Two Distinct Serotonin Receptors Co-mediate Non-photic Signals to the Circadian Clock

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Abstract. Several lines of evidence indicate that serotonin type 7 (5-HT7) receptors play a critical role for non-photic resetting of the mammalian circadian clock; however, the contributions of other types of 5-HT receptors to non-photic entrainment are not yet clarified. The present study demonstrates that MKC-242, a selective 5-HT1A receptor agonist, can evoke a non-photic-like phase-response in hamsters in vivo. This phase-shifting response to MKC-242 was antagonized not only by the selective 5-HT1A receptor blocker WAY100635 but also by the selective 5-HT7 receptor blocker DR4004. These suggest that synchronous activation of 5-HT1A and 5-HT7 receptors mediates non-photic signals to the hamster circadian clock.

Keywords: circadian clock, serotonin, non-photic

The suprachiasmatic nucleus (SCN) contains a master pacemaker responsible for various circadian functions (1). This pacemaker maintains synchrony with the external environment by receiving photic signals sent from the retina during subjective night (1). Non-photic stimuli, such as scheduled exercise or sleep deprivation, also can reset the circadian pacemaker (2). When non-photic manipulations are presented during subjective day, the midbrain raphe nuclei (RN) and the thalamic intergeniculate leaflet (IGL) conveys non-photic information to the pacemaker in a serotonergic activity–dependent manner, leading to the phase-shifting of circadian rhythms (3, 4).

It is well-documented that serotonergic clock-resetting is dependent on activity of serotonin type 7 (5-HT7) receptors (5). In studies using knockout (KO) mice, several reports show that serotonergic resetting is attenuated in 5-HT7 KO mice (6, 7); however, another report shows that 5-HT7 KO mice exhibit a normal restoring response to non-photic stimuli in the SCN firing activity rhythms (8). Interestingly, pharmacological blockade of 5-HT1A receptors almost completely inhibits the serotonergic phase shift in 5-HT7 KO mice (6, 8). Lack of 5-HT1A receptors abolishes a 5-HT1A/7 receptor agonist–induced phase advance in the mouse wheel-running activity rhythm, suggesting that 5-HT1A receptors participate in serotonergic clock-resetting (9). This is in contrast to previous studies (5).

A recent study reported that 5-HT7 receptors form heterodimers with 5-HT1A receptors both in vitro and in vivo and that the heterodimerization reduces the 5-HT1A receptor–mediated signaling (10). This finding raises the possibility that absence of 5-HT1A or 5-HT7 receptors alters phase responses to non-photic stimuli through the functional changes in respective heterodimerizing partners. Therefore, to avoid this issue, the present study was undertaken by using hamsters to assess the role of 5-HT1A receptors in the transmission of non-photic signals in vivo.

Four-week-old male hamsters were obtained from Takasugi Laboratory Animals Co., Ltd. (Saitama). All manipulations were approved by the Experimental Animal Welfare Committee at Waseda University and were conducted in accordance with the Law (No. 105) and Notification (No. 6) of the Japanese Government. After 2-week acclimatization to our laboratory conditions (12-h light–12-h dark cycle, lights on at 8:30, light intensity of 100 – 150 lux at cage level, room tempera-
ture of 23°C ± 2°C), they were housed singly in a transparent plastic cage (36 × 20 × 20 cm) containing a 13-cm-diameter running wheel and a micro-switch to monitor circadian wheel-running activity. The number of wheel rotations was recorded by the automated system. Food and water were available ad libitum. After free-running rhythms were achieved in constant darkness, the effect of serotonergic agonists was evaluated on wheel-running activity rhythms at various circadian times (CT; CT12 is defined as the onset time of wheel-running activity). Hamsters were intraperitoneally injected with vehicle or serotonergic agents and then kept in their individual cages for an additional 7 – 14 days. In the antagonism experiments, they were intraperitoneally injected with serotonergic antagonists 10 – 15 min prior to the administration of serotonergic agonists. The phase shift of wheel-running activity rhythm was quantitatively estimated as previously described (11). Data were analyzed using one-way ANOVA followed by the Tukey-Kramer test or the Student’s t-test. Veh: vehicle, MKC: MKC-242, R-DPAT: (R)-8-OH-DPAT, TSP: tandospirone, WAY: WAY100635, DR: DR4004.

Fig. 1. Effect of MKC-242 on the hamster wheel-running activity rhythm under constant darkness. A) Representative actograms show wheel-running activity records of hamsters injected with (a) vehicle, (b) MKC-242 (3.0 mg/kg) and MKC-242 (3.0 mg/kg), and (d) DR4004 (10 mg/kg) and MKC-242 (3.0 mg/kg) at CT6 (arrowheads in the figure). B) Treatment with MKC-242 at CT6 induced a dose-dependent phase advance (**P < 0.01 vs. vehicle). C) Phase-response curve for MKC-242 or R(+)-8-OH-DPAT. The open box and its shaded area represent a sensitive time to R(+)-8-OH-DPAT (CT2-8: **P < 0.01 vs. vehicle) and MKC-242 (CT2-6: **P < 0.01 vs. vehicle), respectively. Vehicle: open circles, n = 8 – 13. MKC-242: 3.0 mg/kg, closed circles, n = 4 – 13. R(+)-8-OH-DPAT: 2.5 mg/kg, open squares, n = 4 – 9. D) MKC-242-induced phase advance (3.0 mg/kg, **P < 0.01 vs. vehicle) was attenuated by WAY100635 or DR4004 in a dose-dependent manner (**P < 0.01 vs. MKC-242). E) Tandospirone-induced phase advance (30 mg/kg, **P < 0.01 vs. vehicle) was blocked by WAY100635 (10 mg/kg) or DR4004 (10 mg/kg) (**P < 0.01 vs. tandospirone). Numbers in parentheses indicate the number of experiments. For statistical analysis, one-way ANOVA followed by the Tukey-Kramer test or the Student’s t-test was applied.
expressed as means ± S.E.M. These values were analyzed with Student’s t-test or a one-way analysis of variance (ANOVA) followed by the Tukey-Kramer test. A P-value of < 0.05 implied statistical significance.

The following drugs were used in this study: MKC-242 (osemozotan; Mitsubishi Tanabe Pharma Co., Tokyo); racemic, (R)- and (S)-8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) (Sigma, St. Louis, MO, USA); tandospirone (sediel; Dainippon Sumitomo Pharma Co., Ltd., Tokyo); triazolam (Sigma); WAY100635 (Meiji Seika Pharma Co., Ltd., Tokyo); DR4004 (Meiji Seika). MKC-242 and tandospirone were suspended in 0.5% carboxymethyl cellulose solution (Tokyo Kasei, Tokyo). Triazolam was dissolved in dimethyl sulfoxide (Wako, Osaka). DR4004 was dissolved in 1% Tween 80 (Nacalai Tesque, Kyoto). Other drugs were dissolved in saline.

To determine whether activation of 5-HT1A receptors causes circadian phase-resetting, we assessed the effect of a selective the 5-HT1A receptor agonist MKC-242 on the hamster wheel-running activity rhythm. Representative wheel-running activity records of hamsters injected with the serotonergic agonist (and its blockers) or vehicle at CT6 are shown in Fig. 1A. Administration of MKC-242 at CT6 induced a phase advance in a dose-dependent manner (Fig. 1B). The phase advance part of the phase–response curve (PRC) to MKC-242 (3.0 mg/kg) showed a narrow circadian time, but the present PRC was similar to that established by the principal 5-HT1A7 receptor agonist R(+)-8-OH-DPAT (2.5 mg/kg) (Fig. 1C). Thus, activation of 5-HT1A receptors can reset the circadian clock of hamsters in vivo. The phase-advancing action of MKC-242 was dose-dependently attenuated by pretreatment with not only the selective 5-HT1A receptor blocker WAY100635 but also the selective 5-HT7 receptor blocker DR4004 (Fig. 1D), suggesting that this clock-resetting occurs through indirect activation of 5-HT7 receptors because MKC-242 possesses almost no direct agonistic activity at 5-HT7 receptors (12, 13). Similarly, a daytime (CT6) injection of tandospirone (30 mg/kg), a partial 5-HT1A receptor agonist, evoked a phase advance, which was blocked by WAY100635 (10 mg/kg) or DR4004 (10 mg/kg) (*P < 0.05, **P < 0.01 vs. triazolam). Treatment with WAY100635 or DR4004 alone did not cause any phase shifts.

Fig. 2. Blockade of either 5-HT1A or 5-HT7 receptors suppresses a phase-resetting response to 8-OH-DPAT or triazolam. A – C) The phase-advancing action of each 8-OH-DPAT enantiomer was observed when administered at CT6 (**P < 0.01 vs. vehicle). Injection of R(+)-8-OH-DPAT (2.5 mg/kg) (A) at CT6 induced a larger phase advance than that of racemic (5.0 mg/kg) (*P < 0.05 vs. R(+)-8-OH-DPAT) (B) or S(−)-8-OH-DPAT (5.0 mg/kg) (*P < 0.05 vs. R(+)-8-OH-DPAT) (C). The phase advance by each 8-OH-DPAT enantiomer was inhibited by DR4004 (10 mg/kg) or WAY100635 (10 mg/kg) [*P < 0.05, **P < 0.01 vs. R(+)-8-OH-DPAT (A), racemic 8-OH-DPAT (B), or S(−)-8-OH-DPAT (C)]. D) Triazolam-induced phase advance (20 mg/kg, **P < 0.01 vs. vehicle) was blocked by WAY100635 (10 mg/kg) or DR4004 (10 mg/kg) (*P < 0.05, **P < 0.01 vs. triazolam). Numbers in parentheses indicate the number of experiments. For statistical analysis, one-way ANOVA followed by the Tukey-Kramer test or Student’s t-test was applied. Veh: vehicle, R-DPAT: R(+)-8-OH-DPAT, R,S-DPAT: racemic 8-OH-DPAT, S-DPAT: S(−)-8-OH-DPAT, TRZ: triazolam, WAY: WAY100635, DR: DR4004.
(5). The phase-resetting by each 8-OH-DPAT enantiomer was prevented not only with DR4004 (10 mg/kg) but also with WAY100635 (10 mg/kg) (Fig. 2: A – C). These findings suggest that all of its enantiomers exert each phase-advancing action via a 5-HT-responsive multi-synaptic pathway and that the activation of 5-HT_{1A} as well as 5-HT_{7} receptors is a key step in serotonergic phase-resetting processes. Blockade of either 5-HT_{1A} or 5-HT_{7} receptors completely suppressed a phase-resetting response to triazolam (20 mg/kg), a short-acting benzodiazepine, as well (Fig. 2D). Thus, co-activation of 5-HT_{1A} and 5-HT_{7} receptors also contributes to GABA_{A} receptor–mediated phase shifts in vivo.

The present report offers interesting insights into the neural events that convey non-photic information with serotonergic activity. In our study, the findings revealed that activation of 5-HT_{1A} receptors causes clock-resetting under constant darkness, and, in addition, is a key step in serotonergic phase-resetting processes. This clock-resetting is accompanied by activation of 5-HT_{7} receptors. This is the first report that 5-HT_{1A} and 5-HT_{7} receptors co-mediate non-photic signals under constant darkness. On the other hand, these two kinds of receptors mediate GABAergic phase shifts as well. This supports previous reports showing that the serotonergic afferents to the SCN and IGL are necessary for GABAergic clock-resetting (4) and suggests that 5-HT_{1A} and 5-HT_{7} receptors co-regulate serotonergic activity to relay GABAergic signals in vivo.

It is well-known that \( R^{(+)} \)-8-OH-DPAT resets the circadian clock by activating 5-HT_{7} receptors (5, 11). Several studies have suggested the dorsal and median RN and the IGL (rather than the SCN) as the possible target sites for \( R^{(+)} \)-8-OH-DPAT (12, 13), which seems to cause differences in chronobiotic actions between MKC-242 and \( R^{(+)} \)-8-OH-DPAT–induced phase-resetting in vivo. On the other hand, the target area(s) and mechanism for MKC-242–induced phase advance are unclear. As shown in Fig. 1C, the phase-advancing action of \( R^{(+)} \)-8-OH-DPAT is most robust at CT8, which is blocked by WAY100625 or DR4004 (data not shown). In contrast, treatment with MKC-242 at CT8 has no impact on wheel-running activity rhythm. This might mean that MKC-242 uses a different pathway(s) and/or mechanism from that of \( R^{(+)} \)-8-OH-DPAT to induce phase shifts. In fact, the \( K_{i} \) values of MKC-242 for 5-HT_{1A} and 5-HT_{7} receptors are 0.35 nM and > 100 nM, respectively (12, 13), which seems to cause differences in chronobiotic actions between MKC-242 and \( R^{(+)} \)-8-OH-DPAT. Our previous study indicates that, unlike \( R^{(+)} \)-8-OH-DPAT, MKC-242 potentiates photic entrainment by acting possibly on 5-HT_{1A} autoreceptors in the RN (13). More-over, MKC-242 attenuates triazolam-induced phase advance via presynaptic 5-HT_{1A} receptors, suggesting that MKC-242 and \( R^{(+)} \)-8-OH-DPAT have different action on GABAergic neurotransmission (14). However, the present study can not rule out the possibility that MKC-242 and \( R^{(+)} \)-8-OH-DPAT act on a common neural pathway(s) to convey non-photic information. Therefore, further studies are needed to clarify this issue.

It has been reported that systemic administration of 8-OH-DPAT causes a large non-photic phase shift in behavioral activity by activating 5-HT_{1A} receptors on the SCN when exposed to short-term constant light (15). This phase-shifting is associated with the decreased 5-HT release in the SCN and is not blocked by 5-HT_{7} receptor antagonists. In our experimental conditions (constant darkness), 8-OH-DPAT exerts its phase-shifting action via co-activation of 5-HT_{1A} and 5-HT_{7} receptors in vivo. Thus, circadian-related serotonergic signals might be mediated to the circadian clock through a distinct pathway(s) in response to environmental lighting conditions.

In conclusion, the present study demonstrates that serotonergic or GABAergic stimuli elicit non-photic phase shifts via co-activation of 5-HT_{1A} and 5-HT_{7} receptors under constant darkness. This is the first report that two distinct 5-HT receptors co-operatively mediate non-photic signals to the hamster circadian clock in vivo.

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