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Toluene exposure during brain growth spurt and adolescence produces differential effects on N-methyl-D-aspartate receptor-mediated currents in rat hippocampus

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ABSTRACT

Toluene, an industrial organic solvent, is voluntarily inhaled as drug of abuse. Because inhibition of N-methyl-D-aspartate (NMDA) receptors is one of the possible mechanisms underlying developmental neurotoxicity of toluene, the purpose of the present study was to examine the effects of toluene exposure during two major neurodevelopmental stages, brain growth spurt and adolescence, on NMDA receptor-mediated current. Rats were administered with toluene (500 mg/kg, i.p.) or corn oil daily over postnatal days (PN) 4–9 (brain growth spurt) or PN 21–26 (early adolescence). Intracellular electrophysiological recordings employing in CA1 pyramidal neurons in the hippocampal slices were performed during PN 30–38. Toluene exposure during brain growth spurt enhanced NMDA receptor-mediated excitatory postsynaptic currents (EPSCs) by electrical stimulation, but impaired the paired-pulse facilitation and NMDA response by exogenous application of NMDA. Toluene exposure during adolescence resulted in an increase in NMDA receptor-mediated EPSCs and a decrease in exogenous NMDA-induced currents, while lack of any effect on paired-pulse facilitation. These findings suggest that toluene exposure during brain growth spurt and adolescence might result in an increase in synaptic NMDA receptor responsiveness and a decrease in extrasynaptic NMDA receptor responsiveness, while only toluene exposure during brain growth spurt can produce presynaptic modulation in CA1 pyramidal neurons. The functional changes in NMDA receptor-mediated transmission underlying developmental toluene exposure may lead to the neurobehavioral disturbances.

1. Introduction

The chronic abuse of volatile solvents is a growing problem among children and adolescents at social risk worldwide. Toluene is one of the most commonly abused solvents. Since a large number of abusers are adolescent and young adult women in their childbearing years, there are raising concerns about the potential negative impact of intentionally inhaled organic solvents on the unborns.

Abuse of toluene by pregnant women can lead to an embryopathy, also referred to as the fetal solvent syndrome. Characteristics of toluene embryopathy include particular craniofacial features, growth retardation, and central nervous system dysfunctions such as microcephaly, brain malformation, and motor and intellectual disability (Pearson et al., 1994). Nevertheless, not all offspring of mothers exposed to toluene show evident physical features and structural damage. Those who exposed to lower doses or shorter duration of toluene might still have important but subtle impairment in synaptic circuitry, reflecting as neurobehavioral disturbance. However, neuron developmental evaluations of these children have not been reported.

The N-methyl-D-aspartate (NMDA) receptor plays an important role in neurodevelopment, neuroplasticity, neuroendocrine regulation, and neuronal death (Contestabile, 2000; Cull-Candy et al., 2001; Scheetz and Constantine-Paton, 1994). Experimental evidence indicates that the effects of toluene on neuronal activity and behavior might be due to its inhibition of NMDA receptor-mediated currents (Cruz et al., 1998). Our previous studies demonstrated that exposure to toluene during brain growth spurt enhances the NMDA-induced seizure susceptibility (Chen and Lee, 2002), increases the immunoreactivity of NR2A subunit in the hippocampus and cerebellum (Lee et al., 2005), and reduces NMDA antagonist-induced locomotor activity, motor incoordination and hypnosis (Chien et al., 2005) in juveniles. We also demonstrated that neonatal toluene exposure dose-dependently reduced intracellular Ca^{2+} signals in response to glutamate/glycine and NMDA/glycine in cultured cerebellar granule neuron (Chen et al., 2005). Therefore, it is possible that dysregulation of NMDA

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receptor-mediated transmission may play an important role in the pathophysiology of toluene-related neurodevelopmental disorders.

The period of brain growth spurt that largely occurs during the third trimester of human fetal development but occurs during the early postnatal period in the rats (Dobbing and Sands, 1979), is a dynamic period of central nervous system (CNS) development that has been shown to be particularly vulnerable to a variety of neurotoxicants. On the other hand, adolescence is also a time of extensive pruning of synapses and of reorganization of many neurotransmitter systems (Spear, 2000). Temporary interference with the function of neurons with NMDA receptors by toluene exposure during brain growth spurt or adolescence is likely to disturb the normal development of CNS, subsequently resulting in long-lasting functional changes in NMDA receptors. The purpose of this study was to test the effects of toluene exposure during brain growth spurt or early adolescence on NMDA receptor-mediated excitatory response. To differentiate the influence of toluene on the synaptic and extrasynaptic NMDA receptor-mediated currents, electrical stimulation and exogenous application of NMDA were used to evoke excitatory postsynaptic currents (EPSCs). In addition, a paired-pulse paradigm was employed to reveal the involvement of presynaptic mechanism.

2. Materials and methods

2.1. Materials

Toluene (HPLC grade, 99.8%) was obtained from Mallinckrodt Baker (Phillipsburg, NJ, USA). Glycine was purchased from J.T. Baker (Mallinckrodt Baker, Inc., Kentucky, USA). All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Animal treatment

Pregnant female Sprague-Dawley rats were supplied from the Laboratory Animal Center of Tzu Chi University (Hualien, Taiwan). Rats were housed individually on a 12/12 light-dark cycle at 22 °C. All experiments were performed in accordance with the Republic of China animal protection law (Chapter III: Scientific Application of Animals) and approved by Review Committee of the Tzu Chi University.

2.3. Toluene exposure

The day of birth was considered to be postnatal day (PN) 0. The litters were culled to 10–12 pups and each litter was randomly assigned to toluene or control group. Male animals received 500 mg/kg of toluene (0.1 g/ml in corn oil) or corn oil (0.1 ml/10 g) by intraperitoneal injection daily over brain growth spurt (PN 4–9) or adolescence (PN 21–26). A modified 26 G needle (6 mm long) was used for the pups to prevent tissue damage. The mother did not reject the pups treated with toluene. All the pups were weaned on PN 21.

In general, human exposed to toluene by inhalation. Continuous exposure to toluene vapors is usually used in rodent models to mimic the exposure in industrial workers. However, abusers do not continuously sniff glue for long period, but rather prefer to titer their dose by repeatedly 'huffing' very-high-exposure concentrations for only seconds to minutes. This is hard to be conducted in animal models. Actually, intraperitoneal injection of toluene to rodents, like inhalation exposure, produced biphasic locomotor activity and stereotypic behaviors, which resemble behavioral signs observed in toluene abusers (Chan et al., 2004; Riegel and French, 1999) and full substitution for inhaled toluene in drug discrimination (Shelton and Slavova-Hernandez, 2009). The concentration of toluene in the animal tissues at the time of testing determined the behavioral performance no matter the route of administration (Shelton and Slavova-Hernandez, 2009). Furthermore, intraperitoneal injection can produce low variation of toluene concentrations in blood and this route of administration is also routinely used in animals to study drugs commonly abused by inhalation (e.g., cannabinoids and nicotine). Therefore, toluene was administered by intraperitoneal injection.

The toluene dose (500 mg/kg) used in this study was based on our previous studies. Rats subjected to a similar toluene exposure dose and paradigm (PN 4–9) manifested increasing NMDA-induced seizure susceptibility, reducing behavioral responses to NMDA antagonists, and blood toluene concentrations, from blood sample taken 1 and 3 h following the last injection of toluene, were 27.4 ± 5.1 μg/ml and 7.8 ± 1.5 μg/ml, respectively (Chien et al., 2005; Lee et al., 2005). These levels are in the range obtained from toluene abusers (0.1–74.7 μg/ml) (Garriott et al., 1981; Park et al., 1998; Zanatta et al., 1996). In addition, the placenta penetration efficiency for toluene is greater than 90% (Shumilina, 1991).

2.4. In vitro electrophysiology, stimulation, and drug application

Experiments were performed on hippocampal slices obtained from control or toluene-exposed rats at PN 30–38. The brain was quickly removed from the skull and placed in an ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl 120, KCl 3.5, $MgCl_2$ 1.2, $CaCl_2$ 2.5, NaH_2PO_4 1.2, glucose 11.5, $NaHCO_3$ 25, saturated with 95% O_2 and 5% CO_2, pH 7.4. Transverse hippocampal slices (500 μm) were cut with a vibratome and stored at room temperature in holding the same ACSF solution as above. After a recovery period of at least 1 h, an individual slice was transferred to the recording chamber where it was continuously superfused with oxygenated ACSF at a rate of 2–3 ml/min. CA1 pyramidal neurons were voltage clamped at −60 mV to record NMDA receptor-mediated EPSCs. Patch pipettes were filled with a solution containing (in mM): K gluconate 122, NaCl 5, $CaCl_2$ 0.3; $MgCl_2$ 2, EGTA 1, HEPES 1, Na_2ATP 5, NaGTP 2, amphotericin B 0.4 (pH 7.25, resistance 9–12 M). Orthodromic stimuli were delivered with square-wave pulses (5–16 V; 0.1 ms) via a concentric bipolar electrode which was placed in stratum radiatum to activate Schaffer collaterals. Current signals and applied voltages were generated and recorded with an Axoclamp 200B amplifier (Axon Instruments, USA). Whole-cell recording are acquired with a switch frequency of 5–6 kHz (30% duty cycle), gain of 3–8 nA/mV, time constant 20 ms. Tracings shown in figures represent the average of three consecutive sweep. Output signals were stored on an IBM-compatible computer after digitalization with a DigiData-1200 Series Interface using acquisition and analysis software (pClamp, v. 8.10). In order to isolate NMDA receptor-mediated monosynaptic EPSCs, the AMPA/kainate receptor antagonist DNQX (50 μM), γ-aminobutyric acid A ($GABA_A$) receptor antagonists bicuculline (10 μM) and picrotoxin (10 μM), and $GABA_B$ receptor antagonist CGP35348 (200 μM) were applied together as a cocktail. NMDA (10, 20 and 50 μM) and glycine (10 μM) applied by bath superfusion to achieve steady-state concentrations within the 1.0-ml recording chamber.

For paired-pulse facilitation, two stimuli (15 V) were delivered with an interstimulus interval of 40–200 ms. The facilitation was calculated as the current ratio ($EPSC_2$//$EPSC_1$).

2.5. Statistics

All values were given as mean ± SEM. Two-way mixed designed ANOVA was used for the stimulus-induced NMDA current and paired-pulse facilitation. Two-way ANOVA was used for exogenous NMDA-elicited currents. Multiple comparisons were performed using the Student–Newman–Keuls test. $p < 0.05$ was considered statistically significant.

3. Results

3.1. Body weight gain

During the time of toluene exposure (PN 4–9 or PN 21–26), the body weight gain of the toluene-exposed (12.5 ± 1.2 g; 28.8 ± 1.7 g) and control rats (12.3 ± 1.3 g; 27.6 ± 2.0 g) was similar.

3.2. Effects of toluene exposure on NMDA receptor-mediated EPSCs

The effect of toluene exposure during brain growth spurt on NMDA receptor-mediated EPSCs was examined by voltage clamp recordings after appropriate pharmacological isolation to block the AMPA/kainate receptor-mediated components of the EPSCs and GABA-mediated components of the inhibitory postsynaptic currents (IPSCs). The composite EPSCs provoked by electrical stimulation were blocked by the NMDA receptor inhibitor D(−)-2-amino-5-phosphonopentanoic acid (D-APV, 50 μM) (Fig. 1B). Two-way mixed designed ANOVA revealed a main effect of treatment ($F_{1,216} = 22.68$, $p < 0.001$) and stimulus intensity ($F_{8,216} = 67.24$, $p < 0.001$) with significant interaction ($F_{8,189} = 8.19$, $p < 0.001$). *Post hoc* analysis indicated EPSCs in response to 8–16 V stimuli were significantly elevated in the slice from toluene-exposed rats (Fig. 1A and B).

In the adolescent exposure study, the NMDA receptor-mediated EPSCs were significantly increased (*Treatment*: $F_{1,198} = 4.47$, $p = 0.045$; *Stimulus intensity*: $F_{8,198} = 56.82$, $p < 0.001$; *Interaction:* $F_{8,198} = 2.87$, $p = 0.042$) by toluene exposure. *Post hoc* analysis showed that the currents in response to 12–16 V stimuli were significantly higher in the slice from toluene-exposed rats than controls (Fig. 1C).