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Modulation of *mPer1* gene expression by anxiolytic drugs in mouse cerebellum

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1 The *mPer1* and *mPer2* genes are putative mouse clock genes that regulate circadian oscillator present in the suprachiasmatic nucleus (SCN) neuron. While they are also expressed in the granular cell layer in the cerebellum, their function is unknown. In a first step to verify the physiological roles of *mPer1* and *mPer2* genes in the cerebellum, we examined the effects of benzodiazepines on the expression of the *mPer1* and *mPer2* genes.

2 *mPer2* mRNA expression was higher at ZT16 than ZT4 in the mouse cerebellum.

3 High-dose administration of diazepam (10 mg kg^{-1}) or triazolam (1 mg kg^{-1}) reduced *mPer1* mRNA level 1 h after treatment in the cerebellum.

4 Reduced expression of *mPer1* by diazepam treatment was transient. No difference of *mPer1* mRNA level between diazepam (10 mg kg^{-1})- and vehicle-treated group was observed 6 h after treatment.

5 Administration of high doses of tandospirone (30 mg kg^{-1}), a non-benzodiazepine anxiolytic also reduced *mPer1* mRNA expression 1 h after treatment.

6 Administration of high doses of clozapine (5 mg kg^{-1}) or haloperidol (1 mg kg^{-1}) impaired the rota-rod performance without affecting on *mPer1* mRNA level.

7 Diazepam and tandospirone inhibited the expression of *mPer1* mRNA in the primary cultured cerebellum granule cells.

8 Transient reductions of *mPer1* mRNA levels by various benzodiazepines and tandospirone is associated with impairment of coordinated movement, such as rota-rod performance and equilibrium.

Keywords: *mPer1*; cerebellum; anxiolytics; diazepam; rota-rod; tandospirone

Abbreviations: SCN, suprachiasmatic nucleus, ZT, zeitgeber time

Introduction

Circadian rhythms are endogenously generated rhythms that persist under constant conditions with a period of about 24 h (Edmunds, 1988). The biological clock of mammals, which located in the suprachiasmatic nucleus (SCN) of hypothalamus, controls a variety of behavioural and physiological rhythmic phenomena, such as locomotor activity, sleep-wakefulness and secretion of various hormones (Hastings, 1997). Recent isolation of three mammalian homologues of the *Drosophila* clock gene, *period (dPer)*, from mammals [*Per1* (Tei *et al*., 1997; Sun *et al*., 1997), *Per2* (Albrecht *et al*., 1997; Shearman *et al*., 1997; Takumi *et al*., 1998a) and *Per3* (Zylka *et al*., 1998b; Takumi *et al*., 1998b)] and their circadian expression in the SCN suggests that molecular components and some mechanisms of mammalian circadian clock are evolutionarily conserved. This insight was further reinforced with recent isolation of other common clock components like *timeless* (Sangoram *et al*., 1998; Zylka *et al*., 1998a) or *Clock* (Allada *et al*, 1998) from *Drosophila* and mouse (Darlington *et al*., 1998). Interestingly, like *dPer*, all three *mPer* genes are expressed in various organs (Tei *et al*., 1997; Zylka *et al*., 1998a,b). In the mouse brain, *mPer* genes are most extensively expressed in the SCN but they are also expressed in the pyramidal cells of hippocampus and granule cells of cerebellum (Shearman *et al*., 1997). Circadian fluctuation of *mPer1* mRNA in the hippocampus and cerebellum has also been reported (Sun *et al*., 1997). However, it is still unknown why these *mPer* genes are expressed in various regions of the brain and organs.

It is well known that normal function of cerebellum is required to perform co-ordinated motor movements (Thach *et al*., 1992 for review). The α1 and α6 subunits of $GABA_A$ receptor have been reported to express in granule cells of cerebellum (Khan *et al*., 1996). In addition, it is reported that benzodiazepine-induced motor impairment links to point mutation in cerebellar $GABA_A$ receptor containing α6 subunit (Korpi *et al*., 1993). Recently, it has been demonstrated that benzodiazepine reduces $GABA_A$ receptor α1 subunit protein expression in primary cultured cerebellar granule cells (Brown & Bristow, 1996). Thus, benzodiazepines may cause an impairment of co-ordinated motor activity through an interaction with $GABA_A$ receptor in the granule cells of the cerebellum. In a first step to clarify physiological roles of *mPer* genes in brain regions other than the SCN, we examined the *mPer1* and *mPer2* expression in the cerebellum when mice exhibited an impairment of rota-rod performance and equilibrium following high dose administration of anxiolytic drugs, such as benzodiazepines and tandospirone, and antipsychotic drugs, such as clozapine and haloperidol. We have shown here that *mPer1* mRNA

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level was reduced in the cerebellum by diazepam, triazolam and tandospirone, but not by clozapine and haloperidol, even though high doses of these latter drugs also caused a deficit of co-ordinated motor movements as observed in the rota-rod and fixed bar tests. Interestingly, diazepam and tandospirone also reduced *mPer1* mRNA level in the cultured cerebellar granule cells.

Methods

Animals

For all experiments, 4–6 week old male *ddY* mice (Takasugi, Japan) were group housed (8–10 per cage) and maintained at least 2 weeks under a 12 : 12 h light dark cycle with a light on at 8 : 30 h at 23°C. Animals were given food and water *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) 0 was designated as light on and ZT12 as light off.

Fixed-bar test

Mice were trained to stay on a fixed bar (8 or 20 mm in diameter, 50 cm in height) just before drug treatments. Only mice that stayed on the bar over 3 min were used for the subsequent tests. Groups of mice ($n = 5–8$) were administered test compounds and put on the bar and tested for their ability to stay on the bar for 3 min. They were put on a bar again 1, 2, 6 and 12 h after the treatment and tested for staying for 3 min.

Rota-rod test

Mice were trained to stay on a rod (25 mm in diameter, 25 cm in height) rotating at 15 r.p.m. just before drug treatments. Only mice that stayed on the rod over 3 min were used for the subsequent tests. Groups of mice ($n = 5–15$) were administered test compounds and put on the rod and tested for staying on the rod for 3 min. They were again put on the rota-rod 1, 2, 6 and 12 h after the treatment and tested for staying on the rod for 3 min. The mice that dropped within 3 min were again tested immediately under the same conditions and the better score in the two tests was used for analysis.

Drugs

Diazepam (Wako, Japan), triazolam (Sigma), tandospirone citrate (Sumitomo Pharmaceuticals, Japan), clozapine (Sigma) and haloperidol (Wako, Japan) were administered intraperioneally in a volume of 10 ml kg^{-1} to mice. All compounds were suspended in 0.5% sodium carboxymethylcellulose (vehicle) (Tokyo Kasei, Japan). In the *in vitro* experiment, diazepam, tandospirone and nifedipine (Wako, Japan) were dissolved into DMSO with a final concentration of DMSO (1%) which had no effect on *mPer1* mRNA level (data not shown).

RT–PCR analysis

The effects of anxiolytic drugs on *mPer1* and *mPer2* expression in the cerebellum were examined by RT–PCR. Mice entrained to the light dark cycle for 2 weeks were transferred to constant darkness for one extra daily cycle and mice were administered drugs or vehicle at ZT4 or ZT16 on the next day. An hour after injection, mice were deeply anaesthetized with ether and killed by decapitation. Some mice were killed 6 h after administration. The sagittal slices of 1 mm thickness were dissected from the middle vermis of cerebellum by razor blade. Total RNA from the cerebellum of individual animal ($n = 3–7$) was extracted separately by Trizol Reagent (BRL). A One-Step RT–PCR System (BRL) was used for RT–PCR reaction of 100 ng of RNA. *mPer1*, *mPer2* and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNAs were amplified simultaneously in a single tube using a GeneAmp 9900 (Perkin Elmer). Following the reverse transcription at 50°C for 30 min, samples were amplified by following parameters: 23 cycles of 94°C for 15 s, 55°C for 30 s, 72°C for 1 min and a 4°C hold. The primer pairs used for the amplification of each product are as follows: 5′-CCA GGC CCG GAG AAC CTT TTT-3′ and 5′-CGA AGT TTG AGC TCC CGA AGT G-3′ (*mPer1*); 5′-ACA CCA CCC CTT ACA AGC TTC-3′ and CGC TGG ATG ATG TCT GGC TC-3′ (*mPer2*); 5′-GAC CTC AAC TAC ATG GTC TAC A-3′ and TGG CCG TGA TGG CAT GGA CT-3′ (GAPDH). We found no amplification product without reverse transcription under this condition. The sizes of the PCR products of *mPer1*, *mPer2* and GAPDH were 402, 779 and 436 bp, respectively. PCR products were run on 3% agarose gels and DNA in the corresponding bands was detected with an EDAS-120 system (Kodak).

Preparation of cultured granule neurons and treatment of cultures

A primary culture of mouse cerebellar granule neurons was prepared from 7–8 day old *ICR* mice (Takasugi, Japan), as previously reported (Fujita *et al*., 1999). In brief, cerebella were isolated and cut with small pieces and tripsinized at 37°C. The dissociated cells were seeded at about 3×10^5 onto the poly-L-lysine coated culture dish (35 mm diameter) (Iwaki, Japan). The cells were grown at 25 mM KCl in DMEM supplemented with 10% FCS at 37°C. After 2 days, the cells were given fresh medium with 10% FCS supplemented with 10 mM cytosine arabinoside and cultured for additional 2 days. Nifedipine, diazepam, tandospirone and vehicle were added directly to the medium. Total cellular RNAs were prepared 1.5 h after addition and analysed by RT–PCR.

Statistics

Statistical differences between drug treated groups were analysed by one way analysis of variance (ANOVA). After positive ANOVA results, individual differences between groups were tested by the Dunnett's two-tailed test. In cases where two groups are compared, their statistical differences were tested by Student's *t*-test. In the fixed bar tests and the rota-rod test, Fisher's test was used for statistical analysis.

Results

Expression of mPer1 *and* mPer2 *in the cerebellum at day and night*

Amounts of *mPer1* and the *mPer2* mRNA in the mouse cerebellum at ZT4 and ZT16 were measured by RT–PCR and normalized to GAPDH mRNA (Figure 1). GAPDH mRNA level was constant under the light-dark cycle in the cerebellum. In order to compare the present results with SCN data, we decided to use of a same reported RT–PCR method (Akiyama *et al*., 1999) to analyse the amounts of *mPer* mRNAs. While

mPer1 mRNA was not so different between ZT4 and ZT16, *mPer2* mRNA level was higher at ZT16 than at ZT4 (Figure 1b) ($P<0.01$, Student's *t*-test).

Effects of diazepam administration on mPer *genes expression at day and night*

mPer1 and *mPer2* mRNA levels in the cerebellum 1 h after intraperitoneal administration of diazepam at ZT4 and ZT16 were examined. Diazepam treatment (10 mg kg^{-1}) caused significant reduction of *mPer1* mRNA in the cerebellum 1 h after treatment at both ZT (Figure 2b) ($P<0.01$ at ZT4 and $P<0.05$ at ZT16; Student's *t*-test). On the other hand, *mPer2* mRNA levels 1 h after treatment were barely affected by same doses of diazepam administrations at both ZT.

Temporal effects of diazepam on mPer *genes expression*

To confirm whether reduction of *mPer1* mRNA level after diazepam treatment is transient, *mPer1* and *mPer2* mRNA levels were examined 1 and 6 h after administration of diazepam at ZT16. Diazepam treatment (10 mg kg^{-1}) caused a severe deficit of rota-rod performance and equilibrium 1 h after administration at ZT16 (Figure 3a). Thereafter, impairment was slowly restored to normal 6–12 h after administration. While both diazepam and vehicle-treated mice showed reduced *mPer1* mRNA expression 1 h after injection ($P<0.01$, Student's *t*-test), no significant differences of *mPer1* and *mPer2*mRNA levels were observed 6 h after administration (Figure 3b,c).

Effects of anxiolytic drugs on mPer *gene expression* in vivo *and rota-rod performance*

Table 1 shows effects of various anxiolytic drugs (diazepam, triazolam and tandospirone) on performance of rota-rod test and fixed bar test. Administration of tandospirone (15 mg kg^{-1}) showed no deficit of co-ordinated motor activity. In this case, *mPer1* and *mPer2* expression is barely inhibited (Figure 4). On the other hand, high dose administration of diazepam (10 mg kg^{-1}), tandospirone (30 mg kg^{-1}) and triazolam (1 mg kg^{-1}) in that administration lead to significant depression of co-ordinated motor activity caused reduction of *mPer1* mRNA but not *mPer2* mRNA (Figure 4). Diazepam (1 mg kg^{-1}) treatment that caused small inhibition of motor activity also reduced *mPer1* mRNA level. These

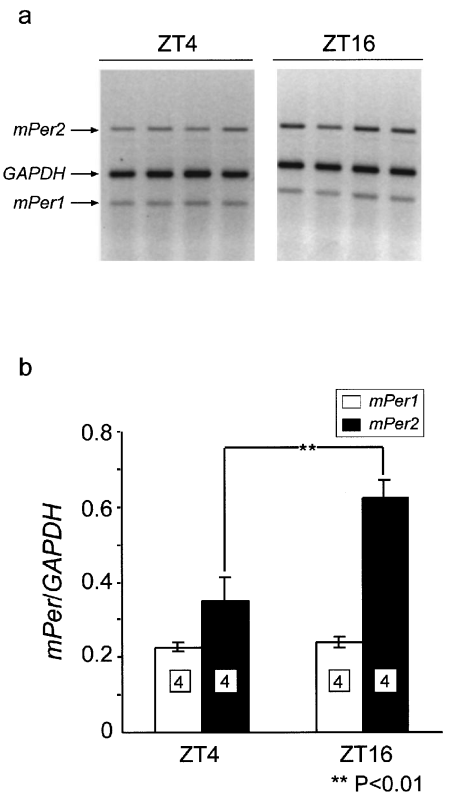

Figure 1 Expression of the *mPer1* and *mPer2* gene in the mouse cerebellum at ZT4 and ZT16. (a) Representative electrophoretic image of RT–PCR products of *mPer1*, *mPer2* and GAPDH mRNA from ZT4 and ZT16. (b) Relative mRNA levels of *mPer1* and *mPer2* at ZT4 and ZT16 were shown by ratio to GAPDH mRNA levels. Numbers in boxes in the columns indicate the number of experiments. *mPer2* mRNA is highly expressed at ZT16 than at ZT4 (**$P>0.01$, Student's *t*-test).

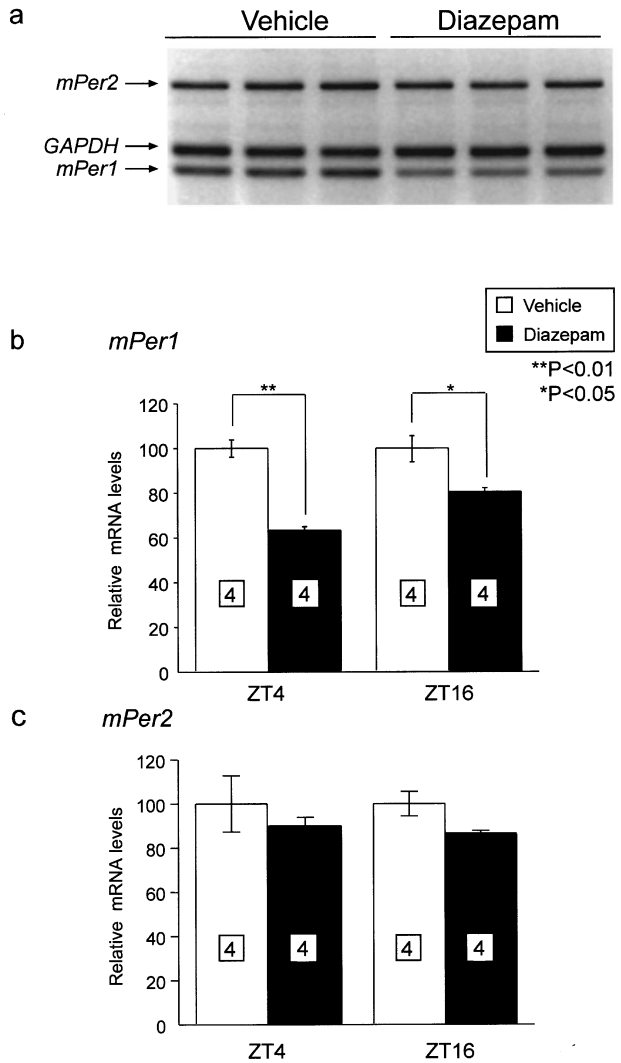

Figure 2 Effect of diazepam treatment on the expression of *mPer1* and *mPer2* at ZT4 and ZT16. (a) Representative example of electrophoretic image of RT–PCR products of *mPer1*, *mPer2* and GAPDH mRNA from vehicle- or diazepam-treated mice at ZT16. mRNA levels of *mPer1* (b) and *mPer2* (c) of vehicle- or diazepam-treated group 1 h after injection at ZT4 and ZT16 were normalized to GAPDH mRNA. Vehicle-treated group was set to 100 in both ZT for avoiding the effects of daily change of *mPer* mRNA. Numbers in boxes in the columns indicate the number of experiments. *mPer1* mRNA level was reduced by diazepam treatment at both ZT (**$P<0.01$ at ZT4, *$P<0.05$ at ZT16, Student's *t*-test).

results showed that transient reduction of *mPer1* mRNA levels by benzodiazepines correlated with an impairment of co-ordinated motor movements.

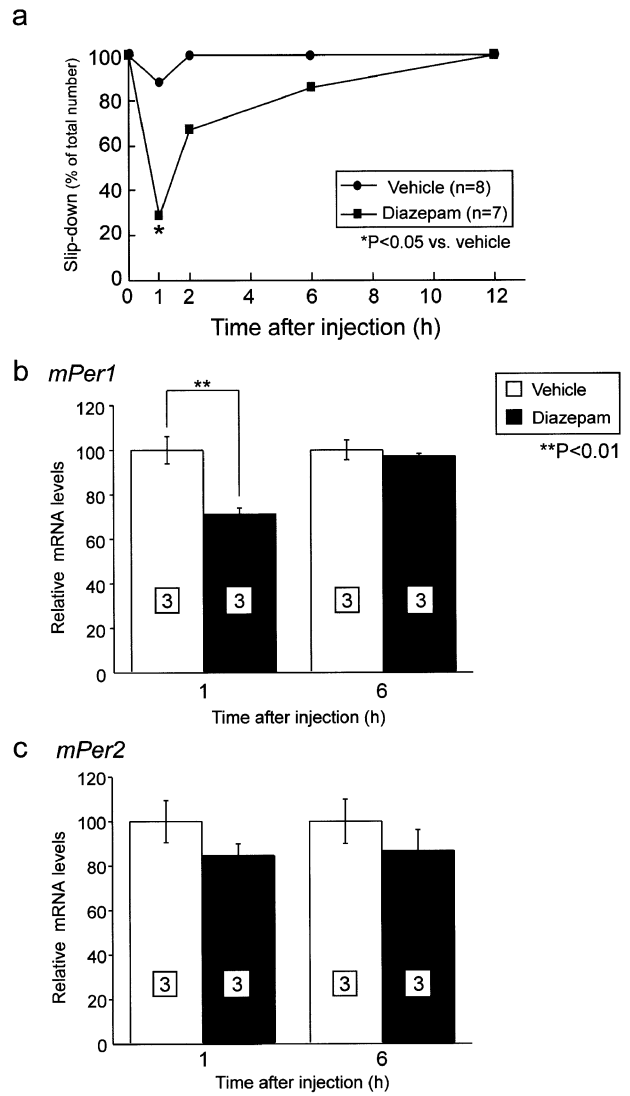

Figure 3 Temporal effect of diazepam administration on the expression of *mPer1* and *mPer2*. (a) Temporal inhibition of cooperative motor activity by diazepam administration was examined by rota-rod test. Most severe inhibition of locomotor activity was observed at 1 h after injection. (*$P<0.05$ vs vehicle, Fisher's test) mRNA levels of *mPer1* (b) and *mPer2* (c) of vehicle- or diazepam-treated group 1 and 6 h after injection of vehicle or diazepam (10 mg kg^{-1}) were normalized by GAPDH mRNA. Vehicle-treated group was set to 100 at both experiments. Numbers in boxes in the columns indicate the number of experiments. Reduction of *mPer1* mRNA by diazepam (*$P<0.01$, Student's *t*-test) restored after 6 h after treatment.

Effects of antipsychotic drugs on mPer *gene expression* in vivo *and rota-rod performance*

Table 1 shows effects of clozapine and haloperidol on performance of rota-rod test. Administration of clozapine (0.5 or 2.5 mg kg^{-1}) and haloperidol (0.2 mg kg^{-1}) showed no defict of co-ordinated motor activity. In all cases, *mPer1* and *mPer2* expression is barely inhibited (Figure 4). High dose clozapine (10 mg kg^{-1}) administration or haloperidol administration (0.2 or 1 mg kg^{-1}) led to significant depression of co-ordinated motor activity but did not reduce *mPer1* and *mPer2* mRNA level.

Effects of diazepam and tandospirone on mPer *genes expression* in vitro

As diazepam and tandospirone reduced *mPer1* gene expression *in vivo*, we tried to see effects of these drugs on *mPer1* and *mPer2* mRNA levels using the primary granule cell culture of mouse cerebellum (Figure 5). We demonstrated that *mPer1* expression in the cultured mouse cerebellar cells was reduced by nifedipine (10 μM) and transiently increased by addition of horse serum (50%) similar to rat-1 fibroblast cell culture as previously reported (Balsalobre *et al*., 1998). Addition of high concentrations of diazepam or tandospirone (each 100 μM) significantly reduced *mPer1* expression (Figure 5).

Discussion

The present study demonstrated that the expression of *mPer1* mRNA was rapidly reduced in the cerebellum by acute intraperitoneal injection of diazepam, triazolam and tandospirone, but not by clozapine and haloperidol. In addition, the present data showed that a transient reduction of *mPer1* was mRNA levels by diazepam, triazolam and tandospirone associated with an impairment of co-ordinated motor movements. These results demonstrated the first evidence indicating a potential function for the *mPer* genes outside the SCN. *mPer1* and *mPer2* mRNA are rapidly induced after light exposure during the subjective night in the SCN (Shigeyoshi *et al*., 1997; Zylka *et al*., 1998b). A recent study showed that *Per1* and *Per2* mRNA are induced by transient application of a high concentration of serum in a few mammalian cell lines (Balsalobre *et al*., 1998). Their temporal induction of *mPer1* and *mPer2* mRNA is similar to those of light induction in the SCN. In our culture experiment, we observed a similar increase of *mPer1* and *mPer2* mRNA in the cerebellar granule cell culture after application of high concentration of serum. However, the mechanism of acute induction after light exposure or incubation in the presence of high concentration of serum is still unknown. It has been shown that the

Table 1 Effects of various drugs on rota-rod performance and equilibrium

Drugs	*Vehicle*		*Diazepam*		*Triazolam*	*Tandospirone*			*Clozapine*			*Haloperidol*	
Dose (mg kg^{-1})		*0.5*	*1*	*10*	*1*	*15*	*30*	*0.5*	*2.5*	*5*	*0.2*	*1*
Fixed bar (8 mm)	8/8	–	6/7	4/7$^{\#}$	1/6**	7/7	4/6	–	–	–	–	–
Fixed bar (20 mm)	8/8	–	7/7	7/7	1/6**	7/7	5/6	–	–	–	–	–
Rota-rod	14/15	4/5	4/7$^{\#}$	2/7**	1/6*	5/7	0/6**	5/5	4/5	2/7**	4/8$^{\#}$	2/8**

Inhibition of coordinated motor activity by diazepam, triazolam, tandospirone, clozapine and haloperidol 1 h after injection were examined by rota-rod test and fixed-bar test (8 and 20 mm in diameters). The number in the second line in the Table indicated the amounts (mg kg^{-1}) of administered drugs. Fractional numbers indicate the number of animals stayed on the bar or rod vs number tested. Administration of diazepam (10 mg kg^{-1}), triazolam 1 mg kg^{-1}), tandospirone (30 mg kg^{-1}), clozapine (5 mg kg^{-1}) and haloperidol (0.2 or 1 mg kg^{-1}) impaired motor coordination (**$P<0.01$, *$P<0.05$, #$P<0.1$, Fisher's test).

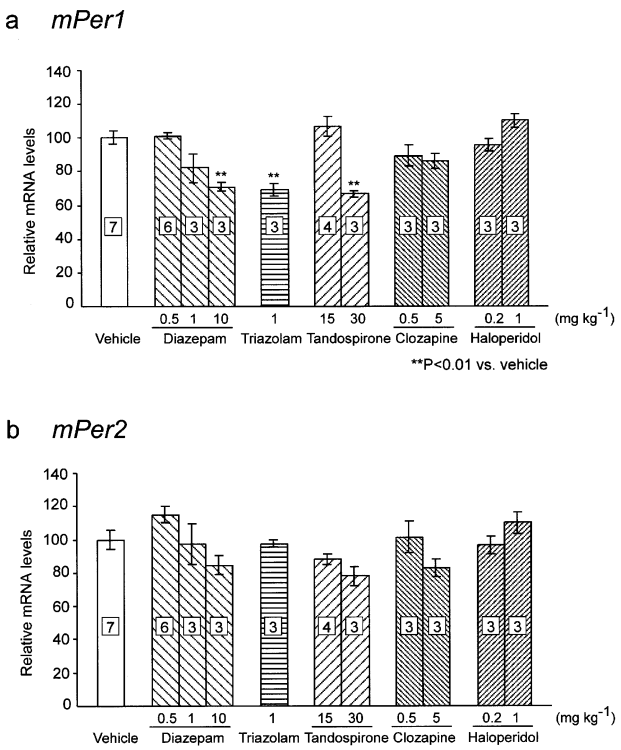

Figure 4 Effects of various drugs on *mPer1* (a) and *mPer2* (b) expression 1 h after administration. mRNA levels of *mPer1* and *mPer2* of vehicle-, diazepam-, triazolam-, tandospirone-, clozapine- and haloperidol-treated group 1 h after injection were normalized to GAPDH mRNA levels. The number in the Figure indicates the amounts (mg kg^{-1}) of administered drugs. Numbers in boxes indicate the number of experiments. Vehicle-treated group was set to 100. Numbers in boxes in the columns indicate the number of experiments. High dose administration of diazepam (10 mg kg^{-1}), triazolam (1 mg kg^{-1}) and tandospirone (30 mg kg^{-1}) reduced *mPer1* expression (**$P<0.01$ vs vehicle, Student's *t*-test).

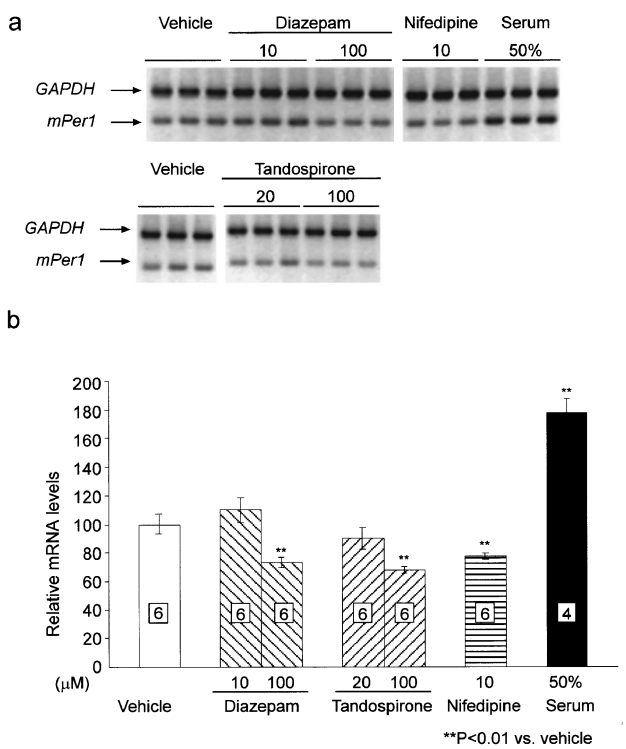

Figure 5 Effects of diazepam, tandospirone, nifedipine and 50% serum on *mPer1* expression in cerebellar granule cell culture. (a) Representative example of electrophoretic image of RT–PCR products of *mPer1*, *mPer2* and GAPDH mRNA from vehicle-diazepam- nifedipine- or high concentration of serum-treated granule cell culture. (b) mRNA levels of *mPer1* 1 h after treatment were normalized to GAPDH mRNA. Vehicle-treated group was set to 100. Numbers in boxes in the columns indicate the number of experiments. High dose treatment of diazepam (100 μM), tandospirone (100 μM) or nifedipine (10 μM) reduced *mPer1* expression (**$P<0.01$ vs vehicle, Student's *t*-test).

activation of N-methyl-D-aspartate receptor is required to produce the phase shift of SCN neuronal circadian rhythm (Shibata *et al*., 1994) as well as behavioural rhythms (Colwell *et al*., 1990). Thus, facilitation of glutamatergic receptors may be related to induction of *mPer1* mRNA after light exposure and serum shock. In contrast to excitatory influence, activation of $GABA_A$ receptors by muscimol and benzodiazepines caused the inhibition of SCN neuronal firing (Tominaga *et al*., 1994; Liou *et al*., 1990). Thus, administration of benzodiazepines may inhibit granular cell activity in the cerebellum through the activation of $GABA_A$ receptor subunit 6. Although the level of *mPer1* mRNA in the cerebellum was dose-dependently reduced by *in vivo* administration of diazepam, application of this drug *in vitro* required a high concentration (100 μM) to reduce the *mPer1* mRNA. There are several reasons for the discrepancy, but at present we can not be precise. In the present culture conditions, there might be an insufficient number of benzodiazepine receptors to cause the inhibition of *mPer1* mRNA. Actually, in the cultured granular cells, *mPer1* mRNA was highly expressed, because extracellular KCl concentration was kept high to keep the neurons alive. It is well known that cerebellum receives several inputs from other brain areas such as cerebrum, hindbrain and spinal cord (Voogd & Glickstein, 1998). An alternative explanation for the disagreement may be that the normal morphological structure of cerebellum or secondary influence *via* inputs from other brain regions is necessary to cause the reduction of *mPer1* mRNA by benzodiazepines.

What does the reduction of *mPer1* mRNA expression mean? Amounts of *mPer1* and *mPer2* mRNA are oscillated in the SCN (Tei *et al*., 1997; Zylka *et al*., 1998b). A recent study showed that activation of *mPer1* transcription in circadian oscillation is mediated by binding CLOCK-BMAL1 hetero-duplexes to E-box elements in the promoter region of the *mPer1* gene (Darlington *et al*., 1998). Increased expression of *mPer1* is thought to induce an increase of mPER1 protein and in turn inhibit the CLOCK's activity and down-regulate *mPer1* transcription (Darlington *et al*., 1998). Therefore, reduction of *mPer1* mRNA by anxiolytics may cause a disinhibition upon the CLOCK's activity. Thus, anxiolytic drugs can produce an impairment of circadian clock mechanism in the cerebellum, suggesting that these drugs may also impair clock-controlled functions such as neuronal activity. Effects of anxiolytic drugs on *mPer2* mRNA expression are much smaller than those on *mPer1* mRNA expression. Light induction of *mPer2* mRNA in the SCN is about 30 min slower than *mPer1* (Takumi *et al*., 1998a). These results taken together, suggest that various regulatory mechanisms exist in the expression of the *mPer1* and *mPer2* gene in different cell groups.

It has been proposed that the role of the cerebellar cortex is to combine simpler elements of movement into complex coordinated action (Thach *et al*., 1992). In the rota-rod test, the mGluR1, GluRδ2 and NMDA2A/NMDA2C receptor mutant mice have been shown to fall off the rota-rod with a deficit of long-term depression in the cerebellum (Aiba *et al*., 1994; Kashiwabuchi *et al*., 1995; Kadotani *et al*., 1996). Thus, normal function of glutamatergic neurotransmission in mossy

fibre-granule cell synapses may be important for performance of motor co-ordination. Further genetic dissection of *mPer1* gene, possibly with knockout mice is useful to identify the role of these genes in the cerebellum.

The high dose of tandospirone, a $5HT_{1A}$ receptor agonist, caused a reduction of *mPer1* mRNA expression in the cerebellum *in vivo* as well as *in vitro* again with an impairment of rota-rod performance, however its low doses affected neither *mPer1* mRNA nor co-ordinated motor activity and equilibrium. The administration of high doses of haloperidol and clozapine caused an impairment of rota-rod performance and the dose range used in the present experimental study is well in accordance with a previous paper by Millan *et al.* (1999). However, haloperidol and clozapine did not affect the expression of *mPer1* mRNA in the cerebellum. The present result suggested that the reduction *mPer1* mRNA in the cerebellum may not be related to the anxiolytic activity, because small dose of tandospirone (15 mg kg^{-1}) is sufficient to produce the anxiolytic activity (Shimizu *et al.*, 1992) and clozapine is known to possess anxiolytic properties in certain experimental models (Wiley *et al.*, 1993). Why did high dose of tandospirone reduce both *mPer1* mRNA and rota-rod performance? Tandospirone exhibits the high affinity to 5-HT_{1A} receptors (Shimizu *et al.*, 1988), but clozapine shows mild agonistic properties at these receptors (Newman-Tancredi *et al.*, 1996). Although there is a report that shows a low expression of 5-HT_{1A} receptors in the cerebellum (Kia *et al.*, 1996), tandospirone reduced *mPer1* mRNA expression *in vivo* and *in vitro*. Therefore, the present results suggest that a high dose of tandospirone affects the cerebellar function. Alternatively, high dose of tandospirone may affect 5-HT_{1A} receptors in the hindbrain and/or spinal cord to cause an impairment of motor coordination. In the present experiment, haloperidol did not affect the expression of *mPer1* mRNA in the cerebellum, although this chemical impaired the rota-rod performance. Therefore, it is suggested that impairment of rota-rod performance itself did not affect the expression of *mPer1* mRNA. Although *Yokoyama et al.* (1994) reported the high expression of D2 receptors in the cerebellum and extrapyramidal, brain areas such as striatum may be responsible for haloperidol-induced impairment of rota-rod performance. Actually, the haloperidol induced decrement in co-ordinated locomotor activity was observed only after a high dose was injected into the cerebellum (Barik & de Beaurepaire, 1996). Thus, we conclude that there is no contribution of D2 receptors on the *mPer1* mRNA expression in the cerebellum.

In summary, present study showed a reduction of *mPer1* mRNA level in the cerebellum by benzodiazepines and a 5-HT1A receptor agonist, but not by antipsychotics closely associated with impairment of co-ordinated motor activity.

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