Effects of the Abused Solvent Toluene on Recombinant N-Methyl-D-Aspartate and non-N-Methyl-D-Aspartate Receptors Expressed in *Xenopus* Oocytes

SILVIA L. CRUZ, TOORAJ MIRSHAHI, BRIAN THOMAS, ROBERT L. BALSTER and JOHN J. WOODWARD

Department of Pharmacology and Toxicology, Medical College of Virginia Campus, Virginia Commonwealth University, Richmond, Virginia (T.M., R.L.B., J.J.W.); Departamento de Farmacología y Toxicología, CINVESTAV, IPN, Apartado Postal 22026, 14000 Mexico DF, Mexico (S.L.C.) and Center for Chemistry and Life Sciences, Research Triangle Institute, Research Triangle Park, North Carolina (B.T.)

ABSTRACT

Previous studies have shown that toluene, which is commonly abused, depresses neuronal activity and causes behavioral effects in both animals and man similar to those observed for ethanol. In this study, the oocyte expression system was used to test the hypothesis that toluene, like ethanol, inhibits the function of ionotropic glutamate receptors. Oocytes were injected with mRNA for specific N-methyl-D-aspartate (NMDA) or non-NMDA subunits and currents were recorded using conventional two-electrode voltage clamp. To enhance the low water solubility of toluene, drug solutions were prepared by mixing toluene with alkamuls (ethoxylated castor oil) at a 1:1 ratio (v:v) and diluting this mixture to the appropriate concentration with barium-containing normal frog Ringer solution. Alkamuls, up to 0.1%, had no significant effects on membrane leak currents or on NMDA-induced currents. Toluene, up to ~9 mM, had only minor effects on membrane leak currents but dose-dependently inhibited NMDA-mediated currents in oocytes. The inhibition of NMDA receptor currents by toluene was rapid, reversible and the potency for toluene's effects was subunit dependent. The NR1/2B subunit combination was the most sensitive with an IC_{50} value for toluene-induced inhibition of 0.17 mM. The NR1/2A and NR1/2C receptors were 6- and 12-fold less sensitive with IC_{50} values of 1.4 and 2.1 mM, respectively. In contrast, toluene up to ~9 mM did not inhibit kainate-induced currents in oocytes expressing GluR1, GluR1+R2 or GluR6 subunits. These results suggest that some of the effects of toluene on neuronal activity and behavior may be mediated by inhibition of NMDA receptors.

Toluene is a widely used industrial solvent and is a major component of adhesives, paint thinners and gasoline. In addition to these industrial uses, many toluene-containing products are abused via inhalation (Streicher *et al.*, 1981). Although inhalant abuse is a world wide drug abuse problem (Kozel *et al.*, 1995), relatively little is known about the neurobehavioral basis for the effects of abused inhalants (Balster, 1997). The volatile solvents including toluene and related compounds represent one of the largest classes of abused inhalants, and previous research has suggested several mechanisms for their actions on brain function and behavior.

Abused solvents such as toluene share a pharmacological profile with other abused depressant drugs including ethanol, barbiturates and benzodiazepines. Clinically, toluene intoxication resembles alcohol intoxication (Echeverria *et al.*, 1991), and in animal studies abused solvents act as central nervous system depressants (Evans and Balster, 1991). For instance, toluene produces motor impairment at moderate to high concentrations (Tegeris and Balster, 1994), has anticonvulsant effects (Silva-Filho *et al.*, 1991; Wood *et al.*, 1984), antianxiety drug-like effects (Wood *et al.*, 1984; Bowen *et al.*, 1996) and shares discriminative stimulus effects with barbiturates and ethanol (Rees *et al.*, 1987; Knisely *et al.*, 1990). This has led to the hypothesis that some abused inhalants may share common mechanisms of action with other abused depressant drugs.

There are substantial data demonstrating that ethanol alters the function of ligand-gated channels expressed in brain neurons (Grant and Lovinger, 1995). One of the best studied examples of this interaction is with the NMDA and non-NMDA family of ionotropic neuronal glutamate receptors. The NMDA subtype of glutamate receptor is a calcium-permeable ligand-gated channel which plays a critical role in synaptic transmission and is involved in modulating a variety of complex events including neuronal plasticity and excitotoxicity (Nakanishi, 1992). Molecular cloning studies have

**ABBREVIATIONS:** AMPA, DL-α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; Ba-NFR, barium-containing normal frog Ringer; HEPES, N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid; MS222, tricaine methanesulfonate; NMDA, N-methyl-D-aspartate.

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revealed that NMDA receptors are made up of multiple subunits which fall into the NR1 and NR2 (A-D) families (for a review see Hollman and Heinemann, 1994). Acute exposure of both native and recombinant NMDA receptors to ethanol (10–100 mM) inhibits ion flux (Lovinger et al., 1989; Gonzales and Woodward, 1990; Mirshahi and Woodward, 1995). Data from animal behavior studies suggest that ethanol inhibition of NMDA receptors is an important determinant of acute ethanol intoxication (Grant et al., 1991).

The AMPA/kainate subtype of glutamate receptors are also ionotropic glutamate receptors and their activation underlies most of the fast synaptic transmission in the brain. AMPA-selective channels form from four subunits (GluR1-GluR4) although kainate-selective channels are formed by the GluR5-7 subunits (Hollman and Heinemann, 1994). Recombinant non-NMDA receptors expressed in oocytes and HEK293 cells are also inhibited by ethanol (Dildy-Mayfield and Harris, 1995) although native receptors expressed in neurons appear to be less sensitive (Lovinger et al., 1989).

In this study, we have used the oocyte expression system to test the hypothesis that toluene, like ethanol, inhibits the function of NMDA and non-NMDA receptors. The results indicate that toluene antagonizes the function of NMDA receptors expressed in oocytes in a subunit-selective fashion but has little effect on non-NMDA receptors.

Materials and Methods

NMDA, glycine, kainic acid, tricine methanesulfonate (MS222) and collagenase were purchased from Sigma Chemical Co. (St. Louis, MO), concanavalin A from ICN (Costa Mesa, CA), toluene HPLC grade from Aldrich (Milwaukee, WI) and alkamuls EL-620 (ethoxylated castor oil) from Rhone-Poulenc (Princeton, NJ). NMDA receptor cDNA clones in Bluescript vectors were kindly provided by S. Nakanishi (Kyoto University, Kyoto, Japan), P. Seeburg (University of Heidelberg, Heidelberg, Germany) and M. Mishina (University of Tokyo, Tokyo, Japan). GluR1, GluR2 and GluR6 cDNA clones in pBluescript were generous gifts of S. Heinemann (Salk Institute, La Jolla, CA).

Synthesis of mRNA. All clones were linearized downstream from the coding sequence with the appropriate restriction enzyme, purified by phenol/chloroform extraction, precipitated with ethanol and resuspended in RNase-free distilled water before being used in the in vitro transcription reaction (Ambion, Austin, TX). Formaldehyde gels were used to confirm the quality and the size of the synthesized mRNA.

Oocyte preparation and microinjections. Adult Xenopus laevis female frogs were purchased from Xenopus I (Ann Arbor, MI). Frogs were anesthetized before surgery by immersion in a 0.2% MS-222 solution. Stage V and VI eggs were dissected and treated for 45 to 60 min with a collagenase (1 mg/ml) containing solution before mRNA injection (Variable Nanodot, Drummond Scientific Co., Broomall, PA). Oocytes were injected with either 1) 5 to 10 ng of NR1 and NR2 mRNA at a ratio of 1:1 for NMDA receptors, 2) 10 to 20 ng for GluR1, 3) 10 ng GluR1 + 50 ng GluR2 or 4) 10–20 ng GluR6. Oocytes were maintained at 18°C in L-15 media at pH 7.4 supplemented with 10,000 U/liter penicillin G, 10 mg/liter streptomycin and 15.5 mg/liter gentamycin for up to 7 days before recording.

Drug solutions. Fresh solutions were used in all the experiments. Ba-NFR, in mM: NaCl (115), KCl (2.5), HEPES (10) and BaCl2 (1.8); pH 7.2, was used to eliminate the activation of endogenous calcium-dependent chloride channels during NMDA perfusion (Leonard and Keino, 1990). Rainic acid and concanavalin A solutions were made by dissolving the substance directly into the Ba-NFR at the desired concentration. Because of its limited solubility in aqueous solvents, toluene solutions were made using alkamuls as vehicle (Knisely et al., 1990). Very homogenous suspensions were obtained by mixing toluene with alkamuls at a ratio of 1:1 (v:v). Dilutions of this mixture using Ba-NFR were made to obtain the following solutions: 0.1% (9.39 mM), 0.05% (4.69 mM), 0.025% (2.35 mM), 0.012% (1.17 mM), 0.006% (0.56 mM), 0.003% (0.28 mM). A 1:10 dilution of the 0.012% solution was made to achieve the 0.001% (0.11 mM) concentration. For reasons of clarity, the closest rounded mM concentration are expressed in graphs.

To determine the stability of these solutions over time, a 1 mM solution of toluene in alkamuls was prepared in Ba-NFR as described above, stored in an open container and sampled every 15 min. The toluene concentration in the samples was determined by gas chromatography/mass spectrometry (GC/MS). Briefly, a 1-μl sample was injected into the GC/MS (Hewlett-Packard) and selected-ion monitoring was performed on m/z 91 (tropolium ion) for quantitation of toluene. Expressed as a percent of the initial time zero control value, toluene concentrations at 15, 30, 45 and 60 min were (mean ± S.E.; n = 3) 77.9 ± 15; 75.7 ± 15.4; 73.0 ± 19.3 and 70.8 ± 22.9, respectively.

To minimize variability in the oocyte recording studies due to loss of solutions, solutions were placed in the open perfusion containers at least 15 min before recordings were made. All concentrations of toluene shown are uncorrected for loss of sample to evaporation.

Electrophysiological recordings. Eggs were placed in a 200-μl recording chamber and continuously perfused with Ba-NFR at a flow rate of 4 to 6 ml/min. Oocytes were impaled with two microelectrodes (0.1–0.8 ΜΩ) filled with 3 M KCl and agarose 0.8% (Schreibmayer et al., 1994) and voltage-clamped at −80 mV using a Geneclamp amplifier (Axon Instruments Inc., Foster City, CA). Data were acquired and analyzed on a Macintosh Centris 650 computer equipped with an Instutech ITC-16 computer interface and Pulse Control, version 4.3 (Herrington and Bookman, 1994) and Igor Pro, version 3 (WaveMetrics) software. NMDA receptors were stimulated by switching the perfusion solution to one containing NMDA (100 μM) plus glycine (10 μM): Similarly, non-NMDA receptors were stimulated by switching the perfusion solution to one containing either 400 μM kainate (GluR1 and GluR1+GluR2 combinations) or 10 μM kainate (GluR6 receptors). To prevent desensitization, GluR6 receptors were pretreated with concanavalin A (10 μM) for 5 min before recording. In all cases, non-drug-treated responses were obtained before and after each toluene concentration and were averaged to give the control response. Net agonist-stimulated current responses (in nA) were expressed as percentage of the average control value to reduce variability associated with the varying levels of expression among different batches of oocytes.

Data and statistical analysis. Data from each oocyte represent a single observation and oocytes from at least two different frogs were tested for each experimental condition. Dose response curves were analyzed using the ALLFIT program. Comparisons between two means were performed using the Student’s t test and comparisons among several groups were made using a one-way analysis of variance procedure with post hoc testing where appropriate. Differences between means were considered significant when P < .05.

Results

Alkamuls, up to the highest concentration used (0.1%), had no significant effects on membrane leak currents of oocytes clamped at −80 mV (fig. 1A). Toluene up to the highest concentration tested (0.1%; 9 mM) slightly increased the leak current (fig. 1B) by 7.2 ± 1.8 nA (mean ± S.E.). This effect was rapidly reversed when the toluene-containing solution was switched back to Ba-NFR. Alkamuls (0.1%) had no effect on the magnitude and/or the shape of NMDA-induced currents in oocytes expressing recombinant NMDA receptors, as represented by the sample tracing for the R1/2A receptors.
In contrast, as shown in figure 2, toluene dramatically inhibited NMDA-induced currents in oocytes. Each panel in figure 2 shows a representative example of currents induced by 100 μM NMDA and 10 μM glycine in oocytes expressing different receptor subunits. The first trace in each panel corresponds to a control current induced by the agonist, followed by an agonist plus toluene (9 mM for NR1/2A and NR1/2C, and 1 mM for NR1/2B), followed by the second control response obtained within 3 min after washing out the toluene. Note that the toluene-induced inhibition was rapid and that control currents were fully recovered during the 3-min washout period. In addition, the effects of toluene on NMDA receptor function were subunit-selective with the NR1/2B receptors being significantly more sensitive than either the NR1/2A or NR1/2C subtype combinations. This is more clearly demonstrated by the concentration-response curves for the inhibitory effects of toluene on the different receptor combinations shown in figure 3. For all subunit combinations tested, toluene dose-dependently inhibited NMDA-induced currents with NR1/2A and NR1/2B being almost completely inhibited. The toluene inhibition of NR1/2C receptors was not maximal even at the highest concentration tested (9 mM). Higher concentrations could not be reliably tested due to irreversible effects of toluene on oocyte membrane leak currents. The IC50 values for toluene’s inhibition of NMDA-induced currents were 1.4 mM (NR1/2A), 0.17 mM (NR1/2B) and 2.1 mM (NR1/2C).
NMAD-activated currents were 0.17 ± 0.01 mM for NR1/2B, 1.40 ± 0.17 mM for NR1/2A and 2.13 ± 0.27 mM for NR1/2C. Although the Hill coefficients for NR1/2A and NR1/2C were near unity (0.76 ± 0.07 and 1.1 ± 0.1, respectively) that for NR1/2B was significantly higher (3.8 ± 0.5).

In some cases, the block of NMAD-induced currents by lower concentrations of toluene was biphasic consisting of a peak effect and a steady-state block that developed during the course of the drug administration (fig. 4; compare traces 1 and 2). These results suggested that at lower concentrations, toluene may slowly gain access to its blocking site. To further investigate this possibility, the toluene-containing solution was introduced before NMAD receptor activation. As shown in figure 4 (trace 3), a 20-sec exposure to toluene alone had no effect on the membrane leak current. However, this preexposure protocol significantly reduced the magnitude of the peak effect of toluene on NMAD-induced currents as compared with the effects of toluene without preexposure (compare traces 2 and 3). The preexposure protocol did not significantly change the percent inhibition of NMAD-induced currents by toluene measured at the end of the NMAD/toluene perfusion period (with preexposure, 28.9% ± 3.4 vs. without preexposure, 29.4% ± 3.5; n = 5). It should be noted that the toluene dose response curves shown in figure 3 represent the steady-state inhibition obtained during the course of the toluene exposure.

A series of experiments were performed using the NR1/2B subunit combination to investigate possible mechanisms of action for toluene’s inhibition of NMAD receptors. As summarized in figure 5, the inhibition of NMAD-stimulated currents by 1 mM toluene was not affected by increasing the concentrations of either NMAD (1 mM) or glycine (100 μM) as would be expected if toluene acted as a competitive NMAD or glycine site antagonist. To assess the voltage-dependence of the block, current-voltage (IV) curves were generated in the absence and presence of toluene by slowly ramping the holding membrane potential from −80 to +20 mV after establishing a steady state NMAD activated current (fig. 6). As expected, the control IV curve for the NR1/2B receptor in the magnesium-free recording solution was linear with a reversal potential near zero. Toluene’s (1 mM) inhibition of the magnesium-free recording solution was linear with a reversal potential near unity (0.76 ± 0.07 and 1.1 ± 0.1, respectively) that for NR1/2B was significantly higher (3.8 ± 0.5).

Finally, the effects of toluene on GluR1, GluR1+GluR2 and GluR6 receptors expressed in oocytes were investigated to assess the receptor selectivity of toluene’s effects. Figure 7A shows a representative IV curve demonstrating the inward rectifying nature of the homomeric GluR1 receptor during stimulation with 400 μM kainate. This IV relationship was converted to a linear IV curve when the GluR1 was coexpressed with the GluR2 subunit as previously described by Hollmann et al. (1991). Figure 7B shows that toluene (1–5 mM) had no effect on kainate-induced currents in oocytes expressing homomeric GluR1 receptors. However, at the highest concentration tested (9 mM), toluene produced a statistically significant potentiation (68% increase) of kainate-induced currents. At this concentration, toluene had no effect on kainate-induced currents in oocytes expressing GluR1+R2 receptors although it slightly (15–20%) enhanced kainate-activated currents in GluR6-injected oocytes (fig. 7C).
The major finding of this work is that toluene inhibited recombinant NMDA receptors in a dose-dependent and subunit-dependent manner. This inhibition occurred at concentrations of toluene (<10 mM) that did not significantly alter the resting membrane conductance of un.injected oocytes. These findings strongly suggest that the inhibitory effects of toluene on NMDA-induced currents were not due to a nonspecific disruption of the oocyte membrane or activation of endogenous ion channels. Such a disruption may have been expected to induce membrane currents in the absence of any receptor stimulation. No evidence of this was found although higher concentrations of toluene (>20 mM) could induce an irreversible increase in the membrane leak current suggesting that membrane integrity was compromised.

At concentrations that significantly inhibited currents carried by NMDA receptors, toluene had only minor effects on non-NMDA GluR1, GluR1+R2 and GluR6 receptors. The potentiation of GluR1 receptor-mediated currents by 9 mM toluene was statistically significant but it is unlikely that this effect is physiologically relevant with respect to toluene's neurobehavioral actions. Lethal concentrations of toluene are on the order of 1 mM and enhanced non-NMDA receptor function by toluene would be expected to produce seizures and/or convulsions which is counter to the anticonvulsant actions of this compound observed by others (Silva-Filho et al., 1991; Wood et al., 1984). The differential effects of toluene on ionotropic glutamate receptors also support the conclusion that toluene's effects on NMDA receptors were not due to a nonspecific membrane interaction.

Despite these findings, changes in membrane integrity and fluidization have been proposed as a possible mechanism of action for solvents. According to some authors, in vitro exposure to toluene at concentrations above 1 mM produces an increase in synaptosomal membrane fluidity (Edelfors and Raven-Jansen, 1989; Engelke et al., 1992). However, Le Bel and Schatz (1988) reported that toluene (0.5–5 mM) had no effect in the same experimental preparation. Our results show that toluene, up to 9 mM, does not have an important effect on the membrane integrity of oocytes as measured by changes in membrane conductance.

**Toluene: comparison to ethanol.** The major hypothesis that was tested in this study was that toluene would exert effects on recombinant NMDA receptors that were similar to those previously observed for ethanol. Ethanol, at concentrations that are associated with intoxication (10–100 mM), inhibits the function of native and recombinant NMDA receptors (Lovinger et al., 1989; Kuner et al., 1993). Ethanol has also been shown in behavioral studies to produce discriminative stimulus effects similar to those of NMDA antagonists suggesting that it may also act as an NMDA antagonist in vivo (Grant et al., 1991). Although our results support the hypothesis that toluene also inhibits NMDA receptor activity, it is clear that toluene is more potent than ethanol in producing these effects.

In animal behavioral studies, toluene was found to substitute for ethanol in animals trained to discriminate between ethanol and saline (Rees et al., 1987) and is more potent than ethanol for acute effects on learned behavior (Moser and Balster, 1986). Our results support these observations and show that the estimated IC$_{50}$ of toluene for inhibiting NMDA-induced currents in oocytes is in the range from 0.2 to 2 mM depending on the subunit combination expressed. These values are approximately 50 to 500 times less than the IC$_{50}$ values reported for ethanol (100 mM) using the same experimental preparation (Mirshahi and Woodward, 1995). It should be noted that in those studies, ethanol was dissolved directly in the perfusion buffer although an alkamuls-containing solution was used in our study to enhance the solubility of toluene in aqueous solutions. Recent experiments indicate that the ethanol sensitivity of recombinant NMDA receptors expressed in oocytes is not altered by the presence of 0.1% alkamuls (Woodward J, unpublished observations). These findings suggest that the enhanced potency of toluene as compared to ethanol is not due to an effect of the vehicle but is likely due to a greater affinity of toluene for its site of action.

Similar findings have been reported for long-chain alcohols that also inhibit the NMDA receptor with potencies that are directly correlated with their chain length and therefore hy-
drophobicity (Peoples and Weight, 1995). Interestingly, the antagonist potency of these alcohols on NMDA receptor function does not increase past 12 carbons despite greater hydrophobicity. These findings suggest that toluene, like ethanol and other long-chain alcohols, may alter NMDA receptor function via an interaction with a hydrophobic site. Although it is not known where such a site might exist, it is clear that toluene and ethanol do not act as simple competitive inhibitors of glutamate or glycine binding as their inhibition persists in the presence of high concentrations of the agonists. The inhibitory effects of these agents are also not voltage-dependent suggesting that they are not acting as direct channel blockers such as is found for magnesium. It is possible that toluene, ethanol and other long-chain alcohols may disrupt receptor gating processes by interfering with the subtle movements of transmembrane domains that mark the transition between closed and open states of the channel.

Subunit selectivity. The effects of toluene on NMDA receptor function were clearly subunit-dependent with NR1/2B receptors being approximately 6 to 12 times more sensitive to toluene than the NR1/2A and NR1/2C combinations. The NR1/2C receptor was approximately 1.5 times less sensitive to inhibition by toluene as compared to NR1/2A. These differences in subunit sensitivity to toluene are more marked than those found for ethanol. In most studies the NR1/2A and NR1/2B combinations are similarly inhibited by ethanol although the NR1/2C and NR1/2D receptors are less sensitive (Masood et al., 1994; Mirshahi and Woodward, 1995; but see Kuner et al., 1993). In addition, the shapes of the dose-response curves for ethanol-induced inhibition of NMDA receptors are similar to one another although the dose-response curve for toluene’s inhibition of the NR1/2B receptors was very steep. Thus, it was almost possible to fully inhibit NR1/2B receptors at a concentration of toluene (0.3 mM) that only slightly reduced currents carried by NR1/2A or NR1/2C receptors. The differences in Hill coefficients between the toluene concentration-response curves for the different receptor subtypes suggest that toluene’s interaction with NR1/2B might involve more than a single site of action or some kind of cooperativity.

Although the physiological significance of these findings remains to be determined, they suggest that toluene which is inhaled as an abused solvent may preferentially inhibit those NMDA receptors comprised of the NR1/2B subunits. NR2B subunits are highly expressed in the forebrain of embryonic and neonatal rodents with the NR2A subunit showing increased expression during development (Monyer et al., 1994). Exposure of both humans (Pearson et al., 1994) and mice (Jones et al., 1997) to toluene in utero is associated with antaomical and neurobehavioral deficits that are manifested during postnatal development. Many of these changes are similar to those observed in fetal alcohol syndrome. Although speculative, our results suggest that some of the prenatal effects of toluene may be related to its inhibition of NR2B containing receptors.

Toluene: comparison to volatile anesthetics. In addition to producing ethanol-like pharmacological and behavioral effects, toluene and other solvents have effects in common with volatile anesthetics such as halothane (Evans and Balster, 1991). At high concentrations, toluene can produce an anesthetic-like state in mice (Tegeris and Balster, 1994). Anesthetics also produce behavioral effects in animals at subanesthetic concentrations that resemble those produced by toluene and other abused solvents (Moser and Balster, 1986). Thus, it is not surprising that volatile anesthetics, such as toluene, are also subject to abuse (Yamashita et al., 1984).

Although most research on the effects of anesthetics on ligand-gated ion channels has focused on the enhancement of GABA-mediated inhibition (Harris et al., 1995), there have been some studies showing effects on glutamate ionotropic receptors as well (Franks and Lieb, 1994). Insufficient data exist to directly compare the cellular actions of abused solvents and anesthetics, but it may not be surprising to find some common mechanisms for their effects.

Relevance to the behavioral effects of toluene. All NMDA subunit combinations in this study were significantly inhibited by toluene concentrations from 0.1 to 1 mM. However, it is difficult to know whether these concentrations are similar to those that cause behavioral effects in vivo. In mice, toluene produces ethanol-like discriminative stimulus effects at vapor concentrations of approximately 1000 ppm (Rees et al., 1987). Although blood or brain levels were not measured in the Rees study, Benignus et al. (1991) measured toluene concentrations in rats exposed via inhalation. Blood and brain levels were 10.5 and 18.0 ppm (100–200 μM, approximately) after exposure to 575 ppm toluene. In humans, concentrations of toluene in the range of 10 to 100 μM have been found in blood samples from inhalant abusers (King, 1982; Morton, 1987; Meredith et al., 1989). Finally, the brain toluene concentration of a worker who died after acute intoxication was 80 μg/g (80 ppm; 1 mM, approximately; Takeichi et al., 1986). The lowest concentration of toluene that significantly affected NMDA receptor function in this study was approximately 100 μM although it should be noted that this value is likely to be lower because there was some loss of toluene (about 25%) from the experimental solutions due to evaporation. Taken together with the animal data discussed above and the steep dose response of the NR1/2B receptor (toluene IC₅₀, 170 μM), the concentrations of toluene found in this study to inhibit NMDA receptors appear to be relevant to those associated with behavioral effects of toluene in both animals and man.

Summary

Our results are the first direct evidence that toluene directly alters the function of a neurotransmitter-gated ion channel. The effects of toluene on the NMDA receptor were similar to those produced by ethanol although they occurred at concentrations at least 100 times lower. The lack of a dose-dependent effect of toluene on oocyte leak currents or on non-NMDA glutamate ionotropic receptors argues against a nonspecific membrane disruption mechanism of action for this solvent. These results suggest that some of the behavioral effects of toluene and other abused solvents may be due to its inhibition of neuronal NMDA receptors.

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References


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