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Investigation of calcium-stimulated adenylyl cyclases 1 and 8 on toluene and ethanol neurobehavioral actions ☆

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ABSTRACT

The abused inhalant toluene has potent behavioral effects, but only recently has progress been made in understanding the molecular pathways that mediate the action of toluene in the brain. Toluene and ethanol induce similar behavioral effects and share some targets including NMDA and GABA receptors. In studies examining neuronal actions of ethanol, mice lacking the calcium-stimulated adenylyl cyclases (ACs), AC1 and AC8 (DKO), show increased sedation durations and impaired protein kinase A (PKA) phosphorylation following acute ethanol treatment. Therefore, using DKO mice, we compared the neurobehavioral responses following toluene exposure to that of ethanol exposure to determine if these abused substances share molecular mechanisms of action. In the present study, acute sensitivity to toluene- or ethanol-induced changes in locomotor activity was evaluated in DKO and wild type (WT) mice. Mice were exposed to toluene vapor (0, 500, 1000, 2000, 6000, or 8000 ppm) for 30 min in static exposure chambers equipped with activity monitors. Both WT and DKO mice demonstrated increased ambulatory distance during exposure to a 2000-ppm concentration of toluene compared to respective air-exposed (0 ppm) controls. Significant increases in locomotor activity were also observed during an air-only recovery period following toluene exposure in WT and DKO mice that had been exposed to 2000 ppm of toluene compared to respective air controls. Sedative effects of toluene were equivalent in WT and DKO mice, both during exposure and afterwards during recovery. Although no significant differences in locomotor activity were detected in DKO compared to WT mice at individual doses tested, a significant main effect of toluene was achieved, with DKO mice demonstrating a generalized reduction in locomotor activity during the post-toluene recovery period compared to WT mice (when analyzing all doses collectively). For comparison to toluene, additional WT and DKO mice were treated with 1.0 or 2.0 g/kg ethanol (i.p.) and monitored for locomotor activation. In WT mice, both doses of ethanol increased distance traveled compared to saline controls. Conversely, DKO mice demonstrated no increase in locomotor activation at 1.0 g/kg, with significantly reduced distances traveled at both doses compared to ethanol-treated WT mice. These behavioral activity results suggest that acute effects of ethanol and toluene are distinct in the mechanisms by which they induce acute sedating effects with respect to AC1 and AC8 activity, but may be similar in the mechanisms subserving locomotor stimulation.

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1. Introduction

Toluene is a widely used industrial solvent that is also deliberately inhaled for its euphoric and intoxicating effects (Cruz and Bowen, 2008; Howard et al., 2011). Found in adhesives, paint and cleaning fluids, toluene is readily available, inexpensive and legal to obtain, making it a popular inhalant among adolescents. The most recent *Monitoring the Future* survey revealed that lifetime prevalence of volatile substance misuse among 8th graders is 14.9%, higher than all other illicit drugs in this age group with the exception of marijuana (15.7%) (Johnston et al., 2010). A recent National Survey on Drug Use and Health (SAMHSA) indicates that more than 22 million Americans have intentionally misused volatile substances at least once in

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their lifetimes (SAMHSA, 2007). In humans, acute exposure to high concentrations of toluene results in dizziness, slurred speech, and ataxia, which can persist for hours with repeated exposure (Anderson and Loomis, 2003; Brouette and Anton, 2001; Flanagan and Ives, 1994; Kurtzman et al., 2001). Further, permanent neural injury, including brainstem and cerebellar damage, cognitive impairments, and peripheral neuropathy, has been demonstrated after chronic toluene exposure (Ehyai and Freemon, 1983; Hormes et al., 1986; Kiyokawa et al., 1999; Rosenberg et al., 1988). Despite the neurobehavioral and neurotoxic consequences of toluene exposure and abuse, knowledge regarding the mechanism of action of toluene remains limited.

Several animal studies have demonstrated similarities between the behavioral effects of toluene and central nervous system (CNS) stimulants, like amphetamine or cocaine (Bowen, 2006; Bowen et al., 2007; Gerasimov et al., 2002) and CNS depressants, like ethanol. For example, acute ethanol exposure produces dose-dependent, reversible alterations in behaviors, such as locomotor activity (for review, see Phillips and Shen, 1996) which are also observed following acute inhalant administration (Bowen and Balster, 1996, 1998). Specifically, animals demonstrate a biphasic concentration-effect curve in general locomotor activity after acute toluene exposure with excitatory stimulation at low concentrations and sedative or depressive effects at higher concentrations (for review, see Bowen et al., 2006; Evans and Balster, 1991). Excitatory stimulation of locomotor activity has also been reported during the "recovery" period following high dose exposure that is indirect and secondary to the direct effects observed with exposure (Batis et al., 2010).

Similarities in the behavioral effects of toluene and ethanol suggest that they share molecular mechanisms, however, few studies have sought to investigate this. For example, similar to ethanol, toluene inhibits NMDA receptor-mediated currents while potentiating currents associated with GABA and glycine receptors (Bale et al., 2005; Beckstead et al., 2000; Cruz et al., 1998). Likewise, both toluene and ethanol have been shown to modulate intracellular calcium concentrations and calcium currents. Specifically, toluene has been shown to release intracellular calcium stores and to inhibit voltage-sensitive calcium channels in neurons exposed to clinically-relevant concentrations of toluene, which are similar to the effects observed *in vitro* with ethanol exposure (Kerschbaum and Hermann, 1997; MacIver, 2009; Shafer et al., 2005; Tillar et al., 2002). The shared effects of toluene and ethanol on calcium suggest common downstream mechanisms of action. The calcium/calmodulin-stimulated adenylyl cyclases, enzymes that catalyze the production of cAMP, may represent one such mechanism.

The cAMP signaling pathway is recognized as an important modulator of neurobehavioral sensitivity to ethanol. Reductions in cAMP signaling have been shown to increase behavioral sensitivity to ethanol in mice (Maas et al., 2005; Wand et al., 2001). Specifically, mice lacking the calcium/calmodulin-stimulated adenylyl cyclases 1 and 8 (AC1 and AC8, respectively) exhibit increased durations of ethanol-induced sedation compared to controls (Maas et al., 2005). The role of AC1 and AC8 in ethanol- and toluene-induced locomotor activation or toluene-induced sedation, however, is yet unknown. AC1 and AC8 generate cAMP from ATP and are the only AC isoforms primarily stimulated by calcium via calmodulin activation (i.e., not direct G-protein activation) (Cali et al., 1994; Tang et al., 1991; Wang et al., 2003). Previous studies have demonstrated that toluene has differential effects on G-protein-coupled receptor-stimulated signal transduction mechanisms involving cAMP production, depending on the receptor activated (Tsuga et al., 1999), however these data were not specific to AC1 and AC8. AC1 and AC8 are localized synaptically, in proximity to NMDA receptors (Mons et al., 1995), suggesting their involvement in NMDA-mediated events such as toluene and ethanol action. The ability of NMDA receptors to mediate calcium entry represents a pathway by which NMDA can regulate AC1/8 activity (Woodward, 2000). This observation, along with the ability of toluene and ethanol to impact intracellular calcium concentrations through the mechanisms described above, suggest that AC1 and AC8 could mediate the behavioral response to these drugs. To examine this possibility, we used C57BL/6-derived mice deficient in AC1 and AC8 (DKO), to investigate the ability for AC1 and AC8 to mediate alterations in locomotor activity in response to stimulating (1.0 and 2.0 g/kg) doses of ethanol, or stimulating (500–2000 ppm) and depressing (6000–8000 ppm) concentrations of inhaled toluene both during toluene exposure and afterward, during a recovery period.

2. Materials and methods

All animal procedures had prior approval by the Wayne State University Institutional Animal Care and Use Committee and were conducted in accordance with the NIH "Guide for the Care and Use of Laboratory Animals" (Institute of Laboratory Animal Resources, National Academy Press 1996; NIH publication no. 85–23, revised 1996).

2.1. Subjects

All mice (N = 256 (141 for toluene study; 115 for ethanol study)) were back-crossed a minimum of ten generations to wild type C57BL/6 (WT) mice from The Jackson Laboratory (Bar Harbor, ME). To generate mice for these experiments, we used progeny of homozygous mutants (DKO × DKO) and WT (WT × WT) mice from The Jackson Laboratory bred in our colony. DKO mice have been genetically-altered to have a constitutive deletion of both AC1 and AC8. All experiments were performed using male mice between 2 and 4 months of age. Mice were housed in groups of 3–5 in polypropylene cages (18 cm × 29 cm × 13 cm) with corn cob bedding and steel-wire tops. Mice were allowed *ad libitum* access to Rodent Lab Diet 5001 (PMI, Nutrition International, Inc., Brentwood, MO) and water when in their home cages. The Association for Assessment and Accreditation of Laboratory Animal Care-approved vivarium was kept on a 12-h light/dark cycle (lights on 0600 h). Animals were transported to the laboratory 45 min before all behavioral testing for acclimation to the testing environment. Testing was carried out during the light phase of the diurnal cycle (1200–1700 h).

2.2. Toluene inhalation exposure procedures

Exposure procedures were identical to those previously described (Bowen et al., 2010). Briefly, vapor exposures were given in one of 6 sealed 26-liter cylindrical glass jars housed within a fume hood. During exposure, one mouse was placed into the bottom of the chamber ~38 cm from a vapor diffuser with a fan in the Plexiglas lid. For toluene exposures, a calculated amount of the solvent was injected into the diffuser and the fan was turned on to volatilize and distribute the toluene within the exposure chamber. Toluene vapor concentrations were confirmed periodically by single wavelength-monitoring infrared spectrometry (Miran 1A, Foxboro Analytical). Mean concentrations of toluene were within 3% of nominal levels ~2.5 min after toluene was added, and remained within 2% of nominal levels throughout the 30-min exposure. For air-only (0 ppm) exposures, the lid was sealed, nothing was injected into the diffuser, and the fan was turned on. Levels of waste gasses (i.e., water vapor and CO_2) were monitored during pilot studies (N = 8) and changes following 30-min sessions were negligible (mean CO_2 < 900 ppm). After the 30-min exposure, the lids were unsealed, opened and toluene was vented. Toluene vapor was not detectable after 1 min by single wavelength-monitoring infrared spectrometry (Miran 1A, Foxboro Analytical). Locomotor activity was measured for an additional 30 min during the recovery period. After this 30-min recovery period, mice were returned to their home cages.

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