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Sarcosine attenuates toluene-induced motor incoordination, memory impairment, and hypothermia but not brain stimulation reward enhancement in mice

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ABSTRACT

Toluene, a widely used and commonly abused organic solvent, produces various behavioral disturbances, including motor incoordination and cognitive impairment. Toluene alters the function of a large number of receptors and ion channels. Blockade of *N*-methyl-D-aspartate (NMDA) receptors has been suggested to play a critical role in toluene-induced behavioral manifestations. The present study determined the effects of various toluene doses on motor coordination, recognition memory, body temperature, and intracranial self-stimulation (ICSS) thresholds in mice. Additionally, the effects of sarcosine on the behavioral and physiological effects induced by toluene were evaluated. Sarcosine may reverse toluene-induced behavioral manifestations by acting as an NMDA receptor co-agonist and by inhibiting the effects of the type I glycine transporter (GlyT1). Mice were treated with toluene alone or combined with sarcosine pretreatment and assessed for rotarod performance, object recognition memory, rectal temperature, and ICSS thresholds. Toluene dose-dependently induced motor incoordination, recognition memory impairment, and hypothermia and lowered ICSS thresholds. Sarcosine pretreatment reversed toluene-induced changes in rotarod performance, novel object recognition, and rectal temperature but not ICSS thresholds. These findings suggest that the sarcosine-induced potentiation of NMDA receptors may reverse motor incoordination, memory impairment, and hypothermia but not the enhancement of brain stimulation reward function associated with toluene exposure. Sarcosine may be a promising compound to prevent acute toluene intoxications by occupational or intentional exposure.

Introduction

Toluene is a widely used solvent. In addition to occupational exposure, a particular type of intoxication is caused by its intentional inhalation for recreational purposes, such as glue sniffing (Anderson and Loomis, 2003; Flanagan and Fisher, 2008; Howard et al., 2011). Toluene is frequently abused for its euphoric and hallucinating effects (Garland and Howard, 2010). However, toluene abuse also produces several severe adverse effects, including motor incoordination, hypothermia, and mental confusion, such as delusions and amnesia (Andersen et al., 1983; Chouaniere et al., 2002; Meulenbelt et al., 1990; Saito and Wada, 1993). Behavioral disturbances that result from toluene use, including motor incoordination, cognitive impairment (Lo et al., 2009), and hypothermia (Gordon et al., 2010), have been reported in rats. Additionally, the reward-enhancing effects of toluene have been studied using the intracranial self-stimulation (ICSS) procedure in rats. In general, a lowering of ICSS thresholds reflects increased brain reward sensitivity induced by the administration of drugs of abuse. However, toluene has been shown to increase the threshold current of self-stimulation in the study of Yavich and Zvartau (1994), using a rate-intensity protocol. By contrast auto-titration procedure that offers a rate-independent assessment of the ICSS thresholds demonstrated that toluene significantly reduced ICSS thresholds in rats (Bespalov et al., 2003).

An improved understanding of the mechanisms of action of toluene may aid in the identification of therapeutic approaches to counter the problem of toluene abuse. Mice may be an important tool in the investigation of the neurobiological effects of toluene with the recent advances that have been made in genetic engineering in this species. Several studies have used mice to study the acute effects of toluene and found that toluene exposure could result in changes in locomotor activity (Bowen and Balster, 1998; Bowen et al., 2010; Bushnell et al., 1985; Conti et al., 2012; Wood and Colotla, 1990), disturbances of gait, mobility, and righting reflex, impaired psychomotor coordination (Tegeris and Balster, 1994) and recognition memory (Win-Shwe and Fujimaki, 2012). However, the effects of toluene on rotarod test, rectal temperature, and brain stimulation reward in mice remain to be

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revealed. In the present study, the dose-dependent effects of toluene on ICSS thresholds in mice were determined by a discrete-trial current-intensity procedure which provides a current-intensity-threshold measure to avoid the problems inherent in rate-dependent studies. Additionally, the effects of toluene on rotarod test, rectal temperature, and novel object recognition test in mice were assessed.

Toluene alters the function of a large number of receptors and ion channels (Lubman et al., 2008). However, the mechanisms that underlie toluene-induced behavioral and physiological responses remain unclear, although the effects of toluene have been suggested to be at least partially mediated through the inhibition of *N*-methyl-D-aspartate (NMDA) receptor activity. Toluene blocked NMDA receptor-mediated currents in vitro (Cruz et al., 1998) and also induced partial substitution for the discriminative properties of the NMDA receptor antagonist phencyclidine (PCP) (Bowen et al., 1999). A recent study from our laboratory demonstrated that toluene-induced locomotor hyperactivity, motor incoordination, and memory impairment in rats was reversed by D-serine, a selective co-agonist for the glycine site of NMDA receptors (Lo et al., 2009). These findings suggest that the positive modulation of NMDA receptors may effectively block several behavioral disturbances induced by toluene. However, remaining unknown is whether the positive modulation of NMDA receptors blocks the stimulation reward-enhancing and hypothermic effects of toluene.

Sarcosine is an NMDA receptor co-agonist (Zhang et al., 2009), a competitive inhibitor of the type I glycine transporter (GlyT1) (Eulenburg et al., 2005; Lopez-Corcuera et al., 1998; Smith et al., 1992), and an important intermediate in one-carbon metabolism (Chen et al., 2010; Wittwer and Wagner, 1981). Sarcosine induced less NMDA receptor desensitization and larger increases in intracellular Ca^{2+} levels compared with glycine (Zhang et al., 2009). Additionally, sarcosine is more potent than D-serine in reducing the anti-seizure effect of MK-801 (Long et al., 2006) and as an add-on treatment for schizophrenia (Lane et al., 2010). Sarcosine appears to act as a potent positive allosteric NMDA receptor modulator in vitro and effectively exerts its effect in vivo. Thus, the present study assessed the effects of sarcosine on toluene-induced motor incoordination and recognition memory impairment to test the hypothesis that positive modulation of NMDA receptors by sarcosine administration in mice suppresses acute behavioral responses elicited by toluene. Furthermore, the effects of sarcosine on toluene-induced stimulation reward enhancement and hypothermia were evaluated.

Materials and methods

Animals and drugs. Male NMRI mice (8–9 weeks, 33–40 g) were supplied by the Laboratory Animal Center of Tzu Chi University (Hualien, Taiwan) and housed in groups of four to five mice per cage on a 12 h/12 h light/dark cycle with ad libitum access to water and food. The experiments conducted at Tzu Chi University were performed in accordance with the Republic of China animal protection law (Chapter III: Scientific Application of Animals) and approved by the Review Committee of Tzu Chi University. The ICSS experiments were conducted at the University of California, San Diego, in accordance with the guidelines of the American Association for the Accreditation of Laboratory Animal Care, and the National Research Council's Guide for Care and Use of Laboratory (NIH Publication No. 85-23, revised 1996) and approved by the University of California San Diego Institutional Animal Care and Use Committee. Male C57BL/6J mice were used for the ICSS experiments and purchased from Jackson Laboratories (Sacramento, CA, USA).

Toluene (high-performance liquid chromatography grade, 99.8%, Mallinckrodt Baker Inc., KY, USA) was diluted in corn oil (0, 250, 500, and 750 mg/kg) to achieve an injection volume of 10 ml/kg and administered intraperitoneally. Sarcosine (Sigma, St. Louis, MO, USA) was dissolved in saline and intraperitoneally injected in volumes of 10 ml/kg and administered 30 min prior to toluene treatment.

Naive mice were used in each experiment, in which each mouse underwent one procedure only. To determine the effects of various doses of toluene on rotarod performance, novel object recognition memory, and rectal temperature, groups of six mice per dose of toluene (0, 250, 500, and 750 mg/kg) were used for each experiment. The dose range of toluene used was based on the study in rats (Lo et al., 2009). To test whether sarcosine pretreatment reverses the effects of toluene in these tests, 40 mice were divided into five groups (control, 300 mg/kg sarcosine + oil, saline + toluene, 100 mg/kg sarcosine + toluene, and 300 mg/kg sarcosine + toluene) in each experiment. The doses of sarcosine were selected based on the previous study, which are effective to enhance the prepulse inhibition in mGluR5 KO mice (Chen et al., 2010). The ICSS experiments were conducted using a within-subjects Latin square design, such that each mouse received all of the treatments. Nine and eight mice were used to determine the dose-dependent effects of toluene and sarcosine pretreatment, respectively.

Rotarod motor coordination test. Motor coordination was assessed using an automated rotarod apparatus (TSE systems, Bad Homburg, Germany). A computer recorded the latency to fall in seconds. The mice were first trained on the rotarod at a constant speed of 20 rotations per minute (rpm) until all of the mice were able to spend at least 3 min on the rod. The mice were then treated with toluene (250, 500, or 750 mg/kg) or vehicle 30 min prior to testing since the brain toluene level reached the peak at 30 min after injection and returned to the basal level after 2 h (Win-Shwe et al., 2007). The mice were tested at 20 rpm at 15 min intervals for 120 min. To test the effects of sarcosine pretreatment, sarcosine (0, 100, and 300 mg/kg) was administered 30 min prior to the toluene (750 mg/kg) or corn oil (vehicle) injection.

Novel object recognition test. The experimental apparatus consisted of a Plexiglas open field box (50 × 50 × 25 cm) located in a sound-attenuated room and illuminated with a 20-W light bulb. The novel object recognition procedure consisted of habituation, training, and retention sessions. A video camera recorded behavior during the training and retention phases. Habituation was conducted in three consecutive daily sessions, during which each mouse was allowed to individually explore the box without objects for 10 min. During the training session, each animal was placed in the box, and after 5 min, two identical objects (plastic items) were simultaneously introduced in two corners. Each animal was allowed to explore the objects for 5 min. An animal was considered to explore the object when its head was facing the object at a distance of approximately 1 cm or less between the head and object or when it was touching or sniffing the object. The time spent exploring each object was recorded using stopwatches by an experimenter blind to the treatment condition. After the training session, the mice were immediately returned to their home cages. The retention session was conducted 24 h after the training session. The animals were returned to the same box as during the training session, and one of the two objects of the training session was replaced with a novel object. The animals were allowed to explore the box freely for 5 min, and the time spent exploring each object was recorded as described above. The objects and chambers were cleaned with 70% ethanol after each use. The preference index in the retention session, defined as the ratio of the amount of time spent exploring the novel object and total time spent exploring both objects, was used to evaluate recognition memory. In the training session, the preference index was defined as the ratio of the time spent exploring the object that replaced the original object in the retention session and the total exploration time. The mice received toluene (250, 500, and 750 mg/kg) or corn oil (vehicle) 30 min prior to initiating the training session. To test the effect of sarcosine pretreatment, sarcosine (0, 100, and 300 mg/kg) was administered 30 min prior to the toluene (750 mg/kg) or corn oil (vehicle) injection.

Body temperature. Rectal temperature was measured by a thermistor probe and digital thermometer (Singa Technology, Taipei, Taiwan). Baseline rectal temperatures were recorded before the mice were