Short Communication

Inhibitory Action of 5-HT_{1A} Agonist MKC-242 on Triazolam-Induced Phase Advances in Hamster Circadian Activity Rhythms

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Abstract. Mammalian circadian rhythms can be entrained by photic and non-photic stimuli. Although we know that non-photic entrainment interferes with photic entrainment signals, there is no information about whether photic entrainment interferes with non-photic entraining signals. We examined whether triazolam (TRZ), a non-photic form of entrainment, could be attenuated by pre-treatment with (*S*)-5-[3-[(1,4-benzodioxan-2-ylmethyl)amino]propoxy]-1,3-benzodioxole hydrochloride (MKC-242), a photic enhancing drug. We found that TRZ-induced phase advances in hamster behavioral circadian rhythms and the increase of locomotor activity were both significantly attenuated by MKC-242. Thus, in hamsters, the photic enhancing drug MKC-242 seemed to hinder benzodiazepine-induced non-photic entrainment.

Keywords: circadian rhythm, non-photic, benzodiazepine

Mammalian circadian rhythms are generated and maintained by a biological clock located in the supra-chiasmatic nucleus (SCN) of the hypothalamus (1, 2). While external photic signals during subjected night strongly reset the central clock, other kinds of non-photic stimuli, such as novel wheel-running or sleep deprivation, produce phase advances in the circadian pacemaker when performed during subjective day (1 – 3).

The SCN receives neuronal input in the form of serotonin (5-HT) from the midbrain raphe nuclei, the location of one of the highest concentrations of 5-HT in the forebrain (4). A wide variety of experiments indicate the involvement of 5-HT in both the response of SCN neurons to light and in the phase-resetting properties of non-photic stimuli. For example, daytime injection of 8-hydroxy-dipropylaminotetralin (8-OH-DPAT), an agonist of both 5-HT_{1A} and 5-HT₇ receptors, produces a phase advance in hamster behavioral circadian rhythms (5). In addition to 8-OH-DPAT, injection of benzodiaz-

epine drugs such as triazolam (TRZ) and brotizolam during the mid-subjective day causes phase advances in behavioral circadian rhythms (6, 7). Since a TRZ-induced phase shift is attenuated by lesion of the midbrain raphe nucleus (6), the 5-HT mechanism should be involved in the TRZ-induced phase shift. Brotizolam and 8-OH-DPAT reduce *Per1* and *Per2* gene expression in the hamster SCN (7, 8), suggesting that TRZ may cause a phase shift through the acute reduction of *Per1* and *Per2* gene expression in the SCN. Interestingly, light-induced *Per1* and *Per2* gene expression in the SCN is attenuated by pre-treatment with brotizolam and 8-OH-DPAT (7). Thus, non-photic signals seem to interfere with a light-induced phase shift.

On the other hand, it has yet to be elucidated whether photic entraining signals interfere with non-photic entrainment. As non-photic entrainment is carried out during the daytime (light period), it is hard to use light exposure as the photic stimulus. Recently, we demonstrated that (*S*)-5-[3-[(1,4-benzodioxan-2-ylmethyl)amino]propoxy]-1,3-benzodioxole hydrochloride

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(MKC-242), a selective 5-HT_{1A}-receptor agonist, augmented photic entrainment of the hamster wheel-running rhythm and light-induced *Per1* and *Per2* gene expression in the SCN (9, 10). Therefore, we examined the effect of pre-treatment with MKC-242, a photic enhancer, on TRZ-induced phase advances in hamster wheel-running rhythm.

Male golden hamsters (*Mesocricetus auratus*) (Tokyo Laboratory Animal Science Co., Ltd., Tokyo) weighing between 120–200 g were used for this study. All animals were maintained under controlled environmental conditions ($23 \pm 2^\circ\text{C}$ room temperature; 12 h – 12 h light-dark cycle, lights on at 08:30) for at least 2 weeks before use in our experiments. There was an approximately 200 lux light intensity at the level of the animal cage. Food and water were provided ad libitum. Animals were treated in accordance with the guidelines for experimental animal care at the School of Human Sciences, Waseda University. To assess wheel running activity, hamsters were housed individually in transparent plastic cages ($36 \times 20 \times 20$ cm) equipped with a running wheel, 15 cm in diameter. The running wheel closed a micro-switch with each revolution so that the number of wheel rotations could be measured. Data was stored on a personal computer. For free-running conditions under constant darkness, circadian time (CT) was used instead of ZT, and CT12 referred to the onset of wheel-running.

All drugs were freshly prepared before the experiment. MKC-242 (a gift from Mitsubishi Chemical Co., Tokyo) was suspended in 0.5% carboxymethylcellulose (CMC) at a dose of 0.1, 1.0, or 3.0 mg/kg. TRZ (a gift from Boehringer-Ingelheim, Ingelheim, Germany) was dissolved in dimethyl sulfoxide at a dose of 20 mg/kg. The doses of TRZ and dissolving vehicle used in this study were established during prior investigation (6). Hamsters were randomly assigned to an intraperitoneal (i.p.) injection of MKC-242 or vehicle at CT7.5 and then an i.p. injection of TRZ or vehicle at CT8, before they were returned to their individual cages. The phase of the rhythm was assessed visually by applying a straight edge to the onset of activity on successive days before and after drug injection and determining the difference in phases on the day of drug injection (11). To establish the activity index, the total number of wheel revolutions was calculated for 4 h from CT8 to CT12, a time interval falling before subjective night and after TRZ injection. The values were expressed as means $\pm$ S.E.M. For statistical analysis, one-way ANOVA followed by Dunnett's test or the Student's *t*-test was applied.

Vehicle administration at CT8 did not lead to any change in phase (Fig. 1A), but TRZ (20 mg/kg) admin-

Fig. 1. Double-plotted wheel-running activity records showing representative profiles from the four treatment groups. Days are indicated vertically from top to bottom, and time is indicated horizontally. The phase of the rhythm was assessed visually by applying a straight edge to the onset of activity on successive days before and after drug injection and determining the difference in phases on the day of drug injection. An asterisk indicates the day of drug injection. A: Vehicle control (CMC and DMSO treatment groups); B: groups of CMC (vehicle of MKC-242) and triazolam treatment; C: groups of MKC-242 (3 mg/kg, i.p.) and triazolam (20 mg/kg, i.p.) treatment; D: groups of MKC-242 (3 mg/kg, i.p.) and DMSO (vehicle of triazolam) treatment.

istration at CT8 produced a clear phase advance that was accompanied by an increase in the number of wheel revolutions (Figs. 1B and 2). Pre-treatment with MKC-242 attenuated the TRZ-induced phase advances in a dose-dependent manner, and significant inhibition was observed at a dose of 3 mg/kg (Figs. 1C and 2). MKC-242 inhibited the number of wheel revolutions after TRZ injection at CT8, and a significant effect was observed at a dose of 1 mg/kg. However, MKC-242 (3 mg/kg) itself

Fig. 2. Effects of MKC-242, a selective 5-HT_{1A}-receptor agonist, on triazolam-induced phase resetting in hamster wheel-running rhythm. Hamsters received either vehicle or MKC-242 (0.1, 1, or 3 mg/kg, i.p.) 30 min prior to the triazolam (20 mg/kg, i.p.) injection during subjective night (at CT8). Dose-dependent effects on triazolam-induced phase advances (A) and increase of wheel revolutions (B) in hamster wheel-running rhythm. Values are given as means $\pm$ S.E.M. (n = 5–10). * P < 0.05, compared with groups of CMC (vehicle of MKC-242) and triazolam treatment (Student's t -test). * P < 0.05, compared with groups of CMC (vehicle of MKC-242) and MKC-242 treatment (one-way ANOVA followed by Dunnett's test).

did not affect either the phase advances or increase of locomotor activity in the hamsters (Figs. 1D and 2).

In the present experiment, we demonstrated that both TRZ-induced phase advances and increased activity were attenuated by pretreatment with MKC-242. Thus, MKC-242, a drug that enhances photic entrainment, attenuated non-photic entrainment with TRZ. We previously reported that photic entrainment was attenuated by non-photic entraining treatment (7). The present results combined with our previous findings support the idea that photic and non-photic entrainment interfere

with each other when used simultaneously.

The action mechanism of 8-OH-DPAT and MKC-242 may differ in circadian clock systems such that 8-OH-DPAT has an affinity for 5-HT_{1A} and 5-HT₇ receptors, while MKC-242 only has an affinity for 5-HT_{1A}. For example, MKC-242, but not 8-OH-DPAT, decreased the contents of 5-HT in the SCN (9), although the precise mechanism underlying such differences remains unknown. In the previous study, light-induced *Per1* and *Per2* gene expression in the SCN was augmented by MKC-242 but not by 8-OH-DPAT (10), and 8-OH-DPAT caused non-photic entrainment but MKC-242 did not (8; present results). Also, pre-treatment with 8-OH-DPAT did not attenuate a brotizolam-induced phase advance in hamster wheel-running activity rhythm (7). The K_i values of MKC-242 for 5-HT_{1A} and 5-HT₇ receptors are 0.35 nM and >100 nM, respectively (12), and 8-OH-DPAT has both 5-HT_{1A} and 5-HT₇ receptor affinities (13). The above papers (12, 13) suggest that MKC-242 may preferentially act on presynaptic 5-HT_{1A} located in the raphe nucleus, while 8-OH-DPAT can act on both 5-HT_{1A} and 5-HT₇ receptors in the SCN. An activity decrease of 5-HT neurons by MKC-242 may result in attenuation of both the TRZ-induced phase advance and motor activity increase. Such an explanation is supported by a previous investigation in which lesion to the midbrain raphe nucleus attenuated TRZ-induced phase advances (6). As MKC-242 is reported to augment light-induced *Per1* and *Per2* gene expression in the SCN (7), MKC-242 may attenuate the TRZ-induced reduction of *Per1* and *Per2* gene expression in the SCN. In the present study, MKC-242 preferentially attenuated the TRZ-induced increase of wheel-running activity rather than the TRZ-induced phase advances. Thus, activity decrease by MKC-242 was not required to attenuate the effect of MKC-242 on TRZ-induced phase advances. Our present results are physiologically relevant in that we should be more aware of environmental lighting conditions and their pharmacological actions when melatonin and TRZ are taken to reduce jet-lag syndrome.

In summary, we conclude that non-photic entrainment could be attenuated by MKC-242, a drug that enhances photic entrainment, and that intact 5-HTergic activity was necessary for TRZ-induced non-photic entrainment.

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