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Short communication

# Inhibition of gap junction currents by the abused solvent toluene

Angelo M. Del Re, John J. Woodward*

*Department of Physiology and Neuroscience, Center for Drug and Alcohol Programs, 173 Ashley Avenue, P.O. Box 250510, Medical University of South Carolina, Charleston, SC 29425, USA*

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**Abstract**

Abused inhalants are a large class of compounds that are inhaled for their intoxicating and mood altering effects. They include chemicals with known therapeutic uses such as anesthetic gases as well as volatile organic solvents like toluene that are found in paint thinners and adhesives. Because of their widespread commercial use and availability, inhalants are often among the first drugs that children encounter and use of these compounds is often associated with adverse acute and long-term consequences. The cellular and molecular sites of action for abused inhalants is not well known although recent studies report that toluene and other organic solvents alter the activity of specific ligand- and voltage-gated ion channels that regulate cellular excitability. As part of an ongoing effort to define molecular sites of action for abused inhalants, this study examined the effect of toluene on the function of gap junction proteins endogenously expressed in human embryonic kidney (HEK 293) cells. Gap junctions allow cell-to-cell electrical communication as well as passage of small molecular weight substances and are critical for synchronizing cellular activity in certain tissues. Gap junction currents in HEK 293 cells were measured during brief voltage steps using patch-clamp electrophysiology and were blocked by known gap junction blockers confirming expression of connexin proteins in these cells. Toluene dose-dependently inhibited these conductances with threshold effects appearing at approximately 0.4 mM and near complete inhibition occurring at concentrations of 1 mM and higher. The estimated $EC_{50}$ value for toluene inhibition of gap junction currents in HEK 293 cells was 0.57 mM. The results of these studies suggest that volatile solvents including toluene may produce some of their effects by disrupting inter-cellular communication mediated by gap junction proteins.
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## 1. Introduction

The voluntary inhalation of volatile solvents for their intoxicating effects is a worldwide drug abuse problem and is particularly popular among children and adolescents. This is likely due to the relative ease in obtaining these compounds and their low cost as compared to illicit drugs. Although many different types of volatile chemicals are inhaled as drugs of abuse, toluene, or methylbenzene, is considered a prototype for this class as it is a component in common commercial products such as paints and adhesives. Although the physiological and behavioral responses to toluene are similar to those of central nervous system depressants such as alcohol, until recently, relatively little was known regarding its cellular and molecular sites of action.

Previous studies from this lab and others have shown that abused solvents alter the function of several important ion channels that regulate cellular excitability. For example, toluene and related alkylbenzenes inhibit *N*-methyl-D-aspartate (NMDA) receptors at concentrations that do not affect non-NMDA ionotropic glutamatergic receptors (Cruz et al., 1998, 2000). In addition, toluene and other volatile solvents potentiate currents carried by glycine, $GABA_A$ and $5HT_3$ receptors (Beckstead et al., 2000; Lopreato et al., 2003) whereas nicotinic acetylcholine receptors are inhibited (Bale et al., 2002). Recently, toluene has also been shown to inhibit voltage-dependent calcium and sodium currents (Tillar et al., 2002; Cruz et al., 2003) and to cause subunit-dependent effects on ATP-gated P2X receptors (Woodward et al., 2004).

* Corresponding author. Tel.: +1 843 792 5225; fax: +1 843 792 7353.
*E-mail address:* woodward@musc.edu (J.J. Woodward).

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Toluene's action on these various channel subtypes would be expected to profoundly affect excitable cells such as neurons and cardiac myocytes that rely upon these ion channels for activity and signaling processes. In addition to these ligand and voltage-gated ion channels, many cells utilize another major class of membrane spanning proteins called connexins for intercellular signaling. Connexins are a large family of proteins that oligomerize from groups of six polypeptides to form an aqueous pore known as a connexon. Gap junctions form when two connexons on adjacent cells align with one another through interactions involving their extracellular loop domains. Functioning gap junctions permit the intercellular transfer of ions, metabolites and signaling molecules that are needed to regulate coordinated cellular activity in many physiological systems (Simpson et al., 1977; Sáez et al., 2003).

The sensitivity of gap junctions to abused solvents such as toluene is unknown. However, compounds such as halothane, heptanol, octanol have been previously reported to directly close gap junctions expressed in various cell lines (Rozental et al., 2001). Additionally, ethanol has been shown to indirectly close gap junctions via a second messenger system involving elevated intracellular calcium (Mustonen, 2004). Since toluene shares many of the same pharmacological actions as these compounds, we tested whether physiologically relevant concentrations of toluene would have a similar effect on gap junctions. In this study, an electrophysiological approach was used to determine whether endogenous gap junction proteins are affected by concentrations of toluene known to alter the activity of ion channel proteins.

## 2. Methods

### 2.1. Cell culture, drugs and chemicals

Human embryonic kidney (HEK) 293 cells were cultured as previously described (Blevins et al., 1997). Cells were split weekly and plated on 35 mm polyornithine-treated dishes 1–2 days prior to use. Unless otherwise noted, all experimental compounds were purchased from Sigma Chemical Co. (St. Louis, MO).

### 2.2. Electrophysiology

Cells were perfused with recording solution that contained (in mM): 135 NaCl, 5 KCl, 1.8 $CaCl_2$, 10 glucose, and buffered with 5 HEPES (pH adjusted to 7.2 with NaOH and to 325 mOsm with sucrose). The pipette recording solution was (in mM): 140 CsCl, 2.5 EGTA, 2 $MgCl_2$, 10 HEPES, 2 TEA, and 4 $K_2ATP$ (pH was adjusted to 7.4 with NaOH). Whole cell patch-clamp recordings were made using an Axopatch 200B amplifier (Axon Instruments, Union City, CA, USA) in conjunction with Clampex or Axograph software. Patch electrodes were pulled from standard wall borosilicate glass (o.d. = 1.5 mm, i.d. = 0.86 mm) with filament to an open resistance of 5–7 MΩ). HEK 293 cells were voltage clamped at −50 mV and voltage pulses (+10 mV, 200 ms duration) were applied every 30 s to evoke capacitative currents. Toluene-containing solutions were prepared fresh daily by diluting toluene directly into the recording buffer and were kept in glass-stoppered reservoirs. Solutions were applied directly to cells through a square glass capillary pipet (800 μm per side) using a pressurized glass and Teflon perfusion system. Each cell was tested for only one concentration of toluene and different dishes of cells were used for each experiment. Currents measured in the presence of toluene were normalized as a percent of the control current for each cell that was calculated by averaging the control currents obtained during the first three pulses.

### 2.3. Data analysis

Data for the toluene concentration–response curve were expressed as a percent of the pre-toluene control–response and are shown as the mean ± S.E.M. Dose–response data was analyzed using non-linear regression (Prism 4.0; GraphPad Software, San Diego, CA) with upper and lower limits set to 100 and 0%, respectively.

## 3. Results

The degree of electrical coupling between cells was measured by applying voltage pulses (+10 mV; 200 ms duration) to voltage-clamped HEK cells growing alone or in clusters. In single cells, the voltage step resulted in a rapid capacitative current transient that quickly decayed to a small steady-state current (Fig. 1a). In a cell that was growing in a cluster, the capacitative transient decayed into a larger steady-state current that persisted for the duration of the voltage pulse. The amplitude of this steady-state current was monitored as an indication of the degree of electrical coupling between cells (Fig. 1a; second trace). In the absence of drug, the amplitude of this current was relatively stable over time. As reported previously (Harks et al., 2003), application of the gap junction blocker 2-aminoethoxydiphenyl borate (2-APB; 40 μM) to a single cell had no effect (data not shown). However, when applied to a cell growing in a cluster, 2-APB reduced currents to the level observed for single cells (Fig. 1b). Following washout of 2-APB from the bath solution, there was a significant recovery of current to approximately 75% of the pre-drug level. Similar results were obtained with other gap junction blockers including niflumic acid (data not shown).

Using this experimental protocol, the steady-state current response evoked at 30 s intervals was measured before, during and after perfusion of cells with solutions containing different concentrations of toluene. As shown in Fig. 1d, steady-state currents elicited by the +10 mV voltage pulse were relatively stable over a 20-min recording period. Toluene, at concentrations of 0.1 and 0.3 mM produced negligible effects on steady-state currents. At a concentration of 0.4 mM

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