

5-HT_{1A} autoreceptor antagonist-induced 5-HT release in the hamster suprachiasmatic nuclei: effects on circadian clock resetting

Michael C. Antle^a, J. David Glass^b, Ralph E. Mistlberger^{a,*}

^aDepartment of Psychology, Simon Fraser University, Burnaby, B.C., Canada, V5A 1S6

^bDepartment of Biological Sciences, Kent State University, Kent, Ohio, OH, 44242, USA

Received 27 December 1999; received in revised form 27 January 2000; accepted 27 January 2000

Abstract

Serotonin (5-HT) has been implicated in phase shifting of mammalian circadian rhythms by non-photic stimuli. This study tests whether pharmacological induction of endogenous 5-HT release can shift circadian phase in the Syrian hamster. Systemic injections of the 5-HT_{1A} antagonist WAY100635 during the mid-subjective day significantly increased 5-HT in dialysate from the hamster suprachiasmatic nucleus (SCN) circadian pacemaker by ~50% for 40–60 min. However, this was not associated with phase shifts or with potentiation of phase shifts induced by a 3 h bout of running. These results indicate that enhanced 5-HT release in the SCN or possibly other regions is not sufficient to induce phase shifts in the subjective day. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Circadian rhythms; Serotonin; WAY100635; Non-photic entrainment; Microdialysis; Wheel running

Locomotor activity stimulated in the mid-subjective day (rest phase) of hamsters can induce significant phase advance shifts of circadian rhythms [6]. The master pacemaker controlling rhythmicity in mammals is located in the suprachiasmatic nucleus (SCN). The SCN receive photic input via a glutamatergic pathway from the retina, and are thought to receive inputs relating to non-photic stimuli (defined here as locomotor activity) via a neuropeptide Y (NPY) pathway from the intergeniculate leaflet (IGL) and possibly also a serotonergic (5-HT) pathway from the median raphe (MR) [20]. The body of evidence supporting a role for NPY in non-photic phase resetting is internally consistent. However, the case for 5-HT is mixed [19,20]. In Syrian hamsters, extracellular 5-HT levels in the SCN increase during spontaneous nocturnal activity and during bouts of running stimulated by confinement to a novel wheel in the mid-subjective day [8]. At this circadian phase, small phase advances can be induced in this species by systemic injections of the 5-HT agonist 8-OH-DPAT [18] and by electrical stimulation of the MR [17]. In the rat SCN slice, 5-HT agonists induce large phase advances via the 5-HT₇ receptor, and similar shifts have also been reported following intraventricular injections in vivo [10,19]. Microdrop

applications of 5-HT as brief as 5 min have proven effective [14]. In mice, neurotoxic lesions that deplete SCN 5-HT prevent entrainment of circadian rhythms to daily schedules of forced or voluntary activity [13]. However, activity-induced phase shifts in hamsters are not affected by 5-HT lesions or receptor antagonists [2,6,16]. Moreover, 5-HT agonists induce either very small [7] or no phase shifts when applied directly to the hamster SCN in vivo [11,18].

The present study was designed to further assess the role of 5-HT in the induction of non-photic phase shifts in the Syrian hamster. The potent and selective 5-HT_{1A} antagonist WAY100635 [3] was used to increase endogenous 5-HT release in the SCN of hamsters at rest or during 3 h of running in a novel wheel. Changes in SCN 5-HT were assessed in a separate group using in vivo microdialysis. If 5-HT release within the SCN mediates non-photic phase shifts, then WAY100635, at doses that raise endogenous SCN 5-HT, should mimic these phase shifts and might also potentiate activity-induced shifts.

Young adult male hamsters (80–100; 100–120 g for cannulation experiments) were obtained from Charles River, (Montreal, Quebec) or raised from breeding pairs obtained from Harlan–Sprague–Dawley (Madison, IL). For behavioral testing, animals were maintained in isolation cabinets in plastic cages equipped with 17-cm running wheels connected to an interface and microcomputer that summed wheel revolutions in 10-min bins (Activity Count-

* Corresponding author. Tel.: +1-604-291-3462; fax: +1-604-291-3427.

E-mail address: mistlber@sfu.ca (R.E. Mistlberger)

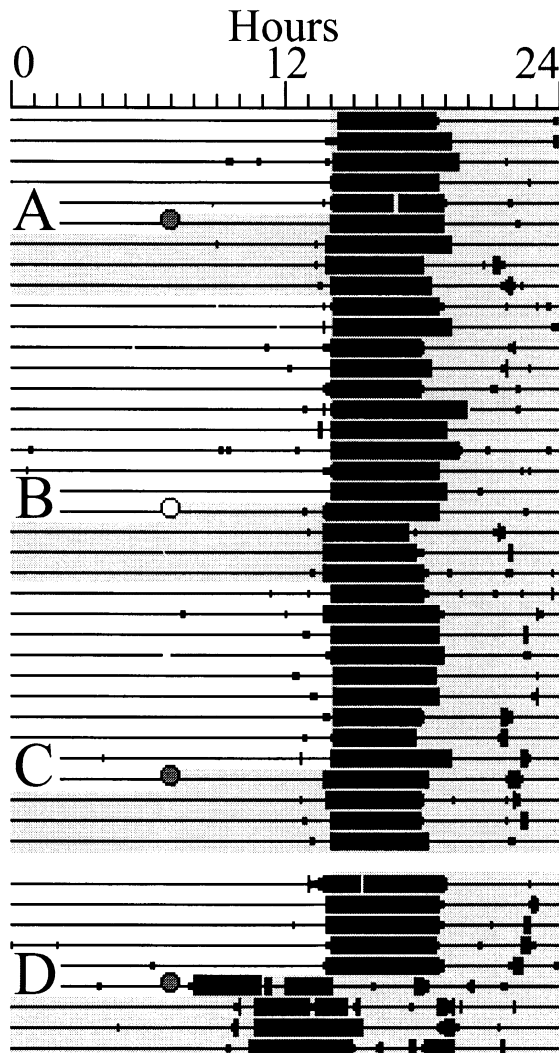


Fig. 1. Wheel running activity of a representative hamster that received i.p. injections at zt5 of (A) 10 mg/kg WAY100635 in PBS; (B) PBS vehicle control, and (C) 4.5 mg/kg WAY100635 in PBS. In these cases, constant darkness was initiated at the time of injection. (D) Wheel running activity of a representative hamster that received an i.p. injection 4.5 mg/kg WAY100635 in PBS at ZT5.5 with constant darkness and a 3 h wheel confinement both starting at ZT6. Each line of the chart represents 24 h, plotted in 10 min bins from left to right. Vertical deflections on each line indicate bins during which wheel counts were registered. Lights-out is indicated by shading.

ing System, Simon Fraser University). Food and water were available ad libitum under LD 14:10 (~50 lux:0 lux).

A modified Aschoff type II design was used to test the phase shifting properties of WAY100635 (Wyeth Ayerst) at zeitgeber time (ZT) 5 or ZT8 (i.e. 7 or 4 h before normal lights-out, where lights-out is designated ZT12, by convention). Animals were entrained to the LD cycle for 10 days prior to each manipulation. On the test day, the drug or vehicle control phosphate-buffered saline (PBS) was administered at the appropriate phase after which the lights were turned out for the next 3 full days. To quantify phase shifts,

activity onset (the first 10-min bin with 50 counts following 4 h below this threshold) on the second full day of DD was compared to the average onset during the last 5 days of LD.

Two sets of 12 animals were used to test doses of 0, 4.5, and 10 mg/kg of WAY100635, one set for each of the two phases tested. At ZT8, mean phase shifts ($\pm$ SD) to these doses were 18 ± 18 , 13 ± 16 min and 16 ± 15 min, respectively ($F_{2,21} = 0.295$, $P = 0.747$). At ZT5, mean phase shifts were 10 ± 7 , 9 ± 9 and 16 ± 15 min, respectively ($F_{2,21} = 1.163$, $P = 0.332$; Fig. 1). In another set of 16 animals doses of 0.16 and 0.45 mg/kg also did not induce significant shifts (14 ± 23 and 22 ± 32 min, respectively; $P > 0.1$ compared to PBS).

At ZT5, extracellular 5-HT levels in the SCN were significantly elevated for 20 min following the 4.5 mg/kg dose ($F_{3,16} = 41.9$, $P = 0.0001$), and for 40 min following the 10 mg/kg dose ($F_{5,20} = 6.863$, $P = 0.0007$; Fig. 2). In some animals, baseline levels were not restored until after 60 min, post-injection.

Confinement of hamsters to novel running wheels for 3 h beginning at ZT5 induced phase advance shifts of 146 ± 51 min. Pretreatment with WAY100635 at doses of 0.16, 0.45 and 4.5 mg/kg had no significant effect on shift size ($F_{4,29} = 2.37$, $P > 0.05$; Fig. 1D) or on the amount of running ($F_{4,29} = 2.07$, $P > 0.1$). There was a trend for smaller shifts and less activity in the drug conditions, but the dose-response curve relating shift size to the amount of running was not altered.

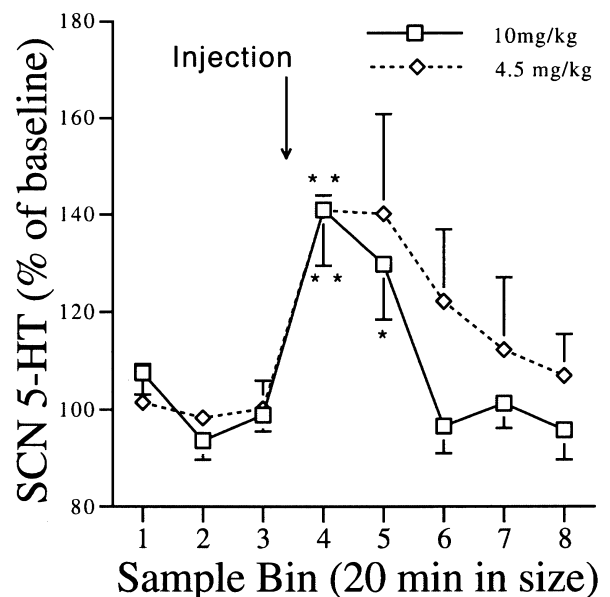


Fig. 2. Mean levels ($\pm$ SEM) of extracellular 5-HT in the region of the SCN after treatment with WAY100635 at doses of 4.5 mg/kg or 10 mg/kg as assessed by microdialysis. Levels are expressed as % of baseline, which was assessed during the hour prior to administration of WAY100635 (i.e. during bins 1–3). **above the plot = $P < 0.01$ compared to baseline for the dose of 4.5 mg/kg. **below the plot = $P < 0.01$ compared to baseline for the dose of 10 mg/kg. *below the plot = $P < 0.05$ compared to baseline for the dose of 10 mg/kg.

Wheel-running activity during the mid-subjective day in hamsters is associated with a significant (~35%) increase in 5-HT levels in the SCN [8], and with large phase advances in the circadian rhythm of locomotor activity (e.g. [2,4–6,16,19]). 5-HT agonists can also induce phase advance shifts, both in vivo and in vitro, suggesting that phase shifts to locomotor activity may be mediated by endogenous 5-HT release in the SCN. The results of the present study, however, do not support this hypothesis. At doses of 4.5 and 10.0 mg/kg, WAY100635 increased endogenous 5-HT in the SCN for 40 min (and in some individuals for 60 min), presumably by blocking inhibitory raphe 5-HT_{1A} autoreceptors [3]. However, the drug, tested over a wide range of doses at two circadian phases, did not induce phase shifts. In addition, the drug did not potentiate or inhibit the phase shift response to running in a novel cage. Neither the mean shift nor the dose-response relation between shifting and activity level were altered by pretreatment with WAY100635. These results indicate that, at least in Syrian hamsters, endogenous serotonergic activity within the SCN in the mid-subjective day is not sufficient to alter circadian phase and likely does not mediate phase shifts induced by wheel running.

One interpretation of these results is that while WAY100635 may induce significant 5-HT release in the SCN pacemaker, it may also block post-synaptic receptors in the SCN that are necessary for phase shifting. However, this is considered unlikely, because WAY100635 has a high specificity for 5-HT_{1A} receptors, and other antagonists of 5-HT_{1A} do not block 5-HT-induced phase shifts in vitro [3,12]. The critical receptor for serotonergic circadian phase advancing is thought to be the 5-HT₇ subtype [12].

Another interpretation of these results is that SCN 5-HT release in response to WAY100635 was inadequate in magnitude and/or duration to induce phase shifting. However, the magnitude of the increase in SCN 5-HT induced by the two highest doses was comparable to the magnitude of 5-HT release during electrical stimulation of the median raphe [9] and during running in a novel wheel [8]. Sixty-min bouts of these stimuli in the mid-subjective day are sufficient to induce significant phase shifts [5,17], and large shifts can be induced in vitro by bath applications of 5-HT as short as 5 min [15]. Thus, both the magnitude and the duration of 5-HT release in the SCN either matches or exceeds the parameters of 5-HT release associated with complex non-photoc stimuli (running or electrical stimulation) that do produce phase shifts.

The data reported here are compatible with the results of two previous studies showing that intra-SCN microinjections of the 5HT_{1A/7} agonist 8-OH-DPAT in the mid-subjective day do not induce significant phase shifts of hamster rhythms in vivo [11,18]. In one of those studies, the biological activity of 8-OH-DPAT and the accuracy of the cannula placements were confirmed by demonstrating that phase shifts to light pulses in the subjective night could be attenuated by injections of 8-OH-DPAT through

the same cannulae that were used to assess the phase shifting effects of the drug in the subjective day [11]. 8-OH-DPAT and 5-HT applied to SCN slices in the mid-subjective day in vitro typically induce very large, activity-like phase advances in the peak of the circadian rhythm of SCN neuronal firing rate [21]. This discrepancy between the results of in vivo and in vitro studies may reflect 5-HT supersensitivity in the slice preparation, caused by loss of 5-HT afferents to the SCN. Our preliminary evidence indicates that phase shifts to intra-SCN 8-OH-DPAT are larger in animals pretreated with p-CPA to induce 5-HT receptor supersensitivity in vivo [11].

There is one report that microinjections of 8-OH-DPAT to the SCN of hamsters in the mid-subjective day can induce significant phase advance shifts in vivo [7]. However, these shifts were only about 1/10 the size of shifts induced by 8-OH-DPAT pulses in vitro [21]. The use of bilateral, as opposed to unilateral, injection procedures does not likely explain the different results across in vivo studies, because unilateral microinjections of 8-OH-DPAT in vivo diffuse throughout both SCN bilaterally within 10 min [22]. Regardless of the different statistical outcomes of the in vivo studies, the combined results highlight the differential sensitivity of the SCN pacemaker to serotonergic stimuli in vivo and in vitro. Species difference may also contribute to these discrepancies, as most of the in vivo work has utilized Syrian hamsters, while in vitro work has used rat SCN tissue slices.

The failure of WAY100635 to induce phase shifts despite stimulating SCN 5-HT release has implications for interpreting the mechanism by which electrical stimulation of the MR induces phase advance shifts in the subjective day. The MR is the sole source of 5-HT input to the SCN in Syrian hamsters. However, at least 50% of the MR pathway is non-serotonergic. Electrical stimulation presumably activates the entire pathway, and the present results suggest that non-serotonergic fibers (e.g. γ -aminobutyric acid containing) may mediate phase shifting, either by direct actions on the SCN, or by modulating other inputs to the SCN. This is consonant with evidence that loss of non-serotonergic cells may account for some of the effects of MR ablation on circadian rhythms in hamsters [15].

Systemic 8-OH-DPAT may also induce phase shifts by modulating non-serotonergic inputs to the SCN. 8-OH-DPAT activates raphe somatodendritic autoreceptors, and thereby inhibits MR and dorsal raphe (DR) 5-HT neuronal activity [3]. If the DR inhibits the IGL, then 8-OH-DPAT would disinhibit IGL neurons and increase the release of NPY in the SCN, as proposed by Mintz et al. [18]. This NPY pathway has been strongly implicated as necessary for non-photoc shifting in hamsters [1,4]. The results of the present study demonstrate that if IGL disinhibition is the route by which systemic 8-OH-DPAT induces phase shifts in hamsters, then the relevant 5-HT receptor in the IGL cannot be of the 5-HT_{1A} subtype, because WAY100635 blocks this receptor. If tonic IGL inhibition was mediated by

this receptor, then WAY100635 alone should induce a non-photopic type phase shift by transiently releasing these cells from serotonergic inhibition.

In closing, systemic administration of the selective 5-HT_{1A} antagonist WAY100635 significantly raises SCN 5-HT levels, but alters neither circadian phase nor the phase-shifting effect of scheduled wheel running. If a prolonged elevation of 5-HT is necessary to evoke phase shifts, then WAY100635 is of limited utility, because its effects on 5-HT release, at even high doses, do not exceed 40–60 min. Finally, the recently-reported phase-shifting effects of raphe nucleus electrical stimulation [17] may not be entirely, or at all, dependent upon 5-HT release alone, because although brief raphe stimulation and WAY100635 have comparable effects on SCN 5-HT, the latter treatment does not induce phase shifts.

Supported by NIH (NS35229, JDG) and NSERC, Canada (REM, MCA). We are grateful to Tom Dudley for technical assistance.

- [1] Albers, H.E. and Ferris, C.F., Neuropeptide Y: role in light-dark cycle entrainment of hamster circadian rhythms. *Neurosci. Lett.*, 50 (1984) 163–168.
- [2] Antle, M.C., Marchant, E.G., Niel, L. and Mistlberger, R.E., Serotonin antagonists do not attenuate activity-induced phase shifts of circadian rhythms in the Syrian hamster. *Brain Res.*, 813 (1998) 139–149.
- [3] Assie, M.B. and Koek, W., Effects of 5-HT_{1A} receptor antagonists on hippocampal 5-hydroxytryptamine levels: (S)-WAY100135, but not WAY100635, has partial agonist properties. *Eur. J. Pharmacol.*, 23 (1996) 15–21.
- [4] Biello, S.M., Janik, D. and Mrosovsky, N., Neuropeptide Y and behaviorally induced phase shifts. *Neuroscience*, 62 (1994) 273–279.
- [5] Bobrzynska, K.J. and Mrosovsky, N., Phase shifting by novelty-induced running: activity dose-response curves at different circadian times. *J. Comp. Physiol. A*, 182 (1998) 251–258.
- [6] Bobrzynska, K.J., Vrang, N. and Mrosovsky, N., Persistence of non-photopic phase shifts in hamsters after serotonin depletion in the suprachiasmatic nucleus. *Brain Res.*, 741 (1996) 205–214.
- [7] Challet, E., Scarbrough, K., Penev, P.D. and Turek, F.W., Roles of suprachiasmatic nuclei and intergeniculate leaflets in mediating the phase-shifting effects of a serotonergic agonist and their photic modulation during subjective day. *J. Biol. Rhythms*, 13 (1998) 410–421.
- [8] Dudley, T.E., DiNardo, L.A. and Glass, J.D., Endogenous regulation of serotonin release in the hamster suprachiasmatic nucleus. *J. Neurosci.*, 18 (1998) 5045–5052.
- [9] Dudley, T.E., Dinardo, L.A. and Glass, J.D., In vivo assessment of the midbrain raphe nuclear regulation of serotonin release in the hamster suprachiasmatic nucleus. *J. Neurophysiol.*, 81 (1999) 1469–1477.
- [10] Edgar, D.M., Miller, J.D., Prosser, R.A., Dean, R.R. and Dement, W.C., Serotonin and the mammalian circadian system: II. Phase-shifting rat behavioral rhythms with serotonergic agonists. *J. Biol. Rhythms*, 8 (1993) 17–31.
- [11] Ehlen, J.C., Antle, M., Mistlberger, R.E. and Glass, J.D., Phase shifting effects of timed in vivo microdialysis perfusion of the SCN with 8-OH-DPAT. *Soc. Neurosci. Abstr.*, 25 (1999) 1888.
- [12] Lovenberg, T.W., Baron, B.M., de Lecea, L., Miller, J.D., Prosser, R.A., Rea, M.A., Foye, P.E., Racke, M., Slone, A.L., Siegel, B.W., Danielson, P.E., Sutcliffe, J.G. and Erlander, M.G., A novel adenylyl cyclase-activating serotonin receptor (5-HT₇) implicated in the regulation of mammalian circadian rhythms. *Neuron*, 11 (1993) 449–458.
- [13] Marchant, E.G., Watson, N.V. and Mistlberger, R.E., Both neuropeptide Y and serotonin are necessary for entrainment of circadian rhythms in mice by daily treadmill running schedules. *J. Neurosci.*, 17 (1997) 7974–7987.
- [14] Medanic, M. and Gillette, M.U., Serotonin regulates the phase of the rat suprachiasmatic circadian pacemaker in vitro only during the subjective day. *J. Physiol. (Lond.)*, 450 (1992) 629–642.
- [15] Meyer-Bernstein, E.L. and Morin, L.P., Differential serotonergic innervation of the suprachiasmatic nucleus and the intergeniculate leaflet and its role in circadian rhythm modulation. *J. Neurosci.*, 16 (1996) 2097–2111.
- [16] Meyer-Bernstein, E.L. and Morin, L.P., Destruction of serotonergic neurons in the median raphe nucleus blocks circadian rhythm phase shifts to triazolam but not to novel wheel access. *J. Biol. Rhythms*, 13 (1998) 494–505.
- [17] Meyer-Bernstein, E.L. and Morin, L.P., Electrical stimulation of the median or dorsal raphe nuclei reduces light-induced FOS protein in the suprachiasmatic nucleus and causes circadian activity rhythm phase shifts. *Neuroscience*, 92 (1999) 267–279.
- [18] Mintz, E.M., Gillespie, C.F., Marvel, C.L., Huhman, K.L. and Albers, H.E., Serotonergic regulation of circadian rhythms in Syrian hamsters. *Neuroscience*, 79 (1997) 563–569.
- [19] Mistlberger, R.E., Antle, M.C., Glass, J.D., Miller, J.D., Behavioral and serotonergic regulation of circadian rhythms. *Biol. Rhythm Res.* 31 (2000) in press.
- [20] Morin, L.P., Serotonin and the regulation of mammalian circadian rhythmicity. *Ann. Med.*, 31 (1999) 12–33.
- [21] Prosser, R.A., Dean, R.R., Edgar, D.M., Heller, H.C. and Miller, J.D., Serotonin and the mammalian circadian system: I. In vitro phase shifts by serotonergic agonists and antagonists. *J. Biol. Rhythms*, 8 (1993) 1–16.
- [22] Weber, E.T., Gannon, R.L. and Rea, M.A., Local administration of serotonin agonists block light-induced phase advances of the circadian activity rhythm in the hamster. *J. Biol. Rhythm*, 13 (1998) 209–218.