The effect of a typical neuroleptic haloperidol (Hal) on mPer1 gene expression was investigated in mouse suprachiasmatic nuclei (SCN). Hal induced mPer1 mRNA levels both in vivo and in cultured SCN cells. For mechanisms underlying Hal-induced mPer1 expression, N-methyl-D-aspartate (NMDA) glutamate receptor subtype, the phosphorylation form of the transcription factor, and the Ser-133 phosphorylation form of cAMP-responsive element-binding protein (CREB) played an important role, because the induction of mPer1 mRNA significantly decreased after pretreatment with a non-competitive NMDA receptor antagonist, such as MK-801 or CREB antisense. These results suggest that Hal may increase CREB phosphorylation and mPer1 expression according to the activation of the NMDA receptor through the dopaminergic pathways. Although the injection of Hal during the light period increased the amplitude of mPer1 mRNA rhythmicity in a nondrug state, the injection of the drug during the dark period disturbed the rhythmic pattern of mPer1 mRNA. These results suggest that the rhythmicity of clock genes in SCN may be disturbed depending on the dosing time of Hal. On the other hand, because the induction of mPer1 mRNA by Hal seems to be at least partly caused by the NMDA receptor, showing a phase shift or resetting effect of the circadian clock, Hal may also cause such phase shift effects.

The variation of behavior and biological functions in whole animals expressed as circadian rhythms is controlled by the endogenous clock (pacemaker), which resides in the suprachiasmatic nuclei (SCN) (1–3). The pacemaker components such as Clock, Bmal1, Periods (Per1, Per2, and Per3), and Cryptochromes (Cry1 and Cry2) express their circadian rhythms, which are persistent rhythmicity, even under constant environmental conditions and external stimuli that could change the timing of the rhythm. Most of these components are probably controlled rhythmically by integrating the external cues from the entrainment pathway and initiating a variety of signals to the output pathway, which determines the circadian activities of the organism (4, 5).

A typical neuroleptic haloperidol (Hal) is a non-selective dopamine receptor antagonist for the treatment of schizophrenia. The blocking of dopamine 2 (D2) receptors by Hal has been associated with therapeutic action and also with extrapyramidal effects such as catalepsy in rodent. Cataleptic responses to this drug vary throughout the day, with a maximum at mid-light and a minimum at mid-dark (6, 7). It is believed that the extrapyramidal effects are because of the blockage of D2 receptors and could result from the induction of the clock-controlled gene c-fos through the activation of adenylate cyclase and the protein kinase A (PKA) signaling pathway in the striatum (8–12). However, the effect of Hal on Per1 expression in mouse SCN has not been examined. Per1 is particularly interesting because of its very high amplitude of circadian expression (the peak:trough ratio is more than 5 at the mRNA level). In addition, several studies suggest that Per1 is intimately associated with organism activities because there is a similarity in response to light between Per1 mRNA induction and rodent behavior (13, 14). In Drosophila, the mutation according to the loss of functional Per1-encoded protein abolishes the free running rhythm (15). In mice, the mutation of mPer1 leads to a shorter circadian period of locomotor activity, with reduced precision and stability (16). In addition to the induction of Per1 expression by light, the induction of Per1 is also seen in cultured rat fibroblasts after exposure to high concentrations of serum or to the adenylate cyclase activator, forskolin (17, 18). Previous studies have shown that repetitive administration of the antidepressant imipramine abolishes the free running rhythm of mPer1 mRNA expression in the mouse SCN (19). These findings suggest that the expression of Per1 could be altered by various stimuli through various pathways, and this alteration may cause changes in physiological activities of downstream organisms. In another way, the alteration in the expression level of Per1 may relate to the level of susceptibility to the drug.

In the present study, therefore, we investigated the effect of Hal on the expression of mPer1 mRNA in mouse SCN. We also clarified the mechanism underlying Hal-induced mPer1 gene expression by using the non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist MK-801 and the cultured SCN cell transfected with antisense deoxyoligonucleotide directed against CAMP-responsive element-binding protein (CREB) mRNA.

**EXPERIMENTAL PROCEDURES**

**Animals and Treatments**—Six-week-old male ICR mice purchased from Charles River Japan (Kanagawa, Japan) were used. Prior to

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Experiments, the animals were housed 3–6 cages with free access to food and water, under controlled lighting. The light:dark ratio was 12:12 h (7:00 a.m., lights on; 7:00 p.m., lights off), constant temperature was 24 ± 1 °C, and controlled humidity was 60 ± 10%. All mice were kept under these conditions for 1 week before use. Hal (5 mg/ml ampule, Serenace, Dai Nippon Seiyaku Co., Ltd.) or (+/-) MK-801 maleate (Tocris) diluted or dissolved, respectively, in 0.9% saline was administered by intraperitoneal injection at the dose and time indicated below. The volumes of injection were standardized to 1 ml/10 g of body weight. In the dark period, mice were injected, and their brains were removed under dim red illumination.

To study the effect of dosage on mPer1 mRNA levels in SCN regions, groups of 18 mice were each administered saline or 1, 4, or 8 mg/kg Hal before lights on (6:00 a.m.). Thereafter, animals were decapitated at 10:00 a.m., and their brains were removed quickly and placed in Hanks’ balanced salt solution. The coronal sections were cut through the hypothalamus at the level of optic chiasma to obtain sections including the SCN, and the SCN regions were taken out to investigate mPer1 mRNA levels by RT-PCR.

To monitor the time course of mPer1 mRNA levels in SCN regions following Hal administration, groups of nine mice were each administered saline or 4 mg/kg Hal before lights on (6:00 a.m.). This dosage was employed to provide highly significant changes in mPer1 mRNA levels as well as a high suppressor effect on the circadian rhythms. The effect of dosage on mPer1 mRNA levels. In addition, this dosage is similar to the dosage used in our previous study (20). Mice were then decapitated 2, 4, 6, or 16 h after injection, and their SCN regions were dissected out to analyze mPer1 mRNA levels.

To study the influence of administration time on the induction of mPer1 mRNA levels in SCN regions, groups of nine mice were each administered saline or 4 mg/kg Hal at one of the following times: 9:00 a.m., 1:00 p.m., 5:00 p.m., 9:00 p.m., 1:00 a.m., or 5:00 a.m. Mice were decapitated 4 h after injection, and their SCN regions were collected for the measurement of mPer1 mRNA levels. The sampling time was determined to correspond with maximum mPer1 mRNA levels of response to Hal, which resulted from monitoring of mPer1 mRNA levels following the administration of 4 mg/kg Hal. To investigate the possible involvement of NMDA receptors in Hal-induced mPer1 mRNA levels in SCN regions, groups of nine mice each were administrated saline, Hal, MK-801 plus Hal, or MK-801 at 9:00 a.m. The dose of Hal or MK-801 was 4 or 0.1 mg/kg, respectively. For the MK-801 plus Hal-treated group, mice were administered MK-801 prior to Hal to 20 min. All groups of mice were decapitated 2 h after the final injection, and their SCN regions were collected to investigate mPer1 mRNA levels. The dose of MK-801 and sampling time were determined using an earlier study (21).

To study the effect of Hal on the Ser-133 phosphorylation form of CREB in SCN regions, we first investigated the 24-h rhythm in levels of P-CREB in mouse SCN presented. Groups of three mice were decapitated at 9:00 a.m., 1:00 p.m., 5:00 p.m., 9:00 p.m., 1:00 a.m., or 5:00 a.m. After decapitation, the SCN regions were immediately dissected out and analyzed for P-CREB levels by Western blot analysis. Thereafter, the other groups of mice were each administrated saline or 4 mg/kg Hal at 1:00 p.m. and then decapitated 2, 4, or 8 h after injection to monitor P-CREB levels. The injection time was determined using nearby corresponding minimal P-CREB levels that were found from 24-h rhythms of P-CREB study. To study the involvement of CREB in mediating mPer1 mRNA levels by Hal, primary cultures of SCN cells and liposome-antisense deoxyoligonucleotide directed against CREB mRNA (DNA) complex were employed. The cultures were treated with empty liposome (control), liposome-DNA complex, empty liposome plus Hal, or liposome-DNA complex plus Hal. Total RNA was collected for analysis by RT-PCR. To investigate the efficiency of liposome-DNA complex activity, the levels of CREB following CREB antisense transfection were also analyzed by Western blots.

Primary Cultures of Mouse SCN and Treatment of Cultures—Cell isolation and culture procedures were modified from an earlier report (22). Briefly, SCN regions were isolated from 5–7-day-old ICR mouse brains at 9:00 a.m. and incubated in an isotonic salt solution of 0.6 mg/ml protease (Wako Pure Chemical Industries, Ltd., Osaka, Japan) at 37 °C for 15 min. Cells were then rinsed with an isotonic salt solution mixture of 0.2 mg/ml trypsin inhibitor (Sigma) and 0.1 mg/ml DNase I (Wako Pure Chemical Industries, Ltd.) and resuspended in the same solution. Cell suspension was centrifuged at 650 rpm for 10 min. The obtained cell pellet was resuspended (about 40 μg/SNC) in Dulbecco’s modified Eagle’s medium (Nikken Bio Medical Laboratory) containing 15 mM NaHCO3, 10 mM HEPES, and 20 mg/liter kanamycin (Invitro-gen). ~1 ¥ 108 viable cells (100 μl of cell suspension) were plated into 24-well plates (Greiner Labortrchnik, Frickenhausen, Germany) coated with 0.02% poly-t-ornithine. After incubation at 37 °C in a 5% CO2 incubator for 4, 500 μl of Dulbecco’s modified Eagle’s medium supplemented with 15 mM NaHCO3, 10 mM HEPES, 5 μg/ml insulin (Sigma), 100 μg/ml human transferrin (Sigma), 100 μg/ml putrescine (Sigma), 20 μM progesterone (Sigma), 30 mM sodium selenite (Sigma), and 20 mg/liter kanamycin was overlaid, and cultures were incubated under the conditions mentioned above for further study.

For treatment of cells with CREB antisense, the procedure was performed after incubation for 24 h. The treatment was modified from an earlier report (23). The CREB gene corresponding to nucleotides 27–46 (5’-TGG TCA TCT AGT CAC CGG TG-3’) was incubated with an equivalent amount of cationic liposome (Lipofectin reagent, Invitrogen) (1 μg of DNA/5 μl of liposome) for 15 min at room temperature. Cultures were washed with modified Eagle’s minimal essential medium (OPTI-MEM I, Invitrogen) once prior to adding 500 μl of the same medium containing liposome-DNA complex. After a 5-h incubation, the medium was replaced by normal supplemented medium. For treatment of cells with Hal, 50 μM drug was directly added in a medium of empty liposome or liposome-DNA complex-transfected cells after transfection for 72 h. Total RNA was extracted after adding Hal for 2 h. CREB levels of control and CREB antisense-treated cultures were measured at 3 h after transfection, which resulted in the 5% level of probability was considered to be significant.

Effect of Haloperidol on the Clock Gene

To study the influence of Hal administration on the levels of various clock gene mRNAs in mouse SCN tissues or cultures was extracted using TRIzol solution (Invitrogen). Finally, the RNA was resuspended in diethyl pyrocarbonate-treated water and kept at -80 °C. A one-step RT-PCR system (Invitrogen) was used for reverse transcription of 200 ng of RNA, and mPer1 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were used as internal controls. The PCR primers were designed as follows: mPer1-5’-CCC AGG CCC GGA GAA CCT TTT T-3’; mPer1-3’, 5’-GCA AGT TTG AAC TCA GAA GAT T-3’. PCR was conducted in a programmed temperature control system (PC 700, Astec, Fukuoka, Japan) for 25 cycles under the following cycle conditions: 1 min at 94 °C for denaturation, 2 min at 53 °C for annealing, and 2 min at 72 °C for extension. The sizes of the PCR products of mPer1 and GAPDH were 403 and 178 base pairs, respectively. PCR products were run on 3% agarose gels. The band intensity of mPer1 mRNA was analyzed using NIH Image software on a Macintosh computer, and amounts were measured versus GAPDH.

Western Blot Analysis—The procedure of Western blot analysis for CREB and P-CREB from the mouse SCN tissues and cultures was modified from earlier works (24, 25). SCN tissues were resuspended in lysis buffer, pH 7.4, composed of 50 mM β-glycerolphosphate, 1.5 mM EGTA, 0.1 mM Na2VO4, 1 mM dithiothreitol, 10 μM leupeptin, and 1 mM phenylmethylsulfonyl fluoride and sonicated for 10 s. 2% sample buffer in the same volume of sample suspension was then added (20 μg of cell protein for each sample). For primary cultures, cells (1 ¥ 107cells/well) were washed with phosphate-buffered saline, lysed by adding 100 μl of 2× sample buffer, and then sonicated for 10 s. The sample was heated at 95 °C for 5 min. Afterward, the sample was vortexed for 20 s and then centrifuged to remove debris at 13,000 ¥ g for 5 min. The sample was loaded on a SDS-polyacrylamide gel, and proteins were separated at 20 mA for 2 h. Proteins were transferred to nitrocellulose membrane at 100 V for 2 h. Transblotted membrane was incubated at room temperature for 2 h in blocking solution composed of Tris-buffered saline containing 5% skim milk powder and 0.1% Tween 20 and then incubated at 4 °C overnight with primary rabbit antibody (1:1000 for CREB, 1:500 for P-CREB, New England Biolabs Inc., Hertfordshire, UK) in Tris-buffered saline containing 5% bovine serum albumin and 0.1% Tween 20. Thereafter, transblotted membrane was washed three times in washing buffer composed of Tris-buffered saline containing 0.1% Tween 20 prior to incubation at room temperature for 1 h with anti-rabbit secondary antibody conjugated to horseradish peroxidase (1:2000, New England Biolabs Inc.) in blocking solution. Membrane was washed three times in washing buffer and then developed using a chemiluminescence detection kit (New England Biolabs Inc.) prior to exposure on X-ray film (Kodak, NY, Amersham Biosciences, Band.) intensity was analyzed using NIH Image software on a Macintosh computer. Total proteins were verified using a Bio-Rad DC protein assay kit.

Statistics—Data were analyzed using Student’s t test for two independent groups, and analysis of variance was used for multiple comparisons. The 5% level of probability was considered to be significant. All data are expressed as mean ± S.E.
Effect of Haloperidol on the Clock Gene

RESULTS

Effect of Dosage on Levels of mPer1 mRNA in Mouse SCN Regions—Fig. 1 shows data for the levels of mPer1 mRNA followed by acute Hal administration at various doses. These results imply that mRNA levels of mPer1, which is the component of oscillators, obviously elevate following Hal treatment. Furthermore, the relative mPer1 mRNA levels showed marked differences in response to varied doses of Hal. Statistical differences (p < 0.01) were seen between saline- and Hal-treated groups. These differences increased 40, 70, and 110% for groups treated with 1, 4, or 8 mg/kg Hal, respectively, compared with the saline-treated group.

Time Course of Hal-induced mPer1 mRNA in Mouse SCN Regions—As shown in Fig. 2, mPer1 was strongly and temporarily responsive to 4 mg/kg Hal. The transient increase in the levels of mPer1 mRNA, as compared with controls, reached the maximum at 4 h after injection. Levels of mPer1 mRNA declined at 6 h after administration. The dose used on the MK-801-treated group had no significant difference between the saline and 1 mg/kg Hal-treated group (**, p < 0.01). The dose used on the MK-801 plus Hal-treated group, pretreatment with MK-801 significantly reduced the enhancement of mPer1 mRNA by Hal (~30%) compared with the Hal-treated group (p < 0.05). The dose used on the MK-801-treated group had no significant effect on mPer1 mRNA levels compared with the saline-treated group.

Effect of Administration Time on Hal-induced mPer1 mRNA in Mouse SCN Regions—We have shown previously that mPer1 could be induced by neuroleptic Hal. As shown in Fig. 4, a similar pattern of the significant enhancement of mPer1 mRNA following Hal administration compared with the saline-treated group was also obtained in this experiment (p < 0.01). In the MK-801 plus Hal-treated group, pretreatment with MK-801 significantly reduced the enhancement of mPer1 mRNA by Hal (~30%) compared with the Hal-treated group (p < 0.05). The dose used on the MK-801-treated group had no significant effect on mPer1 mRNA levels compared with the saline-treated group.

Effect of NMDA Receptor Antagonist on Hal-induced mPer1 mRNA in Mouse SCN Regions—As shown in Fig. 5, CREB levels did not vary over the time span. The open or filled bar indicates the light or dark period, respectively.

24-Hour Rhythms in P-CREB and the Effect of Hal on P-CREB Levels in Mouse SCN Regions—As shown in Fig. 5, levels of P-CREB exhibited a significant 24-h rhythm under the 12 h light:12 h dark cycle (p < 0.01). Maximum levels were observed from 5:00 to 9:00 a.m. Levels of P-CREB declined during mid-light and then began to rise before dusk. However, CREB levels did not vary over the time span.
The results of the effect of Hal on P-CREB levels is shown in Fig. 6. Remarkably, Hal treatment triggered the temporal induction of P-CREB 2 h after administration by 50% compared with the group of mice treated by saline and sampled at the same time (p < 0.05). Thereafter, P-CREB levels declined until they reached basal levels ~4 h after injection. In the saline-treated group, P-CREB levels 8 h after injection (9:00 p.m.) were significantly higher than at all other sampling time points (p < 0.05).

Effect of CREB Antisense on Hal-induced mPer1 mRNA in Mouse SCN Cultures—In our experiment, the transfection conditions showed that treatment with antisense deoxyoligonucleotide significantly reduced CREB levels in SCN cultures (Fig. 7A). The effect of treatment with CREB antisense on mPer1 mRNA in SCN cultures is shown in Fig. 7, B and C. A significant difference in mPer1 mRNA levels was obtained between control and cultures treated with antisense CREB (p < 0.01). Treatment of control cultures with 50 μM Hal resulted in significant increases in mPer1 mRNA levels (p < 0.05). On the other hand, the stimulation by Hal was significantly reduced when the cultures were pretreated with CREB antisense (p < 0.01). In addition, a significant difference between control and the CREB antisense plus Hal-treated group was seen (p < 0.05).

**DISCUSSION**

The explanation of complex behaviors at the level of gene expression is one of the ultimate goals of neuroscience. A number of studies have shown that mPer1 in SCN may be central in determining organism activities because there is a similarity in behavior in the response to light in mPer1 mRNA induction. Another question is whether the effects of other external cues, such as drug, interfere with the action of dopamine and affect motor activity in mPer1 expression. To answer this question, mPer1 mRNA levels of SCN were monitored in mice given neuroleptic Hal. Interestingly, mPer1 mRNA levels could be strongly and transiently induced by acute administration of Hal in a dose-dependent manner.

We attempted to clarify the possible biochemical pathways that mediate transcriptional induction of mPer1 following acute Hal administration (Fig. 8). We found that Hal could greatly activate (by 50%) the phosphorylation of transcription factor CREB within 2 h after treatment. The obtained results of our present study revealed the maximum influence of Hal on mPer1 4 h after treatment. In addition, the study of the effect of CREB antisense on Hal-induced mPer1 mRNA in the cultured SCN cell showed that the induction levels of mPer1 mRNA by Hal sig-
Effect of CREB antisense on Hal-induced mPer1 mRNA in SCN cultures. Cultures were treated with empty liposome (control), liposome containing CREB antisense, 50 μM Hal, or CREB antisense prior to 50 μM Hal. A, photographs represent the band intensity of CREB of cultures before and after transfection. B, photographs represent the band intensity of RT-PCR products of mPer1 and GAPDH mRNA. C, percentage of relative mPer1 mRNA levels is shown against GAPDH mRNA levels. Relative mRNA levels were determined with the control value being adjusted to 100. Each column represents mean ± S.E. of studies performed in triplicate. Student’s t test indicated the significant difference between control and CREB antisense-treated cultures (**, p < 0.01), between control and Hal-treated cultures (*, p < 0.05), between control and CREB antisense plus Hal-treated cultures (**, p < 0.01), and between the Hal-treated and the CREB antisense plus Hal-treated cultures (***, p < 0.001).

A significant decrease in mPer1 mRNA levels in non-Hal-treated cultures transfected by CREB antisense, as compared with control, was also seen. Four conserved cAMP-responsive elements (TGACGTCA) are present in the 5' flanking regulatory regions of the mPer1 genes (26, 27). The phosphorylated CREB activates cAMP-responsive element-mediated Per1 transcription (25, 26). In addition, it has been reported that the adenylate cyclase (AC) activator, forskolin, increases rPer1 mRNA in rat-1 fibroblast. These results suggest that Hal enhances the phosphorylation of CREB and finally elicits the levels of mPer1 mRNA through P-CREB binding with the cis-element (CaRE/cAMP-responsive element; calcium/CAMP-responsive element).

Another question is how Hal activates and phosphorylates CREB. Hal mediates the pharmacological action mainly via antagonizing D2 receptors. However, Hal also affects D1 receptors, α1 noradrenaline receptors, and serotonin receptors (28–31). Recent studies using DNA microarray technology have identified expression of D2 receptors (32), D1 receptors (33), 5HT1a, 5HT1b, 5HT5a, and 5HT7 serotonin receptors (34, 35), α1 noradrenaline receptors (36), and NMDA receptors (37) in mouse SCN. D2 receptors inhibit AC by coupling with the inhibitory guanine nucleotide-binding protein (G_i protein) (33). The so-called G_i protein couples to inhibitory receptors, finally resulting in inhibition of cAMP formation. Inhibition of D2 receptors with Hal increases cAMP levels, activates the PKA second messenger pathway, and increases CREB phosphorylation and mPer1 expression. On the other hand, the second messenger pathway also increases NMDA receptors function via the 897Ser-NR1 subunit of NMDA receptors phosphorylation, such that NMDA receptors activate Ca^{2+} influx. Ca^{2+} influx activates Ca^{2+}/calmodulin (CaM)-dependent protein kinases and the extracellular signal-regulated kinase-Mitogen-activated protein kinase (ERK-MAPK) pathway (39). The Ca^{2+}/CaM-dependent protein kinases and ERK-MAPK signaling cascade induce CREB phosphorylation and mPer1 mRNA according to the activation of NMDA receptor through the dopaminergic pathways.

As the other possible pathway, D1 receptors stimulate AC by coupling with stimulatory guanine nucleotide-binding protein (G_s protein) (33). The so-called G_s protein couples to stimulatory receptors, ultimately leading to the stimulation of cAMP formation. Both dopamine and the D1 agonist increase CREB phosphorylation, and D1 antagonist blocks the effect in SCN (40, 41). Inhibition of D1 receptors with Hal may decrease cAMP levels, inactivate the PKA second messenger pathway, and decrease CREB phosphorylation. α1 noradrenaline receptors stimulate phospholipase C through guanine nucleotide-binding protein (G protein) (33). The so-called G protein couples to inhibitory receptors, finally resulting in inhibition of cAMP formation. Inhibition of D2 receptors with Hal increases cAMP levels, activates the PKA second messenger pathways, and increases CREB phosphorylation (38). On the other hand, the second messenger pathway also increases NMDA receptors function via the 897Ser-NR1 subunit of NMDA receptors phosphorylation, such that the NMDA receptors activate Ca^{2+} influx (38). Ca^{2+} influx activates Ca^{2+}/CaM-dependent protein kinases and the extracellular signal-regulated kinase-Mitogen-activated protein kinase (ERK-MAPK) pathway (39). The Ca^{2+}/CaM-dependent protein kinases and ERK-MAPK signaling cascade induce CREB phosphorylation and mPer1 mRNA according to the activation of NMDA receptor through the dopaminergic pathways.

FIG. 8. Model illustrates hypothesized mechanism underlying Hal-induced mPer1 mRNA in mouse SCN. There are D2 receptors, 5HT1a and 5HT1b serotonin receptors, and NMDA receptors in the SCN. D2, 5HT1a, and 5HT1b receptors with Hal increases cAMP levels, activates the PKA second messenger pathway, and increases CREB phosphorylation and mPer1 expression. On the other hand, the second messenger pathway also increases NMDA receptor function via the 897Ser-NR1 subunit of NMDA receptors phosphorylation, such that NMDA receptors activate Ca^{2+} influx. Ca^{2+} influx activates Ca^{2+}/CaM-dependent protein kinases and the extracellular signal-regulated kinase-Mitogen-activated protein kinase (ERK-MAPK) pathway (39). The Ca^{2+}/CaM-dependent protein kinases and ERK-MAPK signaling cascade induce CREB phosphorylation and mPer1 mRNA according to the activation of NMDA receptor through the dopaminergic pathways.
receptors with Hal may decrease phospholipase C activation levels, inactivate the protein kinase C second messenger pathway, and decrease CREB phosphorylation. 5HT1a and 5HT1b receptors inhibit AC with a rapid decrease of cAMP levels via G_{i} protein (45). Inhibition of 5HT1a receptors with Hal may increase cAMP levels, activate the PKA second messenger pathway, and increase CREB phosphorylation. 5HT5a and 5HT7 receptors stimulate AC mainly through a stimulatory G_{s} protein (45). 8-Hydroxy-2-(di-n-propylamino)tetratin (8-OH DPAT), a selective agonist at 5HT1a and 5HT5a/7, exhibited a higher affinity for 5HT5/7 receptors than 5HT1a receptors (46, 47). 8-OH DPAT increased cAMP production. Inhibition of 5HT5a and 5HT7 receptors with Hal may decrease cAMP levels, inactivate the PKA second messenger pathway, and decrease CREB phosphorylation. These results suggest that Hal may induce CREB phosphorylation via 5HT1a and 5HT7 serotonin receptors in addition to D2 receptors and NMDA receptors mediated by D2 receptors.

We also found that Hal induced the levels of mPer1 mRNA at every time point. In addition, the amount of induction is minimal at the time corresponding to minimum catalepsy response to our previous study (20). These findings likely indicate that the alteration of mPer1 levels in SCN correlates to the alteration of drug response. However, the induction of mPer1 mRNA at the time corresponding to minimum catalepsy reduction of mPer1 may show a phase shift or resetting effect of the circadian clock.

In conclusion, our results demonstrate that Hal is able to induce the levels of mPer1 mRNA in mouse SCN, both in vivo and in cultured cells. Moreover, our study could clarify the mechanism underlying Hal-induced mPer1. Here we reveal that the NMDA receptor and P-CREB play an important role in the Hal-induced mPer1 phenomenon.

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