ELSEVIER

Pharmacology, Biochemistry and Behavior 71 (2002) 163–169

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

Evaluation of 1,1,1-trichloroethane and flurothyl locomotor effects following diazepam treatment in mice

Jenny L. Wiley*, R. Ellis Fagaldé, Katriona G. Bühler, Kari L. LaVecchia, Robert L. Balster

Department of Pharmacology and Toxicology, Medical College of Virginia, Virginia Commonwealth University, P.O. Box 980613, Richmond, VA 23298-0613, USA

Received 10 April 2001; received in revised form 26 July 2001; accepted 15 August 2001

Abstract

The abused volatile solvent 1,1,1-trichloroethane (TCE) shares many acute behavioral effects with central nervous system (CNS) depressants; however, demonstration of tolerance to these effects has been difficult. The purpose of the present study was to investigate the development of TCE-induced changes in locomotor activity in mice following repeated injections with diazepam. In the initial concentration–effect curve determinations, diazepam decreased locomotor activity at all doses tested and TCE produced a biphasic effect, increasing locomotor activity at lower concentrations with return to control levels at a high (16,000 ppm) concentration. Flurothyl, a vapor with convulsive properties, had no pronounced effects on locomotor activity at subconvulsant concentrations. Following four daily injections with vehicle or with 10 mg/kg/day diazepam, mice were administered the same concentration of drug/inhalant that they received initially and were retested for locomotor activity effects. Concentration–effect curves for diazepam and flurothyl were not altered by this modest regimen of repeated dosing with diazepam. In contrast, sensitization to the locomotor-stimulating effects of TCE was observed in diazepam-treated mice, but not in vehicle-treated mice. These results suggest that the development of sensitization to TCE involves common mechanisms with those that are affected by repeated dosing with the CNS depressant diazepam.

Keywords: Diazepam; Cross-tolerance; Flurothyl; Locomotion; Sensitization; 1,1,1-Trichloroethane

1. Introduction

The abuse of high concentrations of volatile organic solvents contained in many household and industrial products continues to be a public health problem, particularly among children in middle school (National Institute on Drug Abuse, 1995). Although the neural basis for the pharmacological effects of abused inhalants is unknown, previous research suggests that some of them may share properties with various drugs of abuse (Balster, 1998). For example, the acute behavioral properties of toluene and 1,1,1-trichloroethane (TCE), two of the most investigated inhalants that are self-administered by humans, resemble those of central nervous system (CNS) depressants. In animals, these inhalants increase punished responding (Wood et al., 1984), fully or partially substitute and cross-substitute for CNS depressants in drug discrimination studies (Bowen et al., 1999; Knisely et al., 1990; Rees et al., 1987a,b), and produce biphasic effects on locomotor activity (Bowen and Balster, 1996, 1998) and operant responding (Moser and Balster, 1981). In contrast, flurothyl, a volatile chemical with convulsive properties (Adler, 1975), does not share these behavioral effects with toluene and TCE (Bowen et al., 1996a,b, 1999) and is not abused by humans. Indeed, flurothyl substitutes for the convulsant pentylenetetrazol in mice trained to discriminate this drug from saline (Evans and Balster, 1992). Hence, the neural basis for flurothyl's pharmacological effects probably differs from that of TCE and other abused inhalants.

Given that inhalant abuse often involves frequent use, it is important to determine not only the acute pharmacological effects of inhalants, but also their behavioral effects following repeated administration. Inhalant abusers have reported development of pronounced tolerance with repeated use of inhalants (Glaser and Massengale, 1962; Novak, 1980); however, this phenomenon has been difficult to observe in animals. Previous research has shown only modest tolerance

* Corresponding author. Tel.: +1-804-828-2067; fax: +1-804-828-2117.

E-mail address: jwiley@hsc.vcu.edu (J.L. Wiley).

0091-3057/01/$ – see front matter
PII: S0091-3057(01)00645-1

development to the response rate-decreasing effects of TCE under a fixed-ratio schedule (Moser et al., 1985) and no tolerance to the rate-decreasing effects of toluene under a differential reinforcement of low rates schedule (Moser and Balster, 1981). A moderate degree of tolerance developed to the effects of trichloroethylene in a signal detection task (Bushnell and Oshiro, 2000) and to the effects of toluene on accuracy in signaled and unsignaled fixed consecutive number procedures (Rees et al., 1989). This tolerance was primarily behavioral in nature, as the opportunity to practice the behavior during exposure was required. Other studies, however, have reported that repeated exposure to toluene produced sensitization rather than tolerance to initial increases in motor activity or response rates observed following acute administration of this inhalant (Himnan, 1984; Moser and Balster, 1981). One of the reasons that tolerance/sensitization to inhalant effects may be difficult to demonstrate in animals is that the fast rate of clearance of these substances from the body following inhalation may necessitate chronic administration of high concentrations over a long period of exposure. This problem is lessened with repeated administration of CNS depressant drugs that have longer half-lives. Rapid tolerance to the acute motor impairment effects of diazepam has been noted following as little as a single prior injection (Khanna et al., 1998). In the same study, a single diazepam dose also produced cross-tolerance to ethanol. As noted above, we and others have previously demonstrated that TCE shares a profile of acute behavioral effects with diazepam and ethanol. The purpose of the present study was to investigate whether cross-tolerance to the effects of TCE on locomotor activity would develop in animals injected repeatedly with diazepam. Subconvulsant concentrations of flurothyl were also tested as a negative control, as its profile of behavioral effects does not resemble those of the CNS depressants or TCE (Bowen et al., 1996b; Evans and Balster, 1992; Rees et al., 1987c).

2. Methods

2.1. Subjects

Male ICR mice (25–32 g), purchased from Harlan (Dublin, VA), were housed in groups of six in plastic cages with wood chip bedding. All animals were kept in a temperature-controlled (20–22 °C) environment with a 12-h light–dark cycle (lights on at 7:00 a.m.) and received food and water ad libitum. Mice were transported to the laboratory for all testings, which were carried out during the light cycle. Different mice were used for testing each drug dose or inhalant concentration.

2.2. Apparatus

Vapor exposures were conducted in 29-l transparent glass cylindrical jars (47 cm height×35 cm diameter; total floor space=962 cm^2), which have been described previously (Moser and Balster, 1981). Briefly, vapor generation commenced when liquid anesthetic was injected through a port onto filter paper suspended below the sealed lid. A fan, mounted on the inside of the lid, was then turned on, which volatilized and distributed the agent within the chamber. Nominal chamber concentrations did not vary by more than 10% from measured concentrations as determined by single wavelength monitoring infrared spectrometry (Miran 1A; Foxboro Analytical, North Haven, CT). Two pairs of standard photocells, mounted at right angles to each other outside and near the glass bottom of the static exposure chamber, were used to measure locomotor activity. Locomotor activity was defined as the sum of the interruptions of either photocell beam (counts) during each 20-min exposure to air or inhalants. A computer with Med-PC software and interfacing (Med Associates, Georgia, VT) was used to record locomotor counts.

2.3. Procedure

Prior to testing, mice were habituated to the static exposure chambers during three 20-min sessions. During these sessions, they were exposed to air and allowed to traverse freely within the chamber. Following habituation, concentration– and dose–effect curve determinations with diazepam and with each of the inhalants were conducted in separate groups of mice (n=5–6 per dose/concentration). For the initial diazepam dose–effect curve determinations (Day 1), each mouse was injected with a dose of diazepam or vehicle and placed back into their home cage. Fifteen minutes later, the mouse was removed from its home cage and placed in the exposure chamber for 20-min exposure to air. Photocell beam breaks were counted in 5-min bins during exposure. After removal from the chamber, half of the mice in each dose group was assigned to the repeated vehicle treatment group and half was assigned to the repeated diazepam group. Mice in the repeated vehicle group received an injection of vehicle after removal from the chamber and then received a single daily vehicle injection during the next three mornings (Days 2–4). Mice in the repeated diazepam group were injected with a supplemental dose of diazepam following removal from the exposure chamber such that total daily dose of diazepam equaled 10 mg/kg (e.g., vehicle-treated mice received 10 mg/kg and mice treated with 10 mg/kg diazepam received vehicle). Subsequently, they received a single 10-mg/kg dose of diazepam during the next three mornings (Days 2–4). Throughout the study, all diazepam and vehicle injections occurred in the laboratory in which testing was conducted. Locomotor activity sessions were not conducted in either group of mice during repeated dosing with vehicle or diazepam. During the morning of Day 5, a second diazepam dose–effect curve determination was conducted. Each mouse in both repeated dosing regimens was injected with the same acute dose of diazepam that it had received

during the initial dose–effect curve determination on Day 1 and was placed in the chamber for 20-min exposure to air, during which locomotor activity was measured.

Inhalant concentration–effect curves were determined in a similar manner. The mice were habituated to the chambers for 3 days. Then, on Day 1, each mouse was placed in the exposure chamber and exposed to air or to a single concentration of the inhalant. Locomotor activity was measured during the 20-min exposure. After removal from the chamber and on the next three mornings, mice were injected with either 10 mg/kg diazepam or vehicle. On Day 5, each mouse was retested with the same concentration of inhalant that it had received during the initial concentration–effect curve determination.

2.4. Chemicals

Diazepam (Schein Pharmaceuticals, Port Washington, NY), TCE (T391; Fisher Scientific) and flurothyl (28-757-1; Aldrich, Milwaukee, WI) were purchased commercially. The 5-mg/ml stock concentration of diazepam was diluted to desired concentrations with a vehicle of ethanol (10%), propylene glycol (40%) and sterile water (50%). All diazepam and vehicle injections were given intraperitoneally in a volume of 10 ml/kg. Vapor concentrations shown in the figures and table are calculated nominal concentrations. All vapor exposures were 20 min in duration.

2.5. Statistical analysis

Separate split-plot ANOVAs were performed for each drug or inhalant and for each repeated dosing regimen (diazepam vs. vehicle). Locomotor count was the dependent variable. Separate mean (±S.E.M.) numbers of counts were calculated for each 5-min bin of each 20-min session. The repeated measures factors for each ANOVA were Time (Day 1 vs. Day 5) and Bin (1–4). The between-subjects factor for each ANOVA was Dose or Concentration. When the ANOVA was significant, Tukey post hoc tests (α=.05) were used to compare individual means.

3. Results

Fig. 1 shows locomotor counts across doses of diazepam administered before and after repeated administration of diazepam (left panels) or vehicle (right panels). A significant main effect for diazepam was obtained for each treatment condition. When administered prior to the repeated dosing regimen, diazepam decreased locomotor counts in both groups of mice across all 5-min bins of the 20-min test sessions. Following repeated dosing with either diazepam or vehicle, the second diazepam dose–effect curves for each condition showed little change from the first curves.

A significant three-way interaction (Concentration × Bin × Time) was obtained with TCE for each treatment

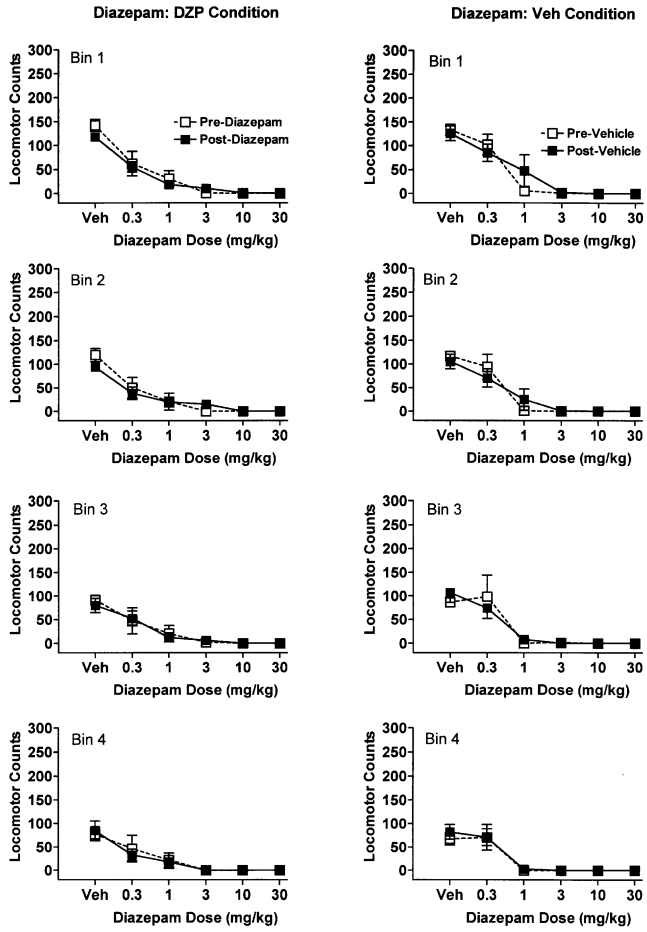

Fig. 1. Effects of diazepam on locomotor activity before (□) and after (■) 4 days of repeated dosing with 10 mg/kg/day diazepam (left panels) or vehicle (right panels). Descending panels present activity counts during consecutive 5-min bins of 20-min test sessions. Each point represents the mean (±S.E.M.) locomotor counts for four to six mice. For mice in the diazepam condition, analysis of the significant main effect for diazepam dose indicated that all doses of diazepam decreased activity compared to the vehicle control. For mice in the vehicle condition, analysis of the significant diazepam dose main effect showed that doses of 1 mg/kg and higher decreased activity compared to vehicle.

condition. As shown in Fig. 2, the initial concentration–effect curves for TCE were shifted upward during the last three bins following repeated daily injections of diazepam (left panels). The concentrations of TCE, at which activity was significantly increased after diazepam treatment (as compared to the initial testing of the concentration), were dependent upon bin. Mice in the diazepam condition were significantly more sensitive to the activity-increasing effects of 8000 ppm TCE following diazepam treatment during Bin 2. After diazepam treatment, they were significantly more sensitive to the effects of 12,000 ppm TCE during the last 10 min of the session (Bins 3 and 4). During the first 10 min of the session (Bins 1 and 2), mice were significantly more resistant to the decrease in activity to control levels obtained following initial exposure to 16,000 ppm TCE, although a floor effect may have prevented detection of significant effects during later bins. In the vehicle treatment group,

significant differences between pre- and post-effects of TCE were not found at any concentration or during any bin.

In addition to the significant interactions, main effects of TCE concentration were obtained for both treatment conditions. TCE had biphasic effects on activity during the last 15 min of the session. In both groups of mice, TCE increased locomotor activity at one or more concentrations between 4000 and 12,000 ppm, returning towards air control levels at higher concentrations. Locomotor activity was not significantly decreased at any concentration of TCE.

Results with flurothyl are shown in Fig. 3. A significant main effect for flurothyl was obtained for each treatment condition. In the diazepam-treated group, flurothyl did not affect locomotor counts compared to vehicle (left panels). In the vehicle-treated group, 900 ppm flurothyl significantly decreased locomotor activity (right panels), although the magnitude of the decrease was small. Pre- and postdiazepam treatment concentration–effect curves for flurothyl did not differ in either group of mice.

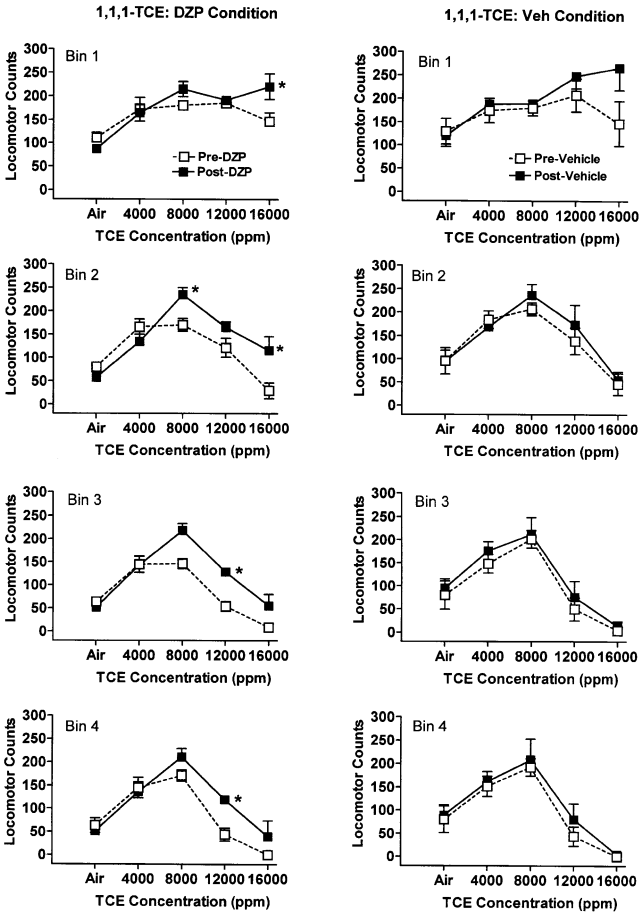

Fig. 2. Effects of TCE on locomotor activity before (□) and after (■) 4 days of repeated dosing with 10 mg/kg/day diazepam (left panels) or vehicle (right panels). Descending panels present activity counts during consecutive 5-min bins of 20-min test sessions. Each point represents the mean (±S.E.M.) locomotor counts for four to six mice. For mice in the diazepam condition, analysis of the significant main effect for TCE concentration indicated that 4000, 8000 and 12,000 ppm TCE increased activity compared to air exposure. For mice in the vehicle condition, analysis of the significant TCE concentration main effect showed that only the 8000 ppm concentration of TCE increased activity compared to air exposure. * Indicates a three-way interaction effect (i.e., a postrepeated dosing mean is significantly different from the corresponding prevehicle or prediazepam mean during the bin).

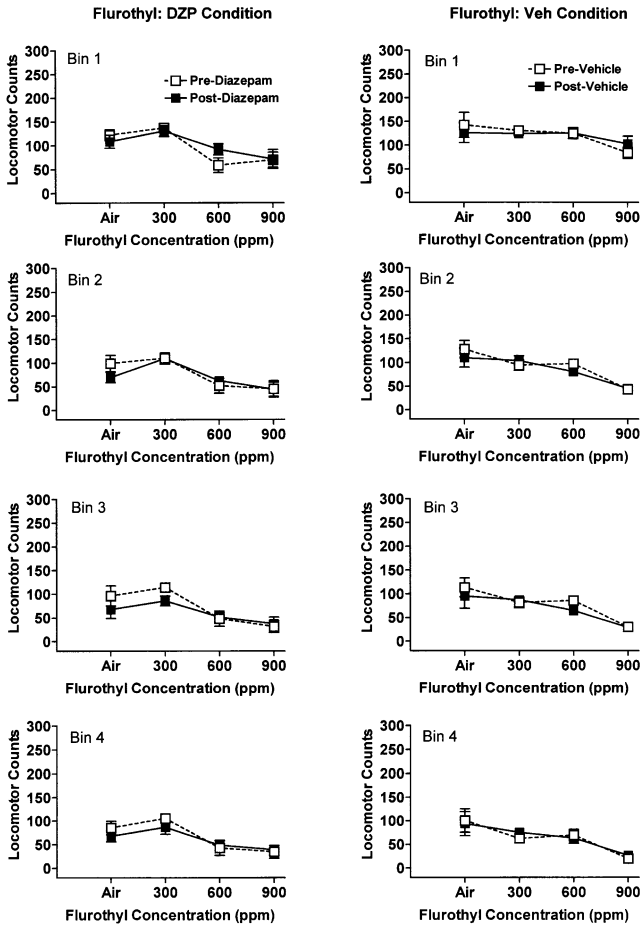

Fig. 3. Effect of flurothyl on locomotor activity before (□) and after (■) 4 days of repeated dosing with 10 mg/kg/day diazepam (left panels) or vehicle (right panels). Descending panels present activity counts during consecutive 5-min bins of 20-min test sessions. Each point represents the mean (±S.E.M.) locomotor counts for four to six mice. Flurothyl did not significantly alter activity for mice in the diazepam condition at any concentration. In the vehicle condition, 900 ppm flurothyl decreased counts compared to air.

4. Discussion

Numerous previous studies have reported that benzodiazepines and other CNS depressants often produce a biphasic effect on locomotor activity with stimulation at low doses and suppression at higher doses (Davies and Steinberg, 1984; Herberg and Williams, 1983; Soderpalm et al., 1991). Biphasic concentration–effect curves for locomotor activity have also been obtained with TCE (Warren et al., 2000), but not with flurothyl (Bowen and Balster, 1998). In the present study, the concentrations of drug/inhalant tested did not always result in a biphasic concentration–effect

curve; hence, examination of the initial level of drug-induced activity was crucial prior to evaluation and interpretation of tolerance development. To this end, it is apparent that acute exposure to diazepam and to each inhalant produced different patterns of effects on activity. Whereas acute dosing with diazepam decreased locomotor counts at all doses tested in both diazepam and vehicle groups, acute exposure to TCE increased locomotor counts in both groups at one or more concentrations. Compared to air exposure conditions, significant decreases in activity following TCE were not observed, although a return to vehicle levels at concentrations above those that stimulated behavior did occur. Higher concentrations of TCE were not tested because of potential lethal effects. In other studies of TCE effects on locomotor activity, concentrations of 16,000 ppm produced substantial decreases in activity after 10–20 min of exposure (Warren et al., 2000). In the present study, examination of the data in 5-min bins revealed that the control levels of activity of high TCE concentrations (12,000–16,000 ppm) reflect activity-increasing effects early in the exposure progressing to decreases (near-anesthesia) by the end of exposure. Acute exposure to flurothyl decreased locomotor counts only in the vehicle group and the magnitude of this decrease was small. In summary, then, acute administration of diazepam depressed activity, acute exposure to TCE increased activity and acute flurothyl did not produce pronounced effects at any of these subconvulsant concentrations.

Several investigators have reported that tolerance to the anxiolytic, discriminative stimulus, rate-decreasing and enhanced exploratory effects of benzodiazepines developed following rigorous repeated dosing schedules (File and Pellow, 1985; Ishihara et al., 1993; McMillan, 1992; Pugh et al., 1992). In contrast, tolerance to diazepam's locomotor suppressant effects failed to develop following the more modest diazepam dosing schedule of four daily injections of 10 mg/kg used in the present study. The pre- and posttreatment diazepam dose–effect curves were nearly identical for mice that received vehicle and those that received diazepam treatment and showed very little variability. Further, locomotor counts for mice in all groups that received vehicle or air on both test days were similar before and after they received repeated diazepam or vehicle injections, suggesting that repeated dosing with diazepam did not have residual effects on locomotor activity 24 h after the final dose. There are at least two possible explanations of the lack of tolerance to diazepam that occurred in this study. First, a more moderate diazepam dosing schedule of four daily injections of 10 mg/kg was used in the present study. While it is possible that increased frequency or duration of diazepam administration would have resulted in tolerance development, benzodiazepine tolerance development in another type of motor task (tilt plane) has been observed previously with as few as one prior injection (Khanna et al., 1998). Second, mice in the present study were placed into their home cages following each of the repeated diazepam injections. Because they were not exposed to the locomotor chambers while under the influence of diazepam except during the test sessions, context-dependent aspects of any behavioral tolerance that may have developed would probably have been masked by the change in environment during the test session.

In contrast to the lack of tolerance with diazepam, the modest repeated diazepam dosing schedule used here affected subsequent response to TCE. The locomotor effects of TCE were dependent on bin. For both treatment groups, activity during the first 5 min of the session was notably different from that in later bins, with the primary difference being the lack of a biphasic concentration–effect curve during the first bin. Warren et al. (2000) have shown that TCE levels in the brain and blood equilibrated rapidly following inhalation. With 6 min of exposure, these levels were at 77% of those at 30 min and remained high for the remainder of the 30-min session. Hence, the differences observed here in activity during the first bin are probably due to insufficient time to reach steady-state TCE levels. For this reason, the remaining part of the discussion of TCE effects focuses on the effects of TCE on locomotion during the last 15 min of the session.

While repeated dosing with vehicle did not alter the concentration-related biphasic effects of TCE during Bins 2–4, repeated dosing with diazepam produced an upward shift in the TCE concentration–effect curve at concentrations of 8000, 12,000 and 16,000 ppm. The effect of each of these concentrations was not attenuated after repeated diazepam administration, but rather, their initial stimulatory effect was enhanced. Hence, these results suggest the development of sensitization, not cross-tolerance. Further, this sensitization occurred following administration of a diazepam regimen that did not produce tolerance or sensitization to diazepam itself. Differential development of tolerance/sensitization to the depressant and stimulant effects of diazepam has previously been noted (File and Pellow, 1985; McMillan, 1992) and suggests differences in underlying mechanisms for the two types of effects. The development of tolerance versus sensitization to toluene shows similar behavioral selectivity in that the same exposure regimen produces tolerance to some of toluene's effects and sensitization to others (Moser and Balster, 1981; Himnan, 1984). Further, the time course for development of tolerance to the effects of toluene differs from that for development of sensitization to its effects (Himnan, 1984). While this study was not specifically designed to investigate behavioral factors involved in tolerance/sensitization to the locomotor effects of TCE, it is unlikely that the observed sensitization was a result of behavioral contingencies in the experiment. Mice in both groups were exposed to TCE twice, but only mice in the diazepam-treated group developed sensitization, suggesting that acute sensitization to the initial TCE exposure cannot account for the results. Further, since diazepam-treated mice experienced the pharmacological effects of diazepam in the home cage and those of TCE in the exposure chamber, opportunity for learning while intoxicated (i.e., behavioral tolerance or sensitization) was limited. Collectively, these results suggest that different

neural mechanisms may underlie the development of tolerance and sensitization to toluene and perhaps other inhalants. The present results are consistent with this hypothesis in that cross-sensitization to the activity-increasing effects of TCE developed in the absence of the development of tolerance to the motor-impairing effects of diazepam.

In contrast to results with TCE, flurothyl concentration–effect curves were not affected by repeated administration of diazepam or vehicle. Previous studies have demonstrated that flurothyl does not share acute behavioral effects with toluene or TCE in a number of procedures, including a functional observational battery (Bowen et al., 1996b), elevated plus maze (Bowen et al., 1996a) and pentobarbital discrimination procedure (Rees et al., 1987c). The effects of flurothyl are more accurately classified as excitatory, rather than inhibitory or depressant, and include pentylenetetrazol-like discriminative stimulus effects (Evans and Balster, 1992) and convulsions at higher concentrations (Adler, 1975). Given these differences between the acute effects of flurothyl and TCE and diazepam, it is not surprising that repeated dosing with diazepam failed to alter flurothyl's effects.

In summary, the results of the present study demonstrate that a modest schedule of repeated diazepam administration that does not induce tolerance to the activity-suppressant effects of diazepam itself is sufficient to produce sensitization to the locomotor-stimulating effects of TCE. This effect was behaviorally selective in that alteration of the concentration–effect curve only occurred in conjunction with initial inhalant-induced increases in activity. Neither sensitization nor tolerance to flurothyl was observed. Previous research has shown that TCE and other abused volatile inhalants, but not flurothyl, share a profile of acute behavioral effects similar to those of CNS depressants, including diazepam. The present results offer support for the hypothesis that sensitization to the locomotor-stimulating effects of TCE involves common mechanisms with those that are altered during repeated injection with diazepam. Further, the specificity of this sensitization for the abused inhalant TCE, but not for flurothyl, suggests that these results may be pertinent to understanding mechanisms involved in chronic inhalant abuse.

Acknowledgments

This research was supported by grants from the National Institute on Drug Abuse (DA-03112) and the National Center for Research Resources (RR-10118).

References

Adler MW. Pharmacology of flurothyl: laboratory and clinical applications. In: Essman W, Valzelli L, editors. Current developments in psychopharmacology. New York: Spectrum Publications, 1975. pp. 31–78.

Balster RL. Neural basis of inhalant abuse. Drug Alcohol Depend 1998; 51:207–214.

Bowen SE, Balster RL. Effects of inhaled 1,1,1-trichloroethane on locomotor activity in mice. Neurotoxicol Teratol 1996;18:77–81.

Bowen SE, Balster RL. A direct comparison of inhalant effects on locomotor activity and schedule-controlled behavior in mice. Exp Clin Psychopharmacol 1998;6:235–247.

Bowen SE, Wiley JL, Balster RL. The effects of abused inhalants on mouse behavior in an elevated plus-maze. Eur J Pharmacol 1996a; 312:131–136.

Bowen SE, Wiley JL, Evans EB, Tokarz ME, Balster RL. Functional observational battery comparing effects of ethanol, 1,1,1-trichloroethane, ether, and flurothyl. Neurotoxicol Teratol 1996b;18:577–585.

Bowen SE, Wiley JL, Jones HE, Balster RL. Phencyclidine- and diazepam-like discriminative stimulus effects of inhalants in mice. Exp Clin Psychopharmacol 1999;7:28–37.

Bushnell PJ, Oshiro WM. Behavioral components of tolerance to repeated inhalation of trichloroethylene (TCE) in rats. Neurotoxicol Teratol 2000; 22:221–229.

Davies C, Steinberg H. A biphasic effect of chlordiazepoxide on animal locomotor activity. Neurosci Lett 1984;46:347–351.

Evans EB, Balster RL. Effects of methoxyflurane and flurothyl in mice trained to discriminate pentylenetetrazol from saline. Behav Pharmacol 1992;3:465–473.

File SE, Pellow S. No cross-tolerance between the stimulatory and depressant actions of benzodiazepines in mice. Behav Brain Res 1985;17:1–7.

Glaser HH, Massengale ON. Glue sniffing in children. Deliberate inhalation of vaporized plastic cements. J Am Med Assoc 1962;181:300–302.

Herberg LJ, Williams SF. Anti-conflict and depressant effects by GABA agonists and antagonists, benzodiazepines and non-GABAergic anticonvulsants on self-stimulation and locomotor activity. Pharmacol, Biochem Behav 1983;19:625–633.

Himnan DJ. Tolerance and reverse tolerance to toluene inhalation: effects open-field behavior. Pharmacol, Biochem Behav 1984;21:625–631.

Ishihara S, Hiramatsu M, Kameyama T, Nabeshima T. Development of tolerance to anxiolytic effects of chlordiazepoxide in elevated plus-maze test and decrease of $GABA_A$ receptors. J Neural Transm: Gen Sect 1993; 91:27–37.

Khanna JM, Kalant H, Chau A, Shah G. Rapid tolerance and crosstolerance to motor impairment effects of benzodiazepines, barbiturates, and ethanol. Pharmacol, Biochem Behav 1998;59:511–519.

Knisely JS, Rees DC, Balster RL. Discriminative stimulus properties of toluene in the rat. Neurotoxicol Teratol 1990;12:129–133.

McMillan DE. Effects of drugs on behavior before and during chronic diazepam administration. Eur J Pharmacol 1992;215:145–152.

Moser VC, Balster RL. The effects of acute and repeated toluene exposure on operant behavior in mice. Neurobehav Toxicol Teratol 1981;3:471–475.

Moser VC, Scimeca JA, Balster RL. Minimal tolerance to the effects of 1,1,1-trichloroethane on fixed-ratio responding in mice. Neurotoxicology 1985;6:35–42.

National Institute on Drug Abuse. Preliminary estimates from the 1994 National Household Survey on Drug Abuse. Rockville (MD): Division of Epidemiology and Statistical Analysis, National Institute on Drug Abuse, 1995.

Novak A. The deliberate inhalation of volatile substances. J Psychedelic Drugs 1980;12:105–122.

Pugh SL, Boone MS, Emmett-Oglesby MW. Tolerance, cross-tolerance and withdrawal in rats made dependent on diazepam. J Pharmacol Exp Ther 1992;262:751–758.

Rees DC, Knisely JS, Breen TJ, Balster RL. Pentobarbital-like discriminative stimulus properties of halothane, 1,1,1-trichloroethane, isoamyl nitrite, flurothyl and oxazepam in mice. J Pharmacol Exp Ther 1987a; 241:507–515.

Rees DC, Knisely JS, Breen TJ, Balster RL. Toluene, halothane, 1,1,1-trichloroethane and oxazepam produce ethanol-like discriminative stimulus effects in mice. J Pharmacol Exp Ther 1987b;243:931–937.

Rees DC, Knisely JS, Jordan S, Balster RL. Discriminative stimulus properties of toluene in the mouse. Toxicol Appl Pharmacol 1987c; 88:97–104.

Rees DC, Wood RW, Laties VG. Evidence of tolerance following repeated exposure to toluene in the rat. Pharmacol, Biochem Behav 1989;32: 283–291.

Soderpalm B, Svensson L, Hulthe P, Johannessen K, Engel JA. Evidence for a role for dopamine in the diazepam locomotor stimulating effect. Psychopharmacology 1991;104:97–102.

Warren DA, Bowen SE, Jennings WB, Dallas CE, Balster RL. Biphasic effects of 1,1,1-trichloroethane on the locomotor activity of mice: relationship to blood and brain solvent concentrations. Toxicol Sci 2000;56: 365–373.

Wood RW, Coleman JB, Schuler R, Cox C. Anticonvulsant and antipunishment effects of toluene. J Pharmacol Exp Ther 1984;230:407–412.