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Lamotrigine blocks repeated high-dose methamphetamine-induced behavioral sensitization to dizocilpine (MK-801), but not methamphetamine in rats

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ABSTRACT

We recently proposed a new psychostimulant animal model of the progressive pathophysiological changes of schizophrenia. Studies using that model produced a treatment strategy for preventing progression. Lamotrigine (LTG) blocks repeated high-dosage methamphetamine (METH)-induced initiation and expression of prepulse inhibition deficit and development of apoptosis in the medial prefrontal cortex (mPFC). Moreover, it inhibits METH-induced increases in extracellular glutamate levels in the mPFC (Nakato et al., 2011, Neurosci. Lett.). Abnormal behavior induced by METH or NMDA receptor antagonists is regarded as an animal model of schizophrenia. This study examined the effects of LTG on the development of behavioral sensitization to METH and cross-sensitization to dizocilpine (MK-801) by repeated administration of high-dose METH (2.5 mg/kg, 10 times s.c.). Rats were injected repeatedly with LTG (30 mg/kg) after 120 min METH administration (2.5 mg/kg). Repeated co-administration of LTG blocked the development of behavioral cross-sensitization to MK-801 (0.15 mg/kg), but it did not prevent behavioral sensitization to METH (0.2 mg/kg). The LTG-induced prevention of increased glutamate by high-dose METH might be related to the former finding. Combined results of our previous studies and this study suggest that LTG is useful to treat schizophrenia, especially at a critical point in its progression.

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Although the precise mechanism of sensitization is unknown, repeated administration of psychostimulants can induce behavioral sensitization, characterized by enhanced locomotor activity, in rodents [18]. We postulated that changes in glutamatergic neural transmission induced by psychostimulants such as methamphetamine (METH) and amphetamine are closely related to this sensitization [1,2,10,16].

All antipsychotics currently on the market have a blocking effect on dopamine D2 receptors. Consequently, reactivity to the dopamine D2 receptor antagonist is an important factor in the treatment of schizophrenia. The pathology of this dopamine D2 receptor antagonist treatment-resistance is classifiable into two types. The first does not respond well to dopamine D2 receptor antagonists from the onset. The second responds well to dopamine D2 receptor antagonists in the first psychotic episode, but the process of psychotic relapse or recurrence engenders treatment resistance [19]. Hyperdopaminergic neurotransmission via dopamine D2 receptors is believed to be involved in the latter type, whereas changes in neurotransmission other than the D2 receptor-mediated dopaminergic neurotransmission are involved in the former type. METH-induced and amphetamine-induced hyperlocomotion respond well to dopamine D2 receptor antagonists, but NMDA receptor antagonist-induced, phencyclidine-induced, and MK-801-induced hyperlocomotion do not [9,20]. To produce a strategy for preventing the progression, our group developed a comprehensive animal model for these progressive pathophysiological changes caused by schizophrenia [1,2]. The model uses repeated administration of METH 2.5 mg/kg. That dosage, but not 1.0 mg/kg (which increases extracellular dopamine levels but not glutamate levels), can increase both extracellular glutamate and dopamine levels in the medial prefrontal cortex (mPFC) [1,2]. It induces schizophrenia-related behavioral and histological abnormalities including cross-sensitization to the NMDA receptor antagonist, a neuroplastic prepulse inhibition (PPI) deficit, and an apoptotic reaction in the mPFC [1,2,10,11,15,16].

Clinically, lamotrigine (LTG) is known as an anticonvulsant [12]. A clinical trial demonstrated that administration of LTG along with stable clozapine elicited beneficial effects on the psychopathological symptoms of clozapine-resistant schizophrenic patients [7,8,23]. Furthermore, LTG pretreatment prevents regional blood oxygenation level-dependent (BOLD) signal changes and psychotic symptoms induced by ketamine in healthy men [6]. In an animal model, LTG plus clozapine decreased hyperlocomotion induced by the NMDA receptor antagonist phencyclidine in rats [25]. Our recent report described that prepulse inhibition deficit and the

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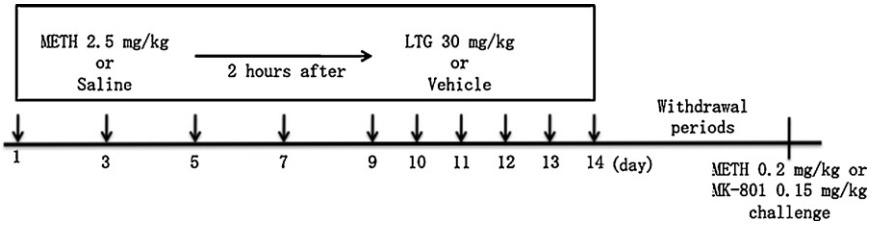


Fig. 1. Schematic representation of a time-line of the experiments.

increase of TUNEL-positive cells in the mPFC induced by repeated administration of 2.5 mg/kg of METH are prevented by repeated co-administration of LTG [15,16]. Moreover, our group presented data showing that LTG inhibits increased extracellular glutamate levels in the mPFC induced by 2.5 mg/kg of METH [16].

This study analyzed the effects of LTG on the development of behavioral cross-sensitization to MK-801 by repeated METH. The METH 2.5 mg/kg induced a delayed increase in the basal levels of glutamate in the mPFC [1,2,16]. Therefore, we administered LTG 120 min after METH injection to block phenomena related to the delayed increase in glutamate levels. If LTG prevents METH-induced cross-sensitization to an NMDA receptor antagonist, MK-801, then it might be a promising treatment strategy to prevent longitudinal pathophysiological progression from dopamine D2 receptor antagonists that are responsive to the resistant pathophysiology of schizophrenia.

Experiments 1 and 2 involved examination of six-week-old male Sprague-Dawley rats (SLC, Inc., Japan) weighing 160–180 g at the start of the experiment. In experiment 1, each group comprised 5–8 animals (28 animals total). In experiment 2, each group comprised 8 animals (32 animals total). The animals were housed individually in plastic cages (30 cm × 25 cm × 18 cm) with a wire mesh top and sawdust bedding. The animal house was maintained under controlled conditions of light (6:30–18:30), temperature (24 °C), and humidity (50%). The animals were provided a standard laboratory diet and tap water. They were handled daily for at least 3 days before the start of the experiment. This study was conducted in accordance with guidelines for the care and use of laboratory animals of the Hokkaido University Graduate School of Medicine and the National Institutes of Health guidelines on animal care.

For this study, METH (Dainippon Sumitomo Pharma Co. Ltd., Japan), after dissolution in sterile physiological saline, was injected subcutaneously at a volume of 1 ml/kg. We dissolved MK-801 (Tocris Bioscience, MO, USA) in distilled water and injected it subcutaneously at a dosage of 1 ml/kg. Then we dissolved LTG (a gift from GlaxoSmithKline, UK) in 10 ml of distilled water with 10 drops of 0.1 N HCl. It was injected intraperitoneally at 4 ml/kg. The respective vehicles for METH and LTG were saline (1 ml/kg) and approximately 0.002 N HCl in distilled water (4 ml/kg). This study used dosages of METH (2.5 mg/kg) and LTG (30 mg/kg) that were chosen after considering the results obtained from previous studies [15,16].

In experiment 1, we observed the effects of LTG on the development of behavioral sensitization to METH. Rats were assigned randomly to one of the following four groups. The METH + LTG group was injected with LTG 30 mg/kg after 120 min METH 2.5 mg/kg administration. The METH + Vehicle group was injected with vehicle after 120 min METH 2.5 mg/kg administration. The Saline + LTG group received LTG 30 mg/kg after 120 min after saline injection. The Saline + Vehicle group received vehicle after 120 min after saline injection. All these treatments were repeated five times on alternate days, and for an additional five times every day, amounting to 10 times in the rats' home cages (Fig. 1) [15]. Then METH 0.2 mg/kg was administered in the home cages to four groups following an 8–9-day period of withdrawal from the repeated treatments.

In experiment 2, we observed the effects of LTG on the development of behavioral sensitization to MK-801. Rats were assigned randomly to one of the following four groups. The METH + LTG group was injected with LTG 30 mg/kg after 120 min METH 2.5 mg/kg administration. The METH + Vehicle group was injected with vehicle after 120 min METH 2.5 mg/kg administration. The Saline + LTG group received LTG 30 mg/kg after 120 min following saline injection. The Saline + Vehicle group received vehicle after 120 min following saline injection. All these treatments were repeated five times on alternate days, and for an additional five times every day, amounting to a total of 10 times in the rats' home cages (Fig. 1). Following a 30–31-day withdrawal period from the repeated treatments, MK-801 0.15 mg/kg was administered in activity chambers to the four groups.

The home cage for each rat was placed under a sensor. Measurements of locomotor activity using an apparatus with an infrared sensor that detects thermal radiation from animals (Supermex; Muromachi Kikai Co. Ltd., Tokyo, Japan) began after a 2-h habituation period, as described previously [17]. Horizontal movements of the rats were digitized and stored in a computer every 10 min.

Data from the locomotor activity were analyzed using repeated-measures two-way analysis of variance (ANOVA), with the treatment group as the between-subjects variable and time as the repeated-measures variable. The respective areas under the curve for the 20–40 min periods in experiment 1 and the 60–80 min periods in experiment 2 were compared among the four groups using one-way ANOVA, followed by Bonferroni testing. Differences in absolute values measured at each time point of collection among the four groups were analyzed using Bonferroni post hoc tests. Differences were considered significant at $p < 0.05$.

Results show that LTG 30 mg/kg did not block the development of repeated METH 2.5 mg/kg administration-induced behavioral sensitization to METH (0.2 mg/kg). However, unexpectedly, it did partly enhance the development of behavioral sensitization to METH (Fig. 2). A repeated-measures two-way ANOVA showed significant effects of time [$F(9, 207) = 54.03$, $p < 0.01$], treatment [$F(3, 23) = 3.95$, $p = 0.02$], and time × treatment interaction [$F(27, 207) = 3.49$, $p < 0.01$]. Bonferroni post hoc tests revealed that locomotor activity of the METH + Vehicle group was significantly higher than that of the Saline + Vehicle group at 30 min, and that of METH + LTG group was significantly higher than that of METH + Vehicle or the Saline + Vehicle group at 40 min. One-way ANOVA indicated a significant main effect of treatment on the area under the curve (20–40 min) for locomotor activity [$F(3, 23) = 5.58$, $p < 0.01$] (data not shown). The area under the curve (20–40 min) for locomotor activity of the METH + LTG treatment group was significantly greater than that of the Saline + LTG group (Bonferroni test; $p < 0.05$), but was not significantly different from that of the METH + Vehicle group.

Administration of LTG 30 mg/kg blocked the development of repeated METH 2.5 mg/kg administration-induced behavioral cross-sensitization to MK-801 (0.15 mg/kg) (Fig. 3). A repeated-measures two-way ANOVA revealed significant effects of time

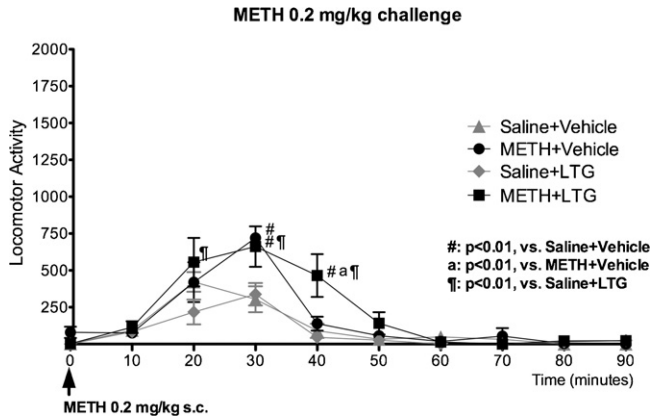


Fig. 2. Effects of a challenge injection of METH 0.2 mg/kg on locomotor activity after 8–9 days of withdrawal from repeated co-administration of LTG 30 mg/kg or vehicle with METH 2.5 mg/kg or saline.

[$F(9, 252) = 35.34$, $p < 0.01$], treatment [$F(3, 28) = 4.55$, $p = 0.01$], and time × treatment interaction [$F(27, 252) = 2.47$, $p < 0.01$]. Bonferroni post hoc testing revealed that locomotion of the METH + Vehicle group was significantly higher than that of Saline + Vehicle group at 60–70 min, and that of METH + LTG group was significantly lower than that of METH + Vehicle group at 70–90 min. One-way ANOVA showed a significant main effect of treatment on the area under the curve (60–80 min) for locomotor activity [$F(3, 28) = 5.42$, $p < 0.01$] (data not shown). The area under the curve (60–80 min) for locomotor activity of the METH + Vehicle treatment group was significantly greater than that of the Saline + Vehicle or METH + LTG group (Bonferroni test; $p < 0.05$).

The repeated administration of METH 2.5 mg/kg induces behavioral sensitization to the locomotion-stimulating effects of METH and behavioral cross-sensitization to MK-801. Repeated co-administration of LTG 30 mg/kg with METH 2.5 mg/kg blocks this behavioral sensitization to MK-801, but not sensitization to METH. Repeated administration of METH, with a main effect of increasing the dopamine levels in the dopaminergic terminals, induces behavioral sensitization [24]. Actually, METH-induced abnormal behavior is regarded as a model for dopamine D2 receptor antagonist-responsive pathogenesis of schizophrenia [21]. This behavioral sensitization is associated with enhanced dopaminergic neurotransmission in the nucleus accumbens [18]. Furthermore, it is inhibited by dopamine D2 receptor antagonists. Abnormal behavior induced by NMDA receptor antagonists such as phencyclidine and MK-801 is also an animal model of schizophrenia. However, this abnormal behavior was not blocked by a dopamine D2 receptor antagonist [5]. The behavioral cross-sensitization to an NMDA receptor antagonist induced by repeated administration of METH 2.5 mg/kg in the present study suggests that the repeated administration of METH 2.5 mg/kg can induce dopamine D2 receptor antagonist-resistant schizophrenia.

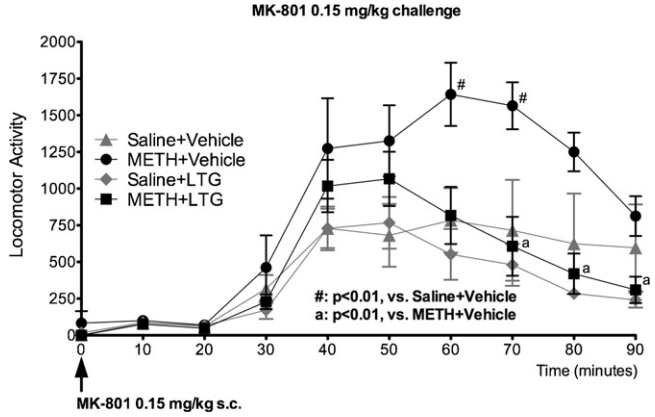


Fig. 3. Effects of a challenge injection of MK-801 0.15 mg/kg on locomotor activity after 30–31 days of withdrawal from repeated co-administration of LTG 30 mg/kg or vehicle with METH 2.5 mg/kg or saline.

Our group also confirmed that 2.5 mg/kg, but not 1.0 mg/kg, of METH induces delayed increases in glutamate levels in the nucleus accumbens and the mPFC [1,2,10,16]. Repeated administration of METH reduces the protein levels of NMDA receptors, including NMDA receptor 1 (NR1), in the hippocampus [22]. In addition, repeated administration of METH reduces immunoreactivities of NR1 and NMDA receptors comprising NMDA receptor 2A and 2B in the striatum [26]. Continuous stimulation of NMDA receptors induces desensitization of this type of receptor [13,14]. Considering these findings, the repeated administration of METH 2.5 mg/kg might desensitize NMDA receptors, thereby inducing hypersensitivity to MK-801. Although this study did not examine the effects of repeated administration of METH on the function of NMDA receptors, this examination confirms the hypothesis presented above. Moreover, this report describes that LTG 30 mg/kg administered 120 min after administration of METH 2.5 mg/kg inhibits the METH 2.5 mg/kg-induced delayed increase in glutamate levels in the mPFC [16]. Based on results of the present study, we speculate that LTG inhibits repeated stimulation of NMDA receptors by METH-induced increases in glutamate. Moreover, LTG prevents the development of desensitization and hypofunction of NMDA receptor to induce behavioral cross-sensitization to MK-801.

Unexpectedly, co-administration of LTG with METH elicited greater behavioral sensitization to METH at 40 min after METH administration than that shown by the METH + Vehicle group. Actually, LTG was administered at 120 min after METH 2.5 mg/kg administration, when locomotion induced by METH 2.5 mg/kg had almost disappeared (data not shown), but the increased extracellular levels of dopamine and glutamate in the mPFC were still induced by METH 2.5 mg/kg [2]. The LTG inhibits hyperlocomotion induced by D-amphetamine [4] and decreases the basal extracellular levels of dopamine in the hippocampus [3], but it remains unclear whether LTG affects the increased extracellular levels of dopamine in the brain induced by METH. The mechanism of the enhancement of behavioral sensitization to METH by co-administration of LTG remains unclear. Future studies are necessary to clarify that point.

In rats, repeated administration of METH 2.5 mg/kg caused pathological conditions resembling the progressive pathological conditions of schizophrenia [1,2,10]. This new animal model will contribute to screening of antipsychotics such as olanzapine, risperidone, and aripiprazole, which can block the progression of schizophrenia pathology [1,2]. Our group earlier reported that LTG blocks the initiation of PPI deficit and apoptosis induced by repeated administration of METH 2.5 mg/kg [15,16]. Considering results of those earlier studies and this study together, we infer that LTG is a useful drug for the treatment of schizophrenia, especially at the critical point of progressive pathology of schizophrenia.

In summary, repeated co-administration of LTG 30 mg/kg with METH 2.5 mg/kg blocked the development of behavioral cross-sensitization to MK-801, but it did not prevent behavioral sensitization to METH. Inhibiting the effects of LTG on increased extracellular glutamate levels induced by METH might prevent neuronal plasticity produced by repeated administration of METH.

Disclosure

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