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Research Report

Neural mechanisms mediating circadian phase resetting by activation of 5-HT$_7$ receptors in the dorsal raphe: Roles of GABAergic and glutamatergic neurotransmission

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ABSTRACT

5-HT$_7$ receptors in the dorsal raphe nucleus (DRN) influence circadian rhythms, sleep, and serotonin release. Because interactions between 5-HT$_7$ receptors and glutamatergic and GABAergic neurons have been demonstrated previously, the current studies tested the hypothesis that GABAergic and/or glutamatergic neurons mediate phase shifts induced by activation of DRN 5-HT$_7$ receptors. Hamsters were fitted with guide cannulae aimed at the DRN, housed in cages with running wheels, and exposed to 14 h light (L):10 h dark (D). In Experiment 1, hamsters received DRN pretreatment with muscimol (87.6 pmol) or vehicle before DRN 8-OH-DPAT (6 pmol) microinjections at ZT6. After exposure to constant darkness (10 days), phase shifts were calculated and animals were re-exposed to 14 L:10D. The procedure was repeated to give each animal the alternate pretreatment. In Experiment 2, hamsters received DRN pretreatment with NMDA (20 pmol) or vehicle before 8-OH-DPAT at ZT 6. Other experiments tested the effects of single DRN microinjections of muscimol, bicuculline (136 pmol), NMDA, MK-801 (10 pmol) or vehicle. Phase shifts (mean ± S.E.M., h) in muscimol/8-OH-DPAT-microinjected hamsters (1.02 ± 0.30) were not different ($P = 0.11$) from those in vehicle/8-OH-DPAT-microinjected hamsters (1.34 ± 0.30), while those in NMDA/8-OH-DPAT-microinjected hamsters (0.67 ± 0.17) were smaller ($P < 0.05$) than those in vehicle/8-OH-DPAT-microinjected hamsters (0.97 ± 0.10). DRN single microinjections of bicuculline, but not muscimol, NMDA, or MK-801 induced phase advances. Bicuculline also potentiated 8-OH-DPAT-induced phase advances ($P < 0.05$). These finding suggest that the mechanism mediating DRN 5-HT$_7$ receptor induction of phase advances involves decreased glutamatergic neurotransmission, and furthermore, that inhibition of DRN GABAergic neurotransmission causes a phase advance.

1. Introduction

The 5-HT$_7$ receptors in the central nervous system modulate a range of physiological and cognitive functions, including circadian timekeeping, paradoxical (rapid eye movement [REM]) sleep, thermoregulation, memory, and affective state (Bonaventure et al., 2007; Duncan et al., 2004; Ehlen et al., 2001; Guscott et al., 2005; Hedlund et al., 2003; Monti and Jantos,

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2006; Roberts et al., 2004a; Thomas et al., 2003). Electrophysiological studies have shown that activation of 5-HT$_7$ receptors can either potentiate neuronal excitability (e.g., in the hippocampus and thalamus) (Bacon and Beck, 2000; Tokarski et al., 2003, 2005) or attenuate the responsiveness to excitatory stimuli (e.g., in the hypothalamic suprachiasmatic nucleus (SCN), the site of the master mammalian circadian pacemaker) (Bacon and Beck, 2000; Quintero and McMahon, 1999; Smith et al., 2001). Although the highest densities of 5-HT$_7$ receptor expression have been detected in hypothalamic, thalamic and limbic regions, studies in rodents and humans have revealed that these receptors are also present, albeit at lower levels, in the dorsal raphe nucleus (DRN) (Duncan and Franklin, 2007; To et al., 1995; Varnas et al., 2004), a major site of serotonergic neurons that innervate the forebrain (Jacobs and Azmitia, 1992; Molliver, 1987). Within the DRN, 5-HT$_7$ receptors regulate serotonin release, REM sleep, and circadian rhythms (Duncan et al., 2004; Monti and Jantos, 2006; Roberts et al., 2004b).

We have previously demonstrated that activation of DRN 5-HT$_7$ receptors alters the phase of circadian locomotor rhythms (Duncan et al., 2004; Duncan and Davis, 2005). For example, local microinjection of 5-HT$_{1A/7}$ receptor agonists, e.g., (±)-8-hydroxy-2-dipropylaminotetralin hydrobromide (8-OH-DPAT) or 5-carboxyimidotryptamine (5-CT), to the hamster DRN during the mid-subjective day induces advances of the circadian locomotor activity rhythm (Duncan et al., 2004; Mintz et al., 1997). This treatment mimics the circadian phase shifting effects of electrical stimulation of the DRN (Meyer-Bernstein and Morin, 1999) and other nonphotic stimuli such as systemic administration of serotonergic drugs or benzodiazepines, and behavioral conditions, including presentation of a novel wheel or sleep deprivation (Antle and Mistlberger, 2000; Turek and Losee-Olson, 1986; Van Reeth et al., 1994). The 5-HT$_7$ receptors mediate the phase advances induced by DRN microinjection of serotonergic drugs because these phase advances can be blocked by DRN co-administration of the selective 5-HT$_7$ receptor antagonist SB-269970 (at doses as low as 50 nanomolar) or by RP-cAMP, an antagonist to cyclic AMP, which is the intracellular second messenger to which 5-HT$_7$ receptors are positively coupled (Bard et al., 1993; Duncan et al., 2004; Duncan and Davis, 2005; Plassat et al., 1993; Ruat et al., 1993). DRN microinjections of 8-bromo-cAMP also induce circadian phase advances (Duncan and Davis, 2005). Furthermore, DRN microinjection of another 5-HT$_7$ receptor antagonist, DR4004, attenuates phase advances stimulated by presentation of a novel running wheel (Glass et al., 2003). Thus, activation of DRN 5-HT$_7$ receptors is sufficient to induce nonphotic phase shifts, and these receptors are necessary for at least one type of nonphotically-induced phase shift.

In contrast to the identification of cAMP as the intracellular signal linked to 5-HT$_7$ receptor activation, the neural mechanisms mediating the effect of DRN 5-HT$_7$ receptor activation on circadian phase shifts or other functions are not well understood. A role for GABA neurons is suggested by findings that the activation of DRN GABA-A receptors attenuates novel wheel induced phase shifts (Glass et al., 2003). Also, changes in either GABAergic or glutamatergic neurotransmission have been reported to mediate the effect of 5-HT$_7$ receptor activation on serotonin release in the DRN in vitro (Harsing et al., 2004; Roberts et al., 2004b). Therefore, we tested the hypotheses that circadian phase shifts induced by activation of DRN 5-HT$_7$ receptors are mediated by (1) GABAergic neurotransmission or (2) glutamatergic neurotransmission. We also investigated whether alterations in GABAergic or glutamatergic neurotransmission alone would alter circadian phase.

2. Results

2.1. *General results*

There was variability among the experiments in the mean phase shifting effects of vehicle (ranging from 0.57 h [Expt. 1b & 2b] to 0.88 h [Expt. 2c]) or 8-OH-DPAT (ranging from 0.97 h [Expt. 1c] to 1.34 h [Expt. 1a]). Based on the within-subjects, randomized order design, the data from each experiment were analyzed using a paired *t*-test to identify significant drug effects. Acute behavioral effects of the drug administration were observed in a few cases, as noted for individual experiments.

2.2. *Investigations of the role of GABA neurotransmission on phase shifts induced by DRN microinjection of 8-OH-DPAT*

2.2.1. *Extp. 1a*

DRN microinjection of 8-OH-DPAT (6 pmol) at zeitgeber time 6 (ZT 6, i.e., 6 h before normal time of lights-off) induced robust phase advances (~ 1.3 h on average) as reported previously (Duncan et al., 2004; Duncan and Davis, 2005), that were not significantly affected by pretreatment of the DRN with the GABA-A receptor agonist, 5-aminomethyl-3-hydroxyisoaxozole (muscimol, 87.6 pmol) ($P=0.110$) (Fig. 1).

2.2.2. *Expt. 1b*

Phase shifts induced by DRN microinjection of muscimol alone were not significantly different from those induced by vehicle alone (Mean ± S.E.M., muscimol: 0.41 ± 0.29 h; vehicle: 0.85 ± 0.20 h; $P=0.14$).

2.2.3. *Expt. 1c*

DRN microinjections of the GABA-A receptor antagonist, R-(R*S*)-5-6-(6,8-dihydro-8-oxofuro[3,4,e-]-1,3-benxodioxol-6-yl)-5,6,7,8-tetrahydro-6,6-dimethyl-1,3-dioxolo[4,5-g]isoqinolinium chloride, (bicuculline, 136 pmol) induced phase advances that were nearly twice as large as those induced by vehicle ($P<0.05$) (Fig. 2). Five of the nine hamsters that received a microinjection of bicuculline exhibited immediate behavioral effects, e.g., vocalization, running around the cage, and jumping, that lasted for up to 10 min, but their phase shifts were not different from those of the other four bicuculline-injected hamsters (phase shifts [h]: behavioral expression, 0.87 ± 0.32 [$N=5$]; no behavioral expression, 1.16 ± 0.39 [$N=4$]; $P=0.293$).

2.2.4. *Expt. 1d*

DRN microinjections of bicuculline mixed with 8-OH-DPAT induced phase shifts that were significantly larger than