Nonphotic Entrainment by 5-HT$_{1A/7}$ Receptor Agonists Accompanied by Reduced *Per1* and *Per2* mRNA Levels in the Suprachiasmatic Nuclei

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In mammals, the environmental light/dark cycle strongly synchronizes the circadian clock within the suprachiasmatic nuclei (SCN) to 24 hr. It is well known that not only photic but also nonphotic stimuli can entrain the SCN clock. Actually, many studies have shown that a daytime injection of 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH DPAT), a serotonin 1A/7 receptor agonist, as a nonphotic stimulus induces phase advances in hamster behavioral circadian rhythms *in vivo*, as well as the neuron activity rhythm of the SCN *in vitro*. Recent reports suggest that mammalian homologs of the *Drosophila* clock gene, *Period* (*Per*), are involved in photic entrainment. Therefore, we examined whether phase advances elicited by 8-OH DPAT were associated with a change of *Period* mRNA levels in the SCN. In this experiment, we cloned partial cDNAs encoding hamster *Per1*, *Per2*, and *Per3* and observed both circadian oscillation and the light responsiveness of *Period*. Furthermore, we found that the inhibitory effect of 8-OH DPAT on hamster *Per1* and *Per2* mRNA levels in the SCN occurred only during the hamster's mid-subjective day, but not during the early subjective day or subjective night. The present findings demonstrate that the acute and circadian time-dependent reduction of *Per1* and/or *Per2* mRNA in the hamster SCN by 8-OH DPAT is strongly correlated with the phase resetting in response to 8-OH DPAT.

Key words: suprachiasmatic nucleus; 8-OH DPAT; Per *mRNA; 5-HT$_{1A/7}$ receptor; hamster; circadian rhythm; NIH Image*

In mammals, the suprachiasmatic nucleus (SCN) of the hypothalamus has been shown to be a primary circadian pacemaker of locomotor activity and various physiological phenomena (Hastings, 1997). Recent studies on the molecular aspects of clock genes have produced a functional model of circadian rhythms (for review, see Dunlap, 1999).

mPer1, *mPer2*, and *mPer3*, cloned as mouse homologs of the *Drosophila* clock gene, *Period* (*Per*), exhibit circadian rhythmic expressions in the SCN (Albrecht et al., 1997; Shearman et al., 1997; Sun et al., 1997; Tei et al., 1997; Takumi et al., 1998a,b; Zylka et al., 1998). Brief exposure to light during subjective night results in a large and rapid induction of *mPer1* expression (Albrecht et al., 1997; Shigeyoshi et al., 1997). *mPer2* mRNA expression in the SCN is also induced in response to light stimuli (Shearman et al., 1997; Takumi et al., 1998a). On the other hand, *mPer3* mRNA levels do not respond to light during either the subjective night or subjective day (Takumi et al., 1998b; Zylka et al., 1998). Recently, we demonstrated that light-induced phase delays in locomotor activity at CT16 were significantly inhibited when mice were pretreated with *mPer1* antisense phosphorothioate oligodeoxynucleotide (ODN) (Akiyama et al., 1999). Therefore, we suggest that the gated expression of *mPer1* may be an important step in causing photic entrainment.

On the other hand, nonphotic manipulation such as novel wheel-running (Reebs and Mrosovsky, 1989), social interaction (Mrosovsky, 1988), and saline injection and/or handling (Mead et al., 1992) reportedly causes big phase advances in the hamster circadian clock when performed during subjective day. Additionally, many studies have shown that a daytime injection of 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH DPAT), a serotonin 1A/7 receptor agonist, induces a phase advance in hamster behavioral circadian rhythms *in vivo* (Tominaga et al., 1992; Edgar et al., 1993; Cutrera et al., 1996; Mintz et al., 1997), as well as the neuron activity rhythm of the SCN *in vitro* (Shibata et al., 1992; Prosser et al., 1993). Thus, serotonin (5-HT) has been implicated in phase shifts of the circadian system during subjective day in response to nonphotic stimuli. Because light exposure induces *mPer1* and *mPer2* expression during subjective night, we questioned whether injection of 8-OH DPAT modifies the *Per* mRNA levels during subjective day. Almost all behavioral experiments investigating nonphotic-induced phase advances were performed using hamsters, so we attempted to clone partial cDNAs encoding the golden hamster *Per1*, *Per2*, and *Per3*. Therefore, we first established that oscillations and light responses of hamster *Per* gene mRNA were similar to what has previously been published in the mouse. Second, we found that injection of 8-OH DPAT at CT6, but not at CT1 or CT20, reduced the amount of *Per1* and *Per2*, but not *Per3* mRNA in the hamster SCN. Third, we demonstrated that nonphotic phase shifting with 8-OH DPAT is strongly correlated with an 8-OH DPAT-dependent, transient decrease in *Per1* and *Per2* mRNA levels.

MATERIALS AND METHODS

Cloning of partial cDNAs encoding hamster Per *genes.* For analysis of *Per* gene expression by *in situ* hybridization, we attempted to clone partial cDNAs encoding the golden hamster *Per* genes. Total RNA was extracted from the golden hamster brain using Trizol (BRL, Bethesda, MD) and was reverse-transcribed using the Superscript one-step RT-PCR system (BRL). RT-PCR was performed using a DNA Thermal Cycler 9600 (Perkin-Elmer, Norwalk, CT) with specific primers derived from mouse and human sequences of *Per* genes. The sequences of the primers were as follows: *Per2* (nucleotide position 822–1601 of *mPer2*; GenBank accession number AF035830): 5′-ACACCACCCCTTACAAGCTTCC-3′, 5′-CGCTGGATGATGTCTGGCTC-3′; *Per3* (nucleotide position 1956–2754 of *mPer3*; GenBank accession number AF050182): 5′-GAACTGTATCG-

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ACAGTGTCATC-3′, 5′-GGCCATATCTTGGAGGGGAAA-3′. The PCR protocol was executed under the following conditions: cDNA synthesis and predenaturation at 50°C for 30 min followed by 94°C for 2 min, PCR amplification for 35 cycles with denaturation at 94°C for 15 sec, annealing at 55°C for 30 sec, extension at 72°C for 1 min, and final extension at 72°C for 5 min. These PCR products were subcloned into the pGEM-T Easy Vector (Promega, Madison, WI) and were sequenced using ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer). A partial cDNA encoding hamster *Per1* corresponding to the nucleotide position 726–1367 of *mPer1* (GenBank accession number AB002108) was also cloned (S. Yamamoto and H. Okamura, data not shown).

Animals. Male golden hamsters (*Mesocricetus auratus*, Tokyo Laboratory Animals Science Co. Ltd., Tokyo, Japan) purchased 6 weeks postpartum were maintained on a 12 hr light/dark cycle with lights on at 8:30 A.M. (room temperature at 23 ± 2°C). Animals were given food and water *ad libitum*. For assessment of wheel-running activity, hamsters were housed individually in transparent plastic cages (36 × 20 × 20 cm) equipped with a running wheel (13 cm in diameter) that closed a microswitch with each revolution. The number of wheel rotations was measured, and data were stored on a personal computer.

Behavioral experiment. Because 8-OH DPAT has an asymmetrical carbon, this compound has two optical isomers: *R*(+) and *S*(−) 8-OH DPAT. A few studies have investigated the relationship between binding affinity of 5-HT_{1A} and 5-HT_7 receptors and the effect of each optical isomer on circadian rhythms (Lovenberg et al., 1993; Eriksson and Evrin, 1996; Miller et al., 1996; Ying and Rusak, 1997). After free-running for 14–20 d in constant darkness, hamsters were randomly assigned to an intraperitoneal injection of 8-OH DPAT (1.0 or 5.0 mg/kg; Research Biochemicals, Natick, MA), (+) 8-OH DPAT (2.5 mg/kg, Research Biochemicals), triazolam (20 mg/kg, Upjohn), or vehicle [sterilized saline for both 8-OH DPAT and (+) 8-OH DPAT or dimethyl sulfoxide (Wako) for triazolam]. Injection was performed at circadian time (CT; CT12: onset time of wheel-running activity) 1, CT6, CT8, CT14, or CT20. Animals were then 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 (Daan and Pittendrigh, 1976).

In situ *hybridization using digoxigenin. In situ* hybridization using digoxigenin (DIG) was applied to determine the semiquantity or histochemical distribution of *Per* mRNA levels in coronal sections of the hypothalamus. Hamsters were entrained to the light/dark cycle for at least 14 d and then kept in constant dark conditions. On the third day of constant darkness at CT6 or CT20, hamsters were intraperitoneally injected with each drug and then deeply anesthetized with ether 1, 2, or 4 hr after injection and intracardially perfused with 0.1 M phosphate buffer (PB) containing 4% paraformaldehyde (PFA). In some cases, hamster brains were obtained for observation of circadian changes in *Per* gene expression in the SCN. To make this observation, light (60 lux, 15 min) was applied at CT14 or CT20, and hamsters were killed 1 or 2 hr after the initiation of light exposure. Brains were removed, post-fixed in 0.1 M PB containing 4% PFA for 24 hr at 4°C, and transferred into 20% sucrose in PBS for 24 hr at 4°C. Frontal sections (40 μm thick) were collected and placed in PBS for 30 min, followed by treatment with 6 × SSC for 30 min. Sections were incubated in hybridization buffer [50% formamide, 6 × SSC, 0.1 mg/ml denatured salmon sperm DNA, 1 × Denhardt's solution (0.02% Ficoll, 0.02% polyvinyl pyrolidone, 0.02% bovine serum albumin), and 10% dextran sulfate] containing labeled cRNA probes overnight at 60°C. DIG-UTP (Roche Molecular Biochemicals, Indianapolis, IN)-labeled antisense cRNA was made using a standard protocol for cRNA synthesis. After hybridization, these sections were rinsed in 2 × SSC/50% formamide for 45 min followed by 15 min at 60°C and treated with RNase A for 30 min at 37°C, 2 × SSC/50% formamide for 2 × 15 min at 60°C, and 0.4 × SSC for 30 min at 60°C. Sections were processed for immunocytochemistry by following the DIG nucleic acid detection kit (Roche Molecular Biochemicals) protocol. Photomicrographs were taken with a Fujix digital camera (HC-300, Fujifilm, Tokyo Japan) and captured with photograb-300 (Fujifilm). The density of *Per* gene expression was semiquantified on a Macintosh computer using the public domain NIH Image program (written by Wayne Rasband, National Institutes of Health).

In situ *hybridization using radioisotope. In situ* hybridization using radioisotope (RI)-labeled probes was applied to determine the quantity of *Per1*, *Per2*, and *Per3* mRNA levels in coronal sections of the hypothalamus. Hamsters were entrained to the light/dark cycle for at least 14 d and then kept in constant dark conditions. On the third day of constant darkness at CT1, CT6, or CT20, hamsters were intraperitoneally injected with each drug and then deeply anesthetized for 2 hr after injection and intracardially perfused with 0.1 M PB containing 4% PFA. Brains were removed, post-fixed in 0.1 M PB containing 4% PFA for 24 hr at 4°C, and transferred into 20% sucrose in PBS for 24 hr at 4°C. Frontal sections (30 μm thick) were collected and placed in 2 × SSC and then treated with proteinase K (1.0 μg/ml, 10 mM Tris buffer, pH 7.5, 10 mM EDTA) for 10 min at 37°C, 4% PFA in 0.1 M PB for 5 min, and 2 × SSC for 5 min followed by 0.25% acetic anhydride in 0.1 M triethanolamine for 10 min and 2 × SSC for 2 × 5 min. RI [α[^{33}P]UTP (New England Nuclear)]-labeled antisense cRNA was made using a standard protocol for cRNA synthesis. Hybridization and posthybridization washing steps were the same as the protocol for DIG *in situ* hybridization. RI *in situ* hybridization images were visualized by autoradiogram and BioMax film (Kodak) and analyzed using an image analyzing system (MCID, Imaging Research Inc.) after conversion into optical density by ^{14}C-autoradiographic microscales (Amersham, Arlington Heights, IL). The values were expressed as means ± SEM. For statistical analysis, one-way ANOVA followed by the Student's *t* test was applied.

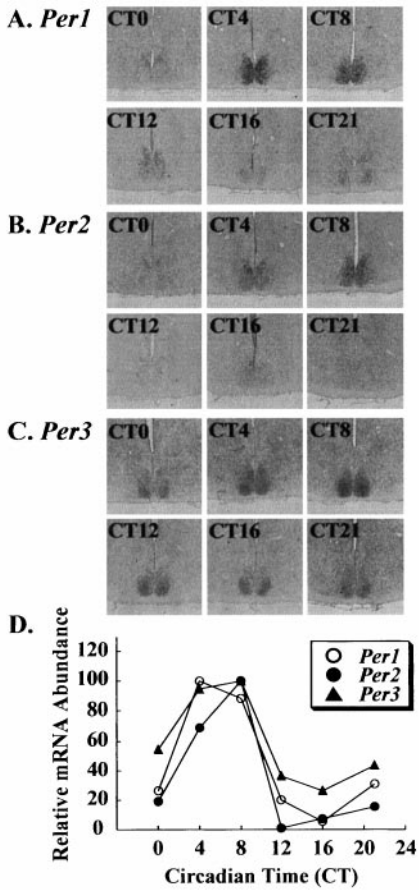

Figure 1. Circadian expressions of (*A*) *Per1*, (*B*) *Per2*, and (*C*) *Per3* in the hamster suprachiasmatic nucleus. mRNA expression was demonstrated by DIG *in situ* hybridization and semiquantified by a Macintosh computer using the public domain NIH Image program (*D*). There are clear circadian rhythms of *Per1*, *Per2*, and *Per3* expression in the hamster SCN with a peak at CT4 for *Per1* and at CT8 for both *Per2* and *Per3*.

RESULTS

Oscillation and light response of SCN *Per* mRNA expression

To evaluate the topographic differences of expression profile in hamster *Per* genes of the SCN, *in situ* hybridizations using digoxigenin-labeled probes of hamster *Per1, Per2*, and *Per3* were performed. DIG *in situ* hybridization showed clear signals with the antisense probes in the hamster SCN (Fig. 1). These antisense probe signals were suppressed by competition experiments using the unlabeled antisense probes. Furthermore, the sense probes demonstrated specificity of the antisense hybridization by exhibiting no signals within the SCN (data not shown). The distribution or expression patterns of hamster *Per* mRNA inside and outside the SCN were consistent with those of already published findings in the mouse (Albrecht et al., 1997; Shearman et al., 1997; Sun et al., 1997; Tei et al., 1997; Takumi et al., 1998a,b; Zylka et al., 1998). Next, we examined the circadian pattern of *Per* gene expression in the hamster SCN. There were clear circadian rhythms of *Per1*, *Per2*, and *Per3* expression in the hamster SCN with a peak at CT4 for *Per1* and at CT8 for both *Per2* and *Per3* (Fig. 1). In nocturnal rodents, it is well established that light pulses administered during the early subjective night cause phase delays of the circadian rhythm, whereas pulses delivered during the latter half of the

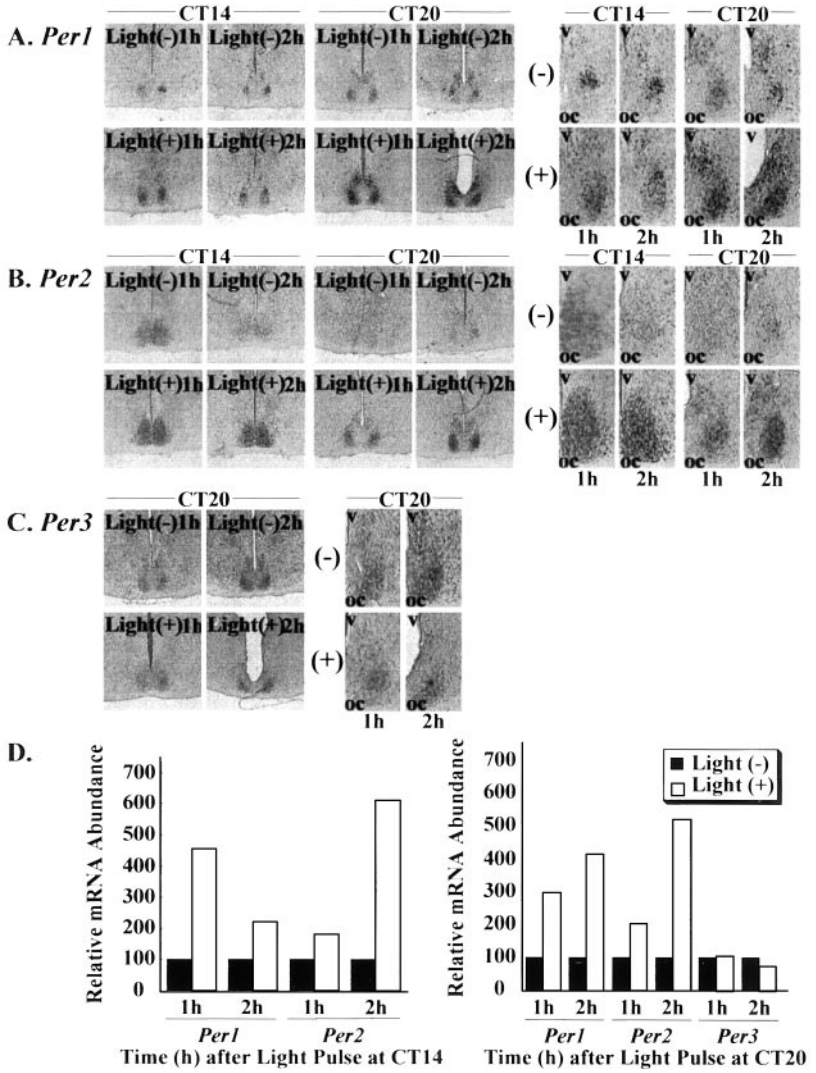

Figure 2. Effect of light exposure on the expression of (*A*) *Per1*, (*B*) *Per2*, and (*C*) *Per3* in the hamster suprachiasmatic nucleus. mRNA expression was demonstrated by DIG *in situ* hybridization, and a more magnified picture is shown. *Per* mRNA expression was semiquantified by a Macintosh computer using the public domain NIH Image program (*D*). Hamster was exposed to light (60 lux, 15 min) at CT14 or CT20 and then killed 60 or 120 min after light pulse. Light at CT14 or CT20 induced *Per1* and *Per2* expression in the SCN; however, *Per3* expression was not affected by light exposure at CT20. *oc*, Optic chiasma; *v*, third ventricle; *(+)*, light pulses were applied; *(−)*, light pulses were not applied.

subjective night cause phase advances. Recent reports indicated that *mPer1* and *mPer2* expression in the mouse SCN was increased rapidly and transiently after brief light exposure during both early and late subjective night, whereas *mPer3* expression was not (Takumi et al., 1998b; Zylka et al., 1998). We also observed that *Per1* and *Per2* expression in the hamster SCN was induced in response to brief light exposure at CT14 or CT20, and *Per3* expression was not affected by light stimulation at CT20 (Fig. 2). In both *Per1* and *Per2* cases, we found that signal intensities were more likely to be stronger in the ventrolateral part of the SCN than in the dorsomedial part. These results were consistent with previous data found in the mouse.

Wheel-running activity in response to 8-OH DPAT

Vehicle administration at CT6 did not show any change in phase (Fig. 3*A*); however, 8-OH DPAT (5.0 mg/kg) administration at CT6 produced a clear phase advance (Fig. 3*B*). Administration of this compound at CT20 did not affect the wheel-running rhythm (Fig. 3*C*). Administration of 8-OH DPAT (5.0 mg/kg) at various CTs (CT1, CT6, CT8, CT14, CT20) was compared with vehicle administration (Fig. 3*D*). Significant phase advances were observed when this compound was administered at CT6 or CT8. There were no significant differences at other CTs. Administration of triazolam (20 mg/kg) at CT6 also caused a phase advance in hamster wheel-running rhythm (data not shown).

Effect of 8-OH DPAT on SCN *Per* mRNA

Administration of 8-OH DPAT (5.0 mg/kg) reduced the levels of *Per1* in the entire SCN and *Per2* preferentially in the ventrolateral part of the SCN (Fig. 4*A*,*B*). Four hours after drug injection, however, reduced *Per1* and *Per2* levels recovered to the control level of vehicle-treated animals. Injection of 8-OH DPAT at CT20 did not change the *Per1* and *Per2* levels in the SCN. (+) 8-OH DPAT (2.5 mg/kg)-induced reduction of *Per1* and *Per2* was similar to 8-OH DPAT (5.0 mg/kg)-induced reductions (Fig. 4*A*,*B*). Injection of 8-OH DPAT did not reduce the *Per3* mRNA levels (Fig. 4*C*). For quantitative measurements of *Per* mRNA levels, *in situ* hybridizations using RI-labeled probes were performed. In Figure 5, the mean values for *Per1*, *Per2*, and *Per3* in the hamster SCN of 8-OH DPAT and vehicle-treated groups at various CTs are shown. Vehicle-treated hamsters exhibit clear circadian rhythms of *Per1* (ANOVA, $F_{(2,12)} = 9.363$, $p = 0.0032$), *Per2* (ANOVA, $F_{(2,13)} = 62.375$, $p = 0.0001$), and *Per3* (ANOVA, $F_{(2,13)} = 39.892$, $p = 0.0001$) expression in the SCN. High expression of these genes was seen at CT6. Two hours after the administration of 8-OH DPAT (5.0 mg/kg) at CT6, the amount of both *Per1* and *Per2* mRNA was significantly reduced in comparison with the group receiving vehicle treatment (Fig. 5*A*,*B*). On the other hand, injection of 8-OH DPAT at CT1 or CT20 did not affect the amount of *Per1* and *Per2* mRNA. Interestingly, this drug did not change the *Per3* mRNA of the SCN at any CTs. In the next experiment, we examined whether 8-OH DPAT reduced *Per1* and *Per2* mRNA in a dose-dependent manner (Fig. 6). Administration of 8-OH DPAT caused a phase advance in hamster wheel-running rhythm in a dose-dependent manner (Fig. 6*A*), and this compound also reduced the amount of both *Per1* and *Per2* mRNA in a dose-related fashion (Fig. 6*B*,*C*). Thus, the effective dose for behavioral phase shift induction and

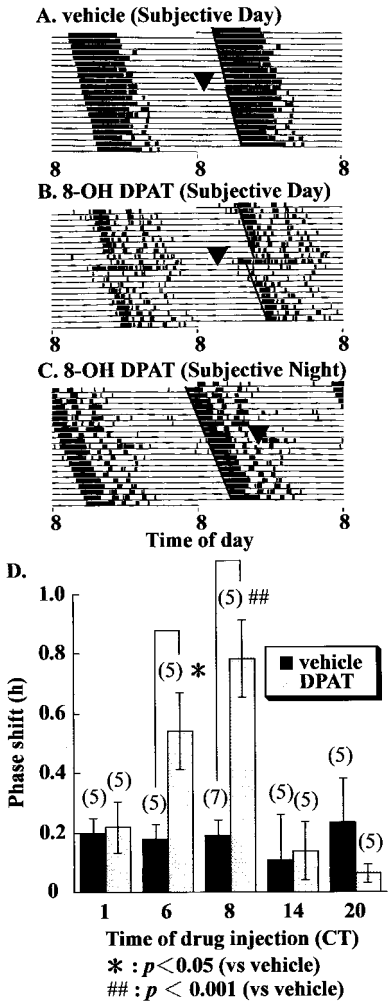

Figure 3. Effects of 8-OH DPAT administration on the hamster circadian wheel-running rhythm. Double-plotted actogram shows wheel-running activity records of (*A*) vehicle and (*B*) 8-OH DPAT (5.0 mg/kg, i.p.)-injected hamster at CT6, and (*C*) 8-OH DPAT (5.0 mg/kg, i.p.) injection at CT20. Each animal was injected at CT6 or CT20 (*arrowheads*) and returned to constant darkness. *D*, Mean phase advances induced by 8-OH DPAT (5.0 mg/kg, i.p.) administration at CT1, CT6, CT8, CT14, and CT20. *Numbers* in *parentheses* indicate the number of experiments. Injection of 8-OH DPAT at CT6 or CT8 induced a significant phase advance ($^{*}p < 0.05$, $^{\#\#}p < 0.001$, Student's *t* test).

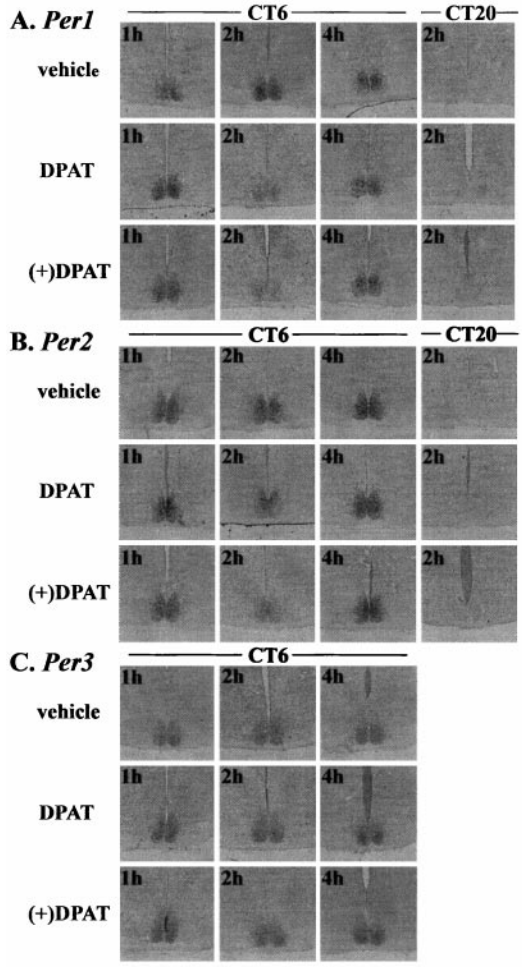

Figure 4. Effects of 8-OH DPAT (5.0 mg/kg, i.p.) or (+) 8-OH DPAT (2.5 mg/kg, i.p.) on the expression of (*A*) *Per1*, (*B*) *Per2*, and (*C*) *Per3* in the hamster suprachiasmatic nucleus. mRNA levels were demonstrated by DIG *in situ* hybridization. Drug was administered at CT6 or CT20, and *Per* mRNA levels were examined 60–240 min after drug injection. 8-OH DPAT reduced *Per1* and *Per2* mRNA levels in the SCN 120 min after injection at CT6, but mRNA levels returned to the control level 240 min after injection. Injection of 8-OH DPAT at CT20 did not affect *Per1* or *Per2* mRNA levels. The amount of *Per3* mRNA was not affected by injection of 8-OH DPAT.

reduction of *Per1* and *Per2* was very similar. Amplitude of *Per1* and *Per2* reduction by (+) 8-OH DPAT (5.0 mg/kg) was similar to that of (+) 8-OH DPAT (2.5 mg/kg) (Fig. 6). Administration of triazolam (20 mg/kg), a central-type benzodiazepine receptor ligand, at CT 6 also reduced the amount of SCN *Per1* and *Per2* but not *Per3* mRNA.

DISCUSSION

In situ hybridizations using digoxigenin-labeled probes of hamster *Per* genes revealed clear circadian expressions of *Per1*, *Per2*, and *Per3* in the hamster SCN. The pattern of expression of these genes is very similar to that observed in the mouse (Albrecht et al., 1997; Shearman et al., 1997; Sun et al., 1997; Tei et al., 1997; Takumi et al., 1998a,b; Zylka et al., 1998) and rat (Sakamoto et al., 1998; Yan et al., 1999) SCN, with peak expression of hamster *Per1*, *Per2*, and *Per3* found at CT4, CT8, and CT8, respectively. Reportedly, light exposure during subjective night causes a rapid induction of *mPer1* and *mPer2* in the SCN (Albrecht et al., 1997; Shearman et al., 1997; Takumi et al., 1998a). In the hamster, we also observed that *Per1* and *Per2* have light-responsive characters similar to mouse *period* genes.

Anatomical subdivisions such as the ventrolateral and dorsomedial parts in the rat SCN have been well established (Moore, 1982). Recently, we reported that light exposure at CT16 induced the expression of rat *Per1* and *Per2* in SCN neurons of the ventrolateral part, although the circadian rat *Per1* and *Per2* mRNA oscillations in light/dark and constant dark conditions occurred strongly in neurons in the dorsomedial part but weakly in neurons in the ventrolateral part of the SCN (Yan et al., 1999). In the hamster SCN, light-induced expression of *Per1* and *Per2* was preferential to the ventrolateral part of the SCN. Although the basic compartment profile of *Per* gene expression may be preserved in the hamster SCN, however, anatomical subdivision of the hamster SCN is vague compared with the rat SCN. Therefore, further experiments are needed to elucidate the compartmentalization of circadian profiles in *Per* genes in the hamster in detail.

In the present experiment, we demonstrated that 8-OH DPAT reduces SCN *Per1* and *Per2* mRNA in a circadian time-dependent manner. Actually, 8-OH DPAT reduced these gene mRNAs when administered at CT6 but not at CT1 or CT20. This result relates to the behavioral result showing a large phase advance at CT6 and CT8 but not at CT1 or CT20. In addition, 8-OH DPAT reduced *Per1* and *Per2* in a dose-dependent fashion. The dose and threshold closely correlated with *Per1* and *Per2* reduction in the SCN. *mPer1* and *mPer2* transcription is rapidly induced by light in a time-of-

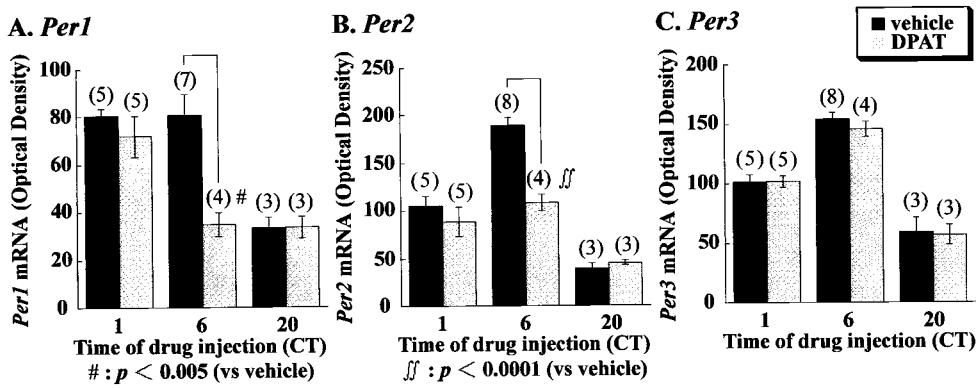

Figure 5. Effect of 8-OH DPAT at various CTs on *Per1*, *Per2*, and *Per3* expression in the hamster suprachiasmatic nucleus. RI *in situ* hybridization was performed for the quantitative analysis. Circadian expression of these genes was observed at (*A*) *Per1* (ANOVA, $F_{(2,12)} = 9.363$, $p = 0.0032$), (*B*) *Per2* (ANOVA, $F_{(2,13)} = 62.375$, $p = 0.0001$), and (*C*) *Per3* (ANOVA, $F_{(2,13)} = 39.892$, $p = 0.0001$). 8-OH DPAT (5.0 mg/kg, i.p.) significantly reduced *Per1* ($^{\#}p < 0.005$, Student's *t* test) and *Per2* ($^{\int\int}p < 0.0001$, Student's *t* test) but not *Per3* mRNA levels 2 hr after drug injection at CT6. *Numbers* in *parentheses* indicate the number of experiments.

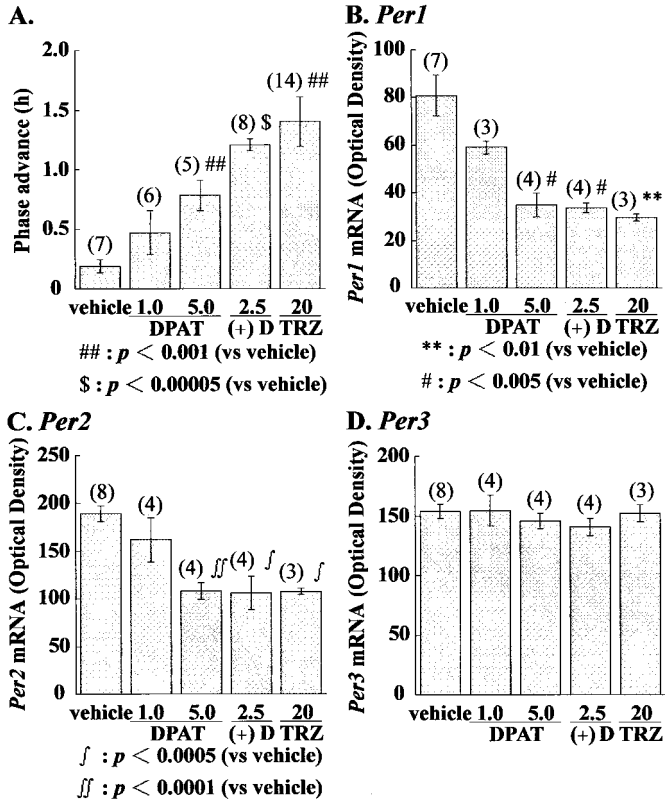

Figure 6. Dose-dependent advance of behavioral rhythms (*A*) and dose-dependent reduction of *Per1* (*B*), *Per2* (*C*), and *Per3* (*D*) mRNA levels by 8-OH DPAT (1.0 or 5.0 mg/kg, i.p.), (+) 8-OH DPAT (2.5 mg/kg, i.p.), or triazolam (20 mg/kg, i.p.). mRNA levels were quantified using RI *in situ* hybridization. $^{**}p < 0.01$, $^{\#}p < 0.005$, $^{\#\#}p < 0.001$, $^{\int}p < 0.0005$, $^{\int\int}p < 0.0001$, $^{\$}p < 0.00005$ versus vehicle alone, Student's *t* test. *Numbers* in *parentheses* indicate the number of experiments. *DPAT*, 8-OH DPAT; *(+)D*, (+) 8-OH DPAT; *TRZ*, triazolam.

day-dependent manner (Shigeyoshi et al., 1997; Takumi et al., 1998b). Gating is known to play a role in the light-induced phase shifts of behavioral rhythms. The responsiveness of *mPer1* mRNA to light is closely related to behavioral phase delays induced by light (Shigeyoshi et al., 1997). In addition, we previously demonstrated that light-induced phase delays in locomotor activity at CT16 were significantly inhibited when the mice were pretreated with *mPer1* antisense ODN before light exposure (Akiyama et al., 1999). Therefore, the gated induction of *mPer1* is a step necessary for producing behavioral phase shifts. These results along with our present results suggest that gated inhibition of *Per1* and/or *Per2* expression by nonphotic stimulation may facilitate *Per* gene reduction resulting in the onset of the next circadian induction of *Per* gene expression.

(+) 8-OH DPAT exhibited a higher affinity for 5-HT_7 receptors than for 5-HT_{1A} receptors, and amplitude of *Per1* and *Per2* reduction by 8-OH DPAT (5 mg/kg) was similar to that of (+) 8-OH DPAT (2.5 mg/kg). Thus, we estimate that the potential effect of (+) 8-OH DPAT is two times higher than that of 8-OH DPAT. The present results suggest that 5-HT_7 receptors, rather than 5-HT_{1A} receptors, have a more important role in phase shifting, as pointed out by Ying and Rusak (1997) during investigation of the inhibitory effect of 5-HT_7 receptors on light-sensitive SCN neurons. Challet et al. (1998) reported that bilateral 8-OH DPAT injections into either the SCN or the intergeniculate leaflet cause significant phase advances in hamster wheel-running activity. Additional studies proposed that perfusion of 8-OH DPAT at CT6–CT8 advances neuron activity rhythm of the SCN *in vitro* (Shibata et al., 1992; Prosser et al., 1993). These reports prefer the direct action of 8-OH DPAT on SCN *Per* gene expression. On the other hand, Schuhler et al. (1998) demonstrated that the 5-HT fibers connecting the median raphe to the SCN are essential for the phase-shifting action of peripheral 8-OH DPAT injections into the SCN using microinjections of 5-HT neurotoxin. The present results demonstrate that 8-OH DPAT reduces *Per1* mRNA levels in the entire SCN and preferentially reduces *Per2* mRNA in the ventrolateral part of the SCN. Because serotonergic fibers from the median raphe nucleus innervate the ventrolateral part of the hamster SCN (Meyer-Bernstein and Morin, 1996; Leander et al., 1998), the reduction of this SCN serotonergic input may be one of the possible outcomes of 8-OH DPAT-induced reductions of *Per* mRNA. It is interesting that triazolam (20 mg/kg) causes not only a big phase advance but also a strong inhibition of *Per1* and *Per2* mRNA. Thus, reduction of *Per1* and *Per2* mRNA correlates well with phase advances induced by 8-OH DPAT as well as triazolam.

The SCN entrains to the environmental light/dark cycle via a retinal projection called the retinohypothalamic tract (RHT). Glutamate, which acts as an RHT transmitter (de Vries et al., 1993), and glutamate and NMDA application to the rat SCN *in vitro* reportedly causes phase delays in SCN firing rhythms when applied during early subjective night (Ding et al., 1994; Shibata et al., 1994; Shirakawa and Moore, 1994; Ding et al., 1997). Excitation of glutamate receptors is reported to facilitate the phosphorylation of cAMP response element binding protein (CREB) (Ding et al., 1997; McNulty et al., 1998). Furthermore, light exposure at night is reported to produce mitogen-activated protein kinase phosphorylation and CREB phosphorylation (Obrietan et al., 1998). Thus, the signal cascade of photic entrainment is well documented, whereas the signal cascade of nonphotic entrainment is obscure at present.

Currently, we do not know the mechanism of *Per1* and *Per2* reduction in the SCN by 8-OH DPAT or triazolam.

Treatments using pituitary adenylate cyclase-activating polypeptide or cAMP during subjective day are reported to induce the phase shift of circadian rhythm, apparently via activation of adenylate cyclase and PKA activity (Prosser and Gillette, 1989; Hannibal et al., 1997; Harrington et al., 1999). 5-HT_7 receptors are positively coupled to adenylate cyclase (Lovenberg et al., 1993; Tsou AP et al., 1994), and activation of both PKA and K^+ channels is necessary for 5-HT-induced phase advances of circadian rhythm (Prosser et al., 1994). Therefore, we speculate that activation of PKA may be involved in the 8-OH DPAT-induced phase advance and transient reduction of *Per* mRNA levels.

In this experiment, we demonstrated that administration of triazolam and 8-OH DPAT during subjective day reduces *Per1* and *Per2* mRNA in the hamster SCN. Nonphotic resetting by benzodiazepine or novel wheel-running requires neuropeptide Y innervation of the SCN from the thalamus (Biello et al., 1994; Maywood et al., 1997). Recently, Maywood et al. (1999) also demonstrated the acute downregulation of SCN *Per1* and *Per2*, whereas there was no significant change in SCN PER1 immunoreactivity by novel wheel-running during the daytime under a light/dark cycle. Our present results are highly consistent with the data of Maywood et al. (1997, 1999). Although we do not know whether the signal transduction mechanism of benzodiazepine-GABA, 8-OH DPAT, and novel wheel-running are identical, it is strongly suggested that nonphotic stimuli presented during subjective day cause a phase advance through the reduction of *Per1* and *Per2* mRNA in the hamster SCN. However, further experiments are needed to investigate the response of other clock elements such as *Clock*, *Bmal1*, *Cry1*, *Cry2* (Gekakis et al., 1998; Thresher et al., 1998; Griffin et al., 1999; Jin et al., 1999; Kume et al., 1999; Miyamoto and Sancar, 1999; Okamura et al., 1999; van der Horst et al., 1999; Vitaterna et al., 1999), or other *Per* proteins in the hamster SCN after nonphotic stimuli, because these assessments will better clarify the cascading effects of nonphotic stimuli on the clock loop.

The levels of *mPer3* mRNA are not affected by light exposure (Takumi et al., 1998b; Zylka et al., 1998). In the present experiment, *Per3* mRNA levels in the hamster SCN were unresponsive to light exposure at CT20 or to administration of 8-OH DPAT at various CTs. Triazolam also did not affect *Per3* mRNA levels. We do not know why *Per3* mRNA levels in the SCN are insensitive to both photic and nonphotic stimuli. It could be that acute changing of the expression of *Per3* is not required for phase shifts in mice and hamster circadian rhythms.

In summary, we found that the acute and circadian time-dependent reduction of *Per1* or *Per2* mRNA, or both, in the hamster SCN by 8-OH DPAT strongly correlates with the phase resetting in response to 8-OH DPAT. Therefore, the present findings suggest that nonphotic shifts involve alteration in *Per1* or *Per2* mRNA levels, or both, in the SCN.

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