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Inhibitory action of brotizolam on circadian and light-induced *Per1* and *Per2* expression in the hamster suprachiasmatic nucleus

[1]Shin-ichi Yokota, [1]Kazumasa Horikawa, [1]Masashi Akiyama, [2]Takahiro Moriya, [3]Shizufumi Ebihara, [3]Goyo Komuro, [4]Tatsuro Ohta & *,[1,2]Shigenobu Shibata

[1]Department of Pharmacology and Brain Science, School of Human Sciences, Waseda University, Tokorozawa, Saitama 359-1192, Japan; [2]ARCHS, School of Human Sciences, Waseda University, Tokorozawa, Saitama 359-1192, Japan; [3]Department of Animal Physiology, Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa, Nagoya 464-0814, Japan and [4]Department of Psychiatry, School of Medical Sciences, Nagoya University, Chikusa, Nagoya 464-0814, Japan

1 Triazolam reportedly causes phase advances in hamster wheel-running rhythm after injection during subjective daytime. However, it is unclear whether benzodiazepine affects the *Per* gene expression accompanying a behavioural phase shift.

2 Brotizolam (0.5–10 mg kg^{-1}) induced large phase advances in hamster rhythm when injected during mid-subjective daytime (circadian time 6 or 9), but not at circadian time 0, 3 or 15.

3 Brotizolam (5 mg kg^{-1}) significantly reduced the expression of *Per1* and *Per2* in the suprachiasmatic nucleus 1 and 2 h after injection at circadian time 6, and slightly reduced them at circadian time 20.

4 Injection of 8-OH-DPAT (5 mg kg^{-1}) at subjective daytime induced similar phase advances with a reduction of *Per1* and *Per2* expression. Co-administration of brotizolam with 8-OH DPAT failed to potentiate the 8-OH DPAT-induced phase advances and reduced *Per* expression.

5 Both phase advance and rapid induction of *Per1* and *Per2* in the suprachiasmatic nucleus after light exposure (5 lux, 15 min) at circadian time 20 was strongly attenuated by co-treatment with brotizolam 5 mg kg^{-1}.

6 The present results strongly suggest that reduction of *Per1* and/or *Per2* expression during subjective daytime by brotizolam may be an important step in causing a behavioural phase advance. The co-administration experiment suggests that common mechanism(s) are involved in brotizolam- or 8-OH DPAT-induced phase advances and the reduction of *Per* gene expression.

7 These results suggest that brotizolam is not only a good drug for insomnia but also a drug capable of facilitating re-entrainment like melatonin.

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Keywords: Suprachiasmatic nucleus; brotizolam; *Per* genes; benzodiazepine; hamster; phase shift

Abbreviations: BRZ, brotizolam; CT, circadian time; IGL, intergeniculate leaflet; 8-OH DPAT, 8-hydroxy dipropylaminotetralin; SCN, suprachiasmatic nucleus; TRZ, triazolam

Introduction

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). Active stimuli, such as a dark pulse, novel wheel-running access, or triazolam (TRZ) (Turek & Losee-Olson, 1986) and chlordiazepoxide (Biello & Mrosovsky, 1993) administration, phase shift mammalian circadian clocks, thereby producing phase advances during the subjective day and small phase delays during the subjective night (Hastings *et al*., 1998, for review). Both TRZ and novel wheel-running access require an intact intergeniculate leaflet (IGL) to modulate the circadian rhythm phase because lesion of the IGL attenuates the non-photic treatments such as TRZ administration or novel wheel-running access (Johnson *et al*., 1988; Janik & Mrosovsky, 1994; Wickland & Turek 1994). Furthermore, there has been suggestion that TRZ-induced phase shifts require an intact serotonergic system for modulation of the rhythm phase (Cutrera *et al*., 1996; Penev *et al*., 1995; Meyer-Bernstein & Morin, 1998). In fact, many studies have showed that daytime injection of 8-OH DPAT [8-hydroxy-2-(di-n-propylamino) tetralin], which is a 5-HT$_{1A/7}$ receptor agonist, induces a phase advance in hamster behavioural circadian rhythms *in vivo* (Tominaga *et al*., 1992; Cutrera *et al*., 1996; Mintz *et al*., 1997), as well as phase advances 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 the phase shifts of the circadian system during subjective day in response to non-photic stimuli.

Recent studies on the molecular aspects of clock genes have produced a functional model for circadian rhythms (Dunlap, 1999 for review). For example, the expression of *mPer1*, *mPer2*, and *mPer3* mRNAs in the SCN occurs in a circadian fashion (Shearman *et al*., 1997; Zylka *et al*., 1998; Takumi *et al*., 1998b). We found that brief exposure to light during the subjective night resulted in a large and rapid induction of *mPer1* expression (Shigeyoshi *et al*., 1997) and *mPer2* mRNA expression in the SCN (Shearman *et al*., 1997; Takumi *et al*., 1998b). On the other hand, *mPer3* mRNA levels did not respond to light during the subjective night or day (Takumi *et al*., 1998a; Zylka *et al*., 1998). Thus, *mPer1*, *mPer2*, and *mPer3* are rhythmically expressed in the SCN, and *mPer1* and *mPer2* exhibit circadian photoresponses. We

*Author for correspondence at: Department of Pharmacology and Brain Science, School of Human Sciences, Waseda University, Tokorozawa, Saitama 359-1192, Japan.
E-mail: shibata@human.waseda.ac.jp

recently demonstrated that a light-induced phase delay in locomotor activity at circadian time (CT) 16 was significantly inhibited when mice were pretreated with *mPer1* antisense oligonucleotide 1 h before light exposure (Akiyama *et al*., 1999b). Therefore, we suggested that gating expression of the *mPer1* gene may be an important step in causing photic entrainment.

Short half-life benzodiazepine hypnotics such as TRZ and brotizolam (BRZ) utilized for several nights at the new sleep time have been recommended for decreasing jet lag symptoms. Therefore, we examined whether BRZ-induced behavioural phase advances in hamster wheel-running accompanied changes in hamster *Per1* and/or *Per2* expression in the SCN. Because light exposure induced *mPer1* and *mPer2* expression during the subjective night, we questioned whether injected BRZ could attenuate both a behavioural phase delay and the induction of *Per* in the SCN after light exposure during subjective night. Almost all previous behavioural experiments on non-photic phase advances utilized hamsters, so we attempted to show circadian expression patterns in golden hamster *Per1*, *Per2* and *Per3*.

Methods

Animals

Male golden hamsters (*Mesocricetus auratus*, Tokyo Laboratory Animal Co. Ltd., Japan) weighing 120–200 g were used for this study. All animals were maintained under controlled environmental conditions ($23 \pm 2^{\circ}$C room temperature; 12/12 h light/dark cycle, lights on at 08:30 h) for at least 2 weeks before use in our experiments. A light intensity of 200 lux was utilized at the level of the animal cage. Food and water were provided *ad libitum*. Animals were treated in accordance with the Law (No. 141) and Notification (No. 6) of the Japanese Government. For assessment of the wheel-running activity, hamsters were housed individually in transparent plastic cages ($36 \times 20 \times 20$ cm) equipped with a running wheel 13 cm in diameter that closed a microswitch with each revolution, so the numbers of wheel rotation could be measured. Data was stored on a personal computer. For free-running conditions under constant darkness, circadian time (CT) was defined, and CT12 referred to the onset of wheel-running.

Drugs

BRZ (a gift from Behringer-Ingelheim) and 8-OH DPAT (Sigma, U.S.A.) were administered intraperitoneally at a volume of 1 ml kg^{-1} to hamsters. BRZ was dissolved into dimethyl sulphoxide (DMSO, WAKO, Japan), and 8-OH DPAT was dissolved into saline. DMSO injection had no effect on behavioural rhythm (Figure 4) or *Per1* mRNA level in the SCN (data not shown).

Behavioural experiment

After free-running for at least 2 weeks in constant darkness, hamsters were randomly assigned to injections of BRZ (0.5–10 mg kg^{-1}), 8-OH DPAT (0.5–10 mg kg^{-1}) or vehicle. Injections were performed at a circadian time (CT; CT12: onset time of wheel-running activity) of CT0, CT3, CT6, CT9, CT12, CT15, CT18, or CT21, then animals were returned to their individual cages. For the co-administration experiment, both drugs (BRZ and 8-OH DPAT) were injected at the same time. For the combined experiment involving light treatment and BRZ administration, BRZ (5 mg kg^{-1}) was injected 30 min before light exposure (5 lux, 15 min) at CT 20. 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 & Pittendrigh, 1976).

The hamster Per *probes*

For analysis of *Per* gene expression using an *in situ* hybridization, we made hamster *Per2* and *Per3* probes according to our previous study (Horikawa *et al*., 2000). A partial cDNA encoding hamster *Per1* gene (nucleotide position 726–1367 of *mPer1*; Gene Bank accession number AB002108) was kindly provided by Dr Hitoshi Okamura.

Sample preparation

In situ hybridization was applied to quantify or determine the histochemical distribution of the expression of *Per1* and *Per2* mRNAs in coronal sections of the hypothalamus. Hamsters were entrained to the light/dark cycle for at least 2 weeks then kept in constant dark conditions. On the second day of constant darkness at CT1, CT6, or CT20, hamsters were i.p. injected with BRZ (5 mg kg^{-1}), 8-OH DPAT (5 mg kg^{-1}), or vehicle, and then deeply anaesthetized with ether 1 or 2 h after injection and intracardially perfused with 0.1 M phosphate buffer (PB) containing 4% paraformaldehyde (PFA). In some cases, hamster brains were obtained for examination of the circadian changes of *Per* gene expression in the SCN. Light (5 lux, 15 min) was also applied at CT20, 30 min after BRZ (5 mg kg^{-1}) administration. In the present experiment, hamsters were sacrificed 1.5 h after the initiation of light exposure. Brains were excised, post-fixed in 0.1 M PB containing 4% PFA for 24 h at 4°C, and transferred into 20% sucrose in phosphate buffer saline for 24 h at 4°C.

In situ *hybridization protocol*

Frontal sections (30 μm thick for Radio isotope) were collected and transferred into phosphate buffer saline for 30 min following treatment with $6 \times$ standard saline citrate (SSC) for 30 min. Sections were incubated in hybridization buffer [50% formamide, $6 \times$SSC, 0.1 mg ml^{-1} denatured salmon sperm DNA, $1 \times$Denhardt's solution (0.02% Ficoll, 0.02% polyvinyl pyrolidone, 0.02% bovine serum albumin) and 10% dextran sulphate] containing labelled cRNA probes overnight at 60°C. Radio isotope [RI: α[^{33}P]-UTP (New England Nuclear, U.S.A.)]-labelled antisense cRNA were made according to a standard protocol for cRNA synthesis. After hybridization, these sections were rinsed in $2 \times$SSC/50% formamide for 45 min and again for 15 min at 60°C, treated with RNase A for 30 min at 37°C, treated twice with $2 \times$SSC/50% formamide for 15 min at 60°C, and then treated with $0.4 \times$SSC for 30 min at 60°C. For RI *in situ* hybridization, tissue sections were collected into $2 \times$SSC and then treated with proteinase K [1.0 μg ml^{-1}, 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, $2 \times$SSC for 5 min followed by 0.25% acetic anhydride in 0.1 M triethanolamine for 10 min, and then treated twice with $2 \times$SSC for 5 min. The radioactivity of each SCN on BioMax MR film (Kodak) was analysed using a microcomputer interface to an image analysis system (MCID, Imaging Research Inc., Canada) after conversion into optical

density by ^{14}C-autoradiographic microscales (Amersham, Buckinhamshire, U.K.). For data analysis, we subtracted the intensities of the optical density in the corpus callosum from those in the SCN of each section and regarded this value as the net intensity in the SCN. The intensity values of the sections from the rostralmost to the caudalmost part of the SCN (five sections per hamster brain) were then summed; the sum was considered to be a measure of the amount of *Per1* and *Per2* mRNA in this region. When we expressed the relative mRNA abundance (Figures 3, 4, and 6), the intensity values of vehicle treatment were adjusted to 100.

Statistics

Values are expressed as the mean ± s.e.mean. The results were analysed by two-way or one-way ANOVA followed by Dunnett's test, Tukey's test, or the Student's *t*-test.

Results

Phase shift effects of BRZ on various CTs

Vehicle (DMSO) administration at CT6 did not show any change in phase (Figure 1A, upper), but BRZ (5.0 mg kg^{-1}) administration produced a clear phase advance (Figure 1A, lower). There was a significant difference in interaction between injection CT and drug ($F7,66 = 7.3$, $P < 0.01$, for two-way ANOVA). Administration of BRZ (5 mg kg^{-1}) caused significant phase advances with injection at CT6 ($P < 0.01$ vs vehicle, Student's *t*-test) and CT9 ($P < 0.01$), and small but significant phase delays at CT18 ($P < 0.01$) and CT21 ($P < 0.05$) (Figure 1B). However, BRZ injection at other CTs such as CT0, 3, 12, and 15 did not change the phase of the free-running rhythm (Figure 1B). In the next experiment, administration of BRZ at various doses was compared with vehicle administration (Figure 1C). Significant phase advances were observed when this compound was administered at CT6 in 5 mg kg^{-1} ($P < 0.01$) and 10 mg kg^{-1} ($P < 0.01$) doses.

Circadian pattern of hamster Per1*,* Per2*, and* Per3 *mRNA expression using* in situ *hybridization*

Figure 2A shows the circadian changes in mean values of *Per1*, *Per2*, or *Per3* expression in the hamster SCN. The data from one-way ANOVA revealed significant circadian oscillations in *Per1* ($F5,18 = 21.5$, $P < 0.01$), *Per2* ($F5,18 = 45.1$, $P < 0.01$), and *Per3* ($F5,18 = 8.1$ $P < 0.01$) expression. Peak *Per* mRNA expression occurred at CT2-10 for *Per1*, CT6-14 for *Per2*, and CT6-14 for *Per3*. Although the experiment was carried out using RI *in situ* hybridization, hybridized signals of *Per1*, *Per2* and *Per3* were expressed throughout almost the entire SCN region (Figure 2B).

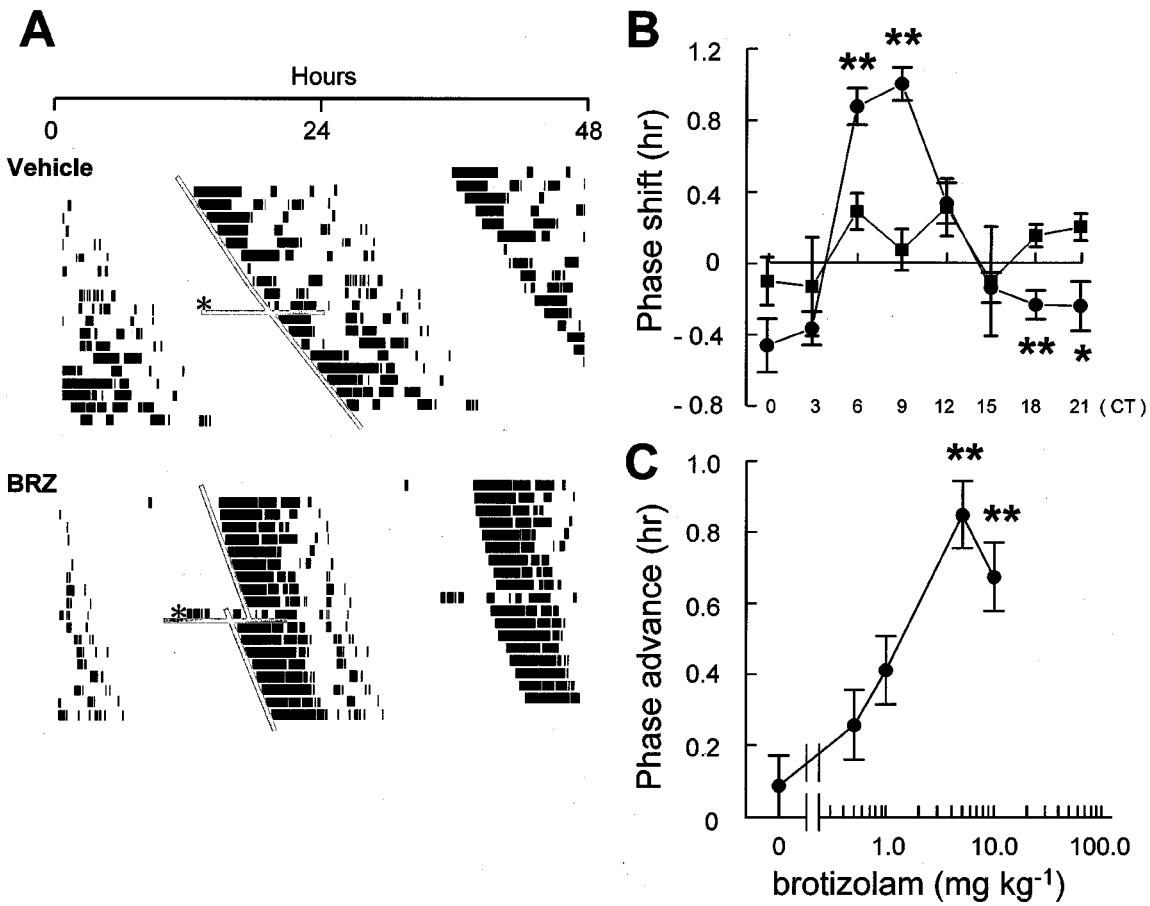

Figure 1 Effects of BRZ administration on hamster circadian wheel-running rhythm. Double-plotted actogram shows wheel-running activity records of vehicle-(A, upper) and BRZ-(5.0 mg kg^{-1}). (A, lower) injected hamsters at CT6. Each animal was injected at CT6 (asterisk in the figure) and returned to constant darkness. (B) Mean phase shifts induced by BRZ (5.0 mg kg^{-1}), i.p.) (circle) or vehicle (triangle) administration at CT0, CT3, CT6, CT9, CT12, CT15, CT18, and CT21. Five to 10 animals made up each point. (*$P < 0.05$, **$P < 0.01$ in comparison with vehicle by Student's *t*-test). (C) Dose-response curve for BRZ administration at CT6. Four to 10 animals made up each point. (**$P < 0.01$ in comparison with vehicle BRZ (0 mg kg^{-1}) by Dunnett's test).

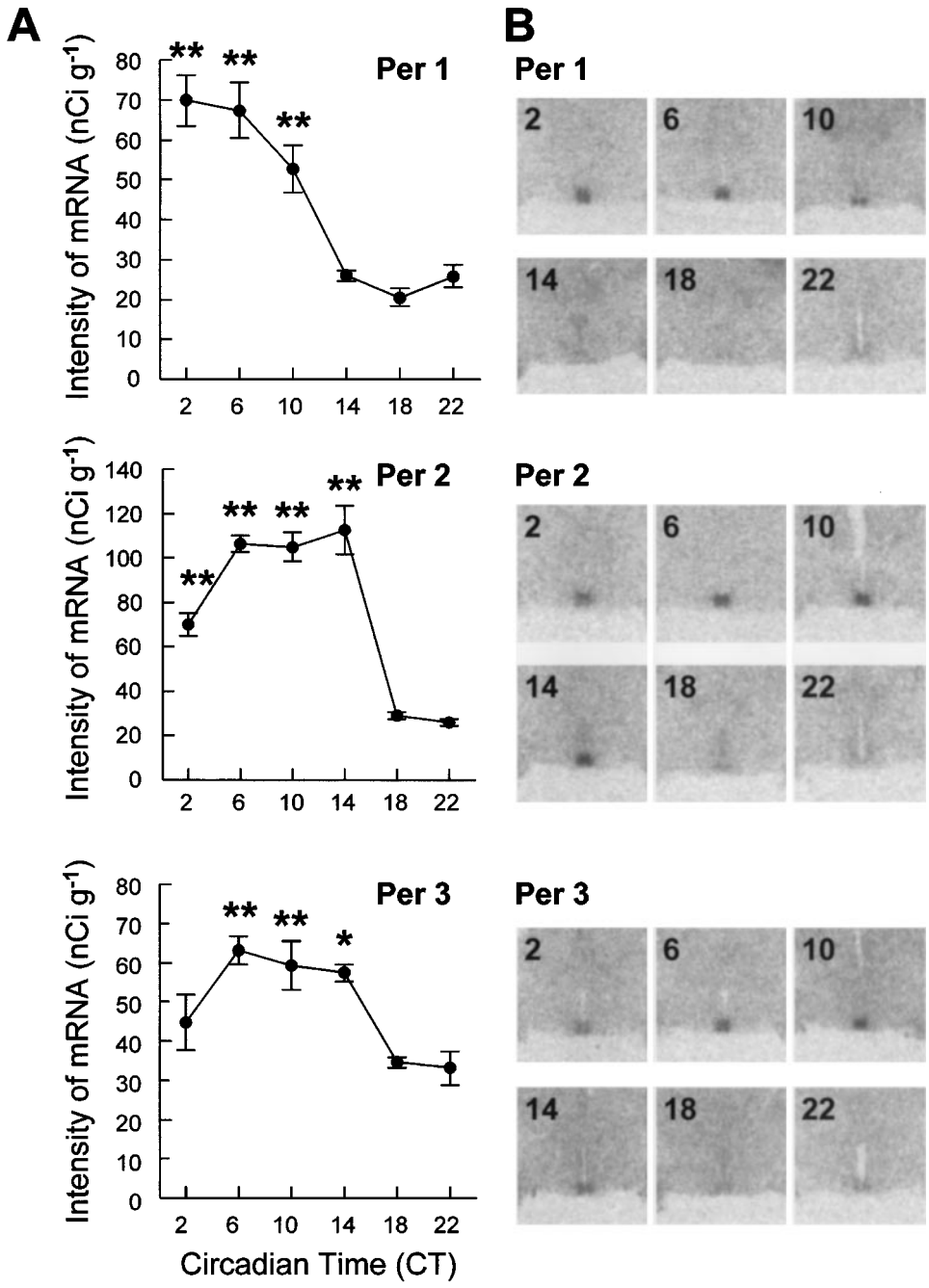

Figure 2 Circadian expression of hamster *Per1*, *Per2*, and *Per3* in the suprachiasmatic nucleus. (A) Circadian mean expression of these *Per* genes. Four animals made up each point. (*$P<0.05$, **$P<0.01$ in comparison with bottom point by Dunnett's test). (B) Hybridized signal of each *Per* gene in the SCN.

Effect of BRZ on hamster Per1*,* Per2*, and* Per3 *mRNA expression using* in situ *hybridization*

In the behavioural experiment, BRZ injection induced a phase advance at CT6 and small delays at CT18 and 21 (Figure 1B); as the next step, the effect of BRZ on hamster *Per* genes was examined 1 and 2 h after injection at CT6, CT1, and CT20. Administration of BRZ at CT6 significantly reduced the expression of *Per1* 1 h ($P<0.01$) and 2 h ($P<0.05$) after injection and that of *Per2* 2 h ($P<0.05$) after injection (Figure 3A). Injection of BRZ at CT1 did not change any *Per* gene expression 1 or 2 h after injection. Injection of BRZ at CT20 caused a reduction of *Per1* expression 1 and 2 h after administration, but the differences between vehicle- and drug-treated hamsters were not significant. When BRZ reduced *Per1* and *Per2* expression, *in situ* hybridization data demonstrated that there were no SCN regional differences in BRZ-induced inhibition (Figure 3B).

Effect of co-administration of BRZ with 8-OH DPAT on behavioural rhythm and Per *gene expression*

Injection of 8-OH DPAT (5 mg kg^{-1}) at CT6 caused a phase advance of hamster wheel-running rhythm, corresponding with the reduction of *Per1* expression in the SCN (Figure 4). Co-administration of BRZ (5 mg kg^{-1}) with 8-OH DPAT (5 mg kg^{-1}) failed to potentiate 8-OH DPAT (5 mg kg^{-1})-induced phase advances (Figure 4A) and the reduction of *Per1* expression in the SCN (Figure 4B).

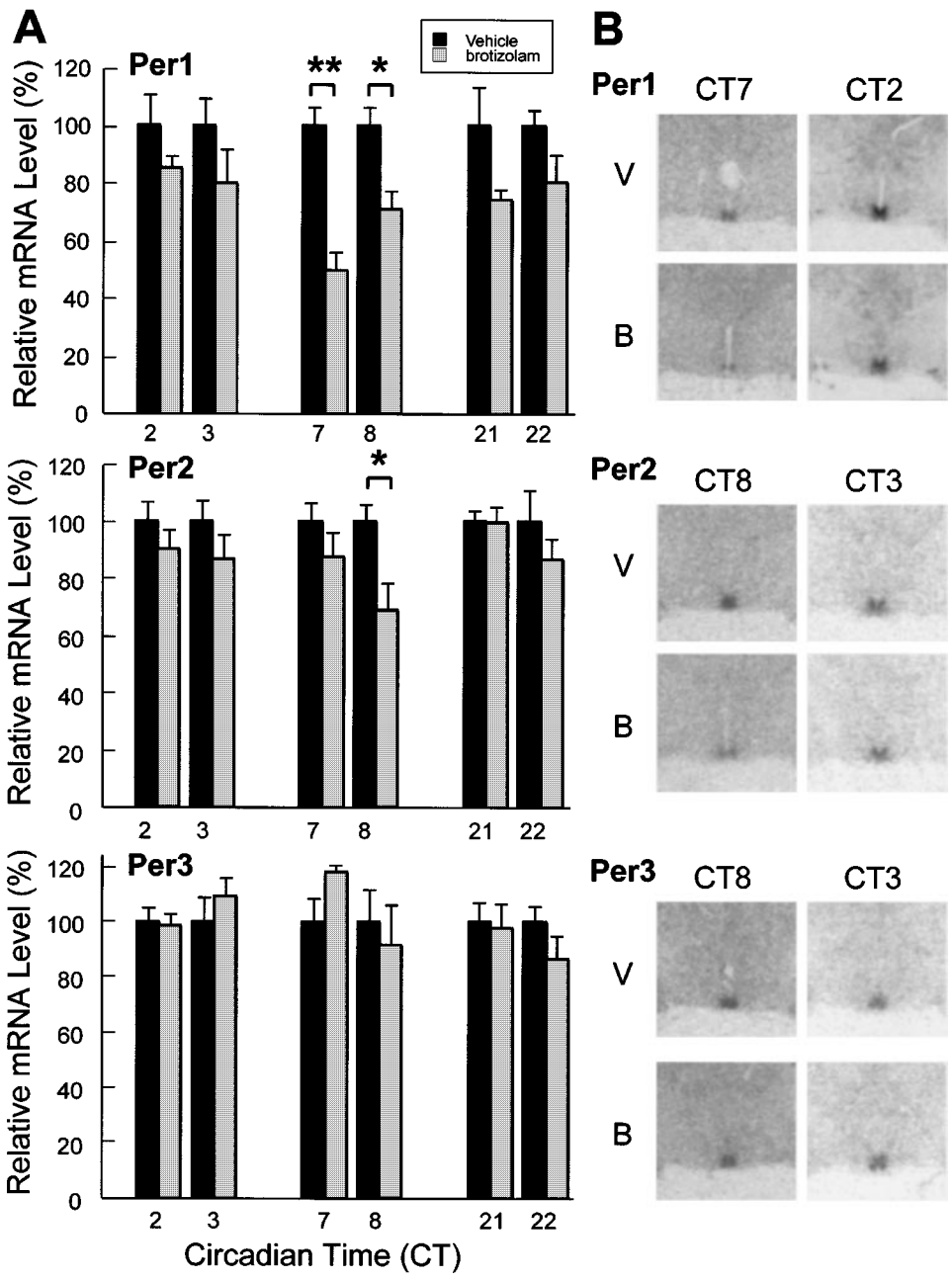

Figure 3 Effect of BRZ injected at CT1, CT6, and CT20 on *Per1*, *Per2*, and *Per3* expression in the hamster suprachiasmatic nucleus. (A) Relative value of *Per* mRNA expression. *Per* expression observed in vehicle-treated animals was set as 100%. Hybridized signals were examined 1 or 2 h after each injection. Four animals made up each point. (*$P<0.05$, **$P<0.01$ in comparison with vehicle by Student's *t*-test). (B) Hybridized signal of each *Per* gene in the SCN. V, vehicle; B, brotizolam.

Inhibitory effect of BRZ on light-induced phase advances

Previous reports have suggested that photic entrainment and non-photic entrainment (Penev *et al*., 1997; Biello *et al*., 1997) interact. In this experiment, we examined whether BRZ attenuates the light-induced phase advances in hamster wheel-running rhythm. Light exposure (5 lux, 15 min) at CT20 caused a phase advance (Figure 5A, upper). Pre-treatment with BRZ (5 mg kg^{-1}) 30 min before light exposure attenuated the light-induced phase advances (Figure 5A, lower). The average of light-induced phase advances (2.56 h) was significantly reduced by pre-treatment with BRZ (1.31 h, $P<0.05$), whereas, light-induced phase delays at CT13.5 were unaffected by BRZ (Figure 5B, lower).

Effect of BRZ on light-induced expression of hamster Per1, Per2 *and* Per3 *mRNAs using* in situ *hybridization*

In order to confirm this interaction on a molecular level, we examined whether BRZ attenuates the light-induced rapid induction of *Per1* and *Per2* mRNA in the SCN. Light exposure strongly induced *Per1* (300% of control) and *Per2* (200% of control) but not *Per3* (120% of control) expression 90 min after light application (Figure 6B). Two-way ANOVA revealed the interaction between light and drug treatment in *Per1* ($F1,16=5.9$, $P<0.05$) and *Per2* ($F1,16=13.9$, $P<0.01$), but not *Per3* ($F1,16=0.07$, $P<0.05$). *Post-hoc* Tukey's test demonstrated that pre-treatment with BRZ (5 mg kg^{-1}) significantly attenuated the light-induced *Per1* ($P<0.05$) and *Per2* ($P<0.01$) induction. Histological analysis demon-

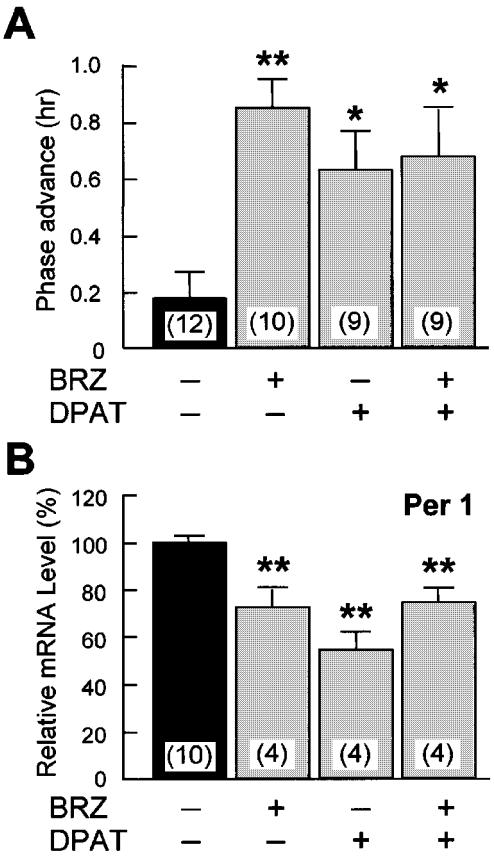

Figure 4 Effect of co-administration of BRZ with 8-OH DPAT on behavioural rhythm and *Per1* expression in the hamster suprachiasmatic nucleus. (A) Behavioural phase advances, BRZ (5 mg kg^{-1}, 8-OH DPAT (5 mg kg^{-1}) or a combination of the two was injected at CT6 under constant dark conditions. The numbers in parenthesis indicate the number of animals. (*$P<0.05$, **$P<0.01$ in comparison with DMSO by Dunnett's test). (B) Relative value of *Per* mRNA expression. *Per* expression observed in vehicle-treated animals was set as 100%. BRZ (5 mg kg^{-1}), 8-OH DPAT (5 mg kg^{-1}) or a combination of two was injected at CT6, then hybridized signals were examined 2 h after injection. Four animals made up each point. The numbers in parenthesis indicate the number of animals. (**$P<0.01$ in comparison with DMSO by Dunnett's test).

strated that reduction of the hybridized signals by BRZ was observed equally in the dorsomedial and ventrolateral SCN where the retinohypothalmic pathways arrive (Figure 6A).

Discussion

In situ hybridizations using RI-labelled probes of hamster *Per* genes revealed clear a circadian expression of *Per1*, *Per2*, and *Per3* in the hamster SCN. The pattern of expression of these genes was very similar to that observed in the mouse (Albrecht *et al*., 1997; Tei *et al*., 1997; Takumi *et al*., 1998b; Zylk *et al*., 1998; Sun *et al*., 1997) and rat (Sakamoto *et al*., 1998; Yan *et al*., 1999) SCN. Thus, hamster *Per1*, *Per2*, and *Per3* mRNA expression using RI *in situ* hybridization is a reliable marker of hamster SCN clock function.

In the present experiment, we demonstrated the BRZ reduced *Per1* and *Per2* in the SCN in a circadian time-dependent manner. Actually, these gene mRNAs were reduced by an injection of BRZ at CT6 and slightly reduced at CT20, but not at CT1. This result is closely related to the behavioural result showing a large phase advance at CT6 and CT9, but a small delay at CT20 and no effect at CT1. Recently, our own investigations (Horikawa *et al*., 2000) as well as Maywood *et al*. (1999) demonstrate a similar reduction in hamster *Per1* and *Per2* with 8-OH DPAT injection at CT9 under constant darkness, and with novel wheel-running for 3 h at mid-daytime under a light/dark cycle, respectively. Although there are some differences in the methods producing non-photic entrainment, both BRZ/8-OH DPAT and novel wheel-running caused a gated reduction of *Per1* and *Per2* in the SCN. Although previous findings suggest there was no significant change in SCN PER1 immunoreactivity with novel wheel-running (Maywood *et al*., 1999), it is important to investigate whether *Per1* and *Per2* changes by BRZ are translated into differences in the levels of Per proteins.

While our present results strongly suggest that BRZ exhibits an inhibitory action on SCN *Per* gene expression, there are also reports suggesting that IGL plays an important role in TRZ-induced phase advances in lesioning experiments (Johnson *et al*., 1988; Wickland & Turek, 1994). Therefore, peripheral injection of BRZ may affect the IGL neurons in which neuropeptide Y and GABA neurons are involved resulting in the reduction of *Per1* and *Per2* expression in the SCN. It was previously demonstrated that there was an induction of Fos protein in the SCN and IGL regions in response to non-photic manipulations such as saline injection and cage change (Edelstein & Amir, 1995) supporting the above idea. However, in an *in vitro* experiment, benzodiazepine facilitated the GABA-induced inhibition of SCN neuron activity (Strecker *et al*., 1999) and muscimol injected into the SCN area and *in vitro* application caused large phase advances in behaviour (Smith *et al*., 1989) and neuron activity rhythms (Tominaga *et al*., 1994), respectively. Our recent study demonstrated that benzodiazepines reduced mouse *Per1* gene expression in the cerebellum (Akiyama *et al*., 1999a). Thus, at present we can not completely exclude SCN as a possible action site for benzodiazepine.

Furthermore, 5-HT innervation from the medial raphe nucleus to IGL is also necessary to produce TRZ-induced phase advances, because TRZ-induced phase advances disappeared in 5-HT fibre lesioned animals (Penev *et al*., 1995; Meyer-Bernstein & Morin, 1998). Challet *et al*. (1998) reported that bilateral 8-OH DPAT injections into either the SCN or the intergeniculate leaflet caused significant phase advances in hamster wheel-running activity. Also it has been reported 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 indirect action of benzodiazepine on SCN *Per* gene expression through the activation of 5-HT neurons. This hypothesis may be supported by the present co-administration experiment which demonstrated that BRZ failed to potentiate 8-OH DPAT-induced behavioural phase advances and the reduction of *Per1* expression in the SCN. Although we do not know whether the signal transduction mechanism of benzodiazepine-GABA, 8-OH DPAT, and novel wheel-running are identical, it is suggested that common mechanism(s) are involved in the 8-OH DPAT- and BRZ-induced reduction of *Per1* expression.

Light exposure during subjective night caused a rapid induction of hamster *Per1* and *Per2* in the SCN. Administration of BRZ strongly attenuated the light-induced *Per1* and *Per2* expression, suggesting an interaction between photic entrainment and non-photic entrainment. It has been reported that 8-OH DPAT, a non-photic stimulus, and diazepam attenuated the light-induced behavioural phase shift (Rea *et al*., 1994; Weber *et al*., 1998) as well as Fos

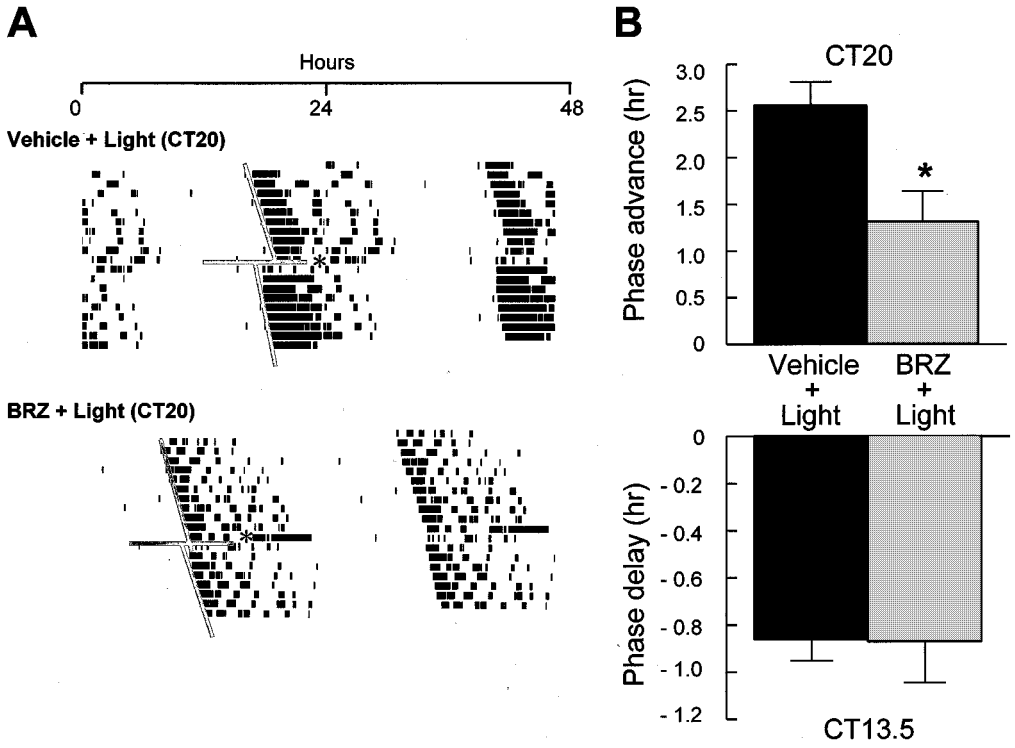

Figure 5 Inhibitory effect of BRZ on light-induced phase advance in behaviour. (A) Double-plotted actogram shows wheel-running activity records in the vehicle-(A, upper) and BRZ-(5.0 mg kg^{-1}, i.p.) (A, lower) injected hamster at CT19.5, and 30 min after injection light was delivered for 15 min (5 lux). (B) Mean value of phase advance (B, upper) and delay (B, lower). Thirty minutes after injection of BRZ (5.0 mg kg^{-1}, i.p.) light was delivered for 15 min at CT20 (advance part) or CT13.5 (delay part). Four to five animals made up each column. (*$P<0.05$, in comparison with vehicle by Student's *t*-test).

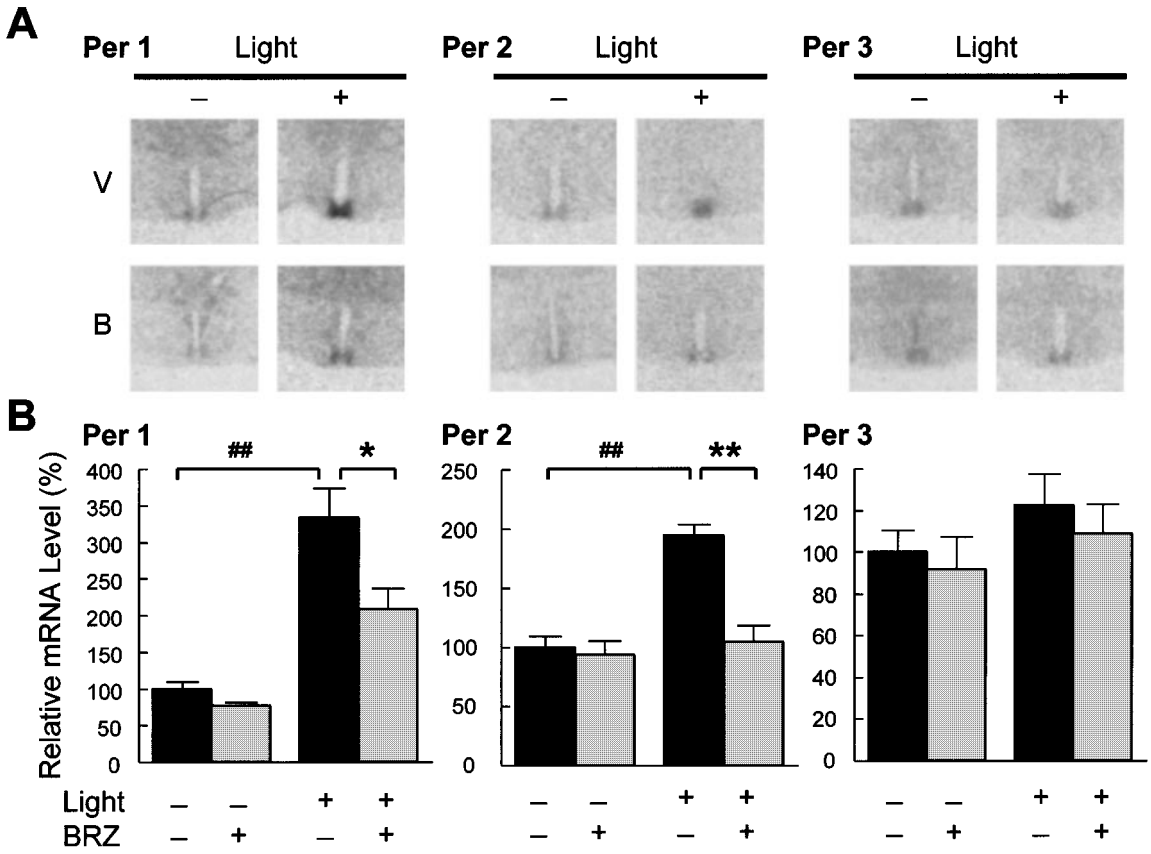

Figure 6 Effect of BRZ on light exposure-induced expression of *Per1*, *Per2*, and *Per3* in the hamster suprachiasmatic nucleus. Upper shows the hybridized signals of *Per1*, *Per2*, and *Per3* in vehicle- and BRZ-(5 mg kg^{-1}) treated animals. Drug was injected at CT19.5 and light was applied at CT20. Animals were then returned to constant darkness. At CT21.5, animals were sacrificed. Lower shows the mean values of hybridized signals. Four animals made up each point. (*$P<0.05$, **$P<0.01$ in comparison with DMSO by Tukey's test; ##$P<0.01$ in comparison with non-light by Tukey's test).

expression in the SCN (Rea *et al*., 1994; Recio *et al*., 1996). Our present results provide new evidence of photic and non-photic interaction in terms of *Per1* and *Per2* expression.

In the present experiment, BRZ did not affect *Per3* expression in the SCN at any CT. It is also well known that the expression of *mPer3* is not affected by light exposure (Takumi *et al*., 1998a; Zylka *et al*., 1998). These results together suggest that changing the expression of *Per3* is not required for phase shifts in mouse and hamster circadian rhythms.

Triazolam and BRZ are widely used as hypnotic drugs in patients with insomnia. These results suggest that BRZ is not only a good drug for insomnia but also a potential drug capable of facilitating re-entrainment after phase advances in the light/dark cycle. Thus, BRZ may decrease jet lag symptoms by facilitating advances in an advanced light-dark cycle. Therefore, the present results call clinical attention to the carrying over of BRZ into the morning, which then disturbs the phase advances induced by morning light. At present, we do not know the reason why BRZ failed to reduce light-induced delays in behavioural rhythm. Interestingly, light-induced phase delays were unaffected by diazepam at doses that significantly blocked phase advances (Ralph & Menaker, 1985), suggesting the possibility that different neurochemical mechanisms (Ding *et al*., 1998) are required to process light-induced phase advances and delays.

In conclusion, it is suggested that the reduction of *Per1* and/or *Per2* in the SCN by BRZ may be an important step in the phase resetting of behaviour and that a gating mechanism may be present in non-photic- as well as photic-induced phase shifts in behavioural rhythm.

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