Pharmacology, Biochemistry and Behavior 103 (2012) 418–424

Contents lists available at SciVerse ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Metabotropic glutamate receptor 5 modulates behavioral and hypothermic responses to toluene in rats

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ARTICLE INFO

Article history:
Received 4 June 2012
Received in revised form 21 September 2012
Accepted 24 September 2012
Available online 29 September 2012

Keywords:
Toluene
mGluR5
NMDA receptor
Motor coordination
Learning
Hypothermia

ABSTRACT

Toluene, a widely used and commonly abused organic solvent, produces various behavioral disturbances in both humans and animals. Blockade of N-methyl-D-aspartate (NMDA) receptors has been suggested to play a critical role in acute toluene-induced behavioral manifestations. Activation of type 5 metabotropic glutamate receptors (mGluR5) attenuates behavioral responses induced by NMDA receptor blockade. The present study elucidated the role of mGluR5 on toluene-induced behavioral and hypothermic responses. Male Sprague–Dawley rats received the mGluR5 agonist (RS)-2-chloro-5-hydroxyphenylglycine (CHPG) or antagonist 6-methyl-2-[phenylethynyl]-pyridine (MPEP) prior to toluene administration. Rotarod test, step-down inhibitory avoidance learning task, and rectal temperature were monitored. Pretreatment of CHPG and MPEP attenuated and potentiated these toluene-induced responses, respectively. In addition, the inhibitory effects of CHPG on toluene-induced motor incoordination, learning impairment, and hypothermia were reversed by the protein kinase C (PKC) inhibitor chelerythrine chloride. These findings suggest that mGluR5 may modulate the neural circuits responsible for motor incoordination, learning impairment, and hypothermic action of toluene through a PKC-dependent signal transduction pathway.

1. Introduction

Inhalant abuse is the deliberate inhalation of a volatile substance to achieve an altered mental state. The prevalence of inhalant abuse is increasing among school students around the world (Dell et al., 2011; Medina-Mora and Real, 2008). Most of the commonly abused inhalants, such as gasoline, paint, varnish, glue, shoe and fingernail polish, and rubber cement, contain varying percentages of toluene; whereas some abusers inhale toluene directly. Toluene abuse has become a serious social and medical problem in children and young adults. However, the neural basis for the abuse-related acute behavioral effects of toluene is poorly understood. In humans, exposure to toluene at low concentrations elicits CNS effects such as fatigue, confusion, incoordination, and impairments in reaction time, perception, and motor control and function. Exposure to extremely high concentrations of toluene results in narcosis and death (Evans and Balster, 1991). Toluene also has been found to produce hypothermic (Gordon et al., 2010), anticonvulsant (Chan et al., 2006; Cruz et al., 2003; Wood et al., 1984) and anxiolytic-like effects (Bowen et al., 1996; Lopez-Rubalcava et al., 2000; Paez-Martinez et al., 2003) in animal studies.

Despite toluene shares considerable overlap with CNS depressants such as barbiturates and benzodiazepines (Evans and Balster, 1991) and has been observed to exert its effects through significant enhancement of $GABA_A$ receptor function (Beckstead et al., 2000), several lines of evidence suggest that NMDA receptor inhibition might be associated with toluene-induced certain behavioral responses. Electrophysiological studies have demonstrated that toluene blocks NMDA receptor-mediated currents *in vitro* and the NR1/2B subunit combination of NMDA receptor is the most sensitive target with an IC50 value of 0.17 mM (Cruz et al., 1998) and dose-dependently inhibits NMDA-mediated EPSCs in the medial PFC (Beckley and Woodward, 2011). The neurobehavioral profiles of toluene including biphasic effect on locomotor activity (Chan et al., 2004; Dell et al., 2011; Riegel et al., 2003; Riegel and French, 1999), ataxia (Lo et al., 2009), and learning impairments (Huerta-Rivas et al., 2012; Lo et al., 2009; Win-Shwe et al., 2010) overlap those of NMDA receptor antagonists, such as phencyclidine (PCP) and ketamine. Toluene produces concentration-related partial substitution for PCP discriminative stimulus effects (Bowen et al., 1999). Furthermore, enhancement of NMDA receptor function by D-serine, a glycine binding site modulator, can effectively suppress the

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http://dx.doi.org/10.1016/j.pbb.2012.09.019

toluene-induced locomotor hyperactivity, motor incoordination, and learning impairment (Lo et al., 2009).

Activation of metabotropic glutamate receptors is another strategy for selectively potentiating NMDA receptor function (Lindsley et al., 2006). Metabotropic glutamate receptor 5 (mGluR5) is linked to the NMDA receptor via Homer, Shank, and postsynaptic density protein of 95 kDa (PSD-95) (Tu et al., 1999), supporting the interaction between these two receptor types (Awad et al., 2000; Pisani et al., 2001). Such linkages allow for reciprocal potentiation of function and synergistic activity between these two receptors. The mGluR5 antagonists 2-methyl-6-(phenylethynyl)-pyridine (MPEP) and 3-[(2-methyl-1, 3-thiazol-4-yl) ethynyl]-pyridine (MTEP), at analgesic doses, induce impairment in cognitive function and rotarod performance, and reduce body temperature and locomotor activity, similar to the effects of non-competitive NMDA receptor antagonists MK-801, PCP, or ketamine (Varty et al., 2005), whereas low doses of MPEP and MTEP augment the behavioral effects induced by NMDA receptor antagonists, including locomotor hyperactivity, repulse inhibition (PPI) deficits, and learning impairments (Campbell et al., 2004; Henry et al., 2002; Homayoun et al., 2004; Kinney et al., 2003; Pietraszek et al., 2005). In contrast, the mGluR5 agonist (RS)-2-chloro-5-hydroxyphenylglycine (CHPG) effectively reverses ketamine-induced anesthesia (Sou et al., 2006), motor incoordination, and the abovementioned behavioral effects (Chan et al., 2008). The mGluR5 positive allosteric modulators, 3,3′-difluorobenzaldazine (DFB) and 3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl)benzamide (CDPPB), also effectively attenuated the memory impairment induced by NMDA receptor antagonists (Chan et al., 2008; Fowler et al., 2011). These findings further suggest a functional interaction between mGluR5 and NMDA receptors in a variety of behaviors including avoidance learning and motor coordination.

Based on the similarity of behavioral responses elicited by toluene and non-competitive NMDA receptor antagonists, it was intriguing to reveal the role of mGluR5 in toluene-induced behavioral and hypothermic responses. In the present study, the effects of mGluR5 agonist CHPG and antagonist MPEP on toluene-induced motor incoordination, learning impairment, and hypothermia were evaluated. Since the enhancing effect of mGluR5 agonists on NMDA responses has been related to the activation of protein kinase C (PKC) (Pisani et al., 1997), after finding that the mGluR5 agonist CHPG effectively reduced the toluene-induced behavioral disturbances and hypothermia, we further examined whether PKC was involved in the inhibitory effects of CHPG on these responses to toluene.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (National Laboratory Animal Center, Taiwan) weighing 300–350 g were used in this study. They arrived at the animal facility at least 5 days prior to the start of the experiments. Rats were housed in groups of 3 on a 12/12 light–dark cycle (lights on 0700 h) at 22 °C. Food and water were available *ad libitum* during the time when the animals were in their home cages. The experimental protocol was approved by the Review Committee of the Tzu Chi University for the Use of Animal Subjects. All experiments were carried out during the light phase (0900–1600 h). Each animal was used in only one behavioral test.

2.2. Drug administration

Toluene (HPLC grade 99.8%, Mallinckrodt Baker, Inc, Kentucky, USA) was diluted in corn oil. Intraperitoneal (IP) injection of toluene was used in the present study since it has been routinely used in animals to study drugs commonly abused by inhalation (*e.g.* nicotine and cannabinoids) and extensively reported to produce the same behavioral signs in rats as does inhalation (Chan et al., 2004; Kondo et al., 1995; Riegel and French, 1999). The dose range of toluene (250 to 750 mg/kg) employed was the same as that used in our previous study (Chan et al., 2004; Lo et al., 2009). Actually, previous reports related to toluene vapor discrimination have demonstrated that inhaled toluene concentrations of 1200 ppm and higher fully substituted for i.p injection of 100 mg/kg toluene and the 560 mg/kg dose produced 99% toluene vapor-lever responding for the 6000 ppm toluene vapor training condition (Shelton, 2007; Shelton and Slavova-Hernandez, 2009). In addition, toluene blood concentrations measured under several exposure conditions which produced full substitution were all nearly identical (Shelton and Slavova-Hernandez, 2009). Accordingly, the doses of toluene used in the present study (250–750 mg/kg, i.p.) might produce interoceptive stimulus effects and toluene blood concentrations equal to 2500–8000 ppm toluene vapor exposure.

CHPG, MPEP, and PKC inhibitor chelerythrine chloride were purchased from Tocris (Northpoint Forth Way Avonmouth, UK). Other chemicals were obtained from Sigma (St Louis, MO, USA). For the preparation of stock solution CHPG was dissolved in 0.5 N NaOH (pH adjusted to 7.0 with 1 N HCl). MPEP was dissolved in a 10%:90% Tween 80/water vehicle, whereas chelerythrine chloride was dissolved in DMSO. Then the individual reagents were diluted in an artificial cerebrospinal fluid (ACSF) containing (in mM) NaCl (120), KCl (5), $CaCl_2$ (1.5), $MgCl_2$ (0.8), Na_2HPO_4 (1.4), and NaH_2PO_4 (0.25) for intracerebroventricular (ICV) injection. MPEP (50 nmol, 5 μl) or CHPG (50 nmol, 5 μl) was injected 10 min prior to administration of toluene. Chelerythrine chloride (1 nmol, 5 μl) was given 20 min prior to CHPG injection. The concentrations of MPEP and chelerythrine chloride were chosen according to their IC50 values (Herbert et al., 1990), whereas the concentration of CHPG was selected on the basis of our previous studies (Chan et al., 2008).

2.3. ICV cannula implantation and injection

Under IP injection of pentobarbital (45 mg/kg) anesthesia, rats were placed in a stereotaxic frame with the incisor bar, and an occipital hole was drilled to the desired position in relation to the bregma, as described below. A stainless steel guide cannula (23-gauge) was stereotaxically placed into the right lateral cerebroventricle using the following stereotaxic coordinates: 1.6 mm lateral to the midline, 0.8 mm caudal to the bregma, and 3.5 mm below the dorsal surface of the brain. The cannula was secured to the skull using two stainless steel screws and dental cement and closed with a removable stylet. Animals were allowed an at least 5-day recovery period before testing. For ICV injections, a 27-gauge stainless steel micropipette was introduced into the ventricle through the cannula and connected to Hamilton microsyringe. Solutions (5 μl) were injected using a syringe pump at a rate of 10 μl/min. To determine correct placement of the guide cannula, the drinking behavior caused by ICV angiotensin II (50 ng, 5 μl) 3 days after surgery was observed (Camargo et al., 1991); the rats that started drinking within 3 min after the injection were used for the following experiments. At least 3 days after administration of angiotensin II, when it was completely metabolized, those rats with a positive drinking response were entered into the study.

2.4. Rotarod test

Motor coordination was assessed by means of an automated rotarod apparatus (TSE systems, Bad Homburg, Germany). Initially, rats were trained on the rotating rod at a constant speed of 15 rpm for three consecutive days. Only the rats which were able to spend on the rod at least for 180 s were used. Then, rats were tested every 10 min for 120 min after toluene treatment. The trial ended when rats fell and the time that rats remained on the rod was recorded automatically. When a rat remained on the rod for 180 s, it was then removed, and the time was recorded as 180 s.

2.5. Step-down inhibitory avoidance learning

The apparatus was a 40×25×25-cm acrylic box with a floor made of parallel 2 mm-caliber stainless steel bars spaced 1 cm apart for administering shock. A 2.5 cm high, 8×25 cm platform was placed on the left extreme of the box. Rats were moved to the testing room for at least 2 h before the experiments. Before each testing the platform and the floor of the apparatus were cleaned with a 70% ethanol solution. At the start of training, the rats were gently placed on the platform. In the training session, immediately upon stepping down, the animal received a 1 s–0.5 mA scrambled footshock. Toluene was given 30 min prior to training. The rats were removed from the chamber about 10 s after receiving the shock and replaced in its home cage. The retention of the avoidance response was tested 24 h later. In the memory retention test session, no footshock was given. The latency for rats to step down by placing four paws on the grid was measured. Staying on platform for 180 s was counted as ceiling response.

2.6. Body temperature

Rectal temperature was measured by a thermistor probe and digital thermometer (Singa Technology Co., Taipei, Taiwan). Readings were taken just before and after injection of toluene per 10-min interval for 120 min. The toluene-induced body temperature change was calculated by the difference in rectal temperature before and after treatment for each rat.

2.7. Statistical analyses

The data are expressed as mean ± SEM. The data obtained in step-down inhibitory avoidance learning task were analyzed by two-way ANOVA. The data from rotarod test and body temperature were analyzed by two-way mixed design ANOVA (with repeated measures on time). *Post hoc* comparisons were done by the Student–Newman–Keuls test. $P<0.05$ was considered statistically significant.

3. Results

3.1. Effects of CHPG and MPEP on toluene-induced motor incoordination

Toluene at a dose of 750 mg/kg which can remarkably impair motor coordination (Lo et al., 2009) was used to assess the effect of CHPG and toluene on rotarod performance. Two-way mixed design ANOVA (with repeated measures on time) revealed a significant main effect of treatment ($F_{3,176}=60.34$, $P<0.001$), and treatment×time interaction ($F_{33,176}=4.409$, $P<0.001$) for the latency to fall from the rotarod. Post-hoc comparisons indicated that CHPG alone did not affect motor function, but significantly attenuated the toluene-induced motor incoordination (Fig. 1A).

In order to examine the potentiating effect of MPEP, a lower dose of toluene (500 mg/kg) was used. Two-way mixed design ANOVA (with repeated measures on time) revealed a significant main effect of treatment ($F_{3,176}=788.417$, $P<0.001$), time ($F_{11,\ 176}=28.742$, $P<0.001$), and treatment×time interaction ($F_{33,176}=29.016$, $P<0.001$) for the motor coordination performance. Post hoc comparisons indicated that MPEP by itself did not affect motor function, but significantly potentiated motor-impairing effect of toluene (Fig. 1B).

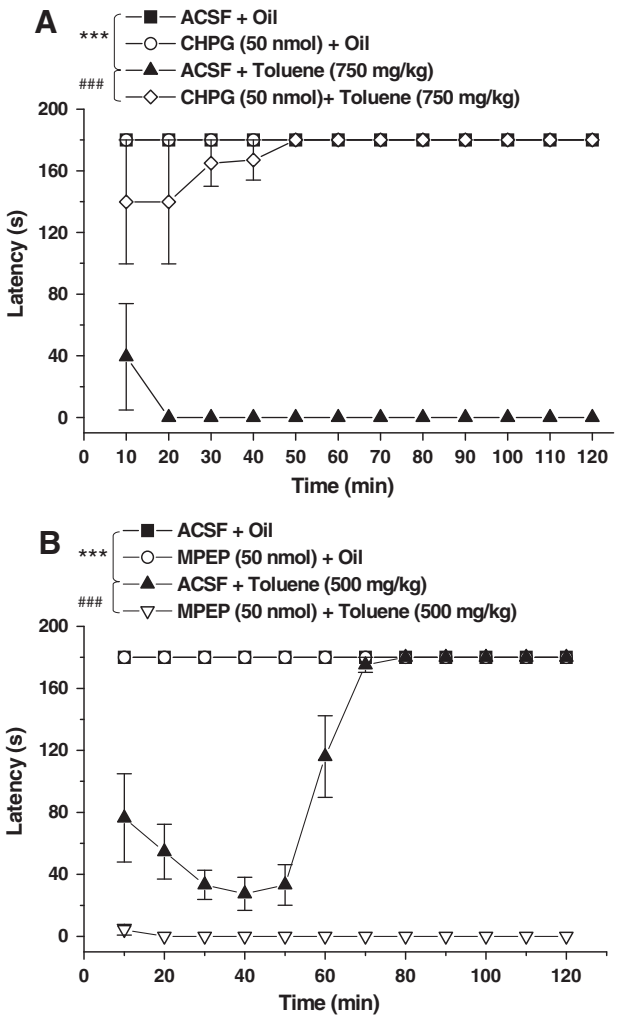

Fig. 1. Effects of CHPG and MPEP on toluene-induced motor incoordination in the rotarod test. Rats were pretreated with ACSF (artificial cerebrospinal fluid), CHPG (50 nmol, icv) (A) or MPEP (50 nmol, icv) (B) with oil or toluene (750 or 500 mg/kg, i.p.) and the latency to fall was recorded every 10 min for 120 min. All values are expressed as the mean ± SEM (n=5). ***P<0.001 vs. ACSF+oil, ###P<0.001 vs. ACSF+toluene (post hoc Student–Newman–Keuls test).

3.2. Effects of CHPG and MPEP on toluene-induced learning impairment

It has been found that a dose-dependent deleterious effect of toluene on performance in the avoidance learning task occurred when it was administered before training session (Lo et al., 2009). Thus, toluene (500 mg/kg) was given before training session to assess the beneficial effect of CHPG on toluene-induced learning impairment. Two-way ANOVA revealed that a significant main effect of toluene ($F_{1,16}=47.474$, $P<0.001$), CHPG ($F_{1,16}=5.774$, $P<0.05$), and a significant interaction between toluene and CHPG ($F_{1,16}=5.774$, $P<0.05$). Post hoc comparisons indicated that CHPG alone did not produce any effect on the avoidance learning task, but significantly antagonized the toluene-induced learning impairment (Fig. 2A).

In order to test the potentiating effects of MPEP, a lower dose of toluene (250 mg/kg) was applied. Two-way ANOVA revealed a significant main effect of toluene ($F_{1,16}=238.8$, $P<0.001$), MPEP ($F_{1,16}=8.497$, $P<0.05$) and a significant interaction between toluene and MPEP ($F_{1,16}=8.497$, $P<0.05$). Post hoc tests showed that MPEP by itself did not affect the avoidance learning, but augmented the learning–impairing effect of toluene (Fig. 2B).

3.3. Effects of CHPG and MPEP on toluene-induced hypothermia

Toluene dose-dependently produced hypothermia (Fig. 3A). Two-way mixed design ANOVA (with repeated measures on time) revealed a significant main effect of toluene dose ($F_{3,176}=33.204$, $P<0.001$), time ($F_{11,176}=7.551$, $P<0.001$), and toluene dose×time interaction ($F_{33,176}=3.342$, $P<0.001$). Toluene (750 mg/kg) was used to assess the effect of CHPG on toluene-induced hypothermia.

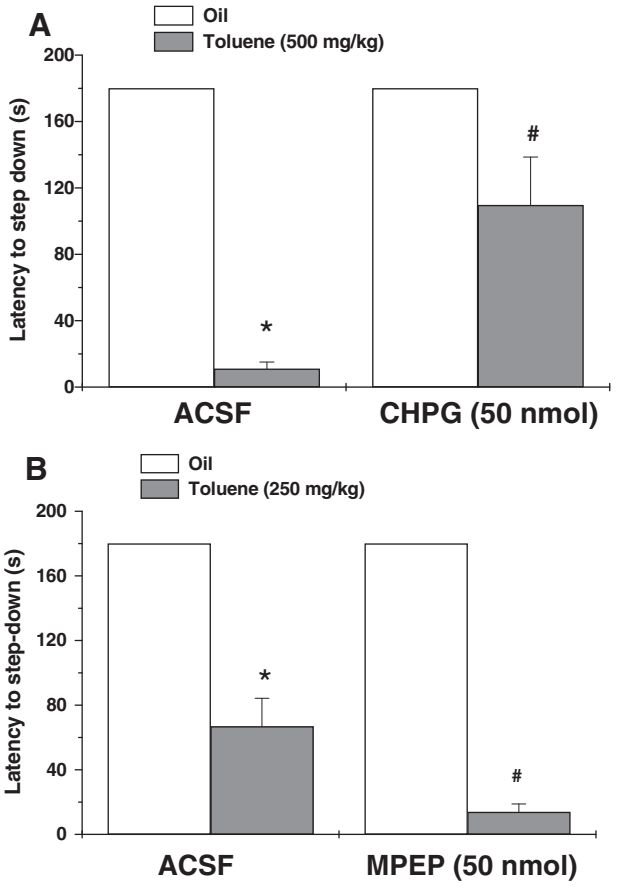

Fig. 2. Effects of CHPG and MPEP on toluene-induced learning impairment in the step-down inhibitory avoidance learning task. Rats were pretreated with ACSF (artificial cerebrospinal fluid), CHPG (50 nmol, icv) (A) or MPEP (50 nmol, icv) (B) 10 min prior to administration of toluene (500 or 250 mg/kg, i.p.). Animals were trained 30 min after toluene administration. After 24 h, the step-down latencies were recorded in the retention test with an upper limit of 180 s. All values are expressed as the mean ± SEM (n = 5). *P<0.05 vs. ACSF + oil, #P<0.05 vs. ACSF + toluene (post hoc Student–Newman–Keuls test).

Two-way mixed design ANOVA (with repeated measures on time) revealed a significant main effect of treatment ($F_{3,176} = 46.466$, $P<0.001$), time ($F_{11,176} = 4.548$) and treatment × time interaction ($F_{33,176} = 15.442$, $P<0.001$). Post hoc comparisons indicated that CHPG did not alter the rectal temperature, but significantly antagonized the hypothermic effect of toluene (Fig. 3B).

A low dose of toluene (250 mg/kg) was used to assess the potentiating effect of MPEP on toluene-induced hypothermia. Two-way mixed design ANOVA (with repeated measures on time) revealed a significant main effect of treatment ($F_{3,176} = 38.468$, $P<0.001$), time ($F_{11,176} = 17.113$, $P<0.001$) and treatment × time interaction ($F_{33,176} = 8.856$, $P<0.001$). *Post hoc* analysis indicated that MPEP alone had no significant effect on the rectal temperature, but significantly augmented the hypothermic effect of toluene (Fig. 3C).

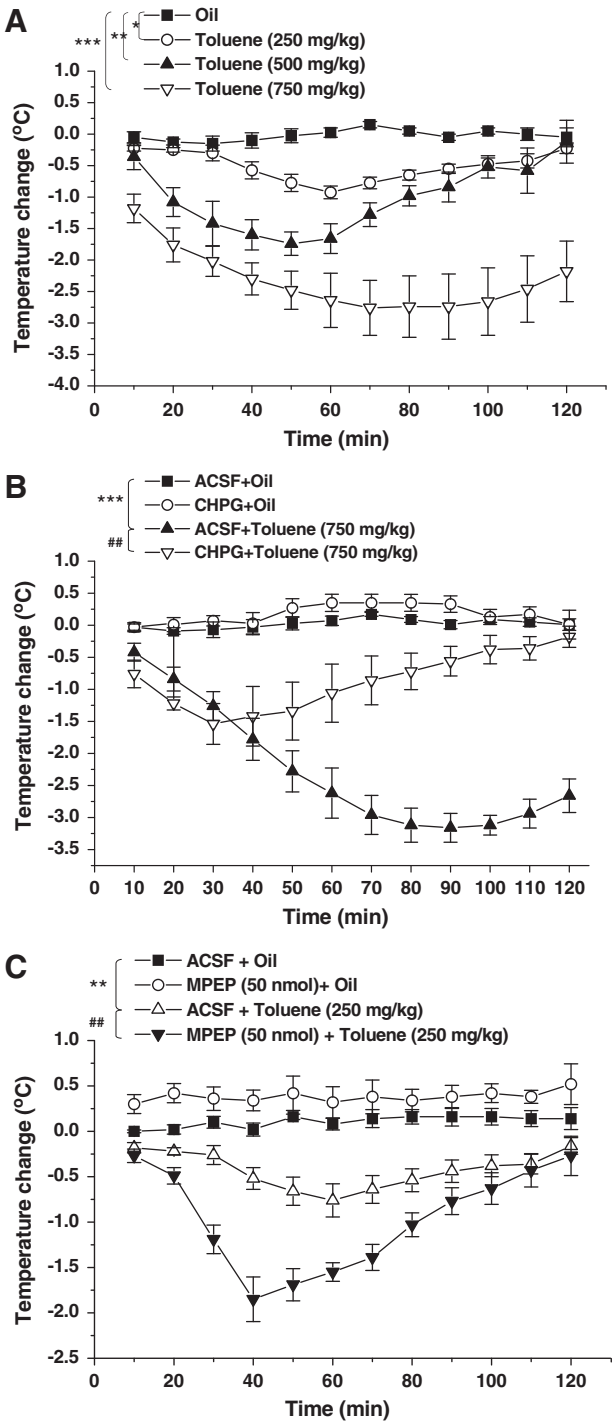

Fig. 3. Dose-dependent effect of toluene on body temperature and the effects of CHPG and MPEP on toluene-induced hypothermia. Rats were given toluene (0–750 mg/kg) (A) or pretreated with ACSF, CHPG (50 nmol, icv) (B) or MPEP (50 nmol, icv) (C) 10 min prior to administration of toluene (500 or 250 mg/kg, i.p.). The rectal temperature changes were recorded every 10 min for 120 min after administration of toluene. All values are expressed as the mean ± SEM (n = 5). *P<0.05, **P<0.01, ***P<0.001 vs. ACSF + oil, ##P<0.01 vs. ACSF + toluene (post hoc Student–Newman–Keuls test).

3.4. Effects of PKC inhibitor chelerythrine chloride on attenuation of toluene-induced motor incoordination, learning impairment, and hypothermia by CHPG

The selective PKC inhibitor chelerythrine chloride was used to reveal the role of PKC in the attenuating effect of CHPG on toluene-induced responses. Two-way mixed design ANOVA (with repeated measures on time) indicated significant effects of treatment ($F_{3,144} = 303.485$, $P<0.001$), time ($F_{9,144} = 22.168$, $P<0.001$) and treatment × time interaction ($F_{27,144} = 65.944$, $P<0.001$) for the performance in the rotarod test. *Post hoc* tests indicated that chelerythrine chloride alone did not affect the toluene's effect, but reversed the attenuating effect of CHPG (Fig. 4A).

In the experiment of step-down avoidance learning task, one-way ANOVA revealed that there was a significant difference between treatment groups ($F_{3,16} = 8.902$, $P<0.01$). *Post hoc* tests indicated that chelerythrine chloride reversed the effect of CHPG on toluene-induced learning deficits (Fig. 4B). With regard to the toluene-induced

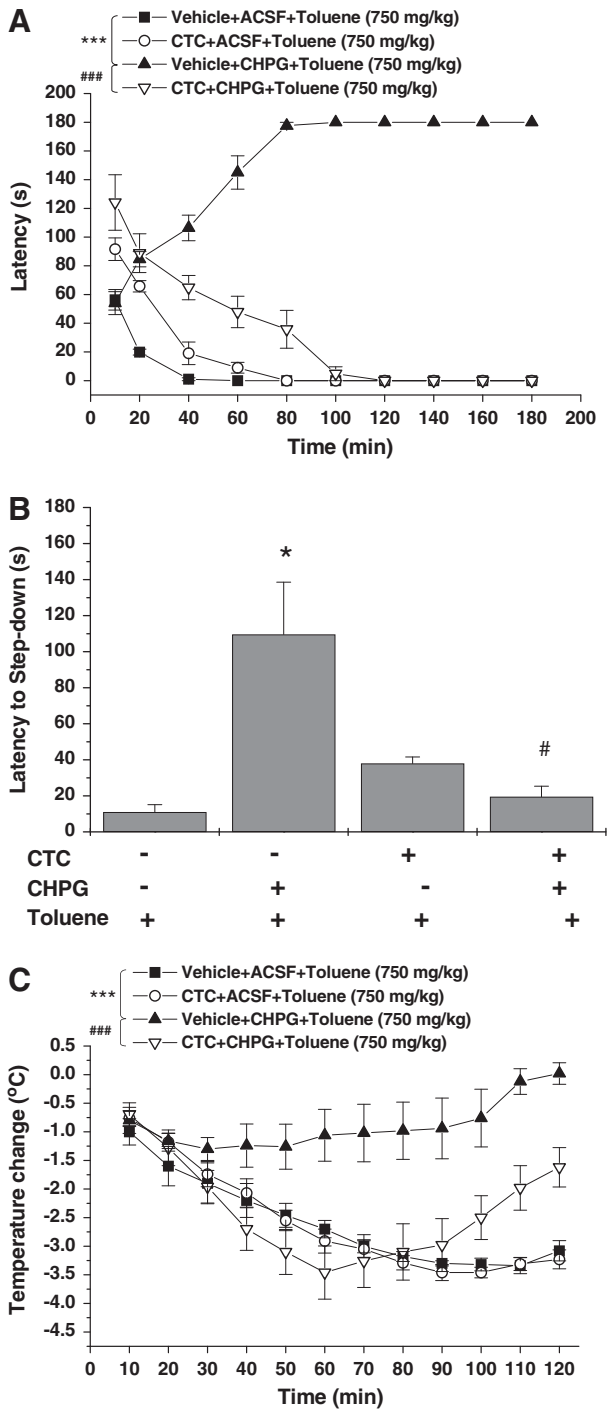

Fig. 4. Effects of PKC inhibitor chelerythrine chloride on attenuation of toluene-induced motor incoordination, learning impairment, and hypothermia by CHPG. Chelerythrine chloride (CTC, 1 nmol, icv) was administered 10 min prior to CHPG (50 nmol, icv). The rotarod test (A), step-down inhibitory avoidance learning task (B), and measurement of rectal temperature (C) were conducted after toluene treatment as previous described. All values are expressed as the mean ± SEM (n = 5). *P<0.05, ***P<0.001 vs. vehicle + ACSF + toluene, #P<0.05, ###P<0.001 vs. vehicle + CHPG + toluene (post hoc Student–Newman–Keuls test).

hypothermia, Two-way mixed design ANOVA with one factor repetition (time) indicated a significant main effect of treatment ($F_{3,176} = 12.908$, $P<0.001$), time ($F_{11,176} = 31.087$, $P<0.001$) and treatment × time interaction ($F_{33,144} = 8.154$, $P<0.001$). *Post hoc* tests demonstrated that PKC inhibitor chelerythrine chloride reversed the inhibitory effect of CHPG on toluene-induced hypothermia (Fig. 4C).

4. Discussion

Consistent with our previous findings (Lo et al., 2009), the present study demonstrated that acute toluene exposure resulted in motor incoordination in the rotarod test and learning impairment in the inhibitory avoidance learning task. In addition, toluene dose-dependently produced hypothermia. The mGluR5 agonist and antagonist, CHPG and MPEP, attenuated and potentiated the behavioral and hypothermic responses induced by toluene, respectively. Furthermore, the PKC inhibitor chelerythrine chloride reversed the inhibitory effects of CHPG on toluene-induced motor incoordination, cognitive impairment, and hypothermia, indicating that PKC might be involved in the mGluR5-mediated modulation of toluene responses.

The time course curves for toluene-induced motor incoordination and hypothermia are not parallel. Body temperature was declined by toluene gradually, whereas motor incoordination developed quickly. Different neuronal circuits are responsible for regulating the motor coordination and body temperature. Vast areas in the cerebral cortex, the basal ganglia, the cerebellum, the brain stem, and the spinal cord are intimately connected and cooperate in initiating, producing, and controlling coordinating movements and maintaining body balance. On the other hand, body temperature is controlled by the thermoregulatory center in the hypothalamus. The sensitivity of target neurons within these brain areas in response to toluene should be different. The time course of CHPG-induced recovery from toluene's impairment of rotarod and hypothermia appears different. It may be attributed to toluene-induced responses with distinct time courses and different sensitivities of CHPG's target sites involved in controlling these two functions.

Although toluene produces analgesic effects in rats (Huerta-Rivas et al., 2012), the electrical shock (0.5 mA) applied in the present study for step-down inhibitory avoidance task was enough to elicit clear aversive responses in all groups because all the rats showed flinch, jump, or vocalization after receiving the aversive electrical stimulus no matter they received toluene or not. This shock intensity used in this study seems equal to the thresholds for flinch and lower than thresholds for jump and vocalization reported previously (Huerta-Rivas et al., 2012). Strain (Sprgue–Dawley vs. Wistar) difference and route of administration (i.p. vs. air exposure) may contribute to the distinct sensitivity of antinociceptive response to toluene.

Despite the selectivity for mGluR5 (Gasparini et al., 1999), MPEP, at concentrations of 20 μM or greater, reduces the NMDA receptor activity (O'Leary et al., 2000). In fact, high-dose MPEP treatment can induce impairment in cognitive function and rotarod performance, and to reduce body temperature and locomotor activity (Varty et al., 2005). However, the dose of MPEP used in the present study appears not to antagonize NMDA receptors because administration of MPEP alone, did not produce any effects on rotarod test, step-down inhibitory avoidance learning task, and body temperature. The potentiating effects of MPEP on toluene-induced behavioral responses are most likely mediated by selective inhibition of mGluR5. However, the possibility for the additive inhibitory effects of MPEP on NMDA receptors with toluene cannot be unequivocally ruled out.

Since our data showed that CHPG and MPEP alone did not produce significant effects on rotarod test, step-down inhibitory avoidance learning task, and body temperature, mGluR5 might not be the direct target of toluene to produce its behavioral and hypothermic responses. In fact, NMDA receptor is one of the most sensitive action sites for toluene (Cruz et al., 1998). Toluene produces behavioral effects similar to those induced by NMDA receptor antagonists, such as ketamine or PCP. The current study further demonstrated that CHPG and MPEP modulated the memory impairment and motor incoordination elicited by toluene in the same way as those induced by NMDA receptor antagonists. Together with our previous findings that D-serine, a modulator of NMDA receptor, could attenuate several toluene-induced behavioral disturbances (Lo et al., 2009), these data

further support that the acute toluene-induced behavioral responses, at least motor and learning impairment, are mediated by blockade of NMDA receptors.

It is hypothesized that mGluR5 modulates the behavioral responses to toluene through regulation of the NMDA receptor function. Activation of mGluR5 stimulates the activity of phospholipase C, thereby generating diacylglycerol, which in turn can activate PKC (Hermans and Challiss, 2001). Actually, the enhancing effect of mGluR5 agonists on NMDA receptor function is mediated by activation of PKC (Pisani et al., 1997). It is further characterized that PKCγ isoform is responsible for mGluR5-elicited phosphorylation ser890 of NMDA receptor NR1 subunit to enhance NMDA receptor function (Sanchez-Perez and Felipo, 2005; Takagi et al., 2010). Therefore, PKCγ might be the main target of chelerythrine to reverse the attenuating effect of CHPG on toluene-induced responses. Our results demonstrate that PKC inhibitor chelerythrine chloride reversed the inhibitory effects of CHPG on toluene-induced motor incoordination, learning impairment, and hypothermia, suggesting that PKC-dependent signaling pathway plays an important role in the modulation of the behavioral responses of toluene by mGluR5 agonists. However, chelerythrine has no specificity to any PKC isoform because it binds to the catalytic domain of PKC and all PKC isoforms share a highly conserved catalytic domain. The critical role of PKCγ in the inhibitory effects of CHPG on toluene-induced responses remains to be verified by inhibitors with selective inhibition of individual PKC isoforms.

We found that toluene elicited hypothermia in a dose-dependent manner in rats, which is consistent with a previous report (Gordon et al., 2010). Our results further demonstrated that mGluR5 inhibition and stimulation could regulate the hypothermic effect of toluene and the involvement of PKC-dependent signaling pathway. To the best of our knowledge, this is the first report related to the possible mechanisms that underlie the toluene-induced hypothermia. Alterations of both central and peripheral thermoregulatory mechanisms may contribute to the toluene-induced hypothermia. Because the mGluR5 modulating compounds and PKC inhibitor chelerythrine chloride were administered by ICV in the present study, toluene might affect the thermoregulatory center of the hypothalamus to produce hypothermic effect through blockade of NMDA receptors. In fact, the NMDA receptor antagonists PCP and ketamine also produce hypothermia (Fahim et al., 1973; Pechnick et al., 1989). Nevertheless, the regulatory role of mGluR5 in the hypothermic effects of PCP and ketamine remains to be elucidated.

It is notable that chelerythrine chloride is not only a selective PKC inhibitor (Herbert et al., 1990), but also produces several PKC-independent responses, such as activation of the MAPK pathways (Yang et al., 2008; Yu et al., 2000) and Bcl-xL (B-cell lymphoma-extra large) inhibition (Chan et al., 2003). In addition to activation of PKC, several mGluR5-induced responses are mediated via activation of the MAPK pathway (Wang et al., 2004). Since chelerythrine chloride is an activator rather than an inhibitor of MAPK, it is unlikely that the effect of CHPG can be reversed by a MAPK activator. Bcl-xL, known as a protector against programmed cell death, regulates mitochondrial membrane conductance and bioenergetics in the synapse and can thereby alter presynaptic transmitter release and the recycling of pools of synaptic vesicles (Jonas, 2006). However, mGluR5 is mostly located at the postsynaptic elements to regulate NMDA receptor function. Thus, chelerythrine chloride reversing the effects of CHPG on toluene-induced responses is, probably, mainly associated with its inhibitory effect on PKC.

Toluene shares similar actions with ethanol and other CNS depressant drugs (Evans and Balster, 1991) besides NMDA receptor antagonists. It has been shown that MPEP significantly enhanced the sedative and hypnotic effects (Sharko and Hodge, 2008), but not the locomotor-stimulating effects of alcohol (Blednov and Harris, 2008). In contrast, MPEP reduces the discriminative stimulus effects of ethanol and ethanol-like stimulus properties of diazepam (Besheer and Hodge, 2005). These findings indicate that mGluR5 may play distinct roles underlying specific behavioral responses to ethanol. Our results showed that MPEP potentiated the motor and learning impairment and hypothermic effects of toluene. Further studies are needed to determine whether other neurobehavioral effects of toluene, such as anxiolytic (Bamat et al., 2005; Bowen et al., 1996), anticonvulsant (Chan et al., 2006; Wood et al., 1984), rewarding (Funada et al., 2002; Gerasimov et al., 2003), and analgesic effects (Huerta-Rivas et al., 2012) are modulated by mGluR5 in the same manner.

5. Conclusion

Toluene toxicity can occur from accidental or deliberate inhalation of fumes. No antidote exists for toluene intoxication by now. The present study shed light on the beneficial effects of positive modulation of mGluR5 on toluene-induced motor and learning impairment as well as hypothermic response and the critical role of PKC-dependent pathway in the mGluR5 action. Attenuation of these responses to toluene by mGluR5 agonist CHPG may provide insight into the development of novel therapeutic interventions for the treatment of toluene intoxication. Together with the suppressing effects of D-serine, a modulator of glycine-binding site of NMDA receptor, on toluene-induced behavioral responses, the present findings further support that blockade of NMDA receptor function might be the main mechanism underlying acute toluene-related motor incoordination, learning impairment, and hypothermia, which in the past has been more typically attributed to general nonspecific mechanisms.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgment

This work was supported by a grant (NSC 96-2314-B-320-004) from the National Scientific Council of Taiwan.

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