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THE ROLE OF *mPer1* IN MORPHINE DEPENDENCE IN MICE

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Abstract—Investigations using *Drosophila melanogaster* have shown that the circadian clock gene *period* can influence behavioral responses to cocaine, and the mouse homologues, *mPer1* and *mPer2*, modulate cocaine sensitization and reward. In the present study, we applied DNAzyme targeting *mPer1* to interfere the expression of *mPer1* in CNS in mice and studied the role of *mPer1* on morphine dependence. We found that the DNAzyme could attenuate the expression of *mPer1* in CNS in mice. Mice treated with DNAzyme and morphine synchronously did not show preference to the morphine-trained side, whereas the control group did. In contrast, mice treated with DNAzyme after morphine showed preference to the morphine-trained side as well as the control group did. These results indicate that drug dependence seems to be influenced at least partially by *mPer1*, but *mPer1* cannot affect morphine dependence that has been formed.

Key words: drug dependence, DNAzyme, learning and memory, circadian, i.c.v.

Circadian clocks are molecular time-keeping mechanisms that reside in a diverse range of cell types in a variety of organisms. The primary role of these cell-autonomous clocks is to maintain their own approximately 24 h molecular rhythm and to drive the rhythmic expression of genes involved in physiology, metabolism and behavior. Components of the endogenous master clock were first identified in the fruit fly *Drosophila melanogaster*. The *Period* (*Per*) encodes one of the essential elements involved in the transcription/translation-based auto-regular loop of the endogenous master clock (Reppert and Weaver, 2001). Three homologues of *Drosophila Per* genes were subsequently identified in mice (*mPer1*, *mPer2*, and *mPer3*) (Albrecht, 2002), leading to great progress in elucidation of the molecular mechanism underlying circadian rhythm in the CNS.

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Abbreviations: CPP, conditioned place preference; ODN, oligonucleotide; *Per*, *Period*; ZT, zeitgeber time.

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It has been shown that repeated administration of methamphetamine caused behavioral sensitization as well as sensitized expression of *mPer1* (Nikaido et al., 2001). Some studies implicate a role for *Per* genes in drug-induced behavioral sensitization processes. This suggestion is supported by investigations using *Drosophila* flies. Flies mutant in the *Per* gene did not sensitize after repeated exposure to volatilized free-base cocaine (Andretic et al., 1999; Hirsh, 2001). In mice, the *mPer1* and *mPer2* genes influence cocaine-induced sensitization and reward in an opposite manner. The lack or dysfunction of the *mPer1* gene abolishes cocaine sensitization and reward whereas the dysfunction of the *mPer2* gene induces a hypersensitized response to cocaine (Abarca et al., 2002).

DNAzyme is a suitable tool for studying gene function. The typical DNAzyme, known as the "10–23" model, is capable of cleaving single-stranded RNA at specific sites. The "10–23" model of DNAzymes has a catalytic domain of 15 highly conserved deoxyribonucleotides, flanked by two substrate-recognition domains, which can cleave effectively between any unpaired purine and pyrimidine of mRNA transcripts (Santoro and Joyce, 1997).

To further understand the role and the mechanism of *mPer1* gene in morphine dependence, we first studied morphine-induced reward to be involved in morphine dependence by blocking the expression of *mPer1* gene with the "10–23" DNAzyme.

EXPERIMENTAL PROCEDURES

Animals

In all experiments, 4- to 6-week-old male BALB/C mice were used. Mice were housed in groups of five and provided with food and water *ad libitum*. Artificial light was provided daily from 8:00 a.m. to 8:00 p.m. with room temperature and humidity kept constant (temperature: 22–24 °C; humidity: 55–65%). All procedures were performed in compliance with the local, international and institutional guidelines. All efforts were made to minimize the number of animals used and their suffering.

Conditioned place preference (CPP)

Place conditioning was conducted as described previously according to Suzuki et al. (1993). The apparatus consisted of a shuttle box (30×15×15 cm: length×width×height) made of an acryl-resin board. The box was divided into two compartments of equal size by means of a sliding partition. One compartment was white with a textured floor, and the other was black with a smooth one. When the CPP was measured, the partition separating the two compartments was raised to 7 cm above the floor. Preference for a particular place was assessed. The time spent in black compartment during a 900-s session was measured automatically. To avoid the introduction of systematic errors, the CPP experiment was carried out in a light and sound-controlled environment.

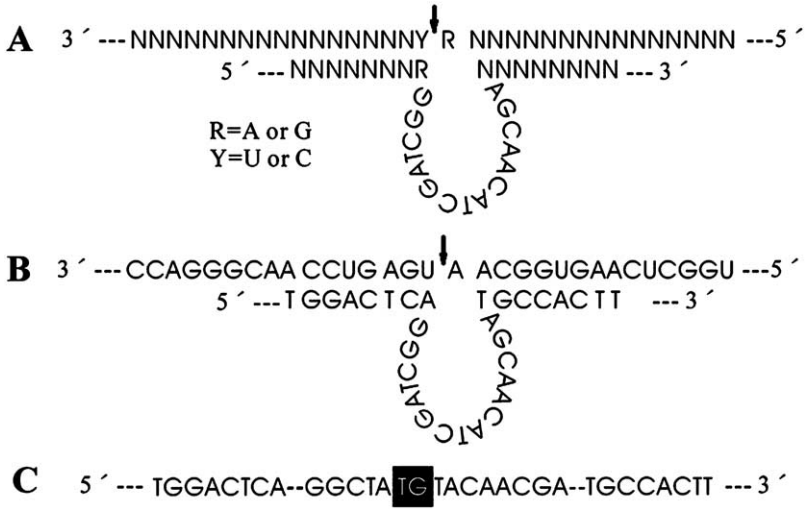

Fig. 1. DNAzyme and its mRNA substrate of *mPer1*. (A) A 10–23 DNAzyme structure with substrate cleavage occurring at the position indicated by the arrow. (B) Sequence and structure of *mPer1* DNAzyme annealed to the *mPer1* substrate. The arrow indicates the cleavage site. (C) Sequence of the control ODN. To inactivate enzymatic activity of the DNAzyme, two nts (black) in the intervening 15-nt catalytic domain were altered.

I.c.v. injection

The i.c.v. injection procedure was adapted from the method described earlier (Mistry et al., 1997). Briefly, the i.c.v. injections were given as follows: under light ether anesthesia, bregma was exposed. An injection volume of 20 μl was delivered over a 60-s period, 2 mm lateral and caudal to bregma at a depth of 2 mm by using a syringe. Proper placement was verified in the experiments by injection and localization of Methylene Blue dye.

DNAzyme

We designed a "10–23" DNAzyme, as described previously by Santoro and Joyce (1997), targeting *mPer1* gene in mice (Fig. 1). The 15-nt catalytic domain is flanked by two eight-nt arms that recognized the *mPer1* mRNA substrate from 287 to 303 nt except 296 nt (GenBank accession number: U49930). The 5′ and 3′ termini of the molecule are protected from exonucleases by a phosphorothioate linkage and a CPG-C3 cap respectively. To inactivate the DNAzyme and to generate a control oligonucleotide (ODN), two nts were changed in the catalytic domain of the DNAzyme (Fig. 1C). Transversion of two nts in the disabled ODN is sufficient to inactivate the catalytic activity (Santoro and Joyce, 1997; Wu et al., 1999; Sriram and Banerjea, 2000; Unwalla and Banerjea, 2001). The DNAzyme and the disabled DNAzyme ODN (control ODN) were synthesized by Invitrogen (Invitrogen, USA).

In vitro transcript of sequence of *mPer1* mRNA for cleavage

A double stranded ODN containing the sequence of the *mPer1* cDNA 140–845 nts (GenBank accession number: U49930) plus appropriate cloning sites was synthesized and introduced into the *Hind*III and *Bam*H I sites of plasmid pBluescript II SK(+). This recombinant was prepared for cleavage experiments.

Cleavage experiments

DNAzyme cleavage experiments were performed as described previously (Santiago et al., 1999). The oligoribonucleotide substrate of the DNAzyme was labeled at the 5′ end with [^{33}P] ATP (2500 Ci/mmol; Amersham Pharmacia Biotech) by using T4 polynucleotide kinase (New England Biolabs). DNAzyme (50 pmol) was added to 4 pmol of the *in vitro* transcript substrate. The reaction was then stopped at several time points. In these cleavage experiments, the cut and uncut substrates were separated by electrophoresis on a 5% urea denaturing polyacrylamide gel and detected by autoradiography at 4 °C. Signals were then scanned by Storm 840 instrument and analyzed by Image-Quant 5.0 software (Molecular Dynamics).

Treated with DNAzyme and morphine synchronously

DNAzyme and control ODN were enclosed with Lipofectamine (Invitrogen) according to the description respectively. After mice's acclimatization, the basic CPP of mice was measured. The animals were divided into two groups (n=30 per group), including DNAzyme group (DMS) and control ODN group (CMS). The animals were given saline (the same volume as morphine, s.c.) at zeitgeber time (ZT) 2 (10:00 a.m.) before placed into black section of the shuttle boxes for 30 min. On the next day, the animals were given morphine (10 mg/kg, s.c.) at ZT2 (10:00 a.m.) and then placed into white section of the shuttle boxes for 30 min. These procedures were repeated four times in 8 days. At ZT12 (8:00 p.m.) mice of different group were injected intracerebroventricularly with DNAzyme and control ODN enclosed with Lipofectamine respectively once a day from the 1st to the 7th day of the experiment according to different group. At ZT8 (4:00 p.m.), the CPP of the mice was measured on the 8th day of the experiment. Thus four mice of the each group were killed at ZT12 (8:00 p.m.) and ZT16 (12:00 p.m.) on the 6th day and at ZT20 (4:00 a.m.), ZT0 (8:00 a.m.), ZT4 (12:00 a.m.) and ZT8 (4:00 p.m.) on the 9th day respectively. The brains of the killed mice were prepared for Western blot.

Treated with DNAzyme after morphine

Mice were also divided into two groups (n=30 per group), including DNAzyme group and (DMA) control ODN group (CMA). The basic CPP of mice was measured. The animals were given saline (the same volume as morphine, s.c.) at ZT2 (10:00 a.m.) before placed into black section of the shuttle boxes for 30 min. On the next day, the animals were given morphine (10 mg/kg, s.c.) at ZT2 (10:00 a.m.) and then placed into white section of the shuttle boxes for 30 min. These procedures were repeated four times in 8 days, and then the mice were injected intracerebroventricularly with DNAzyme and control ODN once a day respectively according to different group in the next 7 days at ZT12 (8:00 p.m.). The remained procedures were the same as the abovementioned.