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Lack of effect of anticonvulsant topiramate on methamphetamine-induced stereotypy and rewarding property in mice

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Abstract

The effects of topiramate, a structurally novel anticonvulsant, on the methamphetamine (METH)-induced expression of stereotypy and conditioned place preference (CPP) in male ICR mice were investigated. After a single administration of METH (10 mg/kg, i.p.), mice showed stereotyped behaviors with a plateau level 25 min after drug challenge. Pretreatment with topiramate (1, 10, and 100 mg/kg, i.p.) 30 min prior to METH challenge had no effect on the expression frequency of stereotypy, compared with saline challenge. No differential effects of topiramate on METH-induced stereotyped behavior (that is, head-bobbing, circling, continuous sniffing, nail and/or wood-chip biting, and vigorous and compulsive grooming) were observed. In saline-challenged groups, the doses of topiramate examined did not induce any stereotyped behaviors. Although mice showed a significant CPP for METH (0.5 mg/kg, i.p.), pretreatment with subchronic topiramate did not affect the magnitude of CPP. Locomotor activity was not affected by the doses of topiramate tested. Conditioned rewarding or aversive effects of topiramate were not observed as indexed by the place preference procedure. These results suggested the lack of effect of topiramate on METH-induced stereotypy and rewarding property in mice.

Keywords: Methamphetamine; Anticonvulsant; Topiramate; Stereotypy; Conditioned place preference

1. Introduction

Methamphetamine (METH) abuse is a serious problem worldwide, and there are no effective medications for its treatment (Kantak, 2003). METH-induced rewarding properties and abnormal behavior such as stereotypy in rodents are considered psychomotor aspects of drug treatments in an animal model similar to some aspects of METH abuse in humans. The animal model is useful to find effective medications for the treatment of METH abuse in humans (for review, see Kitanaka et al., 2006a).

METH interacts with subcellular target components such as the cocaine-sensitive dopamine transporter (DAT), the vesicular monoamine transporter-2 (VMAT-2), and the monoamine oxidase isozymes. Reverse transport of dopamine via DAT and inhibition of VMAT-2 are the primary mechanisms of action of METH, resulting in the massive outflow of dopamine from the presynaptic terminal into the synaptic cleft (for reviews, see Seiden et al., 1993; Sulzer et al., 2005). The activation of dopamine receptors by aberrant levels of released dopamine in mesolimbic and mesocortical areas has been suggested to be closely associated with METH-induced abnormal behavior such as hyperlocomotion and stereotypy and METH reward in rodents and humans (Robinson and Becker, 1986; Seiden et al., 1993; Self and Nestler, 1995; Wise, 2002).

The activation of γ-aminobutylic acid (GABA) receptor signal transduction inhibits the enhancement of mesolimbic dopaminergic transmission. GABA receptor-related ligands have been tested to suppress METH reward such as the augmentation of METH self-administration and the progressive augmentation of locomotor activity in response to METH treatment, a phenomenon referred to as METH-induced

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behavioral sensitization, in rodents. For example, it was reported that baclofen, a $GABA_B$ receptor agonist, significantly attenuated METH self-administration in rats, suggesting that $GABA_B$ receptor agonists may be useful for the treatment of METH abuse (Ranaldi and Poeggel, 2002). Ito et al. (2000) raised the suggestion that $GABA_A$ agonists may prevent the acquisition of behavioral sensitization to METH in rats pretreated with a benzodiazepine ligand, clonazepam.

Topiramate (2,3:4,5-bis-*O*-(1-methylethylidiene)-β-d-fructopyranose sulfamate), a structurally novel anticonvulsant (Maryanoff et al., 1987; Shank et al., 1994), is suggested to show its anticonvulsant effect through GABA-mediated chloride flux, similar to benzodiazepines (White et al., 1997). In addition, topiramate inhibits ionotropic AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionate)/kainate receptor-mediated synaptic currents and voltage-dependent sodium and calcium currents, resulting in a broad spectrum of neurostabilizing effects (Zona et al., 1997; Taverna et al., 1999; Gibbs et al., 2000; Zhang et al., 2000; Gryder and Rogawski, 2003). Topiramate also weakly but effectively inhibits carbonic anhydrase isozymes from humans as well as rodents, and with this activity topiramate is assumed to possess anticonvulsant activity (Maryanoff et al., 1987; Dodgson et al., 2000). With these multiple mechanisms of action such as neurostabilizing effects, topiramate has been tested for the treatment of seizures and drug dependence including alcohol, nicotine, cocaine, and the club drug MDMA (3,4-methylenedioxymethamphetamine, "ecstasy") in pre-clinical experiments and clinical trials (Johnson et al., 2003; Cagetti et al., 2004; Sofuoglu and Kosten, 2005; Akhondzadeh and Hampa, 2005; Sofuoglu et al., 2006). On the basis of these observations, topiramate may be effective as a treatment for drugs of abuse. Systematic clinical trials using topiramate as a treatment have been performed successfully only in alcoholic patients (Johnson et al., 2003; Arnone, 2005), and there is one positive pilot study in cocaine addicts using topiramate (Kampman et al., 2004).

Kampman et al. (2004) reported the effect of topiramate on cocaine dependence in a 13-week, double-blind, placebo-controlled pilot trial as indexed by urine benzoylecgonine test. Analysis of the levels of benzoylecgonine, a major metabolite of cocaine, in urine showed that topiramate-assigned outpatients were more likely to abstain from cocaine compared to subjects assigned to placebo, suggesting that topiramate might be effective for the treatment of cocaine dependence (Kampman et al., 2004). Cocaine enhances extracellular dopamine in the mesolimbic area by blocking DAT activity. The direct action of METH on DAT and VMAT-2 enhances dopamine release from presynaptic terminals into the synaptic cleft (Seiden et al., 1993; Sulzer et al., 2005). In contrast to this, topiramate inhibited the nicotine-induced increase in dopamine and norepinephrine release in the nucleus accumbens of rats (Schiffer et al., 2001), suggesting that topiramate can decrease the release of dopamine stimulated by drugs of abuse. Therefore, there is a possibility that topiramate is effective for the treatment of METH dependence. To address the possibility of whether topiramate inhibits METH-induced abnormal behavior such as stereotypy and the rewarding property of METH, the effects of topiramate on METH-induced stereotypy and conditioned place preference (CPP) were examined in mice. In our CPP apparatus, there is a strong negative correlation between the magnitude of the CPP index and locomotor activity induced by METH (0, 0.5, 1, and 2 mg/kg, i.p.) during the post-conditioning period (unpublished observations). In an attempt to increase the possibility of the expression of CPP in response to the psychomotor stimulant METH at a low dose, the present study used a biased CPP apparatus and subject assignment procedure under the lowest dose of METH (0.5 mg/kg) at which mice show the reliable acquisition of CPP with less hyperactivity compared with that at doses of 1 mg/kg or higher (Kitanaka et al., 2006b). Systemic injection of topiramate has been reported to stimulate locomotor activity in rats and mice (Cagetti et al., 2004; Gremel et al., 2006); therefore, possible conditioned rewarding or aversive effects of topiramate were also investigated as indexed by the place preference procedure.

2. Methods

2.1. Subjects

Male ICR mice (9–10 weeks old; Japan SLC, Shizuoka, Japan) were housed in groups of 8 (cage size, 37 × 22 × 15 cm) in a temperature- (22 ± 2 °C) and humidity- (50 ± 10%) controlled environment under a 12-h light/dark cycle (lights on at 0700 h) with food and water available *ad libitum* except during the experimental observations. Animal handling and care were conducted according to the *Guide for the Care and Use of Laboratory Animals* (7th edition, Institute of Laboratory Animal Resources-National Research Council, National

Table 1
The design of Experiments 1, 2 and 3

Day	1	2	3–8	9	10
Experiment 1: effect of TPM on METH-induced stereotypy					
		Test			
Saline-challenged group (8)	S	TPM/S			
METH-challenged group (8)	S	TPM/METH10			
Experiment 2: effect of TPM on METH CPP					
	Pre-cond.		Conditioning (days 3–8)	Post-cond.	
S/S pairing group (7)	No inj	No inj	(TPM/S–TPM/S)×3 paring days	No inj	No inj
METH/S pairing group (7)	No inj	No inj	(TPM/METH0.5–TPM/S)×3 paring days	No inj	No inj
Experiment 3: TPM CPP					
	Pre-cond.		Conditioning (days 3–8)	Post-cond.	
TPM/S pairing group (8)	No inj	No inj	(TPM–S)×3 paring days	No inj	No inj

Parentheses indicate the number of subjects used per group. CPP, conditioned place preference; inj, injection; METH0.5, 0.5 mg/kg of methamphetamine; METH10, 10 mg/kg of methamphetamine; Post-cond., post-conditioning; Pre-cond., pre-conditioning; S, saline; TPM, doses of topiramate (0 = saline, 1, 10 and 100 mg/kg). In Experiments 1 and 2, topiramate was pretreated 30 min prior to the injection of METH or vehicle.

Academy Press 1996) and all experiments were approved by the Institutional Animal Research Committee. Every effort was made to minimize the number of animals used and their suffering. Mice were used after at least 6 days' habituation in this facility.

2.2. Drug preparation

All drugs were dissolved in sterile saline. Nine milligrams of topiramate (Toronto Research Chemicals, Inc., ON, Canada) was dissolved and sonicated in 1.125 ml of saline. Topiramate solutions were prepared in such a way that the necessary dose could be injected in a volume of 0.125 ml/10 g of body weight by an intraperitoneal (i.p.) route. METH (Dainippon Pharmaceutical Co., Osaka, Japan) was administered i.p. in a volume of 0.1 ml/kg of body weight. The same volume of saline was used for the vehicle. The doses of drugs refer to the weight of salt.

2.3. Experiment 1: effect of topiramate on METH-induced stereotypy in mice

For stereotypy rating, all mice (31–42 g, 10 weeks old, $n=64$) were injected i.p. with 0.1 ml/10 g of sterile saline on day 1 (Table 1). This procedure was required to reduce the variance of the data for locomotor activity on day 2 (Kitanaka et al., 2005). On day 2, mice were divided randomly into eight groups ($n=8$ each), and the mice in each group were subjected to treatment as indicated in Table 1; separate groups of mice were injected with 10 mg/kg of METH or vehicle (*i.e.* 0.1 ml/kg of saline) 30 min after the injection of 0 (*i.e.* 0.125 ml/10 g of saline), 1, 10, or 100 mg/kg of topiramate. The doses of topiramate tested and the treatment period in this study are determined on the basis of the recent literature: Effects of topiramate (i.p. or p.o. route, 0.5–5-h treatment) on several external stimuli-induced seizures were investigated in Swiss Webster mice and Wistar rats (ED_{50} values of anticonvulsant activity evaluated by maximal electroshock seizure test were 47.5 and 24.5 mg/kg, i.p., for mice and rats, respectively) (Shank et al., 1994). Measurements were made by an observer unaware of the treatments. Animals were placed in individual $30\times30\times35$ cm observation chambers, which had the floor spread with approximately 25 g of wood chips, and the transparent acrylic sides. Animals were observed for 120 min described previously (Kitanaka et al., 2005; Tatsuta et al., 2005). Behavior was broken down into 30-s bins, and predominant behavior was recorded for each bin. The behaviors scored were quiet and awake/sleeping, ambulating, rearing, head-bobbing (up-and-down movements of the head), circling, continuous sniffing with apparent exploratory behavior, nail and/or wood-chip biting, and vigorous and compulsive grooming. Ambulating and rearing were considered locomotor/exploratory behaviors and the last five were considered stereotypy. The rationale for using the five distinguishable categories of stereotypy is to examine any possible differential effects of topiramate on the type of stereotypy observed. The cumulative number of bins within every 5 min in which stereotypies were observed is shown (maximal value = 10). All experiments were performed between 0900 h and 1800 h.

2.4. Experiment 2: effect of topiramate on METH-induced CPP in mice

The place preference apparatus was developed at Muromachi Kikai, Co., Ltd. (Tokyo, Japan) using Supermex® sensors (Kitanaka et al., 2006b). The infrared pyroelectric sensors were originally developed to measure horizontal locomotor activity by detecting the body heat of an animal (Kitanaka et al., 2003). The CPP boxes were made of acrylic resin, with two main compartments of $24.3\times15.6\times21.3$ cm, divided by a removable barrier with a doorway (8.0×6.0 cm). A solid barrier without a doorway was used to confine animals to a given side of the compartment. Two sensors were positioned on the top cover of each compartment of the CPP box. The data collected from the sensors were horizontal locomotor activity as well as the time spent in each compartment. When the animal entered and stayed in an overlapping detection area of the two sensors (1.8 cm each from the border of the two compartment floors), the animal was defined as being in a "neutral" position. The collected data were analyzed using a newly developed program (CompACT CPP for Windows, version 1.11, Muromachi Kikai, Co., Ltd) running on a PC computer. The compartments had different visual and texture cues (one was black with a smooth floor and the other was white with 5 g of fresh wood chips on a smooth floor). All compartments were cleaned and wiped dry between the sessions for each animal.

On day 1, mice were weighed (31–44 g, 10–11 weeks old, $n=56$) and placed in CPP boxes for pre-conditioning with no injection (Table 1); the mice were placed into the neutral position and then allowed free access to the two compartments for 10 min. The compartment in which the mice stayed for a shorter time was defined as the conditioning compartment. The definition was based on an individual animal (a biased design).

Mice were then randomly assigned to either the METH/saline conditioning group or saline/saline control group ($n=28$ each). The second phase consisted of six days (three METH/saline or three saline/saline pairings) of conditioning. Two hours after lights on, seven mice in each group received injections of topiramate (0, 1, 10, or 100 mg/kg, once per day for six consecutive days) and were then returned to their home cages. Therefore, all mice were pretreated with topiramate on both METH/saline and saline/saline pairings session. On days 3, 5, and 7, 30 min after topiramate pretreatment, the mice received injections of saline or METH (0.5 mg/kg) in the conditioning compartment determined on day 1 and were then confined to the conditioning compartment for 20 min. On days 4, 6, and 8, all mice were injected with saline and immediately confined to the opposite compartment for 20 min. Control mice therefore received saline in both compartments once per day for six consecutive days in this conditioning phase. On day 10 (post-conditioning day), mice were placed in the neutral position and then allowed 10 min with free access to the two compartments. Time spent in each compartment and locomotor activity (number of signal changes in sensor elements) were recorded using the

computerized system (Kitanaka et al., 2006b). The difference in time spent in the conditioning compartment between post-conditioning (day 10) and pre-conditioning (day 1) sessions for each treatment was analyzed as a CPP index. In this study, the duration spent in the neutral position on days 1 (pre-conditioning day) and 10 (post-conditioning day) was 11 ± 1 s and 10 ± 1 s in a total of 600 s, respectively, and no difference was observed between each treatment group on each day (data not shown). The experimental room was kept quiet, and all experiments were conducted between 0900 h and 1500 h. The data consisted of four completely separate sets of experiments (1–2 mice per group in each experiment), and the results were reproducible. All mice maintained or gained body weight during the experimental period (data not shown).

2.5. Experiment 3: topiramate-induced change in CPP index and locomotor activity in mice

Experiment 3 was designed to examine whether topiramate (1, 10, and 100 mg/kg) *per se* might have effects on non-drug related learning occurring on non-drug conditioning trials. The mice were weighed (48–54 g, 11 weeks old, $n=64$) and placed in CPP boxes for pre-conditioning with no injection (Table 1) as described in Section 2.4. The second phase consisted of 6 days (three topiramate/saline or three saline/saline pairings) of conditioning. Time spent in each compartment and locomotor activity (number of signal changes in sensor elements) were recorded on days 1, 3–8, and 10, as described previously (Kitanaka et al., 2006b).

2.6. Experiment 4: tissue levels of dopamine and homovanillic acid in the striatum and nucleus accumbens of the mouse

Mice ($n=3$ each) were pretreated with topiramate (0, 1, 10, and 100 mg/kg) for 30 min followed by METH or saline challenge. The mice were sacrificed by cervical dislocation and decapitation 20 min after the drug challenge. The brains were immediately removed, and the striata were isolated, weighed, and frozen in liquid nitrogen. Tissue levels of dopamine and homovanillic acid (HVA), a metabolite of dopamine, were quantified by high-performance liquid chromatography (HPLC) with electrochemical detection as described previously (Kitanaka et al., 2005) as follows: Each frozen brain sample was homogenized with a Teflon/glass homogenizer in 10–20 volumes (w/v) of ice-cold 0.1 N perchloric acid with 30-μM Na_2EDTA containing 3,4-dihydroxybenzylamine hydrobromide and isoproterenol as internal standards for the catechols and for the indoles, respectively. The homogenates were centrifuged at 10,000 ×*g* for 10 min at 4 °C and the supernatants were filtered through a 0.20-μm membrane filter (Millipore Co., Bedford, MA, USA). The filtrates (10 μl) were injected directly into a HPLC system (system controller, model SCL-10A; auto-injector, model SIL-10A; pump, model LC-10AD; Shimadzu Co., Kyoto, Japan) equipped with a reversed-phase ODS-column (MCM column 150; 4.6 × 150 mm; MC Medical, Inc., Osaka, Japan) and an electrochemical detector (Coulochem Model 5100A, ESA, Inc., Chelmsford, MA, USA). The column temperature was maintained at 24 °C, and the detector potentials were set at +0.40 V, +0.15 V and −0.35 V on the conditioning cell, and Detectors 1 and 2, respectively. The mobile phase was a 1000:35.2:85.8 (v/v) mixture of a buffer (50 mM Na_2HPO_4, 50 mM citric acid, 4.4 mM 1-heptanesulfonic acid and 0.1 mM Na_2EDTA, pH 3.0), acetonitrile and methanol, and the flow rate was set at 0.9 ml/min.

2.7. Statistics

Values are shown as the means with bars representing the standard errors of the means (S.E.M.). Statistical analysis was performed using one-way, two-way, or three-way analysis of variance (ANOVA) with or without repeated measures followed by Bonferroni/Dunn test. A P value of less than 0.05 was considered significant.

3. Results

3.1. Experiment 1: effect of topiramate on METH-induced stereotypy in mice

A repeated-measures three-way ANOVA (Topiramate Dose × METH Treatment × Time) applied to Fig. 1 yielded significant main effects of METH Treatment ($F(1,56)=1018.8$, $P<0.0001$) and Time ($F(23,1344)=55.561$, $P<0.0001$), but no significant main effect of Topiramate Dose ($F(3,56)=0.060$, $P=0.9808$). This analysis also yielded a significant METH Treatment × Time interaction ($F(23,1344)=51.517$, $P<0.0001$), but no significant Topiramate Dose × METH Treatment, Topiramate Dose × Time, or Topiramate Dose × METH Treatment × Time interactions ($F(3,56)=0.078$, $P=0.9717$, $F(69,1344)=0.343$, $P>0.9999$, and $F(69,1344)=0.371$, $P>0.9999$, respectively). *Post-hoc* comparisons indicated a significant difference between the METH-treated and the saline-treated mice (Bonferroni/Dunn test, $P<0.001$). *Post-hoc* pair-wise

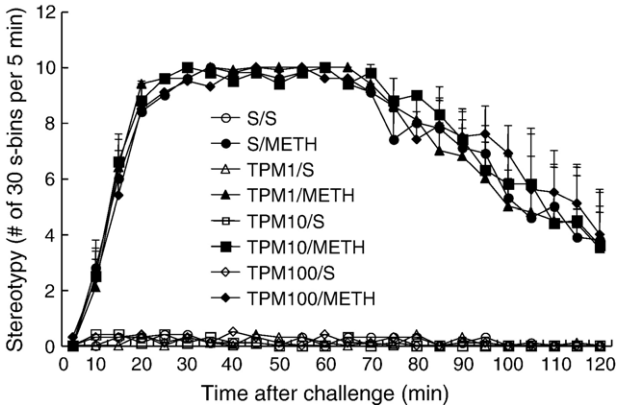

Fig. 1. Effect of topiramate pretreatment on METH-induced stereotyped behavior in mice. Each symbol indicates stereotyped behavior scores within each 5-min interval, and data during 120 min after drug administration are shown. Various doses of topiramate or 0.125 ml/10 g of saline were injected 30 min prior to the injection of 10 mg/kg of METH or vehicle (0.1 ml/10 g of saline). Data are expressed as the means ± S.E.M. ($n=8$ mice per group). METH, methamphetamine; S. saline; TPM1, 1 mg/kg of topiramate; TPM10, 10 mg/kg of topiramate; TPM100, 100 mg/kg of topiramate.

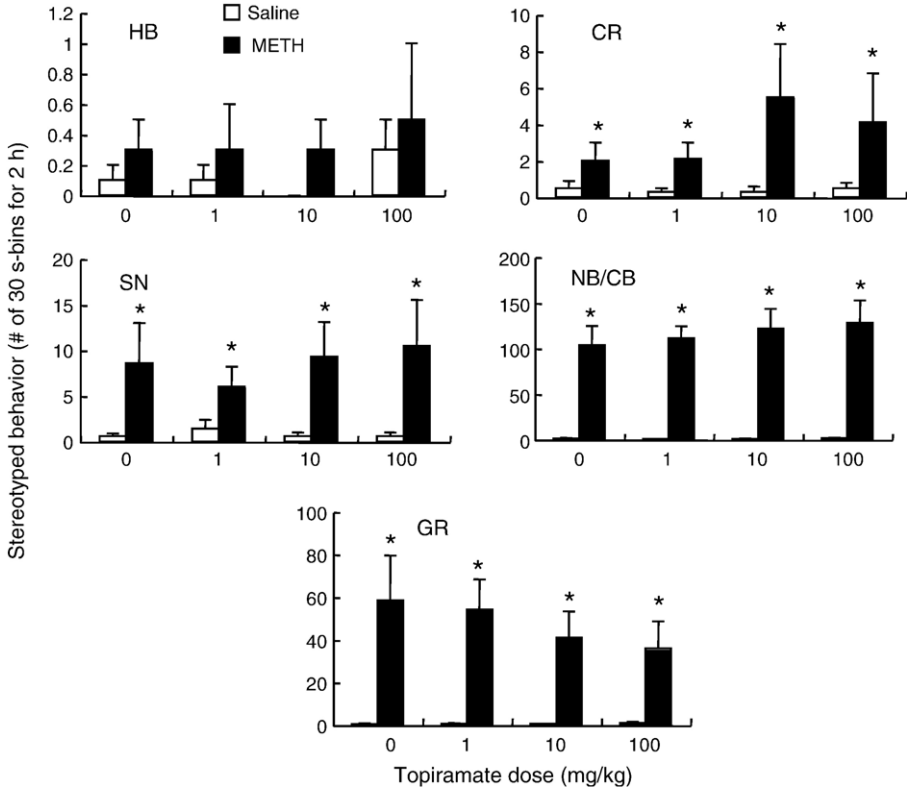

Fig. 2. Stereotyped behavior in response to saline (open column) or 10 mg/kg of METH (solid column) in mice pretreated with 1, 10, and 100 mg/kg of topiramate. Behavior was scored in 30-s bins, and total values for 2 h are shown. HB, head-bobbing; CR, circling; SN, continuous sniffing; NB/CB, nail and/or wood-chip biting; GR, vigorous and compulsive grooming. Values are shown as the means±S.E.M. (n=8 mice per group).*P<0.05, compared with saline-treated mice (Bonferroni/Dunn test).

comparisons also showed significant differences of Time course between the time 5 and the time 10–120, the time 10 and the time 15–120, the time 15 and the time 20–85 and 105–120, the time 20 and the time 45 and 85–120, the time 25–65 and the time 75–120, the time 70 and the time 80–120, the time 75 and the time 90–120, the time 80 and the time 95–120, the time 85–90 and the time 100–120, the time 95 and the time 100–120, the time 100 and 115–120, and the time 105–110 and the time 120 (Bonferroni/Dunn test, P<0.05).

The observed stereotyped behaviors were classified into five groups: head-bobbing (HB), circling (CR), continuous sniffing with apparent exploratory behavior (SN), nail and/or wood-chip biting (NB/CB), and continuous and compulsive grooming (GR) (Fig. 2). METH treatment induced significant stereotypic behaviors except head-bobbing. Topiramate pretreatment did not affect any stereotyped behavior after METH challenge in mice. Topiramate treatment did not induce any stereotyped behavior in mice. These observations were supported by the outcomes of following analyses. Two-way ANOVAs (Topiramate Dose×METH Treatment) were applied separately for each behavioral pattern shown in Fig. 2. The ANOVAs showed significant main effect of METH Treatment (F(1,56)=8.632, P<0.05, F(1,56)=10.540, P<0.01, F(1,56)=80.779, P<0.001, and F(1,56)=60.647, P<0.001 for CR, SN, NB/CB, and GR, respectively; no significant METH effect on HB, F(1,56)=0.840, P=0.3749), and Time (F(23,1344)=55.561, P<0.0001), but no significant main effect of Topiramate Dose (F(3,56)=0.548, P=0.6524, F(3,56)=0.588, P=0.6263, F(3,56)=0.177, P=0.9111, F(3,56)=0.344, P=0.8006, and F(3,56)=0.402, P=0.7520 for HB, CR, SN, NB/CB, and GR, respectively). *Post-hoc* comparisons indicated significant differences of expressed stereotypic behaviors except head-bobbing between the METH-treated and the saline-treated mice (Bonferroni/Dunn test, P<0.05). The ANOVA analysis also indicated no significant Topiramate Dose×METH Treatment interaction (F(3,56)=0.061, P=0.9801, F(3,56)=0.642, P=0.5925, F(3,56)=0.366, P=0.7780, F(3,56)=0.317, P=0.8131, and F

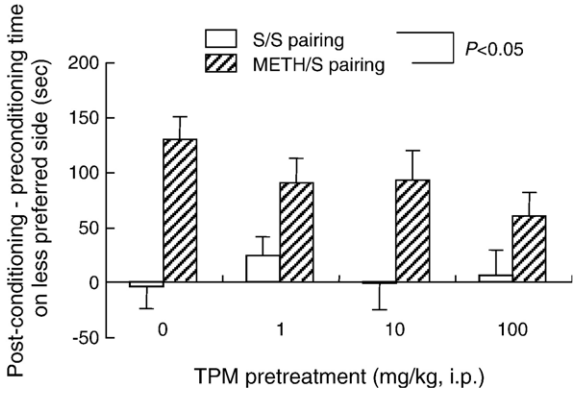

Fig. 3. CPP for METH and the effect of topiramate pretreatment in mice. Place preference was measured as the difference in time spent in the compartment associated with either saline/saline pairing (open column) or METH (0.5 mg/kg, i.p.)/saline pairing group (hatched column) between the post-conditioning and pre-conditioning sessions (mean±S.E.M., n=7 per column). METH, methamphetamine; S, saline; TPM, topiramate.

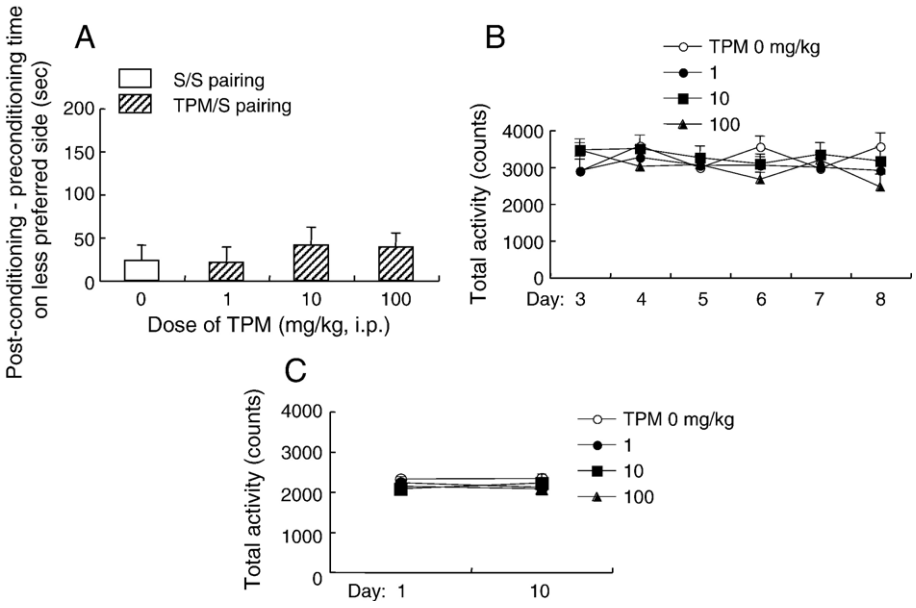

Fig. 4. CPP index for topiramate (A) and the effect of topiramate on locomotor activity on days 3–8 (B) and days 1 and 10 (C) in mice. Place preference was measured as the difference in time spent in compartment associated with either saline/saline (open column) or topiramate (1, 10 and 100 mg/kg, i.p.)/saline pairing group (hatched column) between post-conditioning and pre-conditioning sessions (mean±S.E.M., $n=8$ per column). S, saline; TPM, topiramate. Total locomotor activity was measured simultaneously with CPP apparatus for 20 min in mice confined to one compartment on days 3–8 (B) and for 10 min in mice confined to two compartments accessible from each other on days 1 and 10 (C).

(3,56)=0.430, $P=0.7326$ for HB, CR, SN, NB/CB, and GR, respectively).

3.2. Experiment 2: effect of topiramate on METH-induced CPP in mice

A two-way ANOVA (Topiramate Dose×METH Treatment×Time) applied to Fig. 3 yielded a significant main effect of METH Treatment ($F(1,48)=30.899$, $P<0.0001$), but no significant main effect of Topiramate Dose ($F(3,48)=0.723$, $P=0.5429$). *Post-hoc* comparisons indicated significant differences of CPP index between the METH-treated and the saline-treated mice (Bonferroni/Dunn test, $P<0.0001$). This analysis also yielded no significant METH Treatment×Topiramate Dose interaction ($F(3,48)=1.259$, $P=0.2989$).

3.3. Experiment 3: topiramate-induced change in CPP index and locomotor activity in mice

All three groups of topiramate/saline pairing exhibited no change in CPP, compared with the saline/saline pairing group (Fig. 4A); a one-way ANOVA (Topiramate Dose) applied to Fig. 4A yielded no significant main effect of Topiramate Dose ($F(3,28)=0.323$, $P=0.8088$).

For the locomotion data, a repeated-measures two-way ANOVA (Topiramate Dose×Conditioning Day) applied to Fig. 4B yielded no significant main effects of Topiramate Dose and Conditioning Day ($F(3,168)=0.514$, $P=0.6761$ and $F(5,168)=168$, $P=0.2114$, respectively). The ANOVA analysis also indicated a significant Topiramate Dose×Conditioning Day interaction ($F(15,168)=2.421$, $P<0.05$).

On days 1 (pre-conditioning) and 10 (post-conditioning), the dose of topiramate, the conditioning day, or the dose of topiramate×the conditioning day interaction effect did not affect locomotor activity in mice. These observations were supported by the following ANOVA analysis. A repeated-measures two-way ANOVA (Topiramate Dose×Conditioning Day) applied to Fig. 4C yielded no significant main effects of Topiramate Dose and Conditioning Day ($F(3,56)=1.569$, $P=0.2189$ and $F(1,56)=0.009$, $P=0.9260$, respectively). The ANOVA analysis also indicated no significant Topiramate Dose×Conditioning Day interaction ($F(3,56)=1.731$, $P=0.1835$).

3.4. Experiment 4: tissue levels of dopamine and HVA in the striatum and nucleus accumbens of the mouse

Table 2 shows the tissue contents of dopamine and HVA in the striatum and nucleus accumbens of the mouse. A two-way

Table 2
Tissue levels of dopamine and homovanillic acid in the striatum and nucleus accumbens of the mouse 20 min after challenge

Pretreatment (30 min before challenge)	Challenge			
	Saline		METH	
	Dopamine	HVA	Dopamine	HVA
Vehicle (3)	11.9±1.3	0.53±0.04	13.6±0.8	0.58±0.04
TPM1 (3)	11.3±1.2	0.56±0.04	9.7±2.6	0.51±0.05
TPM10 (3)	11.6±1.2	0.55±0.01	10.3±0.6	0.51±0.11
TPM100 (3)	12.3±1.2	0.67±0.03	11.6±2.0	0.60±0.04

Values are expressed as nanograms per milligram of wet tissues (mean±SEM; $n=3$).
HVA, homovanillic acid.

ANOVA (Topiramate Dose × METH Treatment) applied to Table 2 yielded no significant main effects of Topiramate Dose and METH Treatment in dopamine level ($F(3,16)=0.963$, $P=0.4343$ and $F(1,16)=0.202$, $P=0.6588$, respectively) and in HVA level ($F(3,16)=1.866$, $P=0.1760$ and $F(1,16)=0.593$, $P=0.4525$, respectively). The ANOVA analysis also indicated no significant Topiramate Dose × METH Treatment interaction ($F(3,16)=0.6703$, $P=0.7175$).

4. Discussion

Unexpectedly, the results presented here suggest that topiramate does not affect the METH-induced stereotypy and rewarding effects of METH in animals, although it has a complex cellular mechanism resulting in an overall neurostabilizing effect (White et al., 1997; Zona et al., 1997; Taverna et al., 1999; Zhang et al., 2000; Gryder and Rogawski, 2003). Topiramate failed to attenuate or potentiate METH-induced stereotypy (Fig. 1), indicating no effect of topiramate on METH's stimulant effect for 2 h after the injection, although 61% of the unchanged form of topiramate was found in pooled urine samples of mice 0–24 h after oral administration (Caldwell et al., 2005).

As topiramate shows multiple mechanisms of action and thereby has complex effects on neural activity, it was of interest to examine the effect of topiramate pretreatment on METH-induced stereotyped behavior patterns (that is, continuous sniffing, nail biting, and so on). However, no differential effects of topiramate on METH-induced stereotyped behavior were observed (Fig. 2), suggesting that the molecular mechanism(s) of topiramate did not influence neural activity involved in METH-induced stereotyped behavior patterns.

The doses of topiramate tested and the treatment period in this study are comparable with *in vivo* experiments of seizures in the recent literature (Shank et al., 2000). There is evidence that the doses of topiramate stimulated locomotor activity in rats and mice (Cagetti et al., 2004; Gremel et al., 2006). Provided that topiramate enhanced conditioned locomotor activity or long-term effects on motor activity that were present during the post-conditioning period, it might obscure METH-induced enhancement of the CPP index. However, there was no significant change in locomotor activity among mice treated with doses of topiramate tested, analyzed by ANOVA followed by a *post-hoc* test (Fig. 4B and C). In addition, topiramate administration had no rewarding or aversive effects that might affect the bias procedure independent of its possible effects on METH reward (Fig. 4A). Therefore, the levels of preference expressed (Fig. 1) were attributed primarily to the METH effect, independent of the action of topiramate. The different effects of topiramate on locomotor activity between our result and the reported observations of increased locomotor activity in rodents (Cagetti et al., 2004; Gremel et al., 2006) cannot be well explained at present, although the possibility that strain differences might affect the topiramate effect on locomotor activity should be examined. In mice, the effect of topiramate on locomotor activity was substantially similar in two inbred strains DBA/2J and C57BL/6J (Gremel et al., 2006), while different from the result in an ICR (Fig. 4B and C).

The neural mechanism of METH reward has been explained by the activation of mesolimbic dopaminergic pathway (Wise, 2002; Gardner, 2004); this reflects the fact that drugs of abuse such as amphetamine, cocaine, morphine, nicotine, and alcohol increase the extracellular levels of dopamine in mesolimbic areas in rats (Di Chiara and Imperato, 1988). The activation of dopamine receptors by increased dopamine in the mesolimbic and mesocortical areas is closely related to the rewarding property of the drugs of abuse or the motivation towards the reward. Evidence that topiramate inhibited the nicotine-induced release of dopamine in the nucleus accumbens (Schiffer et al., 2001) prompted an evaluation of the possible inhibitory effect of topiramate on the rewarding property of the drugs of abuse. Johnson et al. (2003) proposed that topiramate would antagonize alcohol's rewarding property by inhibiting mesolimbic and mesocortical dopamine release through the activation of GABA and the inhibition of glutamate systems, respectively. This perspective is supported by evidence that $GABA_A$ antagonists, inverse agonists, attenuate alcohol's stimulant effects in rodents (for reviews, see Mehta and Ticku, 1999; Chester and Cunningham, 2002). However, topiramate reduced alcohol preference in mice using a continuous access, two-bottle choice procedure, likely through elevated water intake, and probably not through the decreased motivation of the reward (Gabriel and Cunningham, 2005). Furthermore, topiramate did not affect the rewarding property of alcohol in mice using the CPP procedure (Gremel et al., 2006), suggesting that positive observations of the effect of topiramate on the treatment of alcoholism in clinical trials (Johnson et al., 2003) might be attributed to mechanism(s) other than the neural circuits participating in the alcohol reward.

The neurostabilizing effect of anticonvulsants including topiramate is highlighted in the treatment of dependence of drugs such as alcohol and cocaine in humans (Johnson et al., 2003; Zullino et al., 2004; Kampman et al., 2004; Sofuoglu and Kosten, 2005). The purpose of an experimenter-administration procedure in the present study is not to mimic human METH abuse phenomenologically *per se*, but rather to induce METH-linked place preference that may be relevant to METH abuse in rodents. Therefore, additional testing of topiramate against behavior associated with an apparent increase in the index of METH preference (for example, self-administration procedure) other than the forced drug injection procedure should add to our understanding of topiramate's action on rewarding properties of METH in humans.

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