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Attenuation of toluene-induced brain stimulation reward enhancement and behavioral disturbances by N-acetylcysteine in mice

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ABSTRACT

Toluene, a commonly used organic solvent, produces a variety of behavioral disturbances in both humans and animals comparable to noncompetitive N-methyl-D-aspartate receptor (NMDARs) antagonists, such as phencyclidine (PCP). N-acetylcysteine (NAC) is capable of reversing the psychotomimetic effects of PCP via activation of cystine-glutamate antiporters (xCT). The present study examined whether NAC is capable of attenuating the toluene-induced brain stimulation reward enhancement and behavioral manifestations. Male mice received various doses of NAC prior to toluene exposure for assessment of intracranial self-stimulation (ICSS) thresholds, rotarod test, novel object recognition task and social interaction test. NAC ameliorated the lowering of ICSS thresholds, motor incoordination, object recognition memory impairments and social withdrawal induced by toluene. Furthermore, the capacity of NAC to ameliorate acute toluene-induced deficits in object recognition and social interaction was blocked by the xCT inhibitor (S)-4-carboxyphenylglycine and the mGluR2/3 antagonist LY341495. These results indicate that NAC could prevent toluene-induced reward facilitation and behavioral disturbances and its beneficial effects, at least for cognitive function and social interaction, are associated with activation of the xCT and mGluR2/3. These findings show the potential promise for NAC to treat toluene dependence and to prevent toluene intoxication caused by unintentional or deliberate inhalation.

1. Introduction

Toluene-containing products are often deliberately inhaled for recreational use, such as glue sniffing, to produce intense euphoria. In addition, industrial workers might be accidentally exposed to toxic levels of toluene. Clinical features of acute intoxication with toluene include lightheadedness, euphoria, incoordination, disequilibrium, confusion, and cognitive and memory deficits (Andersen et al., 1983; Meulenbelt et al., 1990; Saito and Wada, 1993). In rodent studies, the reward-enhancing effects of toluene have been demonstrated using intracranial self-stimulation (ICSS) (Bespalov et al., 2003; Chan et al., 2012; Tracy et al., 2014). The behavioral characteristics of toluene including a biphasic effect on locomotor activity (Chan et al., 2004; Riegel et al., 2003; Riegel and French, 1999b), motor incoordination (Chan et al., 2012; Lo et al., 2009), learning impairment (Huerta-Rivas et al., 2012; Lo et al., 2009; Win-Shwe et al., 2010), and social withdrawal (Chan et al., 2015), resemble those of NMDA receptor (NMDAR) channel blockers, such as PCP, MK-801, and ketamine.

In fact, toluene suppresses the NMDAR-mediated currents (Cruz et al., 1998). Furthermore, it partially substitutes for the discriminative stimulus effect of PCP (Bowen et al., 1999). Thus, interfering with NMDAR function is one of the leading hypotheses to explain the acute behavioral responses to toluene. The psychotomimetic behaviors induced by NMDAR antagonists have been associated with the cortical disinhibition, resulting in excessive glutamate release (Farber, 2003). Modulation of excitatory transmission by activation of group II metabotropic glutamate receptors (mGluR) including mGluR2/3 has been proven to be an effective strategy to reverse the psychotomimetic effects of NMDAR antagonists (Imre et al., 2006). Similarly, toluene-induced decreases in brain stimulation reward thresholds and behavioral disturbances are reduced by mGluR2/3 agonist LY379268 (Chan et al., 2015).

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N-acetylcysteine (NAC) is a widely used mucolytic drug, an antidote for acetaminophen overdose and a prodrug of cysteine. NAC modulates the glutamatergic transmission by acting at the cystine-glutamate antiporter (xCT) of glial cells, concomitant with the non-vesicular glutamate release in extrasynaptic space to stimulate proximal receptors, such as mGluR2/3 (Baker et al., 2002; Moran et al., 2005). In fact, the blunting effects of NAC on PCP-induced psychotomimetic behaviors have been suggested to be associated with negative modulation of synaptic release of glutamate through activation of presynaptic mGluR2/3 (Baker et al., 2008). Taken together, it is hypothesized that NAC is also capable of counteracting the toluene-induced behavioral disturbances and lowering of ICSS reward thresholds in the same manner.

In this paper, we demonstrated that NAC significantly counteracted the behavioral effects of toluene, and we further examined the combined effects of NAC and the xCT inhibitor (S)-4-carboxyphenylglycine (CPG) or the mGluR2/3 antagonist (2S)-2-amino-2-[(1S,2S)-2-carboxycycloprop-1-yl]-3-(xanth-9-yl) propanoic acid (LY341495) on toluene-induced cognitive deficits and social withdrawal to delineate the roles of xCT and mGluR2/3 in the beneficial effects of NAC.

2. Materials and methods

2.1. Animals and chemicals

Male C57BL/6 J mice (7–8 weeks of age) supplied from Jackson Laboratories (Sacramento, CA, USA) were used for the ICSS experiments. The ICSS experiments were performed at the University of California, San Diego and approved by the Institutional Animal Care and Use Committee of University of California San Diego. All experiments, except ICSS, were conducted at Tzu Chi University and approved by the Institutional Animal Care and Use Committee of Tzu Chi University. C57BL/6 J mice is relatively anxious to explore the objects in the novel object recognition test. With more active object exploration, NMRI mice were used for the novel object recognition test and other behavioral tests. Male NMRI mice (7–8 weeks of age) were obtained from the Laboratory Animal Center of Tzu Chi University, (Hualien, Taiwan). Four mice were kept in a cage with food and water access ad libitum and 12/12 light-dark cycle.

Toluene (99.8%; Mallinckrodt Baker) was diluted in corn oil. NAC (Sigma-Aldrich) and LY341495 (Tocris Bioscience) were dissolved in saline. Toluene, NAC, and LY341495 were administered by intraperitoneal (ip) injection with the injection volume of 10 ml/kg body weight. CPG (Tocris Bioscience) was dissolved in saline and adjusted into pH 7.4, then diluted to the final concentrations and given by intracerebroventricular (icv) injection.

Within-subject Latin square design was used in ICSS experiments. Between-subjects design was used in other behavioral tests, in which each mouse received only one treatment. The doses of toluene for different behavioral tests were based on our previous work (Chan et al., 2012, 2015).

2.2. Electrode implantation and ICSS procedure

The surgical and ICSS operating procedures in mice were performed as mentioned previously (Chan et al., 2012; Stoker et al., 2008). Briefly, the stainless steel bipolar electrodes were implanted into the medial forebrain bundle (AP: + 1.58 mm, ML: 1.0 mm, DV: 5.3 mm). One week after surgery, the mice were trained and tested in the discrete-trial current-threshold procedure in the operant chambers (Med Associates, Inc). Two variables were assessed in the operant chamber, including threshold and response latency. The threshold for each test session was defined as the mean of overall thresholds for each individual series. The response latency was obtained by averaging the duration between the electrical stimulus delivery and the positive response for all trials.

The effects of NAC and toluene were assessed after ICSS thresholds were established stably. Two ICSS sessions were performed daily, with a 2-hr interval. The ICSS threshold received in the second session was presented as a percentage of the baseline values from a drug-naive state collected in the first session. Animals were received NAC (0, 30 or 100 mg/kg, ip) 60 min prior to toluene (500 mg/kg, ip) or corn oil administration and 75 min before the initiation of second session. The test interval was 48 h. On the day between test days, ICSS procedure was performed twice daily. Mice received saline and corn oil 75 and 15 min prior to the second session, respectively.

2.3. Rotarod test

An automated rotarod apparatus (TSE Systems) was used to assess motor coordination as previously described (Chan et al., 2012, 2015). Mice were trained on the rotarod at a constant speed of 20 rpm until they could remain on for at least 3 min. NAC (0, 30, or 100 mg/kg) was administered 60 min prior to the toluene (750 mg/kg) or corn oil injection. The test was initiated 30 min after toluene treatment. Six testing time points separated by a 20-min interval. The latency to fall from the rotating rod was recorded.

2.4. Novel object recognition test

The novel object recognition was tested according to previous studies (Chan et al., 2012). Briefly, each mouse was habituated to a testing chamber for 10 min daily for 3 days. On test day, the mouse was first stayed in the empty chamber for 5 min. Then, two identical objects were introduced in the corners. The mouse was allowed to explore the objects for 5 min, called sample phase session, followed by two retention sessions 30 min and 24 h later. For the first retention session, one of the identical objects was replaced with a novel object. During the second retention session, the novel object in the first session was replaced with another novel object, while the original object remained at the same position. The mouse was allowed to explore for 5 min for each retention session. The time spent on exploring each object was recorded. A preference index, a ratio of the time spent exploring the original object that was replaced in the retention session (sample phase) or the novel object (retention session) over the total time spent exploring both objects, was used to evaluate recognition memory.

Mice were pretreated with NAC (0, 30, or 100 mg/kg, ip) 60 min prior to toluene (750 mg/kg, ip) or corn oil injection. Thirty min after treatment of toluene or corn oil, sample phase was initiated. Mice failed to explore the two objects for 10 s during sample phase were excluded from data analysis.

2.5. Social interaction test

Social interaction test was performed as described in our previous studies (Chan et al., 2015). Two unfamiliar mice from the same treatment group were placed in an open-field box (35 × 35 × 30 cm). The duration of social contacts including sniffing and grooming the partner, following, mounting, and crawling under or over the partner was recorded. Mice received NAC (0, 30, or 100 mg/kg, ip) 60 min prior to toluene (750 mg/kg, ip) or corn oil treatment and tested 30 min after toluene application.

In order to determine the role of xCT and mGluR2/3 in the effects of NAC, LY341495 (0, 1 or 3 mg/kg, ip) was administered 20 min after NAC (100 mg/kg) or (S)-4-carboxyphenylglycine (CPG) (0, 50 or 100 nmol/ 5 µl, icv) was microinjected 2 min prior to NAC (100 mg/kg, ip) or saline injection. Both novel object recognition test and social interaction test were performed 30 min after toluene exposure.

2.6. Locomotor activity

NAC (0, 30 or 100 mg/kg, ip) was administered 60 min prior to toluene (750 mg/kg, ip) or corn oil application. Each mouse was put into an activity chamber (TruScan Mouse chamber, Coulbourn