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Research Report

An NMDA antagonist inhibits light but not GRP-induced phase shifts when administered after the phase-shifting stimulus

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ABSTRACT

Brief light pulses or microinjection of gastrin-releasing peptide (GRP) into the third ventricle or near the suprachiasmatic nucleus (SCN) induce phase shifts of circadian rhythms during the subjective night. It has previously been reported that these effects are strongly influenced by the activation of *N*-methyl-D-aspartate (NMDA) receptors and the availability of glutamate. We hypothesized that the photic signaling pathway in the SCN was dependent on glutamate neurotransmission even after the completion of a photic stimulus. Adult male Syrian hamsters equipped with a surgically implanted guide cannula aimed at the SCN region were housed in constant darkness until stable free-running rhythms of wheel-running activity were apparent. Light pulses administered in the early night induced phase delays of circadian rhythms which were attenuated by the co-administration of (±)-2-amino-5-phosphonopentanoic acid (AP5), an NMDA antagonist. Microinjection of AP5 also inhibited light-induced shifts, to a lesser extent, immediately after and 15 min after, but not 30 min after the light pulse. A second experiment was designed to test whether AP5 would be able to attenuate GRP-induced shifts 15 min following microinjection of GRP. Phase shifts of animals that received microinjection of AP5 15 min after the administration of GRP were not different from those that received microinjection of GRP and vehicle. These data suggest that glutamate signaling remains necessary for a full photic response in the SCN even after the termination of the photic signal, but that this dependency ends once GRP-dependent signaling is complete.

1. Introduction

The mammalian suprachiasmatic nucleus (SCN) contains an endogenous clock from which overt rhythms of physiology and behavior are driven (Moore and Eichler, 1972; Stephan and Zucker, 1972). SCN rhythms are entrained by photic and non-photic signals and these rhythms occur in roughly 24-h (circadian) intervals (Inouye and Kawamura, 1979; Moore, 1983). One of the most studied entraining signals is photic stimulation. Photic signals are transmitted to the clock via the retinohypothalamic tract (RHT); a monosynaptic projection from the retinas of the eyes to the SCN that primarily uses glutamate (Castel et al., 1993) to synchronize circadian rhythms with the environmental light/dark schedule (Hendrickson et al., 1972; Pickard, 1982).

Glutamatergic signaling is essential for photic entrainment to occur (for a review see (Ebling, 1996)). Microinjection of *N*-methyl-D-aspartate (NMDA), a glutamate agonist (Mintz et al., 1999; Mintz and Albers, 1997) *in vivo* and the administration of glutamate to the hypothalamic slice preparation (Ding et al., 1994) *in vitro* elicits

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phase shifts of the circadian clock in a manner similar to light, with the amplitude and direction of the response (advance of rhythms vs. delay) dependent on the time of administration. Light or NMDA induces phase delays during the early subjective night and phase advances during the late subjective night; no shifts occur during the subjective day in response to light or NMDA. For animals kept in constant conditions, a light pulse (Ginty et al., 1993; Rea et al., 1993), or the ventricular administration of NMDA (Ebling et al., 1991) induces the immediate-early gene c-fos in the SCN which can be attenuated by the administration of glutamate antagonists (Abe and Rusak, 1994; Rea et al., 1993).

In Syrian hamsters, a cluster of gastrin-releasing peptide (GRP) immunoreactive cells are located dorsal to, but substantially overlapping, the area containing VIP cells (Antle et al., 2005b). Microinjection of GRP into the SCN region induces c-fos expression in the dorsolateral SCN (Piggins et al., 2005) as well as the expression of phosphorylated extracellular-related kinase (Antle et al., 2005a). The administration of GRP *in vitro* (McArthur et al., 2000) or *in vivo* (Albers et al., 1991; Antle et al., 2005a; Kallingal and Mintz, 2007; Piggins et al., 1995) elicits phase shifts during the subjective night in a manner similar to light. Light pulses during the subjective night induce the expression of c-fos in SCN cells that are immunoreactive for GRP (Earnest et al., 1993; Romijn et al., 1996). These data indicate that GRP plays an integral role in photic entrainment.

Previous reports have indicated that glutamate receptor activation is a necessary step for light- (Colwell and Menaker, 1992) and GRP- (Kallingal and Mintz, 2006) induced phase shifts. These reports suggest that GRP acts downstream of glutamate receptor activation in the photic phase-shifting pathway. A combination *in vivo-in vitro* approach has also indicated that neuropeptide Y, when applied to the hypothalamic slice preparation, can remarkably attenuate shifts induced *in vivo* by light up to 30 min following a light pulse (Yannielli and Harrington, 2000). Furthermore, novel wheel running or NPY administration has been shown to reduce the magnitude of light-induced shifts up to 60 min following exposure to light (Lall and Biello, 2002). It is apparent that the photic signaling pathway can be disrupted following termination of the photic signal, but it is not known if the simple inhibition of glutamatergic signaling following a light pulse or GRP administration could similarly impact the magnitude of the resulting shifts. In this study, we tested the effects of microinjection of an NMDA antagonist on the behavioral response to light before and at various time points after exposure to a light pulse. We additionally tested the effects of an NMDA antagonist on GRP-induced shifts 15 min after administration of GRP.

2. Results

Animals received microinjections of (±)-2-amino-5-phosphonopentanoic acid (AP5) or 0.9% saline (SAL) before, immediately after, 15 min after, or 30 min after a light pulse of either 2400 or 300 lux intensity at CT 13±15 min (Figs. 1 and 2). Significant main effects of treatment, time of injection, and light intensity were detected (treatment ($F_{1,141}=27.19$, $p<.001$); injection time ($F_{3,141}=8.24$, $p<0.001$); and light intensity ($F_{1,141}=12.28$, $p<0.001$)). There was a significant interaction between treatment and injection time ($F_{3,141}=6.40$, $p<0.001$). AP5 significantly inhibited light-induced shifts before, after, and 15 min after the light pulse across both light intensities (Tukey–Kramer test, $p<0.05$, Figs. 3 and 4). There were no significant interactions between treatment and light intensity ($F_{1,141}=0.07$, $p=0.80$), light intensity and injection time ($F_{3,141}=0.17$, $p=0.91$), or treatment, time of injection, and light intensities ($F_{3,141}=0.38$, $p=0.77$).

Because our data revealed that AP5 can attenuate light-induced shifts of the circadian activity rhythm up to 15 min after the end of a light pulse, and since a previous report indicated that AP5 can attenuate GRP-induced shifts (Kallingal and Mintz, 2006), an additional experiment was designed to test whether AP5 can similarly block GRP-induced shifts 15 min following microinjection of GRP into the SCN region. Animals received an initial microinjection of GRP at CT 13±15 min with a follow-up microinjection of either AP5 ($n=9$) or SAL ($n=10$) 15 min later (Fig. 5). There was no significant difference between GRP/AP5 (–0.70±0.09 h) or GRP/SAL (–0.63±0.09 h) in the magnitude of the GRP-induced phase delay ($t_{17}=0.59$, $p=0.56$) (Fig. 6).

3. Discussion

The present study finds that microinjection of an NMDA antagonist into the SCN region reduces the phase-delaying effects of light when given up to 15 min after the end of a 15-min light pulse. Conversely, an NMDA antagonist does not attenuate GRP-induced phase shifts 15 min after central administration of GRP. Previous studies have shown that exposure to light (DeCoursey, 1960), or GRP administration into the third ventricle (Antle et al., 2005a; Kallingal and Mintz, 2006) or near the SCN (Albers et al., 1991; Kallingal and Mintz, 2007; Piggins et al., 1995) can induce shifts of circadian rhythms during the subjective night. The responses to light (Colwell and Menaker, 1992) and GRP (Kallingal and Mintz, 2006) can be attenuated by administration of glutamate receptor antagonists. However, this is the first examination of the effectiveness of NMDA antagonists given after the phase shifting stimulus has already been delivered.

In the light pulse experiment, AP5 was able to block light-induced shifts up to 15 min following a light pulse at both high and low intensities of light. Light information reaches the SCN through a population of retinal ganglion cells that utilize glutamate as a primary transmitter at the retinohypothalamic tract/SCN synaptic junction (for review see (Morin and Allen, 2006; Wang et al., 2008)). The release of glutamate activates NMDA receptors (Cull-Candy et al., 2001; McBain and Mayer, 1994) which triggers signaling cascades that lead to shifts of the circadian clock (Wang et al., 2008). While it is clear that photic stimuli increase NMDA receptor activity, it is unknown if these heightened levels of activity persist following the presentation of a photic signal or if the blockade of NMDA receptors after the presentation of a light pulse can affect the magnitude of light-induced shifts. Because AP5 was able to attenuate light-induced shifts even 15 min following a light pulse, our findings suggest that either retinal ganglion cells continue to fire even after a light pulse has been presented or that other sources of glutamate provide some stimulation that is necessary even after the lights are turned off.