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Modulation of Photic Response by the Metabotropic Glutamate Receptor Agonist t-ACPD

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Abstract

Glutamate is the primary excitatory transmitter in the hypothalamus. It conveys photic information to the suprachiasmatic nucleus of the hypothalamus, thereby entraining the circadian clock to environmental light cycles. While ionotropic glutamate receptors have been implicated in the transduction of photic information in suprachiasmatic nucleus cells, there is evidence that metabotropic glutamate receptors play a significant modulatory role. We investigated the effects of the metabotropic glutamate agonist ($\pm$)-1-Aminocyclopentane-trans-1,3-dicarboxylic acid (ACPD) on light-evoked phase responses in Syrian hamsters at three phase points: circadian time 6, a time when light has no effect on the circadian timing system; circadian time 13.5, when light evokes the maximum phase delay; and circadian time 19, the maximum phase advance. We found that ACPD significantly increased the light-evoked phase shift at circadian time 13.5, and had no effect at other phase points tested. These data support a role for metabotropic glutamate receptors in the circadian photic signal transduction system.

Keywords

circadian; suprachiasmatic; hamster; entrainment

INTRODUCTION

Mammals exhibit circadian rhythms generated by an endogenous clock. This clock is located in the hypothalamic suprachiasmatic nucleus (SCN). The SCN regulates the timing of many physiological processes including locomotor activity, sleep-wake and hormonal cycles. While the circadian clock in the SCN free-runs in the absence of external time cues, it can be stably entrained by phase advances or delays induced by environmental or pharmacological stimulation [1;2]. Light appears to be the strongest clock-entraining stimulus.

The retinohypothalamic tract (RHT) carries photic information to the SCN [3;4], and there is considerable evidence that the effects of light are mediated by the release of glutamate onto SCN cells [5-7]. Furthermore, calcium imaging in brain slices indicates that optic nerve stimulation evokes calcium rises in specific cells, and may involve both ionotropic and metabotropic glutamate receptors [8]. While glutamate appears critical in entrainment of the clock to light [9], the receptors involved and how they couple to cellular transduction pathways are not fully described.

Metabotropic glutamate receptors (mGluRs) are divided into three groups based on sequence homology of the cloned receptors and by pharmacology and second messenger pathways [10]. All three mGluR groups (types I, II, and III) are expressed in the SCN [11], although there is little, if any, information available on the spatial distribution of the mGluRs within the SCN. It appears that glutamate released by the RHT acts on ionotropic glutamate receptors (iGluRs), since microinjection of n-methyl-d-aspartate (NMDA) into the SCN region mimics the effects of light [12;13], and kainate and NMDA receptor antagonists block the effects of light [6;14]. However, modulation of iGluRs by mGluR activation may be an important means of regulating SCN responsiveness to glutamate, not just through effects on neurons but also on astrocytes [15]. In this paper, we describe modulation of the phase-shifting effects of light by the type I/II mGluR agonist trans-(±)-1-amino-cyclopentanedicarboxylic acid (ACPD).

MATERIALS AND METHODS

Animals

Adult male Syrian hamsters (*Mesocricetus auratus*) were purchased from Charles River Labs. Hamsters were maintained on a 14:10 light-dark cycle, and food and water were available ad libitum. They were housed individually in 20×40×20 cm Plexiglas cages equipped with 16 cm diameter running wheels. Each revolution of the running wheel activated a microswitch on the outside of the cage. Switch activity was continuously monitored using DataCol software (MiniMitter, Sun River, OR). All procedures complied with Public Health Service policies on the care and use of laboratory animals and were approved by the Georgia State University institutional animal care and use committee.

Surgery

Hamsters (130-160g) were deeply anesthetized using sodium pentobarbital (90 mg/kg), and were stereotactically implanted with an 11 mm × 26-gauge stainless-steel guide cannula aimed at the SCN. With bregma and lambda level, measuring from bregma the stereotaxic coordinates were 0.8 mm anterior, 1.7 mm lateral, 7.5 mm below dura, with a 10° angle towards the midline. After surgery, hamsters were housed in constant darkness (DD).

Microinjections

Microinjections began once hamsters had established 7-10 days of stable, free-running circadian activity rhythms. Hamsters were gently restrained by hand in dim red illumination, and injections were given using a 16-mm, 32-gauge needle attached by polyethylene tubing to a 1 µl Hamilton syringe. The needle was left in for 15 sec after each injection to provide for substrate diffusion. Injections were given at circadian time (CT) 6, 13.5, and 19. In hamsters, CT 12 is defined as the onset of nocturnal activity on a running wheel. Therefore, CT 6 corresponds to the middle of the subjective day, CT 13.5 is in the early subjective night, and CT 19 in the late subjective night. We chose these times because light has no effect on behavioral circadian phase at CT 6, produces phase delays at CT 13.5, and produces phase advances at CT 19 [1]. Microinjections consisted of 200 nl of 1.5 mM ACPD (Research Biochemicals, Natick, MA) dissolved in 0.9% saline, or saline alone. Each hamster received up to three injections, and a minimum of 9 days separated each injection. Each hamster was used for injections at only one of the three circadian time points, and the order of injections was counterbalanced for hamsters within each circadian time grouping. Immediately after an injection, the animal (in its own home cage) was moved to a separate room and exposed to 170 lux incandescent light for 15 minutes to produce a non-saturating light pulse [16]. Sham light pulses were performed in the same manner but with the light turned off. Following the light or sham treatment, animals were moved back into their original rooms.

Histology

After completion of the experiment, hamsters were injected with an overdose of sodium pentobarbital. Animals were perfused intracardially with 10% formalin (100 ml), and then brains were removed and stored in 10% formalin until sectioning. Tissue sections (50 μ m) obtained using a vibratome, counterstained with cresyl violet, and injection sites were verified by examination with light microscopy. Only animals with sites within 500 μ m of the SCN and not penetrating the third ventricle were included (Fig 1) in the results, which matches the criteria used in numerous previous studies [12;13;17;18].

Phase-shift analysis

Locomotor activity phase-shifts were quantified using the linear regression method described by Daan and Pittendrigh [1]. This method estimates circadian period and phase by fitting regression lines through daily activity onsets (CT 12). Lines are fitted to daily onsets of activity for 6-7 days prior to and for 8-10 days following each injection. To avoid transient onsets, the first three days following an injection were not included. For each injection, the data was only included in the analysis if the standard error of estimate for both regression lines was less than 15 minutes. A phase shift was measured as the difference between predicted activity onset before and after the injection. Results are expressed as group mean $\pm$ SEM. Because not all animals received all treatments, this experiment is analyzed as a between-subjects design. Statistical comparisons were planned only between drug and saline treatments within each circadian time group, as the phase dependence of light-induced phase shifts is well established. Two-tailed t-tests were used to test significance between drug and saline treatments.

RESULTS

Photic phase shifts

Phase shifts to light preceded by the injection of saline are similar to those already reported for light alone in hamsters [19]. Hamsters did not show significant phase shifts at any of the time points tested when no light pulse was presented (Fig 2).

ACPD modulation of photic phase shifts

Microinjection of ACPD into the SCN region prior to a light pulse at CT 13.5 significantly increased the resulting phase delay (light + ACPD: -59.0 ± 6.7 min; light + saline: -34.4 ± 4.3 min; $t_{10} = 3.246$, $p < 0.01$) (Fig 2, 3). In contrast, at CT 19 microinjection of ACPD did not alter the phase advance in response to light (light + ACPD: -49.0 ± 23.4 ; light + saline: -50.3 ± 11.3 min; $t_{11} = 0.052$, $p > 0.05$). There were no significant differences between the effects of ACPD and vehicle in hamsters not exposed to light pulses at all time points tested.

DISCUSSION

These results demonstrate that metabotropic glutamate receptors are involved in modulating the responsiveness of the circadian clock to light. The type I/II mGluR agonist ACPD significantly increased the light-evoked phase delay at CT 13.5 by 71%. The ability of ACPD to alter the phase-shifting effects of light is phase dependent, as there was no effect of ACPD administration on light-induced phase shifts at CT 19, and ACPD did not induce phase shifts when administered at CT 6.

It is possible that ACPD is exerting its effects on light-induced phase delays through inhibition of GABAergic activity, as GABA antagonists have similar effects on light-induced phase shifts. Microinjection of the GABA_A antagonist bicuculline or the GABA_B antagonist CGP-35348 into the SCN region at CT 13.5 increases the amplitude of light-induced phase

delays, whereas microinjection of either antagonist into the SCN region at CT 19 fails to alter light-induced phase advances [20;21].

There is strong evidence from culture systems that activation of mGluRs inhibits presynaptic GABA-ergic activity. Application of a variety of mGluR agonists depresses evoked inhibitory postsynaptic potentials in single cultured SCN neurons without altering postsynaptic GABA receptor responses [22]. This suggests that mGluR activation inhibits the release of GABA. This hypothesis is supported by evidence from other hypothalamic systems. mGluRs inhibit GABAergic input to GnRH neurons in the preoptic area and ventral hypothalamus [23] as well as input to the supraoptic nucleus (SON) [24]. mGluR activation in the SON has been shown to enhance glutamate release in the SON [25], an effect that, if present in the SCN, would support an increase in light-induced phase shifts. Inhibition of GABAergic transmission would also help to explain an apparent conflict between these results and the finding that ACPD inhibits NMDA-induced calcium influx in SCN explant cultures [26]. Inhibition of calcium influx would be expected to inhibit the phase shifting effects of light, since activation of NMDA receptors is a critical step in light-induced phase shifts [12;13]. However, inhibition of GABAergic transmission increases the phase delaying effects of light [20;21] *in vivo*. The effects of ACPD *in vivo* may simply be more pronounced on the GABAergic system than on the glutamatergic system, or the elimination of many GABAergic connections in the SCN explant cultures may have unmasked the effects of ACPD on NMDA-induced calcium influx.

Other signal transduction pathways may contribute to the effects of ACPD on light-induced phase shifts, notably the phospholipase C (PLC) pathway. Metabotropic glutamate receptors are involved in translating rhythms in gene expression into rhythms of neuronal firing via linkage to the PLC signaling pathway [27]. ACPD increases spontaneous firing of SCN neurons in wild-type mice but not in PLC- $\beta 4$ (an isozyme of PLC) knockout mice, and the knockouts have a reduced-amplitude circadian rhythm of neuronal firing rate [27]. Inhibition of PLC reduces VIP-induced increases in *per1* and *per2* mRNA expression [28]. ACPD could enhance light-induced phase shifts through a similar mechanism, activating the PLC $\beta 4$ pathway and increasing light-induced period gene expression, resulting in a phase shift. However, the demonstrated effects of the PLC inhibitor on VIP-induced period gene expression occurred at CT19, when ACPD has no effect on light-induced phase shifts. Therefore, this mechanism can not fully explain the phase-dependence of ACPD's effects.

This study provides new information on the signals that comprise the photic input to the SCN. Photic stimulation releases glutamate [5], evokes intracellular calcium rises in SCN cells [8], has phase-dependent effects on neural firing [29], and gene transcription [30;31]. Since GluRs play an integral role in transducing photic information, it is likely that they are also critically involved in coordinating the timing of SCN clock cells with environmental light cycles. While it appears that iGluRs are primarily responsible for transducing photic signals [6;7;13], results from this study provide support for the idea that mGluRs may play a significant role in the circadian gating of photic signals.

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Figure 1. Cresyl violet-stained section (50 μ m) showing the SCN and the microinjection site. The SCN is the darkly stained bilateral nucleus located just above the optic chiasm (OX) and on either side of the third ventricle (3V). The injection site (tip) is visible as the darkly stained gliosed needle track just alongside the SCN.

Figure 2. Mean phase-shift $\pm$ SEM are shown for all treatments. Negative numbers indicate phase delays; positive numbers indicate phase advances. The numbers above and below the bars indicate sample sizes. *ACPD + light differs significantly from saline + light at CT 13.5.

Figure 3.

Double-plotted actogram of a representative animal running in constant darkness that shows different phase-shifts to light + saline and light + ACPD at CT 13.5. Each horizontal line represents two 24 hr periods; successive days are displayed vertically. Running wheel activity is denoted by the height of the bars extending upwards from the horizontal lines. Injections were given in dim red light on the days indicated by pointers and at the times indicated by an "x".