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Potentiating action of MKC-242, a selective 5-HT_{1A} receptor agonist, on the photic entrainment of the circadian activity rhythm in hamsters

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1 Serotonergic projections from the midbrain raphe nuclei to the suprachiasmatic nuclei (SCN) are known to regulate the photic entrainment of circadian clocks. However, it is not known which 5-hydroxytryptamine (5-HT) receptor subtypes are involved in the circadian regulation. In order to verify the role of 5-HT_{1A} receptors, we examined the effects of 5-{3-[((2S)-1,4-benzodioxan-2-ylmethyl)amino]-propoxy}-1,3-benzodioxole HCl (MKC-242), a selective 5-HT_{1A} receptor agonist, on photic entrainment of wheel-running circadian rhythms of hamsters.

2 MKC-242 (3 mg kg^{-1}, i.p.) significantly accelerated the re-entrainment of wheel-running rhythms to a new 8 h delayed or advanced light-dark cycle.

3 MKC-242 (3 mg kg^{-1}, i.p.) also potentiated the phase advance of the wheel-running rhythm produced by low (5 lux) or high (60 lux) intensity light pulses. In contrast, 8-hydroxy-dipropylaminotetralin (8-OH-DPAT)(5 mg kg^{-1}, i.p.), a well known 5-HT_{1A}/5-HT_7 receptor agonist, only suppressed low intensity (5 lux) light-induced phase advances.

4 The potentiating actions of MKC-242 on light pulse-induced phase advances were observed even when injected 20 or 60 min after the light exposure.

5 The potentiating action of MKC-242 was antagonized by WAY100635, a selective 5-HT_{1A} receptor blocker, but not by ritanserin, a 5-HT_2/5-HT_7 receptor blocker, indicating that MKC-242 is activating 5-HT_{1A} receptors.

6 Light pulse-induced c-*fos* expression in the SCN and the intergeniculate leaflet (IGL) were unaffected by MKC-242 (3 mg kg^{-1}, i.p.).

7 HPLC analysis demonstrated that MKC-242 (3 mg kg^{-1}, i.p.) decreased the 5-HIAA content in the SCN.

8 The present results suggest that presynaptic 5-HT_{1A} receptor activation may be involved in the potentiation of photic entrainment by MKC-242 in hamsters.

Keywords: Circadian rhythm; light-entrainment; 5-Hydroxytryptamine (5-HT); 5-HT_{1A} receptor

Introduction

The biological clocks of mammals, which are located in the suprachiasmatic nuclei (SCN) of the hypothalamus, control various physiological daily rhythms such as feeding, drinking, locomotor activity, sleep-wakefulness, plasma adrenal corticosterone levels and the body temperature cycle (Inouye & Shibata, 1994). It is well known that daily light-dark cycles strongly entrain the circadian rhythms generated by the biological clock. Light signals for photic entrainments reach the SCN *via* a direct projection from the retina (retinohypothalamic tract (RHT)) and *via* an indirect projection from the retina through the intergeniculate leaflet (IGL) (geniculohypothalamic tract (GHT)) (Inouye & Shibata, 1994).

Both the SCN and the IGL are innervated by 5-hydroxytryptamine (serotonin, 5-HT) neurons in the midbrain raphe nuclei in rats (Cagampang *et al*., 1993; Cagampang & Inouye, 1994), and in hamsters (Meyer-Bernstein & Morin, 1996). Systemic or local injections of agonists for 5-HT receptor subtypes suppress the light-induced phase shifts of hamster activity rhythms (Rea *et al*., 1994; Pickard *et al*., 1996; Mintz *et al*., 1997), light-induced c-*fos* expression in the hamster SCN (Selim *et al*., 1993; Glass *et al*., 1994, 1995; Pickard *et al*., 1996), the extracellular glutamate concentration (Selim *et al*., 1993; Srkalovi *et al*., 1994) and the firing rates of light responsible cells in the hamster SCN (Ying & Rusak, 1994). In *in vitro* studies, Liou *et al*. (1986) and Rea *et al*. (1994) reported that 5-HT suppressed the optic nerve stimulation-evoked field potentials in the rat SCN and hamster SCN respectively. Furthermore, Morin & Blanchard (1991) reported that depletion of hamster brain serotonin increased the circadian activity rhythm response to light. This evidence suggests that 5-HT neurons from midbrain raphe nuclei regulate the photic entrainment of the biological clock in mammals in an inhibitory manner.

However, it remains to be clarified how various 5-HT receptor subtypes are involved in circadian regulation. Recently, Pickard *et al*. (1996, 1997) reported that selective 5-HT_{1B} receptor agonists inhibit the light-induced phase shift of hamster wheel-running activity rhythm and light-induced c-*fos* expression in the SCN. Bilateral enucleation reduces the density of 5-HT_{1B} receptors in the SCN. Based on these observations, they suggested that activation of 5-HT_{1B} receptors, which are localized presynaptically on retinal terminals in the SCN, suppress the photic entrainment of the biological clock.

On the other hand, the roles of 5-HT_{1A} or 5-HT_7 receptor are still vague because of a lack of subtype-specific ligands. For example, 8-hydroxy-dipropylaminotetralin (8-OH-DPAT),

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which is an agonist for both 5-HT_{1A} and 5-HT_7 receptors suppressed photic responses of the circadian clock, and these actions are antagonized by both 5-HT_{1A} and 5-HT_7 receptors antagonists (Weber *et al*., 1996). In contrast, Ying & Rusak (1997) reported that the suppression of the firing rates of light-responsive SCN neurons induced by 8-OH-DPAT were mediated *via* activation of 5-HT_7 receptors but not 5-HT_{1A} receptors. Furthermore, 5-HT_{1A} receptor mRNA is sparsely distributed in the rat SCN (Roca *et al*., 1993).

In order to establish the role of 5-HT_{1A} receptors in regulating the photic entrainment of the biological clock, we examined the effects of 5-{3-[((2S)-1,4-benzodioxan-2-ylmethyl)amino]propoxy}-1,3,-benzodioxole HCl (MKC-242), a selective 5-HT_{1A} receptor agonist (Matsuda *et al*., 1995a,b; Suzuki *et al*., 1995; Abe *et al*., 1996; Asano *et al*., 1997) on the photic entrainment of the hamster wheel-running rhythm. The K_i values of MKC-242 for 5-HT_{1A} and 5-HT_7 receptors are 0.35 nM (Matsuda *et al*., 1995a) and >100 nM (personal communication from Mitsubishi Chemical Co.), respectively. We also investigated the effects of MKC-242 on light-induced c-*fos* expression in the SCN and the IGL, and on 5-HT turnover in the SCN to confirm neuronal and cellular mechanisms of serotonergic regulation of photic entrainment.

Methods

Animals

Male Syrian hamsters (*Mesocricetus auratus*) weighing 120–200 g were maintained under controlled environmental conditions ($23\pm2^{\circ}$C room temperature; 12-12 h light-dark cycle, lights on at 08:30 h) for at least 2 weeks before being used for the experiments. The light intensity was almost 200 lux at the level of the animal cage. Food and water were provided *ad libitum*. Animals were treated in accordance with the Law (No. 105) and Notification (No. 6) of the Japanese Government. Under the light-dark cycle, zeitgeber time (ZT) referred to animal colony light-dark cycle. ZT0 was designated as light-on and ZT12 as light-off. In free-running conditions under constant darkness, circadian time (CT) was defined instead of ZT, and CT12 referred to the onset of wheel-running.

Recording of wheel-running rhythm

Hamsters were housed individually in transparent plastic cages ($35\times20\times20$ cm), each equipped with a running wheel of 15 cm diameter, which closes a microswitch on each revolution. Wheel-running activity was continuously recorded in 6 min epochs by a PC-9801 computer.

Fos immunohistochemistry

After 2 days of constant darkness, hamsters were injected intraperitoneally with drugs 30 min prior to a light pulse (0, 5 or 60 lux for 15 min) at projected ZT 20 and returned to darkness. Sixty minutes after the light pulse onset, the animals were deeply anaesthetized with Nembutal and perfused intracardially with 100 ml of saline (37°C), followed by 100 ml of 4% paraformaldehyde in 0.1 M phosphate buffered saline (PBS; pH 7.2; 4°C). Brains were removed from the skull and fixed with 50 ml of 4% paraformaldehyde in 0.1 M PBS and transferred to 20 and 30% sucrose solutions in 0.1 M PBS for 24 and 48 h, respectively. Brains were cut into 40 μm slices from rostral to caudal SCN or IGL with a freezing microtome. Alternate sections were incubated for 48 h with anti-Fos antibody (OA-11-824, Cambridge Research Biochemical. U.S.A.) diluted to 1 : 1000 with 0.1 M PBS containing 1% normal rabbit serum and 0.3% Triton X-100 (PBSRT). All sections were then washed three times with 0.1 M PBS (10 min each) and incubated for 2 h with biotinylated anti-sheep rabbit antibody (diluted to 1 : 200 with PBSRT; Vectastain). The sections were washed three times with 0.1 M PBS and incubated for 2 h in an avidin-biotin complex solution (Vectastain ABC kit). After three washes with 0.1 M PBS, sections were visualized with diaminobenzidine as a chromogen and mounted on gelatin-coated glass slides. All procedures were performed at room temperature. The number of cells which expressed Fos immunoreactivity was counted by an unnotified observer. Average cell numbers in the bilateral SCN or the IGL per one slice were calculated.

Measurement of 5-HT and 5-HIAA content

Measurements of 5-HT and 5-HIAA contents in the SCN were done by HPLC as previously reported (Ono *et al*., 1996). Hamsters were anaesthetized with ether and killed by decapitation. The brain was rapidly removed from the skull and the SCN was dissected free. Monoamines in the SCN was extracted with 200 μl of 0.5 M $HClO_4$ by sonication on ice. After centrifugation at 15,000 r.p.m. for 10 min at 4°C, supernatants were collected for measurement of monoamine contents. Eicompak MA-50DS (4.6×150 mm) (Eicom, Kyoto) and an electrochemical detector (ECD-300, Eicom) were used for 5-HT and 5-HIAA assays. The mobile phases were as follows: 50 mM sodium-acetate-citrate buffer (pH 3.9) containing 80 mg l^{-1} sodium 1-octanesulphonate, 5 mg l^{-1} EDTA and 10 (v/v)% methanol. The data were analysed with a Powerchrom 2.0.6 system.

Drugs and reagents

MKC-242 and WAY100635 (N-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-N-(2-pyridinyl) cyclohexanecarboxamide trihydrochloride) were a kind gift from Mitsubishi Chemical Co. (Yokohama, Japan). (±)8-OH-DPAT and ritanserin were purchased from Research Biochemicals (Natick, MA, U.S.A.). All other chemicals were of the highest grade.

All drugs were freshly prepared. MKC-242 was suspended in 0.5% carboxymethylcellulose (CMC) and injected i.p. at the dose indicated (1–3 ml kg^{-1}). All other drugs were dissolved in saline and injected i.p. at the indicated doses (1 ml kg^{-1}).

Data and Statistical analysis

The data are presented as means ± s.e.mean. Statistical analysis was conducted by one- or two-way ANOVA followed by Dunnett's test or Student's *t*-test. *P* values of 5% or less were considered as statistically significant.

Results

Effect of MKC-242 on re-entrainment of wheel-running activity rhythms to a new light-dark cycle

Figure 1 shows a representative actogram of the re-entrainment of wheel-running activity to an 8 h advance of light-dark cycle in hamsters. When hamsters were injected with vehicle, it took approximately 2 weeks for complete re-entrainment to a new light-dark cycle. On the other hand, it took only 2–3 days for re-entrainment, when animals were

injected with MKC-242 (3 mg kg^{-1}) at ZT20 on the day of light exposure. Advance and delay experiments were carried out using different animals. MKC-242 (3 mg kg^{-1}) significantly accelerated the re-entrainment of wheel-running activity to both an 8-h-delayed (-6.3 ± 0.3 h for MKC-242 and -4.8 ± 0.3 h for vehicle on 4th day, $P<0.05$, Student's *t*-test) or -advanced (6.7 ± 0.79 h for MKC-242 and 4.3 ± 0.47 h for vehicle on the 4th day, $P<0.05$, Student's *t*-test) light-dark cycle.

Effect of MKC-242 on light pulse-induced phase shifts of wheel-running activity in constant darkness

To confirm that MKC-242 accelerated the re-entrainment to new light-dark cycle by potentiating the effects of environmental light, we next examined the effect of MKC-242 on the light pulse-induced phase shift of wheel-running activity in hamsters maintained in constant darkness. As shown in Figure 2a and Table 1, exposure to a light pulse for 15 min (light intensity: 60 lux) at circadian time 20 (CT; CT 12: onset time of wheel-running) caused a phase advance (average values: 1.98 ± 0.13 h) of wheel-running activity in hamsters. One-way ANOVA revealed the significant potentiation of MKC-242 in light-induced phase advance ($F_{4,35}=6.4$, $P<0.01$). Injection of MKC-242 (3 mg kg^{-1}), but not 0.1 or 1.0 mg kg^{-1} 30 min prior to light exposure dramatically potentiated the phase advance of wheel-running activity induced by a light pulse at CT 20 (60 lux) (Dunnett's test, $P<0.05$) (Figure 2b and Table 1). Two to three days are required to get stable phase shifts, when light phase advances the activity rhythm of mammals (Figure 2a). MKC-242 did not affect the time required to complete a phase shift induced by a light pulse at CT20 (data not shown). Whereas, MKC-242 application without light exposure did not affect the phase of wheel-running activity rhythm (Figure 2c and Table 1).

The potentiating action of MKC-242 was dependent on the intensity of the light exposure (Table 2). In the case of vehicle, the phase advance of wheel-running activity increased with an increase of light intensity between 5 and 60 lux. A high

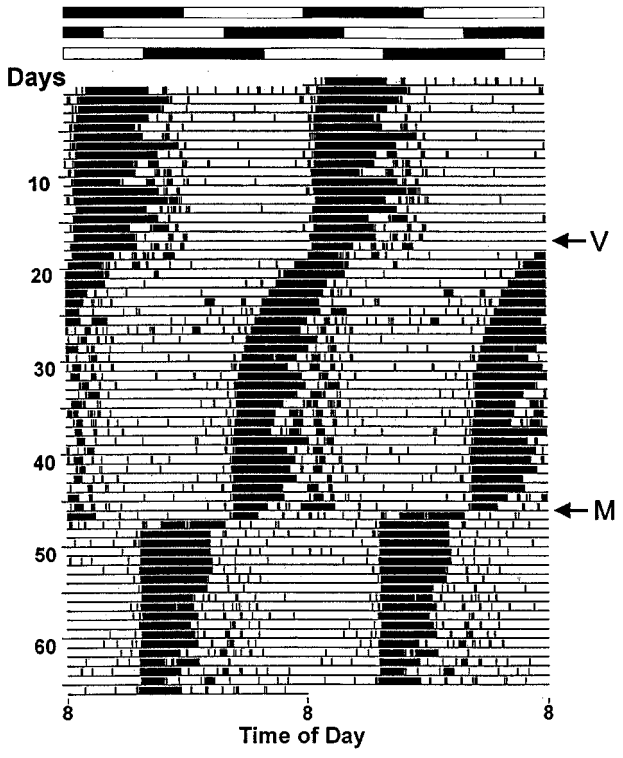

Figure 1 Representative double plot-actogram demonstrating the potentiating effect of MKC-242 on re-entrainment of the wheel-running activity to an 8 h advanced light-dark cycle. Time of day is indicated horizontally and consecutive days vertically. Upper, middle and lower bars on the top of actogram show the light (open bar)-dark (solid bar) cycle during day 1–18, day 19–47 and day 48–66, respectively. Hamsters were maintained in a 12:12 h light-dark cycle at least for 15 days, the light-dark cycle was advanced 8 h (indicated by arrows), then MKC-242 (M) (3 mg kg^{-1}, i.p.) or vehicle (V) were administered at ZT20 of former light-dark cycle for 2 continuous days.

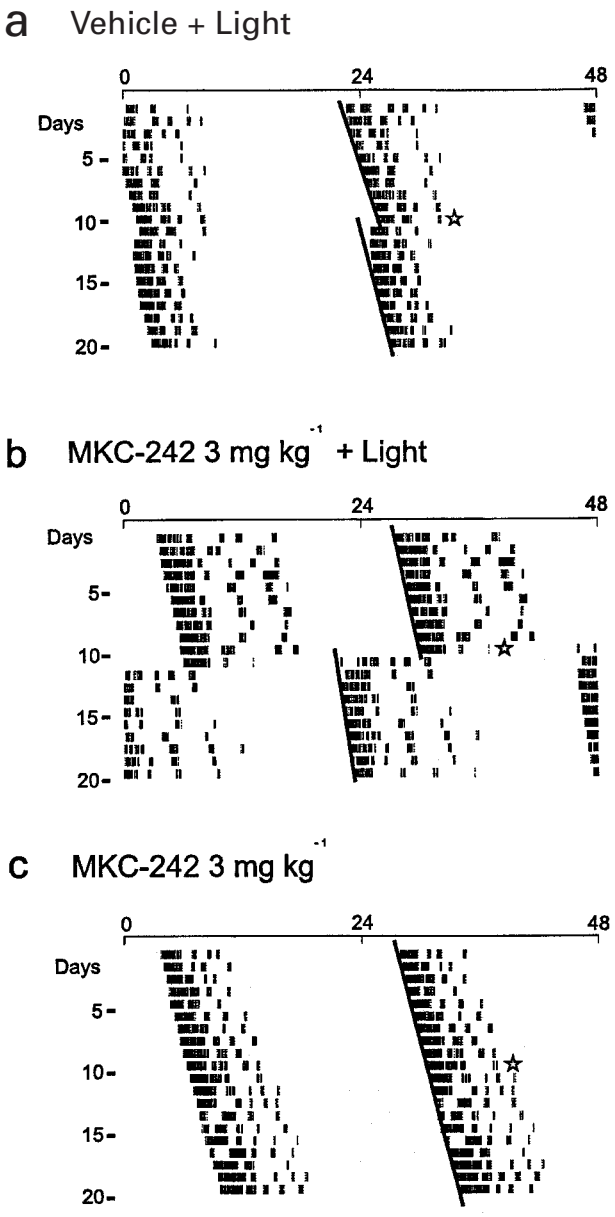

Figure 2 Representative double plot-actograms demonstrating the potentiating effects of MKC-242 on the light pulse-induced phase advance of wheel-running activity of hamsters. The time of day is indicated horizontally and consecutive days vertically. Hamsters were maintained in constant darkness until a stable free-running rhythm was observed for at least 10 days. Hamsters then received an intraperitoneal injection of vehicle (a) or MKC-242 (3 mg kg^{-1}) (b,c) 30 min prior to light exposure (60 lux, 15 min) at CT20 (a,b) or were handled the same without receiving a light pulse (c). Eye-fitted lines to activity onset were also shown in each actogram, and the differences between these two lines were designated as phase changes (h). Approximate treatment times are indicated by stars.

Table 1 Dose-dependent effects of MKC-242 on the light pulse-induced phase advances of wheel-running activity of hamsters

MKC-242 (mg kg^{-1})	*Light pulse (CT20 for 15 min)*	*Phase advance induced by light pulse (h)*
0	+	1.98±0.13 (7)
0.1	+	2.78±0.51 (6)
1	+	3.17±0.63 (8)
3	+	4.25±0.65 (13)*
3	−	0.23±0.21 (6)

Detail of the methods were described in the legend for Figure 2. Data are expressed as means±s.e.mean. The number of animals are shown in parentheses. *, Significant difference ($P<0.05$) compared to vehicle (one-way ANOVA followed by Dunnett's test).

Table 2 Light pulse intensity-dependence for the potentiating actions of MKC-242 on light pulse-induced phase advances of wheel-running activity of hamsters

	Phase advance induced by light pulse (CT20 for 15 min) (h)	
Light intensity (lux)	*Vehicle*	*MKC-242* (3 mg kg^{-1})
5	0.83±0.34 (7)	3.12±0.84 (10)*
60	1.98±0.13 (7)	4.25±0.65 (13)*
200	2.30±0.10 (5)	Not tested

Hamsters in constant darkness received either vehicle or MKC-242 (3 mg kg^{-1}) 30 min before a light pulse (5, 60, 200 lux for 15 min at CT20). High intensity (200 lux) light-induced phase shifts were tested for the vehicle group only. Data are expressed as means±s.e.mean. The number of animals are shown in parentheses. *, Significant difference ($P<0.05$) compared to vehicle (unpaired Student's *t*-test).

Table 3 Effect of injection timing on the potentiation action of MKC-242 on light pulse-induced phase advances of wheel-running activity

	Phase advamce induced by light pulse (CT20 for 15 min) (h)	
Time of injection after light pulse onset (min)	*Vehicle*	*MKC-242* (3 mg kg^{-1})
−30	1.98±0.13 (7)	4.25±0.65 (13)*
20	2.78±0.51 (5)	5.65±0.85 (6)*
60	2.72±0.33 (6)	5.62±0.096 (6)*

Hamsters maintained in constant darkness received either vehicle or MKC-242 (3 mg kg^{-1}) 30 min before or 20, 60 min after light exposure (60 lux for 15 min at CT20). Data are expressed as means±s.e.mean. The number of animals are shown in parentheses. *, Significant difference ($P<0.05$) compared to vehicle (unpaired Student's *t*-test).

Table 4 Effect of 8-OH-DPAT on light pulse-induced phase advance of wheel-running activity of hamsters maintained in constant darkness

	Phase advance induced by light pulse (CT20 for 15 min) (h)	
Light intensity (lux)	*Vehicle*	*8-OH-DPAT* (5 mg kg^{-1})
5	1.13±0.30 (10)	0.25±0.10 (6)*
20	2.28±0.40 (6)	1.52±0.58 (7)
60	2.61±0.44 (6)	2.25±0.50 (6)

Data are expressed as means±s.e.mean. The number of animals are shown in parentheses. *, Significant difference ($P<0.05$) compared to vehicle (unpaired Student's *t*-test).

intensity light pulse (200 lux) caused a ceiling effect on the phase advance (2 h), because more high intensity of light ($10^{11}-10^{15}$ photons cm^{-1} s^{-1}) has reported to cause almost same degree of advance (2 h) (Boulos, 1995). MKC-242 (3 mg kg^{-1}) potentiated not only low (5 lux), but also high (60 lux) intensity light-induced phase advances. MKC-242 caused a large phase advance beyond the ceiling effect, when a 60 lux light pulse was applied. In the next experiment, we observed the effect of 8-OH DPAT on light-induced phase advance. Two-way ANOVA revealed no significant differences between Drug × Intensity of light ($F_{2,35}=0.23$, $P>0.05$), but there are significant increase of phase advance with intensity-dependent manner ($F_{1,35}=10.5$, one-way ANOVA, $P<0.01$). As shown in Table 4, 8-OH-DPAT suppressed low intensity (5 lux) light-induced phase advances, but did not affect high intensity (20 and 60 lux) light-induced phase advances.

In the next experiments, we examined the importance of the timing of MKC-242 injection on the potentiating action on a light pulse-induced phase advance (Table 3). There are significant differences in drug effect ($F_{1,37}=20.6$, $P<0.01$), but in injection timing ($F_{2,37}=1.7$, $P>0.05$). Injection of MKC-242 (3 mg kg^{-1}) 30 min prior to light pulse onset significantly potentiated the phase advance of wheel-running activity, as shown in Figure 3. Furthermore, an injection of MKC-242 (3 mg kg^{-1}) 20 min or 60 min after the light onset, when the light has been turned off, resulted in a strong potentiating action on the light pulse-induced phase advance of wheel-running activity.

As shown in Figure 3, pre-injection of WAY100635 (3–10 mg kg^{-1}), a selective 5-HT$_{1A}$ receptor blocker, antagonized the potentiating action of MKC-242 in a dose-dependent manner. However, WAY100635 (10 mg kg^{-1}) itself did not affect the light pulse-induced phase advance of wheel-running activity. On the other hand, ritanserin (10 mg kg^{-1}), a 5-HT$_2$/5-HT$_7$ blocker failed to affect the potentiating action of MKC-242 on light pulse (60 lux for 15 min at CT20)-induced phase advances (vehicle + vehicle; 2.40±0.08 h ($n=5$), vehicle + MKC-242 (3 mg kg^{-1});

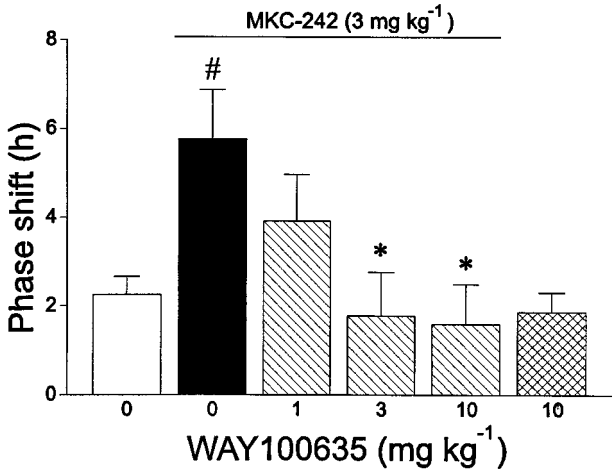

Figure 3 Effect of WAY100635, a selective 5-HT$_{1A}$ receptor blocker, on the potentiating action of MKC-242 on the light pulse-induced phase advance of wheel-running activity. Hamsters received either vehicle or WAY100635 45 min prior to the light pulse, followed by vehicle or MKC-242 (3 mg kg^{-1}) 30 min prior to the light pulse (60 lux for 15 min at CT20). Values are given as means±s.e.mean ($n=6-12$). Cross-hatched column exhibits the injection of WAY100635 without MKC-242. *$P<0.05$, compared with MKC-242 (3 mg kg^{-1}) (closed column) (one-way ANOVA followed by Dunnett's test). #:$P<0.05$, compared with vehicle (open column) (one-way ANOVA followed by Dunnett's test).

5.21±0.66 h ($n=13$), ritanserin (10 mg kg^{-1})+MKC-242 (3 mg kg^{-1}); 4.81±0.61 h ($n=9$), ritanserin (10 mg kg^{-1})+vehicle; 2.73±0.58 h ($n=5$)).

Effect of MKC-242 on light pulse-induced c-fos expression in the SCN and the IGL

Figure 4 shows the effect of MKC-242 on light pulse-induced c-*fos* expression in the SCN and the IGL. Exposure to a light pulse at CT20 for 15 min significantly increased c-*fos* immunoreactive cells both in the SCN ($F_{2,16}=15.7$, $P<0.01$) and the IGL ($F_{2,7}=13.1$, $P<0.01$) (Figure 4a,b) in an intensity-dependent manner. However, pre-injection of MKC-242 (3 mg kg^{-1}) did not alter the light pulse (5 lux and 60 lux)-induced c-*fos* expression in the SCN nor in the IGL.

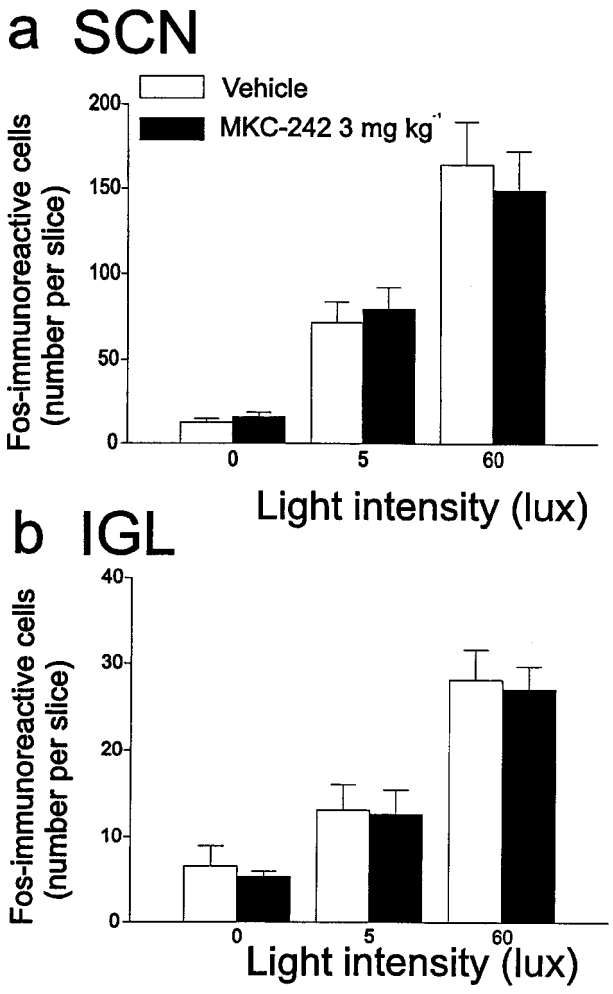

Figure 4 Effect of MKC-242 on light pulse-induced c-*fos* expression in the SCN (a) or the IGL (b) in hamsters. Hamsters maintained on a light-dark cycle were transferred to constant darkness. After 2 days in constant darkness, hamsters were received either vehicle or MKC-242 (3 mg kg^{-1}) 30 min prior to the light pulse (60 lux for 15 min at CT20). Sixty minutes after light pulse onset, the animals were anaesthetized with an overdose of pentobarbital (80 mg kg^{-1}) and perfused transcardially with saline followed by 4% paraformaldehyde. Coronal sections (40 μm) through the SCN or the IGL were processed for immunohistochemistry. Values are given as means± s.e.mean ($n=5-9$ for the SCN; $n=3-4$ for the IGL). No significant differences were observed when compared to vehicle group (Student's *t*-test).

Table 5 Effect of MKC-242 on the 5-HT and 5-HIAA concentrations in the SCN of hamsters

	Vehicle	*MKC-242* (3 mg kg^{-1})
5-HT (ng mg protein^{-1})	60.46±7.61 (7)	59.83±12.17 (8)
5-HIAA (ng mg protein^{-1})	28.50±3.35 (7)	18.89±2.62 (8)*
5-HIAA/5-HT ratio	0.52±0.09 (7)	0.38±0.07 (8)

Hamsters maintained under a light-dark cycle were transferred to a light room at ZT16 (8 h light–dark cycle shift) and received vehicle or MKC-242 (3 mg kg^{-1}) at ZT20 (4 h after transfer to light room). Sixty minutes after injection, brains were prepared and monoamine contents were measured by h.p.l.c. as described in Methods. Data are expressed as means±s.e.mean. The number of animals are shown in parentheses. *, Significant difference ($P<0.05$) compared to vehicle (unpaired Student's *t*-test).

Effect of MKC-242 on 5-HT and 5-HIAA concentrations in the hamster SCN

Table 5 shows the effect of MKC-242 on the 5-HT and its metabolite, 5-HIAA concentrations in the hamster SCN. MKC-242 (3 mg kg^{-1}) decreased 5-HIAA content to 73% of control and did not affect the 5-HT content in the SCN.

Discussion

In the present experiments, we demonstrated that MKC-242, a selective 5-HT$_{1A}$ receptor agonist, potentiated the photic entrainment of wheel-running activity in hamsters in a c-*fos* expression-independent manner. Furthermore, MKC-242 decreased the turnover of 5-HT in the SCN.

In contrast to MKC-242, 8-OH-DPAT, a well known 5-HT$_{1A}$ receptor agonist, suppressed the photic entrainment. The differences in actions of MKC-242 and 8-OH-DPAT, however may reflect the specificity of these chemicals for 5-HT receptor subtypes. MKC-242 has been reported to have high affinity for 5-HT$_{1A}$ receptors ($K_i=0.35$ nM, Matsuda *et al.*, 1995a) and a relative low affinity for 5-HT$_7$ receptor ($K_i>100$ nM; personal communication from Mitsubishi Chemical Co.). In addition, the potentiating action of MKC-242 on the photic entrainment was not reversed by co-administration of ritanserin, a 5-HT$_2$/5-HT$_7$ receptor blocker. On the other hand, 8-OH-DPAT has a high affinity for both 5-HT$_{1A}$ and 5-HT$_7$ receptors (Lovenberg *et al.*, 1993). The importance of 5-HT$_7$ receptors in regulating photic and non-photic entrainment of the biological clock is becoming abundantly clear. Ying & Rusak (1997) reported that 8-OH-DPAT suppressed firing rates of light-responsible SCN neurons *via* activation of 5-HT$_7$ receptors in the SCN. Furthermore, the phase advancing action of 8-OH-DPAT on firing rhythms in the SCN slice were abolished by the 5-HT$_2$/5-HT$_7$ blocker, ritanserin, but not the 5-HT$_{1A}$ antagonist, pindolol, suggesting a functional role of 5-HT$_7$ receptors in the SCN. It may, therefore, be possible that 8-OH-DPAT suppresses photic entrainment *via* 5-HT$_7$ receptor activation.

The site of action or the intra/intercellular mechanism of the potentiating actions of MKC-242 on photic entrainment are still unclear. Although c-*fos* expression in the SCN by light is known to be a biochemical marker of photic entrainment, in the present results, MKC-242 did not affect c-*fos* expression in the SCN. Weber *et al.* (1995) reported that a nitric oxide synthase (NOS) inhibitor blocks light-induced phase shifts of wheel-running activity, but not c-*fos* expression in the hamster SCN. Therefore, the potentiated photic entrainment by MKC-

242 may be the result of an augmented c-*fos*-independent light signal pathway, such as NOS activation. In addition to this possibility, an alternate explanation is that MKC-242 acts downstream of the light-induced c-*fos* expression in the SCN.

We can rule out the following possibility that MKC-242 increases the sensitivity for light in the photo-recipient organ, i.e. retina, because MKC-242 is still able to potentiate photic entrainment, even when it was administered after turning off the light pulse.

5-HT_{1A} receptors were reported to be present in the SCN, IGL and raphe nuclei, all of which are involved in regulating photic entrainment of the biological clocks (Wright *et al*., 1995). The role of SCN 5-HT_{1A} receptors in photic entrainment is not established at present, although some reports have suggested a suppressing action of 5-HT_{1A} receptor activation on photic entrainment (Rea *et al*., 1994; Moriya *et al*., 1996). MKC-242, however, did not suppress but potentiated the photic entrainment by low and high intensity light pulse, suggesting that MKC-242 failed to act on 5-HT_{1A} receptors in the SCN.

The GHT pathway has been reported to inhibitory regulate the photic entrainment of the biological clock *via* the release of neuropeptide Y (NPY) and gamma-aminobutyric acid (GABA) in the SCN (Ying *et al*., 1993; Biello & Mrosovsky, 1995). The IGL, a relay area of GHT, was reported to be innervated by abundant 5-HT neurons from the raphe nuclei and a 5-HT_{1A} receptor agonist potently suppressed both the spontaneous and light-induced activity of IGL neurons (Ying *et al*., 1993). Therefore, MKC-242 may act at 5-HT_{1A} receptors in the IGL and block the light signal communicated *via* the GHT. Suppression of GHT activity could potentiate photic entrainment of the biological clock by decreasing the NPY and GABA releases to the SCN. MKC-242, however, failed to affect light-induced c-*fos* expression in the IGL, therefore we believe that MKC-242 is acting in brain region other than the IGL. Alternative explanation is that MKC-242 acts on the IGL-included pathways not involving c-*fos* expression.

MKC-242 may selectively activate 5-HT_{1A} receptors in the midbrain raphe nuclei, which are widely known to possess autoreceptors suppressing the activity of 5-HT neurons. In the present experiments, we showed that MKC-242 suppressed 5-HT turnover in the SCN. Systemic injection of 5-HT_{1A} receptor antagonist, NAN-190 or 5-HT-terminal destruction restricted to the SCN resulted in a potentiation of photic entrainment of the biological clock in rodents (Rea *et al*., 1995; Bradbury *et al*., 1997). Furthermore, the light pulse-induced phase shift of activity rhythms was dramatically increased after monoamine (including serotonin) depletion by reserpine (Penev *et al*., 1993). This evidence suggests that serotonergic innervation to the SCN has tonic suppressing actions on photic entrainment of the biological clock. Therefore, we propose that MKC-242 activate 5-HT_{1A} autoreceptors in the midbrain raphe and suppress the activity of 5-HT neurons projecting to the SCN, and that the reduction of 5-HT activity in the SCN leads to potentiation of the photic entrainment. To confirm this hypothesis, further experiments, i.e. microinjection study, will be required.

MKC-242 has been reported to possess anti-depressant effects (Matsuda *et al*., 1995a). Therefore, the circadian rhythm disorders observed in depressed subjects may be improved by treatment with MKC-242.

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