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Do NMDA receptors mediate the effects of light on circadian behavior?

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We report here the results of experiments designed to evaluate whether a specific NMDA receptor antagonist, (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,b]cyclohepten-5,10-imine maleate (MK-801), blocks the phase shifting effects of light on the circadian rhythm of wheel-running activity in golden hamsters. Intraperitoneal administration of (+)-MK-801 produced a dose-dependent blockade of both light-induced phase advances and delays. The effect was stereoselective and treatment with related compounds, phenylcyclidine and ketamine, also blocked light-induced phase shifts. MK-801, by itself, did not cause any consistent effect on the phase of the rhythm. These data, coupled with previous findings, indicate that excitatory amino acid receptors play an important role in the transmission of light information from the retina to the circadian system.

INTRODUCTION

Circadian rhythms in behavior and physiology are ubiquitous among eukaryotic organisms. Under constant conditions, these rhythms continue to be expressed with periods of approximately 24 h. A defining feature of these endogenous oscillations, and one that confers major adaptive significance, is that they can be synchronized (entrained) to periodic signals from the environment. The daily light–dark cycle entrains all known circadian oscillators[13] and in the golden hamster a brief flash of light (e.g. 1 s) presented once a day is sufficient[4]. In mammals, this effect of light on the circadian system is mediated by photoreceptors that are located in the retina and project to the hypothalamus, at least in part, via a monosynaptic fiber tract known as the retinohypothalamic tract or RHT[12,15,20]. The hypothalamic site at which these fibers terminate, the suprachiasmatic nucleus (SCN), is known to function as the dominant pacemaker of the mammalian circadian system[12,14,15,20].

The neurotransmitters that mediate the RHT to SCN synaptic connection are unidentified; however, recent work suggests that excitatory amino acids (EAA) are involved. Stimulation of the optic nerve in an in vitro hypothalamic slice preparation that contains the SCN has been shown to induce a calcium-dependent release of [^{3}H]glutamate and [^{3}H]aspartate[9]. Furthermore, in vitro studies on rat and mouse hypothalami provide evidence suggesting that retinal input to the SCN is mediated at least in part by EAA receptors[2,3,16]. Finally, glutamate injected into the area of the SCN causes phase shifts in the circadian rhythm of wheel-running in hamsters[10].

If L-glutamate and related excitatory amino acids do mediate the effects of light on the circadian system, then antagonists to EAA receptors should block light-induced phase shifts in behavioral rhythms such as that of wheel-running. Three major subtypes of postsynaptic EAA receptors have been identified in the central nervous system and named for their specific agonists: *N*-methyl-D-aspartate (NMDA), kainate (KA), and quisqualate (QUIS)[21]. MK-801 ((+)-5-methyl-10,11-dihydro-5H-dibenzo[a,b]cyclohepten-5,10-imine maleate) is a potent and specific antagonist of the NMDA receptor, which crosses the blood–brain barrier[22]. Its mechanism of action is thought to involve blockade of the ion channel associated with the NMDA receptor[5]. Other compounds which share this site of action but are less specific and potent include phenylcyclidine (PCP) and ketamine[1,5]. In the study reported here, we sought to determine whether MK-801 would block the phase shifting effects of light pulses on the behavioral circadian rhythm of locomotor activity of golden hamsters.

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MATERIALS AND METHODS

Male golden hamsters (*Mesocricetus auratus*, LVG-outbred), obtained from Lakeview, Charles River at 5 weeks of age, were housed individually and their wheel-running activity recorded. The animals were exposed to a 14:10 h light–dark cycle for 2 weeks and then placed in constant dark (DD) for 10 days to assess their free-running activity pattern. Hamsters remained in DD and were subjected to one of 5 treatments: (1) single quantified light pulse; (2) i.p. injection of experimental drug plus light pulse; (3) i.p. injection of vehicle plus light pulse; (4) i.p. injection of experimental drug alone; (5) i.p. injection of vehicle alone. The light pulses were delivered either 1.5 h after the onset of activity (i.e. circadian time or (CT) 13.5, onset of activity is defined as CT 12 for nocturnal animals) when light would normally induce a phase delay or 6 h after activity onset (i.e. CT 18) when light would normally induce a phase advance. Following each treatment, the animals were allowed to free-run undisturbed in DD for 10 days before receiving another treatment. No animal was treated more than twice.

The light stimulus used to induce phase shifts was a 15-min pulse of monochromatic light (515 nm) at an intensity of 8.5×10^{-2} $\mu W/cm^2$. The apparatus used to produce the stimulus has been previously described[18]. The stimulus parameters (duration, irradiance and wavelength) were chosen to produce submaximal phase shifts. Stimulus intensity (irradiance) was measured before each trial with a UDT radiometer. Drugs, in either dimethylsulfoxide (DMSO) or isotonic saline, were administered by i.p. injection. Injection volumes were either 0.1 or 0.2 ml of solution. All handling and injection of animals was carried out in complete darkness with the aid of an FJW infrared viewer. PCP was purchased from Sigma, St. Louis, MO; all other drugs were purchased from RBI, Natick, MA.

Three sets of experiments were performed. In Expt. 1, the effect of (+)-MK-801 on light-induced phase shifts at CT 13.5 and CT 18 was investigated. Experimental animals received an i.p. injection of MK-801 (4.8 mg/kg) in DMSO vehicle 60 min prior to the light pulse. Control groups were treated as described above. The stereospecificity of this drug's effect was investigated by using (−)-MK-801 in combination with a light pulse.

In Expt. 2, the dose–response function of the effect of MK-801 on light-induced phase shifts was investigated at both circadian times. Six doses of MK-801 were tested (1.2, 2.0, 2.4, 3.2, 4.8, 6.0 mg/kg). Drug alone controls were obtained at each dose. The ability of a higher intensity of light (6.0×10^{-1} μW) to overcome the effects of 4.8 mg/kg of MK-801 on light-induced phase shifts was also investigated.

In Expt. 3, the blocking effects of other EAA receptor antagonists were assessed. Experimental animals received an i.p. injection of ketamine (40 mg/kg) or phenylcyclidine (30 mg/kg) in saline 30 min prior to the light pulse. Four control groups were employed as described above.

Phase shifts in the activity rhythm were determined by measuring the phase difference between eye-fitted lines connecting the onsets of activity for a period of 7 days before and 10 days after an experimental manipulation. In order to estimate the steady-state phase shifts produced, one day of data after treatments which caused phase delays and 4 days of data after treatments which caused phase advances were excluded from the analysis. In other respects the method for calculating phase shifts was the same as has been reported elsewhere[18]. The effects of the drugs on the phase-shifting effects of light pulses were evaluated using ANOVA. Values were considered significantly different if $P < 0.05$.

RESULTS

The i.p. administration of MK-801 blocked light-induced phase advances as well as light-induced phase delays in the circadian rhythm of wheel-running activity

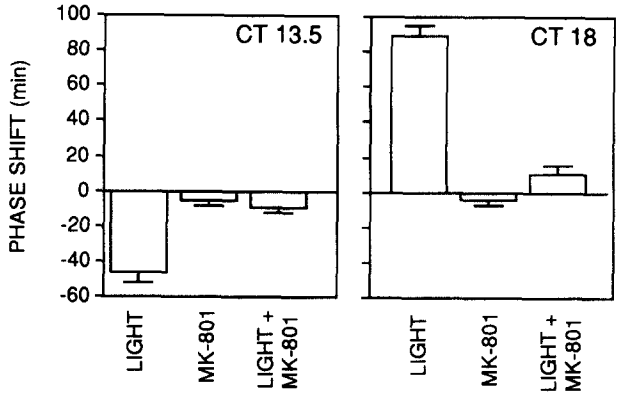

Fig. 1. Mean phase shift in the rhythm of locomotor activity in hamsters in constant darkness that received a treatment of either light + vehicle (labeled LIGHT), MK-801, or light + MK-801. Left: MK-801 or vehicle were administered 0.5 h after activity onset while the light pulses were delivered 60 min later at CT 13.5. Right: drug or vehicle was administered 5.0 h after the onset of activity. The light pulses were delivered 60 min later at CT 18.0. n = 6–8 for all points, verticle bar represents S.E.M.

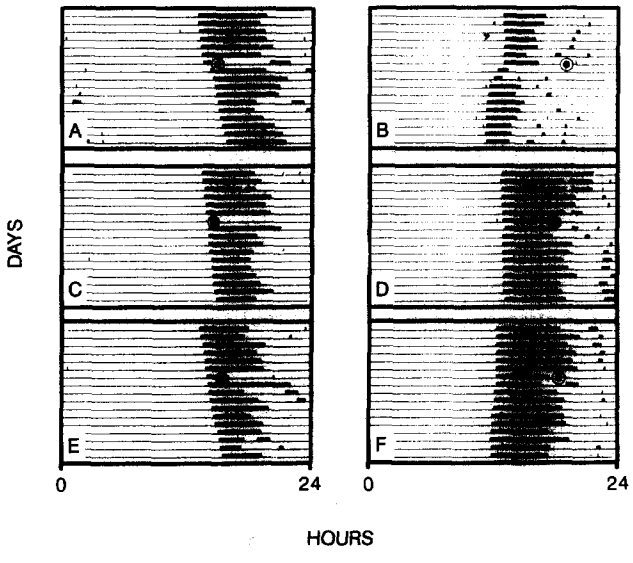

Fig. 2. Locomotor activity records from experimental and control animals maintained in constant darkness. Each horizontal line represents the activity record for a 24-h day and successive days are plotted from top to bottom. Circles represent the time of light and/or drug treatment. A: activity record illustrating the phase delaying effects of a 15-min light pulse (515 nm, $I = 8.5 \times 10^{-2}$ μW) given at CT 13.5. This animal also received an injection of vehicle (DMSO) 1 h earlier as a control. B: activity record illustrating the phase advance which results from a light pulse given at CT 18. C: activity record illustrating the lack of effect of an injection of MK-801 (4.8 mg/kg) at CT 12.5 on the phase of the circadian rhythm in locomotor activity. D: activity record illustrating that an injection of MK-801 (4.8 mg/kg) at CT 17 had no effect on the phase of the circadian rhythm of locomotor activity. E: activity record illustrating the blockade of light-induced phase delay by an injection of MK-801 (4.8 mg/kg) 60 min prior to a light pulse given at CT 13.5. F: activity record illustrating the blockade of light-induced phase advances by an injection of MK-801 (4.8 mg/kg) 60 min prior to a light pulse given at CT 18.

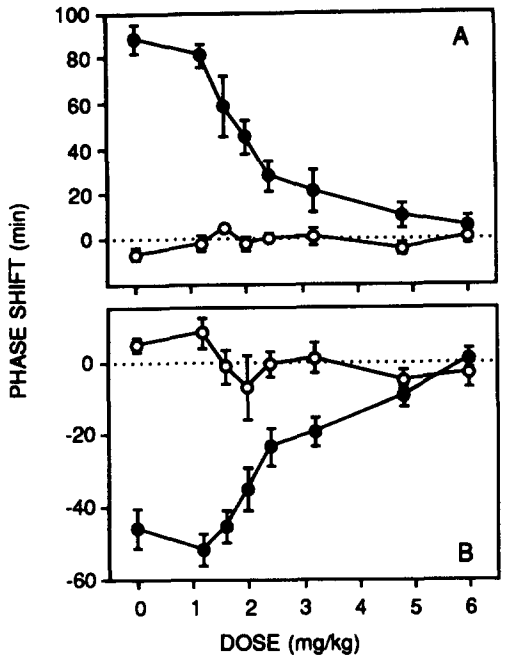

Fig. 3. Dose–response curves for the blockade of light-induced phase advances (A) and phase delays (B) by MK-801. Open circles represent groups which received MK-801 alone; closed circles represent groups which received light and MK-801. The effects of light + vehicle injection are shown at 0 mg/kg. n = 6–8 for all points, verticle bar represents S.E.M.

in hamsters (Fig. 1). Control injections of MK-801 or vehicle administered in the absence of a light pulse had no significant effect on the phase of the free-running rhythm. Vehicle alone injections had no significant effect on the amplitude of light-induced phase advances or delays. Examples of activity records from experimental and control animals are shown in Fig. 2A–F. Light-induced phase shifts were not affected by treatment with the optical isomer (–)-MK-801. The phase advance induced by light plus vehicle was 89.1 ± 15.9 min (mean ± S.E.M.) and the phase advance induced by light plus (–)-MK-801 at a dose of 4.8 mg/kg was 80.7 ± 19.8 min.

MK-801's blockade of light-induced phase shifts was dose dependent (Fig. 3). Doses lower than 1.6 mg/kg had no effect at the light intensity employed in these experiments. The reduction of phase advances was dose dependent between 1.6 and 6.0 mg/kg with an ED_{50} of 2.0 mg/kg; the reduction of phase delays was dose dependent between 2.0 and 6.0 mg/kg with an ED_{50} of 2.5 mg/kg. Increasing the intensity of the light stimulus by an order of magnitude (from 8.5×10^{-2} μW to 6.0×10^{-1} μW) was sufficient to completely overcome MK-801's (4.8 mg/kg) blockade of light-induced phase shifts. The phase advances induced by the higher intensity of light and by light plus MK-801 (4.8 mg/kg) were 115.8 ± 12.9 and 115.0 ± 8.6, respectively.

Treatment with ketamine or PCP did not block light-induced phase shifts at a dose of 4.8 mg/kg. However, when dosage was increased (PCP, 30 mg/kg; ketamine, 40 mg/kg), both drugs blocked light-induced phase shifts. The results of these experiments are shown in Table I. In no case did the drug or vehicle alone produce any significant effect of the phase of the locomotor activity rhythm.

TABLE I

The effects of ketamine and phenylcyclidine on light-induced phase shifts of hamster locomotor rhythms

Drugs were administered by i.p. injection 30 min prior to the light pulse. Light pulses were delivered either 1.5 h after the onset of activity (i.e. CT 13.5) when light would normally induce a phase delay or (delays are indicated by –) 6 h after activity onset (i.e. CT 18) when light would normally induce a phase advance. Significance was tested by ANOVA and the number within parentheses indicates the number of trails for each treatment group.

Treatment	*Phase shift (min ± S.E.M.).*	
	Light (CT 13.5)	*Light (CT 18.0)*
Vehicle	–55.4 ± 4.4 (11)	96.7 ± 6.8 (9)
Ketamine 6.0 mg/kg	–45.8 ± 8.1 (6)	90.0 ± 11.7 (5)
Ketamine 40.0 mg/kg	–4.2 ± 2.9* (6)	–0.3 ± 5.6* (6)
PCP 6.0 mg/kg	–57.5 ± 8.5 (6)	107.0 ± 10.5 (5)
PCP 30.0 mg/kg	–7.8 ± 3.0* (7)	15.0 ± 8.0* (5)

* Significant ($P < 0.05$) compared to vehicle + light controls.

DISCUSSION

Our results demonstrate that an excitatory amino acid receptor antagonist can pharmacologically block the effects of light on the circadian rhythm of locomotor activity in the hamster. These results complement an earlier behavioral study which demonstrated that glutamate injection into the area of the SCN produced phase shifts in this rhythm[10]. Interpretation of the effects of the antagonist is not complicated, as is interpretation of the effects of direct injection of glutamate, by the EAA's almost ubiquitous excitatory effect in the mammalian CNS. EAA antagonists have now been shown to block both the phase shifting effects of light on behavioral rhythmicity and the field potentials recorded in the SCN in response to optic nerve stimulation in vitro[2,3,16]. These findings strongly suggest that EAAs mediate the effects of light on the circadian system in rodents.

MK-801 has been reported to be a potent and selective antagonist of the NMDA receptor class[5,22] and it is likely that this is the mechanism by which this agent acts on the circadian system. The drug's effects in the present study were dose dependent over a range of doses comparable to those reported to specifically block NMDA-mediated responses in other in vivo systems[17,29,23]. Additionally, the behavioral effects of MK-801 appear to be stereose-

lective. (–)-MK-801 completely failed to block light-induced phase shifts at the same dose at which (+)-MK-801 completely blocked the effects of light on wheel-running activity. Finally, although ketamine and PCP are known to be less potent than MK-801, they both antagonize NMDA responses — presumably by blocking the NMDA receptor associated ion channel in a manner similar to MK-801[1,5]. The rank order of the potency of these compounds in blocking light-induced phase shifts ((+)-MK-801 > PCP > ketamine) is the same as that observed in binding experiments[22] and in other in vivo studies[1,8,17,19]. These results support our suggestion that the EAA receptors involved in mediating the effects of light on the circadian system are of the NMDA type.

The site of action of MK-801's effect on light-induced phase shifts is not known. The observation that this drug blocks both light-induced phase advances and delays but does not, by itself, produce any phase shift suggests that it is acting on a light input pathway to the SCN or within the SCN itself. Evidence for EAA neurotransmission has also been uncovered in other visual pathways including the cat retinogeniculate[6] and the goldfish retinotectal projection[7] and NMDA receptors have been localized to the lateral geniculate nucleus and the superior colliculus[11]. The data reported here, and earlier studies which found evidence that EAA receptors mediate SCN responses to RHT input[2,3,16], lead us to the hypothesis that the RHT/SCN synapse may be the site of MK-801's action on the circadian system.

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