, 1998).

The nucleoside messenger, adenosine, is a potent modulator of excitatory neurotransmission in the central nervous system (Dunwiddie, 1985). Adenosine is released from both neurons and glia (Dunwiddie, 1985; White and Hoehn, 1991), and accumulates in the extracellular space during periods of high metabolic demand, including seizure activity (Winn et al., 1980; Abdul-Ghani et al., 1997) and ischemia (Kleihues et al., 1974; Winn et al., 1979). Under these conditions, the nucleoside is thought to serve a neuroprotective role, primarily by inhibiting the release of glutamate (Dunwiddie, 1985). This effect of adenosine is mediated by presynaptic adenosine A_1 receptors located on or near glutamatergic nerve terminals (Dunwiddie and Haas, 1985; Flagmeyer et al., 1997).

Although modulatory effects of adenosine have been observed in many brain regions (Dunwiddie, 1985; Greene and Haas, 1991), the mechanism by which adenosine regulates neuronal excitability has been best characterized in the hippocampus (Proctor and Dunwiddie, 1987; Lupica et al., 1992; Thompson et al., 1992; Mitchell et al., 1993; Manzoni et al., 1994; Dunwiddie and Diao, 1994; Brundege and Dunwiddie, 1996). Transsynaptic activation of CA1 pyramidal neurons by glutamate results in the release of physiologically significant quantities of adenosine, leading to the subsequent inhibition of glutamate release through the activation of presynaptic adenosine A_1 receptors (Lupica et al., 1992; Mitchell et al., 1993; Brundege and Dunwiddie, 1996). Furthermore, adenosine released from a single pyramidal cell is sufficient to significantly inhibit the release of glutamate from excitatory synapses afferent to the same cell, suggesting that adenosine may function as a retrograde messenger to dynamically regulate excitatory input to the same cell from which it was released (Brundege and Dunwiddie, 1996). In addition, NMDA-receptor mediated adenosine production by hippocampal interneurons is sufficient to modulate glutamate release at excitatory synapses on pyramidal cells (Manzoni et al., 1994), indicating that adenosine can also influence the excitability of neurons located at some distance from the site of production.

The current study investigated the hypothesis that adenosine regulates the response of the circadian clock to the phase adjusting effects of light. Preliminary reports (Watanabe et al., 1996; Elliott et al., 1997) have indicated that systemic administration of the adenosine A_1 receptor agonist, N^6-cyclohexyladenosine (CHA), inhibits light-induced phase delays in hamsters, as well as light-induced *c-fos* expression in the rat suprachiasmatic nucleus. Herein, we report a more detailed characterization of the effects of selective adenosine receptor agonists on light-induced phase shifts of the circadian rhythm in wheel running behavior in the Syrian hamster.

2. Materials and methods

2.1. Animals

Male, Syrian hamsters (*Mesocricetus auratus*; Charles River Labs, Wilmington, MA) were housed in groups of 6 under a cycle of 14 h of light (beginning at 1200 h) and 10 h of total darkness. After at least 14 days in the 14:10, light:dark cycle, hamsters (approximately 16 to 22 weeks old) were transferred to cages equipped with 17-cm-diameter running wheels and maintained under constant total darkness. For the remainder of the experiment, wheel-running activity was monitored continuously using an Intel 486-based computer running Dataquest III data acquisition software (Minimitter, Sunriver, OR). Animal care and experimental procedures were accomplished with the aid of night vision goggles under infrared illumination ($>$ 850 nm). Food and water were available ad libitum.

2.2. Circadian activity rhythms

After 7–10 days in constant darkness, hamsters displayed robust circadian rhythms in wheel running activity (Fig. 1). Animals were not manipulated until the period of this "free-running" rhythm remained stable for at least seven consecutive days. Circadian period was determined as the average interval between daily activity onsets (see Rea et al., 1993). The onset of wheel running activity served as an indicator of circadian phase, which was expressed in "circadian time" (i.e., one circadian hour is defined as 1/24th of the free-running period). In this way, specific phase points relative to activity onset (denoted as circadian time 12) were normalized for individual variability in free-running period. At either two circadian hours after activity onset (circadian time 14), or seven circadian hours after activity onset (circadian time 19), hamsters were removed from their cages, transferred to a light exposure apparatus (Rea, 1998), and exposed to 20 lx of white light for 10 min. After light exposure, hamsters were returned to their wheel cages under constant darkness and the circadian rhythm in wheel running activity was monitored for an additional 10–14 days.

2.3. Light-induced phase shifts

Light exposure-induced alterations in the phase of the circadian activity rhythm were determined as previously described (Rea et al., 1993). Phase shifts were defined as the difference between the anticipated and the observed times of activity onset on the day after light exposure. These values were obtained by fitting regression lines through activity onsets on the 5 days prior to exposure, and days 4 through 9 after exposure, and extrapolating these lines to the day after light exposure (Rea et al., 1993).

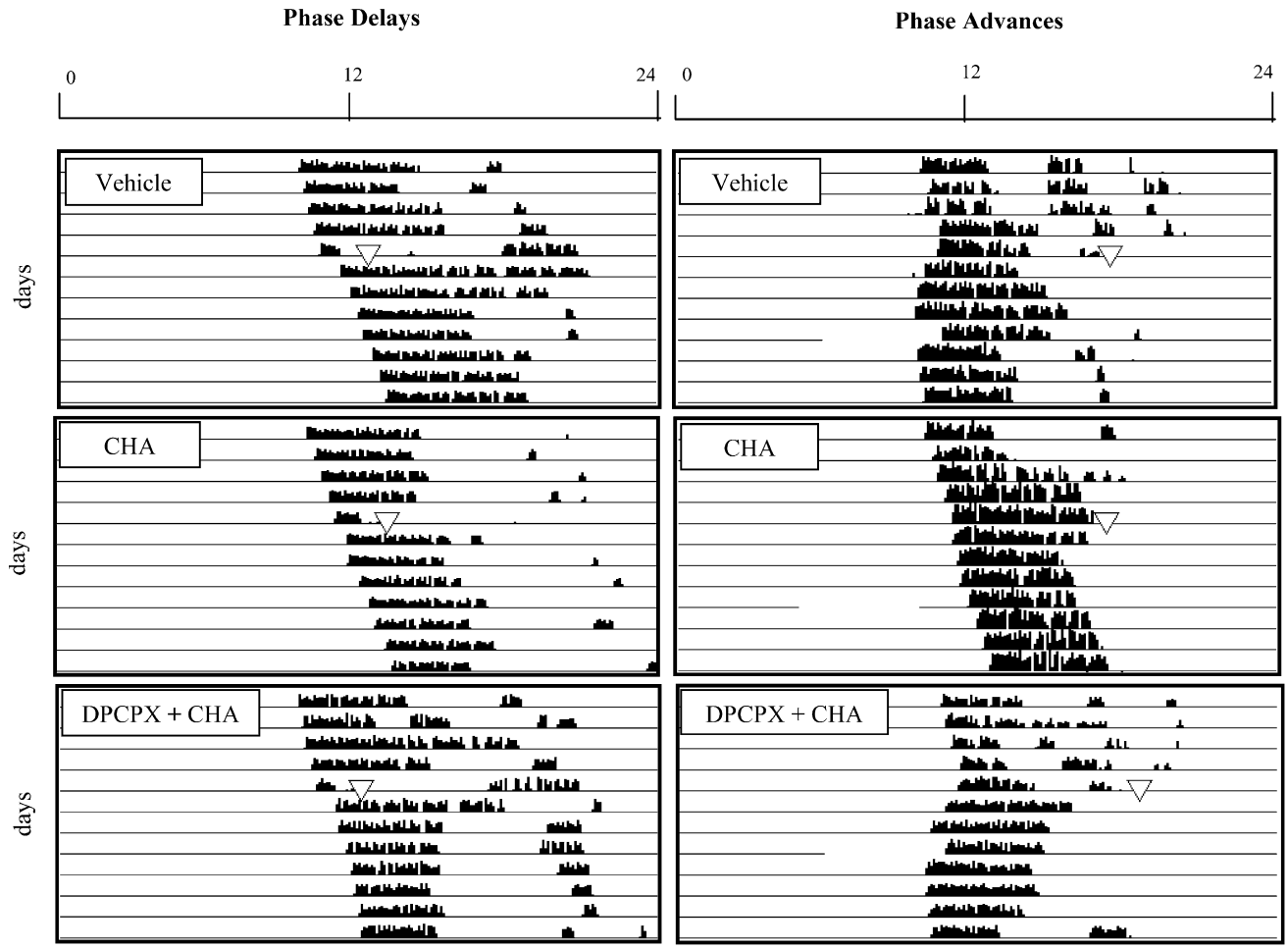

Fig. 1. The adenosine A_1 receptor agonist, CHA, reversibly attenuates light-induced phase shifts. Shown are representative actograms illustrating the effects of vehicle (DMSO i.p.; top panels), CHA (0.5 mg/kg i.p.; middle panels), and DPCPX (1.0 mg/kg i.p) followed by CHA (bottom panels) on light-induced phase delays (left panels) and phase advances (right panels) of the circadian rhythm in wheel running activity of hamsters maintained under constant darkness. In each actogram, the horizontal lines represent successive 24-h periods of a continuous activity record, and the height of the vertical bars is proportional to the number of wheel revolutions that occurred during each 6-min bin. Phase delays or advances were induced by a 10-min exposure to 20 lx of white light (inverted triangles) at circadian time 14 (panel A) or circadian time 19 (panel B), respectively. The phase shifts are apparent as abrupt changes in the timing of activity onset. Phase delays (left panels) of wheel running behavior are usually complete within 1–2 days, while phase advances (right panels) often require 3–4 days to achieve full expression. Administration of CHA 30 min prior to the beginning of the light pulse (middle panels) attenuates both light-induced phase delays and advances (middle panels). The inhibitory effect of CHA is completely reversed by prior (40 min) administration of the adenosine A_1 receptor antagonist, DPCPX (bottom panels). The gaps in the phase advance actograms on day 9 are the result of a computer malfunction.

2.4. Effects of systemic injections of adenosine receptor agonists on photic phase shifts

Hamsters were housed in wheel cages under constant darkness as described above. After stable free-running activity rhythms were established, hamsters received intraperitoneal (i.p.) injections of either N^6-cyclohexyladenosine (CHA; 0.5 mg/kg; Research Biochemicals, Natick, MA) or the agonist vehicle (25% dimethyl sulfoxide (DMSO) in saline) 30 min prior to light exposure (20 lx white light for 10 min) at either circadian time 14 or circadian time 19. Some animals received i.p. injections of either 8-cyclopentyl-1,3-dipropylxanthine (DPCPX; 1.0 mg/kg; Research Biochemicals) or vehicle (DMSO at 1 ml/kg) given 40 min prior to injection of either agonist or agonist vehicle. In order to ensure that each animal received an equivalent "dose" of light, all animals were observed during the light exposure session, and were encouraged to remain awake during light treatment by gentle manipulation.

Selective agonists for the adenosine A_{2A} and A_3 receptor subtypes were systemically administered 30 min prior to light exposure (20 lx for 10 min) at circadian time 19 in hamsters maintained under conditions identical to those described above. Animals that received drug injections without light treatment (drug alone) were placed in the light exposure chamber for 10 min beginning at circadian time19, but were not exposed to light.

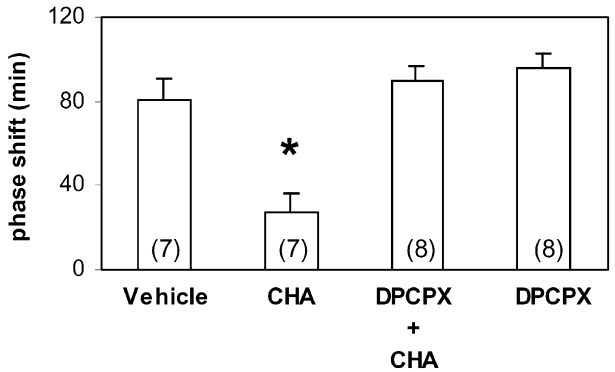

Fig. 2. CHA attenuates light-induced phase advances and this effect is blocked by prior administration of DPCPX. Data represent the mean ± S.E.M. of the number of determinations indicated in parentheses. CHA (0.5 mg/kg) and DPCPX (1.0 mg/kg) were administered systemically as described in Section 2. Asterisks (*) indicate statistically significant differences ($P < 0.05$) relative to all other groups shown as determined by one-way ANOVA followed by the Fisher test.

2.5. Stereotaxic surgery

Male, Syrian hamsters (160–230 g and approximately 26 weeks old at the time of surgery) were anesthetized with ketamine (175 mg/kg), xylazine (17 mg/kg) and acepromazine maleate (2 mg/kg) by i.p. injection. Cannula guides were implanted using stereotaxic technique and fixed to the skull with fine machine screws and cranioplastic cement. Cannula guides were aimed at the suprachiasmatic nucleus at a 10° angle from the sagittal plane (1.0 mm anterior to bregma, 1.6 mm lateral to the midline, 3.1 mm below the dura, upper incisor bar at 0). After recovery from surgery, animals were transferred to individual cages with running wheels, maintained for six to eight days under the 14:10 light:dark cycle, then in constant darkness for the remainder of the experiment.

2.6. Intracranial drug injections

Drugs for intracranial injection were prepared in a solution of 0.1% DMSO in artificial cerebrospinal fluid (122 mM NaCl, 3.8 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, and 1.2 mM $CaCl_2$). After 8–14 days in constant darkness, groups of hamsters received 0.3-μl injections into the suprachiasmatic region of either vehicle, 10 μM CHA, 100 μM DPCPX, or a combination of 10 μM CHA + 100 μM DPCPX. Intracranial drug administration was achieved using a 33-gauge infusion cannula attached to a 1 μl Hamilton syringe, and performed under infrared illumination with the aid of night vision goggles. The infusion cannula extended 4.3 mm beyond the tip of the cannula guide to a position near the dorsolateral border of the right suprachiasmatic nucleus. Animals were gently restrained for approximately 10 to 15 s during the injection, and the infusion cannula remained in place for approximately 30 s after the injection. Drugs were administered 10 min prior to circadian time 19. Following injection, some animals were exposed to white light (20 lx) for 10 min beginning at circadian time 19. After treatment, the hamsters were returned to their wheel cages and maintained under constant darkness for an additional 10–14 days. Phase shifts were calculated as described above. After data collection, the location of each injection site was verified histologically by examination of 100-μm-thick vibratome sections cut through the injection site.

2.7. Drugs

N^6-cyclohexyladenosine (CHA), 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), 2-[[*p*-(2-carboxyethyl)-phenethyl]amino]-5′-*N*-ethylcarboxyamidoadenosine (CGS-21680), and N^6-(benzyl)-5′-*N*-ethylcarboxyamidoadenosine (N^6-benzyl-NECA), and 2-chloro-N^6-cyclopentyladenosine (CCPA) were obtained from Research Biochemical. Ketamine (125 mg/ml) was purchased from Fort Dodge Laboratories (Fort Dodge, IA), xylazine (20 and 100 mg/ml) was obtained from Miles Laboratories (Shawnee Mission, KS) and acepromazine maleate (10 mg/kg) was purchased from Fermenta Animal Health (Kansas City, KS).

3. Results

3.1. Effects of CHA and DPCPX on photic phase shifts

Representative activity records from each dual injection series are presented in Fig. 1. In vehicle-injected hamsters, light exposure at circadian time 19 produced stable phase advances (81 ± 10 min, $n = 7$; Fig. 2), while exposure at circadian time 14 resulted in stable phase delays (−41 ± 4 min, $n = 7$; Fig. 3). Systemic injection of CHA (0.5 mg/kg) resulted in a 67% reduction of the mean phase advance (27 ± 9 min, $n = 7$; $P < 0.05$) relative to the

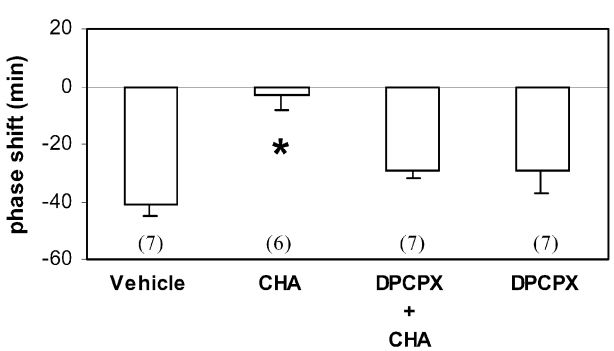

Fig. 3. CHA attenuates light-induced phase delays and this effect is blocked by prior administration of DPCPX. Data represent the mean ± S.E.M. of the number of determinations indicated in parentheses. CHA (0.5 mg/kg) and DPCPX (1.0 mg/kg) were administered systemically as described in Section 2. Asterisks (*) indicate statistically significant differences ($P < 0.05$) relative to all other groups shown as determined by one-way ANOVA followed by the Fisher test.

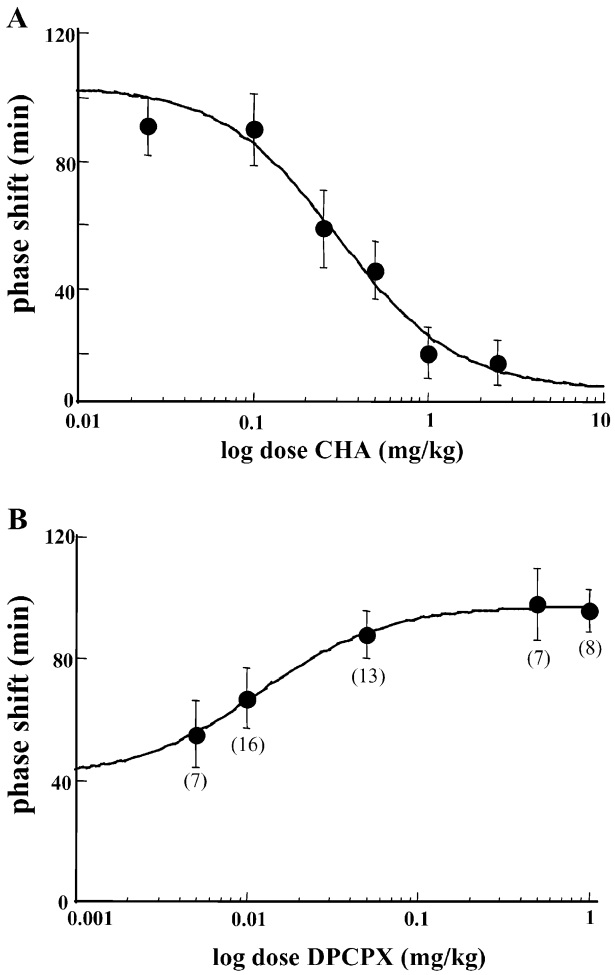

Fig. 4. The effects of both CHA and DPCPX are dose dependent. (A) Dose response curve for CHA. Data represent the mean ± S.E.M. of eight animals per group. The mean phase advance for the vehicle + light group ($n = 9$) was 89 ± 10 min. The dose that resulted in 50% inhibition of light-induced phase advances for CHA was approximately 0.3 mg/kg. (B) Dose response curve for DPCPX. Data represent the mean ± S.E.M. of the number of determinations indicated in parentheses. Increasing doses of DPCPX were administered 40 min prior to 0.3 mg/kg CHA (i.p.). In this case, the mean phase advance for the vehicle + light group ($n = 13$) was 119 ± 8 min. The average phase shift for the CHA + light group ($n = 7$) was 41 ± 14 min. The dose of DPCPX that results in 50% reversal of inhibition of light-induced phase advances by 0.3 mg/kg CHA was approximately 0.012 mg/kg.

vehicle-injected group. Prior administration of DPCPX reversed the inhibition of photic phase advances by CHA (90 ± 7 min, $n = 8$), while DPCPX + vehicle injections did not significantly alter light-induced phase advances (96 ± 7 min, $n = 8$). Similarly, CHA (0.5 mg/kg) completely inhibited light-induced phase delays of the circadian activity rhythm (−3 ± 5 min, $n = 6$; $P < 0.05$) and this inhibition was reversed by prior injection of 1.0 mg/kg DPCPX (−29 ± 3 min, $n = 7$; $P > 0.2$ relative to vehicle + light group). DPCPX did not significantly alter light-induced phase delays (−29 ± 8 min, $n = 7$). Administration of CHA (0.5 mg/kg), DPCPX (1.0 mg/kg), or vehicle, followed by transfer of the animals to the light stimulation chamber for 10 min without light exposure at either circadian time did not significantly alter circadian phase (data not shown).

High doses of CHA (> 2.5 mg/kg) caused noticeable lethargy in most animals. However, even after receiving as much as 5 mg/kg, animals responded to vigilance promoting manipulations, and remained awake with eyes open during the entire light exposure period. Furthermore, wheel running activity during the 2-h period after CHA injection was not significantly different from vehicle-injected animals (vehicle + light = 54 ± 24 turns; CHA + light = 14 ± 10 turns; $n = 6$), although DPCPX treatment significantly enhanced wheel running activity in most animals (380 ± 125 turns; $P < 0.05$ relative to vehicle + light group; $n = 6$).

3.2. Effects of CHA and DPCPX are dose-dependent

CHA inhibited light-induced phase advances in a dose dependent manner over a dose range of 0.025 to 2.5 mg/kg (Fig. 4A). The estimated ED_{50} for CHA was approximately 0.3 mg/kg. Similarly, DPCPX dose-dependently antagonized the inhibitory effect of 0.3 mg/kg CHA (Fig. 4B) with an apparent ED_{50} of 0.012 mg/kg.

3.3. Effects of other adenosine receptor agonists on light-induced phase advances

Injection of the adenosine A_1 receptor agonist CCPA (Fig. 5) also resulted in significant ($P < 0.05$) inhibition of light-induced phase advances compared to vehicle controls (vehicle + light = 97 ± 10 min, $n = 8$; CCPA + light = 45

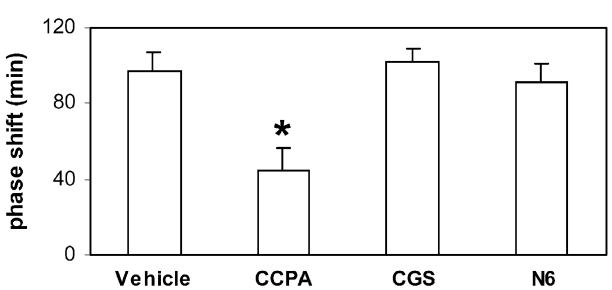

Fig. 5. Adenosine A_1 receptor agonists inhibit light-induced phase advances while agonists for the adenosine A_{2A} and A_3 receptors do not. All agonists were administered systemically 30 min prior to light exposure at circadian time 19 (CCPA = 0.2 mg/kg; CGS-21680 = 3 mg/kg; N^6-benzyl-NECA = 1 mg/kg). Data represent the mean ± S.E.M. of eight animals per group. Asterisks (*) indicate statistically significant differences ($P < 0.05$) relative to all other groups shown as determined by one-way ANOVA followed by the Fisher test. The adenosine A_1 receptor agonist, CCPA, significantly attenuates light-induced phase advances. Phase advances similar to vehicle are observed after administration of the adenosine A_{2A} receptor agonist, CGS-21680 (CGS) and the adenosine A_3 receptor agonist, N^6-benzyl-NECA (N6).

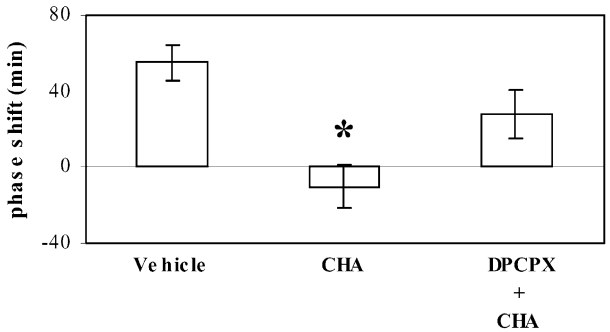

Fig. 6. Local injection of CHA into the suprachiasmatic region reversibly inhibits light-induced phase advances. Data represent the mean ± S.E.M. of six animals per group. CHA (10 μM) and/or DPCPX (100 μM) were administered 10 min prior to light exposure (20 lx for 10 min) at circadian time 19. Asterisks (*) indicate statistically significant differences ($P < 0.05$) relative to all other groups as determined by one-way ANOVA followed by the Fisher test. Co-administration of DPCPX with CHA results in reversal of the inhibition of light-induced phase advances by CHA alone.

± 12 min, $n = 8$). However, neither the adenosine A_{2A} receptor agonist, CGS-21680 (102 ± 7 min, $n = 8$), nor the selective adenosine A_3 receptor agonist, N^6-benzyl-NECA (91 ± 10 min, $n = 8$) significantly attenuated light-induced phase advances.

3.4. Effect of intra-suprachiasmatic nucleus injections of CHA

Local microinjection of 10 μM (3 pmol) CHA into the suprachiasmatic region completely inhibited light-induced phase advances (vehicle + light = 55 ± 9 min, $n = 6$; CHA + light = −10 ± 11 min, $n = 6$; $P < 0.05$; Fig. 6). Co-administration of 100 μM (30 pmol) DPCPX with 10 μM CHA reversed the inhibition (28 ± 13 min, $n = 6$; $P > 0.05$ relative to the vehicle + light group). CHA (10 μM) administered without light exposure did not alter circadian phase (not shown). Phase advances after DPCPX (100 μM) administration and light exposure (40 min and 72 min, $n = 2$) were comparable to those observed after vehicle injection with light exposure.

4. Discussion

The current report presents pharmacological evidence suggesting the involvement of adenosine A_1 receptors in the regulation of the response of the circadian clock to light. The adenosine A_1 receptor agonist, CHA, significantly inhibited both light-induced phase advances and delays of the circadian wheel-running rhythm, and these effects were blocked by prior administration of DPCPX, a highly selective adenosine A_1 receptor antagonist (Lohse et al., 1987). The effects of both CHA and DPCPX were dose-dependent over a dose range that is consistent with the involvement of adenosine A_1 receptors. Furthermore, only selective adenosine A_1 receptor agonists inhibited light-induced phase advances, while systemic injection of selective agonists for the adenosine A_{2A} and A_3 receptors had no effect at the doses employed. Light-induced phase advances were also reversibly inhibited by local administration of CHA indicating that the receptors responsible for this effect are located in the suprachiasmatic region. Together, these findings indicate that the inhibition of light-induced phase shifts by adenosine occurs through the activation of adenosine A_1 receptors located in the region of the suprachiasmatic nucleus, probably in association with proximal elements of the photic entrainment pathway.

The results of the current investigation are in agreement with the work of Watanabe et al. (1996). These investigators reported that a similar dose of CHA (0.5 mg/kg) inhibited light-induced phase delays in hamsters. Additionally, a selective agonist for the adenosine A_2 receptor, N^6-[2-(3,5-dimethoxyphenyl)-2-(2-methyl-phenyl)-ethyl]-adenosine, did not inhibit light-induced phase delays, even at high doses. The results of the current investigation extend these observations by establishing that (1) CHA administration inhibits both light induced phase advances and delays, (2) the effects of CHA on both light-induced phase advances and phase delays are reversible by prior application of a selective adenosine A_1 receptor antagonist, DPCPX, and (3) the actions of CHA and DPCPX on the transmission of light information to the circadian clock are localized to the region of the suprachiasmatic nucleus.

Although CHA and related adenosine A1 receptor agonists are somnogenic at high doses, it is unlikely that the effect of CHA on light-induced phase shifts is due to the somnogenic nature of the drug. All animals were observed during light delivery and encouraged to remain awake with eyes open. Furthermore, CHA significantly attenuated photic phase shifts at doses that were not somnogenic. In addition, local administration of sub-picomole amounts of CHA into the SCN region completely blocked light-induced phase advances. No evidence of drug-induced lethargy was observed in these animals.

Both the production of adenosine (Chagoya de Sanchez et al., 1993) and the density of adenosine receptors in the brain (Virus et al., 1984; Florio et al., 1991) have been reported to show diurnal fluctuations, suggesting that aspects of adenosine function are under circadian control. However, few studies concerning the neurophysiology of adenosine in the suprachiasmatic nucleus, the anatomical site of localization of the mammalian circadian clock, have been reported (Watanabe et al., 1996; Chen and Van den Pol, 1997; Elliott et al., 1997). The lack of information regarding the effects of adenosine in the suprachiasmatic nucleus may be due to the relatively low abundance of adenosine A_1 receptor binding (Fastbom et al., 1987), and adenosine A_1 receptor mRNA (Mahan et al., 1991; Reppert et al., 1991) in the hypothalamus. Nonetheless, Chen and Van den Pol (1997) recently reported that some 70%

of cultured suprachiasmatic nucleus neurons, most of which are gamma-aminobutyric acid (GABA)-ergic (Moore and Speh, 1993), respond to exogenous adenosine. In this preparation, adenosine reversibly inhibited whole cell barium currents under voltage clamp conditions through the activation of both adenosine A_1 and A_2 receptors. Furthermore, adenosine and selective adenosine receptor agonists inhibited GABA release through the activation of adenosine A_1 and A_2 receptors colocalized on presynaptic terminals of autaptic suprachiasmatic nucleus neurons in micro-island culture (Chen and Van den Pol, 1997). Thus, one mechanism by which adenosine A_1 receptor agonists could inhibit light-induced phase shifts and gene expression, as shown in the current study, might be through the inhibition of GABA release in the suprachiasmatic nucleus. Pharmacological manipulations that alter GABA neurotransmission have been shown to modulate the response of the circadian clock to light (Ralph and Menaker, 1985, 1989; Golombek and Ralph, 1994; Gillespie et al., 1996, 1997). However, Gillespie et al. (1996, 1997) reported that microinjection of selective agonists for either the $GABA_A$ or the $GABA_B$ receptor into the suprachiasmatic region inhibited both light-induced phase advances and phase delays, while local administration of selective $GABA_A$ or $GABA_B$ receptor antagonists either augmented light-induced phase shifts, or failed to alter the response to light. Therefore, the inhibitory effect of adenosine A_1 receptor agonists on light-induced phase shifts is probably not due to the inhibition of GABA release in the suprachiasmatic nucleus, since a decrease in GABA release would be expected to have the opposite effect.

A more likely mechanism for the inhibitory effect of adenosine agonists on light-induced phase shifts is the activation of presynaptic adenosine A_1 receptors located on retinohypothalamic tract terminals leading to a reduction in glutamate release in response to retinal illumination. Adenosine A_1 receptor binding in the superior colliculus, which receives direct retinal innervation, disappears 21 days after removal of the contralateral eye, suggesting that adenosine A_1 receptors are associated with the terminal processes of retinal afferents (Goodman et al., 1983). Consistent with this interpretation, Kvanta et al. (1997) have shown that adenosine A_1 receptor mRNA is expressed in a population of retinal ganglion cells. Watanabe et al. (1996) reported that an adenosine A_1 receptor agonist blocked excitatory field potentials in the rat suprachiasmatic nucleus evoked by electrical stimulation of the optic nerve in vitro. However, neither this observation, nor the results of the current study eliminate the possibility of a postsynaptic mechanism of action (Obrietan et al., 1995). A more detailed neurophysiological investigation of the effects of adenosine receptor agonists on retinohypothalamic tract-evoked responses of suprachiasmatic nucleus neurons is necessary to resolve this issue.

Adenosine is one of several neuroactive substances that accumulate in the brain during sustained wakefulness (Chagoya de Sanchez et al., 1993; Porkka-Heiskanen et al., 1997). In fact, the extracellular concentration of adenosine in the basal forebrain of the cat is directly correlated with the extent of wakefulness (Porkka-Heiskanen et al., 1997). Adenosine A_1 receptor agonists induce electroencephalographic activity resembling that observed during sleep deprivation (Benington et al., 1995) and increase rapid eye movement (REM) sleep in cats (Portas et al., 1997). Furthermore, adenosine A_1 receptor antagonists, including caffeine and theophylline, promote alertness even after prolonged sleep deprivation (Snyder et al., 1981; Radulovacki et al., 1984). These observations suggest that adenosine may function as a natural sleep-promoting agent (Radulovacki, 1985; Benington and Heller, 1995), accumulating during periods of sustained wakefulness to reduce neuronal excitability in a manner consistent with the proposed role of the nucleoside as a neuroprotective agent.

Recently, Mistlberger et al. (1997) reported that as little as 6 h of sleep deprivation significantly attenuated light-induced phase delays in the hamster. It is tempting to speculate that this decrease in the response to light may be due to the accumulation of adenosine in the suprachiasmatic region during sustained wakefulness. Thus, adenosine tonus could provide information to the circadian clock regarding the state of fatigue of the organism, possibly resulting in an altered response to the daily, light-induced phase adjustment of the clock. In this way, the temporal program of wakefulness and sleep, which is under control of the circadian clock, could be adjusted to accommodate day-to-day variation in the quantity and/or quality of sleep.

In summary, systemic administration of the selective adenosine A_1 receptor agonist, CHA, reversibly and dose-dependently inhibits light-induced phase advances and delays of the circadian rhythm in wheel-running behavior in the Syrian hamster. Selective agonists for the adenosine A_{2A} and A_3 receptors are without effect. The inhibitory effect of CHA is dose-dependently attenuated by the selective adenosine A_1 receptor antagonist, DPCPX. Finally, similar results were obtained after injection of CHA into the suprachiasmatic region. It is concluded that adenosine A_1 receptors located in the region of the suprachiasmatic nucleus regulate the response of the circadian clock to the phase-adjusting effect of light.

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